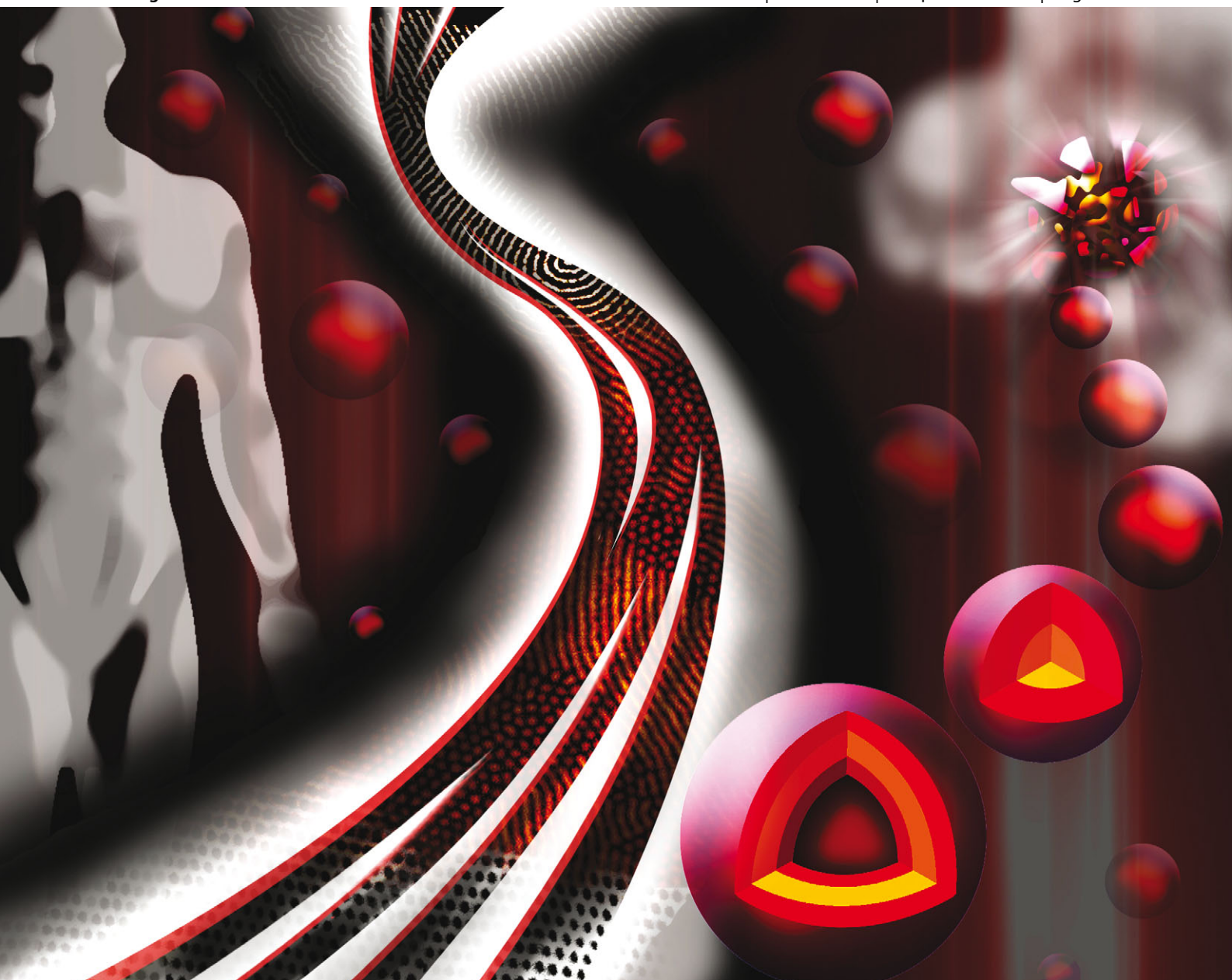


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TUTORIAL REVIEW: Stimuli-responsive copolymer solution and surface assemblies for biomedical applications
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Stimuli-responsive copolymer solution and surface assemblies for biomedical applications

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Stimuli-responsive polymeric materials is one of the fastest growing fields of the 21st century, with the annual number of papers published more than quadrupling in the last ten years. The responsiveness of polymer solution assemblies and surfaces to biological stimuli (e.g. pH, reduction–oxidation, enzymes, glucose) and externally applied triggers (e.g. temperature, light, solvent quality) shows particular promise for various biomedical applications including drug delivery, tissue engineering, medical diagnostics, and bioseparations. Furthermore, the integration of copolymer architectures into stimuli-responsive materials design enables exquisite control over the locations of responsive sites within self-assembled nanostructures. The combination of new synthesis techniques and well-defined copolymer self-assembly has facilitated substantial developments in stimuli-responsive materials in recent years. In this tutorial review, we discuss several methods that have been employed to synthesize self-assembling and stimuli-responsive copolymers for biomedical applications, and we identify common themes in the response mechanisms among the targeted stimuli. Additionally, we highlight parallels between the chemistries used for generating solution assemblies and those employed for creating copolymer surfaces.

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Key learning points

- (1) Describe the potential roles of stimuli-responsive copolymer assemblies in various biomedical applications.
- (2) Identify the response mechanisms of copolymer assemblies in solution and on surfaces to biological and external stimuli.
- (3) Detail the common chemistries associated with the design of copolymers that are responsive to selected stimuli (biological: pH, reduction–oxidation [redox], enzymes, glucose; external: temperature, light, solvent quality).

Introduction

'Smart' synthetic materials that respond to applied stimuli represent an exciting and rapidly growing area of polymer science. In particular, solution assemblies and polymer surfaces designed to respond to biological stimuli (e.g. pH, reduction–oxidation, enzymes, glucose) and externally applied triggers (e.g. temperature, light, solvent quality) show promise for a variety of biomedical applications including drug delivery, medical diagnostics and imaging, tissue engineering, biosensors (e.g. lab-on-a-chip), and bioseparations. Many of these applications require features on biologically relevant length scales (~10–100 nm). Hence, copolymer self-assembly is an attractive

approach to responsive materials design, as copolymer self-assembly schemes provide a robust and tunable means for creating solution^{1,2} and surface³ structures on nanometer length scales. The morphologies and sizes of these nanostructured assemblies can be tuned by controlling copolymer architecture (e.g. linear, branched, graft), co-monomer sequence (e.g. random, gradient, block), composition, interactions between constituent monomers, and overall copolymer molecular weight.^{1–3} Furthermore, stimuli-responsive moieties can be incorporated into copolymer architectures using functional monomers or linkers to trigger nanostructure assembly/disassembly *via* bond cleavage or conformational/solubility changes, depending on the stimulus and the type of chemistry (see Fig. 1).

Controlled bolus delivery is one of the most common application goals for solution assemblies.⁴ Similar to small molecule surfactants, amphiphilic copolymers self-assemble into a variety of nanostructures in which the hydrophobic domains are shielded from the aqueous environment and the hydrophilic domains form a hydrated corona.^{1,2} Copolymers with comparable

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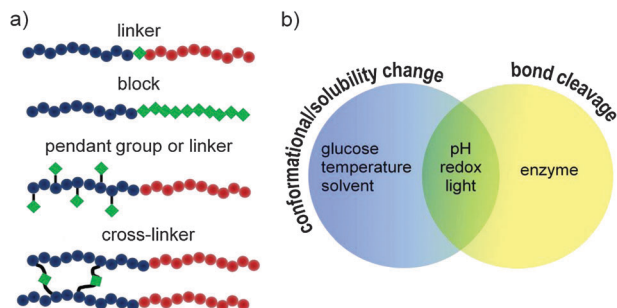


Fig. 1 (a) Schematic representation of copolymer architectures used in stimuli-responsive materials, where the response is localized to the units shown as green diamonds. (b) Chemical and physical changes of copolymers in response to a stimulus can grant access to one or multiple response modes (i.e. bond cleavage or conformational/solubility change).

hydrophobic and hydrophilic volume fractions self-assemble into bilayers or vesicles, and increasing the hydrophilic volume

fraction favors the formation of structures with greater interfacial curvature, such as cylindrical or spherical micelles.^{1,2} These nanostructures can be used to encapsulate therapeutics, such as small molecules or proteins, to protect the therapeutic agent (upon injection into the body) and to improve circulation times, thereby increasing the amount of active drug that reaches the targeted site (see Fig. 2).⁴ However, once the nanocarrier reaches its target site, the drug must be released to accomplish its therapeutic goal. The contradictory demands of carrier stability during circulation and rapid drug release at the target site have motivated significant research in stimuli-responsive nanostructure design for drug delivery. In self-assembled materials, rapid release of the therapeutic molecules is facilitated by stimulus-triggered changes to the micelle or vesicle nanostructure.⁴

In addition to solution assemblies, interest is growing in the development of polymers for surface-mediated drug delivery or regenerative medicine and cell culture applications in which



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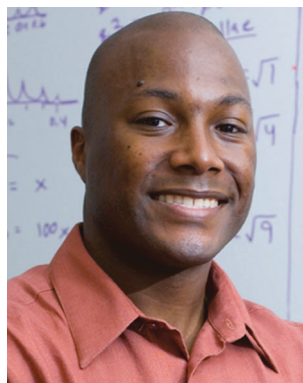
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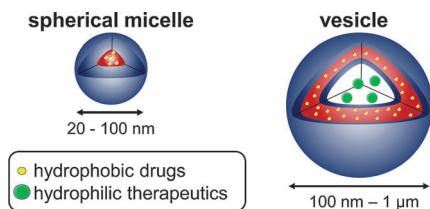


Fig. 2 Typical copolymer solution assemblies used in drug delivery applications. Red and blue regions represent the hydrophobic and hydrophilic domains, respectively. Hydrophobic drugs are shown in yellow, and hydrophilic therapeutics are shown in green.

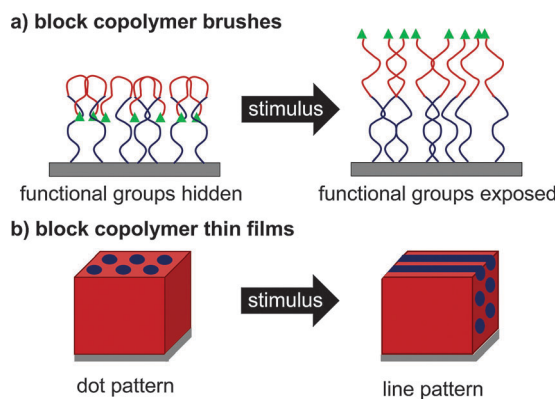


Fig. 3 (a) Prior to the application of a stimulus, functional groups (green triangles) are hidden within the collapsed block copolymer brush. Upon application of a stimulus, chain extension exposes the functional groups to the free surface. (b) Nanostructure reorientation within a block copolymer thin film in response to a stimulus permits control over surface patterns.

the response can trigger cellular adhesion and/or release from a surface (see Fig. 3a).^{5,6} For these applications, stimuli-responsive surfaces typically employ copolymer brushes in which copolymer chains are end-tethered to a substrate to form a brush that may expand or collapse following application of the stimulus.^{5,6} Additionally, responsive surfaces may utilize the nanoscale patterns formed by block copolymers in thin film geometries. Like their solution-assembled counterparts, copolymers with similar block volume fractions self-assemble into lamellae, and asymmetric compositions favor the formation of structures with greater interfacial curvature such as cylinders, gyroid networks, and spheres.^{2,3} In block copolymer thin films, the thin film morphology and nanostructure orientation also are influenced by copolymer interactions with the substrate and free surfaces.⁷ Thus, responsive surfaces often take advantage of nanostructure rearrangements at the free surface of the film (see Fig. 3b), which can be exploited to pattern proteins in nanoscale arrays for biosensors or cell proliferation and migration studies on surfaces.^{3,7}

Advances in polymer chemistry over the last decade have facilitated the synthesis of stimuli-responsive polymeric materials. Developments in controlled polymerization techniques such as reversible addition-fragmentation chain-transfer (RAFT) polymerization, atom transfer radical polymerization (ATRP), nitroxide-mediated radical polymerization (NMP), and ring-opening metathesis polymerization (ROMP) allow for the

synthesis of polymers with well-defined architectures, reproducible molecular weights, low polydispersities, and end-group fidelity.⁸ Many of these polymerizations proceed under mild conditions and are tolerant to functional groups, enabling the direct polymerization of responsive monomers or the incorporation of responsive end-groups through the use of functional chain transfer agents (CTA)s or initiators. Additionally, the proliferation of ‘click’ reactions (e.g. thiol-ene, azide-alkyne) provides highly efficient and orthogonal coupling chemistries to create well-defined copolymers or to incorporate responsive groups into copolymer architectures in a near-quantitative fashion.^{8,9} Coupled with advances in solid phase synthesis of biomolecules (e.g. peptides, DNA), these chemistries allow for facile incorporation of functional peptides or peptidomimetics (e.g. peptoids) into copolymer architectures.^{8,10} Also, advances in *N*-carboxyanhydride (NCA) ring-opening polymerization have facilitated the synthesis of high molecular weight polypeptides¹¹ and polypeptoids,¹² providing access to biohybrid copolymers for biomedical applications. Moreover, recent developments in polymer drug (polymers that degrade into a bioactive drug) synthesis offer new opportunities in polymer-based drug delivery.¹³

This tutorial review highlights prominent trends in the design of stimuli-responsive copolymers, primarily block copolymers, for biomedical applications. These materials combine decades of fundamental research on copolymer solution- and surface-assembly with recent innovations in polymer synthesis. Herein, we identify common stimulus-response mechanisms, and we relate the stimulus-induced changes in polymer chemistry and/or molecular conformation to transformations in self-assembled nanostructures and, ultimately, to material performance. We focus on examples in which the copolymer architecture is key to material functionality, and we refer the reader to additional sources for discussions on other stimuli-responsive polymeric systems involved in biomedical research, such as layer-by-layer assemblies,¹⁴ hydrogels,¹⁵ and polymer-grafted nanoparticles.¹⁶ Also, for more general reviews of stimuli-responsive materials, including other applications of stimuli-responsive polymers, we refer the reader to several recent works.^{17–19}

This tutorial review is organized by stimulus, focusing on systems that respond to various biological stimuli (first section), external stimuli (second section), and multiple stimuli (third section). For each stimulus, we address the stated key learning points by discussing [1] the relevant biomedical applications (*i.e.* drug delivery, medical diagnostics and imaging, tissue engineering, control of *in vitro* cell adhesion, or biosensing); [2] the copolymer response mechanisms (*i.e.* solution nanostructure assembly/disassembly or surface reconstruction); and [3] the key trends in materials design and synthesis (*i.e.* incorporation of cleavable functional groups in copolymer assemblies, use of polymers that exhibit conformational or solubility responses, and/or integration of biomolecules into copolymer architectures). We note that the literature related to copolymers that respond to biological stimuli is dominated by systems that self-assemble in solution, whereas the work related to external stimuli draws primarily

from the design of responsive surfaces. We suspect that this difference arises in part from [1] the difficulty in applying an external stimulus to solution assemblies designed for *in vivo* drug delivery, and [2] the spatial and temporal control afforded by the *ex vivo* application of external stimuli that readily lends these stimuli to surface-oriented applications like peptide arrays and cell culture. The specific examples we have chosen identify important design principles and synthesis techniques common to both solution and surface assemblies.

1. Biological stimuli

The generation of materials that respond to stimuli encountered in cells and organisms is highly desirable for biomedical applications. However, biological environments place extreme demands on materials design and polymer chemistry due to the myriad of highly specific and highly localized signals in cellular and multicellular systems. Also, the materials should be sensitive to biological cues at millimolar (or lower) levels, as many chemical stimuli of interest are naturally present at these low concentrations. Simultaneously, the materials should not elicit unwanted local or systemic responses. In the following sections, we highlight recent developments in copolymer design and synthesis that meet these sometimes-contradictory demands.

1.1. pH-responsive

For many biological applications, pH-sensitive materials should be stable at physiological pH ($\text{pH} \approx 7.4$) and responsive to slightly lower pH ($\text{pH} \approx 5.0$ – 6.5).²⁰ For drug delivery, this design facilitates the release of therapeutics within the cell (*versus* in the surrounding tissue), as the endolysosomal compartments formed upon internalization of many drug carriers gradually acidify ($\text{pH} \approx 5.0$ – 6.5).²⁰ Additionally, the pH in tumor tissues ($\text{pH} \approx 6.5$ – 7.2) is slightly lower than the pH in normal tissues,²⁰ making pH-responsiveness an ideal way to target tumors with chemotherapeutics. For other biomedical applications, such as anti-biofouling, bioseparations, and biofiltration, the pH ranges for stability *versus* response are case-specific,²¹ with a key parameter being the use of protein-friendly conditions that maintain bioactivity.

Two primary strategies have been identified for the design of pH-responsive copolymer assemblies. In the first, acid-labile functional groups are incorporated into the backbone of the copolymer or used to conjugate active drugs to copolymer side-chains. In the second, the molecular pH response initiates a conformational change in all or part of the copolymer that disrupts (or otherwise alters) the self-assembled nanostructures.

Many pH-sensitive drug carriers incorporate acid-labile moieties at key points along the copolymer chain or throughout the copolymer backbone, such as main-chain ester groups [e.g. poly(ϵ -caprolactone) (PCL), poly(lactic acid) (PLA), polyanhydrides, polycarbonates, polyketals].^{20,22,23} Thus, pH-triggered bond cleavage disrupts the hydrophobic/hydrophilic balance or degrades the copolymer, leading to nanocarrier disassembly and release of encapsulated therapeutics. Degradable nanocarriers are particularly attractive for drug delivery applications

because these materials avoid renal clearance during circulation due to their size (~ 10 to 100 nm), yet the degradation products (< 10 nm) are cleared from the body after pH-triggered drug delivery.^{4,24}

Alternatively, drug molecules can be conjugated to copolymer nanostructures *via* acid-labile linkers. Covalent attachment of drugs to carriers has numerous potential benefits including improved drug stability, enhanced circulation times, altered biodistribution, and reduced systemic drug toxicity. For example, Lee *et al.* assembled micelles from a double-hydrophilic hyperbranched copolymer, poly(ethylene glycol-*hb*-glycerol) (PEG-*hb*-PG), in which the anti-cancer drug doxorubicin was conjugated to the hyperbranched PG units *via* acid-labile hydrazone linkages.²⁵ Attachment of the hydrophobic drug rendered the PG segments hydrophobic, and the resulting amphiphilic copolymer formed micelles in aqueous solutions. Upon cleavage of the hydrazone linkage in solution at $\text{pH} = 5.0$, or in the endolysosomal compartments within human cervical cancer HeLa cells, doxorubicin was released, and the micelles disassembled into relatively benign PEG-*hb*-PG unimers.²⁵ Thus, the combination of pH-cleavable units with a hyperbranched architecture and a biocompatible polymer enhanced drug loading capacity and promoted efficient release, while also providing a potential mechanism for polymer clearance.²⁵ Other noteworthy acid-cleavable chemistries that have been employed in copolymer assemblies include carbamate,²⁵ catechol,²⁶ and Schiff base.²⁷

In the second strategy for responsive assembly design, all or part of the copolymer undergoes a pH-triggered change in hydrophilicity or conformation that disrupts the self-assembled nanostructures. pH-dependent changes in hydrophilicity have been used frequently to stimulate nanostructure disassembly into unimers; for example, an elegant study by Manganiello *et al.* demonstrated the disassembly of micelles into unimers at endosomal pH, and the authors showed that the copolymer architecture could be tuned such that the released unimers destabilized the endosomal membrane and thereby enabled enhanced cytoplasmic delivery of nucleic acids.²⁸ Alterations in the copolymer chains also can be exploited to induce nanostructure rearrangements, which could alleviate concerns over the aggregation of copolymer materials post-delivery. For example, Doncom *et al.* took advantage of pH-dependent protonation of amine groups to increase the hydrophilicity of an amphiphilic copolymer, leading to a pH-induced vesicle to micelle transformation.²⁹ Moreover, the copolymer nanocarriers released a hydrophilic dye during the structural rearrangement, suggesting that they could be used for pH-sensitive drug release within acidic intracellular compartments or tumor tissues.²⁹

Alternatively, polymer-peptide conjugates are an attractive class of materials that undergo pH-dependent conformational changes. These conjugates combine the synthetic versatility of polymeric materials with the well-defined structures inherent to biological macromolecules. Rather than relying exclusively on changes in solubility, polymer-peptide conjugates exploit pH-dependent changes in polypeptide secondary structure (e.g. random coil to α -helix) that lead to pronounced changes

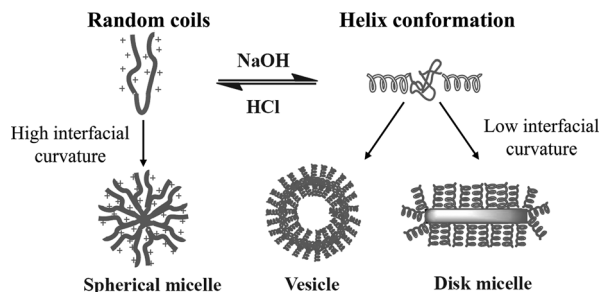


Fig. 4 Schematic of responsive KPK morphologies showing the formation of high interfacial curvature spherical micelles at low pH where the K block exists as a random coil, and low interfacial curvature structures (vesicles or disk micelles) at higher pH where the K block adopts an α -helix conformation. Adapted from ref. 30. Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA.

in the chain configuration and self-assembled nanostructures. For example, Ray *et al.* synthesized poly(L-lysine-*b*-propylene oxide-*b*-L-lysine) (KPK) copolymers in which the K-block underwent a gradual transformation from random coil to α -helix from pH 3 to pH 7.³⁰ The formation of α -helices increased polymer chain rigidity, leading to a more extended chain configuration that favored the formation of morphologies with lower interfacial curvature (see Fig. 4).³⁰ This work highlights the advantages of utilizing defined conformational changes in polymer-peptide conjugates to manipulate self-assembled nanostructures for drug delivery. Moreover, these materials have the potential to provide additional control over nanostructure rearrangement through careful design of the polypeptide sequence.

Surface assemblies with conformational responses to pH typically incorporate a weak polyelectrolyte into a surface-grafted polymer brush to enable pH-mediated control over brush charge and swelling. These polyelectrolyte brushes can reversibly adsorb and release nanoparticles or macromolecules for substrate-mediated drug delivery and bioseparations, enable attachment or release of cells from culture dishes, impart anti-biofouling and contaminant-release functions, and induce microcantilever actuation in biosensors.⁵ A plethora of examples employing surface-grafted homopolymers illustrate the power of these polymeric brushes for accomplishing the abovementioned functions and demonstrate the diversity of polymers available for such applications [e.g. poly(2-vinyl pyridine) (P2VP), poly(acrylic acid) (PAA), poly(2-(dimethylamino)-ethyl methacrylate) (PDMAEMA), poly(2-(dimethylamino)-ethyl acrylate) (PDMAEA)].^{5,6,31}

Simultaneously, our understanding of the unique surface charging properties accessible through copolymer electrolytes has grown immensely. Block copolymer electrolyte brushes offer advantages over their mixed brush counterparts including (1) the avoidance of lateral phase segregation, which can create undesirable chemical heterogeneity at the brush surface, (2) the ability to tune the phase behavior in response to pH, depending on which block tethers the copolymer to the surface, and (3) the capacity to alter the relative lengths of polymer components within the confines of maintaining a responsive surface.⁶ For example, in work by Yu and Han²¹ and references therein, the authors created brushes of P2VP-*b*-PAA copolymers in which

the distinct isoelectric points of the two constituent polymers ($pI_{P2VP} = 6.7$, $pI_{PAA} = 3.2$) enabled reversible pH-induced changes in surface properties over three separate regimes (cationic, dual-charged, or anionic).²¹ The responsiveness of these brushes, as characterized by water contact angle and surface composition measurements, was highly dependent on which block anchored the copolymer to the surface.²¹ The copolymers anchored by the P2VP block (substrate/P2VP-*b*-PAA) exhibited the same pH response as mixed brushes of P2VP and PAA homopolymers. However, the responsiveness of copolymers anchored by the PAA block (substrate/PAA-*b*-P2VP) could be tuned according to the block lengths. The placement of the PAA block adjacent to the substrate and the P2VP block at the free surface provided access to wettability trends as a function of pH that had not been reported previously for mixed brush systems.²¹ Furthermore, the authors had shown in prior work that P2VP-*b*-PAA brush charging in response to pH could be used to regulate the adsorption or non-adsorption of negatively charged polystyrene (PS) nanoparticles on the brush surface, illustrating the potential of these copolymer brushes to bind or repel charged biomolecules such as proteins. Their recent demonstration of block order and block length as accessible parameters for tuning the pH response of surface assemblies is an important consideration for biomedical applications. For example, in bioseparations and biofiltration applications, adsorption of specific species from a mixture of biomolecules at one pH and release at a different pH is desirable and could be accomplished by carefully tuning the copolymer electrolyte brush response.

Finally, copolymer designs are not limited to a single mode of pH response, as recently demonstrated by Du *et al.*³² In this work, two distinct pH-responsive groups, one that exhibited a conformational change and another that was acid-labile, were used to create dual pH-responsive nanoparticles that exploited deviations from physiological pH both in tumor tissues and in endosomal compartments within tumor cells.³² To accomplish this task, the authors synthesized functional polyphosphoester copolymer-doxorubicin conjugates through a combination of ring-opening polymerization, thiol-ene 'click' chemistry, and sulfhydryl chemistry. These drug-linked copolymers self-assembled into negatively charged nanoparticles in water. After incubation at pH = 6.8 (in mimicry of the extracellular pH of tumor tissue), the particles became positively charged, thus stimulating nanoparticle internalization by MDA-MB-231 breast cancer cells.³² Subsequent to cellular uptake, the nanoparticles released doxorubicin in response to endosome acidification *via* cleavage of the hydrazone linkage binding the drug molecule to the copolymer. Thus, this dual-responsive design facilitated tumor cell uptake of drug carriers and improved intracellular release of encapsulated drug molecules.

The versatility of pH-sensitive chemistries affords many opportunities for exploiting pH variations found in biological systems. As illustrated by the examples presented in this section, these chemistries include a variety of acid-labile linkers, biodegradable polymers, polymer-peptide conjugates, and polyelectrolytes. When these chemistries are coupled with

the controlled self-assembly of copolymers, their responses to changing pH conditions influence critical self-assembly parameters. These parameters include the hydrophobic/hydrophilic balance, electrostatic interactions, and molecular weight (through hydrolysis), and their manipulation leads to morphological changes that can be exploited for biomedical applications. In particular, the acidic conditions found in tumor tissues and in intracellular compartments have made solution-assembled pH-responsive copolymer systems ideal for drug delivery applications. Additionally, pH-responsive polyelectrolyte surfaces capable of reversibly binding and releasing biological molecules present promising opportunities in bio-separations and biofiltration. As illustrated in the above-mentioned example from Yu and coworkers,²¹ detailed knowledge of copolymer electrolyte phase behavior and the ability to control pH response through brush architecture can help tune surface properties for these applications.

1.2. Reduction–oxidation (redox)-responsive

For applications in controlled delivery, redox-responsive materials have the potential to exploit the varying concentrations of redox species in intracellular and extracellular compartments to facilitate drug release within diseased cells or tissues. For example, the concentration of glutathione (a reducing agent) is two orders of magnitude higher in the cellular cytosol and nucleus than in extracellular fluids, and intracellular compartments such as endosomes are reducing environments. Additionally, inflammation and many cancers are associated with increased enzyme activity that leads to high concentrations of oxidizing agents such as hydrogen peroxide and superoxide anions. Alternatively, for *ex vivo* biomedical applications, redox-sensitive copolymer materials are desirable due to the need for reversible and biologically friendly methods to control cell adhesion and protein patterning for cell culture, diagnostics, and biosensing.

Because many therapeutics are active in the cytosol or nucleus of cells, there is significant interest in developing glutathione-responsive solution assemblies for drug delivery applications. Many glutathione-responsive materials incorporate similar design principles to those used in pH-responsive systems containing acid-labile moieties. Glutathione-responsive block copolymers often contain a disulfide linkage between the hydrophobic and hydrophilic blocks that is introduced through the use of a disulfide functionalized initiator, thereby creating 'shell-sheddable' micelles.³³ This disulfide linkage between the blocks is cleaved upon exposure to glutathione, destabilizing the micelles, and releasing the therapeutic cargo.³³ Other glutathione-responsive micelles take advantage of thiol-reducible linkers that are used to cross-link the micelle core or shell or employ disulfide linkages to attach drug molecules to one of the polymer blocks. *In vitro* and *in vivo* studies of glutathione-responsive micelles suggest that disulfide linkers have the potential to address the need for a drug carrier that is stable during circulation but rapidly destabilizes upon cellular entry to release therapeutic molecules.³³

While disulfide-containing polymers take advantage of the high concentrations of reducing agents in intracellular compartments, amphiphilic block copolymers containing poly(propylene sulfide) (PPS) as the hydrophobic block are sensitive to the oxidative environment associated with numerous diseases. The sulfide groups oxidize to form hydrophilic sulfoxide and sulfone moieties that disrupt the hydrophobic/hydrophilic balance of the solution assemblies and ultimately lead to nanostructure disassembly.³⁴ In an analogous approach, Ren *et al.* took advantage of oxidation-induced solubility changes of selenium-containing block copolymers to disrupt self-assembled nanostructures.³⁵ These copolymers formed spherical micelles in aqueous solutions with hydrophobic, selenium-containing cores. Upon exposure to hydrogen peroxide (H_2O_2), the selenide groups were oxidized to selenoxide, which increased the hydrophilicity of the selenium-functionalized block and resulted in micelle disassembly (see Fig. 5). The spherical micelles were regenerated upon addition of a reducing agent, demonstrating that these selenium-containing nanocontainers were responsive to both oxidizing and reducing conditions and that their redox-responsive properties were fully recoverable.³⁵

Iron centers are a versatile redox-sensitive group used in materials design that takes advantage of the well-understood and reversible properties of the $\text{Fe}^{\text{II}}/\text{Fe}^{\text{III}}$ couple.³⁶ For example, polyferrocenylsilane (PFS)-based copolymers contain main chain iron centers, and these centers are positively charged when oxidized (see Fig. 6).⁷ Eloi *et al.* exploited this redox-induced charging to trigger the selective adsorption of negatively charged proteins onto nanostructured block copolymer thin films.⁷ By carefully tuning the ionic strength, pH, and protein concentration in solution, they demonstrated selective adsorption of the negatively charged protein ferritin onto the surface of the positively charged, oxidized PFS domains in PS-*b*-PFS thin films. Thus, they created a protein array on the length scale of the self-assembled block copolymers (≈ 30 nm) (see Fig. 6).⁷ Protein arrays are of interest for the development of high-throughput, low-volume assays to screen drug interactions or investigate protein expression in samples with low analyte concentrations. Moreover, nanoscale protein patterns significantly affect cell behavior and are of interest for studying cell adhesion, proliferation, and migration on surfaces. However, creating arrays on the length scale of individual proteins (~ 10 nm) often is difficult to achieve with lithographic methods that typically require expensive and highly specialized equipment.³

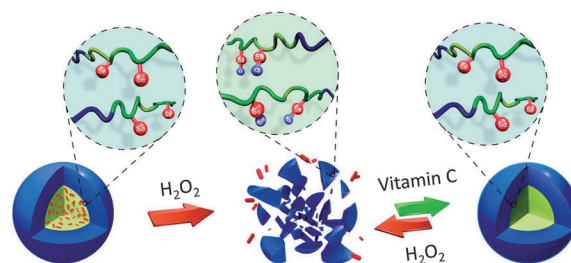


Fig. 5 Cartoon showing redox-responsive assembly and disassembly of selenium-grafted PEG-*b*-PAA polymers (selenium shown in red). Adapted from ref. 35.

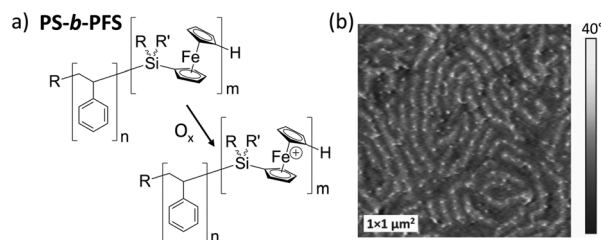


Fig. 6 (a) Structure of PS-*b*-PFS before and after oxidation, where R = CH₃ and R' = CH₂CH₃. (b) AFM phase image showing selective ferritin (white dots) adsorption onto PFS domains (light domains) in an oxidized PS-*b*-PFS thin film. Adapted from ref. 7. Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA.

Thus, self-assembling copolymers provide an ideal medium for controlled and nanoscale protein deposition.

Generally, the chemistries selected for the design of redox-responsive materials utilize disulfide linkages or chemical species with multiple oxidation states (sulfur, selenium, or iron). Like their pH-responsive counterparts, redox-responsive solution assemblies are designed to respond to specific conditions found in cellular and multicellular systems through bond cleavage or solubility changes at the molecular level, which in turn affect the stability of solution-assembled structures. Likewise, oxidation-induced charging in nanoscale surface assemblies is a facile, bottom-up approach to creating protein arrays on the length scale of individual proteins for biosensors, miniaturized assays for low-volume biological tests, and substrates for studying biomolecule–cell interactions.

1.3. Enzyme-responsive

Enzymes are responsible for remodeling biomolecular polymers such as the extracellular matrix (ECM), making them attractive targets for the ‘remodeling’ of synthetic materials for biomedical applications. Also, incorporating enzyme substrates into synthetic systems can facilitate mechanistic investigations of the complex and dynamic interactions between cells and the ECM. Examples of biological enzyme substrates include peptides and DNA, which are highly specific, chemically stable at physiological pH, and tunable (*i.e.* the rate of cleavage can be tuned by controlling the sequence of the substrate). Moreover, enzymes can trigger drug release in targeted tissues, as certain enzymes are overexpressed in diseased cells. Advances in controlled polymerization, solid phase synthesis of biomolecules, and bioconjugation have facilitated the generation of biohybrid copolymers that contain enzyme substrates, enabling the design of new bio-responsive materials.

Although there are few examples of purely synthetic polymers that are enzyme-responsive, several ester-containing polymer systems take advantage of enzyme-catalyzed hydrolysis, such as PCL, PLA, and polycarbonates.³⁷ While these polyesters degrade in acidic solutions in the absence of enzymes (see Section 1.1), acid-catalyzed hydrolysis can be slow, taking weeks to months. For systems that require a faster response, enzyme-catalyzed hydrolysis is an attractive alternative. For example, Sanson *et al.* demonstrated the rapid destabilization of poly(trimethyl carbonate)-*b*-poly(L-glutamic acid) vesicles upon

exposure to lipase, an enzyme known to have activity for ester chain-scission.³⁷ In addition to highlighting the benefits of the enzyme-catalyzed hydrolysis of synthetic polymers, this study demonstrated the capability of enzymes to migrate through the hydrophilic shells of block copolymer vesicles and cleave bonds associated with the hydrophobic vesicle bilayer. This migration and cleavage has promising implications for the design of enzyme-responsive materials.

Currently, the majority of enzyme-responsive systems rely on proteases with broad specificity, or kinases and/or phosphatases that add or remove phosphate groups; however, a desirable advantage of enzyme-responsive materials is the potential for high specificity. Polypeptide-based block copolymers offer the benefit of incorporating natural enzyme substrates into synthetic polymers, thereby increasing the selectivity of the enzyme response. NCA polymerization is a convenient means of synthesizing well-defined polypeptides and copolypeptides with high molecular weights and moderate polydispersities.¹¹ As exemplified by Sparks *et al.* and references therein,³⁸ recent advances in NCA polymerization and ‘click’ chemistries have brought together polypeptide synthesis, surface-initiated polymerization, and surface modification schemes. Although a relatively underdeveloped area, surface grafted polypeptides have the potential to provide greater versatility in chemistry and surface topography as compared to traditional self-assembled monolayer substrates, enabling superior enzyme-induced control over the surface properties that dictate cell responses. Moreover, polypeptides and copolypeptides can be used for enzyme-triggered release of growth factors from surfaces for tissue engineering or small molecules from solution assemblies for drug delivery. For example, Habraken *et al.* utilized a combination of NCA polymerization and NMP to synthesize a series of amphiphilic block copolymers containing a hydrophilic poly(L-glutamic acid-*co*-alanine) [P(Glu-*co*-Ala)] block coupled to a PS or poly(*n*-butylacrylate) (PnBA) hydrophobic block.³⁹ These polymers self-assembled into vesicles or spherical micelles in aqueous solution, and enzymatic degradation of the polypeptide block by thermolysin or elastase eventually led to precipitation of the polymer. Notably, the degradation rate in response to elastase was dependent on the alanine distribution in the copolypeptide, as elastase selectively cleaves peptide bonds between small, hydrophobic amino acids (in this case alanine-alanine). This work demonstrates the potential for not only selectivity, but also tunability in enzyme-responsive systems, thereby enabling both spatial and temporal control over drug release.

In order to further increase the specificity of enzyme-responsive materials, enzyme substrates composed of a specific sequence of amino acids or nucleic acid bases are necessary. Traditionally, these sequence-specific substrates are synthesized using solid phase methods. Incorporating these substrates into synthetic polymers requires high end-group fidelity as well as orthogonal chemistries that will not affect the substrate side-chains that are essential for enzyme recognition. One approach for incorporating sequence-specific enzyme substrates into synthetic polymers is through peptide-functionalized initiators

or peptide macromonomers. For example, Chu *et al.* took advantage of the tolerance of RAFT polymerization toward functional groups and directly copolymerized *N*-(2-hydroxypropyl) methacrylamide (HPMA) with a methacrylamido-terminated peptide macromonomer containing a Cathepsin B cleavage site.⁴⁰ *Note: Cathepsin B is a protease that is active in the endosomal compartments of cells.* The authors demonstrated that the resulting cationic polymers could efficiently condense DNA, yet rapidly release it in the presence of Cathepsin B. However, despite this mechanism for intracellular DNA release, the efficacy of *in vitro* gene transfer was not enhanced. The authors postulated that this lack of efficacy was caused by inefficient escape of DNA from the endosomal compartments and limited trafficking to the nucleus.⁴⁰ This example emphasizes the significant challenges associated with designing carriers to overcome the multiple barriers to drug delivery and highlights the need for a better understanding of both intracellular trafficking and the ultimate fate of drug carriers in cells.

In addition to increasing specificity, copolymers containing sequence-specific enzyme substrates allow for precise control over the final hydrophobic/hydrophilic ratio upon cleavage and therefore can be used to induce well-defined morphology changes. For example, Chien *et al.* reported the intelligent design of an amphiphilic DNA brush copolymer in which site specific cleavage of a portion of the DNA brush reduced the hydrophilic weight fraction of the copolymer and resulted in a transformation of the solution-assembled structures from spherical micelles to cylindrical micelles (see Fig. 7).⁴¹ While many enzyme-responsive systems rely on disassembly or degradation of nanostructures, this example demonstrates a distinct, enzyme-induced morphological transformation that could alleviate concerns regarding copolymer aggregation or precipitation in biological systems during drug release.

The development of self-assembling and enzyme-responsive copolymer materials has paralleled advances in polymer chemistry. NCA polymerization allows for facile synthesis of high molecular weight polypeptides that can be incorporated into copolymer architectures. Also, solid phase biomolecule synthesis methods enable exquisite control over the sequence and diversity of enzyme substrates and therefore substrate specificity and cleavage rate. Incorporating these sequence-specific substrates into copolymer architectures using polymerization techniques that are tolerant to functional groups (*e.g.* RAFT) or post-polymerization modification

chemistries (*e.g.* 'click' chemistries) provides control over copolymer self-assembly that is not afforded easily by purely synthetic polymers.

1.4. Glucose-responsive

As recently reviewed by Cambre and Sumerlin, there is significant interest in developing sugar-responsive boronic acid-containing copolymers for biomedical applications.⁴² Boronic acid-sugar interactions have been exploited in affinity chromatography to purify sugars, nucleotides, and glycoproteins.⁴³ Also, sugar-responsive copolymers have been immobilized on surfaces to promote cell adhesion/release, which could be used to isolate cells.^{42,43} Furthermore, these materials have attracted considerable research attention for glucose sensing applications and, more importantly, for self-regulated insulin delivery systems to treat diabetes.^{42,44}

Advances in controlled polymerization methods have facilitated greatly the synthesis of copolymers containing boronic acid. These copolymers take advantage of the increased hydrophilicity of the boronic acid moieties upon addition of glucose to disrupt solution-assembled nanostructures or to swell polymer brushes. At a pH near the pK_a of boronic acid, glucose addition causes a dramatic shift in the ionization equilibrium and a transition from the hydrophobic and insoluble form to the hydrophilic and soluble form of boronic acid polymers (see Fig 8).^{42–44} Therefore, the glucose sensitivity of boronic acid-based polymers is highly dependent on the pK_a of the specific boronic acid-based functionality ($pK_a \approx 4.5$ –10). Additionally, many of these materials are only glucose-responsive at high pH conditions that have limited relevance for *in vivo* applications.^{42,44}

Fortunately, within the past few years, significant progress has been made in the synthesis of phenylboronic-acid [$Ph-B(OH)_2$]-containing copolymers. The pK_a range of the phenylboronic acid functionalities ($pK_a \approx 8$ –9) imparts glucose-responsiveness near physiologically relevant pH ($pH \approx 7.4$).⁴² Moreover, the pK_a of phenylboronic acids can be tuned by adding various substituents to the phenyl ring, potentially enabling the use of these copolymers for *in vivo* glucose sensing and insulin delivery applications.⁴²

An additional challenge in the design of sugar-responsive materials for *in vivo* applications is tuning the glucose sensitivity at physiological pH. This consideration is important both for surface assemblies designed for glucose-sensing devices and for

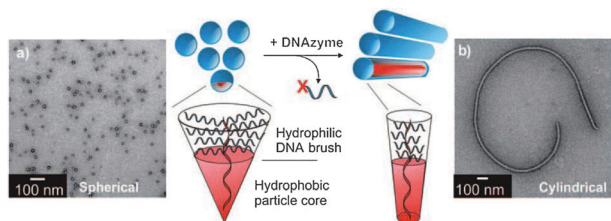


Fig. 7 DNA-brush copolymer structural rearrangement induced by enzymatic cleavage of a portion of the DNA brush. (left) TEM micrograph of spherical micelles formed in aqueous solutions. (middle) Cartoon representation of the changes in copolymer structure that lead to changes in self-assembly. (right) Cylindrical micelles formed following enzymatic cleavage. Adapted from ref. 41. Copyright 2010 Wiley-VCH Verlag GmbH & Co. KGaA.

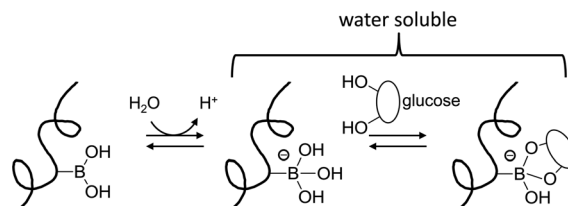


Fig. 8 Ionization equilibrium of boronic acids. In aqueous solutions, the neutral, hydrophobic form of boronic acid is in equilibrium with the anionic, hydrophilic form. The balance of this equilibrium depends on the pH of the solution and the pK_a of the boronic acid moiety, and increasing the concentration of glucose shifts the equilibrium towards the hydrophilic (water-soluble) forms.

solution assemblies designed for controlled insulin release. In response to the demand for more sensitive chemistries, Yao *et al.* took advantage of the relatively low pK_a of phenylboronic ester-containing polymers (PPBDEMA) to synthesize copolymers that were sensitive to glucose at both physiological pH and glucose concentrations (5–20 mM).⁴⁴ They synthesized several block copolymers (PEG-*b*-PPBDEMA) with varying hydrophobic block lengths using ATRP and a PEG-based macroinitiator. At pH = 7.4, these polymers self-assembled into spherical micelles with a hydrophobic PPBDEMA core and a hydrophilic PEG corona. Increasing the PPBDEMA block length increased the encapsulation efficiency and the loading capacity of insulin into these micelles, while also moderating the insulin release in response to glucose. These micelles released insulin gradually over 55 h in response to glucose concentrations as low as 5.6 mM, with higher glucose concentrations resulting in greater cumulative release of the encapsulated insulin over the same period. The tunable loading capacity and release profile of these micelles in response to physiological glucose concentrations demonstrates the promising potential of boronic acid-based copolymers for 'smart' insulin delivery to treat diabetes.

Glucose-responsive copolymer assemblies represent an important niche within stimuli-responsive materials. They exhibit a response mechanism (hydrophobic–hydrophilic shift) that is similar to other biologically-sensitive copolymer assemblies addressed in this review. However, in contrast to the chemical versatility found in systems responding to other stimuli, glucose-responsive materials have primarily used boronic acid chemistries due to their tailorability and specificity for simple sugars. Recent advances in controlled polymerization methods amenable to novel boronic acid-functionalized monomers have permitted the sensitivity of these polymers to be tuned to physiologically relevant conditions, presenting new opportunities in glucose-triggered delivery.^{42,44}

2. External and applied stimuli

Compared to biological stimuli, external stimuli offer the benefits of user-defined spatial and temporal control, and they are ideal for preparation and use of assemblies *ex vivo*. Specifically, the spatial control afforded by these stimuli is beneficial for patterning surface assemblies. Solution-based therapies also can benefit from enhanced spatial control, which can ensure that drugs are released at the targeted site and not into other organs where nanoparticles tend to accumulate (*e.g.* liver and spleen). Furthermore, temporal control is important for timed drug release, reversible biomolecule adsorption or cell adhesion to surfaces, and sequential processing of functional polymeric assemblies. Here we highlight recent examples from the literature that take advantage of applied stimuli in materials design for biomedical applications.

2.1. Temperature-responsive

Thermally-responsive polymers have received attention primarily for applications *ex vivo*. Temperature-responsive surfaces have been used to control cell adhesion, with the goal of either

recovering cells for additional analysis (*e.g.* flow cytometry) or creating surfaces that mimic the stimuli that cells might encounter *in vivo*. Thermally-responsive polymers also are involved in the *ex vivo* fabrication of solution assemblies intended for *in vivo* applications. Additionally, local temperature increases have been accomplished *in vitro* by applying magnetic fields to polymer-coated magnetically-responsive inorganic nanoparticles.⁴⁵ Finally, externally-focused mild hyperthermia is used clinically, in combination with other therapies, for the *in vivo* treatment of large tumor masses.⁴⁶ However, precise manipulation of temperature *in vivo* remains difficult, and the design of polymer assemblies that are sensitive to the limited range of allowable temperature changes *in vivo* presents a significant challenge.⁴⁶ In this section, we discuss recent trends surrounding two of the most popular temperature-responsive synthetic polymers for biomedical applications, poly(*N*-isopropylacrylamide) (PNIPAM) and poly(ethylene glycol) (PEG), and we refer the reader to a recent article by Ward and Georgiou for a more comprehensive review on the topic.⁴⁷ We also consider recent innovations in block copolypeptide materials that combine the self-assembly properties of traditional copolymer architectures with the temperature-sensitivity of biomolecules.

PNIPAM has a lower critical solution temperature (LCST) of $\approx 32^\circ\text{C}$, which lies in the vicinity of physiological temperature ($\approx 37^\circ\text{C}$), making the polymer a popular choice for thermally-responsive assemblies.⁴⁷ By conjugating PNIPAM to other polymers, peptides, and proteins, researchers have tuned the thermal properties of various materials to achieve the desired thermo-responsive effects.⁴⁸ For example, Ebara *et al.* demonstrated temperature-sensitive 'on-off' affinity control of cell adhesion and detachment from PNIPAM-based surfaces.⁴⁸ They assembled poly(NIPAM-*co*-CIPAM) (where CIPAM is 2-carboxyisopropylacrylamide) brushes in which the CIPAM groups were functionalized with an RGD integrin binding sequence to promote cell attachment. Above the LCST of PNIPAM, the collapsed brushes effectively immobilized the RGD units at the free surface of the brush, making them available for cell adhesion. Under these conditions, the spreading of human umbilical vein endothelial cells on the surface was indicative of RGD engagement and cell attachment.⁴⁸ Upon reducing the temperature to 20°C (*i.e.* below the LCST of PNIPAM), hydration of the brush caused the chains to swell and expand, thus shielding the RGD sites from the cells and disrupting cell–surface interactions. Under these conditions, the percentage of spread cells decreased to near zero in less than 1 h.⁴⁸ The ability to bind and, more importantly, to release cells using a temperature stimulus has important implications for biomedical applications. Traditionally, cell removal from surfaces is accomplished using enzymes (*e.g.* trypsin), which requires optimization for individual cell types and is not well tolerated by sensitive cells. However, removing cells by simply cooling the surface may be applicable to all cell types. Moreover, the detached cells are fully functional and can be removed as intact cell sheets containing the deposited ECM, which is useful for tissue engineering and regenerative medicine applications.

Whereas PNIPAM is not a bio-inert polymer (due to the potential for cooperative hydrogen bonding interactions between secondary amide groups on the polymer with amide groups on proteins), PEG, also known as poly(ethylene oxide) (PEO), has a long history in biomedical applications due to its biocompatibility and ability to mitigate protein adsorption.⁴⁹ In addition to resisting protein fouling, PEG surfaces also can be functionalized with protein-binding moieties like reactive NHS groups or biotin. With an experimentally accessible LCST of $\approx 85^\circ\text{C}$ in water, PEG recently has attracted attention as an alternative to PNIPAM for its temperature-responsive properties.⁴⁷ Moreover, copolymerization of PEG with other functional monomers has yielded libraries of copolymers with highly tunable LCSTs.⁵⁰ LCST tunability also has been demonstrated for copolymers of poly[oligo(ethylene glycol) methacrylate] (POEGMA) and poly[2-(2-methoxyethoxy)-ethyl methacrylate] (PMEOMA).⁴⁹ Wischerhoff *et al.* employed a POEGMA-*b*-PMEOMA copolymer with an LCST of 39°C in water and 35°C in phosphate buffered saline (PBS) to fabricate polymer brushes on gold surfaces, and they demonstrated reversible cell adhesion as a function of temperature.⁴⁹ Above the LCST, the copolymer brush adopted a collapsed conformation and fibroblast cells adhered and spread readily. Below the LCST, the copolymer brush expanded into a fully hydrated conformation and became cell-repellent, as evidenced by both the rounded morphology of the fibroblasts and the facile cell removal from the surface by gentle rinsing (see Fig. 9).⁴⁹

In an example of solution-assembled nanostructures, Pietsch *et al.* explored the thermo-responsive behavior of poly(DMAEMA-*b*-DEGMA) (where PDEGMA is a POEGMA with two ethylene glycol units).⁵¹ The authors linked the chemistry of the copolymers (composition and copolymer architecture) to the thermal properties (cloud point temperature, T_{cp}) and self-assembly of the polymer into multilamellar vesicles (MLVs) or unilamellar vesicles (ULVs). A significant finding of their work was the identification of a quasi diblock (gradient-type) copolymer that displayed double-thermo-responsive behavior, assembling into MLVs above the T_{cp} of PDEGMA ($T_{\text{cp,PDEGMA}} = 33^\circ\text{C}$) and then into ULVs as the temperature was increased above the T_{cp} of PDMAEMA ($T_{\text{cp,PDMAEMA}} = 49^\circ\text{C}$).⁵¹ Although not investigated in this work, their system also presents an opportunity to further manipulate the response of the copolymers by using pH to alter the T_{cp} of PDMAEMA, which is a weak polyelectrolyte with pH-responsive properties (see Section 1.1). This aspect of their system offers exciting prospects for using the temperature response to encapsulate drugs *ex vivo* and the pH response to deliver therapeutic agents *in vivo*.

For *in vivo* drug delivery applications, McDaniel *et al.* have developed block copolypeptides capable of responding to localized hyperthermia induced by externally heating solid tumors, thus providing a mechanism for thermally-targeted drug delivery.⁴⁶ Each of their temperature-responsive block copolypeptides included a long, elastin-like polypeptide (ELP) block that exhibited a sequence-dependent LCST phase transition, as well as a shorter polypeptide block that contained cysteine residues. Attachment of doxorubicin to the cysteine groups rendered the shorter block hydrophobic, and the resulting

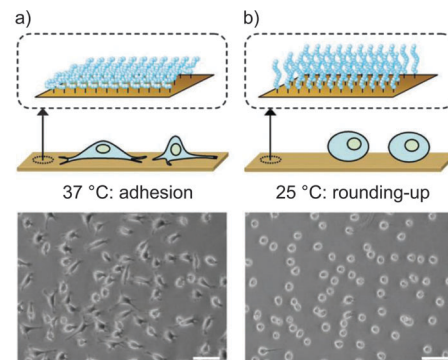


Fig. 9 Reversible cell adhesion via the thermal response of PEG copolymers. (a) Cartoon of adhesive state and optical micrograph of cells with spread morphology. (b) Cartoon of cell-repellent state and optical micrograph of cells with rounded morphology. Scale bars correspond to 100 μm . Adapted from ref. 49. Copyright 2008 Wiley-VCH Verlag GmbH & Co. KGaA.

amphiphilic copolypeptides self-assembled into spherical micelles.⁴⁶ After screening the temperature responses of various copolymer formulations, the authors identified nanocarriers that [1] were cytotoxic at both physiological and mild hyperthermia temperatures and [2] exhibited a transition from stable nanoparticles to large aggregates at a temperature of $\approx 40^\circ\text{C}$ in cell culture media.⁴⁶ Thus, these particles are expected to aggregate and thereby selectively accumulate in hyperthermic tumor tissues *in vivo* to facilitate localized tumor treatment.⁴⁶

The ability to tune the temperature response of PNIPAM- and PEG-based copolymers opens doors to new applications for these materials. Temperature control provides a simple means of detaching cells from a surface, which has led to the commercial promotion of PNIPAM as a coating for tissue culture dishes. Also, new polymer chemistries make it easier to generate temperature-sensitive surfaces and tailor surface properties, which may lead to exciting innovations in tissue engineering and regenerative medicine. In solution, temperature-sensitive copolymers grant access to new routes for controlling the self-assembly of nanostructures that are useful drug carriers (*e.g.* micelles and vesicles). These structures then can be stabilized (*e.g.* by cross-linking) for re-suspension in a medium suitable for biomedical applications. Furthermore, the advanced design of self-assembling block copolypeptides and polypeptoids with tunable thermal properties provides a means for developing temperature-responsive materials for *in vivo* applications. Although the temperature-sensitivity of many polypeptoids represents an on-going area of study, these materials offer an interesting avenue to couple independent advances in synthetic polymer and biopolymer designs. These peptidomimetic polymers are attractive for biomedical applications due to their low cytotoxicity and because LCST tunability (over the temperature ranges of interest for *in vivo* applications) can be achieved through copolymerization.¹²

2.2. Photo-responsive

As an external stimulus, light benefits from three key properties: first, it can be applied locally without suffering from diffusion effects as would a thermal or chemical stimulus; second, it can

be switched 'on' and 'off' easily; and third, the desired response can be tuned according to the wavelength and intensity of light.⁵² Most chemistries respond to ultra-violet (UV) wavelengths (<400 nm); however, recent efforts have investigated near infra-red (NIR)-responsive moieties for biomedical applications, as NIR light penetrates more deeply into tissues and is less harmful to cells.⁵²

Photo-responsive assemblies operate by similar principles as pH-responsive materials: upon application of the light stimulus, photo-cleavable junction(s) or photo-induced conformational changes in molecular structure disrupt the self-assembled morphologies. In Fig. 10, we summarize some of the most popular photo-responsive chemistries, the applicable light stimuli, and the primary mechanism of response. Poly(methacrylates) often are used to incorporate photo-responsive groups into copolymers, as its side chains can accommodate a number of photo-labile groups (*e.g.* *o*-nitrobenzyl, coumarin, pyrenyl) and groups that change conformation in response to light stimuli (*e.g.* azobenzene, spiropyran).⁵²

In an example of photo-responsive solution assemblies for targeted drug delivery, Johnson *et al.* synthesized a versatile class of bottle-brush copolymers using ROMP of branched macromonomers.⁵³ These copolymer chains were structurally similar to a micelle, containing a polynorbornene backbone, azide groups located in the molecule core near the backbone, and PEG chains that extended into solution to form a corona. The authors reacted a photocleavable doxorubicin-alkyne derivative with their polymers using azide-alkyne 'click' chemistry. They demonstrated the successful delivery of doxorubicin to MCF-7 human breast cancer cells in the presence of UV light (365 nm) and showed that these doxorubicin-conjugated copolymers were non-toxic in the absence of UV light.⁵³

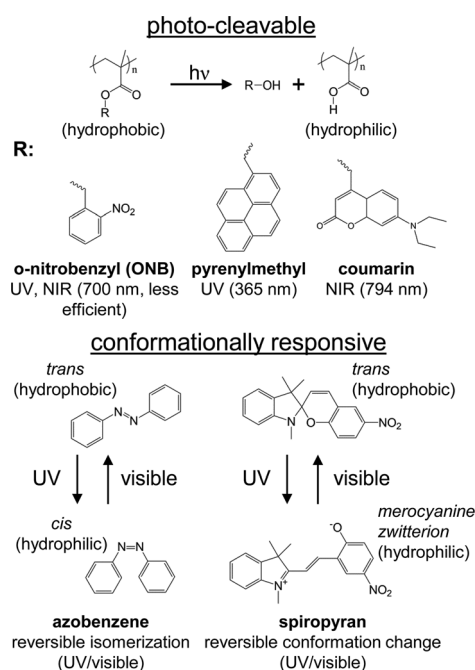


Fig. 10 Common photo-responsive chemistries.

On surfaces, photocleavable systems can be used to remove material and expose new functionalities. For example, Xu *et al.* modified the side-chains of poly(2-hydroxyethyl methacrylate) (PHEMA) brushes with *o*-nitrobenzyl (ONB) groups that were subsequently quaternized to generate a cationic brush.⁵⁴ Light-induced cleavage of the ONB groups exposed anionic carboxylic acid moieties. Thus, the authors were able to photo-pattern cationic/anionic binary brushes to selectively adsorb bovine serum albumin (BSA) and avidin onto the positively and negatively charged regions, respectively (see Fig. 11).⁵⁴

Photo-responsive chemistries provide a versatile toolkit that enables spatial and temporal control over stimulus application and therefore stimulus response. In particular, light provides an external handle by which the hydrophobic/hydrophilic balance and molecular weight (through photo-degradation or cross-linking) of self-assembled copolymers can be influenced to manipulate nanoscale morphologies. Furthermore, the above-mentioned photo-responses can be tailored for specific wavelengths and intensities of light through the attachment of functional groups to the light-absorbing portions of macromolecules.

2.3. Changing solvent quality

The ability to manipulate solvent quality in copolymer systems is a universal consideration among solution and surface assemblies. Changes in solvent quality can cause conformational responses in polymer chains (*e.g.* between extended coil and collapsed globule) or rearrangement of chains in a solution-assembled structure (*e.g.* micelle to free chains to inverse micelle transitions). Many of these responses are

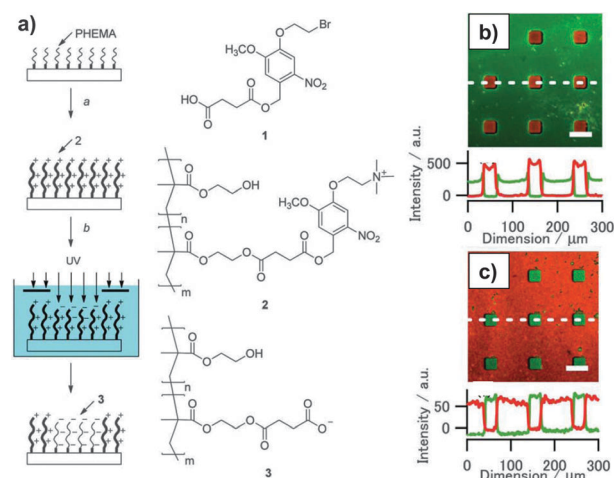


Fig. 11 (a) Synthetic scheme for fabricating copolymer brushes for dual protein patterning. In step 1, PHEMA is grafted to the substrate surface. In step 2, the PHEMA brushes are quaternized, giving them a positive charge. In step 3, the ONB groups are selectively cleaved, exposing negatively charged carboxylic acid groups. (b) Fluorescently-labeled proteins [bovine serum albumin (BSA), green, negatively charged; avidin (AV), red, positively charged] adsorbed onto the oppositely charged areas of the surface. (c) Non-fluorescent BSA and AV were adsorbed onto the surface and immunolabeled with fluorescent antibodies to demonstrate that the adsorbed proteins retained their functionality. Adapted from ref. 54.

similar to those found in other responsive assemblies, but these phenomena are nearly universal and not limited to polymers with dedicated stimuli-sensitive moieties. For biomedical applications, responses to solvent quality typically are used to control the self-assembly of functional copolymer materials *ex vivo*.

A leading application for the manipulation of solvent quality in solution assemblies is the fabrication of polymer templates. In solution, hollow nanoparticles have been produced using solvent quality to selectively expand, collapse, or extract copolymer segments (see Fig. 12).⁵⁵ These templates then can be used as drug carriers. In another example, Kelley *et al.* tailored the structural properties of self-assembled micelles by using cosolvent mixtures to control micelle size, corona thickness, and the solvent accessibility to the core block.⁵⁶ In these systems, the micelle response to solvent quality was described by the change in interfacial tension between the micelle core and the solvent. This ability to control micelle size and corona thickness is critical to the design and optimization of copolymer nanocarriers for drug delivery.

For surface assemblies, the reconstruction of copolymer brushes in response to changes in solvent quality has received much attention. Furthermore, recent advances in preparation methods have facilitated the design of new assemblies that are biocompatible. For example, poly(dimethylsiloxane-*b*-ethylene glycol) (PDMS-*b*-PEG) brushes prepared by Yang *et al.* utilized a grafting-to approach with sequential thiol-ene 'click' reactions to first graft PDMS to the surface and then attach PEG to the PDMS block.⁵⁷ These brushes exhibited changes in surface morphology (*e.g.* collapsed *versus* expanded brushes) as a

function of solvent quality. However, despite progress in the synthesis of biocompatible, solvent-responsive copolymer surface assemblies, adaptation of these materials for direct use in biomedical applications remains an open area for growth. Nevertheless, these model systems represent a useful platform for studying the mechanisms by which surface reconstruction of polymer brushes occurs in response to a stimulus (*e.g.* characteristic time scale of response, effects of grafting density and chain length on magnitude of response) that are important in the design of copolymer brush surfaces that respond to more biologically-relevant stimuli.

Solvent quality provides an important handle for manipulating self-assembly in solution and on surfaces for biomedical applications. Decades of work in this area have yielded a significant library of knowledge regarding solvent selectivity for numerous polymers. However, much of this work was conducted with other applications in mind, and the utilization of this knowledge in designing self-assembling materials for biomedical applications represents a relatively recent, albeit significant, advancement in the field of stimuli-responsive materials.

3. Multiple stimuli

As highlighted above, advances in polymer chemistry have led to exciting developments in the design and synthesis of responsive copolymers for biomedical applications. Yet significant challenges remain in the development of 'smart' synthetic materials that interface with biological systems, as these systems are rich with nanoscale structures that selectively respond to multiple stimuli. Synthetic materials ideally should mimic this complexity; however, creating polymer systems that respond to multiple stimuli requires the rational design of molecular building blocks and synthetic schemes.⁵⁸

While copolymers that respond to multiple stimuli present additional synthetic challenges, these materials have the potential to enhance the tunability of controlled drug release profiles. For example, Han *et al.* created block copolymer micelles that responded to both a biological stimulus (reducing agent) and an applied stimulus (light) (see Fig. 13).⁵⁹ They incorporated alternating photo-responsive ONB and redox-responsive disulfide functionalities into the hydrophobic block *via* a two-step 'click' chemistry approach to achieve rapid release upon exposure to UV light or slow release upon addition of a reducing agent. This combined response resulted in a complex fast/slow release profile, which is desirable for certain drug delivery applications.

Incorporating multiple responsive groups also provides an additional level of control over changes in nanoscale architecture and enables the formation of complex, hierarchal assemblies on surfaces. In one example, Kim *et al.* utilized multi-responsive block copolymers to template protein self-assembly in block copolymer thin films using only aqueous processing conditions (see Fig. 14).³¹ Processing polymer-protein hybrid films in aqueous conditions is highly desirable to preserve protein structure (and therefore function), although designing synthetic schemes in which polymer-protein

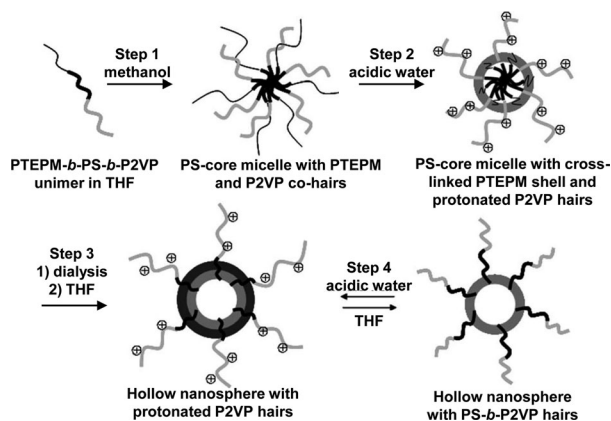


Fig. 12 Hollow nanoparticle templates fabricated by controlling solvent quality during the assembly of a triblock copolymer [PTEPM-*b*-PS-*b*-P2VP; PTEPM: poly((3-triethoxysilyl)propyl methacrylate)]. (step 1) Upon addition of methanol to a solution of unimers in tetrahydrofuran (THF), the triblock self-assembles into a micelle with a PS core and a PTEPM/P2VP corona. (step 2) Addition of acidic water protonates the P2VP block and collapses the PTEPM block onto the PS core where it is subsequently cross-linked. (step 3) When the micelles are returned to a good solvent, the cross-linked PTEPM block prevents dissociation into unimers, and the PS block is extracted from the core of the micelle, producing a hollow nanoparticle. (step 4) Subsequent switching between poor and good solvent conditions reversibly collapses the PS block onto the PTEPM shell and protonates the P2VP block. Adapted from ref. 55. Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA.

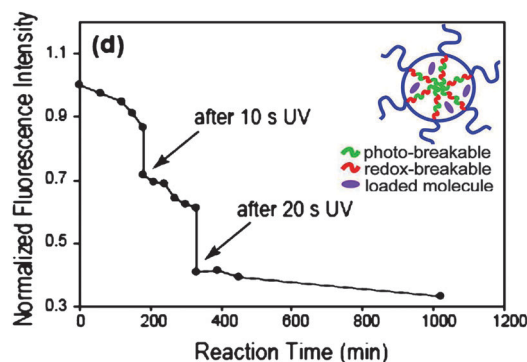


Fig. 13 Release profile of a fluorescent dye in response to both a reducing agent (slow response) and short exposures to UV light (fast response). The reducing agent was added at $t = 0$ min, and the solutions were exposed to UV light at $t = 180$ min and $t = 360$ min. (inset) Schematic representation of block copolymer micelles containing redox-responsive and photo-labile groups. Adapted from ref. 59. Copyright 2012 American Chemical Society.

complexes can be formed and stabilized under these conditions can be difficult. Kim *et al.* overcame these challenges by exploiting the multi-responsive properties of PNIPAM-*b*-PDMAEA. They used ionic interactions between the pH-responsive PDMAEA block and mCherry proteins to create coacervate micelles in aqueous solutions.³¹ Then, thin films of the polymer-protein coacervate micelles were cast onto substrates and immobilized by heating the films above the LCST of the PNIPAM block.³¹ By stabilizing the films in aqueous solutions at physiological temperatures, they were able to study the pH-dependent release of mCherry protein from the polymer films. Furthermore, >80% of the released protein retained its activity due to the mild and aqueous processing conditions employed during film preparation.³¹

Combining new chemistries into multi-responsive copolymers presents exciting opportunities as researchers strive to mimic the complexity of biological systems using synthetic materials. Copolymers that respond to multiple stimuli enable intricate control over drug release profiles and promote the rational design of delivery vehicles to overcome the multiple barriers in drug delivery. Moreover, these materials can be exploited to create complex, hierarchical structures that are not accessible with traditional processing techniques but are of interest for both fundamental studies of copolymer self-assembly and biomedical applications.

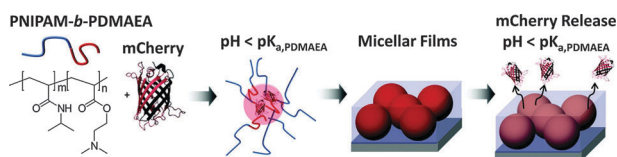


Fig. 14 Schematic representation PNIPAM-*b*-PDMAEA and mCherry protein coacervate micelles formed due to ionic interactions between the positively charged polymer and negatively charged protein. Thin films were cast from these coacervate micelle solutions and stabilized by heating the film above the LCST of the PNIPAM block, allowing for pH-dependent mCherry release. Adapted from ref. 31. Copyright 2012 American Chemical Society.

Summary and future perspectives

Advances in stimuli-responsive copolymer assemblies have paralleled developments in polymer synthesis and macromolecular design. In particular, the advent and optimization of controlled polymerization techniques allows for the synthesis of novel copolymers with well-defined architectures, reproducible molecular weights, low polydispersities, and end-group fidelity, while straightforward conjugation chemistries permit the facile incorporation of responsive groups (or attachment of drugs) into polymer architectures. The combination of controlled polymerization and conjugation methods enables the design and generation of well-defined and tunable nanostructures through copolymer self-assembly. As demonstrated by the examples provided in this review and elsewhere in the literature, exquisite control over polymer morphology on nanometer length scales reliably generates stable and responsive nanoscale structures that can be used to encapsulate and release therapeutics, create reversibly adhesive surfaces for *ex vivo* studies, and pattern proteins on surfaces into well-defined geometries for applications in drug delivery, medical diagnostics and imaging, tissue engineering, and biosensing. These biomedical applications exploit response mechanisms such as solution nanostructure assembly/disassembly or surface reconstruction that are common amongst the various biological and external stimuli described in this tutorial review. In copolymer assemblies, the response mechanisms are facilitated by the incorporation of chemical groups that undergo bond cleavage or exhibit a conformational/solubility change in response to a defined stimulus. Additionally, many of the recently developed synthetic approaches are applicable to materials design for both solution and surface assemblies, leading to significant synergies in, and streamlined optimization of, the overall design of stimuli-responsive materials for biomedical applications.

However, we note that even though substantial progress has been made in the design and generation of novel stimuli-responsive materials, there is a continued need to strengthen the direct links between detailed synthesis and equally detailed *in situ* and *ex situ* nanostructure characterization. To fully glean the true nature and efficacy of these complex self-assembling materials, the specific shapes, sizes, surface properties, density profiles, *etc.* of the solution and surface assemblies must continue to be investigated. A growing number of works demonstrate the importance of these detailed nanostructure characteristics on the performance of these surface and solution assemblies in biomedical applications.^{3,4,24} Additionally, recent advances in macromolecular synthesis have enabled the fabrication of material libraries that will facilitate necessary mechanistic investigations of *in vitro* and *in vivo* cellular uptake. In particular, the major relationships between a nanostructure's physicochemical properties and the material's cytotoxicity, cellular internalization, and intracellular trafficking can be refined significantly using the responsive structures described in this work.⁶⁰ Thus, not only will strong linkages between macromolecular synthesis,

materials nanoscale characterization, and biophysicochemical analysis provide necessary information on the intimate structure–property relationships inherent to polymer systems in biological environments, but these same linkages also will enable the *de novo* design of next-generation ‘smart’ materials for biomedical applications.

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