Overlapping Bio and Nano Technologies

Srinivas Iyer

Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM 87545
siyer@lanl.gov

Abstract

"What is the connection between nanoscience and biology?" "What are biologists doing at Nanotech 2004 and why do you have so many 'bio" related sessions?" "So biologists are also jumping on the nanoscience bandwagon." These are real questions/comments that are brought up even today. In spite of well established, classic hybrid fields such as biochemistry or biophysics, biology has long been perceived as an isolated science. This was clearly evident during the formative stages of Nanotech (erstwhile MSM/ICCN) conference series which brings together cross-disciplinary scientists to provide a forum for a free-flowing exchange of ideas. A lot has changed since then and rapid strides have been made towards merging bio- and nanotechnologies. The nano-bio association is a two-way street. Modern biology is heavily dependent on technological innovations that have improved analytical processes and, in turn, biological molecules and processes have inspired the synthesis of nanomaterials with novel properties and functionality. The purpose of this brief overview is to highlight a few hybrid topics that could reinforce the belief that nano-biotechnology as a field is here to stay and that its benefits will be clearly visible to the public much sooner than was predicted.

Keywords: biotechnology, nanoscience, protein, materials, interfaces

Nano to Bio?

Modern biology is rapidly moving towards systems level studies. Current challenges include understanding structure-function relationships, generating networks of signals that underlie the response to stimuli and developing crosscuts to other disciplines. The sequencing of a large number of complete genomes has led to an explosion in proteomic projects and new insights are being gained into disease processes. Several major advances that we have seen in the past couple of decades are largely due to increasingly sophisticated analytical methods to probe complex systems. A key component of these methods is the scale at which they operate. Most biological molecules and processes fall into the nano-regime in terms of size and time. Being able to view or manipulate these processes at this scale is a tremendous advantage and nanotechnology offers several attractive options. While many definitions for nanotechnology exist, the National Nanotechnology Initiative [1] calls it "nanotechnology" only if it involves all of the following: a) Research and technology development at the atomic, molecular or macromolecular levels, on the scale of approximately 1 - 100 nm range, b) Creating and using structures, devices and systems that have novel properties and functions because of their small and/or intermediate size, and c) Ability to control or manipulate on the atomic scale.

The National Institutes of Health (NIH) has identified "Imaging biological processes and the effects of disease" as one of the leading opportunities for nanoscience in biomedical research. This is not surprising considering the variety of applications that have benefited from novel imaging tools. Multiple color fluorescence imaging spectroscopy using dyes with overlapping spectra has been used to follow pH-sensitive liposomes within cells [2]. Chen *et al*. [3, 4] used two-photon excitation fluorescence resonance energy transfer (FRET) microscopy to image protein interactions in cells and tissues *in vivo*. The dynamics of protein association and distance measurements can be made by examining donor fluorophore lifetime [4] in the presence of acceptor. On the structural front, protein folding dynamics is critical to understanding its function and ameliorating debilitating conditions such as Alzheimer's disease that are caused by protein misfolding. Laser induced temperature-jump relaxation techniques and time-resolved infrared and fluorescence spectroscopies have been used to probe these dynamics in peptides [5] and small proteins. To do such studies however, it is important to be able to provide biologically relevant experimental conditions. 100 nm sized lipid vesicles have been tethered [6] using biotin-streptavidin chemistry to a supported lipid bilayer and proteins were incorporated in the vesicles to

obtain an average 1:1 ratio. Of all biological molecules, DNA is perhaps the most widely studied using imaging tools at the single molecule level. Kim *et al*. [7] have demonstrated the use of scanning near-field optical microscopy to detect damaged bases in DNA at a scale of tens of nanometers. In an elegant experiment, single molecules of fluorescently labeled nucleotides were detected following exonuclease digestion [8]. DNA sequencing has also been achieved using electrochemical detection by passing single-strands through a nanopore and a solid-state nanopore microscope has been described [9] that can probe individual molecules. The application of nanopores in sensors for biomolecular characterization has led to enhanced techniques for fabrication of robust pores with nanometer precision [10].

The sequencing of several complete genomes, significantly the human genome, has revolutionized biology. The emphasis now is on understanding how the various components of a cell interact. Two major approaches stand out in their contributions to enable such studies. The first is mass spectrometry based proteomics, the large scale study of the proteins related to a genome. Proteins are the terminal effectors of the biological functions of genes and can be modified post-translationally, making their study a complex task. While advances in mass spectrometry methods and protein identification software have given protein scientists a great tool to work with, the power of this approach has truly been realized due to efficient, high-throughput upstream separation systems such as capillary electrophoresis (CE) and HPLC with nanoliter flow rates. Multi-dimensional protein identification technology or MudPIT [11] combines strong cation exchange with reversed phase chromatography of tryptic peptides in a nanoflow HPLC format that is coupled either with online nanospray mass spectrometry or with a MALDI-TOF plate spotting robot. CE on the other hand is being used more and more in a microfluidic chip format [12, 13] coupled with mass spectrometry directly. Laser-induced fluorescence is another detection method that is often coupled to CE for protein analysis. The liquid core optical waveguide properties of Teflon™ coated capillaries have been used to perform protein complex separations in fixed two-color laser format using zone electrophoresis [14] or for isoelectric focusing using a rastered laser [15] to image the whole separation. Separations of biomolecules are not typically thought of as a nanoscale science. This is surprising considering that sample volumes can be as low as picoliters, the molecules being analyzed are nanometers in size and

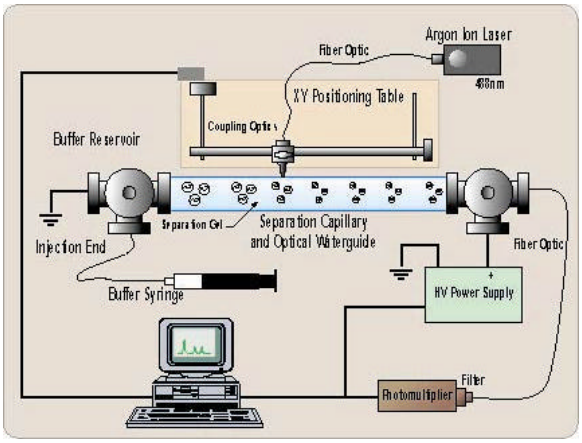

Fig 1: Schematic of a capillary electrophoresis laser-induced fluorescence imaging system. (courtesy of Dr. J.A. Olivares)

that technologies such as interferometric lithography are used to generate these devices. Researchers at the University of New Mexico in Albuquerque have fabricated integrated fluidic systems that allow facile manipulation of fluids and sample injection into nanochannel arrays [16]. The fabrication methods employed are very reproducible and have the potential to yield a new generation of economically viable devices for high throughput biomolecular separation. Most of the advances in microchip separations have their origins in techniques developed in the semiconductor industry. This is now a rapidly growing area of research where a significant amount of sample manipulation is conducted in micro fabricated devices [17] for improved sensitivity, throughput and reproducibility.

Bio to nano?

The transcription of biological designs and functions into robust synthetic systems is one of the key elements of the nanotechnology initiative and a significant research thrust of several agencies including the NIH, DOE etc. Biological systems have the ability to adapt to their environment by their inherent functional flexibility. The design of novel programmable or adaptive materials at the interface of biology and materials science is an exciting area of research with tremendous potential for growth.

Materials with controlled fluid and ionic transport properties are needed in diverse areas such as sensing, drug delivery, fuel cells, artificial photosynthesis and molecular electronics. Although the control of fluidic transport at the nanometer length scales is rarely achieved in synthetic material systems, many biological processes exhibit a remarkable control of both ionic and molecular transport using nanometer-sized pore-channels, coupled exquisitely to specific biomolecular functions. Every cell is enclosed by a plasma membrane that provides a barrier between the environment and the intracellular components. Transmembrane proteins span the lipid bilayer and hence have hydrophobic regions that penetrate the membrane and hydrophilic regions that are exposed to aqueous medium on either side. A significant class of transmembrane proteins form pores in the membrane to allow selective transport of charged species such as ions and macromolecules through the otherwise largely impermeable bilayer. Pore forming proteins display intricate control of transport through them, which is used in several biological systems for a variety of functions. Perhaps the most commonly used feature of such proteins is selective ion transport. This process serves to regulate the electrical potential across the membrane medium by facilitating an active, directional and preferential transport of ions. Channel proteins form water-filled pores across membranes and act as gatekeepers, allowing the flow of ions across the otherwise impermeable membrane. The transport process is not coupled to an energy source and hence is always along the electrochemical concentration gradient. An important feature of ion channels is their selectivity, permitting some ions to pass through but not others. In addition, ion channels are "gated", and their momentary opening and closing is closely regulated in response to specific stimuli. Such gated ion channels have been studied extensively and the synthesis of biomimetic pores in robust materials [18] forms a major area of current research.

While the applications of bio-inspired materials are many, there is a strong thrust by the NIH to develop biomedical applications for such materials. "Biomaterials and tissue engineering" is another of the key opportunities outlined by this agency. These materials may have applications in regenerative medicine due to easier acceptance by the body and maintenance of functionality in that environment. Lipid bilayer vesicles, alluded to earlier, are being developed as drug delivery vehicles [19] in a pH-sensitive format so as to gain control over release of their contents upon cellular internalization. The folate-derivatized liposomes were used to deliver cytosine-b-d-arabinofuranoside to oral cancer cells with elevated folate receptor expression Control over release or activation of specific agents will allow targeted drug delivery and reduce deleterious effects. Sensor development is another area with significant overlaps and of strong interest to several agencies, including the NIH. The integration of molecular recognition elements into portable widgets finds applications in the biothreat reduction world as well as for medical diagnostics. Atomic force microscopy in combination with a recognition based sensor has been reported for the detection of virus particles [20]. This is just one example of several projects that are involved in the development of biosensors (see [21] for an interesting article and a list of companies involved in nanobiotechnology).

Concluding remarks

Of the nine Grand Challenges posed by the NNI focusing on nanotechnology applications with highest potential impact, at least three are directly related to the biosciences. Perhaps the strongest support to this area of research is endorsement from the NIH, without doubt the leading authority in biomedical research. We have barely scratched the surface in terms of the huge potential that this field has to dramatically improve quality of life with advances in areas such as health and novel forms of energy production/conservation. The US Department of Energy is preparing for the nanotechnology revolution by establishing five Nanoscale Research Centers across the country. At least one of these user centers, CINT (Center for Integrated Nanotechnologies, www.cint.lanl.gov) has "Nano-Bio-Micro Interfaces" as one of its key thrust areas. The chief focus of this thrust area is to exploit the conceptual interface between biology and nanoscale materials science. Efforts such as these can only be fruitful with meaningful collaborations across disciplines.

Acknowledgements

In an overview this brief, omissions rather than inclusions are the norm. Hence, apologies are due to groups whose research in this area was not covered. The author acknowledges research support from LANL LDRD and DOE NSET.

References

1. www.nano.gov, *National Nanotechnology Initiative*.

2. Huth, U., et al., *Fourier transformed spectral bio-imaging for studying the intracellular.* Cytometry, 2004. **57A**(1): p. 10-21.
3. Chen, Y., J. Mills, and A. Periasamy, *Protein localization in living cells and tissues using FRET and FLIM.* Differentiation, 2003. **71**(9-10): p. 528-541.
4. Chen, Y. and A. Periasamy, *Characterization of two-photon excitation fluorescence lifetime imaging.* Microscopy research and technique, 2004. **63**(1): p. 72-80.
5. Maness, S., et al., *Nanosecond temperature jump relaxation dynamics of cyclic beta-hairpin.* Biophysical journal, 2003. **84**(6): p. 3874-82.
6. Boukobza, E., A. Sonnenfeld, and G. Haran, *Immobilization in surface-tethered lipid vesicles as a new tool for single biomolecule spectroscopy.* The journal of physical chemistry. B, Materials, surfaces, interfaces, 2001. **105**(48): p. 12165-12170.
7. Kim, J., et al., *Near-field optical imaging of abasic sites on a single DNA molecule.* FEBS letters, 2003. **555**(3): p. 611-5.
8. Werner, J., et al., *Progress towards single-molecule DNA sequencing: a one color.* Journal of biotechnology, 2003. **102**(1): p. 1-14.
9. Li, J., et al., *DNA molecules and configurations in a solid-state nanopore microscope.* Nature materials, 2003. **2**(9): p. 611-5.
10. Storm, A., et al., *Fabrication of solid-state nanopores with single-nanometre precision.* Nature materials, 2003. **2**(8): p. 537-40.
11. Washburn, M.P., D. Wolters, and J.R. Yates, 3rd, *Large-scale analysis of the yeast proteome by multidimensional protein identification technology.* Nat Biotechnol, 2001. **19**(3): p. 242-7.
12. Figeys, D., et al., *An integrated microfluidics-tandem mass spectrometry system for automated protein analysis.* Anal Chem, 1998. **70**(18): p. 3728-34.
13. Figeys, D., et al., *Microfabricated device coupled with an electrospray ionization quadrupole time-of-flight mass spectrometer: protein identifications based on enhanced-resolution mass spectrometry and tandem mass spectrometry data.* Rapid Commun Mass Spectrom, 1998. **12**(20): p. 1435-44.
14. Iyer, S., Stark, P., and Olivares, J., *Two-color Capillary electrophoresis with on-column excitation and wave-guide based fluorescent detection.* JALA, 2003. **8**(4): p. 41-45.
15. Olivares, J.A., P.C. Stark, and P. Jackson, *Liquid core waveguide for full imaging of electrophoretic separations.* Anal Chem, 2002. **74**(9): p. 2008-13.
16. O'Brien, M., et al., *Fabrication of an integrated nanofluidic chip using interferometric lithography.* Journal of vacuum science, 2003. **21**(6): p. 2941-5.
17. Li, J., et al., *Integrated system for high-throughput protein identification using a microfabricated device coupled to capillary electrophoresis/nanoelectrospray mass spectrometry.* Proteomics, 2001. **1**(8): p. 975-86.
18. Steinle, E., et al., *Ion channel mimetic micropore and nanotube membrane sensors.* Analytical chemistry, 2002. **74**(10): p. 2416-22.
19. Sudimack, J.J., et al., *A novel pH-sensitive liposome formulation containing oleyl alcohol.* Biochim Biophys Acta, 2002. **1564**(1): p. 31-7.
20. Nettikadan, S.R., *ViriChip: a solid phase assay for detection and identification of viruses by atomic force microscopy.* Nanotechnology, 2004. **15**(3): p. 383.
21. Stikeman, A., *Nanobiotech makes the diagnosis.* Technology review, 2002. **105**(4): p. 60.