The ENATBIO: a National Conference on Emerging Nanoscience in Technology and Biomedicine was held at Wayne State University (Detroit, Michigan) during 15–16 October 2007. Scientists and researchers gave advanced outstanding presentations and discussions in this conference regarding nanobiotechnology and nanomedicine research. The following is a summary of this conference.

From interfaces to nanoparticle—polyelectrolyte hybrid capsules and back, by Helmuth Möhwald, Max Planck Institute of Colloids and Interfaces

In recent years many efforts have been devoted to the construction of micro- and nanocapsules with defined and designed surfaces and walls. For this a sacrificial colloidal core was coated by alternating adsorption of oppositely charged polyelectrolytes, and after core removal microcapsules with nanodefined walls were obtained. Thus, one arrives at a promising drug delivery system with tailored walls and hence also controlled and switchable permeability and mechanics. One obstacle to arriving at well-defined properties has been that the nonequilibrium nature of the multilayer is responsible for the dependence of properties on the preparation history. This problem can now be circumvented, because in many of the standard multilayers used, glass transitions have been observed in convenient temperature ranges. Adding high salt concentrations can reduce these transition temperatures even below room temperature (25°C). During the glass transition the Young’s modulus changes reversibly by two orders of magnitude. The system is permeable for molecules in the melting state; in the glassy state the wall becomes impermeable, because prior heat treatment also causes defect annealing. Heat- and salt-induced capsule shape transitions wherein the diameter can change up to a factor of four are understood from an equilibrium between hydrophobic contributions to the surface energy favoring shrinking and electrostatic and osmotic energy contributions of the polymer brush tending to expand the surface. In the melting phase capsules can be induced to fuse, thus exchanging their content without leakage. Embedding infrared-absorbing metallic nanoparticles can locally melt them over areas below 50 nm diameter. This can be used for dye release without destruction and also can be remotely controlled within tissue. This process, which may be termed optoporation, provides various advantages over the well-known electroporation and can be as widely used.

Nanoparticles for delivery of drugs, peptides, and siRNA, by Mark Saltzman, Yale University

Drug-loaded nanoparticles, particularly those formed from degradable polymers, have several properties that will make them useful vehicles for drug delivery including high drug loading, efficient entry into cells, and capability for selective targeting. This presentation reviewed these properties, and engineering methods for achieving them with poly(D,L-lactide-co-glycolide) acid materials. The application of nanoparticles for cancer treatment, drug delivery to the brain, and delivery through epithelial cells was reviewed.

Microenvironments for regenerative medicine, by Lonnie Shea, Northwestern University

Tissue development and regeneration are complex processes that occur as a result of a dynamic interplay between the cells and their microenvironment. The
microenvironment contains signals, generally categorized as immobilized (e.g., extracellular matrix, ECM) or diffusible (e.g., growth factors), which orchestrate progenitor cell formation into functional tissues. We combine biomaterials, gene therapy, and drug delivery to control the cellular microenvironment, which is exemplified with ovarian follicle maturation and islet transplantation. A particular approach with potential for controlling the microenvironment involves the delivery of gene therapy vectors from tissue engineering scaffolds. Gene delivery, however, is often limited by the delivery efficiency. Biomaterials can enhance gene transfer relative to more traditional delivery methods (e.g., injection), and our laboratory has identified several design parameters that correlate scaffold and vector properties to the gene transfer. Scaffolds that control the presentation of signals within the cellular microenvironment provide a powerful tool to molecularly dissect tissue formation and to promote functional tissue formation.

New advances in nanomedicine: from bench to bedside, by Chiming Wei, Johns Hopkins University

Nanomedicine consists of medical diagnosis, monitoring, and applying treatment at the level of single molecules or molecular assemblies that provide structure, control, signals, homeostasis, and motility in living cells. It is a very important research direction to understand the cellular mechanisms in living cells and developing advanced technologies for early diagnosis and early treatment of various diseases—at the nano level. Nanomedicine research will exploit and build upon other research in nanotechnology, and apply it to studies of molecular systems in living cells, which contain a multitude of nanoscale structures such as membrane transporters, processes such as self-assembly of protein–nucleic acid complexes, and nanomachines such as molecular motors. Thus far, assessing single-molecule properties in living cells has been restricted by either the size of the probe or the photobleaching of the small fluorescent labels. Quantum dots (QDs) are nanometer-sized fluorescent probes suitable for advanced biological imaging. The unique fluorescent spectral characteristics and photostability of the QDs position them above traditional organic dyes for live cells, in time-resolved imaging applications. Nanotechnology will be very useful for the drug delivery and diagnostic approach. Magnetic nanotechnology is finding wide applications in medicine, most notably in magnetic resonance imaging and magnetic separation. The impedance biosensor is expected to find applications in monitoring cancer and in understanding neurological disease.

The plenary talks were followed by additional oral presentations focusing on one of the conference themes: supramolecular assemblies, nanoparticles and nanomaterials, biomaterials and tissue engineering, and translational nanomedicine.

The talks on supramolecular assemblies are summarized as follows.

Figure 1. Anisotropic shape change in specimen isolated from Canavalia gladiata.

Asymmetric Metal Complexes as Precursors for Responsive Films: Coordination Modes, Electronic and Amphiphilic Properties, by Cláudio N. Verani, Wayne State University. The first part discussed the incorporation of redox-active groups as asymmetric ligands to metal-containing amphiphiles for Langmuir-Blodgett films. The second part discussed the use of ligand design to achieve cyclable redox-driven switching mechanisms aiming at modular self-assembled films.

Forisomes—Natural Prototypes of a Proteinaceous Smart Material, by Winfried S. Peters, Indiana/Purdue University Fort Wayne, and Michael Knoblauch, Washington State University. Legumes possess protein bodies in their sieve tubes that act as reversible micro-valves; we have called these spindle-shaped structures forisomes (gate bodies) and characterized them in vivo and in vitro. Anisotropic contraction in forisomes is Ca^{2+}-mediated but ATP-independent; Figure 1 shows the response in a specimen isolated from Canavalia gladiata. Forisomes attract interest from the materials sciences, microfluidics, and nanotechnology, because they combine several desirable attributes of a proteinaceous microactuator and smart material.

Probing Supramolecular Assemblies with Atomic Force Microscopy, by Guangzhao Mao, Wayne State University. ATM was used to capture the release of DNA from its complexes with responsive polycations. Polycations containing disulfide bonds or temperature-responsive blocks were incorporated into the polycations so that DNA release
could be modulated by external stimuli (Figure 2). Stimuli-responsive polymers are promising components of drug and gene delivery systems.

Study of Cell-Substrate Interactions for Nanomedicine, by Xiangqun Zeng and Zhihong Shen, Oakland University; Mingchuan Huang and Peng G. Wang, Ohio State University. This presentation showed a few examples for study of cell-substrate interactions using real-time label-free sensor technology. Several new sensor recognition elements (i.e., recombinant antibodies, lectins, and carbohydrates) and their use as biosensing materials for fabricating new sensor structures with potential of high capabilities but limited size, weight, and power consumption by using label-free transducers (i.e., quartz crystal microbalance, surface plasmon resonance, and electrochemical impedance spectroscopy) were discussed.

Small-Amplitude Atomic Force Microscopy—Squeezing and Pulling Molecules, by Peter M. Hoffmann, George Matei, Mircea Pantea, Shah Khan, Venkatesh Subba-Rao, and Lindsay Runyan, Wayne State University. These workers introduced their novel AFM technique and presented startling results, ranging from molecular layering in liquid water to dynamically induced liquid-solid transitions in confined organic liquids. They also presented preliminary results of measurements on biological systems, in particular lipid bilayers and protein-protein interactions.

The talks on nanoparticles and nanomaterials are summarized as follows.

Nanoscale Multivalent Antitumor Agents, by Bob A. Howell, Central Michigan University. Treatment of a generation 4.5 (carboxylate surface) poly(amidoamine) (PAMAM) dendrimer with diaquo(1,2-diaminocyclohexene) platinum(II) generates a nanoparticle with 40 platinum moieties attached at the surface. This polymer-drug conjugate displays a very good release profile for the platinum species. Under physiological conditions about 80% of the platinum units are released over the first 24 hours.

Single-Molecule Semiconductor Nanoparticle-Based Biosensors, by Elvin Aleman, Pahan Godakumbura, De Gao, Roselyne N. Lichuma, Vivekanand S. Shete, Marinella G. Sandros, Rui Zhao, David E. Benson, and David Rueda, Wayne State University. In this work the stochastic nature of intermittent fluorescence, or “blinking”, of semiconductor nanoparticles was altered by the interaction of a receptor-attached [RuL(NH3)4](PF6)2 (L = 5-maleimido-1,10-phenanthroline) to the ZnS-coated CdSe (CdSe@ZnS) nanoparticle surface. As opposed to energy transfer–based biosensors, this electron transfer–based biosensor directly correlates single-biosensor “blinking” with ensemble biosensor measurements.

Nanoscale Photonics with Optical Plasmons, by Ivan Avrutsky, Wayne State University. Surface plasmon polaritons at optical frequencies provide strong light confinement at the sub-wavelength scale. The author’s group has shown that much stronger true-nanoscale confinement is possible with the gain-assisted resonant surface plasmon plaritons.

Nonlinear Rotational Frequency Shifts of Driven Magnetic Microspheres and Nanoparticles for Single Bacterial Cell Detection, by Brandon H. McNaughton, Rodney R. Agayan, Raoul Kopelman, and Roy Clarke, University of Michigan. Shifts in the nonlinear rotational frequency of magnetic particles offer a new and dynamic approach for the detection and monitoring of single cells. These authors have demonstrated this capability by
measuring the changes in the nonlinear rotational frequency of magnetic microspheres driven by an external magnetic field. Attachment of an Escherichia coli bacterium to the surface of a 2.0-μm magnetic microsphere affects the drag of the system, thus changing the nonlinear rotation rate (Figure 3).

Dendrimer-Based Nanodevice Platforms for Therapeutic and Imaging Applications, by R.M. Kannan, R. Inapagolla, K. Kotta, B. Rajaguru, Y.E. Turkoglu, R. Iezzi, S. Kannan, L. Matherly, A. Shields, and J. Whittum-Hudson, Wayne State University, Kresge Eye Institute, Children’s Hospital of Michigan, Karmenlos Cancer Institute. The results presented suggest that dendrimers could be excellent candidates for intracellular delivery and multimodal treatment. Specifically, (1) they are capable of transporting and delivering drugs into cells faster than free drugs, with superior therapeutic efficiency; (2) for chemotherapy drugs the conjugates are a factor of 6 to 20 times more effective than the free drug, even in drug-resistant cancer cell lines; (3) for ocular applications the dendrimer-based nanodevices show favorable ocular biodistribution and improved therapeutic efficacy.

The talks on biomaterials and tissue engineering are summarized as follows.

Nanoscale 3D Cues for Neural Cell Growth and Alignment, by Qian Chen, Yuan Fan, Virginia Ayres, and Lalita Udpa, Michigan State University; Roberto Delgado-Rivera, Sally Meiners, Ijaz Ahmed, and Suzan L. Harris, UMDNJ—Robert Wood Johnson Medical School. Recent work in these workers’ laboratories has demonstrated that a scaffold composed of electrospun polyamide nanofibers can mimic the geometry of extracellular matrix/basement membrane (ECM/BM) and that the surface of these matrices can be modified with specific peptides to provide chemical cues for neurite outgrowth and guidance. In this presentation they provided a high-resolution analysis of these modified nanofibrillar surfaces using scanning probe recognition microscopy (SPRM) (Figure 5).

Self-Assembled Monolayers Studies on Gold for Neural Cells Immobilization, by Olena Palyvoda, Erik McCullen, Hitesh Handa, Andrey N. Bordenyuk, Achani K. Yatawara, Alexander V. Benderskii, and Gregory W. Auner, Wayne State University. To assess the suitability of SAMs for biohybrid neuroprosthetic devices systems, correlations of neuron adhesion with the chemical structure at the SAM surface were studied for alkanethiol SAMs (Figure 4). Characterizations using fluorescent microscopy, sum frequency generation vibrational spectroscopy, x-ray photoelectron spectroscopy (XPS), and AFM were conducted. Cells adhesion was not critically affected by the surface roughness or details of the molecular ordering and orientation of the presented terminal groups but only the chemical functionality displayed at the surface.

Nanoscale Biofilm Characterization: Molecular Simulations of Protein/Polypeptide Adsorption on Biomaterial Surfaces, by A. al-Mekhnaqi, M. Mayeed, and G. Newaz, Wayne State University. A virtual-bond united-atom method has been considered to predict the structure of a protein in solutions and on surfaces using Monte Carlo with energy minimization technique. The model has demonstrated that on a graphite surface the random conformation of the original α-helix secondary structures of albumin subdomain has been denatured, which is consistent with the experimental and atomistic results. The talks on translational nanomedicine are summarized as follows.

The Role of Dendrimer-based Nanoconjugates in the Treatment of Retinal Degenerative Diseases, by Raymond Iezzi, Rangaramanujam Kannan, Bharath Raja Guru, Alex Kennedy, and Inna Glybina, Wayne State University. These authors have characterized neuroinflammatory features of age-related macular degeneration conditions in two transgenic rat models. To solve this problem they examined the biodistribution of PAMAM generation 4 dendrimers, conjugated to fluorescein isothiocyanate (D-FITC). Their data show that D-FITC conjugates distribute within the retina and provide sustained release. Distribution was primarily within the eye, with little crossover effect to the other eye after intravitreal administration.

Dendrimer-based Nanodevices for Treatment of Neuroinflammation in Cerebral Palsy, by S. Kannan, F. Saadani-Makki, Diane Chugani, Roberto Romero, K. Kotta, and R.M. Kannan, Wayne State University. The cellular uptake by microglial cells (BV2 mouse microglial cell line), of FITC-labeled PAMAM dendrimers
with different end functionalities was determined using flow cytometry. Based on these results, generation 4 hydroxyl-terminated PAMAM (PAMAM G4-OH) dendrimers were found to be most effectively taken up by microglial cells and were suitable for intracellular drug release.

**STARBURST and Priostar Dendrimers for Enhanced Drug Solubilization**, by Abhay Singh Chauhan, Sonke Svenson, Lori A. Reyna, and Donald A. Tomalia, Dendritic Nanotechnologies, Inc., Mount Pleasant, Michigan. Poly(amidoamine) STARBURST and Priostar dendrimers are core-shell nanostructures with precise architecture and low polydispersity, which are synthesized in a generation-by-generation fashion around a core unit, resulting in high level of control over size, branching points, and surface functionality. The anticancer drug cisplatin and the nonsteroidal anti-inflammatory drug indomethacin have been associated with STARBURST and Priostar dendrimers for enhanced water solubility (see Figure 6), and their association efficiency and release profiles have been studied.

**Nanotechnology Approaches to Genetic Manipulation of Chlamydiae**, by Judith A. Whittum-Hudson, Jayanth Panyam, and Alan P. Hudson, Wayne State University. These authors showed that in cultured human and other cells infected with C. trachomatis (K serovar), expression of genes encoding specific surface receptors is significantly increased over that of uninfected cells. They further showed that labeled nanoparticles that have ligands for those receptors on their surface track rapidly to chlamydiae within the inclusions in infected cells. This was the case with empty nanoparticles or nanoparticles containing an irrelevant plasmid.

**Formulations for the Noninvasive Delivery of Systemically Acting Drugs to and Through the Lungs**, by Libo Wu, P. Robson, S. Peguin, Parthiban Selvam, Balaji Bharatwaj, Mariam Al-Haydari, and Sandro R.P. da Rocha, Wayne State University. This group is interested in developing novel pressurized metered-dose inhaler (pMDI) formulations for the delivery of systemically acting drugs (small and biomolecules) to and through the lungs.

Computer simulations and microscopic experimental information from chemical force/colloidal probe microscopy, and small-angle scattering are used to understand solvation and structure in hydrofluoroalkane propellants.

**Developing Probes for Intracellular Glucose and Pb\(^{2+}\) Ion Concentrations**, by Baikuntha P. Aryal, Shelli Abbott, and David E. Benson, Wayne State University. These authors have developed maltose, glucose, Pb\(^{2+}\) ion, fatty acid, and thrombin biosensors that are based on electron transfer reactions with QDs. Furthermore, these reagents are unimolecular assemblies that allow reversible detection of small-molecule analytes. Fluorescence images revealed heterogeneous emission intensities from InGaP@ZnS nanoparticles, which is due to nanoparticle insolubility at moderate ionic strength. We have addressed this by altering the water-soluble capping group and developing a dual-wavelength emitting biosensor for sensor concentration-independent measurements.

Three graduate students, Hitesh Handa, Balaji Bharatwaj, and Lijun Chen, won the conference poster awards. Their posters are summarized as follows.

**Deposition and Aggregation of Aspirin on Lipid Bilayers**, by Hitesh Handa, Guangzhao Mao, Dongzhong Chen, Wenfei Dong, Dirk G. Kurth, and Helmut Möhwald, Wayne State University and Max Planck Institute of Colloids and Interfaces. The dimyristoylphosphatidylethanolamine
(DMPE) self-assembled nanopattern prevents aspirin crystallization. Instead of forming platelike crystals on bare graphite, aspirin deposits selectively along the hydrophobic core of the DMPE bilayer pattern, which results in a parallel rod overlayer of aspirin. The bilayer nanopattern can be used to control the nucleation and crystallization of aspirin and possibly other drug compounds.

**Toward Novel Inhalation Formulations for the Delivery of Therapeutic Molecules for Chlamydial Respiratory Infections**, by Balaji Bharatwaj, Arulselvi Anbalagan, Jayanth Panyam, Judith A. Whittum-Hudson, Alan P. Hudson, and Sandro R.P. da Rocha, Wayne State University. These authors have developed a dispersion-based pMDI formulation wherein biodegradable nanoparticles are entrapped in a water-soluble biodegradable co-polymer matrix. Their studies indicate that the nanoparticles released from the co-polymer matrix gain access to chlamydial inclusions in infected lung epithelial cells in vitro. These feasibility studies indicate that the proposed formulation can be used as a platform for the delivery of therapeutic molecules for treating chlamydia-related respiratory infections as well as other medically relevant pulmonary disorders.

**Engineering Functional Bile Ducts Using Glycosaminoglycan-Chitosan Nanocomposites**, by Lijun Chen, and Howard W.T. Matthew, Wayne State University. Cholangiocytes (bile duct epithelial cells) perform important biological functions in formation and transport of bile, as well as producing cytokines and chemokines. In this study the authors investigated cholangiocyte behavior on tubular scaffolds made of covalently linked, glycosaminoglycan (GAG)-chitosan nanocomposites. Results showed that cholangiocytes proliferated on and coated GAG-chitosan tubes with variable, GAG-dependent efficiency. The engineered bile duct retained transport functions and thus may be a useful adjunct in the design of an engineered liver system with functional bile ducts.