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

Maike Wiemann[a] and Pascal Jonkheijm[a]

Abstract: 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 review 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 and that 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.

Keywords: cucurbituril · host-guest chemistry · supramolecular chemistry · supramolecular surfaces · stimuli-responsive

1. Introduction

Cucurbit[n]urils 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.[3,4] This condensation reaction yields different CB[n] homologues ranging from CB[5] to CB[8], but also traces of higher homologues were isolated.[3–8] CB[n] have a common depth, but their width and volumes differ progressively with their ring size (Table 1, Figure 1).[3,4] CB[n] are symmetric and possess two identical portal sites conceived to be the most probable binding scenario for a 1:1:1 heteroternary complex or twice the same guest to form a 1:2 homoternary complex.[13,17,18] Much research on CB[n] macrocycles focuses on understanding CB[n]-guest complexation and modulation of the CB[n]-guest binding affinity 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 excellent reviews on CB[n] related host-guest complexation in solution,[11,13,14–16] here we review in detail the latest progress of CB[n]-mediated host-guest complexes on surfaces with special attention to stimuli-responsive studies. Stimulus-responsive systems by means of pH, light or electrochemistry are attractive as such systems offer advantages such as dynamicity and reversibility, so that they are able to function as molecular machines or mimic molecular systems from nature. We first give selected examples on the state-of-the-art on stimuli-responsive CB[n] systems in solution before we describe in detail recent investigations on surface-anchored stimuli-responsive CB[n] systems.

Table 1. Structural parameters for CB[n] homologues

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>portal diameter (Å)</td>
<td>2.4</td>
<td>3.9</td>
<td>5.4</td>
<td>6.9</td>
</tr>
<tr>
<td>cavity diameter (Å)</td>
<td>4.4</td>
<td>5.8</td>
<td>7.3</td>
<td>8.8</td>
</tr>
<tr>
<td>volume (Å³)</td>
<td>82</td>
<td>164</td>
<td>279</td>
<td>870</td>
</tr>
<tr>
<td>height (Å)</td>
<td>9.1</td>
<td>9.1</td>
<td>9.1</td>
<td>9.1</td>
</tr>
</tbody>
</table>

1.1 Host-Guest Chemistry of Cucurbit[n]urils

Increasing the host cavity size from CB[5] to CB[8], increases the size of the guests forming 1:1 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 twice 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 behavior.[10,13] CB[8] hetero-

[a] M. Wiemann, Prof. P. Jonkheijm
Bioinspired Molecular Engineering Laboratory of the MIRA Institute of Biomedical Technology and Technical Medicine and the Molecular Nanofabrication Group of the MESA+ Institute for Nanotechnology, University of Twente, P.O. Box 217, 7500 AE, Enschede, The Netherlands
E-mail: p.jonkheijm@utwente.nl

© 2017 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Wiley Online Library 314
complexation has been exploited for various assembly schemes. An interesting example by Kim and coworkers showed an unusual back-folding complex of a naphthol-methyl viologen derivative, which can lead to a folded or unfolded system with CB[8] (Figure 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]-favoring and disfavoring conformers can be achieved through applying of an external stimulus.[30–33]

The role of water in host-guest complexation has received attention in numerous studies.[34,35] In aqueous solution, water molecules are complexed in the cavities of CB[n]s, 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 CBs,[36,37] released water molecules by guest complexation leads 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 early reported 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] 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 methyl viologen and subsequent binding in a 2:1

Pascal Jonkheijm earned his PhD in macromolecular chemistry from the University of Eindhoven (Netherlands) with Prof. E. W. Meijer and Prof. A. P. H. J. Schenning and he performed his post-doctoral studies at the Max Planck Institute of Molecular Physiology in Dortmund (Germany) with Prof. H. Waldmann. His group aims to develop dynamic chemical strategies to understand, direct and manipulate cellular processes with temporal and spatial control (densities, specificities, separation). Insight into the mechanisms that direct and regulate cellular function will be used to make a new generation of smart biomaterials and to fabricate multifunctional biochips.

Maike Wiemann studied chemistry at the Ruhr-University Bochum (Germany), 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 EGFRTargeting Peptides GE11(2R) and Organometallic GE11(2R) bioconjugates”. In May 2014, she started her PhD program under the supervision of Prof. Pascal Jonkheijm. The aim of her project is the development of responsive bioactive interfaces to study and manipulate cellular processes.
fashion.\textsuperscript{[49]} The mentioned examples gave a first impression of stimuli-responsiveness of molecular switches.

Recently, CB\textit{n}-based hydrogels (Figure 3 and Figure 4a),\textsuperscript{[50,51]} polymer networks\textsuperscript{[51]} and nanoparticles\textsuperscript{[52,53]}, with potential use in biomedical science have been made. For example, Kim and coworkers designed a CB\textit{[6]}-based hydrogel, containing hyaluronic acid. This hydrogel was able to interact with cells within the hydrogel and makes it suitable for 3D cellular engineering. Furthermore, subcutaneous injection CB\textit{[6]}-hyaluronic acid and polyamine-grafted hyaluronic acid led to \textit{in situ} hydrogel formation in mice and finally 11 days lasting fluorescence.\textsuperscript{[50]} Reversibility was not shown here, but pH sensitivity is conceivable by protonation and deprotonation of the polyamine linker.

We have shown a supramolecular strategy for the self-assembly of dual-responsive supramolecular nanoparticles based on heteroternary interactions between azobenzene-modified dendrimers, methyl viologen-modified polymers and CB\textit{[8]} (Figure 4b).\textsuperscript{[52,53]} Using monovalent stoppers supramolecular particles could be stabilized. CB\textit{[n]}-based nanoparticles have recently been reviewed.\textsuperscript{[54]}

\textbf{2. CB\textit{n}-Mediated Host-Guest Chemistry on Surfaces}

The ability to integrate CB\textit{n} in surface-based sensors and devices offers new opportunities to fabricate dynamic surfaces by exploiting stimulus-responsive CB\textit{n} systems. Dynamic surfaces play an important role in many processes in living systems and as such CB\textit{n}-mediated dynamic surfaces could be important in achieving a mimic of dynamic aspects of living systems and subsequently could be utilized in biomaterials, tissue engineering, biosensors and cell biology.\textsuperscript{[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\textit{n} to surfaces and subsequent CB\textit{n}-guest interactions on surfaces has been characterized and studied.\textsuperscript{[58–60]} In general, anchoring of CB\textit{n} to surfaces has been achieved in different ways as depicted in Figure 5.\textsuperscript{[11,57,62–66]} When able to covalently functionalize the CB\textit{[7]} macrocycle via covalent modification of CB\textit{n} in a strongly oxidative environment to generate reactive perallyloxy sidegroups Kim and coworkers set out to employ these functional groups to covalently anchor CB\textit{[6]} and CB\textit{[7]} onto surfaces. Perallyloxy-CB\textit{[6]} could be anchored via the thiol-ene reaction with thiol-functionalized glass slides to form a thioether bond between surface and macrocycle (Figure 5a).\textsuperscript{[62]} Anchoring of partially allyloxylated CB\textit{[7]} was achieved by olefin cross-metathesis reaction with vinyl-terminated SAMs on gold (Figure 5a).\textsuperscript{[62,67]} In both cases suitable guests were specifically complexed to the CB\textit{n} surfaces, however their binding affinities were not verified. To circumvent lengthy, somewhat difficult chemical CB\textit{n} functionalization,\textsuperscript{[62]} Shi and coworkers demonstrated covalent CB\textit{n} anchoring to readily available azide surfaces via a photochemical reaction (Figure 5b).\textsuperscript{[68]} Despite that the surface immobilization mechanism was not fully characterized, guests were complexed specifically.\textsuperscript{[69]} Li and coworkers made non-covalent CB\textit{n} SAMs by spontaneously adsorbing of CB\textit{n} cavities on gold surfaces utilizing the multivalent interactions between the lone-pairs of the carbonyls and the gold surface (Figure 5b).\textsuperscript{[64]} Surface-attached CB\textit{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.\textsuperscript{[64]} This arrangement maintains the recognition properties of CB\textit{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\textit{n} SAMs and requires no chemical functionalization of CB\textit{n}, the coverage of the gold surface was incomplete (48-55 \%) and lead to nonspecific interactions.\textsuperscript{[60,69,70]} Improvements of the surface coverage following this assembly method have recently been reported by the group of Hernandez.\textsuperscript{[71]} Best monolayer quality was achieved when a gold surface was immersed in saturated CB\textit{n} solution without any salts. Incubation time varied between 1 hour for CB\textit{[7]} and up to 4 hours for CB\textit{[6]}.\textsuperscript{[71]}

Another noncovalent approach to anchor CB\textit{n} to surfaces makes use of thiolated guest molecules that can trap CB\textit{n} to the surface.\textsuperscript{[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\textit{n}. Kim and coworkers synthesized a CB\textit{[6]} threaded on a molecular string that consists of a diaminobutane unit as a station for CB\textit{[6]} and a 1,2-dithiolane group as anchor group on gold (Figure 5c).\textsuperscript{[72]} One advantage of this approach, is the tuneability of the CB\textit{n} surface density through varying the concentration of present guest molecules. A disadvantage is that in case of CB\textit{[6]} and CB\textit{[7]} the cavities are already occupied and are not able to bind other guests. An alternative offers surface-bound
pseudorotaxanes containing CB[8], which were formed by a binary complex of methyl viologen-capped SAMs and CB[8].
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 5c).

Figure 4. a) Illustration of the assembly of supramolecular polymer microcapsules using MV2+- and naphthol (Np)-grafted polymers. b) Schematic presentation of programmed assembly and disassembly of nanoparticles (polyethylene imine (PEI), azobenzene (Azo), polyethylene glycol (PEG), polyamidoamine (PAMAM)). Reproduced with permission from [51] and [53], Copyright 2017 American Chemical Society and 2016 Elsevier B.V.

Figure 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 CBs via noncovalent host-guest chemistry by e.g. direct thiol-gold interaction of methyl viologen or azobenzene.
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 5c).[76]

2.1 pH Sensitive Platforms

More selective sensing is required for different types of cancer to advance cancer therapies. A breast cancer gene sensing platform was developed by He and coworkers employing CB[7].[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 worked as 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] was 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 and 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] was shifting to the terminal diaminoalkane and consequently opened the pore to release the loaded molecules.[84–82] To stay within physiological conditions and make it suitable for drug delivery, systems, which are switching in a small pH range are needed.

2.2 Redox Sensitive Platforms

Schoenhoff 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 sensing platforms or nanomaterials, since they can mimic complicated molecular recognition systems in nature (Figure 5c). Usual 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 methyl viologen-grafted polymer into the multilayer by the layer-by-layer technique. Pyridinium-modified anthracene was inserted as second guest and anthracene’s optical properties allowed to follow the redox triggered uptake and release of the guest upon reduction using NaBH₄.[81] Multilayer films containing poly azoelectrolytes and CB[8] were also investigated, which have similar properties as the above described multilayer.[76]

A redox-responsive underwater adhesion system was designed by Kim and coworkers (method Figure 5a, Figure 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 withdraw up to four kilogram. Reduction of the ferrocene led to a loss in binding affinity and opening of the Velcro. The system represents an example where 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 5a, Figure 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 a CB[7]-ferrocenemethylammonium pair as a replacement for the biotin-avidin pair, due to their strong binding properties of 10¹⁵ M⁻¹, which is the first synthetic binding pair overcoming...
Review

the affinity of the biotin-avidin affinity. Unfortunately the redox responsiveness was not shown with an experiment.[67]

We have reported successful ways to anchor 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-labeled proteins, which can be used for oriented positioning of proteins, as for example demonstrated on CB[7] SAMs. Mono- and divalent ferrocenylated yellow fluorescent protein were prepared and these formed stable inclusion complexes on CB[7] SAMs (method Figure 5b).[88,89] We 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 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]/MV\(^{2+}\) SAMs (method Figure 5c, Figure 8) heteroternary complexation of site-selectively naphthol-modified proteins was demonstrated by us.[74] Supramolecular patterning of these proteins was achieved by reactive microcontact printing of a mixture of CB[8] and naphthol-modified proteins on methyl viologen SAMs.[74]

![Figure 8](image)

**Figure 8.** Fluorescence images of CB[8] mediated heteroternary host-guest complexes of methyl viologen and naphthol. a) Pattern of printed lissamine- and YFP-naphthol. b) Disappearance of fluorescence after reduction with zinc. c) Reinstalled fluorescence after oxidation and reincubation of the host-guest complex. Reproduced with permission from ref. [74]. Copyright 2012 American Chemical Society.

Also tryptophan (Trp) based protein immobilization was studied on this type of CB[8]-pseudorotaxane based SAMs.[91,95,97] Specificity and reversibility was typically verified in the experiments to confirm envisioned host-guest interactions to occur. In a recent study we prepared a focused set of integrin-targeting knottin variants with distinct numbers of tryptophans to vary affinities for the pseudorotaxane-based CB[8] SAMs.[91,95] The genetically engineered knottin constructs are able to simultaneously bind integrins and MV\(^{2+}\)/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 tryptophan residues while an increased extent of multilayer formation for the higher valent constructs, which we attributed to homoternary complex formation between tryptophans 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, we have recently metabolically expressed naphthol moieties on the outer surface of non-adherent Jurkat cells (Figure 11c).[93] Specific host-guest was demonstrated on surfaces and the method potentially allows for programmable supramolecular interactions with spatial and temporal control over cell adhesion as well.

Up to now the results reviewed in this section have not fully utilized the potential of including methyl viologen 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 CB[8]-methyl viologen works as a molecular junction and that encapsulation influences the conductance and peak current of methyl viologen.[98] A first example on utilizing the redox responsiveness of CB[8]/MV\(^{2+}\) SAMs was nicely described by Scherman and coworkers in a focused attempt to separate peptides containing aromatic residues.[99,100] They trapped tryptophan containing peptides from a peptide mixture without any aromatic containing peptides and immobilized them on pseudorotaxane-based CB[8]/MV\(^{2+}\) SAMs (method Figure 5c, Figure 9). An electrochemical stimulus led to a one-electron reduction of the MV\(^{2+}\) and release of the separated peptide from the host-guest complex. With this method reversible binding and release was possible over many cycles.[100]

![Figure 9](image)

**Figure 9.** Peptide separation with a CB[8] trapping surface via selective heteroternary complex formation. Reproduced with permission from ref. [100]. Copyright 2010 American Chemical Society.

We have demonstrated a supramolecular approach to attach cells via host-guest chemistry on pseudorotaxane-based CB[8] SAMs (Figure 5c, Figure 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 MV\(^{2+}\) 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 characterized of the surfaces showed same binding affinities as found in solution.[73] Cells specifically adhered to the integrin-binding peptide on the surface and started spreading after 1 h of...
incubation and the supramolecular SAMs were also shown to be functional in a wound assay.\[73\]

Upon reduction of methyl viologen 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 RGD peptide on gold electrodes.\[73\]

Using single-cell force spectroscopy we have recently explored the possibility to estimate the rupture forces of adhered cells on the CB[8] surfaces. We were able to determine rupture forces and found them comparable to the rupture forces of cells adhered to covalent surfaces (Figure 11a, b).\[94\] 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 signaling are subject of future studies and show the potential of this type stimuli-responsive supramolecular surfaces in cell biology and biomaterials.

2.3 Photo Sensitive Platforms

Cowpea chlorotic mottle virus was functionalized with azobenzene moieties on the outer surface and employed for immobilization on pseudorotaxane-based CB[8] SAMs (method Figure 5c, Figure 12c) to form heteroternary complexes.\[96\] Our SPR binding studies of azobenzene-modified virusses to the CB[8] SAM showed a binding constant of $K_a = 1.41 \times 10^6$ M$^{-1}$, in agreement with solution studies. When applying a light stimulus using $\lambda = 365$ nm, the azobenzene isomerized from the trans to the cis state and subsequently dissociated from the CB[8] surface as the more bulky and polar cis-azobenzene was unable to bind to the binary complex of CB[8] and MV$^{2+}$.\[96,101\]

In related work an azobenzene-modified mannose was immobilized on a CB[8] surface. This surface consisted of a
supported lipid bilayer with improved nonfouling properties onto which the pseudorotaxane system MV\(^2^+\)/CB[8] was installed (Figure 12a, b).\(^{[102]}\) The E. coli bacteria strain ORN178 bearing a carbohydrate binding receptor was found to bind mannose, which could be shown by 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.\(^{[99]}\) Local photo-switching from the trans- to cis-azobenzene mannose leads to dissociation of the ligand from the surface. As a consequence nearly 80% of the bacteria were removed from the surface.\(^{[102]}\)

Ravoo and coworkers reported heterocomplexation of arylazopyrazole-modified integrin-binding peptides onto pseudorotaxane-based CB[8] SAMs (Figure 13).\(^{[103]}\) Arylazopyrazoles are of interest to improve the photoswitching characteristics of the CB[8] SAMs. Previous work on arylazopyrazoles demonstrated excellent switchability with a photostationary state up to 96% and half-lifetimes of several days. The properties remain stable also in host-guest complexes.\(^{[104–106]}\)

Myoblast cells were adhered onto the supramolecular surfaces and released by UV induced isomerization of the arylazopyrazoles.\(^{[103]}\)

Another photoswitchable molecule, cinnamamide, has been attached to the surface of silica nanoparticles and undergoes, similar to azobenzene, a trans to cis isomerization after irradiation with light of \(\lambda = 300\) nm. As a consequence CB[7] dissociated away from the surface thereby releasing of guest molecules out of the porous nanoparticles.\(^{[107]}\)

2.4 Competing Guests

Usage of competing guests on interfaces is a straightforward method to adsorb and release compounds on surfaces.\(^{[108]}\) 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 systems was presented by Rotello, Isaacs and coworkers.\(^{[109,111]}\) A diaminohexane functionalized cytotoxic gold nanoparticle surface was covered with CB[7], which lead to a reduced toxicity. After cell uptake a competing adamantane guest was added and removed the CB[7] cover from the gold nanoparticle, which induces apoptosis due to its cytotoxicity.\(^{[109]}\)

A naphtalimide based fluorescence sensor was used for rapid detection of therapeutically relevant drugs. CB[7] increases the fluorescence signal due to encapsulation of the naphtalimide and the fluorescence was reduced when CB[7] was removed from the platform by a relevant drug as competing guest.\(^{[110]}\)

Reversible protein adhesion was demonstrated in the case of a CB[7]-ferrocene binding pair. Dissociation of the ferrocenylated protein away from the surface was observed after washing with a ferrocene derivative with a higher affinity.
Furthermore, we achieved also reversible bacteria adhesion on pseudorotaxane CB[8] surfaces (Figure 14). The bacterial strain of *E. coli* was genetically modified on the outer membrane with tryptophane 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.\(^\text{[92]}\)

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.

### 2.5 Dual Stimuli

A very promising strategy is the integration of multi, orthogonal stimuli in sensing platforms and other surface-related applications. Interesting work to achieve dual stimuli-responsive surfaces was reported by Scherman and coworkers. 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 other work before.\(^\text{[75,101]}\) In their case the azobenzene guest was immobilized on the surface and a surface-bound heteroternary complex formed with CB[8] and a dye-labelled MV\(^{2+}\) (Figure 15).\(^\text{[101]}\) 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 heteroternary complexes to the starting situation. When MV\(^{2+}\) was converted to MV\(^{+*}\) by electrochemistry, homoternary complexes form in solution

![Figure 14](image1.png)

**Figure 14.** a) Surface immobilization of bacteria. b) Adhered bacteria on 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. c) Reduced number of adhered bacteria, due to incubation with competitor (FG6). Reproduced with permission from ref. [92]. Copyright 2015 American Chemical Society.

![Figure 15](image2.png)

**Figure 15.** Dual switching of the heteroternary complex of MV\(^{2+}\) and azobenzene with CB[8] by a redox stimulus and UV light stimulus. Reproduced with permission from ref. [101]. Copyright 2012 Nature Publishing Group.
between two MV$^{2+}$ and CB[8], which was signified by the disappearance of the fluorescence. This process could be reversed.

3. Conclusion

CB[n] have become an essential part of supramolecular chemistry and a large number of possible supramolecular conjugates, surfaces and materials have been made. We reviewed in some detail the latest progress of CB[n]-mediated host-guest complexes on surfaces 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 milieux of the human body. Surely, supramolecular CB[n]-based functional surfaces are now taking a more central place in supramolecular materials and devices.

Acknowledgements

This work was supported by the Netherlands Organisation for Scientific Research (NWO) through a VIDI grant (723.012.106).

References


Received: September 20, 2017
Accepted: November 4, 2017
Published online on November 23, 2017