Nano Approaches To Using Nucleic Acids

One of the most fascinating aspects of nanotechnology comes from the designs that nature provides. The worlds of DNA and RNA nanoscience have presented many examples of how naturally occurring motifs used in the genetic code can also be engineered to design complex and detailed self-assembled structures with molecular precision.

In this issue of *ACS Nano*, Guo and co-workers[1] once again demonstrate the elegance that nature itself can provide with these biomolecular components. Nanomotors are an area of intense interest to our community because they can be used to move, to track, or to guide molecular elements for assembly or manipulation of structures. One nanomotor of particular interest is the phage DNA packaging motor, capable of assembling and packing highly charged double-stranded DNA into a pre-existing viral capsid; although much is known about this motor's individual components, the mechanism for its motion was not understood, and thus, the nanomotor could not be employed for the engineering of new synthetic structures. A barrier in understanding the process related to the idea that charged DNA must be rotated while packaged, which would lead to issues such as friction and supercoiling of the DNA chains. Here, it is revealed that nature has a much more elegant means of moving the DNA double strand forward; through the formation of a hexameric ring scaffold composed of RNA, ATPase, and a protein connector, the chain is gently revolved around and gradually moved forward with each step, without axial rotation of the dsDNA backbone. A series of ratchet-like ATP-powered steps shift the binding of the molecule up and along the helix during the revolution, thus, avoiding the issues and difficulties of packaging *via* a rotational, molecular twisting mechanism; notably, the procession is unidirectional by nature. If we can translate this understanding toward the manipulation of biomolecular building blocks, we may find unique means of generating nanomolecular structures or creating nanostructures that can routinely manipulate large biomolecular species in a controlled fashion. This kind of approach may even potentially be harnessed to generate nucleic acid delivery systems that assemble *in vivo*.

On the other hand, a great deal of effort has recently been focused on the delivery of nucleic acids such as RNAi, DNA, and micro-RNA using synthetic carriers that replace the viral systems used more dominantly in biological and medical research. Ideally, by understanding the nature of nucleic acid encapsulation and release by synthetic vectors, we can better design and engineer them for optimal success. Zuhorn and co-workers[2] provide a critical window into understanding the mechanisms of delivery using a systematic study with the advanced imaging capabilities of spinning disk confocal microscopy. They compare transfection achieved using lipid-based cations like Lipofectamine with polymeric systems with buffering amine groups such as poly(ethyleneimine), and show that there are critical differences in the mechanisms and kinetics of endosomal escape from these two types of vehicles. They observe that the polymeric systems exhibit endosomal bursts yielding large-scale release of nucleic acids following an initial incubation period of 30−50 min; furthermore, these nucleic acids are released free of complexation with the polymer, which is also ejected into the cytoplasm. This result is in stark contrast to release from lipoplexes, which exhibit an immediate but gradual and sustained release from endosomes over time. In both cases, short oligonucleotides diffuse directly and rapidly into the nucleus, whereas plasmids do not readily diffuse to the nucleus and are taken up in much lower amounts. Mechanisms related to membrane pore formation in the presence of the lipoplexes *versus* proton buffering in the case of endosomal systems address many of the proposed mechanisms that have been posited but not fully proven in past literature. Perhaps more importantly, there are potential design principles that may be shaped based on studies such as these. For polyplex systems, which are often deemed safer for *in vivo* applications, it appears that for many cells, as few as one nanoparticle per cell may

Published online May 28, 2013
10.1021/nn402448e

be responsible for transfection; this implies that when targeting multiple genes, multicomponent nanoparticle formulations may be key for efficacy, and achieving high loadings per nanoparticle may also be a factor of consideration.

Of course, there are more direct ways of delivering DNA to cells, and such methods may well be important for *in vitro* cell studies, the design of cell or tissue-on-a-chip platforms, and other dynamic two-dimensional or three-dimensional formats for cell culture. The Melosh research lab[3] presents a highly effective and nondestructive means of transfection by bypassing the membrane altogether. Small diameter "nano-straws" are grown from transparent conducting substrates, and cells are seeded on these platforms in culture. When a low field is applied across the electrode, it is applied across each nanostraw, and nanoelectroporation coupled with diffusion enables solutions of DNA to enter the cell directly from microfluidic channels. This approach enables the introduction of different DNA plasmids in sequence or together, and the precise control of dose. The process only opens the membrane temporarily upon application of field, and removal of the field leads to the resealing of the cell membrane; thus, cell viability is maintained in these systems.

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Professor Paul Mulvaney of the University of Melbourne joins ACS Nano as an associate editor.

Nanoscience continues to provide new means of helping us understand, manipulate, and engineer nucleic acid systems. Whether we use this knowledge to build beautiful and complex nanoscale systems, to generate new biological tools for knowledge, or to design new delivery methods for therapeutic applications, nanoscale methods will play key roles for us now and in the future.

This month, we are delighted to welcome Prof. Paul Mulvaney of the University of Melbourne as an associate editor. Prof. Mulvaney is an expert, and a frequent contributor to *ACS Nano*, on the subject of semiconductor and metallic nanoparticles.[4–8] He is a professor of chemistry at the University of Melbourne.

Disclosure: Views expressed in this editorial are those of the author and not necessarily the views of the ACS.

Paula Hammond
Associate Editor

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