Stimuli-Responsive Nanomaterials for Biomedical Applications

Angela P. Blum, † Jacquelin K. Kammeyer, †§ Anthony M. Rush, †§ Cassandra E. Callmann, † Michael E. Hahn, †‡ and Nathan C. Gianneschi* †‡

ABSTRACT: Nature employs a variety of tactics to precisely time and execute the processes and mechanics of life, relying on sequential sense and response cascades to transduce signaling events over multiple length and time scales. Many of these tactics, such as the activation of a zymogen, involve the direct manipulation of a material by a stimulus. Similarly, effective therapeutics and diagnostics require the selective and efficient homing of material to specific tissues and biomolecular targets with appropriate temporal resolution. These systems must also avoid undesirable or toxic side effects and evade unwanted removal by endogenous clearing mechanisms. Nanoscale delivery vehicles have been developed to package materials with the hope of delivering them to select locations with rates of accumulation and clearance governed by an interplay between the carrier and its cargo. Many modern approaches to drug delivery have taken inspiration from natural activatable materials like zymogens, membrane proteins, and metabolites, whereby stimuli initiate transformations that are required for cargo release, prodrug activation, or selective transport. This Perspective describes key advances in the field of stimuli-responsive nanomaterials while highlighting some of the many challenges faced and opportunities for development. Major hurdles include the increasing need for powerful new tools and strategies for characterizing the dynamics, morphology, and behavior of advanced delivery systems in situ and the perennial problem of identifying truly specific and useful physical or molecular biomarkers that allow a material to autonomously distinguish diseased from normal tissue.

INTRODUCTION

The clinical efficacy of small-molecule therapeutics is limited by many factors, including poor solubility, inefficient cellular uptake, low bioavailability due to rapid renal clearance, and an inability to target desired locations.1,2 Moreover, the side effects of cytotoxic agents, such as those used in classical anti-cancer regimens, are often the direct result of the drug’s inability to discriminate between healthy and diseased tissue.3 Nanoscale drug delivery vehicles have been under frantic development to address these issues, with the promise that such formulations will offer significant advantages over systemically administered small molecules. As a result, there have been notable successes in the clinical translation of nanoparticle therapeutics, most of which are hypothesized to rely on the enhanced permeation and retention (EPR)4 effect as a means to passively accumulate drug-carrying nanomaterial delivery vehicles within diseased tissue.5,6 The EPR effect is thought to facilitate the accumulation of nanoscale structures in the highly fenestrated vasculature (200–800 nm pores) that is characteristic of the rapid angiogenesis seen in cancer,7 inflammation,8 and infection.9 However, given that the EPR effect operates via passive accumulation, it offers little control over the timed release of drugs and generally cannot be invoked for the treatment of pathologies with normal, or approaching normal, vasculature. Furthermore, observations during testing of new, specially designed nanomaterials frequently show behavior that contravenes the commonly held belief that EPR is at play in delivery, resulting in materials that lack desirable properties or fault the thesis entirely. Efforts to include active accumulation and programmed release properties into nanomaterial designs include displaying targeting moieties,10–12 transporting materials with serum proteins,13 disguising synthetic nanoparticles as red blood cells,14 using chemical functionalities invoking efficient cellular uptake,15 labeling particles to enable endosomal release,16,17 and preparing nanostructures imbued with the means for timed release of cargo.18–22

Nature provides inspiration for the creative development of novel drugs and drug delivery platforms. Elaborate and efficient viruses have evolved over time, adapting the ability to enter specific cells, disassemble, deliver proteins and nucleic acids, and ultimately replicate themselves to ensure propagation of the process.23,24 Many of the systems we describe have much in common with the evolved strategies of viruses, albeit to a much simplified and, unfortunately, inefficient degree. At the level of the active small molecule or biomolecule, nature often solves issues of off-target effects by synthesizing these species as inactive or dormant precursors. Indeed, many effective small-molecule drugs are delivered in a deactivated form by chemical conjugation of the active core to a cleavable moiety. Prodrugs often enable enhanced solubility, membrane permeability and/or environment-specific activation of the parent drug. One example is salicylic acid, a β-glucoside that is hydrolyzed by hydrochloric acid in the stomach to yield salicylic acid, the active metabolite of aspirin.25 Similarly, organisms produce many other activatable molecules such as zymogens, deactivated enzymes, that must be activated biochemically (i.e., by cleavage of a peptide fragment) to perform their intended catalytic function. This ensures that the enzyme is only active once it reaches its target destination. For example, pepsin, a highly active proteolytic enzyme that degrades peptides and proteins in the stomach, is synthesized as a
zymogen (pepsinogen) to ensure that the contents of the cell in which the enzyme is synthesized are not degraded. Following secretion from these cells, pepsinogen is activated by the low pH of stomach tissue, where it functions to digest ingested protein. Indeed, life depends on our ability to eat, while not being consumed by the molecules and materials that facilitate digestion. Hence, biology takes a compartmentalization and selective activation approach to harnessing reactivity for temporal and spatial control of chemical processes. These types of concepts have been borrowed in the attempt to develop synthetic small molecules, macromolecules, and nanomaterials capable of interacting with cellular machinery and with biochemical systems.

In recent years, there has been increasing effort in the development of stimuli-responsive nanomaterials with the hope that they will be developed into effective drug delivery or diagnostic vehicles. These synthetic systems utilize an assortment of endogenous or exogenous stimuli to induce a variety of responses that can facilitate targeted drug delivery. Most commonly, effective drug delivery is associated with nanomaterial-facilitated accumulation and/or cellular internalization. This Perspective on progress and future directions in this area is not meant to be a comprehensive review, nor an exhaustive one. Instead, we aim to highlight only certain advances in the field as they relate to stimuli-responsive behavior that is representative of cutting edge attempts to inspire the delivery of therapeutic and diagnostic agents. Many existing review articles describing progress in stimuli-responsive materials are organized around the types of stimuli used. Here, we first briefly describe and attempt to correlate selected commonly employed stimuli with associated lead references (Table 1). To offer a unique perspective on this topic, this Perspective is primarily organized in terms of the type of physical response elicited. Limitations to these behaviors and future directions are discussed throughout. It is a challenge in some cases to characterize certain experimental approaches into a single category, therefore, several of our classifications are subjective and may not reflect a complete description of the entire process. Throughout, we have aimed to present existing data in a new light and to offer a platform for fresh insight and perspective on the field.

### ENDOWENOUS VERSUS EXOGENOUS STIMULI

An assortment of endogenous stimuli are capable of inducing changes in nanomaterial structure and function, many of which exhibit varying expression patterns within certain cellular organelles or in diseased tissue. These stimuli include small molecules, proteins, nucleic acids, peptides, electron transfer reactions, viscosity, osmotic pressure, and local environmental factors, such as pH, temperature, or redox state. Of these, enzymatically catalyzed processes make ideal candidates as triggers for the selective release or accumulation of drugs due to their high specificity for their substrate and their catalytic properties. As with enzymes, other endogenous stimuli, such as the tumor-associated oxidant peroxynitrite, also show high selectivity in cleaving specific chemical motifs, albeit not in a catalytic manner. Indeed, as discussed throughout this Perspective, one of the central problems in designing materials that respond to a given endogenous stimulus is that they will inevitably respond to related stimuli, activating at unwanted times and in unwanted locations. However, examples of highly specific cleavage-based systems do exist in nature and should be taken advantage of including zinc finger nucleases, TALENs, and CRISPR-Cas gene editing.

It is important to note that while many systems seek to take advantage of naturally occurring endogenous stimuli, much effort has been expended on approaches that rely on exogenous stimuli, such as ultrasound, electromagnetism, light, and temperature, which can be applied directly to a tissue of interest to drive localization or release of cargo. Because these stimuli may offer spatiotemporal control over the activation of materials, it is proposed that cargo release can be performed directly at the desired site, minimizing side-effects in surrounding, healthy tissue. Moreover, in these scenarios, the chemistries used for initiating a drug release event, for example, can be truly bioorthogonal as they are decoupled from biological stimuli. However, in these cases, the problems facing selective delivery are deferred from the nanomaterial to the selective application of the exogenously applied stimulus.

When designing a material, it is important to match the clinical application with an appropriate stimulus. Situations in which a high degree of specificity and selectivity are required (such as in the selective killing of a glioblastoma), may be better suited to an enzymatic activation pathway where multiple contact points with the substrate are required, rather than relying on a stimulus that can freely cleave assorted functionalities, such as bulk environmental properties like low pH. In some situations damage to healthy tissue can be minimized by applying an exogenous stimulus directly to the tissue of interest. However, treatments involving the application of an injectable material coupled with activation by ultrasonic waves, advanced light sources, or a strong magnetic field may require elaborate protocols that may not always be practical or cost-effective. Other problems related to the application of an unnatural, exogenous stimulus are related to the depth of penetration. For example, activation by UV-light is primarily limited to regions of the body that can be directly illuminated (i.e., the teeth, skin, or eyes). Low penetration depths (~10 mm) result from strong scattering and absorption in the ultraviolet–visible region (<700 nm) by soft tissue. To expand the scope of tissues that can be accessed by light, either photoreponsive moieties that respond to longer wavelengths of light or two-photon strategies must be employed.

### Table 1. Highlights of Stimuli, Example Responses, and Associated References Broached in this Perspective

| stimulus | examples of responses |
|----------|-----------------------|
| **Endogenous** | |
| pH gradients | direct activation, expansion, gatekeeping, morphology switch |
| redox processes | direct activation, gatekeeping, disassembly, assembly, morphology switch |
| enzymes or proteins | direct activation, gatekeeping, disassembly, assembly, motion |
| nucleic acids or small molecules | gatekeeping, disassembly, assembly, motion |
| **Exogenous** | |
| temperature | expansion, disassembly, assembly, morphology switch |
| light | direct activation, gatekeeping, disassembly, assembly, morphology |
| ultrasound | motion |
| magnetic field | motion |
Using a NIR laser (700–1000 nm) as the trigger enables deeper penetration into tissue as the result of decreased light scattering, decreased absorbance, and minimal harm to tissue. However, even if energetic considerations are overcome, successful execution of strategies involving the use of exogenous stimuli require that they are applied when diseased tissue can be spatially differentiated from healthy tissue, which could prove problematic for certain diseases such as infiltrative neoplasms. Systems responsive to endogenous stimuli must navigate this dilemma in an autonomous fashion, distinguishing friend from foe in a manner preprogrammed into the structure ahead of systemic delivery. Alternatively, another option exists where both exogenous and endogenous stimuli are coupled into a single, elaborate system. Needless to say, there are considerable challenges facing any approach, and we aim to capture some of these through a discussion of intriguing examples.

## THE RESPONSE

### Direct Release or Activation

Given the relative simplicity of a prodrug/zymogen approach, it is not surprising that some of the earliest examples of stimuli-responsive nanomaterials in the literature are those involving a direct cleavage event resulting in the release of therapeutic cargo or the excitation or quenching of a fluorophore for imaging (Figure 1). Indeed, the literature is ripe with examples of this process where drugs are covalently attached to an otherwise inert nanomaterial scaffold via a linker that is susceptible to cleavage by an appropriate stimulus. For example, the lysosomal protease cathepsin B has been used extensively to trigger drug release directly inside of cells when a targeting/internalization agent such as folic acid facilitates the internalization of the nanocarrier.

Other common triggers for direct release strategies include pH and light. Two-step approaches, such as polymer-directed enzyme prodrug therapy, are also employed in which a prodrug and its enzyme effector are chemically conjugated to two separate carriers. There are relatively few examples of the two-step strategy, and most purport to rely on the EPR effect to localize nanoparticles at tumor tissue. Once localized, the protease, which is still active as part of the conjugate, facilitates direct release of the drug from its nanomaterial carrier into tumor tissue, thus minimizing off-target cytotoxicity. Administration of an HMPA–cathepsin B conjugate to tumor-bearing mice, which had been pretreated with an HMPA particle linked to doxorubicin via an enzyme-cleavable peptide substrate, resulted in a 3.6-fold increase in the rate of drug release with improved tumor reduction relative to the polymer–drug conjugate alone.

Fluorescence resonance energy transfer (FRET)-based approaches used in diagnostics also invoke a direct cleavage mechanism. In traditional designs, a fluorescent donor and acceptor flank a peptide substrate that is optimized for degradation by the protease of interest. Cleavage of this linkage facilitates the physical separation of the donor and acceptor in space, resulting in a decrease in FRET. These cleavage events are often mediated by proteases that are dysregulated in a particular pathology, such as cancer, and so the function or abundance of the proteolytic enzyme can be assessed by monitoring changes in FRET efficiency.

Quantum dots (QDs) are a promising set of materials used in nanomaterial diagnostics, especially those utilizing a sense-and-response switching mechanism. QDs are luminescent semiconductor nanocrystals, typically comprised of CdSe, PhSe, or InAs, that have unique photophysical properties that can address many of the limitations encountered in in vivo imaging by traditional small-molecule fluorophores. For example, the photoemission of a QD can be matched for spectral overlap with a given acceptor by simply tuning the size of the nanocrystal. Moreover, QDs have been shown to be more resistant to photobleaching than their small-

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**Figure 1.** Stimuli-driven direct release or activation strategies. (A) Cartoon scheme depicting the direct release of drugs (red circles) or activation of diagnostic agents following initiation by a stimulus. (B) A literature example of a dendrimer (called an activatable cell-penetrating dendrimer, ACPPD) decorated with activatable cell-penetrating peptides (ACPPs) that also contains encapsulated Cy 5 dye for fluorescence imaging or gadolinium cargo for use in MRI diagnostics (yellow circles). In this design, enzymes upregulated in cancer cells (MMPs) facilitate cleavage of the ACPP hairpin, exposing a polyarginine cell penetrating peptide motif, which facilitates the entry of the cargo-carrying nanoparticle. Prior to cleavage, the ACPP forms a hairpin by non-covalent interactions between segments of polyglutamic acid and polyarginine (the cell-penetrating motif) that flank the recognition sequence of the enzyme. Upon cleavage of the peptide hairpin, the polyglutamic acid segment is released, exposing the polyarginine fragment, which can then penetrate cells. (C) Fluorescence images of mice 48 h post-injection of either the cleavable ACPPD with encapsulated Cy 5, or a non-cleavable ACPPD (D-amino acid control) variant. In these images, there is a substantial increase in florescence at tumors only when the particles with the cleavable ACPP are administered, illustrating that this method can be used to target cancer cells and internalize while carrying useful diagnostic or therapeutic cargo. Panels B and C are adapted from Olson et al. with permission from the National Academy of Sciences.
molecule counterparts. Given their exceptional promise, QDs make intriguing diagnostic agents, especially when encoded to respond to a biological stimulus that is a signature for a specific disease type. Medintz et al. described a stimuli-responsive example in which the surface of a QD is modified with peptide sequences that terminate in a fluorescent quencher or acceptor dye. Here, an assortment of peptide linkers are employed that are each activated by one of several clinically relevant proteases, including caspase-1, thrombin, collagenase, and chymotrypsin. In this study, each variation of the assay was shown to be selective for the intended protease. However, it is well documented that QDs with CdSe semiconductor cores can be toxic to cells resulting, in part, from Cd\textsuperscript{2+} contamination or release. Cytotoxicity can be alleviated by modifying the surface of the QD with a shell, such as those comprised of ZnS. Like many small-molecule fluorophores, QDs also suffer from “blinking”, an issue which has not yet been solved and may prove problematic for diagnostic applications.

Upconverting nanoparticles (UCNPs) could also make exceptional diagnostic tools if rendered responsive to disease-associated stimuli. UCNPs function via anti-Stokes emission, converting excitation photons of NIR light into an emission in the visible spectrum. Because they are excited by NIR light, they do not exhibit the photodamage to tissue, background fluorescence, or tissue-induced scattering issues that are typical of small-molecule fluorophores that absorb UV light. UCNPs are generally comprised of a crystalline host matrix that is doped with a lanthanide ion, which defines the photophysical properties of the material (i.e., the excitation and emission wavelengths). They are usually synthesized and studied in organic solvents due to poor water solubility or because of unfavorable interactions between water and the chromophore. In recent years, UCNPs have been prepared as stable aqueous colloidal dispersions via conjugation to water-solubilizing ligands or shells. Moreover, these surfaces are amenable to the conjugation of biomolecules or other stimuli-responsive appendages. To date, there have been several successful attempts to use stimuli to toggle between emissive and “dark”, non-emissive states of UCNPs. Examples include UCNPs that are quenched by UV light or those that act as pH sensors, but none have been rendered sensitive to specific markers of disease, aside from pH. Therefore, UCNPs have untapped potential for use in advanced diagnostic imaging if strategies are developed to render their photophysical properties sensitive to specific disease biomarkers. Yet another alternative to QDs are photoluminescent porous silicon nanoparticles. The Sailor laboratory has demonstrated that these materials exhibit exceptionally long emission lifetimes (\(5-13 \mu s\)), making possible time-gated imaging of tissue \textit{in vivo} to minimize background associated with autofluorescence signatures that typically decay in 1–10 \(\mu s\). Moreover, these materials exhibit low toxicity and are hydrolyzed under physiological conditions, making them exceptional candidates for the development of stimuli-responsive \textit{in vivo} imaging probes.

In general, direct release/activation strategies suffer from issues of specificity. For example, linkages susceptible to cleavage at low pH, such as esters, are typically also cleavable by enzymes like esterases, which are abundant in circulation. Even peptide sequences programmed to be cleaved by a particular protease with a highly specific cleavage propensity may also be generically cleaved by other proteases that have more permissive active sites. For example, peptide substrates typically used by cathepsin B (Gly-Phe-Leu-Gly)\textsuperscript{18} can also be recognized by a number of other enzymes including pepsin, a key digestive protease. Given that the stomach secretes gram quantities of proteases, oral delivery of peptide-based agents is thwarted by proteolytic activation in the stomach, among other potential issues including the low pH environment.\textsuperscript{2} Indeed peptide-based therapeutics are typically injected at the site of interest due to rapid digestion by proteases that are abundant in circulation.\textsuperscript{139} In this regard, materials capable of responding to enzymatic signatures must be designed in such a way that they do not undergo unwanted activation.\textsuperscript{140} Oral delivery remains a key challenge in this type of approach, and one with significant potential to impact and dictate how materials are delivered for \textit{in vivo} use.

In summary, with any direct sense-and-release strategy, care must be taken when choosing the stimulus. For example, extra-cellularly expressed enzymes may be effective at releasing a therapeutic agent in tumor tissue, but they offer less help in delivering the material to the interior of a cell, where most cytotoxic drugs are active. While it is true that these strategies increase the local concentration of drug at the site of interest, it is possible for these agents to return to the general circulation in the absence of a mechanism for efficient and rapid cellular uptake. Therefore, intra-cellularly expressed enzymes like cathepsin B, methionine sulfoxide reductase, glycosidases, or intra-cellular kinases make particularly intriguing stimuli. Alternatively, strategies like those developed by the Tsien laboratory (Figure 1B,C) in which a cell-penetrating motif is masked and activated by enzymes overexpressed in certain tumors (matrix metalloproteinases, MMPs), are also promising approaches for drug delivery and imaging.

Expansion. Expansile particles swell or contract in response to activation by a stimulus. When these nanoparticles swell, they typically become fenestrated or leaky, enabling the release of encapsulated drugs (Figure 2).\textsuperscript{19} pH is often used as the stimulus to invoke expansion, as it can alter the protonation state of basic/acidic functionalities such as tertiary amino or carboxyl groups. Polymeric micelles, polymerosomes, hydrogels, or other scaffolds loaded with these moieties can act as pH sensors whose hydrophobicity, conformation, or electrostatics are altered based on their protonation state (see Figure 2B–D)\textsuperscript{19,48,49,141–145} In one example, polymerosomes composed of poly(-L-glutamic acid)-block-poly(-L-lysine) exchange the identity of their hydrophilic corona and hydrophobic core in response to protonation/deprotonation cycles.\textsuperscript{48} Many other stimuli can also be used to control particle expansion or contraction including temperature\textsuperscript{97,98,144} or the ionic strength of the solution.\textsuperscript{145}

As with any strategy, caution is necessary when designing systems for non-covalent encapsulation, because there is the potential for unintended off-target or burst release upon injection. Efficient encapsulation itself can be a challenging task that is sometimes difficult to quantify, and thus more research into encapsulation-and-release efficiencies, such as those reported by Adams and co-workers,\textsuperscript{146} could help propel these systems further. Additionally, for expansile systems, the efficiency of swelling/contraction needs to be robust enough to release cargo in a reproducible, high-fidelity fashion. Moreover, thus far, expansion of nanoparticles has been primarily triggered only by bulk environmental properties such as ionic strength, temperature, or pH. However, variations in these properties occur in both healthy and diseased tissue. For example, a lowered pH may very well be a marker of cancerous tissue, but
it is also found inside the endosomal/lysosomal compartments of any cell. Therefore, it would be intriguing to develop expansile systems for which more specific triggers, such as enzymes or other known biomarkers, may be employed.

**Gatekeeping.** Gatekeeper strategies rely on an "uncaging" mechanism in which a nanocarrier is coated with a sterically bulky shell or "gate" that encapsulates drug cargo. Upon decaging by removal of the shell or opening of the gate, the entrapped drug molecule is released (Figure 3).

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**Figure 2.** Stimuli-responsive strategies invoking a gatekeeping mechanism. (A) A cartoon illustration of the gatekeeping strategy in which the pores (green cylinders) of mesoporous silica nanoparticles (MSNPs, depicted as blue spheres) are blocked by a gate (yellow crosses). Stimulus activation results in opening of the gate and release of encapsulated imaging agents or therapeutics (red spheres). Note that other nanomaterials such as core−shell particles also employ similar approaches to release cargo. (B) A literature example of a pH-activated MSNP. In this example, a β-cyclodextrin (β-CD) is used to cap the pores of drug- or fluorophore-carrying MSNPs. At physiological pH, the β-CD encapsulates aromatic amines that are appended to the periphery of the MSNP, blocking the nanopore and entrapping cargo. Protonation of the amines following a decrease in pH results in the release of the cyclodextrin gate, enabling free diffusion of the pore contents. (C) Fluorescent images illustrating doxorubicin release from the β-CD-gated MSNPs described in panel B after internalization of the materials into acidified endosomal compartments of KB-13 cells. Release of doxorubicin was also correlated with a decrease in cell viability. Neutralization of lysosomal pH by the addition of NH₄Cl results in inhibition of doxorubicin release and toxicity, providing support for the proposed mechanism of activation. Panels B and C are adapted from Meng et al. with permission from the American Chemical Society.
responsive mesoporous silica nanoparticle (MSNPs) systems often employ a gatekeeper approach. These MSNPs are popular drug delivery vehicles because of their low cytotoxicity, large surface area, tailorable pore volumes, and the ease with which their surfaces can be chemically manipulated. In these systems, a sterically bulky gate, such as β-cycloextrin (β-CD, Figure 3B,C), is installed on the surface of the MSNP via a cleavable linkage to block cargo release (opening of the gate) until activation by a stimulus. An assortment of gates have been employed including rotaxanes, saccharides, gold nanoparticles, and segments of DNA. To further tailor release profiles, the gates are operated by a wide range of endogenous and exogenous stimuli such as pH, redox, UV light, enzymes, and DNA recognition. In one intriguing example, double-stranded DNA is immobilized via an azobenzene linkage. Light-triggered dehybridization/rehybridization of complementary DNA leads to channel opening/gating. When dehybridized, the channel opens and releases entrapped doxorubicin. In addition to MSNPs, a variety of other nanoscale vehicles have been caged by polyethylene glycol (PEG) shells and other bulky or water-solubilizing appendages to shield covalently or non-covalently linked drugs or targeting agents.

As with other encapsulation techniques such as the expandable particles described above, undesired leakage of drug could potentially lead to systemic toxicity. Additionally, any strategy ultimately relying on a cleavage event will likely suffer from a lack of universal specificity because of the difficulty in preparing a linkage that is recognized by only one stimulus found in vivo. Again, here we return to the central, all too common biomarker problem faced by any targeted system from small-molecule drugs to nanoscale carriers. The key and obvious challenge is to solve the problem for responsive systems using truly orthogonal linkers designed to be recognized and cleaved only by a single stimulus.

**Disassembly and Degradation.** Nanoparticles that degrade or otherwise disassemble have been explored as a means to deliver cargo in a spatially and temporally controlled manner. We define disassembly as a process by which a discrete material breaks into pieces via preprogrammed stimuli-triggered events (Figure 4). This is distinct from other processes such as the gatekeeping mechanisms described above where only a shell or portion of a nanoparticle is dispersed in response to the stimulus. There are two main approaches for triggering disassembly: single-event disassembly and multi-step degradation.

In a single-event disassembly process, one recognition event initiates a degradation event, leading to the complete disassembly of the nanostructure, either in a “one-to-one” fashion or via a cascade mechanism. In a one-to-one model, the stimulus acts stoichiometrically to trigger each cleavage event at each cleavable bond within a micro- or nanoparticle. The culmination of multiple cleavage events leads to the dissolution of the material. Mechanistically, this is the simplest degradation strategy, and as such, many of the earliest examples of stimuli-responsive nanomaterials dissociate in this manner. The vast majority of these systems are designed to degrade in response to low pH, which may be advantageous for tumor treatment. Polymers composed of poly(lactic acid) (PLA) or polylactide-co-glycolide (PLGA) are commonly incorporated into degradable nanoparticles, because they contain chemical bonds (e.g., esters) that are susceptible to hydrolysis at acidic pH. Heller et al. were among the first to report a pH-sensitive nanoparticle amenable to controlled release, which was designed to contain acid-sensitive maleic anhydrides.

After then, there have been extensive reports on nanoparticle systems that fully degrade in response to pH. For example, Bae et al. developed a diblock copolymer system comprised of a hydrophilic PEG block, linked through a hydrazone to a hydrophobic poly(N,N-dimethylacrylamide) (PDMA) block. In this report, the self-immolative block was caged with either perylen-3-yl, o-nitrobenzyl, or disulffide moieties (as depicted), which uncage in response to visible light (420 nm), UV light (365 nm), or reductive agents, respectively. Upon activation, the block copolymer depolymerizes into 4-aminobenzyl alcohol, carbon dioxide, and PDMA. These polymersomes were also loaded with doxorubicin or camptothecin, and payload release coincident with depolymerization was observed. This system is stable at pH 7.4, but rapidly disassembles at pH 6.6–7.2, which matches the extra-cellular pH of some tumor tissues.

In the cascade model, a stimulus triggers the complete dissolution of the nanoparticle by initiating either a cascade reaction or a rapid depolymerization (Figure 4B,C). The advantage of a cascade trigger is the possibility of amplification wherein only a single recognition event is needed.
to initiate the degradation process, ultimately leading to payload release. However, complete disassembly requires a great deal of optimization, including kinetic considerations. For example, insufficient exposure to the stimulus at physiologically relevant concentrations may lead to incomplete degradation, and thus a poor result in vivo. Shabat and colleagues developed a dendrimer programmed to degrade in a cascade fashion upon exposure to UV light.153 In this system, an “adaptor” molecule is used to link two or more “reporter” moieties to a photolabile trigger. Upon irradiation with 360 nm light, an o-nitrobenzyl linkage is cleaved, triggering a cascade that results in the degradation of the dendrimer and ultimately the release of multiple cargo units. In this example, disassembly proceeds much slower in the second generation dendrimer than in the first generation variant, likely due to sterics in the larger dendrimer, illustrating the critical need for optimization of these types of systems. More recently, the Almutairi group developed a polymeric system more amenable to biomedical applications in vivo as it is degraded in the near-IR, rather than by irradiation with UV light.39

Other stimuli, such as redox,60–62 temperature changes,99,100 or enzyme triggers,71,72 have also been employed to facilitate nanomaterial degradation by either the one-to-one or cascade mechanisms. For example, the Sumerlin laboratory has explored the use of block copolymers containing boronic acid moieties whose solubility (and thus amphiphilicity) is influenced by either pH or the presence of diol-containing small molecules (i.e., sugars).86 Remarkably, the disassembly of these materials can be triggered in the presence of glucose at physiological pH, which could be an intriguing approach to the treatment of diabetes. If prepared with a thermoresponsive N-isopropylacrylamide block, the assemblies can be rendered sensitive to three separate stimuli: pH, temperature, and sugar concentration.103 Liu et al. has prepared polymersomes that depolymerize in response to UV light, visible light, or glutathione (example shown in Figure 4B,C), depending upon the identity of a small-molecule cap.60 Triggers located in cellular cytosols like glutathione are particularly intriguing because they ensure that the material remains intact until internalized into a cell. Thayumanavan and co-workers have explored protein-binding induced disassembly of dendritic supramolecular assemblies in which a ligand such as biotin is strategically placed on the dendrimer scaffold.74 Binding of a cognate protein like avidin to this ligand then triggers the disassembly of the dendritic complex and coincident payload release. In other work, Caruso and co-workers reported an elegant, programmable strategy utilizing a DNA-based nanoparticle system that incorporates restriction enzyme cut-sites for simultaneous controlled drug release and nanoparticle dissolution.71 This stands out as a key example of the exciting trend73,74 toward designing far more selective nanoparticle systems wherein a material can be truly encoded with information. These materials open up the possibility that such approaches will be more routinely utilized in future schemes to home and respond to patterns of gene expression and to enzymatic activity in vivo.

Degradable nanoparticles that undergo multi-step degradation processes can require two or more orthogonal stimuli acting independently to provide more spatiotemporal control of cargo release. Generally, one stimulus initiates surface degradation while a separate stimulus triggers bulk degradation and subsequent cargo release. Wooley and co-workers recently reported the development of this type of degradable nano-

particle, utilizing redox and hydrolysis through the incorporation of both polycarbonates and disulfides within the same amphiphilic diblock copolymer.154 Pasparakis et al. developed a system that degrades in response to both light and pH and demonstrated a more selective release of chemotherapeutics in cancer cells in vitro than strategies that utilize only one stimulus.43 The Thayumanavan laboratory has worked to develop composite nanostructures comprised of two separate supramolecular assemblies—a block copolymer micelle core and a nanogel shell—that are disassembled fully in response to decreased pH (to degrade the core) and reduction by glutathione (to disassemble the nanogel).155 This same laboratory has also prepared an amphiphilic micelle-like assembly in which a protein is used to trigger the disassembly of the micelle via a protein—ligand binding event and an enzyme cleaves the otherwise inaccessible hydrophobic segment of the exposed amphiphile, releasing cargo.42 Moreover, cross-linking of this micelle via a UV light-sensitive linkage introduced the necessity of a third stimulus to the system, cleverly creating a triply gated material. These types of degradable systems offer a promising combination of approaches, which may lead to better in vivo specificity. However, given the complexity of the approach, it will perhaps require additional effort and optimization to bring these systems to an in vivo context.

Assembly and Aggregation. Assembly is a ubiquitous and necessary process in nature demonstrated elegantly in the ribosomal assembly of proteins from amino acid building blocks, in transcription via the recruitment of transcription factors and RNA polymerase, and in the intricate folding of peptides and proteins. Mimicking this assembly process in the context of drug delivery remains a challenge, but several success stories (albeit none at the level of sophistication of natural systems) serve as inspiration for the future. Many groups have reported successful assembly or aggregation of water-soluble unimers into larger structures by pH,56,57 thermal,105 enzymatic,86,156 or DNA hybridization47,157 triggers in vitro, but few strategies have been translated to in vivo applications.57 Herein, we define assembly as a process by which materials are collected into discrete and ordered structures of larger size in response to a stimulus, whereas aggregation involves the assemblage of amorphous structures from initial monomeric units (Figure 5). These are both distinct from the morphology switches described in this Perspective in which a discrete, well-defined phase undergoes a change in physical architecture in response to a stimulus to generate a new phase, which does not necessarily correlate with an increase in size. Highlighted below are a number of promising drug delivery approaches described in the literature that take advantage of either a nanomaterial assembly event, pretargeting strategy, or thermally controlled aggregation.

A common method for in vivo assembly uses a two-step targeting mechanism, in which the nanomaterial scaffold is first passively “pretargeted” to the tissue of interest via the EPR effect, followed by the systemic administration of a drug that subsequently accumulates on the pretargeted nanomaterial. Many groups have used such a pretargeting strategy to deliver radiolabeled antibodies to cancerous tissue,158–160 as well as to deliver small-molecule drugs or MRI contrast agents via pretargeted gold nanomaterials.161 This strategy is advantageous because it offers the possibility of decreasing systemic toxicity by first administering a non-toxic material to target the tissue of interest and then subsequently administering the
cytotoxic agent. Likewise, in in vivo imaging applications, the two-stage approach leads to decreased background signal. These strategies typically use highly selective or bio-orthogonal chemistry to link the two materials together, such as a biotin−avidin pairing.

The assembly of nanofibers can be induced in vivo by hydrogelation or by assembly of amphiphilic materials. Xu et al. used an enzyme trigger to assemble nanofibers from a “pre-hydrogelator” peptide. Upon cleavage of a phosphate group from the pre-hydrogelator, the resulting peptide assembled into a biodegradable hydrogel. Uljin and co-workers have employed a similar approach utilizing enzyme-triggered reverse hydrolysis to assemble a hydrogel from hydrogelators that could be used for controlled release of encapsulated drugs.

The Stupp laboratory has pioneered the use of masked amphiphilic peptides to assemble nanofibers in solution (demasking) via pH, osmolarity changes, UV light, or enzyme triggers (Figure 5). The properties of the resulting hydrogel can be tuned by changing the length and identity of the hydrophobic tail or by modifying the hydrophilic peptide to contain cross-linking domains, β-sheet-forming domains, or bireponsive units. Covalently linked drugs can be released in a controlled manner via slow hydrolysis of an acid labile linker. Furthermore, therapeutic peptides such as a VEGF mimic or a cytotoxic peptide have been used as part of the nanofiber. In these cases, the resulting nanofiber remarkably maintains the therapeutic properties of the parent peptide.

Other strategies rely on polymeric systems that exhibit pronounced structural differences in response to thermal triggers. Thermoresponsive polymer systems exhibit a lower critical solution temperature (LCST), which defines a transition between hydrophilic and soluble polymers to hydrophobic and amorphous aggregated structures in aqueous solution. This transition can be triggered by heat application at a localized area, such as a tumor, to cause specific aggregation at this location. The assembly of nano bers can be induced in vivo utility of this strategy with elastin-like peptide (ELP) unimers, which have an LCST between body temperature and 42 °C, which is within the approved temperature range for clinical hyperthermia. Using the local heating strategy, a 2-fold increase in accumulation of ELP in tumors was observed by the hyperthermia strategy compared to scenarios in which no heat was applied. ELPs have also been conjugated to cell-penetrating peptides and shown to increase the intra-cellular delivery of therapeutic peptides and doxorubicin in vivo. In addition to amorphous aggregated structures, ELPs can also be formulated so that they aggregate into well-ordered micelles upon heating. It has been demonstrated that the micelles of ELPs labeled with integrin or CD13 receptor-targeted peptides (Arg-Gly-Asp or Asn-Gly-Arg peptides, respectively) show a greater uptake in tumors than their unimers alone, possibly due to ligand multivalency. Furthermore, ELP micelles loaded with doxorubicin aggregate upon heating and have shown an increased accumulation in tumors that were subjected to heating and cooling cycles, compared to those that were either constantly heated or kept at physiological temperature. Moreover, ELPs represent a unique material synthon in that they are genetically encodable, and thus their synthesis can be programmed into a cell via standard genetic engineering approaches.

Others have demonstrated that assembled materials undergo size transitions at temperatures below their LCST. This was demonstrated with oligoethylene glycol-based dendron assemblies that show an increase in encapsulated guest release with decreasing temperature. These materials serve as stable hosts above their LCST, but lose their ability to house guests at lower

Figure 5. Stimuli-induced assembly events. (A) Cartoon illustrating a generic assembly event in which an uncaging event occurs (loss of red blocking unit) to enable the assembly of many unimers (blue octagons). Note that we define an assembly event as a unimer or ill-defined structure assembling into a much larger stucture of higher order. Aggregation is a related process in which unimers or disordered phases assemble into larger, amorphous aggregates. (B) Chemical structures depicting a literature example of an o-nitrobenzyl “caged” amphiphilic peptide that becomes amphiphilic and self-assembles into hydrogel-forming fibers upon removal of the caging group (i.e., the red oval in scheme A) by 350 nm light. (C) Photograph of the caged amphiphile and the hydrogel formed after removal of the o-nitrobenzyl cage. (D) Transmission electron microscopy images of the system before and after exposure to UV light. Panels B–D are adapted from Muraoka et al. with permission from John Wiley and Sons Ltd.
Morphology Switches. The morphology of discrete biological materials often plays a significant role in facilitating molecular and cellular interactions, dictating tissue function, and guiding biodistribution in vivo. At the micrometer length scale, flexible biconcave red blood cells squeeze through capillaries to deliver their oxygen payloads. Genetic abnormalities producing malfunctioning hemoglobin result in deformed erythrocytes, which lack the flexible properties of normal red blood cells and can block blood vessels and lead to infarcts.\(^{168}\) At the nanoscale, bacteriophages bind a host and flex their tail fibers to achieve optimal positioning for a syringe-like injection of genetic material into a bacterium through a rifled helical sheath.\(^ {169}\) Other biological processes, such as HIV fusion, are also dependent on complex nanoscale morphological changes.\(^ {170}\) Over the past 50 years, scientists have made many advances in understanding nanomaterial assembly events\(^ {59,171−175}\) by studying natural and synthetic self-assembling systems through the use of both microscopy and spectroscopy. These advances have paved the way for chemists to synthesize nanomaterials that have unique functions dictated by morphology and composition.\(^ {176,177}\) We have developed various approaches aimed at manipulating the morphologies of synthetic nanomaterials in situ, in the hope of changing material properties and hence switching functionality. Our ability to drive and control these manipulations hinges upon the premise that the morphology of these systems is dictated by kinetic and thermodynamic principles inherent to the building blocks of a material in a particular environment. Ultimately, dramatic changes in morphology achieved in vivo could lead to changes in pharmacokinetics, stability, bioavailability, and biodistribution. For example, Discher and co-workers have demonstrated that flexible filaments composed of polymer micelle assemblies remain in circulation in rodents up to 10 times longer than spherical assemblies composed of similar chemistries.\(^ {176}\) Indeed, the circulation time of soft polymeric fibers is highly dependent on the length of the fiber itself. A stimulus-driven transition between these two morphologies could therefore lead to retention of a drug-loaded nanodelivery vehicle or excretion of a used carrier.

The morphological manipulation of nanomaterials can be achieved with a range of stimuli and with predictable consequences depending on the nanomaterial composition. It should be noted that we define a change in morphology as distinct from assembly or disassembly of a material in that a transition from one ordered phase to another must occur (Figure 6). Scenarios in which a material retains its phase and assembles into a higher order structure of the same phase or begins as an ill-defined or unstructured phase prior to assembly into a much larger and more ordered material are treated here as assembly events and not as morphology switches. Some of the earliest published examples of synthetic materials that change shape in response to a stimulus involve block copolymer nanomaterials comprised of polystyrene and poly(ethylene oxide) that change from rods to vesicles due to the addition of lithium chloride.\(^ {178}\) Shortly after, inorganic materials chemists discovered that gold nanoparticles could be induced to change shape from rods to spheres via a photoannealing process owing to the plasmon resonance of gold nanoparticles.\(^ {178,120}\) Materials decorated with thermoresponsive polymers show dramatic shape changes upon heating, which is caused by a reorganization of solubilizing polymer domains that results primarily from desolvation processes that occur when the materials are heated above their corresponding LCSTs. Indeed, such effects are witnessed in self-assembled materials composed of dendronized oligoether moieties laterally grafted from aromatic scaffolds.\(^ {109}\) Such materials switch from two-dimensional sheets to tubular scrolls due to the dehydration of the oligoether moieties upon heating above the LCST. Assemblies of molecules and polymers are held together by a combination of covalent and non-covalent interactions. Disrupting non-covalent interactions via the introduction of a small molecule capable of hydrogen bonding can significantly disrupt the phase of a given assembly. Work by Zhu and co-workers demonstrates that nanostructures based on polyphosphazene-derived block copolymers switch from network aggregates to multilamellar spheres upon the introduction of the small molecule indomethacin (Figure 6B,C).\(^ {88}\)

Because polymeric nanomaterials composed of amphiphilic subunits assemble into a given shape based largely upon the ratio of hydrophobic and hydrophilic domains in each subunit,
these parameters can be manipulated in situ to achieve shape modulation. Stimuli for such transformations have included light, pH, temperature changes, shear, DNA hybridization, and enzymatic manipulation.

Note, however, that there are few examples of synthetic drug delivery systems whose function is dictated by discrete changes in the morphology of a nanoscale material. Presumably, this is due to the fact that tracking the change in morphology of a nanoscale material in vivo is an extremely challenging task due to the dearth of imaging techniques providing adequate resolution and signal-to-noise in this context. Indeed, in situ imaging techniques are being rapidly developed with significant progress in recent years. Our laboratory and others have worked to contribute to the advancement of in situ imaging techniques using electron microscopy to monitor dynamic structures at high resolution. We look forward to witnessing such advances that enable the preparation and use of more and more complex and dynamic nanomaterials in the years to come.

**Autonomous Motion.** Nanoscale drug delivery systems that function or achieve enhanced function via autonomous motion (Figure 7) represent an attractive approach to advanced materials that are capable of controllable localization, controlled percolation, or deep tissue penetration not otherwise achievable. The ability to direct motion at the nanoscale remains an elusive challenge for chemists and engineers due to difficulties in the synthesis and positioning of functional motors at this length scale.

Nature has evolved complex networks that utilize numerous protein interactions and small-molecule metabolism to drive motion via cooperative changes in conformation or polymerization and depolymerization reactions. Canonical motor proteins, including kinesins, myosins, and dyneins, are involved in chemotaxis, trafficking, signal transduction, and locomotion and take advantage of polymeric scaffolds such as actin and tubulin to coordinate their movements. The keys to achieving motion in synthetic nanomaterials thus far are asymmetric placement of an appropriate motor and propulsion using an adequate fuel. The first reported successful attempt at synthesizing a nanoscale object capable of autonomous motion involves the asymmetric positioning of a platinum-based catalyst within a discrete metallic nanomaterial. In this example, the motive force (on the order of piconewtons) is generated via the establishment of a chemical concentration gradient produced only at the platinum end of a gold–platinum nanorod (approximately 350 × 2000 nm in dimension). The stimulus, or fuel, is hydrogen peroxide, which is rapidly converted to water and oxygen gas by the platinum surface of the nanorod. Other work involving tubular nanorods takes advantage of bubbles formed during electrocatalytic peroxide degradation to propel the nanomaterial. Similar achievements have been demonstrated using enzymes as catalysts and their corresponding substrates as fuel. One could imagine taking advantage of this type of system to direct motion of a delivery vehicle through substrate concentration gradients in vivo. Indeed, delivery of drug cargo to cultured cells has been achieved with reasonable results. Progress toward realization of these goals in an in vivo setting has been demonstrated in a few recent examples and will continue to advance as we gain an understanding of how to control motion in complex environments with fuels limited to endogenous biological components.

A different approach to generating motion of nanoscale devices relies on the formation of transient hydrogen bonds propagated by DNA hybridization/dehybridization and subsequent polymerization reactions. DNA nanomechanical “cranes” have been developed to move cargo taking advantage of Brownian motion and the free energy of hybridization/dehybridization between DNA strands. The Achilles heel of these systems, as functional units in biological settings, will be the enzymatic degradation of the nucleic acid components used to assemble such machines.

To achieve the goal of harnessing motion at the nanoscale for the delivery of therapeutic payloads, stable materials must be synthesized that take advantage of abundant biomolecules in a given environment in order to fuel themselves, while simultaneously achieving active steering. At the present time, external steering provided via acoustic waves or magnetism seems to be the simplest way to spatially direct materials. Alternatively, motion across very small distances could potentially be achieved using machinery implanted in cellular membranes or at the surfaces of intra-

**Figure 7.** Nanomaterials capable of autonomous motion. These materials are often prepared by incorporating motors that use catalysis to generate chemical concentration gradients and propel themselves through solution via self-electrophoresis. (A) Cartoon depiction of the motion of a nanomaterial generated via the depletion of chemical fuel placed on one side of the object. (B) Platinum–copper nanorods that catalyze the reduction of iodine to iodide while oxidizing copper at the opposite end of the rod. The reduction that takes place at the platinum end of the nanorod generates a flow of electrons toward the platinum end of the material. This electron flow generates a charge differential, thus inducing fluid movement toward the platinum segment and propelling the nanorod in the opposite direction of the fluid. (C) Optical microscopy snapshots tracking movement of Pt–Cu nanorods over time (images (g) and (h) depict instances of surface-immobilized nanorods). Panels B and C are adapted from Liu et al. with permission from the American Chemical Society.
cellular organelles, for example. Indeed, there are many issues left to be resolved including the ability to incorporate and release drug payloads at specific locations with temporal control in relevant biological contexts. Further, anchoring materials into precise locations in vivo and controlling or monitoring the function of those materials in live animals has not been demonstrated, to our knowledge. There are, nevertheless, intriguing future prospects in which nanoscale platforms could be used to import or transfer therapeutic agents between extra-cellular and intra-cellular locations or between different tissues in a living organism. Communication with and control over synthetic nanomotors, once placed in the bloodstream or within the peritoneal cavity, for example, will be the key to developing such technologies further.

**DISCUSSION**

The holy grail in the field of drug delivery is to develop drug delivery vehicles that can discriminate between healthy and diseased tissue, internalize directly into cells, and facilitate the efficient delivery of cargo. Such a "magic bullet" has been an elusive target for decades because there are few truly unique biomarkers or stimuli that are present only in specific cells or tissues. Therefore, scientists and engineers are tasked to find new ways to discriminate between cell types and, furthermore, to take advantage of these key differences to actuate a material for drug release. For example, RNA expression profiles serve as transient barcodes distinguishing cell identity and state. Drug delivery devices capable of harnessing this information would have significant impact on the field as a whole. There are also significant challenges in the delivery of materials to relevant cellular compartments with high efficiency. Designing non-toxic materials capable of efficient cellular entry, endosomal escape, and predictable intra-cellular trafficking is a major challenge that will require the engineering of materials capable of responding to various stimuli and orchestrating several specific interactions in a coordinated fashion.

It is likely that strategies relying solely on direct cleavage of a covalently linked drug carried on a nanoparticle platform will suffer from a lack of specificity due to cleavage by indiscriminate triggers present throughout complex biological milieu. Oftentimes materials that show cleavage selectively in a test tube or in cell cultures are less effective in vivo due to the wide range of biological components that hydrolyze or cleave chemical linkages via redox or enzymatic activity. We anticipate that strategies involving a more complex manipulation of nanocarrier properties, such as shape or architecture, could provide opportunities for developing more selective or efficient materials for drug delivery. Moreover, materials that assemble into nanoscale structures directly at the site of interest could avoid the issue of size-dependent reticuloendothelial system uptake that is typical for injected nanomaterials. Encoding materials with biological information in the form of peptides, proteins, and nucleic acids is a promising way to integrate such materials with biological environments as noted in some example strategies presented in this Perspective. In our own laboratory we have developed a strategy for manipulating the aggregation propensities of spherical micellar nanoparticles by encoding them with peptides that are substrates for proteases that are overexpressed in cancerous tissue (i.e., MMPs). The materials are designed to circulate freely as spherical micelles until they encounter MMPs in tumor tissue. Cleavage of the hydrophilic peptide portion of the amphiphilic polymer changes the properties of each amphiphile enough to facilitate aggregation of multiple peptide-based micelles to form a microscale, interconnected net (Figure 8). These aggregates are simply too large to re-enter circulation and remain localized in tumors for days after injection in vivo (Figure 8B). We envision that scaffolds of this sort could be loaded with drugs, which could be triggered for release by a separate stimulus or slowly by non-specific hydrolysis once accumulated. This type of strategy encourages tissue-specific accumulation of material and could be further coupled with stimuli-specific drug release in order to achieve the high specificity provided by multiple stimuli.

Another key concern is the toxicity of the nanomaterial or what remains of the material after it has responded to a stimulus. For example, Doxil, a PEGylated liposomal formulation of the anti-cancer drug doxorubicin, frequently causes palmar-plantar erythrodysesthesia, also known as hand-foot syndrome. The successful implementation of any drug

![Figure 8. Strategy for facilitating direct accumulation of nanomaterial-derived scaffolds in tumor tissue. (A) A general scheme depicting the design of the system consisting of a spherical particle composed of polymeric peptide-amphiphiles, where the hydrophilic portion is a substrate for tumor-associated MMPs. As nanoscale spherical assemblies, the nanoparticles are free to circulate into and out of tissue until they encounter locations with a large abundance of MMPs, indicative of cancer or inflammation. Upon cleavage of the peptide, fragmented amphiphiles reassemble into a large, interconnected scaffold that is too large to re-enter circulation. (B) Fluorescence data of tumor-burdened mice 1 h or 3 days post intra-tumoral injection of the MMP-responsive nanoparticles labeled with AlexaFluor-647. Enzyme-responsive particles (prepared from l-amino acids) are assembled as designed and are retained in the tumor tissue for at least a week, at which point the animal is sacrificed. Non-responsive particles (comprised of d-amino acids) are rapidly cleared from the tumor tissue. We envision that responsive systems such as these could be used to trigger accumulation of drug-loaded particles that could be further activated to release drugs in a tissue-specific fashion or passively via long-term degradation. Figure adapted from Chien et al. with permission from the American Chemical Society.](https://doi.org/10.1021/ja510147n)
delivery agent will clearly require that such concerns be met. Caution and foresight must be used whenever preparing materials with toxic metals or chemical scaffolds that are difficult to degrade in biological milieu.

Finally, we must note that material properties that can potentially be tuned in response to stimuli are not limited to those discussed in this Perspective. For example, it can be envisioned that tuning the elastic modulus or the electromagnetic properties of a nanomaterial in response to a given stimulus may be of significant value in terms of developing advanced materials for biomedical applications in the future.

# TOWARD THE FUTURE

In this Perspective, we have described physical material responses that can be induced to facilitate drug delivery or diagnostic imaging by nanoscale carriers. These responses include direct release or activation, expansion, gatekeeping, disassembly or degradation, assembly or aggregation, switches in morphology, and the induction of autonomous motion. Many of the earliest examples of stimuli-responsive nanomaterials are initiated by the cleavage of a chemical bond, these include direct release, gatekeeping, and disassembly/degradation strategies. Key obstacles for the clinical success of these strategies include identifying linkages that are susceptible to cleavage by only one stimulus and optimizing the kinetics of release to ensure selective, efficient, and timely delivery or activation of the materials. Reponses like expansion, assembly/aggregation, or morphology switching result from changes in the physical properties of the material, such as its amphiphilicity, electrostatics, or sterics, which bring about shape, size, or phase changes. The clear challenge with these approaches is in devising practical strategies to translate complicated designs that work in a test tube into a successful in vivo application. Simpler transitions like expansion must also be triggered by more selective stimuli such as enzymes or other relevant small molecules instead of bulk environmental triggers like pH or redox, which are found in varying abundance in multiple locations throughout healthy or diseased tissue. Motion has yet to be realized in a practical sense for drug delivery or diagnostics, despite key advances, owing to the many challenges facing in vivo use. Key challenges include the identification of relevant and abundant biomolecules for use as fuel sources, achieving active steering in complex biological milieu and the precise positioning/implantation of such devices. However, a key struggle hindering the success of each of these applications is the in vivo characterization of the size, shape, and structure of nanoscale devices due to the limited imaging techniques currently available for such purposes. As such, advances in in situ imaging techniques will likely result in achievements in many of these approaches.

An interesting and potentially useful feat would be to link multiple stimuli or responses together sequentially in order to achieve delivery of a therapeutic payload or a diagnostic agent. Nature often uses multistage events to perform complex and important processes. In neurotransmission, an electrical signal known as an action potential is transmitted between nerve cells via the release of small-molecule neurotransmitters, which traverse the synaptic cleft and bind ligand gated ion channels on the opposing cell. The opening of these channels enables the flow of ions across the membrane of the receiving cell, thus turning a chemical signal back into an electrical signal in the postsynaptic nerve cell. The precise timing of multiple chemical and electrical stimuli and responses in this scenario ensures that signals transmitted are intentional, thus minimizing misfires that could result in overstimulation and a host of physiological consequences. This is but one of the innumerable tightly controlled, multistaged, biological processes that exist. If scientists can create drug delivery vehicles as robust as this, then perhaps truly selective drug delivery may be realized. We should note, however, that the use of stimuli cascades and thresholded responses in the development of nanomaterial platforms for drug delivery is a promising direction fraught with many obstacles. Key to these developments is progress in precise synthesis of programmable nanomaterials and advances in our ability to track and monitor such materials, their stimuli, and their response in the complex milieu ever present in vivo.

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