Expert Opinion

1. Introduction
2. Biomimetic synthesis and assembly
3. Biological assembly and structures as biomimetic systems
4. Biohybrid structures as biomimetic systems
5. Synthetic structures as biomimetic systems
6. Expert opinion

Applications of biomimetic systems in drug delivery

Siddarth Venkatesh, Mark E Byrne, Nicholas A Peppas & J Zach Hilt

This review article highlights recent activities in the field of biomimetic systems and their application in controlled drug delivery. A definition and overview of biomimetic processes is given, with a focus on synthesis and assembly for the creation of novel biomaterials. In particular, systems are classified on the basis of three subsets, which include biological, biohybrid and synthetic structures. Examples focus on the current and proposed clinical significance for systems that mimic processes where the underlying molecular principles are well understood. Biomimetic materials and systems are presented as exceptional candidates for various controlled drug delivery applications and have enormous potential in medicine for the treatment of disease.

Keywords: analyte sensitive gel, biohybrid, biomaterial, biomimetic, configurational biomimesis, conjugated biomaterial, controlled release, drug delivery, molecular imprinting, oligonucleotide, pulsatile release, sustained release, synthetic peptide, targeted delivery

Expert Opin. Drug Deliv. (2005) 2(6):1085-1096

1. Introduction

Biological systems and physiological processes have evolved over millions of years to have precise properties and functions. Today, numerous engineers and scientists have an appreciation for the sophistication of biological systems, and they look to these for inspiration in their own research efforts. By studying and mimicking complex biological structures and processes, researchers design materials with enhanced properties, and can provide solutions to fundamental problems in medicine, pharmaceutics, cell biology and biophysics.

Biomimetic methods and materials are a fundamental example of the synergistic opportunities that are present at the interface of biological systems and nanotechnology, the broad and fascinating field of bionanotechnology [1]. In the context of this article, the authors offer a critical review of recent literature within the biomimetic field, and specifically, provide highlighted examples in drug delivery and direct the reader to key articles and reviews in the literature.

2. Biomimetic synthesis and assembly

In nature, molecular recognition leads to the assembly of macromolecular and multimolecular structures, and orchestrates complex processes through myriads of molecular motors, metabolic pathways and cyto-trafficking species. These recognition processes are tightly regulated, and rely on numerous interactions at the atomic level and cumulative interactions between secondary structures. Proteins, metal ions, nucleic acids, steroids and carbohydrates interact with each other in exquisite mechanisms that are only now coming to light using structural and genetic approaches (e.g., elucidation of various signal transduction processes, binding mechanisms and so on).

To illustrate the sheer efficacy and precision of recognition at the cellular and subcellular level, one need go no further than the highly versatile proteins. Proteins build and repair cellular structures and genetic material. As enzymes they control and catalyse an incredible number of biochemical reactions with high specificity. As hormones, they regulate the signal transduction pathways, which transport respiratory

Ashley Publications
www.ashley-pub.com

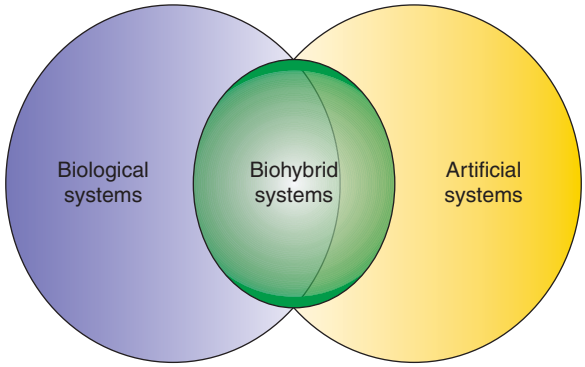

Figure 1. Classification of biomimetic systems. Biomimetic systems can be classified as biological, synthetic, or biohybrid. Biohybrid systems reside at the intersection of artificial and biological systems.

gases and other metabolites and are responsible for muscular contraction. They fashion the adaptive arm of the immune system by forming a vast repertoire of antibodies and serve as the major histocompatibility complex (MHC) molecules, which are displayed on cell surfaces and are responsible for antigen presentation and lymphocyte recognition, offering protection against antigens of varying pathology. They form integrins, which help in cell adhesion and signalling. All this is possible due to the conformational complexity provided by chaperone-mediated protein folding and post-translational modifications.

What principles steer biologically inspired researchers today? Biomimesis is the process of coordinating molecular recognition and interactions to design materials that can be structurally similar to and/or function in similar ways as biological structures [2-8]. Central to the concept of biomimesis is the need of a firm command of the internal machinery of the cell and cellular systems. Vital questions in understanding biomimesis are: how cells grow and divide, how they regulate metabolic fluxes, how they move and interact with one another, how hormones affect signal transduction pathways, and how the cell responds to the environment (i.e., how cells interact with their extracellular matrix and so on).

To clarify the context and definition of biomimetic systems, the authors now describe some of these systems, and their subclassifications (Figure 1). Biomimetic systems may be classified as biological, biohybrid and synthetic structures. Biological structures denote materials consisting of natural biological molecules such as proteins, DNA, RNA, and/or unnatural biomolecules that have been assembled/synthesised by biological systems, such as unnatural amino acids prepared via genetic engineering methods. The biohybrid structures comprise materials that combine synthetic structures (e.g., polymeric chains, metal particles and so on) and natural biological molecules. Lastly, synthetic structures represent materials based on man-made building blocks, such as synthetic polymers and unnatural amino acids (i.e., prepared *in vitro* such as solid-phase synthesised peptides).

To create biomimetic systems, one can use and mimic biological processes and interactions where the underlying molecular principles are understood. This is illustrated by DNA-mediated assembly and nanoconstruction [9-11], where the complementary and well-understood pairing of the genetic alphabet is exploited. These robust interactions, responsible for the faithful preservation of the genetic materials, are excellent targets for biomimesis.

3. Biological assembly and structures as biomimetic systems

Nature arranges the subcellular world in many ways. Mimicking this spontaneous organisation remains the endeavour of scientists and engineers in the field. For the fabrication of novel biomaterials, natural building blocks (e.g., peptides, proteins and lipids) and natural processes (e.g., self-assembly and genetic engineering) have been used. In a recent review, Zhang [8] highlights the use of biological components and processes to fabricate various nanostructured materials, such as nanofibre peptides and protein scaffolds.

Peptides with precise sequences can be synthesised on a solid resin (these are discussed in Section 5), or by genetically engineering bacteria, and these can be made with unusual architectures (e.g., branching and rings) or with unnatural amino acids [7]. In particular, the genetically engineered systems have been demonstrated as a powerful method to generate macromolecules tailored at the molecular level resulting in desired properties [2-5,8]. Novel protein structures that are created within living organisms are expected to have a large impact, facilitating the creation of novel materials with tailored structure for enhanced properties, such as specificity, stimulus-sensitivity and catalytic aptitude. These biomimetic and functional architectures have wide applicability in biomaterials and, specifically, in targeted drug delivery applications.

Ghadiri *et al.* [12,13] synthesised nanotubes from the ring arrangement of cyclic peptides, which show antimicrobial [14] and ion channel properties [15] (Figure 2). A recent success includes the demonstration that cyclic peptides, when used as adaptors in β-barrel shaped α-haemolysin, can be used for single channel detection and quantification of molecules.

For many years, the regeneration of damaged spinal cords has been a research goal. Silva [16] developed a fibrous scaffold, which assembles itself at the site of injection. The scaffold, formed by a fluidic solution of peptide amphiphiles, starts from an epicentre and spreads out in all directions, thus resembling the neuronal morphology. During the self-assembly, major amino acids, essential for neuronal growth, proliferate on the surface of the structure [17]. In addition, growth of astrocytes, which are large neuroglial cells, is inhibited, thus enhancing spinal restoration.

Over the last 20 years, bioadhesion has been a focus area of drug delivery research [18-22], allowing for enhanced control over drug delivery (e.g., site-specific adhesion and increased

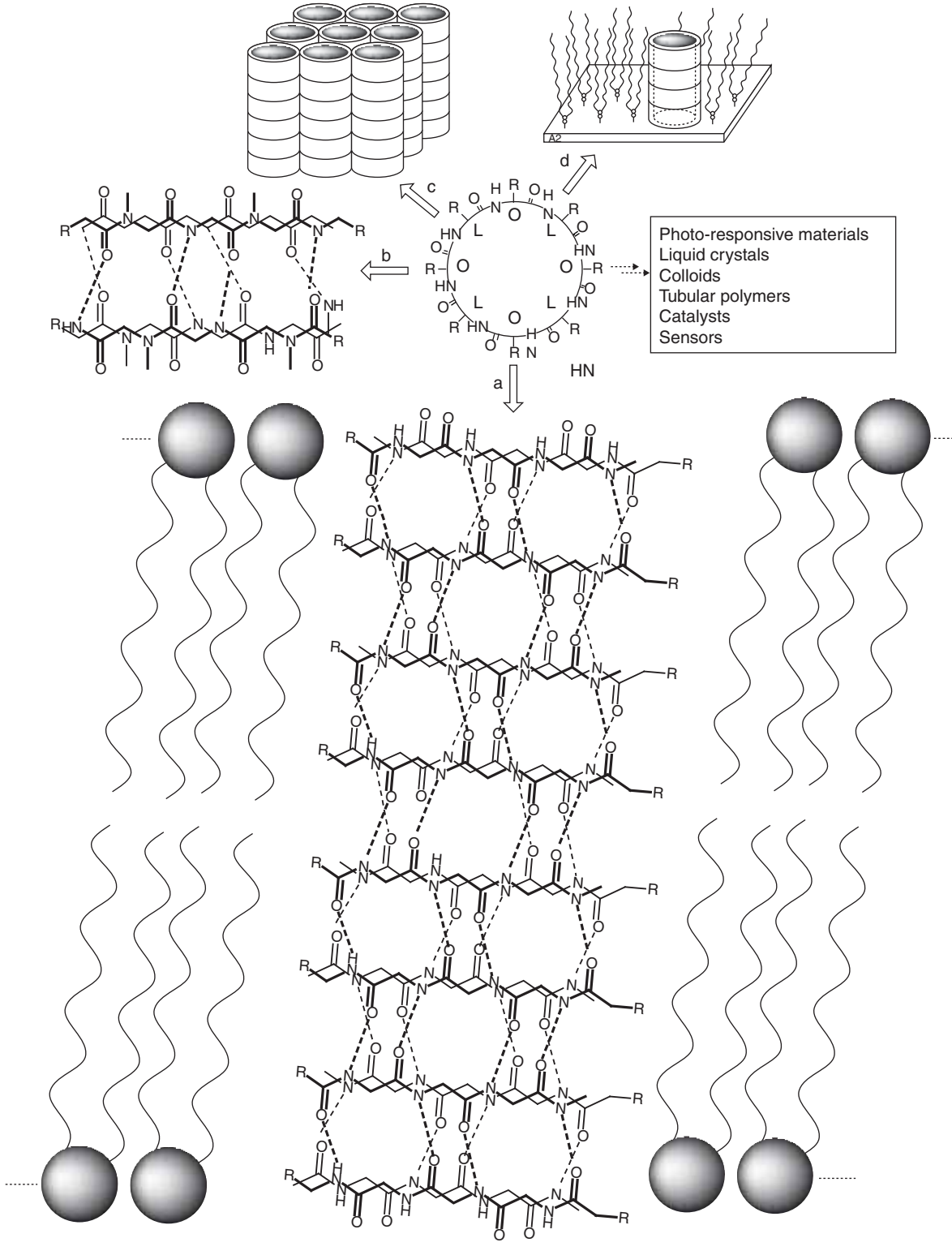

Figure 2. Transmembrane ion channels and pore structures adopted by a ring conformation of cyclic D, L peptides. Cyclic peptides are adaptors used in single molecule detection and quantification. *Chem. Eur. J.* **4**(8):1367-1372 Copyright (1998), with permission from WILEY-VCH Verlag.

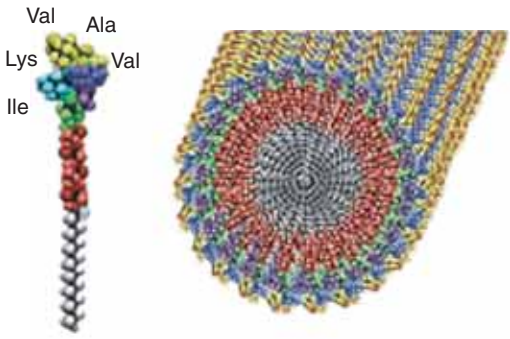

Figure 3. Self-assembled amphiphilic nanostructures. Biomimetic peptide amphiphiles of Ile-Lys-Val-Ala-Val sequences have great potential in tissue regeneration of damaged spinal cords. Reprinted (abstracted/excerpted) with permission from SILVA GA, CZEISLER C, NIECE KL *et al.*: Selective differentiation of neural progenitor cells by high-epitope density nanofibres. *Science* (2004) **303**(5662):1352-1355. Copyright (2004) AAAS.

residence time). Recently, there has been a particular focus on integrins, which are cellular adhesion molecules [23,24]. Yu *et al.* [25] created peptides for studying interactions between amino acids, for testing them as biomaterial coatings and for use as drug delivery devices (Figure 3). Melanoma cells spread indiscriminately on carboxyl-coupled Arg-Gly-Asp amphiphiles and did not spread on amino-coupled Arg-Gly-Asp amphiphiles, showing that they are effective cellular recognition agents.

Wu *et al.* [26] used a single-chain, single-gene approach to produce genetically engineered antibodies (i.e., chimeric structures) to provide immunotherapeutic treatment of cancer. Novel fragmented antibody constructs such as single chain Fv (scFv) with human IgG1 hinge and Fc regions (scFv–Fc dimers) are designed to produce molecules for the delivery of radionucleotides to tumours with reduced immunogenicity, increased circulation life, and gained effector functions [27].

4. Biohybrid structures as biomimetic systems

Integration of biological entities with synthetic materials can create biomimetic systems that use biological mechanisms (i.e., enhanced affinity and specificity of binding, catalysis) while enhancing physiochemical properties such as mechanical stability, *in vivo* lifetime, degradability and so on. Biohybrid structures can also be created by synthesising macromolecules with a combination of natural and unnatural building blocks and/or sequences (i.e., aptamers that are synthetic oligonucleotides, which bind nucleic acids, proteins, small organic compounds and so on).

Biologically active molecules can be incorporated into polymer networks (e.g., physically or chemically entrapped) to produce conjugated biomaterials. In a recent review [28], the field of stimuli-responsive polymers and their bioconjugates is detailed, and their potential applications in drug delivery are highlighted. In many situations, localised delivery is critical for a therapeutic molecule to have optimised effect. Biologically active entities conjugated to polymer networks have applicability in targeted delivery of therapeutic compounds [29]. An example of biomimetic targeting is via glycoprotein mimics. Researchers have synthesised glycopolymers that function as a synthetic multivalent ligand able to facilitate site-specific drug delivery [30].

Lackey *et al.* [31] demonstrated a functional biomimetic system, which involved the use of a pH-responsive polymer as a membrane-disrupting agent that aided the transport of antibody conjugates from the endosome to the cytoplasm. This work highlights the use of endosomal releasing agents for targeted therapeutics that are internalised through receptor-mediated endocytosis.

Wang and colleagues [32] have prepared copolymers of metal-chelating hydrophilic monomers and coiled coils, which are thermo-sensitive due to the latter (Figure 4). The backbone consisted of N-(2-hydroxypropyl)-methacrylamide and the metal chelating (N′,N′-dicarboxymethylaminopropyl)methacrylamide. The thermosensitive coiled coils used to assemble the hydrogels were CC1 and CC2. The CC1 sequence has biological importance, and is part of the sequence of the microtubule motor protein kinesin, which induces cytoskeletal gliding through ATP hydrolysis, essential for fundamental processes such as chromosome motility during mitosis and meiosis. Hence, genetic engineering can aid in the design and tailoring of stimuli-sensitive hydrogels with well-defined volume transitions based on pH and temperature changes in the environment.

Ehrick and colleagues [33] have produced stimuli-responsive hybrid materials consisting of hydrogels and genetically engineered protein. The stimuli-responsive hydrogel exhibited three specific swelling stages induced by conformational changes and binding affinities of the protein in response to various ligands. The authors showed gating and transport of biomolecules across a polymer network, demonstrating a large potential in microfluidics and drug delivery.

Petka and colleagues [34] have prepared thermally reversible hydrogels from proteins obtained from chimeric genes (Figure 5). They inserted custom oligonucleotide sequences into polylinker regions of the cloning vector pUC18 and then directionally ligated into the expression vector PQE9, using EcoRI and HindIII. The genes coded for a protein that consisted of a leucine zipper and polyelectrolyte domains. The triblock structure had a few interesting features, including a terminal cysteine residue, intended for disulfide bridging between individual strands and a His-tag at the N terminus, so that it could be purified by affinity chromatography. They observed coiled-coil formation at low pH, and protonation of the Glu residues at higher pH, leading to dissociation of heterodimer. The triblock protein underwent bridging and

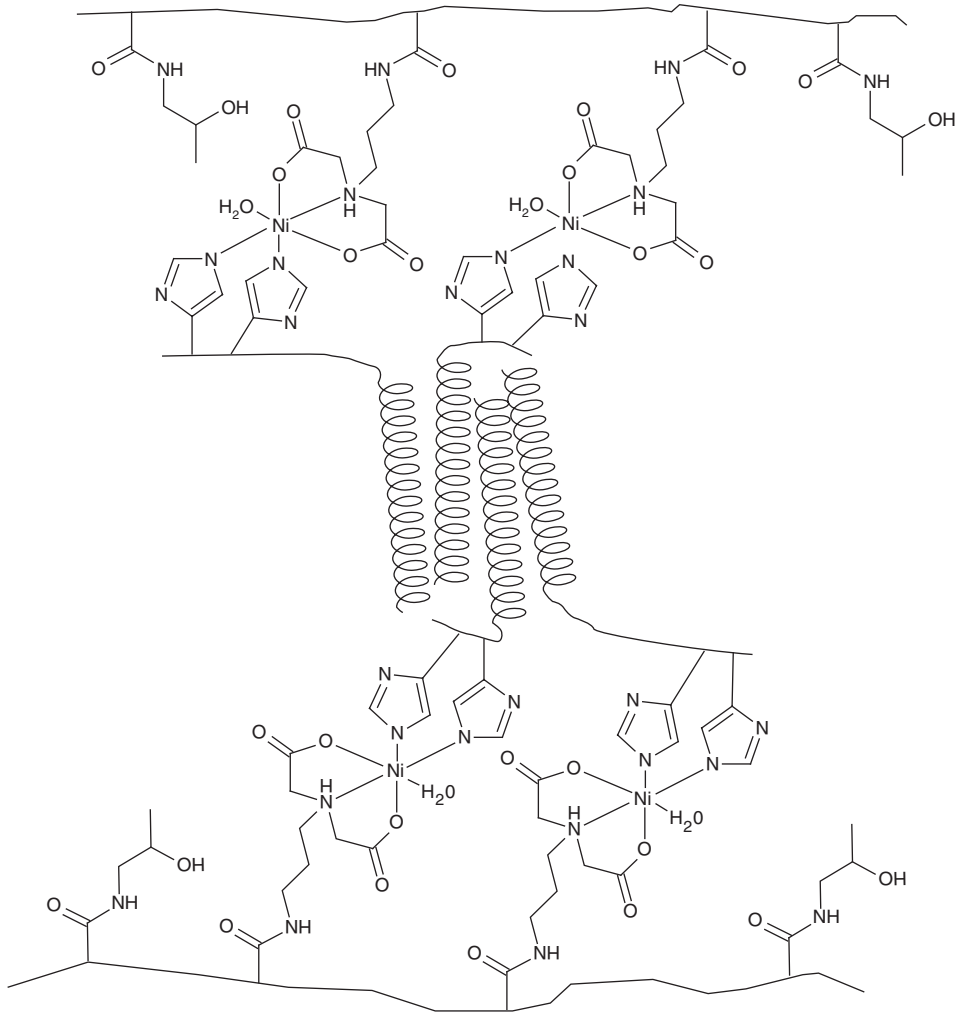

Figure 4. Hydrogels assembled from His-tagged coiled coils. Highly tunable α-helical coiled coils are used as crosslinks to prepare hybrid hydrogels. Reprinted with permission from WANG C, STEWART RJ, KOPECEK J: Hybrid hydrogels assembled from synthetic polymers and coiled-coil protein domains. *Nature* (1999) **397**(6718):417-420. Copyright (1999) NPG.

formed complex networks. The fact that the biocompatibility of these gels was high due to the poly(ethylene glycol) (PEG)-rich region in the polyelectrolyte domain, and that the switching of these gels can be controlled by crafting custom sequences, lends them great potential in controlled release.

Using oligonucleotide bridges, dendrimers can be used as imaging and therapeutic agents. Choi and colleagues [35] have designed a DNA-driven dendrimer assembly (Figure 6). Dendrimers conjugated to different biofunctional moieties were linked together using complementary DNA oligonucleotides, which produced clustered molecules targeting cancer cells that overexpress the high-affinity folate receptor.

In the early 1990s, pioneering work was published [36,37] demonstrating that large libraries of synthetic RNA molecules could be screened *in vitro* to identify and capture ligands with high-binding selectivities and affinities for a specific target. These ligands were termed aptamers [36], and the selection process was called systematic evolution of ligands by exponential enrichment (SELEX) [37]. These groundbreaking developments opened the door to the widespread application of the unique recognition capabilities of aptamers. In particular, they have proven to be valuable therapeutic agents with comparable and some enhanced properties relative to antibodies [38]. Such structures can be easily tagged with proteins [39], fluorophores and antibiotics, and can be chemically ligated with cytotoxic agents and radionucleides for therapy and diagnosis of aberrant cells [40]. Perhaps the greatest implications of aptamers will be felt in gene regulation and ribozyme function where such aptamer domains deactivate or trigger function by the binding of certain small molecules such as ATP and theophylline, and the natural aptamer domain can be mutated for regulating protein expression. Such aptamers, when tagged by fluorophores, are exquisitely sensitive biosensors for proteins and other molecules [41,42], as

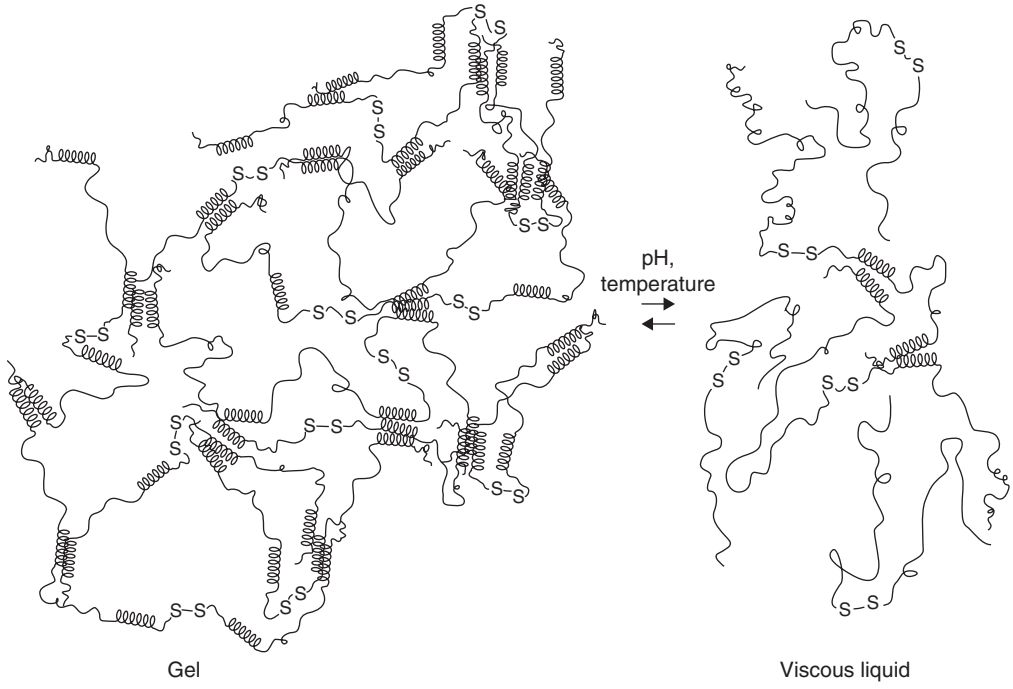

Figure 5. Gelation of a triblock copolymer. Chimeric genes code for a leucine zipper, which shows excellent thermal and pH modulated characteristics. Cysteine residues provide disulfide bridging between individual residues. Reprinted (abstracted/excerpted) with permission from PETKA WA, HARDIN JL, MCGRATH KP, WIRTZ D, TIRRELL DA: Reversible hydrogels from self-assembling artificial proteins. *Science* (1998) **281**(5375):389-392. Copyright (1998) AAAS.

they undergo conformational switches when they bind to their ligands.

5. Synthetic structures as biomimetic systems

Polymers, unnatural amino acids, aptamers and other synthetic molecules or materials based on man-made building blocks, have major roles in biomimetic design. This is primarily due to the ability to tailor properties exactly in the way the molecular designer intends. Synthetic biological building blocks can take advantage of flexibility in design and use various motifs that have programmable conformational states, which can produce novel carriers for drug delivery, designed therapeutics and sensing. α-Helical coiled coils are ubiquitous structural motifs in the proteome, and abound in G proteins, motor proteins, chaperones, transcription factors, cytoskeletal proteins and viral fusion proteins to name just a few. Their amphipathicity has come under scrutiny in recent years by engineers and chemists alike, albeit for different reasons. Organic chemists are interested in the total syntheses of transcription factors, chaperones and other structural elements and, hence, they have studied the dynamics of coiled coils in solution. In addition, these motifs can be characterised better in environments independent of the cellular confines. Engineers are interested in these motifs for tissue scaffolds and drug delivery. Hodges *et al.* have conducted exhaustive investigations of coiled coils over the last 15 years [43-50] by preparing vast libraries of peptides by Merrifield's synthesis. In particular, the studies have dealt with putative leucine zippers (i.e., having Leu in position *d*; Figure 7). In particular, the exquisite conformational switches of the G-protein-coupled receptors, formed primarily of α-helical coiled coils, can be mimicked to develop stimuli-sensitive drug delivery devices.

The terminals of coiled coils are quite amenable to modification, and have been used for solubilisation during protein crystallisation. Weissenhorn *et al.* [51] employed the trimeric coiled-coil domains of GCN-pII to enhance the solubility of the HIV glycoprotein 41. In addition, Kim and colleagues used the Fos/Jun leucine zipper pair to assemble α- and β-chains of DQ2. DQ2 is a MHC class II molecule, which presents gliadin epitopes to CD4 single-positive T cells. The crystal structure obtained can be used to design therapeutics for curing celiac disease, a chronic autoimmune digestive disease due to the ingestion of gluten (i.e., a protein found in wheat, rye and barley) that damages the duodenum and limits nutrient absorption [52]. The crystal structure details the non-covalent interactions responsible for the deamidation of certain residues in gluten peptides, which allows the MHC class II molecule to capture it.

DeCrescenzo and colleagues [53] have studied coiled-coil heterodimer formation in order to create highly specific

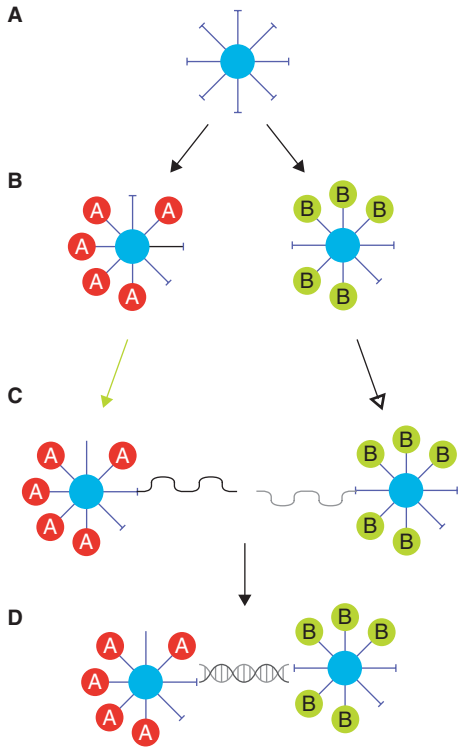

Figure 6. Oligonucleotide-driven dendrimer assembly. Dendrimers **(A)** are functionalised with certain molecules of interest **(B)**, linked with complementary oligonucleotides **(C)**, and then conjugated using the Watson-Crick rules of base pairing **(D)**. A and B (circled) are functional molecules of interest. EISENSTEIN M: DNA helps dendrimers branch out. *Nat. Met.* (2005) **2**(3):156-157. Copyright (2005), with permission from NPG.

biosensors. Here, a Cys-Gly-Gly-tag was added to the N terminus of a peptide (heptad repeat of *a*Val*b*Ser*c*Ala*d*Leu*e*Lys*f*-Glu*g*Lys) to immobilise it on the biosensor surface. α-Helical coiled coil association with another peptide (*a*Val*b*Ser*c*Ala*d*-Leu*e*Glu*f*Lys*g*Glu) was facilitated through Lys–Glu interactions ($e - g'$ and $e' - g$; Figure 7) [53]. Sensorgrams of the refractive index variations were analysed by nonlinear regression and binding affinity was modulated by using peptide pairs of three, four and five heptads.

Coiled coils can also be used for designing more stable antigen-binding sites. Arndt *et al.* have stabilised the traditionally unstable Fv (fragment variable) antibodies using the coiled-coil WinZip A2B1 [54]. In general, coiled coils have well-defined structure and properties and have demonstrated great potential as peptide-based drug delivery vehicles.

Today, polymer scientists and engineers can synthesise a wide variety of macromolecules with precise control of their molecular structure and physiochemical properties. By designing the molecular functionality and structure of a synthetic polymer network to mimic biological systems, three-dimensional network structures exhibiting structural similarities can be created with tailorable drug delivery properties [55,56]. In essence, biomimetic assemblies provide a molecular platform that mimic biological systems with applicability in encapsulating and delivering drug molecules.

Synthetic networks that can be designed to recognise and bind biologically significant molecules can be prepared using template-mediated polymerisation techniques (e.g., molecular imprinting). There have been many excellent reviews on the field of molecular imprinting, and the authors direct the reader to the following papers [57,58]. Only recently researchers have applied imprinting methodology in the design of polymers for the recognition of biologically significant molecules and application as controlled drug delivery systems [59-62]. Of particular interest is a recent review that highlights the wide applicability of these polymer systems in controlled drug delivery such as sustained release, enhanced loading capacity and enantioselective loading or release (Figure 8) [63]. The review also discusses the future of designed recognition, configurational biomimesis within polymeric gels, problems to be solved in the design of synthetic recognition-based networks, and recent efforts toward integrating imprinted polymers in controlled drug delivery systems and sensing devices.

Hammer *et al.* have designed block copolymers structurally similar to the lipid bilayer. Termed polymerosomes, these possess amphipathic properties, and are more robust and 10-fold less impermeable to water than the plasma membrane [64,65]. The high degree of crosslinking makes the cells durable, which makes it easy to dehydrate them into powders, and, hence, increase their shelf life [66]. Such structures can be used to manufacture artificial cells, or to carry medication and nutrition during space missions.

Strict control of molecular structure, which is an inherent advantage of synthetic systems, is of high importance in the design of materials with reproducible and consistent properties. Deming has pioneered the use of transition metals as initiators for the ring-opening polymerisation of *N*-carboxyanhydrides. The use of organonickel as the initiator effectively suppresses the chain termination and transfer reactions, resulting in block copolypeptides of highly controlled chain lengths, which produce novel polypeptide hydrogels [67]. Variation of copolymer chain length and composition produced various hydrogels, characterised by confocal microscopy and transition electron microscopy. At the molecular level, familiar conformations of polypeptides were obtained with poly-(Leu) giving α-helices and poly-(Val) giving β-sheets [68].

Savic and colleagues have synthesised micelles from polycaprolactone-b-poly(ethylene oxide) for the delivery of hydrophobic drugs [69]. The block copolymer was tagged with a fluorophore to study the intracellular distribution of the drug. Confocal microscopy demonstrated that they were endocytosed and localised within various organelles such as the mitochondria and Golgi apparatus but not the nucleus. It was also shown that a noncovalently bound agent to the micelles would be internalised more than the agent alone. Subsequently, the uptake of these micelles in pluripotent P19 mouse embryonal carcinoma cells was studied [70]. It was shown that

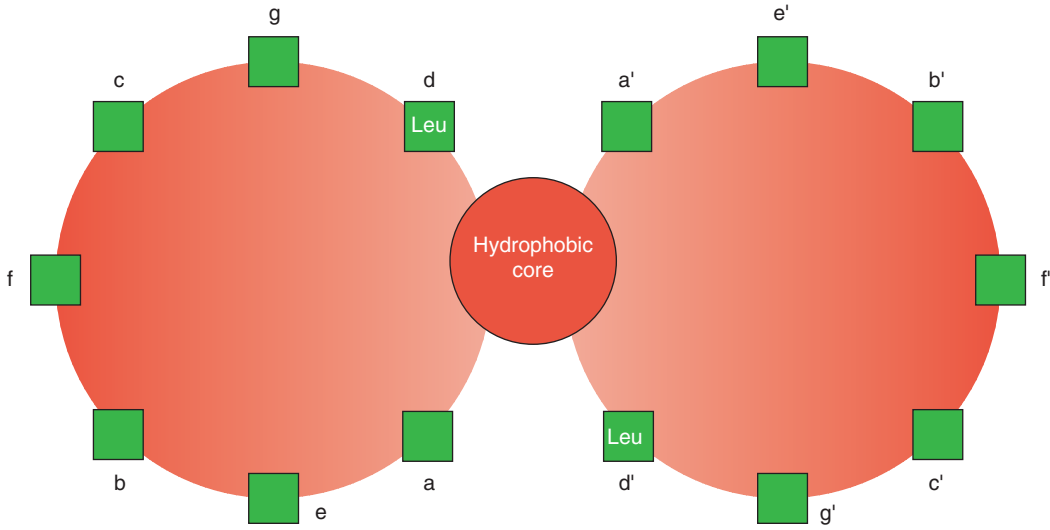

Figure 7. Helical wheel notation of a leucine zipper. The helical wheel notation shows the two α-helices forming a left-handed superhelix. The leucine zipper model consists of a signature 'heptad' sequence with leucine at position *d*. The positions *a* and *d* consists of hydrophobic residues, while *e* – *g'* and *g* – *e'* hold together the coiled coil by electrostatic attractions.

the extent of internalisation was markedly reduced at low temperatures and pH, and in the presence of drugs such as chlorpromazine. Such micelles could be used in the delivery of androgens and oestrogens during hormonal imbalances.

6. Expert opinion

In the last 25 years, there have been significant developments in advanced drug delivery formulations, facilitating the creation of systems that do not simply release a drug at a specific rate, but release the drug in a way that the pharmaceutical scientist and molecular designer has designed [71,72]. Because controlled drug delivery systems can improve safety, efficacy, convenience and patient compliance, novel delivery methods are a major focus of pharmaceutical companies [73].

In addition to the intrinsic activity of a drug, the delivery of it in a specific, controlled manner to the desired site of action is critical to its overall therapeutic effect. For instance, the optimum therapeutic effect depends on the drug arriving at the intended place at the right time and then staying active for a certain period of time. Many drugs can interact with different molecules in the body in ways that lead to adverse side effects, and a physiomimetic delivery system that can respond to the concentrations of certain molecules in the body is invaluable and continues to be the goal for many researchers. Essentially, this involves a mimicking of the natural response of the body/organs/tissues/cells to varying concentration levels of target biomolecules (e.g., release of insulin in response to serum glucose levels or release of therapeutic molecules based on temporal relationships; i.e., chronobiological therapy).

There has been considerable work in the preparation of intelligent biomaterials to address and achieve these desired therapeutic effects and with varying levels of success. Only recently has the potential of biomimetic systems as intelligent biomaterials and in therapeutic applications begun to be realised. These biomimetic structures based on proteins, DNA, RNA, man-made building blocks (i.e., synthetic polymers and unnatural amino acids) or combinations of these have been demonstrated to mimic biological systems and processes. The authors have introduced groundbreaking efforts that span a variety of biomimetic systems, and they expect that this work and the solid foundation it has provided will lead to methods and materials that will revolutionise medicine. In particular, the ability of biomimetic systems to provide biomolecular targeting, sustained release and pulsatile release will facilitate enhanced control over the delivery of therapeutic molecules.

The field of proteomics and protein folding is expected to accelerate the discovery of biomimetic drug carriers. In the early 1990s, genomics became a commercial enterprise with high-throughput sequencing on an industrial scale [74,75]. As the automation, speed and accuracy of sequencing results increased, it dramatically decreased the time to complete the Human Genome Project, which determined the sequences of the 3 billion chemical base pairs and identified ~ 30,000 genes in human DNA as well as patterns of variation across the genome. This was a tremendous accomplishment, but work is now beginning to probe the potential of the sequence code (Box 1) [76]. This work is expected to have a profound influence on the genetic and mechanistic basis for disease and will lead to genotype or phenotype-specific therapeutics.

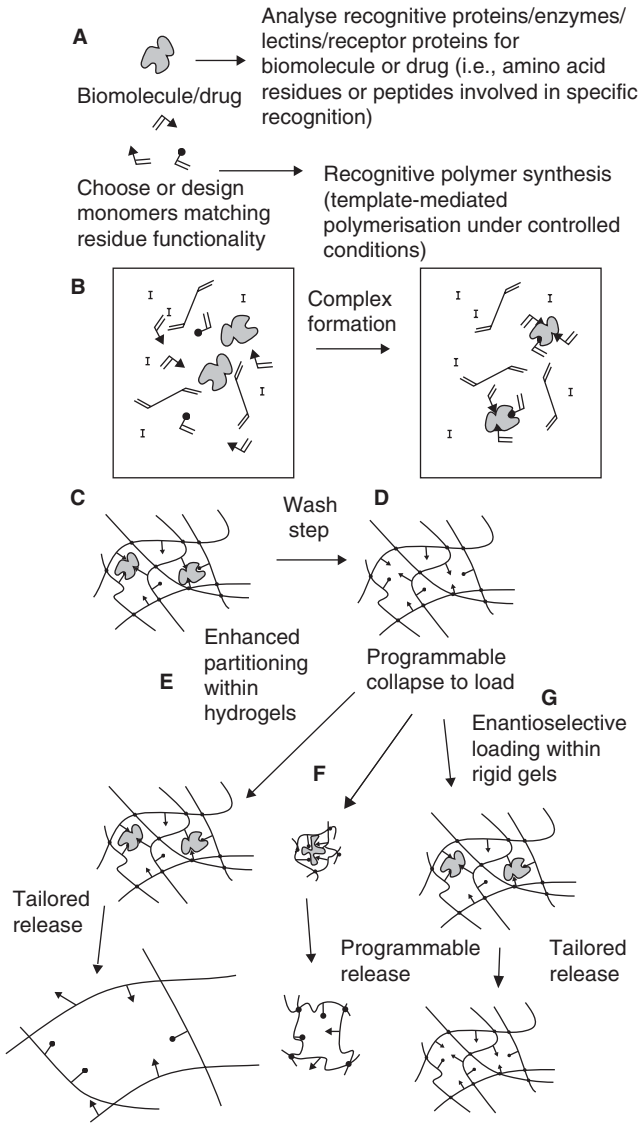

Figure 8. Configurational biomimesis in the production of recognitive networks. A. Mimic recognitive proteins, peptide sequences and enzymes by analysing the amino acids involved in binding a particular molecule and duplicating complexation interactions. **B.** Solution mixture of biomolecule (template), functional monomer(s) (triangles and circles), crosslinking monomer, solvent and initiator (I). The prepolymerisation complex is formed via covalent or noncovalent chemistry. **C.** The formation of the network (imprinting process). **D.** Wash step where original template is removed. **E.** Networks can be designed to increase partitioning or uptake within hydrogels and swellable materials. **F.** Programmable collapse can lead to loading as well as programmable release. Modulation of binding events can occur via pH, temperature, or other stimuli sensitive mechanisms. **G.** Within higher crosslinked matrices, the affinity can alter the release profile and extend release. It can also lead to enantioselective loading of mixtures.

Box 1. Research challenges that remain after sequencing the genome [76]**.**

- Gene number, exact location and functions
- Gene regulation
- DNA sequence organisation
- Chromosomal structure and organisation
- Noncoding DNA types, amount, distribution, information content and functions
- Coordination of gene expression, protein synthesis and post-translational events
- Interaction of proteins in complex molecular machines
- Predicted versus experimentally determined gene function
- Evolutionary conservation among organisms
- Protein conservation (structure and function)
- Proteomes (total protein content and function) in organisms
- Correlation of SNPs (single-base DNA variation among individuals) with health and disease
- Disease-susceptibility prediction based on gene sequence variation
- Genes involved in complex traits and multigene diseases
- Complex systems biology including microbial consortia useful for environmental restoration
- Developmental genetics and genomics

SNP: Single-nucleotide polymorphism.

In the next 25 years, one will begin to see the tailoring of therapeutics based on an individuals genetic predisposition toward disease (i.e., individualised therapeutics). Inherent within these developments will be incorporated drug delivery carriers, which may or may not influence the underlying therapeutic effect.

With the mapping of the human genome complete, the relationship between structure and function as well as the expression of genome sequences is of utmost importance in the rational treatment of disease. Although understanding how a protein folds into its native conformation from a given amino acid sequence is still being studied and the structural basis of several motifs is understood, it is known that much of the protein structure exists as a support for recognition processes (i.e., binding or catalytic sites). In the case of streptavidin (with molecular weight 64,000 Da), several binding sites are present solely for the binding of the relatively small (molecular weight 244 Da) biotin molecule. This complex has been studied for decades and is still of great interest because it has the highest interaction affinity of any noncovalent system known. With the creation of novel synthetic recognition systems using biomimetic strategies, one is beginning to approach these limits of binding affinity. The selection process for the design of these materials as well as the determination of optimal reaction conditions can be accomplished with several methods; trial and error, the configurational biomimetic approach, combinatorial methods and lastly by computational simulation.

The recent 2004 Gordon Research Conference entitled Drug Carriers in Medicine and Biology discussed exciting topics of research involving drug delivery including biomimetic strategies [77]. It is evident that the study of biology and biological processes is creating novel and successful strategies for a number of drugs and carriers in terms of delivery issues such as persistence, biodistribution, penetration, metabolism, systemic clearance, as well as imaging to treat a number of disease or abnormal cellular states.

Of continued importance to biomimetic developments will be the rational design of the constituent chemistry and subsequent linking of various synthetic and biological counterparts. If certain strategies to produce or obtain biological structures are insurmountable and/or inefficient, researchers have used a number ingeniously crafted methods based on fundamental biology to obtain novel biological or hybrid structures. However, the challenge lies in building precise structural alignment as well as tunable or switchable functionality into materials as well as optimising delivery profiles and release constraints.

In view of the various examples cited in this article, it is clear that biomimetic systems can be put to many uses: to investigate diseased cells and tissues; to target such locations with therapeutic formulations; or just to study the internal organisation of the cell. Biomimetic drug carriers are emerging in various clinical applications such as autoimmunity, imaging and targeted therapeutic options for cancer and infectious disease. In addition, the past has seen the use of PEG for its stealth characteristics, but the future may hold novel passivation strategies using biomimetic epitopes that are recognised as self by the immune system effector cells. Further designs of biomimetic systems have the potential to rapidly transform the field of controlled release and greatly enhance the performance of existing pharmaceutical compounds.

The development of micro and nanoscale delivery systems [78] has promised significantly improved treatment regimens that were previously not possible. Within these efforts, major emphasis has been focused toward engineering the architectural design of materials at the molecular level, and biomimetic processes are prime candidates for the creation of enhanced delivery systems at the small scale with tremendous promise to profoundly impact medicine and treatment options for disease. Biomimetic materials and systems are exceptional candidates for various controlled drug delivery applications and have enormous potential to revolutionise medical treatments.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. LANGER R, PEPPAS NA: Advances in biomaterials, drug delivery, and bionanotechnology. *AIChE J.* (2003) 49(12):2990-3006.
•• Thorough review of the literature on the subject with critical analysis of expectations.
2. SANCHEZ C, ARRIBART H, MADELEINE M, GUILLE G: Biomimetism and bioinspiration as tools for the design of innovative materials and systems. *Nat. Mat.* (2005) 4(4):277-288.
•• Excellent review covering various aspects of the biomimetic field.
3. SARIKAYA M: Biomimetics: materials fabrication through biology. *Proc. Natl. Acad. Sci. USA* (1999) 96(25):14183-14185.
4. SARIKAYA M, TAMERLER C, JEN AK, SCHULTEN K, BANEYX F: Molecular biomimetics: nanotechnology through biology. *Nat. Mat.* (2003) 2(9):577-585.
5. SEEMAN NC, BELCHER AM: Emulating biology: building nanostructures from the bottom up. *Proc. Natl. Acad. Sci. USA* (2002) 99(9 Suppl. 2):6451-6455.
6. SHIN H, JO S, MIKOS AG: Biomimetic materials for tissue engineering. *Biomaterials* (2003) 24(24):4353-4364.
•• A seminal contribution towards identification of the important properties of biomimetic scaffolds.
7. TU RS, TIRRELL M: Bottom-up design of biomimetic assemblies. *Adv. Drug Delivery Rev.* (2004) 56(11):1537-1563.
8. ZHANG S: Fabrication of novel biomaterials through molecular self-assembly. *Nat. Biotechnol.* (2003) 21(10):1171-1178.
•• Excellent review covering various aspects of the biomimetic field.
9. ALIVISATOS AP, JOHNSSON KP, PENG X *et al.*: Organization of 'nanocrystal molecules' using DNA. *Nature* (1996) 382(6592):609-611.
•• Seminal contribution on nanocrystal structure.
10. CHEN J, SEEMAN NC: Synthesis from DNA of a molecule with the connectivity of a cube. *Nature* (1991) 350(6319):631-633.
11. MIRKIN CA, LETSINGER RL, MUCIC RC, STORHOFF JJ: A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature* (1996) 382(6592):607-609.
•• This and [9] were published back-to-back in the same issue and were the first reports demonstrating nanoparticle assembly using oligonucleotide base pairing rules.
12. GHADIRI MR, GRANJA JR, BUEHLER LK: Artificial transmembrane ion channels from self-assembling peptide nanotubes. *Nature* (1994) 369(6478):301-304.
13. GHADIRI MR, GRANJA JR, MILLIGAN RA, MCREE DE, KHAZANOVICH N: Self-assembling organic nanotubes based on a cyclic peptide architecture. *Nature* (1993) 366(6453):324-327.
14. FERNANDEZ-LOPEZ S, KIM H-S, CHOI EC *et al.*: Antibacterial agents based on the cyclic D,L-a-peptide architecture. *Nature* (2001) 412(6845):452-456.
15. SANCHEZ-QUESADA J, ISLER MP, GHADIRI MR: Modulating ion channel properties of transmembrane peptide nanotubes through heteromeric supramolecular assemblies. *J. Am. Chem. Soc.* (2002) 124(34):10004-10005.
16. SILVA GA: Introduction to nanotechnology and its applications to medicine. *Surg. Neurol.* (2004) 61(3):216-220.
17. SILVA GA, CZEISLER C, NIECE KL *et al.*: Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science* (2004) 303(5662):1352-1355.

18. PEPPAS NA, HUANG YB: Nanoscale technology of mucoadhesive interactions. *Adv. Drug Delivery Rev.* (2004) 56(11):1675-1687.

19. PEPPAS NA, HUANG Y: Polymers and gels as molecular recognition agents. *Pharm. Res.* (2002) 19(5):578-587.

20. BIES C, LEHR C, WOODLEY JF: Lectin-mediated drug targeting: history and applications. *Adv. Drug Delivery Rev.* (2004) 56:425-435.

21. HAAS J, LEHR CM: Developments in the area of bioadhesive drug delivery systems. *Exp. Opin. Biol. Therapy* (2002) 2(3):287-298.

22. SMART JD: Recent developments in the use of bioadhesive systems for delivery of drugs to the oral cavity. *Crit. Rev. Ther. Drug Carrier Syst.* (2004) 21(4):319-344.

23. HAMMER DA, TIRRELL M: Biological adhesion at interfaces. *Ann. Rev. Mat. Sci.* (1996) 26:651-691.

24. TIRRELL M, KOKKOLI E, BIESALSKI M: The role of surface science in bioengineered materials. *Surf. Sci.* (2002) 500(1-3):61-83.

•• A pioneering contribution in the field of surface science of biomaterials.

25. YU Y-C, BERNDT P, TIRRELL M, FIELDS GB: Self-assembling amphiphiles for construction of protein molecular architecture. *J. Am. Chem. Soc.* (1996) 118(50):12515-12520.

26. WU AM, TAN GJ, SHERMAN MA *et al.*: Multimerization of a chimeric anti-CD20 single-chain Fv-Fc fusion protein is mediated through variable domain exchange. *Protein Eng.* (2001) 14(12):1025-1033.

27. LI L, OLAFSEN T, ANDERSON AL *et al.*: Reduction of kidney uptake in radiometal labeled peptide linkers conjugated to recombinant antibody fragments. Site-specific conjugation of DOTA-peptides to a Cys-diabody. *Bioconjug. Chem.* (2002) 13(5):985-995.

28. GIL ES, HUDSON SM: Stimuli-reponsive polymers and their bioconjugates. *Progress Poly. Sci.* (2004) 29(12):1173.

29. DROTLEFF S, LUNGWITZ U, BREUNIG M *et al.*: Biomimetic polymers in pharmaceutical and biomedical sciences. *Eur. J. Pharm. Biopharm.* (2004) 58(2):385-407.

30. DONG C-M, FAUCHER KM, CHAIKOF EL: Synthesis and properties of biomimetic poly(L-glutamate)-b-poly(2-acryloyloxyethyllactoside)-b-poly(L-glutamate) triblock copolymers. *J. Polym. Sci. Part A: Polym. Chem.* (2004) 42(22):5754-5765.

•• Uses calmodulin for conformation switches.

31. LACKEY CA, PRESS OW, HOFFMAN AS, STAYTON PS: A biomimetic pH-responsive polymer directs endosomal release and intracellular delivery of an endocytosed antibody complex. *Bioconjug. Chem.* (2002) 13(5):996-1001.

•• One of the pioneering papers on biomimetic chemistry and endosomal release.

32. WANG C, STEWART RJ, KOPECEK J: Hybrid hydrogels assembled from synthetic polymers and coiled-coil protein domains. *Nature* (1999) 397(6718):417-420.

•• Uses the leucine zipper domain.

33. EHRICK JD, DEO SK, BROWNING TW *et al.*: Genetically engineered protein in hydrogels tailors stimuli-responsive characteristics. *Nat. Mat.* (2005) 4(4):298-302.

34. PETKA WA, HARDIN JL, MCGRATH KP, WIRTZ D, TIRRELL DA: Reversible hydrogels from self-assembling artificial proteins. *Science* (1998) 281(5375):389-392.

•• Uses the leucine zipper domain.

35. CHOI Y, THOMAS T, KOTLYAR A, ISLAM MT, BAKER JR Jr: Synthesis and functional evaluation of DNA-assembled polyamidoamine dendrimer clusters for cancer cell-specific targeting. *Chem. Biol.* (2005) 12(1):35-43.

36. ELLINGTON AD, SZOSTAK JW: *In vitro* selection of RNA molecules that bind specific ligands. *Nature* (1990) 346(6287):818-822.

•• Groundbreaking paper that helped open the door to the widespread application of aptamers.

37. TUERK C, GOLD L: Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* (1990) 249:505-510.

•• Groundbreaking paper that helped open the door to the widespread application of aptamers.

38. NIMJEE SM, RUSCONI CP, SULLENGER BA: Aptamers: an emerging class of therapeutics. *Ann. Rev. Med.* (2005) 56:555-583.

39. EATON BE: The joys of *in vitro* selection: chemically dressing oligonucleotides to satiate protein targets. *Curr. Opin. Chem. Bio.* (1997) 1(1):10-16.

40. HERMANN T, PATEL DJ: Adaptive recognition by nucleic acid aptamers. *Science* (2000) 287(5454):820-825.

41. HAMAGUCHI N, ELLINGTON A, STANTON M: Aptamer beacons for the direct detection of proteins. *Anal. Biochem.* (2001) 294(2):126-131.

42. JHAVERI S, RAJENDRAN M, ELLINGTON AD: *In vitro* selection of signaling aptamers. *Nat. Biotech.* (2000) 18(12):1293-1297.

43. MONERA OD, KAY CM, HODGES RS: Electrostatic interactions control the parallel and antiparallel orientation of alpha-helical chains in two-stranded alpha-helical coiled-coils. *Biochemistry* (1994) 33(13):3862-3871.

44. SU JY, HODGES RS, KAY CM: Effect of chain length on the formation and stability of synthetic alpha-helical coiled coils. *Biochemistry* (1994) 33(51):15501-15510.

•• Two classic papers by the leading group in α-helical coiled-coil chemistry.

45. TRIPET B, YU L, BAUTISTA DL *et al.*: Engineering a *de novo*-designed coiled-coil heterodimerization domain off the rapid detection, purification and characterization of recombinantly expressed peptides and proteins. *Protein Eng.* (1996) 9(11):1029-1042.

46. WAGSCHAL K, TRIPET B, HODGES RS: *De novo* design of a model peptide sequence to examine the effects of single amino acid substitutions in the hydrophobic core on both stability and oligomerization state of coiled-coils. *J. Mol. Biol.* (1999) 285(2):785-803.

47. KOHN WD, KAY CM, HODGES RS: Orientation, positional, additivity, and oligomerization-state effects of interhelical ion pairs in alpha-helical coiled-coils. *J. Mol. Biol.* (1998) 283(5):993-1012.

48. ZHOU NE, KAY CM, HODGES RS: The role of interhelical ionic interactions in controlling protein folding and stability. *De novo* designed synthetic two-stranded alpha-helical coiled-coils. *J. Mol. Biol.* (1994) 237(4):500-512.

49. ZHOU NE, KAY CM, HODGES RS: Synthetic model proteins. Positional effects of interchain hydrophobic interactions on stability of two-stranded alpha-helical coiled-coils. *J. Biol. Chem.* (1992) 267(4):2664-2670.

50. ZHOU NE, ZHU BY, KAY CM, HODGES RS: The two-stranded alpha-helical coiled-coil is an ideal model for studying protein stability and subunit interactions. *Biopolymers* (1992) 32(4):419-426.

51. WEISSENHORN W, DESSEN A, HARRISON SC, SKEHEL JJ, WILEY DC: Atomic structure of the ectodomain from HIV-1 gp41. *Nature* (1997) 387(6631):426-430.

52. KIM C-Y, QUARSTEN H, BERGSENG E, KHOSLA C, SOLLID LM: Structural basis for HLA-DQ2-mediated presentation of gluten epitopes in celiac disease. *Proc. Natl. Acad. Sci. USA* (2004) 101(12):4175-4179.

53. DE CRESCENZO G, LITOWSKI JR, HODGES RS, O'CONNOR-MCCOURT MD: Real-time monitoring of the interactions of two-stranded *de novo* designed coiled-coils: effect of chain length on the kinetic and thermodynamic constants of binding. *Biochemistry* (2003) 42(6):1754-1763.

54. ARNDT KM, MULLER KM, PLUCKTHUN A: Helix-stabilized Fv (hsFv) antibody fragments: substituting the constant domains of a Fab fragment for a heterodimeric coiled-coil domain. *J. Mol. Biol.* (2001) 312(1):221-228.

55. PEPPAS NA: Devices based on intelligent biopolymers for oral protein delivery. *Intern. J. Pharm.* (2004) 277:11-17.

56. PEPPAS NA, WOOD KM, BLANCHETTE JO: Hydrogels for oral delivery of therapeutic proteins. *Expert Opin. Biol. Ther.* (2004) 4:881-887.

57. SELLERGREN B: Noncovalent molecular imprinting: antibody-like molecular recognition in polymeric network materials. *Trends Anal. Chem.* (1997) 16(6):310-320.

58. WULFF G: Molecular imprinting in crosslinked materials with the aid of molecular templates – a way towards artificial antibodies. *Ang. Che. Int. Edi.* (1995) 34(17):1812-1832.

59. BYRNE M, PARK K, PEPPAS NA: Molecular imprinting within hydrogels. *Adv. Drug Deliv. Revs.* (2002) 54:149-161.
•• The earliest review on the use of hydrogels in biomedical imprinting

60. BYRNE ME, HENTHORN DB, HUANG Y, PEPPAS NA: Micropatterning biomimetic materials for bioadhesion and drug delivery. In: *Biomimetic Materials and Design: Biointerfacial Strategies, Tissue Engineering and Targeted Drug Delivery*, AK Dillow and A Lowman (Eds), Marcel Dekker, New York, NY, USA (2002):443-470.

61. BYRNE ME, ORAL E, HILT JZ, PEPPAS NA: Networks for recognition of biomolecules: molecular imprinting and micropatterning poly(ethylene glycol)-containing films. *Polym. Adv. Technol.* (2002) 13:798-816.

62. ORAL E, PEPPAS NA: Responsive and recognitive hydrogels using star polymers. *J. Biomed. Mater. Res.* (2004) 68A:439-447.

63. HILT JZ, BYRNE MARK E: Configurational biomimesis in drug delivery: molecular imprinting of biologically significant molecules. *Adv. Drug Deliv. Rev.* (2004) 56(11):1599-1620.
•• A comprehensive review of macromolecular recognition of biologically significant molecules.

64. DISCHER BM, BERMUDEZ H, HAMMER DA *et al.*: Cross-linked polymersome membranes: vesicles with broadly adjustable properties. *J. Phy. Che. B* (2002) 106(11):2848-2854.

65. DISCHER BM, WON YY, EGE DS *et al.*: Polymersomes: tough vesicles made from diblock copolymers. *Science* (1999) 284(5417):1143-1146.

66. BERMUDEZ H, BRANNAN AK, HAMMER DA, BATES FS, DISCHER DE: Molecular weight dependence of polymersome membrane structure, elasticity, and stability. *Macromolecules* (2002) 35(21):8203-8208.

67. DEMING TJ: Facile synthesis of block copolypeptides of defined architecture. *Nature* (1997) 390(6658):386-389.
•• An excellent reference on the controlled synthesis of amino acid-based polymeric systems using transition metal catalysts.

68. NOWAK AP, BREEDVELD V, PAKSTIS L *et al.*: Rapidly recovering hydrogel scaffolds from self-assembling diblock copolypeptide amphiphiles. *Nature* (2002) 417(6887):424-428.

69. SAVIC R, LUO L, EISENBERG A, MAYSINGER D: Micellar nanocontainers distribute to defined cytoplasmic organelles. *Science* (2003) 300(5619):615-618.
•• Demonstrates the feasibility of liposomes for hydrophobic drug delivery.

70. LUO L, TAM J, MAYSINGER D, EISENBERG A: Cellular internalization of poly(ethylene oxide)-b-poly(e-caprolactone) diblock copolymer micelles. *Bioconjug. Chem.* (2002) 13(6):1259-1265.
•• Demonstrates the feasibility of liposomes for hydrophobic drug delivery.

71. PEPPAS N, BURES P, LEOBANDUNG W, ICHIKAWA H: Hydrogels in pharmaceutical formulations. *Eur. J. Pharm. Biopharm.* (2000) 50:27-46.
•• A highly cited and leading review in the field highlighting application, characterisation and drug release from hydrogels.

72. LANGER R: Drug delivery and targeting. *Nature* (1998) 392:5-10.

73. HENRY C: Special delivery: alternative methods for delivering drugs improve performance, convenience, and patient compliance. *C &EN* (2000):49-65.

74. GERSHON D: Microarray technology an array of opportunities. *Nature* (2002) 416:885-891.

75. KONONEN J, BUBENDORF L, KALLIONIEMI A *et al.*: Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat. Med.* (1998) 4:844-847.

76. HUMAN GENOME PROGRAM, US DEPARTMENT OF ENERGY: *Human Genome News* (2000) 11(1-2):7.

77. SENTER PD, KOPECEK J: Drug carriers in medicine and biology. *Mol. Pharamaceutics* (2004) 1(6):395-398.

78. LAVAN DA, MCGUIRE T, LANGER R: Small-scale systems for *in vivo* drug delivery. *Nat. Biotechnol.* (2003) 21(10):1184-1191.

Affiliation

Siddarth Venkatesh[1], Mark E Byrne[1], Nicholas A Peppas[2] & J Zach Hilt[†3]
[†]Author for correspondence
[1]Auburn University, Biomimetic and Biohybrid Materials, Biomedical Devices and Drug Delivery Laboratories, Department of Chemical Engineering, Auburn, AL 36849-5127, USA
[2]The University of Texas, Biomaterials, Drug Delivery and Molecular Recognition Laboratories, Departments of Chemical Engineering, Biomedical Engineering and Pharmaceutics, Austin, TX 78712-0231, USA
[3]University of Kentucky, Department of Chemical and Materials Engineering, Lexington, KY 40506-0046, USA
Tel: +1 859 257 9844; Fax: +1 859 323 1929; E-mail: hilt@engr.uky.edu