Maike Wiemann

**BINDING OF AZOBENZENE
DERIVATIVES ONTO
MACROCYCLIC SURFACES**

Maike Wiemann

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Chapter 1

Stimuli-Responsive Cucurbit[n]uril-mediated Host-Guest Complexes on Surfaces

Supramolecular functional surfaces are at the heart of many materials and medical applications. Increasing interest can be seen for devising new supramolecular functionalization strategies of surfaces. In this chapter, we place particular emphasis on the use of cucurbit[n]uril-mediated host-guest complexation as surface functionalization strategy. The state of the art of cucurbit[n]uril-mediated host-guest complexes on surfaces is reviewed. Cucurbit[n]urils (CB[n]) are able to form strong host-guest complexes with affinities that span several orders of magnitudes up to the regime of the biotin-streptavidin pair. The CB[n] host-guest complexes can be modulated by applying remote stimuli provided suitably sensitive guests were selected. Strategies to fabricate stimuli-responsive surfaces creates versatile supramolecular systems and several applications of these types of surfaces are outlined.

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1.1 Introduction

1 Cucurbit[n]urils CB[n] are pumpkin-shaped macromolecules first synthesized by Behrend.^[1,2] CB[n] are methylene bridged glycoluril oligomers that appear in the condensation of glycoluril and formaldehyde.^[3-5] This condensation reaction yields different CB[n] homologues ranging from CB[5] to CB[8], but also traces of higher homologues were isolated by Kim, Day and Li.^[3-8] CB[n] have a common depth, but their width and volumes differ progressively with their ring size (Table 1, Figure 1.1).^[3-5] CB[n] are symmetric and possess two identical portal sites consisting of carbonyl groups. These portals narrow the cavity entrance by approximately 2 Å compared to the inner diameter of the cavity (Table 1).^[7,9] Electrostatic potential maps show a negative potential at the CB[n] portals,^[5,10] which drives the molecular recognition properties by forming ion-dipole and hydrogen bonding interactions with guests.^[5,10,11] Much research on CB[n] macrocycles focusses on understanding CB[n]-guest complexation and modulating binding affinity to CB[n] by external stimuli.^[11-13] These insights in CB[n] host-guest chemistry have recently led to developing bioanalytical and biomedical applications that are based on surface-anchored CB[n] host-guest chemistry. While there are recent reviews on CB[n] related host-guest complexation in solution,^[11,13,14-16] in this chapter latest progress of CB[n]-mediated host-guest complexes on surfaces is reviewed with special attention to stimuli-responsive studies. Stimuli-responsive systems using pH, light or electrochemistry are attractive as such systems offer advantages such as dynamics and reversibility, so that they are able to function as molecular machines or mimic molecular systems from nature. Selected examples to illustrate the state-of-the-art stimuli-responsive CB[n] systems in solution are given before recent investigations on surface-anchored stimuli-responsive CB[n] systems in much more detail.

Figure 1.1 | Structural parameters and space-filling models for CB[n] homologues illustrate their increasing size and volumes with constant height. Reproduced with permission from ref [11]. Copyright 2015 American Chemical Society.

1.2 Host-Guest Chemistry of Cucurbit[n]urils

Increasing the host cavity size from CB[5] to CB[8], increases the size of the guests to form 1:1 host guest complexes.^[5] Interestingly, the CB[8] host offers the possibility of including two guests.^[17,18] CB[8] includes either two different guests to form a 1:1:1 heteroternary complex or two times the same guest to form a 1:2 homoternary complex. Non-cooperative binding is conceived to be the most probable binding scenario for binding of two guests to CB[8].^[13,17,18] In the case of hetero-complexation, the electron-poor guest binds first to the CB[8] followed by the electron-rich guest in agreement with donor-acceptor behaviour.^[10,13] CB[8] hetero-complexation has been exploited for various assembly schemes. An interesting example by Kim and co-workers showed an unusual back-folding complex of a naphthol-methylviologen derivative, which can lead to a folded or unfolded system with CB[8] (Figure 1.2).^[20] As shown by several groups CB[8] can be used for photo-induced chemical reactions inside the cavity,^[21-29] and switching between CB[n]-favouring and disfavouring conformers can be achieved through applying of an external stimulus.^[30-33]

Figure 1.2 | Possible charge-transfer complexes of a naphthol-methylviologen derivative (A: methylviologen, D: naphthol) and CB[8]. Reproduced with permission from ref [20]. Copyright 2002 The Royal Society of Chemistry.

The role of water in host-guest complexation has received attention in numerous studies.^[32-35] In aqueous solution, water molecules are complexed in the cavity of CB[n], whose amount is depending on the cavity size.^[36] Since encapsulated water molecules are of high energy, due to limited hydrogen bonding and weak interaction with the walls of the CB[n],^[36,37] released water molecules by guest complexation lead to positive energy contribution to guest inclusion.^[37,38] Recent studies by several groups demonstrated the potential of applying CB[n]-mediated complexes for anticancer therapies, for example as drug carrier or sensors.^[39-42]

In the design of dynamic systems with on-demand changes in guest binding by stimuli-responsiveness, it is attractive to exploit CB[n] since they are selective binders while being sensitive to changes in molecular structure of the guests.^[43] An earlier example is the shuttling of CB[6] along a triamine chain.^[44] Shuttling of CB[6] was a consequence of changes in pH to deprotonate and protonate the amine groups, which causes CB[6] to unbind and rebind.^[44] Pioneering work by Kim and coworkers shows results of applying control over recognition processes by means of photo- and/or electrochemistry. CB[8] has been frequently used for different purposes, such as vesicle formation, stoichiometry control or CB[8]-mediated chemical reactions.^[17,18,45-49]

Figure 1.3 | Schematic presentation of the in situ formation of a CB[6]-based hydrogel and possible cell-hydrogel interactions. Hyaluronic acid (HA) and polyamines (PA). Reproduced with permission from ref. [50]. Copyright 2012 American Chemical Society.

Based on these interesting properties of CB[8], a molecular machine was designed to demonstrate different guest binding modi in response to appropriate stimuli. A 1:1 host-guest complex of hexamethylene-bridged bisviologen with CB[8] can reversibly form a molecular loop triggered by electrochemical or photochemical stimuli. Both stimuli lead to a one-electron reduction of the methylviologen (MV^{2+}) and subsequent binding in a 2:1 fashion.^[49] The given examples are a first impression of stimuli-responsiveness of molecular switches. Recently, CB[n]-based hydrogels,^[50] polymer networks^[51] and nanoparticles (NPs)^[52,53], with potential use in biomedical science, have been made (Figure 1.3 and 1.4).

Figure 1.4 | Schematic presentation of programmed assembly and disassembly of NPs (polyethylene imine (PEI), azobenzene (Azo), polyethylene glycol (PEG), polyamidoamine (PAMAM)). Reproduced with permission from ref. [53]. Copyright 2017 American Chemical Society and 2016 Elsevier B.V.

For example, Kim and coworkers designed a CB[6]-based hydrogel containing hyaluronic acid. This hydrogel was able to include cells, which makes them suitable for 3D cellular engineering. Furthermore, subcutaneous injection of CB[6]-hyaluronic acid and polyamine-grafted hyaluronic acid led to *in situ* hydrogel formation in mice and finally 11 days lasting fluorescence.^[50] Reversibility was not shown here, but pH sensitivity is conceivable by protonation and deprotonation of the polyamine linker. The groups of Huskens and Jonkheijm have shown a supramolecular strategy for the self-assembly of dual-responsive supramolecular nanoparticles based on heteroternary interactions between azobenzene-modified dendrimers, methylviologen-modified polymers and CB[8] (Figure 4b). Using monovalent stoppers supramolecular particles could be stabilized.^[52,53] CB[n]-based nanoparticles have recently been reviewed.^[54]

1.3. CB[n]-mediated Host-Guest Chemistry on Surfaces

The ability to integrate CB[n] in surface-based sensors and devices offers new opportunities to fabricate dynamic surfaces by exploiting stimulus-responsive CB[n] systems. Such dynamic surfaces play an important role in many processes in living systems and as such CB[n]-mediated dynamic surfaces could be important in achieving mimics of dynamic aspects of living systems and subsequently they could be utilized in biomaterials, tissue engineering, biosensors and cell biology.^[55-57] With sophisticated surface analytical tools and methods such as atomic and dynamic force spectroscopy (AFM and DFS), surface plasmon resonance (SPR), quartz crystal microbalance (QCM) and fluorescence microscopy, the ability to anchor CB[n] to surfaces and subsequent CB[n]-guest interactions on surfaces have been characterized and studied.^[58-61] In general, anchoring of CB[n] to surfaces has been achieved in different ways as depicted in Figure 5.^[11,55,62-66] Being able to covalently functionalize the CB[n] macrocycle in a strongly oxidative environment to generate reactive perallyloxy sidegroups, Kim and coworkers set out to employ these functional groups to covalently anchor CB[6] and CB[7] onto surfaces. Perallyloxy-CB[6] could be anchored *via* the thiol-ene reaction with thiol-functionalized glass slides to form a thioether bond between surface and macrocycle (Figure 1.5a).^[62] Anchoring of partially allyloxylated-CB[7] was achieved by olefin cross-metathesis reaction with vinyl-terminated SAMs on gold (Figure 1.5a).^[62,67] In both cases suitable guests were specifically complexed to the CB[n] surfaces, however their binding affinities were not verified. To circumvent lengthy, somewhat difficult chemical functionalization of CB[n],^[62] Shi and coworkers demonstrated covalent anchoring of CB[n] to readily available azide surfaces *via* a photochemical reaction (Figure 1.5a).^[68] Despite that the surface immobilization mechanism was not fully characterized, guests were complexed specifically.^[68] In contrast, Li and coworkers readily made noncovalent CB[n] SAMs by spontaneously adsorbing of CB[n] cavities on gold surfaces utilizing the multivalent interactions between the lone-pairs of the carbonyls and the gold surface (Figure 5b).^[64] Surface attached CB[n] molecules were found uniform in orientation and hold their carbonyls perpendicular with respect to the gold surface and their cavities open to the outer atmosphere or solution.^[64] This arrangement maintains the recognition properties of CB[n] and facilitates guest molecules to approach the cavities. However, no binding affinities have been reported. Although this method represents an easy way to fabricate CB[n] SAMs and requires no chemical functionalization of CB[n], the coverage of the gold surface is incomplete (48-55 %) and leads to nonspecific interactions.^[60,69,70] Improvements of the surface coverage following this assembly

method have recently been reported by the group of Hernandez.^[71] The best monolayer quality was achieved when a gold surface was immersed in saturated CB[n] solution without any salts. Incubation time varied between 1 hour for CB[7] and up to 4 hours for CB[6].^[71]

Another noncovalent approach to anchor CB[n] to surfaces makes use of thiolated guest molecules that can trap CB[n] to the surface.^[66,72] Due to strong sulfur-gold interactions it is possible to form stable SAMs, which can be functionalized with certain guest molecules for different CB[n]. Kim and coworkers synthesized a CB[6] threaded on a molecular string that consists of a diaminobutane unit as a station for CB[6] and a 1,2-dithiolane group as anchor group on gold (method Figure 1.5c).^[72] One advantage of this approach is the ability to tune the surface density of CB[n] through varying the concentration of guest molecules on the surface. A disadvantage is that, in case of CB[6] and CB[7], the cavities are already occupied and are not able to bind any other guests. An alternative offers surface-bound pseudorotaxanes containing CB[8], which were formed by a binary complex of methylviologen-capped SAMs and CB[8].^[73-75] These pseudorotaxane CB[8] complexes subsequently gave easy access to further surface functionalization by formation of ternary complexes when flowing a suitable second guest. Care must be taken to dissociation of CB[8] and in an attempt to prevent this, Scherman and coworkers recently reported CB[8]-based rotaxanes on gold SAMs, inspired by previous work from the group of Li (Figure 1.5c).^[107,75] These SAMs of CB[8] rotaxanes were accomplished by first complexing a bis-aminoethyl viologen reagent to form a 1:1 binary complex in solution with CB[8] before its reaction with aldehyde-functionalized SAMs. The rotaxanes were able to form ternary complexes following affinities known from solution studies. Other strategies to obtain well-covered SAMs with CB[n], are for example layer-by-layer (LbL) assemblies with polyelectrolytes, which can be as well combined with host-guest chemistry (Figure 1.5c).^[76]

Figure 1.5 | Schematic presentations of different ways to fabricate CB[n]-based monolayers. a) Direct and covalent attachment with functionalized CB[n] derivatives. b) Electrostatic adsorption or UV-mediated attachment on gold. c) Guest-mediated immobilization of CB[n] via noncovalent host-guest chemistry by e.g. direct thiol-gold interaction of thiolated-methylviologen or azobenzene.

1.3.1 pH Sensitive Platforms

A breast cancer gene sensing platform employing CB[7] was developed by He and coworkers.^[77] Homogenous DNA hybridization occurred with ferrocene (Fc)-labeled DNA. The Fc-label on the DNA interacted with CB[7] on a gold-nanosphere, which works as a signal amplifier. Simple pH control led to dissociation of the DNA from the platform and rendered a reusable sensing device, which was shown for several cycles (80% after 5 cycles), indicating good stability.^[77] Utilization of the strong affinity between CB[6] and alkylammonium groups led to several systems in which CB[6] is sliding along an alkylammonium chain and triggered closing of gates or pores, which can be (re)opened by changing the pH as a consequence of lowering the affinity between deprotonated alkylammonium groups and CB[6].^[62,72,78,79] Stoddart, Zink and coworkers have recently reported a drug cargo delivery system based on mesoporous silica nanoparticles.^[80] First trials were done with rhodamine B and propidium iodide loaded nanoparticles. These nanoparticles were functionalized with different ammonium chains on which CB[6] was threaded. Upon delivering the nanoparticles to e.g. the lysosome, which has a pH of 4.5-5.0, the aniline moiety became protonated and CB[6] shifted to the terminal diaminoalkane and consequently opened the pore to release the loaded molecules.^[81,82] To stay within physiological conditions and make it suitable for drug delivery, systems, which are switching in a small pH range, are needed.

1.3.2 Redox Sensitive Platforms

Schönhoff and coworkers were able to form a redox-controlled multilayer biointerface, which could reversibly adsorb and release fluorescent molecules, such as anthracene, upon applying an external redox stimulus.^[83] This type of multilayer films with imprints has potential for application in sensing platforms or nanomaterials, since they can mimic complicated molecular recognition systems in nature (Figure 1.5c). Usually multilayer films can lose their shape and stability over time, however the multilayer films containing CB[8] were found to be more rigid and had specific recognition properties. CB[8] was inserted via a methylviologen-grafted polymer into the multilayer by the layer-by-layer technique. Pyridinium-modified anthracene (AnPy) was inserted as second guest and anthracene's optical properties allowed to follow the redox triggered uptake and release of the guest upon reduction with NaBH₄.^[83] Multilayer films containing polyazoelectrolytes and CB[8] were also investigated, which have similar properties as the above described multilayer.^[76]

Figure 1.6 | “Velcro” underwater adhesion system based on CB[7] and ferrocene interactions. Reproduced from ref. [84]. Copyright 2013 John Wiley and Sons, Inc.

A redox-responsive underwater adhesion system was designed by Kim and coworkers (method Figure 1.5a, Figure 1.6).^[84] The system is based on host-guest binding between ferrocene and CB[7]. When applied on large areas, this single interaction led to a strong adhesive surface, which was able to carry up to four kilograms. Reduction of the ferrocene led to a loss in binding affinity and opening of the Velcro. The system represents an example where a molecular recognition event is present on a macroscopic level.^[84]

The group of Kim has also reported the immobilization of proteins on CB[n] surfaces.^[67,85-87] A redox-responsive ferrocenylated glucose oxidase (Fc-GOX) was immobilized on CB[7] SAMs

(method Figure 1.5a, Figure 1.7). In enzyme activity experiments, Fc-GOX showed a moderately reduced enzyme activity, but compared to covalently immobilized enzymes, the enzymatic activity was similar. Furthermore, they suggested the use of a CB[7]-ferrocencemethylammonium pair as a replacement for the use of the biotin-avidin pair, due to their strong binding properties of 10^{15} M^{-1} , which is the first synthetic binding pair overcoming the affinity of the biotin-avidin affinity. Unfortunately, the redox responsiveness was not shown experimentally.^[67]

Figure 1.7 | Non-covalent protein immobilization on a surface using partially allyloxyated-CB[7] and ferrocene-labelled glucose oxidase. Reproduced with permission from ref. [67]. Copyright 2007 American Chemical Society.

Jonkheijm and coworkers have reported successful ways to adhere proteins, viruses, bacteria and cells using CB[n]-mediated complexation on SAMs.^[73,74,88-97] A focus point has been to employ site-selectively guest-labelled proteins, which can be used for oriented positioning of proteins, as for example demonstrated on CB[7] SAMs. Mono- and divalent ferrocenylated yellow fluorescent proteins (YFP) were prepared and these formed stable inclusion complexes on CB[7] SAMs (method Figure 1.5b).^[88,89] The group of Jonkheijm designed several dynamic supramolecular CB[7] surfaces that are suitable for adhesion of cells.^[88,90] Ferrocenylated-modified integrin-binding Arg-Gly-Asp (RGD) peptides were anchored to CB[7] SAMs.^[90] These supramolecular RGD SAMs were used for adhering human umbilical vein endothelial cells representing an early example of cell adhesion by the supramolecular ferrocene-CB[7] guest-host pair on gold surfaces.^[90]

On pseudorotaxane-based CB[8]/MV²⁺ SAMs (method Figure 1.5c, Figure 1.8) heteroternary complexation of site-selectively naphthol-modified proteins was demonstrated by Jonkheijm and coworkers.^[74] Supramolecular patterning of these proteins was achieved by reactive microcontact printing of a mixture of CB[8] and naphthol-modified proteins on methylviologen SAMs.^[74] Also tryptophan (Trp) based protein immobilization was studied on this type of CB[8]-pseudorotaxane based SAMs.^[91,95,97] Specificity and reversibility were typically verified in experiments to confirm envisioned host-guest interactions to occur.

Figure 1.8 | Fluorescence images of CB[8] mediated heteroternary host-guest complexes of methylviologen and naphthol. a) Pattern of printed lissamine- and YFP-naphthol, b) disappearance of fluorescence after reduction with zinc and reinstalled fluorescence after oxidation and reincubation of the host-guest complex. Reproduced with permission from ref. [74]. Copyright 2012 American Chemical Society.

In a recent study, Sankaran *et al.* prepared a focused set of integrin-targeting knottin variants with distinct numbers of Trps to vary affinities for the pseudorotaxane-based CB[8] SAMs.^[91,95] The genetically engineered knottin constructs were able to simultaneously bind integrins and MV²⁺/CB[8] *via* one, two, three or four Trp heteroternary complexations.^[95] Binding studies showed slightly higher binding affinities for knottins with a larger number of Trp residues while an increased extent of multilayer formation for the higher-valent constructs occurred, which was attributed to homoternary complex formation between Trp of different knottins and CB[8]. Tri- and tetravalent knottin constructs yielded the largest extent of adhered cell elongation and more pronounced focal adhesion formation.^[95] As an alternative to integrin-receptor mediated adhesion of cells on CB[8] SAMs, Jonkheijm and coworkers recently metabolically expressed naphthol moieties on the outer surface of non-adherent Jurkat cells (Figure 1.11c).^[93] Specific host-guest interactions were demonstrated on surfaces and the method potentially allows for programmable supramolecular interactions with spatial and temporal control over cell adhesion.

Up to now the results reviewed in this section have not fully utilized the potential of including methylviologen in surface-based CB[8] systems for redox sensitive applications. Electronic devices could profit from this type of supramolecular surfaces. It has been shown that the host-guest system of CB[8]-methylviologen works as a molecular junction and that encapsulation influences the conductance and peak current of methylviologen.^[98] A first example on utilizing the redox-responsiveness of CB[8]/MV²⁺ SAMs was nicely described by Scherman and coworkers in a focused attempt to separate peptides containing aromatic residues.^[99,100] They trapped Trp containing peptides from a peptide mixture without any aromatic containing peptides and immobilized them on pseudorotaxane-based CB[8]/MV²⁺ SAMs (method Figure 1.5c, Figure 1.9). An electrochemical stimulus led to a one-electron reduction of the MV²⁺ and a release of the separated peptide from the host-guest complex. With this method reversible binding and release was possible over many cycles.^[100]

Figure 1.9 | Peptide separation with a CB[8] trapping surface via selective heteroternary complex formation. Reproduced with permission from ref. [98]. Copyright 2010 American Chemical Society.

Our group has demonstrated a supramolecular approach to attach cells via host-guest chemistry on pseudorotaxane-based CB[8] SAMs (Figure 1.5c, Figure 1.10) and release them with an electrochemical stimulus.^[73] These experiments were carried out on SAMs that consisted of cell-repellent ethylene glycols to which MV²⁺ was attached to interact with CB[8] resulting in pseudorotaxane-based CB[8] SAMs. Subsequently, heteroternary complexation with CB[8] and tryptophan containing integrin-binding peptides occurred. Detailed characterization of the surfaces showed the same binding affinities as found in solution.^[73]

Figure 1.10 | Bright field images of cells before (a) and after (b) electrochemical activation. The white dots on the substrate serve as markers to indicate the same observation area. Scale bar 100 μm . Reproduced with permission from ref. [73]. Copyright 2012 John Wiley and Sons, Inc.

Cells specifically adhered to the integrin-binding peptide on the surface and started spreading after 1h of incubation and the supramolecular SAMs were also shown to be functional in a wound assay.^[73] Upon reduction of MV^{2+} through applying a suitable voltage, the heteroternary complex dissociated and the adhered cells were removed for ca. 90% and remained in rounded morphology, which implies low surface contact. Furthermore, spatial and temporal control over cell detachment was presented using these stimulus-responsive CB[8]-mediated interactions employing patterns of integrin-binding peptides on gold electrodes.^[73] Using single-cell force spectroscopy, our group has recently explored the possibility to estimate the rupture forces of adhered cells on the CB[8] surfaces.^[94] We were able to determine rupture forces and found them comparable to the rupture forces of cells adhered to covalent surfaces (Figure 1.11a, b). The results indicate that cell adhesion on both surface approaches is nearly identical in terms of force generation, but different in ligand dynamics.^[94] Detailed consequences for cell skeleton and cell signalling are subject of future studies and show the potential of this type stimuli-responsive supramolecular surfaces in cell biology and biomaterials.

Figure 1.11 | a) Representative images of cell pick-up experiments. b) Plot of cell areas on different attachment strategies and fluorescence images of covalent and non-covalent attached cells. c) Naphthol-functionalized Jurkat cells assemble on surfaces of CB[8]/ MV^{2+} by formation of a heteroternary host-guest complex. Peracetylated N-azidoacetyl-D-mannosamine (MAN) and naphthol-bicyclononyne (NphBCN). Reproduced from ref. [94] and [91]. Copyright 2017 American Chemical Society.

1.3.3 Photo Sensitive Platforms

Cowpea Chlorotic Mottle Virus (CCMV) was functionalized with azobenzene moieties on the outer surface and employed for immobilization on pseudorotaxane-based CB[8] SAMs (method Figure 1.5c, Figure 1.12c) to form heteroternary complexes.^[96] SPR binding studies of azobenzene-CCMV to the CB[8] SAM showed a binding constant of $K_d = 1.41 \times 10^6 \text{ M}^{-1}$, in agreement with solution studies. When applying a light stimulus using $\lambda = 365 \text{ nm}$, azobenzene isomerizes from the *trans* to the *cis* state and subsequently dissociates from the CB[8] surface as the more bulky and polar *cis*-azobenzene is unable to bind to the binary complex of CB[8] and MV^{2+} .^[96,101] In a related work, an azobenzene-modified mannose was immobilized on a CB[8] surface.^[102] This surface consisted of a supported lipid bilayer with improved nonfouling properties onto which the pseudorotaxane system $\text{MV}^{2+}/\text{CB}[8]$ was installed (Figure 12a,b).^[102] The *E. coli* bacteria strain ORN178, which bears a carbohydrate binding receptor, was found to bind as shown using QCM-D measurements.^[102] In contrast, the control strain ORN208, which lacks the mannose binding receptor, was not able to bind to the mannose bearing surface.^[102] Local photo-switching from the *trans*- to *cis*-azobenzene mannose led to dissociation of the ligand from the surface. As a consequence, nearly 80% of the bacteria were removed from the surface.^[102]

Figure 1.12 | a) Schematic images of bacteria immobilization on a CB[8]/ MV^{2+} bearing supporting lipid bilayer surfaces. Subsequent release by UV light is shown. b) Plots represent results from bacteria release experiments. c) Schematic images of virus immobilization onto and release of a CB[8]/ MV^{2+} functionalized gold surface. Reproduced from ref. [102] and [96]. Copyright 2015 Wiley and Sons, Inc and 2017 The Royal Chemical Society.

1.3.4 Competing Guests

Usage of competing guests on interfaces is a straightforward method to adsorb and release compounds on surfaces.^[103] This approach could have great potential in targeting therapy, drug delivery and biosensing. Kim and coworkers developed a strategy to isolate plasma membrane proteins with a ferrocene/CB[7] ultrastable binding pair on sepharose beads. Purification was done with a competing ferrocene derivative with a higher binding affinity to CB[7], so that the plasma protein was released from the host-guest complex.^[85,86] A first attempt on a therapeutic system was presented by Rotello, Isaacs and coworkers.^[104,106] A diaminohexane functionalized gold nanoparticle surface was covered with CB[7], which led to a reduced toxicity of the nanoparticle. After cell uptake, a competing adamantane guest was added and removed the CB[7] cover from the surface, which induces apoptosis due to its cytotoxicity.^[104]

A naphthalimide-based fluorescence sensor was used for rapid detection of therapeutically relevant drugs. CB[7] increases the fluorescence signal due to encapsulation of the naphthalimide and the fluorescence was reduced when CB[7] was removed from the platform by a relevant drug as competing guest.^[105]

Reversible protein adhesion was demonstrated by the dynamic CB[7]-ferrocene binding pair in the group of Jonkheijm. Dissociation of the ferrocenylated protein away from the surface was observed after washing with a ferrocene derivative with a higher affinity to CB[7].^[88]

Furthermore, reversible bacteria adhesion was achieved on pseudorotaxane CB[8] surfaces (Figure 1.13). The bacterial strain of *E.coli* was genetically modified on the outer membrane with Trp containing knottins, to make it addressable for CB[8]-mediated host-guest binding. The motility of adhered bacteria was shown to be in agreement with their natural motility, previously not achieved using other immobilization techniques. Immediately upon introduction of a competitive CB[8] binding molecule, dissociation of the bacteria from the surface was observed.^[92]

In most of the herein described examples, the competing guests were synthetic molecules. To make this system more suitable for biomedical application, conversion to natural guests, such as amino acids or peptides, would be desirable.

Figure 1.13 | a) Surface immobilization of bacteria. b) Adhered bacteria on a CB[8] surface and negative control of non-modified bacteria (inset). c) *E. coli* and their flagellae imaged with AFM. d) Motility distribution of adhered bacteria. e) Reduced number of adhered bacteria, due to incubation with competitor Phenylalanine-(Glycine)₆ peptide (FG6). Reproduced with permission from ref. [92]. Copyright 2015 American Chemical Society.

1.3.5 Dual Stimuli

A very promising strategy is the integration of dual, orthogonal stimuli in sensing platforms and other surface-related applications. Interesting work to achieve dual stimuli-responsive surfaces was reported by Scherman and coworkers.^[75,101] They made use of the possibility to form heterocomplexes between MV^{2+} , CB[8] and azobenzene. These complexes then consist of a redox- (MV^{2+}) and photo- (azobenzene) responsive moiety as had been used in previous

work.^[101] In their case, the azobenzene guest was immobilized on the surface and a surface-bound heterocomplex was formed with CB[8] and a dye-labelled MV^{2+} (Figure 1.14).^[101] *Cis*-isomerization of the azobenzene led to dissociated binary complexes of MV^{2+} and CB[8], which was signified by a drop in surface-bound fluorescence. *Trans*-isomerization of the azobenzene reinstalled the heterocomplexes to the starting situation. When MV^{2+} was converted to $MV^{+\bullet}$ by electrochemistry, homoternary complexes are formed in solution between two $MV^{+\bullet}$ and CB[8], which was signified by the disappearance of the fluorescence.^[101] This process could be reversed.

Figure 1.14 | Dual switching of the heteroternary complex of MV^{2+} and azobenzene with CB[8] by a redox and a UV light stimulus. Reproduced with permission from ref. [100]. Copyright 2012 Nature Publishing Group.

1.4 Conclusions

CB[n] have become an essential part of supramolecular chemistry and a large number of possible supramolecular conjugates, surfaces and materials have been made. In this chapter, the latest progress of CB[n]-mediated host-guest complexes on surfaces are reviewed with a special attention to stimuli-responsive studies. CB[n]-based surface systems have been exploited to fabricate useful bioanalytical platforms and to interrogate with cells. Undoubtedly these initial studies have demonstrated the feasibility of performing CB[n]-based host-guest chemistry on surfaces and of undertaking relevant studies with biological content.

Much future work could benefit from CB[n]-based systems that entail nontoxic redox responsive guests, near infrared guests, improved understanding of selective host-guest chemistry in

competitive milieus of the human body. Surely, supramolecular CB[n]-based functional surfaces are now taking a more central place in supramolecular materials and devices.

1.5 Thesis Outline

From the reviewed literature in the **introduction**, it becomes clear that stimuli-responsive interfaces based on cucurbit[n]uril host molecules are attractive for the fabrication of dynamic biointerfaces. In this thesis, the design and synthesis of different light-responsive molecules are described and their binding characteristics are analysed and discussed while some of the stimulus-responsive systems are studied in biological systems.

Chapter 2 describes a monovalent arylazopyrazole derivative as suitable photosensitive candidate in heteroternary cucurbit[8]uril complexes. The improved photo-responsiveness of this molecule is demonstrated and first results of photoresponsive cell studies are discussed. **Chapter 3** and **4** introduce the concept of multivalency in combination with light-responsiveness. Dimeric and trimeric azobenzene and azopyridine guests are investigated to bind to cucurbit[8]uril surfaces. Since multiple binding sites can not only enhance specific binding, but can also suffer from steric inhibition, special care is taken in the design of multivalent molecules and their binding behavior on host surfaces. In **chapter 5**, the mobility of multivalent azopyridine molecules on cucurbit[8]uril-based platforms is studied. Patterns made by soft lithography are used for producing sharp boundaries between patterned and unpatterned regions, which are visualized using fluorescence microscopy. **Chapter 6** deals with heterovalent dimeric azobenzene derivatives. Different binding affinities of the binding moieties result in faster and slower binding and might induce rearrangement processes on the surface. Dual stimulus control over bound and unbound states in host-guest chemistry is considered and discussed.

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Chapter 2

Photoresponsive Arylazopyrazoles in CB[8]-mediated Ternary Host-Guest Complexes

A photoswitchable arylazopyrazole (AAP) derivative binds with cucurbit[8]uril (CB[8]) and paraquat to form a 1:1:1 heteroternary host–guest complex with a binding constant of $K_a = 2 \times 10^3 \text{ M}^{-1}$. The excellent photoswitching properties of AAP are preserved in the inclusion complex. Irradiation with light of a wavelength of 365 and 520 nm leads to quantitative E- to Z- isomerization and vice versa, respectively. Formation of the Z-isomer leads to dissociation of the complex as evidenced using ^1H NMR spectroscopy. AAP derivatives are then used to immobilize bioactive molecules and photorelease them on demand. When Arg-Gly-Asp-AAP (AAP–RGD) peptides are attached to surface bound CB[8] and thiolated-methylviologen (MV^{2+}) complexes, cells adhere and can be released upon irradiation. The heteroternary host–guest system offers highly reversible binding properties due to efficient photoswitching and these properties are attractive for designing smart surfaces.

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[#]Shared first authorship.

2.1 Introduction

External control of bioactivity on biointerfaces and coatings has attracted considerable interest across bioanalytical and biomedical applications.^[1–4] In supramolecular chemistry, an important focus lies on the use of light to exploit and direct reversible control over the state of molecular assemblies and complex structures because light does not require additional components and light can be applied with a very high degree of spatio-temporal control.^[5–7] Shinkai et al. proposed to use light for manipulating self-assembled structures. Their self-complementary azobenzene derivative underwent cyclic oligomerization in the *E*-form and led to intramolecular cyclization when irradiated with UV light and subsequent switching to the *Z*-isomer.^[8] Feringa and co-workers used dithienylcyclopentene photochromic switches to tune the viscosity of solutions.^[8, 9] Azobenzene isomerization processes have successfully been employed to enable a large functional change in biomolecules and ligands in a number of instances.^[10, 11] Inclusion of azobenzene derivatives as guests in macrocyclic hosts such as cyclodextrins (CD) and cucurbit[*n*]urils (CB[*n*]) can give rise to photosensitive host–guest complexation to regulate recognition and function.^[12–18] Photosensitive host–guest complexation is currently intensively explored on surfaces and is promising to come yet a step closer to mimic natural cell-extracellular matrix (ECM) interactions on surfaces.^[11] Zhang et al. prepared β CD surfaces modified with azobenzene-containing propyltriethoxysilane guests to tune the wettability properties of surfaces.^[19] We have demonstrated the possibility of photospecific protein assembly via azobenzene-functionalized ligands on β CD surfaces.^[20] Gong and co-workers designed α CD self-assembled monolayers (SAMs) to immobilize azobenzene-modified cell adhesive Arg-Gly-Asp (RGD) peptides and subsequently control cell attachment and release on this surface with UV light.^[21] cucurbit[8]uril-mediated (CB[8]) host–guest heteroternary complexes including photoactive azobenzenes have been used in photomodulation of the assembly of (bio)molecules on surfaces.^[22–29] For example, Scherman et al. reported the photoinduced disassembly of raspberry-shaped colloids, representing these systems as useful tools for cargo delivery.^[22] We have recently assembled these photoresponsive azobenzene-containing heteroternary complexes onto chips and employed them to attract and release proteins, viruses and bacteria by photoisomerization.^[23, 24] Interestingly, when these heteroternary complexes have been constructed with both redox- and light-responsive elements multi stimuli-responsivity becomes available.^[25] Azobenzenes have a thermodynamically stable *E*-isomer and a metastable *Z*-isomer and they can be switched from *E* to *Z* with UV irradiation (light of wavelength $\lambda = 360$ nm) and

back from *Z* to *E* with visible irradiation ($\lambda = 460$ nm).^[26] Unfortunately, the thermodynamic stability of the *Z*-isomer is low and the overlapping absorbance bands lead to incomplete photoswitching with a photostationary state (PSS) of about 70 – 80%.^[6, 12, 27] Increasing the half-life time, while retaining good addressability has been a major challenge and led to the design of new derivatives, such as *o*-methoxy and *o*-fluoro azobenzenes or bridged azobenzenes.^[28, 29] Alternatively, recent literature documents excellent switching efficiencies and improved half-life times of *Z*-isomers in the case one aryl ring in the azobenzene system is exchanged with a five-membered nitrogen based heteroaromatic ring, so-called azoheteroaryl photoswitches.^[30–32] For example, N-substituted arylazopyrazoles (AAPs) show half-life times of 10 to 1000 days at 25 °C.^[31] PSS for both isomers are $\geq 98\%$ upon irradiation with $\lambda = 365$ nm for *E* to *Z* isomerization while employing $\lambda = 520$ nm leads to *Z* to *E* isomerization.^[30] Ravoo and co-workers have recently shown that AAPs form photoresponsive inclusion complexes with β CD.^[33] Light-responsive switching of these β CD-AAP host–guest inclusion complexes occurred more efficient and with a superior thermal half-life time of the *Z*-isomer compared to commonly used azobenzenes.^[33] Incorporation of the AAP in β CD vesicles and nanoparticles revealed excellent photoresponsive aggregation and dispersion.^[33] Since heterocyclic azo-compounds such as AAPs are relatively unknown in host–guest chemistry and its surface-related applications, we describe the novel use of AAP as potent photoswitchable ligand for heteroternary CB[8]-based inclusion complexes in solution and on surfaces (Figure 2.1). We show the potential for fabricating photosensitive bioactive surfaces using an AAP modified integrin binding RGD peptide.

In this chapter we report a novel AAP guest for heteroternary complex formation in CB[8] cavities with improved photoresponsiveness, followed by testing its biocompatibility. Furthermore, we tested the photoresponse of the AAP guest under physiological conditions which was verified by controlled cellular release from the surface.

Figure 2.1 | Strategy of assembling the AAP consisting heteroternary complex on antifouling SAMs (disulfide tri ethylene glycol, disulfide-maleimide ethylene glycol, thiolated-methylviologen (MV²⁺), CB[8] and AAP-RGD). Assembly starts with overnight incubation of the antifouling layer and subsequent stepwise incubation of MV²⁺, CB[8] and AAP-RGD. After cell adhesion, UV irradiation releases AAP-RGD and subsequent detachment of cells.

2.2 Results and Discussion

2.2.1 Ternary Complex Formation in Solution

To verify CB[8]-mediated supramolecular complexation of AAP (see Experimental section 2.5 for synthetic details and characterization of AAP), a solution of *E*-AAP (1 mM) was titrated to a 1:1 solution (0.1 mM) of CB[8] and paraquat while monitoring the change in heat using isothermal titration calorimetry (ITC, Figure 2.2a). An exothermic, 1:1 binding event was observed and a binding constant between CB[8]/paraquat and *E*-AAP was determined to be $K_a = 2.5 \times 10^3 \text{ M}^{-1}$ (Figure 2.2a).

Figure 2.2 | a) Isothermal calorimetry of CB[8]/paraquat with *E*-AAP and the corresponding 1:1 fit. b) ^1H NMR titration with 100 μM CB[8]/paraquat and *E*-AAP. c) Job plot based on a ^1H NMR titration.

Additionally, AAP concentrations in a range of 0.05 to 0.4 mM were titrated to a 0.1 mM solution of CB[8]/paraquat in a ^1H NMR titration (Figure 2.2b, full spectra are given in Figure 2.10). On the basis of the downfield shift of the aromatic signals of paraquat, a binding constant of $K_d = 2.1 \times 10^3 \text{ M}^{-1}$ was obtained. A 1:1 binding stoichiometry was verified using a Job plot (Figure 2.2c, ^1H NMR spectra are given in Figure 2.10).^[35] Upon increasing the AAP concentration, the aromatic signals of AAP as well as both methyl signals of the pyrazole unit of the AAP shifted upfield. These observations are in agreement with CB[8] guest complexation and show that paraquat is interacting with the AAP moiety inside the CB[8] cavity.^[25] The ternary complex was also identified using ESI-ToF mass spectrometry, which showed a signal at $m/z = 974.4$ corresponding to a doubly charged heteroternary complex (Figure 2.9).

To investigate the photoisomerization of AAP in the presence of CB[8]/paraquat, ^1H NMR and UV/vis spectroscopy measurements were performed (Figure 2.3). Upon irradiation with UV light ($\lambda = 365 \text{ nm}$) the AAP shows the characteristic changes in absorbance, which remained detectable when complexed. A significant decrease and a 10 nm blue-shifted $\pi\text{-}\pi^*$ absorbance band at $\lambda = 328 \text{ nm}$ concomitant with an increase and a 10 nm red-shifted $n\text{-}\pi^*$ band at $\lambda = 430 \text{ nm}$ confirmed the isomerization from the *E* to *Z*-isomer (Figure 2.3a).^[33] Moreover, *Z* to *E* isomerization occurred upon irradiation with light of $\lambda = 520 \text{ nm}$, optically similar as observed in the case of non-complexed AAP. Alternating the wavelength of irradiation between $\lambda = 520 \text{ nm}$ and $\lambda = 365 \text{ nm}$ and following the maximal absorption of the *E*-AAP at $\lambda = 311 \text{ nm}$ showed that AAP stably and near-quantitatively switched between the two isomers (Figure 2.3b). Reisomerization was also characterized using ^1H NMR (Figure 2.3c). The methyl signals of the *E*-AAP at $\delta = 2.55 \text{ ppm}$ and 2.41 ppm reversibly changed to $\delta = 1.87 \text{ ppm}$ and 1.54 ppm , due to the formation of the *Z*-isomer. Comparing the spectra of the *E*- and *Z*-isomer in the aromatic region around $\delta = 7.3 \text{ ppm}$, the signals of the AAP sharpened in the *Z*-state, indicating a disassembly of the 1:1:1 complex (Figure 2.3c).

These photo-isomerization properties of AAP, i.e. the separated excitation of the different states, is advantageous for molecular switches and photoresponsive materials and is an improvement when compared to the photo-isomerization properties of azobenzenes. In addition, these results demonstrate that the photoisomerization properties of AAP are unaffected when complexed with CB[8] and paraquat.

Figure 2.3 | a) UV/vis spectrum of the photoisomerization of AAP in the presence of CB[8]/paraquat (1:1:1) at 100 μ M in water, and b) corresponding switching cycles. c) ^1H NMR spectrum of the re-isomerization of a 1:1:1 mixture irradiated with $\lambda = 520, 365$ and 520 nm for 10 min ((*E-Z-E*) from top to bottom) at 100 μ M and scheme of heteroternary inclusion complex formation and dissociation (R_1 : $\text{CH}_2\text{CONH}-(\text{OCH}_2\text{CH}_2)_4\text{-OH}$).

2.2.2 Complex Formation on Surface

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Having established that a heteroternary, photoswitchable complex forms in aqueous solution, we fabricated SAMs that are modified with AAP conjugated ternary complexes (Figure 2.4). To introduce specific cell interactions an integrin-specific binding peptide RGD was attached to the AAP moiety (see experimental section for details). In short, the carboxylic acid functionality of AAP was modified with an tetraethylene glycol chain bearing an azide moiety at the end. This AAP-azide derivative was suitable for strain-induced, metal-free cycloaddition with a purified bicyclononyne-RGD conjugate. The AAP-RGD peptide binds to CB[8] entities through inclusion of the hydrophobic *E*-AAP moiety and to cells via the integrin-binding peptide RGD. SAMs were then prepared on gold sensors for surface plasmon resonance (SPR) and quartz crystal microbalance with dissipation monitoring (QCM-D) with a background layer of antifouling oligo(ethylene glycol) alkanethiols consisting of 1% or 0.1% maleimide groups.⁵ MV²⁺ was conjugated to the maleimide groups and acted as the first guest for CB[8] to bind the macrocycle to the surface (for clarity only explicitly shown in QCM-D plot, Figure 2.4b). SPR spectroscopy and QCM-D measurements confirmed that the formed monolayer is efficiently binding CB[8] and AAP (Figure 2.4a,b and Figure 2.11). Contact angle measurements verified the assembly on the surface (Figure 2.12). A concentration series of AAP over a range of 0.1 – 1 mM was performed at a flow rate of 100 $\mu\text{L}/\text{min}$ and followed by SPR (Figure 2.4a) and QCM-D (Figure 2.4b). The binding constants are $K_a = 1.9 \times 10^3 \text{ M}^{-1}$ ($K_d = 518 \text{ }\mu\text{M}$) and $K_a = 3.5 \times 10^3 \text{ M}^{-1}$ ($K_d = 283 \text{ }\mu\text{M}$) respectively (Figure 2.4d), and these values are in good agreement with the binding constants determined in solution using ITC and ^1H NMR. Control experiments confirmed negligible nonspecific interactions of AAP-RGD and RGD with other surface components. When the smaller host CB[7] was used, AAP showed no binding (Figure 2.11).

Figure 2.4 | Concentration-dependent (0.1, 0.25, 0.5, 0.7, 1 mM) *E*-AAP assembly (■) to a MV²⁺/CB[8] surface by a) SPR and b) QCM-D and subsequent rinsing (●). c) SPR response of flowing *E*- and *Z*-AAP over MV²⁺/CB[8] SAMs. d) Change in SPR angle shift (square) and QCM-D frequency (circle) vs *E*-AAP concentration (1:1 Langmuir fit are shown).

Therefore, we conclude that the selective formation of the MV²⁺/CB[8]/AAP heteroternary complex on the surface occurred as envisioned. Subsequently, photoswitching of surface-bound AAP was studied using SPR. A significant change in SPR angle was observed when flowing a $\lambda = 520 \text{ nm}$ irradiated solution of 1 mM *E*-AAP over a surface of CB[8]/MV²⁺ (Figure 2.4c). This change was absent when the solution was irradiated with UV light ($\lambda = 365 \text{ nm}$) indicating that the binding of the *Z*-isomer to the CB[8]/MV²⁺ surface is negligible (Figure 2.4c). Based on these results that surface-bound CB[8]-mediated heteroternary *N*-substituted AAP ligand complexes were formed, we then performed a set of experiments to demonstrate that these responsive supramolecular layers can be used for the photo-controlled cell adhesion. These experiments were performed on monolayers presenting the AAP-RGD complexes using the above-mentioned assembly strategy.

Figure 2.5 | Fluorescence microscopy image of fixed C2C12 cells seeded for 1h on CB[8]/MV²⁺/AAP-RGD SAMs. Cells were stained for nucleus (blue), actin (red) and vinculin (green). Scale bars represent 100 μm (left column) and 50 μm for the magnified images (right column).

First, substrates were seeded with mouse myoblast C2C12 cells for 1 h in cell culture medium. Control surfaces without RGD, bearing just MV^{2+} or $CB[8]/MV^{2+}$, showed limited cell adhesion, whereas $CB[8]$ -mediated AAP-RGD containing surfaces showed increased number of adhered cells (an overview of images is shown in Figure 2.5 and 2.13). In addition, adhered cells on AAP-RGD surfaces were more elongated and cover a larger area compared to all control surfaces of MV^{2+} and $CB[8]/MV^{2+}$ (Figure 2.6a). Staining in green of focal adhesion protein vinculin revealed well-formed focal adhesions at the ends of the red-stained actin only on the $CB[8]$ -mediated AAP-RGD presenting SAMs indicating efficient integrin-mediated adhesion on this type of surface (inset Figure 2.6a). Similar results were visible on SAMs using 0.1% maleimide groups (Figure 2.13). Having established that specific cell adhesion occurs on self-assembled $CB[8]/MV^{2+}$ /AAP-RGD monolayers, we evaluated cell detachment from the surfaces induced by photoswitching of AAP-RGD. As control surfaces we used a maleimide-SAM to which a cysteine-capped RGD peptide was coupled (cRGD SAM).^[35]

Figure 2.6 | a) Fluorescence microscopy image of fixed C2C12 cells seeded for 1 h on $CB[8]/MV^{2+}/AAP-RGD$ SAMs. Cells were stained for nucleus (blue), actin (red) and vinculin (green), scale bar 100 μm . Inset is a magnified image of the same surface, scale bar 50 μm . b) Quantitative analysis of C2C12 cells before (no UV) and after (UV) irradiation of $\lambda = 365$ nm of the $CB[8]/MV^{2+}/AAP-RGD$ and cRGD SAMs.

After C2C12 cells were seeded for 1 h, these surfaces were irradiated for 10 min with UV light and dipped once in PBS. Cells were imaged (Figure 2.7) and counted before and after irradiation on at least 10 spots on the surfaces. Significantly less cells were counted on the supramolecular AAP-RGD surface after irradiation while this was not the case when irradiation was performed on the control surfaces (Figure 2.6b). This result leads to the conclusion that AAP-RGD is

switchable on surfaces. Shortening the irradiation time to 1 min removed similar amounts of cells from the supramolecular surface while longer irradiation did not improve the results.

Figure 2.7 | Representative fluorescence microscopy images of cell release experiments. Scale bar represents 200 μm .

2.3 Conclusions

In this chapter, a novel CB[8]-mediated photoresponsive heteroternary complex consisting of *N*-substituted AAP compounds is reported. Complexation of the heteroternary complex on the surface has been studied using SPR and QCM-D and yields binding constants that are similar to the values we found in solution studies using ITC and ^1H NMR. We applied this new type of photoresponsive complexes for fabricating bioactive surfaces. Cells adhered to the supramolecularly immobilized AAP-modified RGD peptide and were removed by irradiation with UV light. This type of AAPs with improved photoswitching behavior when compared to the commonly applied azobenzene derivatives are of interest for constructing supramolecular assemblies in solution and on surfaces. Designing dynamic reversible bioactive surfaces opens possibilities for novel innovative schemes including multi-responsive bioactivity.^[25, 36] We presented a system that can simplify the investigation of cell/surface interactions through the use of synthetic ligands and receptors. Such a system provides a bioorthogonal modification of

the cell surface independently from natural cell ligands and with numbers of supramolecular handles that can in principle be modulated. We have demonstrated the successful use of host-guest chemistry to introduce non-natural ligand-receptor interactions while it also enables the specific control over cell adhesion, possibly even on a spatial and temporal scale. This artificial yet biomimetic approach paves the way for quantifying the relation between the number of receptors and the forces involved in the supramolecular cell adhesion.

2.4 Acknowledgements

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2.5 Experimental Section

2.5.1 General Methods

All chemical reagents were of analytical grade and were used without further purification. Thiol-functionalized methylviologen (MV^{2+})^[38], AAP-carboxylic acid and tetraethylene glycol- N_3 were synthesized according to literature procedures.^[33] Chemicals for synthesis were purchased from Acros Organics, Alfa Aesar, Fluka, Merck, TCI, Sigma Aldrich and Wacker. CB[8], fetal bovine serum (FBS), L-glutamine, Dulbecco's modified eagle medium (DMEM), bovine serum albumin (BSA), and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich. Triton X-100 was purchased from Acros Organics. Maleimide-terminated disulfide and tetra(ethylene glycol) disulfide were purchased from Prochimia. Fmoc-protected amino acids and agents for peptide synthesis were purchased from Novabiochem and Multisynthet GmbH chemicals. Gold surfaces (20 and 40 nm) were purchased from Ssens (Enschede, Netherlands). Concentration of CB[8] was assessed by a UV/vis titration against a cobaltocenium salt.^[39] For synthesis MilliQ water ($R = 18.2 \text{ M}\Omega$) was used. The synthesized products were analyzed by ^1H NMR on a Bruker 300 MHz, 400 MHz and 600 MHz, samples were dissolved in deuterated solvent purchased from Cambridge Isotope Laboratories Inc. All chemical shifts (δ) are given in parts per million (ppm) and relative to the residual solvent signal. The coupling constants measured are given in Hertz (Hz). For splitting pattern description, the following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). Mass analysis was done using a matrix-assisted

quant. ^1H NMR (400 MHz, CDCl_3) δ 3.72 - 3.67 (m, 2H), 3.64 (s, 4H), 3.62 (s, 4H), 3.59 - 3.55 (m, 2H), 3.53 - 3.48 (m, 2H), 2.84 (dd, J = 5.4, 4.6 Hz, 2H), 2.73 (s, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 73.14, 73.00, 70.67, 70.58, 70.28, 70.15, 61.53, 41.46. ESI-MS (m/z): calculated for $[\text{C}_8\text{H}_{20}\text{NO}_4]^+$: 194.1, found 194.1.

2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-N-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)acetamide (1)

1 eq. of 2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetic acid (495 mg, 1.916 mmol) was added to an oven dried flask and

it was purged with argon. The sample was diluted with dry DMF/DCM (2:1) and 629 μL Diisopropylamine (470 mg, 3.641 mmol, 1.9 eq.) and O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (799 mg, 2.108 mmol, 1.1 eq.) were added to the flask and stirred at r.t. for 1 h. 407 mg (2.108 mmol, 1.1 eq.) 2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethan-1-ol were added dropwise to the flask and the reaction was allowed to proceed for 12 h. The solvent was removed under reduced pressure. The resulting oil was diluted with DCM and washed with 1 M NaOH, 1 M HCl and brine. The organic layer was dried over MgSO_4 and concentrated in vacuum. For purification a column, using DCM/MeOH (97:3), was run. Yield: 107 mg, 0.247 mmol, 46 %. ^1H NMR (300 MHz, CDCl_3) δ 7.81 - 7.71 (m, 2H), 7.50 - 7.40 (m, 2H), 7.40 - 7.32 (m, 1H), 4.73 (s, 2H), 3.71 - 3.50 (m, 14H), 3.48 - 3.44 (m, 2H), 2.59 (s, 3H), 2.49 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ 166.42, 153.25, 143.22, 132.45, 129.35 (2C), 128.70 (2C), 121.61, 72.28, 70.27, 70.21, 69.92, 69.77, 69.57, 68.91, 61.22, 51.90, 39.19, 13.86, 9.69. ESI-MS (m/z): calculated for $[\text{C}_{21}\text{H}_{31}\text{N}_5\text{NaO}_5]^+$: 456.2, found 456.2.

1-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl 4-methylbenzenesulfonate (2)

0.242 g 2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-N-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)

ethyl)acetamide (0.559 mmol, 1 eq.) were dissolved in dry DCM and 2 eq. Diisopropylamine (190 μL , 1.118 mmol) and 1.2 eq. Tosyl Chloride (128 mg, 0.671 mmol) were added. The reaction mixture was stirred at r.t. over night. The reaction mixture was washed with brine twice and the organic phase was dried over MgSO_4 and concentrated in vacuum. For

purification a column was run (DCM/MeOH, 97:3). Yield: 110 mg, 0.187 mmol, 34 %. ^1H NMR (300 MHz, CDCl_3) δ 7.79 - 7.71 (m, 4H), 7.48 - 7.40 (m, 2H), 7.39 - 7.34 (m, 1H), 7.31 - 7.25 (m, 2H), 4.71 (s, 2H), 4.12 - 4.05 (m, 2H), 3.64 - 3.57 (m, 2H), 3.55 - 3.46 (m, 12H), 3.46 - 3.40 (m, 2H), 2.56 (s, 3H), 2.48 (s, 3H), 2.40 (s, 3H). ESI-MS (m/z): calculated for $[\text{C}_{28}\text{H}_{37}\text{N}_5\text{NaO}_7\text{S}]^+$: 610.2, found 610.2.

N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetamide (3)

methylbenzenesulfonate (110 mg, 0.187 mmol, 1 eq.) was dissolved in EtOH and 2 eq. of NaN_3 (25 mg, 0.374 mmol) were added. The reaction mixture was refluxed overnight. After TLC control 50 mg NaN_3 (4eq., 0.748 mmol) were added. After 3 more hours of reflux the reaction mixture was allowed to cool to r.t. and water was added. The mixture was extracted with DCM three times and the organic phase was washed with water. Purification by column chromatography: DCM with 0 % - 5 % MeOH. Yield: 20 mg, 0.063 mmol, 34 % ^1H NMR (400 MHz, CDCl_3) δ 7.75 - 7.69 (m, 2H), 7.44 - 7.37 (m, 2H), 7.36 - 7.31 (m, 1H), 6.56 (bs, 2H, NH), 4.71 (s, 2H), 3.57 - 3.49 (m, 10H), 3.49 - 3.44 (m, 2H), 3.42 - 3.36 (m, 2H), 3.31 - 3.23 (m, 2H), 2.54 (s, 3H), 2.47 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 166.7, 153.5 (2C), 130.1, 129.2 (2C), 122.1 (2C), 70.8 (3C), 70.6, 70.2, 69.6, 52.3, 50.8, 39.6, 14.1, 10.1. ESI-MS (m/z): calculated for $[\text{C}_{21}\text{H}_{31}\text{N}_8\text{O}_4]^+$: 459.3, found 459.2.

((1S,17S)-17-(((S)-1-((2-amino-2-oxoethyl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamoyl)-1-(1-(1-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)-11-(3-guanidinopropyl)-3,6,9,12,15-pentaoxo-2-oxa-4,7,10,13,16-pentaazonadecan-19-oic acid (4)

50 mg (64 μ mol, 1 eq.) of N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetamide and 29 mg (64 μ mol, 1 eq.) of (11S,17S)-17-(((S)-1-((2-amino-2-oxoethyl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamoyl)-1-(bicyclo[6.1.0]non-4-yn-9-yl)-11-(3-guanidinopropyl)-3,4,9,12,15-pentaoxo-2-oxa-6,7,10,13,16-pentaazanonadecan-19-oic acid were dissolved in water and stirred for 18h at r.t.. Purification by HPLC (ACN/H₂O gradient, reversed phase column) gave the desired product, which was confirmed by ESI-MS. Yield: 3 mg, 2.4 μ mol, 4 %. ESI-MS (m/z): calculated for [C₅₃H₈₁N₁₉O₁₆]²⁺: 619.8, found 619.5.

(11S,17S)-17-(((S)-1-((2-amino-2-oxoethyl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamoyl)-1-(bicyclo[6.1.0]non-4-yn-9-yl)-11-(3-guanidinopropyl)-3,4,9,12,15-pentaoxo-2-oxa-6,7,10,13,16-pentaazanonadecan-19-oic acid (5)

To a solution of bicyclo[6.1.0]non-4-yn-9-ylmethyl(4-nitrophenyl)carbonate (30 mg, 94 μ mol, 1eq.) in dry DMF, (7S, 13S)-13-(((S)-1-((aminomethyl)amino)-3-hydroxy-1-

oxopropan-2-yl)carbamoyl)-1-(12-azanyl)-7-(3-guanidinopropyl)-2,5,8,11-tetraoxo-3,6,9,12-tetraazapentadecan-15-oic acid (57 mg, 94 μ mol, 1eq.) and NEt₃ (60 μ L, 430 μ mol, 4.5 eq.) were added and after the reaction mixture was stirred for 2 h at r.t. the solvent was evaporated. Purification by HPLC (ACN/H₂O gradient, reversed phase column) gave the desired product. Yield: 50 mg, 64 μ mol, 68 %. Retention time t_R (GGRGDSG-Bicyclononyne): 16.5 minutes. ESI-MS (m/z): calculated for [C₃₂H₄₉N₁₁O₁₂]⁺: 779.8, found 780.2.

Peptide Synthesis

Peptide GGRGDSG and GGCGGRGDS (covalent RGD (cRGD)) was synthesized using an automatic solid phase peptide synthesis, following standard Fmoc protocols. Cleavage was performed in a 95/2.5/0.1/2.5 mixture of TFA/TIS/EDT/water for 4h. Purification of the peptide was done by reversed phase HPLC in gradient H₂O/acetonitrile (10/90% to 0/100% in 40 min), and characterized by analytical HPLC and mass spectrometry. Retention time t_R (GGRGDSG): 5 minutes, t_R (GGCGGRGDS): 4 minutes. ESI-MS (m/z): calculated for [C₂₁H₃₇N₁₁O₁₀]⁺: 603.6, found 604.2. Calculated for [C₂₆H₄₅N₁₃O₁₂S]⁺: 763.8, found 764.3.

2.5.3 Complex Formation in Solution and Switching

ESI-MS measurements of the supramolecular complex were taken in a positive ionization mode in D₂O. ESI-MS (m/z): calculated for [CB[8]/paraquat]²⁺: 757.5, found 757.4. Calculated for [CB[8]/paraquat/AAP]²⁺: 974, found 974.7 (Figure 2.9).

Figure 2.9 | ESI mass spectrum of a) CB[8]/paraquat and b) CB[8]/paraquat/AAP at 100 μ M inlet: isotopic pattern.

ITC measurements were carried out using a Microcal VP-ITC with a cell volume of 1.4115 mL. For studying the complexation of AAP to CB[8]/paraquat 10 μ L aliquots of 1 mM solution of AAP were added to 0.1 mM solution of CB[8]/paraquat in the calorimetric cell, monitoring the heat change after each injection.

UV/vis measurements were carried out on a Perkin Elmer UV/vis spectrometer Lambda 850. The photoisomerization behavior of AAP in the heteroternary complex was characterized by UV/vis spectroscopy at room temperature. A solution of CB[8]/paraquat/AAP (1:1:1) was prepared. The samples were dissolved in water and measured against the same water sample. Time-dependent trans-cis isomerization was analyzed by UV/vis spectroscopy by keeping the solution under UV light conditions and measuring the UV absorbance after 0, 15, 30, 45, 75, 105, 135, 165, 210, 300, 390, 510 and 630 seconds. Full conversion to Z-isomer was observed after 510 seconds. Switching cycles were performed 3 times from E-isomer to Z-isomer without changes in absorbance at 331 nm. Full conversion was reached after 5 minutes of irradiation each. UV and visible light irradiation experiments were done with a Hönle bluepoint 2 easycure using a shortpass filter $\lambda \leq 400$ nm and a LSC-G HighPower-LED emitting at 520 nm.

For Binding analysis of the heteroternary complex as well photoisomerization and stoichiometry **NMR** spectroscopy was used. Complexation was studied with a titration of rising AAP amount

(50–400 μM) to a 1:1 CB[8]/paraquat (0.1 mM) solution. Common methods in literature allowed to calculate a binding constant from the shifting NMR signals. Another titration was done to get insight into the stoichiometry of the complex. From a continuous variation titration with a total constant concentration of 100 μM , a Job's plot was obtained.^[35] Therefore the ratios between AAP to CB[8]/paraquat were verified as followed: 0.3, 0.4, 0.5, 0.6, 0.75, 0.9. The photoisomerization was done with 1:1:1 solution of AAP/CB[8]/paraquat at 100 μM . Isomerization was obtained by irradiation with light of 365 nm or 520 nm for 10 minutes.

Figure 2.10 | Full ^1H NMR spectra of a) titration experiment and b) titration of continuous variation (Job's Plot).

2.5.4 Self-assembly on Surface

Gold substrates were first cleaned with piranha solution ($\text{H}_2\text{SO}_4 + 30\% \text{H}_2\text{O}_2$, v/v, 70/30) and following rinsing with water. Substrates were then incubated in a solution of (triethyleneglycol)-undecanyl disulfide $\text{OH}-(\text{EG}_3)-\text{C}_{11}-\text{S}-\text{S}-\text{C}_{11}-(\text{EG}_3)-\text{OH}$ mixed with $\text{HO}-(\text{EG}_3)-\text{C}_{11}-\text{S}-\text{S}-(\text{EG}_6)-$

OCH₂-CONH-Maleimide (0.1 or 1%) overnight at RT. Following incubation of MV²⁺ or covRGD for 1h and rinsing with EtOH and subsequent incubation of the preformed complex CB[8]/AAP or AAP-RGD for 1h at RT. SPR experiments were done using 1% of HO-(EG₃)-C₁₁-S-S-(EG₆)-OCH₂-CONH-Maleimide.

SPR measurements were performed in a two-channel vibrating mirror angle scan setup base on the Kretschmann configuration. Light from a 2 mW HeNe laser is directed onto a prism surface by means of a vibrating mirror. The intensity of the light is measured by means of a large-area photodiode. This setup allows the determination of changes in plasmon angle with an accuracy of 0.0028. The gold substrate with the monolayer was optically matched to the prism using an index matching oil. After stabilization of the SPR signal, titrations were performed by adding five different concentrations of AAP (100, 250, 500, 700 and 1000 μ M) and subsequent rinsing with water.

Figure 2.11 | SPR control experiments a) GGRGDSG peptide on CB[8]/MV²⁺ surface. No unspecific interaction between the RGD peptide and CB[8]/MV²⁺ monolayer, b) AAP on CB[7]/MV²⁺ surface. Only formation of a heteroternary complex with CB[8]/MV²⁺ and no interaction with CB[7]/MV²⁺ c) Z-E photoisomerization upon alternating irradiation with $\lambda = 530$ and 365 nm.

QCM-D measurements were conducted using the Q-sense E1 instrument with a peristaltic pump. Gold coated QCM-D resonators QSX 301 were used for all experiments. All binding

studies were conducted with a flow rate of 100 $\mu\text{L}/\text{min}$ at room temperature. Resonance frequency and dissipation values were determined automatically by the instrument. Resonators were functionalized with a antifouling layer and subsequent functionalization with MV^{2+} . Binding experiments were performed in water at five different concentrations of AAP (see above) and prior attachment of CB[8], and following rinsing with 0.1 mM CB[8]/paraquat. Prior the binding of CB[8] and AAP, surfaces were equilibrated by flowing over water until a stable baseline was obtained.

Water contact angle (WCA) measurements were performed on a Krüss G10 contact angle measuring instrument, equipped with a CCD camera. Images were analysed using the Drop Shape Analysis software. The contact angle was obtained from at least five different spots from the surface and contact angles were averaged. Surfaces were measured directly after functionalization to avoid any oxidation.

Figure 2.12 | Representative WCA values for a) piranha cleaned gold surface (71.2°), b) Mal-EG₄-CB[8]/ MV^{2+} functionalized surface (38°), c) Mal-EG₄-CB[8]/ MV^{2+} /AAP (49°).

2.5.5 Cell Experiments

C2C12 cells were cultured in DMEM supplemented with L-glutamine, 1% streptomycin/penicillin and 10 % FBS. For cell experiments serum was depleted from the medium. Cells between passage 10 and 30 were used at a cell density of 8.000-10.000 cells/ cm^2 . Cells were maintained at incubator at 37°C with 5% CO_2 for 1h and washed with PBS buffer prior work up. Cells were fixated on surface with cold 4% Paraformaldehyde, permeabilized with 0.5% Triton X-100 in PBS for 10 min and blocked in 0.1% Triton X-100 and 5% BSA for 20 min in PBS. Staining was done with vinculin (1:200) and Phalloidin (1:100) for 1h at RT in PBS with 0.1% Triton X-100 and 5% BSA (PBST), followed by washing with PBS 3x. Cell Nuclei were stained with DAPI (1:1000) for 10 min at RT in PBS. Cells were rinsed with PBS and mounted on glass coverslips for imaging. Cell imaging was done using an inverted fluorescence microscope with corresponding excitation and emission filters (Olympus, 1X71, Melville, NY,

USA). Image analysis was done using ImageJ software. Quantitative analysis of cells was based on a minimum of two independent experiments. Detachment of cells was done with UV irradiation for 10 min with Hönle bluepoint 2 easycure with 60% power and subsequent rinsing with PBS.

Figure 2.13 | Representative fluorescence microscopy images of 0.1% maleimide functionalized gold surface. Scale bars represent 100 μm (left column) and 50 μm for the magnified images (right column).

2.6 References

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Chapter 3

Multivalent Azobenzene Guests on CB[8]-mediated Supramolecular Surfaces

The synthesis of multivalent azobenzene guests is described. The binding affinity of the azobenzene with cucurbit[8]uril/thiolated-methylviologen (CB[8]/MV²⁺) host surfaces were measured. The number of azobenzene moieties in the guest molecules was two or three while each of the azobenzenes was equipped with either a tetra ethylene glycol or a longer hexa ethylene glycol linker. Surface plasmon resonance spectroscopy (SPR) and quartz crystal microbalance (QCM) titration data for the bivalent azobenzene assembly were fitted to a 1:1 Langmuir-type model, yielding $K_{LM} = 4.8 - 8.3 \times 10^5 \text{ M}^{-1}$ and $K_{is} = 1.5 \times 10^3 \text{ M}^{-1}$, which compares favorably to the intrinsic binding constant measured in solution for the monovalent interaction of azobenzene with CB[8]/paraquat using ITC and UV/vis but also with literature for the monovalent interaction of azobenzene with CB[8]/MV²⁺ self-assembled monolayers. Binding data of the trivalent azobenzene guest showed a binding affinity similar to the bivalent analogue, which lead us to the assumption that the third binding site is not or only partially contributing to the overall binding. Differences in linker length appear to have only a minor effect on the binding affinities.

3.1 Introduction

Multivalency is ubiquitous in biological processes, such as viral interactions, inflammation or immune responses, but multivalent interactions also play an important role in nanoparticles, dendrimers or polymers.^[1-6] Several reviews dealing with multivalency have been published in the last years to manifest the impact of multivalent interactions in chemical and biological systems.^[7-12] In multivalent supramolecular systems, the molecules are interacting with more than one noncovalent interaction, the first intermolecular binding event is followed by an intramolecular binding event.^[13] With the model of multivalency developed in the group of Huskens, the overall binding affinity was separated into the intrinsic binding constant of each interaction pair, the number of binding sites and the molecular structure.^[14, 15] Herein, the term of effective concentration/molarity (C_{eff}/EM) is introduced and mainly affected by the geometry of the system.^[15] C_{eff} is based on calculated concentrations of all equilibrium complex species and EM indicates whether intramolecular processes prevail over intermolecular ones.^[16]

A perfect multivalent system offers quantitative ways to enhance the binding affinity by additive individual binding contributions. In such a case, a simple linear correlation between the number of binding sites and the binding affinity can be predicted.^[17] Limitations for the additivity of noncovalent interactions can be caused by geometrical mismatches, negative cooperativity or solvent effects.^[18] Increasing the length of the linker changes the value of the C_{eff} from close to zero to a maximum value at which distance the optimum of receptors can be reached and then decreased again (Figure 3.1). The effect of linker properties was discussed in the past, for example by Whitesides and coworkers who suggested to not use a flexible linker, due to loss in conformational entropy following the multivalent binding event. However, several systems are reported with flexible oligo ethylene glycol linkers, which preserve high affinities.^[19]

Solvent effects are well-known and play a major role in supramolecular chemistry.^[17] Solvent molecules can act for example as competitor or they can change the polarizability of guests that their driving forces are reduced. In the context of solvent effects there is often referred to water as the most suitable solvent for supramolecular complexation.^[20] Especially for complexation with cucurbit[n]urils, but also with cyclodextrins (CD), the effect of high energy water has been discussed.^[21] High energy water are water molecules inside the host cavities that give an energy subsidy when replaced by a suitable guest and released to the bulk.^[21] Cooperativity can both enhance as well as decrease the affinity.^[12, 13, 17, 22] If the binding of the first guest enhances the probability to bind a second guest, it is a positive cooperative system ($\text{EM} > C_{\text{eff}}$). On the other

hand, a system is negative cooperative when a binding event is reducing the overall affinity for subsequent binding events ($EM < C_{eff}$).^[12] The majority of studied multivalent systems in literature can be treated as non-cooperative systems and can be explained with multivalency and C_{eff} .^[22]

For the study of multivalent supramolecular interactions, host monolayers offer a number of advantages.^[11, 23, 24] Understanding the interactions at interfaces is important for mimicking many biological processes at for example membrane structures.^[25] Furthermore, immobilized functionalities can be easily varied regarding structure, density or environment and surface plasmon resonance spectroscopy (SPR) or quartz crystal microbalance with dissipation (QCM-D) enable characterization of the multivalent interactions. So far, multivalent interactions on several surfaces utilizing CD, alkanethiolates and Ni(II)NTA, but also reversible phenoxyalkanes are reported.^[11, 26-28] First studies to multivalent interactions on cucurbit[8]uril (CB[8]) surfaces were conducted in our group.^[29] In this study tryptophane-modified knottins were synthesized with 1, 2, 3, or 4 tryptophane guest binding site ranging from 1 to 4 and immobilized on CB[8] surfaces. Beside successful cell adhesion on those surfaces, surface binding studies revealed indecisive binding behavior. Most probably caused by competition and formation of multilayer through homoternary complex formation of the higher valent knottins.^[29] To closer investigate multivalent binding of guests on CB[8] surface, in this chapter azobenzene guests with valencies of two and three were synthesized and their binding onto CB[8] surfaces was characterized using SPR. CB[8] supramolecular surfaces are discussed in detail in chapter 1. We have investigated the implications of spacing between the binding sites by varying the linker length and differences in overall affinity with varying the number of binding sites.

Figure 3.1 | Schematic representation of the concept of C_{eff} for the interaction of a divalent guest on a host monolayer.

3.2 Results and Discussion

3.2.1 Synthesis

Four different multivalent azobenzenes were synthesized using tosylated tetra or hexa ethylene glycols (TEG or HEG), which were modified with 4-phenylazophenol and were subsequently coupled to either 3,5-di- or 3,4,5-trihydroxy benzoic acid methyl ester to yield divalent or trivalent azobenzenes (AZO_{2,3}) respectively. The ethylene glycols act as spacer and enhances flexibility and solubility at the same time. The azobenzene moiety serves as guest group to bind to CB[8] host cavities.^[30] Additionally, the spacing between azobenzene moieties was varied by changing the length of the ethylene glycol spacer (Figure 3.2). Purification was performed using column chromatography and characterization was done using NMR spectroscopy and ESI mass spectrometry and confirmed the purity of the target compounds.

Figure 3.2 | Synthesis route towards multivalent azobenzenes. i: K₂CO₃, MeCN, LiBr, 4-phenylazophenol, 80°C, 48h, ii: TosCl, NEt₃, CH₂Cl₂, 18h, rt, iii: K₂CO₃, MeCN, LiBr, dihydroxy benzoic acid methylester, 80°C, 48h, iv: LiOH, MeOH/H₂O (2:1), 24h, rt, v: K₂CO₃, MeCN, LiBr, trihydroxy benzoic acid methylester, 80°C, 48h, vi: LiOH, MeOH/H₂O (2:1), 24h, rt.

3.2.2 Complex Formation

The hetero-complexation of the multivalent azobenzene derivatives with CB[8] and paraquat was studied in solution using UV/vis titration experiments (Figure 3.3). In these titrations, a solution of CB[8]/paraquat (0.5 mM) was added to a solution of AZO_{2,3} (AZO₂: 0.025 mM and AZO₃: 0.0166 mM) guests. The azobenzene absorbance band was monitored (ΔA_{obs}) upon adding CB[8]/paraquat. This UV/vis band decreased in intensity and, when plotted versus the host/guest ratio. The binding curve was fitted to a 1:1 binding model from which the binding affinities were calculated to be $K_a = 0.6 \times 10^3 \text{ M}^{-1}$ and $K_a = 1.3 \times 10^3 \text{ M}^{-1}$ for AZO₂ and AZO₃ respectively. Additionally, isothermal titration calorimetry (ITC) was used to characterize the complexation process by monitoring changes of heat when CB[8]/paraquat was titrated to AZO₂ (0.05 mM) and AZO₃ (0.0166 mM) solutions (Figure 3.9). Binding affinities derived from ITC ($K_a = 4.9 \times 10^3$ and $7.9 \times 10^3 \text{ M}^{-1}$ for AZO₂ and AZO₃ respectively) were in agreement with the values derived from UV/vis. Stoichiometry values derived from ITC reveal 1:2 complex for AZO₂ and approximately a 1:3 complex for AZO₃.

Figure 3.3 | UV/vis titration experiments of a) AZO₂ and b) AZO₃ with CB[8]/paraquat. Binding curves of c) AZO₂ and d) AZO₃.

To study the binding of guests to host modified substrates, SPR and QCM-D experiments were done. In short, in both experiments, a solution of the respective guest was passed over a host modified surface. Interaction of the guest with the host surface resulted in a change of the reflectivity or frequency. As soon as the equilibrium between unbound guest in solution and bound guest on surface was reached, the signal reached a plateau value. Final washing steps led to partial or complete recovery of the host surface and gave also insight into the dissociation rates. Homogeneously packed surfaces on gold were prepared as before.^[31] CB[8] cavities were complexed to sensors modified with a SAM in which maleimide-tri ethylene glycols were reacted with thiol-modified methylviologen (MV^{2+}).^[31]

As shown in Figure 3.4a, at least five different solutions with increasing concentrations were flowed along the platform while recording changes in frequency and dissipation.

When the changes in signals were plotted against the concentration of the respective guest (Figure 3.4b), the curves were fitted to a 1:1 Langmuir binding isotherm (K_{LM})^[32] (Table 3.2), which indicate that azobenzene moieties are interacting multivalently with CB[8]/ MV^{2+} dimers at the surface.

Figure 3.4 | a) QCM-D sensorgram of HEG-AZO₃ on a CB[8]/ MV^{2+} platform, (■) indicate adsorption steps of 0.2, 0.4, 1, 5, 10 μ M and (●) subsequent rinsing. b) Binding isotherms for all four azobenzene derivatives (Langmuir fits are shown).

Molecule	$K_{LM,d}$ [μ M]	$K_{LM,a}$ [M^{-1}]
HEG-AZO₂	2.1 ± 0.05	4.8×10^5
HEG-AZO₃	8.3 ± 3.8	1.2×10^5
TEG-AZO₂	1.2 ± 0.1	8.3×10^5
TEG-AZO₃	10 ± 4.5	1×10^5

Table 3.1 | Binding constants of multivalent guest molecules on CB[8]/MV²⁺ interfaces determined with QCM-D.

Washing steps with 0.1 mM CB[8]/paraquat at the end of the HEG-AZO₃ titration experiment led only to a partial desorption from the surface, which reflects a thermodynamic equilibrium was reached. The binding affinities show an increase of about two orders of magnitude going from the monovalent AZO to the divalent AZO₂, irrespective whether hexa ethylene glycol or tetra ethylene glycol linkers were used. These results indicate that a more stable binding was found between the divalent AZO₂ and the CB[8]/MV²⁺ surface. The overall Langmuir binding constant K_{LM} can be related to the intrinsic binding constant at the surface for monovalent azobenzene/CB[8]/MV²⁺ interaction ($K_{i,s}$) and the maximum effective concentration at the surface ($C_{eff,max}$), as in $K_{LM} = EM^{n-1}K_{i,s}^n$ with EM in the order of 0.2 M as commonly found for closed packed surfaces ($n = 1, 2, 3$ for valency)^[27], $K_{i,s}$ was calculated to be $1.5 \times 10^3 M^{-1}$ which is close to the $K_{i,s}$ value of $2.9 \times 10^3 M^{-1}$ given for the interaction of monovalent azobenzene with CB[8]/MV²⁺ surfaces and the $K_{i,s}$ values of 4.9 to $7.9 \times 10^3 M^{-1}$ (ITC) and 0.6 to $1.3 \times 10^3 M^{-1}$ (UV/vis) for monovalent azobenzene with CB[8]/paraquat in solution. For the trivalent AZO₃ guest, one would predict to find an overall binding constant that would reach 10^8 - $10^9 M^{-1}$, however the overall binding affinity of the trivalent guests were found weaker compared to their dimeric analogues. These observations may be caused by mismatches in chain length and/or steric hindrance of the three bulky binding moieties.

Figure 3.5 | Schematic representation of binding site distances according to Chem3D.

GUEST	Max. distance a [nm]	Max. distance b [nm]	Oligo ethylene glycol chain length in ttg [nm]
HEG-AZO ₂	-	4.25	1.68
HEG-AZO ₃	2.46	4.25	1.68
TEG-AZO ₂	-	3.04	1.12
TEG-AZO ₃	1.75	3.18	1.12

Table 3.2 | Azobenzene based multivalent binding molecules with varying (through space) distances between each binding moiety and chain length in *trans-trans-gauche* (ttg).

Figure 3.5 and Table 3.2 depict the geometrical information of the synthesized azobenzene derivatives and the approximate chain length of tetra ethylene glycol and hexa ethylene glycol linkers. The given distances are estimated with Chem3D software without influences of water.

Figure 3.6 | CB[8] adsorption on 0.1, 0.5 and 1% functionalized maleimide monolayer.

The mass of host molecules adsorbed on the monolayer was estimated from the changes in frequency (Δf), the overtone (n) and the sensitivity C of $17.7 \text{ ng Hz}^{-1} \text{ cm}^{-2}$ to be $1.3 \times 10^{11} \text{ mol cm}^{-2}$. Since this value is close to values found in literature for β CD surfaces, we also assume a hexagonal packing with a lattice constant of 2 nm for CB[8] surfaces.^[34] Three amounts of maleimide-functionalities were used to form CB[8]/MV²⁺ monolayers and this gave the same adsorbed CB[8] mass (Figure 3.6).

The divalent guest with both ethylene glycol linkers can bind with each of its azobenzenes to two hosts without steric restrictions. However, for the trivalent AZO₃ to bind the third azobenzene moiety, there are steric restrictions and even in the case of the hexa ethylene glycol linker optimal trivalent binding will be beyond reach (Figure 3.7). This in contrast to the reported multivalent adamantane- β CD system in which a trivalent binding was observed even with tetra ethylene glycol as linker.^[14,35,36] One possible explanation for this distinction between the two systems could be the orientation of the guest inside the cavity. For optimal binding, the guest needs a tight fit inside the CB[8] cavity, especially in ternary complexes the guest needs to be in an upright orientation in the cavity. A tilted guest orientation decreases the charge-transfer interaction with MV²⁺, which is the main driving force of ternary complex formation, and possibly leading to steric restrictions and repulsive interactions at the portals of CB[8].

Figure 3.7 | Geometrical estimations for the binding of divalent and trivalent guest molecules and core-to-core distance. Adapted from ref. [39].

3

The structure of polyethylene glycol (PEG) in water has been previously studied.^[37–39] It is shown that PEG chains are highly flexible which results in unwanted coiling.^[38] PEG chains are barely found in completely stretched *trans-trans-trans* (*ttt*) configuration, but most likely in a bended *trans-trans-gauche* (*ttg*) configuration. Water molecules can form bridges with the oxygen atoms in the chain when the bonds are in *ttg* configuration. One ethylene glycol (EG) unit in *ttg* has a length of 0.28 nm. If the chain is stretched, the water bridge is broken and the EG adopt a *ttt* configuration, with a unitary length of 0.358 nm, but with an energy penalty of approx. 7.5 kJ/mol.^[37] When both divalent AZO₂ guests are bound to the surface it is possible in *ttg*, because they are able to bridge a distance of approx. 2.24 nm, which is sufficient for a CB[8] distance of 2 nm. However, in the case of the trivalent TEG-AZO₃ guest four EG units must cover a distance of 1.21 nm instead of 1.0 nm for the dimeric case. Subsequently, one EG unit must obtain the *ttt* conformation, that full binding is possible. The distances of binding sites depicted in Figure 3.5 also confirm, that both divalent guest molecules are able to cover the distance for two CB[8] cavities immobilized on a surface. However, the structure prediction of both trivalent guest molecules show a marginal or even too short binding site distance of a, which cannot span the CB[8] distance of 2.4 nm. For this reason, only two binding sites are interacting with the host surface out of three possible.

3.3 Conclusions

Multivalent azobenzene guest molecules have been synthesized. Stable assemblies on CB[8] surfaces have been prepared by employing multivalent supramolecular interactions. Improved surface binding has been found going from a monovalent interaction to a divalent interaction

and the experimentally derived divalent surface binding affinities were in agreement with the monovalent intrinsic binding affinities as found in solution binding studies of the monovalent interaction of azobenzene and CB[8]/paraquat. The observed binding affinity of AZO_3 is indicative for interaction of two azobenzene moieties with two surface binding sites, which is also supported by computational visualization of the molecular structure. A more branched or a longer linker system would be needed to reveal trivalent binding, with simultaneous consideration of the entropy-enthalpy compensation.^[40]

This feature of using multivalent binding systems for supramolecular CB[8]-mediated interfaces is further exploited in the following chapters.

3.4 Acknowledgments

Prof. Jens Voskuhl is acknowledged for starting-up this project and his fruitful discussions on synthesis and host-guest chemistry.

3.5 Experimental Section

3.5.1 General Methods

Chemicals were purchased from Sigma Aldrich or from Acros Organics and used without further purification. Reactions were carried out using dried solvent and under an atmosphere of argon. Reactions were monitored by thin-layer chromatography (TLC), which was performed on 0.2 mm Merck precoated silica gel 60 F254 aluminium sheets. Spots were visualized by treatment with basic KMnO_4 solution or under UV light irradiation. Column chromatography was carried out on silica gel 60 (0.063-0.2 mm, Merck). Thiol-functionalized methylviologen (MV^{2+}), was synthesized according to literature procedures.^[41] CB[8] was purchased from Sigma-Aldrich and Maleimide-terminated disulfide and tetra(ethylene glycol) disulfide were purchased from Prochimia. Gold surfaces (40-50 nm) were purchased from Ssens (Enschede, Netherlands). Concentration of CB[8] was assessed by a UV/vis titration against a cobaltocenium salt.^[42] MilliQ water ($R = 18.2 \text{ M}\Omega$) was used in synthesis and experiments. The synthesized products were analyzed by ^1H NMR on a Bruker spectrometer 400 MHz, samples were dissolved in deuterated solvent purchased from Cambridge Isotope Laboratories Inc. All chemical shifts (δ) are given in parts per million (ppm) and relative to the residual solvent signal. For splitting pattern description, the following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). Mass analysis was done using electrospray ionization using a Voyager DE-RP and a micromass LCT from water.

3.5.2 Synthesis Procedure

Tetra ethylene glycol monotosylate (1a)**Hexa ethylene glycol monotosylate (1b)**

To a stirred solution of tetra/hexa ethylene glycol (1 eq.) in CH_2Cl_2 was added triethylamine (NEt_3) (1 eq.) and a catalytical amount of 4-(dimethylamino) pyridine (DMAP) (50 mg). The mixture was stirred for 30 minutes. After that time p-toluenesulfonylchloride was added in portions (1 eq.). The reaction mixture was stirred overnight followed by evaporation of the solvent. The residue was redissolved in CHCl_3 and extracted three times with water. The organic layer was separated and dried over MgSO_4 . The solvent was removed under reduced pressure. The title compounds were obtained as colorless oils. Yield: 70-78%. ^1H NMR **1a** (400 MHz, CDCl_3): δ = 2.37 (s, 3H), 3.44-3.51 (m, 12H), 3.61 (t, 4H), 4.09 (s, 1H), 7.27 (d, 2H), 7.73 (d, 2H). ^1H NMR **1b** (400 MHz, CDCl_3): δ = 2.37 (s, 3H), 3.44-3.51 (m, 20H), 3.61 (t, 4H), 4.09 (s, 1H), 7.27 (d, 2H), 7.75 (d, 2H). ESI-MS (m/z): calculated for $[\text{C}_{15}\text{H}_{24}\text{O}_7\text{S}]$: 348.4, found: 371.4, $[\text{C}_{19}\text{H}_{32}\text{O}_9\text{S}]$: 436.2, found: 459.4.

Azobenzene tetra ethylene glycol (2a)**Azobenzene hexa ethylene glycol (2b)**

To a stirred solution of **1a** or **1b** (1 eq.) in 150 ml of dry acetonitrile (MeCN), containing of K_2CO_3 (5 eq.) and catalytic amounts of lithium bromide (LiBr), (1 eq.) of 4-phenylazophenol (1 eq.) dissolved in 50 ml of MeCN was added and the reaction mixture was refluxed for 2 days under argon. It was then allowed to cool to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in 100 ml of CH_2Cl_2 , washed once with 100 ml of water and thrice with 100 ml of brine. The organic phase was dried over MgSO_4 and concentrated. The residue was purified by silica gel column chromatography (DCM/MeOH 95:5) to afford of the title compounds. Yield: 80-91 %. ^1H NMR **2a** (400 MHz, CDCl_3): δ = 3.44-3.51 (m, 10H), 3.61 (t, 4H), 4.09 (s, 1H), 4.2 (t, 2H), 7.03 (d, 2H), 7.32 (m, 1H), 7.50 – 7.35 (m, 2H), 7.92 – 7.80 (m, 4H). ^1H NMR **2b** (400 MHz, CDCl_3): δ = 3.44-3.51 (m, 18H), 3.61 (t, 4H), 4.09 (s, 1H), 4.2 (t, 2H), 7.03 (d, 2H), 7.32 (m, 1H), 7.50 – 7.35 (m, 2H), 7.92 – 7.80 (m, 4H). ESI-MS (m/z): calculated for $[\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_5]$: 374.2, found: 397.6, $[\text{C}_{24}\text{H}_{34}\text{N}_2\text{O}_7]$: 462.5, found: 485.4.

Azobenzene tetra ethylene glycol monotosylate (3a)**Azobenzene hexa ethylene glycol monotosylate (3b)**

To a stirred solution of **2a** or **2b** (1 eq.) in CH_2Cl_2 was added NEt_3 (1 eq.) and a catalytical amount of DMAP (50 mg). The mixture was stirred for 30 minutes. After that time p-toluenesulfonylchloride was added in portions (1 eq.). The reaction mixture was stirred overnight followed by evaporation of the solvent. The residue was redissolved in chloroform and extracted 3 times with water. The organic layer was separated and dried over MgSO_4 . The solvent was removed under reduced pressure. The residue was subjected to silica column chromatography (CHCl_3). The products were obtained as orange oils. Yield: 33-74.5 %. ^1H NMR **3a** (400 MHz, CDCl_3): δ = 2.5 (s, 3H), 3.44-3.51 (m, 10H), 3.61 (t, 4H), 4.2 (t, 2H), 7.03 (d, 2H), 7.32 (m, 1H), 7.50 – 7.35 (m, 4H), 7.92 – 7.80 (m, 6H). ^1H NMR **3b** (400 MHz, CDCl_3): δ = 2.5 (s, 3H), 3.44-3.51 (m, 18H), 3.61 (t, 4H), 4.2 (t, 2H), 7.03 (d, 2H), 7.32 (m, 1H), 7.50 – 7.35 (m, 4H), 7.92 – 7.80 (m, 6H). ESI-MS (m/z): calculated for $[\text{C}_{27}\text{H}_{32}\text{N}_2\text{O}_7\text{S}]$: 528.2, found: 551.6, $[\text{C}_{31}\text{H}_{40}\text{N}_2\text{O}_9\text{S}]$: 616.3, found: 639.3.

Bis-azobenzene-carboxylic acid-methylester (4a and 4b)

Methyl-3,5-dihydroxybenzoate (1 eq.) was dissolved in 50 mL of MeCN followed by **3a** or **3b** (3. eq), dried K_2CO_3 (excess) and a catalytic amount of LiBr (20 mg). The suspension was refluxed for 48 h under an atmosphere of argon. After that time the solvent was removed in vacuo and the residue was redissolved in 50 mL of CH_2Cl_2 . The crude mixture was extracted twice with brine (50 mL) and twice with water (50 mL). The combined organic layers were dried over MgSO_4 and the solvent was removed in vacuo. The crude product was purified by silica gel column chromatography (DCM/EtOH – 9:1, R_f = 0,51). After removal of the solvent, the products were obtained as orange, viscous oils. Yield: 30-98%, ^1H NMR **4a** (400 MHz, CDCl_3): δ = 3.91 (s, 3H), 3.44-3.51 (m, 16H), 3.61 (t, 8H), 4.3 (t, 8H), 6.68 (s, 1H), 7.0 (d, 4H), 7.22 (s, 2H), 7.34 (dd, 2H), 7.58 (m, 4H), 7.8-7.92 (d, 8H). ^1H NMR **4b** (400 MHz, CDCl_3): 3.91 (s, 3H), 3.44-3.51 (m, 32H), 3.61 (t, 8H), 4.3 (t, 8H), 6.68 (s, 1H), 7.0 (d, 4H), 7.22 (s, 2H), 7.34 (dd, 2H), 7.58 (m, 4H), 7.8-7.92 (d, 8H). ESI-MS (m/z): calculated for $[\text{C}_{48}\text{H}_{56}\text{N}_4\text{O}_{12}\text{Na}]^+$: 903.4, found: 904.6, $[\text{C}_{56}\text{H}_{72}\text{N}_4\text{O}_{16}\text{Na}]^+$: 1079.6, found: 1080.0.

Bis-azobenzene-carboxylic acid (5a and 5b)

Lithiumhydroxide (LiOH) (8 eq.) was dissolved in a mixture of MeOH/H₂O (2:1, 30 mL). **4a** or **4b** (1 eq.) was added and the mixture was stirred for 24 h at room temperature. After that time, all solvents were evaporated and the residue was redissolved in CH₂Cl₂. The solution was extracted three times with 20 mL of water and the organic layer was dried over MgSO₄. After evaporation, the crude products were obtained as orange oils and these were further purified by silica gel column chromatography (DCM/EtOH - 9:1, R_f = 0,15). Yield: 80-88 %, ¹H NMR **5a** (400 MHz, CDCl₃): δ = 3.44-3.51 (m, 16H), 3.61 (t, 8H), 4.3 (t, 8H), 5.2 (s, 1H), 6.68 (s, 1H), 7.0 (d, 4H), 7.22 (s, 2H), 7.34 (dd, 2H), 7.58 (m, 4H), 7.8-7.92 (d, 8H). ¹H NMR **5b** (400 MHz, CDCl₃): δ = 3.44-3.51 (m, 32H), 3.61 (t, 8H), 4.3 (t, 8H), 5.2 (s, 1H), 6.68 (s, 1H), 7.0 (d, 4H), 7.22 (s, 2H), 7.34 (dd, 2H), 7.58 (m, 4H), 7.8-7.92 (d, 8H). ¹³C NMR **5a** (100 MHz, CDCl₃): δ = 166.5, 159.8, 153.5, 151.7, 143.7, 140.4, 135.7, 135.3, 131.7, 129.8, 129.3, 128.8, 128.2, 125.6, 122.2, 107, 106, 70.8-70.3, 69.6, 69.4, 67.7, 67.6. ¹³C NMR **5b** (100 MHz, CDCl₃): δ = 166.5, 159.8, 153.5, 151.7, 143.7, 140.4, 135.7, 135.3, 131.7, 129.8, 129.3, 128.8, 128.2, 125.6, 122.2, 107, 106, 70.8-70.3, 69.6, 69.4, 67.7, 67.6. ESI-MS (m/z): calculated for [C₄₇H₅₄N₄O₁₂]: 866.4, found: 889.4, [C₅₅H₇₀N₄O₁₆]: 1043.2, found: 1056.8

Tri-azobenzene-carboxylic acid-methylester (6a and 6b)

Methyl-3,4,5-trihydroxybenzoate (1 eq.) was dissolved in 50 mL of MeCN followed by **3a** or **3b** (4 eq.), dried K₂CO₃ (excess) and a catalytic amount of LiBr (20 mg). The suspension was refluxed for 48 h under an atmosphere of argon. After that time, the solvent was removed in vacuo and the residue was redissolved in 50 mL of CH₂Cl₂. The crude mixture was extracted twice with brine (50 mL) and twice with water (50 mL). The combined organic layers were dried over MgSO₄ and the solvent was removed in vacuo. The crude product was purified by silica gel

column chromatography (DCM/EtOH - 9:1, R_f = 0,51). After removal of the solvent, the products were obtained as orange viscous oils. Yield: 30%, ¹H NMR **6a** (400 MHz, CDCl₃): δ = 2.9 (s, 3H), 3.44-3.51 (m, 24H), 3.61 (t, 12H), 4.3 (t, 12H), 7.0 (d, 6H), 7.22 (s, 2H), 7.34 (dd, 3H), 7.58 (m, 6H), 7.8-7.92 (d, 12H). ¹H NMR **6b** (400 MHz, CDCl₃): δ = 2.9 (s, 3H), 3.44-3.51

(m, 48H), 3.61 (t, 12H), 4.3 (t, 12H), 7.0 (d, 6H), 7.12 (s, 2H), 7.34 (dd, 3H), 7.58 (m, 6H), 7.8-7.92 (d, 12H). ESI-MS (m/z): calculated for $[C_{68}H_{80}N_6O_{17}]$: 1253.6, found: 1254.6, $[C_{80}H_{104}N_6O_{23}]$: 1517.8, found: 1518.6.

Tri-azobenzene-carboxylic acid (**7a** and **7b**)

LiOH (8 eq.) was dissolved in a mixture of MeOH/H₂O (2:1, 30 mL). **6a** or **6b** (1 eq.) was added and the mixture was stirred for 24 h at room temperature. After that time, all solvents were evaporated and the residue was redissolved in CH₂Cl₂. The solution was extracted three times with 20 mL of water and the organic layer was dried over MgSO₄. After evaporation, the crude products were obtained as orange oils and these were further purified by silica gel column chromatography (DCM/EtOH - 9:1, R_f = 0,15). Yield: 70-95%, ¹H NMR **7a** (400 MHz, CDCl₃): δ =

3.44-3.51 (m, 24H), 3.61 (t, 12H), 4.3 (t, 12H), 7.0 (d, 6H), 7.22 (s, 2H), 7.34 (dd, 3H), 7.58 (m, 6H), 7.8-7.92 (d, 12H). ¹H NMR **7b** (400 MHz, CDCl₃): δ = 3.44-3.51 (m, 48H), 3.61 (t, 12H), 4.3 (t, 12H), 7.0 (d, 6H), 7.22 (s, 2H), 7.34 (dd, 3H), 7.58 (m, 6H), 7.8-7.92 (d, 12H). ¹³C NMR **7a** (100 MHz, CDCl₃): δ = 161.3, 152.5, 152.3, 147.2, 144.8, 133.0, 130.5, 129, 124.6, 122.2, 114.8, 71.0-70.5, 69.6, 69.2, 68.7, 67.7. ¹³C NMR **7b** (100 MHz, CDCl₃): δ = 161.3, 152.6, 152.2, 146.9, 144.8, 130.5, 129.0, 124.8, 122.5, 119.9, 114.8, 71.0-70.5, 70.2, 69.6, 68.8, 67.7. ESI-MS (m/z): calculated for $[C_{67}H_{78}N_6O_{17}]$: 1239.4, found: 1239.9, $[C_{79}H_{102}N_6O_{23}]$: 1502.8, found: 1503.6.

Figure 3.8 | ¹H NMR spectra of final multivalent azobenzene guest molecules, a) bis-azobenzene-carboxylic acid **5a** and **5b** and b) tri-azobenzene-carboxylic acid **7a** and **7b**.

3.5.3 Self-assembly in Solution

ITC measurements were carried out using a Microcal VP-ITC with a cell volume of 1.4115 mL. For studying the complexation of AZO_{2,3} with CB[8]/paraquat 5 μ L aliquots of CB[8]/ excess paraquat solution (1 mM) were added to AZO_{2,3} (AZO₂ 0.05 mM and AZO₃ 0.016 mM) in the calorimetric cell, monitoring the heat change after each injection. This high amount of paraquat is necessary to overcome the solubility limitations of CB[8] and enlarge the working concentration range.

Figure 3.9 | Isothermal calorimetry titration with a) AZO₂ (50 μ M) and b) AZO₃ (16 μ M) and 1 mM CB[8]/paraquat.

UV/vis measurements were carried out on a Perkin Elmer UV/vis spectrometer Lambda 850. A solution of AZO_{2,3} of 25 and 16 μ M was prepared and a solution of CB[8]/paraquat of 100 μ M was prepared. The samples were dissolved in water and measured against the same water sample. The total volume was kept at 500 μ L and the total amount of our guest molecules was also kept constant during titration. Subsequently, aliquots of 60 μ L CB[8]/paraquat solution were titrated to the AZO_{2,3} guest molecules. The change in absorption with increasing amount of CB[8]/paraquat was recorded.

3.5.4 Self-assembly on Surfaces

Gold substrates were first cleaned with piranha solution (H₂SO₄ + 30% H₂O₂, v/v, 70/30) and following rinsing with water. Substrates were then incubated in a solution of (triethyleneglycol)-

undecanyl disulfide OH-(EG₃)-C₁₁-S-S-C₁₁-(EG₃)-OH mixed with HO-(EG₃)-C₁₁-S-S-(EG₆)-OCH₂-CONH-Maleimide (1%) overnight at rt. Following incubation of thiolated-MV²⁺ (MV²⁺) for 1h and rinsing with EtOH and water.

SPR measurements were performed on a Resonant Probes GmbH instrument. The instrument is in a two-channel vibrating mirror angle scan setup based on the Kretschmann configuration. Light from a 2 mW HeNe laser (JDS Uniphase, $\lambda = 632.8$ nm) is directed onto a prism surface by means of a vibrating mirror. The intensity of the light is measured by a large-area photodiode. This setup allows the determination of changes in plasmon angle with an accuracy of 0.0028. The gold substrate with the monolayer mounted on a Teflon cell with a volume of 800 μ L, was optically matched to the prism using an index matching oil (Cargille, series B; $n_D^{25^\circ\text{C}} = 1.7000 \pm 0.0002$). An O-ring was placed in between flow cell and gold substrate to avoid leakage. After stabilization of the SPR signal, titrations were performed by adding at least five different concentrations of the responding guests and subsequent rinsing with buffer medium.

QCM-D measurements were conducted using the Q-sense E4 instrument with a peristaltic pump. Gold coated QCM-D resonators QSX 301 were used for all experiments. All binding studies were conducted with a flow rate of 100 μ L/min at rt. Binding experiments were performed in water and at least five different concentrations of the responding AZO_{2,3} guest. Prior the binding experiment, surfaces were equilibrated by flowing over water until a stable baseline was obtained. Resonance frequency and dissipation values were determined automatically by the instrument. Resonators were functionalized with an antifouling layer and subsequent functionalization with MV²⁺. After equilibration CB[8] attachment was monitored, followed by the guest molecules.

3.6 References

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Chapter 4

Multivalent Azopyridine Guests in Heteroternary CB[8] Complex Formation on Surfaces

This chapter describes the spatial confinement of multivalent azopyridines (APY) guests on cucurbit[8]uril (CB[8]) functionalized gold surfaces as well on cyclic olefin copolymer (COP) surfaces. The binding of a monovalent APY derivative with a preformed complex of CB[8] and paraquat to form a heteroternary host–guest complex in solution was confirmed using ^1H NMR and isothermal titration calorimetry (ITC) experiments, and a binding constant of $K_a = 1.7 \times 10^4 \text{ M}^{-1}$ was found. On CB[8] surfaces with thiolated methylviologen (MV^{2+}), binding constants were derived from surface plasmon resonance spectroscopy (SPR) or quartz crystal microbalance (QCM-D) titration experiments, and range from $K_a = 7 \times 10^3$, 7×10^5 to $9 \times 10^5 \text{ M}^{-1}$ for the mono-, bi- and trivalent APY derivatives, respectively. In which the monovalent APY binding on surface seems to be in agreement with solution binding and the bivalent APY_2 seems to indicate that APY_2 binds two CB[8]/ MV^{2+} sites on the surface, trivalent APY_3 does not bind stronger presumably due to steric reasons. Microcontact printing (μCP) and micromolding in capillaries (MIMIC) were performed on COP surfaces functionalized with a multilayer consisting of poly-L-lysine-oligo(ethylene glycol)-maleimide (PLL-OEG-Mal) with 20% grafted OEG and 3% maleimide moieties, MV^{2+} and CB[8]. Fluorescence microscopy was used to visualize micrometer-sized patterns of multivalent APY derivatives. Both patterning techniques gave homogeneous patterns as a result of heteroternary complex formation. Supramolecular exchange experiments were performed on these surface bound heterocomplexes to fabricate cross-patterns. But results were observed when low affinity APY was first microcontact printed and the higher affinity APY was deposited by MIMIC.

4.1 Introduction

Engineered microscopic surfaces are frequently incorporated in chemical or biological sensors and therefore their fabrication is of interest.^[1] The use of supramolecular assemblies in these types of sensor applications has been growing owing to their attractive features such as specificity, controlled affinity and reversibility.^[2, 3] The binding as well as desorption of these supramolecular assemblies can be fine-tuned by their chemical design, which allows high flexibility in the chemical functions that can be employed.^[2, 4]

Multivalency allows to tune the binding strength by additivity of several individual interactions, resulting in concomitant increase in affinity and stability of the formed complex.^[5] Cucurbit[8]uril (CB[8]) is well-known for the ability to form ternary complexes.^[6] The cavity of CB[8] enables to include two guest molecules that can either form homoternary complexes, when the same guest is included twice, or heteroternary complexes, when two different guests are included.^[6] CB[8] binds paraquat with an equilibrium constant of $K_a = 10^5 - 10^6 \text{ M}^{-1}$ and the resulting CB[8]/paraquat complex can efficiently include a second electron-rich aromatic guest.^[6] Multivalent CB[8] complexes in solution, have been reported and used for polymeric or polyamide scaffolds.^[6] For example, Urbach and coworkers have synthesized complementary scaffolds with CB[8]/methylviologen and tryptophane moieties, which can interact in a divalent fashion to form 2:2 complexes.^[6] A similar approach to form multivalent carbohydrate-lectin conjugates was described by Scherman and coworkers, who utilized a naphthol-grafted polymer and preformed CB[8]/paraquat.^[7] For example, Scherman reported an orthogonal stimuli-responsive CB[8] heteroternary complex consisting of paraquat and surface-mounted azobenzene.^[8] Stimuli-responsive systems allow controlled disassembly and re-assembly of CB[8]-mediated heteroternary complexes and offers a new tool for sensor surfaces and advanced materials.^[9] Azobenzene derivatives offer photo-responsiveness that can be utilized to introduce dynamics in supramolecular systems. The main drawback of azobenzene as a molecular switch is its inefficient photo-isomerization, which reaches a photostationary state of approximately 70-80%. Improved photo-isomerization properties could be achieved with N-substituted azobenzene derivatives.^[10-14]

The concept of placing molecules at a desired position at a surface is of high interest. The concept of orthogonal supramolecular assembly was first introduced by Wrighton and Whitesides.^[15, 16] Since then, several patterning strategies have been reported to achieve orthogonally patterned surfaces.^[15-19] The fabrication of two-component micro-arrayed

polymers^[20], particles^[21], carbohydrates^[22] and proteins^[23,24], is mostly achieved using advanced lithographic technologies. Most studies employ chemically modified bifunctional surfaces to achieve orthogonal patterns.^[25, 26] A very recently example is the orthogonal patterning of two proteins was achieved by utilizing nanoimprint lithography (NIL).^[24] In that study, histidine-tagged proteins and adamantyl-functionalized proteins were assembled in a stepwise manner onto bifunctional β -cyclodextrin (β CD) and Ni(II)-nitrilotris(acetic acid) (Ni(II)NTA)-bearing self-assembled monolayers (SAMs).^[24] Another lithographic technique that is often used to pattern surfaces is supramolecular microcontact printing (μ CP). μ CP utilizes stamps that are made by replica molding of a soft polymer such as polydimethylsiloxane (PDMS) using a hard master (silicon).^[29,30] In supramolecular μ CP, guest molecules that were adsorbed onto a stamp can be transferred to a host-functionalized surface to give specific host-guest assemblies.^[4] Crucial parameters of μ CP patterning are how long the stamp has been in contact with the surface what ink concentration and ink-transfer mechanism were chosen. Other soft lithography methods, that use the same type of PDMS stamps are, micromolding in capillaries (MIMIC),^[31,32] replica molding (REM),^[29] microtransfer molding (μ TM),^[33] and solvent-assisted micromolding (SAMIM).^[34] A combination of μ CP and MIMIC as patterning technique offers high versatility and high accuracy on the sub-micrometer level.^[35]

In this chapter, azopyridines (APY) are described as novel suitable guests for ternary complexation with CB[8] and paraquat. We demonstrate not only the successful patterning of APY on thiolated methylviologen (MV^{2+})-based SAMs with CB[8] by heteroternary complex formation, but also the improved binding behavior due to the multivalent character of the APY₁₋₃ derivatives. The stimuli-responsiveness of these guests is characterized both in solution and when immobilized on a surface. Dye-labelled APY₁₋₃ derivatives were used to study their complexation and surface binding behaviour with soft lithographic techniques on new CB[8]-functionalized cyclic olefin polymer (COP) platforms. Fluorescent patterns were visualized to demonstrate improved binding affinity by multivalent APYs, and the ability to substitute weaker guests from the CB[8] surface.

4.2 Results and Discussion

4.2.1. Heteroternary Complex Formation in Solution

The compounds used in this chapter are depicted in Figure 4.1. APY guests contain tetra(ethylene glycol) chains to improve water solubility of APY and to create spacing between the APY moieties to allow for multivalent CB[8] binding. Paraquat was functionalized with an alkanethiol for surface assembly experiments on gold.^[36]

Figure 4.1 | Overview of compounds used in this study.

The formation of the heteroternary complex of APY₁ and CB[8]/paraquat in aqueous solution was studied with several techniques, such as isothermal titration calorimetry (ITC), UV/vis spectroscopy and ¹H NMR spectroscopy (Figures 4.2 and 4.3). Nuclear Overhauser Effect Spectroscopy (NOESY) is a useful tool to determine spatial correlations of protons in close proximity up to 4–6 Å.^[37] The ¹H NOESY spectrum for the host-guest complex was recorded in a molar ratio of 1:1:1 for APY₁/CB[8]/paraquat, (Figure 4.2) and shows clear NOE cross peaks between the paraquat protons (H₁, H₂) and the aromatic APY₁ protons (H_a, H_c), which is indicative that paraquat is in close proximity to APY₁.

Figure 4.2 | Complexation of APY₁ with CB[8]/paraquat. NOESY spectrum of the heteroternary complex CB[8]/paraquat/APY₁.

The intrinsic binding constant for the formation of the heteroternary inclusion complex between preformed CB[8]/paraquat and APY₁ was estimated by ITC. A 1 mM solution of APY₁ in PBS was titrated to an equimolar mixture of CB[8]/paraquat of 0.1 mM. The observed binding curve has an inflection point at a molar ratio of 1 indicating the (exothermic) formation of a 1:1:1 heteroternary complex in solution (Figure 4.3c), which is in agreement with other types of heteroternary complexes.^[8,38] The titration data was fitted to a 1:1 binding model, and a binding constant K_a was estimated to be $1.7 \times 10^4 \text{ M}^{-1}$, which is comparable to previous estimated binding constants of azobenzenes and arylazopyrazoles. UV/vis and ¹H NMR titrations verified the formation of the heteroternary complex (Figure 4.3a,b). Upon addition of increasing quantities of APY₁ to a 0.1 mM aqueous solution of CB[8]/paraquat, upfield shifts were observed for the aromatic and aliphatic proton signals of paraquat compared with preformed 1:1 complexes of CB[8]/paraquat. Moreover, the aromatic proton signals of the APY₁ became broad and shifted upfield compared to free APY₁ in solution (Figure 4.3b), indicating that the hydrophobic part of APY₁ resides in the cavity of the CB[8] which is in line with previous reports on the formation of heterocomplexes between CB[8], paraquat and analogous azobenzene derivatives.^[8] The UV/vis spectrum confirmed the formation of a charge-transfer (CT) complex (Figure 4.3a). The decreasing intensity of the π - π^* absorption band at $\lambda = 323 \text{ nm}$ and the bathochromic shift upon titration with increasing amounts CB[8]/paraquat are indicative for the formation of CT complexes.

Figure 4.3 | a) Equilibrium between preformed CB[8]/paraquat and free APY₁ and the corresponding heteroternary complex. Titrations of APY₁ (1 mM) to CB[8]/paraquat (0.1 mM) studied with b) UV/vis spectroscopy, c) ^1H NMR spectroscopy and d) ITC.

To investigate the photoisomerization of APY₁, UV/vis measurements were performed and the characteristic changes in absorbance upon UV irradiation were evident in the region of the intense π - π^* and the weak n - π^* transitions. Alternating the wavelength of irradiation between $\lambda = 365$ and ambient light, while following the maximal absorption of the *E*-APY₁ at $\lambda = 323$ nm, showed that the APY₁ continuously isomerizes and reaches the photostationary state (PSS) within seconds of irradiation, while thermal reisomerization occurred practically instantaneously following switching off UV light irradiation (Figure 4.4a).

However, performing similar experiments in the presence of 2 eq. of CB[8]/paraquat yielded a significantly smaller change of the maximum absorbance as can be seen in Figure 4.4b. Previous photoisomerization studies on azobenzenes or other azoheteroarenes, such as methylated arylazopyrazoles, resulted in a similar absorbance of the isomerization process

irrespective whether the azobenzene derivatives were in the presence or absence of CB[8]/paraquat.^[8,38] One could conclude that the nitrogen of the pyridine plays a role here. The free electron pair of the pyridine nitrogen is in an sp^2 -hybridized orbital, which is perpendicularly oriented with respect to the p-orbitals of the π -system and enables interaction with electron deficient components. While complexed with CB[8] and paraquat, APY₁ is adjacent to the positively charged paraquat and close enough to interact with it, resulting in stabilization of the CT-complex. Nevertheless, computational studies have reported the isomerization of APYs by two possible ways.^[39-41] First, a rotational mechanism allows the movement of the pyridine out of the plane resulting in changing the C-N=N-C angle from 180° to 0°. Second, a concerted inversion changes one N=N-C angle from 120° to 240°, so that this movement can take place within a restricted space, as reported for an cyclodextrin inclusion complex. In the case of the latter, a higher energy consumption is assumed.^[41]

Figure 4.4 | Photoisomerization of APY₁ (50 μ M) upon irradiation with $\lambda = 365$ nm and instantaneous reisomerization upon stopping UV irradiation in a) noncomplexed state and b) complexed state with CB[8]/paraquat (100 μ M).

4.2.2 Complex Formation on Surfaces

Figure 4.5 | Schematic outline of CB[8] layer formation on gold (type 1) and COP (type 2) surfaces and heteroternary complex formation. Assembly starts with i) overnight incubation of the disulfide-OEG/disulfide-OEG-Mal (surface type 1) in EtOH at rt, ii) PLL-OEG-Mal (n = 4, x = 20%, y = 3%) incubation on oxygen plasma-activated COP (surface type 2) in PBS for 5-10 min at rt, iii) functionalization with thiolated-methylviologen (MV²⁺) for 1h, iv) incubation with 50 μM CB[8] and v) assembly of a labelled APY₁₋₃ derivative.

To study the formation of the heteroternary complexes with APY guests on surfaces, surface plasmon resonance spectroscopy (SPR) and quartz crystal microbalance with dissipation monitoring (QCM-D) titrations were performed. The first type of surfaces (type 1) consisted of a mixed SAM of thiols on gold. These type 1 surfaces were fabricated by incubation of gold sensors with a mixture of antifouling oligo(ethylene glycol) alkanethiols with 1% containing maleimide groups (Figure 4.5 top).^[43] The maleimide moieties were then functionalized by a Michael-type addition reaction with the thiol-substituted MV²⁺. Successful immobilization of

CB[8] and the formation of ternary complexes on surface type 1 was reported previously in our group.^[44] SPR on surface type 1 confirmed that the MV²⁺-functionalized SAMs were efficiently binding CB[8] and APY₁₋₃ (Figure 4.6). Initially, the change in reflectivity was measured after the binding of pure CB[8] in water and this change was in agreement with previous measurements.^[44] Subsequently, aqueous solutions containing 50 μM CB[8] and increasing concentrations of APY₁₋₃ were sequentially added and gradual shifts of the resonance angle were observed, indicating adsorption of APY₁₋₃ on the surface. After reaching signal saturation at each concentration, the system was equilibrated for around 10 min. Then the SAMs were rinsed with 50 μM CB[8] shifting back the signal, which indicates the desorption of the bound APY₁₋₃ (Figure 4.6a). The change in resonance angle at each concentration of APY₁₋₃ was used to derive the equilibrium binding constant for the formation of the heteroternary complex on the gold surface assuming a Langmuir 1:1 binding. This binding constant of APY₁ $K_a = 6.5 \times 10^3 \text{ M}^{-1}$ (Figure 4.6b) is in reasonable agreement with the one estimated in solution indicating that the thermodynamic stabilities of the monovalent heteroternary complexes in solution and on gold surfaces are comparable.

Figure 4.6 | Self-assembly on surfaces studied using SPR on a type 1, thiol SAM-based substrate: a) concentration dependent E -APY₁ assembly as follows (■) CB[8], 25, 50, 100, 250, 500, 1,000 μM and subsequent rinsing steps (●) with 100 μM CB[8]/paraquat, b) change in resonance angle vs concentration of all three APY₁₋₃ (1:1 Langmuir fits are shown).

In order to form more stable complexes and gain insight into the effect that several APY moieties have on the overall stability of these supramolecular heteroternary complexes on the surface, we performed similar experiments with the bi- and trivalent APY₂₋₃ on type 1 surfaces. Both APY₂₋₃ bind with an increased affinity to the CB[8] surfaces compared to APY₁, i.e.

$K_a = 6.7 \times 10^5 \text{ M}^{-1}$ for APY₂ and $K_a = 9.1 \times 10^5 \text{ M}^{-1}$ for APY₃ (Figure 4.6b). The two order of magnitude increase in affinity going from a monovalent interaction to a bivalent is indicative for the interaction of two binding sites on the platform. The binding affinity of APY₃ is on the same order of magnitude as for APY₂, which suggests that bivalent binding prevails for binding the trivalent guest to the CB[8]/MV²⁺ SAM. Mismatches between the steric requirements to bind all three guest moieties of the trivalent guest to the surface, is a possible explanation for the lower than expected binding affinity and stoichiometry. The same phenomenon was observed for the trivalent guest in chapter 3. A too low density of CB[8] would also lead to the same lower binding affinity.

A careful interpretation of reflectivity angle changes, which is proportional to adsorbed mass on the surface, supports the observations. Going from APY₁ to APY₂ shows a twofold higher change in reflectivity, which is in agreement with the derived binding affinity. However, the change in reflectivity for APY₃ is less than for APY₁ and APY₂, indicating that less mass was adsorbed during the titration of APY₃.

In order to allow surface patterning to be visualized by fluorescence imaging and thus to avoid fluorescence quenching on gold,^[45] the assembly of the heteroternary complexes was investigated on optically transparent cyclic olefin copolymer (COP) surfaces. The schematic outline of the fabrication of the heteroternary complexes on COP surfaces (surface type 2) is given in the bottom part of Figure 4.5. Prior to substrate incubation, the surface was made hydrophilic by exposing the surface to oxygen plasma to assure good surface wettability and electrostatic adhesion of PLL-OEG-Mal to the COP substrates. These PLL-OEG-Mal surfaces were then allowed to react with a thiol-functionalized MV²⁺. Finally, the MV²⁺-modified surfaces were incubated with 50 μM CB[8] followed by incubation of APY₁₋₃ (10 μM). Each incubation step was monitored by water contact angle measurements and resulted in significant changes in contact angles starting at COP (96°), to oxygen plasma activated COP (8°), PLL-OEG-Mal (30°), MV²⁺ (41°) and CB[8] (54°) (Figure 4.16).

CB[8] adsorption on these surfaces was studied with QCM-D (Figure 4.7). The linear relationship between oscillatory frequency and the adsorbed mass enables to calculate the adsorbed mass density, Δm , of molecules on the surface. For thin and rigid films adsorbed on the surface we can apply the Sauerbrey relation for the mass calculation,^[46]

$$\Delta m = \frac{C \cdot \Delta f}{n}$$

where C is the sensitivity of $17.7 \text{ ng Hz}^{-1} \text{ cm}^{-2}$ for a 5 MHz quartz crystal and Δf is the change in frequency at an overtone of $n = 5$. The molar density can be derived by relating the mass to the molecular weight. The CB[8] surface density for type 2 surfaces was calculated to be $1.1 \times 10^{-11} \text{ mol cm}^{-2}$ and is comparable to the surface density on type 1 surfaces consisting of 1 % maleimide groups of $1.3 \times 10^{-11} \text{ mol cm}^{-2}$.

Figure 4.7 | CB[8] adsorption on PLL-OEG-Mal-MV²⁺ SAM (surface type 2) after establishing baseline for 10 minutes monitored with QCM-D.

4.2.3 Micropatterning of Azopyridine Derivatives

Figure 4.8 | Schematic illustration of rhodamine and fluorescein-labelled APY₁₋₃ derivatives used for patterning experiments.

APY₁₋₃ patterning was attempted using two well-established patterning methods, either by μ CP with APY₁₋₃-inked polydimethylsiloxane (PDMS) stamps or by depositing APY₁₋₃ directly using MIMIC. The patterns produced by these two methods were studied using fluorescence microscopy taking advantage of the fluorescent moieties attached to the APY₁₋₃ compounds (Figure 4.8).

Figure 4.9 | Control μ CP experiments of APY₁ (10 μ M) on: a) COP, b) COP with PLL-OEG-Mal, and c) COP with PLL-OEG-Mal-MV²⁺ and intensity profiles. Inking time: 15 min, printing time: 5 min, followed by a rinsing step with water and drying in N₂, imaging setting: ISO400, 100% light intensity. Scale bars 200 μ m.

To verify the specific interaction between printed APY₁ on CB[8]/MV²⁺, several control experiments were performed (Figure 4.9 and 4.17). Nonspecific interactions were observed when fluorescein-APY₁ was printed onto either the charged PLL-OEG-Mal or MV²⁺-modified COP surfaces (Figure 4.9b,c) and in the case of printing rhodamine-labelled APY₁ onto oxygen plasma activated COP surfaces (Figure 4.17). Nonspecific interactions are presumably caused by electrostatic interactions between positively and negatively charged components. μ CP of all three APY₁₋₃ derivatives on PLL-OEG-Mal with CB[8]/MV²⁺ resulted in distinct patterns that were more intense, and had much larger contrasts compared to the control surfaces (Figure 4.10, 4.18a). No differences in the quality of the patterns were observed when either oxidized or nonoxidized stamps were used for inking and printing. Intensity profiles of printed patterns show equidistant lines of $100 \pm 3 \mu\text{m}$ between printed and nonprinted areas (Figure 4.10).

Figure 4.10 | Fluorescence microscopy images of μ CP of APY₁₋₃ (in PBS) onto CB[8]-functionalized surface type 2 and corresponding intensity profiles. Stamps were incubated for 15 min, dried in N₂ and time of printing was 5 min, followed by rinsing with water and drying in N₂. Imaging setting ISO400, 100% light intensity. Scale bar 200 μ m.

Figure 4.11 | Control experiments of MIMIC patterning: a) oxidized COP, b) COP with PLL-OEG-Mal, and c) COP with PLL-OEG-Mal-MV²⁺ and corresponding intensity profiles (insets). Ink time: 15 min, MIMIC time: 5 min, followed by final rinsing and drying with N₂, imaging setting: ISO400, 100% light intensity. Scale bar 200 μm .

The procedure to fabricate CB[8]/MV²⁺-modified PLL-OEG-Mal surfaces on COP for MIMIC was the same as in the case of μCP experiments. Also, here control experiments were performed to verify specific interactions (Figure 4.11, 4.17). The observations were comparable to the case of μCP . We observed nonspecific interactions on the PLL-OEG-Mal and PLL-OEG-Mal functionalized with MV²⁺, probably due to electrostatic interactions (Figure 4.11b,c). Formation of a well-formed CB[8] layer was found essential for observing specific surface patterning (Figure 4.12 and 4.18b). The MIMIC stamp was placed with the channels facing the surface and depending on the size of the used stamps, 2.5-5 μL was used for depositing APY₁₋₃ for 5 minutes. MIMIC fluorescent patterns were 100 $\pm$ 25 μm wide, which is in agreement with the width of the bottom of the PDMS channels of 129 $\pm$ 3 μm (Figure 4.19).

Using μCP , APY₁₋₃ derivatives were printed on CB[8]/MV²⁺-modified COP surfaces resulting in patterns without considerable nonspecific interactions. MIMIC deposition of APY₁₋₃ derivatives onto CB[8]/MV²⁺-modified COP surfaces was also suitable to fabricate patterns of heteroternary complexes, but after final rinsing weak fluorescence was observed in between the MIMIC features (Figure 4.12). Leakage of the molecules after rinsing may occur by desorption and redeposition of physisorbed molecules from the primary patterns of APY₁₋₃. The rinsing step may thus transfer non-specifically bound molecules from the pattern to the free receptor binding sites in the nonpatterned areas. Obviously, this may be a greater issue on MIMIC surfaces than on μCP surfaces, due to more material available through the MIMIC patterning process. Intensity profiles of μCP show a higher intensity compared to MIMIC pattern. Furthermore, the contrast is greater of μCP pattern than for MIMIC pattern, which

seems to be reasoned by the rinsing step and the transfer of unbound molecules to empty surface sites when the patterns are established by MIMIC. Final rinsing for 10 and 30 s for the monovalent and multivalent patterns, respectively, did not lead to significant desorption from the surface, indicating that the patterns are stable. Using rhodamine as fluorescent label seemed to result in more well-defined patterns with less background signal in both printing and MIMIC deposition of APY₁ (Figure 4.18).

Figure 4.12 | Fluorescence microscopy image of APY₁₋₃ patterns on CB[8]/MV²⁺-modified COP (surface type 2). The patterns were produced by direct deposition of APY₁₋₃ (in PBS) on CB[8]/MV²⁺ platforms using MIMIC for 5 min and subsequent rinsing with water and drying in N₂. Imaging setting: ISO400, 100% light intensity. Scale bar 200 μm.

As a next step cross-patterning experiments were performed to study the influence of multivalency on the stability of surface adhered APY₁₋₃ inks. To this end, cross-patterns of heteroternary complexes were produced by cross patterning of two differently labelled APY₁₋₃ inks. Figure 4.13 shows the process of making supramolecular cross-patterns by two sequential deposition steps. μ CP was used to produce lines in one direction, followed by MIMIC deposition of the second APY ink in orthogonal direction. This process yielded the perpendicularly oriented cross-patterns imaged in Figure 4.13.

In detail, PDMS stamps for μ CP were incubated for 15 min with a 10 μ M solution of APY₁, dried in a stream of N₂ and printed for 5 min. After rinsing with water for 10 seconds and drying in N₂, the patterns were verified using fluorescence microscopy (Figure 4.13, 1a-4a). For the second deposition, a new PDMS stamp was placed perpendicular to the first pattern to deposit APY₂ or APY₃ (10 μ M) for 5 min by MIMIC. After subsequent rinsing for 30 s and drying under N₂, fluorescence images were recorded (Figure 4.13, 1c-4c). On these images, line patterns of APY₁-rhodamine and well-defined areas of fluorescein-labelled bi- and trivalent APY_{2,3} were visible. These results demonstrate successful patterning of the two inks.

Following the upper part of Figure 4.13, which is deposition of rhodamine-APY₁ via μ CP on a prefunctionalized CB[8]/MV²⁺ platform, using the same μ CP procedure as described above, followed by MIMIC deposition of a multivalent ink. The exchange process shown in Figure 4.14 is expected as a consequence of the differences in the stability of monovalent and multivalent complexes, based on the surface studies described in section 4.2.2. Determining factor is the dissociation rate of the monovalent ternary complex. Furthermore, since APY_{2,3} bind with a similar order of magnitude we expect no or only little difference between their behavior after deposition. Images 1a and 2a in Figure 4.13 show the line pattern of rhodamine-APY₁, followed by the line pattern after perpendicular patterning of APY_{2,3} in Figure 4.13, 1b and 2b. Both images in b show the expected checkerboard pattern, which we attribute to a lower coverage of rhodamine-APY₁. Images in 1c and 2c are the line patterns of APY_{2,3} deposited with MIMIC. Also, in this fluorescent channel checkerboard patterns were observed. From this series of experiments, we concluded that APY_{2,3} were able to displace monovalent APY₁ partially or fully. The more complete disappearance of APY₁ in image 2b can be explained by the initially lower fluorescence intensity from the start of the μ CP experiment. When the order of incubation was reversed, i.e. μ CP the stronger interacting APY_{2,3} followed by MIMIC of the lower affinity APY₁, we expected equal or only marginally diminished

fluorescence intensities of APY_{2,3} before and after deposition of APY₁. The fluorescence image Figure 4.13, 3b has also a checkerboard pattern, but green fluorescence remained visible in the darker areas, which is indicative for remaining complexes of APY₂ at the surface. In case of APY₃ in Figure 4.13, image 4b a checkerboard pattern was not observed after deposition of APY₁. However, red fluorescence was observed partially due to physisorbed APY₁ molecules, in a layer on top of surface bound APY₃.

In summary, the results seem to indicate that we can employ the enhanced stability of bi- and trivalent APY_{2,3} compared to monovalent APY₁ in patterning and exchange processes. Nevertheless, we can only draw a qualitative picture. More investigations with varying concentrations or incubation times of the inks are necessary to obtain more quantitative data and to gain better insight in the cross -pattern formation process. When APY₁ was covered by MIMIC deposition of APY_{2,3}, a decrease in fluorescence intensity of APY₁ was observed to be 60% and 67% on APY₂ and APY₃ areas, respectively. On the other hand, we observed 58% lower APY₁ fluorescence intensity in APY₂ areas and 85% lower APY₁ fluorescence intensity in APY₃ contact areas, when APY₁ was MIMIC deposited during the second ink step. But also for APY₂ we observed a decrease in fluorescence intensity when MIMIC deposited an APY₁, which supports partial substitution of APY₂ by APY₁.

Figure 4.13 | Scheme illustrating the fabrication of cross-patterns by two sequential patterning steps on CB[8]. a) First μCP of rhodamine APY_1 (1a, 2a) and then MIMIC of APY_2 (1b, 1c) and APY_3 (2b, 2c) and merged channels (1d, 2d). b) First μCP of fluorescein APY_2 (3a) and fluorescein APY_3 (4a) followed by MIMIC patterning of rhodamine APY_1 (3b-c, 4b-c) and merged channels (3d, 4d).

After verifying the successful complex formation of $\text{CB}[8]/\text{MV}^{2+}/\text{APY}_1$ -functionalized surfaces, we attempted to demonstrate photoswitching of APY assemblies on the surface (Figure 4.14). Differences in the PSS between azobenzenes (AZOs) and APYs could be utilized to selectively deplete or remove one species from a surface and to develop local gradients by local or masked irradiation. Photoresponsive *trans-cis* (*E-Z*) isomerization is a well-known mechanism of common AZOs and its derivatives.^[42] The quantity and stability of the *Z*-isomers are mainly influenced by the electronic structure of the molecule, and the photoinduced isomerization results in changes of the physical properties, such as molecular geometry or dipole moment. The thermal half-life of APYs of $t_{1/2} = 2.7$ s is comparatively short to the thermal half-life time of AZOs (several hours). Both guests APY_1 and AZO_1 isomerize to the

Z-isomer upon irradiation with UV light. The short thermal half-life of APY₁ is causing a fast reisomerization to the *E*-isomer and may thus lead to subsequent fast rebinding to the host platform. Since the *Z*-isomer of AZO₁ is more stable and only reisomerizes over hours, it can be depleted from the surface by APY₁.

To perform photoswitching experiments on the surface we incubated a mixture of APY₁:AZO₁ (1:1) on a surface type 2 platform. The platform was covered with water while irradiating with UV light, followed by rinsing and drying. Experiments were performed in duplicate with alternating rhodamine and fluorescein labelled APY₁ and AZO₁ to exclude the enhanced bleaching of the fluorescein dye, so that the qualitative analysis of the photoisomerization was done with rhodamine labelled molecules. Checking the fluorescence intensity prior to and after UV irradiation and taking the average intensities gave an impression of photoinduced changes in the local surface concentrations of APY₁ and AZO₁. As can be seen from Figure 4.15, the fluorescence intensity of APY₁ remained constant before and after UV irradiation whereas the fluorescence intensity of AZO₁ decreased upon UV irradiation, subsequently resulting in decreasing AZO₁ concentrations on the surface.

Two possibilities allow APY₁ to remain on the surface: first, UV-induced isomerization takes place and fast reisomerization due to the short thermal half life time allows fast reformation of the heteroternary complex, second, only a minor amount of APY₁ is isomerizing and the majority remains complexed due to interaction with the CB[8]/MV²⁺.

Figure 4.14 | Fluorescence intensities of APY₁ vs. AZO₁ prior to and after UV irradiation.

4.3 Conclusions

In this chapter, stimuli-responsive, multivalent APY₁₋₃ CB[8]-mediated heteroternary complexes are reported. Interactions between APY₁₋₃ and CB[8] showed moderate to good binding affinities in solution and on surfaces, and Langmuir-type binding isotherms showed increased affinities for APY₂ and APY₃ on surfaces.

Both soft lithographic methods μ CP and MIMIC were utilized to prepare CB[8]-mediated fluorescent patterns in the micrometer regime on PLL-OEG-Mal supports. Furthermore, ligand exchange experiments showed the successful substitution of surface bound APY₁ by MIMIC deposited higher valent APY_{2,3} derivatives. To verify the efficiency of the photoresponsiveness when complexed with CB[8]/paraquat more investigations are needed in the case of APY derivatives. Thus, we found that our multivalent APY₁₋₃ guests are suitable for surface immobilization and common patterning strategies, which might allow the application of those guests to elucidate some key mechanisms of CB[8]-mediated immobilization. The fundamental understanding of multivalent self-assembly on surfaces play a major role in the design of more advanced nanostructures or sensor surfaces.

4.4 Acknowledgements

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4.5 Experimental Section

4.5.1 General Methods

All chemical reagents were of analytical grade and were used without further purification. Thiol-functionalized methylviologen (MV²⁺), APY₁₋₃ were synthesized according to literature procedures.^[36,47] Chemicals for synthesis were purchased from Acros Organics, Alfa Aesar, Fluka, Merck, TCI, Sigma Aldrich and Wacker. CB[8] and phosphate buffered saline (PBS) were purchased from Sigma Aldrich. Maleimide-terminated disulfide and tetra(ethylene glycol) disulfide were purchased from Prochimia. Gold surfaces (20 and 40 nm) were purchased from Ssens (Enschede, Netherlands). COP surfaces were test stripes received from Zeonor®. MilliQ

water ($R = 18.2 \text{ M}\Omega$) was used. The synthesized products were analysed by ^1H NMR on a Bruker 300 MHz, 400 MHz and 600 MHz, samples were dissolved in deuterated solvent purchased from Cambridge Isotope Laboratories Inc. All chemical shifts (δ) are given in parts per million (ppm) and relative to the residual solvent signal.

4.5.2 Complex Formation in Solution and Switching

ITC measurements were carried out using a Microcal VP-ITC with a cell volume of 1.4115 mL. For studying the complexation of APY₁ to CB[8]/paraquat 10 μL aliquots of 1 mM solution of APY₁ were added to 0.1 mM solution of CB[8]/paraquat in the calorimetric cell, monitoring the heat change after each injection.

UV/vis spectra were recorded using quartz cuvettes on a Varian Cary 50 or a Varian Cary 60 spectrophotometer equipped with a Peltier thermostated cell holder at $25 \pm 0.1 \text{ }^\circ\text{C}$. The photoisomerization behaviour of APY₁ in the heteroternary complex was characterized by UV/vis spectroscopy at room temperature. A solution of CB[8]/paraquat/APY₁ (1:1:1) was prepared. The samples were dissolved in water and measured against the same water sample. Time-dependent *trans-cis* isomerization was analysed by UV/vis spectroscopy by keeping the solution under UV light conditions and measuring the UV absorbance. Irradiation experiments were done with a Hönle bluepoint 2 easycure using a shortpass filter $\lambda \leq 400 \text{ nm}$ and a LSC-G HighPower-LED emitting at 520 nm.

For binding analysis of the heteroternary complex **NMR** spectroscopy was used. Complexation was studied with a titration of rising APY₁ amount (50-400 μM) to a 1:1 CB[8]/paraquat (0.1 mM) solution.

4.5.3 Self Assembly on Surface

Surface type 1 were prepared on gold substrates. Firstly, cleaned with piranha solution ($\text{H}_2\text{SO}_4 + 30\% \text{ H}_2\text{O}_2$, v/v, 70/30) and following rinsing with MilliQ water. Substrates were then incubated in a solution of (triethyleneglycol)-undecanyl disulfide $\text{OH}-(\text{EG}_3)\text{-C}_{11}\text{-S-S-C}_{11}\text{-(EG}_3\text{)-OH}$ mixed with $\text{HO}-(\text{EG}_3)\text{-C}_{11}\text{-S-S-(EG}_6\text{)-OCH}_2\text{-CONH-Maleimide}$ (1%) overnight at rt. Following incubation of MV^{2+} for 1h and rinsing with EtOH and water.

Surface type 2 preparation on CB[8] functionalized SAMs was done on COP surfaces. COP was cutted in appropriate slides, rinsed and sonicated for 10 min in EtOH, followed by oxygen plasma activation for 60 seconds. Stepwise incubation of PLL (5-10 μL , 5 mg/mL in PBS) for

5-10 min, MV^{2+} (100 μL , 1 mM) for 30 min and CB[8] (100 μL , 50 μM) for 10 min yielded in the platforms for μCP .

SPR measurements were performed in a two channel vibrating mirror angle scan setup base on the Kretschmann configuration. Light from a 2 mW HeNe laser is directed onto a prism surface by means of a vibrating mirror. The intensity of the light is measured by means of a large area photodiode. This setup allows the determination of changes in plasmon angle with an accuracy of 0.0028. The gold substrate with the monolayer was optically matched to the prism using an index matching oil. After stabilization of the SPR signal, titrations were performed by adding five different concentrations of APY_1 (100, 250, 500, 700 and 1.000 μM), APY_2 (concentration range: 1, 5, 10, 50, 100 μM) and APY_3 (concentration range: 0.1, 0.5, 1, 5, and 10 μM) and subsequent rinsing with 0.1 mM CB[8]/paraquat.

QCM-D measurements were conducted using the Q-sense E4 instrument with a peristaltic pump. Quartz and gold coated QCM-D resonators (QSX 303 and QSX 301) were used for all experiments. All binding studies were conducted with a flow rate of 100 $\mu\text{L}/\text{min}$ at room temperature. Resonance frequency and dissipation values were determined automatically by the instrument. Binding experiments were performed in water at five different concentrations, followed by rinsing with 0.1 mM CB[8]/paraquat. Prior flowing APY, the surfaces were equilibrated by flowing over water until a stable baseline was established followed by the attachment of CB[8] (50 μM).

Figure 4.15 | PLL-OEG-Mal adsorption on quartz QCM sensor (■), followed by water rinsing step and two hour incubation with MV^{2+} /CB[8] (●) followed by water rinsing.

Water contact angle (WCA) measurements were performed on a Krüss G10 contact angle measuring instrument, equipped with a CCD camera. Images were analysed using the Drop

Shape Analysis software. The contact angle was obtained from at least five different spots from the surface and contact angles were averaged. Surfaces were measured directly after functionalization to avoid any oxidation.

Figure 4.16 | Representative WCA values of incubation steps. a) COP surface rinsed with water and EtOH ($96^\circ \pm 1$), b) oxygen plasma activated COP surface (8°), c) COP surface functionalized with PLL-OEG(20%)-Mal(3%) ($30^\circ \pm 3$), d) COP-PLL modified with MV²⁺ ($41^\circ \pm 2$), e) COP-PLL-MV²⁺ incubated with CB[8] ($54^\circ \pm 1$).

4.5.4 μ CP and MIMIC Experiments

PDMS stamps were prepared from commercially available Sylgard-184. The curing agent and the prepolymer were manually mixed in a 1:10 volume ratio and cured over night at 60°C against the silicon master. Silicon master of $100 \times 100 \mu\text{m}$ line features were fabricated with photolithography. PDMS stamps were rinsed with EtOH and sonicated in EtOH for 15 min and dried under a stream of N_2 . Subsequent up-side-down incubation with $100 \mu\text{L}$ fluorescent APY₁₋₃ and drying with N_2 .

For **microcontact printing (μ CP)** stamps and COP surfaces were washed with EtOH and dried with N_2 . The COP surfaces were activated with oxygen plasma for 60 seconds. After that time, the COP substrates were incubated with PLL-OEG(20%)-Mal(3%) for 5-10 minutes. Afterwards the surfaces were rinsed thoroughly with water and PLL incubated surfaces are further immobilized with MV²⁺ of 1 mM concentration for 30 min and rinsed with water. The complete surface was incubated with $50 \mu\text{M}$ solution of CB[8], followed by printing the APY₁.

₃ molecules. Prior printing, the PDMS stamps were incubated in a sonicated solution to avoid aggregation of the APY₁₋₃ molecules, dried under N₂ stream and brought in conformal contact with the surface for 5 min. The concentration of the APY₁₋₃ molecules was in all cases 10 μ M. After printing the surfaces were rinsed with water to remove any physisorbed molecules (APY₁: 10 sec, APY₂₋₃: 30 sec). For **MIMIC** PDMS elastomer stamps (100 x 100 μ m) were cut to make the channels accessible for APY₁₋₃. The surface preparation and post-processing is the same as for μ CP. For APY₁₋₃ deposition the stamp was placed with the channels facing the surface. Depending on size of surface and stamp 2.5-5 μ L of APY₁₋₃ were injected at the edge of the stamp consequently capillary forces are pulling in the solution. Inking time is 5 min.

4.5.5 Fluorescence Microscopy

Fluorescence microscopy images were taken using an Olympus inverted research microscope XI. Imaging of the fluorescein was performed with excitation filter: $460 \leq \lambda_{\text{ex}} \leq 490$ nm and green emission $\lambda_{\text{em}} > 525$ nm. Imaging of rhodamine excitation filter: $510 \leq \lambda_{\text{ex}} \leq 550$ nm, and red emission $\lambda_{\text{em}} > 590$ nm. Control experiments on non-functionalized COP surfaces, showed slightly unspecific interaction with fluorescein-APY₁₋₃, but highly nonspecific interaction with rhodamine- functionalized APY₁. On PLL functionalized COP the behaviour is the opposite, here the rhodamine-APY₁ show almost no interaction, but the fluorescein APY₁₋₃ is interacting with the PLL on the surface. On COP-PLL-MV²⁺ the nonspecific interaction is less but leads still to moderate background fluorescence.

Figure 4.17 | Control experiments of rhodamine labelled APY₁ on a surface type 2 with different grades of functionalization a) COP, b) COP-PLL-OEG-Mal and c) COP-PLL-OEG-Mal-MV²⁺. imaging setting: ISO400, 50% light intensity. Scale bar 200 μ m.

Figure 4.18 | Surface pattern of rhodamine functionalized APY₁ on surface type 2 deposited with a) μ CP and b) MIMIC. imaging setting: ISO400, 50% light intensity. Scale bar 200 μ m.

Fluorescence images of μ CP and MIMIC patterns show differences in line width, originated from tapered PDMS stamps. Profile images of the PDMS stamp clearly show the tapered shape of the stamps, responsible for MIMIC line pattern broader than 100 μ m.

Figure 4.19 | a) Bright field image and b) intensity profile of used PDMS stamp. Scale bar 200 μ m.

4.6 References

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Chapter 5

Diffusion of Multivalent Azopyridines on CB[8] Platforms

In this chapter, the spreading behavior of microcontact printed mono-, di-, and trivalent azopyridine (APY) guest molecules on cucurbit[8]uril/methylviologen (CB[8]/MV²⁺) platforms is discussed. This study provides insight into the dynamics of multivalent ligands on CB[8] platforms, *e.g.* under which conditions multivalent molecules are mobile, which is an important aspect for the design of biointerfaces or smart materials. Supramolecular fluorescent patterns were made by microcontact printing (μ CP) of multivalent APYs onto a cyclic olefin polymer (COP) surface functionalized with a poly-L-lysine grafted with 20% oligoethylene glycol and 3% maleimide groups (PLL-OEG-Mal). After successful patterning, the mobility of immobilized APY₁₋₃ was investigated under several conditions by adding increasing amounts of competitor (CB[8]/paraquat) to the surrounding medium. Changes in fluorescence intensity of the patterns in time were monitored by fluorescence microscopy. Taking the changes in width of the printed line patterns vs. time, provided an image of the mobility of APY₁₋₃. The resulting slope of the changes gives the spreading rate. Spreading was observed in aqueous media for both APY₂ and APY₃, whereas the monitored changes of APY₁ only implied desorption instead of spreading. Estimated spreading rates for APY_{2,3} were up to 120 nm/min. Upon addition of CB[8]/paraquat, the spreading rate of APY_{2,3} dropped but a clear dependence on concentration was not observed. Reasons for the observed spreading behavior are probably based on the transition to a different diffusion mechanism and on the used competitor concentration, which could be too low to induce competition with the surface. Also for these complexes walking, hopping and flying mechanisms are expected.

5.1 Introduction

Multivalent interactions at interfaces are involved in many biological processes, such as interactions between cells, viruses, proteins and enzymes.^[1-5] Multivalency causes enhancement in binding strength, due to the collective effect of multiple individual, noncovalent interactions.^[6] Defining the factors that govern multivalent binding at interfaces is important for the understanding of biological processes or the design of nanostructures.^[7-9] In the context of multivalency, the concept of effective concentration (C_{eff}) describes the probability for intramolecular bond formation, which is influenced by affinity, architecture and flexibility of the multivalent system.^[10-12] High effective concentrations combined with strong binding affinities force a molecule or binding site to quickly rebind to the surface, and thus the system shows little dynamics, whereas additional weaker interactions between guest and surface may aid diffusion and allow motion along the surface, while minimizing the detachment into the bulk solution. Too low effective concentrations lead to high desorption rates and molecular dissociation.^[11]

Recently, our group published several articles on the mimicry of biological receptor surfaces utilizing cucurbit[8]uril (CB[8]) as immobilized receptor on an interface, and we successfully simulated cell-receptor interactions.^[13] CB[8] is of increasing interest since it enables the inclusion of several cationic or neutral guests by hydrophobic and ion-dipole interactions.^[14] A special feature of CB[8] is the ability to include two guest molecules to either form a homoternary, if both guests are identical, or a heteroternary complex, if two different guests are included. Such interfaces, when functionalized with CB[8] cavities, can function as multivalent binding platforms for host-guest chemistry.^[14] However, most of these studies are using monovalent ligands.^[15-22] Most notably, knowledge of the kinetics of multivalent CB[8] host-guest interactions is immature, whereas the thermodynamics has been studied to some extent.

It is crucial to match the binding behavior of the multivalent guest with the properties of the target interface to achieve multivalent binding.^[23] Strength and selectivity can be tuned by varying the number of binding receptor or ligand sites and the design of the system.^[23] Kinetic studies with multivalent interactions on interfaces have been performed with β -cyclodextrin (β CD) printboards.^[24, 25] In these studies, multivalent adamantane ligands were printed on β CD self-assembled monolayers (SAMs), and the mobility of the ligands was studied upon addition of a competitor (native β CD) in the surrounding solution. The competitor in solution enhanced the mobility of surface-bound guests, and a dependence between competitor concentration and spreading rate was observed.^[25] Other kinetic investigations included the interaction of

concanavalin (ConA) and mannose.^[26, 27] Here, ConA was immobilized on a gold surface and the real-time binding of three dendritic mannose guests was monitored with surface plasmon resonance (SPR) spectroscopy. Early association and late dissociation phases provide information of the binding efficiency and real-time data of the binding event.^[26] The group of Ravoo investigated the kinetics of multivalent binding between β CD vesicles (CDVs), ConA and sugar-decorated adamantane guests with optical density measurements.^[27] The initial rate of aggregation scaled linearly with ConA as well as the CDV concentration, which means that a critical density of components is necessary for multivalent binding.^[27]

Since kinetic studies on CB[8] platforms are lacking, information to understand the multivalent interactions on CB[8] surfaces is required. We utilize microcontact printing (μ CP) as a valuable tool to design patterned SAMs with defined properties in size and shape.^[28-30] This method enables to achieve and replicate surface patterns with high versatility and sub-micrometer accuracy. Defined patterns allow the investigation of dynamics of multivalent ligands on thiolated methylviologen (MV^{2+})/CB[8] platforms under different conditions to obtain insight in their kinetics. In this chapter, we combine spreading experiments and analytical treatment of multivalent guest binding to provide initial insights into the mobility of multivalent guests on CB[8] surfaces.

5.2 Results and Discussion

5.2.1 CB[8]/ MV^{2+} Platform

In order to study the dynamics of multivalent azopyridine-based (APY) molecules on CB[8] interfaces mono-, di-, and trivalent guest molecules (APY_{1-3}) functionalized with a fluorescein dye were printed on a CB[8]/ MV^{2+} platform. Long and flexible tetra ethylene glycol-based linkers connect the APY moieties to the fluorescent head group and provide solubility in aqueous solution (Figure 5.1). The linker enables enough flexibility to allow multivalent interactions on those platforms, which was confirmed in Chapter 4. SPR experiments were performed on a thiol-based self-assembled monolayer (SAM) (surface type 1, Chapter 4), due to the need of a gold surface for surface plasmon resonance monitoring. SPR measurements, as described in chapter 4 confirmed heteroternary complex formation and revealed binding affinities of $K_d(APY_1) = 6.5 \times 10^3 \text{ M}^{-1}$, $K_d(APY_2) = 5.7 \times 10^5 \text{ M}^{-1}$ and $K_d(APY_3) = 9.1 \times 10^5 \text{ M}^{-1}$, respectively.

Figure 5.1 | Schematic outline of CB[8] receptor layer formation on cyclic olefin polymer (COP) surfaces and heteroternary complex formation. a) overview of compounds used in this study, b) assembly strategy starts with i) poly-L-lysine-oligo(ethylene glycol)*-maleimide (PLL-OEG*-Mal, * tetra ethylene glycol, $n = 4$, $x = 20\%$, $y = 3\%$) incubation on oxygen plasma-activated COP in PBS for 5-10 min at rt, ii) functionalization with 1mM thiolated methylviologen (MV²⁺) for 30 min, iii) incubation with 50 μ M CB[8] and iv) subsequent μ CP of the labelled azopyridine (APY₁₋₃) derivatives using polydimethylsiloxane (PDMS) stamps.

We employed the poly-L-lysine (PLL) layer on COP platform (see Chapter 4) to allow fluorescent imaging of diffusion processes occurring on these substrates. In short, the binary CB[8]/MV²⁺ complex was installed on self-assembled monolayers of PLL, which was grafted with oligo(ethylene glycol) (20%) and maleimide-oligo(ethylene glycol) (3%) (PLL-OEG-Mal). PLL-OEG-Mal was electrostatically adsorbed on the oxidized COP support. See for more details on the surface preparation in the experimental section.

The APY₁₋₃ molecules were microcontact printed on the the CB[8]/MV2+/PLL-OEG-Mal. For μ CP PDMS elastomer stamps (lines of 100 μ m wide, spaced by 100 μ m) were used (Figure 5.2). The PDMS stamps were incubated with a solution of APY₁₋₃ molecules (10 μ M) and dried under a stream of N₂ before bringing them in conformal contact with the surface for 5 min. After printing, the surfaces were rinsed with water to remove physisorbed molecules.

Figure 5.2 | a) Average intensity profile of μ CP lines. Indicating I_{max} and I_{min} as the maximum and minimum and w indicating half-width of the intensity profile. b) Fluorescence microscopy image of microcontact printed lines of fluorescein-labelled APY₁ on the CB[8]/MV2⁺ platform. c) Schematic representation of μ CP of fluorescent guests onto a CB[8] SAM. d) Molecular representation of fluorescein-APY₂ onto a PLL-OEG-Mal SAM with CB[8].

5.2.2 Surface Spreading Experiments

For spreading experiments, a frame and a glass cover slip were placed on top of the patterned surface to avoid leakage and evaporation of the incubating medium. The incubating medium was 80 μL of water or an aqueous solution with various concentration of CB[8]/paraquat. After each incubation step, the surface was rinsed quickly, blown dry, and images were recorded on different spots within the frame, followed by the same procedure for the next incubation step. Fluorescence intensity profiles (Figure 5.3 and 5.4) provided all necessary parameters to analyze changes in the intensities and widths of the supramolecular patterns. The spreading rate was derived from changes in peak width (w) against the time using a linear fit. The fluorescent patterns were stable in dry state and did not show spreading. Host-induced competition is expected to affect the mobility of surface-bound APY₁₋₃.^[23] Therefore, diffusion of the molecules was studied under four different conditions, i.e. in pure water and aqueous solutions of 25, 50 and 100 μM CB[8]/paraquat over a time range of 0-60 min (except when stated otherwise).

Control experiments verify that we can exclude nonspecific interactions between CB[8], CB[8]/paraquat and fluorescein (Figure 5.10 and 5.11). A fluorescence titration experiment of 0.5 μM fluorescein with increasing concentrations of CB[8] and CB[8]/paraquat resulted in a small quenching effect of the fluorescence. However in a ITC titration experiment between the same components and concentrations, no heat effects were observed supporting that nonspecific binding of fluorescein and CB[8] and CB[8]/paraquat is negligible in solution.

A typical example of a spreading experiment is shown in Figure 5.3, for which patterned substrates of APY₁₋₃ were incubated with water. For all samples, the fluorescent lines faded away with time. The fluorescent line patterns were converted to intensity profiles (Figure 5.3e). For each guest molecule, the changes in line width and intensity as well as the spreading rate were estimated at varying conditions, and plots of width and intensity as a function of time and for different surrounding solutions are shown in Figure 5.4.

Figure 5.3 | Fluorescence micrographs of APY₁₋₃ guests microcontact printed on CB[8]/MV²⁺ layer and immersed in water at a) 0 min, b) 10 min, c) 30 min, d) 60 min and e) corresponding intensity profiles. For printing, stamp incubation in guest ink solution was 15 min, followed by drying in N₂ and printing. Printing time was 5 min, followed by a rinsing step by water, and drying in N₂. Images were taken with ISO400, 100% light intensity, scale bar 200 μm.

Figure 5.4 | Spreading on a CB[8]/MV²⁺ platform of a) APY₁, b) APY₂ and c) APY₃ under four different conditions (water, 25, 50, 100 μM CB[8]/paraquat) as a function of time. (Error bars represent a single standard deviation over at least three independent measurements).

The patterns of monovalent APY₁ showed neither diffusion in water nor with competitor as visualized by a stable line width (Figure 5.4a). At the same time, we observed a rapid desorption in the first 10 min, which then seemed to level off (Figure 5.4a). Similar experiments were carried out using APY₂. Here we observed spreading under all conditions (Figure 5.4b). Broadening of the lines up to 6 μm was observed. Interestingly, the spreading rates, defined as the slope of the change in width vs. time ($r = \delta W / \delta t$) (Figure 5.5), did not show a dependency on the

concentration of competitor in solution. The divalent APY₂ showed the highest spreading rate of 120 nm/min when surrounded by water, and a significant drop in spreading rate was observed when covered with any CB[8]/paraquat solution. The trivalent APY₃ showed increased line widths for all conditions and at the same time less desorption compared to APY₁ and APY₂ (Figure 5.4c). The spreading rate followed the same trend as for APY₂, a maximum spreading rate of around 110 nm/min in water and decreasing rates when covered with CB[8]/paraquat (Figure 5.5). The similar behavior compared to APY₂ can be attributed to the divalent binding fashion of the trimer, according to the observations made in Chapter 4, although the lesser extent of desorption may be attributed to the presence of the extra binding site compared to APY₂.

Graphs visualizing the change in intensity vs. time show a rapid initial desorption for all systems, which levels off over time (Figure 5.4 right). A possible explanation could be that the rapid desorption of physisorbed molecules allows fast solvation of these molecules but slows down when reaching the chemisorbed layer. Another contribution could arise from mass transport limitation. The rapid initial desorption of chemisorbed guests causes accumulation of guest molecules near the interface, creating a retention zone close to the surface sites that allows dissociated molecules to rebind to empty surface sites before they can escape into the bulk. Supported by the changes in total fluorescence intensity we can conclude that APY₁ is dissolving faster compared to the more hydrophobic and more multivalent APY_{2,3}, which is not contradicting either of the two possibilities.

Another factor that needs to be discussed here is the observation that no dependency on the competitor concentration occurred. Both multivalent guests showed practically constant spreading rates of around 40 nm/min and 65 nm/min for APY₂ and APY₃, respectively, at all CB[8]/paraquat concentrations. This can be possibly attributed to a too low competitor concentration so that the competitor is not notably contributing to the spreading behavior.

Summarizing, both multivalent APY_{2,3} showed increased spreading rates compared to the monovalent APY₁ (Figure 5.5). This is probably caused by the higher probability of rebinding of APY_{2,3} compared to APY₁, which allows lateral motion, and by faster desorption of APY₁ into the bulk. The spreading rate of APY₁ appeared negligible under all conditions. Both multivalent APY_{2,3} showed spreading rates above 110 nm/min in water, which was decreased by approx. a factor 2 in solutions with competing CB[8]/paraquat.

Figure 5.5 | Spreading rates of APY₁₋₃. Spreading rate plotted against the concentration of CB[8]/paraquat in the surrounding medium. (Error bars represent a single standard deviation). Spreading rates have been determined from linearly fitting the changes in width vs. time, as presented in Figure 5.4.

5.2.3 Mechanistic Considerations

Figure 5.6 | Proposed spreading mechanism and equilibria of a) a monovalent guest and b) multivalent guests on a SAM including different surface and solution bound species.

In order to provide insight into the diffusion mechanisms involved in the surface diffusion of monovalent and multivalent guests on CB[8] surfaces, we need to consider the elementary steps that are involved in the possible association and dissociation processes. Figure 5.6 shows these processes for mono- and di/trivalent guests, both in absence and presence of competitor and the evolution of several solution and surface-bound species.

The monovalent APY₁ can appear as three different species during the experiment (Figure 5.6a): First there is the surface-bound APY₁. Upon equilibrating with the surrounding solution, a free unbound APY₁ or a solution-bound APY₁ can evolve depending on the surrounding solution. Due to the multivalent nature of APY_{2,3} different surface-bound and free and solution-bound species can exist (Figure 5.6b). The divalent and trivalent guests bind with a significantly higher binding affinity to the CB[8]/MV²⁺ platform than APY₁ according to the results described in Chapter 4 ($K_d(\text{APY}_1) = 6.5 \times 10^3 \text{ M}^{-1}$, $K_d(\text{APY}_2) = 5.7 \times 10^5 \text{ M}^{-1}$ and $K_d(\text{APY}_3) = 9.1 \times 10^5 \text{ M}^{-1}$).

Three possible spreading mechanisms of multivalent guests on supramolecular platforms have been proposed by Huskens *et al.*^[25] ‘Walking’, ‘hopping’ and ‘flying’ mechanisms have been proposed for dynamic surface bound guest molecules.^[25] Measurable spreading is only observed when laterally moving guests readsorb onto the surface at a different position. Due to the monovalent nature of APY₁, spreading can only occur through hopping or flying, by complete desorption from the platform and subsequent rebinding. Desorption is caused by equilibration of the bound molecules with the surrounding medium. In the case of desorption, the molecules are diffusing into the solution; if they stay close to the surface and the association rate constant is high enough, the probability to rebind is high at the same location to closely empty surface sites by hopping. Partially and fully noncomplexed molecules are initially kept at a sub-nanometer distance from surface, confirming a relatively high probability for rebinding. If the molecules diffuse into the bulk the probability is low that directed motion is observed, most likely they will be rinsed away with the next step, stay bound to solution hosts or they bind anywhere else.

Additionally, a ‘walking’ mechanism is proposed for multivalent guests^[25], such as APY₂ and APY₃ in our case. Walking multivalent guests are always bound to the surface with at least one binding site, while the free guest site(s) is/are binding at a different location. This mechanism is also possible without competitor in solution. For β CD surfaces, this was the slowest mechanism observed. A second possible mechanism proposed for multivalent guests is the ‘hopping’ mechanism. Here the competitor binds to the dissociating guest site from the surface-bound

guest, and the guest molecules needs to ‘hop’ from one surface site to a neighbouring one. It is only possible when the desorbed complex stays in close proximity to the surface and rebinds quickly to any available host there. High competitor concentrations in solution or small association rates support a flying mechanism, which allows the molecule to dissociate completely from the surface, and eventually rebind far away or diffuse into the bulk. Usually this mechanism will lead to high spreading rates, if molecules are diffusing over a long distance and rebind.^[25] Yet, decreased spreading rates can be observed when the lifetime of the solution-bound species is too long, so that it can diffuse into the bulk.

Regarding the results shown in Figure 5.4a, we did not observe line broadening for the monovalent APY₁ which would be indicative for surface diffusion, so it is not possible to categorize the mobility of APY₁ to one of the proposed diffusion mechanisms. According to the intensity changes and the changes in line width in Figure 5.4b and 5.4c, we propose a walking mechanism for both multivalent APY_{2,3} guests, and possibly also hopping occurs. Both diffusion mechanisms allow mobility over a short distance, which is supported by the maximum spreading rate in Figure 5.5. If a competitor supported diffusion mechanism would be preferred, a clear relation to the CB[8]/paraquat concentration could be drawn, which is not the case due to possible reasons, which will be explained below. The decrease in spreading rate observed for both APY_{2,3} guests upon switching from pure water to a competitor solution is, however, an indication that walking is the main mechanism observed, as the presence of competitor decreases the concentration of species with partially unbound guest sites and thus the frequency of walking events. In contrast, hopping is usually connected to accelerated diffusion, which is not observed here.

Limitations in spreading are probably caused by low reaction rate constants. The association rate constant is determined by the formation of a host-guest complex. The association rate is high when the formed complex is preferred over the noncomplexed species. Advantageous interactions, when for example a hydrophobic guest moiety is included in a hydrophobic host cavity, force the guest to remain complexed, subsequently leading to a high association rate constant and a low dissociation rate constant. If dissociation takes place, a fast rebinding is expected either to free surface sites or when CB[8]/paraquat is present also to the in solution competing host. As proposed by Scherman and coworkers, the association rate constant is ‘host-controlled’, whereas the dissociation rate constant is ‘guest-controlled’ of CB[8] ternary complexes.^[31] Reported association rate constants are in the range of $k_{on} = 10^7 \text{ M}^{-1}\text{s}^{-1}$ and are

similar to those reported for CD host-guest complexes. Corresponding dissociation rate constant showed a direct relationship to the thermodynamic binding constant, resulting in lower rates for energetically more stable complexes.^[31] Furthermore, for the hydrophobic APY moiety, it is disadvantageous to dissociate from the ternary complex, due to the polar surrounding medium. The dissociation related activation barrier is much larger compared to the association of the complex.^[31]

The fact that APY₁₋₃ has a high association rate and a comparatively low dissociation rate can also explain why the highest spreading rate was observed in water. In the case when CB[8]/paraquat is present in solution, a dissociation of the surface-bound complex would directly lead to fast rebinding to the competitor in solution. Based on this explanation we would expect a controversy spreading behaviour of CB[8] systems compared to the in literature reported binary CD system.^[25]

5.3 Conclusions

Spreading experiments showed significant differences in desorption and spreading rates between mono-, di-, and trivalent guest molecules. Both multivalent guests showed spreading rates up to 120 nm/min in water, whereas the monomer spreading rate was negligible. Decreasing changes in intensities were observed for all three species with the fastest change for the monomer followed by the dimer and trimer. The main contribution to the fading fluorescence is desorption of physisorbed and/or chemisorbed molecules from the surface and going into the bulk, followed by bleaching/quenching and spreading of the molecules. The influence from CB[8] on fluorescein dyes was studied and a small quenching effect was observed. A surprising fact is, that the dynamics of the divalent and trivalent guest are the fastest in water as surrounding media as well, which are contrary to reported systems in literature.^[25] Addition of CB[8]/paraquat to the surrounding medium led to decrease in spreading rate but not depending on the amount of CB[8]/paraquat and smaller changes in line broadening against the time, most probably due to the low dissociation rate constants of those host-guest complexes. Therefore, the observed spreading rates and the small increase in line widths imply that there is a preference for a walking mechanism but not for a hopping mechanism for both multivalent guest molecules. Most likely, hopping can be enhanced when higher competitor concentrations would be accessible.

Future research directions could include the further characterization of the layer. Establishing a pure chemisorbed layer is a requirement for reliable diffusion experiments. For that, flow cell

experiments seem to be a suitable alternative to clearly distinguish between physisorbed and chemisorbed molecules. Establishing a stable baseline is indicative for a chemisorbed layer after washing away physisorbed molecules. Furthermore, we would overcome mass transport limitation due to avoiding a retention zone above the surface. After verification of a chemisorbed monolayer purely diffusion-based motion is observed. Secondly, changing the fluorescent headgroup to one that is less prone to bleaching, such as rhodamine, could be advantageous and elongate the measurement time. Since the dissociation rate constant is rather low, visualization over a longer time period would be desired. The herein presented results show that cucurbit[n]uril chemistry is behaving differently in spreading studies compared to previous studied β CD based spreading studies. This makes it necessary to get a more detailed molecular understanding of the kinetics of those CB[8]-mediated platforms to understand the involved recognition processes.

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5.5 Experimental Section

5.5.1 General Methods

All chemical reagents were of analytical purification grade and were used without further purification. Thiol-functionalized methylviologen (MV^{2+}) was synthesized according to literature procedures.^[32] PBS and CB[8] were purchased from Sigma Aldrich. Maleimide-terminated disulfide and tetra ethylene glycol disulfide were purchased from Prochimia. Concentration of CB[8] was assessed by a UV/vis titration against a cobaltocenium salt.^[33] COP surfaces were test stripes received from Zeonor®. For measurements MilliQ water ($R = 18.2 \text{ M}\Omega$) was used. Microscopic images were taken using an inverted research microscope (Olympus, 1X71, Melville NY, USA) equipped with a mercury burner U-Rfl-T as a light source and a digital camera with 12.5 million-pixel (Olympus DP70) for acquisition. Imaging of the fluorescein labelled APY₁₋₃ was performed with blue excitation light ($460 \leq \lambda \leq 490 \text{ nm}$) and emitter of 525 nm.

5.5.2 Synthetic Procedure

Synthesis of APY₁₋₃ derivatives used in this study was already described in the previous Chapter 4.

5.5.3 Diffusion Experiments

Substrate preparation of CB[8] functionalized SAMs was done on COP surfaces. COP was cutted in appropriate slides, rinsed and sonicated for 10 min in EtOH, followed by oxygen plasma activation for 60 seconds. Stepwise incubation of PLL-OEG-Mal (5-10 μ L, 5 mg/mL in PBS) for 5-10 min, MV²⁺ (100 μ L, 1 mM) for 30 min and CB[8] (100 μ L, 50 μ M) for 10 min yielded in the platforms for microcontact printing. Polydimethylsiloxane (PDMS) stamps were prepared from commercially available Sylgard-184. The curing agent and the prepolymer were manually mixed in a 1:10 volume ratio and cured over night at 60 °C against the silicon master. Silicon master of 100 x 100 μ m line features were fabricated with photolithography. PDMS stamps were rinsed with EtOH and sonicated in EtOH for 15 min and dried under a stream of N₂. Subsequent up-side-down incubation with 100 μ L fluorescent APY₁₋₃ and drying with N₂. APY₁₋₃ solution were sonicated prior usage to avoid aggregation.

For **microcontact printing (μ CP)** incubated and dried stamps were brought in conformal contact with CB[8]/MV²⁺ functionalized platforms and remained there for 5 min. The samples with pattered APY₁₋₃ were rinsed right after the printing with continuous flow of water to remove physisorbed molecules (APY₁: 10 sec, APY₂₋₃: 30 sec).

For **spreading experiments** 80 μ L aqueous solution of either water or CB[8]/paraquat were applied on top of patterned surfaces confined with an adhesion badge. The system was immediately covered with a microscopy glass slide to avoid evaporation and contamination. After expired time (5, 10, 30 and 60 minutes), the surface was blown dry and images were taken on different spots to avoid photobleaching.

5.5.4 Control Experiments

Fluorescence spectroscopy was used to verify the interaction of fluorescein and CB[8] and CB[8]/paraquat using a Perkin Elmer LS 55. Aliquots with a constant concentration of fluorescein (0.5 μ M) and increasing amounts of CB[8] (0, 1, 10, 25, 50 μ M) and CB[8]/paraquat (0, 1, 10, 25, 50, 100 μ M) were measured and analyzed regarding their maxima and shifts. Fluorescence spectra show a decrease in fluorescence intensity upon increasing amount of CB[8]

or CB[8]/paraquat respectively. A shift of maxima could be not detected (see table for values). Table 5.1 summarizes the wavelength of maxima and the intensity of maxima. No blue or red shifts were observed.

Figure 5.7 | Fluorescence spectra of 0.5 μM Fluorescein titrated with a) 0, 1, 10, 25 and 50 μM CB[8] and b) 0, 1, 10, 25, 50 and 100 μM CB[8]/paraquat.

CONCENTRATION [μM]	CB[8]		CB[8]/PARAQUAT	
	WAVELENGTH		WAVELENGTH	
	MAXIMUM		MAXIMUM	
0	511.4	505.3	512.1	452.2
1	511.6	500.4	512.1	453.5
10	511.7	431.5	512	430.5
25	511.8	246.7	511.9	423.6
50	511.8	176.8	511.9	293.2
100			511.6	247.0

Table 5.1 | Wavelength and intensities of maxima of a titration of Fluorescein to CB[8] and CB[8]/paraquat.

ITC measurements were carried out using a Microcal VP-ITC with a cell volume of 1.4115 mL. For studying the interactions of fluorescein with CB[8] and CB[8]/paraquat 10 μL aliquots of 50 μM and 100 μM CB[8] and CB[8]/paraquat were added to a 0.5 μM solution of fluorescein in the calorimetric cell, monitoring the evolution of heat after each injection. ITC could not verify an interaction neither with CB[8] nor CB[8]/paraquat. A significant evolution of heat was not observed.

Figure 5.8 | ITC titration of 0.5 μM Fluorescein in PBS to a) 0.05 mM CB[8], b) 0.1 mM CB[8]/paraquat.

5.6 References

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Chapter 6

Binding Studies of Heterobivalent Stimuli-Responsive Guests on β CD and CB[7] Monolayers

Changing the affinities of host-guest interactions on surfaces by applying an external stimulus has potential for the development of smart (bio)materials. In this chapter, heterobivalent guest molecules were synthesized that incorporate light, redox or chemical responsive moieties. The binding behavior and stimuli-responsiveness of these heterobivalent azobenzene derivatives were investigated on β -cyclodextrin (β CD) and cucurbit[7]uril (CB[7]) surfaces. Binding studies were performed using surface plasmon resonance spectroscopy (SPR) and the estimated binding affinities were found to be significantly higher compared to their monovalent analogues. The calculated overall binding affinities according to a multivalency model gave a clear indication whether a system binds monovalently or bivalently. From the binding studies, monovalent binding was found on CB[7] surfaces, while mostly divalent binding was found on β CD surfaces. The experimentally derived affinities for AZO-NP, AZO-FC and AZO₂ on β CD of $K_a = 1.3 \times 10^5$, 1×10^6 , $5.6 \times 10^6 \text{ M}^{-1}$ respectively, were in agreement with the calculated affinities of $K_{a,MM} = 1.2 \times 10^5$, 1.4×10^6 and $6.8 \times 10^6 \text{ M}^{-1}$ based on solution monovalent affinities. In contrast, binding studies on the CB[7] monolayers resulted in binding affinities of $K_a = 1.1 \times 10^7$ and $1.2 \times 10^6 \text{ M}^{-1}$, which are three orders of magnitude lower than expected from the multivalent binding model based on monovalent affinities. Furthermore, buckled shaped adsorption curves on CB[7] surfaces were measured and these could be indicative of nonspecific surface interactions. The different stimuli-responsive moieties of the heterobivalent constructs were found to be sensitive to different stimuli, which can be utilized for developing dual stimuli-responsive systems. Applying a stimulus to decrease the affinity of one of the guest moieties of the heterobivalent constructs led to accelerated dissociation from the host platforms.

6.1 Introduction

Supramolecular constructs are consisting of at least two molecules connected by attractive and repulsive interactions.^[1, 2] These are generally weak interactions, such as hydrogen bonding, electrostatic, aromatic, charge-transfer or metal-ligand interactions.^[1-3] As these noncovalent interactions are generally reversible, the assembly and disassembly of these constructs are reversible as well.^[4] Environmental properties, such as polarity, temperature or pressure are affecting noncovalent interactions and can strengthen or weaken them, which changes the structure of supramolecular assemblies.^[5-7] Stimuli-responsive properties can also be a useful tool to change the structure of supramolecular assemblies.^[8, 9] Stimuli-responsive changes can be induced by changing pH, redox state, magnetic field, light or by adding competitors.^[10] Stimuli-responsiveness is well-established in various solution-based systems,^[11, 12] for example in gelation,^[13, 14] polymerization,^[15] self-healing processes^[16, 17] and assembly and disassembly of supramolecular assemblies.^[18, 19]

Macrocyclic host molecules, such as for example cyclodextrins (CD), cucurbit[n]urils (CB[n]) and calix[n]arenes, are useful building blocks for the design of stimuli-responsive supramolecular assemblies on surfaces.^[20] State-of-the-art research focusses on developing switchable systems that are sensitive to dual- or even multi-responsive stimuli.^[21] For example, Scherman and coworkers described the UV or redox-induced disassembly of host-guest complexes away from the surface.^[22] In this case cucurbit[8]uril (CB[8]) linked a fluorescent dye modified with a redox-sensitive methylviologen to surface-bound photo-responsive azobenzenes.^[22] Very recently, multi-stimuli-responsive nanoparticles and vesicles have been reported in which three stimuli-responsive switches were incorporated, which allows to simultaneously or sequentially change the affinity of the molecule and thereby change the assemblies.^[21, 23] Of particular note is a multi-responsive mono-component host-guest systems reported by Cheng *et al.*^[24] The mono-component system consists of azobenzene-modified β CD, which is undergoing gelation at a critical temperature and forming a hydrogel which has possible potential as storage cargo in drug delivery. When the hydrogel is formed, disassembly can be achieved by heating, UV irradiation and host or guest induced competition.^[24] A host-guest based single stimulus triple-responsive system was reported by Zhao *et al.* in which a pillar[n]arene trimer and a bis-methylviologen undergo conformational changes at three critical concentrations. Going from low (>1 mM) to high concentrations (> 5 mM) led to stepwise transformation of spherical and tubular objects and stacked layer.^[25] The group of Ravoo reported a trifunctional azobenzene-ferrocene cross-

linker with an ethylenediamine spacer which mediates aggregation of β CD amphiphilic vesicles by host guest chemistry. Upon applying of a single stimulus of either metal ion, light or oxidation induces dispersion of the aggregates.^[26] The reported combined responsive systems are mostly proof-of-principle studies and future efforts shall be directed to chemical and biological applications with high complexity.

As already described in the previous chapters, multivalent binding can play an important role in many biological processes.^[27] Understanding of multivalent binding events helps to understand and control many processes, not only biological interactions between proteins, cells and viruses, but also supramolecular systems for advanced materials engineering.^[28] Interestingly, reported divalent guests that are used for assembly on surfaces are homodivalent, which means that the binding sites are the same and that a suitably applied stimuli exerts its action on both binding sites, which potentially leads to a complete change in affinity of the homodivalent guests with their host surface. Herein, we investigate the binding behavior of supramolecular heterodivalent guests, i.e. guests with two different binding sites on β CD and CB[7] surfaces, of which one motif is responsive to a light stimulus and the other motif to a redox stimulus. In this way a partial change in affinity of the heterodivalent guests with their host surface could be achieved. First, we discuss the surface binding of these heterodivalent guests on self-assembled monolayers. We also highlight the photo- and redox-responsiveness and subsequent changes in binding behavior.

Figure 6.1 | Guest molecules used in this chapter. a) AZO-FC, b) AZO-AD, c) AZO-NP and d) AZO₂.

6.2 Results and Discussion

6.2.1 Platforms and Molecules

The heterodivalent molecules employed in this chapter are composed of different binding moieties (Figure 6.1). Flexible tetra ethylene glycols serve as spacer between binding sites and enhance water solubility at the same time. Synthetic details are given in the experimental section. The intrinsic monovalent binding affinities of our selected guest moieties with β CD and CB[7] as host cavities were found in literature or determined here (experimental section).^[22, 29-36] The binding moieties show weak to moderate binding constants K_a in M^{-1} , which are listed in table 6.1.

BINDING MOIETY	K_a (M^{-1}) on β CD		K_a (M^{-1}) on CB[7]	
	Solution	Surface	Solution	Surface ^a
ADAMANTANE (AD)	10^4	4.6×10^4	10^8 - 10^{10}	7.6×10^5
AZOBENZENE (AZO)	10^3	5.8×10^3		1×10^4
FERROCENE (FC)	10^3 - 10^4	1.2×10^3	10^9	3.8×10^5
NAPHTHOL (NP)	10^1 - 10^3	10^3	-	-

Table 6.1 | Literature data^[22,29-36] of binding constants K_a in M^{-1} for selected binding moieties and respective host cavities in solution and on self-assembled host monolayers. ^a Experimental data in this chapter.

The binding affinities of the heterobivalent guest with either β CD or CB[7] host functionalized platforms were determined using surface plasmon resonance spectroscopy (SPR). In short, a solution with guests was flowed through a fluidic chip that was covered with the supramolecular host platform. Interaction between the guest and the host molecules resulted in a change of signal, which is proportional to the surface coverage of adsorbed material. Monitoring at least five different guest concentrations on a platform until saturation, enables to estimate the binding constant K_a . After each concentration and prior to the next, the platform was restored by rinsing with a competitor solution at high concentration. CB[7] spontaneously adsorbs onto gold surfaces (Figure 6.3a).^[37, 38] Electrostatic interactions between the carbonyl rim and the gold lead to monolayer formation with only minor defects following the procedure described by Hernandez.^[37] Incubation for 1h in a saturated CB[7] solution (1 mM) gave already satisfying monolayer qualities and reduced the risk of multilayer formation as it is known from longer deposition times (6.16b).^[37, 38] β CD hosts were attached to an antifouling layer through a thiol-

maleimide coupling, as it is described for the methylviologen attachment in Chapter 3 and 4 and yielding β CD surfaces with coverages similar to CB[8] (Figure 6.16a).

6.2.2 Surface Binding on β CD and CB[7] SAMs

The sensorgrams in Figure 6.2 show the real-time adsorption of divalent guest molecules onto a β CD platform. The graphs show the change in resonance angle as a function of time, while guest molecules were flowed over the surface. In the case of AZO-NP, AZO-FC and AZO₂, solutions of the guests were flowed over the surface after saturation of the signal and restoring the host-surface. In the case of AZO-AD, a new sensor surface was used for each concentration due to a low signal-to-noise ratio. The host surfaces were restored by flowing a 10 mM β CD solution and water after each concentration. Large changes in signal when flowing 10 mM β CD solutions were caused by optical effects. For all four divalent guests, changes in the resonance angle were plotted as function of concentrations (Figure 6.3).

Figure 6.2 | SPR titration data of a) AZO-AD (1, 10, 25, 100, 500 μ M), b) AZO-NP (5, 10, 25, 50, 100 μ M), c) AZO-FC (0.5, 1, 2.5, 5, 10, 25 μ M) and d) AZO₂ (0.5, 1, 5, 10, 20 μ M) (■) on β CD platforms and subsequent rinsing with 10 mM β CD solution and water (●).

The SPR data were fitted to the 1:1 Langmuir binding model, which assumes that the two guest moieties of the heterodivalent guests simultaneously interact with β CD cavities at the surface. As shown in Figure 6.3, the SPR data were satisfactorily fitted to yield overall binding constants (K_{LM}) (Table 6.2).

Figure 6.3 | Changes of SPR angle plotted against the corresponding guest concentrations and fitted with a Langmuir 1:1 binding model for a) AZO-AD, b) AZO-NP, c) AZO-FC and d) AZO₂ on β CD SAMs.

We conducted the same type of SPR titration experiments for the four guests on CB[7] functionalized monolayers. CB[7] was adsorbed on gold surfaces following previous methods.^[37, 38] Whereas incubation of the gold sensors for 1h revealed amorphous monolayers of CB[7], longer incubation times resulted in multilayer formation or relocation of gold atoms due to interaction with CB[7] and limits guest binding properties.^[37, 39] Inhomogeneous and incomplete surface coverage can lead to steric hindrance during guest binding and reduce multivalent interactions. A uniform orientation of the CB[7] molecules where they keep their carbonyls perpendicular to the gold surface and leaving their cavities open to solution is preferred.^[39] To assure the same quality of the monolayer, freshly prepared SAMs were used for each concentration. SPR graphs in Figure 6.4 show that the reflectivity shifted while different

concentrations were flowed over the sensors. Dissociation from the surface was observed in all cases when a solution of CB[7] was flowed indicating reversible interactions of the guests with the CB[7] SAMs.

Figure 6.4 | SPR titration data of a) AZO-AD (0.05, 0.1, 0.25, 0.5, 1 μ M) and b) AZO-FC (0.5, 1, 2.5, 5, 10 μ M) on a CB[7] monolayer. After each concentration, a solution of 0.1 mM CB[7] (●) was added.

The SPR binding curves of AZO-AD and AZO-FC on CB[7], but also AZO-AD on β CD surfaces have an unusual appearance. Usually for AZO-NP, AZO-FC and AZO₂ guests on β CD SAMs, smooth gradual curves were observed (Figure 6.2b-d), while in the other cases of heterobivalent guests on CB[7] SAMs, the binding curve displayed a point after which the gradual change of the curve slowed down. These unusual curves reveal binding affinities that are more in agreement with intrinsic monovalent interactions with the host instead of bivalent binding. The different curvature of the adsorption profile can be due a less optimal structural fit to interact with two guest moieties of the same molecule at the same time. Another explanation could be nonspecific binding of AZO-AD and AZO-FC to the gold surface, which were observed in dynamic force spectroscopy measurements on inhomogeneous monolayers of CB[7] in earlier studies.^[40] Furthermore, it is conceivable that a strong first binding event of the adamantane or ferrocene binding site with a CB[7] cavity is followed by a weaker (nonspecific) interaction of the second motif with *e.g.* the bare gold surface or CB[7]. (Figure 6.5).

Figure 6.5 | Schematic representation of equilibria for heterodivalent guest binding to surface bound host. The probability taking the upper or lower intermediate can be estimated from the ratio between the intrinsic affinity constants of both guest motifs, according to equation [1].

The estimated Langmuir binding constants reveal a moderate binding affinity of the heterodivalent guests to the β CD monolayer and the values are one to two orders of magnitude higher compared to the affinity constants of their monovalent motifs. As expected, all four guests are reversibly bound to the β CD surface. Binding curves (Figure 6.6) were also constructed for CB[7] SAMs and a K_d of 93 nM and 0.82 μ M for AZO-AD and AZO-FC, respectively were derived. Since the occurrence of the curvature is not clearly interpretable and to exclude possible nonspecific interactions from the data fitting process SPR angle shifts at 40 and 20 minutes of the AZO-AD and AZO-FC adsorption were taken to get the binding fit.

Figure 6.6 | Changes of SPR angle shifts fitted with a Langmuir 1:1 binding model of a) AZO-AD and b) AZO-FC. Langmuir affinity constants calculated from fitting the SPR data.

The binding affinities of both divalent guests are two orders of magnitude higher compared to the monovalent ones on the CB[7] monolayer (Figure 6.17), but lower than overall binding constants calculated according to equation 2. The overall binding constant can be related to the

intrinsic binding constant at the surface for monovalent host-guest interactions and the maximum effective concentration at the surface ($C_{\text{eff,max}}$), as shown in equation 1.^[41]

$$K_{ov} = K_{i,1} \times K_{i,2} \times EM \quad [2]$$

With $C_{\text{eff,max}} = 0.2$ M and the intrinsic monovalent interactions (K_i) known from literature (table 6.1) the overall binding constant ($K_{\text{MM,ov}}$) at the SAMs were calculated.^[32, 42, 43] Table 6.2 shows the binding constants (K_{ov}) according to both Langmuir and multivalency model and confirms that the guests interact mainly with two moieties to the β CD surface.

β CD SAMs	$K_{\text{LM,d}}$ [μM]	$K_{\text{LM,a}}$ [M^{-1}]	$K_{\text{MM,ov}}$ [M^{-1}]
AZO-AD	30	3.3×10^5	5.3×10^7
AZO-NP	76	1.3×10^5	1.2×10^5
AZO-FC	10	1×10^6	1.4×10^6
AZO₂	1.8	5.6×10^6	6.8×10^6
CB[7] SAMs	$K_{\text{LM,d}}$ [μM]	$K_{\text{LM,a}}$ [M^{-1}]	$K_{\text{MM,ov}}$ [M^{-1}]
AZO-AD	0.093	1.08×10^7	1.5×10^{10}
AZO-FC	0.82	1.22×10^6	7.6×10^9

Table 6.2 | Binding constants of divalent guests on β CD and CB[7] surfaces calculated according to the Langmuir binding model and multivalency model (equation [2]).

Discrepancies between the affinities in solution and the here derived lower affinities on the surface can have different explanations. The guests that interact in solution with CB[7] protrude through the carbonyl rim of CB[7], however the direct proximity of the CB[7] portal to the gold sensor hinders optimal location of the guests in the CB[7] cavity and may lower the interaction strength.^[44, 45] A study of single molecule detection and identification via recognition tunneling revealed optimized molecular structures of three inclusion complexes with amantadine, aminoferrocene and ferrocene and CB[7] (Figure 6.7).^[39] Furthermore, they observed that the interaction strength between gold and CB[7] is reduced upon guest inclusion, due to competing interactions with the carbonyl rim. Especially when the guest contains an amino group, the competition is remarkably strong, because the amino group is located in the vicinity of the carbonyls (Figure 6.7b,c).^[39]

Figure 6.7 | Charge density distribution of CB[7] inclusion complexes with a) ferrocene, b) aminoferrocene and c) amantadine. With permission from ref. [39].

6.2.3 Stimuli-Responsiveness

Figure 6.8 | Scheme of supramolecular host guest functionalization followed by stimuli-responsive stepwise dissociation.

As a next step, the photo-isomerization properties of the homodivalent AZO_2 was investigated using UV/vis spectroscopy. Upon irradiation with UV light ($\lambda = 365$ nm), azobenzene isomerizes into the *cis*-form (Figure 6.9a). The *cis*-azobenzene is sterically hindered to fit within the host cavity, resulting in a reduced binding affinity and subsequent dissociation of the host-guest complex. In water, AZO_2 (50 μ M) displays a characteristic absorption band of *trans*-azobenzene, with a maximum absorption at $\lambda_{\max} = 366$ nm (Figure 6.9b). Upon irradiation with UV light, *trans-cis* isomerization occurred and the π - π^* absorption band at $\lambda_{\max} = 366$ nm decreased, while the characteristic absorption band at $\lambda_{\max} = 440$ nm increased. The solution was irradiated for 15 min with UV light, followed by 20 sec stepwise irradiations with ambient light until no changes were recorded.

Figure 6.9 | a) Azobenzene *trans-cis* isomerization based on UV stimulus and reisomerization, b) photoisomerization of azobenzene in water at 25 μ M before (black) and after UV (red) irradiation and stepwise reisomerization to *trans-AZO*₂.

Next, the binding behavior between *trans*- and *cis*-forms of *AZO*₂ on the β CD monolayer was investigated. For this purpose, a solution of *AZO*₂ was irradiated with UV light for 15 min prior to injection into the SPR flow chamber. As expected, a significant drop in resonance angle was observed between *cis*- and *trans-AZO*₂. However, binding to the platform was also observed for the irradiated species. This could possibly be explained by the incomplete photo-isomerization of the azobenzene moiety, which is known to be about 75-80% efficient.^[46] The red dots on the binding curve (Figure 6.10) indicate that non-irradiated solutions are in line with previous binding experiments described in section 6.2.2. For irradiated solutions containing the *cis*-isomer, a reduced affinity for *AZO*₂ was detected to be 0.5 μ M instead of 2.5 μ M. From the binding curve, the *trans/cis* ratio is calculated to be 75/25 for *AZO*₂, which is in accordance with literature.^[46]

Figure 6.10 | Switching of *AZO*₂ and subsequent binding to a β CD surface. a) adsorption of 2.5 μ M *trans-AZO*₂ (gray) and *cis-AZO*₂ (black) (■), followed by a rinsing step (●) and b) data point of *cis-AZO*₂ (black dot) and *trans-AZO*₂ (gray dot) included in a titration series of *trans-AZO*₂ on β CD (black square).

Upon switching of the AZO-NP guest with UV light to yield *cis*-AZO-NP on a β CD surface, a small decrease in affinity was observed (Figure 6.11a). The adsorption of the partially switched *cis*-AZO-NP starts similar to the non-switched AZO-NP, but is reaching equilibrium around 30 min, whereas AZO-NP is approaching equilibrium at 50 minutes. The adsorption curve of *cis*-AZO-NP is more similar to non-switched AZO-NP than to the monovalent naphthol interaction. A similar experiment was conducted with the AZO-AD guest on a β CD surface (Figure 6.11b). Also, in this case we observed a similar behavior of the UV switched *cis*-AZO-AD compared to the non-switched AZO-AD. The almost stable binding affinity of both partially switched guests is due to the remaining single binding motif, which is considerably binding as a monovalent guest. Furthermore, residual *trans*-azobenzene moieties can still interact with the surface. (Figure 6.11b).

Figure 6.11 | Switching of 50 μ M AZO-NP and 25 μ M AZO-AD on a β CD surface. a) Adsorption of monovalent naphthol (light gray), UV irradiated partially switched *cis*-AZO-NP (gray) and non-switched AZO-NP (black) (■), followed by rinsing with β CD and water (●) and b) adsorption of *cis*-AZO-AD (gray) and *trans*-AZO-AD (black) (■) and subsequent rinsing with water (●).

Lastly, the switching behavior of the heterodivalent AZO-FC guest on a CB[7] monolayer was investigated. AZO-FC is responsive to both UV- and redox-based stimuli (Figure 6.12).

Figure 6.12 | Stimuli-induced changes of AZO-FC upon applying UV and redox stimuli.

Using SPR spectroscopy, the binding of the partially switched and fully switched guest were investigated on a CB[7] monolayer. For oxidation of the ferrocene to the ferrocenium cation (Fc^+), thereby reducing its binding affinity to CB[7] over three orders of magnitude as it was measured in solution,^[47, 48] a sodium hypochlorite (NaClO) solution was used as oxidizing reagent. As shown in Figure 6.13, incubation of a CB[7] SAM with a pre-mixed (for 2h) solution of $10\ \mu\text{M}$ AZO-FC in $10\ \text{mM}$ NaClO , showed the same interaction strength when compared to the control measurement with AZO-FC in water. However, elongated pre-mixing (for 24h) of $10\ \mu\text{M}$ AZO-FC in NaClO , prior to flowing this solution over CB[7] SAMs, resulted in observing a 50-fold lower binding affinity of $K_d = 46\ \mu\text{M}$, compared to AZO-FC with $K_d = 0.82\ \mu\text{M}$ (Figure 6.13a), but is approximately two times higher than the monovalent AZO affinity to CB[7] SAMs. Considering that the ferrocenium cation is still weakly interacting with CB[7] through electrostatic interactions with the cavity or carbonyl rim, a slightly enhanced overall affinity could be achieved.

When both stimuli are applied to the guest, we observed no binding after flowing along the CB[7] platform. When the solution was exposed to ambient light, weak binding was observed, caused by the re-isomerization of the azobenzene moiety from *cis*- to *trans*-state (Figure 6.13b, indication at 20 min).

Figure 6.13 | a) Titration series of partially switched AZO-FC (10 μ M) in 10 mM NaClO plotted with a Langmuir binding model and yielding a dissociation constant of $K_d = 46 \mu$ M. b) Adsorption of non-switched, partially switched and fully switched AZO-FC (■) on a CB[7] monolayer and subsequent rinsing (●).

6.3 Conclusions

In this chapter various heterobivalent azobenzene derivatives have been synthesized and their binding behaviour on surfaces was investigated by SPR. Heterovalency was introduced by using two different binding moieties, which are sensitive to different stimuli and vary in affinity to the responding surface. SPR binding studies on β CD and CB[7] SAMs revealed overall binding affinities larger than the monovalent interactions and those on β CD follow bivalent binding modes as expected. In the case of different affinities of both motifs an unusual curvature of the adsorption was observed in some cases, mostly on CB[7] SAMs, and the derived binding affinities imply monovalent interactions instead of divalent. Several aspects and issues which may cause this unusual curvature need to be considered. Possibly, different binding affinities of both motifs allow a sequential binding/interaction process or a rearrangement process after initial binding. According to that, a first strong binding event occurs followed by weaker second probably nonspecific interaction. Also feasible would be a rearrangement process on the surface, where the weaker motif is substituted by the stronger one. Furthermore, the surface properties are playing an important role in multivalent binding. From literature it is known, that CB[7] SAMs are rarely of the same quality as e.g. β CD SAMs. One more explanation could be that the CB[7] does not allow a divalent binding, due to inhomogeneous surface coverage. This would also support monovalent binding of the stronger motif and enhances the probability of nonspecific interactions of the weaker motif with the bare gold surface.

Utilization of the stimuli-responsiveness enabled to tune the binding affinity over several orders of magnitude up to complete dissociation. Reversible dissociation and association can be induced by irradiation with UV or visible light, whereas irreversibly dissociation occurs following chemical reduction.

Overall, developing and incorporating dynamic molecules into synthetic systems based on stimuli-responsive moieties emerge as a relevant tool to design dynamic supramolecular systems. The responsive and partially dual responsive character of our guests could make them a promising candidate for the design of those systems.

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6.5 Experimental Section

6.5.1 General Methods

Chemicals were purchased from Sigma Aldrich or from Acros Organics and used without further purification. Reactions were carried out using dried solvent and under an atmosphere of argon. Reactions were monitored by thin-layer chromatography (TLC), which was performed on 0.2 mm Merck precoated silica gel 60 F254 aluminium sheets. Spots were visualized by treatment with basic KMnO_4 solution or under UV light irradiation. Column chromatography was carried out on silica gel 60 (0.063-0.2 mm, Merck). Thioether- β CD was synthesized according to literature procedures.^[49] CB[7] was purchased from Sigma-Aldrich. Gold surfaces (40-50 nm) were purchased from Ssens (Enschede, Netherlands). MilliQ water ($R = 18.2 \text{ M}\Omega$) was used for synthesis and measurements. The synthesized products were analyzed by ^1H NMR on a Bruker spectrometer 400 MHz, samples were dissolved in deuterated solvent purchased from Cambridge Isotope Laboratories Inc. All chemical shifts (δ) are given in parts per million (ppm) and relative to the residual solvent signal. For splitting pattern description, the following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). Mass analysis was done using electrospray ionization using a Voyager DE-RP and a micromass LCT from water.

Figure 6.14 | Synthesis route of the tetra ethylene glycol modified binding moieties. (i) TosCl , NEt_3 , DMAP , CH_2Cl_2 , 18h, rt, (ii) K_2CO_3 , MeCN , LiBr , 80°C , 48h, (iii) Tetra ethylene glycol, NEt_3 , 150°C , 24h, (iv) THF , KOtBu , propargyl amide, 24h, (v) CH_3COOH , NaN_3 , 50°C , 3h, (vi) $\text{DMF}/\text{EtOH}/\text{H}_2\text{O}$, CuSO_4 , NaAsc , 18h, Ar.

2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (1)

To a stirred solution of tetra ethylene glycol (30.0 g, 154 mmol, 1 eq.) in CH_2Cl_2 was added triethylamine (NEt_3) (1.2 mL, 10 mmol, 1 eq.)

and a catalytical amount of Dimethylamino-pyridine (DMAP) (50 mg). The mixture was stirred for 30 minutes. After that time p-toluenesulfonylchloride (TosCl) was added in portions (7.24 g, 39 mmol, 1 eq.). The reaction mixture was stirred overnight followed by evaporation of the solvent. The residue was redissolved in CHCl_3 and extracted three times with water. The organic layer was separated and dried over MgSO_4 . The solvent was removed under reduced pressure. The title compound was obtained as colorless oil. Yield: 9.51 g, (70 %). ^1H NMR (400 MHz, CDCl_3): δ = 2.37 (s, 3H), 3.44-3.51 (m, 10H), 3.61 (t, 2H), 4.09 (t, 2H), 7.27 (d, 2H), 7.73 (d, 2H). ESI-MS (m/z): calculated for $[\text{C}_{15}\text{H}_{24}\text{O}_7\text{S}]$: 348.4, found: 371.2

(E)-2-(2-(2-(2-(4-(phenyldiazenyl)phenoxy)ethoxy)ethoxy)ethoxy)ethanol (2)

To a stirred solution of **1** (5.23 g, 15 mmol) in 150 ml of dry acetonitrile (MeCN), containing of K_2CO_3 (10.37 g, 75 mmol) and catalytic amounts of lithium bromide (LiBr), 4-phenylazophenol (3.17 g, 16 mmol), dissolved in 50 ml of MeCN, was added and the reaction mixture was refluxed for 2 days under argon. It was then allowed to cool to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in 100 ml of CH_2Cl_2 , washed once with 100 ml of water and thrice with 100 ml of brine. The organic phase was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2 /methanol 95:5) to afford of the title compound. Yield: 5.11 g, (91 %). 1H NMR (400 MHz, $CDCl_3$): δ = 2.57 (s, 1H), 3.58 – 3.50 (m, 2H), 3.72 – 3.61 (m, 10H), 3.83 (m, 2H), 4.16 (m, 2H), 7.03 – 6.95 (m, 2H), 7.50 – 7.35 (m, 3H), 7.92 – 7.80 (m, 4H). ESI-MS (m/z): calculated for $[C_{20}H_{26}N_2O_5]$: 374.4, found: 397.7.

(E)-2-(2-(2-(4-(phenyldiazenyl)phenoxy)ethoxy)ethoxy)ethoxy)ethyl-4-methylbenzensulfonate (3)

To a stirred solution of **2** (3.00 g, 10 mmol, 1 eq.) in CH_2Cl_2 was added NEt_3 (1.2 mL, 10 mmol, 1 eq.) and a catalytical amount of DMAP (50 mg). The mixture was stirred for 30 minutes. After that time p-toluenesulfonylchloride was added portionwise (2.07 g, 10 mmol, 1 eq.). The reaction mixture was stirred overnight followed by evaporation of the solvent. The residue was redissolved in $CHCl_3$ and extracted 3 times with water. The organic layer was separated and dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was subjected to silica column chromatography ($CHCl_3$). The product was obtained as orange oil. Yield: 1.78 g, (33 %). 1H NMR (400 MHz, $CDCl_3$): δ = 7.89-7.84 (m, 4H); 7.76-7.74 (d, 2H); 7.48-7.45 (m, 2H); 7.42-7.40 (m, 1H); 7.29-7.27 (m, 2H); 7.00-6.98 (m, 2H); 4.18-4.16 (m, 2H); 4.13-4.10 (m, 2H); 3.86-3.83 (m, 2H); 3.68-3.54 (m, 10H); 2.37 (s, 3H). ESI-MS (m/z): calculated for $[C_{27}H_{32}N_2O_7S]$: 528.2, found: 529.2.

2-(2-(2-(2-(naphthalen-2-yloxy)ethoxy)ethoxy)ethoxy)ethan-1-ol (4)

2-naphtol (1.28 g, 8.9 mmol, 1 eq.) was dissolved in MeCN (50 mL) followed by the addition of **1** (3.1g, 8.9 mmol, 1 eq.), K_2CO_3 (6.14 g, 44.5 mmol, 5 eq.) and a catalytical amount

of LiBr (50 mg). The obtained solution was stirred for 24 h, at 80°C. After that time, the solvent was removed in vacuo. The residue was re-suspended in CHCl_3 (50 mL) and was extracted 3 times with 20 mL of 10% HCl and finally thrice with brine (20 mL). The organic layer was dried over MgSO_4 and the solvent was removed in vacuo. The residue was subjected to column chromatography on silica gel ($\text{CHCl}_3/\text{EtOH}$ 19:1, $R_f = 0.7$). The product was obtained as brown oil. Yield: 1.12 g, (39%). ^1H NMR (400 MHz, CDCl_3): $\delta = 7.79\text{--}7.73$ (m, 3H); 7.47–7.43 (m, 1H); 7.37–7.33 (m, 1H); 7.22–7.16 (m, 2H); 4.28 (t, 2H); 3.95 (t, 2H); 3.79–3.61 (m, 12H), 2.60 (s, 1H). ESI-MS (m/z): calculated for $[\text{C}_{18}\text{H}_{24}\text{O}_5]$: 320.4, found: 343.2.

2-(2-(2-(naphthalen-2-yloxy)ethoxy)ethoxy)ethoxy)ethan-4-methylbenzenesulfonate (5)

To a stirred solution of **4** (980 mg, 3 mmol, 1 eq.) in 30 mL of CH_2Cl_2 was added a catalytical amount of DMAP (20 mg) and NEt_3 (0.41 g, 3.6 mmol, 1.2 eq.). After 10 minutes of stirring under ice cooling TosCl (628 mg, 3.3 mmol, 1.1 eq.) was added in portions over a period of 30 minutes. The resulting clear solution was stirred overnight followed by extraction with 10% HCl (3 x 20 mL) and brine (3 x 20 mL). The organic layer was dried over MgSO_4 and the solvent was removed in vacuo. The brown, oily residue was subjected to column chromatography on silica (hexane/ EtOAc 1:1, $R_f = 0.51$). The product was obtained as slightly yellow oil. Yield: 955 mg (69 %), ^1H NMR (400 MHz, CDCl_3): $\delta = 7.79\text{--}7.68$ (m, 5H); 7.43–7.39 (m, 1H); 7.33–7.28 (m, 3H); 7.16–7.11 (m, 2H); 4.23 (t, 2H); 4.12 (t, 2H); 3.90 (t, 2H); 3.73–3.71 (m, 2H); 3.66–3.62 (m, 4H); 3.59–3.56 (m, 4H); 2.40 (s, 3H). ESI-MS (m/z): calculated for $[\text{C}_{25}\text{H}_{30}\text{O}_7\text{S}]$: 474.6, found: 475.7.

Adamantyl-(1-yl-oxy)ethoxy)ethoxy)ethoxy)ethanol (6)

To a stirred solution of 1-adamantylbromide (2.5 g, 10 mmol), in tetra ethylene glycol (20 mL) was added dropwise NEt_3 (8 mL) and the obtained clear solution was stirred for 24 h at 150°C. After that time the resulting brown solution was diluted with CH_2Cl_2 and was extracted three times with 10% HCl (50 mL) and three times with brine (50 mL). The combined organic layers were dried over MgSO_4 . After evaporation of all solvent the desired product was obtained as light brown oil. Yield: 2.61 g, (79%) ^1H NMR (400 MHz, CDCl_3 , 298 K): $\delta = 1.70$ (t, 6H); 1.85–1.90 (t, 9H); 3.51–3.61 (m, 14H), 3.70 (t, 2H). ESI-MS (m/z): calculated for $[\text{C}_{18}\text{H}_{32}\text{O}_5]$: 328.5, found: 329.5.

Adamantyl-(1-yl-oxy)ethoxy)ethoxy)ethoxy)ethoxy)ethan-4-methylbenzenesulfonate (7)

1-Tetra ethylene glycol adamantane **6** (2.5 g, 7.5 mmol) and a catalytical amount of DMAP (50 mg) were dissolved in CH_2Cl_2 (100 mL).

At 0 °C, NEt_3 (1.65 mL, 12 mmol) and then TosCl (1.58 g, 8.4 mmol) was added dropwise. The solution was stirred overnight. Afterwards it was washed two times with water (50 mL), then one time with 10% HCl (50 mL) and dried over MgSO_4 . The solvents were evaporated to yield a yellow-brown oil. Yield: 3.55 g, (79%). $^1\text{H NMR}$ (400 MHz, CDCl_3): δ = 7.76 (d, 2H); 7.30 (d, 2H); 4.11 (t, 2H); 3.76 (t, 2H); 3.60-3.50 (m, 12H); 2.41 (s, 3H); 2.09 (bs, 3H); 1.71-1.68 (m, 6H); 1.65-1.50 (m, 6H). ESI-MS (m/z): calculated for $[\text{C}_{25}\text{H}_{38}\text{O}_7\text{S}]$: 482.6, found: 505.5.

3,6,9,12-tetraoxapentadec-14-yn-1-ol (8)

Tetra ethylene glycol (10 g, 74 mmol, 3.5 eq.) was dissolved in 150 mL of THF followed by

the addition of KOtBu (2.3 g, 19.5 mmol, 1 eq.). The solution was stirred for 10 minutes under ice cooling followed by the dropwise addition of propargylbromide (2.12 mL, 19.5 mmol, 1 eq.) followed by stirring for 24 h. After that time, the solvent was evaporated to dryness and the residue was redissolved in 50 mL of CH_2Cl_2 and extracted three times with 10 % HCl and brine (50 mL). The organic layer was dried over MgSO_4 and the solvent was removed in vacuo to yield the desired product as colorless oil. Yield: 2.35 g, (52 %). $^1\text{H NMR}$ (400 MHz, CDCl_3): δ = 4.17 (d, 2H); 3.70- 3.62 (m, 14H); 3.57 (t, 2H); 2.91 (s, 1H), 2.40 (t, 1H). ESI-MS (m/z): calculated for $[\text{C}_{11}\text{H}_{20}\text{O}_5]$: 232.1, found: 233.1.

3,6,9,12-tetraoxapentadec-14-yn-1-yl 4-methylbenzenesulfonate (9)

To a stirred solution of **8** (2 g, 8.6 mmol, 1 eq.) in 20 mL of CH_2Cl_2 was added NEt_3 (1.04 g, 10.3 mmol, 1.2 eq.) and a catalytical

amount of DMAP (20 mg). The reaction was cooled down to 0°C and TosCl (1.97 g, 10.3 mmol, 1.2 eq.) was added in portions over a period of 30 minutes. The mixture was continued stirring for 18 h followed by extraction with 10 % HCl (3 x 20 mL) and brine (3 x 20 mL). The organic layer was dried over MgSO_4 and the solvent was removed in vacuo. The residue was subjected to column chromatography on silica gel (CHCl_3 , R_f = 0.43). Yield: 2.33 g (70%). $^1\text{H NMR}$ (400 MHz, CDCl_3): δ = 7.77 (d, 2H), 7.32 (d, 2H); 4.17 (d, 2H), 4.13 (t, 2H), 3.67-3.56 (m, 14H); 2.42 (s, 3H); 2.40 (t, 1H). ESI-MS (m/z): calculated for $[\text{C}_{18}\text{H}_{25}\text{O}_7\text{S}]$: 386.1, found: 387.1.

Ferrocenylmethylazide (10)

To a stirred solution of ferrocenemethanol (200 mg, 0.92 mmol, 1 eq.) was added sodiumazide (400 mg, 6.01 mmol, 6 eq.) and acetic acid (10 mL). The mixture was heated to 50°C for 3 h followed by dilution with CH_2Cl_2 (30 mL). The mixture was washed three times with saturated NaHCO_3 solution and the organic layer was dried over MgSO_4 . After evaporation of the solvent, the crude product was further purified by silica gel column chromatography (EtOAc /hexane 1:2) yielding an orange oil. Yield: 180 mg (81 %). ^1H NMR (400 MHz, CDCl_3): δ = 4.22 (t, 2H); 4.18 (t, 2H); 4.15 (bs, 5H); 4.10 (bs, 2H). ^{13}C NMR (100 MHz, CDCl_3): δ = 82.54, 69.18, 69.04, 51.13. ESI-MS (m/z): calculated for $[\text{C}_{11}\text{H}_{11}\text{FeN}_3]$: 241.0, found: 242.1

3,6,9,12-tetraoxapentadec-14-triazoylferrocenyl-1-yl4-methylbenzenesulfonate (11)

Ferrocenylmethylazide **10** (170 mg, 0.92 mmol, 1 eq.) was dissolved in a mixture of DMF/MeOH/ H_2O (5 mL) followed by the addition of **9** (400 mg, 1.47 mmol, 1.5 eq.).

The resulting solution was degassed by bubbling argon through the yellow solution for 10 minutes. After that time, a mixture of CuSO_4 (10 mg) and sodium ascorbate (40 mg) in degassed water was added and the solution was stirred under an atmosphere of argon for 18h. The resulting solution was evaporated to dryness and the residue was subjected to column chromatography on silica gel (EtOAc , R_f = 0.71). The product was obtained as red-brown oil. Yield: 220 mg (41%). ^1H NMR (400 MHz, CDCl_3): δ = 7.75 (d, 2H); 7.46 (s, 1H); 7.30 (d, 2H); 5.23 (s, 2H); 4.58 (s, 2H); 4.42 (t, 2H); 4.17 (t, 2H); 4.14 (bs, 5H, 3CH); 4.09 (bs, 2H); 3.62-3.52 (m, 16H); 2.39 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3): δ = 171.17, 144.93, 144.87, 132.95, 129.89, 127.99, 122.11, 80.88, 70.58, 70.49, 69.35, 69.08, 69.02, 68.94, 68.66, 64.55, 60.41, 50.05, 21.69. ESI-MS (m/z): calculated for $[\text{C}_{29}\text{H}_{37}\text{FeN}_3\text{O}_7\text{S}]$: 627.5, found: 650.8.

Figure 6.15 | Synthesis route to the azide modified heterovalent azobenzene derivatives. i) MeCN, K_2CO_3 , LiBr, 48h, $85^\circ C$, ii) THF/MeOH/ H_2O (1:2:1), LiOH, 3h, $40^\circ C$, iii) DMF, HOBT, EDC, NEt_3 , 24h, rt.

Azobenzene-carboxylic methylester (12)

To a stirred solution of **3** (1.2 g, 2.3 mmol, 1 eq.) in MeCN (30 mL) was added 4-methyldihydroxybenzoate (954 mg, 5.7 mmol, 2.5 eq.) followed by the addition of dry K_2CO_3 (1.9 g, 13.6 mmol, 6 eq.) and a catalytical amount of LiBr (20 mg). The reaction was refluxed under an atmosphere of argon for 36h. After that time, the solvent was removed under reduced pressure. The residue was redissolved in a mixture of CH_2Cl_2 (40 mL) and water (40 mL).

The organic layer was extracted three times with brine (3 x 30 mL) and the organic layer was dried over $MgSO_4$ and the solvent was removed in vacuo. The residue was subjected to column chromatography on silica gel (EtOAc / hexane 1:2, R_f = 0.3). The product was obtained as orange waxy residue. Yield: 525 mg (43 %). 1H NMR (400 MHz, $CDCl_3$): δ = 7.89-7.85 (m, 4H); 7.50-7.46 (m, 2H); 7.44-7.42 (m, 1H); 7.08-7.06 (m, 2H); 6.99- 6.96 (m, 2H); 6.64 (t, 1H); 4.18-4.16 (m, 2H); 4.09-4.06 (m, 2H); 3.88-3.85 (m, 2H); 3.84 (s, 3H); 3.80-3.78 (m, 2H); 3.74-3.72 (m, 2H); 3.70-3.65 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.02, 161.58, 160.02, 157.30, 152.68, 147.11, 132.17, 130.69, 129.28, 125.10, 122.77, 115.08, 109.88, 107.86, 107.79, 77.35, 71.07, 70.95, 70.84, 70.80, 69.86, 69.79, 68.06, 67.90, 60.65, 52.42. ESI-MS: calculated for $[C_{28}H_{32}N_2O_8]$: 524.2, found: 547.2.

Bis-azobenzene-carboxylic methylester (13)

4-Methyldihydroxybenzoate (217 mg, 1.28 mmol) was dissolved in 50 mL of MeCN followed by dried K_2CO_3 (1.80 g, 75 mmol) and a catalytic amount of LiBr (20 mg). The suspension was refluxed for 48h under an atmosphere of argon. After that time, the solvent was removed in vacuo and the residue was redissolved in 50 mL of CH_2Cl_2 . The crude mixture was extracted twice with brine (50 mL) and twice with water (50 mL). The combined organic layers were dried over

$MgSO_4$ and the solvent was removed in vacuo. The crude product was purified by silica gel column chromatography (CH_2Cl_2 /EtOH – 9:1, R_f = 0.51). After removal of the solvent, the product was obtained as orange viscous oil. Yield: 1,18 g, (98 %). 1H NMR (400 MHz, $CDCl_3$): δ = 7.89-7.84 (m, 8H); 7.49-7.45 (m, 4H); 7.43-7.41 (m, 2H); 7.16 (d, 2H); 6.99 (d, 4H); 6.66 (t, 1H); 4.17 (t, 4H); 4.10 (t, 4H); 3.86 (t, 4H); 3.85 (s, 3H); 3.82 (t, 4H); 3.72-3.66 (m, 16H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.95, 161.51, 159.93, 152.85, 147.19, 132.07, 130.59, 129.23,

124.95, 122.75, 115.03, 108.18, 107.07, 77.43, 71.06, 71.03, 70.88, 70.86, 69.80, 69.77, 67.92, 52.44. ESI-MS: calculated for $[C_{48}H_{56}N_4O_{12}]$: 881.0, found: 882.0.

Bis-azobenzene-carboxylic acid (14)

Lithium hydroxide (LiOH) (100 mg, 4.1 mmol) was dissolved in a mixture of MeOH/H₂O (2:1, 30 mL). **4** (500 mg, 0.56 mmol) was added and the mixture was stirred for 24h at room temperature. After that time all solvents were evaporated and the residue was redissolved in CH₂Cl₂. The solution was extracted three times with 20 mL of water and the organic layer was dried over MgSO₄. After evaporation, the crude product was obtained as orange oil and was further purified by silica gel column chromatography (CH₂Cl₂/EtOH - 9:1, R_f = 0,15). Yield: 422 mg, (88 %). ¹H NMR (400 MHz, CDCl₃): δ = 7.89-7.83 (m, 8H); 7.49- 7.45 (m, 4H); 7.42-7.40 (m, 2H); 7.20 (d, 2H); 6.99 (d, 4H); 6.68 (t, 1H), 4.17 (t, 4H); 4.09 (t, 4H); 3.86 (t, 4H); 3.81 (t, 4H); 3.73-3.67 (m, 16H). ¹³C NMR (100 MHz, CDCl₃): δ = 170.20, 161.49, 159.96, 152.89, 147.22, 131.35, 130.58, 129.23, 124.93, 122.76, 115.03, 108.67, 107.69, 77.43, 71.06, 71.01, 70.87, 70.86, 69.81, 69.79, 67.91. ESI-MS: calculated for $[C_{47}H_{54}N_4O_{12}]$: 867.0, found: 889.8.

2-Azido-ethylamine (15)

To a solution of 1-bromoethylaminehydrobromide (4.00 g, 19.52 mmol, 1 eq.) in 20 mL of distilled water was added NaN₃ (3.80 g, 59 mmol, 3 eq.). The solution was heated to 75°C for 48h. After that time, the mixture was cooled to 0°C followed by the careful addition of KOH (6.00 g). The aqueous solution was extracted thrice with 20 mL of Et₂O. The organic layer was dried over MgSO₄ and the solvent was carefully removed in vacuo. The title compound was obtained as colorless liquid. Yield: 1.13 g (67 %). ¹H NMR (400 MHz, CDCl₃, 298 K): δ = 3.31 (t, 2H, CH₂); 2.82 (t, 2H, CH₂), 1.35 (s, 2H, NH₂). ¹³C NMR (100 MHz, CDCl₃, 298 K): δ = 53.66, 40.38.

Azobenzene-naphthyl carboxylic methylester (16)

To a stirred solution of **12** (150 mg) in 20 mL of dry MeCN was added dried K_2CO_3 (1.80 g, 75 mmol) and **5** as well as a catalytical amount of LiBr (20 mg) the obtained suspension was stirred for 48h at 85 °C. After that time, the suspension was filtered and the solvent was evaporated yielding a crude orange oil which was further purified by silica gel chromatography (EtOAc/hexane 1:1, R_f = 0.31). The title compound was obtained as bright orange oil. Yield: 174 mg (72.5 %). ^1H NMR (400 MHz, CDCl_3): δ = 7.89-7.84 (m, 4H); 7.74-7.67 (m, 3H); 7.49-7.45 (t, 2H); 7.41-4.37 (m, 2H); 7.32-7.28 (m, 1H); 7.15 (d, 2H); 7.14-7.10 (m, 2H); 6.99 (d, 2H); 6.66 (t, 1H); 4.21 (t, 2H); 4.17 (t, 2H); 4.11 (m, 4H); 3.91-3.86 (m, 4H); 3.85 (s, 3H); 3.82-3.79 (m, 4H); 3.71-3.64 (m, 16H). ^{13}C NMR (100 MHz, CDCl_3): δ = 166.62, 161.15, 159.61, 156.60, 152.59, 146.91, 134.34, 131.75, 129.22, 128.87, 126.62, 126.19, 124.58, 123.50, 122.42, 118.88, 114.70, 107.88, 107.86, 106.75, 106.58, 77.12, 70.75, 70.72, 70.71, 70.56, 70.54, 69.60, 69.49, 69.44, 67.59, 67.27, 52.06. ESI-MS: calculated for $[\text{C}_{46}\text{H}_{54}\text{N}_2\text{O}_{12}]$: 829.9, found: 832.1.

Azobenzene-adamantyl carboxylic methylester (17)

To a stirred solution of **12** (100 mg) in 20 mL of dry MeCN was added dried K_2CO_3 (1.80 g, 75 mmol) and **7** as well as an catalytical amount of LiBr (20 mg) the obtained suspension was stirred for 48h at 85 °C. After that time, the suspension was filtered and the solvent was evaporated yielding a crude orange oil that was further purified by silica gel chromatography (EtOAc/hexane 1:1, R_f = 0.29). The title compound was obtained as bright orange oil. Yield: 134 mg (84 %). ^1H NMR (400 MHz, CDCl_3): δ = 7.88-7.83 (m, 4H); 7.49-7.40 (m, 3H); 7.16 (d, 2H); 6.98 (d, 2H); 6.66 (t, 1H); 4.17 (t, 2H); 4.12-4.08 (m, 4H); 3.89-3.86 (m, 2H); 3.85 (s, 3H); 3.83-3.80 (m, 4H); 3.71-3.62 (m, 16H); 3.57-3.52 (m, 4H); 2.10 (bs, 3H); 1.71 (s, 6H); 1.61-1.52 (m, 6H). ^{13}C NMR (100 MHz, CDCl_3): δ = 166.71, 161.25, 159.73, 159.71, 152.69, 147.02, 131.84, 130.33, 128.99, 124.67, 122.52, 114.79, 107.99, 107.95, 106.85, 77.23, 72.22, 71.23, 70.67, 70.55, 69.59, 69.54, 67.69, 59.21, 52.19, 41.42, 36.41, 30.45. ESI-MS: calculated for $[\text{C}_{46}\text{H}_{62}\text{N}_2\text{O}_{12}]$: 835.0, found: 836.1.

Azobenzene-ferrocenyl carboxylic methylester (18)

To a stirred solution of **12** (100 mg) in 20 mL of dry MeCN was added dried K_2CO_3 (1.80 g, 75 mmol) and **11** as well as a catalytical amount of LiBr (20 mg) the obtained suspension was stirred for 48h at 85 °C. After that time, the suspension was filtered and the solvent was evaporated yielding a crude orange oil that was further purified by silica gel chromatography (EtOAc/hexane 1:1, R_f = 0.11). The title compound was obtained as brown-orange oil. Yield: 153 mg (82 %), 1H NMR (400

MHz, $CDCl_3$): δ = 7.86-7.81 (m, 4H); 7.45-7.37 (m, 4H); 7.13 (bs, 2H); 6.96 (d, 2H); 6.63 (s, 1H); 5.18 (s, 2H); 4.57 (s, 2H); 4.23-4.03 (m, 11H); 3.85 (s, 3H); 3.81-3.77 (m, 8H); 3.67-3.57 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.84, 166.43, 161.01, 159.48, 152.43, 146.74, 144.68, 131.60, 130.11, 128.75, 128.67, 124.42, 123.32, 122.27, 121.73, 119.52, 114.56, 114.01, 107.73, 107.72, 106.58, 80.55, 77.11, 70.51, 70.40, 70.39, 70.30, 70.25, 70.19, 69.29, 68.79, 68.64, 67.46, 64.38, 60.09, 51.96, 49.73. ESI-MS: calculated for $[C_{50}H_{61}FeN_5O_{12}]$: 980.0, found: 981.0.

Azobenzene-naphthyl carboxylic acid (19)

Compound **16** (174 mg, 0.21 mmol, 1 eq.) was dissolved in a mixture of THF/MeOH/ H_2O (1:2:1, 10 mL) followed by the addition of LiOH (27 mg, 1.05 mmol, 5eq.). The mixture was stirred for 3h at 40°C. After that time, the solution was diluted with 30 mL of 10% HCl followed by the extraction with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried over $MgSO_4$ and all solvents were removed in vacuo. The crude residue was subjected to silica column chromatography (EtOAc,

R_f = 0.45). The desired compound was obtained as orange oil. Yield: 158 mg (91 %) 1H NMR (400 MHz, $CDCl_3$): δ = 7.84-7.82 (m, 4H); 7.69-7.63 (m, 3H); 7.45 (t, 2H); 7.39-4.34 (m, 2H); 7.28-7.24 (m, 1H); 7.15-7.03 (m, 4H); 6.92 (bs, 2H); 6.81-6.79 (m, 1H); 4.21-3.50 (m, 32H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.15, 156.58, 153.84, 152.64, 146.88, 134.40, 130.30, 129.24, 128.97, 128.89, 127.51, 126.90, 126.72, 126.23, 124.61, 123.54, 122.49, 119.74, 118.87, 114.70, 114.17, 106.62, 77.23, 70.57, 70.41, 69.41, 67.63, 67.31, 67.20, ESI-MS: calculated for $[C_{45}H_{52}N_2O_{12}]$: 812.9, found: 835.9.

Azobenzene-adamantyl carboxylic acid (20)

Compound **17** (134 mg, 0.16 mmol, 1 eq.) was dissolved in a mixture of THF/MeOH/H₂O (1:2:1, 10 mL) followed by the addition of LiOH (20 mg, 0.80 mmol, 5 eq.). The mixture was stirred for 3h at 40°C. After that time, the solution was diluted with 30 mL of 10% HCl followed by the extraction with CH₂Cl₂ (3 x 20 mL). The combined organic layers were dried over MgSO₄ and all solvents were removed in vacuo. The crude residue was subjected to silica column chromatography (EtOAc, R_f = 0.40). The desired compound was obtained as orange oil. Yield:

108 mg (81 %), ¹H NMR (400 MHz, CDCl₃): δ = 7.84-7.81 (m, 4H); 7.45 (t, 2H); 7.40-7.37 (m, 1H), 7.13-7.09 (m, 2H); 6.99-6.92 (m, 2H); 6.83-6.76 (m, 1H), 4.18-3.41 (m, 32H); 2.06 (bs, 3H); 1.67 (s, 6H); 1.58-1.49 (m, 6H). ¹³C NMR (100 MHz, CDCl₃): δ = 171.20, 161.24, 159.30, 158.28, 152.71, 146.97, 130.34, 129.02, 128.96, 126.95, 124.68, 124.68, 123.62, 122.56, 119.81, 114.79, 114.26, 10.10, 105.62, 77.28, 72.36, 71.19, 70.58, 70.25, 69.70, 69.59, 69.52, 67.72, 67.55, 67.37, 60.42, 59.20, 41.38, 36.40, 30.46. ESI-MS: calculated for [C₄₅H₆₀N₂O₁₂]: 821.0, found: 822.0.

Azobenzene-ferrocenyl carboxylic acid (21)

Compound **17** (154 mg, 0.16 mmol, 1eq.) was dissolved in a mixture of THF/MeOH/H₂O (1:2:1, 10 mL) followed by the addition of LiOH (20 mg, 0.80 mmol, 5eq.). The mixture was stirred for 3h at 40°C. After that time, the solution was diluted with 30 mL of 10% HCl followed by the extraction with CH₂Cl₂ (3 x 20 mL). The combined organic layers were dried over MgSO₄ and all solvents were removed in vacuo. The crude residue was subjected to silica column chromatography (EtOAc, R_f = 0.15). The desired compound was obtained as brown oil. Yield:

101 mg (66 %), ¹H NMR (400 MHz, CDCl₃): δ = 7.87-7.82 (m, 4H); 7.47-7.44 (m, 3H); 7.41-7.39 (m, 1H); 7.20 (bs, 2H); 6.98 (d, J = 9.0 Hz, 2H); 6.65 (s, 1H); 5.21 (s, 2H); 4.61 (s, 2H); 4.22 (t, 2H); 4.17-4.08 (m, 13H); 3.86 (t, 2H); 3.81-3.78 (m, 4H); 3.71-3.59 (m, 20H). ¹³C NMR (100 MHz, CDCl₃): δ = 161.74, 160.17, 153.17, 147.48, 145.31, 130.84, 129.49, 129.43, 125.17, 124.12, 123.01, 122.59, 120.29, 115.30, 114.76, 108.93, 108.84, 107.67, 81.13, 77.75, 71.24, 71.09, 71.01, 70.94, 70.86, 69.54, 69.35, 68.17, 64.92, 50.53. ESI-MS: calculated for [C₄₉H₅₉FeN₅O₁₂]: 965.8, found: 966.8.

Azobenzene-naphthyl-azide (22)

19 (150 mg, 0.18 mmol, 1 eq.) was dissolved in 10 mL of DMF followed by the addition of 1-Hydroxybenzotriazole (HOBt) (30 mg, 0.22 mmol, 1.2 eq.) and N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride EDC (45 mg, 0.22 mmol). The reaction mixture was stirred for 30 minutes and **(25)** (100 mg, 1.20 mmol) and NEt₃ (50 mg, 0.14 mmol) was added followed by stirring for 24 h. After that time the solvent was removed in vacuo and the

residue was redissolved in CH₂Cl₂. The mixture was extracted three times with water (50 mL). The organic layer was dried over MgSO₄ and the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography (EtOAc, R_f = 0.56). Yield: 40 mg (27 %), ¹H NMR (400 MHz, CDCl₃): δ = 7.91-7.85 (m, 4H); 7.73-7.67 (m, 3H); 7.51-7.47 (m, 2H); 7.42-4.38 (m, 2H); 7.33-7.28 (m, 1H); 7.15 – 7.09 (m, 2H); 6.98 (d, 2H); 6.90 (d, 2H) 6.62 (t, 1H); 6.58 (t, 1H); 4.22 (t, 2H); 4.17 (t, 2H); 4.11-4.07 (m, 4H); 3.90-3.85 (m, 4H); 3.82-3.79 (m, 4H); 3.72-3.63 (m, 16H); 3.53 (t, 2H); 3.50-3.46 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ = 170.77, 167.68, 160.18, 156.89, 152.72, 147.12, 136.37, 134.66, 130.65, 129.43, 129.20, 127.82, 126.95, 126.54, 125.06, 123.85, 122.75, 119.17, 115.07, 106.92, 106.07, 105.23, 77.41, 71.06, 71.03, 70.96, 70.85, 69.93, 69.81, 67.93, 67.60, 51.04, 50.98, 39.64, 39.21. ESI-MS: calculated for [C₄₇H₅₆N₆O₁₁]: 881.0, found: 903.4.

Azobenzene-adamantyl-azide (23)

20 (100 mg, 0.14 mmol, 1 eq.) was dissolved in 10 mL of DMF followed by the addition of HOBt (20 mg, 0.18 mmol, 1.2 eq.) and EDC (45 mg, 0.22 mmol). The reaction mixture was stirred for 30 minutes and **(25)** (100 mg, 1.20 mmol) and NEt₃ (50 mg, 0.14 mmol, 1.2 eq.) was added followed by stirring for 24 h. After that time the solvent was removed in vacuo and the residue was redissolved in CH₂Cl₂. The mixture was extracted three times with water (50 mL). The organic layer was dried over MgSO₄ and the solvent was removed in vacuo. The residue was

subjected to silica gel column chromatography (EtOAc, R_f = 0.53). Yield: 30 mg (30 %), ¹H NMR (400 MHz, CDCl₃): δ = 7.92-7.86 (m, 4H); 7.50-7.46 (m, 3H); 6.99 (d, 2H); 6.92 (t, 2H); 6.59 (t, 1H); 6.66 (t, 1H); 4.18 (t, 2H); 4.12-4.10 (m, 4H); 3.87 (t, 2H); 3.83-3.83-3.80 (m, 4H); 3.71-3.62 (m, 16H); 3.57-3.50 (m, 8H); 2.10 (bs, 3H); 1.71 (s, 6H); 1.62-1.53 (m, 6H). ¹³C NMR

(100 MHz, CDCl_3): δ = 167.66, 160.20, 136.35, 129.28, 125.29, 125.29, 122.77, 115.13, 106.11, 105.30, 77.43, 72.58, 70.98, 70.75, 69.82, 68.00, 67.96, 59.45, 51.02, 41.64, 39.65, 36.63, 30.69. ESI-MS: calculated for $[\text{C}_{47}\text{H}_{64}\text{N}_6\text{O}_{11}]$: 889.1, found: 911.4.

Azobenzene-ferrocenyl-azide (**24**)

21 (80 mg, 0.08 mmol, 1 eq.) was dissolved in 10 mL of DMF followed by the addition of HOBT (20 mg, 0.10 mmol, 1.2 eq.) and EDC (30 mg, 0.10 mmol). The reaction mixture was stirred for 30 minutes and **(25)** (100 mg, 1.20 mmol) and NEt_3 (50 mg, 0.14 mmol, 1.2 eq.) was added followed by stirring for 24 h. After that time the solvent was removed in vacuo and the residue was redissolved in CH_2Cl_2 . The mixture was extracted three times with water (50 mL). The organic layer was dried over MgSO_4 and the solvent was removed in vacuo. The residue was

subjected to silica gel column chromatography (EtOAc, R_f = 0.33). Yield: 45 mg (56 %), ^1H NMR (400 MHz, CDCl_3): δ = 7.88-7.84 (m, 4H); 7.49-7.46 (m, 3H); 7.43-7.39 (m, 1H); 6.99 (d, J = 7.5 Hz, 2H); 6.89 (bs, 2H); 6.76 (bs, 1H); 6.59 (bs, 1H); 5.06 (s, 2H); 4.71-3.95 (m, 15H); 3.91-3.38 (m, 30H). ^{13}C NMR (100 MHz, CDCl_3): δ = 167.58, 161.36, 160.09, 152.82, 147.15, 136.36, 130.50, 129.15, 124.82, 122.66, 114.98, 106.33, 106.17, 105.29, 71.05, 70.99, 70.85, 70.78, 70.72, 69.99, 69.88, 69.80, 68.14, 68.05, 67.93, 65.19, 51.08, 39.75. ESI-MS: calculated for $[\text{C}_{51}\text{H}_{63}\text{FeN}_9\text{O}_{11}]$: 1033.9, found: 1056.4.

6.5.2 Self Assembly on Surfaces

Monolayer **gold substrates** (40-50 nm) were cleaned and activated with piranha for 60 sec followed by thorough rinsing with water and drying with N_2 .

- Substrates were incubated overnight in a mixed 1 mM ethanolic solution of disulfide- $(\text{C}_{11}\text{-EG}_4\text{-OH})_2$ and disulfide- $(\text{C}_{11}\text{-EG}_4\text{-OH})\text{-(C}_{11}\text{-EG}_6\text{-CONH-Mal)}$ in a 99:1 (v/v) ratio under protection of air. A freshly prepared 1 mM solution βCDSH was then reacted with the maleimide-function for 1h.
- Substrates were immersed in a 1 mM solution of CB[7] for 1h to yield a regular monolayer and avoid multilayer formation.

Figure 6.16 | QCM-D of a) (■) β CDSH attachment on a 1% maleimide functionalized SAM and (●) subsequent rinsing with water and b) (■) CB[7] adsorption on a gold sensor, (●) alternating rinsing steps with water. (line) frequency change, (dot) dissipation change.

UV/vis measurements were carried out on a Perkin Elmer UV/Vis spectrometer Lambda 850. The photoisomerization behavior of AZO was characterized by UV/Vis spectroscopy at room temperature. The sample was dissolved in water and measured against the same water sample. UV and visible light irradiation experiments were done with a Hönle bluepoint 2 easycure using a shortpass filter $\lambda \leq 400$ nm and a LSC-G HighPower-LED emitting at 520 nm.

SPR measurements were performed on a Resonant Probes GmbH instrument. The instrument is in a two-channel vibrating mirror angle scan setup based on the Kretschmann configuration. Light from a 2 mW HeNe laser (JDS Uniphase, $\lambda = 632.8$ nm) is directed onto a prism surface by means of a vibrating mirror. The intensity of the light is measured by means of a large-area photodiode. This setup allows the determination of changes in plasmon angle with an accuracy of 0.0028. The gold substrate with the monolayer mounted on a Teflon cell with a volume of 39 μ L, was optically matched to the prism using an index matching oil (Cargille, series B; $n_D^{25^\circ\text{C}} = 1.7000 \pm 0.0002$). After stabilization of the SPR signal, titrations were performed by adding at least five different concentrations of the responding guests and subsequent rinsing with buffer medium.

QCM-D measurements were conducted using the Q-sense E4 instrument with a peristaltic pump. Quartz and gold coated QCM-D resonator (QSX 301) were used for all experiments. All binding studies were conducted with a flow rate of 100 μ L/min at room temperature. Resonance frequency and dissipation values were determined automatically by the instrument. Resonators were functionalized as described above. Binding experiments were performed in water at five

different concentrations. Prior the binding, surfaces were equilibrated by flowing over water until a stable baseline was obtained.

6.5.3 Monovalent Interactions on CB[7]

Figure 6.17 | Monovalent interactions on CB[7] SAMs of a) azobenzene 10-500 μ M, b) Langmuir binding model $K_d = 97 \mu$ M, c) adamantane 5-100 μ M, d) Langmuir binding model $K_d = 13.1 \mu$ M, e) ferrocene 5-100 μ M and f) Langmuir binding model $K_d = 26 \mu$ M. (line) frequency change, (dot) dissipation change.

6.6 References

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Summary

The research described in this thesis is focused on stimuli-responsive macrocyclic supramolecular surfaces. Several macrocyclic host systems such as cucurbit[n]uril and cyclodextrin systems have been employed to host mono-, bi- and trivalent guests that are sensitive to stimuli such as light and electrochemistry. Such supramolecular stimuli-responsive surfaces are of fundamental interest and have potential for application in cell-instructive interfaces.

Cucurbit[n]urils are in the focus of interest since two decades and enjoy increasing enthusiasm due to their potential application in fabricating biological surfaces, which was reviewed in chapter 1. In chapter 2, a new heteroternary complex was described using arylazopyrazole based light switches as guests for CB[8]. The potential for biological application of these complexes was shown with cellular immobilization and release experiments. Multivalent azobenzene derivatives were synthesized and their binding to CB[8] based surfaces was characterized in chapter 3. Whereas bivalent binding was evident, trivalent binding was hampered by the molecular design. Chapter 4 describes the efforts to achieve patterning of multivalent ligands on antifouling surfaces. To this end, CB[8]-mediated assembly and patterning of guest molecules was achieved on optically transparent cyclic olefin polymer platforms using oligo ethylene glycol and maleimide grafted poly-L-lysine as the starting surface. In chapter 5, patterns of CB[8]-mediated heteroternary complexes were made and used to investigate their dynamics on surfaces. Although more experiments are needed for a complete picture of the dynamics on CB[8] surfaces, azopyridine guests were moving slowly along the surface, resulting in maximum spreading rates that were two times slower when compared to analogous dynamics studies that were done in the past for adamantane guests diffusing on β -cyclodextrin SAMs. Strategies to selectively tune surface properties using stimuli-responsiveness were investigated in chapter 6. Application of different stimuli to surface-bound heterodivalent molecules have influence on their binding strength, orientation and thus as well on the surface properties.

Overall, this thesis presents a perspective on several dynamic macrocyclic supramolecular systems. The work on the stimuli-responsive and multivalent guests demonstrates the potential to tune the interaction strength of supramolecular assemblies on surfaces and enables

controlled association and dissociation. Combined with lithographic techniques, such as microcontact printing and micromolding in capillaries, new approaches for supramolecular architectures on interfaces involving CB[8] are described and can be integrated in the future design of molecular devices and dynamic materials.

Samenvatting

Het onderzoek dat is beschreven in dit proefschrift is gebaseerd op stimuli-responsieve macrocyclische supramoleculaire oppervlakken. Oppervlakken kunnen bedekt worden met macrocyclische moleculen zoals cucurbit[n]uril en cyclodextrine, die bekend zijn om hun capaciteit om andere moleculen als gast in te sluiten. Deze complexen kunnen gevoelig zijn voor bepaalde stimuli zoals licht of electriciteit. Zulke supramoleculaire oppervlakken zijn van groot belang in de wetenschap omdat ze gebruikt kunnen worden voor biologisch actieve of technologische materialen.

Cucurbit[n]urils (CB[n]) zijn al twintig jaar belangrijk in de wetenschap, maar de interesse in deze macrocyclische verbinding groeit nog steeds. Dit komt omdat ze veel potentie hebben voor de ontwikkeling van biologische oppervlakken zoals is weergegeven in het literatuuroverzicht in hoofdstuk 1. In hoofdstuk 2 wordt een licht-gevoelige arylazopyrazool derivaat gepresenteerd als mogelijk nieuw, tweede elektronen-rijk gast molecuul de voor CB[8] macrocyclische verbinding in de aanwezigheid van een bekend, eerste elektronen-arm gast molecuul. Deze nieuwe ternaire complexen werden gekarakteriseerd zowel in oplossing als op oppervlakken. In dynamische cel experimenten werd het licht-gevoelige gedeelte van het arylazopyrazool molecuul gebruikt om cellen, gebonden aan het oppervlak, te verwijderen.

In hoofdstuk 3 worden moleculen beschreven waaraan een verschillend aantal gast moleculen is verbonden met korte of lange linkers. Zulke multi-valente moleculen zijn nuttig om multi-valente zelf-assemblage op de CB[8]-gebaseerde oppervlakken te kunnen onderzoeken. Het is gebleken dat de valentie van het gast molecuul de sterkte van de bindingsaffiniteit met het CB[8] sterk beïnvloed, echter als de valentie te hoog werd, beperkte dat de bewegingsvrijheid van het gast molecuul en werd de optimale interactie met het oppervlak niet bereikt en de bindingsaffiniteit steeg daardoor niet verder. Ook in hoofdstuk 4 is multi-valentie op CB[8] oppervlakken onderzocht, maar met behulp van een ander ontwerp van de gast moleculen en ook op gedeeltelijk bedekte oppervlakken. In dit hoofdstuk zijn nieuwe, licht-gevoelige gast moleculen met een azopyridine groep gebruikt. In dit hoofdstuk is ook aandacht besteed aan het ontwikkelen van strategieën om macrocyclische moleculen te kunnen aanhechten aan optisch transparante oppervlakken om multi-valente zelf-assemblage te kunnen bestuderen met fluorescentie spectroscopie. Met behulp van microcontact stempelen zijn de gast moleculen in

contact gebracht met de CB[8] bedekte oppervlakken. Speciale aandacht in hoofdstuk 5 heeft ook de dynamische karakteristieken van supramoleculaire macrocyclische systemen gekregen. De bindingen in zo'n system zijn niet covalent, maar onderhouden een evenwicht. Onder bepaalde condities kunnen supramoleculair gebonden moleculen meerdere nanometers per seconde verplaatsen.

In hoofdstuk 6 werd er gebruik gemaakt van bivalente moleculen die echter nu verschillende stimuli-responsiviteit bezitten, bijvoorbeeld de ene gast-groep kan licht-gevoelig zijn terwijl de andere gast-groep redox-gevoelig is. Hiermee kunnen tweevoudig schakelbare gedeelten in de supramoleculaire complexen op oppervlakken worden geïntroduceerd.

Dit werk maakt deel uit van een ontwikkeling die op dit moment plaats vindt in de supramoleculaire chemie, dat er naar streeft systemen nog beter te controleren. Het onderzoek naar de bindingsaffiniteiten en de mogelijkheid om deze te veranderen, demonstrenen de moleculaire toepassingen van de systemen. Toekomstig werk zal erop gericht zijn deze systemen geschikt te maken voor technologische en fysiologische toepassingen.

Zusammenfassung

Diese wissenschaftliche Arbeit über multifunktionale supramolekulare Oberflächen thematisiert schaltbare Komponenten, die mittels Stimulus die Oberflächeneigenschaften verändern können. Oberflächenbasierende Wirtssysteme, bestehend aus Cucurbit[n]urilen (CB[n]) oder Cyclodextrinen, sind in der Lage multivalente Gastmoleküle zu binden. Die Reaktivität des Gastmoleküls gegenüber Licht, elektrischer Spannung oder anderer äußerlicher Stimuli ermöglicht diesem reversibel zu binden, sodass ein entsprechender Reiz die Freisetzung des Gastmoleküls hervorruft. Diese Eigenschaft eröffnet eine Vielzahl an Möglichkeiten für die Entwicklung von Sensoroberflächen oder neuen technologisch wertvollen Materialien.

Im ersten Kapitel wird der heutige Stand der schaltbaren CB[n] Oberflächensysteme diskutiert. Aufgrund ihrer geringen Toxizität und sehr guten Komplexierungseigenschaften eignen sich CB[n] Oberflächen sowohl für medizinische als auch technologische Zwecke und stehen seit Jahren im Fokus der Forschung. Darauf basierend, wird in Kapitel 2 ein photo-reaktives Gastmolekül untersucht, welches sowohl in Lösung, als auch auf Oberflächen eine starke (Bindungs-)Affinität zu Cucurbit[8]uril (CB[8]) aufweist. Nach Funktionalisierung mit einem bioaktiven Peptid, das an Zellmembranen bindet, konnten die Zellbindungseigenschaften der funktionalisierten Oberflächen evaluiert werden. Nach Bestrahlung der photoaktiven Gastmoleküle mit UV-Licht konnten die gebundenen Zellen wieder von der Oberfläche abgelöst werden.

Der große Vorteil der supramolekularen Chemie ist der hohe Grad an Kontrolle den man über die Bindungseigenschaften vieler Systeme ausüben kann. Bindungsstärken können durch das Verändern der Valenz der Gastmoleküle beeinflusst werden, solange man exzessive sterische Hinderung vermeidet. Zu diesem Zweck wurden im dritten Kapitel vier verschiedene Gastmoleküle synthetisiert, die sich hinsichtlich ihrer Bindungsarmlänge und Valenz unterscheiden. Oberflächenbasierende Bindungsstudien mit zwei- und dreiarmligen Gastmolekülen ergaben, dass es möglich ist mit dem von uns gewählten molekularen Design die Bindungsstärke zu verdoppeln, jedoch nicht zu verdreifachen. Diese Untersuchung wurde im vierten Kapitel weitergeführt. Die hier gewählten Gastmoleküle unterscheiden sich zwar in ihrer Bindungsgruppe von den vorherigen Gastmolekülen aus Kapitel 3, haben aber trotzdem vergleichbare Komplexierungseigenschaften. Der Vorteil dieser Moleküle ist, dass sie mit

einem Farbstoff markiert sind und ihr Bindungsverhalten mittels Fluoreszenzspektroskopie untersucht werden kann.

Eine weitere außergewöhnliche Eigenschaft supramolekular gebundener Moleküle, ist ihre Dynamik. Die Bindungen in supramolekularen Komplexen, sind nicht kovalent und unterliegen somit einem dynamischen Gleichgewicht. Dies führt dazu, dass sich oberflächengebundene Moleküle, abgesehen von kontinuierlicher Adsorption und Desorption, auch entlang der Oberfläche bewegen können. Abhängig von der Bindungsstärke und der Reaktionsgeschwindigkeit können die Moleküle einige Nanometer pro Sekunde zurücklegen. Diese dynamischen Eigenschaften, der hier untersuchten supramolekularen Systeme, sind im fünften Kapitel präsentiert.

In den vorherigen Kapiteln hat sich herausgestellt, dass bivalente Gastmoleküle ein gutes Verhältnis zwischen Bindungsstärke und Dynamik auf supramolekularen Oberflächen aufweisen. Daher wurden im letzten Kapitel die Bindungen von bivalenten Gastmolekülen mit zwei verschiedenen Bindungsgruppen auf Wirtoberflächen untersucht. Eine Besonderheit dieser Moleküle ist deren Multifunktionalität, die Reaktivität gegenüber mehreren Stimuli, ermöglicht einen äußerst hohen Grad an Kontrolle über ihre Bindung. In den Versuchen konnten wir zeigen, dass Bindungsstärken über mehrere Größenordnungen gesteuert werden können, während die Reversibilität der Wirt-Gast-Systeme erhalten bleibt.

Diese Arbeit ist Teil einer Entwicklung hin zu dynamischen supramolekularen Systemen, die wissenschaftlich und technologisch in Zukunft von großer Bedeutung sein werden. Die hier präsentierten Ergebnisse hinsichtlich divergierender Bindungsstärke und Schaltbarkeit demonstrieren, dass mit Hilfe der supramolekularen Chemie, Kontrolle über die Molekülbindung erhalten werden kann. In Verbindung mit Kontaktdrucktechnologien kann dies noch verfeinert werden und die Ergebnisse, die in dieser Arbeit beschrieben werden, können in Zukunft zur Entwicklung von neuer Oberflächen- und Sensortechnik beitragen.

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About the Author

Maïke Wiemann was born in Moers, Germany on November 8th, 1988. She studied chemistry at the Ruhr-University Bochum, where she received her bachelor degree in September 2011 and also her master degree in October 2013. During her master thesis she worked on the “Synthesis and Characterization of EGFR Targeting Peptides GE11(2R) and Organometallic GE11(2R)bioconjugates”. In May 2014 she started her PhD program in the Molecular Nanofabrication group under the supervision of Prof. Dr. Pascal Jonkheijm. The aim of her project is the development of responsive bioactive interfaces in order to study and manipulate cellular processes.

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