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TUTORIAL

Synthetic biology for nanotechnology

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Abstract
Synthetic biology—the redesign of biological molecules, structures and organisms—is potentially one of the most powerful emerging technologies today. The modification of biological structures has already been pursued for a variety of nanotechnological objectives; but synthetic biology could provide the tools and understanding needed both to develop 'nanobiotechnology' in a more systematic manner and to expand the scope of what it might achieve. In this article I shall review what has been attained so far in this field, and look at some of the nanoscale possibilities that an engineering approach to cell biology might herald.

1. Introduction

The benefits of biomimesis in nanotechnology are widely recognized: evolution has already encountered and solved many of the challenges that nanotechnologists face, and even if there is no guarantee that nature's solutions can be translated to a technological setting, nevertheless biology does seem be an abundant storehouse of ideas [1]. There is, furthermore, a grey area where biomimesis merges with bioengineering—where the pre-existing nanoscale devices and structures of the cell can be adapted to suit technological goals. This too is an avenue that now has a substantial and fertile (if relatively recent) history of exploration, for example in the use of protein molecular motors to achieve directed transport of nanoscale particles [2, 3].

Such studies display a spirit of 'bringing biology into nanotech'—which is perhaps another way of saying that they tend not to hold much intrinsic appeal to the molecular or cell biologist. Yet there is now an emerging area of research that one can regard as moving in the other direction: rooted in biology, it reaches out to embrace the biological strands of nanotechnology. This is the field of synthetic biology [4, 5]. It has at some level one of the boldest and most controversial agendas in fundamental biological research: to turn biology into an applied, engineering science, ultimately to the degree that entirely new organisms will be designed and chemically synthesized from scratch [6].

It is worth asking, even (especially?) at this nascent stage in the field's development, what synthetic biology has to offer the nanotechnologist. At present, 'borrowing' from biology has tended to happen in a rather piecemeal, opportunistic manner, and it generally fails to take advantage of the extraordinary degree and hierarchy of organization that biology is clearly capable of generating. If synthetic biology realizes even a part of its promise, the implications for nanotechnology could be profound. What has already been achieved, and what might be in store?

2. What is synthetic biology?

In retrospect, synthetic biology seems an inevitable enterprise. Indeed, the notion was debated at least 16 years ago [7]. One could even argue that any manipulation of living organisms introduces a 'synthetic' element into biology, and from that perspective one might have to admit medical prosthesis (which has a history at least two millennia old) as an aspect of the field. Moreover, the advent of recombinant DNA technology in the 1970s made it possible to 'synthesize' new genetic configurations, giving rise to the discipline of genetic engineering, which, in its very name, acknowledges an element of artificiality.

Simon [8] has proposed four criteria for 'artificial sciences' that distinguish them from the natural sciences.

(1) Artificial things are synthesized (though not always or usually with full forethought) by human beings.
(2) Artificial things may imitate appearances in natural things while lacking, in one or many respects, the reality of the latter.

(3) Artificial things can be characterized in terms of functions, goals, adaptation.
(4) Artificial things are often discussed, particularly when they are being designed, in terms of imperatives as well as descriptives.

Although it is not clear precisely what *degree* of human synthesis Simon had in mind for item 1 (clearly, engineering often uses 'natural' materials, while physics includes the study of 'artificial' matter), he acknowledges that his artificial sciences are 'closely akin to a science of engineering'—to which the key is the notion of function and purpose.

The peculiar thing about biology as a natural science, however, is that it already exhibits function and purpose. It is conventional, and completely meaningful, to speak of 'biological design', despite the absence of a designer. The mechanism of evolution—random mutation combined with natural selection—is now generally recognized as an alternative to rational planning as a means of providing engineered components and structures with well defined functions. The use of combinatorial methods in drug discovery [9] and materials exploration [10], and of genetic algorithms in computer science, acknowledges that for some problems of design (primarily those for which the number of variables and permutations is immense) evolutionary approaches can be vastly more effective than any attempt to find solutions from first principles.

The 'designed' aspect of biology has long been recognized at the organismal level: studying organisms from the viewpoint of the engineer dates back at least as far as the early 20th century [11]. But an explicit statement to the effect that the same considerations apply at the cellular and molecular scales is rather more recent. Monod and Jacob couched their discovery of the operon, groups of genes that include self-regulatory elements, in the 1960s [12] in terms that invoked electrical engineering: they spoke of regulatory circuits, of the logic of the cell, of interactions between transmitters and receivers [13]. In the archetypal *lac* operon of *Escherichia coli* (figure 1), a gene called *lacZ* has a default 'off' setting (it is 'constitutively off') because of a repressor protein that binds to an 'operator' gene and prevents transcription of *lacZ*. The repressor is encoded by another gene in the operon unit, and so in this way one gene 'controls' another. The *lacZ* gene is activated, allowing a sugar-degrading enzyme to be transcribed, by the binding of lactose, the 'inducer' molecule, to the repressor protein, which inhibits it from attaching to DNA. This operon functions like a logic gate, where the inputs are chemical signals and the output is the state of the *lacZ* gene: on or off.

Much of the work in molecular biology over the past two decades has been concerned with uncovering and understanding these gene- and protein-based logic operations. But this has typically been done by studying such operations in isolation—for example, by mapping out gene and protein interactions involved in a specific cell-signalling pathway, which might govern the cell's response to, say, a hormone molecule. In recent years, technologies have become available (in particular, DNA microarrays [14]) that enable biologists to take a broader view of the logic of the cell: to deduce how certain groups of genes act in combination to regulate and modulate one another's activity, and how these 'modules'

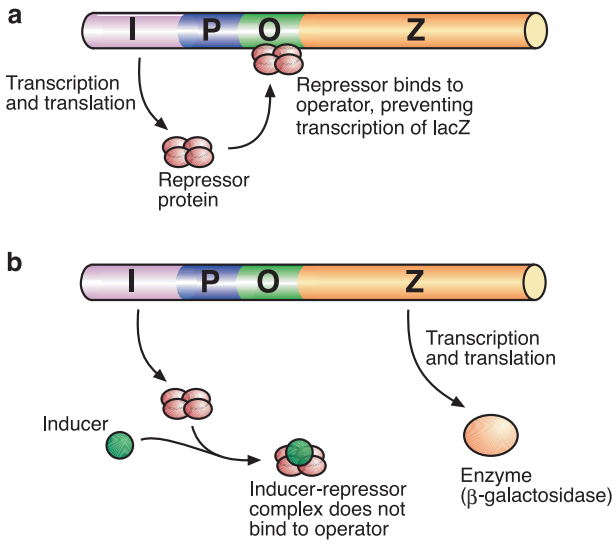

Figure 1. The *lac* operon. (a) The repressor protein encoded by the gene *lacI* binds to the operator *lacO* and suppresses transcription of *lacZ*, which encodes a sugar-degrading enzyme. (b) When the inducer molecule binds to the repressor protein, *lacZ* is 'switched on' and its protein product is expressed.

are wired together in the cell. This has brought about a revitalization of the 'electronics' analogy of Jacob and Monod, in the discipline known as systems biology [15, 16].

Hartwell *et al* [17] have pointed out that the evolutionary 'design' of gene circuitry makes it natural to regard systems biology as akin to engineering science. 'What really distinguishes biology from physics', they say, 'are survival and reproduction, and the concomitant notion of function. Therefore, in our opinion, the most effective language to describe functional [gene] modules and their interactions will be derived from the synthetic sciences, such as computer science or engineering, in which function appears naturally'.

For systems biologists, the challenge is to deduce the circuit diagram of the cell by reverse engineering. Even in simple organisms with perhaps several hundred genes, this is an immensely difficult problem. As Hartwell *et al* say [17],

> Although an electrical engineer could design many different circuits that would amplify signals, he would find it difficult to deduce the circuit diagram of an unknown amplifier by correlating its outputs with its inputs. It is thus unlikely that we can deduce the circuitry or a higher-level description of a module solely from genome-wide information about gene expression and physical interactions between proteins. Solving this problem is likely to require additional types of information and finding general principles that govern the structure and function of modules.

That problem is attracting a diverse community to systems biology: geneticists and molecular biologists are teaming up with computer scientists, electronic engineers, physicists and chemists. While the goal is primarily to understand how natural cells function, it is unsurprising that engineers involved in this project have imported a 'synthetic' perspective that asks whether these circuits can be redesigned, and if so, what can

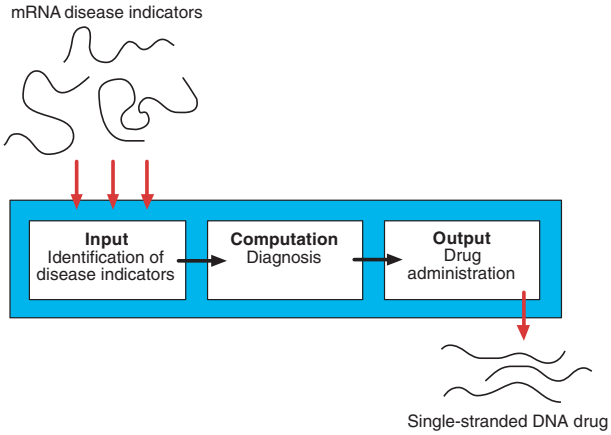

Figure 2. The DNA-based autonomous molecular computer constructed by Benenson *et al* analyses potential disease indicators (mRNA molecules) and, if the diagnosis computation warrants it, triggers production of antisense single-stranded DNA molecules with therapeutic activity.

be made. Moreover, some researchers believe that synthetic gene modules might provide the tools needed to unravel natural wiring patterns, for example by enabling gene modules to be decoupled from one another.

One of the first demonstrations of the potential of this synthetic perspective was provided by Elowitz and Leibler [18], who designed an artificial 'oscillator' module from three mutually regulating genes, which they called a repressilator. Wired into the circuits of *E. coli*, the repressilator induced periodic on–off switching of genes, which was revealed by coupling the module to the synthesis of the green fluorescent protein. The cells flashed on and off rhythmically with a period of several hours.

Other gene modules used for controlling the behaviour of bacterial cells include a toggle switch [19], which can be flipped into a persistent on or off state by an environmental signal (such as a DNA-damaging agent), and a quorum sensor that monitors cell density via a gene that triggers synthesis of a diffusing small molecule—in effect enabling the cells to communicate in a non-native 'language' [20]. Another target that has been postulated is a 'counter' module that keeps track of successive rounds of cell division, thus performing a primitive computation that might be used, for example, as a safety measure to initiate cell suicide after a specified number of divisions.

Since cell processes are increasingly being interpreted and described in terms of computation, this too becomes the natural language for the kind of interventions that synthetic biology permits. Weiss *et al* [21] have combined genetic bistable switches such that they can perform Boolean operations and function as logic devices such as NOT and AND gates. Benenson *et al* [22] have constructed an autonomous 'molecular computer' from DNA which is capable of controlling gene expression for the treatment of disease. The device is comprised of several DNA molecules which perform distinct tasks: sensing, computation (diagnosis) and response (releasing drug molecules) (figure 2). The computer analyses cell function by sensing levels of messenger RNA from specified genes, and responds by producing single-stranded anti-sense DNA molecules that regulate gene expression. For example, Benenson *et al* synthesized versions of the computer that sensed the expression levels of mRNA from genes known to be related to lung and prostate cancers, performed an analysis to determine if these levels indicated a disease state, and then, if appropriate, generated antisense DNA that acted as an anticancer drug by inhibiting the synthesis of crucial proteins involved in the development of the disease. In effect, this could bring the operations of a medical diagnostic laboratory directly into the cell, allowing these processes to be conducted continuously and autonomously at the molecular scale.

3. New parts and materials

Although the engineering of genetic circuits for the redirection of living cells and organisms represents a major focus of synthetic biology, it embraces more modest objectives too. This kind of redesigning of life is likely to need a toolbox with a wider store of fundamental components than those provided by nature. At present, for example, the manipulation of genes for biotechnological processes such as gene splicing or amplification by the polymerase chain reaction is conducted by means of enzymes (restriction enzymes, ligases, polymerases) taken from natural organisms. These are undoubtedly ingenious molecules, but they are limited in terms of, for example, the substrates they will accept and the environments they will tolerate. So synthetic biology is seeking to broaden this range of molecular tools. At root, these efforts are exploring the question of biology's *plasticity*: how far can it be reshaped to accommodate unfamiliar materials, circumstances and tasks? This exploration is already finding applications in nanotechnology.

One of the long-standing dreams of protein chemists has been to be able to design proteins from scratch—for example, to make artificial enzymes and new protein-based materials. There has been some success in designing peptide materials [23–25], as well as creating de novo peptides with specified folds [26]. But achieving novel catalytic function in artificial proteins with an efficacy and a specificity to match that of natural enzymes is a challenge of another order—most efforts so far have tended to use the natural combinatorial mechanism of the immune system to develop antibodies with catalytic functions [27]. Recently, however, advances in computational methods have been exploited to enable the remarkable feat of transforming a non-catalytic protein receptor into a mimic of a natural enzyme by rationally mutating several residues in the binding site [28]. This work offers the encouraging message that rational protein design does not necessarily have to be conducted wholly de novo—one can use existing protein folds for the 'scaffolding', and focus simply on retooling the active site. More modest, perhaps, but equally useful, is the demonstration that proteins can be rationally modified to bind to new, non-natural substrates, including explosives [29].

These developments in protein design have been adapted for nanotechnological uses. For example, the versatility of the immune system has been used to generate antibodies that will recognize and bind to fullerenes [30], carbon nanotubes [31], and a variety of crystal surfaces [32]. Nature shows how soluble molecules capable of recognizing and binding to

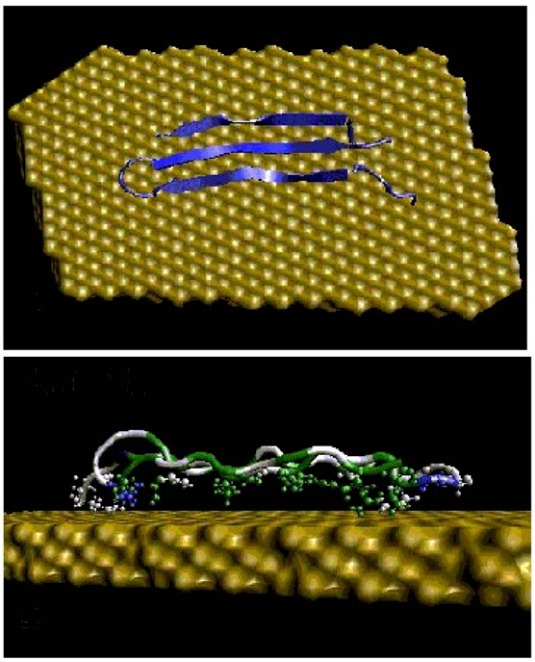

Figure 3. A synthetic polypeptide that binds to the gold(111) surface. In this simulation, polar residues are shown in green, charged residues in blue, and hydrophobic residues in white [37]. Reprinted with permission from Sarikaya M *et al* 2003 *Nat. Mater.* **2** 577.

specific materials can be used to shape and control the growth of crystals and other nanostructures, for example in the way that macromolecules seem to govern the self-assembly of biominerals [33] or the action of antifreeze proteins in suppressing the growth of ice crystals, or of promoting their nucleation [34].

There is no need to rely on the complexity of the immune system in order to conduct combinatorial searches for new peptides of this sort. Whaley *et al* [35] used an *in vitro* evolutionary approach to screen a combinatorial library of 12-residue peptide molecules and identify sequences that would selectively bind to a range of inorganic semiconductor surfaces. The peptides were expressed in the protein coats of bacteriophage, which provided both a vector for the recognition sequences and a marker that signalled binding to the respective surfaces. In this way, peptide 12-mers could be identified that bind to specific crystal faces of GaAs, as well as to the surfaces of GaN, ZnS, CdS, Fe_3O_4 and $CaCO_3$. These recognition peptides might provide selective 'glues' for assembling inorganic nanocrystals into complex arrangements, or for attaching them to other biomolecules for labelling or transport.

Brown's work on polypeptides that will bind to specific metals [36] has been extended by Sarikaya and coworkers [37] to make so-called GEPIs (genetically engineered polypeptides for inorganics) that bind a host of materials (figure 3). Again, the peptides are prepared by combinatorial shuffling of sequences, coupled to a phage-display screening process. Some of these GEPIs exhibit the ability to modify crystal growth, for example switching the morphology of gold nanocrystals from cubo-octahedral (the equilibrium form) to flat triangular or pseudo-hexagonal forms [38].

One of the encouraging messages to emerge from such efforts to use essentially biological structures for nanotechnology is that the potential hurdle of interfacing seems not to be a problem: that is to say, biology is clearly 'plastic' enough to accommodate unfamiliar materials from the inorganic world.

As we become more adept at modifying proteins not just for binding but for catalysis, the nanotechnologist can begin to glimpse some rather dizzying prospects. Can one design an enzyme that constructs carbon nanotubes [39], perhaps even with a specified diameter and chirality (and hence electronic structure)? Could such a molecule then be fitted with a recognition tag that will ensure it does its job of construction only at a particular location in a semiconductor landscape?

Natural proteins and protein-based assemblies have shown considerable potential for nanotechnological applications. The light-activated proton pump bacteriorhodopsin, a membrane protein that regulates the pH of some bacterial cells, is perhaps the prototype, having been used over 10 years ago as a material for optical molecular data storage [40]. More recently, Meier *et al* [41] have shown that this and other membrane proteins will retain their structure and function when immobilized in thin, robust films of crosslinked copolymers with a hydrophilic–hydrophobic–hydrophilic sandwich structure, mimicking the environment of lipid membranes. Ho *et al* [42] used bacteriorhodopsin immobilized in such a polymer membrane to actively pump protons against a pH gradient and thereby to reduce hydrogen-ion leakage across the proton exchange membrane of a fuel cell. Similarly, Montemagno and coworkers [43] are investigating the use of the water-transporting protein channel aquaporin for developing water filtration membranes. Furthermore, Zhang *et al* [44] have shown that peptide-based surfactants can provide a membrane-mimetic environment that maintains the integrity of the entire photosystem I of spinach leaves, so that these assemblies of proteins and pigment molecules can effect light-activated electron transport, with applications in photovoltaic technology.

Audette *et al* find that modified forms of the pilin proteins that constitute the filamentary pili of bacteria will self-assemble into nanotubes about 6 nm in diameter and up to 100 μm long [45] (figure 4). These pilin proteins are produced by a genetically engineered strain of *Pseudomonas aeruginosa*, and lack the first 28 hydrophobic residues of the wild-type form. Although this makes the proteins highly soluble, they aggregate into linear filaments which then wrap around one another in a helical fashion to form the nanotubes. These will bind DNA, suggesting that the nanotubes might be used to make biocomposite nanostructures. Additional engineering of the proteins might allow tuning of the mechanical and chemical properties of the tubes.

Proteins that produce mechanical motion in the cell have been used *in vitro* to transport nanoscale objects in a directional manner, for example propelling protein filaments called microtubules down lithographically defined tracks [46]. Moreover, Soong *et al* [47] showed that the enzyme ATP synthase, a membrane protein with a 'head' that rotates as it converts ADP to ATP (or vice versa), can be used as a molecular motor to drive rotary motion at the nanoscale. Already some of these proteins have required minor chemical modification to enable them to function in nanotechnological devices (for example, to anchor them to a substrate). Liu *et al* [48] went

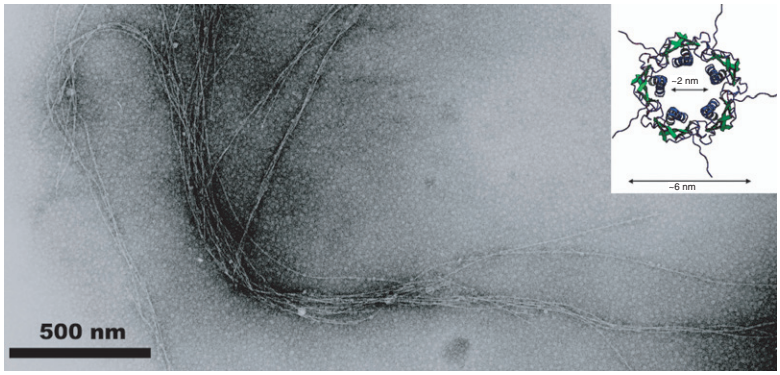

Figure 4. Electron micrograph of protein nanotubes formed from ΔK122-4, an engineered form of the pilin protein of *Pseudomonas aeruginosa*. (Inset) Hypothesized cross-sectional structure of the nanotubes [45]. Reprinted with permission from Audette G F *et al* 2004 *Nano Lett.* **4** 1897. Copyright 2004 Am. Chem. Soc.

a stage further by designing a zinc-binding domain into ATP synthase that acted as a zinc-activated switch to turn the motion on and off. Synthetic biology promises to elucidate some of the design criteria that may be needed for more ambitious adaptations of these natural proteins.

All this can be regarded as a kind of synthetic biology in that it involves the reshaping and redirecting of natural molecular systems, typically using the tools of protein and genetic engineering. But there is no obvious reason why the redesign of proteins need be bound by nature's conservatism of building blocks. Non-natural amino acids could provide new substrate specificities, opportunities for covalent crosslinking of proteins, different electronic properties, enhanced thermal stabilities, and, especially once the principles are better understood, entirely new folds and tertiary structures [49]. Methods for persuading cells to incorporate non-natural amino acids into proteins, and even to biosynthesize such new building blocks from basic carbon sources, are now well established [50, 51]. But the combinatorial design space of peptides is already astronomical just with 20 amino acids, so that adding more of them is probably going to be tractable only if done in a highly directed manner.

Proteins are not the only 'natural' fabrics for nanotechnology. In particular, DNA has been extensively exploited as a 'programmable' building material for constructing complex nanoscale structures [52] and devices [52–59]. This in itself is hardly a form of synthetic biology—DNA is here purely a convenient polymer, although it is true that enzymes and biotechnological processes are typically used both for construction and for analysis of the constructs. Yet the potential for overlap with synthetic biology is plain. For example, rather than relying on a complicated sequence of manual interventions to make these DNA-based assemblies, one might consider engineering cells to produce the requisite sequences and assembly enzymes in the right order and the right place. The design of agents that bind selectively to DNA sequences, such as proteins based on the zinc-finger motif [60], is being pursued to augment the synthetic biological toolbox, and one can imagine such agents proving useful for nanotechnological DNA design too, for instance for decorating a DNA scaffold in a highly programmed way. It is not obviously outrageous to conjecture about synthetic organisms with a nanostructured genome—an alternative, perhaps, to the intricate and still poorly understood packing of the eukaryotic chromosomes. Or maybe the computational logic being explored in DNA nanotechnology [61] might suggest new ways to regulate gene interactions, along the lines demonstrated already in DNA 'molecular computers' [22].

Here, too, there is room to expand the materials basis. Non-natural nucleotides have been successfully incorporated by polymerases into DNA, for example [62–64], and enzymes that tolerate such innovations could be used for a non-natural DNA nanotechnology.

4. Rebuilding viruses

If the idea of transferring nanotechnology to the organismal (whole-cell) level presents a rather daunting challenge, viruses provide a more accessible halfway house. Whether they qualify as living or not, they are undoubtedly an arresting demonstration of what a genetically controlled nanostructure might look like. Viruses are highly organized and sometimes beautiful supramolecular arrays put together by a combination of non-covalent self-assembly and genetic programming. Above all, perhaps, they argue for the literally frightening potential of introducing an evolutionary, adaptive element into the question of nanoscale structure and function. They are truly Darwinian nanomachines.

Currently, the use of viruses in nanotechnology has barely exploited this potential. In some cases, they serve as little more than conveniently shaped nanoparticles, rendered inert by the removal of their genome. Thus, for example, the empty protein shells of viruses (virions) have been used as templates or microreactors for moulding inorganic nanoparticles [65]. But one of the attractions of viruses as nanostructured materials is that their surface chemistry is highly amenable to delicate, site-specific and *inheritable* modification: the proteins that constitute the coat can be altered by introducing the appropriate sequence into the gene that encodes them. Mao *et al* [66, 67] added recognition peptides [35] to the surfaces of M13 bacteriophage so that they bound ZnS or CdS, acting as templates for the synthesis of polynanocrystalline nanowires. Francis *et al* [68] made similar modifications to the tubular protein sheath of the tobacco mosaic virus (TMV) so that it can bind metal ions such as cobalt, potentially enabling the virus to template magnetic nanowires and nanotubes. Each TMV tube is 300 nm long (figure 5(a)), and is made up of 2100 identical

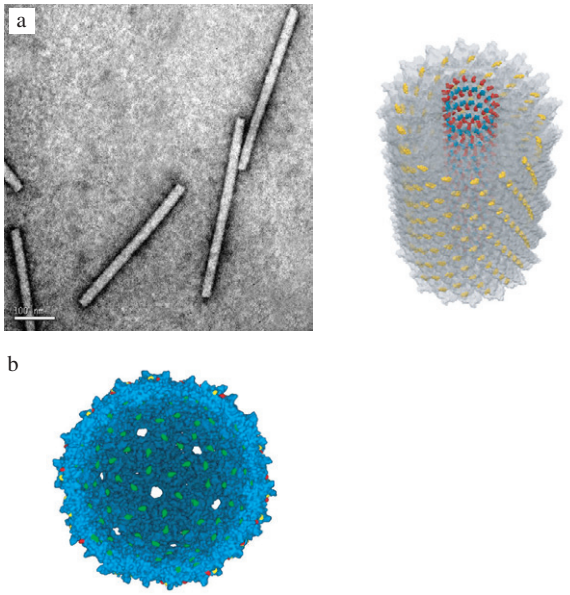

Figure 5. (a) The tubular protein coat of the tobacco mosaic virus, which can serve as a template for inorganic nanotubes. The image on the right shows (in yellow, blue and red) the sites modified by Francis *et al* [68]. (b) The spherical protein coat of the MS2 bacteriophage, showing positions (in yellow) that have been modified by Hooker *et al* to enable covalent attachment of drug molecules [69]. (Images kindly provided by M Francis.)

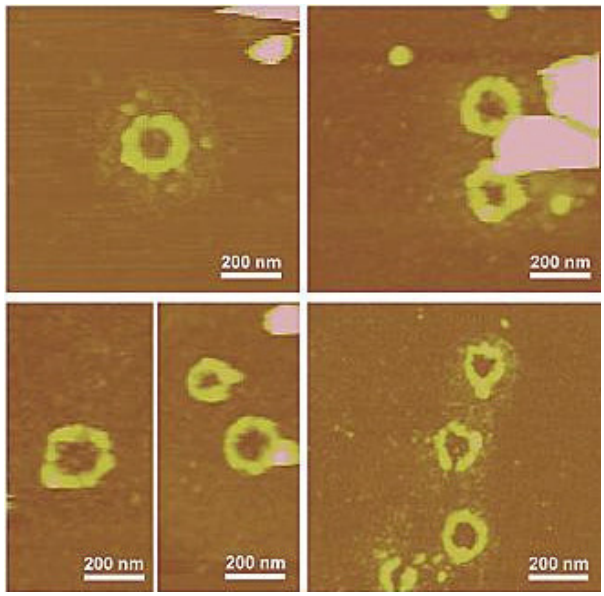

Figure 6. AFM images of ring structures formed by joining the ends of the M13 virus with a linker molecule [71]. Reprinted (in part) with permission from Nam K T *et al* 2004 *Nano Lett.* **4** 23. Copyright 2004 Am. Chem. Soc.

protein subunits. One of the attractions of this self-assembling nanostructure is that the wedge-shaped proteins can also form a variety of other potential template structures, such as shorter tubes and disks, depending on parameters such as pH and ionic strength. Functionalization of these structures with chromophores could provide mimics of the disk-shaped light-harvesting complexes of photosynthetic bacteria. Francis *et al* [69] have also attached acid-labile chemical linker groups to the inside of the spherical virion of the bacteriophage MS2 (figure 5(b)) so that it can be covalently linked to taxol, making the virus a potential vector for transporting this anti-tumour drug in the body.

Lee *et al* [70] combined recognition peptides with the self-organizing property of rod-shaped M13 bacteriophage to arrange inorganic nanocrystals into an ordered superstructure. The viruses spontaneously pack in concentrated solution into a layered, tilted smectic liquid-crystalline phase. When their coats are tipped with a peptide 9-mer that binds to ZnS, the viruses act as 'handles' for arranging ZnS nanocrystals into composite layers with a roughly 700 nm periodicity. Nam *et al* [71] created ring-shaped viruses from genetically modified M13 with two different binding peptides at each end. When a bifunctional linker molecule which binds to the two peptides was added, it secured the flexible rod-shaped viruses into rings about 200 nm in diameter (figure 6). These could be used, for example, as templates for making nanoscale magnetic rings, which are of interest for magnetic data storage.

5. A cell-programming approach to integrated nanosystems

One of the major challenges for nanotechnology is that of reliably and reproducibly synthesizing diverse sets of components and organizing them into functional superstructures. As has been often stressed, this is precisely why biology represents a nanotechnology *par excellence* [37]. Yet little of the work on bio-related nanotechnology to date has given much consideration to the ways that meso- or cell-scale biological organization might be adapted. Perhaps this is not surprising, since the principles of cell architecture and dynamics at these scales are themselves still barely understood—for example, how DNA is packed and unpacked in chromatin. What does seem clear is that the cell is much more than a genetic blueprint for the construction of nanoscale machinery: it also succeeds somehow in combining those components into an integrated system in which time and space are exquisitely managed so that a wide range of functions can be orchestrated and synchronized.

Synthetic biology has the potential to provide access to this super-nanoscale organization—a scale that nanotechnologists will surely need to be able to control. Already, molecular engineers are starting to appreciate this challenge as they seek to integrate several components in functional systems. For example, the ATP synthase rotors devised by Montemagno and coworkers [47] could be powered with fuel generated *in situ* by the same enzyme, perhaps driven by light energy harvested by bacteriorhodopsin and converted to a proton gradient. Can a bacterial cell be adapted to arrange all those components in the necessary manner? More ambitiously, can we imagine designing a cell that will build a genuine photovoltaic cell based on the chloroplast, or a versatile and programmable polymer synthesis factory based on the ribosome?

Mesoscale organization of components may offer not only a way to rationally integrate and coordinate their operation but also improvements in functionality. For example, a spiralling supramolecular arrangement of chlorophyll molecules in the photosynthetic complexes of the algae known as chlorosomes seems to hold the key to their extraordinary light-harvesting capability. These organisms live at very low light levels underneath polar ice, and their antenna arrays can channel light

energy to the photosynthetic centre with barely a single photon wasted [72, 73]. But there is really rather little known so far about how such a superstructure is put together.

One of the lessons from synthetic biology is that the business of control becomes rapidly more difficult as the number of components in the system increases. Attempts to retool *E. coli* so that it synthesizes the antimalarial drug artemisinin, for example, require the expression of ten or so genes at precisely balanced levels [74]. This entails much more than simply adding a synthetic plasmid to the bacteria—one needs to understand the cell circuitry in order to direct the flow of information and energy appropriately.

Yet there has already been a suggestion that engineered cells might be regarded as 'wet nano-robots' [75] that can be programmed with instructions downloaded in genetic cassettes into their genome. To ask whether such micron-scale structures would be truly 'nanotechnological' would be rather to miss the point; the fact is that for many of the prospective functions of such systems, scale is not really the issue. It is not at all hard to envisage bacteria or viruses acting as sensor devices that detect and signal (by fluorescence, say) traces of certain substances in their environment. More startling, perhaps, are possibilities such as programming cells to reproduce the algorithms of cellular automata—an ironic reversal of the metaphor—so that they interact with their neighbours in tightly prescribed ways, allowing them to develop spontaneous patterns, collective and multicelled behaviour, and even forms of computing [76].

It may turn out that, in view of the complexity of most natural cells, more progress can be made by constructing such programmed organisms from the bottom up, from 'minimal genomes' from which all redundant genetic machinery has been removed. The smallest bacterial genome known so far is that of *Mycoplasma genitalium* [77], which has just 517 genes encoded in a genome of 580 000 nucleotides—a good starting point for identifying the smallest genome that will sustain a viable single-celled organism. DNA synthesis technology is now very close to being able to generate a genome this big, and it cannot be too long before a bacterial cell is constructed 'from scratch'. In a minimal organism, the genetic circuit diagram might be simple enough to allow for systematic, rational design of a wide range of functions, such as hydrogen synthesis.

There can be no question that an ability to redesign life is a double-edged sword: it is not difficult to postulate misuses and abuses of synthetic biology that have the potential to wreak unspeakable harm. Engineered cells are precisely the 'green goo' that some environmental groups have presented as nanotechnology's most dangerous and disturbing manifestation [78], and it will surely escape no one's notice that the rogue 'nanobots' of Michael Crichton's thriller *Prey* [79] were manufactured partly with the aid of bacteria. As the young field of synthetic biology emerges and evolves, it will urgently need to address issues of safety, security and regulation [80]. Yet if it fulfils its promise of harnessing the versatility, artistry and inventiveness of life to the engineer's skills of design and planning, nanotechnology is just one of the applied sciences that might never look the same again.

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