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Tesi di Laurea

Photoswitchable systems as supramolecular templates: enzyme-mediated and light-controlled selective synthesis of cyclodextrins with azobenzene templates

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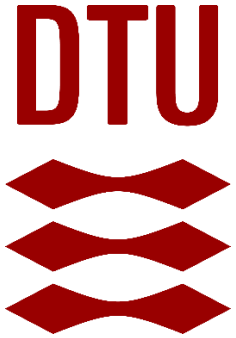

This thesis work has been designed, developed, and realized under the guidance of Prof. Sophie R. Beeren as Supervisor and Dott. Dennis Larsen as a Tutor at the laboratories of the Department of Chemistry of the Technical University of Denmark (DTU), until the lockdown due to COVID-19.

Riassunto in italiano

Nelle strutture supramolecolari, le interazioni *host-guest* sono uno dei principali fenomeni di riconoscimento che spesso coinvolgono macrocicli organici (*host*) in combinazione con una vasta gamma di molecole ospite (*guest*), in cui l'associazione tra le specie è tipicamente guidata da interazioni deboli di tipo attrattivo o dall'effetto idrofobico. Uno dei complessi *host-guest* più studiati ed utilizzati in applicazioni industriali, commerciali e farmaceutiche è il sistema Azobenzene/Ciclodestrine (Azo/CDs). La funzionalità azobenzenica può essere isomerizzata selettivamente dalla configurazione *trans* a quella *cis* mediante radiazioni luminose dell'UV e viceversa, da *cis* a *trans,* attraverso luce visibile o calore. L'isomerizzazione inoltre, determina il cambiamento delle proprietà molecolari quali la geometria e la polarità. Utilizzando le proprietà delle molecole azobenzeniche alle diverse lunghezze d'onda per la creazione di intermedi supramolecolari *host-guest* in sistemi dinamici per la sintesi enzimatica di ciclodestrine, è possibile manipolare e controllare tali reazioni al fine di ottenere prodotti specifici quali α-CD, β-CD o γ-CD.

Lo scopo di questo lavoro di tesi è stato quello di sviluppare nuove molecole azobenzeniche aventi differenti proprietà steriche e di solubilità in ambiente acquoso attraverso la modifica dei sostituenti del sistema aromatico dell'azocomposto. Utilizzando una reazione enzimatica per generare una miscela dinamica di α-1,4-glucani lineari e ciclici ed impiegando l'azobenzene come templante, è possibile ottenere e studiare le derivanti librerie combinatoriali enzima-mediate (DCL) per definire l'effetto degli azo-templanti nella sintesi ciclodestrinica, a seguito delle diverse condizioni di irraggiamento.

A causa dell'emergenza Covid-19, il presente lavoro è stato interrotto nella fase sintetica delle molecole azobenzeniche e non è stato possibile completare gli obiettivi precedentemente riportati. Per completare la tesi è stato quindi condotto uno studio approfondito su sistemi *host-guest* foto-modulabili costituiti da ciclodestrine ed azobenzene, analizzando i meccanismi di isomerizzazione delle varie specie e le loro applicazioni recenti e future in numerosi campi scientifici quali la medicina, la farmaceutica, l'ingegneria, la sensoristica e la catalisi.

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Abstract

In supramolecular assemblies, host-guest interactions are one of the major recognitions phenomena that often involves host macrocycles in combination with a wide range of guest molecules where the association is typically driven by a multitude of weak attractive interactions or by the hydrophobic effect. One of the most studied host-guest complexes used in a variety of industrial, commercial, and pharmaceutical applications is azobenzene/cyclodextrin (Azo/CD). The azobenzene moiety can be photoswitched selectively from the *trans* to *cis* configuration by UV light, and reversely from *cis* to *trans* by visible light, and the isomerization is accompanied by the change in its properties such as geometry and polarity. Exploiting the properties of the azobenzene molecules under different wavelengths for the creation of host-guest supramolecular intermediates in a dynamic system for the enzymatic synthesis of cyclodextrins, it is possible to manipulate and control the enzymatic reaction to direct the formation of specific products (α-CD, β-CD or γ-CD).

The aim of this study was to develop new azobenzene molecules with different steric properties and solubility in aqueous solution through the modification of the substituents present on the azobenzene ring system. Using an enzyme-mediated reaction to generate a mixture of interconverting linear and cyclic α-1,4-glucans and employing an azobenzene photoswitch as a template, it will be possible to obtain enzyme-mediated combinatorial libraries (DCL) of cyclodextrins to define the effect of the azobenzene templates under different conditions of irradiation.

Due to the Covid-19 emergency, the present work has been interrupted in the synthetic phase of the azobenzene molecules and it has not been possible to complete the objectives previously reported. To complete the thesis, an in-depth study was therefore carried out on host-guest photoswitchable systems consisting of cyclodextrins and azobenzene, analysing the isomerization mechanisms of the various species and their recent and future applications in numerous fields such as medicine, pharmaceuticals, engineering, sensory, and catalysis. This material will be the basis for a review manuscript that soon will be submitted to a scientific journal for publication.

1. Introduction

1.1. Supramolecular chemistry

Supramolecular chemistry shows astonishing examples in the living biological systems where supramolecular self-assemblies play critical roles in a variety of biological processes. In Nature, enzymes, antibodies, and many other biological systems are able to selectively bind specific substances through non-covalent intermolecular interactions. Supramolecular coordination chemistry that involves metal centers offered in the recent years many examples of mimicking the cage-like nature of an enzyme active site, whereby it is possible to construct structures of various geometries, which can accommodate molecules with specific shapes and sizes.

1.1.1. From the discovery to the development of supramolecular chemistry

In 1967, Charles J. Pedersen, during the study of the effects of bi- and multidentate phenolic ligands on the catalytic properties of the vanadyl group (VO), obtained as a reaction by-product an 18-membered ring molecule: the dibenzo-18-crown-6 (Figure 1.a). This new class of macrocyclic polyethers, called crown ethers due to their shape, are capable of binding alkali metal ions with high binding affinities. The crown ether motif has been widely used due to its high specificity to guest molecules, and thus potential for implementation in several applications. Pedersen's discovery has allowed the birth and the development of a new field of chemistry, later called "supramolecular chemistry".

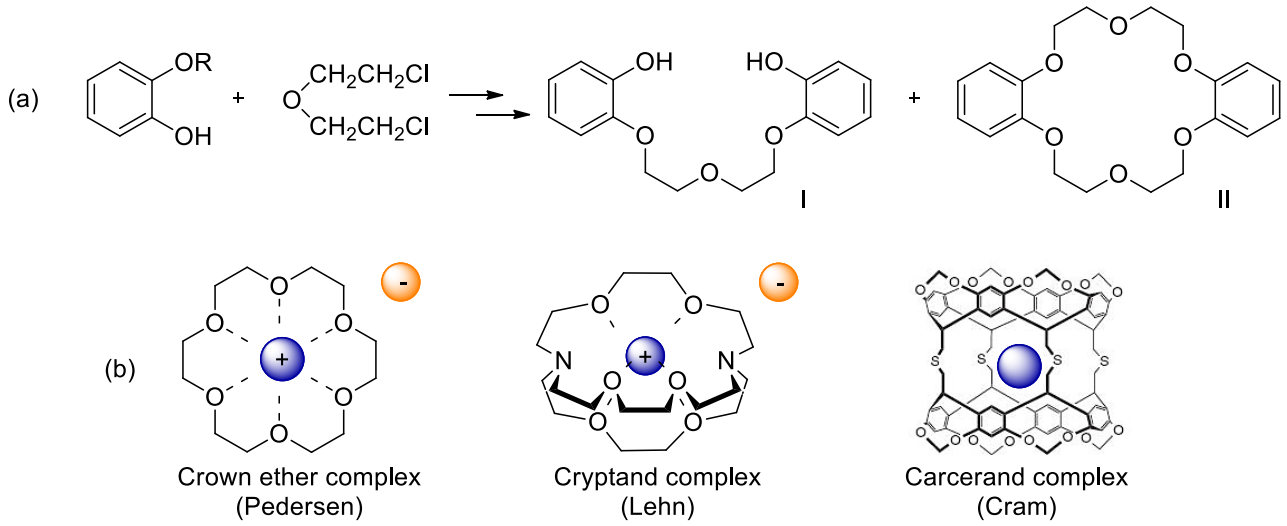


Figure 1 (a) Schematic representation of the reaction conducted by Pedersen which allowed the discovery of the crown ethers (II); (b) Chemical structures of crown ethers, cryptands, and carcerands.

Between 1969 and 1974, Jean-Marie Lehn designed and prepared systems that could incorporate new types of atoms and molecules with different sizes and shapes and the molecules he developed, called

cryptands, 'recognized' specific alkali metals based on the precise spatial organization of the cage's structure [1]. Lehn coined the term "supramolecular chemistry", referred to ordered molecular aggregates held together by non-covalent intermolecular forces. Originally, he proposed the concept of a ''molecule beyond the molecule'' and this was defined in terms of the non-covalent interaction between a ''*host*'' and a ''*guest*'' molecule. These reversible non-covalent interactions can span from electrostatic effects, to hydrogen bonds, metal coordination, aromatic stacking, hydrophobic and van der Waals forces [2]. After the discovery of crown ethers and cryptands by Pedersen and Lehn, Donald Cram succeeded in building molecules with the ability to recognize specific molecules (Figure 1.b). He synthesized molecules that took this chemistry into three dimensions, creating an array of differently shaped structures that could interact selectively with other chemicals due of their complementary three-dimensional architectures. He invented a whole new language with words such as spherands, cavitands and carcerands to describe these new systems. The term *host-guest chemistry* in fact, was first introduced by Donald Cram in 1976 to describe the chemistry of complexes formed by two or more molecules or ions that are held together in certain structures through non-covalent interactions. Host is defined as an organic molecule or ion whose binding sites converge in the complex, while guest is defined as any molecule or ion whose binding sites diverge in the complex. These systems are based on specific molecular recognition of a guest by its corresponding host molecule and the bonds are based on the transient association of a molecule containing a cavity (a cavitand) with suitable molecular guests [3]. In these definitions, hosts are synthetic counterparts of the receptor sites of biological chemistry, and guests, the counterparts of substrates, inhibitors, or co-factors. Lehn, Pedersen, and Cram discoveries demonstrated the opportunities of recognition chemistry to the development of synthetic receptor molecules for cationic, anionic, or neutral guests by organic, inorganic, or biological hosts by means of various weak interactions. Nowadays this branch of chemistry is an extensively evolving interdisciplinary field of research focused on the association of individual molecules giving rise to more complex chemical systems formed through intermolecular interactions, with applications in material science, sensor developments, drug delivery systems, smart compounds and many others.

1.1.2. The dynamic concept of supramolecular chemistry

Supramolecular chemistry has become a perfect bridge to combine biology and chemistry and the great interest that this field of chemistry has found in the recent years arises from the idea of building up complex molecular structures from simple building blocks, which often have rather different properties with respect to those of the precursors. With the revolutionary work of Lehn, Cram, and

Pedersen, supramolecular chemistry has introduced the concept of reversibility in the recognition phenomena where weak intermolecular forces such as hydrogen bonding, halogen bonding, van der Waals forces, hydrophobic, hydrophilic and π-π interactions, offer unlimited possibilities for the construction of original supramolecular assemblies [4]. The capacity for spontaneous and reversible binding between molecular species is the driving force in the development of structured, dynamic and self-healing frameworks by the exchange and selection of molecular components for the generation of a given supramolecular entity from a diverse collection of building blocks to obtain the ‘‘best’’ structure (under a thermodynamic or kinetic point of view)[2]. In fact, supramolecular chemistry is intrinsically a dynamic chemistry in view of the lability of the non-covalent interactions connecting the molecular components of a supramolecular entity. The resulting ability of supramolecular species to reversibly dissociate and associate, deconstruct and reconstruct, allows them to rearrange their molecular components. These characteristics can be exploited by comprehending how molecular recognition and self-assembly operate within biological systems and applying this knowledge towards targeted organic synthesis [5]. Host-guest interactions are one of the major types of interactions involved in supramolecular assembly. Indeed, there is an equilibrium between the unbound state, in which host and guest are separate from each other, and the bound state, in which there is a structurally defined host–guest complex (Figure 2).

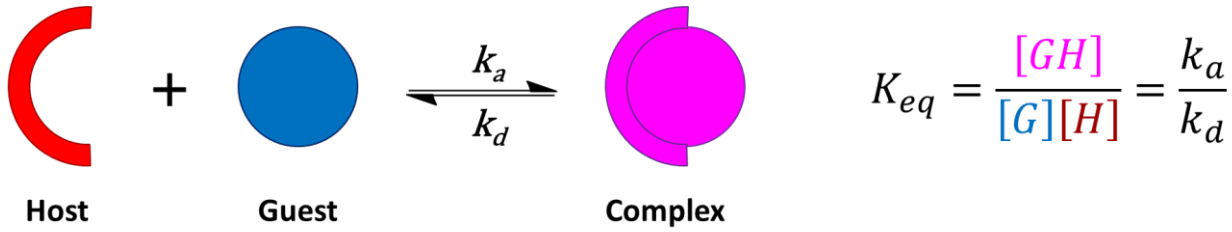


Figure 2 Schematic representation of the *host-guest* mechanism and its association constant.

From a dynamic and thermodynamic point of view, it is possible to define an equilibrium binding affinity constant (abbreviated as K_{eq}), which is sometimes written as an association constant (K_a), or dissociation constant (K_d, which is K_{eq}^{-1}) [4]. It is a quantity defined by a ratio between the concentrations of the formed complex and the individual substituents at equilibrium, as shown in the Figure 2 and below:

$$K_{eq} = \frac{[GH]}{[G][H]} = \frac{k_a}{k_d} \sim \frac{k_{on}}{k_{off}}$$

where *[H]* is the molar concentration of the host molecule, *[G]* is the molar concentration of the guest molecule, and *[HG]* is the molar concentration of the host–guest complex. The equilibrium binding constant is also proportional to the rates of formation and rupture of the complex. For most host-guest interactions, the rate of formation (k_{on}) is approximately diffusion limited, and the lifetime over which

the complex exists is defined by its rate of separation (k_{off}) [6]. This quantity measuring the affinity of an interaction, defines binding systems that exist as a direct function of concentration. Larger values of K_{eq} indicate a higher binding affinity between host and guest.

1.1.3. Supramolecular chemistry from a geometrical point of view

Molecular recognition is a process in which molecules can utilize complementary functionalities to interact in a well-defined and precise manner via intermolecular forces and this concept has been well-established for biological systems and in the synthesis of the biomacromolecules such as DNA, RNA, and proteins. In the 1890 Emil Fisher described for the first time the idea of a "*lock-and -key*" principle to describe the interactions between the enzyme and its substrate prior its chemical transformation [7]. The simplest systems use a cavity that can complex molecules, leading to assembly through non-covalent interactions bringing the molecule in close proximity to the catalytic group (Figure 3).

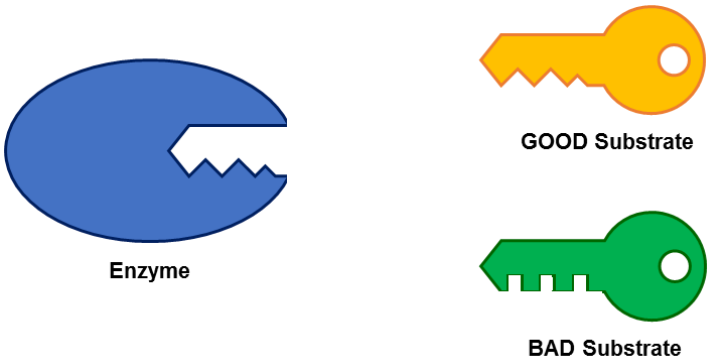


Figure 3 Schematic representation of the "lock-and -key" mechanism.

Understanding the mechanisms that Nature selected over the ages, it is possible to develop a strategy for the mimicking of enzyme activity and that could be applied into different fields of chemistry. The design and synthesis of enzyme-mimicking host compounds remains one of the most challenging and stimulating problems of organic chemistry. The first examples of simple enzyme mimics were described by Cram and collaborators based on chiral host thiols (thiobinapthyl crown ether, Figure 4) to catalyze transacylation reactions (thiolysis). This catalyst uses the ability of a crown ether to bind the ammonium cation of the substrate and subsequently employs the nearby thiol function to cleave the ester.

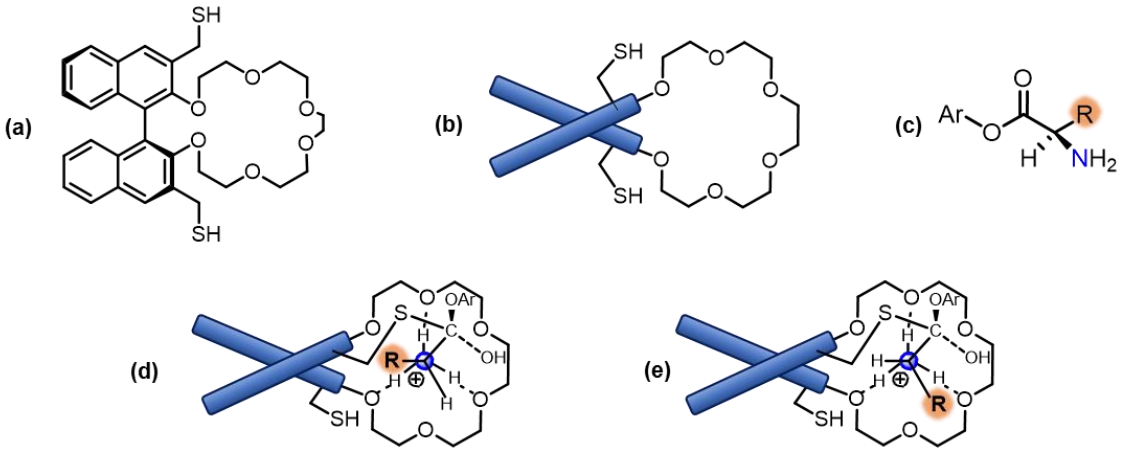


Figure 4 Chemical structure of the molecules used by Cram: (a) The the enzyme-mimic chiral host thiol;(b) Cross section of the chiral host thiol; (c) The substrates of the transacylation reactions (R: H, Cy or Ph); Steric relationship between the less stable host-guest complex (d, due to steric hindrance of R) and the more stable host-guest intermediate (e).

The lock-and-key principle is still a valuable starting point for the understanding and the design of synthetic supramolecular complexes. The enzyme active site shows shape complementarity with the substrate and create interactions with the substrate leading to its chemical activation. In most cases an induced adjustment of the host always takes place after cavity-substrate binding, with the lock-and-key principle that still describe at first sight the necessary geometric fit between the host and guest molecules [8].

1.1.4. Supramolecular chemistry from an energy point of view

The formation of supramolecular complexes do not depend only on an optimal geometric fit between host and guest: phenomena such as the desolvation of the molecules before the complex formation can contribute to a gain of free energy and modify the kind of interaction occurring between the species [9]. Host-guest binding in fact, can also be understood according to the enthalpy and entropy associated with the complex process formation. In a liquid phase it is possible for solvent molecules to access the cavities of hydrophobic host molecules by interacting with their external surfaces or with the inner walls (Figure 5). Since the cavities are confined spaces, the energetic states of encapsulated solvent molecules are different from those in the bulk solvent. The confined space inside the inner cavities of host molecules can be filled with solvent molecules in the absence or presence of guest molecules. In general, the entrapment of water molecules inside hydrophobic cavities can be achieved through a combination of both favourable and unfavourable entropic changes [10]. The release of water from the cavities of host molecules and their replacement with guest molecules is considered a crucial factor in host-guest interactions. When the host-guest recognition occurs in

solution, where all the species are solvated, the final complexes present less surface for solvent contacts with respect to separated hosts plus guests. Therefore, complexes are less solvated than their non-complexed partners and, in many cases, host-guest complexes are more rigid than the corresponding hosts and guests separated in solution [8]. For the hydrophobic theory, the orientation of water molecules near a hydrophobic solute is more ordered and the entropy of the system is reduced. For this reason, apolar solutes tend to be lumped together to minimize the number of water molecules around them. The area of the apolar interface is much larger in the unfolded state and after hydrophobic association, the entropy is increased [11]. If host-guest complementarity is well-aligned, complexation of host and guest is enthalpically favoured. However, the formation of an assembled complex from two dispersed molecules is inherently unfavourable in terms of their entropy. When the host-guest system is formed, a strong enthalpic interaction between multiple binding centres (large enthalpy advantage), normally leads to greater limitations on degrees of freedom and thus to greater losses of entropy[4]. The balance of these favorable and unfavourable thermodynamic drivers gives rise to an understanding of the enthalpy-entropy compensation that occurs in host-guest systems and allows the creation and formation of supramolecular structures having various and different characteristics applicable in many fields of chemistry.

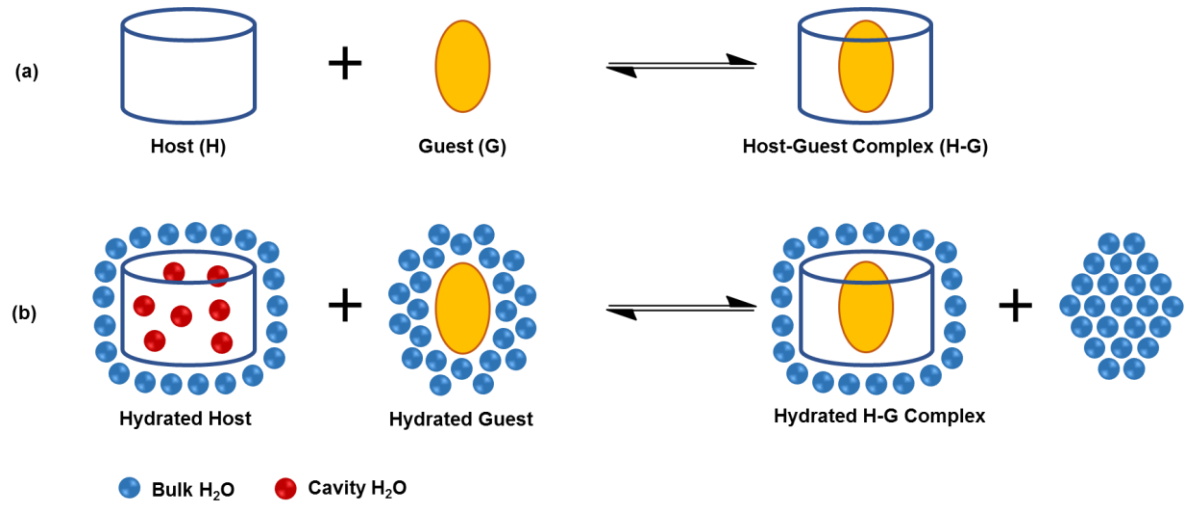


Figure 5 Schematic representation of the host-guest mechanism: theoretical mechanism (a) and in a solvated environment (b).

1.2. Cavitands

In Nature, complex structure such as enzymes, play the important role of providing concave surfaces to which are attached convergent functional groups that can bind substrates and catalyze specific reactions. Taking inspiration from these systems, it is possible to designed supramolecular systems that performs numerous functions similarly to biological molecules. The chemistry of molecular recognition started by Cram[12], Pedersen[13], and Lehn[14], has been developed through the discovery of macrocycles such as crown ethers, calixarenens and curcurbiturils and over the years, various supramolecular architectures have been constructed. Macrocyclic compounds in fact, due to their structure, molecular weight, and size, play a major role as host molecules or building blocks for the construction of supramolecular systems. They usually contain cavities, clefts, and pockets with appropriate size and shape that provide the framework for hosting substrate species by multiple non-covalent interactions. In 1983, Cram proposed the name of cavitand for "synthetic organic compounds that contain enforced cavities large enough to accommodate simple molecules or ions" [15]. These molecules are designed to have rigid cavities of dimensions at least equal to those of ions, atoms, or molecules that can accommodate. These macrocycles can bind various inorganic, organic or biological molecules in both solution and the solid state [16]. The family of cavitands includes both naturally derived (e.g. cyclodextrin) and synthetic (e.g., crown ethers, cucurbit[n]urils, calix[n]arenes, and pillar[n]arenes) macrocycles (Figure 6) and their characteristics can be influenced by the chemical properties of the building blocks used for their synthesis and by their structural shape. The host's inner cavity offers an isolated microenvironment wherein encapsulated guests are exposed to a reduced number of interactions compared to the bulk solution (bound guests may interact only with the host or other co-encapsulated guests). In the bulk, guests display a high number of interactions with solvent molecules or other guests and, as a consequence of the interaction with the host, their properties are usually modified [17]. The close contact in space between the host and guest, strengthen the supramolecular interactions owing to the short distance between the species. Furthermore, macrocyclic supramolecular systems are usually consisting of more than one functional group that can simultaneously interact with the substrates: this property should improve the characteristics of building blocks through an integrated effect. The weak intermolecular forces between hosts and guests, such as hydrogen bonding, halogen bonding, van der Waals forces, hydrophobic, hydrophilic and π-π interactions, offers unlimited possibilities for the construction of original supramolecular assemblies and this dynamic process can contribute to the multistep regulation of various molecular functions such as the catalysis of chemical reactions, transport of materials, control of reaction pathways, and cooperative and responsive phenomena [18]. As a result of these factors, macrocyclic supramolecular systems can exhibit a wide range of properties and application due to the delicate

balance of intermolecular interactions and owing to the supramolecular nature of these interactions, the formation of a host-guest system is always dynamic and reversible: this mean that there is the possibility of controlling the assembly/disassembly process by external stimuli such pH, light, and solvents.

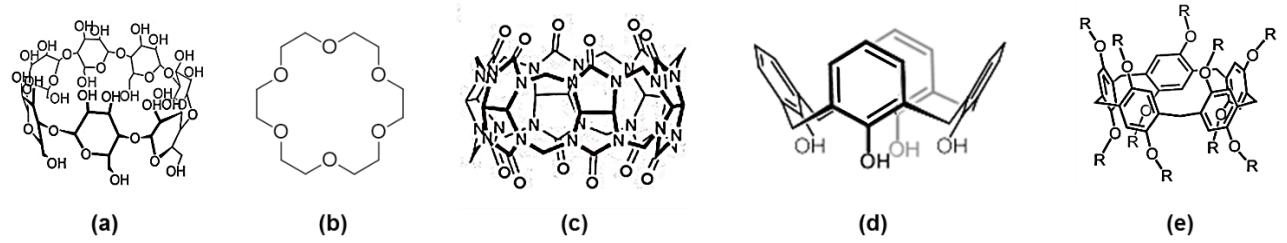


Figure 6 Chemical structures of the cyclodextrins (a), crown ethers (b), cucurbit[n]urils (c), calix[n]arenes, and pillar[n]arenes (d).

1.2.1. Natural cavitands: the Cyclodextrins

Cyclodextrins (CDs) constitute an important building block for supramolecular systems. This kind of molecule in aqueous solution can form inclusion complexes with hydrophobic guest and this ability has been exploited in different fields of chemistry, biology and material science with examples in drug delivery, nano-structures, supramolecular polymers, self-healing materials, amphiphiles or bioactive materials [19]. CDs have been for many years promising macrocyclic hosts since they are inexpensive, water-soluble natural products, non-toxic, easily functionalized and commercially available.

1.2.2. History and synthesis of CDs

The first reference to CD was reported by Villiers in 1891, who isolated a crystalline substance following bacterial digestion of cellulose [20]. Experimental results indicated that the substance was a dextrin and Villiers named it "*cellulosine*". After Viller's discoveries, Franz Schardinger described two crystalline compounds α-dextrin and β-dextrin which he isolated from a bacterial digest of potato starch. Schardinger identified β-dextrin as Villiers' "*cellulosine*" [21]. From 1935 to 1955 Freudenberg, Cramer and their co-workers identified the chemical structure of CDs, their general physicochemical properties, and their abilities to form complexes. Enzymic degradation of starch generally results in the production of glucose, maltose and a long series of linear or branched chain malto-oligomers, due to the hydrolytic process arising from the splitting of the glycosidic linkage. The malto-oligomers obtained are amorphous and hygroscopic substances present in products like beer and bread, known as dextrins [22]. If the starch is degraded by a specific enzyme, the primary product of the chain splitting undergoes an intramolecular reaction and a cyclic product is formed,

characterized by an 1, 4 intramolecular link (transglycosylation reaction): these molecules are known as CDs (Figure 7). The enzymatic product is generally a mixture of CDs including α-, β-, and γ-CDs consisting of six, seven, or eight glucose units, respectively [21].

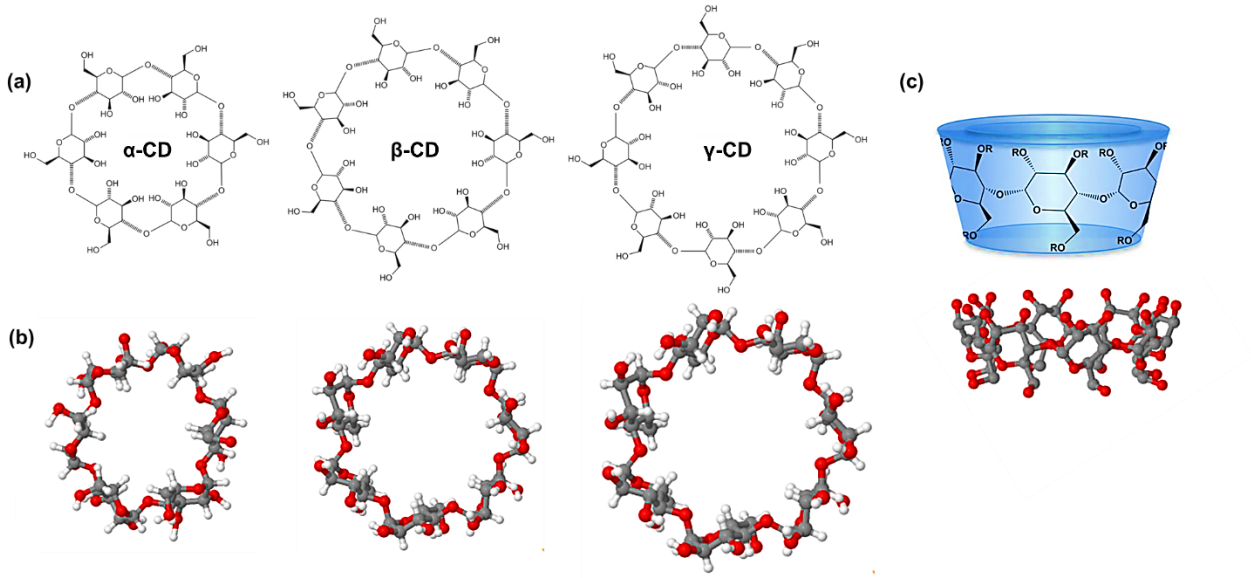
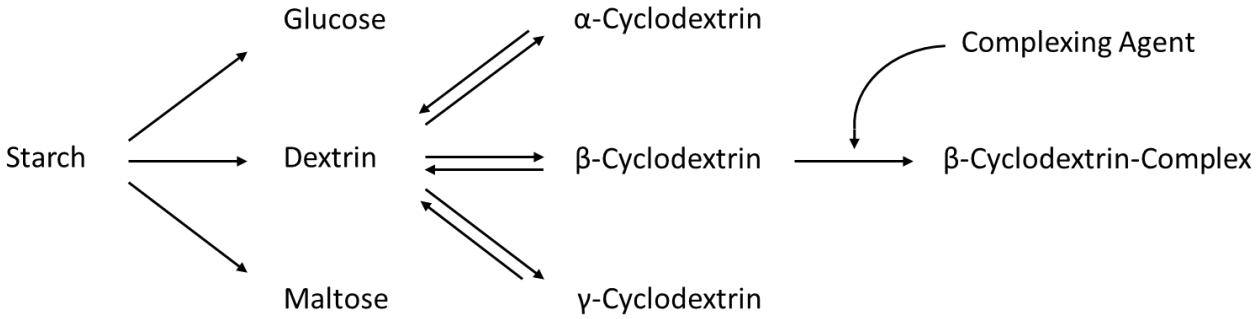


Figure 7 Chemical structures of native cyclodextrins (a); ball-and-stick molecular structures of native cyclodextrins; (c) cross section of CDs.

These three categories of macrocycles are classified as "native cyclodextrins", but in the years following the first Ville's discovery, molecules with hundreds of glucose monomer units were found (LR-CDs, large ring CDs)[23]. Cyclodextrin glucanotransferase (CGTase, 1,4-α-D-glucan, EC 2.4.1.19) is a unique enzyme capable of converting starch and related substrates into CDs. These enzymes recognize the 6, 7 or 8 glucopyranose units from the non-reducing terminus of amylose chain and alters the adjacent α-1,4-linkage, transferring it to the C-4 position to produce α-CD, β-CD or γ-CD. The cyclodextrin glucosyl transferase is produced by many microorganisms, like *Bacillus macrons*, *Klebsiella oxytoca*, *Bacillus circulans*, and *Alkalophylic bacillus* [24]. These kinds of enzyme are in general unspecific with respect to the ring size of the cyclodextrins: CGTase catalyzes both reversible inter- and intramolecular glycosyl transfers of α-1,4-glucans and irreversible hydrolysis of linear and cyclic α -1,4-glucans. Using a specific precipitation agent, it is possible to obtain and isolate from the reaction mixture with high purity the α-CD, β-CD or γ-CDs [25] (Scheme 1). Since the various cyclodextrins are interconverted by CGTases, the final product is the one that is continuously removed from the reaction mixture by selective precipitation [26]. Over the years, numerous types of precipitating agents have been exploited: Cramer initially used a tetrachloroethylene-tetrachloroethane mixture, followed by the addition of p-cymene to isolate native CDs. Subsequently, cyclohexane, fluorobenzene and anthracene were tested to isolate α-CD, β-CD and γ-CDs in high yields [27]. Nowadays, the use of genetic engineering has made possible the

isolation of different types of CGTases that are both more active and more specific towards production of predominantly α-CD, β-CD or γ-CDs and the specific precipitation agents are n-octanol, toluene, and cyclohexadec-8-en-1-one, respectively.



Scheme 1 Schematic representation of the synthesis process of CDs.

1.2.3. Cyclodextrins and their capacity to form inclusion complexes

In recent years CD complexes have proven to be a functional tool for the generation of complex macromolecular architectures. Structurally, cyclodextrin is a truncated cone-like structure due to the chair conformation of the glucopyranose units. The larger rim of the cone is usually called “head”, while the smaller one “tail”. This shape is characterized by a hydrophobic internal cavity and an external hydrophilic surface. The central cavity is constituted by a structure deriving from the glucose residues (carbons and ethereal-oxygens), which makes it much less hydrophilic than the external environment (Figure 8). On the other hand, the exterior portion of the cavity is characterized by hydroxyl groups, giving to the cyclodextrin a hydrophilic character [28]. As a consequence, cyclodextrins are moderately soluble in polar solvents and their cavity can accept non-polar guests to form inclusion complexes, thus leading to increased apparent solubilities for the guest. From a thermodynamic point of view, the cyclodextrin cavity has a slightly nonpolar nature which is not favoured by the presence of water molecules around in terms of energy (polar-nonpolar interaction) and these are spontaneously replaced by suitable guest molecules, less polar than water molecules.

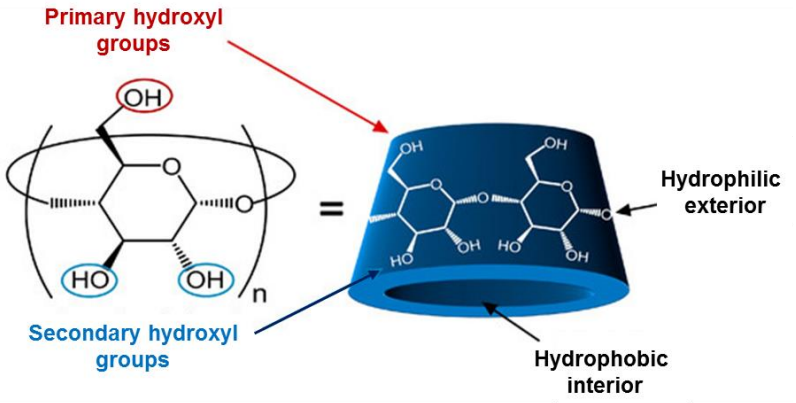


Figure 8 Chemical structure of cyclodextrin.

The formation of the host-guest complex in CDs is a dynamic equilibrium driven by the replacement of the high enthalpy water molecules from the inner cavity. The ability of a cyclodextrin to form an inclusion complex with a guest molecule is driven by two key factors. The first is related to steric requirements and depends on the relative size of the cyclodextrin with respect to the size of the guest molecule: if the guest has the wrong size, it will not fit properly into the cyclodextrin cavity (lock-key mechanism). The second factor is the thermodynamic interactions between the different components of the system: the repulsive forces between the water molecules and the apolar cavity of cyclodextrin and between the water molecules in the bulk and the apolar guest, are the two main components that regulate the inclusion mechanism of the host molecules. Water molecules are displaced by more hydrophobic guest molecules present in the solution to attain an apolar-apolar association and decrease of cyclodextrin ring strain resulting in a more stable lower energy state equilibrium. As a result, an equilibrium among dissociated and associated molecular species is established that can be expressed by the complex stability constant (K_a) [24]. The association of CDs and guest molecules is governed by the thermodynamic equilibrium as presented in Figure 9. During the formation of the inclusion complex, non-covalent bonds are broken or formed.

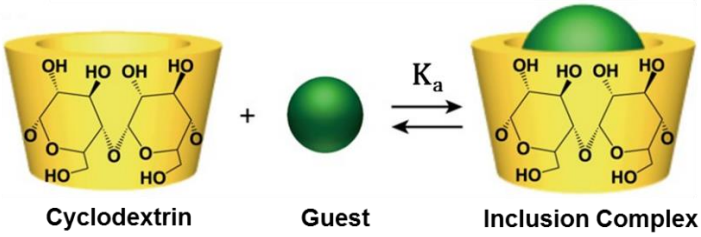


Figure 9 Schematic representation of the formation of the inclusion complex between cyclodextrins and guest molecules.

Once inside the cyclodextrin cavity, the guest molecule makes conformational adjustments to maximize the weak interactions (van der Waals forces, electrostatic interactions, hydrogen bonding and charge-transfer interaction) and the balance in the formation of the complex is reached in a short

time (often within minutes). Dissociation of the inclusion complex is a relatively rapid process usually driven by a large increase in the number of water molecules in the surrounding environment: in fact, in highly dilute and dynamic systems, the guest has difficulty finding another cyclodextrin to reform the complex and is left free in solution. The inclusion process exerts a profound effect on the physicochemical properties of guest molecules as they are temporarily locked or caged within the host cavity. This gives rise to beneficial modifications of guest molecules, which are not achievable otherwise. The properties induced by the formation of the complex are: stabilization of labile guests against the degradative effects of oxidation, visible or UV light and heat, solubility enhancement of highly insoluble guests, physical isolation of incompatible compounds, chromatographic separations, taste modification, and controlled release of drugs and flavours [29]. In this supramolecular phenomenon, more than one cyclodextrin moiety can participate by entrapping one or more guest molecules: the most common stoichiometry observed is the 1:1 but however, sometimes a more complex phenomenon does exist such as 2:1, 1:2, 2:2 (Figure 10).

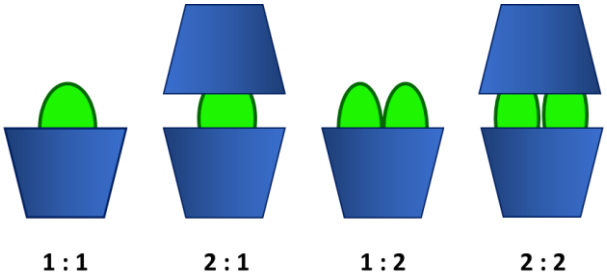


Figure 10 Schematic representation of the possible complexes that can form between cyclodextrins and guest molecules.

1.2.4. Applications of cyclodextrins

The remarkable encapsulation properties of CDs lead to a “host-guest” type relationship that can modify and/or improve the physical, chemical, and/or biological characteristics of the guest molecule. Furthermore, CDs could form supramolecular architectures with linear and branched chain polymeric and inorganic material (for example hyaluronic acid, chitosan, alginate, hydroxyapatite, and calcium phosphate), improving the biocompatibility of native material and the viscoelastic properties due to an additional crosslinking through weak interactions. This particular feature has been widely used over the years for the development of new technologies and devices in numerous fields such as medicine, pharmaceuticals, engineering, sensor development, and catalysis: they are used as solubilizers or drug transporters for pharmaceutical applications (stabilizing of the inclusive guest-complexes for drug encapsulation or enhancing the deep penetration of oligonucleotides into cells), as static phase in chromatography for the separation of chiral entities (enantioselective separation of

enantiomers in gas chromatography), as stabilizers and taste protectors in food and cosmetic industries, in catalytic reaction (they bind substrates and catalyse chemical reactions with high selectivity as well as transfer hydrophobic molecules into water by supramolecular interactions), in nanotechnologies (nanoparticles, nanosponges, nanomicelles, nanovesicles), in biotechnology, and they are proving attractive in new fields such as the treatment of pollutants in the environment (Figure 11). The versatility of CDs is demonstrated by the numerous applications and nowadays they are still the subject of numerous studies, both theoretical and applied [27].

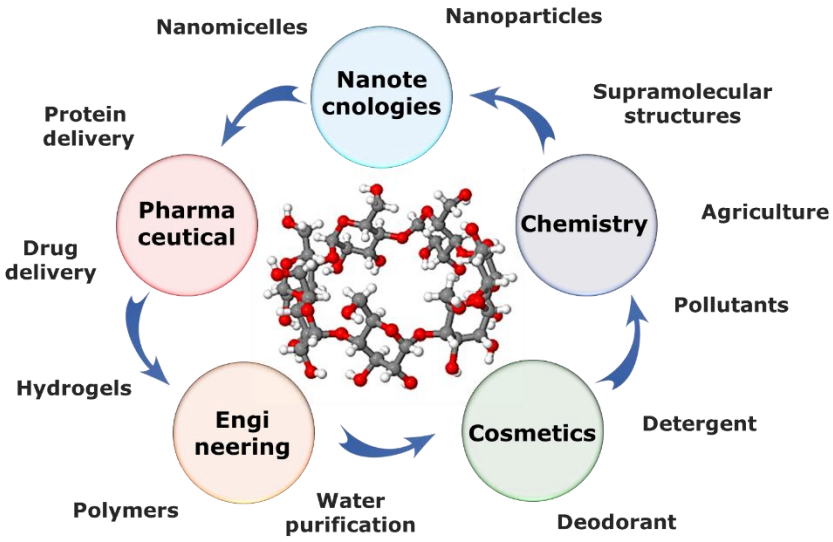


Figure 11 Schematic illustration of the various applications of cyclodextrins.

1.3. Photochromism and light-responsive molecules

In nature, plenty of materials and systems could reversibly adjust their structures and properties in response to environmental stimuli. Light when interacts with matter can cause formation or dissociation of covalent bonds, as well as reversible changes in geometry, polarity, or rigidity of particular molecular systems. Molecular photochromism is a phenomenon that was first described by Fritzsche in 1867 and it is characterized by a light-induced reversible change of the colour of a compound. He observed the discoloration of orange tetracene in daylight and its recolouring at night. Numerous studies have been conducted on this phenomenon and today photochromism defines a reversible transformation of a chemical species between two forms, exhibiting different UV/vis spectra by absorption of electromagnetic radiation in one or both ways [30]. This transformation affects various molecular properties, such as geometry, rigidity, dielectric constant, or refractive index and the molecules that reversibly transforms their characteristics due to the energy of light are called "*molecular photoswitches*". In order to describe photochromism, the most common model is a

simple two-way reaction between two molecular species A and B (Figure 12). Sometimes, it may involve another species but in this model the reaction is assumed to be unimolecular and, in all cases, atoms or molecules that are stable in two forms A and B, with different molecular or electronic configurations. separated by a potential barrier (ΔG). If this barrier is low, B is metastable and can revert back spontaneously to A.

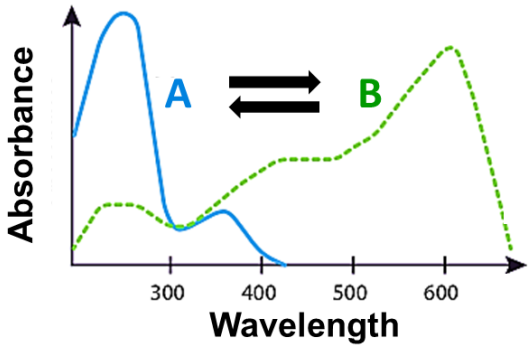


Figure 12 Photochromism: representation of the UV spectra of a molecular photoswitch in two stable states (A and B) characterized by different absorption spectra.

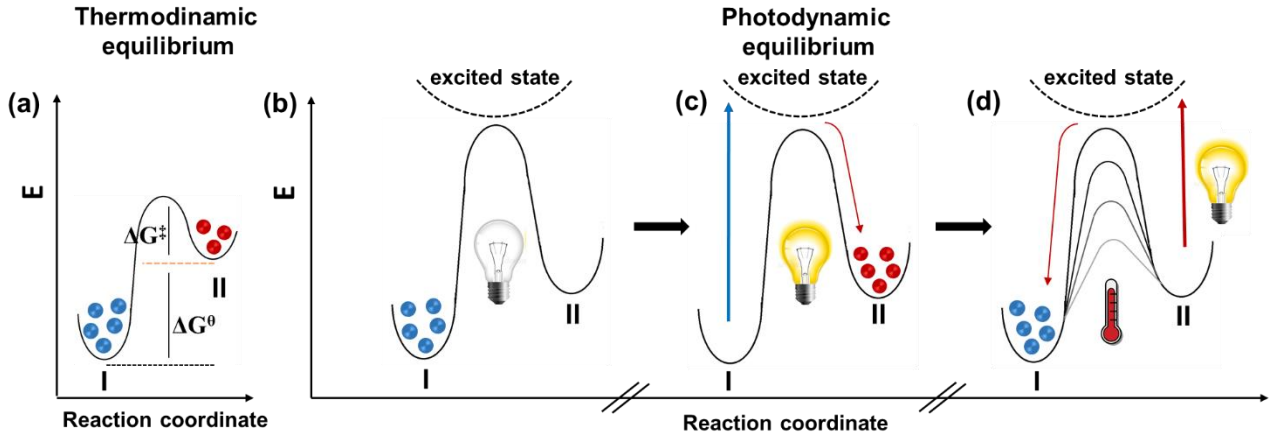


Figure 13 Representation of the thermodynamic (a) and photodynamic (b–d) equilibria: (a) energy levels I and II are populated according to a Boltzmann distribution; (b) without illumination, only I (ground state) is occupied, (c) light with proper energy allow the transition from I in an excited state, from which they relax to II; (d) the back reaction from II to I.

The energy difference between the two states is given by the Gibbs free energy ΔG^{θ} (Figure 13a). Without illumination only the ground state (I) is occupied because the thermal energy barrier $\Delta G^{\ddagger}$ is too high to be overcome at a given temperature. Light with a specific energy allows the passage of molecules from the ground state to an excited state, from which they subsequently relax to II. The return to the initial state I (thermodynamically more stable) can be achieved with another photoexcitation and/or thermally, depending on the energy barrier for the back reaction (Figure 13b-d) [31]. Photochromic systems can be classified in two categories, depending on the thermal stability

of the photogenerated isomer: P-type (photochemically reversible type) and T-type (thermally reversible type). T-type photochromism molecules are compounds for which one isomer is thermally unstable and reverts to the initial stable form. These types of compounds usually cannot be transformed into both species with light but interrupting the irradiation it is possible to reverse the course of the process. P-type photochromism, involves switches for which both isomers are thermally stable and do not revert to the initial isomer, even at elevated temperatures. The typical criterion used to differentiate between the two classes is the thermal half-life ($t^{1/2}$) of the thermodynamically less stable isomer [32]. Several classes of photochromic molecules have been developed such as azobenzenes, stilbenes, spiropyranes, fulgides and diarylethenes and the photochromic processes that take place when these compounds are illuminated can be divided into three different classes as a function of the molecules involved: the *trans-cis-trans* isomerisation, the photo-induced ring-closing reactions and the photo-tautomerism (Figure 14). The *cis-trans* isomerisation is the most common photochromic transformation and involves molecules characterized by double bonds such as stilbene and azobenzene derivates.

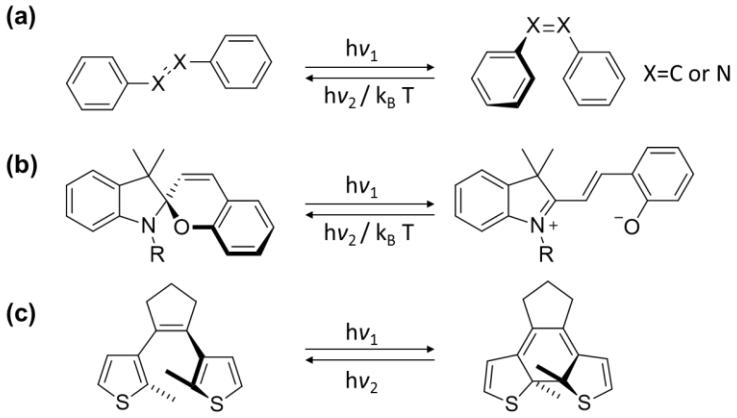


Figure 14 Chemical structures of photochromic molecules and their isomerisation mechanism: (a) azobenzene/stilbene; (b) spiropyran; (c) diarylethene.

1.3.1. Photochromism based on *E-Z* isomerization: azobenzene molecules

Among the photoswitchable compounds, azobenzenes (Azo) have proven to be the most popularly used photo-responsive compound in many applications due to its simple synthesis, rapid response, and high efficiency isomerization. Azobenzene is an aromatic molecule with two phenyl rings linked together by an N=N (azo) bond. The photo-responsive isomerization of azobenzene was first discovered in 1937 by Hartley, after observing a lack of reproducibility in absorbance measurements when azobenzene was exposed to light. These kinds of molecules are photochromic T-type systems and have two isomeric forms: the Z form is the *cis* isomer and the E form is the *trans* isomer (Figure

15a). The *E* form with a near linear conformation is the thermally stable state and has a dipole moment near zero. It can switch to the metastable *Z* form upon UV irradiation (340 nm). The *cis* isomer adopts a bent conformation with its phenyl rings twisted ~55° out of plane from the azo group. It has a dipole moment of 3 Debye and it can revert to *E* form thermally or after visible-light irradiation (450 nm). The end-to-end distance of each isomer is also different: the distance between the carbons at the *para* positions of the phenyl rings is 9 Å for the *E* form, and 7 Å for the *Z* form (Figure 15b and Figure 16)[33]. Moreover, the *trans* configuration of azobenzene is 10-12 kcal mol^{-1} more stable than the *cis* isomer so that, in the dark at equilibrium, *trans* is the dominant isomer (>99.99 %).

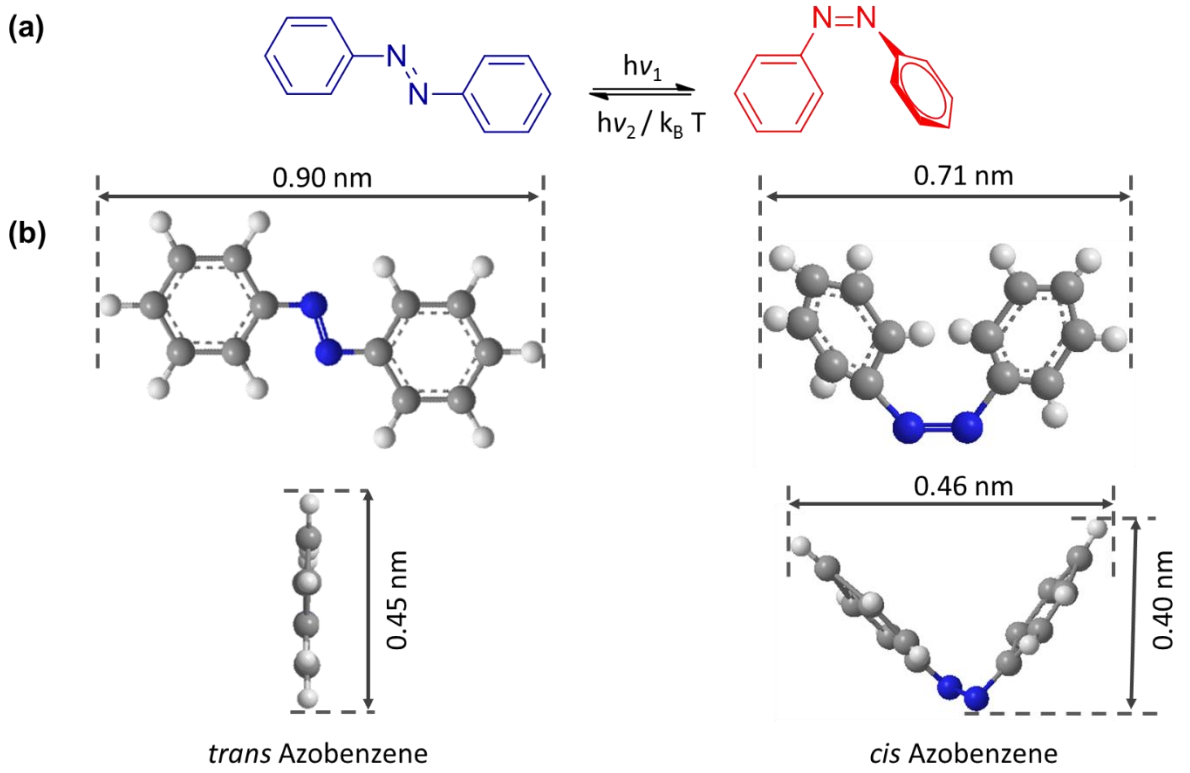


Figure 15 (a) Schematic representation of the isomerisation mechanism of azobenzene; (b) ball-and-stick models and dimensions of the azobenzene isomers.

1.3.2. Photo- and thermal isomerization of azobenzene

The absorption spectrum of the *trans* azobenzene isomer (Figure 16) shows one strong allowed π-π^* transition at approximately at 320 nm and a weak forbidden transition (n- π^*) at approximately 450 nm. The *cis* isomer shows the opposite phenomenon; the π-π^* at approximately 275 nm is weak and the n- π^* band at approximately 450 nm is strong compared to the *trans* π-π^* and n- π^* bands. Excitation of *E*-Azo from its S_0 ground electronic state to its first singlet excited state S_1 is a weakly allowed n-π^* transition in the visible region ($\lambda \approx 450$ nm), while populating the second excited state S_2 is an intense symmetry-allowed π-π^* transition deep in the UV range ($\lambda \approx 300$ nm). Excitation of

these bands at specific wavelengths promotes azobenzene from the S_0 state to the S_1 (n-π*) and S_2 (π-π*) states. This excitation allows for the *trans-cis* photo-isomerization by irradiation of the π-π* band at 350 nm and the *cis-trans* photo-isomerization by subsequent irradiation of the n- π* band at 445 nm. Isomerization is accompanied by changes in volume, polarity and light absorption, the latter in the form of an intensification (and slight shift) of the n-π* band and attenuation (and marked blue-shift) of the π-π* band [34].

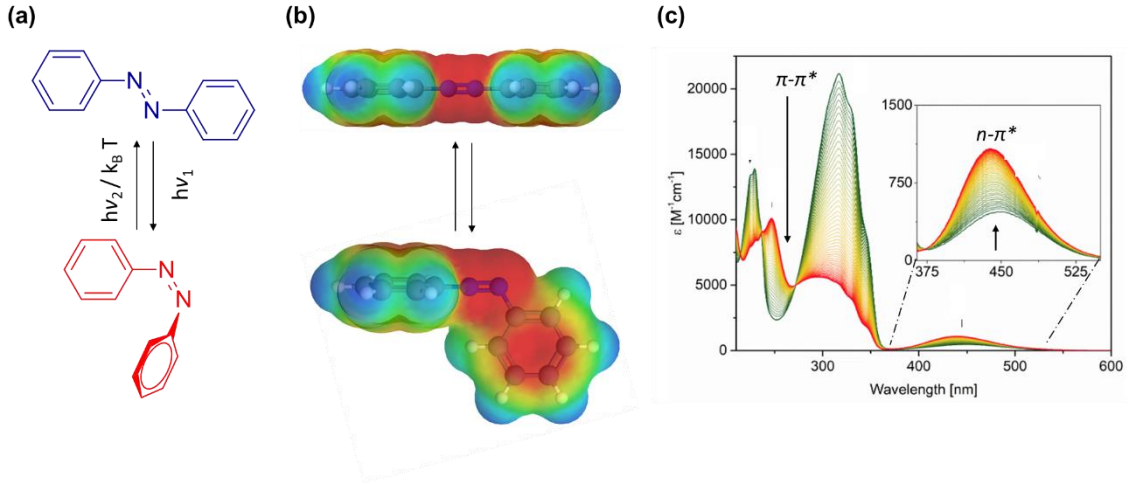
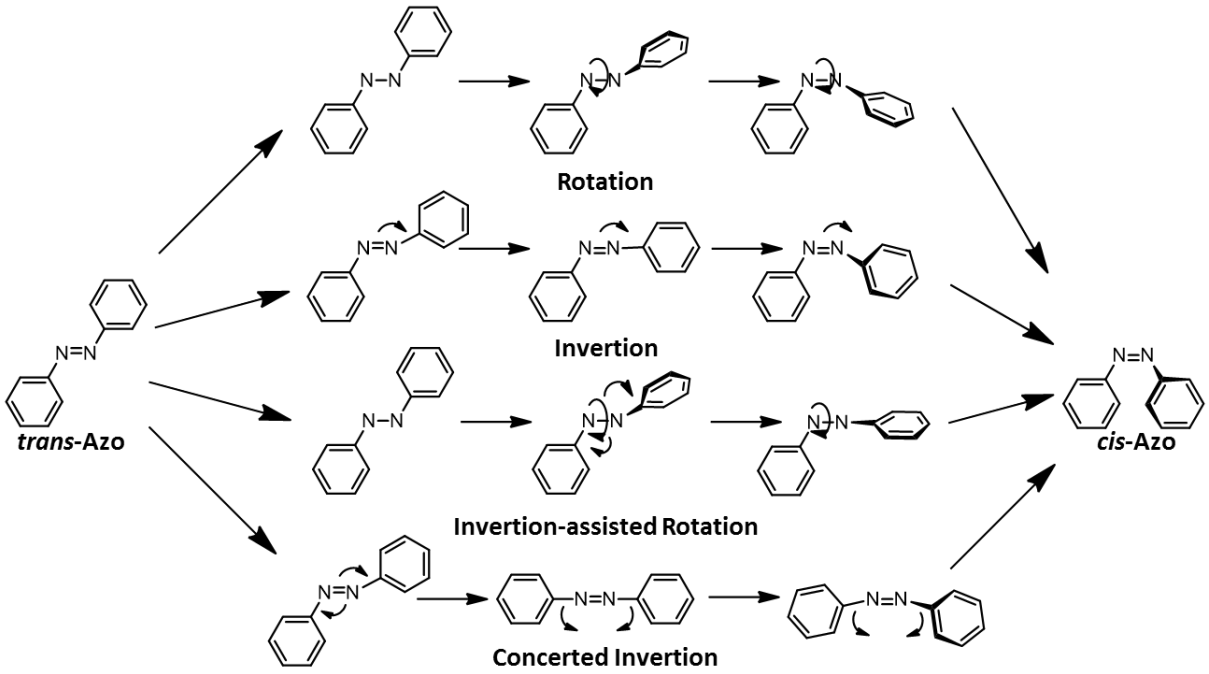


Figure 16 Representation of the changes in shape (a), polarity (b) and light absorption due to the Azo-isomerization process.

The photoisomerization of azobenzene proceeds by an inversion-assisted torsional pathway involving in-plane motion and out-of-plane rotation but the exact mechanism of isomerization is not fully understood. An overview of proposed mechanisms is depicted in Scheme 2: the combination of rotation and inversion leads to significant changes of all angles around the nitrogen double bond and the dipole moments resulting in the transition states [35].



Scheme 2 Schematic representation of the proposed mechanisms of azobenzene photoisomerization.

Four different parameters can describe the performance of a photoswitchable molecules. The first is the wavelength of maximum light absorption and the quantum yield the ensuing isomerization (directly related to the nature of the transition activated by the irradiation and the reaction pathways of the excited state). The second and the third parameters are respectively the thermal stability of the two isomers and the reproducibility of photochemical switching. In fact, the two different isomers may have different lifetimes (τ) or half-lives ($t^{1/2}$) after the isomerization process. The fourth characteristic parameter of a photoswitch is the steady-state relative abundance of *E* and *Z* isomers when the photoswitch is exposed to a given light source [34]. The isomerization properties of azobenzene, including the absorption wavelength for photoisomerization and the half-life of the *cis* isomer, are directly affected by substitutions on azobenzenes. An appropriate modification of the substitution of the azobenzene core is one of the main factors that allows modulating the thermal relaxation rate of azo-dyes and, therefore, determines the response time of the photochromic molecular switch. Simply introducing π-donors and π-acceptors gives rise to hypsochromic (lower λ_{max}) and bathochromic (higher λ_{max}) shifts of the main absorption band (increase or reduction of the HOMO-LUMO gap). The increase of the degree of electron donation at the ortho and para positions on the benzene moiety lead to a red shift of the absorption maximum of *trans* azobenzene, while electron-donating substituents give a red-shift effect of the trans isomer and accelerate the rate of thermal isomerization of the *cis* isomer. A classical way to lower the gap of azobenzene is to introduce electron-donating groups (EDGs as methoxy groups) and/or electron-withdrawing groups (EWGs as fluorine or carboxyl groups) in ortho or para to the N=N. Another possibility for visible-light addressability is the introduction of an ethylene linker in ortho position (bridged azobenzene) or a

BF_2 moieties to constitute a BF_2-coordinated azo compound (Figure 17)[32]. In addition to substituent effects, intramolecular and intermolecular interactions can also perturb the absorption.

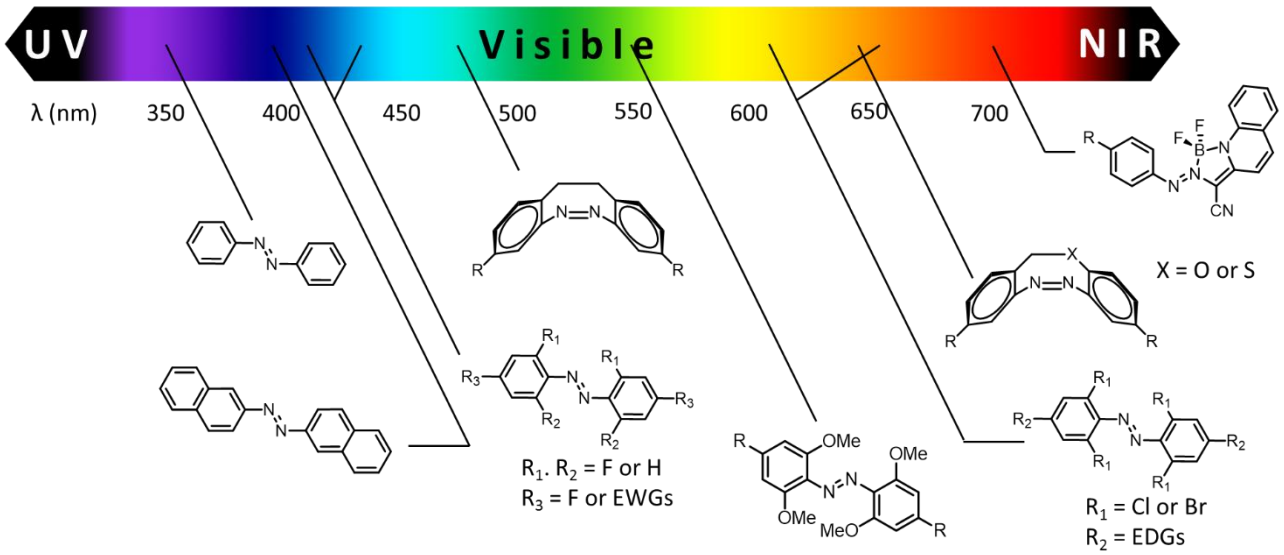
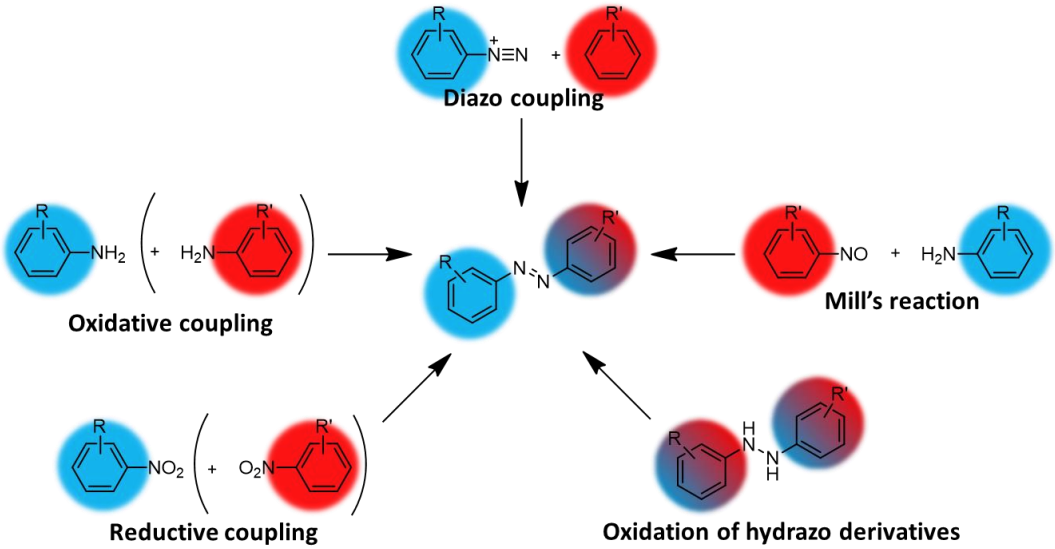


Figure 17 Representation of the maximum irradiation wavelength for isomerization of different types of azobenzene.

1.3.3. Synthesis and applications of azobenzene molecules

There are numerous synthetic pathways for the synthesis of azobenzene molecules, both for symmetric and non-symmetric Azo compounds. An overview of the main techniques is presented in Scheme 3: the oxidative and reductive coupling procedures of anilines and nitrobenzenes, the Mills reaction, and the Azo coupling via diazonium salts. In the oxidative coupling of aromatic amines, many oxidizing agents and reaction conditions are used to generate azo derivatives such as metal oxides ($KMnO_4$), hypervalent iodide, and H_2O_2 [36]. The oxidising agent and the reaction conditions are usually chosen depending the substituents present in the amine molecules. With this synthetic route, symmetric azo compounds with two equivalent aromatic groups are obtained, and sometimes also hetero-coupling with different amines is feasible. Reductive coupling also leads to the formation of symmetric azo compounds, using zinc, aluminum, magnesium, $LiAlH_4$, $NaBH_4$ or ruthenium nanoparticles as reductive agents [37]. The Mill's reaction, which couples primary arylamines with nitroso compounds in acid media, usually with AcOH, is a simple and widely applied method for the synthesis of dissymmetrical azobenzenes with the further advantage of being compatible with many substituents. The fourth method for azobenzene synthesis is the diazo coupling via diazonium salts followed by an aromatic nucleophilic substitution reaction. The diazonium salt is converted from aromatic amines and $NaNO_2$ in acidic media. In this reaction, the aryldiazonium cation acts as the electrophile and an activated arene is the nucleophile. This is an important method for the synthesis

of dissymmetrical azobenzene derivatives. Finally, azo compounds can be prepared by oxidation of hydrazo-derivatives with a variety of oxidizing agents such as H_2O_2, O_2, MnO_2 or HgO [38].



Scheme 3 Schematic representation of the possible synthetic routes for the synthesis of azobenzene derivatives.

The ease with which various azobenzene derivatives can be synthesized is advantageous for controlling the photochemistry, which is highly dependent on the substitution pattern of the Azo molecules. The change in the physical properties of azobenzene molecules are exploited to use azobenzene as a photoresponsive molecule in smart materials and thanks to their properties, they are used in various fields of life and material sciences. Azobenzene may be blended in polymer matrices or covalently integrated in their main or side chains aiming to control many properties, such as the tensile strength, elasticity, or glass transition temperature, to develop stimuli-responsive materials. It can be used for the synthesis of self-regenerating materials, as a derivative of nanoparticles, for the development of solar-thermal fuel and in the medical field, and for the targeted release of biologically active small molecules. The limitations of using UV light in vivo allowed to synthesize azo compounds that operate with light of longer wavelengths, exploiting the red-shift properties of various substituents and enabling the switching within a therapeutic spectral window suitable for applications in the nascent field of photopharmacology.

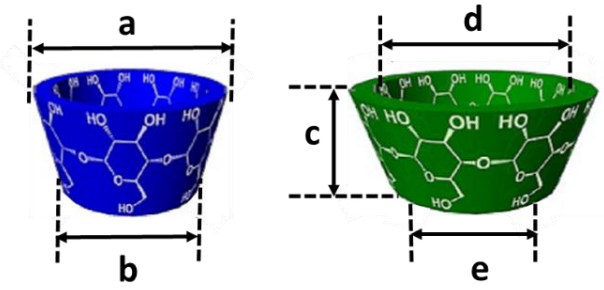
1.4. Host-guest interactions between azobenzene and CDs

In the last decade, several photocontrolled supramolecular systems have been successfully constructed using azobenzene (Azo) and CD components. The reversible photoisomerization of the isomers and/or the different binding behaviours of *trans*- and *cis*-azobenzenes with CDs play major roles: the reversible process of the inclusion complex affords the "on-off" characteristics and the

opportunity to regulate the supramolecular systems. Moreover, azobenzene groups are relatively easy to anchor to targets such as small molecules, polymers, inorganic nanoparticles, and substrates. These systems have been widely used in building molecular shuttles, machines, surfactants, ion channels, hydrogels, and to develop smart materials such as photoswitchable supramolecular hydrogel, photoresponsive artificial muscle, and photodriven pseudorotaxane (discussed at length later in this thesis). However, these studies have focused mainly on the photocontrolled assembly/disassembly process, while the morphological conversion of supramolecular assembly switched by light irradiation has rarely been reported.

1.4.1. Photoswitchable Azo-CD host-guest complex

Azobenzene and CD have been extensively investigated for designing photoresponsive supramolecular materials. The azobenzene moiety can be included into the inner hydrophobic cavities of cyclodextrin, creating a supramolecular complex that can associate/disassociate due to the stimuli of light. Host-guest interactions between azobenzenes and CDs were first reported in 1980, where the azobenzene molecule was encapsulated into the hydrophobic cavity of *β*-CD. After a light stimulus, the *trans*-to-*cis* photoisomerization led to the disassembly of the host-guest complex [39]. The hydrophobic interactions and the release of high-enthalpy water molecules from the cavity of CDs are the major driving forces for the host-guest complexation. Azobenzene containing molecules can interact with the internal cavity of CDs, but the formation of the supramolecular complex is strictly related to the molecular size of the two species and the interactions that take place between them: *α*, *β* and *γ*-CD have different structures and shapes due to the number of D-glucose units and the shape of the azobenzene molecules is related to the type of isomer (Figure 18).



Dimensions (nm)	α-CD	β-CD	γ-CD
a	1.37	1.53	1.69
b	1.32	1.49	1.61
c	0.78	0.78	0.78
d	0.57	0.78	0.95
e	0.45	0.61	0.77

Figure 18 Molecular structures and dimensions of native CDs.

Under dark or visible light irradiation, *trans*-azobenzene fits into the cavity of *α*-CD very well and form a strong 1:1 host-guest complex [40]. However, when it is transformed to the *cis* form by photoisomerization upon UV light, the *cis*-azobenzene cannot be included by *α*-CD anymore due to the mismatch between the host and guest molecules and to the hydrophilicity of the *cis*-isomer.

Compared to *trans* azobenzene, *cis* Azo is more hydrophilic and this implies that the *cis*-Azo/CD complex is unstable in the aqueous environment and easily dissociates. Another reason for the Azo/CD complex disassembly after UV light irradiation is the three-dimensional structure of the Azo-molecules: *cis*- isomer show a dihedral angle of ~60°, compared to the *trans* isomer which has a nearly planar structure, and does not fit the α-CD cavity [41]. Considering the other types of CDs, β and γ-CD are larger than α-CD and can host both kinds of isomers in the internal cavity. However, while α and β-CD are slightly larger than *trans*-Azo, γ-CD is too large and form an unstable complex. The introduction of substituents on benzene rings leads to changes in the shape and size of azobenzene molecules, making it impossible to create host-guest systems with small CDs, such as the α-CD (Figure 19).

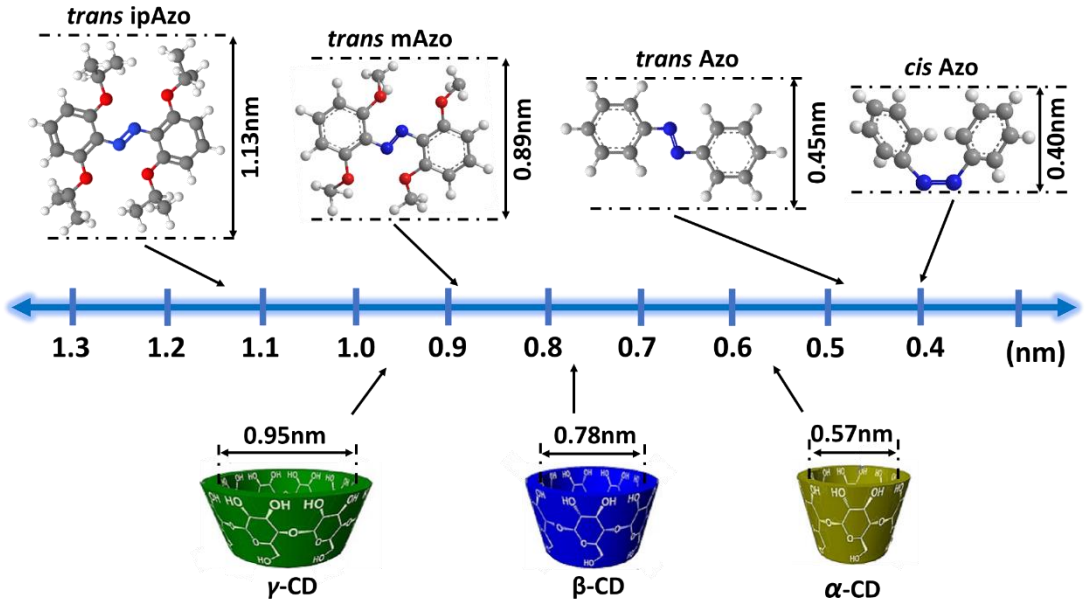


Figure 19 Comparison between the molecular sizes of CD cavities and Azo molecules.

The host-guest interaction between azobenzene molecules and CDs can be quantified by measuring the association constants (K_a) of the host-guest complexes. It is possible to determine the association constants using various spectroscopic techniques involving UV-Vis, fluorescence, infrared, and NMR. With these techniques it is possible to apply the Benesi-Hildebrand's equation, or a modified form, to calculate the K_a as a result of a spectroscopic titration. Moreover, in nuclear magnetic resonance spectroscopy, monitoring the change in diffusion coefficient (D) as a function of the host–guest ratio allows the determination of association constants and the use of D replaces the more usual chemical shift [42]. Using another approach, Meier and Beeren described a new method for the determination of binding constants in complex mixtures determined from a single NMR titration [43]. The association constants between azobenzene group and CDs found in the literature are listed in Table 1. In this table are reported some values of K_a found in the literature: it is not possible to find

univocal K_a because the association constants vary according to the type of technique used, the experimental conditions, and the reagents used.

		Guest	
		***trans*-Azo**	***cis*-Azo**
Host	***α*-CD**	2000.0	35.0
	***β*-CD**	770.0	280.0
	α-CD	1744.7	11.9
	***β*-CD**	892.1	363.4
	***α*-CD**	1340.0	25.4
	***γ*-CD**	149.0	16.0

Table 1 Some association constants (K_a, M^{-1}) between native CDs and *trans*-/*cis*-Azo found in literature[53][54].

From the above results, strong host-guest complexes could only be formed between *trans*-Azo and *α*-CD due to the large difference between the K_a values for complexation of *α*-CD with *trans*-Azo or *cis*-Azo. The introduction of substituents and the modification of the steric properties of the azobenzene molecules modify the values of K_a and determine new properties of the system.

1.5. The dynamic nature of the chemical transformations

In the dynamic processes, host–guest interactions may contribute not only to a multistep regulation of various molecular functions such as the catalysis of chemical reactions, transport of materials and control of reaction pathways, but also in cooperative and responsive phenomena where molecules are engaged together to constitute a macromolecular dynamic system. These molecules can communicate intermolecularly and intramolecularly to give rise to cooperative systems and this phenomenon plays an essential role in the development of complex systems and in the creation of intermediates, products and functions that cannot be achieved by using the single functional units. Whereas *molecular chemistry* essentially deals with the covalent bonding of atoms, *supramolecular chemistry* is predominantly involved in the study of the weaker intermolecular interactions resulting in the association and self-organization of several components to form larger aggregates and during the last decades, chemists have developed new strategies to design molecular systems that are based on non-covalent interactions between individual molecules.

1.5.1. Molecular recognition and self-assembly process

Specific combination of the intermolecular interactions such as hydrogen bonding, electrostatic interaction, coordination bonding and hydrophobic effect, gives rise to well-organized host-guest complexes and supramolecular assemblies from a mixture of the individual molecular components [18]. The introduction of the dynamic features into molecular chemistry requires the shifting from static to ‘‘dynamic’’ bonds and the driving forces in the development of these supramolecular systems are the self-assembly and molecular recognition processes. Molecular self-assembly, by definition, can be defined as the spontaneous and reversible organization of molecular units into ordered structures by non-covalent interactions and under thermodynamic equilibrium conditions. Indeed, if the self-assembly take place without intervention of external forces, the process must lead to a lower Gibbs free energy and thus self-assembled structures are thermodynamically more stable than the single unassembled components [44]. Molecular self-assembly is a key concept in supramolecular chemistry and in nature there are many types of self-assembly systems that vary in dimensions from the molecular level to the macroscopic size: it is exhibited in the self-assembly of phospholipids to form the double layer membranes, in the creation of micelles, vesicles from amphiphilic molecules and in the assembly of proteins to form quaternary structures. The main elements in molecular self-assembly are the chemical complementarity and the structural compatibility between the species that constitute the system. Lehn defined the molecular recognition as "a process involving both the binding and the selection of substrate (or substrates) by a given receptor molecule having a specific function”, and this mechanism has been described for the first time by Fischer with the “*key and lock*” model, involving interactions between rigid molecules with structural complementarity [45]. Knowing the chemical and physical properties of the building blocks in the considered system, it is possible to address these spontaneous phenomena for the purposes of design and generate specific supramolecular architectures with predefined properties and characteristics.

1.5.2. Dynamic combinatorial chemistry (DCC) and dynamic combinatorial library (DCL)

The resulting ability of supramolecular species to reversibly dissociate and associate, deconstruct and reconstruct, allows to incorporate and rearrange their molecular components until obtaining the best structure (kinetically or thermodynamically). These types of interaction characterize a particular field of supramolecular chemistry: The dynamic combinatorial/covalent chemistry (DCC). DCC applies

reversible chemical reactions to form libraries (a set of interconverting supramolecular or molecular entities) under thermodynamic control, which reach the equilibrium state (Figure 20)[46].

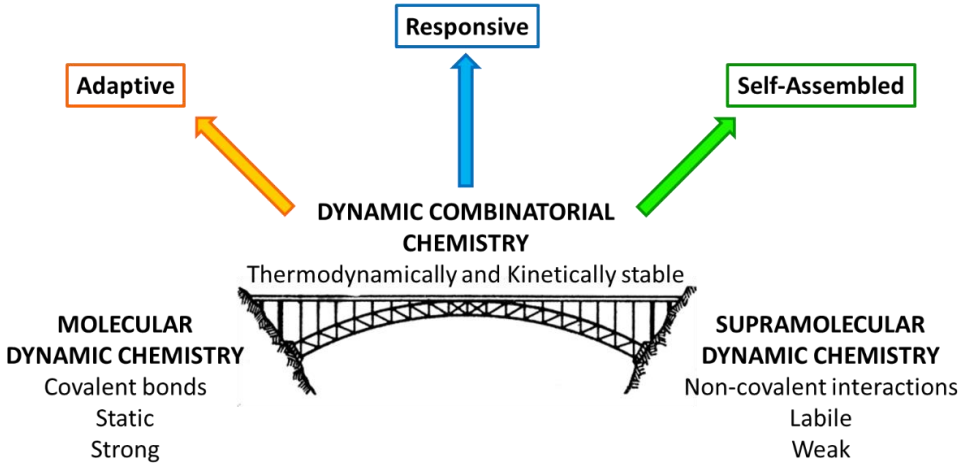


Figure 20 Representation of the dynamic combinatorial chemistry as a link between supramolecular (right) and molecular (left) dynamic chemistry.

In a dynamic combinatorial library (DCL), building blocks and products are continuously interconverting and this enables to shift the composition of the system in any time applying an external stimulus. In this way, it is possible to change the system's conditions to obtain the desired product (or products). All library members are connected through a set of equilibrium reactions and any change to the stability of one member will affect all the others with the consequent modification in the distribution of the products. In the DCL, the final equilibrium distribution is determined by the thermodynamic stabilities of all species in the mixture. However, this mechanism is a time-dependent process and may displays a kinetic control, generating kinetic products before reaching the thermodynamic ones [47]. The "*lock-key* model" is a simple way to explain the dynamics that can occur in the dynamic combinatorial chemistry (Figure 21). With the goal of getting the perfect key (or keys) that fits in the lock, a specific dynamic combinatorial library is built using different building blocks. Under thermodynamic selection, it is possible to express and obtain the key that presents the strongest interaction with the lock, or by kinetic selection, find the key that forms fastest within the lock. In both cases, the supramolecular lock-key recognition interactions direct the process [5].

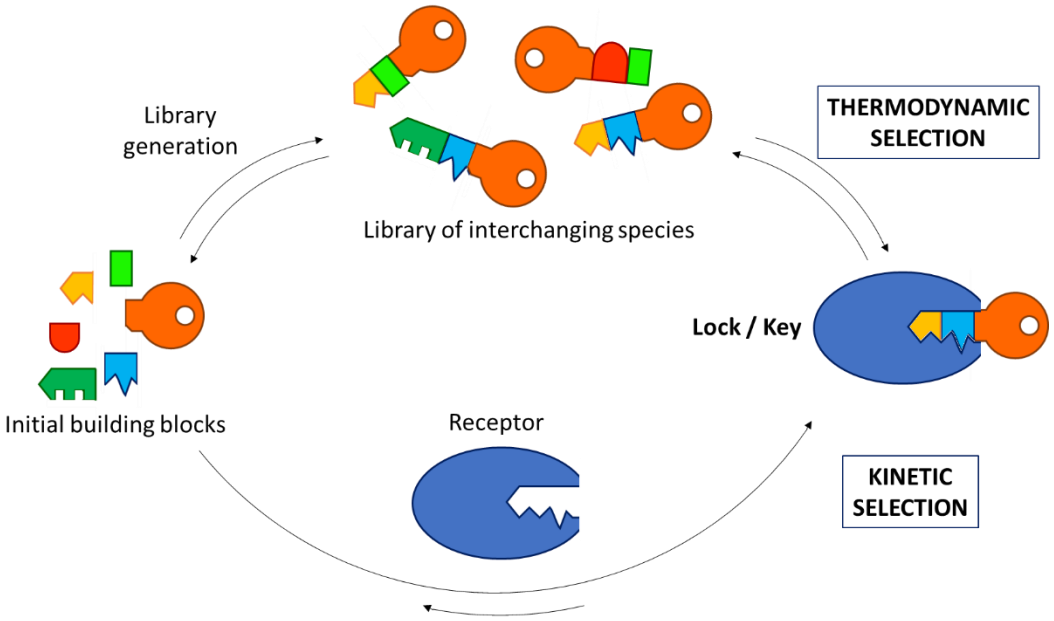


Figure 21 Schematic illustration of the dynamic combinatorial chemistry with the lock-key mechanism.

Systems that increase multiple dynamics provide access to a rich set of properties and may extend to the modulation of the features of supramolecular assemblies. In the biological field, enzymes can increase the reaction rate and quickly reach the thermodynamic equilibrium state. Thanks to their properties of catalysing both the direct reaction and the reverse reaction, they may generate dynamic libraries to direct the self-assembly of specific products in an enzyme-mediated dynamic system.

1.5.3. Target-direct dynamic combinatorial chemistry

Target-directed DCC (tdDCC) is a particular application of the dynamic combinatorial chemistry in *host-guest* systems, where a specific macromolecular molecule, either *host* or *guest*, interacts in a non-covalent way with selected library components, obtaining the most stable thermodynamically product and amplifying the concentrations of constituents that have high-affinity with the target at the expense of weak binders [46]. Thermodynamic control in DCLs implies that changing the conditions of the experiment may induce changes in the library composition: in this way it is possible to predict and direct the formation of the desired product using external influences. The most diffuse approaches to influence the product distribution in DCLs utilize intermolecular interactions (with template molecules or between the DCL members themselves) and physical stimuli (or chemical-physical). Given that the system is at equilibrium and there are a continuous exchange between the library members, a given template could shift the equilibrium in favour of the library member which is its best binder: the template selects and constructs its own "receptor" [48]. The template induces the creation of a specific product by directing the reactive sites in a favourable orientation for the

formation of covalent bonds and these sites are hold in the preferred bond-forming orientation with non-covalent, intermolecular bonds. According to Le Châtelier's principle, upon addition of a template, the system reorganize itself so that species that bind are favourably formed and are thus amplified (Figure 22). This reorganization can be used to identify within a library of molecules the members with a high affinity for the template [49].

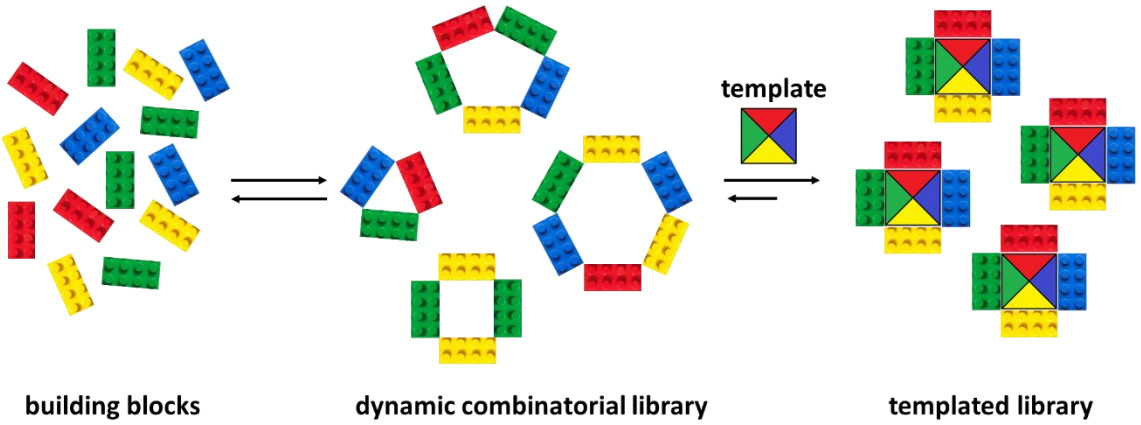


Figure 22 Schematic representation of a dynamic combinatorial library and amplification of the best binder in the presence of a template.

1.5.4. Light controlled DCLs

Dynamic supramolecular self-assembly of specific product can be controlled either by changing the external environment or by *in-situ* morphological transformation of building blocks. Modulation of DCLs by light is a simple way to structurally modifying DCL members. The use or the incorporation of photochromic molecules as guest allows to continuously modify the formation of products, varying the wavelength of the radiation. Light is an efficient stimulus, does not leave chemical sub-products, and it can be applied reversibly without addition of any chemical substance. These characteristics make light an ideal external input to establish this strategy [50]. In contrast to the other physical means, light can selectively target one specific type of bond and promote the isomerization process or the cleavage of covalent bonds. The use of a photoswitchable template as a guest in target-directed DCC, the *cis*-*trans* isomerization of double bonds by visible light (in depth and applied in this thesis work) can lead to remarkable configurational changes, which, in turn, might affect the guest binding properties of a dynamic system. If two photoisomers absorb at different wavelengths, full-controlled two-state photoswitchable molecular systems (guests) can be created and consequently, two or more different products can be obtained. As seen in previous chapters, photoswitchable azobenzene molecules are well-known for UV-light-induced *trans*-to-*cis* isomerization and blue-light or heat-induced *cis*-to-*trans* isomerization and these transformations characterize changes in the shape and physical-chemical properties of the single isomers [35], and consequently in the host formation

around them. This example highlight how photoirradiation can effect significant changes in the architecture of DCLs, at both the molecular and the supramolecular levels. Due to these properties, photoresponsive systems allow the creation of target self-assemblies with high selectivity and provide the access to a rich set of properties.

2. Aim of the work

In a recent work, Sophie R. B. and Dennis L. demonstrated for the first time the possibility of manipulating and controlling such enzymatic reactions using specific template molecules to direct the self-assembly of specific products in an enzyme-mediated dynamic system. They were able to alter the distribution of the different cyclodextrins formed with high selectivity based on the templating effect imparted by the azo guest and by the proper light irradiation [51]. Using this discovery as a new starting point for the selective synthesis of cyclodextrin systems, they used azobenzene as a photo-responsive template to select with light the desired product in an enzyme-mediated dynamic system (Figure 23)[52].

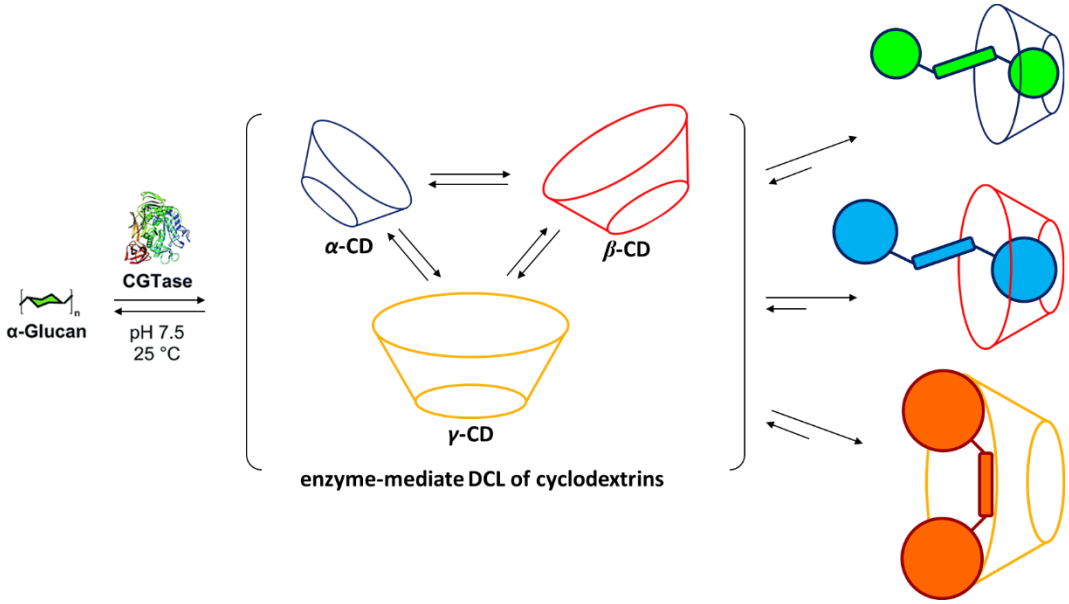


Figure 23 Light-controlled selective enzyme-mediated synthesis of cyclodextrins with azobenzene molecules as templates.

The aim of this thesis work was to investigate the possibility of modulating the selective synthesis of different CDs using light and new different photoswitchable azobenzene as templates to further expand the potentialities of this dynamic photo-templated enzymatic synthesis of CDs. The first approach is to synthesize new types of azobenzene molecules with different steric-properties and solubility in aqueous solution through bearing different substituents (Figure 24). Subsequently, analyze the light-induced isomerization of each molecule and determine the binding strength between photoresponsive molecules and α-CD, β-CD and γ-CD.

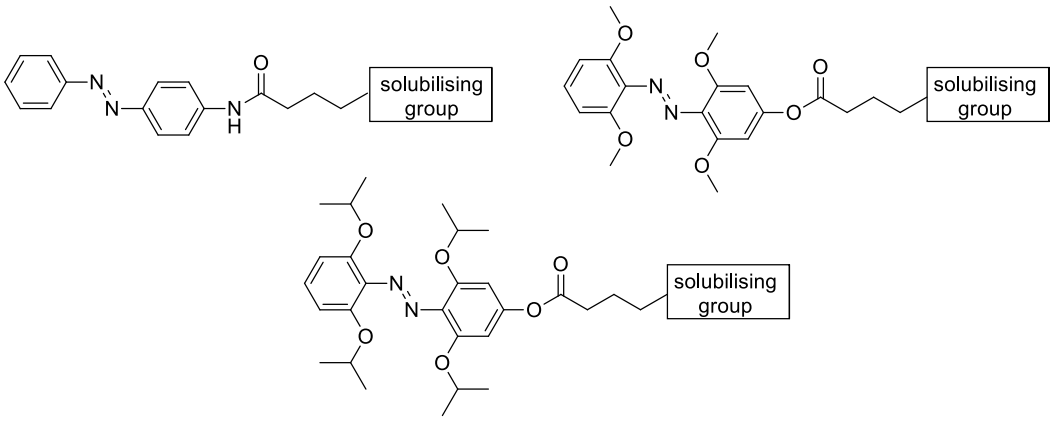


Figure 24 Chemical structure of the water-soluble azobenzene molecules for the photo-templated enzymatic synthesis of CDs.

The second approach is to set up an enzyme-mediated combinatorial libraries (DCL) of cyclodextrins and investigate the effect of the azobenzene templates on DCL under different conditions of irradiation.

Due to the Covid-19 emergency, the present work has been interrupted in the synthetic phase of the azobenzene molecules and it has not been possible to complete the objectives previously reported. An in-depth study was carried out on the host-guest photoswitchable systems consisting of cyclodextrins and azobenzene, analysing the isomerization mechanisms of the various species and their recent and future applications in numerous fields such as medicine, pharmaceuticals, engineering, sensory, and catalysis.

3. Results and discussion

3.1. Enzyme-mediate and light-controlled t-DCC for CDs synthesis

The outcome of enzymatic reactions could be steered and controlled by using artificial template molecules to direct the self-assembly of specific products in an enzyme-mediated dynamic system. By addition of different and specific templates in an enzyme-mediated dynamic combinatorial library of CDs with CGTase, it is possible to promote the selective synthesis of each of the native CDs (with selectivity in the range 89-99%), or entirely alter the outcome of the enzymatic transformation to obtain larger CDs such as the δ-CD and ε-CD, with nine and ten glucose units for macrocycle, respectively, that are industrially obtained in low yields only after extensive chromatographic separations or using a genetic engineered CGTase (Figure 25)[51]. CGTase catalyses the fast, reversible transglycosylation reaction and the slow, irreversible hydrolysis of α-glucan, giving rise to a complex dynamic chemical system in which CDs transiently assemble out-of-equilibrium in a kinetically trapped subsystem (subsystem operates under pseudo-thermodynamic control). In the absence of templates, the transient DCL lasts for a shorter time and CDs convert into short maltooligosaccharides and glucose (the thermodynamic product) due to the irreversible hydrolysis of the glycosidic linkages.

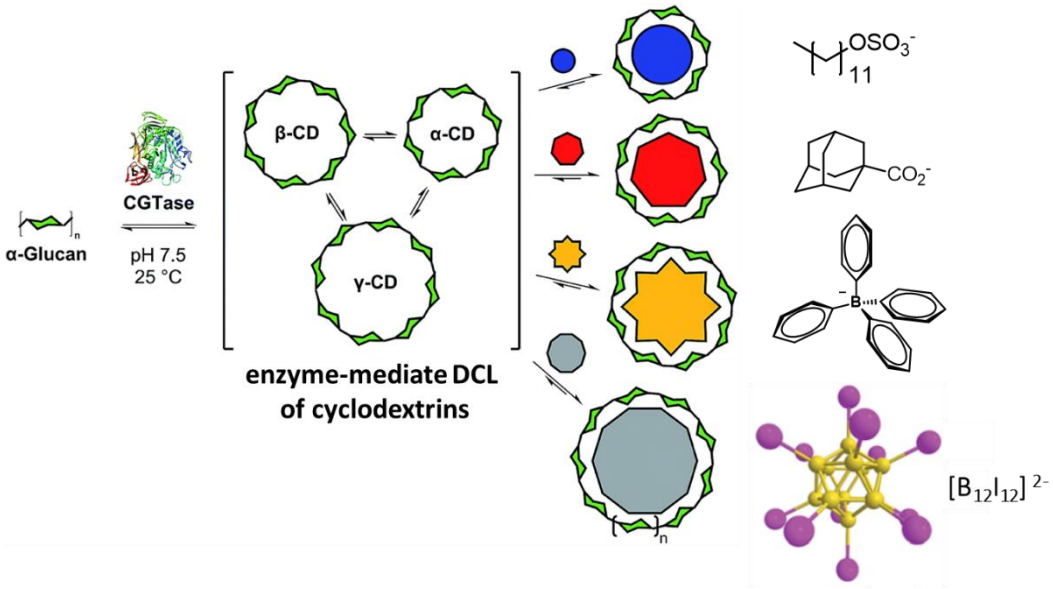


Figure 25 Selective enzyme-mediated synthesis of CDs: the addition of templates induces a change in cyclodextrin distribution and promotes the selective synthesis of different CDs.

With the aim of further expanding the possibilities of this dynamic system, it is possible to control on demand the outcome of an enzymatic process using a stimuli-responsive template to select specific CDs. For this purpose, an azobenzene photoswitch could be used as a template to promote the out-

of-equilibrium assembly of CDs. The key point is the deep understanding on the effects of the geometrical structure of the template, once isomerized in the reaction environment through light stimuli, on the selective synthesis of the desired products. The distribution of the different CDs generated in the presence of a template could be predicted using knowledge on the host-guest interactions and binding constants between the CDs and the photoswitchable guests, in the two isomeric forms.

The control of the outcome of an enzymatic process using light-responsive templates is a new strategy firstly introduced at the end of 2019 by Sophie R. Beeren and Dennis [52]. Being a rather recent innovative synthetic approach, it still has much too reveal and many potentialities are still unexpressed. Specifically, the authors investigated the possibility of modulating the selective synthesis of different CDs using five different types of azobenzene molecules (Figure 26). The composition of each library after two hours reaction showed that the major products were α-CD and β-CD (a little amount of γ-CD was produced as well using low concentrations of α-1,4-glucan). For each azobenzene **1-5**, addition of the template in *trans* configuration without irradiation generated higher concentrations of α-CD, indicating that the *trans* isomer binds preferentially to α-CD. Compared to all other molecules, only photoswitch **1** caused the amplification of β-CD under irradiation, indicating that ***cis*-1** binds preferentially to β-CD, while **trans-1** binds more strongly to α-CD.

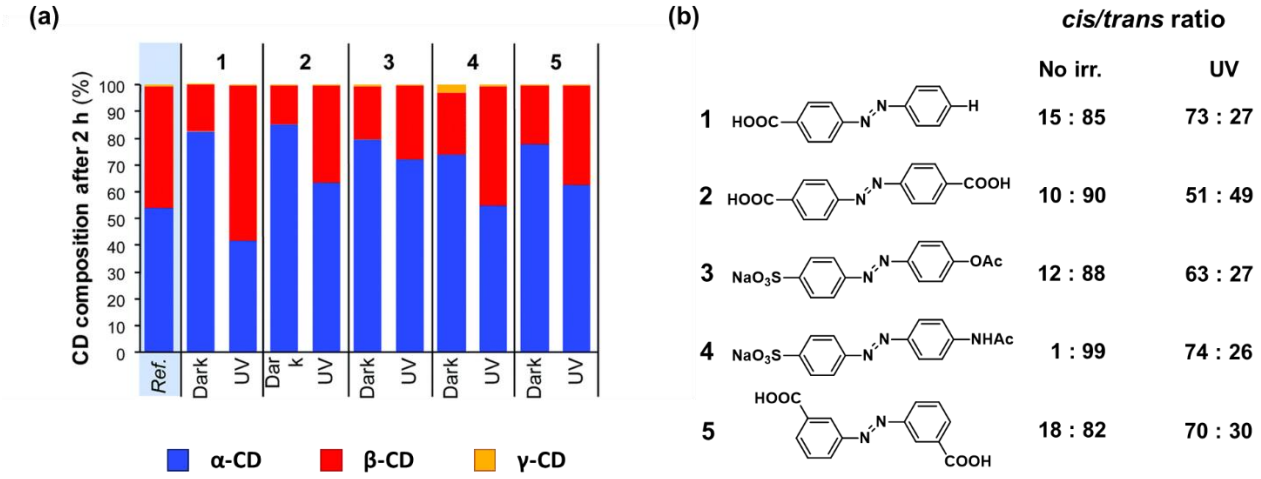


Figure 26 Screening of azobenzenes **1-5** as light-responsive templates:(a) cyclodextrin distributions in a CGTase-mediated DCL templated with azobenzenes **1-5**; (b) physicochemical properties of azobenzene **1-5** [52].

The association constants between CDs and azobenzene molecules (determined by NMR titrations) confirmed the relative affinities of ***cis*-1** and ***trans*-1** for α-CD and β-CD and thus explained the different product distributions with CGTase in the absence or presence of UV irradiation (Table 2). When the enzymatic reaction was carried out in the dark, ***trans*-1** predominated, and since it binds α-

CD approximately twice as strongly as *β*-CD, the production of α-CD was amplified. When exposed to UV light, ***cis*-1** predominated leading to higher production of *β*-CD, due to the 15:1 ratio between the binding constants of ***cis*-1**to *β*-CD *vs.* *α*-CD.

In order to amplify the distribution of the different CDs in the DCLs using light stimuli, new photoswitchable azobenzene molecules have been further synthesized. By modifying the steric properties of the photoresponsive template it was possible to direct the formation of specific CDs within the DCLs: larger templates allowed to obtain CDs having a high number of glucose units and *vice versa*.

	Guest	
Host	*trans*-**1**	*cis*-**1**
α-CD	5000±400	140±70
β-CD	2500±300	2200±300

Table 2 Association constants (K_a, M^{-1}) between *α*-CD or *β*-CD and isomers of **1** [52].

3.1.1. Synthesis of azobenzene derivates

In 2015, Wang and collaborators developed a responsive hydrogel using tetra-ortho-methoxy-substituted azobenzene (**mAzo**, Figure 27a,b) and *β*-CD that showed a gel-to-sol transition under red-light irradiation [53]. They studied the interaction between the two isomers of **mAzo-Py** (the pyridinium residue was added to increase the hydrophilicity of the Azo-molecule) and *α*- and *β*-CDs. The results summarized in Table 3a showed that the supramolecular interactions between **mAzo** and CDs were different from those between the simple azobenzene and CDs: for **mAzo**, *α*-CD showed low association constants with both the *trans* and the *cis* isomers, indicating that this photoswitchable guest could not form a stable host-guest complex with *α*-CD. Conversely, *β*-CD showed high association constant with ***trans* mAzo** and a low association constant with ***cis* mAzo**. The differences in supramolecular interactions came from the different features of **mAzo** and azobenzene, such as the molecular sizes, shapes and hydrophilicity/hydrophobicity balance. The results of this research have established that the tetra-ortho-methoxy-substituted azobenzene and *β*-cyclodextrin spontaneously formed a strong host-guest supramolecular complex.

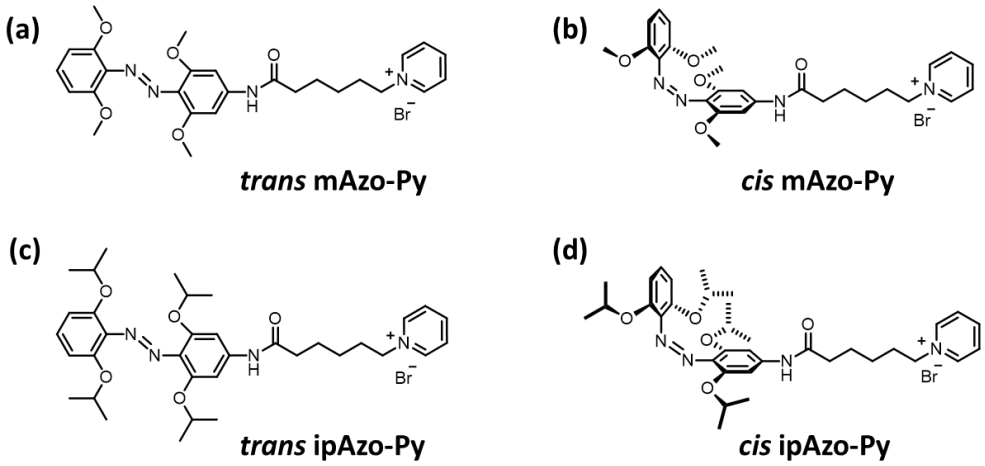


Figure 27 Molecular structures of *trans* and *cis* tetra-ortho-methoxy-substituted azobenzene (a,b) and tetra-ortho-isopropoxy-substituted azobenzene (c,d) isomers.

In another study, the authors synthesized a green-light-responsive tetra-ortho-isopropoxy-substituted azobenzene (**ipAzo**) with four larger substituent groups than **mAzo** (Figure 27c,d)[54]. A strong 1:1 host-guest complex was observed between ***cis* ipAzo** and γ-cyclodextrin (γ-CD), whereas no supramolecular complex was formed between ***trans* ipAzo** and γ-CD. In fact, γ-CD showed a higher association constant with ***cis* ipAzo-Py** compared to the *trans* isomer, while very low association constants were measured for both ***trans*** and ***cis* ipAzo-Py** with α-CD. (Table 3b).

(a)

Host	Guest *trans*-mAzo	Guest *cis*-mAzo
α-CD	145,2	66,9
β-CD	1546	82

(b)

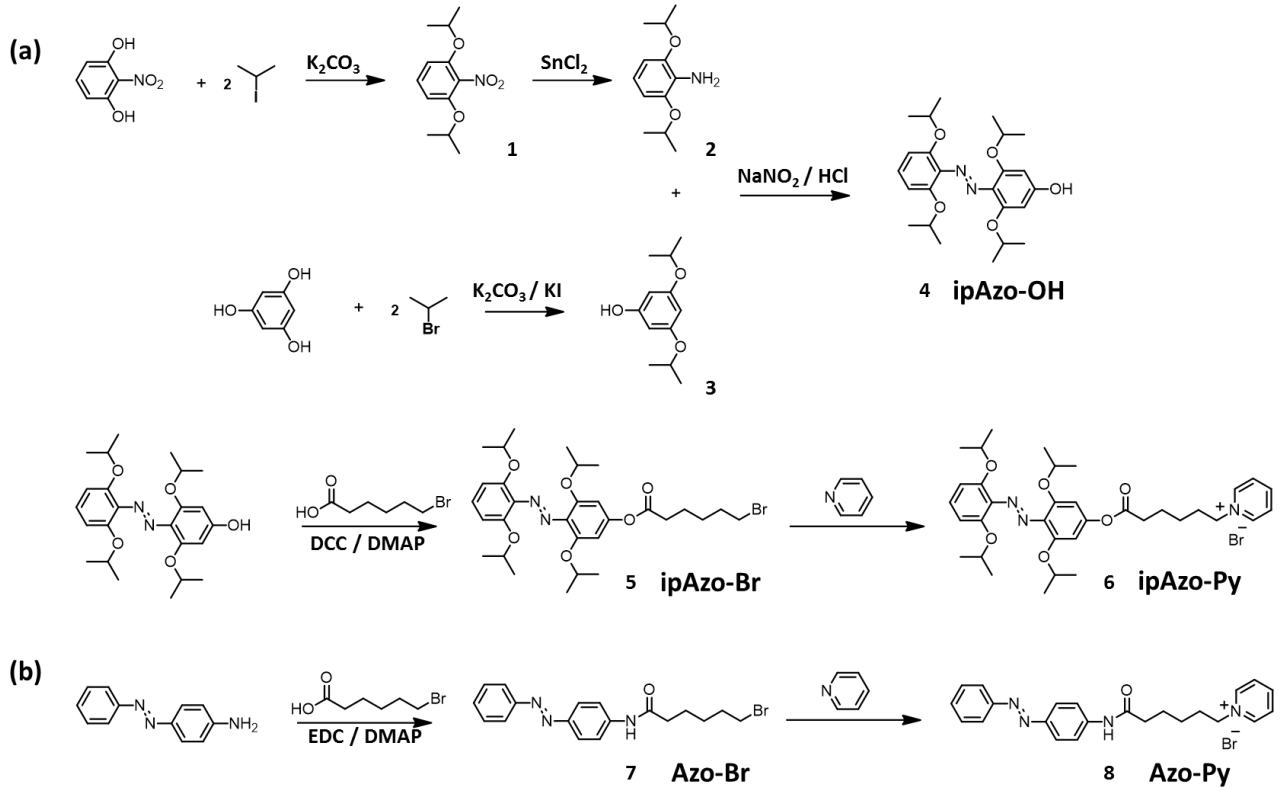
Host	Guest *trans*-ipAzo	Guest *cis*-ipAzo
α-CD	26,4±8,0	28,3±6,9
γ-CD	25,9±5,1	1659,0±57,0

Table 3 Association constants (K_a, M^{-1}) between α-CD, β-CD or γ-CD and **mAzo** (a) and **ipAzo** (b) isomers.

Using these results as a starting point, we planned to synthesize some of these molecules and apply them for expanding and improving the light-induced selectivity of CDs inside an enzyme-mediated dynamic library of CDs: the different CDs showed varying affinities for the *cis* and *trans* configurations and it is expected therefore, that the product distribution in cyclodextrin dynamic covalent libraries will be influences by the azobenzene-based photoswitch driven by the use of UV or visible light. The use of ***trans* mAzo-Py** as a template may significantly increase the production of β-cyclodextrin while the ***cis* ipAzo-Py** molecules could determine the amplification of γ-CD. Both expected results have been hypothesized following the study of the values of the association constants between the CDs and the azobenzene molecules reported in the literature. In this way, the use of artificial templates to selectively access different specific products from a target-direct dynamic

combinatorial library allows to redirect the outcome of enzymatic reaction exploiting the molecular recognition between the light-responsive template and the species in solution.

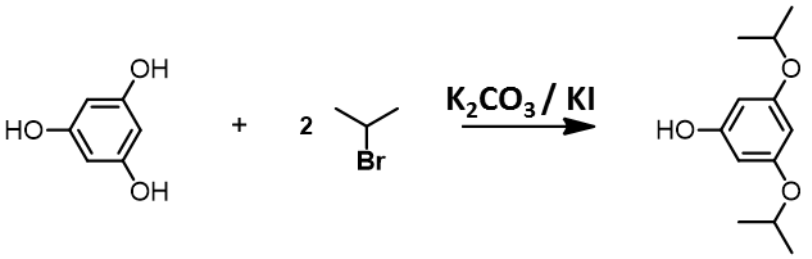
During my Erasmus visit to the lab of prof. Beeren in Copenhagen (Denmark) I started the synthesis of **ipAzo-Py** and **Azo-Py** but, due to the Covid-19 emergency, the synthesis had to be interrupted before reaching the final products. The synthetic route followed are reported in Scheme 4 and the experiments had to stop on the synthesis of products **2** and **3** for the **ipAzo-Py**, and product **7** for the **Azo-Py**.



Scheme 4 Route for the synthesis of **ipAzo-Py** and **Azo-Py**.

3.1.2. Synthesis of ipAzo-Py

3.1.2.1. Synthesis of 1,3-diisopropoxy-2-nitrobenzene (n°1)



Scheme 5 Synthesis of 1,3-diisopropoxy-2-nitrobenzene.

The first step in the synthesis of tetra-ortho-methoxy-substituted azobenzene (Scheme 5) was a bimolecular nucleophilic substitution reaction (Williamson ether synthesis) between 2-iodopropane and 2-nitroresorcinol conducted under basic pH for potassium carbonate. The reaction was carried out in DMF at 90°C for 24 h and the residue was extracted with ethyl acetate and subsequently purified by dry-column chromatography (crude product adsorbed on Celite, eluent *n*-heptane and EtOAc with 3% gradient of EtOAc). The product was obtained as a pale-yellow solid with an 81% yield and the $^{1}H/^{13}C$-NMR and HPLC-MS analyses confirmed the expected molecular structure (Figure 28 and Appendix A).

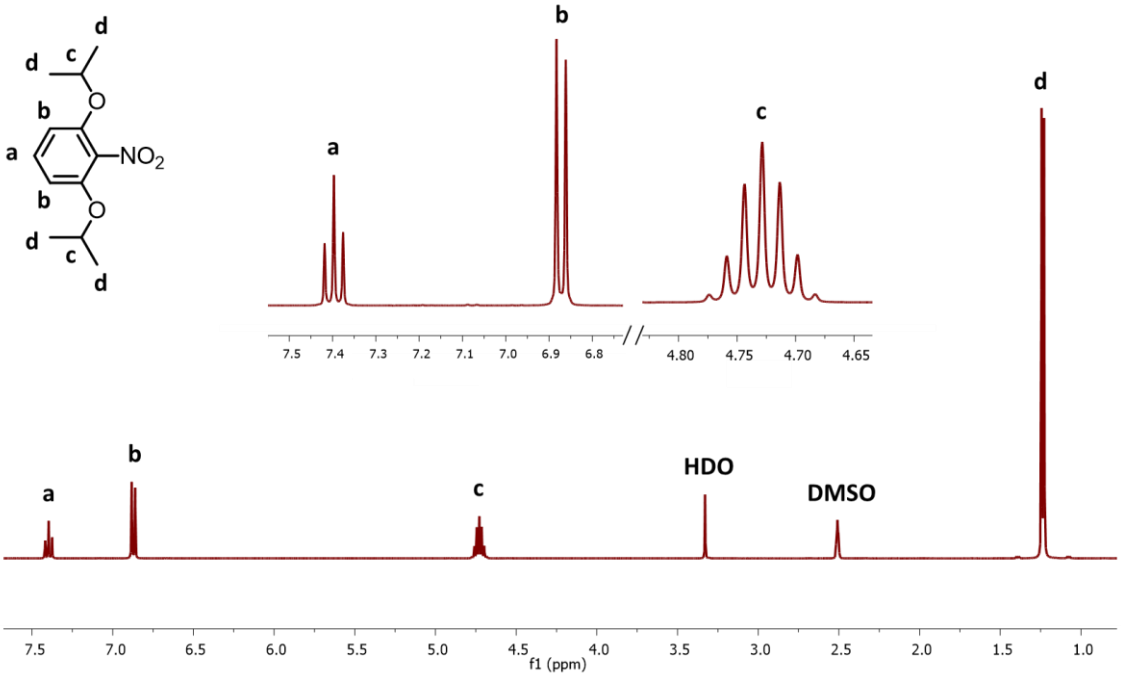
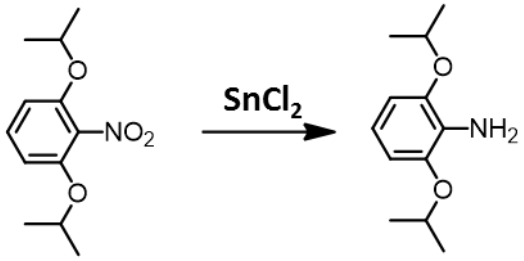


Figure 28 ^{1}H-NMR spectrum (400MHz in DMSO-d_6 at 298 K) of 1,3-diisopropoxy-2-nitrobenzene.

3.1.2.2. Synthesis of 2,6-diisopropoxyaniline (n°2)



Scheme 6 Synthesis of 2,6-diisopropoxyaniline.

In order to reduce the aromatic nitro compound to the corresponding aniline derivate, 1,3-diisopropoxy-2-nitrobenzene was pre-dissolved in dry ethanol and reacted with anhydrous Sn(II) chloride and hydrochloric acid at 80°C for 20 h (Scheme 6). The formation of the product was verified through thin layer chromatography with ninhydrin as specific TLC stain for amines. After aqueous quenching, the product was extracted at pH 11 for NaOH with dichloromethane (Sn hydroxide was separated by filtration on Celite). The product obtained was not purified because all the 1,3-diisopropoxy-2-nitrobenzene reacted only after a further addition of anhydrous Sn(II) chloride. The final product obtained in 80% yield appeared as a yellow-solid and was characterized by $^{1}H/^{13}C$-NMR and HPLC-MS. Numerous difficulties have been encountered in the isolation process due to the presence of the residual nitro compound as unreacted starting material. These difficulties are due to the similar molecular structure of the reagent and the product, and to the polarity of both functional groups and the interactions established with the stationary phase. Between the basic amino groups of the product and the silica groups (stationary phase) there is a natural attraction that can influence the chromatographic separation of the compounds. Sometimes it is necessary to use a competing amine such as triethylamine in the eluant, to inhibit such interactions: the amine molecules temporarily interact with ionized silanol groups with greater affinity than other molecules, in order to protect/deactivate these groups and allow an efficient separation. Unfortunately, in this case the use of triethylamine in the eluent mixture did not allow the separation of the two compounds. To avoid this problem, the reduction of the nitro compound was monitored with HPLC-MS allowing to bring the reaction to completion by successive additions of tin(II) chloride (ratio between reducing agent and starting material equal to 1:6).

The effective reduction of the nitro group was confirmed by ^{1}H-NMR and HPLC-MS analyses: in the ^{1}H-NMR spectrum of 2,6-diisopropoxyaniline (Figure 29) it is clearly distinguished the peak at 4.02 ppm diagnostic for the protons of the amino group and the aromatic protons are characterized by a lower chemical shift with respect to the nitro compounds due to the increased electron density of the

aromatic ring after reduction. Moreover, the molecular structure was further confirmed by the exact mass of the amino compound identified by HPLC-MS analysis (Appendix A).

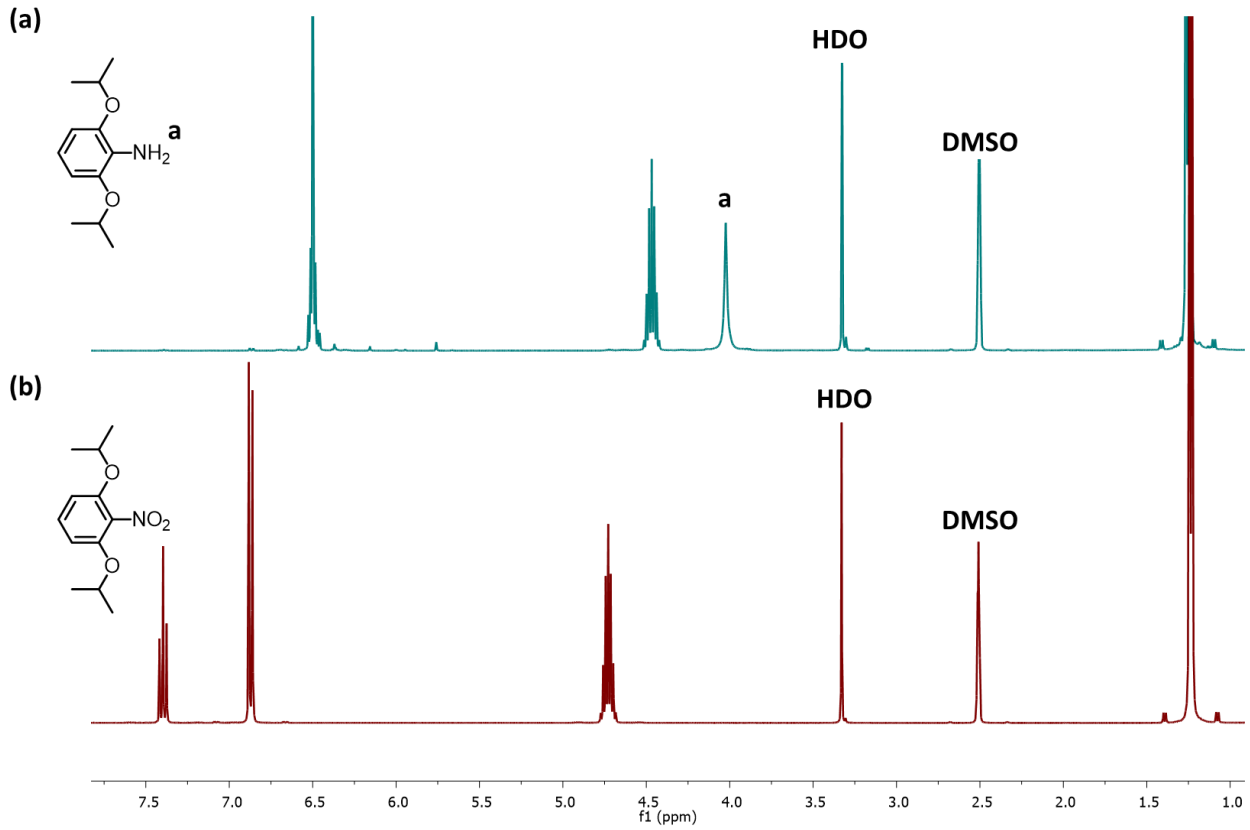
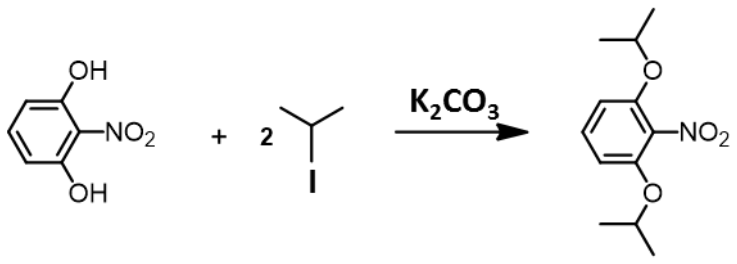


Figure 29 ^{1}H-NMR spectra (400MHz in DMSO-d_6 at 298 K) of 2,6-diisopropoxyaniline (a) and 1,3-diisopropoxy-2-nitrobenzene (b).

3.1.2.3. Synthesis of 3,5-diisopropoxyphenol (n°3)



Scheme 7 Synthesis of 3,5-diisopropoxyphenol.

The 3,5-diisopropoxyphenol was synthesized following a procedure similar to that used in the synthesis of product n°**1** (Scheme 5). The Williamson etherification reaction of phloroglucinol was carried out in a basic environment for potassium carbonate in dry DMF. Differently from the synthesis of the nitro compound, in this case the alkyl halide was added dropwise in order to control the degree of substitution of the phloroglucinol and to interrupt the reaction once the *bis*-substituted product was obtained. To monitor the course of the reaction, TLCs were carried out using $FeCl_3$ as staining agent.

The reaction mixture was stirred at 50 °C for three days under nitrogen inert atmosphere. Dry-column chromatography of the residue on silica, eluting with *n*-hexane and EtOAc (0.1% of EtOAc) provided two different fractions, one as a pale-yellow oil and one as a yellow solid. The products were characterized by ^{1}H-NMR and HPLC-MS analysis (see appendix A). The HPLC-MS analysis of the reaction mixture showed the presence of the expected *bis*- and *tris*-alkylated products which represented a promising result. Nevertheless, the NMR analysis carried out on both collected fractions did not confirm the desired molecular structures: the multiplicity and chemical-shift signals did not match with those of the expected products when compared with literature characterizations [2].

Analyzing the ^{1}H NMR spectra in the range 6.3-4.3 ppm, for the first fraction of the eluate (Figure 30, spectrum **a**) it is possible to distinguish three aromatic signals (two doublets at 6.07 and 5.94 ppm, and one singlets at 6.05 ppm), and three heptets, at 5.25, 4.56 and 4.49 ppm, respectively. Differently, in the spectrum of the second fraction (Figure 30, spectrum **b**), there were only one aromatic signal at 6.08 ppm, and two heptets at 5.28 and 4.49 ppm, respectively. In the chemical shift interval between 1.1 and 1.5 ppm (highlighted in the ^{1}H NMR spectra), in both spectra two types of doublets can be distinguished, belonging to the methyl protons of the isopropyl and isopropoxy groups.

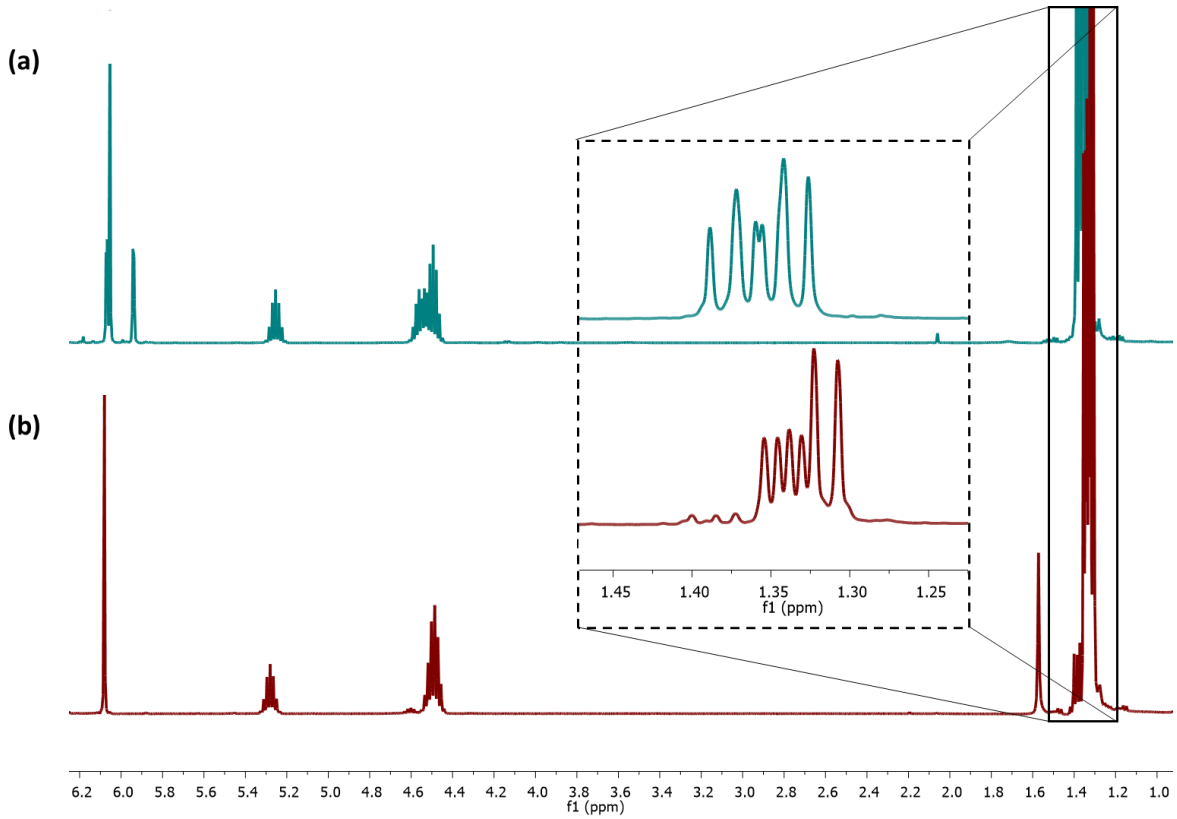


Figure 30 ^{1}H-NMR spectra (400MHz in $CDCl_3$ at 298 K) of the two products obtained from the synthesis of 3,5-diisopropoxyphenol: first (a) and second fractions (b) obtained from the dry-column chromatography.

From the spectra it is likely that fraction **a** contains probably more than one product, while fraction **b** seems to be due to a single product. A possible explanation for the observation of unexpected chemical shifts and multiplicity for the isolated fractions would be that the alkylation reaction occurred not exclusively on the O atoms, but also directly on the aromatic ring by electrophilic aromatic substitution. This hypothesis provides a possible explanation for the different spectra observed with respect to literature characterizations, but at the same time in agreement with MS analyses that confirmed the presence of *bis*- and *tris*-alkylated products. Ring alkylation could be due in particular because of the very high electron density of the aromatic ring bearing oxygen electron activating units, further increased by deprotonation of the phenolic moieties. Possible structures for the unexpected alkylation products are reported in Figure 31.

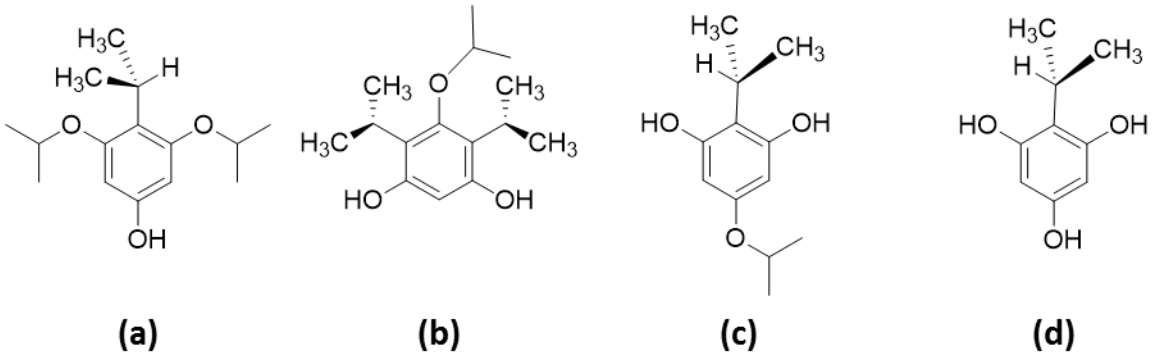


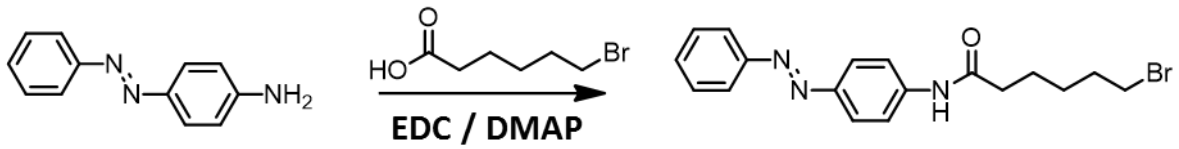
Figure 31 Representation of the possible molecular structures derived from the synthesis of 3,5-diisopropoxyphenol

If we consider molecule (**a**) as one of the possible tris-alkylated products, due to the steric hindrance of the two isopropoxyl groups, the isopropyl group directly connected to the aromatic ring could not rotate around the carbon-carbon bond, leading to an atropoisomeric structure. Remaining in the aromatic plane, because of the anisotropy of the magnetic field induced by the benzene ring, the methyne CH proton of the isopropyl group should be more deshielded than the protons of the CH isopropyl groups on the O atoms. For this effect, its resonance frequency could be higher, justifying the heptet observed at 5.2 ppm. Furthermore, the anisotropy of the molecule defines the signals of the aromatic protons as two doublets with "roof effect" since each proton will be affected by the orientation of the isopropyl group directly bound on the aromatic ring leading to different local environments. The 6.0 ppm singlet can describe aromatic protons of a symmetric non-atropoisomeric molecule like (**b**) while the heptet resonance at 4.5 ppm may be characteristic of an isopropoxyl group's proton. Two extras possible alkylated side products are reported in Figure 31 as structures (**c**) and (**d**).

Overall, due to shortage of time for the synthesis caused by the COVID-19 pandemia, it was not possible to repeat the reaction. The result obtained was not as expected the formation of two new ether bonds, but rather a combination of ether formation and aromatic ring alkylation, giving rise to a complex mixture of products. This point will need further investigation since for the reaction a literature procedure was strictly followed even though a very different final result was observed [54]. All the analysis and the ^{1}H-^{1}H COSY are reported in Appendix A.

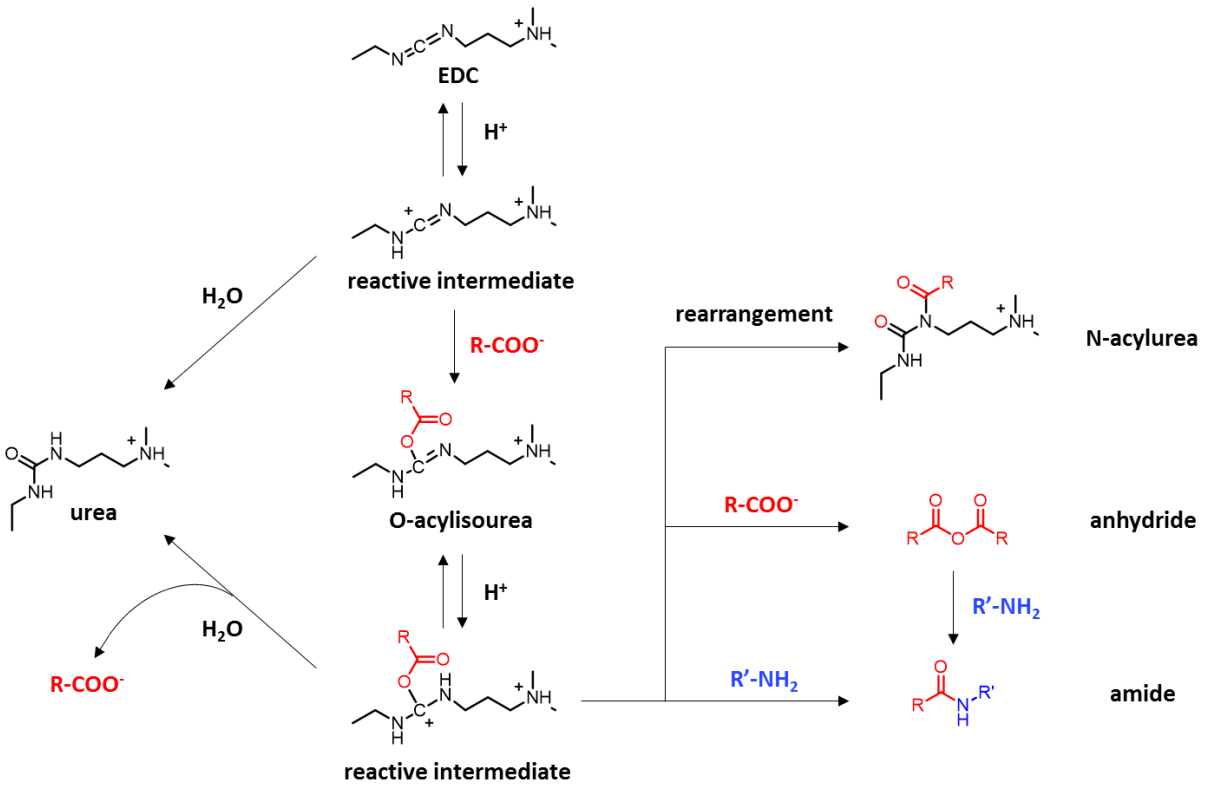
3.1.3. Synthesis of Azo-Py

3.1.3.1. Synthesis of Azo-Br (n°7)



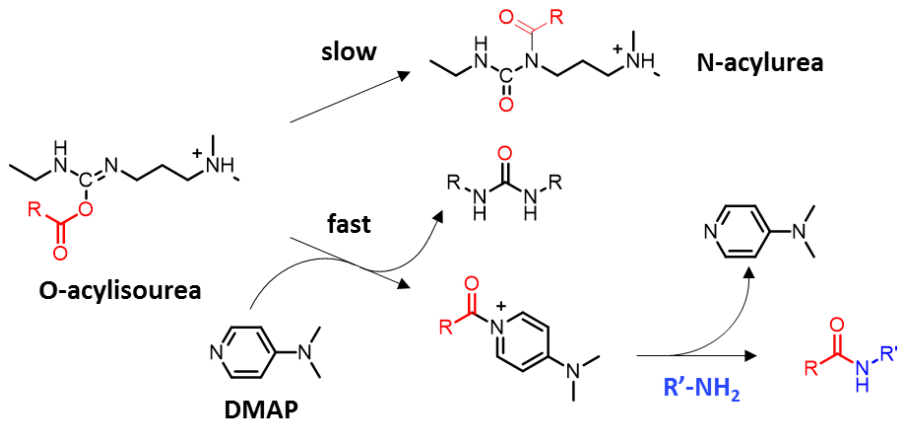
Scheme 8 Synthesis of 6-bromo-N-(4-(phenyldiazenyl)phenyl)hexanamide.

The preparation of **Azo-Py** started from commercial 4-aminoazobenzene that was subjected to a coupling reaction with 6-bromohexanoic acid using 1-Ethyl-3-(3-dimethylaminopropyl carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP) as coupling system (Scheme 8). In this process, the aniline derivative was converted into the corresponding amide. We used EDC to activate the carboxylic group of 6-bromohexanoic acid and DMAP to promote the formation of the most stable amide. In fact, one of the main problems in the use of EDC is the large number of competing side reactions with many secondary paths, which could lead to the formation of undesired by-products as detailed in Scheme 9.



Scheme 9 Reaction paths for EDC coupling.

The main side-reaction that could take place is a 1,3-rearrangement of the *O*-acyl intermediate to an *N*-acyl-urea which cannot further react with the amine. In order to increase the efficiency of EDC coupling and to limit undesired reactions, DMAP was used to form an activated acyl-pyridinium derivative and suppress side reactions, acting as an acyl transfer-reagent as described in Scheme 10.



Scheme 10 Schematic representation of the use of DMAP to increase the efficiency of EDC coupling.

DMAP, as a strong nucleophile, reacts with the *O*-acyl-isourea leading to a reactive amide. This intermediate cannot form intramolecular side products but reacts rapidly with the amino moiety of the 4-aminoazobenzene to give the desired amide-product.

The activation of the carboxylic group of 6-bromohexanoic acid was carried out in dry dichloromethane at 0 °C. Subsequently, at specified time intervals, always keeping the reaction at 0 °C, 4-aminoazobenzene, DMAP and a solution of EDC in DCM were added. The reaction mixture was stirred at 0 °C for three days at rt. Due to the lockdown, it was possible to perform only the HPLC-MS (Figure 32) and NMR (^{1}H-NMR and ^{1}H-^{1}H COSY are reported in appendix A) analyses on the reaction mixture before the purification step by dry-column chromatography, which confirm the formation of the desired product.

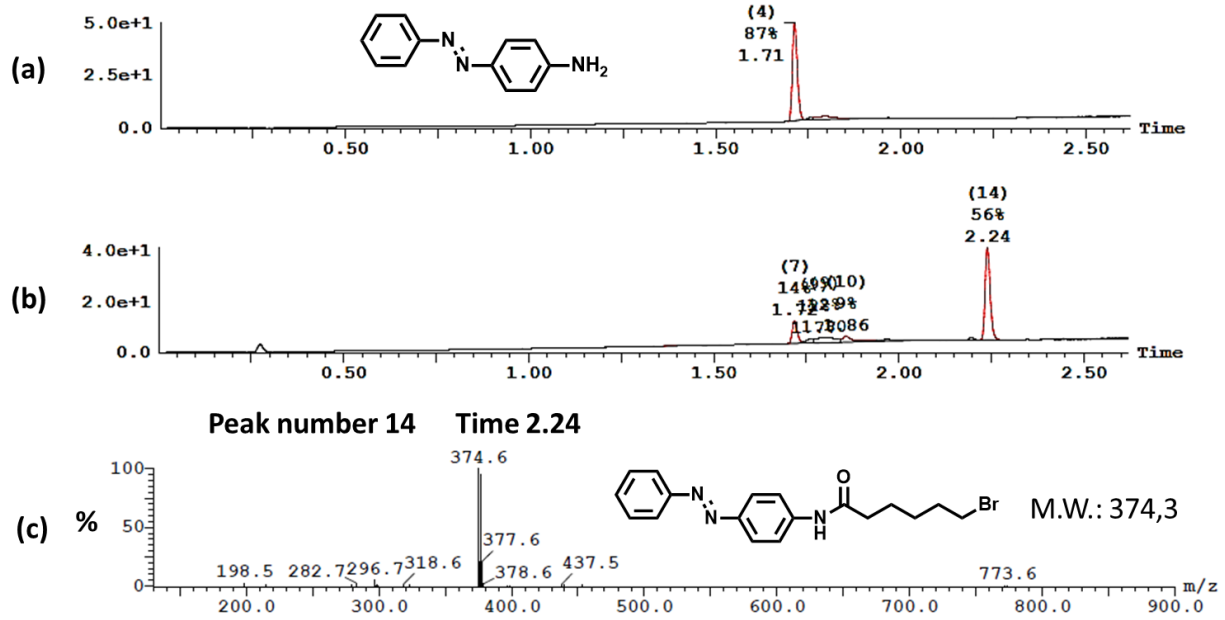


Figure 32 HPLC-MS analyses of the starting reagent and the crude product: (a) chromatogram of 4-aminoazobenzene; (b) chromatogram of the crude product; (c) mass analysis of peak number 14 characteristic of the 6-bromo-N-(4-(phenyldiazenyl)phenyl)hexanamide.

3.2. Design and applications of Azobenzene-CD-based supramolecular systems

Despite being a relatively young field of research, supramolecular chemistry has become a very interest tool in the last few years and contributed to the development of numerous technologies and research subfields such as molecular self-assembly, molecular recognition, sensor design, template-directed synthesis, mechanically interlocked molecular architectures, and molecular machines. Among the frequently encountered photochromic molecules, azobenzene and its water-soluble derivatives are regarded as ideal candidates for the construction of CD-based photoresponsive supramolecular architectures with numerous and practical applications. To date, Azo-CD-based stimuli-responsive supramolecular systems have been widely studied, providing more than four hundred publications (Scopus search on July 2020). In this thesis, it was not possible to report all the works that characterize this increasingly significant interdisciplinary field of research, therefore we restricted the review to the most recent publications, specifically the period 2018-2020 which correspond to the latest publications after the last review appeared in the literature. The contributions presented in this chapter clearly demonstrate the numerous opportunities and practical applications that may arise from the diversity and complexity of Azo-CD stimuli response systems, allowing a continuous development of the implementation of self-assembling smart supramolecular systems.

3.2.1. Azo-CD photoresponsive delivery systems

The non-specific biodistribution of cytotoxic drugs and the associated adverse effects that may arise, often generally limit the efficacy of pharmacological treatments. Using the inclusion complexes between azobenzene molecules and CDs, it is possible to deliver drugs to the cells and tissues, controlling in a targeted and specific way their release by means of light radiations. For this purpose, light-responsive supramolecular materials such as self-assembled micelles, polymers and nanoparticles that can be used thanks to their adjustable on-off properties.

3.2.1.1. Polymeric nanoparticles

Polymeric nanoparticles are biodegradable and non-toxic materials composed of various kinds of polymers either natural or synthetic. These nano-size solid particles with sizes in the range 10 nm–1μm are very popular delivery systems with potential applications in tissue engineering, drug and gene delivery. Zhang and collaborators developed dual-responsive supramolecular polymeric nanoparticles based on poly(α-CDs) and acetal-modified β-CD-azobenzene molecules through the host-guest interactions between α-CD and azobenzene (Figure 33a)[55]. In the aqueous medium, the inclusion complexes further self-assembled to form supramolecular nanoparticles. This system is a

dual stimuli-responsive nano-vehicle for drug-controlled release: if stimulated with UV-light (responsive azobenzene moiety) or by a change of pH (responsive acetal), it could release drugs previously loaded into the *β*-CDs at specific time (UV) and location (pH).

In 2019, Hu and collaborators designed light-responsive nanoparticles consisting of two types of polymers: a poly-azobenzene polymer was used as a stimuli-responsive and a self-assemble unit and a poly(*β*-CD) polymer was employed to construct the nanoparticle by host-guest interactions [56]. Moreover, *β*-CDs were functionalized with folic acid (a target fluorescent molecule with specific protein-binding property) for precise delivery of anticancer drugs. Using light and pH it was possible to modulate the geometric characteristics of the self-assembled systems and consequently the vehicular properties of these systems: after UV irradiation, the self-assembled NPs became more compact (due to the *trans* to *cis* isomerization) and in the acid medium the nanoparticles aggregated, causing a decreased diffusion of drugs in solution (Figure 33b). A similar strategy was used by Song and collaborators in 2020 [57]. Using the host–guest interactions between azobenzene and *β*-CD conjugated to poly (L-glutamic acid)-graft-poly (ethylene glycol) methyl ether (PLG-g-mPEG) to develop NPs, the authors loaded a specific anticancer drug in the nanoparticles. Once reached the cellular environment, the Azo moieties of the NPs could be reduced to aniline derivatives under the catalytic action of nitroreductase (NTR). This class of enzymes is highly expressed in the hypoxic environment of cancer cells, causing the breakage of the nanoparticle structure with the consequent release of the drug (Figure 33c). In this case, the pharmacological action is not light-dependent, and no isomerization occurred.

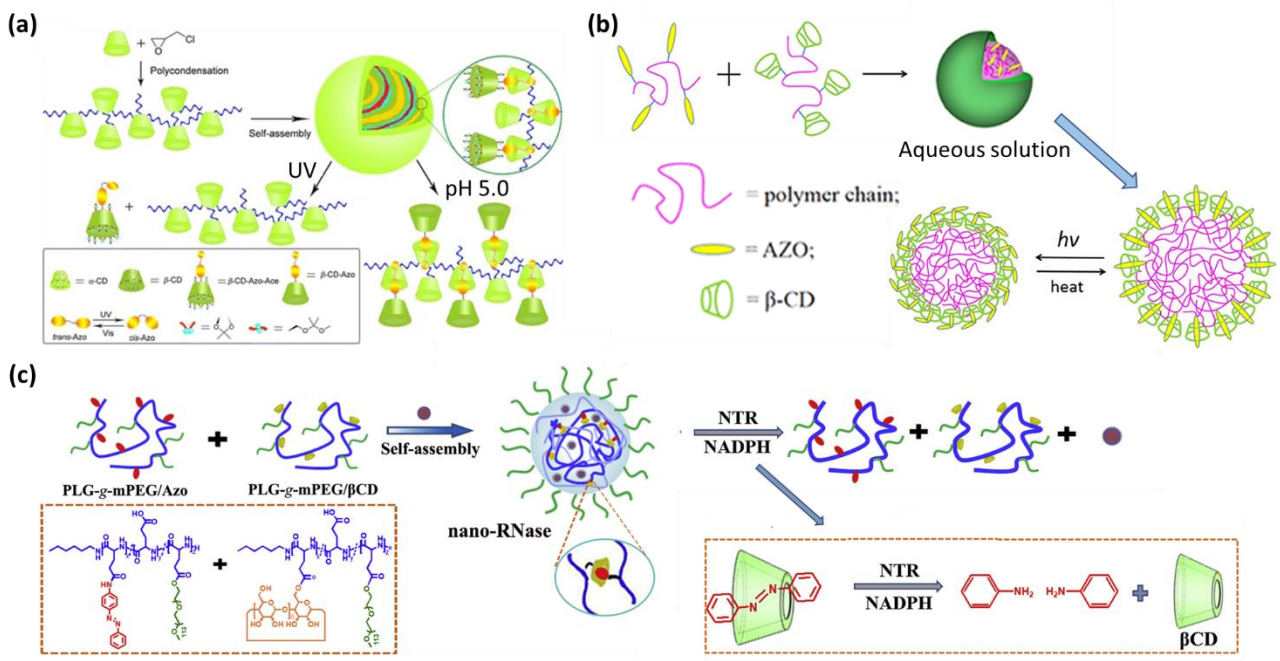


Figure 33 (a) Schematic illustration of the formation of dual stimuli-responsive supramolecular polymeric nanoparticles based on poly(*α*-cyclodextrin) and *β*-CD-Azo-Ace [55]; (b) Self-assembly and UV response mechanism for the light-responsive self-assembled nanoparticles [56]; (c) Schematic illustration of the nano-RNase formation through supramolecular interactions between Azo and *β*-CD grafted onto PLG-g-mPEG and its release mechanism[57].

Always using an external non-radiant stimulus to activate the release of drugs, Fan and collaborators developed an extraordinary target-specific tissue-depth-independent photoisomerization strategy for detecting and simultaneously carry anticancer drugs within cancer cells *in vivo* [58]. They exploited the higher concentration of H_2O_2 in tumours with respect to normal tissues, to activate a chemiluminescent substrate (CLS) which triggers the isomerization of the Azo groups, allowing the release of the previously loaded active principles. In this case, the drug loaded into the system is a chemiluminescent fluorophore (camptothecin, CPT). The host-guest nanoparticles are composed by an azobenzene-pendant polymer and a CD-pendent polymer, and the resulting inclusion complex allows the encapsulation of both molecules. Once the self-assembled system reacts with the hydrogen peroxide molecules inside the cell, the induced chemiluminescence of the CLS isomerize the azobenzene molecules, triggering the partial dissociation of the host–guest carriers and the CPT release. Subsequently, the initially released CPT molecules act as H_2O_2 enhancer to induce a higher hydrogen peroxide level in a tumour cells, establishing a positive feedback mechanism (Figure 34a). This self-luminescence source could precisely activate the Azo-isomerization and drug release, with the specificity to select a target site through high H_2O_2 levels in diseased tissues. With the in-situ-enhanced self-luminescence method it is possible to reach any tissue within the body and carry any type of molecule in a targeted way, without using external light stimuli.

Using a supramolecular nano-assembly composed of α-CD-modified hyaluronic acid and an azobenzene-modified diphenylalanine derivative with a positively charged imidazole group, in 2020, Liu and co-workers developed a highly efficient system to transport RNA based drugs inside the cancer cells (Figure 34b)[59]. Host-guest complexes between the Azo group and CDs allowed the formation of the nanomaterial, which acted as a support to convey the drug molecules: RNA molecules could bind with the imidazole group through electrostatic interactions and their release could be triggered by UV irradiation, allowing an effective delivery into cancer cells and inhibiting their growth.

Another type of nanoparticles usable as drug delivery systems, are those developed by Pu and co-workers [60]. They formulated an amphiphilic copolymer with hydrophilic poly(ethylene glycol) side chains and hydrophobic azobenzene pendant groups (Figure 34c): in aqueous solution, these amphiphilic copolymers could undergo single-chain folding via intramolecular hydrophobic interaction and form single chain polymer nanoparticles (SCPNs). After the addition of CD, the Azo-CD complex could be formed on the pendant groups, making the amphiphilic copolymers hydrophilic and causing the chain unfolding, with the subsequent release in solution of hydrophobic molecules eventually previously loaded inside the nanoparticles.

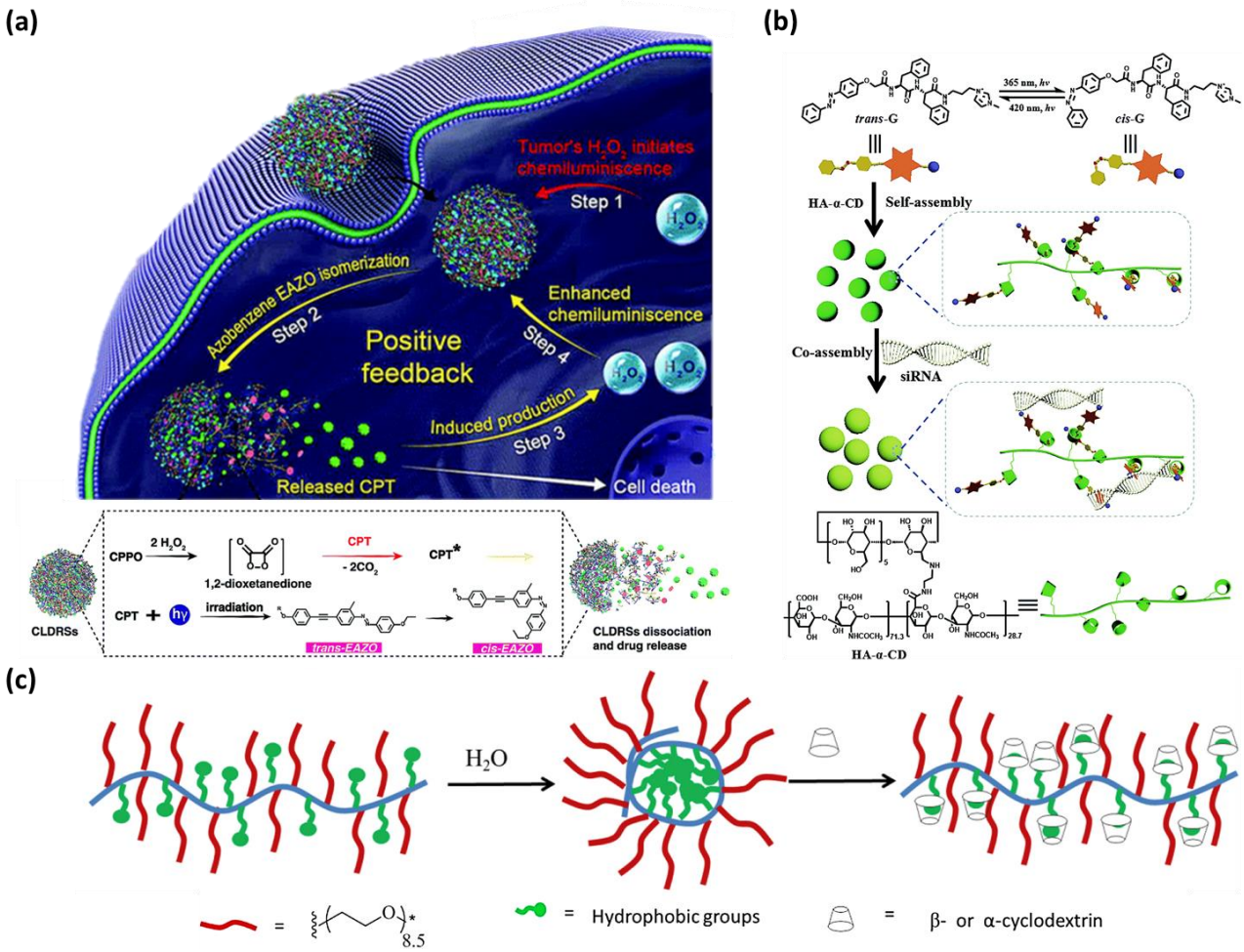


Figure 34 (a) Illustration of the positive-feedback-promoted H_2O_2 production mechanism and amplified isomerization mechanism of EAZO in cells due to the CL-triggered drug release of CLDRSs [58]; (b) Schematic illustration of the trans-G/HA-*α*-CD/siRNA ternary supramolecular nanoassembly [59]; (c) Schematic illustration of the single-chain polymer nanoparticle folding and unfolding mechanism triggered by cyclodextrin[60].

3.2.1.2. Photoresponsive self-assembly systems

Core-shell nanocarriers, containing hydrophilic shells and hydrophobic cores, are widely used for the targeted transport of drugs, due to their advantageous properties. For instance, the hydrophilic shell may ensure prolonged circulation of the carrier into the bloodstream, and the hydrophobic core could increase the efficiency of the drug loading. For this purpose, numerous supramolecular polymer micelles have been developed: through the creation of the host-guest complexes, it was possible to convey drugs within these systems or the same systems may be made up of prodrug molecules (supramolecular prodrug complexes) and perform therapeutic activities as a result of external stimuli such as light, pH changes or for the breaking of specific bonds.

In 2019, Yang's group developed a dual-responsive supramolecular drug carrier based on a *β*-CD amphiphilic polymer for anticancer drug delivery [61]. They used PEG as hydrophilic portions (PEG

and β -CD are linked by disulfide bonds) and poly(ε-caprolactone) chains with azobenzene groups modified on one end as hydrophobic portions (Figure 35a). This system displayed a quick drug release in the presence of two stimuli: light and glutathione (GSH, a tripeptide with antioxidant properties). The effect due to GSH was attributed to the breaking of the disulfide bonds between PEG and β-CD, while the light response, for the simple host-guest interaction between β-CD and azobenzene. Another example of dual-responsive supramolecular micelles is the one developed by Luo and co-workers in early 2020 [62]. They integrated a pH responsive functional groups into supramolecular polymers to change the physicochemical properties of the drug-loaded micelles. These systems are stable in normal physiological conditions and dissociate in acidic environments or under UV-light, thus achieving the specific release of drugs. Both the dual-responsive supramolecular polymers described above are nontoxic and can encapsulate doxorubicin (DOX), a chemotherapy drug for the treatment of many types of tumours, showing a remarkable anticancer activity due to the photo- and pH responsive properties. Indeed, the pH of tumour microenvironments is slightly lower than that of normal human tissues and this property enabled a further stimulus for the targeted release of these specific drugs (Figure 35b).

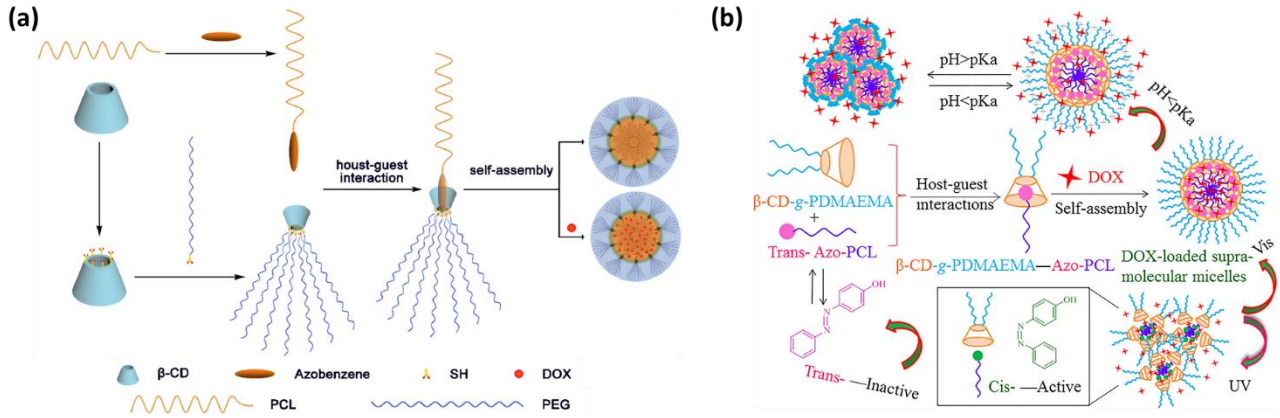


Figure 35 (a) Schematic illustration of the synthesis of dual-responsive supramolecular drug carrier based on β-CD amphiphilic polymer [61]; (b) Schematic illustration of the synthesis and pH- and light-induced drug release mechanism of DOX-β-CD-g-PDMAEMA-Azo-PCL supramolecular self-assembly micelles [62].

Conversely, using a near-infrared (NIR)-sensitive supramolecular conjugated micelles, Zhu's group successfully delivered DOX to tumour site, leading to an efficient anticancer therapy [63]. They developed a host–guest system between a β-CD-grafted hyperbranched conjugated polymer and azobenzene-functionalized poly(ethylene glycol) to build micellar systems and load the drug into the hydrophobic cores (Figure 36a). The photoisomerization of Azo groups from *trans*- to *cis*-isomer was triggered by NIR light via a TP-FRET mechanism (two-photon excited fluorescence resonance energy transfer), which causes the micelle disassembly (Figure 36b). The NIR light is preferable to UV light because UV has a low penetrability through tissues and high cytotoxicity in normal cells.

To avoid the limitations of UV excitation, this system uses NIR light: the hyperbranched conjugated polymer act as a donor and the Azo moiety as an acceptor. Doxorubicin can also be conveyed by linking it directly to CD functionality, realizing self-assembly supramolecular prodrug complexes, through host-guest interactions. Bai and co-workers synthesized a prodrug β-CD-acylhydrazone-DOX and the azobenzene-terminated poly(2-(dimethylamino)ethyl methacrylate) molecules to obtain self-assembles multi-compartment vesicles [64]. The self-assembly morphology transition may be activated with UV light irradiation, generating the formation of complex micelles and a slow release of DOX in solution. Moreover, in the acid environment, the release rate is increased as a result of the breaking of acylhydrazone bond between DOX and β-CD, determining a dual-responsive behaviour (Figure 36c).

Self-assembly systems, as seen above, can be used for the transport and for the target release of specific molecules. Moreover, using the host-guest mechanism, it is possible to fabricate novel vesicles with enantioseparation abilities for the enantioselective release of chiral drugs. Recently, Liu and co-workers described a novel type of M-helix based vesicle derived from the self-assembly of α-CD and azobenzene-containing M-helical quinoline oligoamide foldamer in aqueous solution (Figure 36d)[65]. This kind of vesicle not only showed light-responsive disassembly–reassembly structural transformation due to the isomerization of the Azo-group, but also exhibited enantioselective release performance to racemic drugs thanks to the characteristics of homochiral foldamers in enantiomeric separations. (Figure 36e).

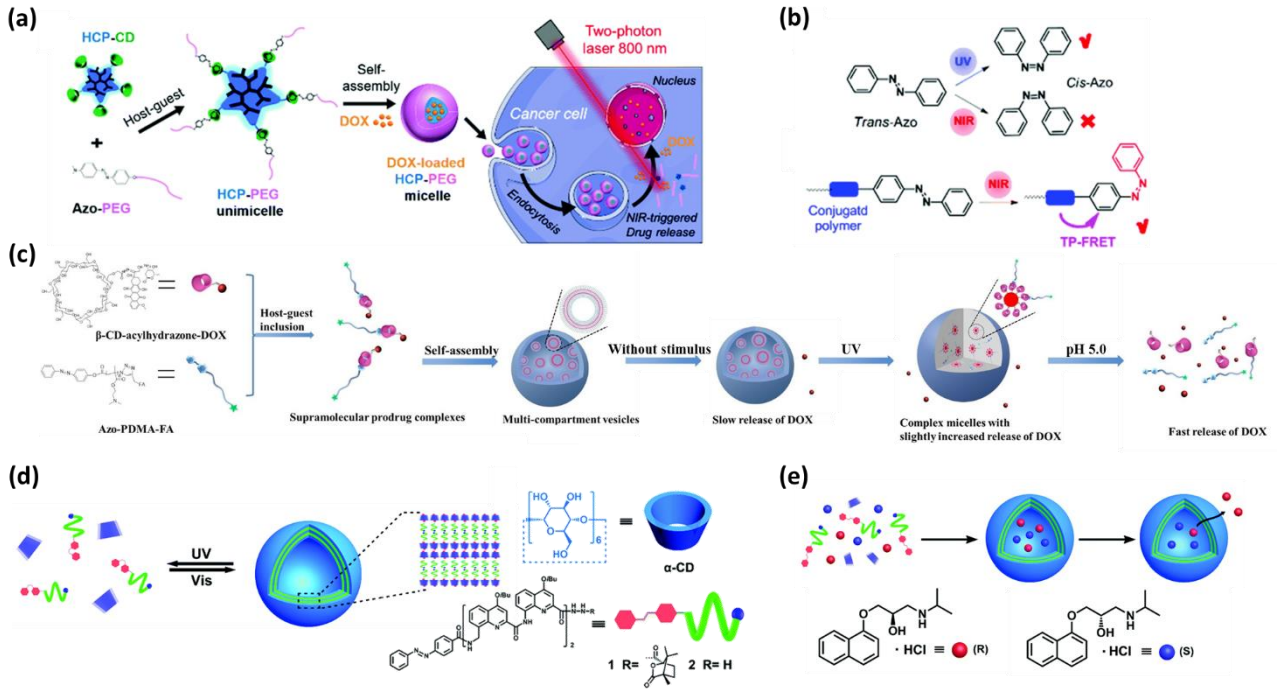


Figure 36 (a) Preparation of supramolecular conjugated HCP-PEG micelles and their NIR-triggered drug release in cancer cells [63]; (b) Schematic illustration of the Azo unit photoisomerization induced by UV light, and NIR light via two-photon (TP)-excited FRET of a conjugated polymer [63]; (c) Schematic illustration of the morphology transition of supramolecular self-assemblies prodrug complexes and their programmed drug release behaviors regulated by UV and pH stimuli [64]; (d) Schematic representation of the M-helix based vesicles and their light-responsive disassembly–reassembly behaviors [65]; (e) Schematic representation of the capture and enantioselective release abilities of the vesicles[65].

3.2.1.3. Light-responsive supramolecular nanovalves

Gated materials are porous materials designed to delivery chemical or biochemical species within the tissues in response to predefined stimuli. These systems are composed mainly of two subunits: a porous inorganic support in which a drug is loaded, and supramolecular entities (nanovalves), generally grafted onto the external surface, which can control mass transport from pores. Based on this concept, many drug delivery systems have been developed but, in this section, we will analyse the systems consisting of host-guest complexes between azobenzene and CDs.

Mesoporous silica nanoparticles (MSNPs) have attracted increasing interest as supports in the design of DDS. Besides their excellent properties as loading supports (large surface area and pore volume), the modification of their external surface with molecular or supramolecular ensembles allows the easy design of gated MSNPs. By filling a tiny, porous silica sphere with a drug and then plugging the pores with specific valves, it is possible to selectively control the release of the drug inside the tissues. The *cis-trans* photoisomerization of azobenzene has been widely applied in light-responsive

supramolecular nanovalves for controlling cargo release. For this purpose, Chen and co-workers developed a light/redox dual-stimuli responsive drug delivery system for targeted therapeutic applications [66]. They used MSNPs to load doxorubicin (DOX) inside the mesopores, which were subsequently capped by *β*-CDs via redox-sensitive disulfide bonds, and an azobenzene/galactose-grafted polymer (GAP) was introduced to functionalize the MSN surface, through host-guest interaction (galactose is a ligand for a specific receptor present in some cancer cells and improves the selectivity of the system). The release of DOX could be realized via dissociation of azobenzene moieties from the *β*-CD cage with UV-irradiation and the subsequent breakdown of the disulfide bonds between MSNPs and CDs (Figure 37a). Instead, in 2019 Bian and colleagues implemented this method using a polymer containing tetra-ortho-methoxy-substituted azobenzene as supramolecular valves, with the ability to activate the release of the drug using a visible light (Figure 37b)[67].

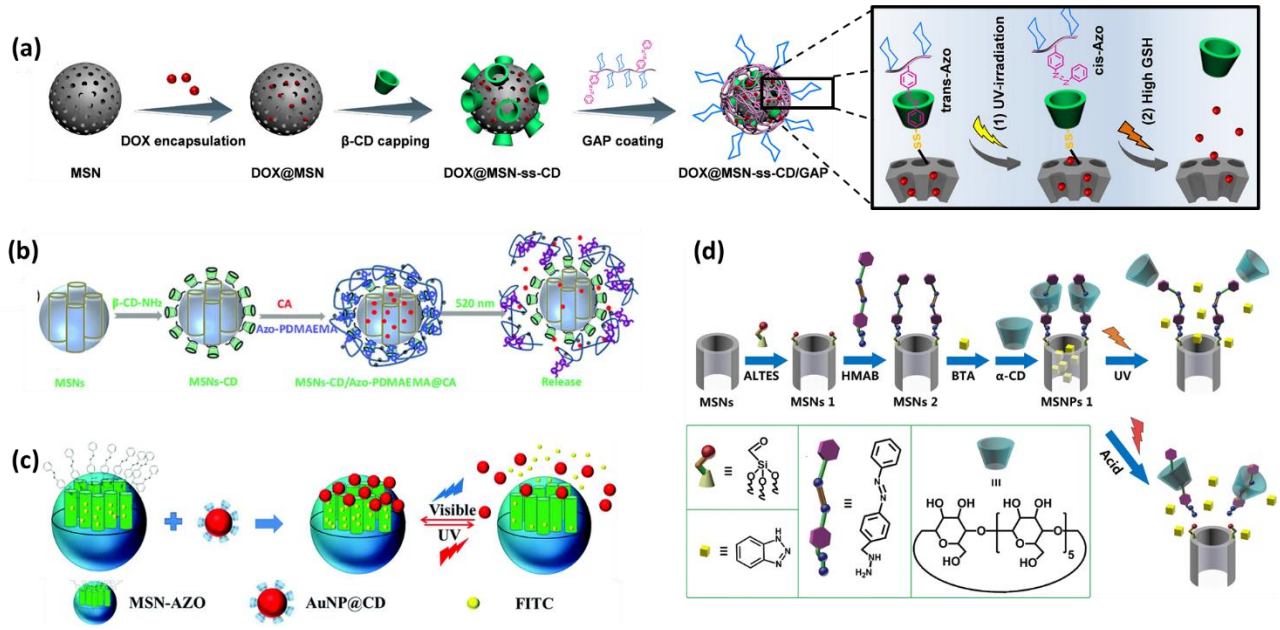


Figure 37 (a) Schematic representation of DOX@MSN-ss-CD/GAP synthesis and its UV/redox dual-triggered DOX release mechanism [66]; (b) Schematic illustration of visible-light triggered cargo release from MSNs-CD/Azo-PDMAEMA [67]; (c) Schematic illustration of the light-responsive controlled assembly system using mesoporous silica nanoparticles capped with Au nanoparticles [68]; (d) Schematic illustration of MSNPs assembly and their controlled molecules release [69].

Functionalizing MSNPs with azobenzene molecules, Yang and co-workers developed a light-responsive assembly system using MSNs-Azo and CD-functionalized Au nanoparticles [68]. Under visible light irradiation, the *trans*-azobenzene could bind *β*-CD on the AuNPs by host-guest interaction, closing the mesopores by the AuNP-CD caps and trapping the cargo molecules into the pores. Under UV irradiation, the azobenzene molecules changed configuration to the *cis*- form, leading to the disaggregation of the caps from the outer surface of the MSNs and releasing in solution

the drug from the pores (Figure 37c). Using this principle, in 2019, Fu's group reported a new dual stimuli-responsive system based on silica nanoparticles [69]. They used supramolecular pseudorotaxanes as nanovalves, consisting of azobenzene and α-CD anchored onto the surface of MSNs by hydrazone bonds. The encapsulated drug molecules could be released simultaneously from MSNPs due to the removal of the supramolecular switches: with UV light decoupling the host-guest complex, or under acid pH, where the acid-sensitive hydrazone bonds was rapidly hydrolysed resulting in the disconnection between pseudorotaxanes and MSNs (Figure 37d). Slight differences in pH between diseased and healthy tissue may allow for precise targeting of the drug and light-triggered controlled release may accelerate the releasing rate, with high potential applications in the DDS field.

These materials can also achieve the on-off drug release because the pores of MSNs could be opened or closed when stimulated by cycling UV or vis light irradiation. However, considering the limitations existing on the penetration depth of light, some DDS could be difficultly applied for drug release in deep tissue.

3.2.1.4. Supramolecular hydrogels as DDS

Hydrogels are widely used in biomedical applications, especially in tissue engineering, due to their good biocompatibility, tissue-like properties, and they allow the transport of nutrients and drugs. They can be fabricated from natural biopolymers, synthetic polymers, or their hybrids. This paragraph reports one of the latest applications in the design and synthesis of Azo-CD biomimetic hydrogels with the aim of regulating the material properties and the drug delivery behaviour. In the next chapters, other applications and types of hydrogels and polymers will be analysed.

In 2018, Rosales and co-workers developed a strategy to reversibly modulate hydrogel properties with light, using supramolecular cross-links formed between *trans*-azobenzene molecules and β-CDs (Figure 38)[70]. They used hyaluronic acid polymers functionalized with the host-guest molecules exploiting the extraordinary water retaining capacity and the ability to form interactions with a variety of cell-surface receptors of hyaluronic acid. The host-guest cross-links can be modulated with mild exposure to UV-light, determining to significant changes in mechanical properties without leading to a gel-sol transition of the material and allowing the release of trapped molecules within the 3D lattice. This result indicates an interesting way for dynamic control of hydrogels in the context of a biomimetic approach to material design and the possibility to tune the release of encapsulated molecules.

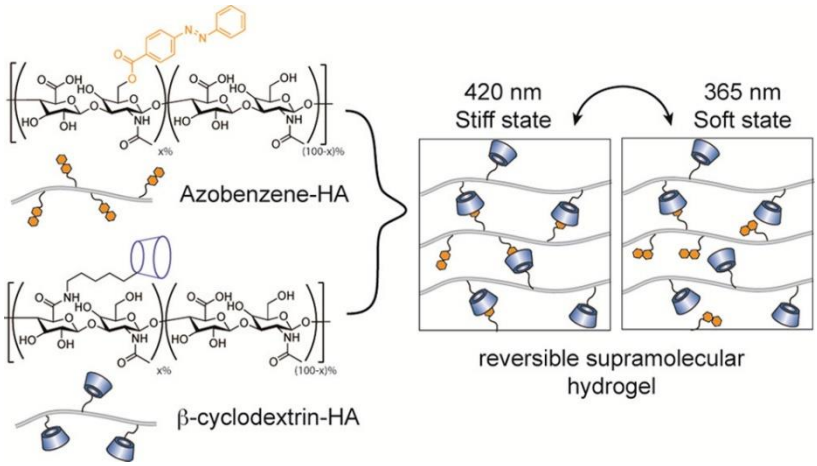


Figure 38 Photoresponsive supramolecular hydrogel system formed by Azo-HA/β-CD-HA host-guest complex [70].

3.2.1.5. Photoresponsive carbon-based materials

Carbon-based nanomaterials such as graphene, are currently considered to be some of the key elements in nanotechnologies and have recently been used to develop nanoplatforms for drug delivery systems. The dispersion of graphene in water has generally been considered a difficult challenge owing to its hydrophobic nature, but using the Azo-CDs inclusion complexes, it is possible to overcome this problem. In recent years, two strategies have been implemented for the development of graphene-based DDS. He and co-workers described an efficient method for the preparation of a light-responsive graphene composite by integration of *β*-CDs onto the surface of reduced graphene oxide (rGO) and the subsequent formation of host-guest complexes with amphiphilic Azo-molecules [71]. The graphene composite exhibits reversible dispersion/aggregation behaviour in aqueous solution triggered by visible and UV light irradiations due to the photoisomerization of the trans and cis forms of the azobenzene molecules (Figure 39a).

In 2018, Hu and colleagues applied a similar strategy to insert smart devices into tissues to protect the cells from oxidative stress [72]. After anchoring *β*-CDs onto graphene surface, they introduced *trans*-Azo-fullerene molecules (Azo-C60) to construct a graphene/C60 nanohybrid material with light-triggered drug release properties and excellent antioxidant activities. Indeed, fullerenes have been extensively used for several biomedical applications, including the photodynamic therapy and drug and gene delivery, but the limited solubility in water and the strong aggregation tendency of C60 derivatives, led to a reduction in its applications inside the cells. Using graphene/CD material as a versatile nanocarrier, it is possible to enhance the cellular uptake and the stability in aqueous environment of the fullerenes: the C60 molecules could loaded and unloaded onto the rGO/*β*-CD platforms in a controlled way by using UV-light irradiation (Figure 39b). This system represents an excellent strategy to combat the oxidative damage inside the cells using smart drug delivery systems.

Because host-guest chemistry is versatile and universally applicable, this nanoplatform could also be applied for the preparation of other photo-sensitive nanohybrids with numerous applications in the transport of different types of molecules.

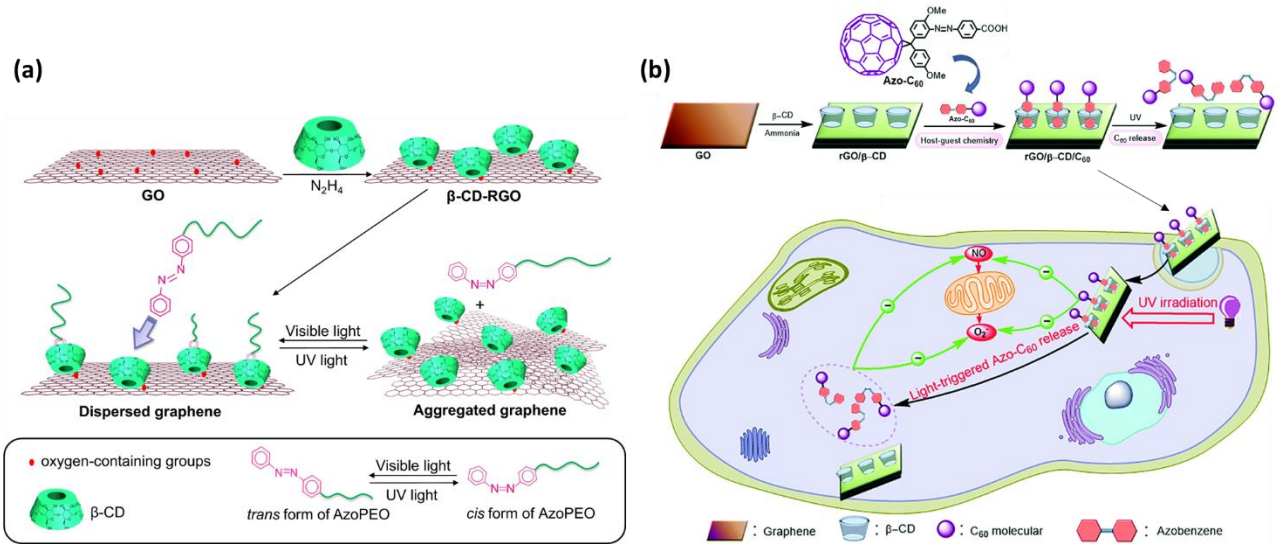


Figure 39 (a) Schematic illustration of the controlled dispersion/aggregation of graphene composite materials in water upon UV and Vis irradiation [71]; (b) Schematic representation of the rGO/*β*-CD/C60 synthesis and the hypothetical mechanism of the cytoprotective effect of the light-responsive rGO/*β*-CD/C60 nanohybrid[72].

3.2.1.6. From UV to NIR-light responsive delivery system

Although photoresponsive supramolecular materials based on azobenzene-CD inclusion complexes are widely investigated, the UV-light responsive mechanism of Azo moiety determines an obstacle especially for in vivo applications. Indeed, when considering the application in medicine, the UV-responsive process might be limited for applications in deep tissue and can cause tissue damages. To avoid the limitations of UV excitation, it is possible to functionalize the aromatic rings of azobenzene to promote absorption in the NIR region (as seen in the previous paragraph) or exploit physical phenomena such as upconversion, FRET or two-photon process. Until now, numerous works have been published describing the applications of these processes in the biomedical field. Recently, the upconversion technology showed interesting applications as a new type of non-invasive treatment used for cancer therapy, called “Photodynamic therapy”.

Upconversion nanoparticles (UCNPs) have emerged as a new class of fluorescent probes for biomedical imaging owing to their ability to convert near-infrared radiations, with lower energy, into ultraviolet radiations using a two- or multi-photon and/or energy transfer processes: in this way, using a lower wavelength light radiation, it is possible to activate the photoisomerization of the Azo-moiety without the use of direct UV radiation. Upconversion materials are usually composed of rare-earth

based lanthanide- or actinide-doped transition metals [73]. Recently, these UCNPs are used as alternative fluorescent labels to traditional fluorophores, showing great potential for applications in vivo bio-imaging, bio-sensing, and nanomedicine due to their highly stable emission and high penetration depth into tissues. For this purpose, in 2018 Zhang and co-workers developed a controlled supramolecular strategy to realize precision bioimaging of tumour tissues (Figure 40a)[74]. When DDS for bioimaging are insert within the organism, in addition to the targeted tissue, uncontrollable assembly of NPs occurs at the liver and spleen with the possibility of hindering the detection. They synthesized a supramolecular structure via host–guest interactions between Azo-modified lanthanide upconversion nanoparticles (UCNP-Azo) and *β*-CD modified downconversion nanoprobes (DCNP-*β*-CD), to enhance the retention of nanoprobes in the tumour area. DCNPs could realize efficient NIR emission under 808 nm excitation, with promising applications for in vivo fluorescence imaging (Figure 40b). However, upon 980 nm irradiation, the UCNPs can convert NIR light into UV light, which can trigger the Azo-isomerization and induce the disassembly of the two NPs. The NIR-laser-controllable nanoprobes disassembly involves a reduction of the long-term cytotoxicity, allowing a rapid disposal of the system from the liver and the organism. Another application for the treatment and detection of tumour tissues was developed by Li and co-workers [75]. They synthesized a cell-based nanoparticle delivery system, taking advantage of host-guest interactions between CD-modified with mesenchymal stem cells (MSCs) and Azo-modified mesoporous silica-coated upconversion nanoparticles. The use of MSCs was due to their specific tumour targeting characteristics and their effects to treat different pathologies, combining these properties with fluorescent UCNPs for a simultaneous image of the target area.

Using the UCNP, it is also possible to interrupt the activity of specific genes by regulating the expression of their encoding proteins. Dou and co-workers used the host-guest interactions to form an upconversion nanoparticle systems to deliver small interfering RNA (siRNA) inside specific cells, with the aim of manipulating their genetic functions [76]. siRNA can induce post-transcriptional gene silencing by interfering with the expression of specific genes and thanks to this characteristic, it is routinely used in molecular biology. Dou's group linked siRNAs to azobenzene, and these molecules were complexed through a host-guest interaction onto the UCNPs surface, previously functionalized with *β*-CDs. In addition, they have introduced some polyethylene glycol polymer chains having Azo-moiety at one end and target-ligands, acid-activatable cell-penetrating peptides or fluorophores in the other, to increase the specificity of the system. The NIR-activated UCNP core can emit UV light, triggering the azobenzene isomerization and releasing the siRNAs molecules within the solution (Figure 40c): this on demand NIR-controlled siRNA release could enable precise and spatiotemporal

gene silencing and promoted the development of nanosystems for the selective activation or release of various biomacromolecules.

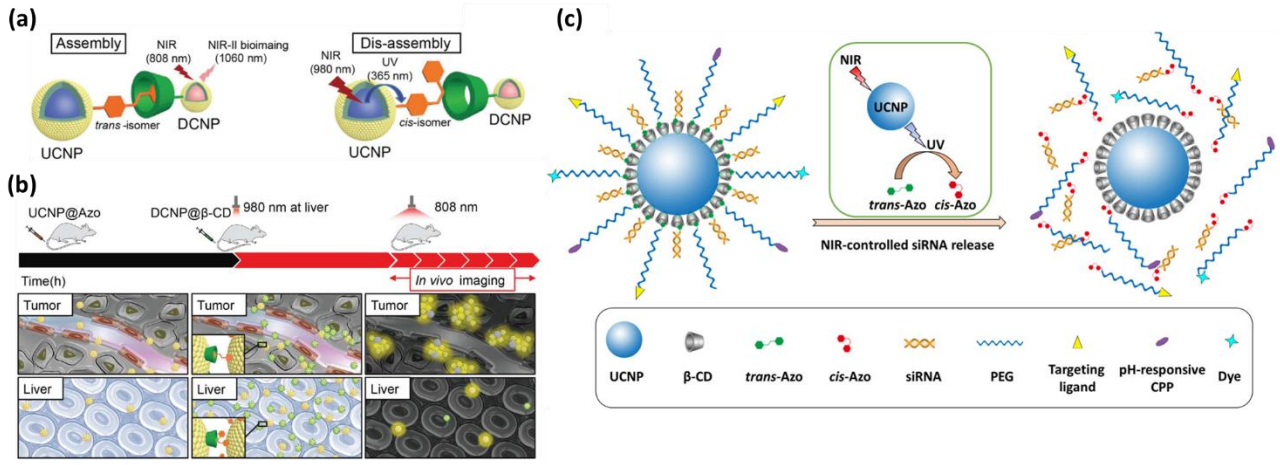


Figure 40 (a) Supramolecular recognition-induced assembly and 980 nm NIR-regulated disassembly of Upconversion and Downconversion nanoparticles [74]; (b) Representation of the *in vivo* assembly of UCNP@Azo and DCNP@*β*-CD with improved tumor targeting [74]; (c) Illustration of NIR-triggered *trans*-to-*cis* photoisomerization of azobenzene, which subsequently leads to siRNA release from the UCNP-(CD/Azo)-siRNA/PEG NPs [76].

3.2.1.7. Azobenzene molecules as a drug: regulation of its activity by host-guest mechanism

Besides the property of forming inclusion complexes, azobenzene functionality can be used directly as a drug. In 2018, Wang and co-workers used CD-bearing micellar nanocarriers to load a photoswitchable microtubule inhibitor consisting in an Azo-derivative (Figure 41a)[77]. The therapeutic activity of this molecule is dependent on its configuration: the *trans* isomer is inactive while the *cis* form is active. The UV-light irradiation can activate the molecule, breaking the host–guest interaction with the *β*-CD inducing a rapid drug release inside the tissue.

Combining the Azo-moiety with specific molecules to construct photo-responsive drugs, through the formation of the inclusion complex and the configuration change, the properties of the systems could be modulated using light stimuli. Liu and co-workers synthesized a photo-switchable azobenzene covalently linked on norfloxacin (Nor), an antibiotic used to treat urinary tract infections [78]. When irradiated by UV light, *trans*-Azo-Nor underwent a conformation change and the *cis*-Azo-Nor exhibited a high antibacterial activity, compared to the *trans* isomer which had a lower antibacterial activity. If *α*-CDs was added in the presence of *trans* isomers, the host-guest complexes were formed, with subsequent deactivation of the antibacterial activity. This supramolecular complex exhibited a higher ‘‘on-off’’ ratio of antibacterial ability compared to azobenzene-norfloxacin alone under UV

irradiation: in fact, the disassembly process between the α-CD and Azo-Nor could always be triggered by UV light (Figure 41b).

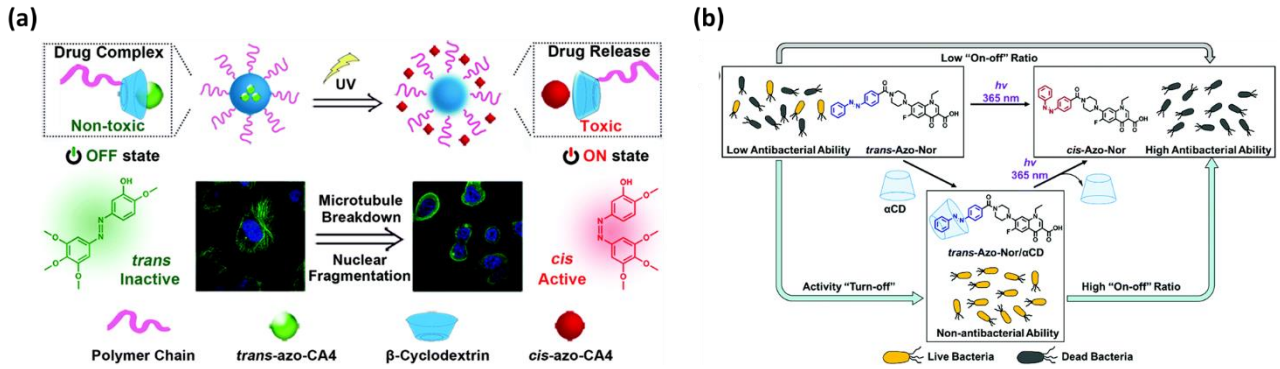


Figure 41 (a) Schematic illustration of simultaneous activation and release of a microtubule inhibitor (Azo-CA4) from β-cyclodextrin-bearing polymer micellar nanocarriers [77]; (b) Schematic representation of supramolecular photo-responsive antibiotic behavior of the Azo-Nor/CD complex [78].

These works offer an approach to efficiently regulate the activity of drugs inside the tissues, favouring targeted applications, thanks to the simple photo-induced activation/deactivation of the Azo isomers. Furthermore, with these strategies it is possible to synthesize new supramolecular photo-responsive drugs and to enhance their administration combining together the supramolecular and light-regulating strategies.

3.2.2. Photoresponsive polymeric materials based on Azo-CD complexes

Supramolecular systems constructed through non-covalent interactions have a high potential in the design of soft and stimuli-responsive materials for applications like the controlled drug delivery, tissue engineering, and self-healing materials. Many nanostructures such as supramolecular polymers, cross-linked hydrogels, catenanes, (pseudo)rotaxanes, and functionalized nanoparticles, can be derived from the self-assembly of CDs with the azobenzene molecules, which have greatly expanded the field of the supramolecular chemistry towards many new applications. In stark contrast to covalent bonds, the dynamic nature of the Azo-CDs supramolecular host-guest interactions provides to the resulting materials additional attractive features, such as reversibility and stimuli-responsiveness, high biocompatibility. In the following chapter, CD-based light-responsive supramolecular materials appeared in the literature in the last three years is classified and discussed.

3.2.2.1. Supramolecular polymers

The host-guest interactions between azobenzene and CD molecules allow the synthesis of numerous supramolecular polymers thanks to their selective recognition, peculiar stimuli-responsiveness, and remarkable capability of forming stable complexes. With these molecules, it is possible to develop materials with sizes ranging from macro- to nano-scale. For example, in 2019, Huang and co-workers introduced the host-guest chemistry into the crosslinking structure of epoxy acrylate polymer to endow the composite with self-healing behaviours [79]. The self-healing process originated thanks to the reversible association of dynamic host-guest interaction, providing numerous new properties (higher rupture stress and strain) and improving the lifetime of the material. Vinyl CDs can form stable inclusion complex with acrylamido azobenzene and this bifunctional complex could be incorporated into the epoxy crosslinking structure via copolymerization reactions. After damaging of the structure by an external event, all the crosslinking points could be unlocked by UV irradiation to increase the chain mobility, enabling the epoxy units to better self-heal. Consequently, the damaged chains were able to recombine via the host-guest mechanism after visible light or heating stimuli (Figure 42a). This strategy could be used to modify the main chain and the cross-linking structures of various polymers, which should be valuable for the design and preparation of novel smart materials, such as degradable composites, shape-memory composites, and controlled release drug carriers (these applications will be explored in the following paragraphs and chapters).

Differently, Zhu and co-workers have changed the thermo-responsive properties of a copolymer using the light and the addition of α-CD [80]. They converted polyacrylamide (PAM), a water-soluble and non-responsive polymer, into a copolymer with temperature-, light-, and host-molecule triple responsiveness by incorporating small amounts of azobenzene. In fact, the hydrophobicity and the solubility in aqueous solution of the copolymer could be altered by irradiation with UV light, modulating the content of azobenzene and the complexation properties with host molecules such as α-CD, that could preferentially complex with *trans*-azobenzene. The polymer chains interacted with each other through a combined effect of hydrogen bonds between the amide groups of the PAM chains, and hydrophobic interactions between azobenzene units. Such interactions could be influenced by the photoisomerization of azobenzene and the host-guest interaction between Azo and α-CD (Figure 42b). Moreover, after irradiation with UV light, the solubility of the polymer increased in the absence of α-CD ,and decreased in the presence of α-CD. The synthesis of polymers with light-switchable hydrophobicity could allow the design of materials which may have numerous potential applications.

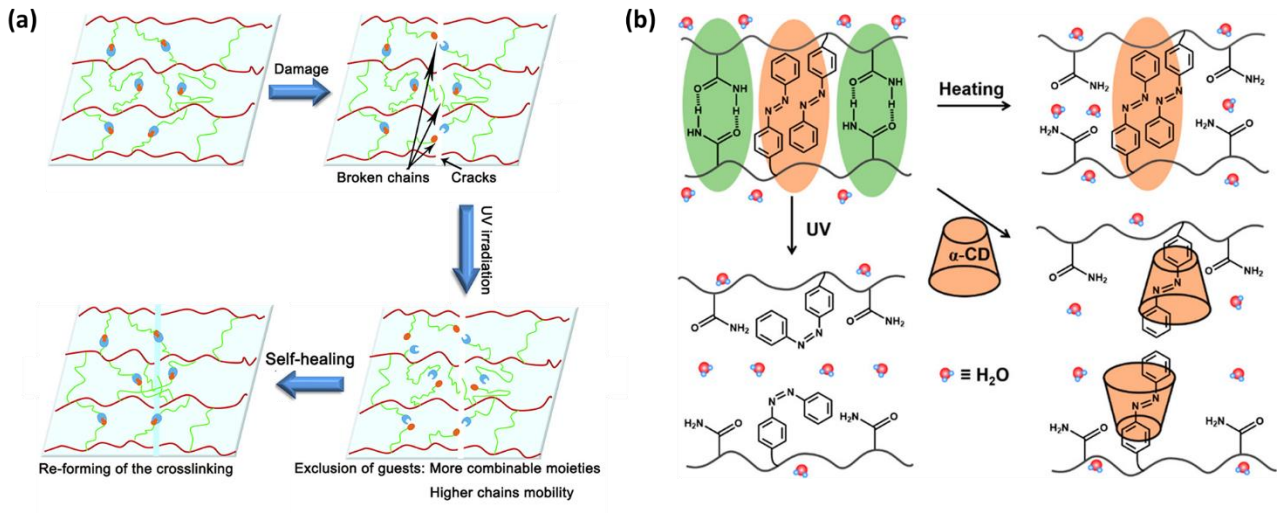


Figure 42 (a) Schematic illustration of the self-healing mechanism based on host-guest supramolecular complexes between Azo and CD molecules [79]; (b) Representation of the mechanism for the responsiveness of P(AM-ABAM) to the stimuli of temperature, light, and a host molecule. The orange and green domains represent the hydrophobic interaction and H-bonds, respectively[80].

Moving from macro to nanostructures, always using the formation of inclusion complexes, it is possible to realize multi stimuli-responsive polymeric materials. For instance, Qiu's group developed a pH and photo dual responsive supramolecular polymer based on the host-guest interactions between two different polymers having respectively azobenzene and *β*-CD molecules [81]. The polymer with Azo-moieties is a pH-responsive polymer containing azobenzene and tertiary amino groups linked to the main chain that could undergo a hydrophilic-to-hydrophobic transition increasing the pH. In water, the combination of pH and UV light or visible light induced the disassembly and the reassembly of the supramolecular polymers, characterizing a twofold property of the material (Figure 43a). In 2019 Wang and co-workers, using a similar strategy, synthesized a multiple stimuli-responsive nanoparticles that could respond to visible-light, pH and host-guest interactions from a tetra-ortho-methoxy-substituted azobenzene-functionalized amphiphilic polymer [82]. In aqueous solution, the polymer self-assembled forming micellar nanoparticles consisting of a hydrophobic mAzo groups as the core. Once formed, the shape and size of the nanoparticles could change according to external stimuli: under green light irradiation, the azobenzene was isomerized with no significant changes of the morphology of the nanoparticles due to the little variations in the polarity of the isomers. In acidic environment, the hydrophilicity of the polymer increased due to the protonation of azo groups and the tertiary amine groups, leading to an increase in its size. Conversely, in alkaline conditions, the hydrophilicity decreased, and the nanoparticle accordingly showed a reduction in size. Finally, the addition of CDs triggered the dissociation of the micellar nanoparticles due to the disruption of hydrophilic-hydrophobic balance. When the system was mixed with CDs and

exposed to green light, the host-guest complexes dissociated, resulting in the formation of micellar nanoparticles from the polymers with the *cis* azobenzene (Figure 43b). Thanks to these characteristics, the light-, pH-, and host-guest-responsive polymeric systems could offer wide applications in the fields of nanotechnology and biotechnology as smart nanomaterials for controlled release.

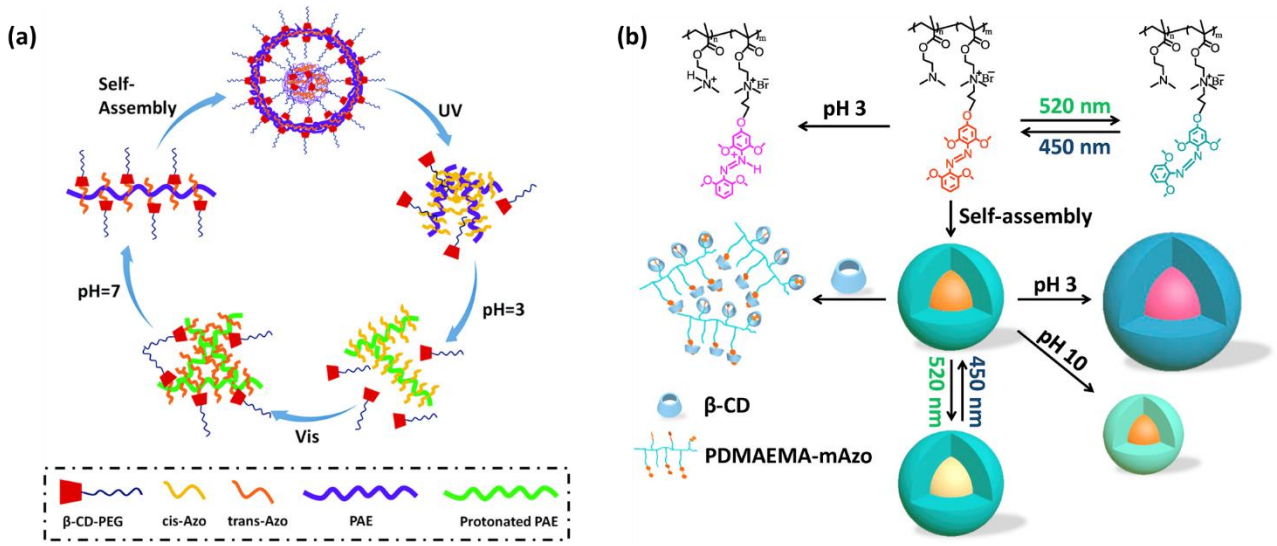


Figure 43 (a)Schematic illustration of the dual-responsive self-assembly behavior of supramolecular polymer PAE-g-Azo/*β*-CD-PEG [81]; (b) Schematic representation of the structural changes of polymer nanoparticles under the stimuli of visible light, pH, and CD [82].

3.2.2.2. Supramolecular hydrogels with photo-controllable properties

Supramolecular hydrogels based on host-guest interactions between CDs and azobenzene molecules are of great interest thanks to their ability to undergo reversible gel-sol transition in response to various environmental stimuli (due to the noncovalent cross-linkages), showing great potential as smart materials. The properties of these materials are closely tied to their supramolecular composition and can exhibit a range of useful properties including stretchability, durability, shear-thinning, self-healing, and stimuli-responsiveness. Through the numerous applications and progress developed over the last three years, this paragraph highlights how the supramolecular interactions that allow the formation of hydrogels also translate into the materials properties.

There are two major methods to form supramolecular hydrogels. One of these is the use of self-assembly of mono-component small molecules that can act simultaneously as host and guest. Using this way, Liao and co-workers have synthesized some azobenzene-modified *β*-CD derivatives with different alkyl lengths, and they discovered that the length of the alkyl chain dramatically influenced the solubility and gelation ability of *β*-CD derivatives in water (Figure 44a)[83]. When the alkyl chain

was eight carbon atoms long, the system exhibited the best gelation properties and through host-guest interactions between β-CD and azobenzene, this derivative could provide supramolecular hydrogels with reversible thermal- and photo-responsive behaviours.

A different approach consists in constructing multi-component systems with at least one macromolecular component. Usually, host molecules and/or guest molecules are grafted to the side chain of polymers and the inclusion complexes formed by host–guest interactions become the physical crosslinking junctions between different polymer chains. Recently, in early 2020, Zheng and co-workers developed a photoresponsive supramolecular material using nanomaterials as crosslinkers [84]. They used natural lignin nanoparticles with α-CD as nano-crosslinkers and a polymer functionalized with azobenzene molecules to construct photoswitchable and photoluminescent ternary supramolecular hydrogels. The reversible sol-gel transition of the supramolecular hydrogels was controlled by UV light (gel to sol) and blue light irradiation (sol to gel). Yan and colleagues, grafted some azobenzene moieties onto a gel matrix, α-CDs, and ionic liquids to synthesize a novel ion-conducting supramolecular hydrogel (Figure 44b)[85]. The photoinduced *trans-cis* isomerization of the azobenzene could switch the host–guest interactions and thus tune the concentration of mobile anions in the system and the conductivity of the hydrogel: in this way, under UV light, the system exhibited high resistance due to the complexation between α-CD and the anionic part of the ionic liquids, while under visible irradiation, more stable α-CD/*trans*-Azo complexes were formed, thereby releasing the bound anions to generate a low-resistive hydrogel. This smart hydrogel was able to act as an intermediary to connect the light signal and electric signal. To prove this characteristic, they applied it to logic circuits to selectively control the on/off states by light, as shown in Figure 44c.

It is also possible to develop a photo-controlled system that features switchable sol-gel transition in response to light and temperature, introducing a competitive photoresponsive guest such as the azobenzene into the polymeric system. Yan and co-workers synthesized an amphiphilic copolymer with charged long alkyl chains and α-CD units, where the temperature could induce a reversible and unusual sol-gel transition due to the binding-release processes between α-CDs and amphiphilic polymer chains [86]. Furthermore, with the introduction of the azobenzene, it was possible to modulate the gelation points in a broad temperature window via light irradiation, providing the system with photo-switchable thermo-responsive ability (Figure 44d).

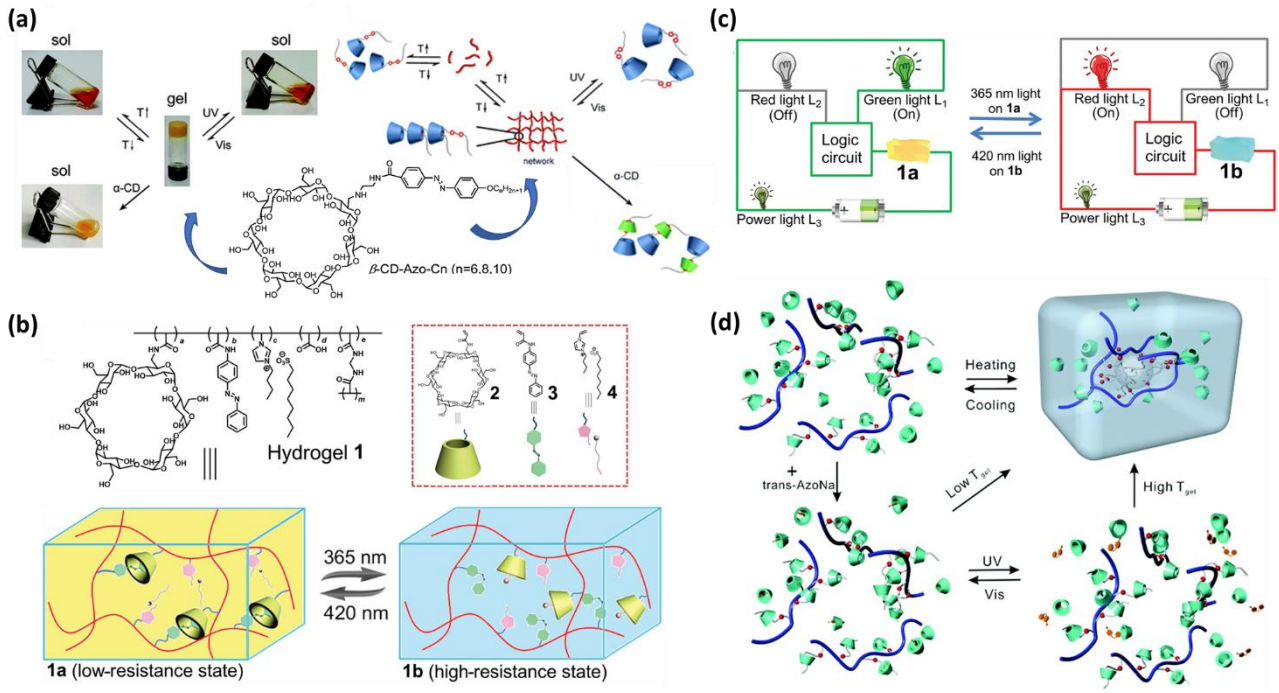


Figure 44 (a) Photographs and schematic illustration of stimuli-responsive behaviors of the β-CD-Azo-C_8 hydrogels [83]; (b) Chemical structure of the ion-conducting supramolecular hydrogel and representation of the host-guest mechanism under irradiation by 365 or 420 nm light [85]; (c) Reversible switching of red and green lights under irradiation by 365 or 420 nm light with the incorporation of the ion-conducting hydrogel in a logic gate circuit [85]; (d) Schematic illustration of the photoswitchable thermogelling copolymer based on host–guest mechanism: the introduction of the azobenzene molecule causes the modification of the gelation points in a broad temperature window via light irradiation [86].

To avoid the use of a UV light source to activate the isomerisation of azobenzene functionality, it was possible to include upconverting nanoparticles within the azobenzene-CD supramolecular hydrogel. For this purpose, Capobianco's group presented this viable alternative to direct UV excitation to achieve a gel-sol transition in an azobenzene-based supramolecular hydrogel, with potential applications in the biomedical field [87].

The use of amphiphilic molecules can enable the synthesis of multi-responsive supramolecular hydrogels. In 2019, Luo and co-workers developed a new series of gemini surfactants containing azobenzene moieties with different alkyl chains which formed stable hydrogels via hydrogen bonding and π-π stacking interactions in aqueous solution [88]. This kind of hydrogel exhibits excellent mechanical property and thermo-reversibility: when α-CDs were added to the system, the corresponding hydrogel displayed a gel-sol transition due to the formation of the inclusion complexes between Azo and CD molecules. Subsequently, irradiation with UV light determined the photoisomerization of the azobenzene with the consequent formation of a new hydrogels (Figure

45a). Furthermore, the visual recognition for *cis-trans* isomerization was achieved by gel-sol-gel transition under cyclic irradiation of UV and visible lights, owing to the host-guest interactions. In the same year, Wu and co-workers have synthesized some surfactants with two identical azobenzene ends separated by a flexible chain but different cationic heads, called "bola-form surfactants" (Figure 45b)[89]. In addition to the *trans*-/*cis*-photoisomerization control via light irradiation, the host–guest inclusion of the CDs triggered the reversible gel-sol transition. Even for this system, after the addition of CDs, the hydrogels were transformed into sols, while the sols were reverted back to gels by the addition of competitive guest molecules which preferentially bound to the CDs.

Besides, Zheng and colleagues developed a supramolecular hydrogel with photo and thermal responsiveness using *β*-CD and asymmetric gemini zwitterionic liquids which contained two types of functional guest groups, one of which was azobenzene [90]. They discovered that one *trans*-Azo group could be included in two *β*-CDs, forming a 1:2 complex and the sol-gel phase transition of this system caused a change in conductive properties, with potential applications as stimuli-responsive hydrogel for electrical sensing materials (Figure 45c). For the systems described above, the hydrogels consisting of molecules with multiple responsive groups have numerous potential applications controllable using external stimuli such as light, heat or the addition of specific molecules, which confer mechanical and physical reversible properties.

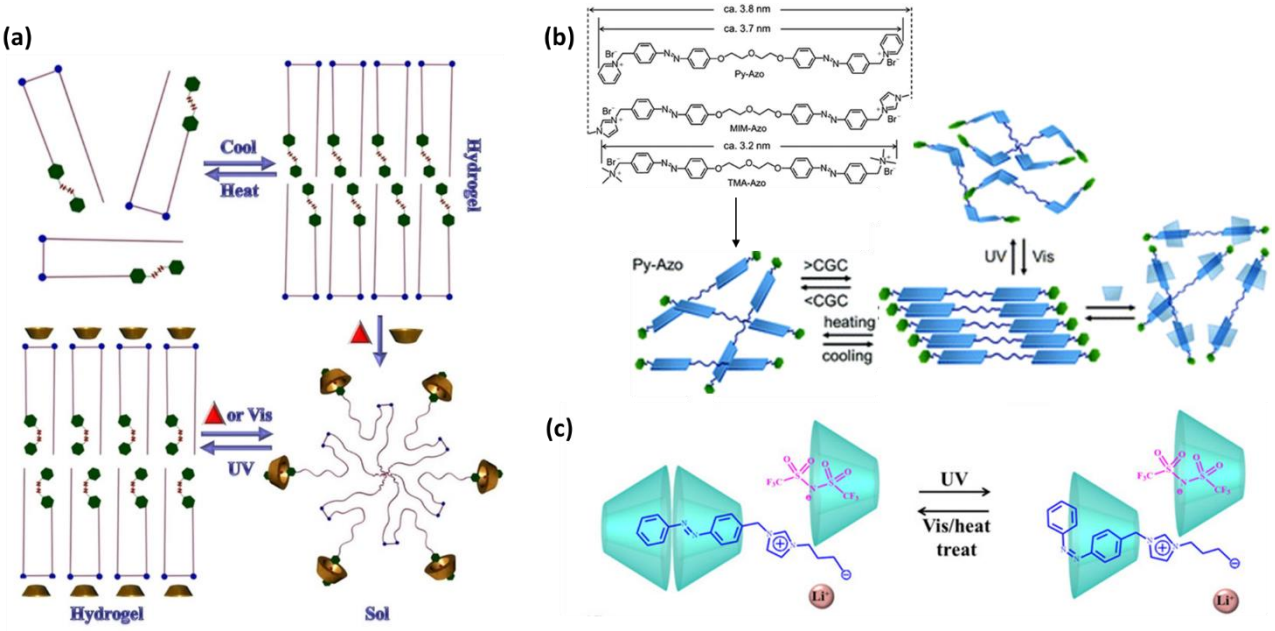


Figure 45 (a) Schematic illustration of the multi-responsive supramolecular hydrogel composed of gemini surfactants with azobenzene moieties [88]; (b) Molecular structures of the bola-form surfactant cations and their structural transformations of under the effects of the external stimuli [89]; (c) Representation of the inclusion complex between *β*-CD and Azo- asymmetric gemini zwitterionic molecules before and after UV irradiation [90].

3.2.2.3. Self-healing and shape-memory supramolecular polymers

Self-healing supramolecular hydrogels have emerged as a novel class of materials that combine hydrogels characteristics with supramolecular chemistry to develop highly functional materials, and owing to their versatility, they could find applications in catalysis, sensors, drug delivery, artificial implants, tissue engineering, etc. In 2020, Zhang and colleagues constructed a novel photoresponsive supramolecular fluorescent hydrogel combining two different water-soluble polymers consisting respectively of *β*-CD and azobenzene groups and tetraphenylethylene (TPE) as a fluorescent unit (Figure 46a)[91]. In the gel-state, the TPE molecules were stuck inside the network and their intramolecular rotations were limited, causing the supramolecular hydrogel to exhibit a cyan fluorescence (TPE is an aggregation-induced emission molecule whose emission is triggered by the formation of aggregates). Under UV light, the photo-inducted isomerization destroyed the physical cross-linked network structure turning off the fluorescence. In addition to the fluorescent properties, this system shoed also self-healing properties due to the formation of the Azo-CD inclusion complexes (Figure 46b). Another example was reported by Sun and co-workers [92]. They synthesized a photoresponsive hydrogel crosslinked by host-guest interactions with high stretchability, strong toughness, and rapid self-healing properties. Moreover, the system is characterized by photochromic properties due to the introduction of two photoswitchable groups: the generation and elimination of the host–guest interactions between Azo and *β*-CD could be induced with light irradiation at different wavelengths and monitored by the colour change of the system, as shown in Figure 46c.

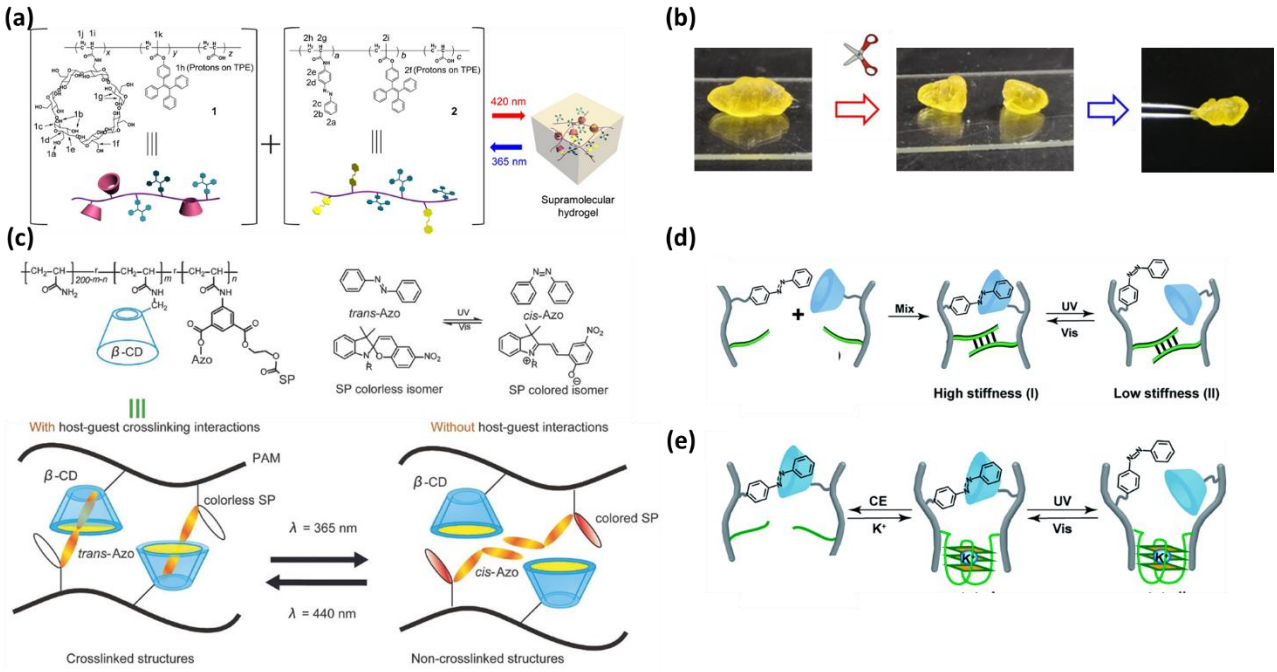


Figure 46 (a) Chemical structures of the water-soluble polymers and representation of the supramolecular fluorescent hydrogel [91]; (b) Images of the supramolecular hydrogel during the entire course of self-healing process under illuminations of 420 nm visible light [91]; (c) Chemical structures and schematic illustrations of the self-healing polymer with two photoswitchable group [92]; (d) (e) Representations of dual stimuli responsive hybrid hydrogels that are crosslinked by nucleic acid bridges/K+-ion-stabilized G-quadruplexes and *trans*-azobenzene/*β*-CD stimuli-responsive bridges [93].

In addition to the self-healing properties, with the use of light, cross-linkers and other molecules, it was possible to modulate numerous characteristics of the supramolecular hydrogels such as their rigidity and shape. One of the main objectives consisted in developing shape memory polymers as materials that can remember temporary shapes under certain conditions and revert to their original shapes as consequence of external stimuli. Such materials can be created introducing two different types of cross-linking agents into the polymer chains, which are activated by different stimuli. Willner's group developed some stimuli-responsive hybrid hydrogels using Azo-CDs and nucleic acids as crosslinkers: one molecular unit acts as the permanent memory and the other acts as the stimuli-responsive crosslinker that controls the stiffness of the hydrogel [93]. Under UV-light, the dissociation of the Azo-CD complexes led to low stiffness values but the presence of duplex nucleic acid bridges maintained the memory of the system (Figure 46d). The same group developed a similar material by introducing nucleic acids rich in guanine (G-quadruplex), which are known for their complexing properties for potassium atoms, forming a stable crosslinker and maintaining the same properties of the first hydrogel (Figure 46e).

In 2019 instead, Zhao and co-workers presented a supramolecular approach to alter the permanent shape of the shape memory polymers with light and with the use of host-guest interactions between azobenzene and α-CD, and ionic coordination between alginate and Ca^{2+} ions [94]. Both interactions are reversible: Azo-CD complex can be opened and closed with the UV and visible light irradiation, and the metal ion-ligand coordination can be dissociated with the addition of EDTA, a competitive chelator for Ca^{2+}. In a hydrogel consisting of polyacrylamide and alginate, the reversible nature of alginate-Ca^{2+} coordination bonds conferred to the hydrogel specific functions such as shape memory and shape recovery, and the light-responsive transition of azobenzene could be applied to generate a new permanent shape by tuning the host-guest complexation via UV light. Through these stimuli it was possible to modify the shape of the hydrogel also in the three dimensions, as shown in Figure 47. These properties allowed the development of new smart materials based on numerous and different interactions could be activated by multiple stimuli, expanding in this way their application areas.

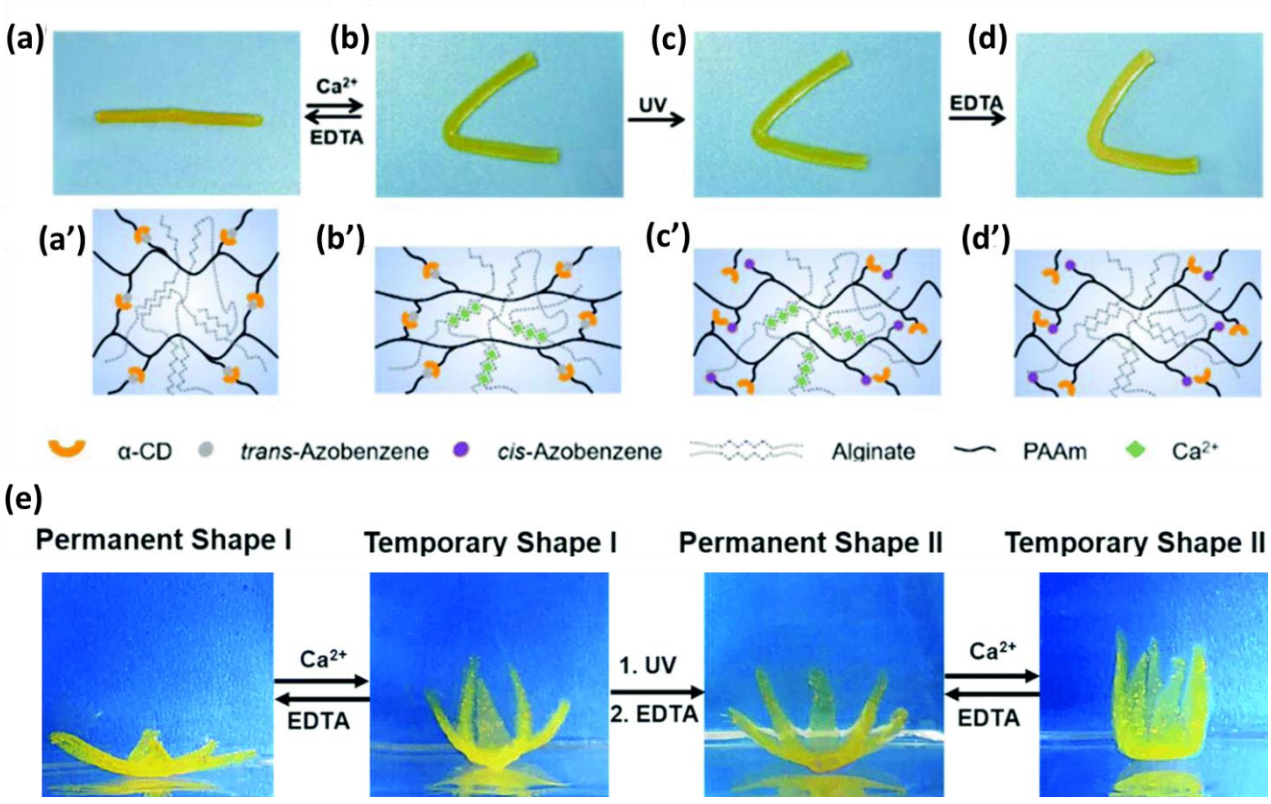


Figure 47 Shape-memory, shape-recovery and shape-erasing behaviors of the dual-supramolecular cross-linked hydrogel (a–d) and schematic illustrations of the network structure (a'–d') [94]. (a) The original shape; (b) the temporary shape fixed with Ca_{2+}; (c) the shape after UV irradiation (365 nm); and (d) the newly formed permanent shape; (e) Example of 3D shape photo-adaption of the host–guest cross-linked PAAm/alginate hydrogel switching between flowers with 6 open petals and 3 open petals.

3.2.2.4. Functional polymers based on interlocked systems

Interlocked molecules are molecular architectures formed from two or more components that are mechanically linked together. Molecules possessing rotor (such as CDs) and axis moieties can form a supramolecular polymer through the formation of intermolecular inclusion complexes. An example of interlocked molecules is rotaxanes: a rotaxane can be considered as a mechanically interlocked wheel-and-axle complex and its components are not connected through covalent bonds. Instead, molecules consisting of an axle with two or more wheels are called polyrotaxanes. CD-based rotaxane have been widely studied as potential materials, sensors and key components for molecular motors and machines due to the possibility to use as axle stimuli-responsive molecules that can change their configurations and properties.

In this regard, in 2018 Harada and co-workers developed photoresponsive polymeric actuators based on a [2]rotaxane consisting of α-CDs, azobenzene derivatives and poly(ethylene glycol) [95]. These systems are materials that were able to modify their shape in response to changes in environmental conditions and are characterized by good mechanical properties. They found that the [2]rotaxane structures acted as movable connections in the polymer network, and the mechanical properties of the material, such as the rupture strain, were enhanced up to 28 times. Irradiation with UV or visible light caused the photoisomerization of the azobenzene moiety leading to the modification of the [2]rotaxane-linker structure and to the deformation of the polymer network (Figure 48a) with a consequent change in its mechanical properties. The use of intermolecular inclusion complexes between "axle" and "wheel" molecules allowed the creation of multifunctional supramolecular polymers thanks to the presence of different chemical and molecular functions in each species. High molecular weight supramolecular polymers usually need noncovalent interactions with high-affinity binding recognition motifs between small molecular monomers but the use of pseudorotaxanes monomer molecules, photoresponsive moieties and host-guest complexes, could be applied for remote control of the resulting supramolecular polymer morphology. For example, with γ-CD and cucurbit[7]uril-based pseudo[3]- rotaxane containing azobenzene and two photoresponsive coumarin moieties, Zou and collaborators synthesized a light-driven non-covalent/covalent switchover linear supramolecular polymer via host–guest molecular recognitions [96]. They formed a linear non-covalent supramolecular polymer through 2:1 host-guest self-assembly between coumarin moieties and γ-CD. The cucurbit[7]uril rings (CB[7]) possess selective recognition toward positively charged species with extremely high binding constants and are exploited for obstructing the movement of γ-CDs in the monomer axis: the γ-CD is characterized by higher binding constant with Azo-molecule compared to coumarin and CB[7] as a stopper, allowing the formation of the host-guest complex

between the CD and two molecules of coumarin (Figure 48b). However, this supramolecular system could also switch between a non-covalent polymer and the corresponding covalent polymer due to the photo-induced reversible cyclodimerization of coumarin units inside the cavity of γ-CD after UV light irradiation, which generated a stable cyclobutane-based dimer. Moreover, the *cis*-azobenzene no longer allowed the shuttling equilibrium of the CB[7] ring along the axle and determined the modification of the shape of the polymer. In this way it was possible to obtain a high molecular weight supramolecular linear polymer by exploiting the ability of CDs to form inclusion complexes and to carry out photo-induced reactions within them.

Molecules possessing rotor and axis moieties, as seen above, can form a supramolecular polymer through the formation of intermolecular inclusion complexes between rotor and axis, but when the latter is capped with a bulky stopper, the product is called as daisy chain. Daisy chain rotaxanes represent a particular class of interlocked molecules that can produce internal sliding movements with a net contraction or extension at the single-molecule level ([c2]daisy chain rotaxanes have been also named as “molecular muscles"). In particular, a wide variety of rotaxanes have been reported to perform programmed intramolecular switching when stimulated by external stimuli and therefore are considered as artificial molecular machines. Exploiting another self-assembly strategy, Easton and co-workers described the synthesis of a daisy chain architecture based on the direct self-assembly of dimeric complexes and their oligomerization in aqueous solution [97]. They synthesized a series of interlocked CD/Azo-based [c2]daisy chains, including a β-CD tetramer, hexamer, octamer and decamer, from the self-assembly of dimeric complexes to produce [c2]rotaxane moieties (Figure 48c). This mechanism involved an intricated balance of competing processes and the interlocked species exhibited switchable behaviour characteristic due the *trans* to *cis* isomerization of the azobenzene: the resulting rotaxanes were able to switch between two limit conformations, contracted and extended, behaving as a molecular muscle.

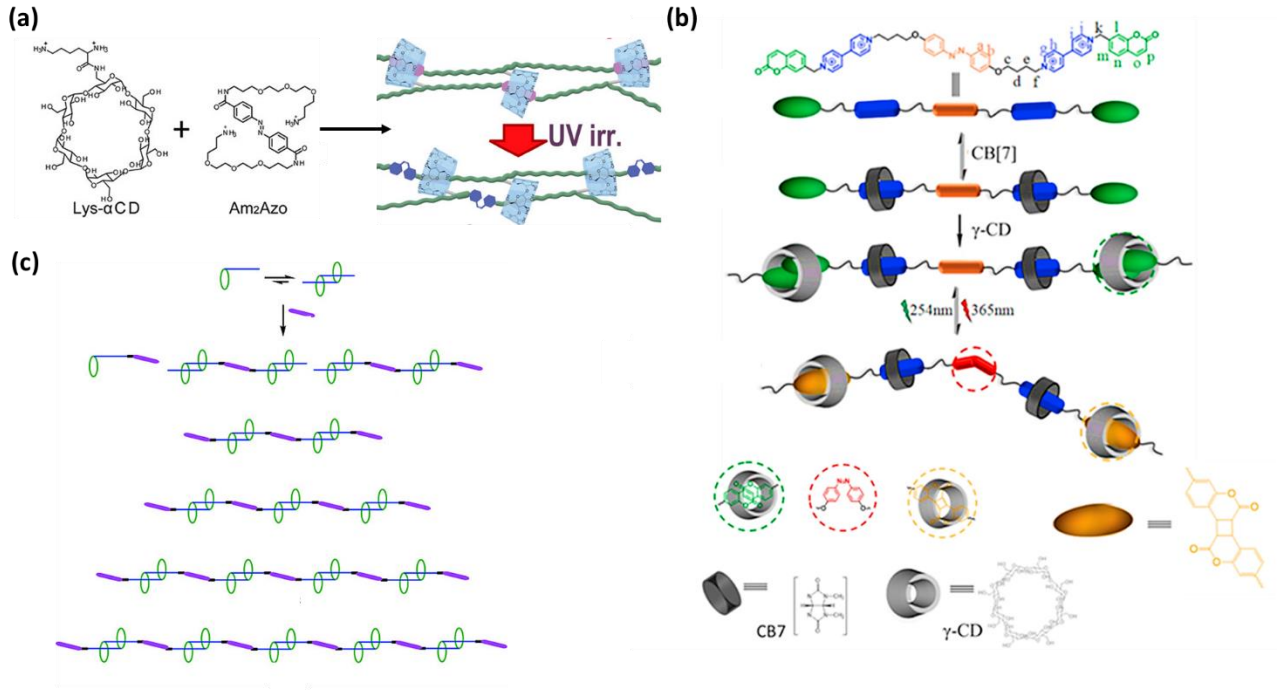


Figure 48 (a) Schematic illustration of the photoresponsive polymeric actuator based on *α*-CDs, azobenzene derivatives and poly(ethylene glycol [95]; (b) Molecular structures of monomers, *γ*-CD, CB[7] and schematic description of the self-assembly process for forming CB[7]-based pseudo[3]rotaxane, the supramolecular polymer with azobenzene in the *trans* configuration, and the corresponding UV-irradiated molecule containing *cis*-azobenzene [96]; (c) Schematic representation of the production of interlocked CD/Azo-based [c2]daisy chains [97].

3.2.3. Azo-CD based supramolecular self-assembly systems

Host-guest interactions can guarantee supramolecular self-assemblies with high adaptability and reversibility, providing them with multi-responsiveness to various external stimuli, such as pH, concentration, temperature, and light. Amphiphilic systems, super-amphiphilic polymers (SAPs) and supramolecular nanoparticles, constructed by host-guest inclusion, can self-assemble into various nanostructures in solution, which could find applications in many fields such as nanodevices, drug delivery, catalysis and template synthesis. For this purpose, numerous supramolecular structures have been studied and developed over the years, having as protagonist the host-guest mechanism between azobenzene molecules and CDs.

Zhao and co-workers developed two photoresponsive supramolecular self-assembling systems based on *β*-CD/Azo complexation. In 2018, they developed a *β*-CD derivative containing an azobenzene terminal group with an oligoethylene glycol linker as a hydrophilic spacer (Azo-EG-*β*CD) [98] and subsequently, in 2019, they replaced the hydrophilic linker with an alkyl chain (Azo-C_6-*β*-CD) [99]. Both molecules exhibited intermolecular host-guest interactions in aqueous solutions: Azo-EG-*β*-CD molecules formed linear supramolecular structures which then became vesicular systems over time,

due to the formations of hydrogen bonds. UV light induced the dissociation of azobenzene in the *β*-CD cavity and the aggregates turned into vesicles (no host-guest interactions were formed between the molecules). Instead, the Azo-C_6-*β*-CD molecules could self-assemble into aggregates in aqueous solution thanks to their amphiphilic nature and after UV irradiation, the photoisomerization of azobenzene groups promoted the formation of vesicles (caused by the dissociation of the host-guest inclusions). For both molecules, the formation and the dissociation processes of host-guest complexes were reversible (Figure 49a).

Super-amphiphilic polymers in aqueous solution can self-assemble into various nanostructures, such as vesicles, nanowire micelles, spherical micelles, worm micelles, spindle micelles. Dong and co-workers designed and synthesized a dual stimuli-responsive amphiphilic polymer (SAP) which contained in the same molecule three different available guests for *β*-CD such as ferrocene (Fc), azobenzene (Azo) and an alkyl chain (Figure 49b)[100]. These SAPs could self-assemble into interesting nanostructures in aqueous solution upon exposure to different external stimuli. After the addition of *β*-CD, the terminal Fc group in polymer chain could be included with *β*-CD to form inclusion complex Fc/*β*-CD, characterized in aqueous solution by nanowire micelles. Subsequently, following oxidation of the ferrocenyl group (Fc^+), *β*-CDs dissociated from the cationic complex Fc^+/β-CD SAP and due to inclusion of the azobenzene molecules form a new complex (Azo/*β*-CD SAP), due to the hydrophilicity of the Fc^+ group, or leaved the amphiphilic molecule and was released back into solution. In this way, the nanowire micelles transformed into spindle micelles. After UV irradiation, the spindle micelles were further transformed into spherical micelles because most of *β*-CDs were excluded from the complex Azo/*β*-CD SAP due to the *trans*- to *cis*-Azo isomerization, forming a new dominant inclusion complex with the alkyl chains (C_{11}/ *β*-CD SAP) (Figure 49c). This work demonstrates for the first time how the application of different stimuli to an amphiphilic system could lead to the formation of new different structures, allowing active control in the self-assembly mechanism through host-guest interactions. Besides, always based on the *β*-CD host–guest molecular recognition, it was possible to develop stimuli-responsive systems that combined different kinds of noncovalent interactions such as the host-guest interactions, ionic bonds and metal–ligand coordination.

In 2019, Wu's group developed an innovative supramolecular self-assembly system based on azobenzene derivatives and functionalized *β*-CDs, mainly driven by hydrophobic, van der Waals and metal-ligand interactions [101]. They synthesized two different azobenzene amphiphilic molecules consisting of one or three alkyl chains (Azo-C and Azo-C_3) and a functionalized *β*-CD with a

terpyridine moiety (Py-CD). Azo-C and Azo-C_3 in aqueous solutions could self-assemble into regular spherical nanovesicles. After the addition of the Py-CD, host-guest interactions between Azo-group and CD gave rise to an increase in the diameter of nanovesicles. Moreover, the irradiation with UV and visible light could determine the reversible disassembly and reassembly of the system. Thanks to the presence of terpyridine groups on the surfaces of supramolecular vesicles, addition of metal ions could trigger the vesicle-vesicle self-assembly from the nanoscale to the microscale. By varying the type of ions coordinated on the terpyridine group, it is possible to modulate the properties of the system and control the growth of the vesicular aggregates, providing the foundations for the development of new artificial membrane structures in the aqueous two-phase systems (Figure 49d).

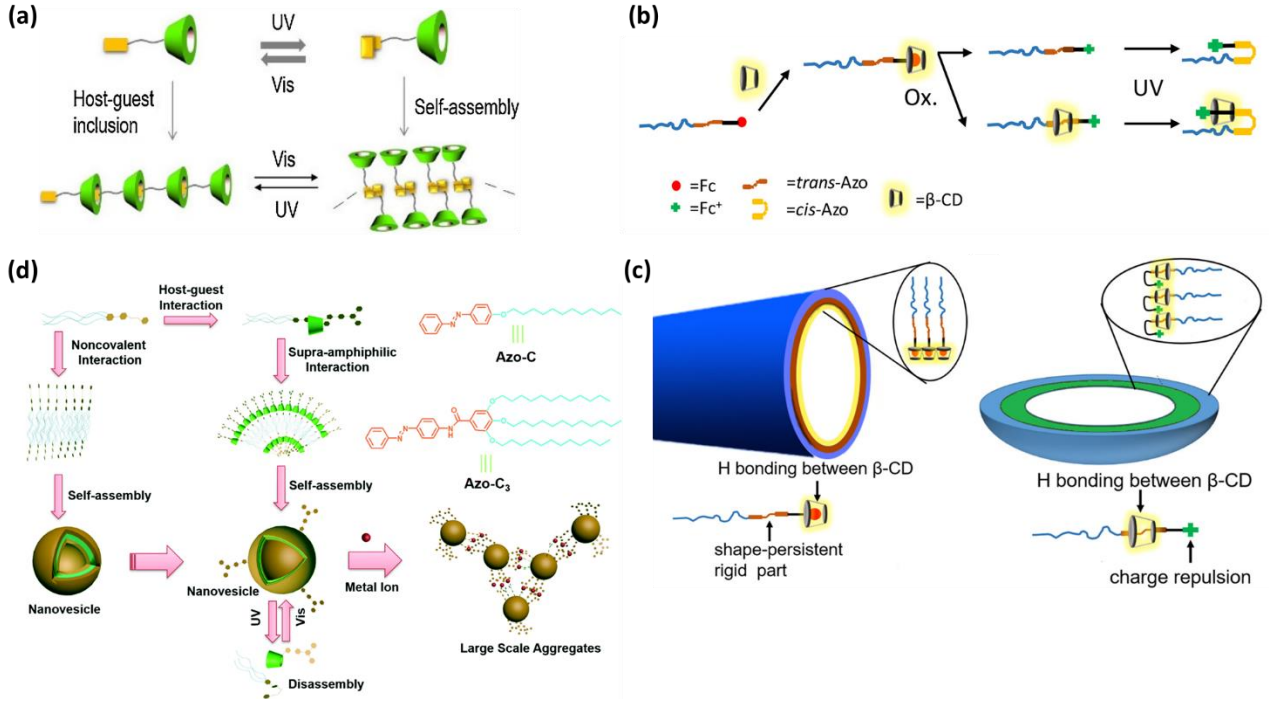


Figure 49 (a)Schematic representation of the self-assembly mechanism and photoresponsive transformation of aggregates of Azo-EG-*β*-CD and Azo-C_6-*β*-CD in water [98] [99]; (b) Representation of stimuli-responsive inclusion complex of Fc/C_{11}AzoPEG with *β*-CD upon exposure to different external stimuli [100]; (c) Schematic illustration of nanowire micelles of Fc/*β*-CD SAP and spindle micelles of Azo/*β*-CD SAP [100]; (d) Chemical structures of building blocks (Azo-C_3 and Py-CD) and schematic illustration of the self-assembly and disassembly processes of supramolecular vesicles under different conditions[101].

Exploiting ionic interactions, Liu and co-workers synthesized water-soluble supramolecular nanoparticles with two opposite charges formed by polycationic CDs and anionic azobenzene-surfactants (Figure 50a)[102]. In this case, the host-guest interaction between Azo-molecules and the internal cavity of the CDs did not take place, but the nanoparticles formed by the self-assembly of the ionic blocking blocks. The size of the resulting supramolecular structures was due to the presence of polycationic CDs and the type of Azo isomer. The azobenzene-containing surfactant having a

hydrophilic sulfonate head and a hydrophobic alkyl tail could self-aggregate in aqueous solution in both isomeric forms. When polycationic-CDs were added to the solution with *trans*-azobenzene derivatives, supramolecular nanoparticles were formed. If subjected to UV radiation, the light-induced *trans*- to *cis*-Azo isomerization led to the disassembly as well as the shrinking of nanoparticles, because *cis*-Azo could not easily assemble with the cationic CDs. It was therefore possible to reduce the dimensions of the self-assembled systems through the light: these systems could shrink under UV irradiation until reaching an average diameter of 90 nm and reversibly resume their original morphology after further exposure to visible light (~181 nm). Due to their photo-triggered growth/shrinkage behaviour, the nanoparticles could also efficiently upload and release dyes and drug substrates in a controlled manner under light stimuli, expanding the application of these systems also as DDS.

The stabilization of the biphasic systems in recent years proved to be very important thanks to the development of green reactions carried out in aqueous environments. For this purpose, Huang and co-workers discovered a new strategy for aqueous two-phase systems to regulate the phase behaviours, for example in emulsions, introducing a photo-trigger molecule in a light-inert aqueous surfactants two-phase system (ASTP), which consisted of two immiscible aqueous phases [103]. In this case, the photo-trigger molecule was an anionic azobenzene derivative and a *β*-CD was used as "Trojan horse" to modify the characteristics of the system. The aqueous surfactant two-phase system was formed by sodium laurate (SL) and dodecyl tributylammonium bromide (DBAB), two commonly used surfactants. In the presence of the *β*-CDs, host-guest complexes were formed between CDs and surfactants, where the alkyl chain of the surfactant was sheltered into the cavity of CD via hydrophobic interaction, characterizing the presence of SL/*β*-CD complex and a two-phase system(Figure 50b). The binding constant between CD and the surfactant was sufficiently low to ensure that the surfactant guest could be replaced by other guests with stronger binding abilities such as Azo compounds. In fact, the addition of azobenzene molecules led to the shift of the previously formed equilibrium between sodium laurate and CDs, with the consequent release of the SL molecules and the formation of a homogeneous system. Azo molecules could undergo *trans*-to-*cis* transition under UV light, whereas a reverse transition occurred under visible light. In fact, under UV irradiation, the ASTP turned quickly into a heterogeneous suspension while under visible light, a homogeneous suspension was reformed (the SL/*β*-CD host-guest complex was restored). This dynamic complex with the ASTP system can be described as a "Trojan horse" that becomes crucial only when the encapsulated SL was triggered to release(Figure 50c). With this strategy it was possible

to separate and obtain insoluble components from aqueous solution with the help of light and conduct reactions in biphasic environments.

Another way to stabilize biphasic systems with the possibility of using them to conduct catalytic reactions consists in the creation of responsive emulsions stabilized by amphiphilic supramolecular polymer particles. In 2019, Gao and co-workers synthesized two different copolymers with *β*-CDs and *trans*-azobenzene molecules at their ends and the self-assembly of supramolecular copolymers occurred through the formation of the host-guest complex between *β*-CD and Azo: due to these interactions, a responsive oil-in-water Pickering emulsion was obtained [104]. Following irradiation by UV light or by the addition of a competitive guest molecule, the formed *β*-CD/azobenzene inclusions disassociated, determining the morphological transition and the demulsification of the system (Figure 50d). In this way, heterogeneous reactions could be carried out within the oil-phase (or at the oil-water interface) and the responsive demulsification allowed the separation of products, the recyclability of emulsifiers and an efficient and sustainable pattern to perform catalytic reactions.

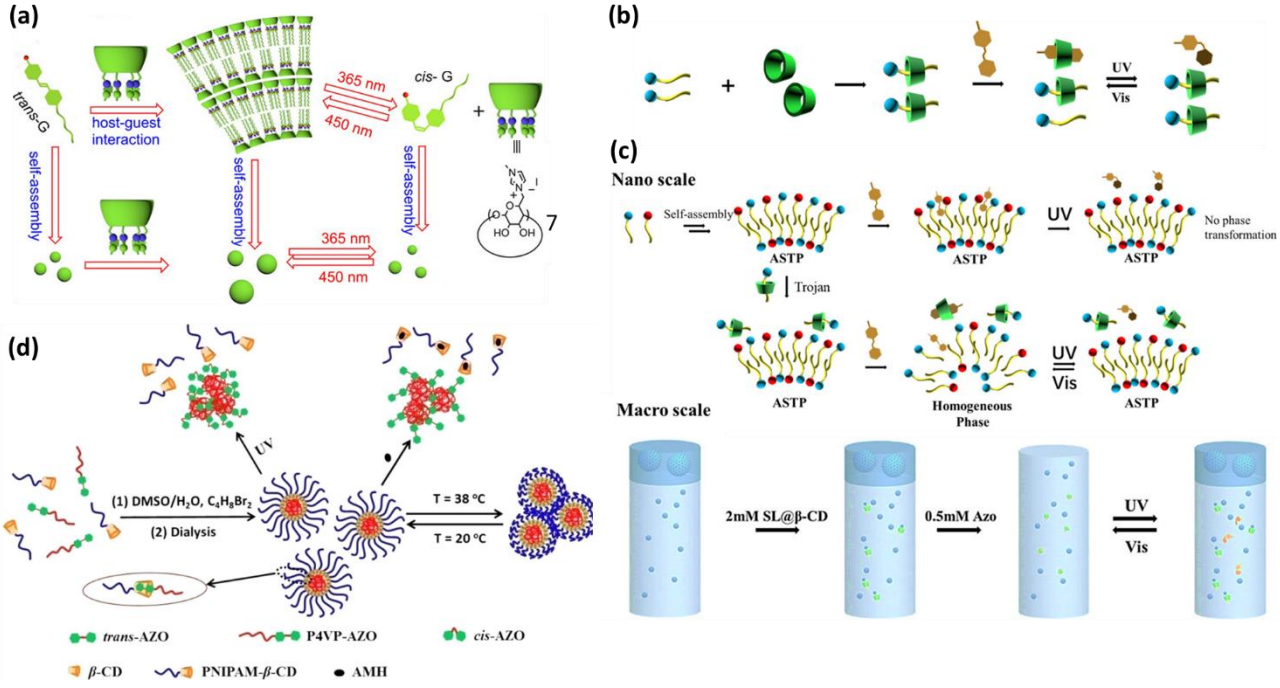


Figure 50 (a) Schematic representation of the reversible formation of photoresponsive self-assembly and disassembly of supramolecular ionic nanoparticles [102]; (b) Schematic illustrations of the equilibrium between β-CD, surfactants and azobenzene molecules [103]; (c) Schematic illustrations of the photoresponsive ASTP system at a multiscale level [103]; (d) Schematic illustration of thermo-, UV light-, and AMH-triggered morphological transitions of amphiphilic core cross-linked supramolecular polymer particles (oil-in-water Pickering emulsion) [104].

3.2.4. Photoresponsive materials based on Azo-CD complexes

3.2.4.1. Graphene-based light-responsive composite materials

Based on the reversible host-guest inclusion/exclusion mechanisms of CD-functionalized graphene oxide (GO-CD) materials and azobenzene-molecules, new kinds of light-responsive nanocomposites have been developed. In addition to mechanical properties, these nanocomposite polymers based on carbon materials have been used to enhance a wide range of properties, giving rise to functional materials for a wide range of high added value applications in fields such as sensors, energy conversion and storage, and materials engineering.

In 2019, Chen and co-workers synthesized a novel composite material with conductive and mechanical self-healing properties, using the host-guest mechanism [105]. They developed a hydrogel consisting of azobenzene molecules, graphene functionalized with β-CDs and hydroxyethyl methyl acrylate, where the Azo-CDs complexes constituted the main structure of the material, also determining the mechanical properties (Figure 51a). This nanocomposite material showed good flexibility, stability, and potential applications as a sensor due to its conductive and strain sensing properties (Figure 51b). Another example of the use of graphene-based nanocomposites was developed by Wang and collaborators in 2019 [106]. They presented a strategy to construct novel graphene oxide nanocomposites with remarkable light-responsive properties to control the gas permeability of the packaging materials. Indeed, gas-barrier polymer materials have great demand in modern electronic and packaging fields due to the control and regulation of oxygen transmission rate and permeability, depending on external circumstances. For this purpose, they combined the nanocage-structured of the azobenzene-terminated polyhedral oligomeric silsesquioxane (Azo-POSS) with the nanosheets of β-CD-functionalized graphene oxide (GO-CD) and introduced these nanocomposites into a PVA-matrix (Figure 51c). This system exhibited a remarkable supramolecular assembly/disassembly behaviour upon UV/vis irradiation, modifying the gas-permeability properties of the material. In this way it was possible to dynamically control the properties of smart gas barrier materials with promising applications for future intelligent packaging.

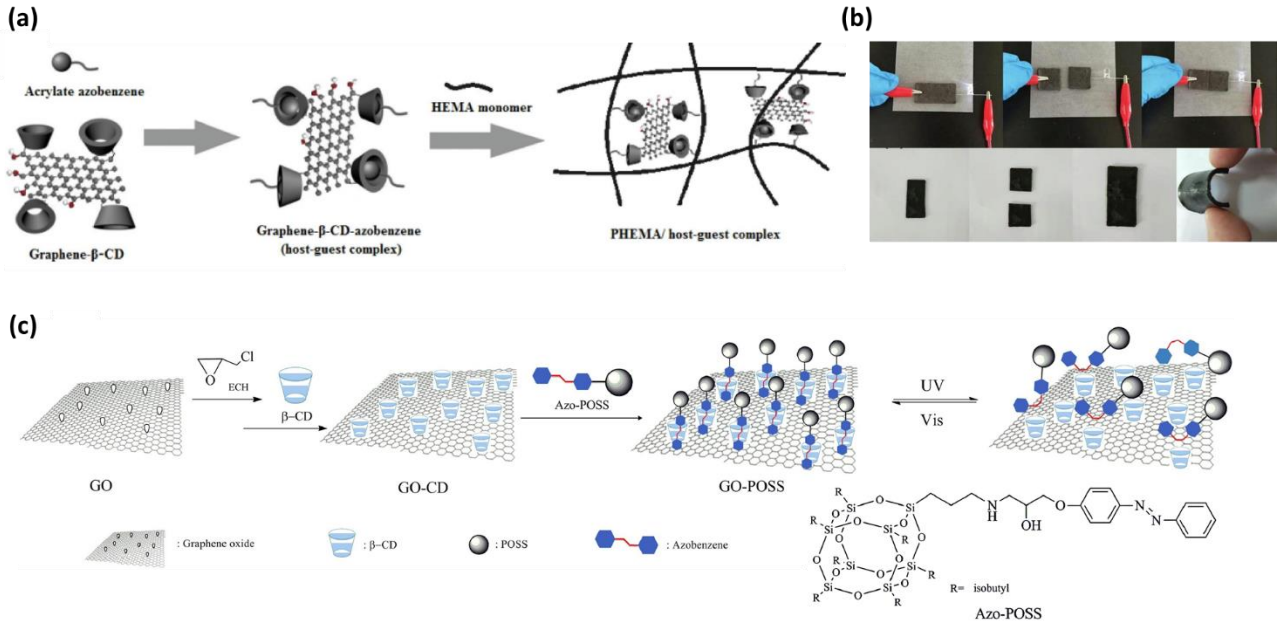


Figure 51 (a) Schematic illustration of the conductive composite graphene-CD-azobenzene nanocomposite synthesis [105]; (b) Images of the electrical, mechanical and self-healing properties of conductive composite graphene-based hydrogel [105]; (c) Schematic representation of the procedure for synthesizing the light-responsive GO-POSS nanocomposite [106].

3.2.4.2. Photoresponsive sensors and capture-materials for environmental applications

The supramolecular systems based on azobenzene-CD complexation have been successfully implemented in numerous scientific fields, including the biological and analytical field and in the treatment of pollutants. Over the years, many researchers have developed self-assembled systems and soft-materials for chemosensory applications and for the removal of organic pollutants. For this purpose, various strategies have been developed: in 2018, Jiao and co-workers prepared a new photosensitive supramolecular polymeric hydrogel via poly-CD (poly-CD) and azobenzene-branched poly(acrylic acid) copolymer (PAA-Azo) in order to use it as a self-healing material and to remove organic molecules that are harmful for the environment, such as bisphenol A (BPA) and methylene blue (MB) [107]. The gel-sol conversion process was due to the isomerization of the azobenzene group, modulated by light and temperature. The prepared gel could bind MB via electrostatic interactions and hydrogen bonding by functional carboxyl groups linked in the polymer molecular chains, and the removal of BPA molecules could be attributed to the host-guest interactions with versatile adsorption process. Recently, in 2020, they designed and synthesized a novel hydrogel material using poly(vinyl alcohol), azobenzene-modified poly-(acrylic acid), and CD-modified poly(acrylic acid) for the removal of organic dyes such as rhodamine and methylene blue (Figure 52a)[108]. Also for this material, the UV light irradiation and temperature changes induced a gel-sol

phase transition in the hydrogel material. Both self-assembled hydrogels could be used as good adsorbents for applications in environmental engineering and wastewater treatment.

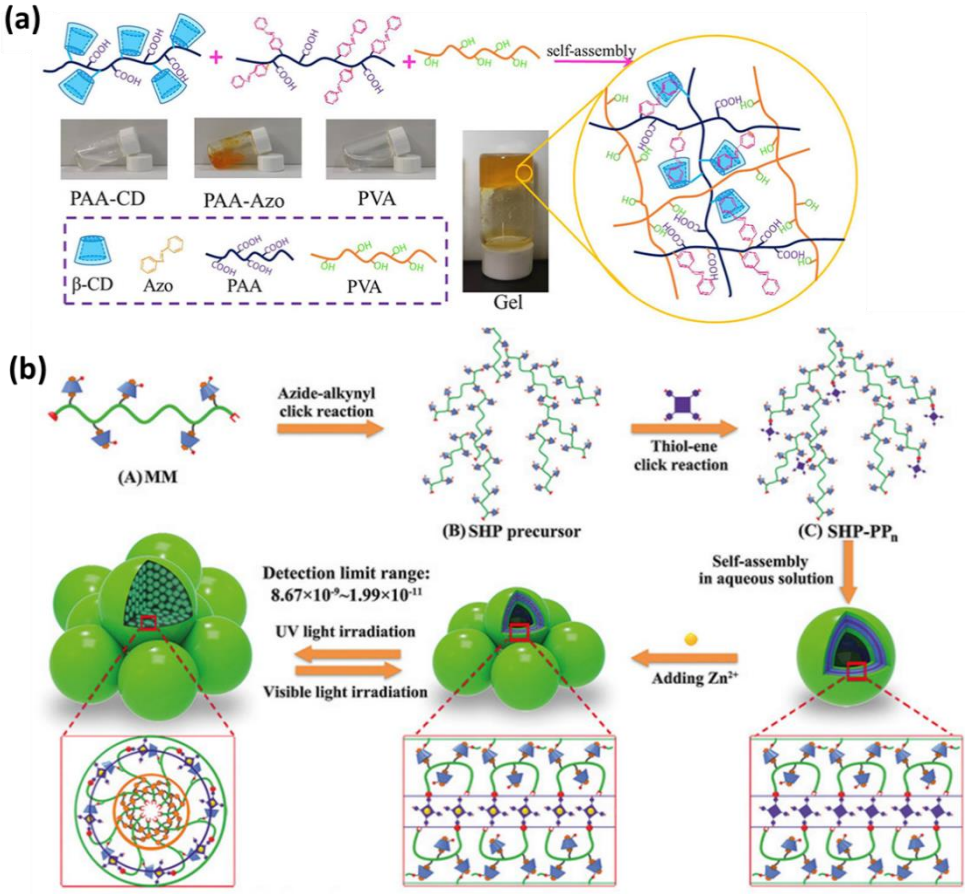


Figure 52 (a) Schematic illustration and images of hydrogel formation [108]; (b) Schematic representation of the construction, sensing process and mechanism of supramolecular polymer vesicles synthesis for Zn^{2+} detection through metal-ligand coordination interactions [109].

In order to detect the presence of pollutants in solution and to determine their concentration, self-assembling vesicular systems and hybrid nanoparticles sensors have been developed over the years. For the first time, in 2018, Tian and co-workers developed a supramolecular polymer vesicle (SPV) chemosensor with a dynamically tunable detection range consisting of porphyrin (PP) moieties and *β*-CD/azobenzene host-guest interactions (Figure 52b) [109]. This sensor was used to detect in fluorescence spectroscopy Zn^{2+} ions in aqueous solution by metal-ligand coordination interactions with high selectivity and sensitivity: the detection limit range was tuned from $8.67 \cdot 10^{-9}$ to $1.99 \cdot 10^{-11}$ mol/L on the basis of the UV-dissociation of the β-CD/Azo host-guest interactions and the association of *β*-CD/PP moieties. The UV-light irradiation caused the formation of bigger multicomponent aggregates composed of large complex micelles, leading to the increase of the fluorescence signal. This method could be used to develop new tunable chemosensors with dynamic detection ranges, since the concentrations of some pollutants may change depending on the conditions of the

environment. Conversely, using nanostructured hybrid assemblies made up of nanoparticle systems and micelles, Deng's group developed a fluorescent sensor for the detection and quantification of Hg^{2+} and Cu^{2+} with detection limits as low as 1.6 and 2.74 μM, respectively [110]. This sensor was constructed by coupling blue-green emission carbon dots (CDs) to a block copolymer micelle-encapsulated red-emission CdSe/ZnS QDs through host-guest interactions (Figure 53a). They demonstrated for the first time the possibility of detecting copper and mercury ions by binding azobenzene-terminated polymeric micelles to *β*-CD units on the surface of *β*-CD-CDs, using dual emission fluorescence under single wavelength excitation. In fact, the presence of these ions determined a fluorescence quenching of QDs-loaded micelles and this mechanism could be used to determine their concentration (Figure 53b). Although a variety of chemosensors as probes have been exploited for the detection of metal ions with high sensitivity and selectivity, the formed probe-metal complexes were hardly suitable for separation, removal, and further recovery. Therefore, efficient detection, removal, and recovery of heavy metal ions from aqueous environments have become an urgent issue.

Chen and co-workers reported a new a method to detect and remove metal ions (Hg^{2+}) from aqueous solutions simultaneously by a fluorescence chemosensor and functional magnetic nanoparticles (Figure 53c)[111]. They developed a novel fluorescent probe based on spirolactam rhodamine B (SRhB), maleic anhydride (MAH), and azobenzene derivative. In this probe, SRhB worked as a fluorophore and MAH as receptor. Azo-molecule was used as the guest group in the formation of an inclusion complex with *β*-CD (*β*-CD)-modified magnetic nanoparticles (CD-MNPs). In fact, after the formation of the stoichiometric complex between Hg^{2+} and SRhBAzo, it was possible to remove the complex from the solution by host-guest interaction: CD-MNP was used as a host material to adsorb the Azo group in the probe-metal binding complex to form an inclusion complex. Then, the complex could be removed using an external magnet and subsequently recovered by UV irradiation, inducing the *trans*/*cis* isomerization of the Azo groups. In this way CD-MNPs could be recycled many times and applied for further detections.

Inspired of the unique host-guest interaction between CDs and *trans* azobenzene, recently Wei and co-workers developed an innovative electrochemical sensor for clinical diagnosis of cancer [112]. Poly(ADP-ribose) polymerase-1 (PARP-1) is an enzyme which could repair DNA damage and it has been found that its presence is larger in tumour cells with respect to normal cells. Therefore, PARP-1 is expected to be a new target of cancer therapy, and it is necessary to explore more sensitive and accurate methods for PARP-1 activity detection. In this application, the dynamic host-guest system

was used to make the electrode recyclable: after immobilizing *β*-CDs on the electrode surface, an Azo-CD complex was generated following the addition of fragments of trans-azobenzene labelled dsDNA. Subsequently, the addition of molybdate ions in solution generated small amounts of $PMo_{12}O_{40}^{3-}$ with the PO_4^{3-} ions present in the dsDNA fragments and a weak electrochemical current was observed with differential pulse voltammetry (DPV). In the presence of PARP-1 and NAD^+, PAR was produced: this molecule contains a large number of PO_4^{3-} which combined with a greater number of MO_4^{2-}. As a result, a strong current was produced, which could be used to detect PARP-1 activity (Figure 53d). Due to the *trans* to *cis* isomerization under UV irradiation, Abz-dsDNA were released from the electrode surface, and it was possible to recycle the electrode for subsequent detections (Figure 53e).

These systems showed how light-controlled isomerization of azobenzene functionality could contribute to the design and synthesis of numerous reusable platforms for the detection of specific analytes in different environmental matrices, providing an excellent starting point for future applications and developments.

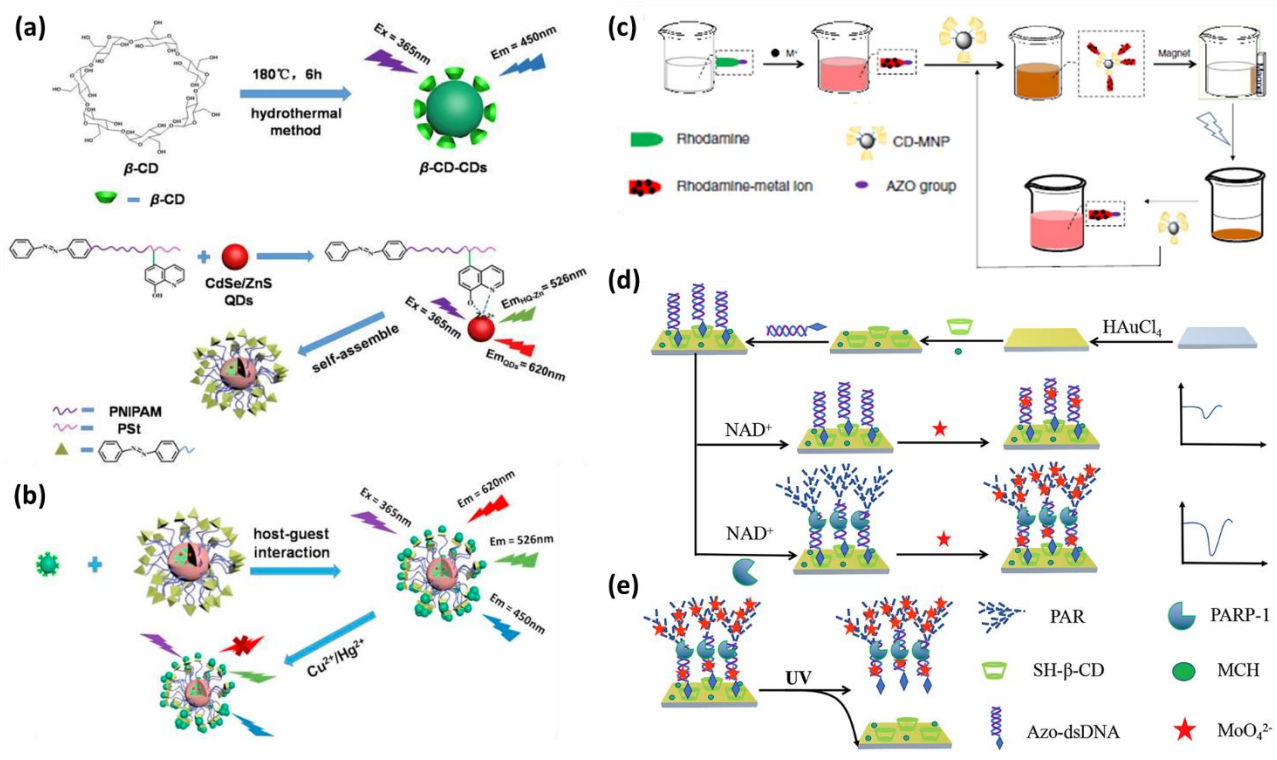


Figure 53 (a) Schematic illustration of the synthetic procedure of *β*-CD-CDs and CdSe/ZnS QDs loaded AZO-BCP micelles [110]; (b) Schematic illustration of the mechanism to detection and quantification mechanism of Hg^{2+} and Cu^{2+} with *β*-CD-CDs/micelles/QDs [110]; (c) Schematic representation of the formation of metal complex, removal, and recovery of the complex by CD-MNP [111]; (d) Schematic illustration of the working mechanism of electrochemical sensor for PARP-1 detection based on host-guest recognition and (e) renewable process of electrode [112].

3.2.4.3. Photoresponsive supramolecular surfaces based on Azo-CD complexes for biological applications

Reversible supramolecular interactions triggered by external stimuli provide a general strategy for controlling surface activity and biorecognition, laying the foundation for a variety of practical applications in the biomedical and biotechnology fields such as biosensors and diagnostic devices. Surfaces having dynamic control of interactions at the biological system-material interface are of high scientific and technological interest and for this purpose, CD-modified substrates together with azobenzene molecules and derivatives can specifically capture and release small molecules, bio-macromolecules and cells. Through the host-guest mechanism, it is therefore possible to imitate the behaviour of cell membranes. Some applications of these supports have been reported in the literature in recent years, which can act as acceptors of bacteria or cells and modulators in cell growth in specific dynamic materials for tissue regeneration, but in each device the host-guest interaction is applied differently.

For the development of supramolecular platforms with switchable multivalent affinity, Chen and co-workers developed a surface that can efficiently capture bacteria and with irradiation at different wavelengths, release them on demand [113]. They synthesized a photoresponsive self-assembled monolayer on a gold substrate, containing azobenzene molecules as ends groups on a hydrophilic spacer (guest) and β-CD-mannose (β-CD-M) conjugates as host. This system exhibited high capacity and specificity for the capture of Gram-negative bacteria, such as Escherichia coli, due to the specific carbohydrate-protein interactions between mannose and the surface proteins of bacteria. Through the simultaneous host-guest inclusion in the CD cavity and the carbohydrate-protein recognition at the mannose moiety, a ternary complex was formed (*trans*-Azo/CD-M/bacteria). The irradiation of UV light on this surface caused the isomerization of Azo from the *trans*-form to the *cis*-form, resulting in the dissociation of the host-guest inclusion complexes and thus the removal of captured bacteria from the surface (Figure 54a). The capture and release process could be repeated for multiple cycles, suggesting good reproducibility and moreover, the possibility to apply this system to other β-CD derivatives with specific biofunctions to capture different bacteria and cells. However, the possible damage that UV light could induce to biological samples and living tissues may limit its applications in vitro or in vivo. To avoid this potential problem, Wang and colleagues developed a new device, functionalized with β-CDs, which used tetra-ortho-methoxy-substituted azobenzene as guest molecule [114]. In this case, host-guest interactions occurred between two different *trans*-azobenzene polymers (polycations and polyanions) and the β-CDs present on the surface. They found that the

polyanionic Azo-polymer exhibited excellent bio-adhesive properties and many bacteria adhered on the surface, while the positive charges of the polycations Azo-polymer exhibited antimicrobial properties. Furthermore, the switch between antibacterial and bio-adhesive behaviour could be realized simply with visible light irradiation: as a result of the Azo-isomerization, the disassembly of the host-guest complex occurred and it was possible to vary the surface characteristics by changing the type of Azo-polymer (Figure 54b). A similar strategy has been used by Gao's group to develop a stimuli-responsive surface to regulate the cell migration in the tissue regeneration [115]. Through the functionalization of a cell adhesion peptide with an azobenzene moiety and the creation of the host-guest complexes with *β*-CD-polymeric platforms, they were able to modulate the growth and the migration of the mesenchymal stem cells on these surfaces. In the presence of cell adhesion peptide, the rate of cell migration was very high but when UV irradiation was used, the release of peptides caused by the configuration transition led to the weakening of cell-substrate interactions and thereby reduced the cell mobility. This stimuli-responsive device provided a new platform for dynamically regulating adhesion and migration of stem cells *in situ* and may be used in dynamic biomaterials for tissue regeneration.

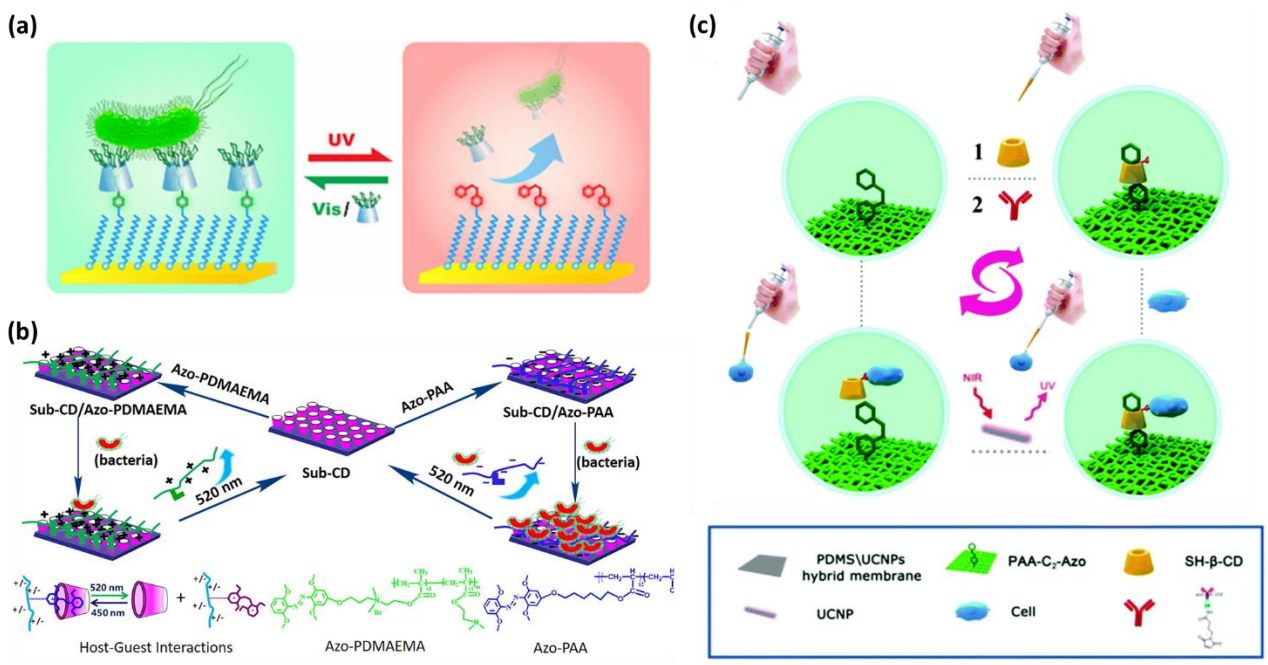


Figure 54 (a) Illustration of the supramolecular platform with switchable multivalent affinity to capture and release bacteria [113]; (b) Schematic illustration of Sub-CD, Sub-CD/Azo-PDMAEMA and Sub-CD/Azo-PAA, and description of visible-light-triggered alternate and reversible switching between antibacterial and bioadhesive behavior [114]; (c) Representation of the working principle of reusable supramolecular ''Pipette Device'': Azo-polymer was deposited as the top layer of the polyelectrolyte multilayers for coupling with the cyclodextrin conjugated antibody. On exposure to 980 nm light, the upconversion nanoparticles can emit UV photons and prompt the transformation from trans-azo to cis-azo, resulting in a fast release of the captured cells [116].

Recently, the regulation of interactions between cells and surfaces with the use of CDs and azobenzene molecules has allowed the development of a revolutionary technology in the identification and removal of cancer cells. In march 2020, Cao and co-workers developed for the first time a light-responsive film to capture and release cancer cells mimicking the mechanism of a laboratory pipette [116]. They used a composite membrane consisting of poly(dimethylsiloxane) and upconversion nanoparticles, whose surface was functionalized with an Azo polymer. Subsequently, following the addition of a *β*-CD modified with an anti-epithelial-cell adhesion-molecule antibody, the host-guest complex was formed and the tumour cells present in solution adhered on the CD portion through the antigen-antibody interaction. If exposed to a NIR-radiation, the upconversion nanoparticles could emit UV photons, leading to the *trans-cis* isomerization of azo units and a rapid release of the CD-cell systems (Figure 54c). In this system the *β*-CD was used as NIR-induced mediators for loading interchangeable capture agents and the composite membrane allowing to reload the antibody molecules for further captures and releases of cancer cells. This technology could be readily extended to produce flexible and diverse thin films with other types of antibodies and may increase the applicability of these materials to other different kind of cells.

The systems described above highlight the possibility of using azo-molecules and CDs as supports for the immobilization of specific cells, allowing their detection, growth, study, and providing new characteristics for the development of diagnostic devices, sensors and smart-materials for the tissue engineering.

3.2.5. Azo-CD based catalytic systems

In recent years, the synthesis and application of photo-responsive dynamic catalysts have allowed the development of new catalytic methodologies both in homogeneous and heterogeneous catalysis. Since access to the catalytic centre is greatly affected by the surrounding steric environment, catalytic functions could be regulated by switching the steric environment around it. Wu and co-workers provided a convenient way to modulate the assembly and disassembly of polyoxometalate (POM) cluster particles with the aim of using these kinds of hybrid POMs in catalytic reactions in different solvents [117]. Polyoxometalates (POMs) are a family of self-assembled molecular anionic metal-oxoclusters, constructed mainly by early transition metals such as V, Nb, Ta, Mo, and W in high oxidation states, with different physical properties, structural features and sizes. The range of size, structure, and elemental composition of polyoxometalates leads to a wide range of properties and a corresponding wide range of potential applications. Furthermore, POMs are typically soluble in water

and in various organic solvents. The authors synthesized a POM that exhibited amphiphilic properties through grafting a spacer group whose end presented an azobenzene group for the formation of an organic-inorganic hybrid cluster (Figure 55a). This molecule showed dynamic reversible transformations via multiple physical and chemical modulations: without any external stimulus, in aqueous environment the hybrid POMs molecules organized forming a self-assembly vesicular system. The subsequent addition of CD molecules (α and β) determined the disassembly of the system due to the formation of host-guest adducts between Azo and CDs molecules. The monodispersed inclusion complex further reassembled into smaller micelles under irradiation with 365 nm light, due to the different geometries between the two Azo-isomers and their steric interactions with the CD-cavities. This transformation was reversibly controlled using 450 nm light irradiation or heating the solution. Moreover, in the case of the POM-Azo/β-CD system, reassembly from the monodispersed state to the vesicular state was achieved by the addition of a competitive guest molecule (1-adamantane carboxylic acid). These results demonstrated that the POM-Azo hybrid cluster could be a multifunctional building block for self-assembly systems and could have catalytic applications in both the homogenous and heterogeneous systems, as a phase-transfer catalyst (when azo molecules are in *cis*-form) or by using the catalytic properties of the POMs functionality. In both applications, it could be possible to obtain the desired products by exploiting the dynamism of the system.

Recently, Gao and colleagues developed a new system that uses the dynamic properties of the host-guest system between Azo and CDs to conduct catalytic reactions in a two-phase environment [104]. They described a triple responsive oil-in-water emulsion that was stabilized by amphiphilic core cross-linked supramolecular polymer particles (called Pickering emulsions) and applied this responsive oil-in-water system as an efficient and recyclable platform for the heterogeneous reaction occurring at the oil-water interface. The use of two immiscible solvents in the biphasic reactions could be a possible solution for the recycling of the solvents since it was possible to advantageously exploit the different solubility of the substrate, the reagent/catalyst and the product to set up an economic and environmental process. In fact, in biphasic catalysis system, the catalyst resides in one phase (e.g. aqueous phase) and the substrate resides in the other immiscible phase or the catalyst and substrates are in the same phase while the product produced is transferred to the second phase. The Pickering emulsions synthesized by Gao and co-workers were oil-in-water emulsions (oil phases generated respectively with styrene, benzene, and chloroform) with a triple stimuli-responsiveness such as temperature, UV light, and presence of competitive guests (Figure 55b). It was composed of two types of self-assembly supramolecular copolymers: the first was constituted by β-CD-poly(N-isopropylacrylamide) (PNIPAM) and the second was an azobenzene-poly(4-vinylpyridine) (P4VP-

AZO). The supramolecular block copolymers were synthesized via the inclusion interaction between *β*-CDs with azobenzene molecules. Following irradiation by UV light or by the addition of a competitive guest molecule, the formed *β*-CD/azobenzene inclusions disassociated, determining the morphological transition and the demulsification of the system. The authors applied this system to perform the heterogeneously catalytic reduction reaction of hydrophobic *p*-nitroanisole by the water soluble $NaBH_4$ and Au-nanoparticles, located at the water-oil interface, in a stabilized chloroform-in-water Pickering emulsion. The reaction was conducted with an efficient and sustainable pattern and besides high reaction efficiency, the responsive demulsification allowed the separation of the products and the recyclability of emulsifiers.

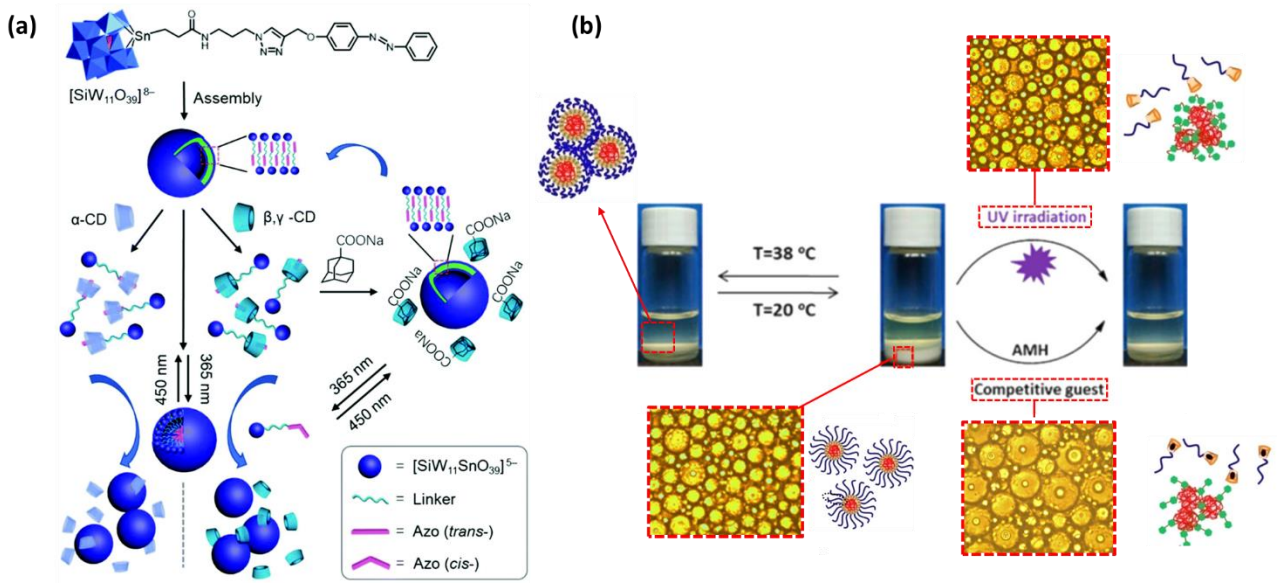


Figure 55 (a) Schematic representation of the formation process of POM-Azo assemblies and their controlled transformations to disassembly systems under multiple stimuli conditions [117]; (b) Schematic illustration and pictures of thermo-, UV light-, and AMH-triggered morphological transitions of Pickering Emulsion [104].

Homogeneous catalysts are usually featured by high activity and high selectivity, but the recovery of homogeneous catalysts from reaction media is sometimes difficult. The most practical solution to avoid such problems is to use heterogeneous catalysts. Although heterogeneous catalysts provide a convenient recovery of the catalyst, the heterogenization of catalytic active species onto supporting matrices inevitably leads to loss of catalytic activity and/or selectivity. For industrial applications, the high efficiency and maximum recyclability of catalysts could be achieved by the construction of a fixed bed. If the catalyst poisoned or damaged itself during the reaction, there would be a problem in either discarding the catalyst together with supporting materials or removing the active catalyst from the supporting materials. Zhu's group, using the reversible host-guest interactions between β-CD and azobenzene, developed a new technique to regenerate the catalytic system by removing the deactivated catalyst with UV-light irradiation and recombining new ones through multivalent

interactions *in situ* [118]. They used a porous nickel support for the fixed bed due to its mechanical strength. The multivalent host-guest interactions between *β*-CD and Azo were used to anchor AuNPs functionalized with *β*-CDs onto the Ni-substrate covered with a silica layer and functionalized with azobenzene-linker molecules, to construct a repairable catalytic bed (Figure 56a). The azobenzene molecules were connected to the support by a linker in order to prevent the aggregation of AuNPs after the creation of the Azo-CD complex. Thanks to the photoisomerization property of azobenzene moiety, the multivalent host-guest interactions between *β*-CD and Azo could be removed by UV irradiation and the gold nanoparticles could be anchored or released from the surface of the porous nickel. This characteristic allowed the regeneration of the catalytic activity of the fixed bed *in situ*, and this approach could provide an effective and regenerative solution for industrial catalysis.

In heterogeneous catalysis, the use of the host-guest complex between photoswitchable molecules and CDs could have extraordinary applications. In addition to industrial applications, it is possible to use such systems to activate reactions in environments difficult to reach, such as intracellular biological environments, where the use of specific transition metal catalysts could rapidly catalyse chemical transformations that could not be realized by natural enzymes. Qu and co-workers proposed a versatile light-controlled catalyst by modifying macroporous silica and Pd-NPs with supramolecular complex of azobenzene and *β*-CD (Figure 56b)[119]. They applied this catalyst with the aim to explore the possibility of catalysing different types of reactions inside the living cells. When the access to the catalytic site of the catalyst was blocked by CDs, the catalytic activity was inhibited, but utilizing the light-isomerization of Azo, the CD blocker could be released and the transition metal could catalyse the desired reactions. Using this principle, different types of reactions were explored, such as the allyl carbamate cleavage reactions (N-alloc-coumarin and protected Rhodamine 110 as weak/no-fluorescent substrates) and the Suzuki-Miyaura cross-coupling reaction, to generate mitochondria-localized fluorescent products for cell imaging (Figure 56c) . Moreover, they were able to activate within the mitochondria a prodrug molecule with the purpose of inducing a targeted and selective cell death after a UV-stimulus. The light-gated host-guest interactions between azobenzene headgroup and β-CD play a critical and important role in reversibly regulation of the catalytic activity: it provides new perspective to customize different heterogeneous catalysts with novel functions, but also has a huge potential in the activation of prodrugs and in the synthesis of active molecules for precision therapy.

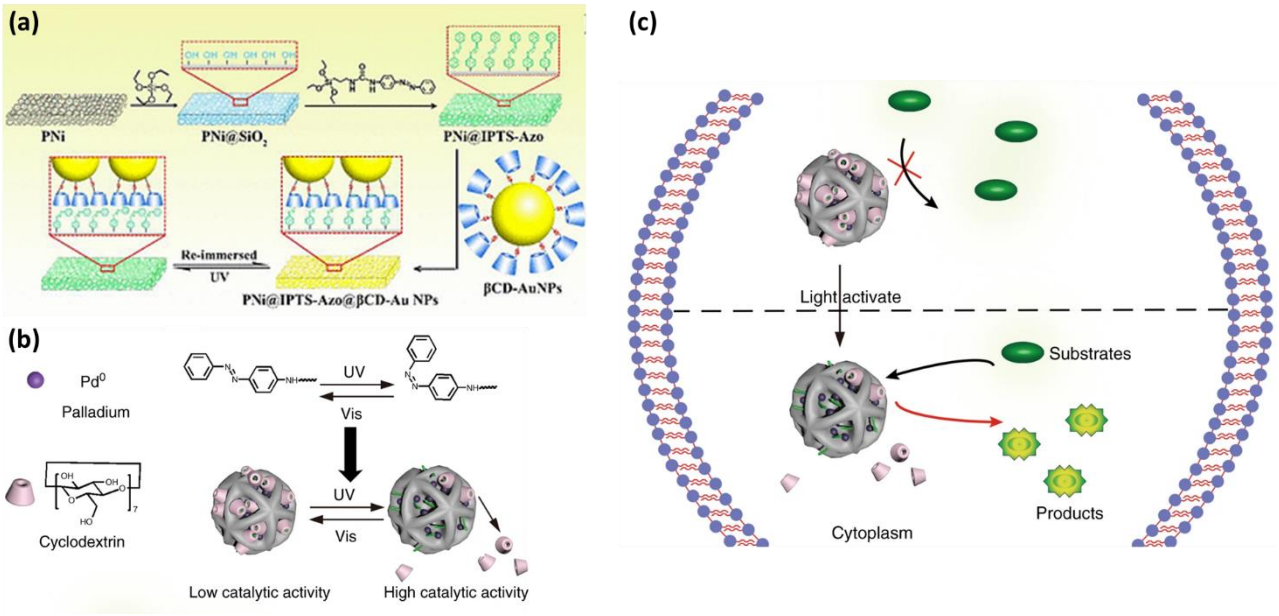


Figure 56 (a) Schematic illustration of the construction process of fixed porous catalyst bed loaded with AuNPs and its photoresponsive property [118]; (b) Representation of the photo-isomerization process between *trans*-azobenzene and *cis*-azobenzene and its effect on the catalytic activity of mesoporous silica/Pd-NPs capped by Azo-modified and CD molecules [119]; (c) Illustration of the light-activated catalytic mechanism of photo-responsive catalyst in living cells [119].

3.2.6. Other applications of Azo-CD supramolecular systems

The inclusion complexes formed between azobenzene and CDs could be used for many other applications, besides those already mentioned in the previous chapters and paragraphs. For example, in 2019 Wilson and co-workers described for the first time the speed manipulation of supramolecular nanomotors via light-responsive valves [120]. Nanomotors are molecular machines that utilize chemical energy to generate physical movement and these systems are often driven by the decomposition of some substrates such as hydrogen peroxide. With this study they demonstrated the possibility to regulate the access of hydrogen peroxide into the motors with the unique possibility to control the speed of these supramolecular systems. They included some PtNPs inside stomatocytes to catalyse the decomposition reaction and attached Azo copolymers onto their surfaces. In the presence of *β*-CDs, strong inclusion complexes were formed, leading to an increased steric impediment, which prevents the easy access of hydrogen peroxide into the inner cavities of the nanomotors. As a result, the speed of nanomotors accordingly decreased due to the lack of fuel. Thanks to the photoisomerization of the Azo compounds, the motion of the nanomotors could be controlled by heating and UV light irradiation, in fact UV-light caused the detachment of the bulky *β*-CDs from the stomatocyte surface and the speeding up of the nanomotors (Figure 57a).

Inspired by biological ion channels, Kong and co-workers, using host-guest interactions between β-CD and azobenzene, developed a bidirectional nanofluidic diode to create a platform with replaceable surface functional groups [121]. These materials exhibited an ion unidirectional transport behaviour and the transport direction of ions could be changed by altering the effective surface charge. Moreover, the system could be applied in various fields, such as photosensitive nanofluidic devices, light-controlled drug transport, pH-activated drug release and devices for optical information storage. Immobilizing β-CDs on a conical nanochannel, the authors modulated its properties exploiting the pH-responsive functional groups attached to the Azo-molecules: using UV light it was possible to modify the properties of the nanochannel walls, characterizing a dual responsive system influenced by light and pH (Figure 57b).

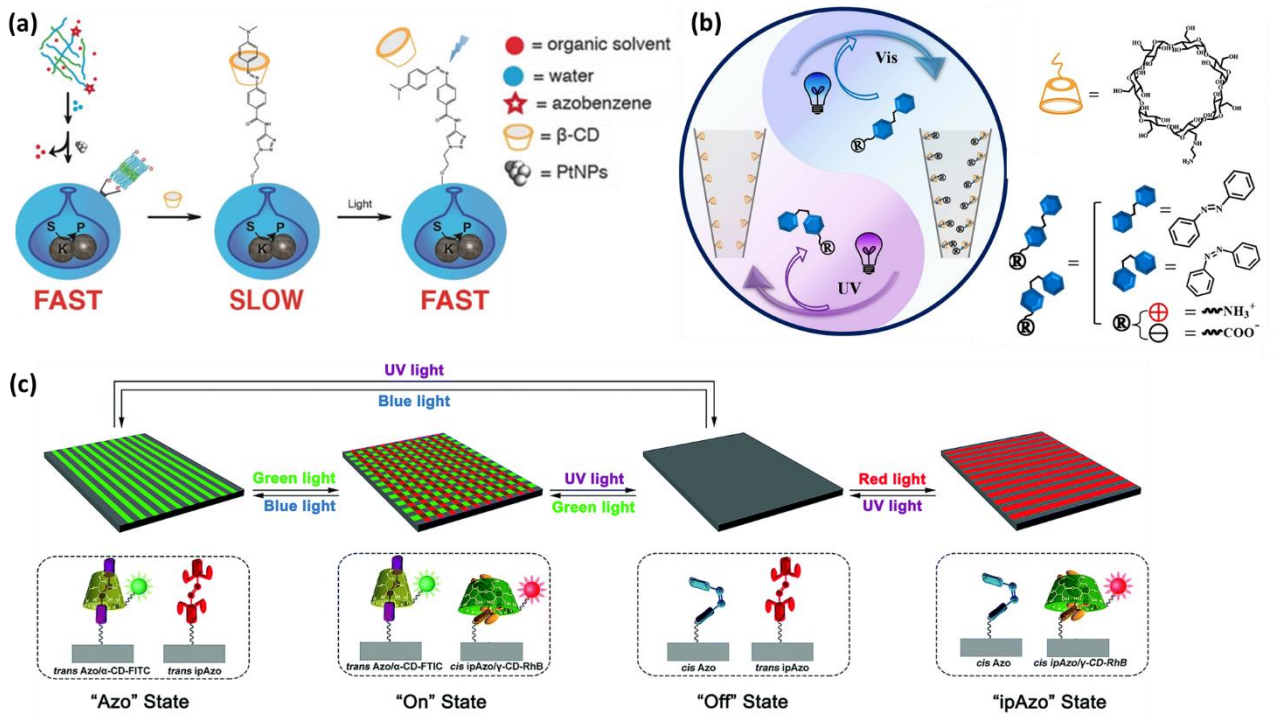


Figure 57 (a) Schematic representation of the stomatocyte nanomotor with a light-responsive valve [120]; (b) Representation of the universal tunable nanofluidic diode controlled via photoresponsive host–guest interactions [121]; (c) Schematic illustration of the photoresponsive supramolecular micropatterned surface: 4 independent states on surface could be obtained and switched under controlling of UV, blue, green and red light irradiations [122].

In 2018, Wu and co-workers demonstrated that the Azo/α-CD and ipAzo/γ-CD host-guest complexes could be used to fabricate photo-switchable supramolecular micropatterned surfaces [122]. Using light with different wavelengths such as UV (365 nm), blue (470 nm), green (530 nm) and red light (625 nm), they identified four independent photostationary states of Azo/ipAzo in combination with CDs, as shown in Figure 57c. This application could have interesting implications for the synthesis of the photoresponsive patterned surfaces, allowing their manufacture with switchable morphology and functions, under precise control of external light irradiation. Moreover, the chemistry of CDs and

azobenzene molecules could be used to load bioactive species in specific positions on the structure of the material during its manufacture, with the possibility of giving the system new properties, moving from the microscopic scale to macroscopic systems.

Finally, through the formation of a supramolecular assembly system based on the host-guest interaction of CB[8], α-CD and a monomer containing coumarin and azobenzene moieties, it was possible to develop a molecular loop-lock system (Figure 58a). Exploiting the self-sorting function of this system, Zou and co-workers synthesized a dynamic lock-key system using the different characteristics and geometric limitations of the host-guest complexes (Figure 58b)[123]. As a result of this study, the use of light and the introduction of α-CDs determined the modification of the system, expanding the ability to synthesize and control systems with defined properties through the formation of inclusion complexes**.**

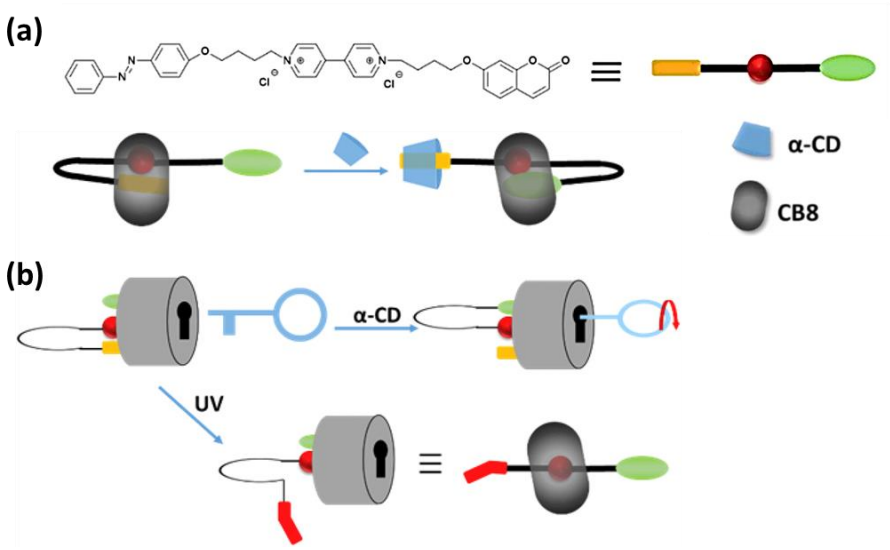


Figure 58 (a) Molecular structures of the linear molecule containing coumarin and azobenzene moieties, α-CD, CB[8], and a schematic description of the self-assembly process [123]; (b) Schematic illustration of the working mode of a molecular loop lock with a key due to the formation of the molecular Azo-viologen-CB[8] complex (locked state) and host-induced formation of ternary complex coumarin-viologen-CB[8] complex(unlocked state[123].

4. Conclusions and perspectives

The aim of this thesis work was to investigate the possibility of modulating the selective synthesis of different CDs using light applied to new different photoswitchable azobenzene as templates, to further expand the potentialities of the CGTase mediated dynamic photo-templated enzymatic synthesis of CDs. The possibility to modulate and control the outcome of enzymatic reactions using artificial photoswitchable templates can offer a new approach to obtain new or difficult-to-access structures and materials in different fields of chemistry and biology. With this strategy, light does not act as activator of substrates or co-factor, but it selects specific products in a dynamic system through the structural changes of photoresponsive templating units. The dynamic nature of the enzymatic reactions allows to obtain numerous molecular structures by modulating *in situ* all the reactive variables.

Unfortunately, it was not possible to achieve the molecular target designed at the beginning of the research: the modification of the experimental conditions of the procedures reported in the literature determined by the irreproducibility of the results has allowed to optimize the synthesis reactions of the synthetic intermediates for the **ipAzo-Py** and **Azo-Py** molecules and due to the failure to achieve the expected result, the synthesis of the 3,5-diisopropoxyphenol will be the subject of a future study. From recent results obtained by Beeren S. R. and Larsen D., we believe that in the future this approach may be extended to a wider range of combinations of enzymes and reactions, directly operating within the enzyme mediated dynamic combinatorial libraries to change the products distribution and to reach the desired molecules. In fact, the host-guest chemistry of cyclodextrin offers high opportunities for the synthesis of specific CDs formed around responsive templates. Especially for the γ-CD, which is so far the least explored among the native cyclodextrins, new discoveries could be made in the future intervening on the geometry and chemical properties of the templates. Furthermore, it will be possible to reach even larger cyclic glucosidic molecules with interesting developments in this emerging field of research.

Light can cause the formation or dissociation of covalent bonds, as well as reversible changes in geometry, polarity or rigidity of particular molecular systems: it is perhaps the most advantageous stimulus as it can be conveniently manipulated enabling the precise selection of the desired wavelength. Based on the properties of the light-responsive moieties, Azo-CD-based supramolecular systems can be designed to be the new frontier for photoswitchable templates in supramolecular chemistry. In fact, over the last three decades, scientists have made significant efforts to understand the supramolecular recognition mechanism between azobenzene and cyclodextrins, toward the design

and synthesis of self-assembling systems, from nano to macro-applications. However, UV-light responsive azobenzene molecules limit the host guest applications, especially biomedical ones, because the UV light shows poor penetrability in the skin and is harmful to cells and tissues. In the future, using the examples reported in this work as a starting point, it will be possible to switch the absorption range from UV light to visible or even better the NIR-light region, allowing the Azo-photoisomerization to occur in a wide interval of wavelengths. Many Azo-CD photoresponsive materials have already been designed: multi-responsive nanocarriers, micelles, nanoparticles, hydrogels, polymers, and self-assembly system, each with interdisciplinary applications. Photochemistry will continue to enrich the development of new smart materials and molecules and the fascinating properties of the stimuli-responsive CD-based systems are promising for many future applications such as green chemistry (synthesis and formulations), materials engineering, environmental science and medicine.

In conclusion, we believe that the applications of these systems will be increased in the next few years and many cross-disciplinary fields will benefit from these technologies, thanks to the facility and versatility of available supramolecular approaches.

This thesis covered the field of photoresponsive azobenzene-CD systems, partially with experimental data carried out at DTU in Copenhagen related to the synthesis of some photochromic units, and partially performing a reviewing activity of the recent literature of the specific field of research due to the COVID-19 lockdown. The reviewing activity pursued during the lockdown period that was carried out on the recent literature covering the period 2018 until date, concerning different applications of azobenzene-CD combinations. This material will be the basis for a review manuscript that soon will be submitted to a scientific journal for publication.

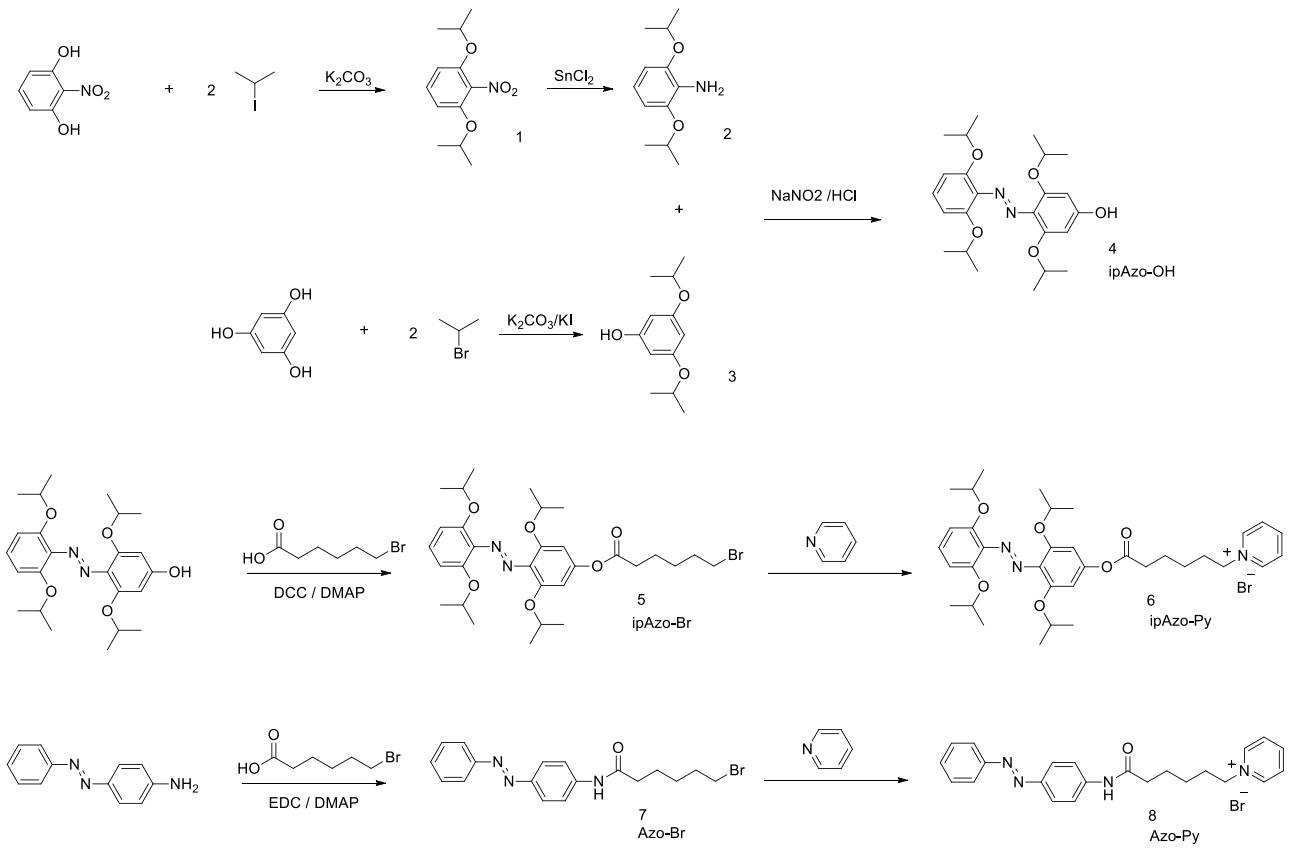
5. Experimental part

5.1. Materials and instruments

All reagents were obtained from commercial suppliers and used as received and all the synthetic procedures are reported in the following paragraphs. Chromatographic analyses were performed on a Thermo Scientific Dionex UltiMate 3000 chromatography. The system was equipped with an autosampler which was maintained at 20 °C and the injection volumes were in the 1.0 - 10.0 μL range. The chromatographic system was connected to an UV and ESI-triple quadrupole-MS detectors. ESI mass spectra were obtained operating in either positive or negative mode. NMR spectra were performed on a Bruker Avance 400 MHz instrument. Chemical shifts were expressed in parts per million (ppm, δ) and coupling constants in Hz. All the instruments are in the laboratories of the Department of Chemistry of the Technical University of Denmark (DTU).

5.2. Synthetic procedures

During my internship it was necessary to modify the experimental procedures reported in the literature (D. Wang, M. Wagner, A. K. Saydjari, and J. Mueller, "A Photoresponsive Orthogonal Supramolecular Complex Based on Host - Guest Interactions," Chem. Eur. J. **2017**, 23, 2628–2634) due to irreproducibility of the results reported. Due to the short time spent in the lab due to COVID-19 lockdown, I was able to optimize only the procedures to prepare products **1** and **2**. Products **3** and **7** were synthesized but not characterized. Products **4, 5, 6** and **8** were only synthesized (Scheme 11).

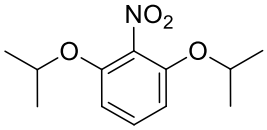


Scheme 11 Route for the synthesis of **ipAzo-Py** and **Azo-Py**.

5.2.1. Synthesis of ipAzo-Py

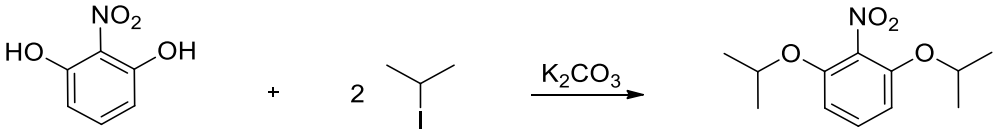
5.2.1.1. Synthesis of 1,3-diisopropoxy-2-nitrobenzene (n°1)

Compound Structure:



Compound Name	1,3-diisopropoxy-2-nitrobenzene

Synthetic Scheme:



Experimental Procedure

Adapted from: D. Wang, M. Wagner, A. K. Saydjari, and J. Mueller, "A Photoresponsive Orthogonal Supramolecular Complex Based on Host – Guest Interactions," Chem. Eur. J. **2017**, 23, 2628–2634

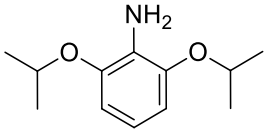
2-Iodopropane (2.3 ml, 23 mmol) was added dropwise to a solution of 2-nitroresorcinol (1550 mg, 10 mmol) and potassium carbonate (4140 mg, 30 mmol) in dry DMF (100 ml). The mixture was stirred at 90 °C for 24 h, cooled to room temperature and the DMF was removed at the rotovapor (black oil). The residue was diluted with water (30 ml) and the crude product was extracted with EtOAc (3 x 20 ml). The combined extracts were dried with $MgSO_4$, filtered, and concentrated (brown-orange oil). The crude product was purified by chromatography (Dry-Column), eluting with *n*-heptane-EtOAc (3% EtOAc gradient) obtaining a pale-yellow solid.

Visual appearance: pale-yellow solid
Yield: 1790mg (7.5 mmol, 75%)
TLC (Rf and eluent): R_f 0.3 (20%EtOAc-80% *n*-heptane)
Exact Mass: m/z calcd. for $C_{12}H_{17}NO_4$ (M+): 239.12; obs: 240.4

^{1}H NMR (DMSO-d_6, 400 MHz, 298 K) δ=7.40 (t, J=8.5Hz,1H), δ=6.87 (d, J=8.6Hz, 2H), δ=4.73 (hept, J=6.0Hz, 2H), δ=1.24 (d, J=6.1Hz, 12H).
^{13}C NMR (DMSO-d_6, 101 MHz; 298 K) δ=149.9, 131.7, 107.4, 72.3, 22.1 ppm.

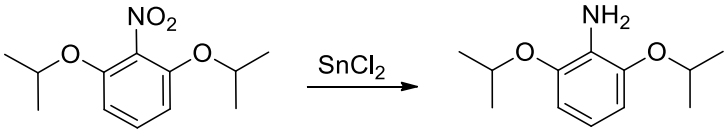
5.2.1.2. Synthesis of 2,6-diisopropoxyaniline (n°2)

Compound Structure:



Compound Name	2,6-diisopropoxyaniline

Synthetic Scheme:



Experimental Procedure

Adapted from: D. Wang, M. Wagner, A. K. Saydjari, and J. Mueller, "A Photoresponsive Orthogonal Supramolecular Complex Based on Host – Guest Interactions," Chem. Eur. J. **2017**, 23, 2628–2634

A solution of 1,3-diisopropoxy-2-nitrobenzene (2300 mg, 10 mmol) in dry EtOH (5 ml) was added dropwise to a solution of anhydrous tin (II) chloride (11377 mg, 60 mmol) and HCl 23M (2 ml)) in dry EtOH (70 ml). The mixture was stirred at 80 °C (reflux) overnight. The disappearance of 1,3-

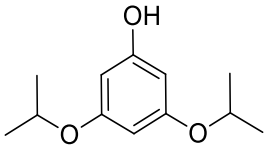
diisopropoxy-2-nitrobenzene and the formation of the amino-product was verified through thin layer chromatography. The solution was cooled to room temperature for 30 min and the reaction quenched with an ice-bath for 30 min. A solution of NaOH 3M was added to the reaction mixture until pH 10/11 (formation of a white precipitate of tin hydroxide). The precipitate was filtered on Celite and the product was extracted from the clear solution with dichloromethane (3 x 20 ml). The combined extracts were dried with $MgSO_4$, filtered, and concentrated providing a white solid. No further purification of the product was necessary (all 1,3-diisopropoxy-2-nitrobenzene must be reacted with an excess of tin (II) chloride anhydrous due to the difficult separation by chromatography of the nitro-compound from the amino-compound).

Visual appearance: white solid
Yield: 1610 mg (7.9 mmol, 80 %)
TLC (Rf and eluent, stain): R_f 0.45 (15 % EtOAc-85 % *n*-heptane, Stain: Ninhydrin for the amine)
Exact Mass: m/z calcd for $C_{12}H_{19}NO_2$ (M+): 209.14; obs: 210.6

^{1}H NMR ($CDCl_3$, 400 MHz, 298 K) δ=6.65 (m, 1H), δ=6.53 (d, *J* = 8.1 Hz, 2H), δ=4.54 (hept, *J* = 6.0 Hz, 2H), δ=3.92 (s, 2H), δ=1.37 (d, *J* = 6.1 Hz, 12H).
^{13}C NMR ($CDCl_3$, 101 MHz; 298 K) δ=145.95, 127.74, 116.73, 106.95, 70.86, 22.41 ppm.

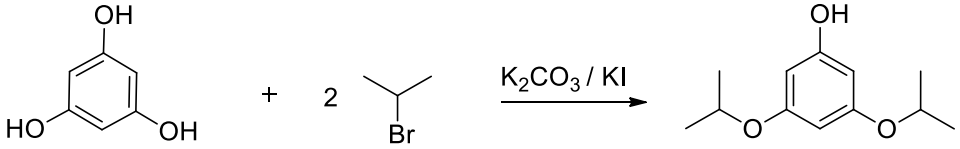
5.2.1.3. Synthesis of 3,5-diisopropoxyphenol (n°3)

Compound Structure:



Compound Name	3,5-diisopropoxyphenol

Synthetic Scheme:



Experimental Procedure

Adapted from: Krishanthi P. Jayasundera, Amy J. Watson, Carol M. Taylor, "Synthesis of a tetrasubstituted arylphosphonate via the anionic phospho-Fries rearrangement", Tetrahedron Letters; **2005** 46,25;4311-4313;2005

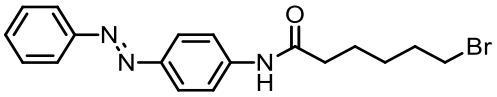
2-Bromopropane (5.6 mL, 7.38 g, 60 mmol) was added dropwise to a solution of phloroglucinol (1.944 g, 12 mmol), K_2CO_3 (8.28 g, 60 mmol) and powdered KI (9.96 g, 60 mmol) in dry DMF (100 mL) under nitrogen atmosphere. The mixture was stirred at 50 °C for 3 days, cooled to rt, diluted with water (100 mL) and extracted with EtOAc (3 x 30 mL). The combined extracts were washed with water (2 x 30 mL) and brine (20 mL), dried over $MgSO_4$, filtered and concentrated. Dry-column chromatography of the residue, eluting with *n*-hexane-EtOAc (0,1% EtOAc gradient), provided two different products, one as a pale-yellow oil and one as a yellow solid. The products were analyzed by ^{1}H-NMR and HPLC-MS analysis

TLC (Rf and eluent, stain): R_f 0.7 (EtOAc-*n*-hexane 1:2, Stain: $FeCl_3$)

5.2.2. Synthesis of Azo-Py

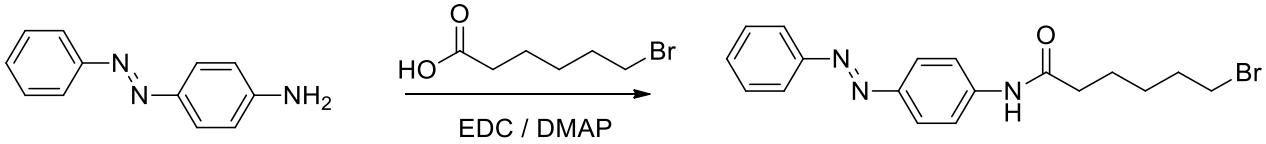
5.2.2.1. Synthesis of Azo-Br (n°7)

Compound Structure:



Compound Name	6-bromo-N-(4-(phenyldiazenyl)phenyl)hexanamide

Synthetic Scheme:



Experimental Procedure

Adapted from: D. Wang, M. Wagner, A. K. Saydjari, and J. Mueller, "A Photoresponsive Orthogonal Supramolecular Complex Based on Host – Guest Interactions," Chem. Eur. J. **2017**, 23, 2628–2634

A solution of 6-Bromohexanoic acid (246 mg, 1.3 mmol) in dry dichloromethane (60 ml) was stirred and cooled at 0 °C for 15min, and then 4-aminoazobenzene (256 mg, 1.3 mmol) was added to the solution. After 10 min at 0 °C, DMAP (20 mg, 0.2 mmol) was added to the solution and after an additional 10 minutes, a solution of EDC (248 mg, 1.3 mmol) in DCM (10 ml) was added dropwise to the reaction mixture. The orange solution was stirred at 0 °C for 1 h and then at rt for 72 h. The

solvent was then removed by evaporation, and the crude product was analysed by NMR spectroscopy and HPLC-MS.

Visual appearance (crude product): orange solid
TLC (Rf and eluent, stain): R_f 0.92 (70% EtOAc-30% *n*-heptane, Stain: Ninhydrin)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (m, 4H), 7.74 (d, *J* = 8.6 Hz, 2H), 7.52 (m, 3H), 3.46 (t, *J* = 6.7 Hz, 2H), 2.47 (t, *J* = 7.5 Hz 2H), 1.95 (quint, *J* = 6.8 Hz, 1H), 1.82 (quint, *J* = 7.3 Hz, 1H), 1.59 (quint, *J* = 7.7 Hz, 1H).

6. Acknowledgements

I wish to express my deepest gratitude to Prof. Sophie R. Beeren, my DTU Supervisor, for giving me the opportunity to do this thesis work in her group in the Department of Chemistry of the Technical University of Denmark. Thank you for trusting me, letting me have this wonderful experience and giving me the opportunity to live and discover the essence of scientific research in an interdisciplinary environment during the whole period I spent in Denmark.

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My sincere thanks go to Dott. Dennis Larsen, my Tutor, and Dott. ssa Giorgia Masciotta. Thank you for welcoming me and making me feel at home during the period in Denmark, for being always available and sharing with me your experience. It was a great pleasure to meet you and working together. Furthermore, I am also grateful to all the Sophie's group, for making me fell part of this beautiful research group.

Finally, I would like to thank my parents and friends for providing me with unfailing support and continuous encouragement throughout my years of study.

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8. Appendix A

1,3-diisopropoxy-2-nitrobenzene

HPLC-MS Analysis:

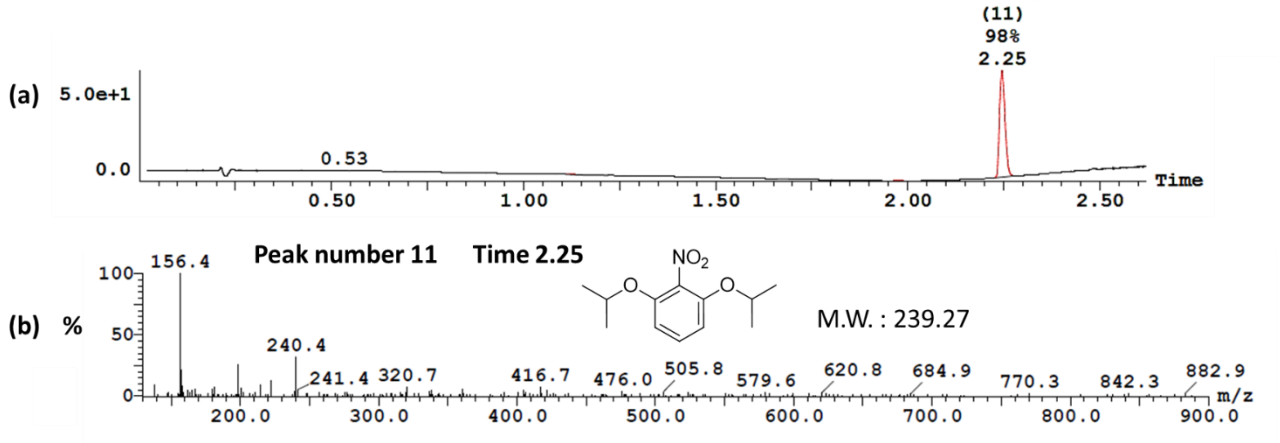

Figure 59 HPLC-MS chromatogram (a) and mass analysis (b) of 1,3-diisopropoxy-2-nitrobenzene.

NMR Spectra:

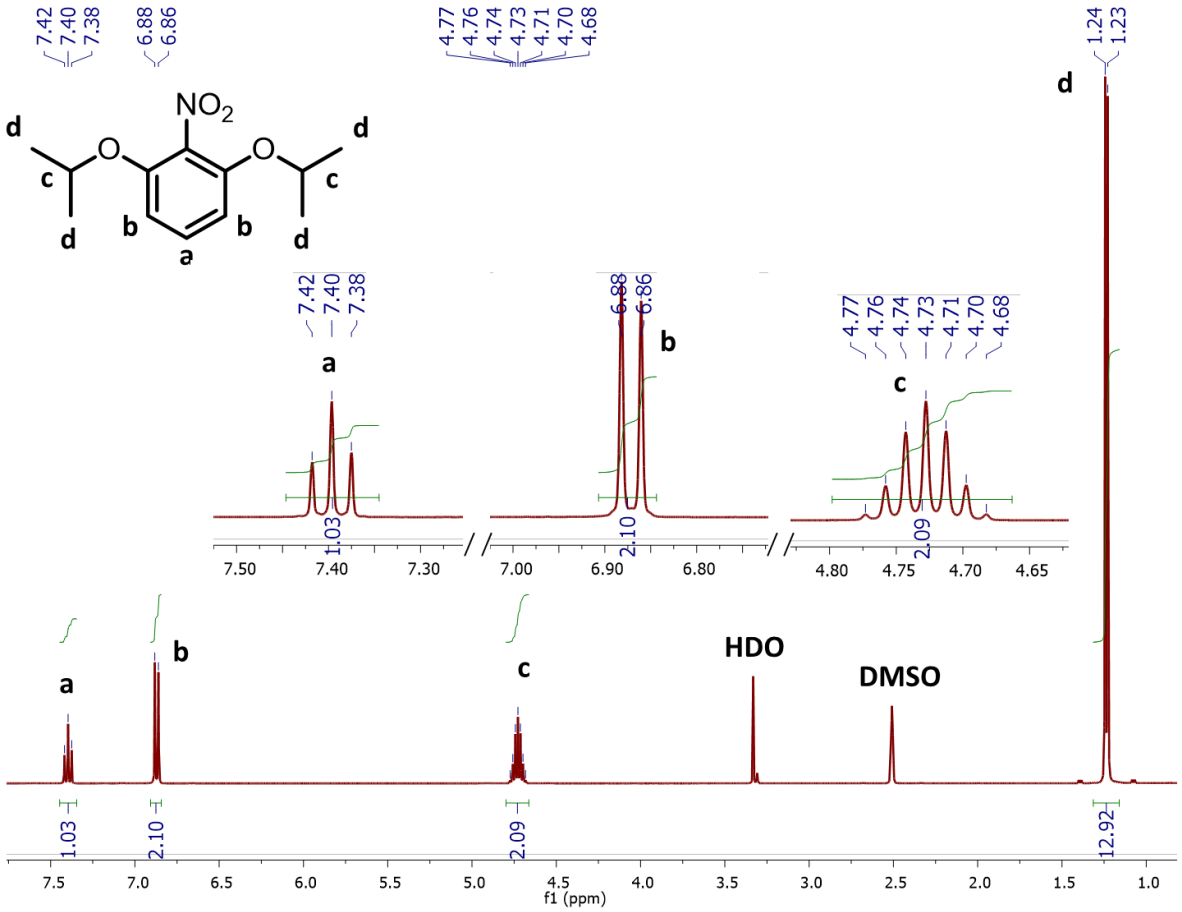

Figure 60 ^{1}H-NMR (DMSO-d_6, 400MHz, 298K) spectrum of 1,3-diisopropoxy-2-nitrobenzene.

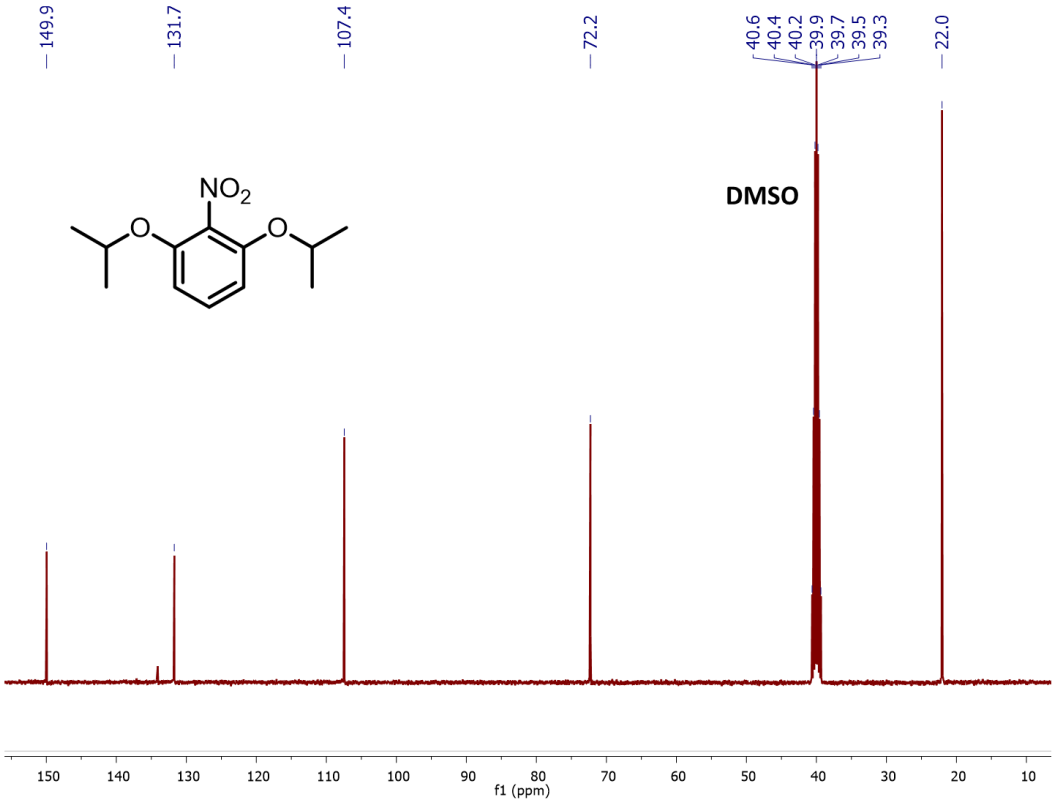

Figure 61 ^{13}C-NMR (DMSO-d_6, 101MHz; 298K) spectrum of 1,3-diisopropoxy-2-nitrobenzene.

2,6-diisopropoxyaniline

HPLC-MS Analysis:

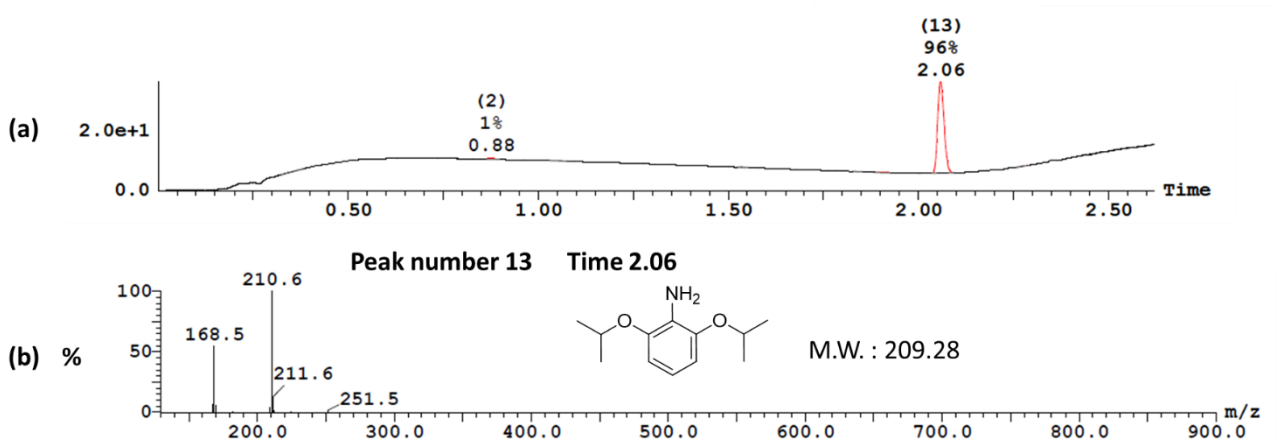

Figure 62 HPLC-MS chromatogram (a) and mass analysis (b) of 2,6-diisopropoxyaniline.

NMR spectra:

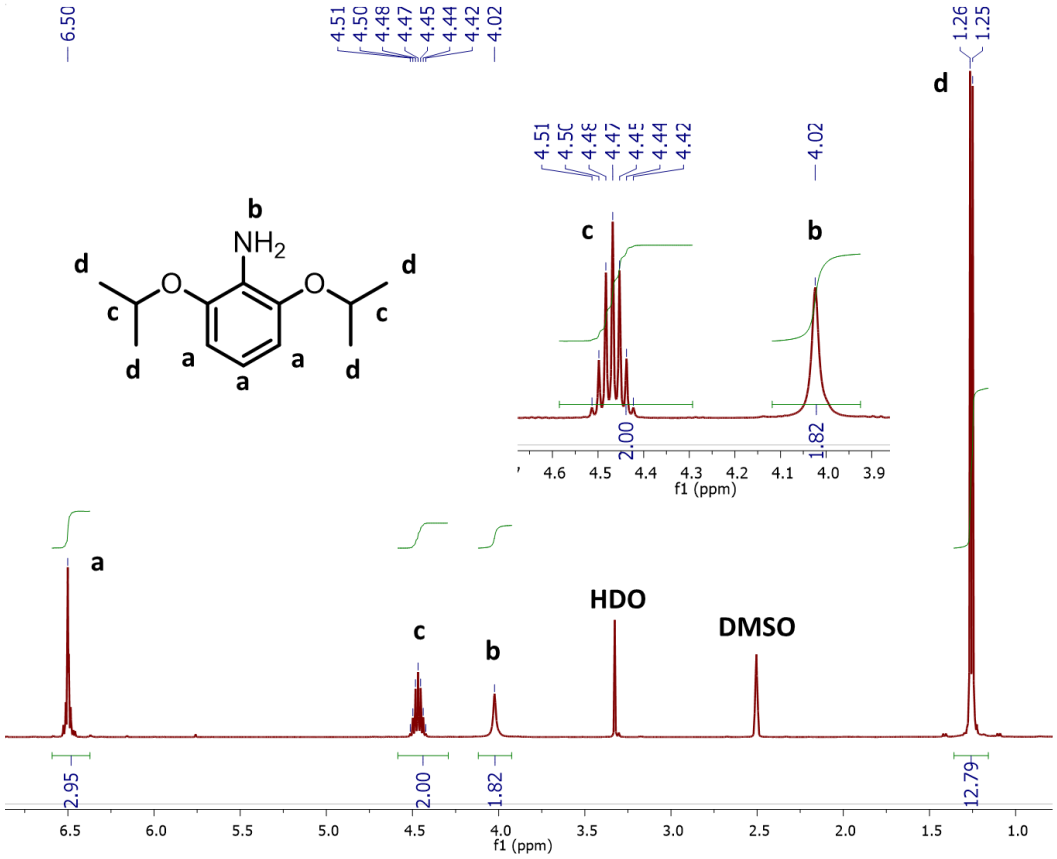

Figure 63 ^{1}H-NMR (DMSO-d_6, 400MHz, 298K) spectrum of 2,6-diisopropoxyaniline.

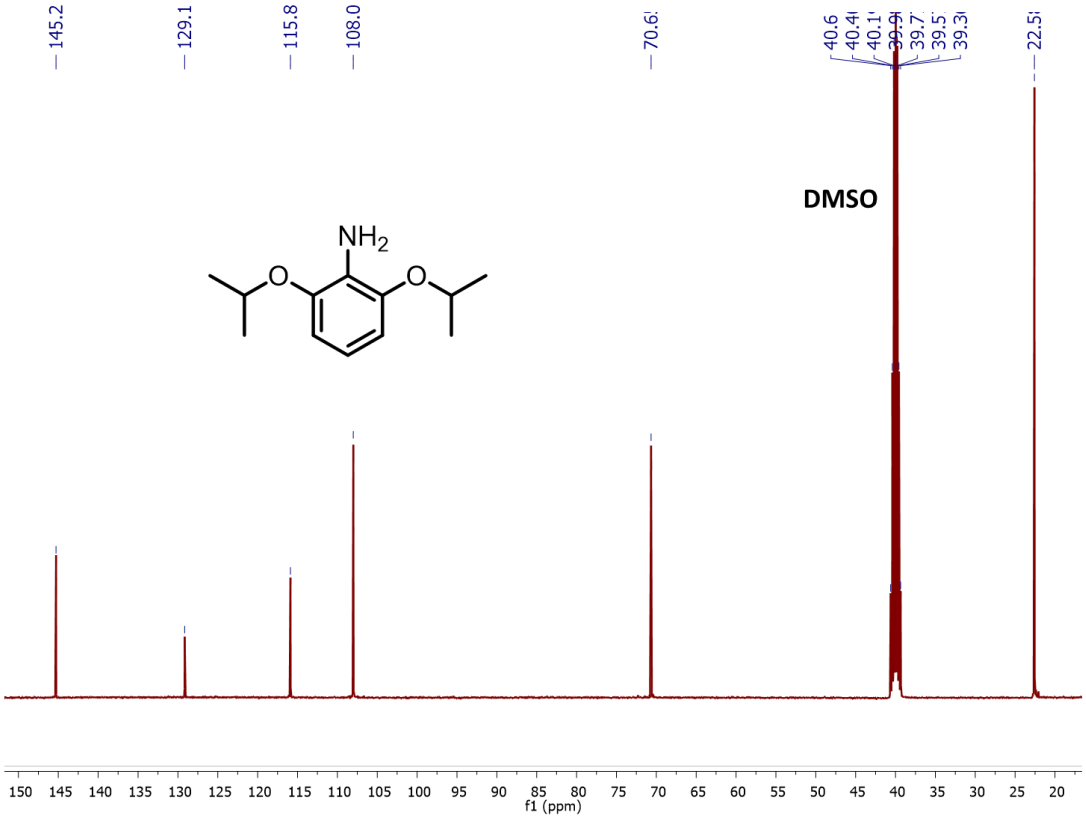

Figure 64 ^{13}C-NMR (DMSO-d_6, 101MHz; 298K) spectrum of 2,6-diisopropoxyaniline.

3,5-diisopropoxyphenol

HPLC-MS Analysis:

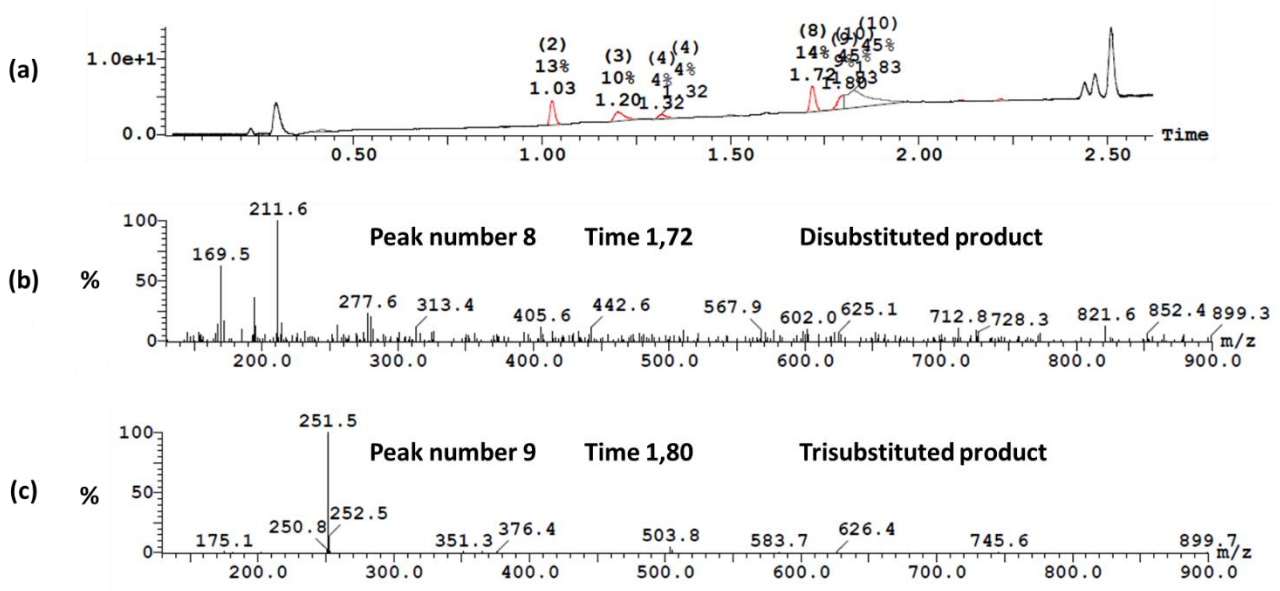

Figure 65 HPLC-MS chromatogram and mass analysis of the 3,5-diisopropoxyphenol synthesis: (a) chromatogram of the crude product; (b) (c) mass analyses of the peaks number 8 and 9 characteristics of the disubstituted and trisubstituted products.

NMR spectra:

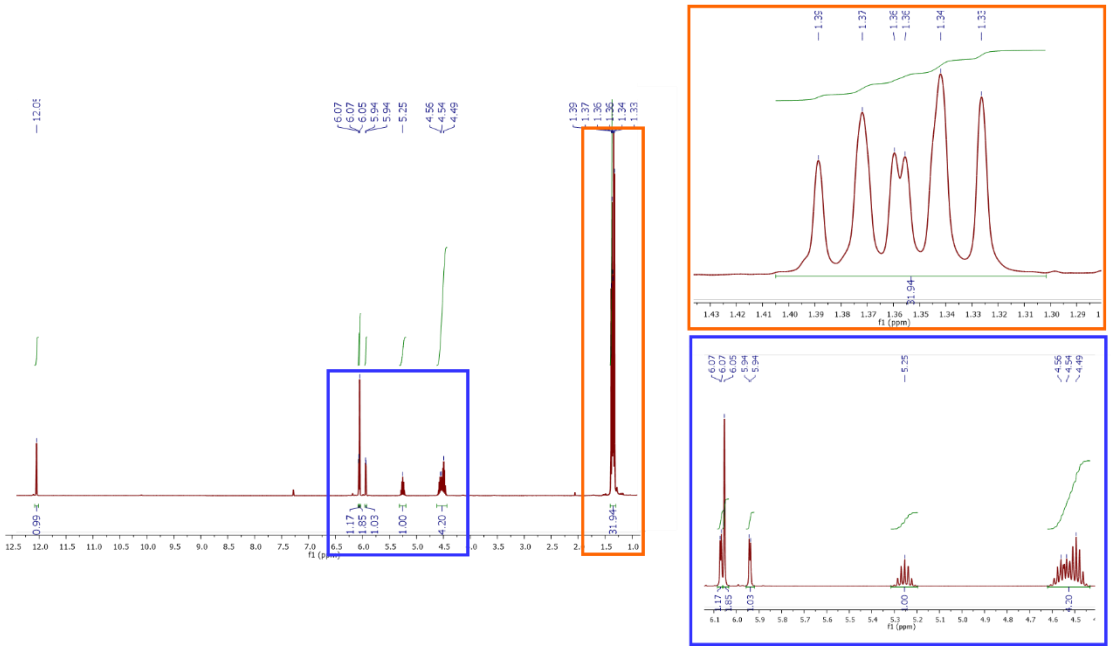

Figure 66 ^{1}H-NMR ($CDCl_3$, 400MHz, 298K) spectrum of the first fraction obtained from the dry-column chromatography with expansion of the aromatic region (frame in lower right corner) and aliphatic region (frame in upper right corner).

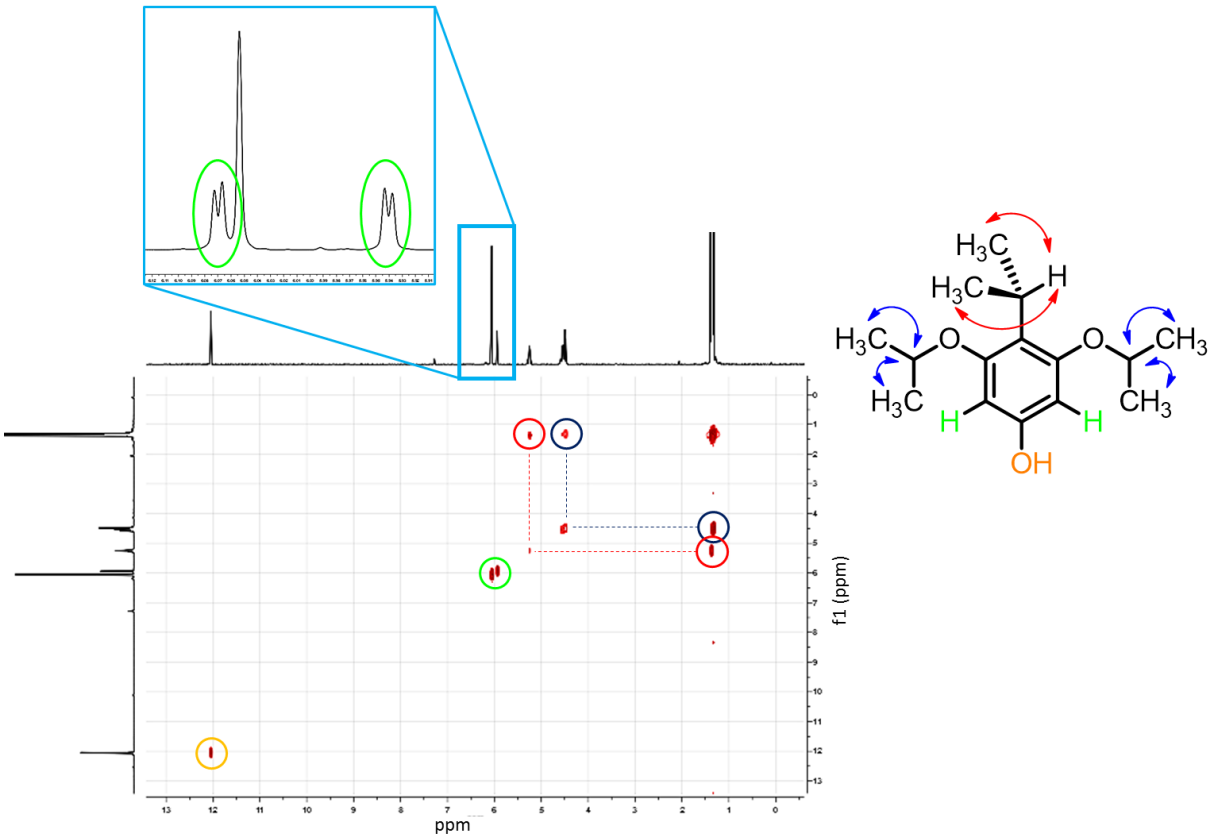

Figure 67 ^{1}H-^{1}H -COSY ($CDCl_3$, 400MHz, 298K) spectrum of the first fraction obtained from the dry-column chromatography with expansion of the aromatic region (frame in upper left corner). In the upper right corner, one of the possible molecular structures derived from the synthesis of 3,5-diisopropoxyphenol is proposed.

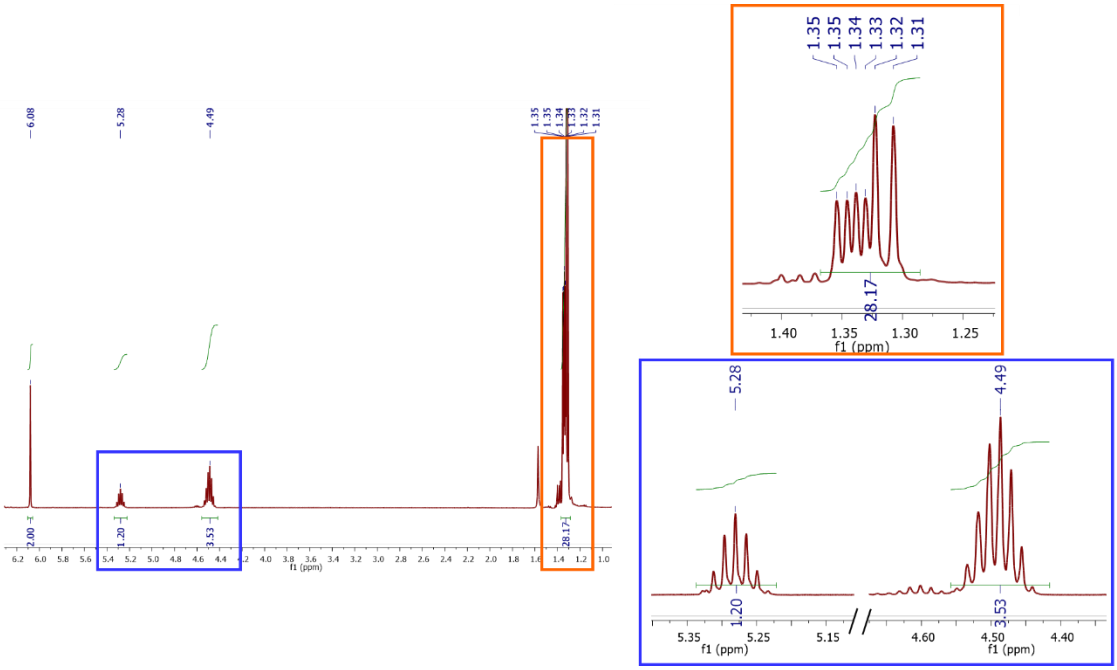

Figure 68 ^{1}H-NMR ($CDCl_3$, 400MHz, 298K) spectrum of the second fraction obtained from the dry-column chromatography with expansion of the characteristic signals of isopropyl and isopropoxyl group's proton (frame in lower and upper right corner)

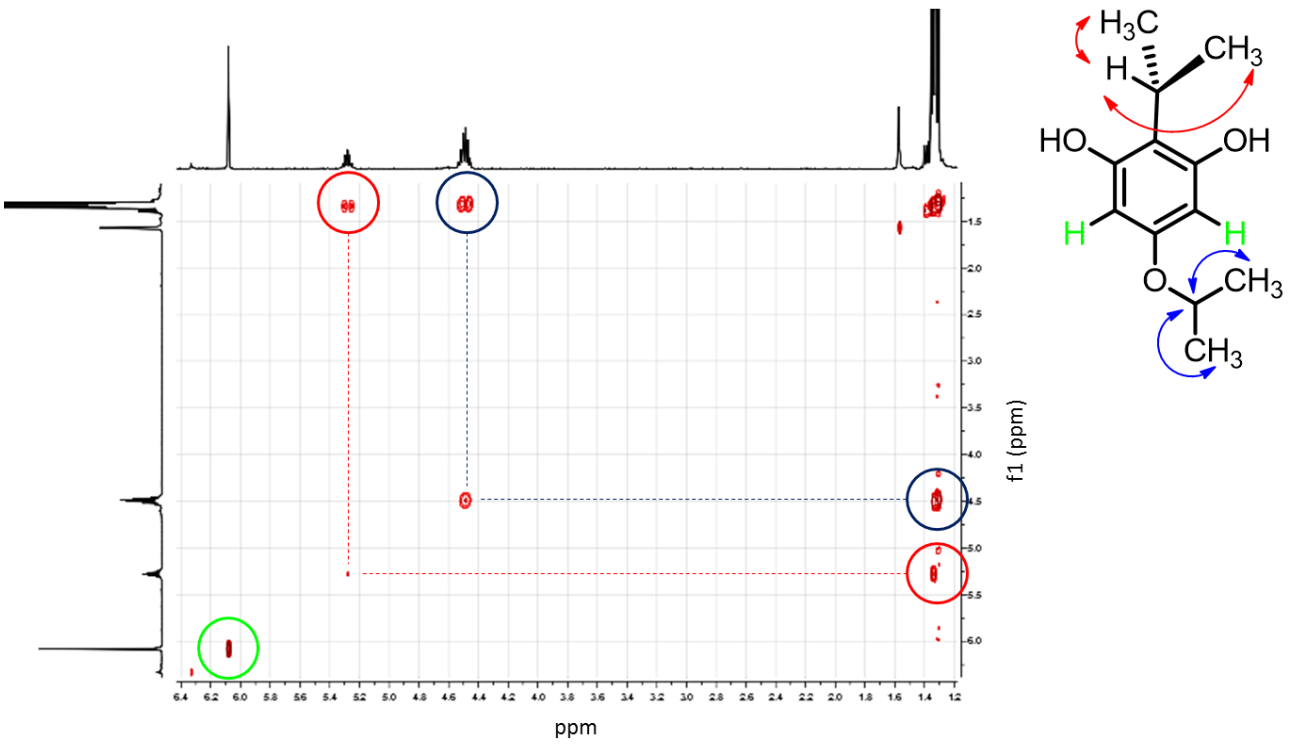

Figure 69 ^{1}H-^{1}H -COSY ($CDCl_3$, 400MHz, 298K) spectrum of the second fraction obtained from the dry-column chromatography. In the upper right corner, one of the possible molecular structures derived from the synthesis of 3,5-diisopropoxyphenol is proposed.

6-bromo-N-(4-(phenyldiazenyl)phenyl)hexanamide

HPLC-MS Analysis:

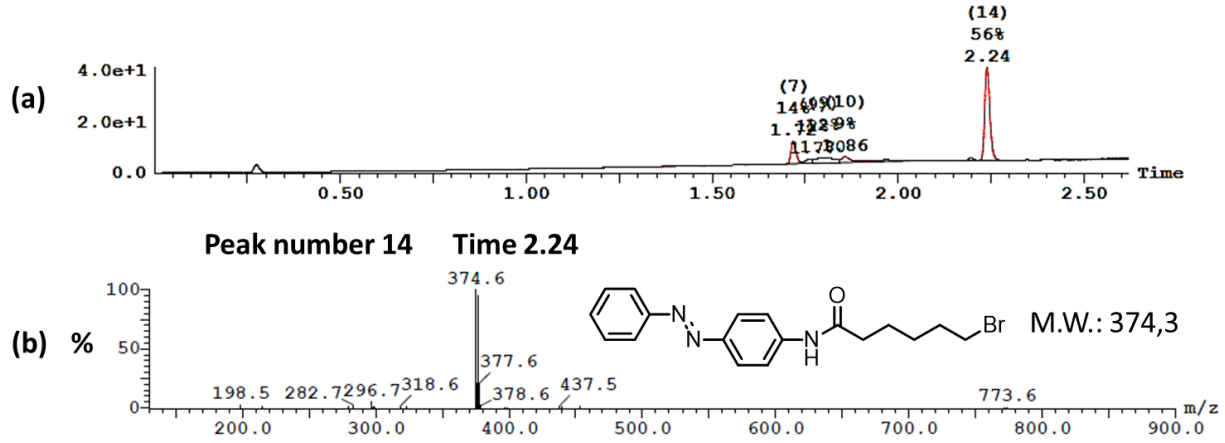

Figure 70 HPLC-MS chromatogram (a) and mass analysis (b) of 6-bromo-N-(4-(phenyldiazenyl)phenyl)hexanamide.

NMR spectra:

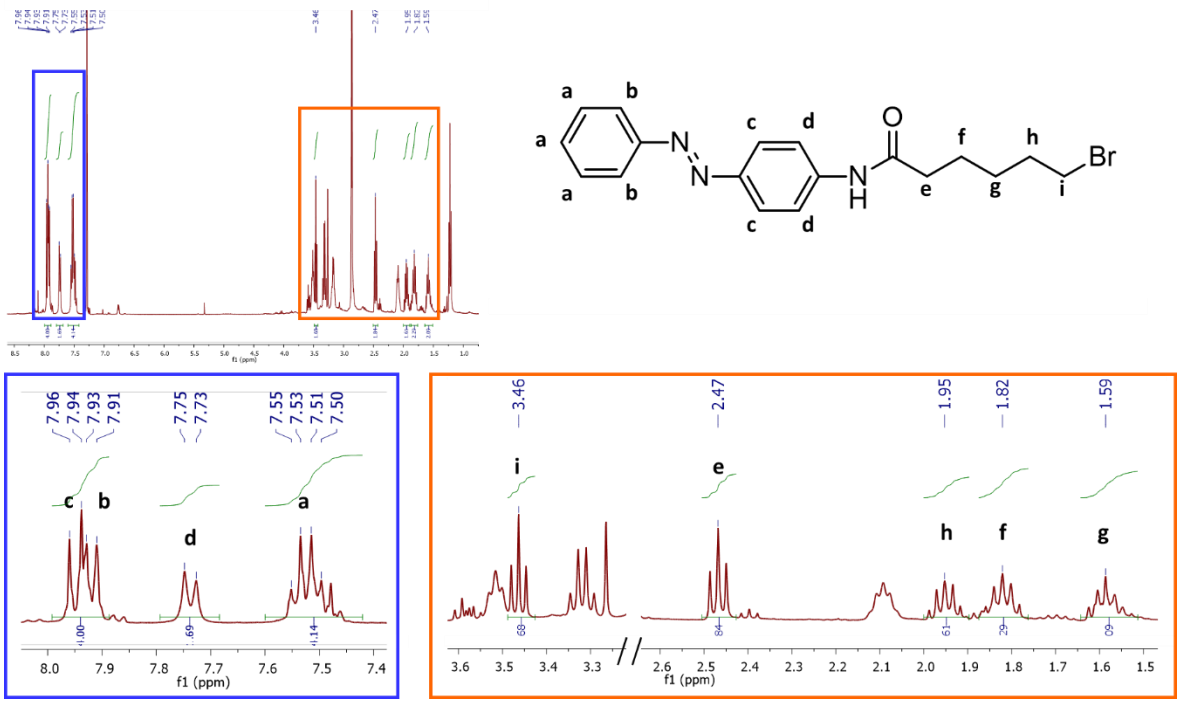

Figure 71 ^{1}H-NMR ($CDCl_3$, 400MHz, 298K) spectrum of 6-bromo-N-(4-(phenyldiazenyl)phenyl)hexanamide with expansion of the aromatic region (frame in lower left corner) and aliphatic region (frame in lower right corner).

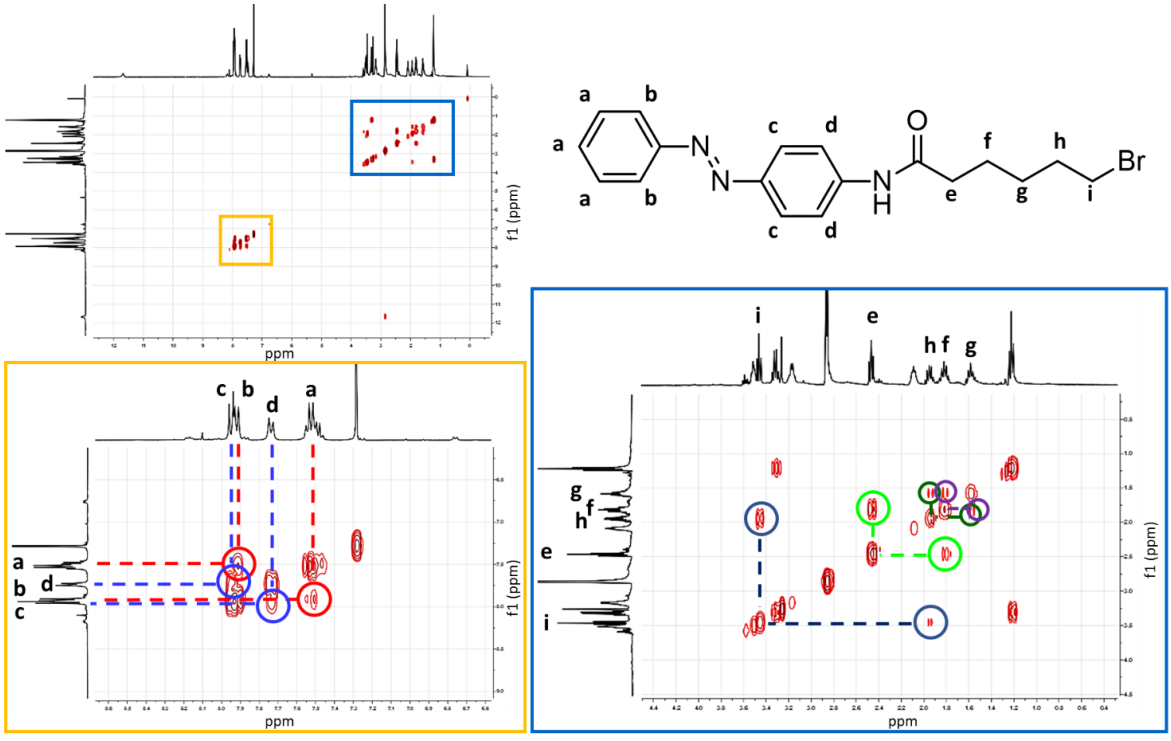

Figure 72 ^{1}H-^{1}H -COSY ($CDCl_3$, 400MHz, 298K) spectrum of 6-bromo-N-(4-(phenyldiazenyl)phenyl)hexanamide with expansion of the aromatic region (frame in lower left corner) and aliphatic region (frame in lower right corner).