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Nanobiotechnology: synthetic biology meets materials science

Editorial overview

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Nanotechnology, the area of science focused on the control of matter in the nanometer scale, allows ground-breaking changes of the fundamental properties of matter that are often radically different compared to those exhibited by the bulk counterparts. In view of the fact that dimensionality plays a key role in determining the qualities of matter, the realization of the great potential of nanotechnology has opened the door to other disciplines such as life sciences and medicine, where the merging between them offers exciting new applications, along with basic science research. The application of nanotechnology in life sciences, nanobiotechnology, is now having a profound impact on biological circuit design, bioproduction systems, synthetic biology, medical diagnostics, disease therapy and drug delivery. This special issue is dedicated to the overview of how we are learning to control biopolymers and biological machines at the molecular- and nanoscale. In addition, it covers far-reaching progress in the design and synthesis of nanoscale materials, thus enabling the construction of integrated systems in which the component blocks are comparable in size to the chemical and biological entities under investigation.

Constructing genetic elements and circuits

Significant progress has been made in the past years on building DNA and RNA structures and circuits. Recent advances expanding the shape space and practical fabrication of structural DNA- based nanotechnology are reviewed by Linko and Dietz. The movement to use one-dimensional functional DNA nanostructures is described by Wang *et al.*, highlighting the potential for their use in positioning enzymes and growing metallic nanowires. Continuing with the theme of controlling DNA, Padirac *et al.* discuss the ability to rationally design nucleic acid circuits and future applications enabled by this capability. Schwartz and Guo describe how advances in RNA nanotechnology may impact therapeutic applications. Each of these articles highlights the potential of using DNA and RNA building blocks to generate useful architectures, and how *in vitro* circuits are emerging as a viable platform for designing, understanding, and exploiting dynamic biochemical circuitry.

Engineering proteins and enzyme cascades

Moving beyond the DNA and RNA level, further advances and applications are being made at the protein level. One particular application area with significant breakthroughs has been genetic code expansion. Budisa discusses how the incorporation of unnatural amino acids into peptides is enabling the design of novel bioactive natural products with therapeutic use. Lee *et al.* describe the emergence of new energy applications for peptide-based materials. Beyond synthesizing individual peptide and protein products, there have been significant developments in controlling protein localization on the nanoscale. For example, the use of scaffold proteins for enzyme cascades is rapidly moving towards potential applications for synthetic biology and green chemistry as reviewed by Idan and Hess. Finally, Dong

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and Dinu highlight the emerging utility of controlling molecular machines *in vitro* for biosensing and biodevice applications. In sum, these articles demonstrate the potential for conferring new properties to proteins for technological goals and fundamental research objectives.

Synthetic compartments, cells, and reactors

Engineering DNA, RNA, and proteins at the molecular level, as described above, is a prerequisite for controlling architectures on longer length scales, including semi-synthetic cells. Indeed, the construction of synthetic compartments for controlling chemical reactions, and as therapeutics is rapidly growing. Smith *et al.* discuss engineered virus-like particles as a foundation for promising new technologies in drug delivery and vaccines. Kim and Tullman-Ercek describe recent advances in engineering protein compartments as specialized organelles for the sequestration of engineered metabolic pathways. Beyond protein compartments for metabolic pathways, compartments that contain the elements necessary and sufficient for life are also being developed. Stano and Luisi describe recent advances in semi-synthetic minimal cells. As an alternative to using compartments for biological applications, nanoreactors hold promise for enabling chemical reactions. Lu and O'Reilly highlight the use of polymer assemblies for macromolecular chemistry. In addition to being able to construct nanoreactors and (semi-)synthetic compartments, there is a need to biophysically probe and study biological systems with nanoscale spatial resolution. Agrawal *et al.* describe how super-resolution nanoscopy imaging techniques are a powerful set of tools that enable direct visualization of organization and dynamics in live cells. The step from the molecular to cellular level shifts focus to the interface of nanomaterials with biological systems, which is covered by the remaining articles.

Interfacing nanomaterials-based systems with living cells and tissues

Nanotechnology allows the fabrication of devices small enough to enable communicating, monitoring and stimulation, of single living cells. The rise in knowledge in controlling nanostructures allows their tailoring to match cellular components, thus offering high level of interfacing to single cells. In this context, recording, and stimulation, of electrical signals from electrogenic cells and tissues is an essential aspect to many areas, ranging from fundamental biophysical studies of the function of the brain and heart, through medical monitoring and intervention. Over the past decades, these studies have been primarily carried out by various well-established techniques that have greatly advanced the field, yet pose handicapping technical limitations.

Patolsky *et al.* report will cover the latest developments in electrophysiology, applying new nanotechnology-based approaches for cellular electrical recordings, both extracellularly and intracellularly. The report illustrates the remarkable progress in the development of experimental techniques for nanostructure-based electrophysiology, which are minimally invasive and have the potential to be used in multiplexed measurements. Key aspects were demonstrated from these pioneering studies. First, such devices were shown to be able to establish effective electrical communication pathways with individual cells, leading to signals significantly greater than those measured using the traditional planar counterparts. These significant findings may be a direct result of the enhanced coupling occurring between the nanodevices and the cell membranes. Second, the inherently superior spatial resolution of the nanoscale devices was exploited for creating device-to-cell electrical connections at the level of individual neurites, single cells, and whole tissues, with resolutions at the subcellular level. Third, large scale arrays of nanodevices had enabled multiplexed real-time electrical recordings, with the final goal being the signal mapping in neuronal and heart tissues. Based on the aforementioned advantageous characteristics, looking into the future, nanomaterial-based electrical devices could potentially serve as actual prosthetic devices.

Loss of tissue or organ function, caused by either trauma or disease, is directly associated with morbidity and mortality. The accepted treatment for organ, or tissue, loss is through transplantation from one individual to another. Unfortunately, the number of available donors is far outnumbered by the number of waiting patients, leading to high mortality rates. This situation motivated the development of the 'tissue engineering' concept, where 3-dimensional biomaterials serve as extracellular matrix (ECM)-like scaffolds to the living cells, enabling the cells to assemble into effective tissue substitutes, that may restore tissue or organ function. After transplantation the scaffolds either degrade or metabolize, eventually leaving a vital tissue instead of the diseased tissue. It is widely accepted that in order for cells to establish natural cell behavior and assemble into a functioning tissue for successful regeneration, scaffolds should provide cells with a microenvironment close to that provided *in vivo*. Since distinct tissues function differently, understanding the complexity and differences between their 3D micro-environments, and fitting the best fabrication technologies for creating the environment hallmarks are prerequisites. Fleischer and Dvir highlight the key players of the natural cellular microenvironment. The report discusses the effect of nanoscale features of the cellular matrix on cell behavior and tissue formation, and the available nanotechnological tools to mimic them. Furthermore, the article underlines the use of inorganic nanomaterials for improving the properties of artificial 3D scaffolds and overcome their limitations. For instance, existing nanotechnologies and their use for engineering functional cardiac tissues are described throughout the manuscript.

Nanotechnology applications in medicine

In recent years, enormous efforts have been invested to translate nanotechnology innovations into medical practice; the main focuses of these efforts being diagnosis and therapy. Since in many instances the sites for imaging and therapy are the same, it became obvious that the design of targeted nanomaterials, which can be imaged *in vivo*, can also be used as a platform for delivering specific drugs to specific targets, thus providing materials for theranostics (the combination of therapeutics and diagnostics) applications. Nanomaterials, which are characterized by high surface-to-volume ratios, are not only excellent scaffolds for loading large amounts of targeting moieties, imaging tags and drugs, but can themselves be used to induce therapeutic effects making them the platform of choice for future combined medical applications. Cohen and Shoushan report outlines some of the recent progress in the synthesis and functionalization of nanomaterials, as well as their application in the field of theranostics. For instance, magnetic nanoparticles (MNPs), which were originally used as vascular contrast agents in magnetic resonance imaging (MRI), are an important class of nanomaterials which have transformed during the last decade, through carefully designed chemical functionalization into useful agents for targeted multimodal imaging and drug delivery systems. These pioneering studies have established the foundations for the transformation of nanomaterials into present, and future, theranostics platforms.

In the context of therapy, the standard care of cancer patients consists of more than one therapeutic agent, leading to complex treatments since several drugs, administered by different routes, need to be carefully coordinated, taking into consideration their side effects and mechanisms of resistance. Drug delivery systems (DDS), such as polymers and liposomes, are designed to improve the pharmacokinetics and efficacy of bioactive agents (drugs, proteins or oligonucleotides), while reducing systemic toxicity. Using DDS for co-delivery of several agents holds great potential since it targets simultaneously synergistic therapeutic agents increasing their selective accumulation at the tumor site and enhancing their activity, allowing administration of lower doses of each agent, thus reducing their side effects. The rational design of nanomedicines can promote a clear advantage for their use in cancer therapy. Using nanosized DDS for combination of therapeutic agents (e.g. drugs, proteins, nucleic acids) holds many advantages such as first, increasing half-life by protecting the compound from degradation in the circulation, second, reducing immunogenicity, third, increasing water solubility of poorly soluble drugs, fourth, enabling targeting to the site of action, fifth, promoting cellular uptake and appropriate intracellular trafficking, sixth, the possibility to form an advanced complex drug delivery system, seventh, promoting synergism between the combined drugs while delivering and releasing them simultaneously at the target site, making use of different mechanisms of action, toxicity and side-effects. All these properties lead to increased efficacy and reduced toxicity, and may enable easier administration and increased patient compliance. Satchi-Fainaro *et al.* report focuses on the latest developments of combination therapy using DDS and the exploitation of DDS for effective delivery of combinations of therapeutic agents in order to improve cancer therapy.

DNA sequencing approaches inspired by nanotechnology

Next generation DNA sequencing (NGS) is revolutionizing all fields of biological research but it fails to extract the full range of information associated with genetic material. Optical mapping of DNA grants access to genetic and epigenetic information on individual DNA molecules up to ~1 Mbp in length. Fluorescent labeling of specific sequence motifs, epigenetic marks and other genomic information on individual DNA molecules generates a high content optical barcode along the DNA. By stretching the DNA to a linear configuration this barcode may be directly visualized by fluorescence microscopy. Nevertheless, developments in nanofabrication technology, nanomaterials and super-resolution optical imaging (nanoscopy) have been harnessed to improve the performance of optical mapping. Three key areas for improvement are throughput, resolution and information content. The introduction of nanochannels for extending DNA via entropic confinement and without the need to immobilize DNA molecules on a surface has the potential to dramatically boost throughput for the analysis of complex eukaryotic genomes. As opposed to scanning large areas over multiple imaging surfaces, nanochannels offer continuous replenishing of the imaged sample by flowing new DNA molecules into the channels form an attached sample reservoir. Levy-Sakin and Ebenstein report covers the latest advances of these methods in light of recent developments in nano-fabrication and super-resolution optical imaging (nanoscopy), and reviews the latest achievements of optical mapping in the context of genomic analysis.

Nanopore technology employs a nanoscale hole in an insulating membrane to stochastically sense with high-throughput individual biomolecules in solution. The generality of the nanopore detection principle and the ease of single-molecule detection suggest many potential applications of nanopores in biotechnology. Recent progress has been made with nanopore fabrication and sophistication, as well as with applications in DNA/protein mapping, biomolecular structure analysis, protein detection, and DNA sequencing. Concepts for DNA sequencing devices have been suggested, showing great potential to revolutionize healthcare, by allowing genomes to be sequenced at permissive costs and speeds. Stoloff and Wanunu report covers recent advances in the applications of nanopores in biotechnology, and in particular for the sequencing of DNA.

Deciphering structure and function of biomolecules by modern spectroscopy methods

The link of structure and dynamics of biomolecules, and their complexes, to their function has driven the quest for their detailed characterization by a variety of biophysical techniques. The protein data bank (PDB) is the largest current compilation of atomic-resolution protein structures, nucleic acid structures and their complexes. Structures are based primarily on contributions from traditional X-ray crystallography and solution NMR. Yet, there are numerous biomolecules and biomolecular complexes that cannot by characterized by these techniques. They appear in the form of large non-soluble complexes and aggregates, embedded in membranes, in the form of fibers and more. In addition, many fine details about the structure and dynamics of crystalline and soluble systems are not captured by crystallographic techniques. The detailed analysis of such systems has benefited significantly in recent years from the application of magic-angle spinning (MAS) solid-state NMR spectroscopy and from its combination with other lower-resolution methods such as cryo-electron microscopy (CryoEM), fiber diffraction, and small angle X-ray scattering (SAXS). Magic-angle spinning solid-state nuclear magnetic resonance spectroscopy provides detailed information on the structural properties of such systems, and in particular contributes invaluable information on non-soluble, large molecular-weight and noncrystalline biomolecules. Goldbourt reports summarizes the recent progress that has been made in the characterization of macromolecular assemblies, viruses, membrane proteins, amyloid fibrils, protein aggregates and more by magic-angle spinning solid-state NMR methods. Beck *et al.* report seeks to highlight some of the major recent achievements in the field of X-ray scattering as being implemented for the investigation of complex biological systems, and the study of *bio-molecular interactions*.

Nanomaterials: toxic or not?

Nanotechnology offers great potential in terms of enabling technological advances across a wide range of industries. However, the acceptance of nanotechnology, and hence nano-enabled products, is reliant on public and consumer confidence in their human and environmental safety. Thus, the analysis of nanomaterials hazard is currently a major research concern for toxicologists, since there is a pressing requirement for a comprehensive understanding of potential hazards due to the wide spectrum of nanomaterials composition, shape and size. The Biologically Effective Doses (BED) of nanoparticles, the dose entity that drives toxicity, include charge, solubility, contaminants, shape and the ability to translocate from the site of deposition in the lungs. Therefore, conventional particle toxicology data is useful and relevant to the determination of the nanoparticle hazard. Donaldson and Poland report discusses the general basis of toxicity for nanoparticles. Generally speaking, to understand the basis of toxicity is to understand the driving component, and how this can be a variable entity between materials of the same, as well as differing, physico-chemical characteristics.

In summary, the collection of articles in this special section demonstrates the potential for nanobiotechnology for a broad array of applications. We believe that these reviews celebrate recent successes, revealing significant potential for near-term and long-term impact. Moreover, we expect these articles to point to new challenges and opportunities that drive future research and advance the field for years to come.