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Enzyme-directed assembly and manipulation of organic nanomaterials

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Abstract

Enzymes are the prime protagonists in the chemistry of living organisms. As such, chemists and biologists have long been fascinated by the array of highly selective transformations possible under biological conditions that are facilitated by enzymecatalyzed reactions. Moreover, enzymes are involved in replicating, repairing and transmitting information in a highly selective and organized fashion through detection and signal amplification cascades. Indeed, because of their selectivity and potential for use outside of biological systems, enzymes have found immense utility in various biochemical assays and are increasingly finding applications in the preparation of small molecules. By contrast, the use of enzymatic reactions to prepare and build supramolecular and nanoscale materials is relatively rare. In this article, we seek to highlight efforts over the past 10 years at taking advantage of enzymatic reactions to assemble and manipulate complex soft, organic materials on the nanoscale. It is tantalizing to think of these processes as mimics of natural systems where enzymes are used in the assembly and transformation of the most complex nanomaterials known, for example, virus capsid assemblies and the myriad array of nanoscale biomolecular machinery.

Enzymes are the workhorses of life's processes, engaged in all manner of interactions, reactions and signalling events within the molecular ecosystems of living organisms and viruses. From the perspective of chemists interested in reactivity, the catalytic reactions they facilitate are highly valuable and interesting in enabling selective reactions to occur under biological conditions. This has driven the search for mimics of these catalysts and the development of model systems for understanding their reactivity.¹ In the context of synthetic chemistry, enzymatic reactions are finding increasing utility in facilitating syntheses of complex natural products and small molecules.² In biology, the ability of enzymes to propagate signalling events through catalytic amplification is of intrinsic importance to living cells, and in biochemical assays (*e.g.* PCR³ and ELISA^{4,5}) that utilize this phenomenon in developing signals from exceptionally low concentrations of analyte. By contrast, predictable reactions for the assembly of complex supramolecular materials are in their infancy. Furthermore, the use of enzymes in manipulating assembled nanostructures is very much under-utilized. Herein, we describe research into this nascent field, with its beginnings in the context of enzymatically biodegradable materials designed for various applications, including drug delivery.^{6–8} However, rather than degradability or the destruction of materials, we are interested here in systems that are *constructed* or *transformed* into supramolecular systems *via* enzyme-based stimuli.

The focus will be on the construction of organic nanoscale materials utilizing enzymatic reactions. In addition, we describe examples of enzyme responsive structures that undergo dramatic changes in morphology. This type of structure offers great potential as a unique platform for interfacing synthetic materials with cells and tissue in normal and/or disease states. We will highlight the enzymatic assembly of nanoparticles, supramolecular or cross-linked hydrogels, and nanofibrils. Various approaches are described in sections delineated in the context of these three types of materials, although the reader will recognize some overlap in certain cases. In particular, where nanofibrils are formed from spherical nanoparticles, or where nano-fibrils can subsequently form gels. In each case, less complex synthons assemble to generate more complex, soft organic, supramolecular entities in response to modification by an enzyme.

Enzyme-driven assembly and enzyme-responsive morphology of nanoscale particles

Organic polymeric and micellar nano-particles have attracted much attention as materials with great promise as carriers of small molecule drugs,⁹ nucleic acids^{10,11} and diagnostic agents.^{7,12–14} Efforts to develop responsive particles of this type have largely focused on pH,^{15–17} temperature,^{18,19} light²⁰ and small molecules²¹ or ions²² as stimuli.^{23–25} However, despite the importance of enzymes as important biomarkers of disease states, enzymatically stimulated assembly and/or morphology switches are rare,^{26,27} with some exciting recent examples highlighting the great potential such approaches may have in the development of programmable nano-structures (Fig. 1). This is important, as truly programmable systems would allow chemists to combine desired elements within nanoscale self-assembled materials and expect predictable outcomes. We are, of course, far from achieving this with synthetic materials, however, we have examples of exactly this in nature, especially evident in the realm of virus self-assembly programmed at the genetic level and built utilizing peptide-based recognition elements.

Herein, we highlight several different modes of action of enzymes in the assembly and manipulation of particles. Firstly, the use of enzymes to initiate the self-assembly of discrete nanoscale particles.²⁶ Secondly, the use of enzymatic reactions to manipulate the morphology of intact nanoscale particles.^{28,29} Thirdly, the use of enzymatic reactions to very selectively manipulate the size of nanoscale particles.³⁰

In the last ten years, several examples of systems capable of undergoing enzymed driven assembly from small molecule synthons have been reported (*vide infra*). However, to our knowledge, only one example of the enzyme-directed assembly of a particle from a synthetic polymeric precursor has been reported (Fig. 1).²⁶ In this work, Hawker and coworkers utilized a hydrophilic block copolymer consisting of a 5 kDa PEG macroinitiator and a block of phosphorylated 4-hydroxystyrene. An amphiphilic block copolymer is generated in response to acid phosphatase activity *via* the removal of the phosphate groups. Following the enzymatic generation of the polymeric amphiphile, micellar nanoparticles spontaneously formed in aqueous solution. This type of approach is particularly interesting given that synthetic amphiphilic polymers offer chemical diversity and a great deal of control over particle morphology. Therefore, this type of approach provides an opportunity to employ a range of functional groups with the potential to respond to various enzymes.

In contrast to synthetic polymers, biomolecular polymers, such as proteins, are of course naturally evolved substrates of enzymes. Therefore, proteins, peptides and nucleic acids provide an interesting range of options in the development of enzyme-responsive biomaterials. In this vein, Jasanoff and coworkers reported the kinase-directed assembly of proteinbased nanoparticles.¹⁴ In their effort to demonstrate a protein-based MRI sensor of

kinase activity, a pair of ferritin (Ft) fusion proteins were generated with a kinase-inducible domain (KID) and a phosphorylation-dependent binding domain (KIX). When iron-loaded KIDFt and KIX-Ft nanoparticles were mixed with protein kinase A (PKA) and ATP, aggregation occurred following phosphorylation, generating larger particles with a concomitant change in relaxivity. This system demonstrates the potential power of utilizing enzyme substrates in the form of proteins, where mixing and matching various components *via* protein engineering allows for the generation of responsive and functional materials.

Several reports describe the formation of nanoparticles from small molecule precursors. These systems are structurally similar to those described in the following section discussing hydrogels and involve peptide-based small molecules. Wang *et al.* describe the formation of particles from an adamantyl–GFFY–OMe peptide phosphorylated at the tyrosine residue.³¹ Upon treatment with alkali phosphatase, particles were spontaneously formed with exceptionally low polydispersity as co-assemblies of the dephosphorylated product and phosphorylated starting material. In a similar example, an Fmoc-protected, phosphorylated dipeptide (Phe–Tyr) was treated with a phosphatase to generate a peptide nanoparticle *via* a reverse microemulsion preparative technique.³² In each of these examples, dephosphorylation of small peptide substrates results in nanoparticle formation primarily driven by hydrophobic aggregation in aqueous solution. However, as with the polymeric system described by Hawker and coworkers, the utility of synthetic organic chemistry in the preparation of enzyme-responsive materials comes from the great diversity in functional groups that one can potentially incorporate. Indeed, recent work by Rao and coworkers³³ demonstrates some of the potential applications that become plausible if the assembly of nanomaterials from small molecule precursors can be linked to enzyme-directed reactions of interest.

Enzymatically driven shifts in soft organic nanoparticle or micelle morphology have been explored in several contexts in recent years. In an early example, Akiyoshi and coworkers described the use of an enzyme-catalyzed polymerization of a sugar-based surfactant.³⁴ A mixture of this surfactant and 1- α -dipalmitoyl phosphatidyl choline (DPPC) yields 20 nm micelles, as observed by DLS. Upon polymerization of the sugar moiety, this surfactant increases in hydrophilicity and leaves the micellar phase, resulting in a morphology shift to larger liposomal particles containing DPPC in excess of 100 nm in diameter.

Employing short phosphorylated peptides, Ulijn and coworkers have reported two examples of particles that undergo changes in three-dimensional morphology.^{35,36} In one example, a simple phosphorylated dipeptide (Fmoc–FY) exists as a micellar particle in aqueous solution.³⁶ Treatment with a phosphatase generates a gel-phase consisting of a nanofiber morphology formed by the uni-directional assembly of the dephosphorylated Fmoc–FY peptide. A similar micelle-to-hydrogel shift has been described by Goto and coworkers, directed by protease activity, resulting in cleavage of a twelve amino acid peptide.³⁷ The peptide consists of a gelator sequence appended to a gelation-prevention moiety. Upon cleavage of the sequence, peptide micelles shift to spontaneously generate a hydrogel. Therefore, these systems utilize the common motif of a self-assembling short peptide sequence to drive a phase shift in organic nanomaterials upon enzymatic cleavage of gelation inhibiting functional groups.

In our own work, we sought to explore the potential of biopolymer hybrid copolymer amphiphiles in programming micellar nanoparticle morphology. To begin developing systems capable of interfacing with enzymes of interest in specific disease states, we designed a set of peptide substrate conjugated polymers.²⁸ In this case, the peptides were designed as substrates for proteases, a kinase (PKA) and a phosphatase. Proteolytic cleavage resulted in a shift from spherical micelles approximately 20 nm in diameter to micron-scale

network architectures. In the case of enzymatic phosphorylation, the micelles switched phase to larger aggregates. Intriguingly, this process occurred with an almost quantitative yield of phosphorylation that could, in turn, be quantitatively reversed by the addition of phosphatase. The dephosphorylation resulted in reversion of the material to the well-defined spherical phase with low polydispersity. This type of design provides a platform for the development of nanoscale particles capable of responding to disease-associated enzymes *via* well-defined shifts in morphology, with the potential to control how particles accumulate and clear from specific tissues.

Inspired by the utility of nucleic acids in the preparation of complex nanomaterials,³⁸ we designed a brush copolymer of DNA conjugated to a polynorbornene backbone.²⁹ This system was amphiphilic, with DNA as the polar head group^{39,40} spontaneously aggregating to yield well-defined spherical micelles in aqueous buffer. Therefore, sequence selective DNzyme-directed cleavage reactions within the polar head group could be used to tune the amphiphilicity of the copolymer, resulting in a dramatic change in particle morphology from spherical micelles to cylindrical phase nanofibrils (Fig. 1B). This type of truly programmable morphology is tremendously powerful, coupling the predictability of biomolecular recognition chemistry with the chemical diversity inherent to synthetic polymers. Indeed, nucleic acids are natural substrates to myriad enzymes, providing further flexibility for potential extension to a range of other systems.

With the goal of exploring nucleic acids as tools in controlling the assembly of nanomaterials, Herrmann and coworkers have described a range of DNA-polymer conjugates capable of forming micelles and studied them in several contexts.³⁹ Particularly interesting examples with regard to enzyme-responsive particles come from their work describing the use of naturally evolved enzymatic machinery for manipulating nucleic acids.^{30,41} In one of these reports, the authors describe enzymatic control over micelle size by treating DNA-copolymer micelles with terminal deoxynucleotidyl transferase (TdT).³⁰ This is an enzyme evolved for the generation of random genetic information required for the function of adaptive immunity in vertebrates. They utilize this chemistry to add deoxynucleotidyl triphosphates to the 32-hydroxy terminus of their DNA conjugated polymers. In doing so, the particles more than doubled in size, illustrating the potential of utilizing selective enzymes, together with their natural substrates conjugated to synthetic elements, to manipulate structural features on the nanoscale with predictability and precision.

Enzyme-driven assembly of hydrogels

Hydrogels are a network of molecular or supramolecular chains capable of gelling aqueous solutions with a water content of up to 99.9% by weight.⁴² Herein, we highlight examples of supramolecular hydrogels assembled from small organic molecules and include enzymatically-driven cross-linking of polymeric hydrogels. To illustrate the broader capabilities of the strategy, we focus on three distinct modes of action of enzymes in the formation of hydrogels (Fig. 2).

Hydrogels have found widespread use in biomedical practice and research, ranging from contact lenses to drug delivery systems.⁴³ One prominent area of highly active research is the preparation of hydrogels as scaffolds for tissue engineering.^{44,45} Such hydrogels can be formed from different arrangements of hydrogelator molecules, for example, covalently cross-linked macromolecular polymers or by the self assembly of smaller hydrogelator molecules. These approaches have provided hydrogels for tissue engineering that have proven useful in controlling cell viability, stem cell fate and resultant tissue properties.^{44,45} As pointed out by Stupp and colleagues, such scaffolds would ideally provide both structural

support and signalling capabilities to instruct resident stem cells to differentiate into the desired cell type and morphology.⁴⁵ Advancing this concept to higher levels of complexity requires two-way communication between the resident cells and the scaffold. Indeed, several examples of stimuli-responsive hydrogels already exist that are capable of altering their physical properties in response to pH, salt concentration, analyte composition, light and temperature, some of which can be modulated by the growing tissue itself.⁴³ One potential means by which this two-way communication may take place in a very specific way is by engineering enzyme responsive capabilities into hydrogel scaffolds. That is, enzymes expressed on the surface of, or secreted by, cells in contact with a hydrogel scaffold could then act upon the hydrogel itself to change its properties.

Towards hydrogel materials capable of two-way interactions with biological systems, researchers have taken the first steps over the last decade in creating increasingly more sophisticated enzyme responsive hydrogels by synthesizing water-soluble molecules that are themselves acted upon by enzymes to trigger hydrogel formation (Fig. 2).^{46,47} Early reports describe the use of transglutaminase (TGase), an enzyme that catalyzes acyl-transfer between the side chains of glutamine and lysine residues on peptide or protein chains to form cross-linked structures *via* interstrand isopeptide linkages.^{48–51} Sperinde and Griffith, as well as Hu and Messersmith each reported peptide substrates containing glutamine and lysine grafted to polyethylene glycol (PEG) polymers of various structures.^{50,51} These PEG-peptide graft polymers were molecularly solvated in aqueous media, but upon the addition of TGase, they were inter-molecularly cross-linked leading to hydrogel formation.

Citing the benefits of working with completely natural substrates, including a ready source of inexpensive starting materials and low potential for immunogenicity/toxicity, Payne and co-workers used TGase to prepare hydrogels of cross-linked gelatin in the presence and absence of chitosan.^{48,49} When the polysaccharide chitosan was present, the properties of the resultant gel were altered, leading to stronger gels, thereby demonstrating a potentially generalizable principle; namely, the inclusion of additives may lead to further customization of gel properties. Payne and colleagues also demonstrated that *E. coli* cells entrapped in a cross-linked gelatin hydrogel maintained viability and protein expression capabilities. Other enzymes, including peroxidases^{52,53} and tyrosinases^{48,52} have also been used to covalently cross-link appropriately derivatized molecules into hydrogels, demonstrating the robustness of this general approach of enzyme-regulated hydrogel formation toward reactants and catalysts.

Extending the development of these approaches beyond proteins and peptidepolymer conjugates to nucleic acids, Luo and colleagues prepared hydrogels composed entirely of DNA that form upon enzymatic cross-linking by DNA ligase.⁵⁴ The key to their design was synthetic branched DNA molecules (BDMs) with mutually complementary sticky ends that served as substrates for DNA ligase. The properties of the resultant gels were readily tuned by altering the structure of the BDMs. These enzymatically-triggered DNA hydrogels show great promise in controlled drug release, mammalian cell encapsulation and as scaffold and substrate for a markedly enhanced *in vitro* protein synthesis system.^{54,55}

Xu and co-workers have taken the concept of enzyme-instructed hydrogel formation another step forward in their pioneering studies on small molecule, supramolecular enzyme-responsive hydro-gelation (Fig. 2).^{46,47} These systems are distinguished from those discussed above in that the enzyme-catalyzed step leads to covalent modification of a non-gelling small molecule (herein termed a *prehydrogelator*), tuning its amphiphilicity and thus its ability to aggregate with other identical molecules *non-covalently* to form a *supramolecular* structure capable of supporting hydrogelation. The advantages of this approach include the facile and economical synthesis of the simple small molecule pre-

hydrogelators that serve as enzyme substrates, as well as the potential for readily controllable reversibility of hydrogelation. As discussed below, sol-to-gel and gel-to-sol cycles can be iteratively undertaken without the need to make or break structural covalent bonds that link together the gel, since these gels are held together by supramolecular forces.^{56,57}

The first example of enzyme-directed supramolecular small molecule hydrogelation appeared in 2004 when Xu and colleagues described the dephosphorylation of Fmoc-phosphotyrosine by alkaline phosphatase to generate hydrogels of Fmoc-tyrosine.⁵⁸ Yang and Xu used a similar system, swapping alkaline phosphatase for acid phosphatase, to demonstrate the potential for drug screening with the formation of the hydrogel as the readout, which is visible to the naked eye and thus requires no equipment for preliminary screening analysis.⁵⁹ Additional examples of phosphatase-triggered hydrogelation,^{36,57,60} as well as an ever-expanding list of other enzymes, including proteases such as tumor-associated MMPs,^{37,61} β -lactamase⁶² and esterases,^{56,63,64} have been successfully used to convert other appropriately designed soluble pre-hydrogelators *via* hydrolysis into hydrogelators.

Of particular interest are reports from the Xu laboratory demonstrating that enzyme-driven hydrogelation can occur intracellularly in both bacterial and mammalian cells, leading to inhibition of growth or the induction of apoptosis, respectively.^{60,63} Xu and colleagues also demonstrated that enzyme-driven hydrogelation can occur in the extracellular subcutaneous tissue of live mice without substantial toxicity, paving the way toward future *in vivo* use of this class of enzyme responsive materials.⁵⁷ In yet another application, Ulijn and co-workers demonstrated that a dried hydrogel, *i.e.* a xerogel, that had been synthesized *via* enzyme-triggered ester hydrolysis to form Fmoc-L₃ can support electronic conductivity, which may have future utility in bio-electronics.⁶⁵

The Ulijn laboratory has pioneered a related route for enzyme-triggered hydrogel formation. Instead of hydrolytic cleavage of a pre-gelator, they demonstrated that enzyme-driven fragment coupling can generate hydrogelators *in situ*, as shown schematically in Fig. 2.⁶⁶ Taking their cue from classical protein engineering approaches,^{67,68} they designed systems that could be enzymatically ligated *via* protease-catalyzed reverse proteolysis.^{56,66,69,70} The driving force for the protease in these systems to *ligate* rather than *hydrolyse* peptide bonds is that ligation produces a hydrogelator that, in turn, leads to supramolecular hydrogel formation, thus driving the equilibrium of the enzyme catalyzed ligation forward as it is coupled to the energetically favourable hydrogel formation. The first such example involved the thermolysin catalyzed ligation of Fmoc-Phe to dipeptide Phe-Phe to form Fmoc-Phe₃, which functioned well as a hydrogelator.⁶⁶ This type of system operates under thermodynamic control and, therefore, when presented with a mixture of substrate fragments, the system will generate a dynamic combinatorial library of coupled fragments that will evolve and self-select the most thermodynamically favourable products.^{69,70}

In two particularly compelling reports, the Xu and Ulijn laboratories have independently demonstrated cyclical reversibility of hydrogel formation and destruction by utilizing coupled enzyme systems, analogous to that described above for the phosphatase/kinase cycling of nanoparticle morphology.^{28,56,57} For example, Yang *et al.* employed the hydrogelator Nap-FFGEY, which when phosphorylated by a tyrosine kinase led to dissolution of the hydrogel, resulting in a clear, freely flowing solution.⁵⁷ The hydrogel was then reformed upon dephosphorylation of tyrosine by the addition of alkaline phosphatase. Indeed, this type of reversible reaction facilitated by enzymes is a potentially general strategy, finding utility in reversibly shifting discrete micellar nanoparticle morphology in an example described in the previous section of this highlight.²⁸

Enzyme-driven assembly of supramolecular fibrils

In this section, we will consider the recent efforts utilizing enzyme-triggered self-assembly of supramolecular organic nanofibrils that do not subsequently form hydrogels under the conditions described by the authors (Fig. 3). The most recent examples build upon work by the Mutter and DeGrado groups, who independently developed the “switch” concept whereby peptide secondary structure could be controlled by various means, including enzyme catalyzed transformations upon the peptides themselves.^{71–73}

Signarvic and DeGrado designed a short peptide, termed Lac-RRS, based on grafting a recognition site for protein kinase A (PKA)-mediated phosphorylation onto a short segment of the Lac repressor tetramerization domain.⁷¹ Owing to the incorporation of mutations designed to destabilize secondary structure elements of the non-phosphorylated form of Lac-RRS, this peptide was largely monomeric in the non-phosphorylated state. Upon phosphorylation by PKA, Lac-RRpS regained structural stability and formed α -helices that self-assembled into a well-defined anti-parallel fourhelix bundle. While this bundle is formally not a nanofibril, we consider this study to be one of the first to introduce the enzymatic switch concept for the control of the secondary structure of designed peptides, thus laying the groundwork for the discussion of subsequent publications below that utilize the same general concept.

Mutter *et al.* designed switch peptides of primary peptide sequences that are known to have intrinsic β -sheet propensity.⁷² By judicious placement of a secondary structure destabilizing switch element (**S**) within the peptide, the designed molecules display random coil conformation, but when the **S** element is manipulated by a trigger, including various enzymes such as proteases and esterases, the peptide transitions to a distinctly structured conformation. Critical in the design of these systems was the nature of the **S** element itself, which consists of a synthetically incorporated temporary structural defect of the polypeptide chain. In the cases triggered by enzymatic action, the defect is the replacement of a single natural peptide bond by an O-acyl isopeptide linkage. That is, the main chain of the peptide is grafted onto the β -hydroxyl of a serine residue while the α -amine is temporarily protected by an enzyme labile linkage. Upon enzymatic cleavage of the N-protecting group, rapid O- to N-acyl transfer takes place, thus restoring the native primary structure of the peptide.⁷⁴ This triggers the onset of secondary structure formation, leading to β -sheets that may self-assemble into nanofibrils.

In a subsequent report, Dos Santos *et al.* described a switch peptide based on the amyloid forming A β (1–42) peptide that is known to play a pathological role in Alzheimer’s disease.⁷³ Upon triggering of two **S** elements placed internally within A β (1–42), a random coil to β -sheet transition with subsequent fibril formation of the native A β (1–42) occurred. In related work, Broncel *et al.* demonstrated that a synthetically phosphorylated amyloidogenic peptide underwent a complex phosphatase-triggered structural transition starting from a random coil and ending in a nanofiber-generating β -sheet conformation.⁷⁵ These approaches may prove useful in screening for drugs to combat Alzheimer’s disease and other related amyloidopathies, as well as aiding in the understanding of the basic biochemical underpinnings of amyloid pathology.^{76,77}

While the examples discussed above use peptides to accomplish enzyme-triggered transformation of molecularly solvated subunits into nanofibers, Kühnle and Börner extended this concept to include polymer–peptide conjugates in their so-called BioSwitch concept (Fig. 3).⁷⁸ Applying the same general strategy of installing secondary structure destabilizing modifications, in this case phosphorylation of threonine residues, they accomplished acid phosphatase-triggered transformation of a molecularly solvated random

coil phosphorylated peptide-PEO bioconjugate to the non-phosphorylated form. These structures formed β -sheets in the peptide domain yielding self-assembled nanofibrils. The self-assembling peptides are located within the center of the fibrillar structure, while the PEO polymer units are arranged along the outer surface, creating a core-shell morphology.

Conclusion

In conclusion, the specificity and diversity of reactions catalyzed by enzymes have been under-utilized in the development and manipulation of nanomaterials in general and especially in the context of the assembly of soft nanomaterials. However, the last ten years have witnessed a dramatic increase in the number of approaches describing enzymes in the assembly and manipulation of the morphology of nanomaterials. As highlighted here, this is particularly interesting in the context of building up complexity in soft materials, although it should be noted that recent examples in the programmed *disassembly* of related materials are becoming increasingly sophisticated.^{79–82} This is a rich field with implications for how we interface future materials with biological systems, especially where two-way communication and feedback is of importance in regulating synergistic behaviour. One such enticing possibility is the idea that nanomaterials may alter their function in response to signals from diseased and/or healthy tissue and hence adapt to their environment. This type of development is critical if we hope to approach the complexity, nuances and adaptability inherent to living molecular ecosystems. Therefore, a key challenge to the field includes extension to a greatly diversified array of enzymatic processes because the majority of systems studied to date utilize only kinases and/or phosphatases. Furthermore, as with all nanoscale materials, novel characterization methods are needed to elucidate structures with high resolution, especially if our goal is to develop systems of increasing complexity. Certainly, the next decade of research into nanomaterials in general will involve the development of greater levels of predictable control over their preparation and an understanding of their dynamic possibilities.

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Biographies

Michael E. Hahn

Michael E. Hahn attended Brandeis University where he received his BS under Prof. John Lowenstein studying classical biochemistry. He then went on to join the Tri-Institutional MD-PhD Program, receiving his PhD from The Rockefeller University in synthetic protein chemistry under Prof. Tom Muir and his MD from Cornell University. Following a medical internship at Memorial Sloan-Kettering Cancer Center, Dr Hahn joined UCSD in 2010 as part of a hybrid clinical/research radiology residency where he currently designs and synthesizes novel molecular imaging agents under the tutelage of Profs. Nathan Gianneschi (chemistry and biochemistry) and Robert Mattrey (radiology).

Nathan C. Gianneschi

Nathan C. Gianneschi received his BSc(Hons) at the University of Adelaide in 1999. In 2005 he completed his PhD at Northwestern University. Following a Dow Chemical postdoctoral fellowship at The Scripps Research Institute, in 2008 he became an assistant professor at the University of California, San Diego. The Gianneschi group takes an interdisciplinary approach to nanomaterials research with a focus on multifunctional materials with interests that include biomedical applications, programmed interactions with biomolecules and cells, and basic research into nanoscale materials design, synthesis and characterization.

Enzyme-driven assembly and enzyme-responsive morphology of nanoscale particles

Fig. 1. (A) Three approaches that have been explored for the manipulation of soft, organic nanoparticles by enzymatically catalyzed reactions. (B) TEM images from ref. 29, illustrating a phosphodiesterase-driven morphology switch utilizing DNA-copolymer micelles.

Enzyme-driven assembly of hydrogels^a **Fig. 2.**

(A) Three approaches that have been explored in the enzyme-driven formation of hydrogel materials. (B) An example from ref. 61, of the enzyme-directed cleavage of a peptide that results in formation of a hydrogel. ^aReferences are given as examples of each of the three systems – see main text for other relevant examples. Photographic images are of vials containing gels or aqueous solutions of peptide, adapted from ref. 61.

Enzyme-driven assembly of supramolecular fibrils

Reference	Method	Schematic
Borner and coworker (2009) Ref: 78	Enzyme-driven modification of a polymer-substrate conjugate results in directional assembly and formation of unidimensional fibrils

Fig. 3.

The enzyme-driven assembly of nanoscale fibrils. In this example, a phosphatase catalyzes the dephosphorylation of a phosphorylated peptide substrate conjugated to a polyethylene oxide polymer. This reaction generates a dephosphorylated peptide designed for β -sheet formation, leading to polymer aggregation and subsequent assembly of nanofibrils. TEM modified from ref. 78.