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Challenges in Soft Nanotechnology

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Introduction

Why "Soft Nanotechnology"? The word betrays its origins in the confluence of two ideas. The first is the emergence of soft condensed matter as an interdisciplinary area of physics, chemistry and materials science. The term "soft matter" is associated with Pierre Gilles de Gennes, as an umbrella term for all those states of matter – polymers, colloids, liquid crystals, etc – in which typical energies of interaction are comparable to thermal energies. The second idea is the notion of "nanotechnology" itself; this word is associated with the endeavour of making potentially useful structures and devices from components on the scale of atoms and molecules. Thus, in soft nanotechnology, we seek to use our knowledge of the behaviour of soft matter to make from such components useful nanostructures and devices.

The relationship between soft nanotechnology and cell and molecular biology is important and should be stressed at the outset. The structures and mechanisms of cell biology present compelling existence proofs that sophisticated, highly functional nano-scale devices are possible[1, 2]: cell biology is indeed nanotechnology that works. But to understand the mechanisms of cell biology we need ideas and concepts from soft matter physics, together with an appreciation that biological systems possess a complexity not found in synthetic systems.

This suggests that there are two complementary ways of thinking about soft nanotechnology. On the one hand, we can ask, what useful nano-scale constructs and devices can we make from the repertoire of components familiar from soft matter science – for example, polymers, amphiphiles, block copolymers, polymer brushes, colloidal particles, etc. On the other hand, we might also look at the functional features of living cells, and ask which of those features we might hope to emulate in synthetic systems[3].

Whichever way one frames the challenges of soft nanotechnology, one has to appreciate the nature of the physical environment in which one is trying to operate. Assuming that we are operating in water or another liquid solvent, at around 300 K, the dominant feature will be Brownian motion. Thus transport will be essentially diffusive in character, and we expect extended objects such as polymer chains to show a high degree of conformational flexibility. At the nano-scale, strong surface

forces will be the rule. The physics of these situations is characterised by Langevin equations; hydrodynamics is at very low Reynolds numbers and any charge interactions are likely to be strongly screened. A variety of forces of entropic origin will be in play, such as the entropic elasticity of polymer chains, Helfrich forces between fluctuating membranes, and the osmotic effects that underlie phenomenon such as depletion forces. Together, these effects add up to an operating environment very different from anything encountered in macroscopic engineering, and this dictates the need to embrace entirely different design principles.

Some design principles of soft nanotechnology

One ubiquitous theme of soft nanotechnology is the importance of self-assembly as a powerful and scalable method of making nano-scale structures. Equilibrium self-assembly, exemplified by the complex phase diagrams of amphiphiles and block copolymers, is now well understood theoretically (at least in principle, though considerable practical difficulties may still stand in the way of calculating phase diagrams of complex systems). Some of the most elegant and powerful implementations of this principle are now to be found in the field of DNA nanotechnology, where the simplicity and tractability of the base-pair interaction allows complex structures in two and three dimensions to be designed and executed[4]. Biological inspiration also lies beneath the increasing use of proteins and designed synthetic peptides to exploit the motifs of protein folding[5].

Self-assembly can very usefully be thought of in terms of information. Equilibrium self-assembly is defined by the condition that all the information required to make the structure must be encoded in the molecules themselves. Many powerful variants of self-assembly relax this principle in various ways. Templating methods, precursor routes, layer-by-layer assembly, and combinations of self-assembly with top-down patterning, all, in different ways, use external interventions to impose extra information on the system, yielding considerable extra flexibility on the kinds of structures that can be formed. As always, biology offers powerful models; one example is the way tough and insoluble collagen fibrils are formed from the hierarchical self-assembly and subsequent chemical modification of soluble pre-collagen precursors.

To be distinguished both from self-assembly at equilibrium, and in various conditions of restricted equilibrium, are a number of methods of forming nano-scale structures by various types of non-equilibrium pattern formation. These include the intricate structures formed in bio-mineralisation and its synthetic analogues by the interaction of growing crystals with adsorbing macromolecules, and structures that arise as a result of reaction-diffusion systems[6].

Soft matter is characterised by weak interactions – interactions whose energy scale is comparable to that of thermal energy – and it is the shifting balance between different weak interactions in the face of subtle changes in external conditions that gives soft matter is characteristic mutability, leading to organisational and

conformational changes in response to changes in the environment. In aqueous systems, hydrogen bonding plays a central role, both in its direct importance for molecular recognition, and more indirectly through the hydrophobic interaction. It is the subtle interplay of these interactions, together with screened charge interactions, which underlie the phenomenon of protein folding. For an example of a much simpler macromolecular conformational transition, which still illustrates the complexity of these kinds of problem, consider a responsive polyelectrolyte brush – a layer of weak poly-acid or poly-base molecules tethered by their ends to a planar surface, and immersed in an aqueous solution of controlled pH and ionic strength[7]. In outline, the behaviour of such a system is simple to understand – a poly-acid brush in conditions of low pH will be un-ionised; the chains will be relatively hydrophobic and will tend to form a dense layer, collapsed close to the substrate. As the pH is increased, the ionisation equilibrium will shift, the chains will become charged and will stretch away from the surface to form a diffuse and extended layer. But to account for this behaviour in detail is surprisingly complex; one has to take into account the screening of the charge interactions by the counter-ions, the osmotic pressure of those counter-ions, the entropic elasticity of the chains, all in the light of the fact that the degree of ionisation of the chains can vary spatially along the chain, as well as in a global way in response to the applied bulk solution conditions[8].

Mimicking the features of cell biology

There are, of course, a huge variety of living cells in biology, which between them display great diversity of structures and capabilities. A very incomplete list of the sorts of features of cell biology one might, in nanotechnology, wish to emulate might begin with *containment*. Cells are defined by a membrane, which encloses an interior space in which chemical components and systems can be maintained out of equilibrium with the external environment. Containment cannot be complete, of course; the operations of the cell require that both energy and molecules can enter and exit the cell. This traffic must be selective and controlled. The simplest way of achieving some selectivity is by relying on the difference in diffusion coefficient through the membrane between molecules of different sizes and chemical types. Much more sophisticated control of traffic is obtained by selective pores and mechanisms for active transport, as well as mechanisms such as endocytosis by which nanoscale objects are engulfed by invaginations of the membrane, often triggered by very specific molecular recognition events.

Within the cell, the contained chemical species are not merely inert cargo, but undertake a series of complex and linked chemical reactions, which together define the cell's metabolism. An important part of the metabolism is devoted to creating more of the molecules that form the components of the cell. This network of reactions needs a continuous source of free energy.

A living cell, then, is defined by constant flows of energy and matter. Flows of information are important, too; all but the most rudimentary organisms are able

detect aspects of their environment and respond to this. This response may take the form of modifications of their own metabolism (for example the classic example of the lac repressor), of modifications of the environment itself (for example, by the formation of a biofilm) or by the cell physically taking itself off to find a new and better environment, if it is capable of autonomous motility. Typically such a response begins with a sensor molecule, using molecular recognition to detect a certain chemical species.

The response to an environmental cue is medated by chemical signals, and these signals are themselves processed by other molecules. Bray pointed out some years ago[9] that many proteins in cells seem to have as their purpose the processing of information rather than the catalysis of chemical reactions or as structural elements; the property of allostery means that an individual protein molecule can behave as a logic gate, with its catalytic activity being turned on or off by the binding of a regulatory molecule. Such logic gates can be linked together in networks – chemical circuits that can carry out computational tasks of some complexity in response to the original detection of an environmental signal.

What progress has been made in mimicking some of these features of cell biology? The prototype of a biomimetic containment system is the phospholipid vesicle, or liposome, which are now very well studied and used. Analogues of liposomes made from amphiphilic block copolymers – polymersomes – have been attracting increasing interest recently[10]. The variety of different chemistries available and the possibility of controlling the degree of polymerisation of the blocks make possible the rational design of polymersomes. For example, the wall thickness, and thus the permeability to molecular species of various sizes, is directly related to the degree of polymerisation of the hydrophobic block[11, 12]. This is another example of the way that in objects built by self-assembly the specification of the object is encoded in the architecture of the component of molecules. An interesting feature of all types of vesicles is that there is not a strong selection mechanism for the overall size of the object. Thus self-assembly is not by itself sufficient for making a population of vesicles with controlled size distribution; this distribution depends on the details of the preparation technique and is often very broad. One way of achieving a narrow and controlled size distribution of polymersomes is to combine self-assembly with top-down patterning[13]. A block copolymer film is cast onto a substrate with hydrophobic and oleophobic patches; this pattern is reproduced in the polymer film by dewetting. When the film is rehydrated, the surface area of each vesicle is set by the size of the patches on the patterned substrate.

As already mentioned, some degree of control of transport in and out of vesicles can be achieved by varying the thickness and the chemical properties of the wall. To go beyond this, one can envisage incorporating pores in the walls, which it might be possible to open and close in response to chemical signals. An exemplar of this approach encapsulated a cell-free protein expression system derived from E. coli within phospholipid vesicles incorporating pore-forming proteins [14]. A wider variety of nano- and micro- scale enclosed reaction systems is reviewed in [15].

Moving from metabolism to molecular information processing, some examples of synthetic molecular logic devices have been reported [16]. These are likely to lead to new sensors of increasing sophistication. However, many of these systems are characterised by the fact that their output takes the form of a fluorescent signal, rather than a chemical signal. This limits the extent to which such logic elements could be built up into large-scale networks like the cell signalling networks of biology. Synthetic DNA-based systems currently seem to offer the best hope for building molecular logic systems [17].

The particular problems of motility at the micro- and nano- scale stem from the special features of hydrodynamics at low Reynolds number, as emphasised in Purcell's classic paper. This emphasises the need to break time symmetry in order to achieve motion; recently a number of elegant theoretical papers have explored various ways of achieving this. The most interesting types of synthetic micro- and nano- scale motors will use chemical energy to drive directional motion, as biological motor proteins use the energy of ATP[18]. One potential class of synthetic systems is built on the fascinating chemistry of catenanes and rotaxanes[19], while exciting progress is being made demonstrating motors based on DNA[20], which provide a different implementation of basic idea underlying the operation of protein molecular motors – a coupling of the conformational change of a macromolecule with the catalysis by that macromolecule of a chemical reaction. The coupling of macromolecular conformational change with a cyclic chemical reaction also underlies experiments in which responsive polymers, such as weak polyelectrolytes, change shape in response to a cyclic chemical reaction[21, 22]. In these systems, however, the coupling between the chemical reaction and the conformational change is only indirect, in contrast both to the DNA-based systems and biological motors.

One other class of systems that can convert chemical energy into mechanical motion of micro-scale objects relies on a phoretic response to a self-generated chemical gradient that arises from an asymmetrically localised chemical reaction[23-25]. The mechanisms of this motion may be electrophoretic or diffusiophoretic in character; in the case of motion driven by self-diffusiophoresis there is some theoretical understanding which is at least consistent with experimental data[26]. These autophoretic motions result in the propulsion of a particle at a velocity which depends on the rate at which reaction products are generated, but it is important to recall that this process takes place in a Brownian environment, in which the orientation of the particle randomly changes over a rotational diffusion time which has a strong dependence on particle radius. This means that if one characterises the motion of such particles, one sees a cross-over in the type of motion[25]. At short times, transport is ballistic, but at the rotational diffusion time there is a crossover to diffusive transport, resulting from a random walk with a step size proportional to the propulsion velocity, and a diffusion coefficient that may be substantially enhanced over the classical Stokes-Einstein value. The degree of this enhancement, and the length of the window of time in which ballistic behaviour is observed,

depends strongly on the size of the particle. Thus to use these mechanisms for particles whose size starts to fall below the micro- to the nano- scale will require chemical reactions that generate products at a considerably higher rate than the reactions that have been looked at so far. The other great challenge is to achieve some degree of directionality and purpose to the motion. Mimicking the ability of some bacteria to undergo chemotaxis, for example, poses an attractive target to which some progress has already been reported[27].

What soft nanotechnology can and cannot now do

One way in which the comparison between soft nanotechnology and the structures and mechanisms of cell biology is helpful, though sobering, is that it emphasises the gulf between what must be possible in principle, as demonstrated by the example of biology, and what we can actually do.

The use of self-assembly in its various forms to generate useful and interesting nanostructures is now well developed, backed by considerable theoretical understanding and a growing set of design rules. There is progress towards designing nano- and micro- scale encapsulating systems, while the principles of using conformational change and osmotic effects such as phoresis to generate motility are beginning to be explored. But the development of analogues of biological systems for chemical sensing and information processing has only just begun. A number of fundamental theoretical and practical issues in soft nanotechnology remain to be addressed; the theoretical basis for understanding small systems driven far from equilibrium remains underdeveloped. It seems likely that the design of complex bio-mimetic nano-systems will require the use of evolutionary design methods, given the size of the configuration spaces that need to be explored. Finally, the intricate mechanisms that underlie the ability of biological systems to self-replicate seem, currently, to be quite out of reach to any synthetic system.

To conclude, it is worth reflecting on the "technology" aspects of soft nanotechnology – those areas of potential application that will drive the development of some of these ideas to become the basis of useful products. The science of soft matter originally found its applications in the chemical industry, and in applications such as home and personal care. In these areas, the scalability of self-assembly is what makes it possible to contemplate what is really quite sophisticated control of nanostructure, and in some cases a degree of environmental responsiveness, in products that are sold at very low cost. Higher margins are possible in materials that are used in information technology, and we are seeing the use of self-assembling structures in materials like high performance dielectrics and for information storage. The drive to decarbonise our energy economies will put a premium on being able to make scalable and cheap nanostructures for applications such as batteries, fuel cells and new photovoltaic materials. But one of the most compelling answers to the question "why nano?" must take us back again to biology. The most basic operations of cell biology take place at the nano-scale, so the nano-

scale is the appropriate length scale for intervening in biology – this is the fundamental motivation for the idea of nanomedicine. Thus we can expect to see the most compelling applications of soft nanotechnology in medicine. We are already seeing applications in areas such as drug delivery, regenerative medicine and sensors and diagnostics. However, one should not underestimate the difficulties, and the timescales for applications of some of these ideas may be long.

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