

Editorial overview:

Nanobiotechnology: Baby steps and giant strides towards molecular mastery

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This special issue reflects the span of bionanotechnology, from the fine-tuning of an individual hydrogen bond or hydrophobic interaction for adding new letters to the genetic code, to the collective behavior of fluid bilayers leading to micron-scale motion. Through this issue we have tried to represent some of the breakthroughs and remaining challenges for the field, looking at questions ranging from the origin of life to how to enhance disease diagnosis and vaccination.

Teamwork at the molecular level:

The re-engineering of metabolism is a key challenge for biotechnology. Metabolic engineering requires efficient individual enzymes, but can be greatly enhanced by organizing different enzymes to increase flux, improve enzyme stability, and decrease cell toxicity. Plegaria and Kerfeld describe the recent insights on how bacterial microcompartments self-assemble from protein units and provide selectively permeable compartments. These microcompartments have multiple different parts assembling to form the shell, so have substantially greater complexity than the widely studied virus like particles. Naturally occurring microcompartments are important for carbon dioxide fixation, as well as a range of catabolic pathways. This review describes successes in transplanting bacterial microcompartments from different bacterial species into *Escherichia coli*. Also, encapsulation peptides have been identified to target new proteins inside the microcompartments. The selective permeability of bacterial microcompartments for small molecules can now be engineered, as well as introducing new properties to the pores, such as electron conduction. These breakthroughs set the scene for major impact from engineering of these nanoreactors. Banerjee and Howarth take a musical analogy, contemplating the state of the art for assembling 'protein orchestras'. Different technologies are evaluated for precise and stable assembly, so that multiple protein units can function together. The chemistry and practical feasibility of split intein, sortase, SpyTag/SpyCatcher, and unnatural amino acid approaches are described in detail. New enzymatic approaches to protein ligation, butelase and OaAEP1, are also considered. Key examples of tailored assemblies are cellulose-degrading enzyme teams or toxic polyproteins co-ligating Death Receptors and Growth Factor Receptors. Modular decoration of virus-like particles has potential to accelerate vaccine development. In addition, bespoke biomaterials should open new landscapes for tissue patterning. Moving beyond nanomachines based entirely on proteins, Wang et al. describe the integration of protein nanopores with membranes, to allow sensing, sequencing and diagnosis. Nanopores provide efficient access to single molecule sensitivity, while avoiding challenges of analyte labeling. Electrical detection can be efficiently miniaturized, multiplexed and automated. Protein nanopores have been engineered based on molecular motors or toxins. The nanopores can be used to sense nucleic acids, proteins or small molecules. Sequencing DNA or RNA with nanopores has been part of the revolution in cheap and long-read sequencing, although much can still be done to reduce the error-rate.

However, the huge potential for nanopore sensing of other molecules has not yet been fully realized. Work on detecting explosives or protein biomarkers of cancer are described, along with the insertion of adaptors in the pore to achieve extra recognition possibilities. Looking beyond the language of proteins and nucleic acids, the wise bionanotechnologist should also think about the opportunities from polysaccharide engineering. Li et al. describe elegantly how carbohydrate

discrimination is fundamental to activation of the immune system. A range of glycan-binding proteins identify polysaccharide patterns from bacteria or fungi, activating relevant defence mechanisms. Also, cancer cells often show altered glycosylation, presenting opportunities for immunotherapy and vaccination. Individual interactions to carbohydrates are often weak. Therefore, various multivalent scaffolds for polysaccharides are described to stimulate strong binding and cellular activation. There are intriguing proofs-of-principle for such nanoassemblies against a range of diseases. Improvements in our understanding of signaling pathways and in patterning specific polysaccharide features should make possible a new genre of therapeutics.

Nature may not know best: the promise of xenobiology:

There are few things more fundamental to biology than base-pair complementarity. Lee et al. describe the different types of unnatural base pairs and how they function in replication, transcription and translation. A breakthrough has been replication of these unnatural base pairs with high fidelity. Semi-synthetic organisms have now been created with a six-letter alphabet, leading to a major expansion in the information-carrying capacity of nucleic acids. These new pairings have fundamental importance for our understanding of how life evolved. The unnatural base pairs dispel the dogma that hydrogen bonds are needed for faithful base pairing. Unnatural base pairs also bring biotechnological opportunities, such as enhanced DNA aptamers for specific recognition of proteins or cells. Genetic alphabet expansion with in vitro evolution led to sequences with exceptional affinity for vascular endothelial growth factor or cytokines. Triphosphates of unnatural base pairs can now be imported efficiently into *E. coli* and faithfully copy a plasmid containing an unnatural base pair. This 'synthetic xenobiology' could lead to new diagnostic and therapeutic products and provide an extra layer of safety for application of genetically modified organisms (GMOs). Xenobiology also relates to the nascent field of designing synthetic cells. Synthetic cells are defined by the ability to replicate both an information carrying molecule and the container of that molecule. Simplicity is crucial here, looking for small numbers of components yielding complex behavior. Spoelstra et al. survey the different scaffolds able to form a synthetic cell: membranous structures, emulsions, and membrane-free coacervates. Scaffolds are not constrained by what is plausible for the origin of life: any synthetic molecules are allowed that achieve the desired behavior. There is particular focus on the dynamics of the candidate synthetic cells: liposome shape can be modified by simple machineries from bacterial division proteins or actin/myosin. Fatty acid vesicles can undergo spontaneous cycles of growth and division. Coacervates have recently enjoyed great attention as membrane-less compartments in eukaryotic cells.

Synthetic coacervates may display complex changes in size and shape or may fuse, with triggers including kinase-mediated phosphorylation or pH change. Synthetic cell design will give fundamental insight into cell organization and may also lead to innovations in drug delivery.

Time for a change: the design of molecular motors and accelerating evolution: Furuta and Furuta start their account at the Industrial Revolution, a turning point in human history for top down assembly of machines. They then introduce the different bottom-up approach to assemble cellular motors and how events at the nanometer scale can lead to motion at the meter scale through collective behaviors. Small changes to a molecular motor like kinesin have altered how far the motor carries on walking down a track, or even made the motor walk in the opposite direction. Myosin directionality can now be switched with unnatural signals such as DNA or light. Abstracting from natural motors further, they describe walkers constructed entirely of DNA and delivery of cargo from place to place on a DNA origami surface. Other surprising concepts are walkers that bias their motion by destroying their tracks as they move, or teams of linear motors achieving collective motility. Nano-organization of motors has led to synthetic assemblies mimicking flagellar beating or altering the shape of micrometer-sized liposomes. Finally the authors consider obstacles to progress, before controlled motor behavior and hierarchical organization can lead to molecular machines underpinning a new Industrial Revolution. All the natural wonders that bionanotechnology strives to adapt are testament to the power of evolution. If there is suitable diversity and enough rounds of selection, survival of the fittest can make extraordinary properties not just possible but likely. Brödel et al. review recent developments in accelerating molecular evolution. The use of phages to evolve proteins in vivo has been a breakthrough for directed evolution. Phages have been developed that conditionally replicate amidst a continuous supply of uninfected host cells. This approach harvests all the benefits of in vivo evolution, without worrying about the evolution of the host genes (which would quickly make the cell resistant to the phage). Previous selections were limited by co-evolution, where any gene to be evolved inside a cell had to cope with evolution of the other host genes (since all genes could contribute to fitness). This limited the pace of evolution to what an organism of a given genome size could tolerate. With phage selection, the mutation rate can be much higher: host genes are effectively frozen, so that the phage genome size becomes the limiting factor in evolution. Initial selections changed the DNA-binding specificity of an RNA polymerase. Subsequent screens evolved a toxin widely used in agriculture, to overcome resistance of different insects. The use of phage transduction systems allows pushing even further the acceleration of evolution because the phage genes could also be frozen. This will be particularly useful for the challenge of de novo evolution of proteins in vivo, a still unmet challenge. The range of future opportunities for accelerated evolution is considered, as well as the challenges in evolving complex post-translationally modified targets.

Concluding remarks:

We are grateful to all the authors of this special issue of Current Opinion in Biotechnology, who have conveyed the diverse scope and exciting frontiers in Nanobiotechnology. Initial investigation in a new area is always a little slow and

can seem esoteric to outsiders. However, examples in this issue show how brave explorations in design, evolution and assembly for biomolecules and compartments can sometimes make a technology explode in importance. Readers of this issue may assist several of the other technologies to make such a transition.