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Sweetness and Light: Design and Applications of Photo-Responsive Glycoconjugates.

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Carbohydrate-protein binding is a supramolecular recognition process that underpins myriad biological events. However, the precise conformational and configurational requirements for biomolecular recognition are often poorly understood, since such phenomena often occur in a strongly spatiotemporal manner. Such challenges have prompted the development of photoswitchable glycoconjugates for modulating glycan structure and conformation and have emerged as promising investigational tools for probing carbohydrate-protein recognition processes and modulating binding affinities for whole bacterial cells. Reversible photoisomerisation, in particular that of azobenzene glycoconjugates, has also been exploited as a promising strategy for controlling supramolecular self-assembly and macroscopic properties, and has emerged as a promising strategy for the development of responsive carbohydrate-based materials. The following review will highlight the recent advances in the design and applications of photoswitchable glycoconjugates, paying particular attention to the application of light as a stimulus for modulating protein and cellular adhesion, amphiphilicity and supramolecular assembly of carbohydrate-based materials.

1.0 Introduction

The binding of a carbohydrate to its protein receptor is a supramolecular recognition process that modulates crucial biological functions.¹⁻³ The presentation of a carbohydrate ligand to its cognate receptor is dependent on the correct configurational and conformational arrangement of the ligand, which occurs in a strongly spatiotemporal manner.⁴ Such processes are often poorly understood and have prompted the development of stimuli-responsive glycoconjugates for reversibly modulating glycan conformation. Of the stimuli that can be employed, including changes in pH and temperature, light is of particular advantage as it is a ‘clean’ stimulus that requires only photons of a specific wavelength to elicit a molecular response.⁵⁻⁷ To this end, various strategies have been reported for controlling the properties of biomolecules using light, such as the irreversible cleavage of light-sensitive protective groups or ‘cages’.⁸⁻¹⁰ The reversible photoisomerisation of photochromic small molecules (or ‘photoswitches’) between at least two photostationary states offers an attractive alternative, as it provides clean,

spatiotemporal control over the molecular structure and properties, and can be cycled many times without loss of function.^{8, 11} To date, this strategy has been exploited as a tool for modulating supramolecular assembly processes, for probing biomolecular interactions and controlling macroscopic properties of functional materials.¹² Such light-responsive materials have attracted considerable interest as self-healing polymers, optical devices, hydrogels, surfactants, and liquid crystals.

Of the photoswitches available, azobenzene has been the most widely studied.^{13, 14} This is not coincidental, as its robust nature, simple synthesis and intense UV-visible absorption properties provide accessible photoswitching wavelengths, and has led to more than a century of study in these dye molecules.¹⁵ Azobenzene undergoes facile and reversible *trans-cis* isomerization upon near-UV irradiation, which occurs in high quantum yields and is accompanied by a significant change in molecular geometry (Fig. 1).^{11, 16} This results in a large variation in dipole moment: the thermodynamically less stable *cis* isomer exhibits a greater dipole moment ($\mu_{cis} = 3.1$

Debye) than the *trans* isomer ($\mu_{trans} = 0.5$ Debye) and accounts for the hydrophilic and hydrophobic nature of these isomers, respectively.⁸ In addition to the significant geometric changes affecting polarity and spatial orientation of attached groups, the readily accessible covalent modification of azobenzene ring(s) provides a means for fine-tuning electronic properties, and hence photochemistry, which can be tailored to provide a desired response.

Although receiving less attention than other classes of biomolecules, the covalent modification of carbohydrates with photoswitchable moieties has emerged as an extremely promising strategy for probing the topological requirements for carbohydrate-protein recognition, and as a mechanism for modulating the macroscopic properties of carbohydrate-based materials with precise spatiotemporal control. As such, these smart, sugar-substituted materials have attracted significant interest for their potential as responsive materials with diverse biomedical and technological applications.

This review will highlight the recent advances in this burgeoning area, paying particular attention to the design and application of azobenzene glycoconjugates as photoswitchable lectin inhibitors, amphiphiles and nanomaterials. This review does not aim to provide an exhaustive account of small molecule photoswitches, nor their application for the development of advanced materials, which are subjects of previous, excellent reviews.^{11-13, 17}

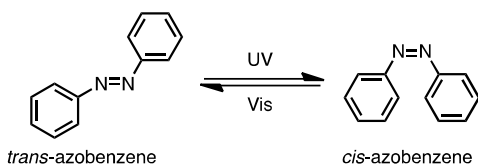


Figure 1. Reversible *trans-cis* photo-isomerization of azobenzene.

2.0 Applications of azobenzene glycoconjugates

2.1 Photoswitchable Lectin inhibitors

Carbohydrate-protein interactions are essential for myriad biological functions, yet individual binding interactions are typically weak in water ($K_a < 10^{-3} \text{ M}^{-1}$).¹⁸ In order to enhance binding avidity, multiple copies of carbohydrate ligands are often presented to the receptor for binding, a phenomenon known as the “multivalent effect” or “glycoside cluster effect.”¹⁹ Such interactions display fast binding kinetics and frequently occur at the fluid-membrane interface, suggesting that a degree of flexibility and adaptability is required for binding.²⁰ The correct presentation of a carbohydrate ligand to a cognate receptor is a critical aspect of molecular recognition and is often complicated by the three-dimensional shape and orientation of highly branched carbohydrate ligands. In order to improve our understanding of these supramolecular processes,

multivalent neoglycoconjugates have been actively pursued as biomolecular probes, antimicrobial drug candidates and biofilm inhibitors.²¹⁻²⁵ In this respect, photoswitchable glycoconjugates have emerged as particularly promising tools for reversibly modulating the spatial orientation of glycan ligands using light irradiation.²⁶⁻²⁹ In their pioneering work, Jayaraman and co-workers reported the synthesis and photoisomerisation properties of a suite of azobenzene cluster lactosides and mannosides, and measured binding affinities for the high-affinity lectin peanut agglutinin (PNA) and concanavalin A (ConA), respectively.^{28, 29} In addition to enhancing the binding avidity relative to monomeric analogues, UV irradiation resulted in a significant change in molecular geometry with a concomitant enhancement in binding affinity compared to the resting *trans* photostationary state.²⁸ Given the relatively short distances between each carbohydrate ligand, a glycoside cluster effect could not be detected. However, a much higher binding constant for the low affinity part of the interaction was observed ($K_{b1} = 5.1 \times 10^4 \text{ M}^{-1}$) for the bivalent lactoside analogue compared with the monovalent analogues, suggesting a co-operative effect on ligand binding, which was more pronounced for the *cis*-azobenzene isomer.

The photo-triggered change in glycan conformation has recently been applied for probing the effect of carbohydrate conformation in relation to bacterial adhesion. Lindhorst and co-workers recently reported the synthesis and photoisomerisation of an azobenzene mannoside and investigated the affinity of the *cis*- and *trans*-azobenzene isomers toward the high-affinity *E. coli* lectin, FimH.³⁰ In this example, FimH was shown to be insensitive to conformational changes in the glycan, with both *cis* and *trans* isomers displaying equal inhibitory potency, with similar IC_{50} values to the well-studied inhibitor, *p*-nitrophenyl α -D-mannoside.

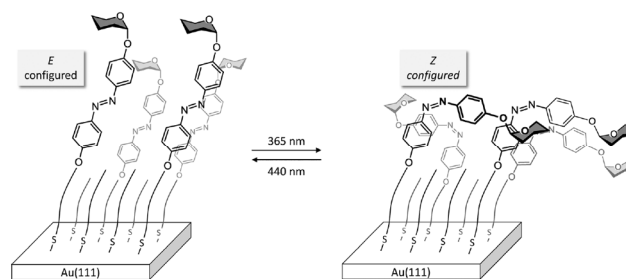


Figure 2. Fabrication of glyco-SAMs with photoswitchable azobenzene mannosides.³¹ Reproduced with permission from reference 31. Copyright 2013 Wiley-VCH.

In an effort to probe the spatial requirements for lectin binding at the fluid-surface interface, with a view of controlling cellular adhesion using light^{12, 32}, the Lindhorst group have recently reported the fabrication and photoswitching properties of a self-assembled monolayer (SAM) comprising Au(111) nanoparticles immobilized with azobenzene glycosides (Fig. 2).³¹ The *trans-cis* photoisomerisation of solid-supported azobenzene glycosides was studied using infrared reflection

absorption spectroscopy (IRRAS). Upon photoirradiation of SAMs at 365 nm, a characteristic reduction in intensity of the prominent band at *ca.* 1250 cm^{-1} was observed, which reflected the $\text{C}_{\text{aryl}}\text{-O}_{\text{Man}}$ stretching frequency. This spectral change was attributed to a change in molecular geometry following *trans-cis* photoisomerisation. Conversely, irradiation of the glycoSAM at 440 cm^{-1} resulted in restoration of the absorption band at 1250 cm^{-1} , thus indicating successful reversion to the *trans* photostationary state. This result demonstrates the feasibility of photoswitching azobenzene glycosides on solid surfaces, and paves the way for the future development of light-controlled glycan arrays for high-throughput diagnostic and screening applications.

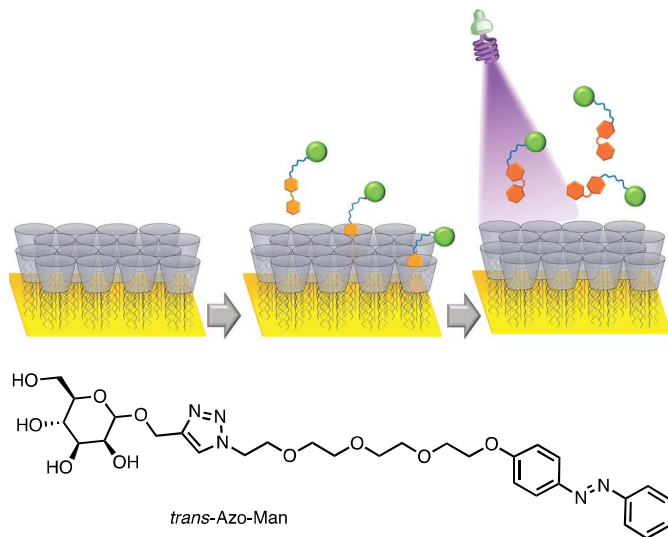


Figure 3. Photo-controlled functionalisation of a β -CD fabricated SAM.³³ Reproduced with permission from reference 33. Copyright 2014 Royal Chemical Society.

Cyclodextrins (CDs) are naturally occurring carbohydrate macrocycles and supramolecular hosts that have found long-term industrial use as excipients and carriers, owing to their ability to form stable host-guest inclusion complexes with hydrophobic guests in water. Both α -CD and β -CD have long been known to form stable host-guest inclusion complexes with azobenzene guests in aqueous systems, and interestingly, CDs show greater thermodynamic preference for *trans*-azobenzene over *cis*-azobenzene. Recently, CDs have been employed as supramolecular hosts for reversibly binding photoswitchable guests, thus providing a mechanism for attenuating lectin binding and microbial adhesion using light irradiation.

In their recent communication, Jonkheijm and co-workers reported the photo-controlled immobilisation of proteins and bacteria onto β -CD modified surfaces.³³ Supramolecular self-assembled monolayers (SAMs) were prepared by functionalising gold surfaces with amphiphilic β -CD, which served as supramolecular anchor points for a photoswitchable *trans*-azobenzene mannoside (*trans*-Azo-Man) through host-guest inclusion complexation (Fig. 3). Photoisomerisation of

the supramolecular SAM provided a trigger for releasing the glycoconjugate from the inclusion complex, thus releasing it from the surface. The photocontrolled functionalisation of SAMs with *trans*-AzoMan was next employed to attenuate binding affinity to the high affinity lectin ConA. Binding affinities to the functionalised surfaces were quantified using quartz crystal microbalance (QCM) measurements ($K_a = 5.8 \times 10^3 \text{ M}^{-1}$) and were shown to be in good agreement with values obtained in solution using isothermal calorimetry (ITC). The photo-induced assembly of whole *E. coli* cells onto β -CD functionalised SAMs was also performed and visualised using fluorescent microscopy.

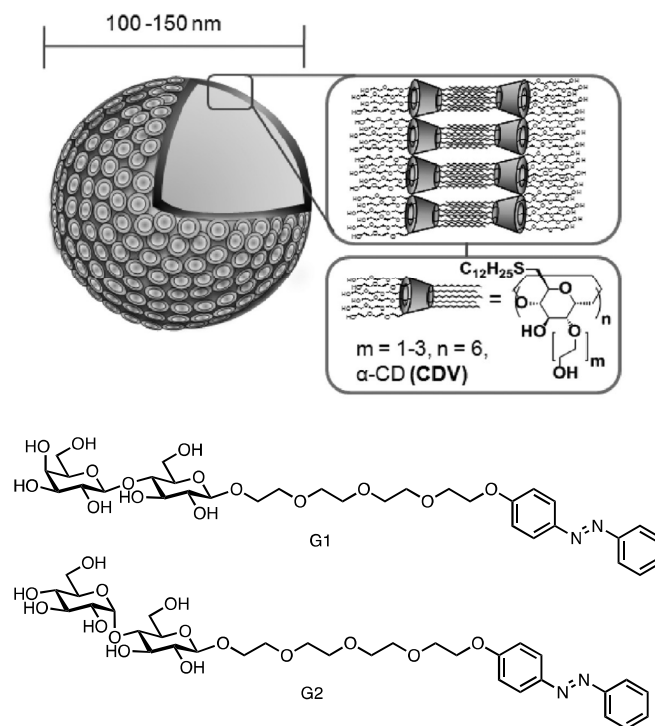


Figure 4. Photocontrolled lectin binding of supramolecular ternary complex.³⁵ Reproduced with permission from reference 35. Copyright 2012 American Chemical Society.

The conformational prerequisites for lectin binding have also been investigated using photoswitchable systems employing multivalent scaffolds in bulk solution. Hartmann and co-workers have recently reported the synthesis of monodisperse, photoswitchable di- and tri-valent glycoooligomers as multivalent tools for studying lectin binding in solution.³⁴ Glycoooligomers were comprised of monosaccharide ligands appended to a polymer, with up to two azobenzene units embedded within the backbone. Photoswitchable glycoooligomers were capable of undergoing reversible photoisomerisation to modulate the intramolecular distance between carbohydrate ligands, and hence the binding affinity to lectins with multiple binding domains. The relative binding affinities of the *cis*- and *trans*-azobenzene configured oligomers varied considerably ($\text{IC}_{50[\text{AZO-Gal}(1,3)\text{-}3 \text{ E}]} = 5.7 \text{ }\mu\text{M}$,

$IC_{50}[\text{AZO-Gal}(1,3)\text{-}3 \text{ PSS}] = 9.4 \mu\text{M}$, $IC_{50}[\text{AZO-Gal}(1,3,5)\text{-}5 \text{ E}] = 3.4 \mu\text{M}$, $IC_{50}[\text{AZO-Gal}(1,3,5)\text{-}5 \text{ PSS}] = 4.1 \mu\text{M}$), demonstrating the capability of light for controlling the conformational dynamics of multivalent glycoconjugates for probing carbohydrate-protein recognition.

Protein immobilisation using azobenzene glycosides has also been reported using photoswitchable supramolecular ternary complexes. Recently, Jan Ravoo and colleagues reported the phototriggered release of lectins from self-assembled ternary complexes (Fig. 4).³⁵ These ternary complexes consisted of azobenzene lactosides non-covalently bound through host-guest inclusion complexation to multiple cyclodextrin (CD) hosts presented on a self-assembled bilayer vesicle. In this manner, the multivalent presentation of an azobenzene lactoside (**G1**) and maltoside (**G2**) facilitated immobilization of the respective lectin protein, thus promoting self-assembly of the respective photoswitchable multilamellar complex. The binding affinity of supramolecular complexes to the lectin was quantified using isothermal titration calorimetry (ITC) ($K_{aG1} = 8.42 \times 10^3 \text{ M}^{-1}$, $K_{aG2} = 8.43 \times 10^3 \text{ M}^{-1}$). UV irradiation resulted in a *trans-cis* photoisomerisation of the azobenzene linker, thus triggering its release from the CD host with concomitant disaggregation of the supramolecular complex, releasing the protein. Furthermore, the photochemical control of lectin binding was shown to be fully reversible, as demonstrated by UV/Vis spectroscopy, dynamic light scattering and transmission electron microscopy techniques.

2.2 Photoswitchable hydrogelators

Glycolipids are ubiquitous natural products comprising a polar carbohydrate head group and hydrophobic tail group. They mediate a number of key biological processes, including membrane formation, immune regulation and microbial infection.³⁶ Amphiphilic carbohydrates have found widespread industrial application, owing to their favourable biocompatibility and physicochemical properties. Carbohydrates are powerful polar head groups as they are abundant natural products endowed with rich stereochemical diversity and functionality, providing access to a virtually unlimited number of designer colloids with tailored properties. The supramolecular assembly and self-organization of carbohydrate amphiphiles in water gives rise to diverse functional materials, such as hydrogels, surfactants and liquid crystals.³⁷ The coupling of carbohydrate head groups to hydrophobic azobenzene tail groups is a recent but exciting development in the field, since it gives rise to photo-responsive systems for reversibly modulating key colloidal properties such as aggregation, viscosity and phase stability. The azobenzene moiety delivers a strong driving force for aggregation in water through both hydrophobic attractions and π - π stacking interactions, providing also a mechanism for controlling amphiphilicity via *trans-cis* isomerisation.

Carbohydrate hydrogelators are low molecular weight amphiphiles that undergo hierarchical self-assembly through non-covalent interactions in water, such as hydrogen bonding,

π - π stacking and van der Waals interactions. Their spontaneous self-assembly gives rise to complex, entangled networks of nanofibers, sheets, bundles, *etc.*, with the hydrophilic carbohydrate exposed at the surface. Since the early work of Reinhoudt and Shinkai, who described the first ‘super’ hydrogelator incorporating an azobenzene tail and sugar head groups, there has been considerable interest in the application of light for controlling gel-sol transitions of photoswitchable carbohydrate hydrogelators.³⁸ As the gelation ability of such materials is influenced by photo-induced *trans-cis* photoisomerisation, this allows for precise control over gelation, molecular aggregation and morphological properties.^{39, 40}

Kitoaka and colleagues have recently reported the self-assembly of a panel of hydrogelators based on a variable disaccharide lactone head group appended to azobenzene and a glycine spacer.¹⁸ The carbohydrate hydrogelators were shown to spontaneously self-assemble in water to provide entangled helical nanofibres, which were subsequently utilized as a biomimetic scaffold for lectin binding and cell adhesion studies. Partial photoisomerisation resulted in a gel-sol transition, resulting in ‘chopping’ of long fibres to give short nanofibres. The photochemical control of gel-sol and sol-gel transitions has also recently been demonstrated to facilitate selective removal of aromatic solvents from aqueous emulsions, making these photo-addressable molecules attractive biomaterials for environmental remediation.³⁹

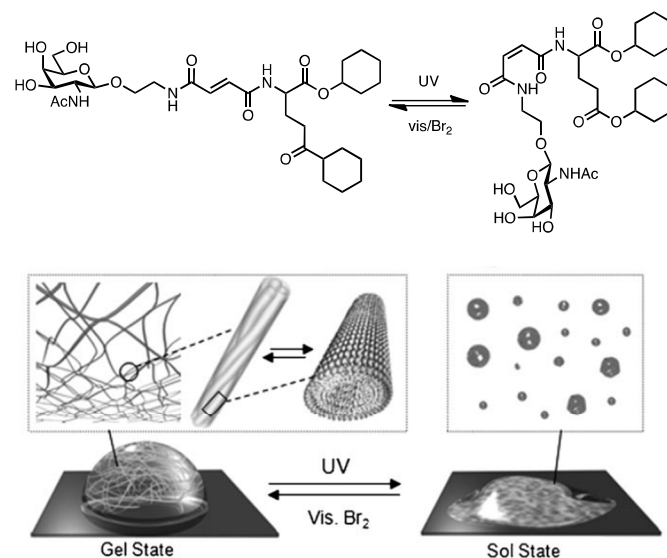


Figure 5. Reversible gel-sol transition of hydrogel induced by UV/Vis irradiation.⁴¹ Reproduced with permission from reference 41. Copyright 2008 Wiley-VCH.

By adopting a similar strategy, Hamachi and colleagues reported the synthesis of a library of fumaric diamide glycosides as photoresponsive hydrogelators (Fig. 5).⁴¹ The glycosides were comprised of variable carbohydrate head groups linked to a hydrophobic alkyl tail, which incorporated a photochromic fumaric diamide unit. Hierarchical self-assembly

of *trans* configured monomers gave rise to hydrogels consisting of extended fibrous networks, which was shown to be strongly dependent on the carbohydrate head group, linker and tail group.⁴¹ Irradiation of hydrogels with UV light resulted in partial isomerisation from the *trans* fumaric diamide to the *cis* maleic diamide form (>50%). The resulting bent molecular geometry caused significant disruption to the hydrogen-bonded network, thus triggering disassociation to result in a gel-sol transition. The gel-sol transition was reversed by irradiation with visible light in the presence of bromine. Subsequent studies focused on the use of an *N*-acetylglucosamine-based hydrogel (Fig. 5) possessing a bis-cyclohexyl tail, as it was shown to provide transparent and shear-stable hydrogels.

2.3 Photoswitchable Surfactants

Carbohydrate-based surfactants of synthetic and natural origin are widely studied amphiphilic materials that combine a hydrophilic carbohydrate head group and a hydrophobic/lipid tail group. Such materials have been shown to give rise to diverse self-assemblies and colloidal structures in aqueous solvents, such as micelles, vesicles, foams and emulsions, *etc.*^{42, 43} Carbohydrates of various size, hydrophilicity and stereochemical configuration provide virtually unlimited options for fine tuning surface and aggregation properties to derive colloidal systems with tailored geometries, properties and applications.

Len and co-workers have recently reported a photoswitchable carbohydrate-based surfactant comprising a sodium glucuronate head group coupled to a butylazobenzene tail.⁴⁴ In this study, the authors demonstrated the phototriggered self-assembly of micelles to facilitate compartmentalization and solubilisation of hydrophobic organic molecules in water. Surfactant micelles were shown to facilitate acylation of hydrophobic amines in water, with similar efficiency to the well-studied surfactant, sodium dodecyl sulfate.

Despite their promising potential, the development and application of photoswitchable, water-soluble carbohydrate-based surfactants has received less attention compared to other classes of carbohydrate amphiphile, with only one example appearing in the literature to date.⁴¹ This may result from the challenges involved in their synthesis or deployment within colloidal systems, as attempting to couple the azobenzene chromophore to conventional tail-group chemistries can result in water-insoluble materials that find limited application.

In order to facilitate the first structure-function investigation of water-soluble, photoresponsive carbohydrate-based surfactants, we recently reported the parallel synthesis of a library of dual pH- and photoswitchable carbohydrate-based surfactants and examined their surface and aggregation properties (Fig. 6).^{45, 46} Using a modular synthetic strategy, carbohydrates of variable size and hydrophilicity, including a pH-sensitive glucuronic acid head group, were introduced onto a butylazobenzene tail group, and the resulting surfactants characterized using small-angled neutron scattering (SANS), tensiometry and UV-visible spectroscopy. A profound effect of

carbohydrate choice on surfactancy, aggregation number and morphology was revealed, with micellar structures ranging from near-spherical to extended cylindrical geometries.

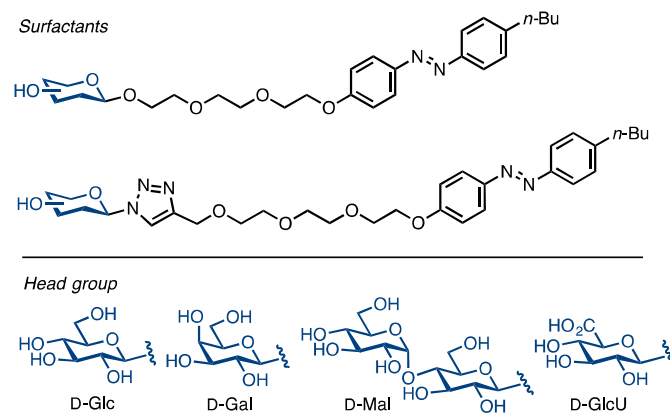


Figure 6. Photo-switchable carbohydrate-based surfactants.⁴⁵ Adapted with permission from reference 45. Copyright 2014 Wiley-VCH.

In recent work, our group has studied the structural evolution of micelle size and composition by time resolved SANS (TR-SANS).⁴⁶ In this study, we identified three distinct micelle behaviours at, near and well above the CMC. Photoisomerisation to the *cis* form at surfactant concentrations just above the CMC resulted in complete disaggregation; however, when the concentration was increased slightly, a rod→sphere transition was observed. Finally, when the concentration was well above the CMC, photoisomerisation resulted in a pronounced thinning of cylindrical or ‘rod-like’ micelles. As rod-like micelles are well known for increasing solution viscosity and providing solution structure, these observations have clear implications for the design of photorheological fluids for drilling and energy applications.

2.4 Liquid crystals

A vast number of glycolipids in nature exhibit thermotropic and lyotropic liquid crystal (LC) phases, owing to their softness and self-organizing properties.^{47, 48} Such properties are believed to confer structural fluidity and adaptability to biological membranes and directly influence their biological properties. Carbohydrates are attractive mesogens for forming LC phases, owing to their high natural abundance, structural diversity, low toxicity and potential to form chiral nanostructures. Carbohydrate LCs have therefore emerged as promising tools for correlating mesophase behavior with biological activity, and for their wide-reaching technological applications.

Azobenzene has been widely used as a mesogen and a photoswitchable chromophore for controlling LC phase behaviour.⁴⁹⁻⁵¹ The addition of the azobenzene chromophore serves to enrich LC phase behaviour, as the π -stacking characteristics have been shown to facilitate a range of smectic, nematic, lamellar and conic LC phases.⁵² A number of groups

have reported the synthesis and properties of amphiphilic LCs consisting of carbohydrates conjugated to azobenzene (Fig. 7a-c).⁵³⁻⁵⁵ In their early work, Goodby and co-workers reported the synthesis and thermotropic LC properties of a panel of amphiphilic azobenzene glycosides (Fig. 7a),⁵³ prepared with various carbohydrates that acted as hydrophilic, microphase segregating and biocompatible head groups. The thermotropic LC properties of the amphiphilic glycosides were investigated using polarised light microscopy, differential scanning calorimetry (DSC) and X-ray diffraction. From this study, achiral smectic lamellar LC-phases were observed, with the only exception being the *D-gluco* configured analogue, which curiously exhibited an achiral lamellar LC phase.

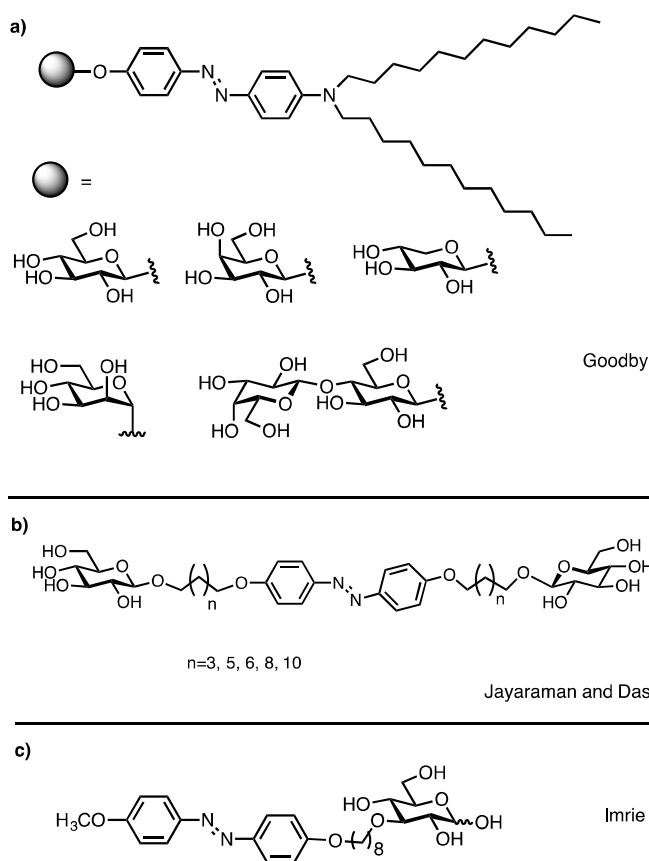


Figure 7. Carbohydrate LCs containing azobenzene mesogens.⁵³⁻⁵⁵ Adapted with permission from references 53, 54, and 55. Copyright 2003 American Chemical Society, 2012 Elsevier and 2005 Wiley-VCH.

Although carbohydrates possess an abundance of chiral centres, carbohydrate LCs incorporating azobenzene mesogens generally display achiral smectic and lamellar phases and do not normally exhibit chiral macroscopic phases. This is believed to arise from the strong hydrogen bonding between carbohydrate head groups, resulting in layered, head-to-head orientations. Although the disruption of such networks can be achieved by site-selective functionalization of the head group, this can result in a pronounced loss of amphiphilicity and LC

properties. Jayaraman and Das have reported the chiral LC phase of a series of azobenzene bolaamphiphile comprising unmodified *D*-glucose head groups linked to an azobenzene core via alkyl spacers of variable length (Fig. 7c).⁵⁵ The LC properties of these amphiphiles were studied using polarised light microscopy, DSC and small-angle X-ray scattering. From this study, a number of bolaamphiphiles were discovered that displayed chiral smectic LC phases, a behaviour that was dependent on the positioning of the azobenzene and length of the spacer group.

Recently, Imrie and co-workers reported the synthesis and LC properties of an amphiphilic azobenzene glycoconjugate (Fig. c).⁵⁴ The amphiphile exhibited glassy behaviour and an interdigitated smectic A phase. The hydrogen bonding strength during both glass and smectic A-isotropic transitions was determined using variable temperature infrared spectroscopy and revealed no marked change from either the glass or smectic A-isotropic phase transition. This suggests that the smectic A-isotropic phase transition was dominated by van der Waals interactions, with hydrogen bonding persisting into the isotropic phase.

It is clear that the combination of azobenzene and carbohydrate mesogens allows for rich and varied LC properties. However, despite the demonstrated potential for controlling LC phase behaviour using light,⁴⁹ the application of this strategy for controlling LC phase behaviour of carbohydrate LCs is yet to be realised, thus presenting exciting opportunities for the future development of photoresponsive, biocompatible colloids.

2.5 Photoswitchable cyclodextrins

The application of an external stimulus for controlling the conformation and dynamics of supramolecular and molecular systems has also gained considerable interest in nanoscience and technology. Light has emerged as particularly useful stimulus for triggering and controlling conformation, which can be further coupled to changes in the physicochemical properties of the material. In the previous section, we described the recent applications of photoswitchable CD host-guest complexes for attenuating lectin binding and microbial adhesion. Reversible *trans-cis* photoisomerisation of intermolecular azobenzene-CD inclusion complexes has also been widely exploited for modulating binding affinity in order to control supramolecular assembly processes.⁵⁶⁻⁵⁹ Such intermolecular host-guest strategies have shown great potential for the development of functional nanodevices and materials, including amphiphilic, tuneable delivery vectors and molecular machines. Such approaches are the subjects of some recent reviews.^{60, 61, 62}

On the other hand, intramolecular host-guest complexes comprising photoswitchable guests covalently attached to the CD ring have emerged as promising materials for accessing light-controlled supramolecular assemblies, including threaded rotaxanes and pseudorotaxanes. Early applications of photoswitchable CDs focused on their use as photoswitchable sensors for low molecular weight organic molecules in water.⁶³

⁶⁴ However, the burgeoning fields of nanoscience and technology have stimulated renewed interest in the application of light for controlling supramolecular assembly through intramolecular host-guest inclusion complexation^{58,65} and this has paved the way for the development of light-responsive assemblies with promising applications as molecular scale machines^{66, 67}, switches⁶⁵, electronics⁶⁸ and gated ion channels.⁶⁹

Work from the Harada group has provided several noteworthy examples where supramolecular assembly is controlled using photoswitchable CDs, in particular azobenzene and stilbene-conjugated CDs using light.^{56, 70, 71} In an early example, a dual thermo- and light-responsive azobenzene CD complex was reported, which comprised a CD host covalently appended to a hydrocinnamoyl linker via a polyethylene glycol spacer that was terminated with an azobenzene host (Fig. 8).⁷⁰ The host-guest complexation of both *cis*- and *trans*-azobenzene isomers was measured using 2D ROESY NMR spectroscopy. The *trans*-configured complex was shown to form a threaded, self-inclusion complex at 60°C, whereby the CD cavity alternately binds *trans*-azobenzene and the hydrocinnamoyl linker, respectively. This inclusion complexation behaviour of the *trans*-configured complex was also shown to be temperature and concentration dependent. Competing intermolecular host-guest inclusion complexation was observed at higher concentrations, giving rise to threaded rotaxane formation. Such behaviour was not mirrored for the corresponding *cis*-photostationary state, which provided only threaded self-inclusion complexes. More recently, the authors reported the application of *trans-cis* photoisomerisation of CD-stilbene complexes for controlling conformation and supramolecular assembly.⁷² Such molecules were shown to undergo broad changes in conformation and host-guest binding on photoisomerisation, leading to a diverse range of threaded and non-threaded supramolecular assemblies.



Figure 8. Proposed conformational changes of 6-Az-PEG600-HyClO- β -CD in aqueous solution using temperature and light.⁷⁰ Reproduced with permission from reference 70. Copyright 2007 American Chemical Society.

Recently, Tian and co-workers have reported the synthesis and light-driven supramolecular assembly of a symmetric [1]rotaxane (Fig. 9).⁶³ The switchable supramolecular system

was comprised on an azobenzene-modified CD dimer bridged by a Co(III) Schiff-base complex. The conformation of the metal coordinated complex was determined before and after UV irradiation. ¹H NMR and induced circular dichroism (ICD) spectroscopy were employed to investigate the movement of the ‘stretch-contraction’ supramolecular system. This study demonstrated the potential for combining host-guest complexation and metal ion coordination for modulating the geometry of light-responsive supramolecular assemblies.

Gin and co-workers have reported a light-controlled gated ion channel employing intramolecular host-guest inclusion complexation (Fig. 10).⁶⁹ To this end, azobenzene was linked to the secondary β -CD face, while multiple hydrophobic alkyl tail groups were attached to primary face to mimic the lipid membrane portion of the channel. UV irradiation of the tethered *trans*-azobenzene resulted in *trans-cis* isomerisation, thus triggering conversion of the threaded self-inclusion complex to give a dissociated structure. In this manner, the self-inclusion complex incorporating the *trans*-azobenzene guest represented the ‘closed’ gate and the dissociated *cis*-azobenzene complex represented the ‘open’ gate. Using UV irradiation to trigger this response resulted in an increase in transport of anions with a concomitant reduction in transport of cations, as determined by a pH-dependent fluorescent assay.

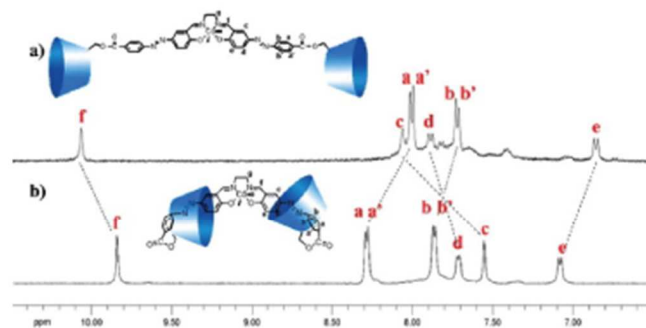


Figure 9. A supramolecular ‘stretch-contraction’ system based on photo-controlled [1]rotaxane formation.⁶³ Reproduced from reference 63. Copyright 2011 Royal Society of Chemistry.

There is considerable interest in the development of molecular scale devices capable of performing information processing, owing to their potential applications as nano-computers and electronics. The development of sophisticated molecular scale devices capable of performing Boolean logic gates (AND, OR, NAND, *etc.*) has long been the primary goal in the field, with many such systems now being realized. To date, such supramolecular systems employ stimulus-responsive rotaxanes and pseudorotaxanes that respond to an external stimulus by changing molecular conformation, producing a recognizable output signal such as fluorescence, UV-visible absorption, circular dichroism, *etc.* Tian and co-workers have recently reported the INHIBIT logic operations of a photoswitchable β -CD pseudo[1]rotaxane using room temperature phosphorescence (RTP) and induced circular dichroism (ICD) output addresses.⁶⁸ To this end, the threaded

pseudo[1]rotaxane was obtained by conjugating an azobenzene chromophore to β -CD in water, forming a threaded self-inclusion complex. UV irradiation of the threaded pseudo[1]rotaxane resulted in *trans-cis* isomerisation of the azobenzene guest to give rise to a de-threaded complex. The reversible threading and de-threading process was addressed using ICD and RTP as outputs, which were coupled to various input stimuli including UV irradiation, temperature, oxygen purging and competing host-guest complexation with phosphor bromonaphthalene (α -BrNp), to derive a simple molecular device capable of INHIBIT molecular logic functions.

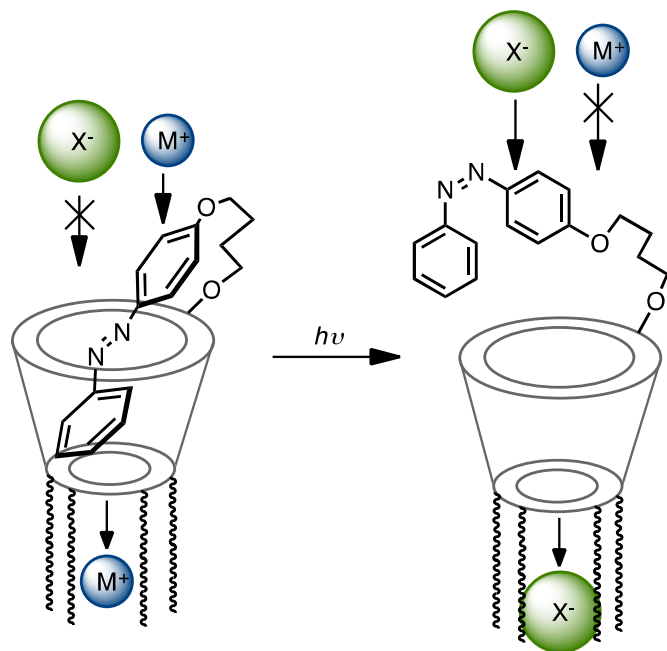


Figure 10. A light-controlled gated ion channel as reported by Gin and co-workers.⁶⁹ Adapted with permission from reference 69. Copyright 2008 American Chemical Society.

3.0 Conclusions and Future directions.

Carbohydrate-protein interactions mediate essential cellular communication events, yet the conformational and configurational basis of these supramolecular interactions is often poorly understood. Such challenges have prompted the development of photoswitchable glycoconjugates for attenuating molecular conformation and configuration in a spatially and temporally resolved manner. Such light-controlled tools have enabled investigations into the structural dynamics of carbohydrate-protein binding with good spatiotemporal control. To date, this strategy has been applied for the photo-controlled immobilisation of lectins and whole cells in solution, or at the liquid-surface interface.

Looking to the future, it is anticipated that this strategy will find broad application in high-throughput screening of carbohydrate-protein interactions, particularly in microarray format, for diagnostic and drug development purposes.

Reversible photoisomerisation has also emerged as an extremely promising strategy for controlling supramolecular assembly and attenuating the macroscopic properties of biocompatible materials, including responsive sugar surfactants and hydrogels. The photocontrolled host-guest inclusion complexation of azobenzene and stilbene guests covalently bound to CD hosts is a promising route to responsive supramolecular assemblies with exciting technological applications, for example as molecular scale machines, computers and electronics. The combination of typically hydrophobic photoisomerisable moieties with hydrophilic carbohydrates offers a vast range of potential amphiphiles with rich, complex and stimulus-responsive adsorption and aggregation properties. An underexplored avenue in this area is the application of photoisomerisation for controlling the phase behaviour of carbohydrate LCs, and this presents opportunities for the development of new, light-responsive, carbohydrate-based LCs and biocompatible colloids.

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Notes and references

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