

EC-US Task Force on Biotechnology Research

**Nanobiotechnology
Workshop**

CONFERENCE PROCEEDINGS

Ispra-Italy 2008

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Acknowledgement:

the pictures and images used in this report are courtesy of JRC-Ispra, University of Twente and CEA.

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EUROPEAN COMMISSION

Workshop on
NANOBIOTECHNOLOGY

Ispra, ITALY

03-04 June 2008

Summary Record

Edited by

Garbiñe Guiu Etxeberria, Lawrence Goldberg, François Rossi

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Luxembourg: Office for Official Publications of the European Communities, 2009

ISBN 978-92-79-12014-5
ISSN 1018-5593-1831-2322

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P R E F A C E

This report summarizes the presentations and discussions of the EC-US Workshop on Nanobiotechnology which was held as a satellite event to the 18th Meeting of the EC-US Task Force on Biotechnology Research on June 3-4, 2008 in ISPRA, Italy. It built on the past nanobiotechnology workshop organised by the EC-US Task Force in 1997.

Since 1997, the field of nanotechnology in general and nanobiotechnology in particular has grown rapidly and has generated many new concepts and high value products in many sectors such as healthcare, environmental monitoring and bioremediation. Significant applications include biocompatibility of medical implants, nanocarriers for drug delivery (diabetes and cancer therapy), water filtration, biopolymers for heavy metal remediation, preventing biofouling, nanodiagnostic devices for early detection (food safety, water quality, environmental monitoring, etc.). The applications also embrace the fields of materials production and energy conversion.

The workshop reviewed developments and advances and identified bottlenecks to further progress in the field. To narrow down the workshop objective, this event did not address the topic of nanomedicine. The workshop brought together 23 scientists from several countries of the European Union and the United States.

The workshop was organized around four emerging areas of research and included discussion on related socio-economic challenges:

- Session 1. Nanobiomaterials and Biointerfaces
- Session 2. Biophysics and Microscopies
- Session 3. Nanopatterning and Self-Organization
- Session 4. Sensor Applications and Microfluidics

This report comprises the contributions made by the scientists present and includes recommendations from the discussion of each session and an executive summary.

The workshop was co-chaired by Dr. Harold Craighead, Director of the Nanobiotechnology Center at Cornell University (a National Science Foundation Science and Technology Center) and Dr. Harald Fuchs, Professor at the University of Muenster, Head of the Center for Nanotechnology (CeNTech). They also contributed to this report's executive summary. We would like to thank them for their outstanding efforts. The coordinators of the activity were Dr. Garbiñe Guiu Etxeberria (EC), Dr. Lawrence Goldberg (US) and Dr. François Rossi (EC).

Timothy Hall, Acting Director
European Commission

Kathie L. Olsen, Deputy Director
US National Science Foundation

The views expressed in this document are those of the workshop participants, and do not necessarily reflect the views of the sponsors or governments.

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EXECUTIVE SUMMARY

This report summarizes the presentations and discussions held at the European Commission – United States (EC-US) Workshop on Nanobiotechnology that took place in Ispra, Italy on 3 - 4 June 2008. The workshop built on a previous nanobiotechnology (NBT) workshop organized by the EC-US Task Force on Biotechnology Research in 1997. At that early stage the opportunity that NBT offered was recognized, but the field was still in its infancy. Since then the field of NBT has grown rapidly with experimental capability greatly expanded.

The current workshop assessed new research initiatives in the area of nanobiotechnology. The aim of the workshop was twofold: to identify strategic opportunities and needs of emerging areas of research, including socio-economic challenges, and to identify opportunities for enhanced EC-US collaboration and scientific exchange. It is expected that the workshop will assist in the definition of Research, Technology, and Development (RTD) programmes in the field of nanobiotechnology on both side of the Atlantic.

For this workshop Professors Harold Craighead and Harald Fuchs co-chaired the meeting and were jointly responsible for its scientific agenda. It is the active participants and contributors in the field who are in the best position to suggest the opportunities and what the agencies and governments can do to facilitate the realization of these opportunities. Twenty three active researchers from the European Union and the United States presented talks and perspectives for the future of NBT research and application in their areas of expertise.

Nanobiotechnology is a broad interdisciplinary field of knowledge and techniques. It exploits capabilities for material modification, manipulation, analysis and imaging at the nanometer scale applied to biomolecules and cells. The combination leads to possibilities for deeper insights into life processes and to devices such as sensors and diagnostic devices based on the combination of nanometer-scale structures and biologically derived components. The interface of both fields has the potential to provide innovative scientific and technical approaches to address existing and new applications.

The field of nanobiotechnology involves many disciplines, research methods, and areas of applications. It is inherently interdisciplinary and involves researchers with backgrounds in physical sciences, engineering, and life sciences. The workshop was structured in five sessions of related applications, scientific goals, or research approaches. The five areas were:

- Societal and ethical issues:
 - The debate on nanobiotechnology suffers from hype-disillusionment cycles that hinder sensible public discussion of real challenges and opportunities;
 - Knowledge of real opportunities and challenges will be crucial in making responsible use of the nanobiotechnology toolbox.
- Nanomaterials and the Biointerfaces:
 - This includes a range of experiments and methods to study how cells explore their environment;
 - Surface patterning and topographical modification are essential components of this area of work;
 - Applications include, for example, biocompatibility of medical implants and biofouling prevention.
- Biophysics and Microscopies:
 - This is a rich area for exploring the fundamentals of life processes at the molecular scale;
 - It utilizes combinations of analytical techniques as well as computational physics and modelling.

- Nanopatterning and Self-Organization:
 - These approaches for material modification and patterning are used across research topics;
 - Numerous converging techniques unite large area and small structure fabrication;
 - Applications include: materials production, energy conversion, water filtration, etc.
- Sensor Applications and Microfluidics:
 - The creation of functional devices involves integration of micro and nanosystems and the combination of inorganic devices with biological molecules and systems
 - Significant applications include diagnostics, food safety, water quality, environmental monitoring, etc.

The following sections of this report include the abstracts of the talks presented in each session, followed by a summary and recommendation for each topic.

Out of the talks and discussion there emerged an identification of an array of tools required for advancing nanobiotechnology. These are being developed for improving the capability to access and utilize biological systems at nanoscale dimensions. Each of these items represents an area of research in the method or tool development as well as an enabling technology for future research and applications. These needed methods and approaches spanning the areas of application and investigation include:

- Topographical and chemical patterning of surfaces
- Computational physics and modeling
- Stable recognition molecules: antibodies, aptamers, other new molecules for selective chemical binding
- High resolution to single molecule characterization and placement methods
- Integration of nanoscale systems for integration of fluids, optics, electronics, and mechanics
- Integration from micro to nanoscale devices and fabrication methods

It was also pointed out that expensive fabrication and analytical machines and capabilities may need to be accessed through shared facilities that may need to be formed or supported.

Some follow-up actions were identified that could be done relatively quickly to foster international collaboration and communication. These included:

- Web database of existing equipment and facilities. As outcome of this workshop a shared website on infrastructure and facilities has been set up at <http://nanoprobenetwork.org/>
- Videoconferencing of currently available seminars and lectures with the possibility for expansion
- Follow up workshops on selected focused topics
- International Training of Interdisciplinary Graduate Student and Postdocs in Nanobiotechnology (6-12 months)

Additional discussion was encouraged to develop funding mechanisms that would help to facilitate the combination of complimentary skills and interest of geographically distributed research groups.

The group of experts participating in the workshop was enthusiastic about the opportunities of nanobiotechnology for increasing our fundamental understanding of biological systems and for the creation of new valuable devices and systems. This will have impact on areas including medicine, environmental monitoring, water quality, food safety, and energy conversion.



PROGRAMME

EC-US Workshop on NANOBIOTECHNOLOGY

03 JUNE 2008

EC/US Introduction and welcome

Kathie L. Olsen, US Co-chair of the EC - US Task Force on Biotechnology Research
Lawrence S. Goldberg, NSF; **Garbiñe Guiu**, EC; **François Rossi**, EC

Workshop Chairs

Harald Fuchs, Center of Nanotechnology, University of Muenster, Germany
Harold Craighead, Nanobiotechnology Center, Cornell University

Setting the scene - Societal and ethical issues of Nanobiotechnology

US expert – David Berube, North Carolina State University
EC expert – Daan Schuurbijs, Delft University of Technology, The Netherlands

Session 1. Nanomaterials and Biointerfaces

US expert - Barbara Baird, Cornell University
EC expert - Michael Grunze, University of Heidelberg, Germany
US expert - Basil Swanson, Los Alamos National Laboratory
EC expert - Viola Vogel, Swiss Federal Institute of Technology
US expert - Jennifer Cha, IBM Corp.
EC expert – John F. Ryan, Oxford University, UK

Discussions session 1 conducted by the Workshop Chairs

Session 2. Biophysics and Microscopies

EC expert - Berenike Maier, Muenster University, Germany
US expert - David Nelson, Harvard University
EC expert - Eugen Georghiu, International Centre of Biodynamics, Romania
US expert - Robert Austin, Princeton University
EC expert - Suzi Jarvis, University College Dublin, Ireland

Discussions session 2 conducted by Workshop Chairs

Key note

Mauro Ferrari, The University of Texas Health Science Center

Thematic visit to the JRC-Ispra Nanobiotechnology labs

04 JUNE 2008

Session 3. Nanopatterning and Self-Organization

US expert - Dawn Bonnell, University of Pennsylvania
EC expert - Flemming Besenbacher, University of Aarhus, Denmark
US expert - Conrad James, Sandia National Laboratory
EC expert - Vinod Subramanian, University of Twente, The Netherlands
US expert - Steven Boxer, Stanford University

Discussions session 3 conducted by Workshop Chairs

Session 4. Sensor Applications and Microfluidics

US expert - Jongyoon Han, MIT
EC expert – François Rossi, JRC Ispra, Italy
US expert - David Walt, Tufts University
EC expert – Patrick Boisseau, CEA, France
US expert – Rashid Bashir, University of Illinois at Urbana-Champaign

Discussions, session 4 conducted by Workshop Chairs

General discussion and sum up conducted by Workshop Chairs

Closing

Lawrence S. Goldberg, NSF; Garbiñe Guiu, EC; François Rossi, EC
Line Matthiessen, EC Executive secretary of the EC-US Task Force on Biotechnology Research



SETTING THE SCENE

Societal and ethical issues of nanobiotechnology

Nanobiotechnology Societal Implications: Breaking the Carbon Barrier

David M. Berube
Department of Communication
Public Communication of Science and Technology Project
North Carolina State University

To the public, biotechnology remains an unknown. Nanotechnology is equally obtuse. Nanobiotechnology seems simply a made-up word. Today, nanobiotechnology is an interdisciplinary field of research integrating engineering and biology through the development of very small physical and biological devices using nano-fabrication techniques. Nanobiotechnology is beginning to generate substantial new insights into how biological systems function, and likewise, nanobiotechnology will lead to the design of entirely new classes of micro- and nanofabricated devices and systems. The use of microfabrication as a method of miniaturizing biological and biomedical devices is just beginning to reach the biotechnology industrial community.

Nanobiotechnology is a relatively young field, and academic projects far outnumber marketed products. According to Front Line Strategic Consulting, Inc., "The estimated market for nanotechnology-based life sciences products is expected to reach over \$3 billion (US) in 2008. New products coming out of development will continue to drive growth through the next five years."

Nanobiotechnology has been associated with concepts including biomimetics and self-organizing systems. Classical examples of such structures are proteinaceous assemblies such as molecular pumps, the strong spider silk proteins, carbohydrate assemblies including cell wall structures and lipid membranes and so forth. Nanobiotechnology is increasingly making hybrids between biological entities and non-biological nano-sized machines. Some postulate one day, due to random mutations, these hybrids can evolve to perform functions which they are not supposed to.

These animals are "replicating nanobots capable of living inside the human body powered by our own metabolic energy." According to blogger Paras Chopra, "If these come into existence then life as we know it will change. This is because our 100% knowledge of life is based on carbon-based life and then we would be dealing with life based on elements other than carbon."

This presentation will examine two applications of nanobiotechnology: environmental remediation and synthetic biology for human enhancement. Nanobiotechnology raises significant ethical concerns in its quest to engineer organisms and manufactured products containing both biological and human-made components.

The first class of applications involves environmental remediation. For example, we are studying the use of biopolymers for heavy metal remediation. Kostal, et al demonstrated the utility of recombinant DNA technologies to design recyclable protein-based nanomaterials with metal-binding domains. Grate, et al have conjectured on remediation strategies involving isolated stable enzymes able to survive in inhospitable environments. Their work with armored enzyme nano-bio-composites, combining a soft bio-organic enzyme core with an inorganic silicate-containing polymer network as the armor seems promising. Kouassi et al synthesized magnetic nanoparticles bound with cholesterol oxidase improving tolerance and storage stability and overall utility of the enzyme in remediation.

Net benefits would seem to favor remediation given the accidental releases of compounds into the environment including polychlorinated biphenyls, sodium cyanide, trichloroethylene, etc. The negative concerns involve release nanobio-engineered products into an open ecosystem and the harms hypothesized from indirect pathway analysis.

The next set of applications discussed involves synthetic biology for human enhancement. These applications link in turn to the powerful argument of dehumanization. Significant advances in biotechnology sustained and accelerated by parallel developments in nanoscience suggest some powerful visions of the future.

Synthetic biology has the potential to produce powerful positive drivers such as enhancement to develop a whole next generation of prosthetics for individual who are ably-challenged. The negative drivers are many of the same associated with unnecessary surgery, especially cosmetic surgery. Others suggest the creation of a new species of humanity with nano-biologically driven adjuncts. Conjecture about a Super Olympics with enhanced athletes as well as Super Babies built with outstanding characteristics to allow a child to dominate her peers have been advanced.

While applications in nanomedicine from nanobiotechnology should be positively embraced by the public given their intense fear of death, their response from cosmetic enhancements and life prolongation driven by nanobiotechnology might be very different. The dehumanization claim associated with advanced science and technology will be powerfully negative. The characteristics that make us human serve as the bases of religious belief systems, national identities and character, and self-identities. Issues of trans-humanism and post-humanism have populated popular culture rather than debates on science and technology, but that may change.

Finally, EC-US cooperation should include drafting a statement on the ethics of synthetic biology for remediation and human enhancement, tracking discoveries including proof of concepts as well as product lines. Finally, we need a legitimate record of events as well as sharing data sets associated with nanobiotechnology.

Promising Technologies - Responsible Use of the Nanobiotechnology Toolbox

D. Schuurbijs

Delft University of Technology, The Netherlands

It is hard to overstate the expectations that have surrounded the emergence of nanotechnology in recent years. It has been hailed as the next major technological revolution, comparable to electrification or the steam engine, providing unparalleled technological and social progress in almost any field imaginable. Despite such promises, or perhaps indeed because of them, others have greeted nanotechnology with great suspicion.

Whether or not they will prove as revolutionary as promised, nanobiotechnologies do seem to pose a new set of questions that warrant special attention: the effects of specific nanoparticles on human and environmental health, ethical issues related to cognitive or physical enhancement, or new regulatory challenges in the area of cosmetics, to name just a few. Apart from posing new questions, the expectations associated with nanotechnology in general have brought back to life more persistent ethical and social concerns. Issues of global equity, sustainability, privacy, and more generally the role of science in society have all found their way back into the debate. These broader issues collectively define the key social challenge for science policy: the responsible development of nanobiotechnologies.

Public opinion research in both Europe and the US on new technologies consistently reveals very similar public concerns about the technological production system¹: the public feels poorly informed and ambivalent about new technologies, wants increased oversight and participation and requires that systems be in place to ensure the accountability of government and industry. These data do not square with the promises made in the name of nanotechnology, that it will revolutionize production, healthcare and ICT for instance. A clear 'disconnect' is visible between what the technological production system

¹ See for example:

- the results of the Nanobio-RAISE Convergence Seminars organized by Sven Ove Hansson and Marion Godman of the Swedish Royal Institute of Technology: <http://files.nanobio-raise.org/Downloads/NBRWP3.pdf>
- the results of the UK Nanojury: <http://www.nanojury.org.uk>
- the results of the US National Citizens Technology Fora: <http://www4.ncsu.edu/~pwhmds/>



is intending for society, and what society perceives to be the intentions of that system. Addressing that disconnect should be the crucial task for the public debate on these developments.

European policy responses have emphasised increasing public knowledge and awareness.² But attention should also be paid to 'symptoms' of the public debate that foster further public concerns (and that reflect the dynamics of the GM-debate): hype, technical fix, narrow views of progress, and failure to take relevant ethical concerns seriously. The challenge is to 'open up' the debate, taking into account the relevant ethical concerns mentioned previously. Restoring public trust depends on critical reflection on existing policies and practices,³ on addressing social challenges as well as technological opportunities: not only to ask 'can we do it?', but above all 'should we do it?'. The recent debate on the toxicity of carbon nanotubes⁴ will serve as a test case for communicating uncertainties and addressing public concerns.

The debate on nanobiotechnology suffers from vicious hype-disillusionment cycles that hinder sensible public discussion of the real challenges and opportunities and, subsequently, evidence-based decision making. Knowledge of the real opportunities and challenges will be crucial in making responsible use of the nanobiotechnology toolbox.

EC-US relations in social research on nanotechnologies

The debate on benefits and risks of nanobiotechnology cannot be limited to any specific part of the world. Technology assessment should therefore keep a global perspective in mind. The EU and the US are of course two major players in this debate, and transatlantic cooperation could prove vital in effectively addressing the ethical and social issues of nanobiotechnologies. The 'real-time technology assessment' research programme of the Center for Nanotechnology in Society in the US for example offers an array of research, education and engagement programmes. Collaborative schemes between CNS and several national technology assessment programmes in Europe, for instance Nanoned in the Netherlands, could offer new fruitful insights in the international assessment of nanobiotechnologies, building on the findings of earlier European projects such as Nanobio-RAISE, Nanologue and Nanodialogue.

² See the European Commission's Action Plan for Nanotechnologies for example.

³ The recently established Code of Conduct for Responsible Nanosciences and Nanotechnologies Research (C(2008) 424 final) offers a step in this direction.

⁴ C.A. Poland et al: Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nature Nanotechnology*, published online: 20 May 2008.



SESSION 1

NANOMATERIALS AND BIOINTERFACES

Nanobiotechnology to Examine and Control Receptor-Mediated Cell Activation

Barbara Baird
Department of Chemistry and Chemical Biology
Cornell University

Research Focus: Nanobiotechnology and engineered materials offer new approaches for investigating spatially regulated properties of receptor-mediated cell activation. Although impressive progress has been made in identifying proteins and pathways involved in cellular signaling, very little is known about how these many components are organized in space to yield a specific response. Exemplifying these intricate systems is IgE receptors (Fc ϵ RI) on mast cells that serve gatekeepers for the allergic immune response. For stimulation, IgE receptors are clustered on the cell surface, typically by a multivalent ligand (antigen), causing their stable association with plasma membrane domains where they initiate transmembrane activation of signaling proteins and engagement of the cytoskeleton. The result is multiple cellular responses, including degranulation to release mediators of allergies and inflammation. My talk will describe our collaborative efforts that incorporate nanobiotechnology to examine the spatial orchestration of these events on the micro and nano length scales at which they occur. These approaches include construction of architecturally defined ligands, fabrication of patterned surfaces, and utilization of zero mode waveguides to capture molecular events.

R&D needs. Several research groups are developing microfabrication to investigate spatial regulation of cellular processes. However, these remain largely restricted to those who can collaborate easily with engineers and materials scientists. Consequently the much larger community of life scientists who are investigating a wide range of cells and cellular processes are not able to take advantage of new opportunities in nanobiotechnology. Increased collaborations between life scientists, physical scientists, and engineers are essential. This will lead to development of commercial products that are easy to use across the life science community without the requirement of direct collaboration. Ongoing and new collaborations will continue to advance technological approaches and commercial products that will extend the capabilities of biological examination and manipulation by tools on micro- and nanoscale. Unfortunately, the current funding climate in the US causes scientists and engineers to become more conservative and focus on core strengths of individual laboratories. There are fewer opportunities for risky collaborative ventures that may lead to substantial multidisciplinary rewards. Moreover, too much scientist/engineer time is taken writing multiple proposals for necessary core funding, rather than using this time and energy to develop collaborations among diverse scientific/engineering groups.

Technical bottlenecks and challenges. Patterned surfaces and other methodologies that probe cells on micron scale are useful for examination of dynamic subcellular structures that involve thousands of proteins or other biomacromolecules. For this purpose, fluorescence microscopy provides a powerful means for detection and analysis. However, many critical cellular events are initiated or proceed in a pathway by a small number of interacting molecules on the nanoscale. Although fabrication on the nanoscale is possible, investigations of molecular interactions are limited by optical resolution. New optical methods combined with nanofabrication can overcome this diffraction limit, and these must continue to be developed for easy and reliable use. Cryo-electron microscopy in conjunction with engineered environments offers new opportunities for nanoscale resolution of responsive cell structures. Current knowledge about cellular organization and processes on the nanoscale is also limited. This will be increased with the development of nanofabricated devices and must be integrated effectively with current cell biology information.



Opportunities for EC-US Cooperation. Effective cooperation will come from identifying the strengths of individual research groups that can synergize to address some of the issues described above. Strong matches among committed individuals will bring good ideas to fruition. Funds must be provided to support exploration that will lead to collaborative advances. A particularly exciting area of potential cooperation is cryo-electron microscopy of responsive cells manipulated by nanofabricated devices. This may also require development of genetically encoded probes.

Interaction of Marinefouling Organisms with Nano-structured Surfaces: Preventing Biofouling by Topography?

Michael Grunze
University of Heidelberg, Germany

Biofouling, the colonization of submerged man-made or natural surfaces by unwanted biological organisms, is a major problem for marine industries. Shipping and leisure vessels, membrane filters, heat exchangers, underwater sensors and aquaculture systems are all subject to its detrimental effects. It is estimated that the world fleet consume an additional 300 million tones of fuel annually as a result of hull fouling^a. The historical paradigm for controlling marine biofouling was to use biocidal products within coating systems to kill colonizing organisms. With the legal implementation of the International Maritime Organization Treaty^b on biocides in 2008 the use of components such as tributyltin (TBT) will be increasingly restricted. Developing environmentally benign fouling-resistant products to fill the gap in the market requires a greater understanding of how the physical and chemical characteristics of a surface influence its tendency to foul. The dimensions of fouling organisms during their settling stages (cells, spores or larvae) are typically 1-100 μ m, but the recognition of surface cues through the relevant sensory structures probably occurs at much smaller length scales (μ m, nm or even the molecular level).

The design of non-toxic and environmentally benign anti-fouling or fouling-release coatings is a highly complex task and requires a systematic approach. Within the Framework 6 EU Integrated Program "AMBIO" we study model surfaces to isolate the influence of a single surface property on the fouling behavior. Selective variation of specific and combined surface properties, whilst keeping the others constant, is a powerful tool to identify cues responsible for biofilm formation, settlement and fouling-release.

As discussed in my talk, a combination of surface topography in the nanometer to micrometer size range, combined with selective chemistry can effectively reduce fouling by marine test organisms. Tailoring of the surface morphology can be achieved by self-assembly, nanolithography, photolithography and micro-contact. Surface roughness and aspect ratio can be varied as well as the order parameter. While certain self-assembled structures reveal low order parameters often on different length scales (e.g. hierarchically organized polyelectrolyte surfaces), photolithography, chemical lithography and micro-contact printing are suited to precisely tune structures and produce regular patterns of well defined shape at the nano- to micrometer length scale. The structure sizes prepared and evaluated within AMBIO for their fouling resistance range from two-dimensional molecular nanostructures created by chemical nanolithography to three-dimensional hydrogel microstructures with high aspect ratios.

Standard analytical methods are routinely utilized to enhance interpretation of the biological assays. Of particular importance are methods for under-water characterization. Moreover, an in-depth structural characterization of materials includes the use of large-scale facilities such as synchrotron and neutron sources.

The anti-fouling and fouling-release properties of both 'model' and 'practical' surfaces within the AMBIO project are characterized with respect to a range of fouling organisms, which must represent the major fouling-groups with which surfaces would be challenged in the natural environment, namely microfoulers or 'slime' (comprising bacteria and microscopic unicellular algae), soft macrofoulers (e.g. macroalgae, anemones and hydroids) and hard macrofoulers (e.g. barnacles, mussels and tubeworms). Microfouling organisms adhere to surfaces by the production of extracellular polymeric substances (EPS), which

are composed of polysaccharides and proteoglycans. The macrofouling alga, *Ulvalinza*, is an intertidal species and the most common ship-fouling alga^c. It colonizes surfaces by means of quadriflagellate motile zoospores, which actively select a settlement site and adhere by secreting a preformed glycoprotein adhesive. The anti-fouling properties of surfaces for hard macrofoulers are characterized using the settlement stage of the tropical barnacle, *Balanus amphitrite*. Cyprid larvae explore a surface on specialized antennular appendages prior to selecting a settlement site and secreting proteinaceous permanent cement.

In addition to conventional laboratory assays which result in “static” information (i.e. degree of settlement, adhesion and/or percentage removal), the study of settlement dynamics is used to reveal subtle variations in how colonizing organisms sense and respond to different surfaces. The exploration of surfaces by barnacle cyprids can be visualized and analyzed by microscopic tracking experiments^d. Small changes in surface properties have been found to critically influence the observed motion patterns. Zoospores of *Ulva* show highly complex, three-dimensional exploration behavior. To record these patterns, digital in-line holography^{e,f} is used. From the holographic scattering data, the motion patterns of marine organisms can be extracted. Furthermore, three dimensional motion data and the behavior in the vicinity of surfaces can be analyzed and quantified^g. The three-dimensional nature of the traces obtained yields quantifiable information on the behavior of colonizing spores, offering a deeper insight into possible sensing mechanisms.

All aspects of the research to prevent or reduce biofouling by environmentally benign surface structures and chemistries research would greatly benefit from a close collaboration with US scientists and institutions. Collaborations would be highly complementary in many aspects, not only in surface functionalization and structuring, but also in biological assays considering the difference in the marine environments. To rationalize the exploration and settlement behavior of spores and different strains of bacteria, detailed information on their extracellular matrix and adhesives is needed, and ultimately a genomic map which allows correlating adhesion ability with the species genome. Specific examples for opportunities in EC-US research cooperation will be given in my presentation.

Acknowledgements: The support for the AMBIO research program by the 6th EU framework program is thankfully acknowledged. Without the enthusiasm, engagement and leadership by Prof. J. Callow and Dr. M. Callow this program would not have happened. This abstract is based on an AMBIO program description by A. Rosenhahn, T. Ederth, M. Pettitt published in *Biointerphases*3, IR1 (2008) (<http://www.biointerphases.org/>)

ⁱ J.J. Corbett and W.H. Koehler, *Journal of Geophysical Research* 2003, 108, 4650.

ⁱⁱ International Maritime Organisation Treaty (2001)

ⁱⁱⁱ ,Biological Adhesives’ Smith A. M.; Callow J. A. (Eds), Springer-Verlag Berlin Heidelberg (2006) pp 63-78

^{iv} J.-P. Marechal, C. Hellio, M. Sebire, A.S. Clare, *Biofouling* 2004, 20, 211-217

^v W. Xu, M.G. Jericho, I.A. Meinertzhagen, H.J. Kreuzer, *Proc. Natl. Acad. Sci. U.S.A.* 2001, 98, 11301

^{vi} Xu, M.H. Jericho, H.J. Kreuzer and I.A. Meinertzhagen, *Opt. Lett.* 2003, 28, 164

^{vii} M. Heydt, A. Rosenhahn, M. Grunze, M. Pettitt, M. Callow, J. Callow, *The Journal of Adhesion* 2007, 83, 417

Biological Sensors Based on Biomimetic Approaches and NanoBio Materials

Basil Swanson
Los Alamos National Laboratory

The autonomous detection of pathogens in the environment remains an unmet grand challenge. Current fielded approaches including the Joint Biological Point Detection System (JBPDS), aimed at troop protection, and Biowatch, the Department of Homeland Security’s (DHS) system for early warning of pathogen release, are both based on conventional off-the-shelf technologies (COTs) and are inadequate. Both Biowatch and JBPDS are little more than sample collectors where confirmation of a pathogen release is performed at off-site laboratories using PCR. The high operating cost of both systems and



the time delay between sample collection and laboratory confirmation (at least 24 hours) do not provide actionable data to drive decisions. What is needed is an autonomous detection system that operates continuously and that can provide strain-specific identification of pathogens in the field. There are many challenges in the development of such an autonomous sensor system (reagent-free transduction to minimize the reagent stream, low false positives and negatives, etc.) and traditional approaches that rely on COTs are not adequate to the challenge.

The Los Alamos Optical Biosensor Team is developing a toolbox of technologies based on nanobio materials that can be used to address this challenge. The keys to autonomous sensing are robust yet specific recognition ligands, sensing surfaces that minimize nonspecific binding and yet are robust enough to be reused, and signal transduction approaches that are reagent free and yet highly specific. Many current reagent free approaches (e.g., surface acoustic wave, surface plasmon resonance, microcantilever) sense anything that interacts with the surface, giving rise to high background signals from nonspecific binding. Recently developed approaches that rely on, for example, protein conformational changes to trigger transduction show more promise. What is needed is a transduction approach where a specific molecular recognition event is the only thing that triggers a signal. We believe that biomimetic approaches that couple recognition with amplified signal transduction, much like cell signaling and mammalian olfaction in nature, and the use of nanobio materials together provide a potential solution to this problem

The heart of our approach is to use molecular recognition to trigger signal transduction at the surface of a single mode planar optical waveguide. We utilize the exponential decay of the evanescent optical field and the modulation of fluorescence from a quantum dot optical reporter that is moving in and out of this evanescent field as the signal. Our preliminary results are based on the modulation of a quantum dot linked to the waveguide surface through ssDNA and the change that occurs upon hybridization. Our goal, however, is to imbed the quantum dot reporter inside a nanoscale chaperonin protein that also serves as the scaffold for attaching recognition ligands. A binding event between the target pathogen (or pathogenic marker) and the ligands that decorate the chaperonin molecule perturbs the modulation of the fluorescence signal that can be recorded sensitively using lock-in techniques. The engineered chaperonin molecule is ideal for this application: 1) the HBLL mutant engineered to have His tags forms stable one-to-one conjugates with small (~ 3 nm) quantum dots where the QD is imbedded inside the chaperonin, 2) the chaperonin-QD conjugate is monodispersed and, therefore, gives a sharp frequency dependence of fluorescence modulation, and 3) multiple ligands can readily be attached to the outer surface of the chaperonin to ensure high affinities and specificities. This ligand-chaperonin-QD construct is conjugated to the surface of the optical waveguide using an intervening self-assembled monolayer that has been designed to minimize nonspecific binding while also providing a robust sensing surface. The preparation and characterization of the ligand-chaperonin-QD conjugate and the self-assembled monolayer sensing films will be discussed along with sensing results using acoustic fields to modulate the nanobio conjugate at a waveguide surface. Progress towards robust ligands and sensing films will also be discussed.

The fact that nature has already solved the problem of coupling highly specific recognition to amplified signal transduction inspires the use of biomimetic approaches to sensing. The problem is that although nature can heal complex molecular machinery when critical "soft" materials (proteins etc.) degrade, man cannot yet do so. Accordingly, there are many areas of potential collaboration between the EU and the US including the development of self-replicating and self-healing materials. Moreover, signaling cascades in nature typically involve the formation of complex architectures that trigger *multiple* amplification stages whereas man's attempts are generally limited to a single amplification stage. Collaborative efforts that target *amplified signal transduction* are, therefore, at the heart of solving many problems in the development of biological sensors whether for environmental detection of pathogens or for medical diagnostics. Although the focus here has been on sensing, the same central problem limits man's ability to, for example, make advances in artificial photosynthesis and the development of advanced photovoltaics where every photon counts (e.g., multicarrier generation in nanomaterials).

Deciphering the Hidden Lives of Stretched Proteins

Viola Vogel

Swiss Federal Institute of Technology, Switzerland

Ample evidence exists that cells and tissues sensitively respond to mechanical stimuli. But how can cells sense and translate a broad range of mechanical forces into distinct sets of biochemical signals that ultimately regulate cellular processes, including adhesion, proliferation, differentiation, and apoptosis? For cell survival, most eukaryotic cells need to be mechanically anchored to their environments. This is done by transmembrane proteins that couple mechanically the extracellular matrix to the interior contractile cytoskeleton. The cellular nanomachinery is thus both subjected to exogenously applied forces, and to cell generated forces that the cells apply to their local extracellular matrix and to neighboring cells.

The advent of nanotech tools, particularly atomic force microscopy and optical tweezers (Kellermayer et al. 1997; Rief et al. 1997; Tskhovrebova et al. 1997), were a major milestone in recognizing the unique mechanical properties of proteins. These first force measurements on single multimodular proteins were performed on titin and revealed that the modules cannot be deformed continuously but that they rupture sequentially. But do cells take advantage of switching protein function mechanically? The first functional significance of protein unfolding upon rapid tissue extension, for example when overstretching a muscle, was seen in them serving as mechanical shock absorbers. Beyond the muscle, do proteins unfold in other force bearing junctions? After a decade of new insights into single molecule mechanics, a new field is emerging: how can force-induced mechanical unfolding of proteins and of other biomolecules switch their structure-function relationship? What might be the physiological significance of the existence of force-stabilized unfolding intermediates (for reviews see (Bershadsky et al. 2003; Bustamante et al. 2004; Discher et al. 2005; Gao et al. 2006; Giannone and Sheetz 2006; Orr et al. 2006; Sawada et al. 2006; Vogel 2006; Vogel and Sheetz 2006; Johnson et al. 2007; Smith et al. 2007))? Tensile force can thereby stabilize proteins in otherwise short-lived structural intermediates. It becomes increasingly important to decipher how the structure/function relationship of proteins is altered by mechanical forces. Since cell-generated forces are sufficient to stretch and unfold proteins, mechanical stretch may be one important motif by which mechanical factors could be translated into biochemical signal changes in a variety of tissues and cell types. Many proteins are involved in force-bearing networks that connect the cell interior with the exterior and they all are potential candidate proteins for mechanosensors. First molecular design principles are also emerging by which mechano-sensory elements are integrated into structural motifs of various proteins whose conformations can be switched by force (as reviewed by (Khan and Sheetz 1997; Silver and Siperko 2003; Bustamante 2004; Martinac 2004; Kung 2005; Shemesh et al. 2005; Sawada et al. 2006; Thomas et al. 2006; Vogel 2006; Johnson et al. 2007; Hytonen and Vogel 2008)).

As cells apply tensile forces to adhesion sites, proteins that are part of such force-bearing networks get stretched out of equilibrium and might display a changed structure-function relationship. How can stretched proteins be identified and imaged in cell culture? How can we decipher the underlying mechanisms how mechanical forces regulate cell and tissue function? Novel tools are needed to identify how force is translated at the molecular level into biochemical signal changes, and ultimately alters signaling pathways. Learning at the nanoscale how some of the biological mechano-chemical switches work is prone to open totally new avenues in biotechnology, systems biology, pharmaceuticals and medicine.

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Advances in Lithography: Opportunities for Bio-Nano Research at US and EU IBM Centers

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As nanoelectronic device features shrink towards a critical limit, new research directions have been sought to resolve the resultant technological issues in a cost-effective manner. Within the electronic industry, extensive research has focused on the development and implementation of new lithographic approaches to fabricate increasingly smaller feature sizes with high yields and minimal defects. A wealth of different lithographic techniques, including immersion, nanoimprint, extreme UV, and electron-beam, have provided ways to manufacture wafer-level, parallel arrays of nanoscale features and architectures. Recently at

IBM, state-of-the art immersion lithography tools allowed the fabrication of sub-30nm lines and spaces.

The techniques that have been developed for future chip manufacturing also enable new opportunities in the bio-nano research space. By merging biological materials with lithographically prepared substrates, these patterned biomaterials can be used to address particular questions and challenges in research areas ranging from medicine to electronics. In this talk, I will show research occurring at two IBM research sites, Almaden (San Jose) and Zurich that uses lithographically generated substrates to template biomolecules to generate wafer-level parallel nanoscale assemblies of proteins and DNA. At IBM Zurich, facile routes to generate complex protein architectures with sub-100nm resolution are being developed to either gain insight into biological phenomena or as materials for biosensors. At IBM Almaden, a variety of substrates generated by optical and e-beam lithography are being used to produce highly parallel arrays of mesoscale DNA scaffolds in a single step. These DNA templates encode multiple nanometer recognition sites that can be further used to generate hierarchical assemblies of both organic and inorganic nanoscale materials. Because a significant challenge of future electronics is the ability to address sub-20nm features, these self-assembled DNA and/or DNA-peptide structures are being explored as potential templates for the assembly and wiring of nanoscale materials for both logic and memory.

Real-time Imaging of Protein Dynamics*

John Ryan
Oxford University, UK

In this talk I will describe recent progress in applying atomic force microscopy (AFM) to investigate the properties of membrane proteins. While scanning probe microscopy has been used successfully to image proteins, the outstanding challenge is to measure their structure and function in a physiological environment. Proteins are in many respects the discrete devices of biological systems: for example, they are responsible for sensory signal input, transduction and processing, and they control and regulate nanoscale mechanical motion. To understand how they function requires a molecular-level description of their dynamics in response to stimulation. Experimentally this requires high spatial resolution (few Å) and high temporal resolution (typically ~ few ms) measurements of functional protein contained in an environment which is as close to physiological as possible; ultimately *in vivo* measurements will be required. I will focus on the following model systems:

(i) Glutamate neuroreceptors - e.g. α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid or AMPA receptors, which are involved in memory and learning.

Glutamate receptors are ligand-gated ion channels that control the flux of K^+ , Na^+ and Ca^{2+} across the post-synaptic membrane. AMPA receptors contain four structural subunits (GluR1-4) which determine its function. For example, Ca^{2+} permeation is greatly reduced when a GluR2 subunit is present which can prevent neuronal damage arising from high stimulation. On the other hand, GluR1-containing AMPA receptors appear to mediate the synaptic strengthening implicated in associative (i.e. content-addressed) memory formation. Unlike logic devices in conventional (digital electronic) information processors, these receptors are *adaptive*: i.e. their *density* and *function*, and thus the *synaptic efficacy*, change in response to input signals. The receptor density is enhanced by “trafficking”, a process which transports receptors from remoter regions of the post-synaptic membrane into the synapse itself.

The nature of the conformational change occurring in AMPA receptors when it binds glutamate neurotransmitter is not known: indeed the full protein structure is not known. To begin with we have explored the role of lipid interactions on receptor function using membranes containing lipid mixtures that approximate the composition of the postsynaptic membrane. The results show a preferential reconstitution into micro-domains or rafts of a well defined thickness (most likely driven by hydrophobic matching of the trans-membrane section of the receptor), which supports the conjecture that lipid rafts in the synapse might act as signalling platforms. Electrical measurements of ion conductance through receptors show that the reconstituted protein remains functional under these conditions, and the data reveal clear inward rectifying behaviour and channel blocking when antagonist molecules bind. High resolution AFM measurements show quite clearly the four sub-domain structure of the receptor. Furthermore, measurements have been made before and after photolysis of caged



glutamate: this process releases neurotransmitter which subsequently binds to and activates the receptor. AFM images provide direct evidence for opening of the ion channel after glutamate binding: a pore forms with a width of ~ 1nm. It should be stressed that these data correspond to the receptor being in the so-called de-sensitized state that occurs a few milliseconds after activation: the channel is no longer able to permeate ions even although a large conformational change persists. The origin of this behaviour is not understood at the present time.

(ii) Photoreceptors – e.g. rhodopsin, which is the primary visual photoreceptor, and its bacterial analogue bacteriorhodopsin (bR), which is a light-activated proton pump.

One of the most important advances in this field has been the recent development of AFM techniques that permit simultaneous high spatial resolution and high temporal resolution in a liquid environment, and so allow for the first time real-time sub-molecular imaging of receptor dynamics. In the case of bR proton transport across the membrane is driven by photo-induced conformational changes of the protein structure on a 10-20ms timescale. The photon energy is used to pump protons from the cytoplasmic region to the extracellular region: protons then re-enters the cell via ATPase which synthesises ATP, the currency of all metabolic energy. Using high speed atomic force microscopy we have directly imaged bR dynamics at the level of individual α -helices on a ~5 ms timescale. Furthermore, we have made quantitative, time-dependent measurements of individual bR protein size variations, re-orientation and lattice relaxation. Inter-molecular effects such as the influence of bR displacements on neighbouring proteins and global membrane dynamics during the photocycle were also measured, providing a unique insight into the correlation between global membrane fluctuations, cooperative effects and single protein activity. We find that thermal fluctuations and structural deformability play crucial roles in the structural dynamics and that the light-activated conformational change is substantially greater than that inferred from crystallography data. The resulting lateral strain induced in the membrane gives rise to distinct cooperative protein dynamics which imposes constraints on the optical energy transduction.

*Work done in collaboration with:

(i) C. Ramanujan (Oxford), N. Kasai (NTT) and K. Torimitsu (NTT); (ii) K. Voitchovsky (Oxford), S. Contera (Oxford), T. Ando (Kanazawa), T. Uchihashi (Kanazawa) and H. Yamashita (Kanazawa).

For discussion: challenges & opportunities

The potential of nanoscale probes for elucidating molecular processes underlying protein function is considerable, but the experimental challenges are severe :

- availability of high speed scanning probe microscopy;
- control and manipulation of proteins in controlled, physiologically-relevant environments, i.e. beyond simple supported bilayer geometries;
- probing single membrane proteins in live cells; and
- understanding the behaviour of the wider network of interacting biomolecules is required: in other words, an integrative approach to understanding biological systems must be pursued.

Opportunities for EC-US collaboration are diverse:

- access to specialised facilities and infrastructure;
- complementary expertise; and
- joint funding initiatives.

The biggest bottleneck to progress is often an inflexible and unresponsive funding environment. Support mechanisms should include:

- travel for students and post docs for short research visits;
- extended visits for post docs and faculty; and
- fully-funded joint projects to address new objectives.

Conclusions & Recommendations

Rapporteurs: Barbara Baird, Cornell University

Michael Grunze, University of Heidelberg, Germany

State of the Art

The biology-materials interface is critical for probing molecular interactions that drive cell function, for developing diagnostic and implantable devices and for controlling biological growth on surfaces. This session discussed progress being made in interfacing nanostructured surfaces with membranes and cells. Current aims include controlling receptor mediated cell activation, influencing cell adhesion by topography, and quantifying of the role of mechanical forces. Some presentations focused on environmental pathogen detection, advances in lithography, and real time protein dynamic observation by AFM. In all talks current limitations and future prospects were considered

Highlights of talks

Barbara Baird ("Nanobiotechnology to Examine and Control Receptor-Mediated Cell Activation") described how it is possible to examine spatial regulation of receptors by engineering the stimulus. For example, architecturally defined ligands can be used to examine structural constraints in early signalling mechanisms. Patterned surfaces can be used to investigate spatial regulation in the plasma membrane and targeting to the membrane of signalling components or secretory vesicles. Nanofabricated surfaces can be coupled with optical methods to overcome the diffraction limit of light and observe individual molecular interactions in living cells. Scientific and technical challenges include limited biological information about molecular interactions within cells as well as limitations of fluorescence and other biophysical methods to measure temporal-spatial regulation on the nanoscale. Further research developments will include highly tunable, engineered environments to probe cellular activities specifically on micro- and nano-scales. Nanofabrication, together with chemistry and biological building materials, can create new classes of defined soluble stimulants and chemically/physically defined surfaces. Diverse new methods must be developed to measure molecular dynamics, interactions, and assemblies within living cell in engineered environment

Michael Grunze ("Interaction of Marine Fouling Organisms with Nano-structured Surfaces: Preventing Biofouling by Topography?") described how a combination of surface topography in the nanometer to micrometer size range, combined with selective chemistry can effectively reduce fouling by marine organisms. Tailoring of the surface morphology can be achieved by self-assembly, nanolithography, photolithography, or micro-contact printing. Surface roughness and aspect ratio can be varied as well as the order parameter. Scientific and technical challenges include routine availability of defined large area nano- and micro-topographies and manufacturing of industrial coatings with defined topographies. On the biology side, there is little known about exploration and settlement strategies of fouling organisms or biochemistry of adhesion. There are limited capacities for biological testing. Further research is needed to develop routine procedures for producing hierarchical topographic surfaces and to establish adequate tests of surface topographies in marine environments. Successful model surfaces must then be translated into products.

Basil Swanson ("Biological Sensors Based on Biomimetic Approaches and NanoBio Materials") described a novel transduction approach where a specific molecular recognition event is the only trigger for a signal: molecular recognition triggers signal transduction at the surface of a single mode planar optical waveguide. This device utilizes the exponential decay of the evanescent optical field, and the modulation of fluorescence from a quantum dot optical reporter that moves in and out of this evanescent field provides the signal. General technical challenges for these types of biosensors include highly specific, *amplified* transduction that requires no added reagents and novel recognition ligands that are robust and specific. Sample handling offers other challenges including stable (reusable?) sensing films and reporters, front-end separation/preconcentration of samples with complex backgrounds, elimination of cross-contamination. Effective biosensors will have all components integrated, be miniaturized, and



require low power.

Viola Vogel (“Deciphering the Hidden Lives of Stretched Proteins”) described how cells and tissues sensitively respond to mechanical stimuli, and addressed the underlying question: How can cells sense and translate a broad range of mechanical forces into distinct sets of biochemical signals that ultimately regulate cellular processes, including adhesion, proliferation, differentiation, and apoptosis? Fluorescence measurements show that cells (via transmembrane proteins) couple mechanically the extracellular matrix to the interior contractile cytoskeleton. The focus of the research is to investigate how proteins respond to mechanical stress and how their function changes, such that the cellular nanomachinery subjected to exogenously applied forces can, in turn, apply forces to local extracellular matrix and to neighboring cells. Scientific and technical challenges include development of hybrid systems to exploit biological nanosystems for their engineering principles. Success in this area requires systems based approaches to biology -- to examine physical/engineering aspects of cell function.

Jennifer Cha (“Advances in Lithography: Opportunities for Bio-Nano Research at US and EU IBM Centers”) described state-of-the-art immersion lithography tools for fabrication of sub-30 nm lines. IBM-Zurich is generating complex protein architectures with sub-100 nm resolution. IBM-Almaden is producing highly parallel arrays of mesoscale DNA scaffolds on substrates generated by optical and e-beam lithography. A major technical challenge is optical limits for characterization at the nanoscale (with high throughput). This is highly problematic when characterizing organic (bio) samples, and it is particularly difficult to obtain information at the nanoscale of biological activity. The complexity of patterned biomolecules makes it difficult to obtain accurate numbers and control the orientation of molecules on surfaces. Another limitation is that nanofabrication of parallel assemblies of arbitrary design and feature sizes is expensive and not accessible to everyone. Progress could be improved with better communication between semiconductor device engineers, physicists, materials scientists, and biologists – this is necessary to move from single type demonstrations to production.

John Ryan (“Real-time imaging of protein dynamics”) described recent progress applying atomic force microscopy (AFM) to investigate the properties of membrane proteins. Although scanning probe microscopy is used successfully to image proteins, the outstanding challenge is to measure their dynamic structure and resulting response to stimulation in a physiological environment. A molecular-level description requires high spatial resolution (a few Å) and high temporal resolution (typically ~ a few ms) measurements of functional protein contained in a physiologically relevant environment; ultimately *in vivo* measurements will be required. Technical and scientific challenges in this research area include availability of high speed scanning probe microscopy and manipulation of proteins in physiologically-relevant environments, i.e., beyond simple supported bilayer geometries. A goal is probing single membrane proteins in live cells. Ultimately this approach can help gain a new understanding of the behavior of the wider network of interacting biomolecules. To this end an integrative approach to elucidating complicated biological systems must be pursued.

Challenges and Research Needs

General research and development needs include availability of highly tunable, engineered environments to probe cellular activities specifically on micro- and nano-scales. Versatile methods must be developed to measure molecular dynamics, interactions, and assemblies within cell in engineered environments. We must overcome current limitations in imaging and biophysical measurements. To apply technical advances to address biological systems we must increase the access of micro- and nanotechnology approaches to larger community of life scientists. For example, it may be possible to standardize sophisticated platforms and provide these in established devices as “kits.” Effective implementation of technology into requires facilitated collaborations among life scientists, physical scientists, and engineers. Successful model systems must be developed into practical applications and products.

EC-US Collaboration Opportunities

Opportunities for EC-US cooperation can come from strong collaborative matches between laboratories committed to common goals that serve the needs of research areas described above. This new level of technology-based science will require specialized facilities, for example: Cryo-electron microscopy/

tomography of supermolecular assemblies and cells; ultra high resolution fluorescence microscopy (STED, PALM, STORM); other methods for imaging biological samples (e.g., synchrotron methods). We need to develop cooperations to characterize and/or manipulate biological materials at the nanoscale with high throughput. To facilitate this effort we should identify strengths within both EC and US in *both* industrial and academic settings.

The biggest bottleneck in international collaborations to progress is often an inflexible and unresponsive funding environment. Support mechanisms and supported efforts at all levels should include: support for two years postdoctoral fellows working jointly with EU/US laboratories collaborating on high profile nanobiotechnology projects; support travel for students and post docs for short research visits; support extended visits for postdoctoral fellows and faculty. Joint, international projects should be fully funded to address new objectives.

To maximize use of expensive equipment needs, we should inventory, prioritize, and support powerful instrumental approaches that promise to be most valuable in nanobiotechnology characterization (e.g., AFM, scanning mass spectrometry, electron tomography). We need to create user friendly facilities that will serve the broader communities, and establish a list of instruments of participating groups that are available for collaboration. At the same time we should support development of theory and computation methods that serve and connect the broad nanobiotechnology community (life scientists, physical scientists, and engineers). Broad training in these methods should be accessible and affordable.

The biological community should prioritize the most valuable instrumental approaches for development and use to characterize biological systems on nano- and microscales. New nanobiology discovered through new nanotools should then be communicated effectively to the broader biological communities (e.g., by publishing in prominent biological journals and presented in biological and biomedical meetings).

To be successful we will need to overcome different funding philosophies and review criteria in the US and the EU. Both researchers and funding agencies will need to avoid "Hype Cycles": Support should be provided and research carried out with a realistic, long term view.



SESSION 2 BIOPHYSICS AND MICROSCOPIES

Controlling Molecular Motors in Living Cells

Berenike Maier
University of Münster, Germany

Biological molecular motors are the basic elements that generate directed movement in living cells. Nanotechnological tools enable the characterization of the physical output of individual molecular motors such as step length, force generation, energy transduction, and directional switching. In particular, the step length of molecular motors has been measured with Angstrom *in vitro* using laser tweezers. However, the application of these tools to characterize the physical output and the regulation of molecular motors in the context of their natural environment has been limited.

Our research group addresses the question how the physical output of molecular motors is controlled by living cells. We are particularly interested in bacterial nanomotors called type 4 pili. Pili are major virulence factors of various bacterial species. A set of about 20 pilus proteins can support various different functions including adhesion to biotic and abiotic surfaces, DNA transport through bacterial cell envelopes, electron transport, surface motility, and force generation. In particular, we have shown that type 4 pili of the human pathogen *Neisseria gonorrhoeae* are remarkably strong molecular motors that generate mechanical force in the range of 100pN. Currently, we address the following questions: Which molecular mechanism underlies force generation and control of motor direction? Is the physical output of the pilus motor conserved between different bacterial species and can parts of the pilus machine be exchanged? How can we control the physical output by genetic modifications? Can we guide bacterial motility using nanotechnological techniques? To address these questions we need to combine techniques for single molecule manipulation with quantitative microscopy and molecular cloning techniques to perturb and characterize the biological networks that control the mechanical elements of the molecular motor.

A current bottleneck is the fact that high-end techniques from nanotechnology, e.g. techniques allowing for quantification of the physical output of molecular motors at the sub-nanometer scale, are still difficult to implement and suffer from day-to-day reliability. On the other hand, interesting biological probes such as force-generating machines in living cells are still challenging in their preparation. The probability of having both the setup and the biological sample working simultaneously is low. It will therefore be a future challenge that requires the collaboration between US and EC researchers to a) improve the day-to-day reliability of high-resolution tools and b) establish direct collaborations with biologists to apply these tools to complex biological systems and address not only mechanical aspects but also the regulation of molecular motors through biological networks. In the long term, this approach will hopefully provide researchers with a molecular level understanding of cellular force regulation in response to external signals, and to design and exchange cellular control modules that switch, tune or coordinate the physical output of molecular motors.

Nanoscience in Biology: Cumping and Buckling of Shells and Guided Folding Pathways

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State of the art of the field

Understanding the structure and stability of particles on curved surfaces is exceeding difficult. For example, even after 100 years, little is known about solutions of the highly nonlinear “Foppl-von Karman equations” that describe thin shells. To quote from Volume 7 of the famous Landau-Lifshitz series on theoretical physics: “These equations are very complicated, and cannot be solved exactly, even in very simple cases”. Exploration of folding pathways, defects and thermal fluctuations in flexible shells, possibly containing nematic, smectic or crystalline order, is still in its infancy. Many of these problems (e.g., virus buckling and folding of pollen grains) arise naturally in a biological context. Practical applications may be possible if insights from theory and from biological examples guide the construction of strong, flexible shells suitable for encapsulated drug delivery, with drug release triggered by shear forces, osmotic pressure or osmotic shock. Hope for progress resides not only in theoretical insights, but also in the exquisite control of assembly of model systems using new experimental developments, such as core-shell technology, a fusion of soft lithography and microfluidics.

The difficulty of constructing ordered states on spheres was recognized by J. J. Thomson, who discovered the electron and then attempted regular tilings of the sphere in an ill-fated attempt to explain the periodic table early in the 20th Century. Protein packings in icosahedral virus shells solve a related “Thomson problem”. The grain boundary scars that appear on drug delivery vehicles called colloidosomes represent another class of solution to this problem. Remarkable modifications in the theory will be necessary to account for thermal fluctuations in polymerosomes and in amorphous shells of spider silk proteins. Some inspiration can be found in the folding strategies and shapes of pollen grains during dehydration when they are released from the anther after maturity. The pollen grain can be modeled as a pressurized high-Young-modulus sphere with a weak sector and a nonzero spontaneous curvature. In the absence of such a weak sector, these shells crumple irreversibly under pressure via a strong first order phase transition. The weak sectors (both one and three-sector pollen grains are found in nature) eliminate the hysteresis and allow easy rehydration at the pollination site, somewhat like the collapse and subsequent reassembly of a folding chair.

Research Needs

To truly understand the strength of small flexible shells, and the role of ordered states on these surfaces, an integrated effort involving (a)experiments on model systems and biological examples, (b)theoretical insights and (c)extensive computer simulations will be necessary.

Opportunities for EC-US Cooperation

Opportunities for cooperation between the EU and the US might include collaborations between pairs of strong experimental/theoretical groups in the US (e.g., Chicago, University of Massachusetts at Amherst, Harvard or the University of Pennsylvania), and the excellent theorists and experimenters who work in Germany (e.g., KFA Juelich, Mainz, or TU Munich) and the Netherlands (e.g., Leiden, AMOLF, or Utrecht).



Time Based Combined Electro-optical Analysis of Affinity / Cellular Platforms; Towards Appraisal of Environmental and Biological Risks of Nanobiotechnology

Eugen Gheorghiu
International Centre of Biodynamics, Bucharest, Romania

State of the art of the field

The concept of sensing and detection has to be readdressed in view of the huge number of analytes & stimuli including the environmental and biological risks of nanobiotechnology, to be assayed. Tremendous progress has been made in the ability to measure particular compounds at very low concentrations. However, evaluation of rare or previously unknown compounds, metabolites and mixtures is still presenting considerable analytical challenges, while being particularly relevant in terms of possible health effects.

With the advent of concerns regarding hazardous effects of engineered (nano)particles and the documented “cocktail effect”, novel analytical and predictive tools based on the interaction with bio-macromolecules or living cells are highly required.

Real-time monitoring of biomolecular recognition processes in living cells is a significant challenge for the next phase of genomics and proteomic technologies [1] leading to improved understanding of cell – environment interactions and to powerful tools for fundamental research and applications.

Whereas common label free analytic assays cope with single techniques e.g., Surface Plasmon Resonance (SPR), Quartz Crystal Microbalance (QCM), Surface Acoustic Wave (SAW), or Impedance Spectroscopy (IS), we are addressing this challenge by developing hybrid sensing platforms able to integrate recognition elements (affine compounds and/or cells immobilized on top of chips) and combined electro-optical analytic systems with Flow Injection Analysis (FIA), suitable for time based assays.

Chemical cues and nano-topographies present on interfaces or in the extracellular medium strongly influence the fate and adhesion of biological cells.

Aiming to assess cell – environment interaction (in particular with noxious agents), we have developed hybrid (bio)affinity and cellular platforms allowing for time based dual electro-optical assays i.e., IS and SPR & Total Internal Reflection Fluorescence Microscopy (TIRF). Such platforms comprising FIA have been advanced to assess the interaction between selected analytes, as well as normal/malignant cells and nano-patterned and/or chemically modified surfaces revealed by the related changes exhibited by cell membrane, morphology, adhesion and monolayer integrity.

Whereas impedance/dielectric analysis of cellular platforms (e.g., epithelial cells) usually ignore the behavior of interconnected cells [2] we aim to relate impedance data both to cell-substrate and to cell monolayer properties using microscopic models of confluent cells (according to cell morphology).

I will present our results on dielectric modeling of interconnected cells as well as the virtues of our electro-optical assays to appraise the effect of minute amounts of detergents, peptides and pathogen cells on confluent epithelial cells and lipid supported membranes.

Research needs & opportunities for US-EC cooperation

Tailoring the interface properties such as to control cell adhesion, cell polarization, as well continuous exchange of the medium beneath cell layer allowing for both electrophysiological techniques (to assess vectorial transport) and SPR or TIRF assays to address the same platform.

In a “nutshell”. ICB skills & and collaboration offer:

- Analytic, platforms integrating several techniques (e.g., IS & SPR, IS & TIRF) and microfluidics able to simultaneously address the same chip.
- Cellular platforms (suitable for epithelial cells adhered on engineered substrates) analyzed by a complementary set of combined assays i.e. AFM, IS, (L)SPR, TIRF, Electro-Physiology, Electrochemistry-detection of Reactive Oxidative Species.

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Large Scale Integrated Desalinization Plant On a Chip: Providing Cheap, Safe Drinking Water from GreyWater Using Nanotechnology

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Princeton University

Nature Magazine recently devoted an entire issue to the issue of Water (Nature 452, 260-261 (2008)). I quote: "As of World Water Day in March 2008, more than billion people around the world still lack access to safe drinking water and two billion have little or no sanitation. As water is sucked up by demands for food and energy, and its distribution on the planet is changed by climate change, what can be done to ensure water availability for the future?" A further issue is the ease of contaminating water with biological agents, surely the most effective means of biological terrorism due to the absolute need for water and the wide distribution of water. There is a real need for dramatic improvements in water purification technologies, especially in light of the present very energy intensive membrane technologies, which operate far from the thermodynamic limit. A further issue is that present desalination plants require rather high grade water input, since the membranes are easily fouled by sub-micron particulates: the ability to recycle "grey water", which at present is disposed in environmentally very damaging ways, is at present out of reach.

I will discuss a revolutionary way to purify and desalinate water based upon technologies developed using microfabrication of asymmetric arrays. These asymmetric arrays have the ability to separate objects based upon their size and/or diffusion coefficient, and are called bump arrays or ratchet arrays depending upon the physics of the device. The challenge we address is how to scale these technologies to include the separation of salt from seawater, at high flow rates with high thermal efficiency. We propose three transformative ideas: (1) a high-throughput room temperature Ratcheting Bump Array (RBA) (a new development that came from a DARPA sponsored Princeton project) for non-clogging particulate removal to the 1 micron scale, followed by: (2) a ratchet-bump array run beyond the supercritical (SC) point of water which will separate inorganic salts which precipitate out of the supercritical water due to the extremely low solubility of inorganic ions in super-critical water. (3) Microfabrication of the entire device on one 8" (200 mm) wafer. There will be no tubes and pipes, and complex electromechanical assemblies connecting parts together in our device will all be on one wafer. Since we envision using conventional 0.5 micron resolution optical lithography through out the design, our aim is to make all components in one lithographic step for the ultimate in compactness and ease of implementation, so that the desalination unit will be exactly like a modern CPU: large scale integration of all components on one wafer. The entire technology to desalinate 60 gallons/hr of water should fit in a 8" x 8" x 4 " box, the size of a Macintosh Mini computer.

The talk will center on a 5-step process for making sterile, potable water from "gray" seawater, which may be possibly contaminated with biowarfare or chemical warfare agents. (1) A Palo Alto Research Center (PARC) microfabricated front filter using centrifugal force created in spiral flow channels to directly separate buoyant and denser particles, while transverse hydrodynamic forces act to separate neutrally buoyant particles into bands which are then diverted for extraction. (2) The prepurified saline water is input into a series of increasingly fine ratcheting bump arrays run at ambient temperature and pressure, which remove particulates to 0.5 microns, effectively removing all bacteria and many viruses. (3) The saline, bumped water is then cycled up via a heat exchanger to beyond the critical point of water local flash heating of the saline water. At this point inorganic salts begin to precipitate out of the supercritical water as microcrystals. (4) The supercritical water is then run through a ratchet-bump array, which transversely removes microcrystals of inorganic salts. The heat and pressure of the supercritical water will also denature and oxidize and biological agents, making the water free of any potential biological warfare agents, which cannot be filtered out in stage 1. (5) The now 100 ppm salt potable and sterile water is now run back through the heat exchanger constructed on chip where the recovered latent heat is used to preheat incoming saline water on chip.

The pressing need for the delivery in a very compact housing of potable, sterile water at minimal



energy cost is surely a project that the United States and Europe can collaborate on. It will require the bringing the present 200 and 300 mm nanolithographic technologies in the electronics industry to the biotechnology arena. The high cost of these large area wafer techniques cannot be carried by academia but will require a consortium of national programs.

Nature Led and Instrumentation Led Research Opportunities in Nanobiotechnology

Suzi Jarvis
University College Dublin, Ireland

In my presentation I will highlight two examples of emerging research areas, one which involves the exploration of materials optimized by nature and one which involves the development of state-of-the-art scanning probe technology for application to biological systems.

Nature Led Nanotechnology

Through the process of evolution, nature has developed solutions to a wide range of industrially relevant problems. Examples include strong light-weight materials, light harvesting materials, aqueous lubricants, adhesives and cements, chemical sensors, and functional coatings and the organisms involved provide specific targets for biodiscovery. However, many of these materials and processes can only be fully understood by investigating them at the nanoscale. If successful, such investigations can lead to biomimicry. By combining two synthetic mimics it is even possible to improve on nature.

In my presentation I will introduce an example from our own work where our investigations at the nanoscale have lead to the discovery of the first mechanistic link between two otherwise unrelated natural adhesives of a marine invertebrate and a unicellular prokaryote.

In the area of targeted biodiscovery, there are significant opportunities for collaboration between the EU and US. The dependence of this field on natural resources, which are often unique to the local environment, consequently reduces competing interests between the two regions and provides the perfect basis for collaboration.

Instrumentation Led Nanobiotechnology

Major developments in instrumentation, including the family of scanning probe microscopes and optical and magnetic tweezers, have underpinned many of the advances in nanoscience and nanotechnology. Whilst biological samples were some of the first samples to be investigated with atomic force microscopy (AFM) over 20 years ago, the microscopes are still not regarded as a routine or an essential part of the biologist's instrumental infrastructure. This is in part due to the technical challenges associated with combining high-performance AFM with biological samples and in part due to the complexity of the instrumentation.

In the second part of my presentation I will introduce a recent design of atomic force microscope which now enables sub-Ångstrom lateral resolution imaging of biological samples under physiological conditions.

Initial commercial developments of the atomic force microscope were dominated by US companies, many originating from University spinouts. In recent years, legal challenges to patent infringements and counter challenges to patent validity have sucked financial resources away from R&D within these companies. This may have contributed to the fact that some developments, like our own have taken place outside the US. Recognizing this, there has been substantial interest from US companies in technological developments outside the US resulting in collaborative development contracts between US companies and European Universities. I will discuss our collaborative research contract with a US-based AFM manufacturer highlighting benefits and drawbacks of this arrangement.

Conclusions & Recommendations

Rapporteurs: Suzi Jarvis, University College Dublin
Robert Austin, Princeton University

State of the Art

The development of new tools and technologies is an essential companion to the development of new ideas in a rapidly growing field of science. The development of new tools and technologies is particularly relevant to biology and biophysics, and has been for many years. One could argue that it has been the tools of the physicist and the chemist which have really driven life sciences forward at an ever increasing rate, from the invention of X-ray crystallography through the development of the polymerase chain reaction to the invention of the gene chip: new technologies and tools have allowed us to study biology at an ever increasing depth, and breadth, with modeling and theory keeping pace with experimental developments. However, we must not fool ourselves into believing like the infamous Head of the US Patent Office in 1899 that everything that can be invented has been invented. Our understanding of the incredible depth of biology is just beginning. We are just starting to expose the vast richness of biology, with currently only limited knowledge of biology both at the local nanoscale and also at the collective scale of ecology, evolution and adaptation. It is difficult to predict what tools and technologies will be critically important in the next 20 years as we pose increasingly deeper questions guided by theory, no doubt the truly transforming ones will be those least expected. Some of the most developed microscopies in the physical sciences, such as 2-photon microscopy and Coherent Anti-Stokes Microscopy are not yet accepted in the life sciences. One of the central problems for researchers as they try to develop new instrumentation and techniques is that until they have been "validated" their initial discoveries can be greeted with skepticism due to the problems of verifying the technique by alternative methods, often the new insights that come from new technologies are at variance or orthogonal to what has been known before, and this can actually slow acceptance. A related problem in the EU and US is the quite different cultural acceptance of new technologies by the public at large. Nanotechnology in particular is a complex issue: in Europe there is much more concern about the long-term effects of nanoparticulates in the biosphere than there is in the US. Toxicology studies are just beginning in the US but are much more advanced in Europe: it is one thing to do studies on cultured cells with nanoparticles, but actually getting them to the market place medically is a vast and extremely expensive undertaking. There was some agreement that there are some very practical global problems, such as access to drinking water, which could potentially benefit from nanotechnology and not face the medical barriers associated with nanotechnology *in vivo*, but to date have not been a major focus for research in the field of Nanobiotechnology. One EU speaker had an existing R&D collaboration with a US company but mentioned that this was logistically difficult to maintain.

Highlights of talks

David Nelson highlighted how the fundamental problem of understanding the structure and stability of particles on curved surfaces has still not been successfully addressed. He described how drug delivery applications might be developed based on the natural phenomena of virus buckling or folding of pollen grains. His stress was on fundamental aspects of materials at the nanoscale, and the difficulty of solving the non-linear equations which govern these processes. A major point here is that if one focuses strictly on development without an emphasis on understanding the theoretical underpinnings of the field then ultimately you stunt the field for lack of depth.

Berenike Maier was also interested in utilizing existing biological processes, in her case, biological molecular motors. She introduced three strategies for their implementation: synthesis of existing biological motors and biohybrid systems and unicellular organisms such as bacteria that act as hosts. Molecular motors are incredibly important to nanobiology: Why? Nanofluidic hydrodynamics is a low Reynold number flow and cannot mix contents because there is no vorticity. Mother Nature is aware of



this problem and has motorized the cytoplasmic fluids: the cytoplasm of eukaryotes is full of motors which pump the fluid around the cell, creating a net circulation within the cell. This active pumping of the cytoplasm solves a number of physics problems: advection of cell contents is now driven by metabolically powered motors rather than a pressure gradient (it is very hard to create pressure gradients in a closed volume!), the cell contents can circulate around the cell rather than just in and out, and the motion can be controlled and directed.

Suzi Jarvis also focused part of her talk on utilizing biological processes, in her case the adhesive strategies of urban biofouling terrestrial algae. She demonstrated how mechanical measurements at the molecular level could help elucidate the underlying natural strategy for adhesion, with the potential for both biomimicry and the development of new anti-biofouling strategies. The second part of her talk introduced new developments in the design of atomic force microscopes for applications in liquid and to biological applications in particular. She demonstrated 90 picometer lateral imaging resolution and 1 piconewton force resolution under physiological conditions.

Eugen Georghiu discussed electro-optical analysis of bio-affinity platforms with the goal of developing non-invasive instrumentation for the rapid analysis of biosystems. In addition to instrumentation they also work on developing 'cellular platforms' to promote the adhesion of specific cell types at specific locations. The importance of developing sample preparation techniques to complement novel instrumentation was highlighted during the presentation.

Bob Austin discussed a way to purify and desalinate water based upon technologies developed using microfabrication of asymmetric arrays. These asymmetric arrays have the ability to separate objects based upon their size and/or diffusion coefficient, and are called bump arrays or ratchet arrays depending upon the physics of the device. The challenge he addressed was how to scale these technologies to include the separation of salt from sea water, at high flow rates with high thermal efficiency. He proposed three transformative ideas: (1) a high-throughput room temperature Ratcheting Bump Array (RBA) (a new development which came from a NSF sponsored project) for non-clogging particulate removal to the 1 micron scale, followed by: (2) a ratchet-bump array run beyond the supercritical point (SC) of water which will separate inorganic salts which precipitate out of the supercritical water (SCW) due to the extremely low solubility of inorganic ions in supercritical water. (3) Microfabrication of the entire device on one 8" (200 mm) wafer. He stipulated that there would be no tubes and pipes and complex electromechanical assemblies connecting parts together in our device, and it would all be on one wafer. Since he envisioned using conventional 0.5 micron resolution optical lithography throughout the design, the aim was to make all components in one lithographic step for the ultimate in compactness and ease of implementation, so that the desalination unit would be exactly like a modern CPU: large scale integration of all components on one wafer. The entire technology to desalinate 500 liters/hr of water should fit in a 8" x 8" x 4" box, the size of a Macintosh Mac Mini computer.

Challenges and Research Needs

High technology science, such as nanoscience, is not cheap. There is a desperate need for the sharing of expensive resources such as supercomputers, X-ray and UV beam lines, electron beam nanofabrication, high resolution imaging, deep sea remote operated vehicles etc. Often industry has these facilities but it is very difficult for the academic researcher to gain access to these tools. There is a strong need to develop agreed upon model systems and to have a balanced attack of both experiment and comparison with theory (including computer simulations). Theory seemed to be under-represented at the workshop; however, theory and simulation are likely to be critically important in the development of the field. The area of simulation of bio-systems is particularly challenging due to their complexity. This will necessitate close collaboration between theorists and experimentalists in order to validate molecular models.

EC-US Collaboration Opportunities

Grand Challenges: 1) Europe has traditionally been the center for theoretical understanding of complex systems, and has a robust culture and infrastructure of summer schools, workshops, and cross-discipline collaborations between theorists. The United States has traditionally been more dominant in applications

of developed technologies to address medical and industrial connections. The two communities should learn from each other: the US should foster the summer school and workshop culture that Europe has, while Europe should try to lower the barriers between academic research and commercial development. 2) Health aspects of nanotechnology: risks and benefits, remains a very difficult area in which there is little collaboration between the US and Europe, and the two regions are almost moving in orthogonal directions. This is a crucial area in which technologies will be hamstrung if there is not some agreement on how to bring nanotechnology into the market safely. 3) The pressing need for the deliver in a very compact housing for potable, sterile water at minimal energy cost is surely a project that the United States and Europe can collaborate on. It will require the bringing of the present 200 and 300 mm nanolithographic technologies in the electronics industry to the biotechnology arena. The high cost of these large area wafer techniques cannot be carried by academia but will require a consortium of national programs. 4) The issue of biofouling emerged in a number of sessions including biophysics and microscopies. It would seem to appear at every level (nano to macro) and across a broad range of NanoBio themes including sensors, implants and MEMS as well as macroscopic marine and urban man-made structures. As biofouling, large or small, appears to be initiated and controlled via nanoscale interactions, it could provide a unifying theme to bring together a diverse range of NanoBio researchers from the US and EC. With regard to urban biofouling, research can be highly region specific due to the location specificity of both building materials and biofouling organisms. However, the solutions to biofouling may be globally applicable, thus providing an incentive for collaboration while investigating different organism-surface interfaces.



SESSION 3 NANOPATTERNING AND SELF-ORGANIZATION

Novel Nanolithography and Approaches to Probing Local Properties

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Significant advances have been made in the last 5 years, both in nanoscale patterning and in the characterization of the behavior of resulting structures. Here perspectives on an aspect in each area will be summarized.

Recent advances in patterning at the nanoscale include micro contact printing, dip pen lithography, and the ever increasing sophistication in silicon fabrication. Self assembly is a powerful strategy that utilizes chemical and physical forces to fabricate ensembles of nanostructures, sometimes in conjunction with patterning approaches. For the most part, self assembly, even in conjunction with electric, magnetic or flow fields, operates on one type of nanostructure producing a layer of molecules, a lattice of particles, templated wires, etc. Approaches that assemble a variety of nanostructures of differing structure and properties would enable the fabrication of functional devices. Ferroelectric Nanolithography is a new approach of directed assembly that positions nanostructures of various compounds into predefined functional configurations. The process relies on domain specific surface electronic structure and consequent chemical reactivity

The ultimate goal of Ferroelectric Nanolithography is to assemble complex structure with potential device behaviour. In this case metallic particles and synthetic proteins are combined. The lithographic approach is illustrated with controlled assembly of an optoelectronic switch made of 3nm - 50 nm metal particles, optically active porphyrins, and functionalized peptide tetramers on an oxide substrate.

As this and other strategies achieve complex multi component nanostructure fabrication, the capability to measure local variations in properties as well as structure becomes necessary to advance fundamental understanding. Of course it has been possible for 20 years to probe electronic structure at atomic resolution with scanning tunnelling microscopy. Equally important have been the parallel advances in probing a much wider range of properties. The interactions between a tip and sample contain information about conductance, resistance, work function, dielectric constant, electromechanical coupling, etc. These properties can be accessed with a combination of interactions at multiple frequencies and harmonic responses. It has now been demonstrated that some of these properties can be measured with spatial resolution $< 1\text{nm}$ and at times with atomic resolution. Furthermore, the combination of scanning probe techniques with optical probes points to the application to biological systems. Several examples will illustrate some possibilities.

Some of these examples are the outcome of US/Germany collaborations that illustrate the potential of such teamwork as well as the challenges in sustaining them. To overcome some of the challenges, the Nano/Bio Interface Center is launching an international network for researchers involve in Nano Probes. This concept will be demonstrated as a framework for discussion on models of international interaction.

PM Studies of DNA Nanostructures and Glucagon

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By using state-of-the-art surface science techniques, such as scanning tunnelling microscope (STM) and atomic force microscope (AFM), quartz crystal microbalance with dissipation (QCM-D) and ellipsometry, we have investigated the behaviour of many important biological molecules upon their adsorption on different surfaces, with an increasing degree of complexity, ranging from amino acids, proteins, hormones (glucagon), individual and complementary DNA nucleobases, to artificial DNA structures.

The self-assembly of individual and complementary DNA nucleobases has been investigated with submolecular resolution in a UHV environment and at the liquid-solid interface where G-C base pairs, G-quartets, and A-T-A-T quartets can be clearly identified [1,2].

Within an implant surface context, the adsorption of relevant blood proteins, including fibrinogen, albumin and fibronectin have been investigated on nanorough tantalum substrates. The main emphasis of these protein adsorption studies has been on elucidating the fundamental mechanisms involved in protein adsorption onto a stochastically random nanorough topography, including protein packing, spreading and potentially changed functionality [3].

The ability of certain polypeptides to form needle-like aggregates and their subsequent deposition at plague sites has been associated with multiple protein folding disorders, including Alzheimer's disease, Huntington's disease, and Parkinson's diseases. We have studied the fibrillation of the hormone glucagon, as it is expected to show many similarities to the often significantly more complex in vivo amyloid systems. By using AFM, we analyzed and mapped the detailed process of glucagon fibrillogenesis, with species including pre-fibrillar, annular and oligomeric states potentially crucial for the mature fibrillar development. [4]

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Field-Gradient Force Techniques and Contact Printing for Micro- and Nano-Structure Assembly

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Current trends in substrate patterning are migrating to new adaptations of techniques that have been in use for years. Gradients in fields (electric, magnetic, acoustic, etc.) have been utilized for chromatographic techniques such as electrophoresis and immunomagnetic separation. The basic forces leveraged in these methods are now being implemented to assemble free standing and thin-film micro- and nano-structures with high degrees of precision and robustness over large surface areas/volumes. Electrophoretic deposition (EPD) is a technique that has been used to deposit nanocrystal films of rare-earth oxide, ferromagnetic, semiconductor, and metallic nanoparticles[1,2]. This method involves both linear (electrophoretic) and non-linear (dielectrophoretic) processes for long distance transport and assembly of nanoparticles. However, the development of robust multifunctional nanoparticle films requires an understanding of the physics and kinetics of the assembly process. The nanoparticle-nanoparticle, nanoparticle-substrate, and nanoparticle-solvent interactions involved in assembly processes are not well understood at the sub-micron scale. In addition, the nonlinear effects of strong electric field gradients ($\sim 10^{20}$ V²/m³) that reside at the interface between the film and the deposition electrode have not been adequately described. Current biotechnology applications for EPD include electrochemical and optical sensors [3].

A second area of interest in nanostructure patterning and assembly is contact printing (CP). This technique involves the production of micro- or nano-structured templates that are replicated with flexible polymers for rapid and cheap molecular pattern transfer to substrates. Various substances such as thiols, proteins, dendrimers, and nanoparticles have been patterned using CP methods, and the technique has also garnered commercial interest for active matrix LCD display applications[4]. Recent interest in CP for biotechnology applications include DNA and protein microarrays and tissue engineering at the level of sub-cellular structures[5,6]. Current limitations of CP include the lack of understanding regarding analyte adsorption to polymer replicates and transfer to target substrates – this leads to trial-and-error patterning processes that suffer from reduced reproducibility. This is an especially important concern for patterning biologically-active molecules that can denature and become inactive during the CP process. A better understanding of the surface, solvation, and analyte interaction forces in the CP process will produce more robust and widely applicable contact printing applications.

For continued progress in electrophoretic deposition of functional structures, integrated electrohydrodynamic models that include electric-field mediated forces and field-induced solute-solvent phase transitions are required. In addition, novel microsystem platforms are needed to investigate the assembly process under well-controlled environmental conditions. This will also require high-resolution imaging and possibly orthogonal interrogation techniques (spectroscopic, impedimetric, etc.) to monitor the assembly process and correlate with computational modeling predictions. These methods would also be useful for investigating analyte deposition and transfer processes in contact printing.

Cooperation between the US and EU should occur through both the governmental and industrial avenues. International research centers funded jointly by the US and EU would provide the infrastructure to facilitate complementary interactions and collaborations. Also, industrial partnerships can provide unique directions for application-driven research and commercialization of new technologies (contact printing and EPD for novel thin-film biosensors, etc.). Specific cooperation in regards complementary use of fabrication/characterization tools and supercomputers should also be pursued to eliminate duplication of capabilities and to maintain a concerted yet competitive effort towards technology development in nanobiosciences.

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Manipulating Biomolecules with Nanotechnology

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Our interests are in quantitative measurements of molecular and cellular biophysical processes. We control and manipulate biomolecular functionality and visualize and quantify dynamic biophysical processes involving multiple molecular interactions in vitro and in living cells at high spatial, temporal, and chemical resolution. Supramolecular associations play a role in several of our projects: disease-related protein aggregation, the functional architecture of protein complexes, patterning of biomolecules, clustering of cell-surface molecules, and protein-nucleic acid interactions. In this presentation I will focus on our work on patterning biomolecules on surfaces.

State of the art:

In collaboration with the Supramolecular Chemistry and Technology and Molecular Nanofabrication groups of Prof. David Reinhoudt and Prof. Jurriaan Huskens respectively at the MESA+ Institute for Nanotechnology, we have used microcontact printing and nanoimprint lithography to create nanopatterned substrates with different chemical functionalities. We have demonstrated the precision placement of the desired proteins on a suitable substrate with the retention of full biological activity, probed by fluorescence microscopy and spectral imaging. We have used electrostatic and specific hexahistidine-NiNTA interactions to drive the directed assembly of visible fluorescent proteins onto patterned substrates¹. We have further demonstrated the directed assembly of the photosynthetic membrane proteins LH1 and LH2 isolated from the purple bacterium *Rhodobacter Sphaeroides* onto chemically patterned substrates using electrostatic interactions² and supramolecular host-guest interactions³. The integrity of the patterned molecules, as reflected by their native optical signatures, is confirmed by spectral imaging using a hybrid scanning probe and single molecule fluorescence microscope.

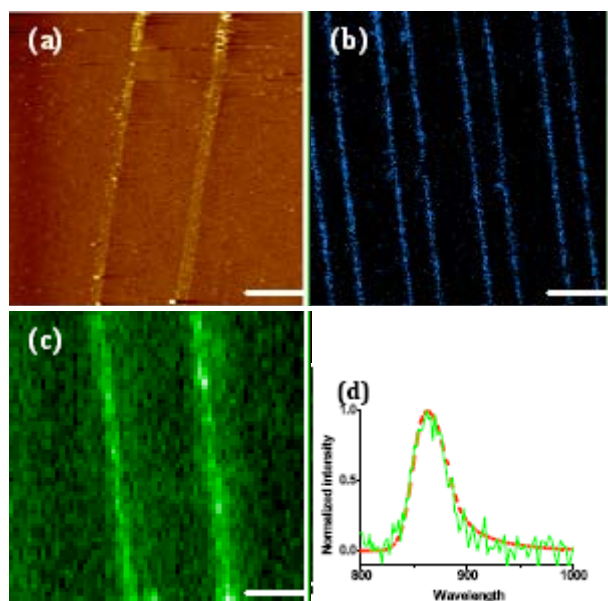


Figure 1. LH2 complexes immobilized onto chemically patterned amino/PEG substrate. (a) AFM height image tapping mode in liquid, scale bar 2 μm , z-scale 25 nm. FWHM of the patterned lines 350 nm with 3 μm separation



(b) (False colour) Fluorescent image of LH2 complexes captured by APD, scale bar 6 μm , 128x128 pixel, separation between the patterned lines either 3 or 5 μm . (c) (False colour) Spectral image, scale bar 1.6 μm , 64x64 pixels, 50ms integration time (d) Comparison of the normalized spectral response of the patterned LH2 complexes (solid-green) with the emission spectral of the respective bulk signal (dashed-red).

Research Needs and Strategic Opportunities:

In order to achieve single molecular scale patterning, advances in driving patterning linewidths to molecular dimensions are required. Homogeneous chemical functionalization and the ability to control interaction strengths (for example by exploiting multivalency) are necessary. Finally, instrumentation that is capable of visualization and *functional imaging* on these molecular length-scales requires further development.

Acknowledgments:

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Imaging and Reaction Dynamics in Model Membranes: Soft Nanoscience

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During the past few years, our lab has developed a wide range of methods for patterning lipid bilayers on solid supports [1]. These 2D fluids are interesting as a model for biological membranes, as a physical system with unusual properties, and as a step towards the creation of controlled interfaces between biological and non-biological surfaces. Methods have been developed for controlling the composition of patterned membrane corrals by variations on microcontact printing and microfluidics. Charged components can be moved around within these fluid surfaces by a form of 2D electrophoresis. Although this is a model membrane system, it provides an excellent platform for the development of advanced imaging and analysis methods, and components displayed in the supported bilayer model membrane can interact with and affect the function of native cell membranes.

The planar geometry of the supported bilayer systems is ideal for surface sensitive imaging methods. We have used imaging mass spectrometry and interferometry. Our lab has developed the application of the NanoSIMS50 (Cameca Instruments) to obtain quantitative composition information on membrane components with sub-100 nm lateral resolution and very high sensitivity [2]. By suitable calibration, high spatial resolution images can be converted into composition images with good precision. We have recently developed a novel membrane interferometer in which free standing membrane is assembled in close proximity to a flat Si mirror to exploit variable incidence angle fluorescence interference contrast microscopy to study nm scale conformational changes of membrane proteins in their native environment [3]. This will be the primary focus of my short talk.

Synthetic or natural vesicles can be tethered to fluid bilayers by short complimentary DNA sequences. Once tethered, vesicles are laterally mobile in the plane of the supported bilayer. Arrays of corrals can be used to tether and sort vesicles displaying the complimentary sequence, and different vesicles can have different contents, lipid composition and/or membrane-associated proteins encoded by the corresponding oligonucleotide sequence. Because the vesicles are laterally mobile, individual vesicle-vesicle interactions, including vesicle fusion [5], mediated by different components on the vesicle surface or in solution, can be observed directly.

Comments on Strategic Opportunities and Research Needs

Nanobiotechnology is a relatively new hybrid concept that means different things to different people. There is a fabrication side (molecules, assemblies, materials), a characterization side (new devices), and combinations related to diagnostics and therapeutics. The hype has been enormous, but it can always be satisfied simply by redefining what is meant. A classic example is provided by a recent presentation I heard (in the context of “space medicine”) of a “nano-satellite” which was about the size of a soccer ball. When I asked how this is “nano”, I was told that it is roughly 10^{-9} times the size of the solar system! So by rescaling the length, everything becomes “nano”. This is a dangerous business, especially when claims of miraculous cures using nano-materials are presented.

At the same time, the fabrication and characterization of materials of increasing complexity largely not based on the creation of covalent bonds (the historical provenance of chemists and a very mature and successful enterprise) does offer exciting prospects. So far, almost nothing is predicted because the rules or relevant parameters are not yet well enough developed (again in contrast to conventional chemical systems). At the fabrication end, this can be a highly creative area. Traditional synthetic chemistry strives (successfully) to produce complex molecules that already exist naturally and is driven by making variations on those frameworks. Some aspects of nanobiotechnology endeavor to mimic natural assemblies or processes, with the possibility of variations that would never exist naturally. Other aspects involve the discovery of new assemblies and materials. Everyone is limited by their ability to characterize what is made or studied, often due to size (e.g. sensitivity & length scale) and often because each copy is different and/or so dynamic that it defies conventional characterization. This latter feature leads to the unpleasant prospect that the traditional duplication of results in different labs may not be simple.

None of this is unique to any country or organization. Large consortia are common now both at the US and in Europe (as well as in Asia & Australia). My view is that this is a good way to support common equipment and fabrication/characterization facilities, but not a good way to support science. Scientists will flock to these centers because they are a pot of gold, but in my experience, these pots of gold are a bit like what is at the end of a rainbow – when you get there, each individual gets very little beyond the common facilities and somehow large sums disappear into activities that have little to do with science or technology. Government agencies, squeezed by the political process, place all sorts of politically-based requirements on the participants in these consortia despite the obvious fact that most of the participating scientists have little training or proven ability to satisfy these goals. To the extent that they do and this is encouraged, natural selection is at work, and scientists increasingly are supported based on their ability to participate in large consortia, with continuous reviewing and outreach, leaving less time for individual creative thought and productivity. It is difficult to find a scientist who describes his or her work as not being interdisciplinary or collaborative. Support for conferences and travel grants for investigators with a common interest can have a positive impact; from my perspective, a lot of the peripheral agendas take a lot of time and money that would be better spent on meetings and travel.

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Conclusions & Recommendations

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State of the Art

Several general themes that evolved from the presentations and discussion of patterning and self-assembly were, in fact, reflected in other sessions as well. Dominant among these are the wide spread use of patterning techniques that utilize topological and chemical features and exploit chemical and biological self assembly, the crucial role of local characterization, and the contribution that can be made by *ex situ* model studies.

Micro contact printing is a flexible tool that has been used to pattern proteins, dendrimers, as well as simpler organic molecules. The approach provides a pathway to construction of platforms that control cellular interactions. To approach molecular length scales, other nanofabrication technologies such as nanoimprint lithography will be required to complement microcontact printing.

In some cases biomolecules are considered as constituents of devices such as those for chemical sensing, opto electronics, and energy harvesting. Patterning approaches that can incorporate a variety of elements with wide ranging properties are necessary for fundamental studies of complex systems as well as for scalable application. Homogeneous patterning capabilities, specific interactions, and multiple orthogonal chemistries were identified as critical needs. Ferroelectric nanolithography illustrates how this can be achieved by taking advantage of domain specific interactions at the surfaces of organic or inorganic substrates. Sequential processing of simple reactions can produce arbitrary configurations of nanostructures consisting of metal or oxide nanoparticles, organic and biomolecular connections in a manner that can be integrated into conventional device processing schemes.

Ex situ studies of molecular interactions are valuable in that they allow the application of experimental approaches with spatial and spectroscopic resolution not yet possible in cellular environments. The extension of these multiparameter microspectroscopic techniques to cellular environments was highlighted. These reductionist approaches yield fundamental insight into molecular interactions at the atomic level that can be used to inform analyses in more complex environments. This is illustrated by STM and AFM imaging of the interaction of individual complementary DNA nucleobases as they assembled on a flat solid substrate. After characterizing a variety of base pair and self-interactions at the submolecular level, the understanding was exploited to control them in a manner that produced pre-designed self-assembled nanostructures. A second illustration is the isolation of single linear motor proteins and the direct observation of the details of motion mechanisms. Actin filaments were suspended on electrodes or attached to a substrate. The insertion of chromophores in the 'legs' of the myosin in conjunction with polarization resolved TIRF allows the details of the myosin 'walk' mechanism to be observed in real time. This insight is used to understand motor motion in the more constrained cellular environment.

Phospholipid membrane patterning and manipulation, and influencing cellular biology (both on the membrane and within the cell) with micro- and nano-patterned surface modifications, using topographical and chemical cues, are being intensively explored and remain a promising area of research and potential interaction between the EU and US.

While significant progress has been made over the last decade in characterizing structure, chemistry and properties at the nanometer scale, the lack of critical capabilities is a roadblock to advance across the board. The ability to characterize the 3-D structure of complex molecules in biological (physiological) environments would immediately advance the state-of-the-art. Strategies toward this goal include polarization and angle resolved TIRF, electron and optical tomography, and the recently developed optical nanoscopy (STED, PALM, STORM) techniques. Combining spectroscopy and spatial imaging to enable *functional* imaging is expected to yield significant added value in imaging function in addition to structure and spatial localization.

Challenges and Research Needs

Many participants indicated the need for further investment in collaborative R&D on membrane patterning and manipulation, striving for pattern dimensions on molecular length scales, orthogonal patterning of multiple classes of molecules, and nanoscale patterning over large areas. An essential complement to the experimental needs defined above is computational modeling for predictive fabrication. Future opportunity areas include micro- and nano- contact printing on tissue and the development of directed assembly techniques to achieve complex three-dimensional structures.

Characterization of patterned and self-organized systems at the atomic and molecular level was identified as a significant challenge. Key elements of this challenge include probing atomic-scale phenomena and further development of single molecule techniques to understand details of molecular interactions at the single molecule level. This field is in its infancy, and has the potential to yield insights into mechanisms that provide a foundation for biological function. For more complex structures, the challenge is to probe buried interfaces rather than being limited to locally accessible surfaces. The measurement of local phenomena is limiting advances across the field, although recent advances imply great opportunity.

Recent advances and near term developments in scanning probe technology offer characterization capability to the biological community but the lack of standards and protocols is inhibiting confidence in these approaches. In addition to structural analysis by atomic force microscopy, property based probes such as scanning ion conductance, local potentiometry, scanning impedance and chemical force mapping would be useful. The combination of ultra-high resolution spatial imaging with spectroscopic and chemical imaging will yield *functional imaging* on molecular length scales. There an opportunity for the international community to collaborate on standardized protocols for probes of biological systems, standards, and demonstrations of reproducibility.

EC-US Collaboration Opportunities

New funding mechanisms for programs are necessary for sustained international collaborations. Several speakers highlighted the need for funds for structural collaborations enabling short-term research exchanges of early-stage researchers (PhD students and postdoctoral fellows) and more senior investigators. It was also noted that our communities are not effectively using electronic venues for community building, an activity that takes resources and effort of the sort that often is not valued in the scientific community. Finally, the mutual use of available large-scale infrastructure could be an important element of EC-US collaborations in this area.



SESSION 4

SENSOR APPLICATIONS AND MICROFLUIDICS

Micro/Nanofluidic Systems for Biomolecule Separation and Concentration

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In many different biological and chemical sensing applications (ranging from biomarker detection to water supply monitoring), we are often faced with the very same challenge of detecting low-abundance targets over large background molecular / matrix backgrounds. While the development of modern sensors (mass spectrometry, nanotechnology-enhanced immunoassays) enabled significant improvement in detection sensitivity and specificity, there is greater potential for improvement for the process of raw sample collection, preparation, and purification, which are often the technological bottlenecks of the sensing problems. Advances in this area would require both better scientific understanding and novel device concepts for manipulating various biomolecules and particles at the nanometer scale.

Recent advances in fabrication techniques allow one to create regular nanofluidic pores and channels down to ~10 nm in critical dimension, with excellent uniformity and size control. This creates unique opportunities for advancing both nanoscience and nanotechnology. Study of nanoscale molecular interaction with surrounding nanostructure has many applications in separation science, membrane engineering, and drug delivery. Nanofluidic channels can provide very uniform, well controlled experimental platform for studying these phenomena. In addition, detailed study of nanoscale transport of ions and molecules through confined environment would lead to ideas for novel nanofluidic devices. In this talk, I will demonstrate some of the examples of nanofluidic devices, which can have impacts both in the science and technology.

One of the important advantages of MEMS-fabricated nanofilter membranes is the flexibility of membrane system design, which is not readily achievable in random nanoporous materials. We have successfully designed and fabricated an anisotropic sieving structure that can be used for size separation of various biomolecules [1]. The sieving structure consists of a two-dimensional periodic array of nanofluidic filter (nanofilter). The bidirectional electrophoretic motion of biomolecules in the sieving structure causes molecules of different sizes to follow radically different paths, leading to efficient separation. Using this sieving structure, we have implemented a high-throughput, continuous-flow biomolecule separation device and evaluated its performance on various biologically relevant molecules [2]. Our device can continuously size-fractionate a wide range of dsDNA fragments (50bp–23kbp) and protein complexes (11kDa–200kDa) in less than 1 min. It has to be recognized that the *anisotropic* sieving properties designed into the system is the key to the operation, and such an operation would not be readily possible with random, isotropic sieving matrix. In addition, this nanofluidic molecular filter array is an ideal experimental platform to study Ogston- and other molecular sieving behavior, with well-defined pore size and shape.

We also demonstrated nanofluidic biomolecule preconcentrator where dilute protein samples can be efficiently concentrated for more efficient downstream detection [3]. In addition to its potential as a signal enhancement strategy for proteomics, the device is a model system for studying nonlinear electrokinetic phenomena and concentration polarization, [4] which has relevance in many perm-selective membrane

applications such as Nafion®. Such a device can be used for enhancing enzyme activity assay by concentrating both substrate and low-abundance enzyme molecules [5].

The current challenges in this area include (i) development of high-throughput solid-state nanofluidic systems, and (ii) Advancing the scientific understanding of complex nanofluidic phenomena, which are often coupled problems in micro-nanofluidic junctions. It is possible that a strong scientific tradition in Europe specifically in the area of electrokinetics and small-scale fluid behavior can be synergistically combined with nano-engineering research activities in US, for solving some of the toughest challenges in the bio/chemical sensing.

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Role of Interface Engineering in NanoBiotechnologies

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NanoBiotechnologies (NBT) are extremely attractive in a wide series of applications such as biosensors, cell culture and tissue engineering^[1,2]. In all these technologies the interface between the biological entities (proteins, oligonucleotides or cells) and the non-biological solid substrate plays the crucial role. In fact, the bio/non-bio interface has a double function of preserving the bio-activity of the biological species immobilized while matching the physico-chemical properties of the non-biological solid substrate.

Surface Functionalization (SF) techniques provide those bio/non-bio interfaces. There are several SF techniques developed during the last decades which can be classified in terms of the production of chemically pure and controlled surfaces, their physical properties (such as roughness), the flexibility (*i.e.* the need of a specific substrate) but also in terms of their production aspects (waste production and the use of hazardous materials).

The main role of SF is to provide a layer which guarantees "the best possible activity of the immobilized interactant"^[3] and from this point of view, the simple control of the surface physico-chemical properties may not be enough. Limitations are due to different effects which are usually driven by the thermodynamics and/or the kinetics of the biomolecules adsorption. To setup the next generation of bio/non-bio interfaces, many approaches have been developed in the last few years: examples may be found in literature and are related to the use of specific covalent binding strategies of the biomolecules, such as the use of special heavy metal ions as biomolecules-surface coupling elements^[4] and the use of "orienting proteins" like protein G or protein A^[5].

An alternative approach is presented here by using *nanostructured surfaces* as a universal platform for bio/non-bio interfaces^[6-9]. The common strategy is to create a bioadhesive/antiadhesive contrast on the surface at the nanoscale. In this way it is possible to induce the biomolecules adsorption in selected areas of the sensor's surface and control the distance between the active domains. As a consequence it is possible to control the thermodynamics and the kinetics of the biomolecules adsorption, improving and triggering the bio-activity of the immobilized species.

Together with these special biomolecules-interaction properties, the nanostructured materials can



be exploited for their intrinsic physico-chemical properties. Some examples include the special electrochemical properties^[10] and optical properties^[11] of nanostructures. The characteristic length scale of the mentioned effects is in the nanometer range and on this length scale, the corresponding material property will occur and is expected to vary with size.

In the present work, nano-structured coatings are provided by plasma processes and self assembled monolayers in combination with Electron Beam Lithography and Colloidal lithography. In particular, micropatterned surfaces were produced by a spatial arrangement of different functional domains by a combination of plasma polymerisation and electron beam lithography: non-fouling patterns were made of poly(ethylene oxide) (PEO)-like polymers obtained by pulsed plasma polymerization of diethylene glycol dimethyl ether while fouling surfaces were composed of Poly-acrylic acid (PAA) from acrylic acid monomer obtained by plasma polymerization, and stabilised by electron beam. PAA nanopillars of 150nm diameter can be obtained in a PEO non-fouling background (Figure 1). Adsorption of IgG on these surfaces show that the proteins attach on the pillars, which results in a higher detection sensitivity in an immunoreaction with anti-IgG. On the other hand, nano-patterns of fouling-antifouling areas have been produced by combining Colloidal Lithography techniques with plasma deposited thin films and SAM's: in particular carboxylic functionalized nano-spots in a PEO-like anti-fouling matrix have been produced. We show that these chemical nano-patterns are able to immobilize proteins selectively in the carboxylic functional nano-domains, leaving the anti-fouling matrix clear. Moreover Enzyme-Linked Immunosorbent Assay and SPR imaging experiments were set-up showing that nano-patterned surface constrains the immobilization of the antibodies in a biological reactive configuration, thus significantly improving the device performances as compared to more conventional non-patterned or disordered patterned surfaces (Figure 2). We show with different methods (SPR, QCM, ELISA) that the detection sensitivity improvement increases as the size of the patterns decreases.

The examples of nanostructured surfaces as advanced bio-interfaces demonstrate the interest of nanopatterning bio interfaces. In the last few years, a strong effort has been done by EU and US research groups in developing and optimizing the nanofabrication techniques. However, there are still opened questions for understanding the mechanisms of nanopattern responses, which could lead to the optimization of the responses observed: what are the optimum size and pattern to control the bio interface response? What pattern, playing with the different interface energy component, is the most efficient in producing a controlled response? Another challenge is to bring these techniques to the "real" applications, in particular in relation to the technological effort that has to be done in terms of fabrication costs and productivity. Finally, another aspect, particularly important for nanotoxicology, is the study of the protein adsorption kinetics and protein denaturation which occurs on nanopatterns, depending on their size and contrast type.

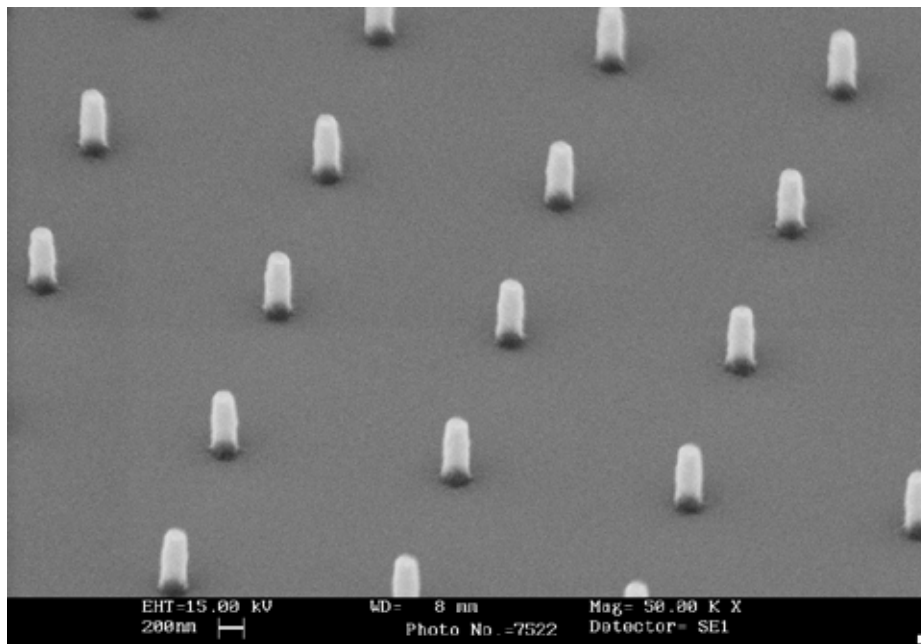


Figure 1. SEM pictures of plasma deposited poly-Acrylic acid in PEO-like background. The pattern has been obtained by electron beam lithography of an unstable plasma deposited polymer.

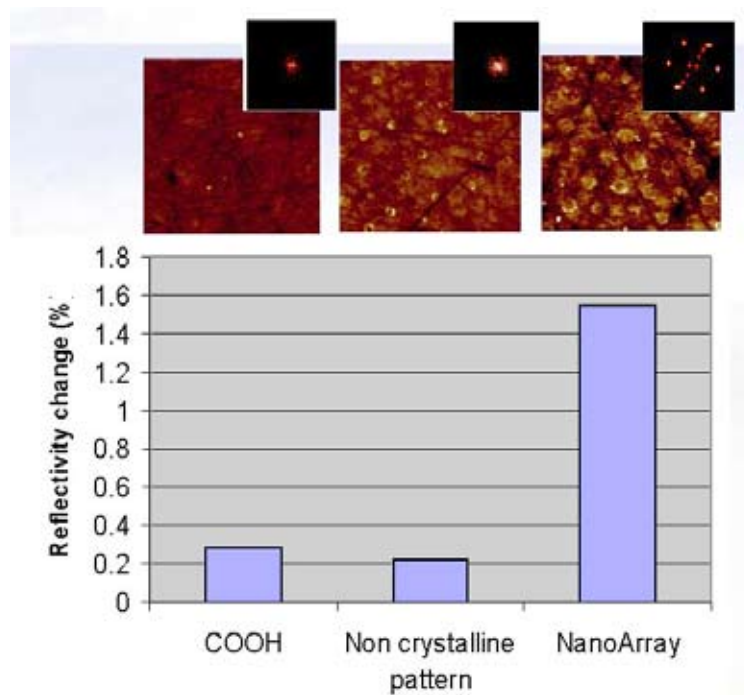


Figure 2. SPR-I response of a flat COOH surface (left), a disordered nanostructured surface (COOH spots in a PEO background: center) and ordered nanostructured surface (COOH/PEO: right) during an immunoreaction.



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Sensors and Sensing Systems

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State of the art of the field

Many interesting sensors are being developed for a variety of applications. Most of these applications have a biological focus. A wide variety of different transduction mechanisms are being investigated for sensors. These sensors tend to employ custom laboratory instrumentation that is unique to each research laboratory and it is difficult to replicate without a significant financial and time investment.

As nanomaterials for sensors and sensing applications become smaller, contain additional functionality, are increasingly selective and more sensitive, they have the potential for improved performance. When such materials are coupled to progressively more sensitive and sophisticated instrumentation, the sensitivity of sensors is moving towards single molecule detection. This increased sensitivity will revolutionize many fields of science. Another aspect of using nanomaterials as sensors is it enables multiplexed sensing using arrays of sensors with thousands of small features. Such arrays may provide the ability to develop "universal" sensors in which all possible specificities are contained in a single array.

Another area of increasing effort and interest is sensors for single cells. While ultimately the application will be in medicine, at present, the focus is on fundamental studies of single cells. By observing single cells, scientists are beginning to understand the diversity of responses in ostensibly identical cells and in cell populations.

Research needs

The ability to recognize analytes is at the core of sensors and new recognition materials are a major need. This need has been one that has limited the advancement of sensors for nearly a decade. New recognition elements have been reported but their penetration into the field has been inadequate.

Integration of sample preparation with sensors and arrays is a critical need. It is important to think about sensing/detection as a *system*. Presently, researchers who develop new sensing technology typically use standard methods to prepare their sample, which can be time-consuming and cumbersome. Microfluidics will play an important part of many sensing systems and must be able to accommodate a

variety of different sensors and arrays. In addition, the detection system needs to be integrated into the system. It is essential that sample preparation, sensing, and detection be viewed as a *system* and that all three subunits be integrated into the sensing process in order to obtain the most value from sensors. Integration of multiplexed sensing on a common platform will facilitate the widespread introduction of the technology.

With single molecule and single cell detection, we are beginning to see stochastic behavior of events at the single molecule and single cell levels. It is important to understand these phenomena and to use this information to develop models of how bulk phenomena manifest themselves at the single molecule or single cell level.

Opportunities for EC-US cooperation

In the US, much of the sensor work is applications oriented; in particular, genomics, proteomics, and diagnostics have been the target of sensor research. The US is good at commercializing discoveries in these areas.

In the EU, sensor research tends to be more fundamental; however, there are also some applications being pursued, primarily in the environmental arena. Sensor groups in the EU tend to be large and are more systems oriented. Collaborations between academic and industrial researchers tend to occur at a much earlier stage in the EU than the US.

There are numerous opportunities for cooperation. Collaborations between laboratories could facilitate research. Student and faculty exchanges on joint projects would benefit both the individuals and the research projects. On the less obvious side, cultural and social aspects of research collaboration and technology transfer could be shared to maximize the research investment.

From Macro to Nano in Biosystems on Chips

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Miniaturisation from micro to nano offers new exciting opportunities to revisit biological sensing, thanks to physical laws and properties specific to the nano scale: incredible surface/volume ratio, faster reaction time, capillary forces stronger than gravity and so on. Research in the microfluidic fields continue to exploit these properties in designing new sensing devices.

However, in the perspective of developing marketable analytical devices in biotech applications or medicine, like Lab-on-Chip, the *full analytical and processing chain should be revisited* in the light of practical use. How to sample millilitres of blood (macro-scale) and then analyse molecular species present in it in a nano-size reactor? How to manage (biological) samples from the macro-, to micro- and then ultimately to the nanoscale? This very practical example of blood analysis at the point of care is in some way comparable to the detection of rare pathogen species in large volume of air or water, for environment monitoring purposes. Examples will be given in the lecture.

The improvement of microfluidic requires technological developments in the design of separation/concentration modules, in mathematical modelling of fluids behaviour, in data management (Signal/Noise), and in manufacturing processes.

One of the great challenges faced by technology developers today remains the *integration of several processing steps in the analytical pathway into a single device*, ready to use by untrained personnel. This is the biggest bottleneck foreseen by the industry in order to commercialize these new developments in the nano field into a useful product. This could explain partially the limited number of nanotech based analytical systems in mass markets. The example of the In-Check platform will be detailed.



Interfacing Biology and Silicon at the Micro and Nano Scale

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State of the Art of the Field

Nanotechnology and BioMEMS will have a significant impact on medicine and biology in the areas of single cell detection, diagnosis and combating disease, providing specificity of drug delivery for therapy, and avoiding time consuming steps to provide faster results and solutions to the patient. Integration of biology and silicon at the micro and nano scale offers tremendous opportunities for solving important problems in biology and medicine and to enable a wide range of applications in diagnostics, therapeutics, and tissue engineering. In this talk, we will present an overview of our work in Silicon-Based BioMEMS and Bionanotechnology and discuss the state of the art and the future challenges and opportunities. We will review a range of projects in our group integrating micro-systems engineering with biology, focused towards developing rapid detection of biological entities and developing point of care devices using electrical or mechanical phenomenon at the micro and nano scale. Towards this end, we will present our work on developing silicon-based petri dishes-on-a-chip, silicon based nano-pores for detection of DNA, silicon field-effect sensors for detection of DNA and proteins, and use of mechanical sensors for characterization of living cells.

Research Needs

Specific areas of future research include:

- **Clinical Applications of Chip-Based Microsensors:** There is a clear need to further develop integrated biochips for food and environmental sample monitoring and apply these devices for detection of bacteria, viruses, and cells and also towards clinical samples for point-of-care applications. Silicon based devices offer a specific opportunity for realizing integrated devices for point of care and personalized monitoring of state of health. These devices need to focus on extracting bacteria and viruses from body fluids including blood, urine, and sputum. Such devices can be of great importance to public health and the global health applications, including infectious diseases such as HIV, TB, Malaria, etc. Even though sensors for detection of such entities can be developed, the extraction of the target analytes from complex samples and concentration of these analytes pose the next challenge in successful realization of such integrated devices.
- **Top-Down Fabricated Biomolecular Sensors:** the promise of label free biomolecular analysis of single cells and its intracellular components has not been fully realized and BioMEMS and Nanotechnology hold a lot of promise for the analysis of single cells and the study of their functions in real time. Micro and nano-scale systems and sensors could allow us to sequence DNA molecules or to precisely measure the protein, mRNA, and chemical profiles of cells in real time, as a function of controlled stimulus and increase our understanding of signaling pathways inside the cell. Current expression analysis is performed from an aggregate of cells, lysed at specific time points when the mRNAs are analyzed. The development of micro-environments where cells can be precisely placed, manipulated, lysed, and then analyzed using micro and nano-sensors in 'real-time', would have a significant impact on systems biology.
- **Integration of Living Cells and Microstructures:** Mammalian cells represent one of the most remarkably engineered biochemical systems. The use of micro/nanosystems and structures and their integration with mammalian cells represents a tremendous area of opportunity for basic cell biology, and also for developing novel biomimetically engineered devices. Specifically, the area of cellular and molecular mechanics is a very promising area of research to investigate how cells communicate (mechanical and chemically) amongst themselves and with the extra-cellular matrix. Microfluidics and bionanotechnology can be used for studying these phenomenon and shed light on basic cell biology including growth of single cells by measuring a change in mass, cell differentiation, and physical properties of cells like

stretching, motility, etc. for many applications including drug screening. The use of specific bio-hybrid structures that integrate living cells could potentially be used for power generation, and harvesting of biological energy.

Opportunities for EC-US Cooperation

There are tremendous strengths and opportunities for collaboration between EU and US in the area of bionanotechnology, BioMEMS, and Microfluidics. The cooperation could focus on the following specific aspects.

1. The areas of expertise are wide and diverse and overlapping across the continents. Steps need to be taken to identify the core strengths of various regions to identify technical areas of excellence and areas that might need to be further developed.
2. A process needs to be developed for identifying the specific grand challenges facing the global communities today that could potentially be addressed via the use of nanotechnology and biotechnology. The obvious ones include health-care, disease monitoring, energy, environment, and shortage of food and water and other resources. How can bionanotechnology be used to address some or part of these global challenges?
3. There is an opportunity to develop joint education and research programs that could be co-funded by the EU and US funding agencies in order to develop the next generation of global scientific leaders. Funds could specifically be used for establishing joint Centers for Excellence in specific areas of research themes where the exchange of researchers and investigators can further fuel additional collaborations and joint projects.

Conclusions & Recommendations

Rapporteurs: Jongyoon Han, MIT
Patrick Boisseau, CEA, France

State of the Art

A strong case for public interest and support for nanobiotechnology can be made related to sensor applications and microfluidic systems. Examples include global health initiative (healthcare in remote/disaster areas), early diagnosis of common diseases, water/environmental monitoring, public health issues (pandemic disease monitoring), food safety, and anti-terrorism/chemical-biological warfare agent detection. The commercial sector is responding to these opportunities by translating lab technologies to the market. One good example is the In-Check™ platform for influenza virus detection, which is the collaboration between STMicroelectronics and Veredus Laboratories.

There has been significant progress in biosensor and chemical sensor engineering during the last decade, many of them are critically dependent on various nanoscale phenomena and nanotechnology. Nanoparticles are now actively considered as the therapeutic agents as well as sensors. Safety of such in-vivo systems are currently monitored by government labs, and standards are being established on various materials. Various types of nanobiosensors have been developed, including nanomechanical sensors, nanopore systems, nanoelectrode arrays and confined optical chambers, taking advantage of technological benefits provided by small length scale of the system. Overall, we now have many different sensing platforms that can provide much better sensitivity than existing standard assays, AND can be miniaturized / integrated into microfluidic systems for portable hand-held sensing. Often, the detection limit is pushed toward the single molecule and single cell limit, which could provide us new information on the biological systems under study.

Many groups are now focusing more on the microfluidic system integration in order to realize the promise of miniaturized, on-site monitoring of chemical and biological signals. Several participants agreed that the sample preparation processes, from complex, raw biofluids and environmental samples, is one of the current challenges in the field. Several sample preparation tools, including biomolecule preconcentration systems and fractionation systems/filters and membranes have been developed, which can enhance the sensor performance significantly. However, seamless integration between sensors and sample



preparation/collection systems is required.

Massive parallelization of sensors (array format) is another important direction to go, especially in terms of more informative bioassay for biological sciences and disease monitoring. Use of nanomaterials as biosensors enables multiplexed sensing toward thousands of different targets. Such arrays may provide the ability to develop “universal” sensors in which all possible specificities are contained in a single array.

Challenges and Research Needs

Almost all discussants agreed that the focus and challenge of this area is going from individual sensor elements toward sensor systems integration. The followings are the list of issues discussed.

- 1) Microfluidic systems are still promising as the platform for integrated sensor elements, but reaching that goal requires sustained, longer-term engineering efforts. Several public challenges mentioned above could serve as an initiator or driver for such collective research efforts.
- 2) Microfluidic sensor integration requires assembling fluidic, electronic, and biochemical components, which is a unique challenge different from any other MEMS engineering. Many fabrication and processing issues, such as packaging, chemical/biological interface, biocompatibility requires further optimization and standardization.
- 3) Different sensing applications demand dissimilar engineering constraints, such as dynamic range of raw sample, sample volume to be processed, macro-to-micro interfacing, and low-abundance biomolecule sampling issue.
- 4) Instead of concentration sensitivity of the sensor as the sole figure of merit, other factors should be considered in the system design. Important merits are; frequency of sensing, sample consumption, actual biomolecule activity (over chemical concentration), portability and automation, reliability and repeatability, and the information content of the sensing signal.
- 5) With single molecule and single cell detection, we are beginning to see stochastic behavior of events at the single molecule and single cell levels. It is important to understand these phenomena and to use this information to develop models of how bulk phenomena manifest themselves at the single molecule or single cell level.
- 6) There is also a great need to interface microfluidic sensor systems and/or bioMEMS devices for studying biological systems in more quantitative ways. Manipulation and measurement of cellular systems in microfluidic systems could generate both scientific advances and advanced diagnostic methods.

EC-US Collaboration Opportunities

The areas of expertise are wide and diverse and overlapping across the continents. Steps need to be taken to identify the core strengths of various regions to identify technical areas of excellence and areas that might need to be further developed. While researchers on both continents are actively addressing the challenges mentioned above, there are some subtle differences in terms of the research and funding environments, cultural and social aspects of research collaboration and technology transfer, and even the public sentiment and response to nanobiotechnology.

While it is hard to generalize, EU tends to have strengths in fundamental research, while the US is a bit more focused at specific applications. Strategies to produce synergies between the two continents should be developed.

Student and faculty exchanges on joint projects would benefit both the individuals and the research projects. Existing opportunities to promote such exchange should be further enlarged and utilized. Funds could specifically be used for establishing joint Centers of Excellence in specific areas of research themes where the exchange of researchers and investigators can further fuel additional collaborations and joint projects. Establishment of web-based seminars and workshops, and any other low-cost networking efforts, could be influential in moving the collaboration forward.

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EUR 23895 EN -

EC-US TASKFORCE COLLECTION

Workshop on NANOBIO TECHNOLOGY *Ispra, (03-04 June 2008)*

2009 — pp. 55 — format 21.0 x 29.7 cm

ISBN 978-92-79-12014-5

ISSN 1018-5593 - 1831-2322



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This report summarizes the presentations and discussions held at the European Commission – United States (EC-US) Workshop on Nanobiotechnology that took place in Ispra, Italy on 3 - 4 June 2008.

The workshop assessed new research initiatives in the area of nanobiotechnology. The aim was twofold: to identify strategic opportunities and needs of emerging areas of research, including socio-economic challenges, and to identify opportunities for enhanced EC-US collaboration and scientific exchange.

As outcome of the workshop some follow-up actions were identified that could be done relatively quickly to foster international collaboration and communication.

Additional discussion was encouraged to develop mechanisms that would help to facilitate the combination of complimentary skills and interest of geographically distributed research groups.

ISBN 978-92-79-12014-5

