cyclic-di GMP observed by Hoffman *et al.* is the opposite of that observed in most prior studies. The prevailing, though not exclusive view, is that elevated intracellular levels of cyclic-di-GMP are associated with increased synthesis of extracellular polysaccharides and enhanced autoaggregation or biofilm accumulation. Hoffman and colleagues acknowledge this discrepancy and suggest that the regulatory function of cyclic-di-GMP is complex. Given this complexity, it may be premature to prescribe therapeutic manipulation of cyclic-di-GMP for managing infections.

How might Arr contribute to biofilm formation and biofilm biology? A mutation in *arr* does not appear to influence biofilm development via effects on flagella or pili, two types of surface appendages involved in biofilm formation by *P. aeruginosa*. Nor does *arr* appear to alter the rate of formation of small colony variants, another factor that can contribute to biofilm formation and biofilm antibiotic resistance[7]. The authors demonstrated no apparent contribution of the polysaccharide alginate, but they did not rule out the possibility that Arr regulates the production of extracellular polysaccharide matrix encoded by two recently discovered loci[8,9]. It is also possible that tobramycin stimulates a previously uncharacterized biofilm production pathway in *P. aeruginosa*—a hypothesis that remains to be tested.

Mutation of *arr* has no apparent effect on the ability of *P. aeruginosa* to form a biofilm, at least as assessed in a static model of biofilm formation. Interestingly, in addition to the lack of stimulation in the *arr* strain upon treatment with low levels of antibiotic, the biofilm produced by an *arr* mutant was ~100-fold more susceptible to killing by high levels of tobramycin than the biofilm formed by the wild-type bacterium. There was no difference in planktonic resistance of the wild-type bacterium and the *arr* mutant. Therefore, *arr* provides a second example of a gene involved in a biofilm-specific pathway of antibiotic resistance[10]. In contrast with the *ndvB* locus, which encodes a glucosyltransferase required for the synthesis of periplasmic glucans, it is not clear how Arr mediates this resistance.

The widespread use of implants in modern medicine means that addressing the issue of biofilm-based infections is of increasing importance. Although little is known regarding mechanisms of biofilm tolerance, it is becoming increasingly clear that the mechanisms underlying this protection are distinct from classical mechanisms of antibiotic resistance described for planktonic cells. As long as large pharmaceutical companies continue to abandon their anti-infective programs and medical device companies are reluctant to invest in novel anti-infective coatings, it will remain the purview of small, innovative biotechnology companies to discover new approaches to eliminating biofilm-based infections.

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A classic assembly of nanobiomaterials

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A recent multidisciplinary conference in Crete underscored advances in protein and peptide self-assembly in a variety of biotechnological applications.

In antiquity, Crete was known as the crossroads of the Mediterranean. Minoan culture was enriched and invigorated by its diverse mix of cultures and thriving commerce with Africa, Europe and Asia. This ancient crossroads was somewhat fitting therefore as a backdrop for a conference that sought to bring together scientists from several different disciplines—biology, chemistry, physics and engineering—and from different continents to discuss diverse aspects of self-assembling biomaterials[1]. The result was very much more than the sum of the parts. And despite the nascent nature of the field, already several tangible applications appear within reach in areas as diverse as materials and biological patterning, adhesives, antimicrobial agents and hydrogels.

In order for peptide self-assembly[2] to be better understood, it will be important to understand how small proteins fold *in vivo* and *in vitro*. One means of accomplishing this is to obtain atomic resolution structures of the entire folding pathway from the denatured state, through to the intermediate and transition states, to the final, native state by incorporating nuclear magnetic resonance (NMR), systematic mutational analysis and molecular dynamics simulation. This combination of methods makes it possible to ascertain protein folding conformations even though most transitional states are fluctuating rapidly and can be so short-lived that they cannot be observed by NMR alone. Using this multidisciplinary approach, the structure of an unfolded protein can be determined under physiological (nondenaturing) conditions—an essential step for understanding how proteins unravel in the cell (Alan Fersht, University of Cambridge, Cambridge, UK). Because most diseases involving protein misfolding result from a native protein that first unfolds and then refolds incorrectly or aggregates, the application of this approach to small self-assembling peptides may facilitate the design of drugs that inhibit unfolding and therefore stabilize the native form of the protein. Thus far, there has been excellent agreement between experimental benchmarks and previous simulations[3]. In a related area, parallel studies on folding and assembly of natural triple-β-stranded proteins extracted from viruses have led to the

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discovery of unusual fibrous folds. X-ray diffraction coupled with Raman spectroscopy suggests that cross-β-amyloid-like conformations exist in these proteins. These can serve as synthetic peptide models for investigating amyloid proteins.

From a biotechnological standpoint, there is hope that this increased knowledge of the processes underlying protein fibril and aggregate formation can be exploited in the design of novel artificial biomaterials. By precisely controlling dimensions and properties[4], it is hoped that proteins can be coaxed to adopt the form of fibrils, tubes, sheets or monolayers. One intriguing application mentioned at the meeting is the use of protein fibrils as biological scaffolds for cell attachment, biomineralization or metallization templates (Anna Mitraki, University of Crete, Greece). In one example of the approach, nanotubes assembled from a dipeptide from the Alzheimer β-amyloid diphenylalanine were later used to cast silver nanowires[5].

Material patterning can also be achieved using the crystalline bacterial cell surface proteins or S-layers, which conveniently form monomolecular arrays with the inherent capacity to crystallize on different surfaces (including silicon wafers) and interfaces. Such functionalized S-layers are being exploited for the development of supramolecular materials and to enable the patterning of nanoscale devices, as demonstrated by the well-defined deposition of metal nanoparticles across macroscopic substrate areas for use in nonlinear optics and molecular electronics[6] (Uwe Sletyr, University of Natural Resources and Applied Life Science, Vienna, Austria). One advantage of this approach is that it can be precisely controlled at the tens of nanometers scale.

Apart from patterning, there is also optimism that self-assembling nanobiomaterials may produce substances with novel elastic or adhesive properties. By studying the biochemistry of the unique sessile crustacean sea barnacle *Megabalanus rosa*, advances have been made in designing adhesives for use at a liquid-solid interface. Barnacles attach very tightly to a variety of surfaces using a self-assembled six-protein complex. Each protein specializes in at least one function and together they cooperate to achieve underwater attachment. Three such recombinant proteins (Mrcp-16k, Mrcp-20k and Mrcp-19k) have been prepared under physiological conditions and shown to have distinct functions, such as specific adsorption to calcite following roughening of the substrate (Kei Kamino, Marine Biology Institute, Heita, Japan). This bioadhesive protein is a good model upon which to design self-assembling peptides used to strongly bind heterogeneous surfaces in aqueous environments, such as dental and other tissues, to facilitate protein localization in water-bathed surfaces in a controlled orientation, or to bind heterogeneous materials such as metals and plastics underwater.

Cross-disciplinary science of classical quality. The attendees of the Fourth Multidisciplinary Workshop: Self-assembling Peptide and Protein Systems in Biology, Medicine and Engineering.

Another area of application for synthetic self-assembling peptides is antimicrobial agents. For instance, the designed cyclic D,L-α-peptides that self-assemble into nanotubes can selectively target bacterial membranes or viral invasion pathways[7]. One barrier to such therapeutics is the cost of synthesis—a problem that is being addressed by designing facially amphiphilic antimicrobial polymers[8] (William DeGrado, University of Pennsylvania School of Medicine, Philadelphia, PA, USA). These polymers mimic the effects of traditional peptides but can be inexpensively synthesized from simple chemical monomers, such as arylamide. In addition, amphiphilic self-assembling beta-tape forming peptides have been observed to assemble into transmembrane porin-like beta-barrels that act as ion channels. These peptides not only induce well-defined ion currents in model lipid bilayers but also exhibit ion-selectivity that is dependent on the peptide primary structure and can be particularly useful for understanding amyloid diseases[9] (Amalia Aggeli, University of Leeds, Leeds, UK).

Self-assembling peptides with a high propensity to form transmembrane ion channels in bacterial cell membranes could lead to a new generation of antimicrobial gel formulations. Alternatively, those peptides with a low tendency to form channels could potentially be used in the production of hydrogels made of entangled micrometer-long tapes and higher order aggregates, such as ribbons and fibrils. Such hydrogels are currently being used as biocompatible matrices for tissue engineering and in the creation of three-dimensional culture systems for tumors (Ingemar Ernberg, Karolinska Institute, Sweden).

The experimental techniques and computational and theoretical methods discussed during this multifaceted meeting were as diverse as the backgrounds of the contributors. The meeting emphasized perhaps that the biological and physical disciplines are coming to their own crossroads, one that will enable nanobiomaterials research to become more mature and more vibrant. The citizens of ancient Heraklion would surely have approved.

As the quest for bioderived biomimetic and bioinspired materials continues, it becomes clear that no one traditional scientific domain dominates. The fusion of disciplines has already begun in this emerging field of inquiry. Dare we call it self-assembly?

1. Fourth Multidisciplinary Workshop: Self-assembling Peptide and Protein Systems in Biology, Medicine and Engineering, Capsis Hotel, Heraklion, Crete, Greece, June 25–28, 2005. http://web.mit.edu/lms/www/lmshome.htm
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