The Center for Biological and Environmental Nanotechnology (CBEN)

Annual Report to the National Science Foundation (NSF)

Report Year: 2003
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3. Project Summary

The Center for Biological and Environmental Nanotechnology, or CBEN, aims to discover and develop nanomaterials that enable new medical and environmental technologies. This mission is inspired by the fact that nanomaterials interact with and control biological systems in ways completely distinct from bulk substances. Located at Rice University, CBEN engages in collaborative, interdisciplinary research activities with over twenty participants from ten departments. Nanoscience is important in CBEN because an understanding of the wet/dry interface between nanomaterials, complex aqueous systems, and ultimately the environment is necessary for intelligent material design. This knowledge supports engineering efforts to integrate nanomaterials into systems that solve outstanding problems in environmental and biological engineering. Our centerwide programs develop the technical workforce of the future, ensure efficient transfer of CBEN research into the marketplace, and communicate the implications of CBEN research to policymakers and media professionals.

The intellectual merits of our activities are illustrated by our diverse research accomplishments which have resulted in over 60 peer-reviewed publications. Some highlights from this year include new uses for dual-purpose nanoshell materials in imaging and therapeutic applications (West and Drezek); reactive ferroxane membranes for the removal and remediation of contaminants in water (Wiesner and Barron); the development of Fluid Electrical Force Microscopy, a new scanning probe microscopy technique that measures charge in biological systems at the nanometer scale (Hafner and Natelson); and the discovery by Colvin, West and Hughes that functionalization of environmentally active fullerene nanocrystals is a simple and effective method for detoxifying them, a result with far-reaching implications for minimizing the potential environmental impacts of many types of commercially useful nanomaterials. Already, CBEN researchers are working on a similar protocol to assess and minimize the eco-toxicology of other candidate nanomaterials, including nanocrystalline titanium dioxide.

The broader impacts of CBEN programs are substantial. Teacher training initiatives for ninth grade science have engaged over fifty local teachers from Houston, Dallas, and Laredo, Texas with CBEN members. The innovative imagery of Nanokids™, developed by CBEN organic chemist Tour, has been successfully piloted and evaluated in the Houston school district. Through these and other K-12 outreach efforts CBEN has in a relatively short time had an impact on hundreds of teachers and students. Over seventy undergraduate and graduate students have been involved in CBEN research projects and a larger number benefit from the many new courses offered at Rice as a result of our programs. We have made great strides in developing a diverse faculty and student population of underrepresented minorities, and now meet or exceed the national averages in most of our membership categories. Finally, CBEN is creating a new broad-based coalition to address and minimize nanomaterial risk called ICON: The International Council on Nanotechnology. This organization will include industry, academics, government officials, and representatives of environmental organizations in a collective effort to assess, communicate, and reduce environmental and health risks associated with nanotechnology.

Through our knowledge transfer activities, CBEN has reached thousands of people through popular articles, informed lawmakers’ work on legislation that codifies the national nanotechnology initiative, engaged with industrial and government partners to assess and minimize nanomaterial risk and worked with policymakers in a variety of federal departments and agencies to ensure that nanotechnology continues to develop responsibly and with strong public support.
## 4. Quantifiable Outputs

### Publications resulted from NSEC Support

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### NSEC Influence on Curriculum (if applicable)

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* Printed, In Press, Submitted

** Implemented – CBEN contributed to 2 of the 3 new Sloan Professional Masters programs now underway at Rice: Nanoscale Physics prog.; Environmental Decision Making prog.

*** Cumulative total reflects only Printed/Implemented
5. Mission and Broader Impact

CBEN’s mission is to discover and develop nanomaterials that enable new medical and environmental technologies. The mission is accomplished by the following:

- **Fundamental examination of the ‘wet/dry’ interface** between nanomaterials, complex aqueous systems, and ultimately our environment (Theme 1).
- **Engineering research** that focuses on integrating nanomaterials into systems that solve problems in environmental and biological engineering (Themes 2, 3).
- **Educational programs that develop teachers, students, and citizens** who are well informed and enthusiastic about nanotechnology.
- **Innovative knowledge transfer activities** that recognize the importance of communicating nanotechnology facts to media, policymakers, and the general public.

This mission is inspired by the observation that because of their small size and unique properties, nanomaterials interact with and control biological systems in entirely new ways. Our research exploits these novel capabilities to develop innovative biomedical and environmental technologies. To ensure that our technologies flourish, our outreach addresses broader issues such as technology transfer, public acceptance, and workforce training.

In our center’s first thirty months, we have achieved many diverse research accomplishments ranging from a novel photothermal treatment for cancer to the first characterization of the environmental implications of \( C_{60} \) (refer to Section 8.0). Some highlights from this year include the development of Fluid Electrical Force Microscopy, a new form of scanning probe microscopy (Hafner), new uses for dual-purpose nanoshell materials in imaging and therapeutic applications (West and Drezek); reactive ferroxane membranes for the removal and remediation of contaminants in water (Wiesner and Barron) and the discovery by Colvin, West and Hughes that functionalization of environmentally active fullerene nanocrystals is a simple and effective method for detoxifying them. Finally, we note that basic science in CBEN is quickly transitioning into our engineering systems. Last year CBEN researchers Weisman and Smalley reported the first observations of SWNT near-infrared emission. In large part because of CBEN’s mission, they have quickly teamed with experts from the Texas Medical Center to use nanotube fluorescence in medical imaging applications. Recently they have demonstrated *in-vitro* imaging from nanotube labeled cells.

CBEN has made significant progress in developing scientifically literate students and teachers at the K-12 level; a focus on this younger generation is essential for the long-term development of a technical workforce. Much of our work here is aimed at increasing the quality and quantity of scientifically literate students and teachers at the pre-college level and is described in Section 9.0. Our ninth grade science program develops better teachers through a CBEN organized content class, CBEN summer fellowships, and a sabbatical for curriculum development. We have worked with over one hundred local teachers to date, and also created more concentrated classes for communities throughout Texas and the nation. CBEN also reaches out directly to students using the innovative imagery of Nanokids™; this effort, led by CBEN organic chemist Tour, takes advantage of the visual appeal of animated molecules to teach the concepts of nanoscience and engineering. Our local school district has completed an enormously successful beta test of Nanokids™ content in its middle school classrooms using CBEN
sponsored materials. Through these and other K-12 outreach efforts CBEN has, in a relatively short time, had impact on hundreds of teachers and students (refer to Section 9).

Near-term, it is vital that CBEN train the next generation of nanotechnologists to sustain and grow the industries spawned by our center’s efforts. Over seventy undergraduate and graduate students have been involved in CBEN research projects and a greater number benefit from the many new courses offered at Rice as a result of CBEN’s programs. Our nanotechnology curriculum has taken on new directions with Rice’s Sloan Professional Master’s programs in nanoscale physics and environmental analysis and decision-making.

We strive to increase diversity in all of our training programs. Our K-12 educational outreach programs serve the largely minority Houston school district; the majority of our participants are underrepresented minorities. CBEN’s graduate student population is nearly half female, which far exceeds the national average in science and engineering. CBEN is making strides in involving underrepresented minorities in both our faculty and student populations as compared to national averages. We welcome Hispanic-American environmental engineer Pedro Alvarez to our center, and he will assume a leadership role in Theme 3 research. His involvement in CBEN will be instrumental in strengthening our ties to the Hispanic community. In the last year, we succeeded in recruiting two minority students to CBEN-funded projects; we now meet the national averages for African-American and Hispanic graduate students. While these numbers are encouraging for our relatively new start, we are setting far more ambitious long-term goals for diversity in our center. Our summer undergraduate research program is the centerpiece of this effort, and we are pleased that our recruiting efforts this year resulted in a class that has several underrepresented minorities. In addition, we are developing new relationships with minority-serving institutions such as Prairie View A&M University, Texas Southern University, and University of Houston through outreach activities and a nascent partnership with Clarkson Aerospace, a local minority-owned technology development company.

Our knowledge transfer activities include aggressive outreach to both established and new companies. We have expanded our industrial affiliates program this year with the addition of Dupont and L’Oréal; at the encouragement of these and our other affiliates we are in the late planning stages for a new broad-based coalition of industry, government, non-governmental and academics partners to assess and minimize risks associated with nanomaterials. We anticipate that this organization, which we call ICON: The International Council on Nanotechnology, will be the central resource for information on responsible nanotechnology development.

Like biotechnology, nanotechnology is developing primarily in high-risk, high-payoff start-up companies associated with academic intellectual property. Therefore, we prepare our students for this by offering a graduate level course in entrepreneurial management. In this course, PhD scientists and engineers work with MBAs to develop business plans. We also sponsor forums to expose business experts to CBEN investment opportunities. This model has proven successful in attracting the interest of the business community. Over five hundred people have attended CBEN forums where new business ideas from Rice faculty were presented. This year, one new company, Oxane Materials, Inc., has been founded by CBEN faculty at least in part because of CBEN support of both research and academic entrepreneurship.
Finally, we have invested in knowledge transfer activities that reflect our interest and commitment to nanotechnology and the environment. The increasing public concerns with the dangers of nanotechnology and our research on the implications of nanotechnology motivated us to develop media and governmental outreach as a cornerstone of our strategic plan. (See Section 10.) Various CBEN members now interact almost weekly with media and policymakers; in a coordinated strategy we inform them and the public about nanotechnology and inject hard data into the emotional debate on ‘risks.’ Our position as a center has been essential here, as it gives us access to a wide set of research data both from CBEN and around the world. Our message on the management of risk in emerging technologies, like nanotechnology, has been widely disseminated in talks, media interviews, and congressional hearings.
6. Nuggets

6.1. Simple fix found for toxicity of fullerene clusters in water

Much attention has been paid recently to the toxic effects of nanomaterials, especially a water-soluble form of the molecule known alternately as C\textsubscript{60}, buckminsterfullerene or, simply, buckyball. These hollow, soccer-ball shaped molecules can cluster together into nanoscale crystals—dubbed “nano-C\textsubscript{60}”—that become active in watery environments. Soluble nano-C\textsubscript{60} clusters have been shown in the lab to be cytotoxic, that is, to kill cells in culture and have been linked by a CBEN collaborator to brain damage in large-mouth bass. Researchers Vicki Colvin, Jennifer West and Joe Hughes at Rice University’s Center for Biological and Environmental Nanotechnology have discovered a simple way to turn the toxicity off, like sliding a dimmer switch on a lamp, by attaching small molecular fragments to the surface of the hollow cages in a process known as functionalization. Figure 6.1 shows the differential cytotoxicity of a series of nano-C\textsubscript{60} clusters where the C\textsubscript{60} molecules have been functionalized to varying degrees. Non-functionalized fullerenes, the form used in the fish study, are toxic to half of the cells in culture at a concentration of 20 parts per billion (ppb). This is more toxic than many other organic molecules including paraquat, which is used as a pesticide. However, by attaching an increasing number of small molecular fragments to the surface of the cages, the cytotoxicity can be made to decrease steadily until, for a fully functionalized fullerene, it was not possible to add enough nano-C\textsubscript{60} to kill half the cells. This important study demonstrates that the risks of nanomaterials are highly dependent on the specific form of the material, and that simple strategies can be found to minimize these risks.

6.2. Ultrasensitive probe to create charge maps of individual molecules in solution

Researcher Jason Hafner at Rice University’s Center for Biological and Environmental Nanotechnology has developed a new technique to measure charges in biological systems at the nanometer scale, and to apply these techniques to the development of biological nanosensors. The technique, a new form of scanning probe microscopy called Fluid Electrical Force Microscopy (FEFM), can create a map of tiny charges on a single molecule in a manner that is nondestructive and demonstrates high sensitivity. FEFM has already been applied to the study of a number of biological systems, including the first direct observation of so-called “lipid rafts” in biomembranes. The molecular components of

Figure 6.1 Differential cytotoxicity of variably functionalized fullerenes.

Figure 6.2: Topography of a mica-supported DOTAP/DOPC bilayer in 3 mM NaCl. The darker areas imply regions of more positive charge density.
biomembranes are in a fluid phase, rapidly moving past one another. Biochemical evidence suggests that within the fluid biomembrane, groups of lipids cluster together in a gel phase to form “rafts” floating in the lipid “sea”. These rafts have been implicated in several biological processes, but have never been directly observed in a cell. We have observed FEFM contrast between the fluid and gel phase regions of a model membrane containing lipid rafts.

6.3. **Researchers develop method for processing nanotubes**

Single-walled carbon nanotubes, one of the most exciting nanomaterials around because of its exceptional strength, elasticity, and conductivity, have historically fallen far short of their promise because of how difficult it is to process them. Researchers Richard Smalley and Matteo Pasquali at Rice University’s Center for Biological and Environmental Nanotechnology (CBEN) have discovered that superacids, such as sulfuric acid with excess sulfur trioxide, effectively disperse the nanotubes into an easily processed form. This form, which can range from individually dissolved nanotubes to a liquid crystal, can serve as a starting material for extruded, aligned fibers. This material is a natural precursor for macroscopic objects made entirely of single-walled carbon nanotubes.

![Figure 6.3: SWNTs in and from superacids. (Upper left) Birefringent domains demonstrating nematic liquid crystal behavior. (Upper right) Aligned species, termed ailwives, formed by phase-precipitating the SWNTs by adding moisture. (Lower left) Extruded fibers of pure SWNTs with 20:1 alignment. (Lower right) a bucky-paper of entangled ropes formed by quenching into ether and filtering.](image)

6.4. **Nanoparticles do double duty in combined imaging/therapy application**

Researchers Jennifer West and Rebekah Drezek at Rice University’s Center for Biological and Environmental Nanotechnology have created miniscule particles of gold-coated glass structures called nanoshells that improve both the detection and treatment of diseased tissue. In Figure 6.4, the top two images demonstrate the power of the nanoshells to image diseased tissue more readily. Image A is a dark-field microscope image of a control sample containing no nanoshells. In Image B, which contains cells that have been treated with nanoshells, the tissue shows up as a light image against the dark background. The bottom two images demonstrate the power of the nanoshells to kill cancerous tissue by converting near-infrared light to heat energy which then “cooks” the cancer cells past the point of viability. Images C and D show samples of cancer cells that have been treated with a stain that glows green when the cells are alive. In Image C these cells have not been exposed to nanoshells, therefore, the laser light has no effect as it passes over them. Image D shows a sample of cells that have been exposed to nanoshells and then illuminated with a near-infrared laser light source. The large dark circle in the middle of Image D is where the laser light passed through the sample, was absorbed by the nanoshells, and then killed the cells in its path.

![Figure 6.4 Nanoshells can be used to both image and kill cancerous tissue](image)
6.5. Panning golden molecules from nanoparticle slurries.

CBEN researchers Colvin and Wong along with graduate student Ali Al’Somali have demonstrated a way to pick out specially sized gold clusters from a broadly distributed nanoparticle sample. They used for the first time recycling chromatography on a very bad nanocrystalline gold sample which had a large mix of sizes and shapes. By cycling the nanocrystal solution multiple times through a size exclusion chromatography column, they were able separate very specially sized gold clusters. The chromatogram (left) shows the special golden clusters which appear as sharp peaks. Peaks one and three, for example, could be collected from the column and correspond to gold nanocrystals different in size by only 6 angstroms, or the size a small organic molecule. Recycling chromatography gives nanochemistry researchers a new way to select the best materials. It also allows allows the solvents used to make nanocrystals to be recycled leading to a greener and more environmentally friendly manufacturing process.

Figure 6.5 First recycled chromatogram of gold nanocrystals. Each peak corresponds to a specific size of gold particle that can be collected and purified. This technique can separate nanocrystals that differ in size by only 6 angstroms.

6.6. CBEN launches new international effort to ensure responsible development of nanotechnology

CBEN is fostering the creation of a new broad-based coalition to address and minimize nanomaterial risk called the International Council on Nanotechnology (ICON). The mission of this organization is to assess, communicate, and reduce environmental and health risks associated with nanotechnology while in turn maximizing its benefits to society. ICON represents a new approach to an industrial affiliates program in that it welcomes not only corporate members, but also government, non-governmental organizations (NGOs) and other academics. This broader partnership is vital to our core mission of creating a sustainable nanotechnology industry that, in contrast to technology development goals, requires meaningful and organized interactions among stakeholders. ICON’s activities span technical research in nano-cell interactions, policy projects such as development of nanomaterial standards and terminology, and social studies of risk perception and communication. By pooling the resources of these diverse partners, ICON can effectively provide a wide range of synergistic projects that serve the interests of all stakeholders. There is widespread enthusiasm for this organization, which will create new knowledge of use to government and industry researchers and serve as a central clearinghouse for information related to health and environmental aspects of nanomaterials. By catalyzing the formation of ICON, we are taking the first, early steps to ensuring that CBEN creates a legacy that lives beyond its ten-year NSF funding cycle.
7. **Strategic Research Plan**

CBEN discovers and develops nanomaterials to enable new medical and environmental technologies. Nanotechnologies generally have great potential to revolutionize how we treat disease and clean our environment. CBEN focuses on those technologies that use chemically prepared, soluble nanomaterials. These materials are the same size as naturally occurring biomolecules, and can be designed for specific functions in biological and environmental systems. Successful applications require fine manipulation of the interface between inorganic nanostructures (the dry side) and biological systems (the wet side). CBEN has termed this the ‘wet/dry interface’ and its control is the organizing theme of all of CBEN’s science and engineering research.

Though unified intellectually by the wet/dry interface, our research programs are oriented toward tangible technological outcomes, or engineered systems. These are:

- Nanoparticles that detect and treat disease
- Effective, high performance water purification systems

Each of these goals carries substantial technical challenges specific to its particular objectives that are addressed in CBEN’s environmental (Theme 3) and bioengineering (Theme 2) thrust areas. We have also identified two potential roadblocks to these technologies that lie outside of their specific technical areas. They are the development of sustainable manufacturing methods for nanoparticles and the stewardship of nanotechnology-based products. We address the first roadblock in the third objective, which cuts across CBEN. The second roadblock is not entirely technical, but as has become recently apparent, remains a critical issue to address. For this reason, we retain both a strong research effort on health effects and environmental studies of engineered nanomaterials as well as substantial outreach focus on educating the public on real versus perceived risks of nanotechnology.

Every year, CBEN members participate in a workshop to update our strategic plan, the text of which follows this summary. This document serves to provide guidance to members when developing and reporting on projects, as well as to introduce interested partners to the goals and operations of CBEN. Finally, it highlights emerging technical needs in the center. The prominent conclusions of this year’s discussion:

- CBEN will catalyze an alliance of other academic centers, industry, government and non-governmental partners on nanotechnology stewardship.
- Theme 3 will choose a specific water treatment problem and partner(s) for its detailed systems engineering goals by the end of year 4.
- CBEN’s strategic plan should be altered to reflect our efforts to overcome commercialization barriers, as well as our systems engineering goals.
- Theme 2 raises external funding for systems-level (e.g. animal) work and should use CBEN funds to overcome roadblocks and evaluate new candidate nanomaterials.
7.1. CBEN Organization

Research in CBEN is organized into distinct but highly interrelated projects; we strongly select for interdisciplinary teams of at least two and more typically three and four investigators. These groups work on problems that can be grounded in fundamental science, focused on enabling technologies, or a combination of the two. In all cases they must be clearly related to the engineering systems goals as articulated in this plan for CBEN.

All projects in CBEN have strong interactions with other efforts in the center. However, for management reasons we group efforts roughly based on their objective: theme 1 for materials development, manufacturing, and fundamental science, theme 2 for bioengineering oriented projects and theme 3 for environmental engineering efforts. Also, CBEN has evolved a number of common objectives that are apparent in distinct engineering efforts. For example, in many projects the need to produce water-soluble and stable nanoparticles of various types is a major goal.

7.2. Systems-level Goals

CBEN devotes significant resources to two equally important parts of the technology development and commercialization pipeline: developing the engineered systems and addressing significant commercialization barriers. The technology development efforts are the first organizing principle in CBEN. They prioritize the research efforts and define which commercialization barriers are of most importance. They are ambitious and at the early stages of CBEN it is unlikely that all participants will be working directly on a systems-level goal. Still, all enabling technologies and fundamental science in CBEN are directed from considerations of these tangible outcomes. Center members and especially project leaders can articulate clearly how and when their work will be used in developing engineered systems.

In technology development, CBEN has picked two systems-level goals from among the multitude of possible ways to use nanomaterials in biological and environmental engineering. These goals define and structure our current research:

**Nanoparticles that detect and treat disease.** Nanoparticles can be three-dimensional biomaterials whose small size permits them to access regions of the body not available to conventional devices. We explore a variety of particle types, and detection and treatment schemes. Drug delivery, photothermal cancer treatments and imaging contrast agents (magnetic, optical, etc.) are some examples where soluble nanoparticles play a key role.

**Nanomaterials for water purification.** Nanoscale materials, whether they are porous membranes or nanoscale catalytic particles, can offer the means to both remove and remediate waste. We support projects that aim to build multifunctionality into these systems, and that articulate specific and relevant environmental problems (*for example*, arsenic removal) as their long-term goal.

CBEN research also responds to the significant roadblocks ahead for commercialization of nanotechnology generally, with attention to issues most relevant in and bioengineering and environmental engineering applications. This year, we have adjusted our strategic plan to account for these activities in a top-down manner strongly inspired by the systems-engineering approach to organizing technology-development-focused research centers. At the top level of this analysis, we have identified the primary barriers to successful commercialization to be
inexpensive, environmentally-friendly synthesis of nanoparticles on bulk scales, and stewardship of nanotechnology-based products and research in a climate where public fears of new, invisible technologies are of serious concern to consumers. In fact, it is our center’s leadership role in these areas that has motivated our industrial affiliates to work with CBEN.

**Sustainable methods for nanomanufacturing.** It is critical to consider ways to make nanostructures that are practical, scalable, green, and cost-effective. Projects that seek to address one of the three elements of sustainability (economic, environmental and social) are encouraged by CBEN. This is both an enabling technology for all other systems goals, as well as an engineered system alone.

**Nanotechnology stewardship.** With the rapid pace of nanotechnology’s growth, it is essential to evaluate the potential risks from its application. Such information enhances public communication about nanotechnology, and permits policymakers to make well grounded decisions about risk management in this new industry. Projects that address the biological and environmental effects on nanotechnology, remediation strategies for potentially hazardous materials, and the social aspects of technology acceptance are required to address this commercialization barrier.
7.3. **CBEN's Three-Plane Chart: Technology Development**

In keeping with the engineering research center’s model for center organization, CBEN’s efforts can be described with a three plane chart, as shown in Figure 7.1. All critical needs for CBEN as well as potential roadblocks to our systems engineering goals were considered in order to develop the research tasks. These were then organized based on whether they involved fundamental science, enabling technology, or systems engineering (specific planes). Because of our focus on soluble nanostructures, our systems engineering efforts share many enabling technologies. More detailed descriptions of the chart entries are available in section 7.6.
7.4. Technology Development Milestone Chart

The following is a list of planned milestones and deliverables:

1. NPs with tunable, strong near-infrared absorption/scattering/emission
2. Conjugation strategies for coupling NPs to water-soluble coatings
3. Localized heating of tissue due to near-infrared absorption from NPs
4. Biocompatible coatings that minimize non-specific adsorption/aggregation
5. Characterization methods for bio-nanoconjugate orientation, stoichiometry, and activity
6. Bioavailability of NPs in extracellular matrix
7. Soluble and stable (physically and chemically) NPs in biological fluids
8. Bioconjugation strategies for coupling inorganic particles to proteins
9. Controlled NP aggregates with tunable pore size and thickness
10. NPs with efficient photogeneration of OH radicals/catalytic sites for dehydrogenation
11. Understanding of bioavailability, fate and transport of NPs in living organisms
12. Understanding of bioactivity of bio-nanoconjugates
13. Light delivery system for efficient illumination of tissue with near-infrared light
14. NPs that efficiently clear from the body
15. Toxicological understanding of select nanoparticles
16. Integrated therapeutic process for laser tissue welding
17. NPs that effectively target particular cells \textit{in vivo} with high selectivity
18. NPs with tunable, large permanent magnetic dipole moments
19. Integrated therapeutic process for targeted photothermal cancer therapy
20. Imaging system for near-infrared detection
21. NPs that sorb target contaminants with high selectivity
22. Water purification systems based on ultra- and nanofiltration membranes
23. Light delivery methods for photothermal and photoactivated remediation
24. Integrated system for bioimaging using NP-scattered near-infrared light
25. Integrated system for bioimaging using NP-emitted near-infrared light
26. Magnetic separation systems for treatment of arsenic and pesticides in water
27. Systems for simultaneous pumping and treatment of contaminated groundwater
7.5. **CBEN’s Three-Plane Chart: Roadblocks**

CBEN’s efforts to address the primary roadblocks to commercialization and public acceptance of nanotechnology can also be described by a three plane chart, as shown in Figure 7.3. All critical needs for CBEN as well as potential roadblocks to our systems engineering goals were considered in order to develop the research tasks. These were then organized based on whether they involved fundamental science, enabling activities, or full roadblocks (specific planes). The overlap between several of the fundamental science and enabling activities entries between this three-plane chart and the one for technology development demonstrates the dramatic synergy among the various research goals. More detailed descriptions of the chart entries are available in section 7.6.
7.6. Three-Plane Chart Reference: CBEN research tasks

7.6.1. Systems goal #1: Nanoparticles that detect and cure disease.

The use of nanoparticles in medicine is a very recent development, but in the last few years there has been an explosion of research activity. This work, from CBEN researchers and others, demonstrates the high value of soluble nanomaterials in biomedical technologies ranging from drug delivery to cancer therapy.

Enabling technologies
- Nanoparticle chemistry
  - Nanoparticles that effectively target particular cells \textit{in vivo} with high selectivity
  - Soluble and stable (physically and chemically) nanoparticles in biological fluids
  - Nanoparticles with tunable properties including:
    - Efficient and stable near-infrared emission
    - Strong near-infrared absorption
    - Large permanent magnetic dipole moments
    - Biocompatible coatings for nanoparticles that minimize non-specific adsorption of proteins in biological fluids
    - Responsive nanoparticle composites that alter properties in response to physiological conditions
- Biomedical engineering
  - Localized heating of tissue due to near-infrared absorption from nanoparticles
  - Bioavailability of nanoparticles in extracellular matrix
  - Light delivery systems for efficient illumination of tissue with near-infrared light
  - Imaging systems for scattering/emissive nanoparticle detection of cancer

Fundamental Science
- Bioconjugation strategies for coupling inorganic particles to proteins
- Theoretical models for nanoparticle solubilization in water
- Predictive models for nanoparticle properties
- Methods to characterize nanoparticle-biomolecule conjugates to determine orientation, stoichiometry, and activity
- Discovery of new nanomaterials with tunable near-infrared photonic and/or magnetic properties
- Separation schemes for distinguishing between nanoparticle size, aspect ratio, surface chemistry and helicity (for example, SWNT)
- Basic understanding of how and why biological activity is influenced by nanoparticle conjugation
- Characterization of the bioavailability, fate, and transport of nanoparticles in living organisms
- Responsive nanoparticle coatings to allow for switching of sorptive behavior in water
7.6.2. Systems goal #2: Effective, high performance water purification systems

*Enabling Technology*

- **Nanoparticle chemistry**
  - Nanoparticles that sorb target contaminants in wastewater with high selectivity
  - Soluble and stable (physically and chemically) nanoparticles in water
  - Nanoparticles with high surface areas and tunable properties including:
    - Efficient generation of OH radicals with visible light
    - Catalytic sites for dehydrogenation reactions
    - Large permanent magnetic dipole moments
    - Biocompatible coatings for nanoparticles that minimize non-specific adsorption of organic matter in waste and ground water
    - Responsive nanoparticles that alter aggregation states in response to chemical additives
    - Controlled nanoparticle aggregates with tunable pore size and thickness

- **Environmental engineering**
  - Water treatment systems based on ultra- and nanofiltration membranes
  - Magnetic separation systems for treatment of arsenic and pesticides in water
  - Water treatment systems for simultaneous pumping and treatment of contaminated groundwater
  - Efficient light delivery methods for photothermal and photoactivated remediation of environmental contaminants

*Fundamental Science*

- Fundamental understanding of membrane fouling processes
- Characterization of how grain size influences magnetic separation processes
- Evaluation and predictive models for arsenic sorption to nanoparticle surfaces
- Determination of the mechanism of activity of bimetallic catalysts
- Identification of factors that produce more effective photocatalytic nanomaterials
7.6.3. Overcoming roadblock #1: Sustainable manufacturing systems for nanoparticle production

*Enabling technology*

- **Nanoparticle chemistry**
  - Predictive models for nanoparticle formation processes in batch reactions
  - Online characterization methods for rapid analysis of nanoparticle quality
  - Soluble and stable (physically and chemically) nanoparticles in water
  - Biocompatible coatings for nanoparticles that minimize aggregation in water
- **Reactor design engineering**
  - Staged batch reactors for high yield formation of nanoparticles
  - Fast and efficient methods for separating nanoparticles for solvent recycling
  - Feedback controlled reactors for optimizing nanoparticle quality
  - Continuous flow reactors with automated separation and analysis schemes

*Fundamental Science*

- Conjugation strategies for coupling inorganic particles to water soluble coatings
- Theoretical and general models for crystallization in nanoscale solids
- New characterization methods for rapid evaluation of nanoparticle quality
- Separation schemes for distinguishing between nanoparticle size, aspect ratio, surface chemistry and helicity (for example, SWNT)
- Characterization of the bioavailability, fate, and transport of nanoparticles in living organisms
- Development of life-cycle assessment for target CBEN nanomaterials
7.6.4. Overcoming roadblock #2: Stewardship for nanotechnology products

**Enabling activity**

- **Social science**
  - Identification of factors that lead to public trust in new technologies
  - Understanding of why technologies can generate unfounded public fear
  - Characterization and monitoring of consumer confidence in nanotechnology
- **Community-building**
  - Launch of a public-private partnership on nanotechnology stewardship
  - Sustained knowledge transfer to journalists on nanotechnology and risk
  - Updated public web page with commented peer-reviewed publications
- **Technological solutions**
  - Fate and transport model for engineered nanomaterials in water
  - General strategies for rendering engineered nanomaterials non-toxic
  - Definition of which engineered nanomaterial features (e.g. size, surface chemistry, composition) should guide hazard assessments

**Basic Science**

- Characterization of the bioavailability, fate, and transport of nanoparticles in living organisms
- Development of life-cycle assessment for target CBEN nanomaterials
- Fundamental understanding of nanoparticle-cell interactions
- Determination of the biodegradation rates of nanostructures in natural waters
- Toxicological characterization of engineered nanomaterials (partnerships)
8. Research Program, Accomplishments, and Plans

Summary of research organization and objectives

Nature provides us with breathtaking examples of elegant and functional nanosystems. From magnetic bacteria that sense the earth's magnetic field using nanosized bar magnets, to the nanoparticle-mediated transport of inorganic material in wastewater, the world around us is filled with examples of nanomaterials in action. These examples illustrate that the interaction between nanosystems and biosystems can be a strong and important one, a lesson that nanoscientists are just beginning to explore in the design of artificial, chemically prepared nano-biosystems. Our Center's research seeks to understand and ultimately manipulate this interaction, which we term the wet/dry interface, as it manifests over a wide range of length scales, ranging from biomolecules to the earth's environment.

CBEN's research program is oriented towards specific engineered systems that exemplify how nano-biosystems can be used to solve real world problems. One systems goal is the development of hybrid bio-nanomaterials that can detect and ultimately treat disease. In Theme 2, CBEN researchers under the direction of theme leader Dr. West pursue both near-term enabling technologies and as well as longer term testbeds (e.g. animal studies) for using nanomaterials to solve problems in bioengineering. A second engineered system goal is the development of more efficient and higher performance water treatment systems. This research is carried out in Theme 3 and is led now by a two theme directors, Dr. Joe Hughes who has recently made a move to Georgia Tech, and Dr. Pedro Alvarez, a new faculty member joining Rice as a chaired professor in environmental engineering. By exploiting state-of-the-art nanochemistry these projects demonstrate that nanomaterials can revolutionize how we treat water and remediate its waste. Themes 2 and 3 share also several cross-cutting projects that seek to address major roadblocks to nanomaterial commercialization- namely their manufacturing and characterization of their environmental and health effects. While grounded in different engineering disciplines (biological and environmental), these two theme areas also share many of the same fundamental science needs. Center projects in Theme 1 address these basic questions in chemistry, physics and biology by studying bio-nano interactions over multiple length scales and systems.
While we divide the research into themes for easier management, our engineered systems have multiple interaction points and share numerous enabling technologies. For example, researchers in Theme 1 provide researchers in Themes 2 and 3 with new varieties of nanomaterials for desired applications. Near-infrared emitting SWNT are now being used to image cells for example. Also we have carefully chosen our portfolio to have overlapping near-term objectives. For example work on solvating nanoparticles (Theme 1) is complemented by work on nanoparticle surface chemistry, aggregation, and contaminant adsorption in the cross-cutting environmental impact project (Theme 3). Much of the fundamental chemistry and biology in Themes 1 and 2 has great relevance to topics in Theme 3. This year, our ongoing studies of the environmental chemistry of C$_{60}$, for example, intersected directly with the efforts of new Theme 2 participant, Lon Wilson, who develops C$_{60}$ for medical applications.

**Summary of major research accomplishments**

The majority of CBEN projects began in 2001/2002 as "new start" collaborations in which investigators had no prior collaborative work. The pay-off from investing in these new areas is clear now, as in this last year alone CBEN work has resulted in over forty published or in-press publications (section 4); our research productivity will only continue to grow as indicators of future performance (papers submitted, provisional patents) are equally sizable.

Our research highlights include

- **The creation of a multifunctional nanoparticle** that can be used in both the detection and treatment of disease. These smart structures can lead to a revolution in medical care wherein a person’s illness can be detected and treated in a single office visit.

- **The development of an ultrasensitive probe** to create charge maps of individual molecules in solution at the nanometer scale. Such technology can be applied to the development of biological nanosensors.

- **The discovery of a simple way to reduce the toxicity** of class of water-soluble nanoparticles. This can lead to broadly applicable strategies for ensuring that nanoparticles do not have adverse impacts on the environment.

- **The development of a high resolution method** to separate nanocrystals from waste streams as well as improve and purify the materials.

- **The demonstration of a reactive ferroxane membrane** capable of degrading organic contaminants in water streams.
8.1. Theme 1: Nanoscience at the Wet/Dry Interface

Table 8.1.1: Summary of Theme 1 Allocations

<table>
<thead>
<tr>
<th>Project</th>
<th>Leader</th>
<th>Investigators (name, dept.)</th>
<th>Disciplines Involved</th>
<th>Number of Students and Post-docs</th>
<th>Current Year Budget (including overhead)</th>
<th>Proposed Budget</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1.1 Activity of Bionanoconjugates</td>
<td>Colvin, Chemistry</td>
<td>Matthews, Biochemistry Tao, Biochemistry Mackenzie, Biochemistry</td>
<td>Inorganic Chemistry, Biochemistry, Biology</td>
<td>1,1</td>
<td>$94,933</td>
<td>$85,363</td>
</tr>
<tr>
<td>8.1.2 Imaging Biological Charge Distributions</td>
<td>Hafner, Physics</td>
<td>Natelson, Physics</td>
<td>Physics, Biology</td>
<td>1.0</td>
<td>$36,665</td>
<td>$43,680</td>
</tr>
<tr>
<td>8.1.3 Biomedical Apps. of SWNTs</td>
<td>Weisman, Chemistry</td>
<td>Smalley, Chemistry Tao, Chemistry Hauge, Chemistry</td>
<td>Chemistry, Biology, Physics, Bioengineering</td>
<td>3,1</td>
<td>$209,526</td>
<td>$204,956</td>
</tr>
<tr>
<td>8.1.4 Biomedical Apps. of SWNTs</td>
<td>Weisman, Chemistry</td>
<td>Smalley, Chemistry</td>
<td>Chemistry, Biology, Physics</td>
<td>0.0</td>
<td>$63,609</td>
<td>$</td>
</tr>
<tr>
<td>8.1.5 Fullerene/SWNT Theory</td>
<td>Scuseria, Chemistry</td>
<td>Yakobson, Materials Science</td>
<td>Chemistry, Physics</td>
<td>0.1</td>
<td>$70,984</td>
<td>$70,715</td>
</tr>
<tr>
<td>8.1.6 Nanomanufacturing</td>
<td>Wong, Chemical Engineering</td>
<td>Zygourakis, Chemical Engineering Pasquali, Chemical Engineering Colvin, Chemistry Mantzaris, Chemical Engineering West, Bioengineering</td>
<td>Chemistry, Chemical Engineering</td>
<td>1,1</td>
<td>$143,476</td>
<td>$114,680</td>
</tr>
</tbody>
</table>

1 Budgetary information includes both NSF and matching funds, and includes overhead.
2 For convenience, compound department titles have been shortened to the most appropriate component. For example, the Department of Physics and Astronomy is listed simply as 'Physics'.
3 Four projects cross theme-area boundaries; they are listed and described only once, but their budgetary information is split equally between the two theme area totals. Personnel are listed only in the theme in which the projects are described. The project, "Biomedical Apps. of SWNTs," is in the process of transitioning from theme 1 to theme 2, so its budgetary information is split equally between the theme 1 total and the theme 2 total. Similarly, the project, "Environmental Exposure Routes," is split equally between themes 1 and 3, and the projects, "Nanomanufacturing," and "Nano-cell Interactions," are split across all three theme areas.

Nature is filled with examples of intricate carbon-based machinery optimized to perform complex chemical, physical, and biological processes. If these biosystems can be harnessed to promote nanoscale synthesis and assembly, the resulting “wet nanoscience” will transform both nanochemistry and biology. Biological materials have proven increasingly useful as tools to solve nanotechnology problems, and bio-nanomaterials have been applied extensively to study biology. These applications raise fundamental questions about how inorganic nanomaterials and biological systems interact and the best means for controlling these interactions. Nanomaterials are, in most cases, foreign materials in biology. How they affect biochemical and cellular processes is a crucial question for the rational design of hybrid nano-biosystems, the topic of Theme 2 as well as for developing a complete picture of their environmental impact (Theme 3). In this theme area, the research approaches these issues from diverse biological and chemical viewpoints which taken together will define how nanomaterials fit within the study of living organisms.

The fundamental science efforts in this theme are diverse, ranging from computational projects to biochemical systems. Examples include new methods for forming, purifying, and analyzing bio-nanoconjugates (Section 8.1.1) and the study of the near-infrared emission from SWNT (Section 8.1.3). CBEN’s mission provides a great motivation for research in this area to quickly transition into related themes. For example, in the previous reporting cycle two separate publications in Science magazine on SWNTemission have become among the most heavily cited recent nanotechnology papers. Because this basic spectroscopy project was initiated in CBEN, it has quickly started a transition from Theme 1 to Theme 2 and the systems under investigation have moved from biocompatible aqueous systems to live macrophages.
8.1.1. **Activity of Bionanoconjugates (Colvin, Matthews, Tao, MacKenzie)**

**Summary**

In all CBEN projects control over the interface between nanostructures and biological systems is central. This project uses a model protein/nanoparticle system to better develop chemical methods as well as characterization tools for examining the wet/dry interface. The fundamental data gathered here has wide impact on projects throughout the center including analysis of SWNT/bioconjugates and the development of biocompatible and non-fouling interfaces for nanoshells and fullerenes.

Our specific focus has been on the effect on biomolecular function of covalent conjugation to nanoparticles. Specifically, gold nanoparticles, as a relatively inert model system, have been conjugated with lactose repressor (LacI), a well characterized and easily studied biomolecule. In this study, we have applied for the first time analytical ultracentrifugation (AU) to the problem of characterizing solution-phase nanomaterial samples. It provides information complementary to typical microscopies that require sample to be dried and mounted. Analytical ultracentrifugation revealed differential sedimentation among the species of interest; the gold nanocrystals sediment rapidly, free mutated or wild type (WT) LacI proteins show little sedimentation, and conjugated gold-LacI species sediment at intermediate rates with more than one boundary, indicating the presence of free protein in the sample. Further, the sedimentation rates of the gold-conjugated mutant and wild-type protein can be distinguished, supporting the hypothesis that the two proteins conjugate either specifically or non-specifically.

With this quantitative method for characterizing the samples in hand, we are now able to effectively determine the extent and mode of binding in bionanoconjugates. The bioactivity of the resulting conjugates can then be evaluated by standard operator DNA binding assays. We observed a dramatic decrease in binding affinity for the WT LacI, but little-to-no decrease for the T334C form. This indicates that by appropriate engineering of the nanoparticle-biomolecule conjugation, the activity of the conjugate can be controlled and in some cases completely reduced.

We have further developed this system to evaluate using analytical ultracentrifugation to determine the extent of non-specific protein sorption to particle surfaces. We find that the sedimentation coefficient of gold particles stabilized by ionic groups changes significantly when exposed to proteins, indicating substantial non-specific sorption. In contrast, gold particles stabilized by a biocompatible surface coating of poly(ethylene glycol) or PEG show only small changes upon exposure to proteins. This work illustrates that analytical ultracentrifugation can be used generally to evaluate the biocompatibility and fouling of engineered nanoparticle interfaces.

![Figure 8.1.1: Placement of T334C mutation. Threonine 334, shown in green, was chosen for mutation because it is solvent accessible and will not obstruct the DNA binding domain or the inducer sugar pocket when bound to nanocrystals.](image-url)
Results

Understanding the nature of nanoparticle-protein interactions is essential to the development of nanomedicine. Recently, positively charged lysine dipeptides were used to assemble three-dimensional structures of gold nanoparticles. These results indicate that charged regions on the protein surface may play a role in non-specific protein-AuNC interactions. We have utilized the lactose repressor, a genetic regulatory protein with small molecule and DNA binding functions, to examine these interactions with nanoparticles in more detail.

Among the biggest challenges for the assessment of nanoparticle-biomolecule binding methodologies is the difficulty with characterizing the final assembly. Nanoparticles are typically imaged with electron or atomic force microscopy. These methods provide information about nanoparticle size, shape and heterogeneity, but require the sample to be dried and mounted thus leaving little information about biomolecular components. We have developed analytical ultracentrifugation to provide quantitative information about biomolecule/nanoparticle assemblies directly in solution. The sedimentation rates of several samples of previously characterized AuNC were followed at 280 nm (Figure 8.1.2). The 10-nm diameter AuNC sediment slower than the larger 20-nm AuNC as expected from simple considerations of mass.

Gold nanocrystals have a well documented strong interaction with the sulfhydryl group of cysteine residues. Solvent-exposed cysteines present a facile means to conjugate proteins to AuNC in a spatially oriented fashion. To that end a Lacl mutant, T334C, was created to introduce a unique site on the protein surface for specific conjugation via a cysteine residue. Threonine 334 was chosen for mutation because it is solvent accessible and distant from the DNA binding domain. This protein exhibits DNA binding behavior similar to the wild-type protein.

The sedimentation profiles change enormously once particles are bound to proteins because the frictional coefficients typically track with the surface coating and density. Sedimentation velocity experiments of free protein, free AuNC and conjugates monitored with absorbance at 280 nm showed that there is differential sedimentation among the three species. The AuNC sediment rapidly at 3000 and 6000 rpm whereas there is little to no sedimentation of free T334C or WT protein at these rotor speeds. Conjugated T334C and WT show an intermediate sedimentation rate with more than one boundary, indicating the presence of free protein in the sample. Similar experiments tracked absorbance at 530 nm (Figure 8.1.3). Differential conjugate sedimentation is also observed at this wavelength. These curves verify that the absorbances from retarded species seen in the conjugate 280-nm scans contain AuNC and protein. Interestingly, the T334C conjugates sediment slower than the WT conjugates. If WT and T334C are conjugating through different modes, i.e., specifically or non-specifically, then the overall shapes of the conjugates are dissimilar and could give rise to the observed rates of
sedimentation. From these data, AU can distinguish between the AuNC interactions of two structurally similar proteins.

The AU data indicate that T334C and WT LacI interact differentially with AuNC, but whether or not T334C is interacting via cysteine 334 cannot be determined directly. If T334C is oriented to the AuNC surface via the C-terminus, then this protein should retain its DNA binding function. Operator DNA binding assays of T334C and WT conjugates show that conjugation has a much greater effect on operator affinity of WT LacI than T334C. Controls (first four bars) show only small effects on operator binding. The next two bars show the operator binding affinities of conjugated repressors. A dramatic decrease in the operator binding affinity of WT LacI occurs upon conjugation, whereas T334C shows little to no decrease in operator binding compared to the control. WT LacI may conjugate to AuNC in a non-specific manner via lysines in the DNA binding domain, thereby explaining effects on WT DNA binding affinity.

This work has shown that proteins are capable of interacting with AuNC specifically and non-specifically. A thorough understanding of protein nanoparticle interactions will allow for control and manipulation of the behavior, aiding in the development of new applications.

Finally, we are developing analytical ultracentrifugation as a measure of the non-specific fouling of nanoparticle surfaces to proteins. This is an extremely important question when designing the wet/dry interface; nanoparticle surfaces that do become coated with incidental proteins will have much less effective interactions with target biomolecules, and may experience irreversible changes in their physical size that influence their distribution in organisms. We have therefore also evaluated LacI interactions with PEG-coated, and hence anti-fouling, gold particles. The aggregation...
state of the PEG-coated particles cannot be determined by color change, complicating detection. Figure 8.1.5 shows a comparison of 5 nm PEG-coated gold particles alone and with 250 mM LacI. Both samples have similar s-values, indicating that there is little interaction between the coated nanoparticles and the protein. These results demonstrate that in the previous experiments LacI is interacting with the gold surface specifically to alter the rate of particle sedimentation.

Figure 8.1.5: S-values for PEG-coated particles with and without LacI. The black line is the PEG-coated particles only, and the gray line is particles in the presence of 250 mM LacI. The rotor speed for both samples is 8,000 rpm, which is significantly faster than that used for uncoated particles because the PEG coating decreases density and may alter frictional properties, decreasing the rate of sedimentation. S-values are derived with an assumed density of 4.0 g/cm³.
8.1.2. Imaging Biological Charge Distributions (Hafner, Natelson)

Summary

This project aims to develop new modes of scanning probe microscopy to measure charge distribution and charge dynamics in biological systems at the nanometer scale. A force-based method was developed, which we call Fluid Electrical Force Microscopy (FEFM), in which local charge density is measured in biological samples in solution through long range electric double layer interactions. The tip-sample force, when measured at a constant separation using Lift Mode, is linearly proportional to the sample charge density. This technique provides some degree of chemical contrast in fluid AFM without friction or adhesive interactions, in contrast to chemical force microscopy. The “gentleness” of FEFM was demonstrated by mapping charge density on fluid phase supported lipid bilayers, and the high sensitivity was demonstrated by mapping the charge density around a single molecule: λ-DNA. These results were published in *Langmuir*.

FEFM has been applied to a number of biological systems. (1) Lipid rafts, postulated gel phases in fluid biomembranes and implicated in several biological processes, have been directly observed for the first time by this technique. Work is underway to directly observe these rafts in living cells. (2) Zwitterionic lipids in biomembranes (DOPC and sphingomyelin) have also been investigated, surprisingly revealing that these model systems have a small net negative charge. We have concluded that the phosphate group has a larger ionization constant. (3) We have initiated a collaboration with Bioforce Nanosciences to use FEFM for nanoscale gene chip analysis. This approach offers an advantage over standard fluorescence detection by obviating the need to attach fluorophores to the probe DNA, and allowing miniaturization of the gene chips.

Additionally, we have developed a single particle microspectrometer to characterize individual nanostructures. With this system we have measured the scattering spectra of individual gold nanoshells, and have found that the dipole radiation has a much narrower linewidth than predicted by Mie theory. The spectral range of the instrument is currently being extended to 1800 nm, and the system will be available for use by researchers throughout CBEN.

Results

The goals of this project were to develop new modes of scanning probe microscopy to measure nanoscale charge distributions and dynamics in biological systems. A force-based method was developed, which we call Fluid Electric Force Microscopy (FEFM). In FEFM, local charge density is measured in biological samples in solution through long-range electric double layer interactions. The expression for this tip-sample force has been derived:
where \( z \) is the tip-sample separation, \( R \) is the tip radius of curvature, \( \lambda \) is the Debye length, \( \sigma_t \) is the tip surface charge density, \( \sigma_s \) is the sample surface charge density, and \( \epsilon \) is the dielectric constant of the medium. When the double layer force is measured at a constant tip-sample separation using Lift Mode, the measured force is linearly proportional to the sample charge density since all other parameters in the above expression remain constant. FEFM gives some degree of chemical contrast in fluid AFM without friction or adhesive interactions (as in chemical force microscopy—CFM), which could perturb soft biological samples. The “gentleness” of FEFM was demonstrated by mapping charge density on fluid phase supported lipid bilayers. The high sensitivity of FEFM was demonstrated by mapping the charge density around a single molecule: \( \lambda \)-DNA. These results were published in *Langmuir* in January 2004.

We have applied FEFM to the study of lipid rafts in biomembranes. The molecular components of biomembranes are in a fluid phase, rapidly diffusing past one another. Biochemical evidence suggests that within the fluid biomembrane, groups of lipids cluster into gel phase “rafts” floating in the lipid “sea”. These rafts have been implicated in several biological processes, but have never been directly observed in a cell. Lipid rafts have been widely studied in model biomembranes – mixtures of synthetic lipids that mimic the content of the biomembrane. We have observed FEFM contrast between the fluid and gel phase regions of a model membrane containing lipid rafts. We are now preparing to carry out FEFM on cultured MDCK cells in a Bioscope AFM to attempt to directly observe lipid rafts in living cells.

While we demonstrated FEFM on cationic (DOTAP) and anionic (DOPS) supported lipid bilayers as described above, the lipids in model biomembranes are zwitterionic (DOPC and sphingomyelin). The head groups of these lipids contain a negative charge on the phosphate group and a positively charged amine at the lipid-water interface. This structure necessitated a reinvestigation of the FEFM contrast mechanism over zwitterionic lipids. Force curve analysis over the DOPC and sphingomyelin lipids revealed that the simple charge density model is still accurate, and that the lipid bilayer surface has a small net negative charge. This is somewhat surprising since the negative charge sits farther from the tip than the positive. We believe the phosphate group has a larger ionization equilibrium constant, resulting in a net negative charge on the bilayer. These results are in preparation for submission to the Biophysical Journal.

We have also initiated a collaboration with Bioforce Nanosciences to use FEFM for nanoscale gene chip analysis. Standard gene chips detect hybridization of probe DNA to surface-bound complementary DNA by fluorescence detection. FEFM could detect hybridization based on an increase in surface charge density due to the phosphate backbone in DNA. This would avoid the need to attach fluorophores to the probe DNA, and allow miniaturization of the gene chips. Bioforce Nanosciences have developed methods to fabricate sub-micron gene chips and are sending them for FEFM analysis.

Attempts to detect biological charge dynamics through capacitive tip-sample measurements were unsuccessful. The background capacitance between the AFM tip chip and sample is simply too large compared to fluctuations in capacitance at the tip-sample interface. We therefore focused on the force-based methods described above.

In addition to scanned probe measurements on biological systems, a CBEN student constructed a single particle microspectrometer to characterize individual plasmon resonant nanostructures. As part of a large group of researchers at Rice lead by Naomi Halas, we are developing the application of plasmonic nanostructures as probes of biological systems in an
advanced scanned probe instrument. To do this, we must be able to characterize the optical properties of single nanostructures, rather than bulk properties in a UV-Vis spectrometer. A single-particle spectrometer has been constructed based on a Zeiss Axiovert 200 inverted microscope, a Spectrapro 150 imaging spectrometer, and a Hamamatsu Orca ER II ccd camera. With this system we have measured the scattering spectra of single gold nanoshells and compared them to spectra calculated by Mie theory. Surprisingly, we find that the dipole radiation from the nanoshell has a much narrower linewidth than predicted, even without enhanced surface scattering terms typically entered into the theory for nanoparticles. These results are still being interpreted and will be submitted to Physical Review B. We have recently acquired a second spectrometer and an InGaAs array detector to extend the spectral range to 1800 nm. This system could be made available to other researchers in CBEN.
8.1.3. Biomedical Applications of SWNT (Weisman, Smalley, Tao, Hauge)

Summary

The goal of this project is to develop single-walled carbon nanotubes (SWNT) as fluorescent bioimaging contrast agents, utilizing the unusual near-infrared fluorescent properties of SWNT. During the past year, progress has been made both in developing spectroscopic analysis methods and in conducting the first studies of nanotube spectra in biological media. We have precisely fit our previous data on the energies of $E_{11}$ and $E_{22}$ spectral transitions of 33 distinct surfactant-suspended semiconducting SWNT species to empirical formulas. These formulas, reported in Nano Letters, coupled with our assignment of Raman and optical spectroscopic features for metallic SWNT, reported in Nano Letters, transform optical spectroscopy into the premier method for qualitative and quantitative analysis of SWNT samples. For a SWNT sample grown using a new supported catalyst method at the University of Oklahoma, we found at least one-half of the semiconducting SWNT content contained in just two $(n,m)$ species. This type of detailed spectroscopic analysis, reported in JACS, provides very valuable feedback for optimizing SWNT production methods so that they can selectively generate species that are most useful for bio-imaging. Most recently, we have succeeded in imaging the fluorescence of SWNT inside mouse macrophage cells. Optical microscope images from a specially adapted system clearly revealed that the SWNT are localized in many small vesicles within the cells. The data indicate that nanotubes are actively ingested through phagocytosis and are held in phagosomes within the macrophages. Cell viability appears unaffected by nanotube uptake under the experimental conditions, and the nanotubes remain detectably fluorescent in the harshly oxidizing environment of the phagosomes. High contrast near-infrared detection of single-walled carbon nanotubes should lead to valuable methods for tracing the interactions of nanotubes with organisms, and may form the basis for future families of biological contrast agents and fluorescence markers.

To cut SWNT to shorter lengths, fluorination and ozonation are currently under investigation. Suspensions of longer nanotubes in biologically compatible surfactants and polymers have been supplied to the Weisman group to test their ability to fluoresce in biological media. We have demonstrated selective functionalization of specific electronic types of SWNT by protonation and diazonium addition, as reported in Science and the Journal of Physical Chemistry B. We reported a mechanistic understanding of the role of surfactants in the debundling of SWNT during ultrasonication in Nano Letters and the Journal of Nanoscience and Nanotechnology. And the ability to separate individually solubilized SWNT from bundles by capillary electrophoresis was reported in the Journal of Physical Chemistry B.
Overview

The goal of this project is to explore interactions between single-walled carbon nanotubes (SWNT) and biological systems in order to pave the way for SWNT applications in biology and medicine. Our strategy centers on exploiting the unusual near-infrared fluorescent properties of SWNT. This fluorescence should allow the selective detection and imaging of nanotubes that are present in low concentrations in complex biological surroundings.

During the past year, progress has been made both in developing spectroscopic analysis methods and in conducting the first studies of nanotube spectra in biological media. We have precisely fit our previous data on the energies of E_{11} and E_{22} spectral transitions of 33 distinct surfactant-suspended semiconducting SWNT species to empirical formulas. These formulas allow the reliable prediction of transition frequencies for any semiconducting SWNT species having a diameter of at least 0.5 nm. They represent an important step for the development of optical spectroscopy into the premier method for qualitative and quantitative analysis of SWNT samples. In another project, we have used spectrofluorimetry to analyze the composition of a SWNT sample grown using a new supported-catalyst method at the University of Oklahoma. We found that this sample has a very narrow distribution in chiral angles and diameters, with at least one-half of the semiconducting SWNT content contained in just two (n,m) species. This type of detailed spectroscopic analysis provides very valuable feedback for optimizing SWNT production methods so that they can selectively generate species that are most useful for bio-imaging.

Most recently, we have succeeded in imaging the fluorescence of SWNT inside biological cells (Figure 8.1.9). Mouse macrophage cells were incubated in a solution that contained pluronic-suspended SWNT. The carefully washed macrophage cells showed near-infrared fluorescence characteristic of nanotubes. In addition, the strength of this fluorescence increased nearly linearly with time of incubation and with SWNT concentration in the growth medium. When SWNT-incubated macrophage cells were examined under an optical microscope that had been specially adapted for exciting and imaging near-infrared fluorescence, clear images were obtained. These show not only that nanotubes are present inside the macrophage cells, but also that they are localized in many small vesicles. The data indicate that nanotubes are actively ingested through phagocytosis and are held in phagosomes within the macrophages. It is notable that the nanotubes remain detectably fluorescent in the harshly oxidizing environment of the phagosomes. Cell viability appears not to be affected by nanotube uptake under the experimental conditions. High contrast near-infrared detection of single-walled carbon nanotubes should lead to valuable methods for tracing the interactions of nanotubes with organisms, and may form the basis for future families of biological

Figure 8.1.8: Skeleton (left) and space filling (right) models of a PEGylated 10-nm long carbon nanotube.
contrast agents and fluorescence markers.

Research in the Smalley and Hauge group on this project seeks to develop a process for producing short biologically compatible single-walled carbon nanotubes that fluoresce in the near-infrared. We envision ca. 10-nm long nanotubes with polyethylene glycol (PEG) end functionalities (Figure 8.1.8) that will wrap back around the nanotube as depicted in the figure above. Over the past year, we have been exploring various methods by which nanotubes can be cut to lengths of 10-20 nm. The two main areas of exploration are fluorination and ozonation. Fluorination is now known to cut nanotubes to lengths shorter than 100 nm. However, the main obstacle to overcome is debundling of short nanotubes. We can use the method developed in this lab to produce individually surfactant-suspended nanotubes in aqueous media through a process of high-shear mixing, ultrasonication, and ultracentrifugation, but the yield is very low (less than 1%). The current yield is, however, high enough for the Weisman group to begin their fluorescence measurements.

Ozonation is another method being explored for cutting of nanotubes to lengths less than 100 nm. The mechanism for ozone cutting is currently under investigation, and the goal is to scale-up the method to produce short nanotubes in usable quantities.

Finally, suspensions of longer nanotubes in biologically compatible surfactants and polymers, such as the Brij and Pluronic series, have been supplied to the Weisman group to test their ability to fluoresce in biological media.
8.1.4. SWNT Modeling in Micelles (Ma, Smalley)

Summary
This project, scheduled to end with the current funding cycle, aims to develop a molecular-level understanding of the structural and dynamic properties of nanotube-surfactant complexes. As an initial step to understand this system, we modeled with molecular dynamics simulations the process of aggregation of pure sodium dodecylsulfate (SDS) surfactants from random configurations into spherical micelles in water. In all cases where the simulation parameters would reasonably have resulted in single micelles, it successfully produced such structures with properties similar to both experimental and earlier simulation results. The aggregation process consisted of rapid clustering into small aggregates within the first nanosecond, followed by a slower combination of the aggregates. This slow combination was mediated by the formation of salt bridges, allowing neighboring surfactant molecules to reorganize and accommodate merging. This work has been submitted to the Journal of Physical Chemistry B. Our results clearly demonstrate that the current computational capacity is fully capable of studying long-time behavior of the surfactant systems. The established protocols will be used in our study of the structural and dynamic properties of systems containing nanotubes and SDS surfactants.

Figure 8.1.9: Spontaneous aggregation of SDS into a spherical micelle. From a random configuration at 0 ns, small clusters form within 1 ns and only two larger clusters remain at about 3 ns. It needs about 10-50 ns for the remaining two clusters to merge into the final micelle.
Results

Recently, Professor Smalley’s group has successfully realized the formation of individual nanotubes coated with sodium dodecylsulfate (SDS) monolayer micelles by sonication, and obtained a rich set of spectroscopic and physicochemical data for the nanotube-SDS micelles. This is a milestone in the production of biologically-friendly nanomaterials.

As the first step toward our understanding these complicated surfactant-based systems, we have studied the processes of aggregation of pure SDS surfactants from random configurations into spherical micelles in water using molecular dynamics simulations.

The simulation protocol was very similar to prior aggregation simulations. Periodic boundary conditions were used with the temperature and pressure maintained at 300 K and 1 atm, respectively, using standard coupling techniques. All bonds were fixed using either the LINCS or SETTLE algorithms, which allow for a five-femtosecond time step. Non-bonded interactions were calculated using a twin-range cutoff of 0.9 and 1.4 nm, while long-range electrostatics was determined using the particle mesh Ewald method (PME). Finally, the simulations were calculated using the GROMACS software.

A total of five separate simulations of varying size were carried out to obtain statistical averages, while also considering different sized systems. Each simulation was completed at approximately 0.5 M concentrations, which is much greater than the critical micelle concentration (CMC) of 0.008 M for SDS surfactants. However, results are still expected to reasonably reproduce micelles just above the CMC as previous studies utilized similar concentrations. All systems were initiated in random configurations within a cubic simulation cell.

The simulation of smaller systems, which were expected to create only one micelle, successfully produced a single micelle with structural properties similar to both experimental and earlier simulation results. Formation of these micelles required ~8 nanoseconds, while a second simulation required >50 nanoseconds for aggregates to combine into a single micelle. Fig. 8.1.8 shows the typical results of spontaneous aggregation of SDS surfactants into a spherical micelle during the simulation. The aggregation process consisted of quick clustering into small aggregates within the first nanosecond, followed by a much slower combination of larger aggregates. Interestingly, the combination of large aggregates was mediated by the formation of salt bridges, which allow neighboring surfactants to reorganize and accommodate merging. Three larger simulations produced multiple micelles that were smaller than expected, but as the simulations increased in size, the micelles were nearer the expected size of 60 SDS; when 150 SDS aggregated, three micelles were produced averaging 50 SDS and were structurally very close to expected sizes. The observation of micelles that were too small is most likely attributed to both the limited size and time frame that can be studied using molecular dynamics simulations.

Our results clearly demonstrated that the current computational capacity is fully capable of studying long-time behavior of the surfactant systems. The established protocols will be used in our study of the structural and dynamic properties of systems containing nanotubes and SDS surfactants. The results shall form a basis for understanding the fundamental principles of nanotube-surfactant interactions.
8.1.5. Fullerene/SWNT Theory (Scuseria, Yakobson)

Summary

This project aims to develop a complete understanding of the electronic structure of single-walled carbon nanotubes (SWNT). Since the optical properties of SWNT, which are vital to the development of their bioengineering applications, depend strongly on their electronic structure, we have over the last year pursued the effects of chemical functionalization on SWNT electronic properties. Hybrid density functionals containing a portion of the Hartree-Fock exchange have been shown to provide an excellent description of the electronic structure of semiconducting SWNT, which are those that possess near-infrared absorptive and fluorescent properties. We have found a chemisorption of atomic hydrogen in the outer walls of the SWNT studied with a binding energy of 1.5 eV. The resulting changes in density of states demonstrate a high sensitivity of the electronic structure to the presence of hydrogen. This work was published in the Journal of Chemical Physics. We have presented a detailed investigation of the effects of oxygen chemisorption on the energy gap of SWNT, in both epoxy and etheric structures. The energy gap of both types of oxidation decreases on zigzag tubes, whereas the energy gap of chiral nanotubes remains nearly constant upon oxidation at an etheric site, but decreases considerably at the epoxy site. However, the binding energy of oxygen to the chiral nanotube at the epoxy site is nearly 1 eV lower than at the etheric sites, leading us to conclude that the electronic transport properties of chiral tubes should not change drastically upon oxidation. This suggests that experimentally observed changes in electronic properties of semiconducting nanotubes upon oxidation is likely due to oxidation at topological defects or contamination from the purification procedure. This work has been submitted for publication. Future work will focus on extending our understanding of the effects of functionalizing the sidewalls of SWNTs to other functional groups. The attachment of functional groups may significantly modify their optical properties, which would affect potential bioimaging applications, and can directly affect mechanical stability, which would be highly useful for “cutting” techniques.

Figure 8.1.10: Density of states of SWNTs with and without H chemisorption.
Overview

Because of the singular electronic properties of fullerenes and single-walled carbon nanotubes (SWNT), they have been envisioned for a large number of applications. Therefore, a complete understanding of the electronic structure of fullerenes and SWNT is essential. For instance, chemical functionalization of SWNT can change dramatically the transport properties of the tubes, narrow SWNT have been reported to behave differently than larger diameter tubes, etc.

Density functional theory is a unique computational tool to study these systems. Particularly, hybrid density functionals, \textit{i.e.}, those that contain a portion of the Hartree-Fock exchange, have been shown to provide an excellent description of the electronic structure of semiconducting SWNT. We perform electronic structure calculations utilizing a development version of the \textit{Gaussian} suite of programs. This program performs molecular and periodic boundary conditions calculations using all-electron Gaussian basis sets and different density functional approximations: local density approximation (LSDA), generalized gradient approximation (GGA), hybrid density functionals, and meta-GGA functionals.

Results

\textit{Interaction of atomic hydrogen with single-walled carbon nanotubes: A density functional theory study}

We have studied the interaction of atomic hydrogen with the (5,5) and (10,0) SWNT. We have presented a systematic comparison between different density functionals: LSDA, GGA, and a screened exchange hybrid functionals. Such a study is presented for the first time utilizing hybrid functionals. The (5,5) and (10,0) SWNT were chosen as model systems for metallic and semiconducting nanotubes since they have a unit cell of moderate size and have similar radii (approximately 4 Å).

We have found a weak chemisorption in the outer wall without relaxing the SWNT geometric structure. A geometry relaxation produces a binding energy of about 1.5 eV (HSE). When the SWNT are completely covered by hydrogen, the binding energy is enlarged by about 0.3 eV. Screened hybrid functionals predict binding energies similar to the PBE values for the cases studied in this work.

In Figure 8.1.10, we present the change of the density of states (DOS) with different hydrogen decoration patterns. From our results, we observe the high sensitivity of the electronic structure to the presence of a H atom. This sensitivity is clearly manifested in the total DOS and could be utilized to design custom electronic devices.

\textit{Effect of oxygen chemisorption on the energy band gap of a chiral semiconducting single-walled carbon nanotube.}

We have presented a detailed investigation of the effects of oxidation on the energy gap of both a zigzag (10,0) and a chiral (8,4) single-walled carbon nanotubes. The chemisorption of an oxygen atom leads to the formation of epoxy and etheric structures in several possible sites on the outer wall of a nanotube. Two such sites are available on the (10,0) (Scheme I) whereas three are possible on the chiral (8,4) nanotube (Scheme II). We have characterized these sites using geometry optimizations with the PBE generalized gradient approximation density functional and then calculated binding energies and energy gaps using the PBE functional as well as the HSE screened exchange hybrid functional. In the (10,0) nanotube, oxygen chemisorption at both possible sites drastically decreases the energy gap.
The chiral (8,4) nanotube exhibits a different behavior. The energy gap remains almost constant upon oxidation at two of the three available sites but decreases considerably for the third site. The binding energy of the epoxy compound at the $C-C_b$ bond is almost 1 eV lower than the corresponding energy of the ether at the $C-C_a$ bond. Therefore, we expect that chiral SWNT do not exhibit a significant change in their transport properties upon oxidation. We attribute the extreme sensitivity observed in previous experiments either to the presence of topological defects or contaminants from the purification procedure. These defects could favor energetically the epoxidation at the $C-C_b$ bond thus changing dramatically the transport properties of these nanotubes.

Following the line of work presented above, we plan to investigate the effects of the chemisorption of different functional groups on the electronic properties of SWNT. We also plan to perform a detailed characterization of the electronic properties of narrow SWNT using hybrid density functionals. Such a study is lacking in the literature and is essential to describe the electronic changes produced by the strong $\sigma-\pi$ hybridization present in small diameter SWNT. To understand and predict important cooperative effects of the functional groups (for either spectroscopy or SWNT cutting), one also needs to evaluate their mobility. For this, transition state barriers must be accurately computed. Attachment of the functional groups (H-, F-, HO-, or more complex units) can directly affect mechanical stability as well, and lead to SWNT opening which can be very useful for developing “cutting” techniques.

Additionally, we plan to calculate the nuclear magnetic resonance spectrum of the $C_{70}$ molecule, for which not only the chemical shifts but also the carbon–carbon indirect spin-spin coupling constants, $J_{CC}$, have been measured.

<table>
<thead>
<tr>
<th>Position</th>
<th>$E_b$ (eV)</th>
<th>$E_g$ (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10,0)</td>
<td>PBE HSE</td>
<td>Exp. PBE HSE</td>
</tr>
<tr>
<td>Isolated</td>
<td>--- ---</td>
<td>1.1 0.76 0.96</td>
</tr>
<tr>
<td>Epoxy-a</td>
<td>-6.22 -5.74</td>
<td>0.56 0.75</td>
</tr>
<tr>
<td>Ether-b</td>
<td>-6.25 -5.73</td>
<td>0.41 0.52</td>
</tr>
<tr>
<td>(8,4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolated</td>
<td>--- ---</td>
<td>1.114 0.79 0.99</td>
</tr>
<tr>
<td>Ether-a</td>
<td>-6.54 -6.04</td>
<td>0.69 0.86</td>
</tr>
<tr>
<td>Epoxy-b</td>
<td>-5.64 -5.05</td>
<td>0.28 0.38</td>
</tr>
<tr>
<td>Epoxy-c</td>
<td>-5.69 -5.10</td>
<td>0.71 0.89</td>
</tr>
</tbody>
</table>
8.1.6. Nanomanufacturing (Wong, Zygourakis, Pasquali, Colvin, Mantzaris, West)

**Summary**

This project has as its system-level goal the design of continuous phase processes for forming nanocrystalline materials. All nanostructures used in CBEN formed in liquid phase systems are generated in highly dilute batch reactors. While suitable for research efforts, this manufacturing approach is not suitable for scaling up as it wastes substantial energy and solvent. Our goal is the formation of a continuous flow reactor that can generate high quality materials using environmentally benign methods. Cadmium selenide (CdSe) nanoparticles are our model system for developing the synthetic techniques, reactor designs, theoretical models and separation tools need for our complete system.

Over the past year we have transformed the classic CdSe nanoparticle formation process into a reliable, low-cost procedure well suited for routine production of large quantities of material. *We have developed a quenching method, wherein room-temperature solvent is added to the reaction vessel immediately after injecting the selenium precursor into hot cadmium-containing hot solvent, lowering the reaction temperature rapidly to conditions that favor rapid growth but not nucleation.* This approach has significantly improved particle size distributions for large batch sizes. We have filed a provisional patent application covering this technique. The traditional solvent for semiconductor nanocrystal formation, trioctylphosphine oxide (TOPO), is exceptionally expensive, significantly driving up the projected cost of bulk synthesis. *We have found that CdSe quantum dots (QD) can be synthesized with a family of commercially available compounds called Dowtherm fluids.* We have filed a provisional patent application covering this technique.

Theoretical models of nanoparticle formation are essential to realize our goal, and our efforts have thus focused on the model system of CdSe QD. This systems is an ideal material for developing particle growth models since the size of the nanoparticle correlates with easily measured optical signatures. The general principles that underlie this scaleup process are extendable to a wide variety of other nanoparticles systems. By combining absorption and photoluminescence data on the growing QD, we are working to extract average particle size and particle size distribution as a function of time during the growth process. *We have simulated the particle size distributions under conditions of diffusion-limited and reaction-limited kinetic regimes, including the effects of focusing and Ostwald ripening, and have qualitatively matched previous reports.*

Separation methods for analysis and preparation of nanocrystal materials have also been studied within this project area. To reach our ultimate systems goal of a continuous flow reactor requires on-line analysis of particle size, as well as methods to separate nanoparticles from waste streams. *We have reported in one publication under review the first application of alternate recycling chromatography to nanoparticle concentration and analysis.* Additionally, chromatographic methods have never before been used for quantum dots in solution because of strong interactions between particles and columns. *We overcome this enthalpic interaction by using mixed solvents and report in a second publication the first size-exclusion chromatograms of these materials.*
Overview

Liquid-phase synthetic routes to nanoparticles offer size and shape uniformity, non-aggregation, and controlled surface chemistry, all features that are difficult to achieve in gas-phase synthetic routes. While gas-phase methods have been scaled up for the large-scale production and manufacture of particular nanoparticle compositions, the liquid-phase methods have not. The overall objective of this proposed research is the development of a general methodology towards the large-scale, liquid-phase production of high-quality nanoparticles. Cadmium selenide nanoparticles (CdSe quantum dots, or QD) are our model system for the experimental and computational studies.

Results

Experimental

For a large reactor, we decided to construct a two-stage reactor as an improvement over a single-stage batch reactor (Figure 8.1.11). We found the separation of nucleation and growth processes of CdSe QD formation to be a critical reaction engineering design criterion, and the separation to be difficult to achieve in large single-stage reactors. We quickly came upon a significant hurdle that could not be overcome. The first stage was constructed successfully with a valve at the bottom of the 1-L pyrex reactor equipped with an automatic injector, but flow measurements and calculations indicated that the transfer of the reaction volume out of the first reactor and into the second was too slow (~5-10 seconds). Unfortunately, Teflon valves with larger bores were not available. We carried out several iterations of reactor design and construction (e.g., use inert gas to blow out reaction volume), but were unsuccessful in significantly improving transfer times.

We then focused on the question of whether there are other methods that we can use to maximize the separation of nucleation and growth. We came up with the idea of quenching the reaction volume using a “cold” solvent. Adding a room-temperature solvent immediately after injecting the Se precursor into the Cd-containing hot solvent (300 °C) would lower the reaction temperature rapidly and would also dilute the precursors, reaction conditions that favor particle growth but not nucleation. The reaction mixture would then contain small, stable CdSe QD that can then be grown to larger diameters by raising the reaction temperature. As far as we know, this concept has not been applied to QD synthesis; we recently filed a provisional patent application on this matter. We found that the “quench method” improved the particle size distributions based on a qualitative evaluation of the UV-vis absorbance spectra.

A practical problem came up with the quench method: the solvents used were expensive. TOPO is commonly used as the solvent for QD synthesis due to its high boiling point and its ability to ligate the CdSe particle surface and prevent particle aggregation. ODE has been shown to operate successfully as an alternative solvent for QD synthesis (although a small amount of TOPO is still required to solubilize the Cd precursor). Both TOPO and ODE are relatively expensive, and so we sought cheaper organic solvents (Table 8.1.4). We found that CdSe QD could be synthesized with a family of commercially available compounds called Dowtherm...
fluids. These compounds are typically used as heat transfer fluids in industrial heat-exchange applications, due to their high boiling point and chemical stability. These same properties, in addition to their cost, make them ideal alternative solvents. As far as we know, the use of such compounds in QD synthesis has not been reported in papers or patents; we recently filed a provisional patent application on this matter also.

The experimental study also encompassed the collection of particle size-growth time data from CdSe QD synthesized at different temperatures. Such data were needed as real inputs to validate the computational model of CdSe QD formation. We modified the method of Peng and co-workers for this set of experiments. Four runs were carried out using the quenching method, in which each involved heating the quenched reaction to different temperatures: 140 °C, 180 °C, 220 °C, and 260 °C. The runs were carried for >4 hrs, and aliquots were taken every few minutes (during the heating step and at the steady-state temperature). The aliquots were diluted with more ODE for collection of UV-vis and photoluminescence spectra.

One of the requirements for scaling up the QD synthesis process was to design control strategies for the optimal operation of the reactor system. To achieve this, we proposed real-time collection and analysis of spectral data of the reaction medium. Average particle size (first moment) could be derived from the first exciton peak of the UV-vis absorbance spectrum, but particle size distribution (second moment) could only be inferred from photoluminescence peak width; determination of skewness (third moment) was not possible from spectral data. We reasoned that all three moments could be derived from UV-vis absorbance spectra (specifically the onset, Figure 8.1.12) if the appropriate mathematical transformation was applied. We modified one such transformation given by Stebe and co-workers. Whereas they assumed that absorbance at a given particle size was proportional to particle volume and that the extinction coefficient was constant, we accounted for the particle size-dependence of the extinction coefficient using the data of Peng and co-workers. An important input into the transformation was the bandgap energy-particle size relationship. The effective mass model provided one such \( E_g \)-diameter relationship, but it did not yield an accurate prediction of average particle size. We settled upon deriving \( E_g \)’s from photoluminescence-particle size data from the Peng et al. 1998

### Table 8.1.3: Comparison of different organic solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Upper temperature limit (°C)</th>
<th>Price ($/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOPO (99%)</td>
<td>~400</td>
<td>1200</td>
</tr>
<tr>
<td>ODE (97%)</td>
<td>~350</td>
<td>420</td>
</tr>
<tr>
<td>Dowtherm A (diphenyl ether + biphenyl)</td>
<td>~400</td>
<td>2</td>
</tr>
</tbody>
</table>

![Figure 8.1.12: Transforming UV-vis spectra into particle size distributions (PSDs).](image)
JACS paper. By using the photoluminescence-derived $E_g$'s, we were able to calculate average particle sizes from the UV-vis onset that matched average particle sizes from the UV-vis exciton peak.

We have not verified the calculated PSDs (Figure 8.1.15) with TEM analysis of the synthesized CdSe QD yet. We have a suspicion that the observed asymmetry (PSD cuts off to the left and tails off to the right) does not reflect skewness in the actual PSD; rather, it may be an artifact of the UV-vis absorbance spectral shape. Nevertheless, qualitative trends could be observed for these data. The CdSe QD did not grow at 140 °C after quenching. At the higher growth temperatures, the QD grew in size but they also defocused or Ostwald ripened (note that the relative standard deviation is standard deviation divided by mean particle size). Talapin et al. (2001) indicated this behavior was evidence of defocused particle growth. These results suggest that the precursors were too diluted for “focused” growth in the quench experiments; we predict that injection of additional precursors would narrow the PSD.

**Computational**

We set up the population balance and particle growth equations and developed a generalized mathematical model to mimic growth behavior of an ensemble of colloidal particles. Using one adjustable parameter, we computed the PSDs under conditions of diffusion-limited and reaction-limited kinetic regimes. We simulated the focusing and Ostwald ripening effects on PSDs under these two regimes, and qualitatively matched the Monte Carlo simulations of Talapin et al. We are currently inputting the particle growth data, along with particle concentration and Cd precursor concentrations, into the model to extract kinetic parameters for particle growth.

**Separations and Chromatography**

We have developed for the first time size exclusion chromatography for the analysis of quantum dots. Figure 8.1.16 illustrates size exclusion (HPSEC) chromatograms of two sizes of CdSe and their respective absorption spectra. Contrary to traditional enthalpic mode HPLC, HPSEC separates solutes based on their entropic interaction with the porous stationary phase. Upon entering pores, the conformational degrees of freedom of a solute, in this case n-CdSe, are reduced resulting in a loss of conformational entropy. This diffusion into the porous stationary phase is driven by the solute concentration gradient between the interstitial and pore volume. Large solutes suffer a larger loss in conformational entropy than small solutes.
when entering pores of the stationary phase. As a result, larger solutes do not enter the pores resulting in an overall shorter path length and earlier elution time as seen in Figure 8.1.17. We have quantitatively correlated the elution time to hydrodynamic volume using known standards; the core size as determined by TEM plus the thickness of the capping group, in this case oleic acid, provides an expected hydrodynamic diameter within 5% of that measured from the elution time. This analysis permitted us to confirm that oleic acid, rather than a thiol, was the capping group on the particle surface.

While SEC works in principle to analyze and separate nanocrystals from their solvent, we would also like to apply it to sharpen nanocrystal size distribution. Post-treatment processing of particles may relax some of the stringent conditions required to ensure small nanoparticle distributions directly out of the reactor. For this goal we need a very high resolution chromatographic method, and for this we applied alternate recycling. Using this technique, we were able to achieve a baseline resolution between two well-defined gold nanocrystals differing by only 6 angstroms in size. We demonstrated also that it is practical to collect these fractions from the column and will explore the use of this method for nanomaterials in the next year.

![Figure 8.1.15: Evolution of separation with recycling process of 1-decanethiol-Au nanoparticles at a flowrate of 1.0 ml/min.](image)

(a) SEC chromatogram of cycle 2. Notice that larger particles are separated in this cycle. (b) Four overlaid chromatograms of cycle 8, showing the stability and reproducibility of the system.
8.2. Theme 2: Nanoparticles that Detect and Treat Disease

There may be no division of engineering that places greater constraints on materials performance and characteristics than bioengineering. Thus, developments in materials technologies are closely linked to success in bioengineering. We believe nanomaterials represent a substantial opportunity in this area. Their extremely small size enables them to access a variety of biological environments; their size also endows them with valuable size dependent properties which can be exploited in applications. Finally, their large surface areas are platforms for engineering multifunctional systems capable of recognizing and responding to disease states.

The five projects in this area all reflect approaches to the overall systems engineering goal of 'nanoparticles that detect and treat disease'. Two complementary efforts (8.2.1 and 8.1.3) aim to apply nanoparticles to imaging of cancerous tumors as well as plaque inside of arteries. Beyond imaging, the strong and tunable near-infrared absorption of some nanoparticles can be used to heat tissue deep inside of animals using external light sources. The application of this enabling technology to cancer treatment, developed this year in CBEN research, is explored next year in a testbed project with animal studies. A related seed project (8.2.2) seeks to apply this basic enabling technology to a new problem in biomedical engineering, tissue welding. Within this project area, CBEN is also providing bioengineers with access to new types of near-infrared absorbing and emitting nanostructures for further developments in near-infrared biophotonics. Project 8.2.4 uses nanoparticles to improve tissue replacement materials for disease treatment. This theme area is fortunate to have NIH funding available as leverage and existing projects have received substantial NIH grants to supplement CBEN efforts in testbed development. As a result, additional funds have been made available to bring in a new seed project 8.2.5 from Dr. Lon Wilson which uses C_{60} and SWNT materials as contrast agents. His expertise in fullerene surface chemistry also has synergy with the cross-cutting theme of nanomaterial environmental impact (8.3.5).

### Table 8.2.1. Theme 2 research allocations

<table>
<thead>
<tr>
<th>Project</th>
<th>Thrust Leader</th>
<th>Investigators (name, dept.)</th>
<th>Disciplines Involved</th>
<th>Number of Students and Post-docs</th>
<th>Current Year Budget</th>
<th>Proposed Budget</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2.1 Nanoshell-based Cancer Therapy</td>
<td>West, Bioengineering</td>
<td>Drezek, Bioengineering</td>
<td>Bioengineering, Biochemistry, Inorganic Chemistry</td>
<td>2.0</td>
<td>$194,782</td>
<td>$164,526</td>
</tr>
<tr>
<td>8.2.2 Nanoshell-assisted Tissue Welding</td>
<td>West, Bioengineering</td>
<td>Drezek, Bioengineering</td>
<td>Bioengineering, Inorganic Chemistry</td>
<td>0.1</td>
<td>$17,880</td>
<td>$46,153</td>
</tr>
<tr>
<td>8.2.3 Nanomaterials for Imaging</td>
<td>West, Bioengineering</td>
<td>Drezek, Bioengineering</td>
<td>Bioengineering, Biochemistry, Inorganic Chemistry, Physics</td>
<td>0.1</td>
<td>$59,600</td>
<td>$59,377</td>
</tr>
<tr>
<td>8.2.4 Nanostructured Bone Replacements</td>
<td>Mikos, Bioengineering</td>
<td>Barron, Chemistry, Hartgerink, Chemistry</td>
<td>Chemistry, Bioengineering, Materials Science</td>
<td>3.0</td>
<td>$144,446</td>
<td>$139,997</td>
</tr>
<tr>
<td>8.2.5 Cut SWNT for Therapy</td>
<td>Wilson, Chemistry</td>
<td></td>
<td>Chemistry, Bioengineering, Materials Science</td>
<td>1.0</td>
<td>$ -</td>
<td>$57,920</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td></td>
<td></td>
<td>615,179</td>
<td>$664,431</td>
<td></td>
</tr>
</tbody>
</table>

1. Budgetary information includes both NSF and matching funds, and includes overhead.
2. For convenience, compound department titles have been shortened to the most appropriate component. For example, the Department of Physics and Astronomy is listed simply as ‘Physics’.
3. Four projects cross theme-area boundaries; they are listed and described only once, but their budgetary information is split equally between the two theme area totals. Personnel are listed only in the theme in which the projects are described. The project, "Biomedical Apps. of SWNTs," is in the process of transitioning from theme 1 to theme 2, so its budgetary information is split equally between the theme 1 total and the theme 2 total. Similarly, the project, "Environmental Exposure Routes," is split equally between themes 1 and 3, and the projects, "Nanomanufacturing," and "Nano-cell Interactions," are split across all three theme areas.
8.2.1. Nanoshell-based Cancer Therapy (West, Drezek)

**Summary**

Nanoshells are particles with tunable optical properties that depend on their size, geometry, and composition. For this biomedical application, nanoparticles with a silica core and gold shell have been designed to strongly absorb in the near infrared where penetration of light through tissue is maximal. A new type of cancer therapy is under investigation using nanoshells. Due to their size, nanoshells extravasate and accumulate in tumors due to the leakiness of highly angiogenic tumor vasculature. This is referred to as the “enhanced permeability and retention”, or EPR, effect. Thus, nanoshells with poly(ethylene glycol) conjugated to their surface can be injected intravenously and substantially accumulate in tumors. When the tissue is illuminated with near infrared light, the absorption of light by nanoshells within the tumor results in heating sufficient to destroy the tumor. In a mouse colon carcinoma model, we have demonstrated complete ablation of tumors after IV injection of nanoshells followed 6 hr later by illumination at 805 nm/4 W/cm² for 2 min. By day 10, all nanoshell treated tumors had entirely abated, while tumors in mice in the sham and control groups grew unchecked. Additionally, long term survival of the mice was tracked. All mice in the sham and control groups died by day 21, while all nanoshell-treated mice survived for at least 90 days (all continue to survive at this point) with no tumor regrowth. Biodistribution, clearance, and systemic toxicity of nanoshells have also been evaluated, with no concerns raised. Additionally, nanoshells can be conjugated to targeting antibodies, directed, for instance against oncoproteins or markers on angiogenic endothelium, which should improve specificity of the therapy to the cellular level.

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**Figure 8.2.1**: MR thermal imaging has been used to monitor temperature changes in tumors during near infrared illumination either with (left) or without (right) injection of nanoshells. These illumination conditions cause minimal tissue heating in the absence of nanoshells, while the damage threshold (55°C) is quickly reached in the presence of nanoshells.
Results

This project aims to develop a nanoshell-based thermal ablation therapy (NBTA) as a significant new therapeutic tool for the treatment of cancer. It is ideally applied to otherwise inoperable cancers where surgery is currently accompanied by a high probability of morbidity or mortality. Nanoshells are concentric sphere nanoparticles consisting of a dielectric silica core surrounded by a very thin metallic shell, which is typically gold. Nanoshells possess a strong optical resonance whose wavelength can be tuned across much of the visible and infrared region of the spectrum by varying the relative size of the core and shell layer. By varying the absolute nanoparticle size, the relative contributions of scattering and absorption at a given wavelength of interest can be controlled. NBTA utilizes metal nanoshells to convert externally applied near-infrared (nIR) light into localized heat to destroy cancer cells, minimizing damage to surrounding tissue and avoiding the long-term effects of radiation therapy or chemotherapy.

During our last funding cycle we have demonstrated that systemically injected nanoshells target tumors due to the leaky vasculature and can be used for photothermal tumor ablation. In a colon carcinoma model in mice, we have demonstrated complete ablation of tumors in all nanoshell-treated mice. No tumor regrowth was observed for over 90 days. Untreated controls and laser-only treated animals had rapid tumor growth, and all animals perished by day 21.

We have also assessed biodistribution and clearance of nanoshells in mice. To do this, we have utilized nuclear activation analysis, using the core facility at Texas A&M University. We have observed significant uptake in tumors, with high levels also in the spleen and liver due to RES activity. No significant quantities of nanoshells

Figure 8.2.2: Mean tumor size measured on treatment day and 10 days later for 25 tumors. All tumors which were treated using NAPT showed complete necrosis by day 10. One standard deviation is shown. NAPT treatment group (n=7), sham treatment group (n=8), untreated controls (n=9).

Figure 8.2.3: A survival time plot for the three groups for the first 60 days. The mean survival time for the control group was 10.1 days with a 95% confidence interval of 9.2 to 11.1 days. The mean survival time for the sham group was 12.5 days with a 95% confidence interval of 9.5 to 15.5 days. By 18 days the mean survival time of the treatment group was significantly higher (p<0.001) compared to either the control or the sham group.
were observed in other tissues, including kidney, lung, and brain. Progressive clearance from the spleen and liver was observed over the subsequent 28 days. Histology of the spleen and liver appeared normal, as were liver enzyme levels in the blood.

Finally, while the nanoshells currently being used are well matched for the applications described, other types of nanostructures that are near-infrared absorbers and emitters may extend the general area of near-infrared biophotonics. In particular, highly absorptive near-infrared quantum dot materials may provide some key advantages, such as a much smaller physical size (10-20 nm as opposed to 50-100 nm for nanoshells). Additionally, these systems are also strong near-IR emitters which makes their application in imaging quite promising. These possible advantages must be weighed against the toxicity of the constituent materials, which are Pb chalcogenides. Still if their toxicity can be minimized through appropriate surface derivatizations they could provide capabilities complementary to gold nanoshells because of their smaller size.

Over the past few months, Colvin and Wong in the nanomanufacturing theme have synthesized water soluble near-IR quantum dots for the first time. These materials are highly monodisperse, with tunable absorption and emission throughout the near-infrared as shown in the figure above. These substances are easily phase transferred to water using the same surface coating as used for nanoshells. Preliminary cytotoxicity data on these suspensions finds minimal effects on human dermal fibroblasts, and more exhaustive toxicological testing is underway before applications in bioengineering are explored.

**Plans**

In continuing work for this project, we are assessing damage to collateral tissue to ensure the localization of therapy. Additionally, the studies above used nanoshells coated with PEG but not with targeting antibodies. The high levels of nanoshells that accumulated in the tumors did so due to the “enhanced permeability and retention” (EPR) effect. This will be sufficient for many applications, and is an attractive approach from an FDA regulatory perspective. However, there are cases where this will not be sufficient, or where the proximity of normal, vital cells (such as in brain tumors, which generally have web-like architectures that interweave with normal brain tissue) requires cellular-level targeting. Additionally, we believe that antibody targeting should increase the fraction of the nanoshell dose that binds to the tumor, allowing a lower dosage to be
used. Thus, we are continuing to focus on antibody-targeted therapies. Additional animal studies will be performed to investigate this. We are also going to be investigating ablation of the tumor vasculature with nanoshells targeted to bind to VEGF-receptor on angiogenic endothelium. We have \textit{in vitro} studies underway to confirm targeting to endothelial cells and will also perform \textit{in vivo} studies this year. We believe that a “cocktail” strategy, targeting both tumor cells and their blood supply, is likely to be most promising in clinical application. We also will continue to evaluate and investigate new nanomaterials with interesting near-infrared photonic properties for use both in this class of applications, and others. Finally, we have studies ongoing with Rebekah Drezek to evaluate combining imaging and therapy with nanoshells. These studies will continue and move on to animal trials this year.
8.2.2. Nanoshell Assisted Tissue Welding (West)

Summary

Nanoshells can be designed to strongly absorb near infrared light, as discussed above. Due to their effective heating at wavelengths where tissue absorption of light is minimal, we are investigating their use as exogenous chromophores to induce laser tissue welding in the near infrared. In conventional laser tissue welding, light sources are chosen to maximize absorption by tissue components. While it is possible to achieve sufficient heating in this manner, limitations have arisen, mainly generation of superficial welds due to poor penetration of light through the tissue and excessive damage to surrounding tissues. It should be possible to minimize both of these problems by selecting wavelengths of light that are minimally absorbed by tissue components, such as in the near infrared. However, by doing so, it becomes necessary to provide exogenous chromophores to absorb the light and generate heat at the interface to be welded. For this, nanoshells have been suspended in an albumin solder formulation and applied to cut strips of tissue (muscle), then illuminated with near infrared light. Under optimal conditions, tissue welding proceeded rapidly, and weld strengths were equivalent to uncut tissue. These illumination conditions result in no welding whatsoever in the absence of nanoshells. Preliminary in vivo experiments have been conducted in rats with full thickness skin wounds. In all animals, welds were sufficient to maintain wound closure, and the strength of the welded site progressively increased over the 32-day study.

Figure 8.2.5 Histological section of skin wound site 32 days following nanoshell-assisted laser tissue welding. Nanoshells were suspended in an albumin solder formulation and applied to the cut edges of tissue. Upon near infrared illumination, the nanoshells heat, denaturing proteins to result in tissue welding. Under these illumination conditions, no welding was observed in the absence of nanoshells.
Overview

This project aims to develop nanoshells as a exogenous heat transfer agent for laser tissue welding. This procedure, the joining of two tissues by heat produced from absorption of light, has emerged as a commercially and clinically attractive strategy that offers a number of potential benefits over conventional closure methods (e.g., suturing) such as immediate fluid-tight sealing, improved wound healing, improved mechanical integrity, and speed of procedure. Currently, tissue welding is accomplished when laser light is absorbed by tissue components such as water or hemoglobin, producing sufficient heat to cause denaturation of collagen and other proteins with subsequent entanglement of adjacent protein chains. The laser light used in this traditional approach does not discriminate between the wound surface and other tissue. As a result, the success of laser tissue welding has been limited because of (1) the generation of superficial welds with poor mechanical integrity as a result of poor optical penetration and (2) excessive damage to adjacent tissues. Given these limitations, focus has turned to the investigation of exogenous materials to facilitate the transfer of heat to enable wound closure. The use of metal nanoshells tuned to absorb light in the nIR water window offers a number of advantages over other tissue welding agents.

Results

Nanoshells were suspended in an albumin solder formulation (commonly used in laser tissue welding) and applied to cut strips of tissue (muscle). The effects of solder formulation, nanoshell concentration, and duration/intensity of illumination were investigated. Under optimized conditions, welds with ultimate tensile strengths equal to uncut tissue were achieved (Figure 8.2.5). Again, in the absence of nanoshells, these welding conditions had no effect and formed no welds whatsoever. In our preliminary in vivo studies, we have investigated welding of full thickness skin wounds in rats. Welds were sufficient to maintain wound closure, with strengths at the wound site similar between welded tissue and Vicryl sutured tissue at 1 week. Further, strengths of the welded sites increased with time over the course of 4 weeks.

Plans

Over the next year we will continue in vivo studies in full thickness skin wounds in rats. We will assess mechanical properties, healing, scarring, inflammatory responses, and damage to adjacent tissue. We will run side-by-side comparisons with indocyanine green (ICG). We also plan to perform studies in vessel anastomosis, initially in an ex vivo system, and later, if results are promising, in a rat model.
8.2.3. Nanomaterials for Imaging (Drezek, West)

Summary

Scattering-based optical imaging technologies, such as optical coherence tomography (OCT), are emerging as tools for non-invasive, point-of-care diagnostics for cancer. Scattering-based optical technologies rely on changes in the tissue index of refraction with disease progression. Such changes might be induced by altered nuclear morphology or mitochondrial activity, for example. Though these strategies have proven valuable in several early cancer screening applications, enhanced sensitivity and potentially imaging of molecular markers may be possible with the use of exogenous contrast agents. Nanoshells possess highly tunable optical properties: they can be designed to either absorb or scatter light at wavelengths across most of the visible and infrared regions of the electromagnetic spectrum. Nanoshells designed to strongly scatter in the near infrared are attractive for this application. In vitro studies have been conducted using dark field microscopy, a form of microscopy sensitive only to scattered light. These studies were conducted using SKBR3 carcinoma cells that overexpress the tumor marker HER2 on their surface. When cells were incubated with nanoshells conjugated to an anti-HER2 antibody, visualization of the cells under dark field was greatly enhanced due to scattering provided by the nanoshells. These molecular imaging approaches can be easily extended to other nanomaterials with useful optical properties such as near infrared fluorescence as they become available. Initial in vivo studies have also been conducted to evaluate the effectiveness of nanoshells as a contrast agent for OCT. These studies were performed by injecting nanoshells intravenously in mice then monitoring image contrast in the skin over time. Contrast increased over the course of the 50-min. study. Nanoshells can also be designed such that their extinction is due in part to absorption and in part to scattering. We are evaluating these types of nanoshells for combined imaging/therapy applications to allow immediate treatment of cancerous or pre-cancerous lesions upon detection.

Figure 8.2.6 Dark field images of carcinoma cells incubated with non-targeted nanoshells (left) or with anti-HER2 targeted nanoshells (right). Nanoshell binding improves scattering at the cell surfaces, leading to brightening of the image.
Overview

Advances in scattering-based optical imaging technologies offer a new approach to non-invasive point-of-care detection, diagnosis, and monitoring of cancer. Emerging photonics technologies provide a cost-effective means to image tissue in vivo with high resolution in real-time. Further enhancing the clinical potential of these imaging strategies requires development of optical contrast agents targeted to specific molecular signatures of disease. Over the past year, our CBEN project has focused on testing a novel class of contrast agents based on nanoshell bioconjugates for molecular imaging in living cells. Nanoshell bioconjugates offer significant advantages over conventional imaging probes including continuous and broad wavelength tunability, far greater scattering and absorption coefficients, increased chemical stability, and improved biocompatibility. In a proof of principle experiment, we show nanoshell bioconjugates can be used to image HER2, a clinically relevant cancer biomarker, in live human breast carcinoma cells. Furthermore, we provide the first experimental evidence that nanoshells provide sufficient optical signal for small animal in vivo imaging and have started to identify optimal imaging conditions to facilitate more extensive in vivo work.

Optical imaging tools such as reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) offer the potential for non-invasive, high-resolution in vivo imaging at competitive costs relative to current imaging modalities. Scattering-based optical technologies rely on inherent changes in tissue index of refraction due to organelles such as mitochondria and cell nuclei for image contrast. Strategies that depend only on intrinsic optical contrast have proven clinically valuable in many screening applications including early cancer detection; however, such techniques are not sensitive enough to resolve an image based solely on the presence of biomarkers of disease. In the case of cancer, where early detection is critical to reducing morbidity and mortality, the use of molecular-specific contrast agents provides the capacity to optically sense and image abnormalities long before pathologic changes occur at the anatomic level. In addition, imaging based on molecular-specific targets enables real-time in vivo monitoring of treatment course and can provide fundamental insights into cancer biology. A recent demonstration of scattering-based optical molecular imaging used gold colloid conjugated to antibodies to the epidermal growth factor receptor as a contrast agent in imaging cervical cancer cells and biopsy samples. While gold colloid conjugates are highly valuable as contrast agents for detecting superficial epithelial cancers with visible light, there is particular need for contrast agents in the near infrared (NIR) region of the spectrum. This is the spectral region where tissue transmission is greatest, allowing imaging of deeper tissue structures. It is also the region already exploited by NIR optical imaging techniques, such as OCT; thus, contrast agents would provide greatly needed enhancement wherever OCT is utilized.

Over the past two years, other nanostructures such as quantum dots have demonstrated significant promise as bioconjugates for targeted optical imaging applications. Advantages of quantum dot-based molecular imaging include narrow spectral emission that facilitates multicolor labeling and bright optical signals. Potential disadvantages inherent in any fluorescence-based imaging modality include high tissue autofluorescence background, slower imaging rates of fluorescence-based as compared to reflectance-based imaging technologies, and high cost. Gold nanoshells provide appealing optical properties for use in conjunction with reflectance-based optical imaging methods. Furthermore, gold is inert and highly biocompatible, thus facilitating in vivo use.
Results

Scattering nanoshell fabrication and antibody conjugation: Nanoshells with a 120-nm silica core radius and a 35-nm thick gold shell were fabricated as previously described. Nanoshells of these dimensions provide spectral characteristics (Figure 8.2.7) which facilitate imaging both in the visible region and throughout the NIR “optical window,” enabling the nanoshell conjugates to be used as contrast agents in imaging modalities including RCM, OCT, and diffuse optical tomography. Either HER2 (specific) or IgG (non-specific) antibodies were conjugated onto nanoshell surfaces using OPSS-PEG-NHS polymer. Unbound antibody was removed by centrifugation, supernatant removal, and resuspending in potassium carbonate. Following antibody conjugation, nanoshell surfaces were further modified with PEG-thiol in order to block non-specific adsorption sites and to enhance biocompatibility.

Cell culture and cytotoxicity assay: HER2-positive SKBR3 human mammary adenocarcinoma cells were cultured in McCoy’s 5A modified medium supplemented with 10% FBS and antibiotics. HER2-negative MCF7 human mammary adenocarcinoma cells were cultured in Eagle’s minimum essential medium supplemented with 10% FBS and 0.01 mg/ml of bovine insulin. Cells were maintained at 37°C and 5% CO₂. Calcein-AM staining was used to measure the effect of HER2-targeted nanoshells on cell viability. No differences in cytotoxicity were found between SKBR3 cells targeted with HER2-conjugated nanoshells compared to cells receiving no nanoshell exposure.

Molecular imaging of the HER2 receptor: Under darkfield microscopy, a form of microscopy sensitive only to scattered light, significantly increased optical contrast due to HER2 expression was observed in HER2-positive SKBR3 breast cancer cells targeted with HER2-labeled nanoshells compared to cells targeted by either nanoshells non-specifically labelled with IgG or control cells which were not exposed to nanoshell conjugates. Using a qualitative silver stain capable of detecting the presence of gold on cell surfaces, greater staining intensity was seen in HER2-targeted cells, providing additional evidence that the increased contrast seen under darkfield is specifically attributable to nanoshell targeting of the HER2 receptor (Figure 8.2.6). Finally, we observed no differences under darkfield or silver stain in HER2 and IgG-targeted nanoshells using the HER2-negative MCF7 breast cancer cell line.
Knowledge of potential molecular targets for diagnosis and therapy of disease continues to expand at a rapid rate. However, translating knowledge of potential targets into new diagnostic and therapeutic techniques requires the development of methods to image molecular targets, or the effects of therapeutic interventions on these targets, \textit{in vivo}, in real-time, in a cost-effective manner. Nanoshell-based molecular contrast agents offer unique advantages including nIR-tunability, size flexibility, and systematic control of optical scattering and absorption properties. In this study, we demonstrated that nanoshell bioconjugates can provide molecular optical contrast enhancement without cytotoxicity. Darkfield microscopy was used to demonstrate the feasibility of nanoshell bioconjugates for molecular imaging in living cells. While darkfield microscopy is appropriate for \textit{in vitro} imaging applications, use of nanoshell conjugates \textit{in vivo} will require more sophisticated imaging techniques. Current results encourage future work assessing nanoshell contrast agents \textit{in vivo} in animal models using scattering-based optical systems including RCM and OCT (see Figure 8.2.8 for initial results in this area). Furthermore, the high level of control over nanoshell properties achievable through systematic manipulation of design parameters suggest the potential for biomedical applications requiring more complex functionalities including integrated cancer imaging and therapy. The combination of targeted optical contrast agents and photonics imaging technologies has the potential to play a vital role in the future of cancer screening and diagnosis, in designing and monitoring therapeutic interventions, and in fundamental studies of carcinogenesis.

**Plans**

Over the upcoming year we will extend the CBEN-sponsored work begun this year. Our efforts will be focused in two areas: (1) continuing to expand our \textit{in vivo} OCT animal imaging studies, and (2) completing proof-of-principle \textit{in vitro} studies using nanoshell bioconjugates designed to enable combined imaging (via scattering) and therapy (via absorption). The integrated imaging and therapy experiments will connect CBEN’s Theme 2 work in the areas of imaging and therapy, which have progressed in parallel over the past several years.
8.2.4. Nanostructured Bone Replacements (Mikos, Hartgerink, Barron)

Summary

Bone healing is insufficient in certain instances where the defect size is very large due to tumor resection or trauma. Tissue engineering strategies are being investigated for these types of applications. A novel nanocomposite has been developed using biodegradable poly(propylene fumarate)/poly(propylene fumarate)-diacylate and surface modified alumoxane nanoparticles. When the nanoparticles were modified with both a surfactant and a reactive group, composites with over a 3-fold improvement in flexural modulus could be fabricated. In another aspect of this work, biomimetic alternatives to the biodegradable polymer used for the tissue engineering scaffold are being developed. Nanostructured, collagen-mimetic materials have been generated from short peptides. These short peptides self assemble into the helical structure of collagen and can polymerize in a head-to-tail fashion to produce long (100’s of nanometers) peptide helices. In the next year, these collagen-mimetic materials will be combined with nanoparticles to generate new nanocomposites with properties suitable for bone tissue engineering.

Figure 8.2.9: SEM images of fracture planes of nanocomposite samples after flexural testing (1 wt % loading): (a) unmodified boehmite crystals in polymer, bar is 1 µm; (b) diacyrloyl lysine-alumoxane nanocomposite (Activated), bar is 10 µm; (c) stearic acid-alumoxane nanocomposite (surfactant), bar is 100 nm; (d) acryloyl undecanoic amino acid-alumoxane nanocomposite (hybrid), bar is 1 µm.
Results

Nanocomposites

A novel composite material has been fabricated for bone tissue engineering scaffolds utilizing the biodegradable polymer poly(propylene fumarate)/poly(propylene fumarate)-diacrylate (PPF/PPF-DA) and surface-modified carboxylate alumoxane nanoparticles. Various surface-modified nanoparticles were added to polymer including: a surfactant alumoxane (stearic acid-alumoxane); an activated alumoxane (diacryloyl lysine-alumoxane); a mixed alumoxane containing both activated and surfactant groups; and a hybrid alumoxane containing both groups within the same substituent (acryloyl undecanoic amino acid-alumoxane). These nanocomposites, as well as polymer resin and unmodified boehmite composites, underwent flexural and compressive mechanical testing and were examined using electron microscopy. Hybrid alumoxane nanoparticles dispersed in PPF/PPF-DA exhibited over a three-fold increase in flexural modulus at 1 wt. % loading compared to polymer resin alone (Figure 8.2.10). No significant loss of flexural or compressive strength was observed with increased loading. These dramatic improvements in flexural properties are attributed to the fine dispersion of nanoparticles into polymer (Figure 8.2.9) and increased covalent interaction between polymer chains and surface modifications of nanoparticles. Work in progress in our laboratory aims (i) to assess the cytotoxicity of PPF/PPF-DA/alumoxane nanocomposites at various stages of degradation in-vitro and (ii) to evaluate the in-vivo bone and soft-tissue response to PPF/PPF-DA/alumoxane nanocomposites in their initial state and at various stages of degradation.

Biomimetic Scaffolds

The goal of this project is to create scaffolds for bone repair and regeneration that more closely match the chemical and mechanical properties of natural bone. Our hypothesis is that by mimicking the nanostructure of natural bone we will simultaneously be matching the

Figure 8.2.10: Flexural modulus of the different nanocomposites tested as a function of nanoparticle loading weight percentage. Error bars represent mean ± standard deviation for n = 5. The symbol "***" indicates a statistically significant difference compared to the pure polymer resin (p < 0.05).

Figure 8.2.11: Scheme showing three levels of collagen hierarchy. From left to right, the collagen triple helix, collagen fibril, and collagen fiber.
desired chemical and mechanical properties necessary to repair hard tissues. Bone is a composite material whose primary organic component is collagen and whose primary inorganic component is hydroxyapatite. Both phases are structured at the nanoscale and are interwoven in a very specific fashion. The first step of our project is to mimic the nanostructure of collagen (Figure 8.2.11). We now have our first data that suggests we have succeeded in this goal. The next step will involve the biomimetic mineralization of our artificial collagen to produce a bone mimic.

Short, 21-amino-acid peptides were synthesized with the following characteristics: a repeating proline-hydroxyproline-glycine repeat, an N-terminal cysteine and a C-terminal thioester. The three amino acid repeat allows the peptide to self-assemble into the secondary structure of collagen—the triple helix—while the N- and C- terminal ends allow the triple helices to polymerize in a head-to-tail fashion and produce extremely long peptide triple helices (100’s of nanometers). The helicity of the peptides was confirmed by circular dichroism spectroscopy and TEM revealed that the helices assemble laterally to form fibril like structures as shown in Figure 8.2.12.

This is a major step forward in preparing nanostructured mimics of bone and sets the stage for the next phase of this work which will be mineralization of the fibrils.
8.2.5. Cut Carbon Nanotubes as Capsules for Radiotherapeutic Agents (Wilson)

Summary

Radioimmunotherapy for treatment of cancer shows great promise, but is hampered by the ability to effectively target radiation to cancerous tissue. The total radiation dose that can be administered is limited by damage to vital normal tissues such as the bone marrow and liver. Additionally, technologies developed to date have focused on β-emitters, which are optimal for treatment of large solid tumors. Treatment of micrometastases, leukemias, and lymphomas may be better suited to α-emitters, which combine high cytotoxicity with very short tissue penetration ranges. The goal of this project is thus to entrap α-emitters within nanotubes that can be conjugated to a tumor targeting antibody; this new seed project will start in Y04.

Figure 8.2.13: Synthetic scheme for SPDP-derivatized US-tubes.
Plans

Radioimmunotherapy (RIT) for treatment of cancer is currently a rapidly expanding field. Recent developments, such as the high efficacy demonstrated by Bexxar™, an ¹³¹I-labeled B1 monoclonal antibody (MAb), illustrate the effectiveness of the technique in selected applications. In another study, RituxiMAb™, a similar MAb labeled with Y-90, also showed promising results, and both of these products have been recently approved for general use.

A major problem yet to be solved in RIT is how to deliver enough radiation to the target cells without causing excessive radiation damage to healthy tissues. Currently, radiation damage to the bone marrow, liver, and cardiopulmonary system limits the overall radiation dose to levels that are incapable of killing most tumors. This presents a formidable and complicated problem for which many potential solutions are being investigated, including the development of new radionuclides and their delivery systems to provide a better match to the particular cancer being targeted. This approach is the subject of this proposed research whereby ultra-short (20 nm) single-walled carbon nanotubes (hereafter US-tubes) will be developed to superecede the metal chelate compounds and carbon-halogen bonds presently used to deliver radiotherapeutic agents for RIT. The advantage of employing US-tubes over existing radionuclide delivery systems could be revolutionary for antibody-targeted radiotherapy. This is especially true for α-RIT where no satisfactory containment system presently exists for important α-emitters such as At-211 and Ac-225.

Margrave and coworkers at Rice have recently reported that fluorination of purified HiPco single-walled carbon nanotubes (SWNTs) followed by pyrolysis up to 1000 ºC "cuts" the nanotubes into short lengths, with 40% being US-tubes at 20 nm or less with some of the ends "open". In comparison, full-length MAbs used in RIT are up to 100 nm in length with MW ca. 2 x 10^5 Daltons. Thus, 20-nm US-tubes are likely small (short) enough that a properly-designed [(US-tube)-(linker)-MAb] construct would still possess the specific cell-binding properties necessary for antibody-targeted therapy.

α-Radioimmunotherapy

Ideally, the type of radionuclide used for RIT should be matched to the type of cancer treated. Solid tumors are best treated with β-emitting isotopes, which have a tissue penetration range of several millimeters. However, the tissue range of β-emitters is not optimal for the treatment of single-cells, small cell clusters, micrometastases, leukemias, and lymphomas. These diseases are more efficiently treated with α-emitting radionuclides, which combine high cytotoxicity with short tissue penetration range of several cell diameters. Accordingly, considerable effort has been invested in the development of the α-emitters At-211 (t½ = 7.2 hr), Bi-212 (t½ = 60 min), Bi-213 (t½ = 45 min) and Ac-225 (t½ = 10d). Compared to most β-emitters, however, these α-emitters suffer a serious common problem for α-RIT: major release or "leakage" of the radionuclide before the radiolabeled-antibody conjugate is on target, resulting in unacceptable radiation damage to bystander healthy tissue. The α-emitters are far more notorious than β-emitters in this regard because of their larger metal-ion size (and resulting smaller stability constants with existing chelating ligands) and weaker carbon-halogen bonds (e.g., carbon-At bond weaker than the carbon-I bond). Containment of all therapeutic radionuclide within US-tubes during RIT could eliminate this "leakage" problem, and thus, generally improve both α-RIT and β-RIT, although the greater improvement is likely to be for
α-RIT. Hence, this research project will concentrate on α-RIT and more specifically on (\(^{211}\)At@US-tube) α-RIT.

We have recently used carbon nanostructures as carriers for radionuclides by synthesizing and using \(^{166}\)Ho@metallofullerenes as radiotracers in a mammal model. These radiotraceres were prepared by neutron activation of \(^{165}\)Ho inside \(C_{82}\) fullerene cages in a high-flux neutron beam that achieves high enough specific activity for imaging but not therapeutic purposes. For therapeutics, highly-enriched radionuclides are required before loading of the carbon nanostructure and, thus, open-ended US-tubes are seemingly a much better choice (versus closed fullerenes) for radionuclide loading and delivery in targeted radiotherapy. This realization has led to the current proposal.

**Functionalized US-tube generation**

SWNT produced by the HiPco process will be fluorinated and pyrolitically cut by techniques developed at Rice University. We have successfully used size-exclusion chromatography to select a sample of US-tubes with lengths in the range 10 nm-40 nm; for larger tubes lengths, our recent successes with nanopore filtration will be built upon.

Experimentally, some of the cut tubes are observed to be open-ended. Any closed-tubes can be opened and polycarboxylated by oxidation with HNO\(_3\). It is likely that these polycarboxylated US-tubes will be soluble enough in water or DMF(10%)/water solutions for the antibody coupling reactions. The open-ended, cut nanotubes can be further derivatized at the carboxylic acid groups to make amides, esters, etc., as desired. If needed, the sidewalls of the cut tubes can also be derivatized by first re-fluorinating the cut tubes and then using the same reactions as those used on the long, original tubes. For example, serinolamidinated, cut US-tubes could be produced by reaction with serinol; this derivative should be extremely soluble in water and, therefore, especially useful for antibody coupling.

Figure 8.2.13 displays a synthesis for a derivatized US-tube ready for coupling to the ZME-018 antibody to be used in this study (see below). In STEP 1, the US-tubes are opened and derivatized with terminal polycarboxylate groups using nitric acid. In STEP 2, the US-tube acid chloride is created by the usual procedure in dry DMF. In STEP 3, an asymmetrically protected diamine is then reacted stoichiometrically (1:1) with the US-tube acid chloride derivative in dry DMF to produce an amide of the protected amine, and in STEP 4, the protected amine is deprotected with trifluoroacetic acid and reacted with N-succinimidyl 3(2-pyridylthio)propionate (or SPDP) to produce the final product, US-tube-(SPDP)(COOH)\(_{x+(y-1)}\). This final reaction product is then poised to be loaded internally with “cold” dopants or with At-211 radionuclide species and coupled to the ZME-018 antibody.

**Loading US-tubes with Dopants**

Once the cut nanotubes are separated into distinct lengths (≤ 20 nm; 20-50 nm etc.), various types of reactions will be explored to fill the tubes with dopants by low-temperature gas adsorption, low-temperature gas reactions, capillary action to introduce liquids, and gaseous diffusion.

In this proposed use of filled US-tubes (nanocapsules) for biomedical applications, it is essential that the nanocapsules do not lose their interior contents. Preliminary results from our laboratories, using a NaI-125 radiotracer, have shown that: 1) the nanocapsules easily fill with I\(^-\) ions in aqueous solution, and 2) the ions do not leach out post-encapsulation. Remarkably, in our experiments, within 1.0 minute virtually all of the \(^{125}\)I activity was with the solid US-tubes, with ≤ 1.0% remaining in the water. This experiment shows how quickly US-tubes take up ions.
from water, a solvent in which the US-tubes themselves are not soluble. Further preliminary experiments gave a strong indication that the interior (radioactive) iodine could not be re-dissolved out of US-tubes.

To minimize the problems with dehalogenation in vivo of At-211 ($\alpha$, $t_{1/2} = 7.2$ hrs) and to overcome the difficulty of this nucleotide’s MAb conjugate chemistry, [US-tubes/MAb] conjugates loaded with $^{211}$AtI will be our target system of choice. Both $I_2$ (I-I bond: 2.66 Å) and $^{211}$AtI (At-I bond: 2.8 Å (est.)) should easily fit in the open-ended US-tube (ca. 7 Å dia.) of Figure 8.2.13. In fact, in a preliminary study we have already successfully loaded US-tubes(COOH)$_{x+y}$ with $I_2$ using an $I_2/CS_2$ solution since $I_2$ (and undoubtedly $^{211}$AtI) is soluble in organic solvents. We will investigate chemically bonding the At-211 to lipophilic moieties that can be sequestered inside of the US-tube in an aqueous environment. Thus, an astatinated lipophilic molecule would be dissolved in DMF and dripped into a stirred aqueous solution containing the prefabricated MAb/US-tube conjugate.

Sublimation is also a possibility because $I_2$ has been successfully loaded into uncut tubes by this method. Temperature, pressure, and solvent will be varied to optimize loading conditions. In this case, the At$^0$ or At$^-$ would be mixed with the $I_2$ carrier molecule prior to sublimation. Elemental astatine, At$^0$, will be obtained from an irradiated bismuth target. While it is unlikely that At$^2$ will be formed due to the very small number of At atoms present, mixed halogens are very likely (e.g. At-I) and it is also likely that these species would sublime readily.

**US-tube Immunoconjugates**

The antibody designated as ZME-018 has been chosen for the US-tube immunoconjugate studies. The antibody targets melanoma cells and studies by the Dr. Michael Rosenblum group have demonstrated that immunoconjugates composed of ZME-018 and various toxins are rapidly internalized into human melanoma cells in culture. Moreover, these conjugates effectively localize into melanoma xenografts after systemic administration and demonstrate impressive cytotoxic effects against established tumors in orthotopic models.

To prepare the US-tube/ZME-018 immunoconjugate, the antibody will first be derivatized with 2-imino-thiolane such that five thiol groups on the Fc fragment are made available to the US-tube(SDPD)(COOH)$_{x+(y-1)}$ product of Figure 8.2.13. A 5-10% solution of this US-tube species in DMF will be then added slowly to ZME-018 in aqueous solution. Upon conjugation, the US-tube species will couple to the antibody by disulfide bond formation, with elimination of the pyridine-2-thione leaving group of SPDP. We are presently using this same strategy with clinical collaborators to couple a C$_{60}$(SPDP) derivative to ZME-018 with some success, and we have confidence in the approach for the US-tube immunoconjugates.
8.3. Theme 3: Effective, High-Performance Water Purification Systems

Table 8.3.1: Summary of research Theme 3 allocations

<table>
<thead>
<tr>
<th>Project</th>
<th>Leader(s)</th>
<th>Investigators (name, dept(s))</th>
<th>Disciplines Involved</th>
<th>Number of Students and Post-docs</th>
<th>Current Year Budget</th>
<th>Proposed Budget</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.3.1 Nanostructured Membranes</td>
<td>Barron, Chemistry</td>
<td>Wiesner, Environmental Engineering</td>
<td>Inorganic Chemistry, Physical Chemistry, Environmental Engineering</td>
<td>3.1</td>
<td>$212,024</td>
<td>$197,355</td>
</tr>
<tr>
<td>8.3.2 Polymer Flow on Nanoscale</td>
<td>Pasquali, Chemical Engineering</td>
<td>Kolomeisky, Chemical Engineering</td>
<td>Physical Chemistry, Chemical Engineering</td>
<td>0.0</td>
<td>$ -</td>
<td>$ -</td>
</tr>
<tr>
<td>8.3.3 Nanocatalysts for Pollutant Treatment</td>
<td>Wong, Chemical Engineering</td>
<td>Colvin, Chemistry, Hughes, Environmental Engineering</td>
<td>Inorganic Chemistry, Environmental Engineering</td>
<td>2.0</td>
<td>$113,932</td>
<td>$109,102</td>
</tr>
<tr>
<td>8.3.4 Sequestration of Contaminants</td>
<td>Tomson, Environmental Engineering</td>
<td>Colvin, Chemistry, Labide, Chemical Engineering</td>
<td>Physical Chemistry, Inorganic Chemistry, Environmental Engineering</td>
<td>4.0</td>
<td>$202,286</td>
<td>$178,909</td>
</tr>
<tr>
<td>8.3.5 Nano-cell interactions³</td>
<td>Alvarez, Environmental Engineering</td>
<td>West, Bioengineering, Hughes, Environmental Engineering, Colvin, Chemistry, Tao, Biochemistry</td>
<td>Chemistry, Biology, Biochemistry, Environmental Engineering</td>
<td>3.1</td>
<td>$137,648</td>
<td>$167,261</td>
</tr>
<tr>
<td>8.3.6 Environmental Exposure Routes³</td>
<td>Hughes, Environmental Engineering</td>
<td>Wiesner, Environmental Engineering, Environmental Engineering, Luetge, Geology</td>
<td>Physical Chemistry, Environmental Engineering</td>
<td>3.1</td>
<td>$246,699</td>
<td>$160,584</td>
</tr>
</tbody>
</table>

| Totals                           |                                    |                                                       |                                                          | 3                                | $745,299           | $659,638         |

1 Budgetary information includes both NSF and matching funds, and includes overhead.
2 For convenience, compound department titles have been shortened to the most appropriate component.
3 Four projects cross theme-area boundaries; they are listed and described only once, but their budgetary information is split equally between the two theme area totals. Personnel are listed only in the theme in which the projects are described. The project, "Biomedical Apps. of SWNTs," is in the process of transitioning from theme 1 to theme 2, so its budgetary information is split equally between the theme 1 total and the theme 2 total. Similarly, the project, "Environmental Exposure Routes," is split equally between themes 1 and 3, and the projects, "Nanomanufacturing," and "Nano-cell interactions," are split across all three theme areas.

Water is one of the most important resources for human existence, and ensuring access to cheap and clean sources is emerging as one of the great challenges of this century. While this problem is particularly acute in developing nations, even here in the United States increased revision of drinking water standards is pushing the envelope for current water treatment methods. This problem will only grow as the world’s population increases, and agriculture draws more and more of the potable water supply. Thus our systems engineering goal of cost-effective and high-performance water treatment systems meets an important social need for this country. Nanostructures can both offer substantial improvements to existing water purification paradigms, and introduce new ones, both of which CBEN exploits.

Our strategy for reaching this goal recognizes the fact that engineered nanomaterials are a very new class of materials, relatively unfamiliar to the environmental engineering community and water treatment businesses. This climate requires very strong evidence of the scope and performance of our enabling nanotechnologies both to target these systems to the appropriate problem, as well as to launch lasting partnerships for testing and knowledge transfer. Additionally, the economic reality of this industry requires particular attention to cost-effective solutions, which has not been a factor in the academic research in the area. Thus, we have followed our strategic plan, which provides substantial time, i.e., the first four years of CBEN’s lifespan, to synthesize candidate nanomaterials, characterize and optimize their performance, and develop an understanding of their mode of action.

This year, our accomplishments have been in the development of enabling technologies for our systems goal. We have developed reactive membranes that are capable of remediating...
organic waste in water. These ferroxane membranes build from our work with alumoxane systems that led to the formation this year of a start-up company, Oxane Materials, Inc. We have also developed high-performance nanoscale catalysts for treating particularly challenging contaminants in water that must be removed to a very low level. While nanoscale catalysts are widely used in improving air quality, their application for water treatment is much less extensive. Finally, with the inclusion of a magnetic separations expert into the center last year (Laibinis) we have developed nanoscale magnetic particles that have strong sorption interactions with arsenic species. The research productivity of these projects has been quite high, comparable to themes 2 and 1, and we are confident that throughout Year 4 these projects will continue to develop promising enabling technologies for our systems goal.

The integration of our enabling technologies represented in the core projects of this area will be of increasing concern to us over the next year. The membrane systems described in Section 8.3.1 provide a general treatment system and platform for water purification. These materials will likely drop into the end of a conventional treatment system for final polishing. Ultrafiltration (UF) and nanofiltration (NF) membranes are already used this way in existing applications so this integration should be straightforward. The incorporation of nanocatalysts into water treatment presents more options. Projects 8.3.2 and 8.3.3 provide nanoscale catalysts and sorbents for specific contaminants of great concern. Such materials could be used in very focused applications where a contaminated groundwater is being treated, and thus specialized reactors would be required to activate the catalysts (via light or hydrogen). In more conventional water purification, these materials could be treated as an additive in the treatment process and recovered by NF membranes or magnetic separators.

In year 3 we have begun to take concrete steps to identify and recruit partners for the systems-level testing and design that will serve as an increasing focus for this theme. In our most mature project, 8.3.1 or nanostructured membranes, this systems-level focus will be provided by a start-up company founded by CBEN members Barron and Wiesner. Oxane Materials, Inc. is confronting the scale-up challenges faced in forming larger membrane structures and evaluating the many diverse markets for these materials. We are also building partnerships with government organizations, such as Pacific Northwest National Laboratory and Oakridge National Laboratory, to provide access to specific water treatment problems found in these DOE facilities. We also expect to have exploratory collaborations with one or two companies in year 4; of particular value is the ability of engineered nanomaterials to remove intractable species such as TCE, arsenic and lead. Finally, we have been approached by private foundations interested in directing nanotechnology research towards problems in developing nations. Potable water is of significant concern in those settings, and nanotechnology may offer practical and unique solutions for water purification where power is not available. While we are exploring all of these options for systems-level partnerships, we anticipate choosing quite carefully only one or two problems and this choice will define much of the systems-focus plan in our renewal proposal.

Concurrent with this technology development is a strong effort aimed at examining the potential environmental consequences of engineered nanoparticle use. These cross-cutting projects evaluates the health and environmental effects of potential water treatment materials and other engineered systems in CBEN. As we have already found this year, such information early in the trajectory of our technology development work allows us to design non-toxic and biocompatible materials better suited for our ultimate application.
8.3.1. Nanostructured Membranes and their Applications (Barron, Wiesner, Colvin)

Summary

The aim of this collaborative project, funded for 2.5 years, is to develop nanostructured ceramic membranes that treat and purify water both actively and passively. This year we have developed a reactive membrane from ferroxanes using methods pioneered by our group previously in CBEN; our publication from this effort represents the first successful attempt to prepare iron oxide ceramic membranes. Because of the unique chemistry of iron, particularly Fe (II), these systems now provide us a platform for not simply removing contaminants from water but also remediating them. We also have shown that these ferroxane materials can decompose a model contaminant, benzoic acid, using Fenton chemistry. Our efforts on our initial ceramic membrane material, alumoxanes, have focused on the casting process of the membrane. We have demonstrated that membrane thickness, pore size distribution, permeability, and surface chemistry can now be tailored through simple changes in the initial deposition of alumoxane particles. Finally, we have recently begun to explore metal-ion doping in both the ferroxane and alumoxane membrane systems to increase the functionality of the membranes and alter their thermal characteristics. By using doped-alumoxanes to form aluminate membranes we can now create a wide range of pore sizes to be formed under identical thermal conditions. Over the next year, our systems-level engineering focus will be tightly coupled with a new start-up company spun out of this project, Oxane Materials, Inc. (Barron, Wiesner). Oxane has received a substantial start-up investment and is using the intellectual property and results from CBEN research to develop these membranes for commercial applications.

This project has also incorporated the Colvin group’s expertise in templating chemistry into its engineering to provide entirely new and more effective membrane architectures to be developed. In particular, the construction of hierarchical pore distributions is advantageous because it lessens fouling of membranes during their use. Using colloidal deposits as frameworks, we have reported in a paper now under review that membranes whose pore distributions vary from their surface to their base can be constructed. We also used similar concepts in forming highly porous membranes of hollow alumoxane spheres in which two length scales of porosity were built into the membrane. Such bimodal pore distributions offer the chance to develop high selective separations without the need for substantial driving pressures.
**Results**

**Ceramic membranes**

Our aim is to develop nanostructured ceramic membranes utilizing an environmentally benign pathway for ceramic fabrication. These membranes will be tailored to applications in the separation of hazardous materials, environmental separations in extreme environments, and reactive environmental separations. The scope of the project includes fundamental research into aqueous processing of metal nanoparticle precursors (alumoxanes and ferroxanes) and their processing into a membrane film with desirable characteristics of thickness, pore size distribution, permeability, and surface chemistry. The ability to cast these materials on suitable support materials was developed along with a control over pore size and pore size distribution. The advantage of this approach to ultra- and nano-filtration membranes is the chemical control over the pore size.

Ceramic membranes are usually fabricated in layers: a substrate or support of large pore size gives mechanical strength while a thin layer of smaller pore size material act as an effective filtration layer. Substrates are fabricated from metal oxide powders that are pressed, extruded or slip cast and then sintered. Pores obtained with this method vary from 5 to 15 µm. Smaller pore sizes can be obtained by the sol-gel process. Although the sol-gel process yields an acceptable product, sensitive control of the process is required and the reagents used are not without environmental impact. In the initial years of this project, we developed an alternative process using alumoxane precursors that has proven to be an environmentally benign alternative to sol-gel fabrication. Specific advantages include lower cost reagents, greater stability of processing suspensions, and the ability to cast membranes in the ultra- to nano-filtration range in a single step.

The alumoxane nanoparticles are deposited on a substrate and converted to alumina upon sintering. Alumoxane derived membranes have an average pore size of 10 nm; the BET surface area is 120 m²/g; and their molecular weight cut off is in the order of 40,000 daltons. Over the last year, we developed an analogous procedure for making ceramic membranes from iron-based nanoparticles; FeOOH and Fe₂O₃ are more resistant to acidic, corrosive and oxidant conditions than AlOOH and alumina, making them better candidates for PEM's, and opening the possibility of creating reactive membranes. Though ferroxane-derived membranes exhibit a rougher surface than their alumoxane analogs (Figure 8.3.1) we find that their performance in separations is still quite acceptable. The mean pore size and molecular weight cutoff of the ferroxane-derived membranes that we have fabricated to date is also somewhat larger than their alumoxane-derived counterparts. The sharpness of the cut off is a consequence of the relatively narrow size distribution of the ferroxane nanoparticles. This molecular weight cut-off (MWCO) corresponds to pore diameters between 14.9 nm and 17.3 nm.

The use of electrical impedance spectroscopy to characterize membranes is being developed, both as a basis for evaluating the proton transfer characteristics of the metal-oxane-derived membranes and as a general tool for characterizing membrane charge. The conductivity of the alumoxane membranes was compared with that of other membranes fabricated as proton transfer membranes for fuel cells. The results show that while the ferroxane and alumoxane-derived membranes fabricated to date do not have as high a conductivity as the benchmark Nafion membranes, metal-oxane-derived membranes are similar or superior to other ceramic membrane previously reported by others (Table 8.3.3). In particular, the ferroxane-derived
membrane shows order of magnitude improvement over existing ceramic membranes for proton transfer in possible fuel cell applications.

We have investigated the required composition and processing to allow for large area crack-free films of ceramics to be prepared using the alumoxane nanoparticles. This work is readily extended to the ferroxanes. Using two types of alumoxane with similar particle sizes but different ceramic yields, we have determined the effects on the shrinkage and cracking of the dried green bodies as well as the final ceramic films. By a combination of chemical and physical processes we are able to make relatively large areas of unsupported ceramic membranes. These results will be used to enhance the ability to make crack free ferroxane membranes.

Given the desire to produce alumoxane and ferroxane nanoparticles under environmentally benign conditions, we have developed a solvent free approach. Carboxylate-substituted alumina nanoparticles are produced solvent free using a Rheomixer. The general nature of this method has been demonstrated for L-lysine-, stearate-, and p-hydroxybenzoate-derived materials. The reaction rate and particle size is controlled by a combination of temperature and shear rate. The nanoparticles are spectroscopically equivalent to those reported from aqueous syntheses, however, the average particle size can be decreased and the particle size distribution narrowed depending on the reaction conditions.

**Tempering methods for controlling membrane architecture**

The objective of the second sub-project is to develop nano-porous membranes of a highly controlled architecture through the procedure of templating these membranes from deposits of nanoparticles. The resulting membranes will be evaluated in the context of their application to environmental separations such as water treatment and analytical separations. The utility of a porous material depends on the internal pore diameter, the pore size distribution, functionality, and morphology. Traditional methods for the fabrication of porous materials can produce membranes with one dimensional channel structures and have very little control over the pore size distribution. The template-derived process provides the means to fabricate three dimensional porous membranes that have well defined and well controlled pore sizes, completely interconnected pores and highly ordered structures, and to design the morphology of the pore structure to the nanosize scale; they can be prepared in a wide variety of materials, pore sizes and pore structures. The deposition of colloidal particles may occur by different mechanisms. The self-assembly technique by capillary forces provides precise control of the thickness of the film through sphere size and concentration in solution. An alternative approach is to deposit sequential layers of nanoparticles from Langmuir-Blodgett films. This year, the Colvin group

<table>
<thead>
<tr>
<th>Table 8.3.2: Conductivity measurements of membranes at 100% humidity.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alumoxane-derived ceramic</td>
</tr>
<tr>
<td>Ferroxane-derived ceramic</td>
</tr>
<tr>
<td>Nafion 117 (Sone, 1997)</td>
</tr>
<tr>
<td>(Sumner, 1998)</td>
</tr>
<tr>
<td>(Kopitzke, 2000)</td>
</tr>
<tr>
<td>Sol-gel Al_{2}O_{3}(Vichi, 1999)</td>
</tr>
<tr>
<td>SiO_{2} glasses (Nogami, 1998)</td>
</tr>
</tbody>
</table>
completed their knowledge transfer of templating methods to both the Barron and Wiesner labs; their role in future years will be reduced as the fundamental techniques are now well established.

One unusual membrane architecture is the production of hierarchical pore structures that consist of large voids connected by smaller pores; this structure has many advantages for separations and can also minimize fouling (Figure 8.3.3). We formed such materials by initially creating hollow alumina spheres by coating polystyrene beads of 3 µm and 50 - 80 µm diameter with carboxylic acid functionalized alumina nanoparticles (alumoxanes) from 2 - 8 wt% aqueous solutions. The resulting coated beads were heated to 220 °C to calcine the alumoxane to porous amorphous alumina before washing with toluene to remove the polystyrene from inside the ceramic coating. The resulting hollow spheres were then sintered at 1000 °C to form α-alumina. The α-alumina spheres show the hardness of the hollow alumina sphere (1900 ±100) approaches that of corundum (ca. 2000).

We also have substantial work now on using silica particles to define membrane pores. This method starts with the generation of deposits of silica particles ranging in size between 50 nm and 300 nm. Particles were prepared in the laboratory following the Stöber-Fink-Bohn method; also, in some cases silica particles were obtained from Nissan Chemical Industries, Ltd. The particle average sizes, standard deviation of the size distribution and sources are shown in Table 8.3.4.

Particles were deposited using the self assembly procedure of immersion of a glass slide in the suspension followed by evaporation. The conditions of evaporation were varied with the goal of speeding this process without sacrificing control over the deposit. Ionic strength of the solution and solvent (water with ionic strength adjustments or ethanol) were varied to control particle deposition and therefore template structure. The commercially supplied particles appeared to have a stabilizing agent associated with the surface of these particles that likely reduced the level of control obtainable through variations in solution chemistry. The higher the ionic strength, the stickier the particles become, and more particles deposit as aggregates resulting in a more disorganized, asymmetric structure. Figure 8.3.4 (a-c) shows the SEM images corresponding to deposits of silica nanoparticles of 244 nm average diameter. Case a is a deposit from ethanol; b is from an aqueous solution of 0.15 M ionic strength; and c from an aqueous solution of 1.5 M ionic strength.

<table>
<thead>
<tr>
<th>Particle Type</th>
<th>Mean Particle Size (nm)</th>
<th>Standard Deviation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si 300</td>
<td>305</td>
<td>9 %</td>
<td>(1)</td>
</tr>
<tr>
<td>Si 250</td>
<td>244</td>
<td>7 %</td>
<td>(1)</td>
</tr>
<tr>
<td>PST 1</td>
<td>88</td>
<td>21 %</td>
<td>(2)</td>
</tr>
</tbody>
</table>
In the case of ethanol, the particles suffer a net repulsive electrostatic force in the bulk approach the glass surface individually to form the deposit. For high ionic strength solvents (case b and c) the particles have formed aggregates while still in suspension and approach the glass and deposit as such. Figure 8.3.4 (d-f) shows deposits of 88nm particles: d is a deposit from pure water, e from a low ionic strength suspension and f from higher ionic strength. The deposits in d and e have similar, well organized structures; we would have expected the water case to be more organized, but the relatively high standard deviation of the particle size distribution in this case prevented the particles from forming a more organized arrangement. Case f indicates that some aggregation has occurred for this particle size and thus, the more disorder structured was formed. Figure 8.3.4 (g-i) illustrates the different deposits obtained from water, Figure 8.3.4 g, and from ethanol, Figure 8.3.4 h. Although in both cases, there should be no particle aggregation, the difference in solvent tension results in higher attractive capillary forces in the ethanol case, and thus, a more compact deposit. Figure 8.3.4 i shows again the open deposits obtained from high ionic strength solvents.

Porous polystyrene films were cast from these templates yielding a structure that presents very few pores on one side of the film feeding into many pores on the opposite side of the film (Figure 8.3.5). The creation of these structures in an important first step in creating membranes with hierarchical structures that may be an improved format for reactive membranes.

A second variety of membrane asymmetry was desired in which membrane templates would be composed of a layer of larger particles underlying a layer of smaller particles. The resulting membrane cast from such a template would have a structure more closely resembling current asymmetric membranes, only with greater control over the size and regularity of structures within each layer than that obtainable via conventional membrane casting techniques. Initial
attempts to create asymmetric membrane templates involved using the self-assembly method in sequential step. This produced templates with little order between the particle layers. The Langmuir-Blodgett for depositing particles layer by layer yield much better results but required some optimization of the process. The first approach pursued was the deposition of smaller particles on top of foundation of layers of larger particles. This method yielded imperfections in the small particle coat with the underlying large particle layers jutting through the small particle layer. This problem was corrected by inverting the deposition process and putting down a layer of small particles onto which was deposited the larger particle “support” layers (Figure 8.3.5).

**Plans**

- Development of metal/ferroxane composites that may be used as intelligent membranes with the ability to perform functions such as monitoring membrane integrity and detecting specific compounds.
- Testing of current generation of membranes for performance in a range of water treatment applications ranging from removal of arsenic, natural organic matter, and oils.
- Evaluation of doped membranes in performing catalysis on bench-mark reactions.
- Transfer our experience in making small particle size alumoxanes by a non-solvent route to the ferroxanes.
- Functionalization of templated membranes
- Evaluate function/structure trade-offs in a series of separations using polar and non-polar organic contaminants over a range a molecular weights.
- Fabricate templates and membranes from nanoparticles that will move cutoffs in to nano-filtration range (1 nm or less)
8.3.2. Polymer Flow on the Nanoscale (Pasquali, Kolomeisky)

**Summary**

This seed project was funded by CBEN for the period 9/2001 to 9/2003. The main thrust of the work was devoted towards the study of the translocation of polymer molecules through nanopores as a theoretical basis for developing new and improved membrane geometries. The phenomenon of translocation of a polymer molecule through a highly confined geometry is relevant in both chemical and biochemical processes. Experiments have been performed in recent years on DNA and RNA molecules driven electrophoretically in membrane channel. DNA and RNA translocation is important in the characterization of viral injection and in the design of sequencing techniques. More generally, the DNA molecule can be seen as a model for synthetic linear polymers, similarly to what has been done in the study of polymer dynamics in unconfined flows.

The dynamics of translocation and its dependence on the various parameter for a generic linear macromolecule may therefore be inferred from the results of these DNA experiments. In such a coarse-grained perspective, the chemical details of the DNA and RNA chains and of the pore are less relevant, and nanoscale computational models can provide relevant insight, and help bridging experimental results and theoretical understanding. *Results from such coarse-grained models developed in this project agreed well with published data by the groups of Kasianovic and Meller, and with the simple phenomenological theory of Slonkina and Kolomeisky.* During the time on this grant, Pasquali received a NSF CAREER award to continue this work on DNA visualization through pores under flow, and the projects were were co-funded for the last year. Joint funding was appropriate because all projects shared common focus on sub-microscopic scale behavior of biomolecules and polymers. Moreover, the nanopore project and the DNA collapse project shared a relevant portion of theoretical and computational tools. *In one case, DNA collapse induced by charge inversion was directly observed as was the visualization of single DNA molecules in micro-scale free surface flows.*

In a separate sub-project, with liquid-phase processing of single-walled carbon nanotubes (SWNTs) central to developing these materials for a variety of applications, and given their negligible solubility in standard solvents, understanding their flow behavior in those few solvents that successfully disperse them individually is vital. In work published as the cover article in the journal *Macromolecules*, SWNTs were successfully dispersed at concentrations up to 10 wt% in superacids, over 10 times the highest concentration ever achieved with wrapping or stabilization by surfactants. At low concentrations the tubes behave as Brownian rods, but at higher concentrations they form a highly unusual nematic phase consisting of self-assembled supermolecular strands of mobile, solvated tubes in equilibrium with a dilute isotropic phase. At even higher concentrations, these strands self-assemble into a polydomain nematic liquid crystal.
Results

Dynamics of Polymer Translocation through Nanopore: Theory meets Experiments

The dynamics of translocation of polymer molecules through nanopores was investigated via coarse-grained Molecular Dynamics simulations. We found that a minimalist computational model for the system was sufficient to capture all the experimentally observed trends and scaling behavior, nominally i) the two translocation regimes depending on the ratio between pore length and polymer length, ii) three different regimes for the probability of translocation depending on applied voltage, iii) an exponential dependence of translocation velocity on applied voltage, and iv) an exponential decrease of the translocation time with temperature. Each of the observed trends can be simply explained with a free energy analysis: the entropy of the polymer grows as the polymer approaches the nanopore because the wall of the membrane reduces the number of accessible configurations to the molecule; once the polymer enters the pore, it feels the electrostatic potential and is drawn further inside. The computational results compare well with published data by the groups of Kasianovic and Meller, and with the simple phenomenological theory of Slonkina and Kolomeisky.

Collapse of a semiflexible polymer in poor solvent

The dynamics and pathways of collapse of a single, semiflexible polymer in a poor solvent were investigated via three-dimensional Brownian Dynamics simulations. The dynamics of collapse of semiflexible molecules is relevant for understanding DNA condensation. DNA frequently forms condensed structures in vivo, e.g., in DNA replication, viral transfection, and compaction within sperm heads and nucleosomes. Controllable in vitro DNA condensation is involved in nonviral gene therapy—which is used in the treatment of various kinds of disease, including cancer—for packaging in, and transfection from gene delivery vehicles. Multivalent cations, in particular polyamines, have been used to induce condensation in vitro, showing that condensed DNA exists in toroidal and rodlike structures. Polyamines present ubiquitously in living cells in millimolar concentrations (e.g., putrescine and spermidine) are also believed to induce condensation in vivo. A better understanding of the dynamics of condensation would lead to improvements in gene delivery technology, as it may help defining the best conditions and protocols to obtain DNA condensation.

Earlier work indicates that the collapse of semiflexible polymers generically proceeds via a cascade through metastable racquet-shaped, long-lived intermediates towards the stable torus state. We investigated the rate of decay of uncollapsed states, analyzed the preferential pathways of condensation, and described the likelihood and lifespan of the different metastable states. The semiflexible chain collapse was studied as a function of the three relevant length scales of the phenomenon (the total chain length, the persistence length, and the condensation length). Two dimensionless ratios are found to suffice to describe the dimensionless decay rate of uncollapsed states. The condensation sequence is described in terms of the time series of the well separated energy levels associated with each metastable collapsed state. The collapsed states are described quantitatively through the spatial correlation of tangent vectors along the chain. Finally, we showed preliminary results that indicate that macroscopic steady shear flow would speed up the early stages of collapse but may lead to less regular final structures.

Visualization of Individual DNA Molecules in a Small-scale Coating Flow

Microscale free surface flows of dilute polymer solutions are important in various industrial and biological applications. In these flows, the velocity gradient stretches the polymer
molecules, and in turn the stretched polymer molecules change dramatically the behavior of the flow. Understanding the interplay of flow and polymer micro and nanostructure in complex flows is key for designing and controlling important processes such as ink-jet printing, spraying, and coating, as well as understanding biological processes such as deformation of blood cells and air displacement in pulmonary alveoli during respiration.

Individual DNA molecules in an ultradilute solution were observed with a fluorescence microscope, as they flow between a scaled-down rotating roll and a stationary glass knife. The roll picked up a thin layer of liquid from a pool and dragged it to the knife, establishing a bead delimited by two menisci. At low roll speed the flow was premetered and recirculations were present. The DNA experienced a nearly rectilinear shear flow at the minimum gap position where there was a zero velocity surface. We found that the measured mean and the distribution of fractional extension of DNA molecules in this region agreed with earlier literature results in a similar, simpler flow. Interestingly, no polymer distortion was observed at the two menisci. At high roll speed, capillarity was not strong enough to drive a back flow; the big recirculation broke into two smaller recirculations and two separation surfaces arose, upstream and downstream of the location of the minimum gap. At the upstream separation surface, most DNA molecules were extended parallel to the knife as they traversed the field of view. We reported the distribution of DNA extension and shape in this flow region. Slow, nodular recirculations were present under the upstream and downstream free surfaces. Notably, most DNA molecules stretched axially as they moved in these slow recirculating regions.

**Phase Behavior and Rheology of SWNTs in Superacids**

The protonation of single-walled carbon nanotubes in superacids allows them to be dispersed at high concentration, more than an order of magnitude higher than typical concentrations achieved in surfactants or organic solvents. The phase behavior of SWNTs in acid shows many parallels with that of lyotropic nematogenic rodlike polymer solutions. The notable distinction is that in the biphasic region the anisotropic phase consists of extremely long self-assembled strands. At sufficiently high concentrations, approximately 4 vol % in 102% H2SO4, a novel type of single phase nematic liquid crystal is formed. The nematic structure of SWNT/acid solutions is evidenced by the ability to form aligned species, birefringence, and rheological signatures including a maximum in the viscosity vs concentration curve, changes in sign of the first normal stress difference, and long oscillatory transients. In addition, the viscosity vs shear rate curve is suggestive of the three-region behavior associated with many lyotropic nematic polymeric liquid crystals. Exploiting successfully this phase behavior will be key to designing and optimizing a process for forming macroscopic objects comprised solely of highly aligned SWNTs.

Figure 8.3.7: SWNTs in and from superacids. (Upper left) Birefringent domains demonstrating nematic liquid crystal behavior. (Upper right) Aligned species, termed ailwives, formed by phase-precipitating the SWNTs by adding moisture. (Lower left) Extruded fibers of pure SWNTs with 20:1 alignment. (Lower right) a bucky-paper of entangled ropes formed by quenching into ether and filtering.
8.3.3. Nanocatalysts for Remediation of Environmental Pollutants (Wong, Colvin, Hughes)

Summary

The overarching goal of this proposed research, funded for the last nine months, is to develop, evaluate, and demonstrate new nanoscale catalysts for use in environmental applications. The membrane systems described above are an important element in water treatment systems, and in their passive form can serve to concentrate and remove contaminants from water. However, catalytic particles either dispersed homogeneously in solution or deposited onto membrane structures of the type developed in 8.3.1, can ensure that pollutants are chemically degraded and not simply moved somewhere else. We focus our efforts on the catalytic treatment of polluted water for which existing technologies are poorly efficient or cost prohibitive. After several years, our efforts will provide library of nanocatalyst systems to choose from so that water treatment systems can be designed to match the specific needs of a particular location.

Of particular interest here is the removal of trichloroethylene and organic aromatic contaminants, mainly pesticides, from groundwater. Each system requires a different catalyst and overall remediation strategy, but in both cases nanoscale engineering of materials permits the design of more efficient systems. The cooperative effect of doped metals in improving catalytic activity is well known in other examples, and we applied it to the problem of trichloroethylene (TCE) removal in groundwater. In one case, bimetallic catalyst particles supported on alumina have been demonstrated to have four to five times higher efficiency than pure metals alone in the hydrodechlorination of TCE. Organic aromatic contaminants present another species of concern for removal in groundwater. Our approach for this class of materials has been to rely on light-activated photocatalytic materials for their oxidation. Most of our work this year has centered on titania which under uv illumination is capable of photodissociating a variety of molecules. We have developed and submitted for publication a new way to produce high surface area (> 250 m$^2$/gm) nanocrystalline titania specifically for this application. We have begun detailed characterization of these systems and discovered that the (101) face of titania, most prevalent in our rod-like systems, is four to five times higher in its activity than other crystal faces. Additionally, ongoing work on the environmental implications of fullerenes, particularly C$_{60}$, led us to hypothesize that the oxygen radical production capabilities of nanoscale C$_{60}$ aggregates (nano-C$_{60}$) in water could be leveraged for degradation of contaminants. We have discovered in our preliminary data that nano-C$_{60}$ is a very efficient photocatalyst; it is a hundred to a thousand times more efficient than equivalent commercially available titania materials.

<table>
<thead>
<tr>
<th>Catalysts</th>
<th>Pd content (wt%)</th>
<th>$k_{\text{obs}}$ (gPd$^{-1}$s$^{-1}$)</th>
<th>Relative reaction rate</th>
<th>Selectivity$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd black</td>
<td>100</td>
<td>0.2</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>Pd NP</td>
<td>100</td>
<td>6.8</td>
<td>34</td>
<td>0.05</td>
</tr>
<tr>
<td>Pd-on-Au NPs</td>
<td>35.1</td>
<td>24.7</td>
<td>124</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>15.3</td>
<td>55.1</td>
<td>276</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>1.9</td>
<td>289.8</td>
<td>1450</td>
<td>2.16</td>
</tr>
<tr>
<td>Au NP</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ [Ethylene]/[ethane]
Results

Hydrodechlorination of chlorinated compounds

Palladium (Pd) is the most used metal for catalyzing hydrodehalogenation reactions, and Pd-based materials have been shown to catalyze the hydrodechlorination (HDC, $C_xH_yCl_z + zH_2 \rightarrow C_xH_y+z + zHCl$) of chlorinated organic compounds in water. Reinhard and co-workers performed field tests using Pd metal supported on alumina (Pd/Al$_2$O$_3$), which gave promising results for the HDC of trichloroethylene (TCE). Improvements in reaction rate and deactivation resistance should be possible by combining Pd with another metal (to create a bimetallic catalyst), as modification of the catalytic properties by a second metal is a known phenomenon. One well-studied material for HDC of contaminated water is composed of Pd and Fe, although it is not a reusable catalyst because the iron metal irreversibly decomposes during the reaction. Little work has been done on Pd-based bimetallic catalysts for the HDC of contaminated water.

Bimetallic catalysts for TCE reduction

Our approach was to focus on the Pd metal as the catalytic material, eliminating the use of the Al$_2$O$_3$ support. Thus, we initiated our investigation by synthesizing Pd nanoparticles (NPs). The synthesis method called for refluxing methanol, water, polyvinylpyrrolidone, and a Pd chloride salt for 3 hr. The methanol reduced the Pd$^{2+}$ to Pd$^0$ and the polymer provided steric stabilization to the colloidal suspension; the Pd NPs were ~10 nm in diameter. We hypothesized that Pd NPs would be more active than Pd metal because more Pd atoms (per gram of Pd) would be exposed at the particle surface. We compared Pd NPs and Pd metal (in the form of commercially available Pd black) for TCE HDC (Table 8.3.6). As we expected, Pd NPs were >10 times more active than Pd black, on a per-gram basis. We also estimated rates of reaction normalized to Pd surface area; the surface Pd atoms of Pd NPs appear to be intrinsically more active than those on Pd black and Pd/Al$_2$O$_3$. Commercially available Pd/Al$_2$O$_3$ was more active that Pd black and less active than Pd NPs, as the Pd metal on the Al$_2$O$_3$ support was dispersed in the form of surface-bound NPs. The Pd black and Pd/Al$_2$O$_3$ reaction rates were close to values determined by Reinhard and co-workers, giving us confidence that our experimental procedures did not give spurious results.

That we were able to run the TCE HDC reaction with a colloidal sol of Pd NPs motivated us to support Pd on well-defined colloidal metal NPs. We synthesized ~20-nm Au NPs using the citrate reduction method (in which citrate, Au chloride salt, and water were refluxed for a few minutes). We then reduced a controlled amount of Pd onto

<table>
<thead>
<tr>
<th>Pd catalyst</th>
<th>Surface area (m$^2$/g)</th>
<th>Pd content (wt%)</th>
<th>$k_{obs}$ (gPd$^{-1}$s$^{-1}$)</th>
<th>$k_{obs}$ (mPd$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd NPs</td>
<td>53$^a$</td>
<td>100</td>
<td>6.8</td>
<td>1.3×10$^{-1}$</td>
</tr>
<tr>
<td>Pd black</td>
<td>17</td>
<td>100</td>
<td>0.2</td>
<td>1.2×10$^{-2}$</td>
</tr>
<tr>
<td>Pd/Al$_2$O$_3$</td>
<td>177 (71$^b$)</td>
<td>1</td>
<td>2.0</td>
<td>2.8×10$^{-2}$</td>
</tr>
</tbody>
</table>

$^a$ Calculated
$^b$ Calculated from 21% dispersion; surface area of Pd (m$^2$/gPd)

Figure 8.3.8: Initial reaction rates
the Au NP surface using ascorbic acid as the reducing agent to create Pd-on-Au NPs. The final catalysts contained Pd ranging from 2 wt% to 35 wt% of total catalyst weight (with the balance being the weight of the Au). From UV-vis analysis of the colloidal sols, we concluded that Pd was reducing onto the Au NP surface; bulk and surface elemental analyses were not performed.

Under the same reaction conditions, three Pd-on-Au NP catalysts were tested and compared against the control samples of Pd NPs and Au NPs. Au NPs were inactive for TCE HDC, but 2 wt% Pd (equivalent to 1/3 Pd monolayer on the Au NP surface) led to a dramatic increase in initial reaction rate (Figure 8.3.8). Additional amounts of Pd (above the Pd monolayer coverage) decreased the reaction rates, indicating that the exposed Au surface participated somewhere in the TCE HDC reaction pathway. In all of three cases, the Pd-on-Au NP catalyst structure was more active than pure Au and pure Pd (Table 8.3.5). Ethane is the product of the TCE HDC reaction, but interestingly, ethylene was detected as a reaction product with the bimetallic catalysts. The selectivity to ethylene over ethane was highest with the 2 wt% Pd-on-Au catalyst, providing a clue in determining how Au is enhancing the HDC reaction. The effect on ethylene selectivity has been reported for Pd/silver bimetallic catalysts for gas-phase HDC of 1,2-dichloroethane by Heinrichs and co-workers.

We hypothesize that TCE adsorbs on Pd via a π bond-Pd or Cl-Pd interaction. Hydrogen also chemisorbs on Pd but does so dissociatively. The hydrogen atoms break the C-Cl bond, such that a C-H is formed and a Pd-Cl bond is formed. This process continues until all the 3 Cl atoms of TCE are replaced by the H to form ethylene. This would be consistent with data collected by Reinhard and co-workers which indicate that saturated dichloroethanes do not undergo the HDC reaction. Chemisorbed ethylene continues to react, hydrogenating to form ethane. This reaction mechanism (dechlorination followed by hydrogenation) is presumably perturbed with surface Au atoms nearby. The higher overall reaction rate could be due to enhanced dechlorination of TCE by the Pd (with its electronic structure modified by the nearby Au atoms), and the generation of ethylene could be indicative of slower ethylene hydrogenation (the new rate-limiting step) relative to ethylene desorption.

To test the effect of metal NP support material, we synthesized a series of Pd-on-Ag NPs. We found that these materials did not exhibit negligibly enhanced rates as those of Pd NPs. We explored other NP substrate materials, specifically Pd-on-Al₂O₃ NPs and Pd-on-SiO₂ NPs; no improvement was detected. We have started to prepare Au/Pd-based supported on Al₂O₃ materials, as a route to improving the Pd/Al₂O₃ currently being studied for TCE HDC. We impregnated commercially available Pd/Al₂O₃ with Au salt (to create a Au-on-Pd/Al₂O₃ catalyst structure), and performed a sequential impregnation/calcination on γ-Al₂O₃ (to create a Pd-on-Au/Al₂O₃ catalyst structure). Even without complete characterization of the two catalysts, preliminary data indicated that the former material was 4-5 times more active than Pd/Al₂O₃ and the latter material was as active as Pd/Al₂O₃.

**Photocatalysts for treating aqueous contaminants**

The objective of this work is to develop nanoscale materials which under light irradiation degrade aqueous contaminants. Within CBEN, this research provides an important capability to our water treatment and purification system in that it offers a route for directly degrading organic compounds such as turbacil in contaminated waste. In the past year this new project (funding started 09/03) has developed and characterized nanomaterials with active photocatalytic capabilities. Our initial efforts have focused on nanoscale titania; larger titania particles are known to possess photocatalytic properties and our work aimed to examine these properties in smaller nanocrystalline system. *We have developed and submitted for publication a new way to*
produce high surface area (> 250 m²/gm) nanocrystalline titania. The photocatalytic efficiencies of this and several other model systems have been quantitatively characterized and compared. Interestingly, our data indicate that the (101) crystal face of titania is substantially more active than other faces in photocatalysis. Most recently, because of ongoing work in the nano-cell interactions project, we have evaluated the photocatalytic performance of nanoscale fullerene aggregates, or nano-C₆₀. We find nano-C₆₀ at concentrations of only 10 ppm in water has a photocatalytic efficiency comparable to that of a 1000 ppm solution of nanoscale titanias. This new discovery provides an entirely new class of materials for consideration as photocatalysts for water treatment and purification.

Titania is a well known photocatalyst whose application to water treatment has been the topic of extensive literature. Upon photoexcitation by ultraviolet (uv) light, crystalline anatase is thought to generate an electron/hole pair and the eventual trapping of the hole at the particle surface leads to the generation of a hydroxyl radical through the following reaction:

\[ \text{h}^+ + \text{OH}^- (\text{adsorbed}) \rightarrow \text{OH}^* \]

This hydroxyl radical in water is highly reactive and will degrade a variety of organic compounds. A typical reaction for a phenol is given below:

\[ \text{C}_6\text{H}_5\text{OH} + 28 \text{OH}^* \rightarrow 6 \text{CO}_2 + 17 \text{H}_2\text{O} \]

In our work, we used this same basic chemistry to degrade two model dyes, Rose Bengal and Congo Red. An important feature of this process is the requirement that the dye be sorbed to the particle surface in order to maximize its exposure to the short-lived radicals. Thus nanocrystalline titania should provide for a greater degradation rate because of its high surface areas relative to the bulk. Our work addressed this issue and also considered whether other material properties such as crystallinity, surface chemistry, or shape could influence the photocatalytic efficiency.

<table>
<thead>
<tr>
<th>Synthetic method</th>
<th>T (°C) or company</th>
<th>Crystalline phase(s)</th>
<th>Particle shape</th>
<th>d (nm)</th>
<th>Surface area (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrothermal, no surfactant</td>
<td>140</td>
<td>Anatase</td>
<td>Dots</td>
<td>5.4</td>
<td>251</td>
</tr>
<tr>
<td>Hydrothermal, no surfactant</td>
<td>150</td>
<td>Anatase</td>
<td>Dots</td>
<td>6.7</td>
<td>153-161</td>
</tr>
<tr>
<td>Hydrothermal, no surfactant</td>
<td>200</td>
<td>Anatase</td>
<td>Dots</td>
<td>7.7</td>
<td>---</td>
</tr>
<tr>
<td>Hydrothermal, no surfactant</td>
<td>220</td>
<td>Anatase</td>
<td>Dots</td>
<td>8.9</td>
<td>128</td>
</tr>
<tr>
<td>Hydrothermal with low surfactant</td>
<td>90-100 (reflux), 180-190 (hyd. trt.)</td>
<td>Anatase</td>
<td>Dots</td>
<td>50</td>
<td>94.5</td>
</tr>
<tr>
<td>Hydrothermal with high surfactant</td>
<td>90-100 (reflux), 180-190 (hyd. trt.)</td>
<td>Anatase</td>
<td>Rods</td>
<td>200-250 (long axis)</td>
<td>28.0</td>
</tr>
<tr>
<td>Aerosol pyrolysis</td>
<td>Degussa</td>
<td>Anatase (80%), rutile (20%)</td>
<td>---</td>
<td>~ 21</td>
<td>50 ± 15</td>
</tr>
</tbody>
</table>

The synthesis of high quality nanocrystalline titania with tunable physical properties was the focus of the first six months of this project; while synthetic conditions for producing titania with grain sizes in excess of 9 nm are known, the formation of much smaller materials with grain sizes under 5 nm and high crystallinity is a significant challenge. We used a modified hydrothermal methodology in which titania precursors were directly injected in a bomb operating
at 600-1100 psi and 150-220 °C. Because of the control over the nucleation process, this method allowed us to produce titania with grain sizes of 5-9 nm and, correspondingly, high surface areas (Table 8.3.7). We also adapted a hydrothermal method that uses surfactants such as tetramethylammonium hydroxide (TMAH) to generate larger titania rods and dots.

Figure 8.3.9 shows the typical materials produced in this work. We use a variety of techniques to analyze our systems including x-ray diffraction, BET surface area analysis, thermo-gravimetric analysis, Raman spectroscopy and infrared spectroscopy. In particular, we focus carefully on the crystalline phase and quality of our materials. Amorphous titania is not an efficient catalyst and its presence in our materials was minimal. Our publication details the characteristics of our hydrothermally generated materials, and provides us with the raw materials for further photocatalytic characterization.

Most recently we have begun to characterize the photocatalytic properties of these and other nanoscale titania materials. Quantitative assessment is best done using dye molecules whose change in absorption provides a measure of the degradation process. The degradation of this dye only occurs in the presence of titania (uv light alone has no effect); these data can be fit using a Langmuir-Hinshelwood kinetic analysis to extract the rate constant k and adsorption equilibrium constant K. Table 8.3.9 shows our preliminary analysis of this data for several classes of nanoscale titania. On a mass basis, the smaller nanoscale titania dots (k = 4.21 µmol g⁻¹ min⁻¹) are about as effective at photodegradation of Congo Red as the commercially available material from Degussa (k = 4.54 µmol g⁻¹ min⁻¹). However, more interesting is the data for the rod-like anatase particles, in which the (101) surface is dominant. When normalized to surface area, the photocatalytic efficiency of these rods is greater than that of dots. We conclude from this that the (101) face of the crystal structure must be particularly efficient at OH radical production.

<p>| Table 8.3.8: Langmuir-Hinshelwood parameters calculated for Congo Red photodegradation using different nanocrystalline titania samples. |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>k (µmol g⁻¹ min⁻¹)</th>
<th>k (µmol m⁻² min⁻¹)</th>
<th>K (L µmol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small dots</td>
<td>4.21</td>
<td>2.75 × 10⁻²</td>
<td>3.26 × 10⁻¹</td>
</tr>
<tr>
<td>Large dots</td>
<td>3.10</td>
<td>3.28 × 10⁻²</td>
<td>2.81 × 10⁻⁵</td>
</tr>
<tr>
<td>Large rods</td>
<td>1.61</td>
<td>5.75 × 10⁻²</td>
<td>2.54 × 10⁻⁴</td>
</tr>
<tr>
<td>Commercial (Degussa P25)</td>
<td>4.54</td>
<td>9.07 × 10⁻²</td>
<td>4.16 × 10⁻²</td>
</tr>
</tbody>
</table>
Additionally, we have evaluated the photocatalytic efficiency of nano-C$_{60}$ in these model aqueous reactions. As described in section 8.3.5, nano-C$_{60}$ is a potent bactericide as well as a highly cytotoxic species. We show that these features can be correlated with its ability to generate oxygen radical species in solution. This observation suggested the application of nano-C$_{60}$ to photocatalysis. Figure 8.3.10 shows the degradation of Congo Red under UV illumination in the presence of nano-C$_{60}$. On a per gram basis we estimate this aggregated fullerene material is ten to thirty times more effective than nanoscale titania as a photocatalyst. The application of any fullerene to photocatalysis has never been reported, and we are currently exploring the limits of its use in water treatment applications.

**Plans**

**Hydrodechlorination**

We have identified a new material that catalyzes the destructive removal of TCE from water at room temperature at reaction rates orders of magnitude faster than currently available materials. We will determine the amount of surface Pd atoms in the Pd-on-Au NPs; explain the reaction mechanism through spectroscopic analyses and HDC reactions with model chlorinated compounds; and optimize the synthesis of the Pd-on-Au/Al$_2$O$_3$ material.

**Photocatalysis**

Over the next year we will conclude our analysis and optimization of nanoscale titania for photocatalysis. We will evaluate the most promising materials for their ability to remove model pesticides and biologicals from groundwater. We will also provide the first characterization of C$_{60}$ as a photocatalyst, and determine if the material is practical for water treatment and purification.
8.3.4. Sorption of Contaminants onto Engineered Nanomaterials (Tomson, Colvin, Laibinis)

Summary

As pointed out in 8.3.1, general water treatment schemes can remove much waste from water, but targeted treatment schemes are most appropriate for highly toxic compounds that must be removed with high efficiency. For many heavy metal species, catalytic remediation of the type described in project 8.3.3 is not an option for their removal, so water treatment schemes focus instead on sorption onto either polymers or particle additives. In this project, ongoing for just nine months, we target a particularly insidious and toxic contaminant for removal from water: Arsenic. Arsenic from both natural and manmade sources is a common contaminant in water; recent data makes clear the correlation between its consumption and an increased level of bladder and rectal cancers. In response to this new data, the US has lowered the drinking water standard for Arsenic to 10 ppb, a value that is challenging to reach in most water treatment plants. Worldwide, arsenic poisoning is a huge public health disaster in many developing nations, such as Bangladesh, where over 10-20% of the population is now afflicted with arsenic poisoning. Although arsenic contamination is generally associated with third world countries, many drinking water supplies in America exceed the 10 ppb level recommended by public health officials. New technologies which can target and remove heavy metal contaminants, such as arsenic, from drinking water to present and planed levels are desperately required.

For this work, magnetite nanocrystals are the core system for arsenic removal. Not only have iron mineral surfaces shown preferential sorption of arsenic species, but also in the right size range these magnetic particles can be removed from water via magnetic separations. The first need in this project was the development of high quality magnetite materials well suited for aqueous separations. We have developed a strategy for generating monodisperse magnetite which provides for good size control in the 15-20 nm diameter range most useful for magnetic separations. Surface chemical control over these materials is essential as they are produced in organic solvents. For the first time we have applied oxidative cleavage of hydrophobic capping groups on magnetite to yield water-soluble materials with high yield in a single step. We have confirmed that nanoscale magnetite is as effective as bulk iron minerals in its sorption of arsenic. In fact, not only is its sorption capacity higher but the material once associated with the nanoparticles is difficult to remove. Taken together these results indicate that magnetite nanoparticles are very effective adsorbents for Arsenic, especially at low pH, and their irreversible sorption provides an efficient storage sink for collection of the waste. Finally, the magnetic separation of nanoscale particles has been evaluated for these nanomaterials. We have used magnetic filtration to size separate the prepared particles, thereby enhancing their capture yield and ability to be manipulated magnetically.
Results

Materials Formation and Characterization

Our specific goals this year have been:

- To produce high quality and size-controlled magnetite, with diameters from 3-20 nm.
- To develop strategies for transferring these nanocrystals into aqueous solutions.

Particle production. The synthesis of high quality magnetite in nanoscale sizes is a significant challenge. Until 2003, the most popular approach relied on the precipitation of iron hydroxides from water, and their subsequent condensation after heating into poorly crystalline and often agglomerated magnetite. New methodologies for forming these materials use direct decomposition of iron precursors in high temperature organic solvents; the first report of such a reaction yielded particles up to 10 nm in diameter. This is too small to have the large and permanent dipole moment required for separations. We have developed another approach for generating monodisperse magnetite which uses cheaper solvents and a different way of controlling particle nucleation. Examples of the materials produced are shown in the transmission electron micrograph (Figure 8.3.11). X-ray diffraction and surface area analysis all indicate these materials are phase pure, and highly crystalline magnetite and this work is currently being described in a paper under preparation. Of most importance of this project is our ability to tune the particle size with a great deal of precision, particularly in the 15-20 nm range which is thought to be optimal for magnetic separations.

Surface chemistry. While the high temperature routes for particle production afford us a great deal of control over nanoparticle quality and size, they produce materials that are coated with organic functionalities and not soluble in water. For this project, we ultimately aim to create a highly tailored particle interface, but our near-term goal was to produce materials that could be easily solubilized in water for sorption studies. Phase-transfer of the particles using surfactants is a reliable and easy approach to the

Figure 8.3.12: Iron oxide particles in water, stabilized by Tween-20. As the ionic strength of the solution increases the stability of the surfactant coating decreases.

Figure 8.3.13: The scheme to phase transfer iron oxides into water uses a mild oxidizing agent to cleave the double bond of oleic acid, yielding particles covalently capped with a carboxylic acid.
problem; Figure 8.3.12 shows images of the particles in solution. The problem with surfactant stabilization is that the coating is not strongly associated with the particle surface, and in high ionic strength conditions particles condense out of solution. Additionally, a surfactant coating is not an ideal platform to build the multifunctional interfacial polymer we will need to ensure the use of these materials in separations.

Because of the limitations of surfactant coatings, we developed a new strategy to render the particles water soluble. The surface of these materials is coated oleic acid; its double bond is available for oxidative cleavage, yielding a polar carboxylic functionality. To complete this process, particles in oleic acid were treated with an oxidizing agent (KMnO₄) and a phase transfer agent. The resulting materials formed water soluble suspensions in high yield which are well suited for precise design of a polymeric interface. In addition, this in situ synthetic route preserved the surface composition unlike other surfactant replacement procedures. The outer carboxylic acid ends also enabled pH dependant property which allows easy recovery and transfer between different buffers. Further research is now carried out to have a one phase oxidative cleavage process in order to increase speed and efficiency of the reaction via better accessibility of the MnO₄ oxidant. Recent runs showed that small amount of acetic acid catalyzes this cleavage reaction.

**Sorption characteristics of magnetite**

The Tomson group has studied the sorption and desorption of As(V) to large (3 µm, SSA = 4 m²/g) and nano-sized (20 nm, 58 m²/g) magnetite (Fe₃O₄) at two pHs (pH 4.8 and 6.2). The point of zero charge of magnetite particles was determined to be 6.8 with alkalimetric and acidimetric titrations. The adsorption kinetics are very fast for the adsorption of As to both large and nano-sized magnetite crystals. It has been observed that magnetite particles tend to form

![Figure 8.3.14: Plots of (a) adsorption isotherms of As(V) to large magnetite particles at pH 4.8 and 6.2; (b) adsorption isotherms of As(V) to magnetite nanoparticles at pH 4.8 and 6.2; and (c) adsorption isotherms of As(V) to sonicated magnetite nanoparticles at pH 4.8 and 6.2.](image-url)
aggregates, especially when applying a magnetic field to the system. With weak sonication, we are able to disaggregate the magnetite particles and expose more surface area for adsorption. At both pH 6.2 and 4.8, As(V), on a per-mass basis, adsorbs stronger on the sonicated nanoparticles (Figure 8.3.14) than on large crystals. When normalized by surface area, the large sized and nano-sized magnetite showed similar Langmuir adsorption capacity. However, the adsorption slope ($Q_{\text{max}} \cdot b$) is much larger for the nano-sized particle than for the large crystals. Interestingly, this observation contradicts our previous observation of Cd adsorption to anatase nanoparticles. The adsorption of Cd to anatase showed a smaller adsorption slope for the nano-sized particle than for the large crystals (see Table 8.3.10). Furthermore, Aresenic adsorption to magnetite nanoparticles is much stronger at low concentrations. The overall capacity for sorption is much higher than Cd adsorption to anatase. Taken together these results indicate that magnetite nanoparticles are very effective adsorbents for As. The adsorption is stronger at lower pH.

In Figure 8.3.15 we plot the desorption of As from large and nano-sized magnetite crystals at pH 6.2. Strong hysteretic desorption was observed for both magnetite crystals, again, completely different than Cd desorption which was 100% reversible. For the large crystals, about 10-25% As was desorbed in three desorption steps. However, less than 1% of total As was desorbed from magnetite nanoparticles. The solution phase As concentration for desorption step two and three are about the same. The strong and irreversible sorption of Arsenic onto nanoparticles is another indication that these materials will be well suited to removal of arsenic in water; in addition to providing a high capacity for sorption because of the high surface areas, nanoscale magnetite will also retain the toxic material even after removal from waste streams.

These results showed that magnetite nanoparticles could be very efficient adsorbent for water treatment to remove As. Several technical issues need to be resolved, e.g., (1) the adsorption and desorption of As(III), (2) the separation of magnetite aggregates, (3) the effective way to regenerate, reuse, and dispose of magnetite, and (4) the effect of natural occurring humic and fulvic acid (NOM) on As sorption. Natural organic matter itself can also interact with arsenic. Redman (13) found natural organic matter rapidly oxidized arsenite to arsenate, and some natural organic matter also reduced arsenate to arsenite, indicating that NOM may strongly influence arsenic redox speciation. These issues will be the research focus of next year.
Magnetic separations

The Laibinis group has used a variety of synthetic procedures for producing magnetic fluids that are being used in studies of the dispersity and collection of magnetite nanoparticles as augmented by the application and localization of magnetic fields. The magnetite nanoparticles comprising the investigated magnetic fluids have been prepared in our laboratory by chemical precipitation from Fe^{II/III} solutions by the addition of ammonia, with different stabilizers added to the reaction mixture as a way to control particle size. Stabilizers include molecular agents such as lauric acid and polymeric species such as poly(acrylic acid) that coordinate to the nucleated particle to limit their growth and to control their level aggregation; uncoordinated carboxylic acid group are also exposed on the outer surface of the particles and provide future sites for the attachment of coordinating sites for targeted metal ions as need for remediation activities. We have used magnetic filtration to size separate the prepared particles, thereby enhancing their capture yield and ability to be manipulated magnetically. Specifically, we have investigated the movement of magnetic particle-rich fluids into native aqueous phases, where dyes have been used to follow perturbations and mixing caused by the magnetically driven movement of the magnetic fluids into other phases. Different size regimes have been observed whereby the external dimensions can produce environments where external media are swept aside by the boundary layer surrounding the volume of magnetic fluids or are transported through the magnetic fluids to be thoroughly mixed and contacted with the nanoparticles. Continued work will examine these regimes with a target to optimize rapid contact times between phases as well as to target future work toward the localization of chelators on the magnetic particle surface to allow ready extraction of contaminating ions present in water streams to be cleaned.

Plans

- Evaluate the size dependent sorption characteristics of As(V)/As(III) to magnetite nanoparticles produced in both synthetic routes (water versus organic)
- Determine the optimal conditions for the removal of magnetic materials from waste streams.

Figure 8.3.15: Plots of (a) As(V) desorption from large magnetite particles at pH 6.2; and (b) As(V) desorption from magnetite nanoparticles at pH 6.2.
• Evaluate the effects of natural organic matter (NOM) on the removal characteristics of nanoscale magnetite.
• Generate porous polymer coatings on particles for enhanced sorption capacities.
• Determine the separation efficiency of arsenic coated magnetic sorbents in a magnetic separation system.
• Explore the application of these materials to lead removal.
8.3.5. Nano-cell Interactions (Hughes, West, Colvin, Dreher (EPA), Bucher (NIEHS), Howard (FDA), Warheit (DuPont))

Summary

This cross-cutting project spans all three themes within the Center, and is described here because of its strong interactions with the environmental exposures projects. The overall aim of this work is to evaluate the toxicological effects of engineered nanoparticles produced in liquid phase reactions; while there is a substantial literature on the effects of incidentally produced aerosols on living systems, particularly through inhalation exposures, virtually nothing is known about the toxicology of engineered nanoparticles that are produced through liquid phase routes. We use toxicology studies to provide information about the fundamental interactions of engineered nanoparticles with cells, so that appropriate surface coatings can be devised to either minimize or accentuate their effects depending on the needs of the application. In response to recommendations from last year’s site visit group, we have extended this project to include toxicology groups both in government and in industry.

We began our work on the eco-toxicology of nanostructures at the start of CBEN two years ago with a focus on a model engineered nanostructure, C60. We have found and reported in a paper soon to be under review that upon contact with water, a pristine C60 will form nanoscale aggregates with hydrophilic surfaces and remarkable stability. Typical concentrations for these materials, which we term nano-C60 because of their size (50 nm) and internal crystallinity, can reach up to 100 ppm but typically are 10-50 ppm. Another publication reports that these species are quite effective bactericidal agents against both gram positive and negative bacteria with EC50 values of 1 mg/L, or ~ 10 nM. We find a substantial physical association with the membranes of bacteria and anticipate these substances will have large effects on the microbial ecology of natural aqueous systems - a topic for the next year of our research with new CBEN participant, Pedro Alvarez. Against earthworms nano-C60 is moderately to non-toxic, and association is only observed for fullerenes introduced into soil in a solid state. Aquatic toxicology described in a recent publication found nano-C60 exhibited only moderate toxicity, but did oxidize lipids to some extent in the brains of fish.

We have made great progress in understanding and controlling the biological effects of C60 using cytotoxicology studies with human dermal, liver and brain cells. We characterized the toxicology of nano-C60 in culture; in a 48 hour test its LC50 was 20 ppb in human dermal cells which is substantially more toxic than molecules such as paraquat. In a comparative toxicology study, we correlated the toxicity of both nano-C60 and other water soluble fullerene derivatives to their degree of derivatization. We also showed that the toxic fullerene species targets specifically the cell membrane by production of oxygen radicals, and that this effect can be turned off by the appropriate derivatization of the C60 molecule.

We have an ongoing collaboration with two inhalation toxicology groups (Warheit, DuPont) and Dreher (EPA). Specifically nanoscale quartz, titania and labeled iron oxides (57Fe3O4) are being studied in both cell culture and lung instillation studies to evaluate how particle size and surface chemistry influence the biological effects and biodistribution of nanoparticles. Efforts with the National Institutes of Environmental and Health Sciences (NIEHS) and the Federal Drug Administration (FDA) use similar materials, but are focused on dermal routes for engineered nanoparticle exposure and immunotoxicology.
Results

Carbon-60 ($C_{60}$), the prototypical carbon-based nanoparticle, is arguably the most well studied engineered nanoparticle. More generally, impending industrial applications of engineered nanomaterials are spawning a growing scientific and public interest in their environmental and health impacts. $C_{60}$, for instance, has tremendous commercial appeal; it is being considered for applications ranging from fuel cells to lubricants, and 2003 brought the first industrial-scale production of the material in multi-ton quantities. Impact studies on this and other engineered nanomaterials are therefore relevant not just because they provide an understanding of these materials in our environment, but also because they permit companies to develop sustainable manufacturing and disposal practices.

The Environmentally Relevant Form of $C_{60}$

Upon contact with water under a wide range of very mild conditions, underivatized $C_{60}$ forms suspended and water-stable nanoscale aggregates, here termed ‘nano-$C_{60}$.’ In fact, two of the most important environmental release forms of $C_{60}$, solid state $C_{60}$ and organic solution, form dark cloudy mixtures upon contact with water that upon filtration through 200 nm filters leave yellow suspensions of these aggregates (Figure 8.3.16). In the case of solid state $C_{60}$, the aggregates form over the course of several days, but from the organic solutions the aggregates form immediately. This material behaves differently from bulk $C_{60}$ in that it does not re-extract into toluene quickly nor does it adsorb to hydrophobic columns. Although this concentration is too low to be useful for many engineering applications, even very low concentrations of sparingly soluble materials in water can have significant effects on the environment, thus an understanding of this process and the material it generates is essential for characterizing the environmental chemistry of fullerenes. This suspended form of $C_{60}$ has been characterized by a wide range of techniques, revealing that it consists of dense aggregates of $C_{60}$, predominantly in a nanocrystalline form with an average particle size of 50 nm ± 10 nm.
Bacterial Studies

This form of n-C$_{60}$ strongly associates with the biomass of two well studied species of bacteria, *Escherichia coli* DH5$\alpha$ and *Bacillus subtilis* at orders of magnitude of 100 µg/g dried cell mass in *E. coli* and 10 µg/g in *B. subtilis*. Monitoring the respiration of both bacteria in minimal Davis media showed that after the addition of 4 mg/L n-C$_{60}$, respiration rates decrease as would be expected for a bactericidal/bacteriostatic agent. In contrast to literature reports of studies performed on derivitized fullerenes, we found that the presence of light and/or oxygen also had no effect on the observed bacteriocidal/bacteriostatic behavior of n-C$_{60}$. In comparing n-C$_{60}$’s efficiency as a bactericide to known materials, we can conclude that this form of C$_{60}$ is a potent antibacterial agent whose presence at nanomolar levels in natural waters will alter microbial ecology.

Earthworm Studies

We also examined the interactions of n-C$_{60}$ with another indicator organism in terrestrial ecotoxicology, earthworms. Many hydrophobic organic contaminants are concentrated in earthworms, and the hydrophobic composition of n-C$_{60}$ suggested it might be a candidate for bioaccumulation. In contrast to the observations with bacterial systems, no n-C$_{60}$ association is observed in the earthworm species *Lumbricus terrestris* (*L. Terristris*) and *Eisenia fetida* (*E. Fetida*) (ASTM Method E 1676-97). Also, n-C$_{60}$ had no effect on the mortality of *E. Fetida* up to concentrations of 18 mg/kg soil. It may be that the size of the aggregates precludes incorporation into organisms through either skin or GI tract absorption. Alternatively, the negatively charged surface of the particles that provides for their stability in water may reduce the propensity for uptake and storage in lipids as would be predicted from bulk characteristics. However, we note that n-C$_{60}$ has recently been shown to induce lipid peroxidation in the brains of large mouth juvenile bass at relatively low concentrations (1ppm), and induces moderate toxicity against *Daphnia Pulicaria*. In bacterial cultures, when n-C$_{60}$ was precipitated from growth media due to high salt contents (Luria Broth (LB) or Davis media supplemented with 0.2% KNO$_3$) bacterial growth was unaffected. While n-C$_{60}$ is a stable material under a wide range of pH levels and ionic strengths, there may be environmental conditions that neutralize its biological activity.

Cytotoxicity

To evaluate these biological effects more broadly, and in systems more relevant for mammals, we evaluated the response of human cells in culture to n-C$_{60}$. Human dermal fibroblasts (HDF) are a model screening system for evaluating the biocompatibility of new materials. It has been shown that HDF cells are good indicators for whole animal dermal toxicity of pesticides and insecticides. The biological effect of n-C$_{60}$ against this cell line was quantified using a standard 48 hour cytotoxicity screen which utilizes a two-color fluorescence assay. From cell counting under a fluorescence microscope, we can measure the live and dead cells and thus the LC$_{50}$ value, or the concentration at which half the cells are observed to die. We validated our procedure using Paraquat as a control; our measured LC$_{50}$ value for Paraquat is 150 ppm as compared to existing literature reports of LC$_{50}$ values of 220 ppm, a value that is well within the variability expected between different cell lines. Cell death was confirmed in all experiments by verifying the release of lactate dehydrogenase as n-C$_{60}$ concentration increased.

Aqueous suspensions of n-C$_{60}$ are toxic to HDFs in concentrations higher than 100 ppb. The cell viability increases with lower doses of n-C$_{60}$ suspensions and obeys a typical dose-response relationship. The LC$_{50}$, or the dose of n- C$_{60}$ that kills 50% of the HDFs in a 48 hour experiment, extracted from this data is ~20 ppb (p < 0.03). We confirmed that the aggregates
themselves are the causative agent by testing resuspended material purified by centrifugation. Recovered powders of n-C\textsubscript{60} contain up to 10 wt\% of low molecular weight impurities, primarily toluene and THF, and it is reasonable to assume these small molecules are intercalated into the n-C\textsubscript{60}. However pure toluene is not toxic to HDFs at the 10-100 ppb levels observed for the n-C\textsubscript{60} suspensions, and is even less cytotoxic against human dermal cell lines. The measured LC50 of 20 ppb against human dermal fibroblasts is among the lowest known values for any organic compound. The high susceptibility of human dermal fibroblasts as well as Gram positive and negative bacteria to this material suggests that these aggregated C\textsubscript{60} systems possess a general property that enables them to disrupt cellular functions with high efficiency. On the other hand, C\textsubscript{60}(OH)\textsubscript{18} shows very different biological effects than n-C\textsubscript{60} suspensions, having no effect on growth or respiration of bacteria, nor on the viability of HDFs in culture. This data illustrates that if n-C60 is to be developed as a broad spectrum biocidal agent or applied as a therapeutic in medicine, it will be necessary to protect the material from hydroxylation. Alternatively, treatment of fullerene materials so as to hydroxylate their surfaces is one strategy to avoid the unwanted biological and environmental effects of n-C\textsubscript{60} aggregates in surface waters. We have fully correlated the cytotoxicity of C\textsubscript{60} molecules generally to the extent of the dervatization of the cage as well (See Nuggets, section 6).

**Remediation**

Using our cell toxicity assays we surveyed a number of biochemical endpoints to determine the general mechanism for cell death upon exposure to nano-C\textsubscript{60}. Our findings clearly indicated that oxidation of the cell membranes was present in exposed cells, and as a result these membranes were leaky and likely contributed to cell death. In contrast, no damage to internal organelles (DNA, Mitochondria) was observed. We found that further derivatization of the fullerene cage limited its ability to generate oxygen radicals and thus rendered the material non-toxic. We concluded that simple treatments of nano-C\textsubscript{60} waste streams provided a practical method for preventing any unintended effects of this material on aquatic environmental systems.

**Results: Toxicology partnerships**

Inhalation toxicologists have a long history of evaluating the effects of incidentally generated ultra-fine particles on organisms; engineered nanomaterials provide them with the ability to study fundamental particle-organism interactions in systems with highly controlled surface, surface chemistry and chemical composition. **Dr. Kevin Dreher at the EPA** is an inhalation toxicologist with great interest in the effects of inhaled metal particles on human health; by using labeled (Fe-57) iron oxide particles generated and characterized by CBEN students in lung instillation experiments he is evaluating the biodistribution of nanoscale irons in rats. Much of the surface chemical expertise needed for these materials described in section 8.3.3 is directly transferable to this collaboration; using lung surfactant solutions we are finding that particle aggregation in this biological media is common making the exact determination of effective particle size problematic. CBEN students are also engaged in collaborations with **Dr. David Warheit at DuPont**; in this project, we are evaluating the hypothesis that nanoscale particles are more toxic than their bulk counterparts. Our data from one and three month instillation experiments in rats with nanoscale titania and quartz disprove this hypothesis. We are repeating this data set in June, but our initial findings suggest that particle surface chemistry is more important than size in governing the response of the lung to these model ultra-fine particles.
Dermal exposure of engineered nanoparticles is an equivalently important exposure route to consider, especially given that most engineered nanoparticles are produced and handled in the liquid phase. In collaboration with Dr. Paul Howard at the FDA CBEN students are providing highly monodisperse and emissive quantum dots for visualizing how particle size effects the dermal penetration of nanoparticles; using laser capture microdissection techniques, his team is quantifying the dynamics of particle penetration into the skin of mouse models. Preliminary data on direct microinjections into the dermis has verified that these particles initiate an immune response, and are rapidly cleared to the lymph nodes. We anticipate this summer beginning collaborative experiments with Dr. John Bucher and team at NIEHS on the nano-C\textsubscript{60} materials.

**Plan**

- Over the next year, we will continue to investigate the mechanism of nano-C\textsubscript{60} toxicity, the formation mechanism of nano-C\textsubscript{60}, and its structure, with a particular emphasis on surface chemistry.
- We have just started to broaden our team’s efforts at Rice to include the eco-toxicology of other candidate nanomaterials; of particular interest are single-walled carbon nanotubes and nanocrystalline titanium dioxide.
- Our collaborative partnerships with toxicologists will continue with potential student exchanges and expansion of the programs described to include more characterization efforts.
8.3.6. Environmental Exposure Routes (Hughes, Wiesner, Tomson, Luttge)

**Summary**

This project, in operation for 2.5 years, aims to evaluate the effective dose that a biological organism might encounter to engineered nanomaterials that might be released into the environment. In order to predict the speed and efficiency of nanoparticles movement through water and soil, it is vital to understand their fundamental transport properties. *We have observed that nanomaterials exhibit widely differing transport behaviors in model porous media* (Figure 8.3.18). Among carbonaceous nanomaterials, hydroxide functionalized C₆₀ (fullerols) and surfactant-dispersed single-walled carbon nanotubes (SWNT) exhibited the greatest mobility, and nano-C₆₀ (see section 8.3.5) manifested lower but moderate mobility, with almost 50% of the clusters removed from the flow. Oxide nanoparticles exhibited a size- and material-dependent mobility that varied from highly mobile (57 nm silica) to significantly attenuated (303 nm ferroxane). *Unexpectedly, however, the three carbon-based systems exhibited a flow-velocity dependent attachment efficiency.* At high Darcy velocity, in contrast to low velocity and to the predictions of deposition theory, we observed a temporary apparent increase in the affinity of the porous medium for nano-C₆₀, fullerols, and solubilized SWNT. Also unexpectedly, the breakthrough curve plateau is observed to be velocity independent. We term this result an “affinity transition,” an effect that might be the result of a modification of collector surface as nanomaterials accumulate, and conclude that particle transport to the collector surface must be the limiting depositional step only during a transient period at higher velocities. We postulate that the large Hamaker constants and the potential for highly-ordered deposits on the surface of the porous medium are the distinguishing factors that make these systems unusual.

In the last six months, an additional component to evaluate nanoparticles crystal dissolution and growth kinetics has been added to this project. Through state-of-the-art Monte Carlo based computer simulations developed over the past three years, we are able to evaluate kinetic implications to nanoparticles stability under environmental conditions that are implied by their lack of screw dislocations.

**Figure 8.3.18: Breakthrough curves of nanoparticle suspension, showing the change in normalized effluent nanoparticle concentration (C/C₀) as a function of cumulative volume normalized to pore volume (V/Vₚ).**
Results

Nanomaterial transport through porous media

The relative mobility of a particle entrained by flow through a porous medium is linked to its tendency to deposit on immobile surfaces such as sand grains or clays in aquifers. The process of particle deposition is often considered as a sequence of particle transport to the immobile surface or “collector” followed by attachment. While accurate models are available to describe the deposition of particles under conditions where conditions favor particle attachment to the collector, there are currently no satisfactory models to describe attachment efficiency under conditions where attachment is not the default. This is particularly true of nanoparticles, whose dimensions are of the same order as the ranges of the relevant forces that govern attachment.

Assuming that sequential steps of transport and attachment are adequate to describe particle deposition, the attachment efficiency may be treated as an empirical parameter that captures all aspects of particle deposition not described by the more extensively validated particle transport models. A mass balance of particles over a differential volume of porous medium can be integrated over distance within a homogenous medium to yield an expression for the attachment efficiency factor, $\alpha$, as a function of the observed removals, the characteristics of the porous medium and the flow:

$$\alpha = -\frac{4r_c}{3(1 - \varepsilon)\eta_0 L} \ln\left(\frac{C}{C_0}\right)$$

where $r_c$ is the radius of the collector in the porous medium, $\varepsilon$ is the porosity of the medium, $L$ is the length of the medium, $C$ and $C_0$ are respectively the particle concentrations at distance $L$ and at $L=0$, and $\eta_0$ is the clean bed single collector efficiency that describes particle transport to an individual collector before particle accumulation alters the collector geometry. This last parameter can be calculated as a function of known variables. Using experimental $C/C_0$ values (fraction of influent particles remaining) and theoretical $\eta_0$ values, experimental $\alpha$ values can be calculated for a given particle suspension. Theoretically, values of the attachment efficiency calculated from data obtained from experiments with one porous medium can be applied to another porous medium of similar composition, but different grain size, fluid flow rate, and porosity. Although the validity of interpreting $C/C_0$ data to obtain an estimate of attachment efficiency has been established for nano-scale particles this assumption has not yet been evaluated for engineered nano-particles.

Breakthrough curves and thus mobilities of the materials evaluated in this study differed substantially from one another (Figure 8.3.18). Among the fullerene-based materials, the SWNTs and fullerols passed through the porous medium in a fashion more similar to that of the tracer than did the nano-C$_{60}$. In aquatic systems, hydrophobic interactions may enhance the deposition of particles on surfaces. The surfaces of fullerenes are in principle quite hydrophobic and non-reactive in water. Indeed, fullerene hydrophobicity impedes the dispersal of materials such as buckyballs and carbon nanotubes in water. This barrier can be an important limitation in fullerene processing where dispersal or manipulation of fullerenes in aqueous solutions is required. The same hydrophobic forces that prevent fullerene dispersal in water should also lead to relatively limited mobilities in aquatic environments. However, fullerenes may become more hydrophilic through intentional or environmental modifications such as capping or wrapping of carbon nanotubes with surfactants or the functionalization of C$_{60}$. Such modifications are
anticipated to decrease attachment efficiency and thereby increase mobility. Consistent with these expectations, the solubilized fullerene were relatively mobile. After approximately 2 pore volumes, the column effluent concentrations of SWNTs and fullerols were approximately 95% of those in the influent. By comparison, almost 50% of the nano-C$_{60}$ present as clusters 168 nm in diameter were removed from the flow thus manifesting a lower but moderate mobility.

Among the oxide nanomaterials investigated, the smaller silica exhibited little affinity for the porous medium. With the exception of the metal-oxanes, the passage of the other six nanomaterials tended to creep upwards with time suggesting a saturation or blocking of sites within the porous medium where particles deposit. This observation suggests that mobility of these materials may increase over time as deposition sites become saturated over progressively larger distances within the porous medium. At the concentrations of fullerols and SWNTs used in this work, porous medium surfaces were calculated to be covered as much as 6% by these materials after 1.5 pore volumes. Thus, plateau values of fullerene passage in these two cases may reflect some interactions between the nanomaterials and a modified silicate collector surface.

Estimates of particle attachment efficiency, one measure of nanoparticles mobility, are obtained from the plateau values of C/C$_0$. Although theoretical analyses assuming particle attachment in primary energy minima indicate that attachment efficiency should decrease with increasing particle size, experimental evidence typically indicates either little dependence on particle size, or an increase in attachment efficiency with increasing particle size when conditions are unfavorable to attachment. This latter trend appears to be consistent with attachment in secondary minima and should be accompanied by a saturation in particle deposition as the particles in suspension come to equilibrium with those transitioning through deposition states in the secondary minimum. It is therefore interesting to note the very different values obtained for attachment efficiencies for the two silica nanoparticles we used in this work in that the particles are of similar composition, but very different size. The experimental attachment efficiency increased over one order of magnitude from the smaller to the larger silica nanoparticles. In contrast with expectations based on electric double layer interactions such as described by DLVO theory, this increase in attachment efficiency occurred despite an increase in surface potential as estimated from measurements of electrophoretic mobility.

Because polyhydroxylated C$_{60}$ nanoparticles (fullerols) and surfactant modified carbon nanotubes are specifically formulated to enhance their affinity for the aqueous phase, it is not surprising that these materials exhibited comparatively high mobilities. Calculations of initial transport of fullerols and surfactant-modified carbon nanotubes in a sandy aquifer suggest that concentrations would be reduced to 0.1% of the initial concentration over a distance of 9.8 meters and 10 meters for SWNTs and fullerols, respectively. The mobility of the fullerols may be considerably greater than that suggested from a calculation of mean values of C/C$_0$ given that the estimate for the collision efficiency was not statistically different from zero. For the conditions assumed in these calculations, groundwater would travel an approximate distance of 9 meters in 1 day. Despite the very low initial solubility of untreated C$_{60}$ particles in water, the stable suspension of nano-C$_{60}$ aggregates exhibited some mobility although it was nearly 140 times less than that of the fullerols. Similarly, the SWNTs moved 100-times farther than did the n-C60 clusters. Nonetheless, these results suggest that fullerenes, through the mechanism of cluster formation, may have moderate mobilities even without specific functionalization to enhance their solubility in aqueous systems.
Although the electrophoretic mobilities, sizes, and calculated mobilities of the nano-C$_{60}$ and the larger silica particles were similar, the evolution of their breakthrough curves differed. By comparison with the 135 nm silica particles, the nano-C$_{60}$ clusters more rapidly approached the plateau value. Like the fullerols and SWNTs, the concentration of nano-C$_{60}$ clusters reached a plateau value for removal within 2 bed volumes while the concentrations of oxide nanomaterials (with the exception of the ferroxane) continued to slowly increase after 3 bed volumes.

Numerous factors in natural systems may combine to increase nanoparticle mobility. For example, we speculate that naturally occurring polyelectrolytes such as fulvic and humic acids might create a similar enhancement in nanoparticle transport as that produced by the surfactant on the SWNTs by sorbing to the surfaces of particles and reducing attachment efficiencies through steric stabilization and increased hydrophilicity of the particle surface. Light- or bio-activated functionalization of C$_{60}$ in nature may solubilize individual C$_{60}$ molecules to yield molecules with very high mobilities similar to the fullerols examined in this work. Functionalization or clathrate formation may naturally induce nano-C$_{60}$ cluster formation and confer moderate mobility in aqueous environments to these otherwise water-insoluble fullerenes. Moreover, while the porous medium used in these experiments was uniform and tightly packed, groundwater aquifers are likely to have fractures and other heterogeneities that may greatly enhance the mobility of particles beyond the values calculated in this work. Indeed, variability in the the ground water characteristics will introduce great variability in the mobility of particulate materials of all kinds.

Thus, the effects of naturally occurring organic matter associated with nanoparticles and collector surfaces, the evolution of collector surfaces with deposition, as well as variations in solution chemistry, aquifer heterogeneity and flow rate merit attention in future studies. The differences in the environmental transport properties observed for these nanomaterials underscores the need to address environmental impacts of nanomaterials on a case-by-case basis.

**Velocity effects on fullerene and oxide nanoparticles deposition**

Particles smaller than approximately 100 nm are predicted to have very high efficiencies of transport to collector surfaces due to Brownian diffusion. If all particle-collector contacts were to result in particle attachment to the collector, these small particles would be retained to a large extent by the porous medium. However, the column experiments showed that very few of the 57 nm silica nanoparticles were retained by the porous medium (Figure 8.3.19). Particles introduced with the first pore volume passing through the column were not appreciably removed relative to the tracer. After three pore volumes, a plateau value of 97% passage of silica particles through the porous medium ($C/C_0$) was observed at the lower velocity and a similar (statistically indistinguishable) value of 98% was observed at the higher velocity. These small removals imply a small value for the particle collision efficiency factor. Under the conditions imposed in these experiments, a change in flow rate is predicted to have a negligible impact on particle removal due to the very small value of the collision efficiency factor.
efficiency factor ($\log \alpha = -2.1$) calculated from these experiments.

The single collector efficiency calculated for the anatase nanoparticles clusters is approximately half that calculated for the silica nanoparticles due to the larger size of the anatase clusters. Thus, all other factors being equal these particles should be removed to a lesser extent by the porous medium. However, the magnitude of the electrophoretic mobility of the anatase clusters is less than that of the silica particles suggesting that these particles should have less electrostatic repulsion and therefore a smaller energy barrier for particle attachment. At the lower velocity, the fraction of influent anatase nanoparticles clusters exiting the column in the first pore volume was approximately 30% lower than the fraction of tracer (Figure 8.3.20). The anatase concentrations then increased monotonically to a plateau value of 55% passage after four pore volumes. Using this latter value for C/C₀, a value of log $\alpha$ = -0.5 is calculated. The passage of anatase clusters is predicted to increase to 77% at the higher Darcy velocity. In reasonable agreement with this prediction, the observed value for anatase passage through the porous medium at the higher velocity was 73%. Thus, the observed passage of these oxide nanoparticles and aggregates as a function of velocity is consistent with predictions of particle deposition based on theory for particle transport to the collector and a constant attachment efficiency factor.

The predicted dependence on velocity of particle passage through the porous medium was not observed for the fullerene-based nanoparticles. Similar to the anatase clusters, the concentration of nano-C₆₀ clusters exiting with the first pore volume passing through the column was approximately 20% of that introduced to the column (Figure 8.3.21). At the slower velocity, n-C₆₀ clusters increased monotonically in concentration. After 3 pore volumes the fraction of clusters passing through the column reached a relatively stable, yet slightly increasing value of approximately 55%. The plateau value of nano-C₆₀ passage was similar to that observed for the anatase clusters and, because the anatase and nano-C₆₀ clusters were of similar size, a similar value for the collision efficiency factor is calculated despite the very different values of electrophoretic mobility measured for the anatase and nano-C₆₀ aggregates. The higher electrophoretic mobility of the nano-C₆₀ aggregates implies a greater electrostatic repulsion for these aggregates compared with the anatase clusters. Similar values of the attachment efficiency for nano-C₆₀ and anatase clusters suggests that attractive forces for attachment may be higher for the nano-C₆₀ than those for the anatase clusters.

![Figure 8.3.20: Breakthrough curves for anatase particle aggregates at two Darcy velocities.](image)

![Figure 8.3.21: Breakthrough curves for nano-C₆₀ aggregates at two Darcy velocities.](image)
At the higher Darcy velocity, two striking departures from the anatase breakthrough and the behavior anticipated based on deposition theory were observed. First, after one pore volume, the retention of nano-C$_{60}$ on the porous media was observed to increase, resulting in a reduction in concentration leaving the porous medium. This apparent increase in the affinity of the porous medium for the nano-C$_{60}$ was temporary, and the concentration of nano-C$_{60}$ in the column effluent increased over the second and third pore volumes. Contrary to deposition theory, the passage of nano-C$_{60}$ asymptotically approached a value equal to that observed at the lower velocity rather than a higher value as was observed with the anatase. The fact that the higher velocity did not result in a higher plateau value for the nano-C$_{60}$ suggests that this material may be less mobile than predicted from a consideration of transport alone.

We interpret the dip in the breakthrough curve observed during the second pore volume to an increased affinity of the nano-C$_{60}$ for the surface of the glass beads making up the porous medium. Such an “affinity transition” might be produced through modification of the collector surface as fullerenes accumulate such that deposited fullerenes serve as favorable sites for subsequent nano-C$_{60}$ deposition. This hypothesis is supported by a consideration of the van der Waals forces between two fullerene surfaces and those between a fullerene and silica surface. As fullerenes deposit on the collector, subsequent deposition will be influenced by a growing probability of fullerene-fullerene interactions. Modification of the collector surface through nano-C$_{60}$ deposition is predicted to lead to a ten-fold increase in van der Waals forces between properly aligned fullerenes and fullerenes deposited on the collector surface with a corresponding increase in the apparent attachment efficiency.

However, the fraction of collector surfaces covered by nano-C$_{60}$ is calculated to be small. Assuming that 20% of the nano-C$_{60}$ is removed during the first pore volume, less than 1% of the collector surface would be modified for enhanced affinity through fullerene-fullerene interactions. Were the nano-C$_{60}$ to rearrange after deposition, fullerene coverage increases to approximately 10%. It is therefore unclear as to whether the transitional enhancement in affinity for fullerene deposition can be attributed to increasing fullerene-fullerene interactions. It is also unclear as to why the affinity transition was not produced at the lower Darcy velocity. Increasingly favorable conditions for attachment would correspond to shorter time constants for attachment in comparison with transport to the collector surface as nano-C$_{60}$ deposits. Transport to collector surfaces is favored at the lower Darcy velocity approximately by a factor of two as reflected in the calculated clean bed collector efficiency, $\eta_0$. A greater affinity for the nano-C$_{60}$ may have increased the rate of attachment sufficiently to outpace transport at the higher velocity while remaining the limiting step in deposition at the lower velocity.

The fact that a similar plateau value of $C/C_0$ was observed at the two velocities suggests that 1) deposition is not mass transport limited, 2) the collision efficiency factor increases with increased velocity or that, 3) aggregate size changes with velocity. This latter explanation can be ruled out based on independent measurements of a constant aggregate size in various mixing regimes. Alternatively, similar plateau values at the two velocities could be explained by an increase in a from 0.3 at the lower velocity to 0.7 at the higher velocity. However, a velocity effect on attachment efficiency would require that surface forces impeding particle attachment on previously deposited nano-C$_{60}$ be overcome by forces imparted on the particle due to the fluid flow, forces which are orders of magnitude too weak. Thus, it does not appear that a similar removal of the nano-C$_{60}$ at the two different velocities can be explained by a velocity effect on the collision efficiency factor. Rather, it appears that nano-C$_{60}$ deposition is asymptotically
independent of transport. Particle transport to the collector surface appears to be limiting only during a transient period at higher velocities.

One possible explanation for the velocity-independent plateau values is that deposition converges to a process that is limited by an additional step in which a finite time is required for particles to find the correct orientation before attachment can occur. Such growth is hypothesized to occur in layers or islands of highly organized C\(_{60}\). Although this work is the first to consider deposition from water, previous efforts have examined C\(_{60}\) deposition on silicon or silver surfaces after sublimation and the formation of films at an air-water interface. In these instances, fullerenes were observed to organize in highly ordered, terraced or multi-layer structures. If similar structures are formed after transport of nano-C\(_{60}\) to the collector surface, it is possible that the energetics of incorporating the fullerene into a growing crystal-like deposit are rate limiting rather than the transport (and perhaps attachment) steps.

The size, shape, and surface chemistry of the other two forms of fullerene used in these experiments differed greatly from one another and from the nano-C\(_{60}\) aggregates. Fullerol molecules were highly functionalized and did not aggregate. Single wall carbon nanotubes (SWNTs) were initially covered with surfactant and had a large aspect ratio. It is therefore surprising that the breakthrough curves produced by these latter two fullerene materials also exhibited “affinity transitions” similar to that observed with the nano-C\(_{60}\).

Polyhydroxylated C\(_{60}\) nanoparticles (fullerols) and surfactant-modified carbon nanotubes are specifically formulated to enhance their affinity for the aqueous phase. Consistent with their high solubility, after several pore volumes these materials exhibited a comparatively high passage through the porous medium. Indeed, the SWNTs and fullerols closely followed the tracer. After one pore volume, SWNT concentrations were approximately 10% lower than would have occurred in the absence of deposition at both Darcy velocities.

As with the nano-C\(_{60}\), the slower velocity resulted in a breakthrough curve that increased monotonically and arrived at a plateau level of removal after 3 pore volumes. A dip in the breakthrough curve was observed after one pore volume at the higher Darcy velocity followed by an asymptotic rise to a plateau removal of nearly 10% (i.e. 90% passage) at 3 pore volumes. At all times, the breakthrough curve for the higher velocity lay below that for breakthrough at the lower velocity. In shear flow, nanotubes are known to align and are likely to bond length-wise due to Van der Waals forces. The nanotube deposits are anticipated to resemble highly ordered “ropes” analogous to structures observed in nanotube suspensions. Outward growth of such a layer might be limited due to irregularities imposed by particle asymmetry as nanotubes attach to one another.

Fullerols displayed the lowest levels of attachment with approximately 99% passage after 3 pore volumes at both Darcy velocities. Nonetheless, there was a transient enhancement in affinity for the fullerols after one pore volume as in the case of the other two fullerenes. After one pore volume a monolayer of the fullerols removed from suspension has the potential to coat approximately 5% of the collector surfaces. Because the fullerols are partially functionalized, it is possible that these molecules have localized facets that are either more hydrophilic or hydrophobic. Orientation of the initial monolayer of fullerol deposit toward functional groups on the glass bead surface would present a favorable surface for deposition a second fullerol layer with functional groups preferentially oriented outward. Subsequent deposition onto such a bilayer would be small due to charge interactions between deposited fullerols and those moving with the fluid.
The hypothesis that deposition of fullerenes enhances the affinity of the collectors for subsequent fullerene deposition is further supported by data on fullerene breakthrough as a function of influent concentration. In experiments performed with the nanotube and fullerol suspensions, the magnitude of the affinity transition was observed to decrease as concentration decreased. If affinity is enhanced due to the establishment of favorable deposition sites on the collector, lowering the concentration of fullerenes in the influent to the column would be expected to slow the establishment of these growth sites, thereby reducing the mass transport and attachment effects observed at the higher velocity.

Although the three fullerene materials differ greatly in size and surface chemistry, they share two important similarities. First these materials are characterized by large Hamaker constants relative to the mineral nanoparticles. Second, they are highly ordered with considerable potential for directed growth of the deposits. Despite the remarkable similarity between breakthrough curves during the early stages of these experiments, we speculate that the structure of the resulting deposits formed by these materials may be very different in each case. Deposit structure and the integrity of the fullerene aggregates upon deposition will likely play an important role in determining bioavailability and persistence in natural systems while these same factors might be controlled in engineered systems to tailor nanoparticle aggregates for separation or fractionation.

Nanocrystal dissolution and stability under environmental conditions

This project started only about six months ago and results are necessarily preliminary. During the last three years, we have developed Monte Carlo based computer simulations of crystal dissolution and growth kinetics. Although models based on adsorption theory treat crystal dissolution mainly as a one-dimensional process, the current simulations provide a three-dimensional view of the processes that build and destroy the crystal lattice. Monte Carlo simulations can therefore successfully simulate crystal dissolution by treating the process as a many-body problem. A large number of detailed findings for AB and A3B models representing analogs for albite and anorthite feldspar structures have been summarized in several manuscripts.

Based on this progress, we are working to apply our Monte Carlo simulation codes in combination with ab initio and density functional theory (DFT) to the study of nanocrystal dissolution. A major step forward was therefore our ability to conduct simulations with full crystal structures (e.g., albite, anorthite, barite, and others) and the fact that our code is now flexible enough to allow the exploration of a wide range of different structures. We work under the hypothesis that nanocrystals do not contain screw dislocations and thus their dissolution kinetics differs significantly from bulk crystals. Therefore, it was a large advance that we are now able to test the kinetic implications of point and screw dislocations on the dissolution kinetics with our simulations.

First results confirm our working hypothesis by indicating that line defects indeed play a central role by initiating fast dissolution kinetics. This result is in good agreement with experimental results and theoretical considerations of the dissolution behavior of bulk crystal close to equilibrium. Under such special near-equilibrium conditions line defects do not open up into etch pits because the critical $\Delta G$ is not available. In this situation, i.e., in the absence of pits even bulk crystals dissolve dramatically slower than they do with active line defects.

By building on these first results, we intend to continue and extend our theoretical studies and complement them with experimental investigations of the size dependence of crystal dissolution with special attention to nanocrystals. It will be of particular interest at which grain size the dissolution kinetics will change from a bulk crystal behavior to nanocrystal dissolution
kinetics. Our model calculation will be important not only for a deeper understanding of the processes at the molecular level but also for the identification of key experiments that will then test specific model predictions.
9. Education and Human Resources

The goal of our educational outreach and human resource programs is to cultivate a future workforce experienced with using nanoscience and nanoengineering to solve problems in biological and environmental engineering. This objective necessarily echoes one of the primary missions of the NSF, namely training of the technical workforce of the future [1]. It also directly relates to our mission: CBEN’s technical objectives can only be realized if there are well-trained scientists and engineers to discover, develop and manage its nanotechnologies.

CBEN’s educational outreach activities are coordinated by Dr. Kristen Kulinowski, Executive Director for Education and Public Policy. CBEN faculty members and students contribute substantially to these programs, as is apparent in section 9.8, which provides a comprehensive listing of all educational outreach activities. The highlights of our program this year include:

- **Training for teachers of at-risk students** in the diverse Houston Independent School District.
- **Involvement of students from 9th grade through graduate school**, in research, classroom and outreach activities
- **Integration of CBEN research into education** by making links between nanotechnology and the Texas high school science curriculum
- **Development of NanoKids™ DVD** for proof-of-concept testing in middle school classrooms

These activities address both long-term and short-term needs in workforce training. In the near term, the next generation of nanotechnologists will be drawn from current graduate and undergraduate students already in our pipeline. We strive to ensure our students are diverse, and have a substantial percentage (41%) of female graduate students. CBEN now meets, and is working to exceed, the national average for African-American and Hispanic graduate students. We also develop students with the right skills to be highly effective researchers. Our systems engineering focus and collaborative research environment train people who excel at working in large, interdisciplinary teams. Through its center programs CBEN also provides researchers with crucial oral and written communication skills, as well as broader perspectives on the economic, societal and governmental context for their research.

Addressing the longer term challenge of workforce training requires programs that increase the number and quality of people entering the science and engineering pipeline. For this reason CBEN has developed connections to our local school districts, businesses and community. We run twelve formal programs for such outreach activities; they are diverse and involve students as young as ten years old as well as laypeople over sixty. These efforts all exploit the capacity that nanotechnology has to spark a sense of wonder in students and adults alike. Like the space program, we find that nanotechnology is an ideal ‘hook’ for drawing people into science and technology.
9.1. CBEN educational outreach and human resource programs

Continuing CBEN educational programs

1. High school teacher professional development series
   a. Spring Content Course
   b. Summer Internship
   c. pH Model Lab Sabbatical
2. Integrated Physics and Chemistry (IPC) workshop for teachers from around Texas
3. Summer academy for high school students
4. NanoKids™ instructional materials development
5. Undergraduate nanotechnology curriculum development
6. Graduate nanotechnology curriculum development
7. Entrepreneurship education for graduate students
9. Bi-monthly graduate student networking and problem-solving lunches
10. Testing and workshop on NanoKids™ in middle-school
11. Nanotechnology Research Experience for Undergraduates

New activities for 2004-2005

1. NUE award for a new undergraduate course entitled Nanotechnology: Content and Context
2. Workshop on integrating nanotechnology into Advanced Placement® courses for attendees of the School of Continuing Studies Advanced Placement® Summer Institute
3. Partnership with the John P. McGovern Museum of Health & Medical Science to run a nanotech weekend for families
4. Partnership with Prairie View A&M University, a minority-serving institution, and Clarkson Aerospace, a minority-owned small technology business to enhance minority recruitment programs at CBEN.

Summary of participants in CBEN educational activities

Total numbers of people who participated in a CBEN educational outreach program activity for the period January 16, 2003-January 15, 2004 are summarized in the table below.

Table 9.1.1. Summary of participants in CBEN educational outreach activities

<table>
<thead>
<tr>
<th>K-12 Teachers</th>
<th>K-12 Students</th>
<th>Undergraduate/Graduate Students</th>
<th>Community</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>117</td>
<td>690</td>
<td>506</td>
<td>460</td>
<td>1773*</td>
</tr>
</tbody>
</table>

*A participant may be double counted. For example, a graduate student may have taken multiple CBEN courses.

A complete listing of all CBEN educational activities with a breakdown of the number and type of people impacted by each can be found in the appendix following this narrative.
9.2. K-12 Programs

9.2.1. High school teacher professional development series

Our centerpiece K-12 educational outreach program focuses on ninth grade science teachers. We feel that by investing in high quality, long-term teacher training, our center’s investment is multiplied many times over. A single teacher will over a decade influence hundreds of students directly, and influence thousands of others through mentoring of other teachers.* We target ninth grade teachers, rather than the more sophisticated and educated advanced placement teachers, for several reasons unique to Houston and CBEN’s research focus. First, in Texas ninth grade science is an integrated physics and chemistry (IPC) class. The integrated subject content of this class makes it a natural place to use illustrative examples of our center’s research in the classroom. Also, there is an acute need for teacher training at this level. This class is often the first assignment given to young teachers. Most do not have extensive scientific training, yet are expected to succeed if they want to advance to courses that are more "desirable". This results in low teacher retention rates and poor student performance. Improvements made at this level will increase the pool of qualified and motivated students for the more advanced high school science classes.

These teacher-centered challenges are exacerbated by those faced by their students. Not surprisingly, there is a high dropout rate in high school in HISD; statistics from the United States Department of Education show that only 47% of those HISD students who entered 9th grade in 1994 graduated in 1998. As in most other large urban school districts, many HISD students face serious barriers to learning, including poverty (75.4% of students are on free/reduced lunch), migrancy (38.2% mobility rate), and language (26.5% have limited English proficiency). Additionally, investing our efforts in HISD impacts mostly underrepresented groups simply due to its diverse demographic makeup: 56% Hispanic, 31% African American, 10% White, and 3% Asian. [2]

The three phases of our program are:

- **Spring Content Course:** refreshes understanding of core science concepts and connects those concepts to ongoing nanotechnology research.
- **Summer Internship:** reinforces science as a process by placing teachers in research laboratories.
- **pH (Partners for Houston) Model Science Lab II:** a year-long sabbatical residency to introduce constructivist teaching techniques.

* Assuming one teacher has four classes per day with 30 students per class, this amounts to 1200 students in a decade.
CHEM 570: Spring Content Course

This evening course has the dual goals of refreshing the teachers’ understanding of core physics and chemistry concepts, and connecting those core scientific concepts to ongoing nanotechnology research. For the first hour of each evening, basic science concepts are reviewed and extended. As moderator, Dr. Kulinowski uses a variety of teaching formats that address essential physics and chemistry topics while modeling active learning techniques. In the second hour of each class period a center researcher presents on their CBEN research and emphasizes a connection to the scientific concepts discussed in the first hour. For example, after the teachers review the basic concepts of the interaction of light with matter with Dr. Kulinowski, CBEN graduate student John-David Rocha discussed his use of fluorescence spectra to distinguish individual carbon nanotubes. In the third hour, the teachers brainstorm about how to incorporate the research concepts into their classroom. The goal is to leave the teachers with tangible lessons, worksheets, activities, active-learning exercises, or other materials that incorporate center research concepts. Pre-test/post-test assessment from last year’s program demonstrated marked improvement in teacher’s knowledge of their IPC course content and an increased awareness of nanotechnology.

Summer Internships for Teachers

We have tailored our summer program to the needs of 9th-grade Integrated Physics and Chemistry teachers. Rather than have teachers work on independent research, as is generally the case for a Research Experience for Teachers (NSF-RET) program, we use the center’s resources to focus on techniques for translating the center’s research methods and results into a classroom setting. The goals of our summer internship program piloted in the summer of 2002 are to reinforce each teacher’s sense of science as a process, to deepen each teacher’s understanding of the achievements and potential of nanoscience, and to apply lessons learned from the content class to a research setting.

We do this by asking that our summer teachers create a lesson plan that explains the research of the host lab at a level appropriate for a high school teacher or student. Over the course of the four-week program, teachers spend four days per week observing and assisting in research, as well as interviewing lab personnel. On the fifth day of each week, the teachers meet together to share and discuss activities in their respective labs, review existing nanoscience and materials-science instructional resources, and develop a lesson that applies center science to at least one unit in IPC. The teachers’ understanding of nanoscience is demonstrated by the construction of a web page or course materials. Subjects of lessons from summer 2003 are shown in the table below.

<table>
<thead>
<tr>
<th>Teacher</th>
<th>Researcher</th>
<th>Model Lesson</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB</td>
<td>Colvin</td>
<td>Emulsions and Surfactants</td>
</tr>
<tr>
<td>MD</td>
<td>Barron</td>
<td>Composite materials</td>
</tr>
<tr>
<td>KP</td>
<td>Mikos</td>
<td>Using nanomaterials to strengthen bone</td>
</tr>
<tr>
<td>AR</td>
<td>Tour</td>
<td>Comparison of silicon transistors and molecular electronics</td>
</tr>
<tr>
<td>DS</td>
<td>West</td>
<td>Nanotech therapeutics for cancer therapy</td>
</tr>
</tbody>
</table>
**pH Model Science Lab II: A sabbatical for teachers**

The final component of our teacher training program is a sabbatical for teachers in the year after their summer internship. This sabbatical is incorporated into our program because of the success that this approach has found in middle school science teacher training. This program, the Model Science Lab (MSL), was developed at Rice prior to CBEN and is an intense residency program in which eight middle school science teachers spend a sabbatical year in an inner city school studying and class-testing discovery-based approaches to science education. Tangible achievements of the MSL include an almost perfect teacher retention rate (95% after 11 years), the development of a cadre of teacher-leaders who act as peer mentors, and the improvement in student test scores. The students of MSL teachers demonstrated greater improvement on both the state achievement test and on a pre/post modified NAEP (National Assessment of Educational Progress) test than students taught by control teachers in each of their schools. [3]

The pH Model Science Lab II, our 9th grade successor program to this successful 5th-8th-grade program, was piloted in the fall of 2002 with four teachers, three funded by our center and the fourth and the programmatic costs funded by the Cain Foundation. Host Lee High School provides a home for teachers as they develop curricula and inquiry-based demonstrations centered on nanotechnology. The four teachers for this pilot were selected from the participants in the summer internship program described above. Four more teachers are supported in the current reporting year, In future years, further external funding will be sought to increase the complement of teachers to eight.

9.2.2. Training Workshops for High School Teachers

Leveraging the curriculum developed for CHEM 570, described above, the center developed an intensive 3-day workshop for teachers that was piloted June 2002 for a set of 9th-grade IPC teachers from Laredo, TX and taken on the road in July 2003 to the Ellison Miles Geotechnology Institute at Brookhaven Community College in Dallas, TX. EMGI offered the use of their facilities and recruited 11 teachers from local school districts. CBEN graduate students Jennifer Jamison, John-David Rocha, and Adina Boyd prepared and implemented lessons for this workshop under the direction of workshop instructor Kulinowski. *NEW* CBEN will offer a five-day workshop in summer 2004 called Nanotechnology Academy for High School Teachers of Advanced Science Courses. This course will help advanced placement teachers from chemistry, physics and biology make connections between nanotech and their advanced science course content, as well as to see the connections between and among the standard science disciplines.

9.2.3. High-School Summer Academy

Summer science experiences provide an excellent tool for discovery-based pedagogy at the high school level. The excitement and interest that can be generated in students by vibrant fields such as nanotechnology and environmental science can dramatically increase the effectiveness of such a teaching tool. We use the cadre of informed and motivated teachers from the HISD professional development series to draw high school students from underrepresented groups into the research activities of the center. This process begins with a two-week Science
Academy for promising 9th/10th grade students identified by MSL teachers. Structuring the Academy as a science fair workshop in which students learn how to construct and test a hypothesis allows the process of scientific inquiry begun in the MSL intern's classroom to continue. Dr. Mary McHale, a chemistry lecturer and water quality expert, is supported by CBEN to design a curriculum in which students analyze local water samples from various sources for impurities. CBEN research into the role of nanotechnology in water purification has been integrated into the academy through an experiment using nano-TiO$_2$ and sunlight to break down pollutants in water.

At this age, students are at a pivotal time in the evolution of their career planning. If we can continue to excite them about the process of scientific discovery, more of them may consider studying science and engineering in college. Promising graduates of the Academy will be invited back in subsequent summers to work in the research labs as assistants or on their own science fair projects under the mentorship of a center researcher. This summer, CBEN is inviting back a Brazilian-American rising junior to spend time in the research lab of Prof. Andy Barron under the supervision of one of the high school teachers. The impact of student involvement in center activities will be assessed through student interviews or surveys. Each student's progress through high school and beyond will be tracked for the duration of the research grant. The center will coordinate activities and work aggressively with Rice's Coordinator of Minority Recruitment; their staff has been drawn from backgrounds similar to those of the minority students we are targeting, and their mentorship will be important in developing minority student relationships.

9.2.4. Nanokids™ Instructional Materials Development

Center member James Tour has synthesized organic molecules whose structures are visually reminiscent of children. The NanoKids™ program is developing curricular materials that introduce the nanoscale world through animated characters based on these molecules. The central theme of ‘atoms and molecules are our world’ emphasizes that most matter is made up of atoms bonding together to form molecules, which form our environment and us. The program is initially targeting the middle-school level, as that is the point in the normal science curriculum where the concept of molecules is introduced.

The NanoKids™ Proof-of-Concept instructional materials module was introduced to teachers participating in the beta testing in a six-hour workshop June 26, 2003. This module consists of:

- 20-minute Proof-of-Concept DVD – 3D animated video combining two lesson/adventures: “Welcome to the NanoLoft” and “DNA the Blueprint of Life?”
- Interactive digital student workbook – features four ‘rooms’ (the Research Laboratory, the NanoLoft, the DNA Room, the Nanotechnology Room) with information, exercises, educational games, sound-bites, out-of-the-box imagination, and songs.
- Teacher Guide – Step-by-step explanation of DVD, previewing hands-on exercises and discussions, post-viewing hands-on exercises and discussions, a walk through the student workbook, suggested quizzes and experiments, and decks of NanoCards©.
• Bilingual Parent/caregiver Guide – Brief introduction to the subject matter with ongoing updates from the support web site.
• Bilingual support web site with two components – one section for the general public and one section that is password accessible by participants in the project. Online assessments are automatically entered into a database that can export to Excel or Access for quick evaluation results. http://www.nanokids.rice.edu/

The materials were piloted in eight Houston Independent School District (HISD) middle schools, one Spring Branch Independent School District (SBISD) high school, and two private schools in the fall of 2003.

Southwest Educational Development Laboratory of Austin, Texas, conducted the outside evaluation. Recommendations from the evaluation include the following comment, “Based on the findings from this pilot project, there is definite consensus that the Rice University should continue to develop the NanoKids materials! Based on the teachers’ responses, they would eagerly look forward to having resources such as these to use with their students. Students appreciate any effort that helps make science a pleasure to learn or more interesting.” (The full report is available upon request.)

Plans are underway to expand the beta testing of this module to other parts of Texas and several school districts in other states. The next two modules to be developed will explore photosynthesis and viruses and how the chemical functioning of each can be potentially mimicked and utilized in nanoscale engineering.

9.3. Undergraduate Programs

Internal educational activities are intended to enhance the knowledge base and professional skills of our undergraduate and graduate students and post-docs. Our undergraduate educational development programs encompass a Nanotechnology Research Experience for Undergraduates program, new course development, and modification of existing courses.

9.3.1. Nanotechnology Undergraduate Research for Undergraduates (NREU)

Involving students in discovery-based scientific inquiry is the primary objective of our Nanotechnology Research Experience for Undergraduates (NREU) program, offered for the first time in the summer of 2003. This program is modeled after an existing REU program offered by the Rice Quantum Institute, in which undergraduates work on independent research projects in a Rice lab for 10 weeks and present their work at a conference held at the end of the summer. Our NREU program specifically targets a diverse group of students through recruitment trips at regional colleges and universities and at national meetings of professional organizations such as the National Society of Black Physicists. It is possible for advanced high school students from the Academy or MSL teacher’s classes to participate in this program. We also welcome incoming graduate students who arrive early to do research with a CBEN faculty member. In addition to providing a research experience, we have social events in which students can interact with one another and discuss any issues of importance to them regarding their career plans.

Figure 9.2.4 Self-assembled NanoScholar molecules
The number of summer undergraduate participants has gone up from six to fourteen, an increase of 133% over last year.

This NREU program is coordinated with Rice’s existing, exceptionally successful Alliances for Graduate Education and the Professoriate (AGEP) program. The AGEP summer undergraduate research program is specifically designed to give hands-on research experience to undergraduate students in the fields of math, engineering, science, and technology-related disciplines. This AGEP program is itself the successor of a highly effective Spend a Summer with a Scientist (SaS) program, which over its lifetime achieved an impressive 62% enrollment of its undergraduate participants in graduate school, of whom 88% were Hispanic or African American. For comparison, National Center for Educational Statistics demonstrate that less than 9% of Hispanics and African Americans who received baccalaureate degrees entered graduate school over the same period; the percentage is even lower in the specific fields covered by Rice’s SaS program. Ethnic information of CBEN’s summer participants is presented in the table below.

Table 9.3.1 Demographic information on CBEN’s summer undergraduate researchers

<table>
<thead>
<tr>
<th>2003 CBEN REU Participants</th>
<th>2004 CBEN REU Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Ethnicity</td>
</tr>
<tr>
<td>F</td>
<td>Other</td>
</tr>
<tr>
<td>F</td>
<td>C</td>
</tr>
<tr>
<td>M</td>
<td>H</td>
</tr>
<tr>
<td>F</td>
<td>AA</td>
</tr>
<tr>
<td>M</td>
<td>H</td>
</tr>
<tr>
<td>F</td>
<td>AA</td>
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<tr>
<td>M</td>
<td>H</td>
</tr>
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<td>F</td>
<td>AA</td>
</tr>
<tr>
<td>M</td>
<td>A</td>
</tr>
<tr>
<td>F</td>
<td>A</td>
</tr>
<tr>
<td>F</td>
<td>A</td>
</tr>
<tr>
<td>M</td>
<td>C</td>
</tr>
</tbody>
</table>

9.3.2. New Undergraduate Courses

For graduate and undergraduate education, CBEN’s vision is to create a series of interlocking courses in nanoscience and education. This curriculum development is already underway, and will leverage two new programs at Rice. One of these, the Sloan Professional Master’s program, has majors in Nanoscale Physics and Environmental Analysis and Decision Making. The second, the Rice Connexions program to develop web-based materials, started in the Electrical Engineering department, provides staff and a web programming language well-suited for equation display and self-study. Many of the courses described in this section are also available to graduate students, and many of the courses described in the graduate section are also
available to undergraduates. CBEN executive director is also principal investigator on a new NSF Nanotechnology in Undergraduate Education award.

**Nanotechnology: Content and Context (CHEM 235/ANTH 235)**

Kulinowski has been awarded a grant from the NSF to develop and implement a new course on nanotechnology in 2004. The course, entitled “Nanotechnology: Content and Context,” aims to introduce the essential scientific and technical content of nanotechnology and the essential ethical, political and social contexts of nanotechnology research through a one-semester multi-disciplinary undergraduate course. Science/engineering majors and social science/humanities majors alike will benefit by learning about both the specific technology and the social impacts and cultural meaning of science and technology in the same class. Students will engage directly with both working scientists and with the heated public debate over its potential impacts on society. They will be asked to acquire a technical understanding of nanotechnology (e.g., the methods of visualization, experimentation, manufacture, and the evaluation of what is and is not technically feasible) as well as a nuanced understanding of scientific and technical research as a social and political process (issues of ethics, regulation, risk assessment, history, funding, intellectual property, controversy and conflict).

By combining these skills in the classroom the proposed work will cultivate a critical and civil discussion of science and technology in an emerging field amongst a younger generation. Dr. Kulinowski is joined in this work by Prof. Chris Kelty in the Anthropology department and several other faculty members from the schools of science, engineering and social science. CBEN research will be incorporated into the class by center director Vicki Colvin and researcher Matteo Pasquali.

This course was inspired by the Science Education for New Civic Engagements and Responsibilities (SENCER) project, an NSF-funded initiative administered by the American Association of Colleges and Universities (AAC&U), which robustly connects science and civic engagement by teaching “to” basic science “through” complex, contested, current public issues.[6] The current public debate over the benefits and risks of nanotechnology provides a timely platform on which to build a course that engages students in exploring this new field. Dr. Kulinowski has written and presented a backgrounder paper on nanotechnology and its social context for the SENCER Summer Institute (SSI 2003) and will lead a campus effort to design a course to be offered in the 2004 academic year.

**Nanotechnology Module Development**

The Chemistry Department at Rice currently offers its upper-division undergraduate lab courses in the format of half-semester themed modules that integrate skills traditionally developed in separate courses. This format is intended to more closely simulate the systems-level focus that underlies many research projects, especially those spanning traditional divisions within a discipline. In an example of a current module, CHEM 373, students synthesize, separate, and characterize fullerenes using electrochemical and photophysical techniques, thus integrating skills conventionally within the separate domains of organic, analytical, and physical chemistry laboratories. A nanochemistry module piloted in Fall 2002 was refined and offered again in Fall 2003.
• **CHEM 375 Advanced Module in Nanochemistry.** This new half-semester course, inspired entirely by CBEN research, introduces students to synthesis and structure of nanoparticles and their physical characterization via introduction to three microscopy techniques. It was first offered in the Fall 2002 semester by Prof. Vicki **Colvin** and had an enrollment of nine students, which demonstrates strong student interest in the subject. Melanie **Thoms**, an instructor in the Chemistry Department, was supported by CBEN to refine and polish the class, and offer it in Fall 2003 with an enrollment of four students. Enrollment is expected to wax and wane during successive years.

9.3.3. **Undergraduate Courses Modified to include Center Content**
Two pre-existing Rice University courses have been modified to include center research. In addition, some graduate level classes, described below, are available for advanced undergraduates. The modified undergraduate courses include:

- **BIOE 441: Advanced Bioengineering Laboratory.** This course is required of all seniors majoring in bioengineering. We have developed a module where students use metal nanoshells and their associated photothermal phenomena to induce tissue welding. Students learn about the nanostructured materials and optical interactions, and then also use this as an opportunity to use their skills in biomechanics to evaluate the efficacy of nanoparticle-assisted laser tissue welding. Piloted Spring 2003.

- **ENVI 401: Introduction to Environmental Chemistry.** Results of CBEN research included as appropriate examples of concepts discussed in class.

9.4. **Graduate Programs**
Our graduate educational development programs encompass a Student Leadership Council, new course development, modifications of existing courses, development of an entrepreneurship education program, media training, and networking/troubleshooting lunches.

9.4.1. **Student Leadership Council**
During the reporting year, CBEN graduate students self-assembled into a new Student Leadership Council with committees on research, communications, education, and careers. The SLC organizes a twice-monthly series of lunch meetings for graduate students and post-docs, during which information about Centerwide events is disseminated and one or two students give a brief overview of their research via an informal chalk talk. These meetings aim to

- Increase interactions among students working on different CBEN projects;
- Provide an opportunity for open dialog about stumbling blocks encountered in the research lab;
- Inform graduate students about upcoming CBEN events;
- Provide opportunities to hone oral presentation skills.

In general, the PIs do not attend these lunches at the request of the students so as to provide a low pressure forum for discussing problems with research projects. When a student presents a research challenge, other students with whom he or she would not normally interact often ask questions or provide suggestions. Occasionally, a meeting is used to provide information on alternate careers, develop non-technical skills, and discuss societal implications of
nanotechnology research. The SLC has been an important organization for facilitating the involvement of graduate students in CBEN’s educational outreach activities.

9.4.2. New Graduate Courses

There are very few interdisciplinary courses in nanoscience offered by universities at the graduate level. We feel the need for graduate education in this area is particularly pressing given our interest in integrating nanoscience into the broader research community. A two-semester course on nanostructures and nanotechnology, exploring the physics of structures and devices at the nanometer scale, was offered during the 2002-2003 academic year (Natelson) as was the first semester of a two-semester course in experimental techniques for physics (Hafner). A companion course was offered in the chemistry department first in Spring 2004 by center director Colvin. Ultimately, we will produce a textbook and interactive web page that will serve as a resource for courses at other institutions. Non-center physics professor Alex Rimberg developed a course on characterization and fabrication at the nanoscale with CBEN content provided by Jason Hafner. A seminar course covering recent advances in nanoscale science and engineering is planned for 2004. In addition, interactive light propagation software designed for use in courses in biomedical optics will be developed and tested at Rice and then broadly disseminated to the optics community (Drezek). The software will allow students to explore (1) relationships between subcellular biochemical and morphological structure and light scattering using a 3D FDTD (finite-difference time domain) model to computationally solve Maxwell's curl equations and (2) relationships between tissue optical properties, fiber optic delivery and collection geometries, and remitted reflectance and fluorescence signals using a Monte Carlo model.

New courses offered during the reporting period:

- NEW: CHEM 533 Nanostructure and Nanotechnology I An introduction to the basic principles of nanoscience and nanotechnology. Size dependent physical properties of nanoscopic solids are described using solid state physics and molecular orbital theory as a foundation. Wet chemical techniques that produce nanoscale materials (e.g. carbon nanotubes, semiconductor and metallic nanocrystals, dendrimers...) are introduced in the second half of the semester. Colvin
- NEW: NSCI 511 Science Policy and Ethics An introduction to the policy, politics, ethics and legal issues that relate to science and technology- discovery and application. This course identifies ways in which government laws, regulations, programs and actions, as well as business policy and practices can promote or inhibit advances in science and technology and applications for the public good. Case studies will be used, and experts from various fields will be invited to meet with the class. Lane and Kulinowski

New courses developed during a previous reporting period:

- NEW: CENG 630 Chemical Engineering of Nanostructured Materials. This lecture class gives an overview of materials with structural features on the nanometer scale. General concepts of synthesis, characterization and applications are discussed, as are advances stemming from CBEN research and/or found in the recent literature.
- NEW: PHYS 533 Nanostructures and Nanotechnology I. This lecture class gives an overview of physics of structures and devices at the nanometer scale. After a review of solid state physics, topics include nanostructured materials, nanoelectronics, and
nanomagnetism. Emphasis on relevance of nanophysics to current and future technologies.

- **NEW: PHYS 534** Nanostructure and Nanotechnology II. Physics of structures and devices at the nanometer scale. Topics include nanomechanics, bionanotechnology, advanced sensors and photonics. Continuation of PHYS 533.
- **NEW: PHYS 537** Methods of Experimental Physics I (*Partial CBEN content*) This two-semester course familiarizes students with basic experimental techniques that are common to all academic and industrial research laboratories. Topics include lab safety, mechanical design, computer-based data acquisition and experimental control, laboratory electronics, vacuum technology, optics, thermal measurement and control, cryogenics and charged particle optics.

### 9.4.3. Graduate Courses Modified

Several pre-existing Rice University courses have been modified to include center research. These include:

- **BIOE 531: Biomaterials Engineering.** A module has been added to this course focusing on nanostructured materials.
- **BIOE 572: Fundamentals of Systems Physiology.** A new semester project has been added in which students advise a mock venture capital firm about potential investments in novel bioengineering technology. Nanoengineered optical contrast agents are used as the sample technology in class.
- **BIOE 589:** Computational Molecular Bioengineering. Results of CBEN research included as appropriate examples of concepts discussed in class.
- **BIOE 620: Tissue Engineering:** A module has been added to this course focusing on nanostructured materials.
- **CENG 603: Rheology.** Results of CBEN research included as appropriate examples of concepts discussed in class.
- **ELEC 603 Nano-Optics.** This is a seminar-based course for seniors and graduate students where a variety of currently hot areas in subwavelength optics are studied. The main topics include: near field optics, microscopy and sensing, single molecule spectroscopy, nanoparticles and their optical properties, and photonic crystals and arrays.
- **ENVI 534: Transport Phenomena and Environmental Modeling.** Examples illustrating transport of nanoparticles in the environment have been introduced.
- **ENVI 550: Applied Water Chemistry.** Results of CBEN research included as appropriate examples of concepts discussed in class.
- **ENVI 635: Advanced Topics in Water.** Results of CBEN research included as appropriate examples of concepts discussed in class.
- **MSCI 614:** Principles of Nanoscale Mechanics. Results of CBEN research included as appropriate examples of concepts discussed in class.

### 9.4.4. Entrepreneurship Education

An important element of our strategic plan is the realization that in the future many of the most important industrial collaborations for our center members will involve small startup companies. Not only are they becoming a major employer of technical PhDs, but these
businesses are also becoming the route by which high-risk technologies are developed. These trends are due in some part to the bull market of the mid- to late 1990's; however, a principle that has withstood the test of time is that breakthrough technology is best developed by small groups of highly skilled, risk-taking individuals. These companies, which often involve the inventors as owners and partners, have very different concerns than larger corporations and require much more active involvement of inventors. Our entrepreneurship education programs are designed to prepare our students for these job opportunities and to forge strong relationships between the center and those corporations founded by our own members.

The entrepreneurial model for business development demands that inventors have access to the resources and talent of the business community. This requires that academics interact with business professionals, ideally in a forum to present their inventions. CBEN in collaboration with the Jones Graduate School of Management provides such opportunities. The Rice Alliance for Technology and Entrepreneurship is the centerpiece of the University's bold initiative that joins the resources of the Wiess School of Natural Sciences, Brown School of Engineering, and Jones Graduate School of Management together with other academic units and the Office of Technology Transfer. The mission of the Rice Alliance is to promote collaboration among university researchers and technology entrepreneurs, the outcome of which is the formation of new technology firms that bring to the marketplace innovative and useful products and services. In addition to the educational activities described below, our center is collaborating with the Rice Alliance on Innovation Forums, described in section 10.5 of this report.

**Entrepreneurship Education Workshop**

To start new companies, or to interact with existing startups, academic inventors must also have a rudimentary knowledge of business practices and language. We held a two-day workshop in Entrepreneurship Education for Researchers for professors, post-docs, and graduate students. This course on the commercialization of research discoveries in science and engineering was staffed by professors in Rice’s Jones Graduate School of Management and industry participants (e.g., local entrepreneurs and investors) involved in the Rice Alliance or as industrial affiliates. The center sponsored the attendance of its own members and promising junior nanoscience researchers at other academic institutions. Topics covered in this course include intellectual property, evaluation of business plans, negotiation skills, corporate governance in new ventures, common mistakes of entrepreneurs, and university incubators. The first of these workshops was held in conjunction with our first annual conference, in the fall of 2002 and repeated in Fall 2003.

**Graduate Course in Entrepreneurship for Scientists and Engineers**

In keeping with our focus on creating technical Ph.D.s well informed about career options, as well as our mission to enhance our members’ knowledge of business practices, we offer a year-long graduate course entitled "Entrepreneurship for Scientists and Engineers" which is co-taught by center faculty from the science, engineering and management schools. This class has already been developed for chemistry students with the support of the Coleman and Dreyfus Foundations. First semester topics include: venture financing, intellectual property (IP), market research and organization behavior. In the 2nd semester, science and management students work together in teams to perform extensive market and IP research for one or more center inventions, culminating in the creation of a business plan for commercializing a center innovation.
• MGMT 750/CHEM750/MSCI 750: Entrepreneurial Management for Science and Engineering. Results of CBEN research included as appropriate examples of concepts discussed in class.
• MGMT 751/CHEM 751/MSCI 751: New Venture Creation for Science and Engineering. Results of CBEN research included as appropriate examples of concepts discussed in class.

9.5. Community Programs

9.5.1. Continuing Studies Course for the Public
Many of our center's members are already involved in bringing the excitement of nanoscience to the public through a "Frontiers of Science" course offered by the School of Continuing Studies. This eight-week lecture course has been enormously popular with enrollments exceeding 80 attendees. Our center will provide speakers and materials to continue and expand this very successful program in Fall 2004 through a course titled, “Nanotechnology: What's So Big About the Science of the Very Small?”

9.6. Increasing the diversity in CBEN's programs
CBEN is committed to increasing the number of traditionally underrepresented students choosing to study nanoscience and engineering. CBEN now meets the national averages for African-American and Hispanic graduate students and welcomes Hispanic-American faculty member Pedro Alvarez to the center. Alvarez and Gustavo Scuseria represent nearly 10% of the center’s active faculty researchers. CBEN’s educational outreach programs are designed to draw underrepresented groups into center activities beginning with middle school students and teachers. Tables 3 (Section 9) and 4 (Section 12) present available diversity statistics on participants in CBEN’s outreach and research activities, respectively. This section highlights the key features with breakout tables.

9.6.1. Graduate student diversity
CBEN continues to do well at training female graduate students, especially as compared with the national and Rice averages within the natural sciences and engineering. As shown in table 9.4, women comprise 41% of CBEN-funded graduate students. This is not surprising given the fact that women are well represented within the center leadership. CBEN is the only Engineering Research Center and the only Nanoscale Science and Engineering Center with a female director (Colvin). Two of the three theme leaders, Drs. Colvin and West, are women, as is Dean of the Wiess School of Natural Science and center researcher Dr. Kathy Matthews.

Table 9.6.1 CBEN graduate students by gender

<table>
<thead>
<tr>
<th>CBEN Graduate Students</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBEN Graduate Students</td>
<td>59%</td>
<td>41%</td>
</tr>
<tr>
<td>Benchmarks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>70%</td>
<td>30%</td>
</tr>
<tr>
<td>National</td>
<td>68%</td>
<td>32%</td>
</tr>
</tbody>
</table>

Includes only natural science and engineering students

CBEN now meets the national averages for African American and Hispanic graduate student participation. While we are proud of this modest increase achieved in the
current reporting year, we are working hard to implement a long-term strategy for improving our student diversity statistics; Table 9.5 shows our current participants with benchmarks to the university and national averages. This is partly due to the success we have had at obtaining external funding for our diverse graduate students; however, our diverse undergraduate student population should also permit us to improve upon these statistics in the future.

Table 9.6.2 CBEN graduate students by ethnicity

<table>
<thead>
<tr>
<th>CBEN Graduate Students</th>
<th>NA</th>
<th>PI</th>
<th>AA</th>
<th>C</th>
<th>A</th>
<th>NP</th>
<th>Hispanic</th>
<th>Other non-US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>0%</td>
<td>0%</td>
<td>3%*</td>
<td>44%</td>
<td>3%</td>
<td>3%</td>
<td>3%</td>
<td>44%</td>
</tr>
<tr>
<td>National</td>
<td>0%</td>
<td>0%</td>
<td>2%</td>
<td>36%</td>
<td>6%</td>
<td>7%</td>
<td>4%</td>
<td>45%</td>
</tr>
</tbody>
</table>

NA = Native American; PI = Pacific Islander; AA = African American; C = Caucasian; A = Asian; NP = Not provided
*Includes first-year student Jennifer Jamison who has worked on a CBEN project since the summer before she started graduate studies and is now fully supported by CBEN funds. She was supported by a non-CBEN fellowship for her first semester.

Improvements in minority representation are not quickly achieved; for that reason we have a long-term plan for increasing the number of underrepresented students entering graduate school in science and engineering, whether at Rice or another institution. Over time, by mentoring a diverse population of students in our NREU program, CBEN will expand the population of students interested in graduate school. Also, this year CBEN has worked with departments to improve their recruitment of graduate students generally. We have begun and will continue to reach out to the incoming graduate students from underrepresented groups by offering them financial support to do CBEN research and affiliation with the AGEP program (see Section 9.3.1) during the summer before they begin graduate work. This kind of support can reduce attrition caused by the often difficult adjustment to graduate work.

In addition, we have developed strategic relationships with regional minority-serving institutions (MSIs). For the first time, we have received applications to our REU program from students at Prairie View A&M University through a relationship established with the assistance of the AGEP program; we placed one PV-AMU student in the lab of Tony Mikos for Summer 2004 and expect to recruit many more in the future years. Kulinowski visited PV-AMU in Spring 2004 to give an outreach talk on nanotechnology to an audience of about 200 mostly African-American students and mentors, which garnered her an invitation to be the keynote speaker at the Pre-College Research Symposium at Universidad Metropolitana in San Juan, Puerto Rico. We will send CBEN faculty to these and other MSIs during the year to actively recruit candidates for both the REU program, in which slots will be reserved for underrepresented students, and our graduate programs. Such activities can lead to meaningful, long-term relationships with MSIs that ultimately increase the number of underrepresented minorities entering graduate school at Rice and elsewhere.

We are also embarking on a relationship with Clarkson Aerospace, a local minority-owned high-tech company, which is seeking funding to develop outreach programs in technology for a predominantly African American and Hispanic population within Houston. This partnership includes historically black and Hispanic serving institutions such as Texas Southern University, PV-AMU, and Louisiana State University, which provides yet another link between CBEN researchers and a diverse group of future scientists and engineers. We will aggressively recruit these students into CBEN’s undergraduate and graduate programs.
9.6.2. Diversity in K-12 and undergraduate programs

CBEN continues to draw underrepresented groups into its K-12 activities and in its REU program. (See Table 9.6.) The high school teachers who have participated in our three-phase training program and workshop draw heavily from the diverse populations of the Houston (56% Hispanic, 31% African American, 10% White, and 3% Asian) and United (95% Hispanic) ISDs. Given the demography of the HISD student population this diversity is matched or exceeded in the high school student academy program. Our targeted approach to REU recruitment, which includes visits to regional schools and national meetings of groups such as the National Society of Black Physicists has paid off. Last summer, we trained two African-American females, two Caucasian females, and two Hispanic males at the undergraduate level, and incoming graduate students supported during the summer before they began graduate studies included an African-American female, Caucasian female and Hispanic male.

Table 9.6.3 CBEN students and outreach participants by ethnic diversity

<table>
<thead>
<tr>
<th>CBEN REU Students</th>
<th>NA</th>
<th>PI</th>
<th>AA</th>
<th>C</th>
<th>A</th>
<th>NP</th>
<th>Hispanic</th>
<th>Other non-US</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
<td>0%</td>
<td>33%</td>
<td>17%</td>
<td>0%</td>
<td>17%</td>
<td>33%</td>
<td>0%</td>
</tr>
<tr>
<td>Benmarks</td>
<td>Rice</td>
<td>1%</td>
<td>0%</td>
<td>6%</td>
<td>52%</td>
<td>17%</td>
<td>10%</td>
<td>11%</td>
</tr>
<tr>
<td></td>
<td>National</td>
<td>1%</td>
<td>0%</td>
<td>6%</td>
<td>74%</td>
<td>11%</td>
<td>2%</td>
<td>6%</td>
</tr>
<tr>
<td>CBEN High School Summer Academy</td>
<td>0%</td>
<td>0%</td>
<td>35%</td>
<td>0%</td>
<td>10%</td>
<td>0%</td>
<td>55%</td>
<td>-</td>
</tr>
<tr>
<td>Benmarks</td>
<td>HISD</td>
<td>0%</td>
<td>0%</td>
<td>31%</td>
<td>10%</td>
<td>3%</td>
<td>0%</td>
<td>56%</td>
</tr>
</tbody>
</table>

NA = Native American; PI = Pacific Islander; AA = African American; C = Caucasian; A = Asian; NP = Not provided

9.7. References Cited

9.8. Summary of CBEN Educational Outreach Activities

Course for K-12 Teachers

<table>
<thead>
<tr>
<th>Teachers</th>
<th>Date</th>
<th>CBEN participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHEM 570</td>
<td>10 Spring 2003</td>
<td>Kulinowski*, Colvin, West, Ma, James, Ausman, Lamminen (NanoKids), Drezek, Mikos, Hafner, Wiesner</td>
</tr>
<tr>
<td>CHEM 570</td>
<td>10 Spring 2004</td>
<td>Kulinowski*, Colvin, West, Drezek, Mikos, Hafner, Wiesner, Barron, Pasquali, Smalley</td>
</tr>
</tbody>
</table>

*Indicates Course Leader

Courses for Undergraduate Students

<table>
<thead>
<tr>
<th>Topic</th>
<th>Students</th>
<th>Date</th>
<th>New/Modified</th>
<th>CBEN participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIOE 441 Advanced Bioengineering Lab</td>
<td>33</td>
<td>Fall 2003</td>
<td>Modified</td>
<td>West, Drezek</td>
</tr>
<tr>
<td>BIOE 452 Bioengineering Design</td>
<td>32</td>
<td>Spring 2004</td>
<td>Modified</td>
<td>West</td>
</tr>
<tr>
<td>CHEM 373 Fullerene Lab</td>
<td>7</td>
<td>Spring 2004</td>
<td>Existing</td>
<td>Weisman</td>
</tr>
<tr>
<td>CHEM 375 Nanochemistry Lab</td>
<td>4</td>
<td>Fall 2003</td>
<td>NEW</td>
<td>Colvin, Thoms</td>
</tr>
<tr>
<td>CEVE 403 Principle of Environmental Engineering</td>
<td>27</td>
<td>Fall 2003</td>
<td>Modified</td>
<td>Hughes</td>
</tr>
<tr>
<td>CEVE 434 Chemical Transport and Fate in the Environment</td>
<td>12</td>
<td>Fall 2003</td>
<td>Modified</td>
<td>Wiesner</td>
</tr>
</tbody>
</table>

Courses for Graduate and Advanced Undergraduate Students

<table>
<thead>
<tr>
<th>Topic</th>
<th>Students</th>
<th>Date</th>
<th>New/Modified</th>
<th>CBEN participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIOE 531 Biomaterials Engineering</td>
<td>42</td>
<td>Fall 2003</td>
<td>Modified</td>
<td>West, Mikos</td>
</tr>
<tr>
<td>BIOE 572 Fundamental of Systems Physiology</td>
<td>15</td>
<td>Spring 2003</td>
<td>Modified</td>
<td>Drezek</td>
</tr>
<tr>
<td>BIOE 589 Computational Molecular Bioengineering</td>
<td>12</td>
<td>Fall 2003</td>
<td>Modified</td>
<td>Ma</td>
</tr>
<tr>
<td>BIOE 620 Tissue Engineering</td>
<td>27</td>
<td>Spring 2003</td>
<td>Modified</td>
<td>Mikos</td>
</tr>
</tbody>
</table>
### Courses for the Public or General Audiences

<table>
<thead>
<tr>
<th>Topic</th>
<th>Students</th>
<th>Date</th>
<th>CBEN participants</th>
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</thead>
<tbody>
<tr>
<td>Medical Applications of Nanotechnology</td>
<td>50</td>
<td>Feb 2003</td>
<td>Lee Hirsch (West grad student)</td>
</tr>
<tr>
<td>Advances in Tissue Engineering</td>
<td>80</td>
<td>August 2003</td>
<td>Mikos</td>
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### Workshops and Campus Visits

<table>
<thead>
<tr>
<th>Audience</th>
<th>Topic</th>
<th>Teachers</th>
<th>Students</th>
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<tr>
<td>High School Teachers in Dallas, TX</td>
<td>Nanotechnology and the Integrated Physics and Chemistry Curriculum</td>
<td>16</td>
<td></td>
<td>July 2003</td>
<td>Kulinowski, grad students Rocha, Jamison and Boyd</td>
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<tr>
<td>Technology Entrepreneurship Workshop for Scientists, Engineers, and Healthcare Professionals</td>
<td>Taking discoveries from the lab to the market</td>
<td>0</td>
<td>60</td>
<td>September 2003</td>
<td>Currall, other Jones School faculty</td>
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### Lectures on Nanotechnology and Careers for K-12 Teachers and Students

<table>
<thead>
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<th>Teachers</th>
<th>Students</th>
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<th>CBEN participants</th>
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<tr>
<td>Boy Scouts Troop Lecture</td>
<td>0</td>
<td>20</td>
<td>Fall 2003</td>
<td>Natelson</td>
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<tr>
<td>Raymond Academy for Engineering, Houston, TX. What do Chemical Engineers do?</td>
<td>10</td>
<td>250</td>
<td>Oct 2003</td>
<td>Pasquali</td>
</tr>
<tr>
<td>Introduction to Nanotechnology, Lee High School campus visit</td>
<td>4</td>
<td>40</td>
<td>May 2003</td>
<td>Kulinowski</td>
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<tr>
<td>Introduction to Nanotechnology, Science Academy of South Texas</td>
<td>2</td>
<td>30</td>
<td>June 2003</td>
<td>Kulinowski</td>
</tr>
<tr>
<td>Introduction to Nanotechnology, Milby/YES summer academy</td>
<td>2</td>
<td>40</td>
<td>June 2003</td>
<td>Kulinowski</td>
</tr>
<tr>
<td>Gaining Early Awareness and Readiness for Undergraduate Programs (GEAR-UP) middle school outreach presentation.</td>
<td>3</td>
<td>30</td>
<td>October 2003</td>
<td>Kulinowski</td>
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### Lectures on Nanotechnology for Undergraduates, Graduate Students and Post-docs

<table>
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<th>Teachers</th>
<th>Students/Post-docs</th>
<th>Date</th>
<th>CBEN participants</th>
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</thead>
<tbody>
<tr>
<td>Maryville College</td>
<td>5</td>
<td>20</td>
<td>Feb 2003</td>
<td>Mary Turner (Colvin grad student)</td>
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<tr>
<td>AGEP Summer Seminar Series</td>
<td></td>
<td>50</td>
<td>Jun 2003</td>
<td>Kulinowski</td>
</tr>
</tbody>
</table>
10. Outreach and Knowledge Transfer

CBEN continues to reach out to partners in industry, government and non-profits to enhance its activities; we have added substantial new collaborations with industry over the past year. The ongoing public discussion of nanotechnology’s risks and benefits is also an area that CBEN has been active in; we work with a broad set of stakeholders in developing a public dialog regarding policies for responsible nanotechnology. We are pleased to welcome two new industrial affiliates to the center: DuPont and L’Oréal. In response to suggestions made at last year’s site visit, CBEN has begun to formalize a systematic toxicological assessment of nanoparticles by fostering active and substantial collaborations with the National Institute for Environmental Health Sciences (NIEHS), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). We’ve also continued our role in providing information to a broad group of stakeholders, including government policy makers, non-governmental organizations (NGOs) and the media. A new effort in research into the societal impacts of nanotechnology is now underway as a result of supplemental funding received last year. This new program also inspired a successful Nanotechnology in Undergraduate Education grant proposal.

10.1. Industrial Affiliates Program and ICON

CBEN has made great strides in attracting industrial partners to our center’s activities. We have found that companies are interested in CBEN for two distinct reasons: (1) sponsorship of technical research and/or access to our intellectual property; (2) association with the world’s premiere academic center for ensuring responsible nanotechnology policies. L’Oréal, which claims to hold the most patents in nanotechnology of any company in the world, joined CBEN not for our IP, but for our central role in the developing US regulatory and policy arenas. DuPont is the rare company that intersects with both the center’s technical research and community-building activities. Their involvement in CBEN includes direct funding for theme 3 research, which we have applied to a project to evaluate the ecotoxicology of variably coated nanoscale titania. Other prospective industrial partners have invited CBEN personnel to participate in discussions of environmental health and safety issues. Colvin and Kulinowski are members of the Semiconductor Research Corporation/National Nanotechnology Initiative Environmental Health and Safety Working Group, which has monthly conference calls to discuss issues important to the semiconductor industry.

Our current and prospective industrial partners have strongly encouraged us to go beyond the traditional structures of an industrial affiliates program to create a more inclusive and international group. Their enthusiasm has prompted us to develop a program that welcomes not only corporate members, but also government, non-governmental organizations (NGOs) and other academics. This broader partnership is vital to our core mission of creating a sustainable nanotechnology industry that, unlike technology development goals, requires meaningful and organized interactions among stakeholders.
At their request we are in the advanced stages of planning the International Council on Nanotechnology (ICON). The mission of this organization is to assess, communicate, and reduce environmental and health risks associated with nanotechnology while in turn maximizing its benefits to society. To realize this vision, ICON seeks participation from a diverse group of parties including industry, academics, government officials, and representatives of environmental organizations. Its activities span technical research in nano-cell interactions, policy projects such as development of nanomaterial standards and terminology, and social studies of risk perception and communication. By pooling the resources of the nanotechnology industry, governments, and academia, ICON can cost-effectively provide a wide range of synergistic projects that serve the interests of all stakeholders. We have named this organization ICON, as opposed to CBEN, because of our anticipated involvement with other international academic centers in this area. While we plan to manage and launch this group, it is vital that we involve and use the expertise of all academics involved in these issues. There is widespread enthusiasm for this organization, which will create new knowledge of use to government and industry researchers and serve as a central clearinghouse for information related to health and environmental aspects of nanomaterials. By catalyzing the formation of ICON, we are taking the first, early steps to ensuring that CBEN creates a legacy that lives beyond its ten-year NSF funding cycle.

10.1.1. Standard terminology for nanomaterials

One of the first projects ICON will tackle is the creation of a systematic standard of terminology for nanomaterials. This will be useful to all stakeholders and is essential to fostering an informed and supportive public. Research into the properties, synthesis, and applications of nanostructures is growing at an exponential rate, yet to date there does not exist a common language to describe the chemical compositions and physical forms of these new materials. This lack hampers not only technical communication within and across the various fields involved in this highly interdisciplinary enterprise, but also outreach to the public at large as products containing nanomaterials enter the marketplace. This process is essential also for effective governmental oversight and standardization for the industry. However, the development of a useful set of standardized terminology rules for this emerging field is a formidable task, requiring the broad-based involvement of experts from diverse constituencies and fields of expertise. Subsequent adoption of such a rule set will require international support and the cooperation of numerous agencies and organizations. As a broad-based entity, ICON can bring together all the necessary sets of expertise.

CBEN has already garnered the support of the American Chemical Society Committee on Nomenclature, Terminology and Symbols for this effort. After Colvin, Kulinowski, and Nano Letters editor and UC Berkeley nanomaterial researcher Paul Alivisatos presented a proposal to the committee during the March 2004 American Chemical Society national meeting, the committee recognized the importance of the work and agreed to form a subcommittee to begin working on our proposal. The committee also agreed to invite a member of the nanomaterials community to sit on the full committee. We will engage the international community in these efforts by submitting a proposal to the International Union of Pure and Applied Chemistry (IUPAC), which will be encouraged to work together with ACS to produce a set of standards that can be adopted across the globe.
10.2. Interactions with Government

CBEN has been very active over the last year in interacting with US federal agencies as well as international government groups. Here in the US we have begun several strategic collaborations with government scientists at NIEHS, FDA and EPA to promote the systematic study of nanomaterial toxicity. CBEN researchers Colvin and Wong are now collaborators with the FDA/NIEHS, who provide direct research support for collaboration on their ongoing toxicology program. Center director Colvin testified before the President’s Council of Advisors on Science and Technology (PCAST) on responsible nanotechnology policy in March 2004. Entities such as the Woodrow Wilson International Center for Scholars (WWIC) can bring policy issues to the attention of members of Congress and executive branch officials, who ultimately control both the amount of public funding of nanotechnology and the manner in which the funds are disbursed. CBEN has been proactive in communicating with these groups about the importance of continued research in basic nanotechnology as well as its societal impacts.

CBEN’s governmental interactions have not been limited to US officials. CBEN personnel were invited to a Government of Canada (GoC) workshop in October 2003 to address environmental health and safety issues (Colvin) and the need for proactive policies (Kulinowski). The purpose of the workshop was to plan the creation of a Canadian National Nanotechnology Initiative. Colvin participated in a European Commission workshop on “Mapping out Nano Risks” in March 2004. The results of the EC’s efforts to assess and manage nanotechnology risk will likely have an impact and be closely monitored by policy makers here in the US.

10.3. Research into the Societal Impacts of Nanotechnology

Supplemental funding from NSF last year has enabled two new research projects that explore the societal impacts of nanotechnology: Assessing Public Trust and Perceptions of Risk (Currall) and The Ethics and Politics of Nanotechnology (Kelty, Landecker and Roush). Interactions with anthropologists Landecker and Kelty that were enabled by this supplemental funding have inspired (1) the submission of a NSF grant to expand the politics and ethics work described in Section 10.3.2; (2) the new Nanotechnology: Content and Context undergraduate course for which Rice was awarded a NSF Nanotechnology in Undergraduate Education grant (see Section 9); and (3) the participation of Landecker and Kelty on a NSF IGERT submission in Sustainable Nanotechnology, which, if funded, would team technical graduate students with social science graduate students to study the adoption of nanotechnologies in rural areas of developing nations. The societal implications supplement also enabled a special panel and session on societal impacts at our annual symposium.

10.3.1. Nanotechnology: Assessing Public Trust and Perceptions of Risk (Currall)

Public perceptions of nanotechnology will inform the study and utilization of nanotechnology by individuals in the for-profit sector and by academic scientists. Furthermore, the level of the public’s trust will determine whether commercial products containing nanotechnology will be embraced because of their perceived benefits or avoided due to fears. The importance of public trust and acceptance is a lesson already learned by corporations and governments who have found that both real and imagined hazards of nuclear power, genetically modified organisms, biotechnology, and semiconductor manufacturing have played a key role in their development. Public trust in any new technology determines the willingness by individuals to purchase products based on these technologies and their willingness to work in companies
where these technologies are developed or manufactured. Through our research, we will develop an understanding of how the public currently perceives nanotechnology, and this information will in turn provide guidance to corporations and governments who wish to avoid public backlash as has been seen in previous technologies (e.g., genetically modified organisms).

Our research program will contribute to a deeper understanding of the public’s perceptions of nanotechnology by:

1) Determining the level of the public’s trust in nanotechnology.
2) Exploring potential risks and benefits that foster or detract from trust in nanotechnology.
3) Comparing trust in nanotechnology with trust in other technologies.
4) Comparing trust in particular commercial applications of nanotechnology with other applications.
5) Examining how a corporation’s association with nanotechnology impacts public perceptions of the corporation.

As a foundation for our work on nanotechnology, we have developed a theoretical model of trust in new technologies. Based on our studies of trust in a variety of organizational contexts, we define trust as the decision to rely on a target under a condition of risk. Our focus on nanotechnology applications as targets of trust allows us to investigate public perceptions of nanotechnology as well as test a general model of trust and risk perception. This theoretical model proposes that attitudes toward new technologies, societal influences, and individual differences in affect and cognition predict intentions to engage in trusting behaviors. These intentions, in turn, lead to trusting behavior (i.e., using a product developed through nanotechnology).

We are applying our model of trust in new technology in two complementary studies of public trust in nanotechnology. First, we will assess the public’s trust based on data from a representative sample of survey respondents across the United States. This sample also will be used to investigate the psychological factors (i.e., risks and benefits) that contribute to trust or lack of trust in nanotechnology. Respondents will be presented with information about hypothetical commercial products that are described as containing nanotechnology. We will experimentally vary risks and benefits associated with these products to examine how people choose whether or not they would use (i.e., trust) these products. This study will allow us to test the relative importance of the target of risk as well as the risk/benefit ratio in predicting trusting behaviors toward nanotechnology.

Second, our model of trust and risk perception will be tested through an extensive laboratory study that requires participants to evaluate hypothetical nanotechnology products in comparison to each other and to other technologies. We also are studying whether corporations that are seen as associated with nanotechnology will benefit from or be negatively affected by this association. Finally, we will investigate the extent to which organizations associated with nanotechnology can maintain or improve public perceptions.

Across both studies, we will include a baseline measure of the general public’s knowledge of nanotechnology. Initial studies suggest that many people know very little about nanotechnology, but few studies have taken an in-depth look at the characteristics of individuals with varying levels of knowledge about nanotechnology. We will contribute to this literature by
asking participants to describe what they know about nanotechnology as well as the overall positivity or negativity of their feelings toward nanotechnology.

10.3.2. Ethics and Politics of Nanotechnology (Kelty, Landecker and Roush)

A central aim of our project is to ensure that productive, highly engaged conversations about the social and ethical nature of science occur across disciplines that are very different: Anthropology and Nanotechnology. To do this, it is not enough to simply put social scientists in the same room as scientists and expect something to happen; these juxtapositions have rarely worked well in the past. Part of the progress made this year involves the training of social science graduate students to read technical scientific literature in nanotechnology without fear. With each of the interviews, the scientist being interviewed provided the research group ahead of time with a range of materials, from general review-type articles or talks to general audiences, to highly technical papers in membrane or polymer science. We used these materials to generate specific questions about the political, economic, and social aspects of the science being done here at Rice University. For students without a scientific background, this requires overcoming ingrained assumptions about the impossibility of being able to interact with technical or scientific ideas. We have been very successful in training the graduate students to find social scientific questions within scientific activities. It is one goal of this project to innovate social science methodology to better correspond with the realities of emerging sciences, and training a new generation of social scientists is an important component of this work.

Interviewing of scientists engaged in nanotechnology and the writing up of case studies will continue over the summer. These case studies will be used in teaching the undergraduate course, Nanotechnology: Content and Context, and as the basis for a small workshop bringing social scientists and the interviewed scientists together to discuss social and political aspects of nanotechnology. This year we have also applied for further grant support from NSF to continue this research on a larger scale.

10.3.3. Societal Impacts Session at NanoDays

CBEN holds an annual research symposium in October called NanoDays. With the supplemental funding we received last year, we were able to have a special focus on Societal Impacts of Nanotechnology, which included an evening panel and a session of invited talks.

Panel Session: Societal, Ethical and Environmental Implications of Nanotechnology

- Neal Lane, Senior Fellow at the James A. Baker III Institute for Public Policy and University Professor, Rice University
- David Berube, Department of English, University of South Carolina
- Mike Gorman, Department of Systems and Information Engineering, University of Virginia
- Tracy Hester, Bracewell and Patterson, L.L.P.
- Pat Roy Mooney, The ETC Group

Session 1: Societal and Ethical Implications of Nanotechnology

- Mike Gorman: "Societal and Ethical Implications of Nanotechnology: A Framework for Interdisciplinary Collaboration"
- David Berube: “SEIN Research: Just Smoke and Mirrors?”
10.4. Outreach to media professionals

Should the public fear nanotechnology? Responding to that question has expanded the definition of “knowledge transfer” at CBEN. We believe our activities in this area are central to ensuring long-term public acceptance of nanomaterials and nanotechnologies, an issue highlighted in our strategic plan. This acceptance could be in jeopardy as nanotechnology’s “yuck index” rises, due both to the revival of old debates and the emergence of new controversies. As the only NSF center charged with assessing the environmental impact of nanomaterials, CBEN’s efforts have been thrust into the public spotlight. In response to the media barrage, we developed an aggressive media and government relations strategy to introduce hard data into this emotional debate. As a result of these strategies, CBEN has become a major academic source for journalists and policy makers concerned about the public perception of nanotechnology. Our outreach to these non-traditional communities will only increase over the next year as the first toxicology studies of nanomaterials become available.

10.4.1. The public message

While CBEN cannot speak for every one in the center, much discussion and debate has gone into questions of nanotechnology’s risks and what message, if any, CBEN should promote. As the only federally funded center with an emphasis on nanotechnology and the environment, we realized the importance of developing a documented policy position on these issues. In brief, we do not support a moratorium on nanotechnology development and do not believe the gray-goo scenario will come to pass. Nevertheless, we do believe that it is simplistic to assume that all nanotechnology will provide only beneficial impacts on society. The reality is that the consequences of a massive scaleup of nanotechnology research and development are unknown.

CBEN’s central message has evolved over the past year as government funding of implications research has increased. The EPA’s STAR program will fund many new research projects through the focused program Environmental Implications of Engineered Nanomaterials; the Multidisciplinary University Research Initiative of the Department of Defense has awarded CBEN collaborator Günter Oberdörster $5.5M to develop a model that would predict the toxicity of certain nanoparticles. These and other programs will result in a growing body of nano-cell interactions research coming out in the next year. Now the message must shift from raising awareness of the need for more studies to providing a context in which journalists and the public can understand the results of these studies. We have already seen what can happen when research results are released without this important context, as happened recently when Eva Oberdörster (the daughter of Günter Oberdörster) press-released her small study on the impact of fullerene clusters on large-mouth bass. CBEN personnel accommodated many interview requests and put the fish study in context by highlighting CBEN work showing that the toxicity of these particles can be turned off by functionalizing the surfaces.

CBEN will work to ensure that individual toxicology studies are interpreted in a broader context.
of the fullerenes. This new message of “control”, \textit{i.e.}, the ability to tune the toxicity \textit{via} control over surface properties, can be powerful in countering the unfortunate tendency toward over-reaction to a single study. Finally, questions about an emerging technology’s risks must be balanced by the known benefits. Given the wide scope and proven advantages of nanotechnology, the perceived risks are put in a new context.

\textbf{10.4.2. Well-informed journalists and educational articles}

The ultimate sources for science- and business-writers are the researchers themselves; it is thus critical to create an environment in which journalists can learn about the research in depth from the primary sources. In efforts to keep nanotechnologists and the larger communities informed about the important research accomplishments and policy issues facing the field, we have posted a website where links to many of these articles can be found: \url{http://www.ruf.rice.edu/~cben/Media.shtml}. In the near future, ICON will serve as the new clearinghouse for information for the public and the general media, and will contain media packets describing CBEN’s technology applications.

In addition to accommodating individual journalists’ requests for interviews, we continue to engage with them directly in a deep and meaningful manner through the Council for Advancement and Support of Education (CASE) Media Fellowships. Center personnel including Neal Lane, Kulinowski, Wiesner, Barron, West and others met with CASE Media Fellows on two occasions in the last year. Now in its twelfth year, the CASE Media Fellowship program offers opportunities for journalists to learn about new scientific and technological developments in depth from the researchers making the discoveries. The focus of each CASE Media Fellowship and the journalists who attended are:

May 2003: Nanotech Comes of Age in a Wet/Dry World (pictured at right)

- Kenneth Chang, \textit{New York Times}
- Candace Stuart, \textit{Small Times Magazine}
- Alex Witze, \textit{Dallas Morning News}

March 2004: Societal Implications of Nanotechnology

- Adam Aston, \textit{Business Week}
- Bethany Halford, \textit{Chemical \& Engineering News}
- Erika Jonietz, \textit{Technology Review}
- Darrin Schlegel, \textit{Houston Chronicle}

During each Fellowship, the journalists spent two days at Rice hearing about research and policy, meeting faculty and graduate students, touring the research facilities, and seeing demonstrations of key experiments. We have found that journalists who take the time to delve deeply into the research in a stress-free environment absent of the daily pressures of the newsroom end up better informed and write more nuanced and positive articles about nanotechnology. Though CBEN research was the focus of their Fellowship, their costs were not covered by CBEN funds but were borne by Rice University.
10.5. Entrepreneurship Promotion

We believe that, as with biotechnology, many of CBEN’s technologies will find commercialization through entrepreneurial ventures centered on our own academic inventions. We thus have several programs designed to enhance and improve the academic-entrepreneurial activities. Many of these include educational efforts for graduate students and faculty that are described in section 9.4. Here we focus on the portion of our entrepreneur program that aims to develop new business.

In this reporting period, CBEN, through the Rice Alliance for Technology and Entrepreneurship, held one Nanotechnology Innovation Forums. The Rice Alliance is a Rice community resource that, through its Innovation Concept Forums, brings together a talent pool of potential collaborators in entrepreneurship ventures. These include scientists and engineers with the technical innovations at various stages of development, venture capitalists, investment bankers, local entrepreneurs, faculty members, recent MBA graduates, and students. These forums are structured around presentations by inventors, who receive feedback on their innovations and business plans from a panel of experts.

This Nanotechnology Innovation Forum, titled “Nanotechnology: Creating the Next Energy Revolution,” was held on January 16, 2004. Keynote speakers were Dr. Richard Smalley, Nobel Laureate and University Professor at Rice University, and Jim Von Eyr Founder, Chairman, and CEO of Zyvex Corporation. Views on trends and commercialization opportunities in nanotechnology were provided by Rice Faculty, and five early-stage nanotechnology companies were presented for evaluation by a venture capital feedback panel. Five business concepts or early-stage ventures were presented to an audience of 350. The presentations were:

- Andrew Barron: NewCyte – Combining nanomaterials with molecular biology
- Louis Brousseau: Quantum Logic Devices – Nanoelectronic platforms for biomolecular research
- Chris Gintz: NanoComposites – Nanoscale materials science
- John Kane: ACTON Materials – Nanoparticles with or without nanoscale coating/structures
- Waqar Qureshi: MolecularDiamond Technologies – Higher diamondoids

10.6. Centerwide events

CBEN sponsors and co-sponsors numerous local and national events in order to disseminate center research, build relationships among its members, and increase the interactions of CBEN with other academic institutions. This year our annual conference, Nanodays 2003, featured talks from CBEN researchers and seven invited keynote speakers. Associated with this conference is the annual poster session for CBEN students at which CBEN REU undergraduate Michelle Afkhami took first prize.

The ‘nano-vivo’ summit in early 2003 provided a forum for CBEN researchers to share their expertise and technologies with medical professionals from the nearby Texas Medical Center. In addition to these formal events, CBEN sponsors bimonthly meetings for its PIs to discuss ongoing research activities in addition to the graduate student meetings described in the educational outreach section 9.4.1.
## 10.7. Summary of CBEN Knowledge Transfer and Outreach

### 10.7.1. Interactions with elected and other government officials

<table>
<thead>
<tr>
<th>Event</th>
<th>Interaction</th>
<th>Who</th>
<th>Date</th>
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<tbody>
<tr>
<td>Site Visit</td>
<td>NSF NSEC Review: Columbia University</td>
<td>Kulinowski</td>
<td>May 2003</td>
</tr>
<tr>
<td>Dialog</td>
<td>Woodrow Wilson Center Dialog on Nanotech and the Environment</td>
<td>Colvin, Kulinowski</td>
<td>June 2003</td>
</tr>
<tr>
<td>Meeting</td>
<td>Assoc Dir Office of Naval Research International Field Office Japan</td>
<td>Kulinowski</td>
<td>Sep 2003</td>
</tr>
<tr>
<td>Workshop</td>
<td>NSF Nanotechnology Science and Engineering Education</td>
<td>Kulinowski</td>
<td>Sep 2003</td>
</tr>
<tr>
<td>Meeting</td>
<td>National Institute for Environmental and Health Sciences (NIEHS) at Rice, host for four scientists</td>
<td>Colvin &amp; others</td>
<td>Sep 2003</td>
</tr>
<tr>
<td>Dialog</td>
<td>Woodrow Wilson Center Dialog on Nanotech and Federal Regulation</td>
<td>Colvin, Kulinowski</td>
<td>Oct 2003</td>
</tr>
<tr>
<td>Workshop</td>
<td>NNI workshop on nanobiotechnology</td>
<td>Colvin</td>
<td>Oct 2003</td>
</tr>
<tr>
<td>Workshop</td>
<td>Government of Canada workshop on Federal Nanotechnology Initiative</td>
<td>Colvin, Kulinowski</td>
<td>Nov 2003</td>
</tr>
<tr>
<td>Workshop</td>
<td>NNI Societal Implications Nanotechnology II</td>
<td>Kulinowski</td>
<td>Dec 2003</td>
</tr>
<tr>
<td>Meeting</td>
<td>National Center for Toxicological Research</td>
<td>Colvin</td>
<td>Dec 2003</td>
</tr>
<tr>
<td>CBEN Overview: Environmental Nanotechnology</td>
<td>Oakridge National Laboratories</td>
<td>Colvin</td>
<td>Feb 2004</td>
</tr>
<tr>
<td>Meeting</td>
<td>Communications Advisor, National Research Council, Canada</td>
<td>Kulinowski</td>
<td>Feb 2004</td>
</tr>
<tr>
<td>Oral testimony</td>
<td>President’s Council of Advisors on Science and Technology (PCAST)</td>
<td>Colvin</td>
<td>March 2004</td>
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<tr>
<td>Workshop</td>
<td>“Mapping out Nano Risks”, European Commission</td>
<td>Colvin</td>
<td>March 2004</td>
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<td>Workshop</td>
<td>“Nanotechnology and Risk”, Swiss Consulate</td>
<td>Colvin</td>
<td>April 2004</td>
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## 10.7.2. Industrial Interactions

<table>
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<th>Group</th>
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<th>CBEN people involved</th>
<th>Date</th>
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<tr>
<td>Honeywell-UoP</td>
<td>Chicago, IL</td>
<td>Colvin</td>
<td>May 2003</td>
</tr>
<tr>
<td>KX Industries</td>
<td>Orange, CT</td>
<td>Ausman</td>
<td>June 2003</td>
</tr>
<tr>
<td>Millipore, INC</td>
<td>Boston, MA</td>
<td>Ausman</td>
<td>June 2003</td>
</tr>
<tr>
<td>Ionics, Inc.</td>
<td>Watertown, MA</td>
<td>Ausman</td>
<td>June 2003</td>
</tr>
<tr>
<td>Perkin Elmer</td>
<td>Houston, TX</td>
<td>Colvin, Ausman</td>
<td>June 2003</td>
</tr>
<tr>
<td>Millipore, INC</td>
<td>Boston, MA</td>
<td>Colvin</td>
<td>July 2003</td>
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<tr>
<td>KX industries</td>
<td>Groton, CT</td>
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<td>July 2003</td>
</tr>
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<td>Cabot Industries</td>
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<td>July 2003</td>
</tr>
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<td>Perkin Elmer</td>
<td>Groton, CT</td>
<td>Colvin</td>
<td>July 2003</td>
</tr>
<tr>
<td>Intel</td>
<td>San Jose, CA</td>
<td>Colvin</td>
<td>Sep 2003</td>
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<tr>
<td>Nanosys</td>
<td>Palo Alto, CA</td>
<td>Colvin</td>
<td>Sep 2003</td>
</tr>
<tr>
<td>Dixie Chemicals</td>
<td>Houston, TX</td>
<td>Ausman</td>
<td>Sep 2003</td>
</tr>
<tr>
<td>L'Oréal</td>
<td>Paris, France</td>
<td>Colvin, Kulinowski</td>
<td>Dec 2003</td>
</tr>
<tr>
<td>DuPont</td>
<td>Rice University</td>
<td>Colvin, Ausman, Currall, Kulinowski, Tomson</td>
<td>Jan 2004</td>
</tr>
<tr>
<td>Qdot</td>
<td>Hayward, CA</td>
<td>Colvin</td>
<td>Jan 2004</td>
</tr>
<tr>
<td>Intel</td>
<td>Berkeley, CA</td>
<td>Colvin</td>
<td>Jan 2004</td>
</tr>
<tr>
<td>Swiss Re</td>
<td>Zurich, Switzerland</td>
<td>Colvin, Kulinowski</td>
<td>Mar 2004</td>
</tr>
<tr>
<td>Industrial Technology Research Institute, Taiwan</td>
<td>Rice University</td>
<td>Colvin, Kulinowski, Ausman</td>
<td>Mar 2004</td>
</tr>
<tr>
<td>DuPont</td>
<td>Wilmington, DE</td>
<td>Colvin</td>
<td>Apr 2004</td>
</tr>
</tbody>
</table>

## 10.7.3. New Companies Started

<table>
<thead>
<tr>
<th>Company Name</th>
<th>CBEN people involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxane</td>
<td>Barron, Wiesner</td>
</tr>
</tbody>
</table>
10.7.4. Policy Symposia and Papers

<table>
<thead>
<tr>
<th>Title</th>
<th>Venue</th>
<th>Organizer</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanomaterial Regulation?</td>
<td>Environmental Forum</td>
<td>Colvin</td>
<td>Mar 2004</td>
</tr>
</tbody>
</table>

10.7.5. Representative Seminars on CBEN generally at major conferences (only CBEN management)

<table>
<thead>
<tr>
<th>Title</th>
<th>Venue</th>
<th>Who</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Environmental Nanotechnology”</td>
<td>American Physical Society Meeting, Austin TX</td>
<td>Ausman</td>
<td>Mar 2003</td>
</tr>
<tr>
<td>“The Science of ‘the Small’ and Where It’s Going”</td>
<td>The Legal and Business Forum on Nanotechnology</td>
<td>Ausman</td>
<td>June 2003</td>
</tr>
<tr>
<td>CBEN Overview</td>
<td>American Society of Mechanical Engineers</td>
<td>Colvin</td>
<td>Sept 2003</td>
</tr>
<tr>
<td>CBEN Overview</td>
<td>Frontiers in Research, Ottawa, Canada</td>
<td>Colvin</td>
<td>Nov 2003</td>
</tr>
<tr>
<td>“Impact on Environment and Health”</td>
<td>EuroNanoForum, Trieste, Italy</td>
<td>Colvin</td>
<td>Dec 2003</td>
</tr>
</tbody>
</table>
### and Politics of the ‘Next Big Thing’

<table>
<thead>
<tr>
<th>Event</th>
<th>Organizer</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Nanotech: Hip, Hyped or Horrible?”</td>
<td>Minnesota section of the American Chemical Society, St. Paul MN</td>
<td>Kulinowski</td>
</tr>
<tr>
<td>Environmental Implications of Engineered Nanomaterials</td>
<td>Groundbreaking Ceremony, Molecular Foundry, LBL, Berkeley CA</td>
<td>Colvin</td>
</tr>
<tr>
<td>Engineered Nanomaterials and Their Implications</td>
<td>Toxicology Forum</td>
<td>Colvin</td>
</tr>
<tr>
<td>Engineered Nanomaterials: Issues for Toxicologists</td>
<td>Society of Toxicology</td>
<td>Colvin</td>
</tr>
</tbody>
</table>

### 10.7.6. Non-governmental Organization Interactions

<table>
<thead>
<tr>
<th>Group</th>
<th>Meeting Place</th>
<th>CBEN people involved</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NanoBusiness Alliance Health and Environmental Impacts Task Force</td>
<td>Teleconferences</td>
<td>Kulinowski</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Semiconductor Research Corporation/NNI Environmental and Health Impacts Working Group</td>
<td>Teleconferences</td>
<td>Colvin, Kulinowski</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Rockefeller Foundation</td>
<td>New York, NY</td>
<td>Colvin, Kulinowski</td>
<td>Oct 2003</td>
</tr>
<tr>
<td>The ETC Group</td>
<td>Rice University</td>
<td>Various</td>
<td>Oct 2003</td>
</tr>
<tr>
<td>Institute for Life Sciences (ILSI-HESI)</td>
<td>Washington DC</td>
<td>Colvin</td>
<td>Jan 2004</td>
</tr>
<tr>
<td>Nanobioconvergence</td>
<td>Palo Alto, CA</td>
<td>Colvin</td>
<td>Jan 2004</td>
</tr>
</tbody>
</table>
11. Shared Experimental Facilities

CBEN has strengthened the Rice infrastructure for nanoscience and engineering and leveraged its funding internally to create an organized facility for equipment management. The initial CBEN equipment monies have been fully expended and all instruments purchased are now fully operational; these new systems build on an extensive set of equipment purchased since 1996 using $5M of private donor money given to Rice’s Center for Nanoscale Science and Technology (CNST). Additionally, through a new federal grant from the Department of Defense, Rice and CBEN have participated in a statewide consortium for building infrastructure in nanotechnology, called the Strategic Partnership for Research In Nanotechnology (SPRING). This year, a sum of almost $2 million dollars has been made available for equipment purchases. This support was targeted at tools for characterizing the wet/dry interface and include electron microscopes, centrifuges, spectrometers, and optical microscopes. CBEN’s PI, Colvin, also received an equipment grant from the NSF MRI program to purchase a scanning photoemission spectrometer for surface analysis. In addition to drawing in additional federal monies, CBEN also catalyzed the formation of a shared faculty group for equipment management, now in its third year. The shared equipment authority is widely recognized across campus as a model for infrastructure stewardship, and many departments are now enthusiastically transferring operations and management of their facilities to SEA. The highlights of our efforts towards building shared experimental equipment:

- **More than $2 million dollars of federal equipment monies** to augment and extend the CBEN shared equipment facilities.
- **Stewardship of the shared equipment authority**, a faculty-run management organization for shared equipment across Rice.
- **Growth of the shared experimental staff** to include a mass spectrosocist and surface analysis expert for continued expansion in capabilities available to researchers.

Existing equipment

Rice University laid the groundwork for our center’s experimental facilities with their 1993-6 fundraising campaign for nanoscience equipment. Our new nanoscience and technology building, Dell Butcher Hall, houses a state-of-the-art equipment room designed for vibrational and electrical isolation. Equipment located in this facility includes a Phillips environmental SEM, a JEOL electron beam lithography system, two Digital Instruments Atomic Force Microscopes, and a variety of smaller items such as sputter- and spin-coaters. Other instrumentation located elsewhere on campus and available for common use includes a JEOL 2010cx transmission electron microscope, a Nicolet FTIR microscope, a high-resolution X-ray diffractometer and an ISA Raman microscope. Additionally, the Texas Center for X-ray Crystallography is housed at Rice and manages two single-crystal, one powder, and one small-angle X-ray diffractometers.

11.1. Strategic equipment purchases

Approximately $1.3M of internal cost-sharing funds have been expended for CBEN equipment purchases. A new scanning electron microscope is now available to allow for the imaging of biological materials, and nanofiltration and bone replacement polymers. An upgrade
to Rice’s ten-year old transmission electron microscope has made student training more effective. This upgrade retrofits the microscope with a digital camera and software for routine high-resolution imaging and makes hands-on demonstrations of microscope procedures much more effective. CBEN purchased two analytical instruments, atomic emission spectrometers, for the characterization of environmental processes of nanomaterials. CBEN also leveraged $433,377 of its equipment money with a SUR grant from IBM for a state-of-the-art parallel supercomputer with multiprocessor nodes running in shared-memory SEP configuration through high-speed switches. Most of the parallel machines at Rice are obsolete or dedicated to specific groups. This facility supports both the memory applications (e.g. the PDE codes of Pasquali and Zygourakis), and the massively-parallel MPI codes (e.g. the molecular dynamics codes of Ma and Yakobson and the quantum mechanical simulations of Scuseria and Yakobson). Finally, the balance of roughly $280,000 was used toward the purchase of a second transmission electron microscope. This system, which is also a JEOL 2010, has been optimized for use as a cryogenic TEM. Dr. Jane Tao, an assistant professor in biology and an expert in cryo-TEM, is now involved as a characterization expert on projects in both themes 1 and 3. Users have just begun to take the first cryo-TEM images on the Rice campus in the past two weeks, and this tool will address the need for characterization of bio-nanoconjugates throughout our center.

Through the Strategic Partnership for Research in Nanotechnology (SPRING) Rice University with CBEN’s help has successfully drawn nearly $2 million dollars to campus for the purpose of developing nanotechnology infrastructure. This year, SPRING monies were targeted for tools needed for evaluating the wet/dry interface of nanotechnology. Instruments purchased include a field emission TEM, a preparative ultracentrifuge, a near-infrared absorption and emission spectrometer, a confocal optical microscope, and an atomic force microscope (AFM) capable of imaging biological samples in liquids.

11.2. Campus Initiative in Equipment Management: The SEA

The maintenance and management of shared equipment is a serious organizational challenge at any university; typically departments are the only entity given funds for such purposes, leaving shared equipment without any resources once the acquisition dollars have been spent. The obvious solution of levying user fees to offset maintenance costs brings up a different set of organizational concerns. For most shared equipment there is no clear authority in charge of setting and spending user fees, and there is no central administrative organization to keep records of equipment costs and to process and request user fees. University administrators are reluctant to cede control of such activities to independent faculty. These problems undermine the overall effectiveness and value of shared equipment in research.

In order to solve these institutional issues CBEN successfully advocated for Rice University to transfer control of all shared equipment, roughly twenty instruments across campus, to a faculty oversight committee two years ago. This campus organization, called the Shared Equipment Authority (SEA), is chaired by center director, Dr. Vicki Colvin. SEA administers money raised from user fees on equipment, sets user fees on a quarterly basis, and makes recommendations concerning strategic instrument acquisitions. While SEA is denoted as a CBEN initiative in internal Rice documentation, its membership is not limited to CBEN participants. Bimonthly meetings throughout the fall and winter have tackled many important policy issues. A web page describing SEA and its current projects and instrument portfolio can be found at http://www.sea.org.
SEA has continued receiving support from departments, university administrators and students over the past year. Its growth continued, and additional university funds have been directed towards SEA to permit a cleanroom technician and mass spectroscopy expert to be hired. These staff positions do not count as part of CBEN match monies, but they are leveraged from the investment CBEN has made in this critical area. SEA has also developed an efficient web-based sign-up system for all instruments, a tool very popular among students. Perhaps the most surprising development over the past year has been the increasing interest from departments in transferring their equipment into SEA for operation and management; the benefits of pooling the costs and overhead of running and recovering shared instrument costs are extensive, and SEA has inspired confidence that operational decisions will be made fairly and in a transparent fashion.

An important issue for this organization is the strategy for cost recovery. SEA has projected that only 25% of the costs for shared equipment at Rice can be recovered by direct billing of internal and external academic users if hourly fees are kept at the average nationwide values. Also, SEA is working with Rice’s Office for Development to identify individuals and private foundations who would donate money for these purposes. The presence of a NSF center in nanotechnology is good leverage for this type of fundraising, and many of the same people who contributed to the highly successful nanocampaign in 1993-6 are being approached again. However, to maintain faculty confidence in SEA and its management, the university has agreed to maintain the technical staff positions associated with CBEN after the funding cycle of the center, and has committed to sustaining shared equipment infrastructure at Rice as needed.

We will continue to increase the usage of all SEA instruments through a series of new training sessions that recognize the needs of ‘exploratory’ users. To address their issues, we are designing weekend workshops that illustrate how key instruments may be used to solve problems in certain disciplines. A similar workshop in electron microscopy (Guo), and FTIR and Raman microscopy will also be developed. It is our intention that these workshops will motivate researchers to invest the time required to become independent and successful users. If instrument use can be doubled, then cost recovery through user fees will contribute to a larger fraction of maintenance costs.

11.3. Full-time staff for student training and equipment maintenance

Two permanent, full-time technical staff positions hired during Y02 to oversee experimental equipment: **microscopist Dr. Wenhua Guo** and **structural biologist Dr. Sean Moran** continue to train students and contribute to CBEN research. These staffers (See Figure 11.2) share responsibility for instrument maintenance, serve as the contact point for repairs, and offer training to students, post-doctoral associates, and visiting scientists. We have seen dramatic improvements in equipment usage as these staff people have helped students and post-docs use instruments more efficiently. Clearly, well-trained instrument users handle these
expensive and sensitive instruments both more effectively and more frequently. These professionals have also begun to contribute to the research enterprise; Dr. Guo, for example, is an author on several CBEN publications where his expertise in electron microscopy was required. Matching monies have also made available a system administrator for our investment in computational resources. This person is responsible for configuring the machine, adding users, doing periodic upgrades, maintaining a web page for the facility with information on the queuing system and software manuals, and delivering periodic training seminars for new users. In Y05 we expect that these staff positions will become fully shared with SEA in anticipation that their ultimate support will derive from university and not federal funds.

Figure 11.3.1 Dr. Guo training CBEN student on the TEM.
12. Personnel

CBEN’s staff has been consistent over the last year and these personnel are responsible for active administration of the center’s outreach and research programs. CBEN has a physical home located between the engineering and natural science schools on the Rice campus. This 1154 square-foot space provides office space and meeting areas for CBEN staff.

While our management structure remains virtually identical to that of last year, there has been one significant change in personnel over the past twelve months. Dr. Joe Hughes, theme leader for our environmental thrust, has taken a position as Chair of the School of Civil and Environmental Engineering at Georgia Institute of Technology. He will remain an important and active member of Theme 3 but, due to the difficulties of managing a theme remotely, he will now share theme leadership with incoming CBEN researcher Pedro Alvarez who is located at Rice University. Co-leadership should ensure effective management of this important theme. All personnel associated with Dr. Hughes’s CBEN projects (graduate students John Fortner and Delina Lyons) will remain on campus and thus his funding does not need to be directed in a sub-contract.

CBEN management is organized around four activities: strategic planning, resource allocation, assessment and operations.

12.1. Strategic Planning

The strategic planning process involves the entire center, and occurs every January. Our senior advisor, Dr. Neal Lane, and founding director, Dr. Rick Smalley, are important participants in this long-range planning process in that they work in collaboration with the director, Dr. Vicki Colvin, to write an initial draft of the strategic plan. Dr. Lane’s extensive past experience as White House science advisor and the Director of NSF provides an important perspective for our planning process. As founding director of CBEN, Dr. Smalley has a long-
range vision for the field of nanotechnology and the role of CBEN in its development. This year, the draft plan was modified by center members in a series of workshops held throughout January and February 2004. During these meetings, small groups of CBEN faculty discussed the plan and ways to shape the upcoming year in CBEN’s research program. Their comments were incorporated into the final document, which is presented in section 7.

CBEN also works actively to invite and incorporate feedback from external sources, including experts from academia, industry and government. This assessment process is used in several ways throughout the center. This year, for example, the external review board participated in several teleconferences and face-to-face meetings with the center director. (See section and rewritten 12.2.) Their comments were instrumental in the management decision on theme 3 leadership, and also for revisions to CBEN’s strategic plan. CBEN also is finalizing the composition of its external advisory board, chaired by Dr. Lane. Since Dr. Lane is not directly involved in the management or research organizations of CBEN, he can deliver an informed yet objective perspective about the center from within Rice University. The board will meet during planning for our renewal to provide input on CBEN performance and future directions.

12.2. Resource Allocation

Director Dr. Vicki Colvin is responsible for leading the annual resource allocation and project evaluation processes. Research project evaluation begins by inviting existing project leaders to submit a progress report and future funding request. Our internal review process is governed by the internal review board, which includes theme leaders Drs. Colvin, West and Hughes. (Drs. Smalley and Colvin will appoint theme leaders annually based on the evolution of research and faculty availability and interest). They are responsible for rank-ordering all proposals. Outreach projects underwent a similar internal review process, with future budget requests produced through the consensus of the executive directors for education and operations. Once the evaluations are completed, Dr. Colvin is responsible for the final resource allocation and annual budget proposal.

12.3. Assessment

In addition to producing a strategic plan, our long-term planning process continues as we develop concrete measures of center performance and establish targets for these performance indicators. Metrics for project performance in CBEN have been discussed at the strategic planning meetings, and are being finalized for wide circulation amongst the membership. We are now tracking carefully our performance using targets which address the number of interdisciplinary projects started, publications with multiple member authorship, students co-advised by members, industrial connections established, commercial ventures started, and the impact of educational outreach programs. These quantitative goals are balanced by more qualitative evaluations of member participation in extracurricular center activities, such as outreach and seminars, and visibility of the center in the academic and national community. As we approach renewal next year, we will also refine the criteria by which individual center research projects are evaluated. Currently, CBEN projects are judged in our internal and external reviews by potential for relevance to the Center mission, potential for high impact, and probability of success, in that order.
12.4. Operations

The final function of CBEN management is day-to-day operations. The center has a number of responsibilities beyond its research activities, including center-wide programs, internal and external reporting, and industrial and educational outreach. After polling informally several engineering research centers CBEN determined that 13-18% of center funding should go towards these crucial functions, corresponding to 7 to 10 full-time personnel. The executive director for operations, Dr. Kevin Ausman, is responsible for developing industrial outreach programs, coordinating center-wide research activities (such as the annual conference, seminars, and research workshops), and serving as the contact point for NSF questions and reporting. Dr. Ausman’s efforts include active coordination of CBEN faculty to identify industrial affiliates and the development of center-industry partnerships. He works closely with the Rice Office of Technology Transfer. The executive director for education and public policy, Dr. Kristen Kulinowski, has responsibility for all educational and policy programming for the center. In addition, Dr. Kulinowski serves as the center’s communication director, translating center findings to the media, government and public at large. We would like to emphasize that naming a non-PI as director of center education does not reflect a lack of commitment to these outreach programs. All center faculty are required to make significant annual contributions to educational efforts, and many programs are lead by faculty. This kind of volunteerism, however, cannot replace the focus and effectiveness of a full-time professional dedicated to creating and nurturing our educational initiatives.

As originally proposed, CBEN also supports staff for general administrative purposes. Administrative director Jason Bradshaw is responsible for center-wide purchasing, accounts, and financial reporting; accountant/bookkeeper Janine Vails maintains records, tracks center expenses and handles travel reporting; receptionist Jerrie Johnson provides a daily presence in our offices, fielding phone calls and information requests; and staff assistant Teresa Champion performs administrative duties for center director and executive director for education and policy. Part-time undergraduate help during the school year is also available for specific project development. CBEN also supports two PhD-level technical positions for managing shared instrumentation. (See section 11).
13. Publications and Patents

13.1. Publications

Year 3: 46 papers in press or published


Year 3: Papers submitted to journals for review by May, 2004


60. “Velocity effects on fullerene and oxide nanoparticle deposition in porous media,” Hélène F. Lecoanet, Mark R. Wiesner. (Submitted).


**Year 3: Papers in the later stages of preparation**


Year 2

Year 1 


13.2. Patent Applications


13.3. Presentations


5. “Protein-Gold Nanoparticle Interactions and Their Effects on Protein Function,” M. Calabretta, NanoDays, Houston, TX, (October 2003).


46. “Fluorescence of Carbon Nanotubes: Applications in Physics, Chemistry, and Biology,” B. Weisman, *Department of Chemistry, University of Utah (Salt Lake City, Utah)*, (September 2003).


52. “Fluorescence of Carbon Nanotubes: Applications in Physics, Chemistry, and Biology,” B. Weisman, *Department of Physics, Universidade Federal de Minas Gerais (UFMG) Belo Horizonte, Brazil*, (November 2003).


### 13.4. Posters


4. “Optical Imaging for Minimally Invasive Medical Diagnosis,” *Duke University, Department of Biomedical Engineering Seminar Series, Durham, NC,* (April 2004).


14. Biographical Information

 PEDRO J. J. ALVAREZ, Ph.D., P.E., DEE
 Dept. of Civil & Environmental Eng. ♦ Rice University ♦ Houston, TX 77251
 TEL: (713) 348-5903 ♦ FAX: (713) 348-5203 ♦ e-mail: alvarez@rice.edu

 EDUCATION

 B.Eng. 1982 Civil Engineering McGill University, Montréal
 M.S.E 1989 Environmental Engng. University of Michigan, Ann Arbor

 POSITIONS

 2004-present G.R. Brown Professor Rice University, Houston, TX
 2001-2003 Professor The University of Iowa, Iowa City
 1999 Visiting Professor Dept. of Microbiology, EAWAG, Switzerland
 1998-2003t Associate Director Center for Biocatalysis & Bioprocessing
 1997-2003 Associate Professor The University of Iowa, Iowa City
 1993-1997 Assistant Professor The University of Iowa, Iowa City

 SELECTED HONORS AND AWARDS

 2003 Vice-President, Association of Environmental Engineering and Science Professors (AEESP)
 2003 Field Editor, European Journal of Soil Biology
 2002 Research project of the year award, SERDP cleanup division, Department of Defense.
 2002 Editorial Board Member of Bioremediation and Bioavailability, The Scientific World
 2000 Awarded the Button of the City of Valencia, Venezuela, by the Mayor of the City
 1998 The UI Collegiate Excellence in Teaching Award, University of Iowa
 1998 Editorial Board, Journal of Environmental Science and Health
 1996 Editorial Advisory Board, Advances in Environmental Research
 1995 Inducted into the American Academy of Environmental Engineers, Diplomate Status
 1995 Career Award, National Science Foundation.
 1992 The Rackham Predoctoral Fellowship, The University of Michigan, Ann Arbor, MI
 1991 The Outstanding Achievement Award in Environmental Engineering, U. of Michigan

 SELECTED ACTIVITIES

 • Registered Professional Engineer, Michigan License # 35419, Iowa License # 12575, RGP Iowa # 1681
 • Board member, Hazard Assessment and Control of Toxic Substances in Water, IWA, 7/00-present.
 • Member, Publications Committee, American Academy of Environ. Engineers (AAEE)
   International Expert Committee for the Mexican Institute of Petroleum, Biotechnology Program, 2/00 present.
 • Technical Advisor, Government of Nicaragua, 1998-present
 • Expert Advisor, Brownfields Technical Advisory Committee, State of Iowa, 1997
 • NSF and EPA Peer Review Panel Member, 1995-present.

 REPRESENTATIVE RELEVANT PUBLICATIONS (from 70 J. articles and 103 proc, & abstracts)


LON JAMES WILSON

Education:
- Iowa State University, Ames, Iowa, B.S. - 1966
- University of Washington, Seattle, Washington, Ph.D. - 1971
- University of Illinois, Urbana, Illinois, Postdoctoral Fellow, 1971 - 1973

Positions Held:
- Visiting Assistant Professor of Chemistry, University of Illinois, 1971 - 1973
- Assistant Professor, Rice University, August 1973 - June 1978
- Associate Professor, Rice University, July 1978 - June 1985
- Professor, Rice University, July 1985 - present

Honors, Awards:
- National Science Foundation Graduate Fellow (University of Washington)
- National Science Foundation Foreign Exchange Graduate Fellow (University of Florence, Florence, Italy)
- National Institutes of Health Postdoctoral Fellow (University of Illinois)
- Member, Laboratory for Biochemical and Genetic Engineering, Rice University
- Member, Center for Nanoscale Science and Technology, Rice University
- Phi Eta Sigma
- Alpha Chi Sigma
- The American Chemical Society and the Inorganic Division of the A.C.S.
- The Chemical Society of London
- Sigma Xi

Representative Publications:
15. Honors and Awards

Vicki Colvin
- 2004 Key Women in Energy – Global

Steven Currall
- 2003 Vocational Leadership Award for collaboration between the academic and business communities of greater Houston, Rotary Club of West University.

Jeffrey Hartgerink
- 2004 Searle Scholar Award for work in biological chemistry.

Jianpeng Ma
- 2003 – 2008 Award for the Faculty Early Career Development (CAREER) Award, National Science Foundation.

Douglas Natelson
- 2004 National Science Foundation CAREER award.
- 2004 Alfred P. Sloan Foundation Research Fellowship.
- 2003 David and Lucille Packard Foundation Fellowship.

Jennifer West
- 100 Most Innovative Young Scientists, MIT Technology Review TR100.
- “Best Discovery of 2003” for nanoshells cancer therapy, Nanotechnology Now.

Gustavo Scuseria
- 2003 John Simon Guggenheim Fellow.

Boris Yakobson
- 2004 NASA Faculty Fellowship.