Photo-Control of Biological Systems with Azobenzene Polymers

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ABSTRACT: Azobenzene-containing polymers offer tremendous advantages and opportunities over other stimuli-responsive materials to interface with biology. Azobenzene's fast, reversible, and innocuous *cis–trans* geometrical isomerization can be leveraged into dramatic intra- and inter-molecular changes when incorporated in polymeric materials. Azobenzene use has grown from a colorant, through to optical storage materials, and most recently in a variety of biologically themed applications. This review highlights the broad impact this photo-switch has had in recent years and offers a snapshot of the research landscape at the interface between photochemistry and biology. From photo-reversible micelles and peptides to controlled drug release and sensing, the versatility of azobenzene makes it a favored photo-switch found in many emerging applications. © 2013 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2013**, *51*, 3058–3070

KEYWORDS: azo polymers; biological applications of polymers; photochemistry; photophysics; water-soluble polymers; stimuli-sensitive polymers; dyes/pigments

INTRODUCTION Synthetic materials for interfacing with biology have been designed and synthesized for millennia; recently the complexity and capability of these systems has increased significantly.[1] Aided by our growing understanding of how biology interacts with artificial materials, researchers have now assembled a broad toolkit of materials and processes that allows us to fine-tune interactions at the interface, eliciting specific biological responses on demand. These methods of bio-control can be broadly categorized as either chemical (ligand presence, charge, surface groups) or material changes (stiffness, moisture content).

These stimuli techniques work exceedingly well and have recently provided devices that function with high reliability and reproducibility. Biology, however, is dynamic and a new challenge arises in designing materials that can respond to, or trigger, a change in biology with spatial and temporal control. There exists a variety of stimuli that have been used traditionally in designing stimuli responsive polymer systems, each with their own challenges and advantages.[2] Some of these stimuli, such as mechanical or electrochemical response, are sometimes less suitable for *in vivo* biological applications. While temperature, pH, and ionic strength responsive materials have specialized applications, these are often limited by the physiological conditions in which they find themselves. This leaves electric current or voltage, magnetism, and light for allowing for user-determined triggering in a biological environment of fixed pH and temperature. Among these three, visible light is not only the least disruptive to biological systems, but the only stimulus which provides high spatio-temporal selectivity with strong dosage control.[3–5]

Light-responsive materials are usually classified by the chromophore they employ in achieving their photo-responsive properties, and fall into two well-defined classes: irreversible or reversible photoswitches.[6] Single-use chromophores include coumarin cages[7] and nitrobenzyl ethers and amines, favored for their high quantum yield, ease of synthesis and relatively large two-photon cross-section.[8] Additional two-photon sensitive diazonaphthoquinones were pioneered by Frechet for delivery applications[9] and leveraging their large change in hydrophilicity is still an active area of research.[10,11]

Reversible photoswitches can involve dimerization reactions such as those between pairs of cinnamates[12,13] or coumarins,[14] however these tend to be triggered by UV light which may be damaging to tissues. Employing a reversible ring opening and closing reaction, spiropyrans have made an appearance in the literature lately, acting as surface switches for actuating[15] and detecting in a biological setting.[16] This leaves the azobenzene[17] (azo-) and stilbene[18] classes of molecule. Both rely on a *trans*/*cis* isomerization triggered by a single photon; though stilbene (the simplest diarylethene) suffers from a side-reaction yielding phenanthrene which shortens the life-cycle of this chromophore (Fig. 1).[19]

Azobenzene, which does not suffer from this side-reaction, can be isomerized on a timescale of microseconds down to sub-nanoseconds, reversibly 10^5–10^6 times before fatigue.[17] Azobenzene chromophores fall into three general classes

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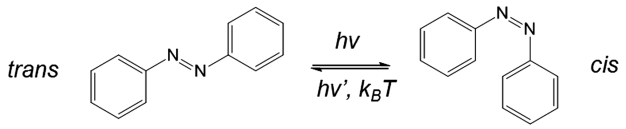

FIGURE 1 Structure of azobenzene: Following irradiation of *trans* azobenzene (left), the *cis* isomer (right) is formed. This isomerization event is reversible either thermally or optically.

(Fig. 2) characterized by the thermal relaxation of the *cis* to *trans* isomerization and the wavelength of absorption triggering the *trans* to *cis* isomerization. Pseudo-stilbenes have a very fast thermal reconversion and a far red shifted absorption, amino-azobenzenes have an intermediate lifetime and slight red shift in the *trans* absorption band, while classical azobenzene absorbs in the UV-violet range and whose *cis* form can be stable for days in the dark.[20] This large variation in properties gives azobenzene a versatility advantage over the previously named chromophores, as chemical substitution has a large effect on the photophysical properties. The variety of possible synthetic routes[21] is another distinct advantage of azobenzene, with the tunability of these properties through chemical substitution allowing for a decoupling of the aforementioned properties. This allows for novel azobenzenes that absorb in the visible with 2-day half-lives.[22]

Regardless of the photophysical properties which trigger the isomerization change, all photoresponsive materials that use azobenzene hinge on the difference between the *cis* and *trans* states. The isomerization leads to a shortening of the end-to-end distance of ~3.5 Å and in the native azobenzene molecule a dipole change of ~3 Debye – two properties, that, when well-engineered, can exhibit impressive changes.[23,24] Azobenzene-containing materials can be found in various applications from photomechanically responsive materials,[25] information storage[26] and reversibly wettable surfaces.[17,27] This review, however, will focus on recent contributions that azo-containing polymers have made in applications specifically related to interfacing with biological and bio-mimetic systems.

SYNTHETIC POLYMERS

Photoresponsive Micelles and Vesicles

Micelles play a critical role in biology, offering a dynamic barrier separating two different environments inside living

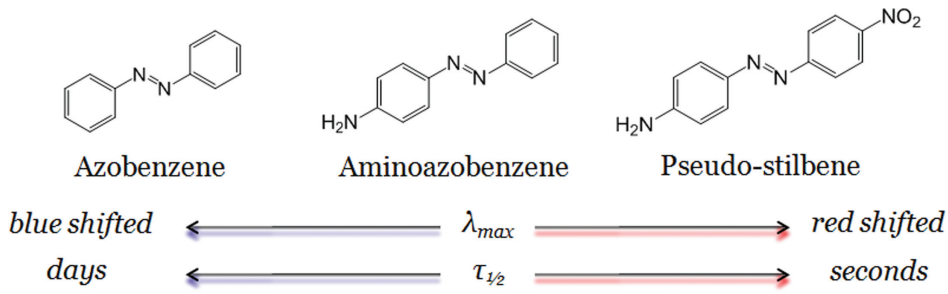

FIGURE 2 The three types of azobenzene and their photophysical trends: classical azobenzene, aminoazobenzene with one electron-donating group and pseudo-stilbenes which possess a strong electron push-pull feature leading to strong red-shifting of the absorbance and shortening of its half-life of thermal reconversion to *trans* from *cis*.

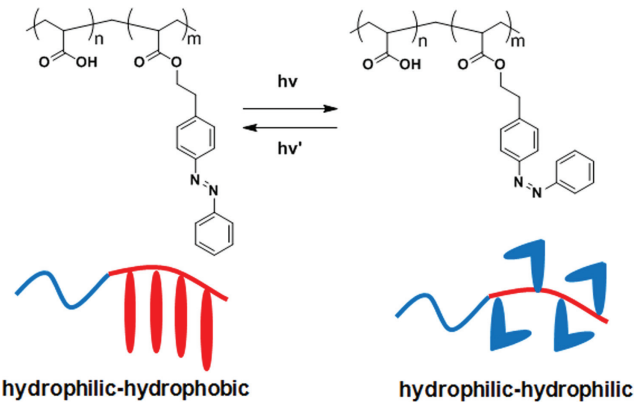

FIGURE 3 Representation of the polarity change in block copolymers containing azobenzene.

organisms. Detergent micelles have been known for centuries, but it was not until 2004 that the idea of introducing stimuli response into synthetic micelles appeared in the literature.[28] Through several iterations and the pioneering work of Zhao, among others, azo-containing polymers have appeared with greater and greater frequency in the literature. In many cases the presence of azobenzene allows for reversible disruption of aggregation which (upon reconversion of the chromophore to the *trans* state) can reform organized structures. These polymers can be classified as either block or random copolymers.

Azobenzene-Containing Block Copolymers

As with many photoresponsive materials, various photo-triggers have been applied to disrupt block-copolymer (BCP) micelles and have been recently reviewed.[29,30] The function of azo-BCP micelles largely hinges on the fact that the *cis* form of azobenzene is more polar that the *trans* form, as shown in Figure 3.[31] As well as exploiting polarity changes, photo-softening of azobenzene materials by isomerization disrupts an ordered micelle into a disordered one, and can now be exploited in allowing cargo release from micelles.[32] The fact that azobenzene is stable and unreactive provides the added advantage that it can be used to create dual-responsive micelles. These can be generated by copolymerizing azobenzene with a second stimuli responsive block such as a thermally responsive segment. In recent work a BCP micelle made up of poly(*N*-isopropylacrylamide) (pNIPAM) and azobenzene copolymer allowed cargo release upon thermal changes, while azobenzene isomerization could change the hydrophobicity of the micelle significantly (Fig. 4).[33]

Amplification of the polarity change in azobenzene isomerization can be achieved by making use of guest-host chemistry. Using the well-known azobenzene and β-cyclodextrin (β-CD) guest-host complex, azobenzene copolymers can be made to undergo enhanced changes in properties. Morphology changes can transform micelles into vesicles as a powerful platform for cargo delivery by using either changes in pH or light-triggered azo assembly with β-CD.[34] The chemical nature of the polymers involved in these assemblies dictates the final form these micelles will have. Two BCPs, each bearing a poly(ethylene glycol) (PEG) segment, one with an azobenzene block and the other with a β-CD block, were allowed to form a micelle and were then chemically cross-linked. This polymeric micelle would remain assembled,

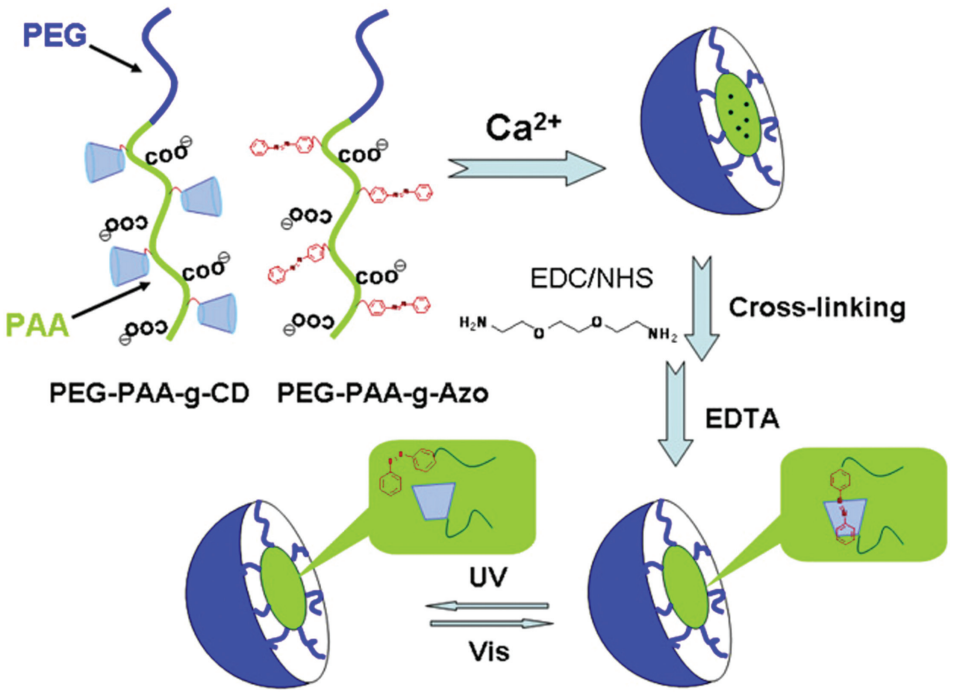

FIGURE 4 Schematic representing the assembly and crosslinking of PEG-*b*-Azo and PEG-*b*- β-CD polymers into drug releasing micelles that do not lose their shape upon cargo release. Reproduced from Ref. 35, with permission from Elsevier.

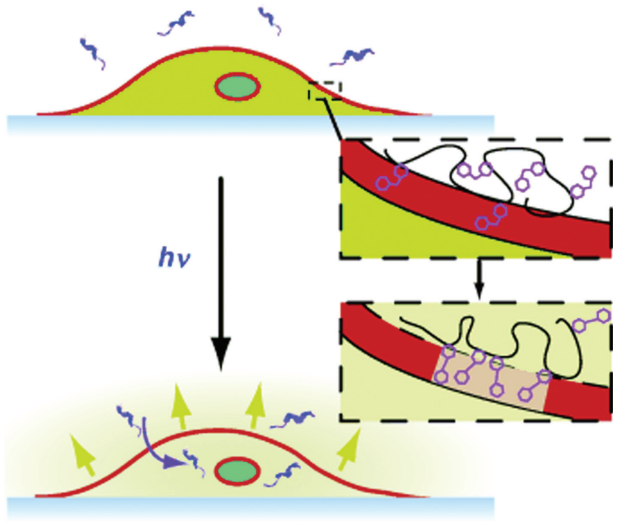

FIGURE 5 Isomerization of *cis* azo to *trans* azo in acrylic acid copolymers embedded in a cell membrane leads to increased permeability allowing for internalization of external cargo, in this case peptides. Reproduced from Ref. 45, with permission from John Wiley and Sons.

releasing the drug upon azo-isomerization acting as a controllable molecular container.[35]

A comparable approach to creating drug-loaded micelles was accomplished by layering polymers of azo-containing poly (acrylic acid) and α-cyclodextrin (α-CD) containing dextran onto particles of $CaCO_3$. These supramolecularly-assembled particles could be loaded with the α-CD-tagged drug model. After removal of the $CaCO_3$ with ethylenediaminetetraacetic acid, The organic capsule could be completely disintegrated using light, releasing the drug.[36] Similar disassembly could be achieved in PEO-*b*-P(6-[4-phenylazo phenoxy]hexyl methacrylate-*co*-2-(dimethylamino)ethyl methacrylate) vesicles in solution, which was transformed to micelles with the addition of *β*-CD. By further increasing *β*-CD concentration, the micelles could be completely disassembled, and re-assembly could then be triggered via azo isomerization to release the *β*-CD from the polymer chains.[37]

Tuning the architecture of the BCPs can have drastic effects on the assembly of these chains in solution. In dendritic diblock copolymers, both the ratio of azo to PEG and the generation number have been found to have a large effect on polymer self-assembly. This generation-dependent aggregation behavior can be disrupted via light irradiation. Given the tunable shape properties from lower generations yielding micelles to higher generations forming nanotubes, these compounds have potential applications in drug delivery.[38]

Micelles in biological systems are dynamic and undergo fusion under the right circumstances: similar behavior can be induced in synthetic micelles triggered by light. In block copolymers containing azopyridine, irradiation with UV light allows these vesicles to undergo photoinduced fusion, disintegration and rearrangement which can be halted upon reconversion to *trans* when irradiated with visible light.[39] Similar behavior can be seen in vesicle fusion of pNIPAM-*b*-PAzoM when light induces isomerization of the outer azo block.[40] This fusion can result in the formation of significantly larger vesicles, as seen in a fivefold increase in vesicle size due to fusion upon irradiation in PEO-*b*-PMeA6AB2 copolymer vesicles.[41] This work in micelle biomimicry shows that dynamic and even complete disruption of micelles is possible using azobenzenes.

Azobenzene-Containing Polymers

Unlike block copolymers, changing chain polarity for non-block copolymers is slightly more difficult. As a result, the tools of supramolecular interactions have to be used: an excellent example of this is using the previously described azobenzene and CD guest-host complex which can amplify azobenzene copolymers' changes in properties. Hyperbranched azo-functionalized polyphosphates containing *β*-CD could be made to form and break micelles reversibly by cycling UV and visible light thus either accepting or removing the host cyclodextrin from the main polymer chain.[42] The effect can also be seen with homopolymers, one bearing azo and the other α-cyclodextrin (α-CD). Mixing these leads to the formation of nanotubes that can be disrupted with light, both of which show promise as nano-carriers in photo-therapy.[43]

A fascinating application of these azo-polymers is the ability to release cargo from a micelle or cell. Copolymers of azobenzene and acrylic acid exposed to micelles or cells in the *cis* form adhere to the surface of the cell membrane and the azo mesogens are able to penetrate the membrane. Upon visible light irradiation, isomerization of the *cis* azo to *trans* increases the membrane permeability (as depicted in Fig. 5) allowing for cargo release[44] or internalization of peptides into living cells.[45] This offers the distinct advantage of added versatility as well as additional temporal control, since the azobenzene group doesn't need to be synthesized into the membrane.

As with living membranes, controlling dynamics is very important in synthetic biological mimics. Pulsating vesicles can be created by the very simple addition of azobenzene-capped PEG, in a tetrahydrofuran (THF)/water solution these vesicles exist in a large monodisperse state when in the *trans* form. Isomerization to *cis* leads to a repeatable shrinking of the vesicle size and expulsion of water, while back-isomerization leads to the reverse effect, causing them to act like biomimetic sensors.[46] This THF/water combination can also create hollow-sphere aggregates with sizes from 168 to 527 nm assembled from an amphiphilic random copolymer of azo and acrylic acid and can be collapsed into nano-rods with UV light, allowing for the release of cargo.[47]

Rheological changes, such as going from a gel to a free-flowing state, can be useful in delivery applications. In an elegant example of simple intermolecular assembly, Yu and co-workers[48] created a carboxylic acid containing azobenzene which through simple hydrogen bonding would gel in the *trans* state and return to a liquid when irradiated to the *cis* form. This followed work in a photo-responsive dextran gel

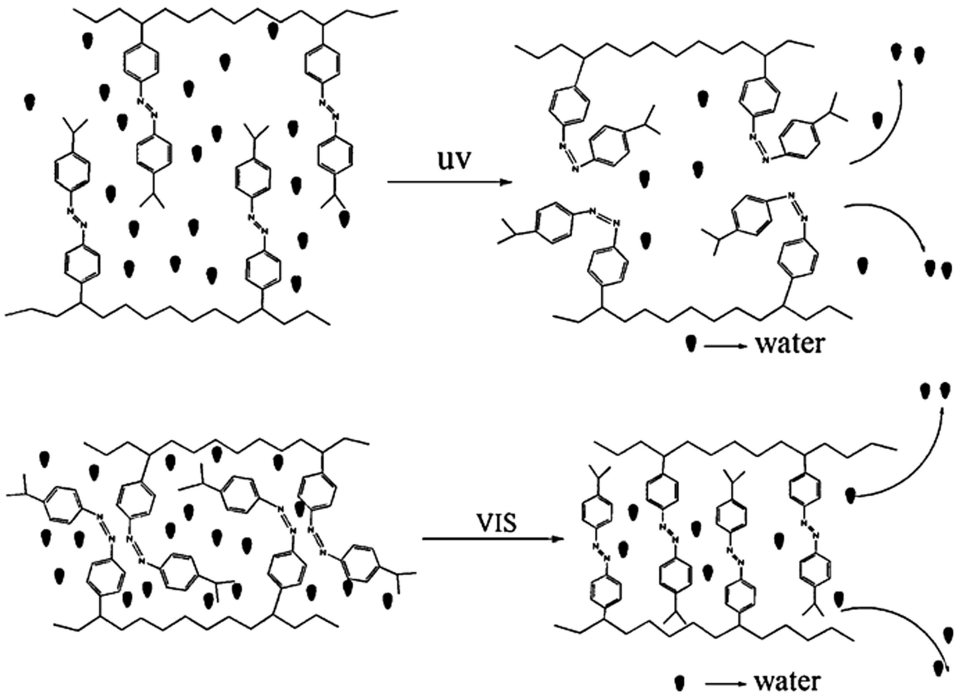

FIGURE 6 Mechanism of drug release from *trans-cis* isomerization (top) and *cis-trans* isomerization (bottom). Both material changes result in drug release. Reproduced from Ref. 50, with permission from Elsevier.

which could be triggered to release drugs in vitro with the release rate being affected by salt concentration and pH.[49] The internal cavity architecture of these azo-hydrogels seems to have a large effect on the release of drugs and uptake of water on isomerization.[50] Isomerization in either direction can result in drug release, since both result in a significant change of the cavity dimensions from the template gel (Fig. 6).

Azo-polymers can also regulate cargo release from inorganic architectures. Liao and co-workers used silica microparticles to template a pAzo shell coating. Removal of the silica with an hydrofluoric acid etch yielded a polymeric microcapsule which allowed diffusion of small molecules; interestingly, this diffusion could be increased by 44% when in the *cis* state. This behavior was reversible, allowing for the possibility of reversible dose control.[51] Additional hollow silica microspheres, functionalized with β-CD using click chemistry, could be drug-loaded and upon addition of an azo-containing polymer: here, the supramolecular interaction creates a polymeric "gate-keeper" driven by the azo- β-CD interaction. Light irradiation to *cis* removed this polymeric barrier allowing drug diffusion out of the microspheres while isomerization back to *trans* caused the barrier to reform around the microspheres.[52] These methods hold promise for photo-reversible drug release applications, aided by recent investigations showing real-time drug release from microcapsules upon azo isomerization.[53]

BIOMACROMOLECULES

Biomolecules have been an exciting area of research in the application of photoresponsive stimuli.[24] Biomacromolecules functionalized with azobenzene such as deoxyribonucleic acid (DNA) and peptides can undergo vast conformational changes upon isomerization, leading to designed and reproducible changes in function. These biomolecules are well suited for disruption with a trigger such as azobenzene, as both will undergo predictable folding or unfolding in a biological context.

Nucleic Acids

Oligonucleotide synthesis has increased greatly since the widespread adoption of automated DNA synthesis. Shortly afterwards Makoto Komiyama's group reported incorporation of an azobenzene nucleobase analogue that could modulate the melting temperature (T_m) of DNA,[54] which was further expanded towards photo-regulation of hybridization and transcription for acceptance by the scientific community at large.[55] This work has since been shown to be applicable to ribonucleic acid (RNA)[56] and peptide nucleic acids with ease (Fig. 7).[57,58]

Improvements to disruption of DNA binding and increases in the T_m of the DNA chains can be induced through modifications of the azobenzene structure via ortho-methylation, leading to increased stability of the *trans* form in the stabilizing duplex.[59] Addition of a methanethiol group at the para position of the methylated ring additionally provides a bathochromic shift to the *trans* absorption band, allowing for separate isomerization of two azo species in one duplex. This provides photoresponsive nanomaterials and allows for duplex disruption at wavelengths above 380 nm.[60] Likewise, addition of a dimethyl amino group at the 4′ position results

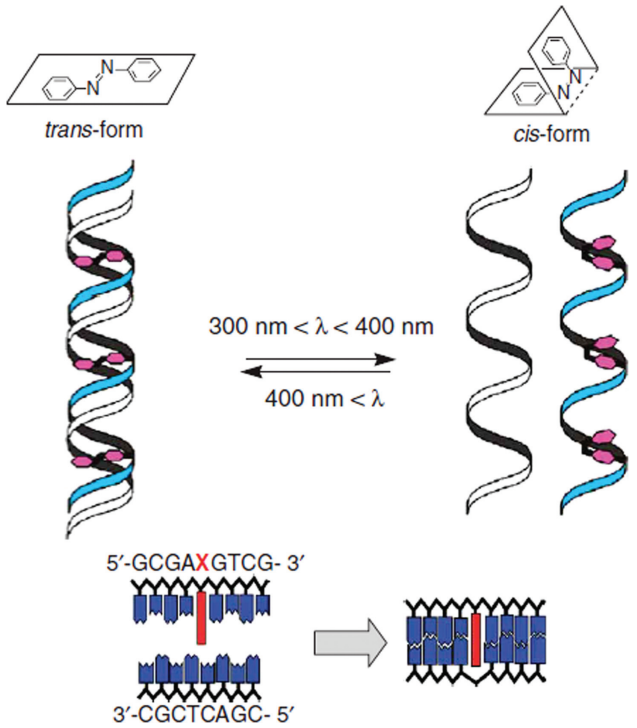

FIGURE 7 Illustration showing how dsDNA can be dehybridized to its single-stranded form by the artificial D-threoninol based azobenzene phosphoramidite monomer, and how this unnatural moiety intercalates into the dsDNA (bottom). Reproduced from Ref. 55, with permission from Nature Publishing Group.

in a further bathochromic shift allowing isomerization at 436 nm (Fig. 8).[61]

This duplex-forming control can be applied to controlling biological function in cells. Transcription can also be regulated through the use of T7 promoters with tethered azobenzenes where a 10-fold reduction in activity could be shown for a polymerase functionalized with two azobenzenes.[62] As well, activity of T4 DNA ligase could be photo-regulated by isomerizing azobenzenes on the DNA chain to the *cis* form when incorporated at the nick site to improve ligation efficiency.[63] By replacing the loop nucleotide with 4,4′-bis(hydroxymethyl)-azobenzene, hairpin structures can be photoregulated as either closed or open by alternatively irradiating with UV or visible light.[64]

Outside of the cellular environment, complex nanostructures such as capsules can be taken apart through judicious engineering of azobenzenes into DNA chains forming polyhedra.[65] Potential applications of these techniques in drug delivery can be seen from Weihong Tan's group who have incorporated this technology into three-dimensional (3D) tetrahedra allowing for potential release of drugs or other cargo.[66] His group has pioneered photo-responsive hydrogels bearing azo-DNA crosslinkers. They then employ the same idea of disrupting a DNA duplex with light to release small drug molecules, proteins, and nanoparticles.[67] Also with therapeutic application, nanoparticles functionalized with DNA containing these photoresponsive azo groups can be triggered to assemble and disassemble, with the potential to have wide applications as sensors in biology (Fig. 9).[68]

All of the examples so far have involved the incorporation of azobenzene directly into the DNA chain. A different approach has been to use so-called "molecular glues" (subject of a recent review)[69] to interact with DNA duplexes. This work is based on the idea of a crosslinker which can interact with the two strands of a double stranded deoxyribonucleic acid, incorporation of an azobenzene chromophore in this linker will lead to photo-reversible duplex formation. By tailoring a flexible linker to recognize G-G mismatches, two strands of pyrene containing DNA can be paired and unpaired allowing for different fluorescence reportings based on the hybridization status of the strands.[70] This work was expanded, allowing for construction of branched DNA networks which could be reversibly assembled and taken apart.[71]

Peptides

Given the predictability of peptides to fold into well determined domains, the ease with which azobenzene photocontrol can be introduced has been aided significantly by computational efforts.[72] Differing reactivity of peptide residues can be exploited to engineer structures (usually helices) that can be further functionalized with photoswitchable functionality.[73,74] With synthetic tools, Woolley and co-workers have found that peptide conformation can be kept in a disordered ground-state, triggered to ordered with light

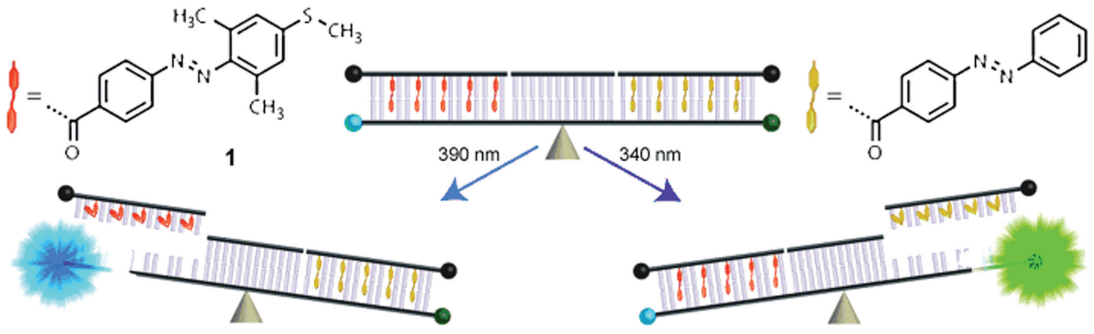

FIGURE 8 Methylation and addition of methanethiol to one of the benzene rings in azobenzene (left) red-shifts the absorbance enough to allow separate irradiation of two azobenzene chromophores in DNA. Reproduced from Ref. 60, with permission from John Wiley and Sons.

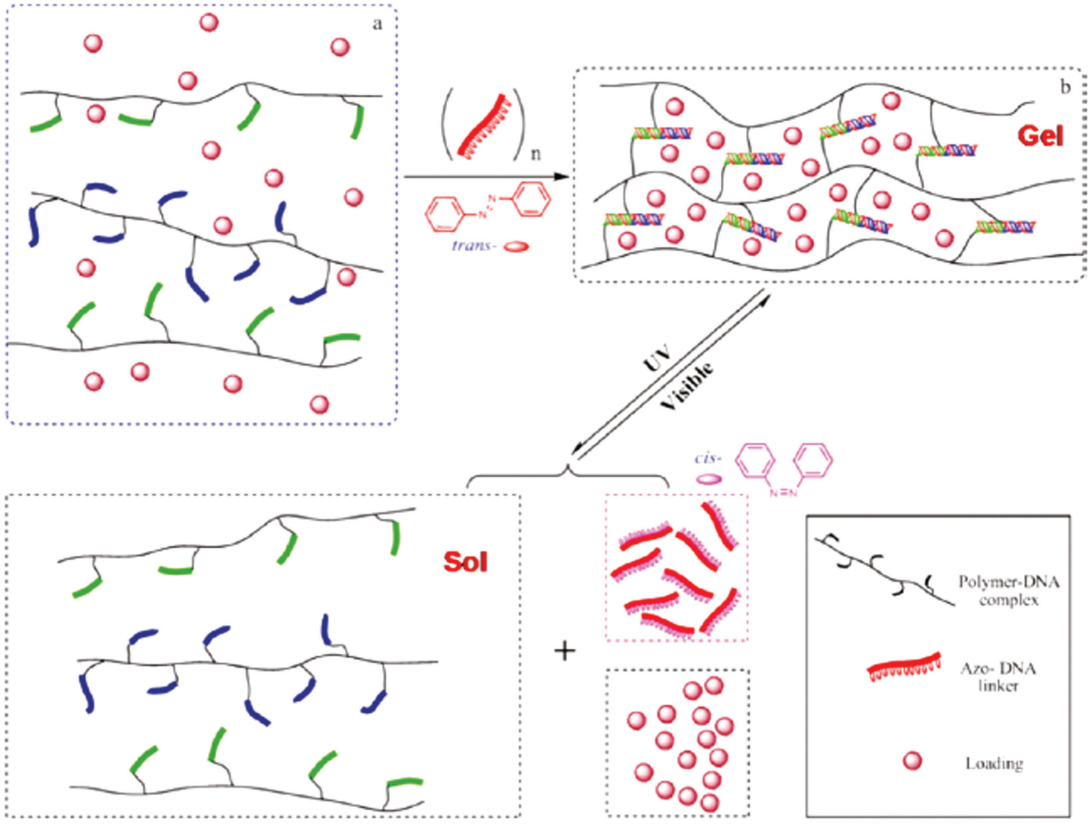

FIGURE 9 Loading and unloading of a DNA functionalized hydrogel with cancer therapies using an azo-DNA crosslinker which triggers a gel-sol transition after light irradiation. Reproduced from Ref. 67, with permission from American Chemical Society.

irradiation,[75] showing that both folded and unfolded peptides can be selected as the ground state. By chemically reacting azobenzene to cysteines situated outside of the secondary protein structure, these protein disruption studies have culminated with *in vivo* fluorescence imaging of the ordered state of peptides in zebrafish. By tethering fluorescein to the end of a peptide helix bridged with an azobenzene, fluorescence could be recorded in an extended state that was quenched upon isomerization, showing *in vivo* application of protein disruption (Fig. 10).[76]

These crosslinking techniques have been expanded from simple peptide chains and can affect global protein structure. Introduction of these crosslinkers in mutated proteins can bring them from globular forms to properly folded (upon *cis* isomerization) showing the applicability to more complex structures.[77] Similar crosslinks can also be used to affect enzyme activity, as seen in the 30 variants of PvuII restriction endonucleases which were generated and crosslinked with azobenzene to determine the optimal placement of azobenzene. The best effect was achieved with multiple azo crosslinks and with placement close to the enzymatic active site resulting in a 16-fold change in activity.[78]

As well as crosslinking the exterior of peptide conformations, azobenzene incorporation in the actual peptide chain can be used as a tool to study the self-assembly of peptide chains. Amyloid-β self-assembly into cross-β amyloid fibrils could be allowed or disallowed based on azobenzene isomerization[79] with an immediate appearance of cytotoxicity determined by whether the peptides existed as fibrils versus oligomers.[80] This structural disassembly has been studied by time-resolved mid-IR spectroscopy to determine how the azobenzene disrupts amyloid formation.[81] Azobenzene has also been shown to disrupt folded β-hairpin motifs that was disrupted within a 1 ns timescale, but took microseconds to reform (Fig. 11).[82,83]

Designing an azobenzene amino acid opens the possibilities for biological incorporation of photo-switches *in situ.* An azobenzene amino acid paired with its own orthogonal aminoacyl transfer RNA synthetase allowed for biological incorporation of azobenzene into the catabolic activator protein of *E. coli* bacteria which showed photoswitchable activity over the wild type.[84] Azoalanine and 4′-carboxyphenylazophenylalanine have also been incorporated into protein structures and can regulate the activity of endonuclease BamHI by preventing the dimers of the protein from assembling properly in the *trans* state, but allowing dimerization in the *cis* state.[85,86]

Interactions between antibody and antigen can be photo-controlled, as has been determined by Freitag and coworkers. They engineered azobenzene into several different positions of the DYKDDDDK (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) peptide backbone.[87] Even protein interactions with metal ions

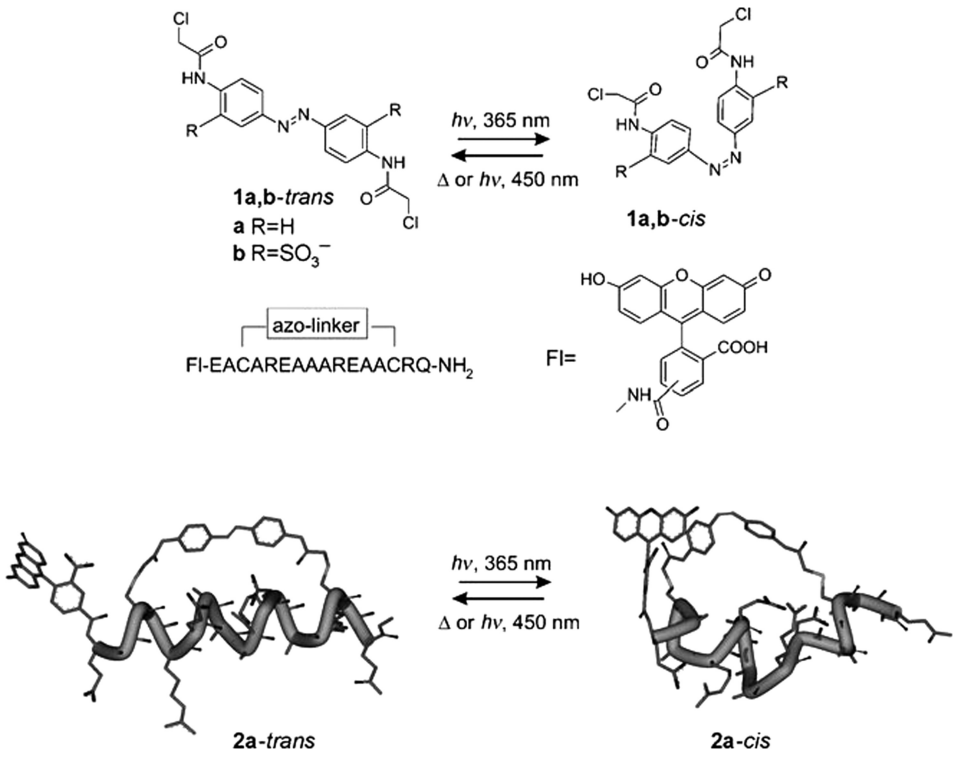

FIGURE 10 A cysteine reactive azobenzene reacts with a designed peptide structure such that UV-triggered isomerization leads to α-helix disruption and fluorescence quenching. Reproduced from Ref. 76, with permission from John Wiley and Sons.

can be regulated on and off with azobenzene as demonstrated with N-(4-phenylazophenyl) maleimide modified calmodulin.[88] Incorporation into a cyclic poly-peptide can have drastic changes on shape recognition of neural NO synthase leading to reversible photocontrol of muscle activity. Beyermann and coworkers applied azobenzene to a peptide and in physiological conditions were able to cease muscle contraction reversibly in C2C12 myotubes and mouse single muscle fibers upon isomerization.[89]

Interactions between DNA and peptides are critical throughout biology and, as a result, photocontrollable peptides that regulate DNA are an exciting area of research. Zinc finger peptides with azobenzene conjugated at the N-terminus afforded a photocontrollable DNA-binding peptide which could block and free a DNA sequence for access by DNA-binding proteins.[90] Similar work in regulating the activity of the transcriptional activator MyoD affected the binding affinity showing that it could be increased by two orders of

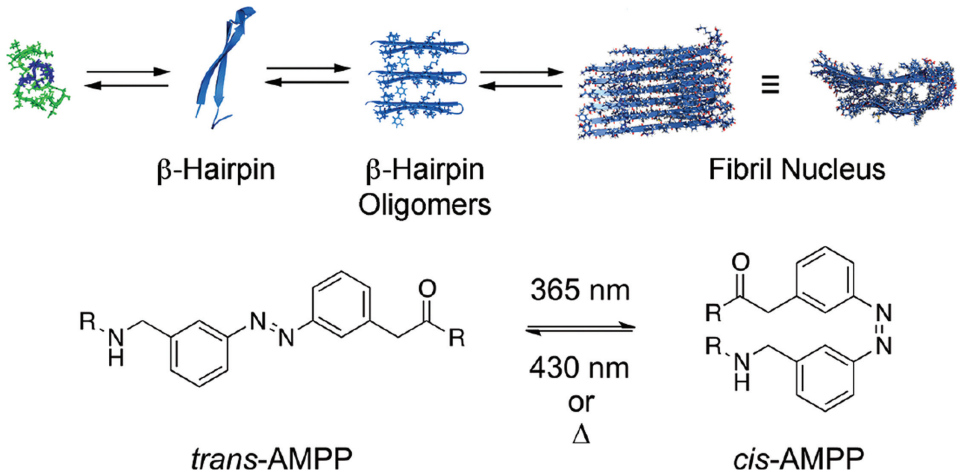

FIGURE 11 Proposed fibrillization mechanism when going from a *trans* azobenzene state to *cis* after UV irradiation in Amyloid-β fibrils. The azobenzene moiety is engineered into what is usually the β-hairpin structure in native Amyloid-β peptides. Reproduced from Ref. 80, with permission from American Chemical Society.

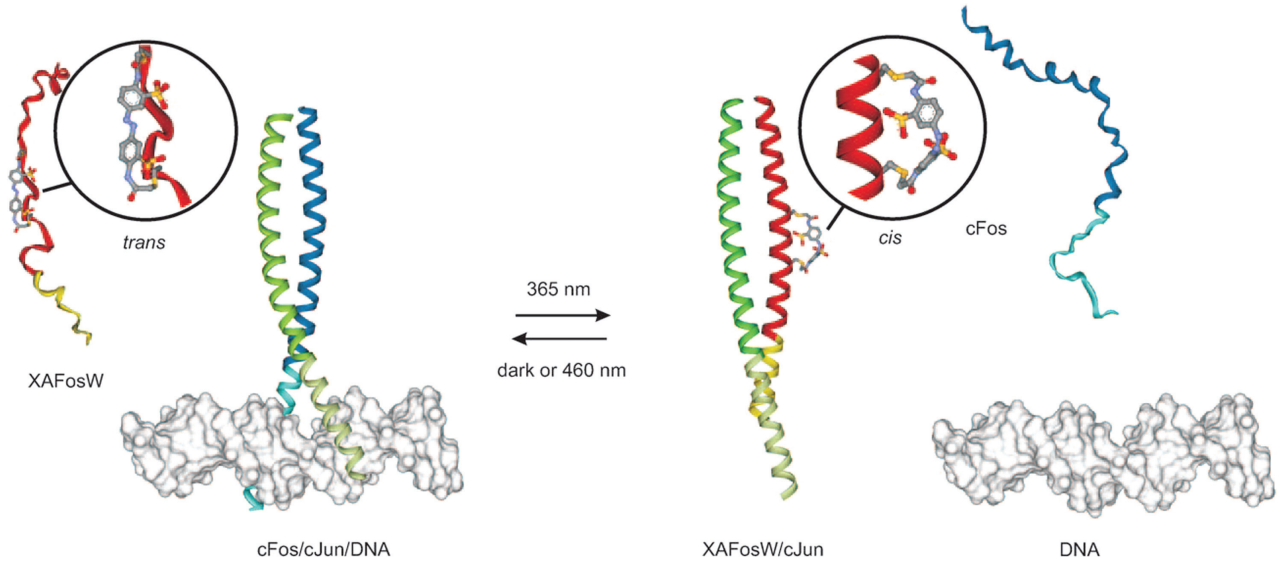

FIGURE 12 A coiled-coil protein inhibits DNA binding which can be removed by isomerization of an azobenzene containing peptide sequence which now pairs preferentially with a second peptide strand, liberating the DNA for replication. Reproduced from Ref. 94, with permission from John Wiley and Sons.

magnitude with the azobenzene in an *cis* irradiated state.[91] This work was followed up with photocontrol of DNA binding by HDH-3[92] and a designed GCN4-bZIP protein[93] as well as control of coiled-coil formation *in vivo* allowing for reversible inhibition of DNA binding.[94] All of which highlights the power of azobenzene in moderating macromolecular interactions in a biological context (Fig. 12).

CELLULAR CONTROL

Photo-control of cellular interactions with azobenzenes on surfaces is a new achievement which can be traced back to pioneering work by Horst Kessler who demonstrated that azobenzene-tethered arginine-glycine-aspartic acid (RGD) peptides grafted onto a silicon surface to achieve photo-reversible control of cell adhesion.[95] Most approaches so far have only been achieved with small molecules.[96] Using polymers, Letournel and coworkers used azobenzene photomobility under irradiation to pattern a biocompatible azo polymer substrate using light. They showed that PC12 cells could sense this nano-topography and were guided down these channels compared to a non-irradiated PLL control.[97] Recent work by our group allowed reversible increases in cell growth by 40% on an appropriately tailored substrate. Using polyelectrolyte multilayers, we were able to design baseline conditions onto which cells survived but did not thrive: light allowed for exposure of RGD ligands which enhanced cell growth via integrin mediated adhesion pathways.[98] These two studies serve as proof of concept that macromolecular azobenzene can act as a powerful substrate for photocontrolled cell culture.

SENSING IN BIOLOGY

Molecularly Imprinted Polymers

Molecularly imprinted polymers offer a tremendous opportunity for biomolecule detection in synthetic materials and are generated by polymerizing the material in the presence of a guest to template a binding pocket. Addition of a photoresponsive element in the binding pocket can affect substrate affinity based on irradiation. The first reporting of a molecularly photo-responsive polymer can be traced back to a 2003 communication by Matsuda and his co-workers who reported uptake and subsequent photo-release of dansylamide from a molecularly imprinted polymer (MIP).[99,100]

As these first reports, the field has enjoyed a marked uptick in productivity in the past couple of years. MIPs of a crosslinked di(ureidoethylenemethacrylate) azobenzene showed differing binding affinities for a methotrexate analogue, but in this case the higher affinity was in the *trans* state; which would mean the ground state would lead to drug release, showing promise as a controlled drug-release material.[101] This remains the only example to date where the *cis* state increases binding affinity for the substrate (Fig. 13).

Polymerization of 4-[(4-methacryloyloxy)phenylazo]benzoic acid in the presence of caffeine yielded a polymer which, upon irradiation to the *cis* state, photo-released 58.3% of bound caffeine and, when returned to *trans,* uptook 96.4% of its released cargo.[102] Paracetamol has also been studied with a similar azobenzene where the carboxylic acid was changed to a sulfonic acid crosslinked with various lengths of bismethacrylamides. Using an optimized *N,N′*-hexylenebismethacrylamide crosslinker, UV irradiation released 83.6% of the azo-receptor bound paracetamol and subsequent irradiation at 440 nm resulted in 94.1% of the released paracetamol to be rebound by the hydrogel again. This process was repeatable in water.[103] Even large molecules such as porphyrin in azo-containing MIPs can undergo reversible recognition.[104]

Melamine can be taken up, detected and released by a molecularly imprinted hydrogel. This azo-hydrogel shows

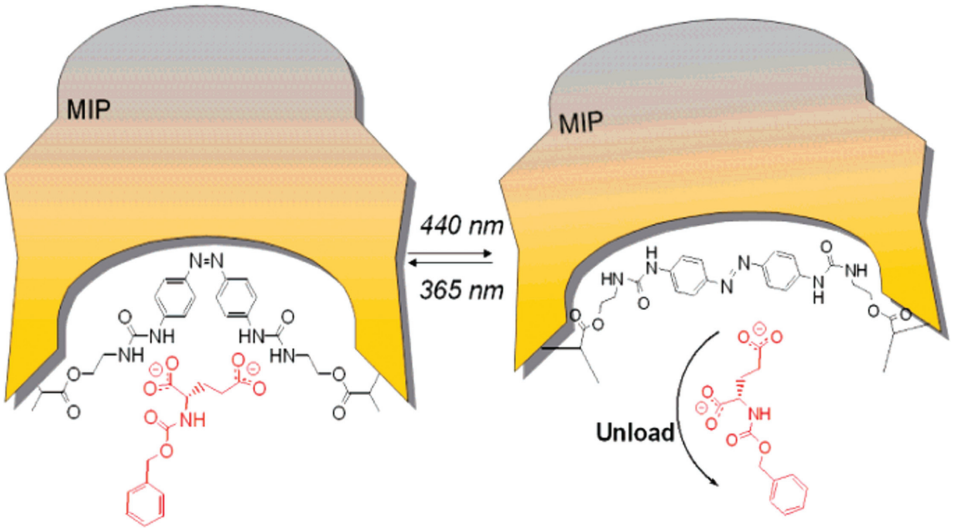

FIGURE 13 A ureido-based azobenzene photoresponsive MIP allows for photoreversible binding of glutamate-based hosts with the *cis* state favoring a bound substrate and the *trans* state expelling it. Reproduced from Ref. 101, with permission from American Chemical Society.

changes in the rate constant of *trans* to *cis* isomerization allowing for effective detection of melamine at ppm concentrations in aqueous or milk environment.[105] This methodology works well enough that the isomerization was monitored in milk as well as milk powder samples spiked with known concentrations of melamine, through which the concentration could be determined by analyzing the *trans-cis* isomerization time (Fig. 14).

Some of these reports demonstrate that specific interactions between the guest and host are not necessarily needed. A highly fluorinated substrate can be taken up and removed with light, as demonstrated with a nonafluoroazobenzene monomer copolymerized with trimethylolpropane trimethacrylate. The resulting MIP exploits the hydrophobic effect in the place of hydrogen bonds to capture 10-octafluorophenazine, showing that the strength of this interactions can add up to 14 kcal/mol and result in the selective binding and release of a substrate.[106]

Changing the monomers to generate silica based organic/inorganic hybrid polymer made with azobenzene and tetraethyl orthosilicate allowed for selective binding of 2,4-dichlorophenoxyacetic acid (2,4-D), where the binding affinity could be regulated by light. As well as modulating binding affinity, the concentration could be determined by monitoring the rate constants of isomerization.[107] Rational design of an MIP recognition polymer of (4-chloro-2-methylphenoxy)acetic acid (MCPA) was synthesized in a similar fashion.[108] Likewise, a sol-gel polymer network with a relatively simple 4,4′-dihydroxylazobenzene showed reversible affinity for and could distinguish between ibuprofen and some of its derivatives.[109]

The 3D architecture of these polymeric detectors can be varied while maintaining the photoresponsive effect. Emulsion polymerization can also afford azo-functionalized microspheres allowing for detection and photo-release of 2,4-D in an easily dispersible form.[110] These have then been functionalized with pNIPAM giving the particles an outer thermally responsive shell. These new microspheres are now dual stimuli-responsive but maintain their light-responsive binding affinity changes for 2,4-D in aqueous media.[111] These have been

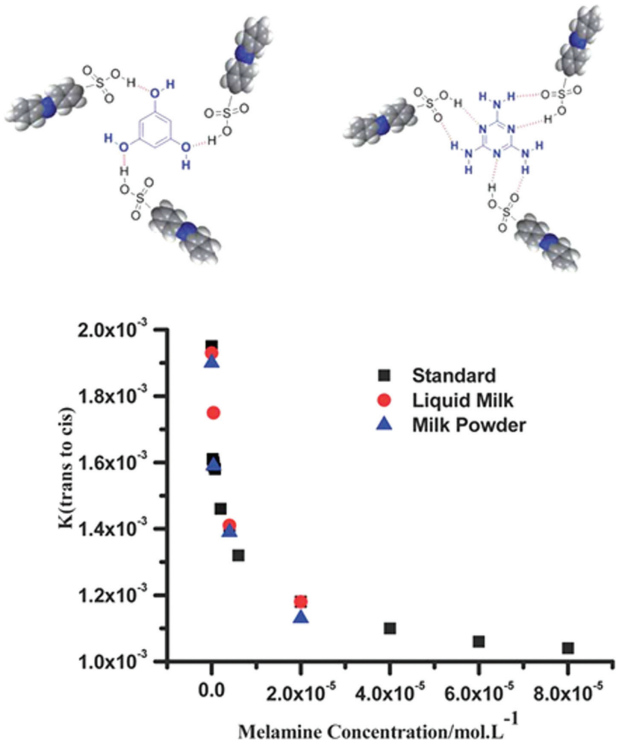

FIGURE 14 A MAPASA-based MIP template on phloroglucinol can reversibly bind melamine. The effect of this binding on the *trans-cis* isomerization rate can be used to determine the amount of melamine in solution. Reproduced from Ref. 105, with permission from Royal Society of Chemistry.

expanded and optimized to bind propranolol and atenolol suggesting promise as drug-delivery materials.[112] Perhaps more interesting for diagnostic applications is the creation of surface functionalized microfibers allowing for photo-reversible binding, release and detection of 4-hydroxybenzoic acid on microfibers of polyethersulfone, polysulfone and polyether ether ketone.[113] This last example is especially notable since the recognition element now is not a cavity, but as close as possible to a flat surface.

CONCLUSIONS AND OUTLOOK

Although the areas of application already covered by azo polymers are broad, there is no easy prediction on what the limits may be in future for stable, bio-compatible, photoswitchable azo polymers. The most interesting areas of future research may not even yet be envisaged, given that these materials are still stimulating the minds of creative scientists and engineers researchers world-wide with new and exciting applications 178 years after the first discovery of azobenzene.[114] The areas of application through this time progressed from tunable dyes and colorants, through to light control of liquid crystals, and electro-optic and photonic fast information switching and reversible storage.

Advancements in chromophore design and synthesis benefit most of the applications discussed, but specifically photocontrolled micelles may be improved greatly. With the tools of modern polymer synthesis, complex polymeric architectures are possible and are less often the limiting factor in the design of new materials. Design and synthesis of novel monomers and supramolecular assemblies becomes the new frontier in this research for stable and efficient nano-carriers.

One of the more mature areas discussed, the photo-control of biomacromolecules, is already being applied in living organisms as well as a tool to study complex diseases such as Alzheimers. With the development of new chromophores with more bathochromic absorptions, deeper tissue applications become possible opening avenues of study *in vivo* as well as potential therapeutic applications. Incorporation of azobenzene into biological transcription pathways opens the door to inclusion as a tool in the growing field of opto-genetics with a widely tunable and versatile chromophore able to regulate many biological interactions in *vivo.*

Polymers interfacing with cells is a growing area of interest; one notable example to follow is the work of Sebai and Tribet in developing azo copolymers that permeabilize cell membranes upon visible light irradiation. This relatively gentle method could be easily expanded to other living models but has already been shown to allow transport of a variety of cargos. These techniques could also be expanded to surface switching of biological function, using polymers as a substrate, which is a nascent area in this field. Although commercial applications may be more difficult to design due to the difficulty of delivering light to the implanted material, there is a lot of potential in exploring cell-surface adhesion and cellular behavior with photocontrolled surfaces bearing azobenzene. These materials fill a currently unfilled niche in an elegant and tunable way.

The final application discussed in this review, photoresponsive MIP, holds promise for future research. Although applications such as photo-released drug delivery may not match the performance of the photo-responsive micelles discussed herein, their capacity to be used as detectors has so far been underdeveloped. Many current biological sensing applications do not function using simple visible light detection techniques and the use of azobenzene offers a marked improvement on this limitation. Rational design of the detecting chromophores coupled with recent reports showing that the MIP cavity is not absolutely necessary for geometric confinement of the guest analyte suggest that *in vivo* detection capability is a very real possibility in the next few years.

One cannot help but acknowledge the large impact these materials are having in research and the growing complexity of the materials being developed. Although certain areas of this research have reached or are entering maturity, the research intensity now turns into deliverable biological or biomedical applications using the materials and techniques discussed. The currently emerging areas reviewed here are centered on the interface to, and reversible control over, biological systems. In future, the energy harvested and stored by the azo molecules may well gain interest in coming decades, as mimics of biological light harvesting polymers, such as retinal/rhodopsin that enables vision, or chlorophyll that enables photosynthesis—the ultimate biological photosystem.

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