

1 **Editorial overview: Nanobiotechnology: baby steps and** 2 **giant strides towards molecular mastery**

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

25 26 **Teamwork at the molecular level**

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28 The re-engineering of metabolism is a key challenge for biotechnology. Metabolic
29 engineering requires efficient individual enzymes, but can be greatly enhanced by
30 organizing different enzymes to increase flux, improve enzyme stability, and decrease
31 cell toxicity. Plegaria and Kerfeld describe the recent insights on how bacterial
32 microcompartments self-assemble from protein units and provide selectively
33 permeable compartments. These microcompartments have multiple different parts
34 assembling to form the shell, so have substantially greater complexity than the widely
35 studied virus-like particles. Naturally occurring microcompartments are important for
36 carbon dioxide fixation, as well as a range of catabolic pathways. This review
37 describes successes in transplanting bacterial microcompartments from different
38 bacterial species into *Escherichia coli*. Also, encapsulation peptides have been
39 identified to target new proteins inside the microcompartments. The selective
40 permeability of bacterial microcompartments for small molecules can now be
41 engineered, as well as introducing new properties to the pores, such as electron
42 conduction. These breakthroughs set the scene for major impact from engineering of
43 these nanoreactors.

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45 Banerjee and Howarth take a musical analogy, contemplating the state of the art for
46 assembling “protein orchestras”. Different technologies are evaluated for precise and
47 stable assembly, so that multiple protein units can function together. The chemistry
48 and practical feasibility of split intein, sortase, SpyTag/SpyCatcher and unnatural
49 amino acid approaches are described in detail. New enzymatic approaches to protein

50 ligation, butelase and OaAEP1, are also considered. Key examples of tailored
51 assemblies are cellulose-degrading enzyme teams or toxic polyproteins co-ligating
52 Death Receptors and Growth Factor Receptors. Modular decoration of virus-like
53 particles has potential to accelerate vaccine development. In addition, bespoke
54 biomaterials should open new landscapes for tissue patterning.

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56 Moving beyond nanomachines based entirely on proteins, Wang et al. describe the
57 integration of protein nanopores with membranes, to allow sensing, sequencing and
58 diagnosis. Nanopores provide efficient access to single molecule sensitivity, while
59 avoiding challenges of analyte labeling. Electrical detection can be efficiently
60 miniaturized, multiplexed and automated. Protein nanopores have been engineered
61 based on molecular motors or toxins. The nanopores can be used to sense nucleic
62 acids, proteins or small molecules. Sequencing DNA or RNA with nanopores has
63 been part of the revolution in cheap and long-read sequencing, although much can
64 still be done to reduce the error-rate. However, the huge potential for nanopore
65 sensing of other molecules has not yet been fully realised. Work on detecting
66 explosives or protein biomarkers of cancer are described, along with the insertion of
67 adaptors in the pore to achieve extra recognition possibilities.

68
69 Looking beyond the language of proteins and nucleic acids, the wise
70 bionanotechnologist should also think about the opportunities from polysaccharide
71 engineering. Li et al. describe elegantly how carbohydrate discrimination is
72 fundamental to activation of the immune system. A range of glycan-binding proteins
73 identify polysaccharide patterns from bacteria or fungi, activating relevant defence
74 mechanisms. Also, cancer cells often show altered glycosylation, presenting
75 opportunities for immunotherapy and vaccination. Individual interactions to
76 carbohydrates are often weak. Therefore, various multivalent scaffolds for
77 polysaccharides are described to stimulate strong binding and cellular activation.
78 There are intriguing proofs-of-principle for such nanoassemblies against a range of
79 diseases. Improvements in our understanding of signaling pathways and in patterning
80 specific polysaccharide features should make possible a new genre of therapeutics.

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82 **Nature may not know best: the promise of xenobiology**

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84 There are few things more fundamental to biology than base-pair complementarity.
85 Lee et al. describe the different types of unnatural base pairs and how they function in
86 replication, transcription and translation. A breakthrough has been replication of these
87 unnatural base pairs with high fidelity. Semi-synthetic organisms have now been
88 created with a six-letter alphabet, leading to a major expansion in the information-
89 carrying capacity of nucleic acids. These new pairings have fundamental importance
90 for our understanding of how life evolved. The unnatural base pairs dispel the dogma
91 that hydrogen bonds are needed for faithful base pairing. Unnatural base pairs also
92 bring biotechnological opportunities, such as enhanced DNA aptamers for specific
93 recognition of proteins or cells. Genetic alphabet expansion with *in vitro* evolution led
94 to sequences with exceptional affinity for vascular endothelial growth factor or
95 cytokines. Triphosphates of unnatural base pairs can now be imported efficiently into
96 *E. coli* and faithfully copy a plasmid containing an unnatural base pair. This
97 “synthetic xenobiology” could lead to new diagnostic and therapeutic products and
98 provide an extra layer of safety for application of genetically modified organisms
99 (GMOs).

100

101 Xenobiology also relates to the nascent field of designing synthetic cells. Synthetic
102 cells are defined by the ability to replicate both an information-carrying molecule and
103 the container of that molecule. Simplicity is crucial here, looking for small numbers
104 of components yielding complex behavior. Spoelstra et al. survey the different
105 scaffolds able to form a synthetic cell: membranous structures, emulsions and
106 membrane-free coacervates. Scaffolds are not constrained by what is plausible for the
107 origin of life: any synthetic molecules are allowed that achieve the desired behavior.
108 There is particular focus on the dynamics of the candidate synthetic cells: liposome
109 shape can be modified by simple machineries from bacterial division proteins or
110 actin/myosin. Fatty acid vesicles can undergo spontaneous cycles of growth and
111 division. Coacervates have recently enjoyed great attention as membrane-less
112 compartments in eukaryotic cells. Synthetic coacervates may display complex
113 changes in size and shape or may fuse, with triggers including kinase-mediated
114 phosphorylation or pH change. Synthetic cell design will give fundamental insight
115 into cell organization and may also lead to innovations in drug delivery.

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117 **Time for a change: the design of molecular motors and accelerating evolution**

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119 Furuta and Furuta start their account at the Industrial Revolution, a turning point in
120 human history for top-down assembly of machines. They then introduce the different
121 bottom-up approach to assemble cellular motors and how events at the nanometer
122 scale can lead to motion at the meter scale through collective behaviors. Small
123 changes to a molecular motor like kinesin have altered how far the motor carries on
124 walking down a track, or even made the motor walk in the opposite direction. Myosin
125 directionality can now be switched with unnatural signals such as DNA or light.
126 Abstracting from natural motors further, they describe walkers constructed entirely of
127 DNA and delivery of cargo from place to place on a DNA origami surface. Other
128 surprising concepts are walkers that bias their motion by destroying their track as they
129 move, or teams of linear motors achieving collective motility. Nano-organization of
130 motors has led to synthetic assemblies mimicking flagellar beating or altering the
131 shape of micrometer-sized liposomes. Finally the authors consider obstacles to
132 progress, before controlled motor behavior and hierarchical organization can lead to
133 molecular machines underpinning a new Industrial Revolution.

134

135 All the natural wonders that bionanotechnology strives to adapt are testament to the
136 power of evolution. If there is suitable diversity and enough rounds of selection,
137 survival of the fittest can make extraordinary properties not just possible but likely.
138 Brödel et al. review recent developments in accelerating molecular evolution. The use
139 of phages to evolve proteins *in vivo* has been a breakthrough for directed evolution.
140 Phage have been developed that conditionally replicate amidst a continuous supply of
141 uninfected host cells. This approach harvests all the benefits of *in vivo* evolution,
142 without worrying about the evolution of the host genes (which would quickly make
143 the cell resistant to the phage). Previous selections were limited by co-evolution,
144 where any gene to be evolved inside a cell had to cope with evolution of the other
145 host genes (since all genes could contribute to fitness). This limited the pace of
146 evolution to what an organism of a given genome size could tolerate. With continuous
147 phage selection, the mutation rate can be much higher: host genes are effectively
148 frozen, so that the phage genome size becomes the limiting factor in evolution. Initial
149 selections changed the DNA-binding specificity of an RNA polymerase. Subsequent

150 screens evolved a toxin widely used in agriculture, to overcome resistance of different
151 insects. The use of phage transduction systems allows pushing even further the
152 acceleration of evolution because the phage genes could also be frozen. This will be
153 particularly useful for the challenge of *de novo* evolution of proteins *in vivo*, a still
154 unmet challenge. The range of future opportunities for accelerated evolution are
155 considered, as well as the challenges in evolving complex post-translationally
156 modified targets.

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158 **Concluding remarks**

159 We are grateful to all the authors of this special issue of Current Opinion in
160 Biotechnology, who have conveyed the diverse scope and exciting frontiers in
161 Nanobiotechnology. Initial investigation in a new area is always a little slow and can
162 seem esoteric to outsiders. However, examples in this issue show how brave
163 explorations in design, evolution and assembly for biomolecules and compartments
164 can sometimes make a technology explode in importance. Readers of this issue may
165 assist several of the other technologies to make such a transition.

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