Stimuli Responsive Systems Constructed Using Cucurbit[7]uril-Type Molecular Containers

Lyle Isaacs*

Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742, United States

CONSPICUOUS: This Account focuses on stimuli responsive systems that function in aqueous solution using examples drawn from the work of the Isaacs group using cucurbit[7]uril (CB[7]) molecular containers as key recognition elements. Our entry into the area of stimuli responsive systems began with the preparation of glycoluril derived molecular clips that efficiently distinguish between self and nonself by H-bonds and π–π interactions even within complex mixtures and therefore undergo self-sorting. We concluded that the selectivity of a wide variety of H-bonded supramolecular assemblies was higher than previously appreciated and that self-sorting is not exceptional behavior. This lead us to examine self-sorting within the context of CB[n] host–guest chemistry in water. We discovered that CB[n] homologues (CB[7] and CB[8]) display remarkably high binding affinity (Kᵣ up to 10¹⁵ M⁻¹) and selectivity (∆ΔG) toward their guests, which renders CB[n]s prime components for the construction of stimuli responsive host–guest systems. The CB[7]-adamantaneammonium ion complex, which is particularly privileged (Kᵣ = 4.2 × 10¹² M⁻¹), was introduced by us as a stimulus to trigger constitutional changes in multicomponent self-sorting systems. For example, we describe how the free energy associated with the formation of host–guest complexes of CB[n]-type receptors can drive conformational changes of included guests like triazene–arylelenfoldamers and cationic calix[4]arenes, as well as induced conformational changes (e.g., ammonium guest size dependent homotropic allostery, metal ion triggered folding, and heterochiral dimerization) of the hosts themselves. Many guests display large pKᵣ shifts within their CB[n]–guest complexes, which we used to promote pH controlled guest swapping and thermal trans-to-cis isomerization of azobenzene derivatives. We also used the high affinity and selectivity of CB[7] toward its guests to outcompete an enzyme (bovine carbonic anhydrase) for a two-faced inhibitor, which allowed stimuli responsive regulation of enzymatic activity. These results prompted us to examine the use of CB[n]-type receptors in both in vitro and in vivo biological systems. We demonstrated that adamantaneammonium ion can be used to intracellularly sequester CB[7] from gold nanoparticles passivated with hexadiammonium ion-CB[7] complexes and thereby trigger cytotoxicity. CB[7] derivatives bearing a biotin targeting group enhance the cytotoxicity of encapsulated oxaliplatin toward L1210FR cells. Finally, acyclic CB[n]-type receptors function as solubilizing excipients for insoluble drugs for drug delivery purposes and as a broad spectrum reversal agent for the neuromuscular blocking agents rocuronium, vecuronium, and cis-atracurium in rats. The work highlights the great potential for integration of CB[n]-type receptors with biological systems.

INTRODUCTION

Herein, I present an Account of research performed in my group directed toward the development of stimuli responsive systems using cucurbit[n]uril (CB[n]) molecular containers and other glycoluril derived systems as key recognition components. In the 1990s, the supramolecular chemistry field began to use its fundamental knowledge of noncovalent interactions (e.g., H-bonds, metal–ligand interactions, electrostatic interactions, and the solvophobic effect) to enable the preparation of more complex multicomponent aggregates formed in self-assembly processes. For the purpose of this Account, we define self-assembly as those processes that result in the transformation of a set of building blocks into one well-defined aggregate under a specific set of conditions (Scheme 1a). Self-sorting can then be defined as the concurrent self-assembly of multiple sets of building blocks into multiple well-defined aggregates under a specific set of conditions (Scheme 1b). The focus of this Account, stimuli responsive systems, is those self-assembling or self-sorting systems whose constituents can be reconfigured in response to suitable stimuli (e.g., pH change, photochemistry, electrochemistry, addition of further constituents). In stimulus responsive systems (Scheme 1c), the most potent stimuli are those that result in large favorable changes in the overall free energy (∆ΔG) after application of the stimulus that can drive substantial changes in constitution.

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Lehn conceptualized self-recognition processes in the context of the self-assembly of a series oligo(bipyridine) compounds (1) in the presence of Cu⁺ ions (Scheme 2a). Individually, compounds 1a–1d self-assemble with Cu⁺ to give the double helicates \([1a_2\cdot Cu_2]\) and \(\cdot [1d_2\cdot Cu_2]\). Remarkably, the mixture of the four structurally similar strands \((1a–d)\) and Cu⁺ gives \([1a_2\cdot Cu_2]\) and \([1d_2\cdot Cu_2]\) in a high fidelity self-recognition process. The Sanders group first used the term self-sorting to describe the behavior of a mixture of building blocks (2 and 3) under thermodynamically controlled transesterification conditions (Scheme 2b). Interestingly, a mixture of the two homomeric macrocycles 4 and 5 is formed in a high fidelity self-sorting process. In both of these pioneering works, the focus was on self-processes where each compound recognizes other identical building blocks within a mixture.

In this Account, we use glycoluril derived systems whose development can be traced to the early work of Mock, Nolte, and Isaacs using milder reaction conditions produced CB[6] in a high yield cucurbit[n]uril (CB[n]); subsequent work by Kim, Day, and Isaacs using milder reaction conditions produced CB[6] also shows high activity, and regulation of \(\text{CB}[n]\) activity, and regulation of \(\text{CB}[n]\) systems including those that display allostery, pH and conformational responsiveness, regulation of enzymatic activity, and regulation of in vitro and in vivo biological activity. As discussed, \(\text{CB}[n]\) compounds are prime components for self-sorting and stimuli responsive systems based on \(\text{CB}[n]\)-type receptors.

**SELF-SORTING SYSTEMS BASED ON SELF-RECOGNITION**

As part of our synthetic and mechanistic studies toward \(\text{CB}[n]\)-type receptors, we prepared molecular clips 7–9, which feature a central glycoluril dimer framed by aromatic walls that display two H-bonding arms in diﬀerent three-dimensional arrangements (Figure 2a). We found that 7–9 individually undergo self-assembly in CDCl₃ to yield homodimeric assemblies 8₁ and 9₂ and heterochiral homodimer (+)–(−)–(−)–7 (Figure 2b) driven by π–π interactions and H-bonds. We found that the three-component mixture 7–9 undergoes high fidelity self-sorting because the hypothetical heterodimers cannot satisfy the geometrical constraints of π–π interactions and simultaneously maximize the number of H-bonds formed.

The results with 7–9 lead us to question whether self-sorting was exceptional behavior or whether the wide variety of H-bonded aggregates known in the literature could simply be mixed together to generate a multicomponent self-sorting system. For this purpose, we selected ten components (7, 8, 10–16, and barium picrate), which are the components of Davis’ self-assembled ionophores, Reinhard’s rosettes, Rebek’s tennis ball and calixarene tetraurea capsule, Meijer’s ureidopyrimidinone, and our molecular clips. We found that the 1H NMR spectrum of the 10-component mixture (Figure 3) is simply equal to the sum of the NMR spectra of its individual self-assembled species (Figure 3A–H), which is the spectroscopic earmark of a self-sorting system. These studies suggested that simply selecting components with diﬀerent H-bond donor/acceptor patterns and spatial orientations whose aggregates feature closed networks of H-bonds would enable the preparation of complex self-sorting systems. This work lead supramolecular chemists to appreciate that the subset of molecular aggregates that are capable of self-sorting was much larger than previously appreciated, which inspired numerous research groups to design and study self-sorting systems.

**SELF-SORTING AND STIMULI RESPONSIVE SYSTEMS BASED ON \(\text{CB}[n]\)-TYPE RECEPTORS**

This section details our use of \(\text{CB}[n]\)-type molecular containers as prime components for the construction of stimuli responsive (self-sorting) systems including those that display allostery, pH and conformational responsiveness, regulation of enzymatic activity, and regulation of in vitro and in vivo biological activity. As discussed, \(\text{CB}[n]\) compounds are prime components for...
self-sorting systems because of their high affinity and high selectivity interactions, which commonly display slow kinetics of exchange on the chemical shift time scale, which allows for convenient monitoring of the constitution of the mixture by $^1$H NMR spectroscopy.

Social Self-Sorting

The self-sorting systems discussed above are based on H-bond driven self-recognition processes in CDCl$_3$ where two or more identical molecules self-assemble. We envisioned that self-sorting processes in water based on host−guest complexation events, social self-sorting, would be both more biologically relevant and stimuli responsive since addition of exogenous guest could trigger transformation from one self-sorted state to another. For our initial demonstration of social self-sorting, we selected several host−guest pairs (CB[6]·6$_{16}$, CB[8]·17, β-cyclodextrin·19, and 20·K$^+$) and other aggregates (21 and 22·23$_3$) and asked whether their components would faithfully reform these aggregates upon mixing or whether alternate host−guest processes would impinge on the self-sorting process (Figure 4).$^{15}$ We found that the $^1$H NMR spectrum of the 12-component mixture is a superposition of the spectra of the six complexes listed above, which means that this mixture is self-sorted. This work showed that self-sorting proceeds efficiently even in biologically relevant H$_2$O as a medium using inherently less directional electrostatic interactions and the hydrophobic effect. Furthermore, the high fidelity self-sorting suggested to us that the high affinity and selectivity observed for CB[6]$^{16}$ would also be observed for CB[n] homologues.

CB[n] Exhibit High Affinity and High Selectivity Host−Guest Interactions

The social self-sorting process described above prompted us to determine the binding constants of CB[7] and CB[8] toward numerous guests by $^1$H NMR competition experiments referenced to absolute $K_a$ values determined by direct UV/vis titrations.$^{17}$ Figure 5a shows a selection of the $K_a$ values determined. For example, we discovered that CB[7] exhibits picomolar binding affinity toward adamantaneammonium and ferrocene ammonium ions, 24 and 25, in 50 mM NaOAc buffered D$_2$O; the high binding affinity of CB[7]·25 was subsequently confirmed by ITC competition experiments performed by Inoue, Kim, and Kaifer.$^{18}$ Equally interesting was the fact that the CB[8]·24 complex is more than 5000-fold
destabilizes the CB[7]-26 complex due to unfavorable steric interactions between the convex surface of the guest and the concave surface of the host. Recent work by Scherman, DeSimone, and Nau suggests that an important factor in the high affinity binding of CB[n] is the release of high energy waters from the CB[n] cavity upon complexation.27 We also measured the $K_a$ values between guest 27 (available from a concurrent study)3 and CB[7] and CB[8]; the encapsulation of both ammonium arms within CB[8]27 leads to 3200-fold higher affinity and conformational control. Intrigued by the high affinity binding of CB[7]-24, several groups sought to reach even higher levels of affinity. In 2007, a collaboration between the Inoue, Kim, Kaifer, Gilson, and Isaacs groups studied the CB[7]-28 complex in pure water and measured $K_a = 3 \times 10^{15} \text{M}^{-1}$, which is comparable to that of avidin–biotin;20 the ability of 28 to engage in ion–dipole interactions with both C=O portals was deemed of critical importance. Additional ferrocene and [2,2.2]bicyclooctane diammonium ions (29) have been explored in this context.21,22 Recently, the Glaser–Majerski–Isaacs team reported that diamantenediammonium ion 30 forms CB[7]-30 with $K_a = 1.4 \text{aM}^{123}$. This remarkable result was attributed to the presence of 14 optimal ion–dipole interactions, the colinearity of the symmetry axes of CB[7] and 30 within CB[7]-30, and the greater hydrophobicity of diamantane relative to adamantane or ferrocene skeletons based on X-ray crystallographic results (Figure 5b).

**Control over Allosteric Processes with CB[n]**

In 2005, we reported that addition of guest 27 to CB[10]-CB[5] gave CB[10]-27, which upon reaction with Ac$_2$O followed by washing delivered free CB[10].8 In searching for guests to fill the voluminous (870 Å$^3$) cavity of CB[10], we found that tetracationic calixarene 32 forms the CB[10]-1,3-alt-32 complex in which 32 preferentially assumes the less stable 1,3-alternate conformation (Scheme 3). Quite interestingly, we found that addition of adamantane derivative 19 resulted in the formation of the CB[10]-cone-32-19, which amounts to a stimuli responsive conformational control of 32 within the macromolecular complex. Cationic adamantane 24 was much less efficient a stimulus than 19 presumably due to unfavorable electrostatic interactions in the CB[10]-cone-32-14 ternary complex. To reverse the conformational change, we added CB[7] as a stimulus, which sequesters the greater hydrophobicity of diamantane relative to adamantane or ferrocene skeletons based on X-ray crystallographic results (Figure 5b).

**Size Dependent Homotropic Allotropy with Bis-nor-seco-CB[10]**

During our studies of the mechanism of CB[n] formation, we isolated bis-nor-CB[10], which is formed by step-growth polymerization.24 Bis-nor-CB[10] features two cavities, each shaped by five glycolurils and connected by one CH$_2$–bridge (Figure 6). Bis-nor-CB[10] forms ternary complexes with a variety of guest molecules including 24, 31, 33, and 34. Molecular modeling shows that the cavity volume (450 to 740 Å$^3$) and H$_2$C–CH$_2$ nonbonded distance (5.5 to 9.3 Å) increase systematically as the size of the included guest increases. Therefore, we hypothesized that the binding of the first guest to give bis-nor-CB[10]–guest complexes would change the size of the second cavity (Figure 6c). Further, we suspected that the second cavity would display a preference to bind a guest of

Figure 4. Social self-sorting in water.

![Figure 4](image)

Figure 5. (a) $K_a$ values for selected CB[7] and CB[8] complexes determined in 50 mM NaOAc buffer and (b) X-ray structure of CB[7]-30.

![Figure 5](image)
similar size by homotropic allostery. To test this hypothesis, we prepared mixtures comprising bis-ns-CB[10] and pairs of guests (e.g., 31 + 33; 24 + 34) and monitored the process by H NMR spectroscopy. We observed that mixtures of small and large guests (e.g., 31 + 33) do indeed undergo size dependent homotropic allostery to yield bis-ns-CB[10]-31 and bis-ns-CB[10]-33; whereas guests of comparable size (24 + 34) form mixtures of the two homomeric and the heteromeric ternary complex bis-ns-CB[10]·24·34. The binding of the first guest acts as a stimulus, which instructs the second binding site to assume a specific size.

**pH Controlled Guest Swapping**

CB[6] compounds bind substantially tighter to cationic guests (GH+) than to the corresponding neutral form (G), which is reflected in a pK¢ shift within the CB[6]-GH+ complex. We designed a four component system comprising two guests and two hosts to undergo controlled guest swapping in response to a change in pH (Scheme 4). As the hosts, we selected β-cyclodextrin (β-CD), which is not selective based on guest charge, and CB[6], which is highly selective for cationic over neutral guests. We selected guest 35H+, which is pH sensitive, and guest 36, which remains cationic over the full pH range. Guests 35H+ and 36 contain adamantaneammonium binding epitopes to promote binding toward β-CD and hexylammonium tails to promote CB[6] binding. At pH 7, an equimolar mixture of all four components mainly forms CB[6]-35H+ and β-CD-36 (84%). At pH 13, 35H+ becomes deprotonated to 35, whereas 36 remains cationic. Accordingly, the dominant species (89%) at pH 13 are CB[6]-36 and β-CD-35 as monitored by 1H NMR spectroscopy. Thermodynamic calculations using Gepasi show that the magnitude of the pK¢ shift (e.g., ΔΔG) between CB[6]-35H+ and 35H+ is the critical parameter governing the guest swapping process.

**Azobenzene Cis–Trans Isomerization**

A more complex pH responsive system comprises CB[7] and azobenzene derivative 37 (Scheme 5a). Azobenzene derivatives typically prefer the trans-form over the cis-form by approximately 10 kcal mol\(^{-1}\). However, when we mixed CB[7] with 37 (D\(_2\)O, pH 4.74), we observed the purple color that is typical of cis-azobenzenes. We performed a pH titration (pH 0.5 to 12.5) of 37 alone and did not observe any purple color; the three pK¢ values of 37-H+ (3.54), 37-2H2+ (2.11), and 37-3H3+ (−0.5) agreed with previous literature reports. Subsequently, we performed a UV/vis titration for the CB[7]-37 complex from pH 0.17 to 13.35. Plots of absorbance (320, 554, and 391 nm) as a function of pH allowed us to extract three pK¢ values for the CB[7]-37-H+ (5.55), CB[7]-37-2H2+ (5.09), and CB[7]-37-3H3+ (3.38) complexes (Scheme 5b). The magnitude of the pK¢ shifts induced upon CB[7] binding for 37-3H3+ and 37-2H2+ are quite substantial and approach the magnitude of shifts observed by Nau\(^{27}\) and Raymond\(^{28}\) for other container complexes. Quite interestingly, decreasing the pH (7 to 5) results in the formation of CB[7]-37-H+ and then CB[7]-trans-37-2H2+, which undergoes thermal isomerization to the CB[7]-cis-37-2H2+ complex (Scheme 5a). We suggest that the very high pK¢ values typically observed for CB[7] complexes and the better match of cis-37-2H2+ than trans-37-2H2+ toward the hydrophobic cavity and electrostatically negative C=O portals of CB[7] provide the potent thermodynamic driving force to overcome the 10 kcal mol\(^{-1}\) preference for trans-azobenzenes. Decreasing pH below 3.4 results in cis-to-trans isomerization to yield CB[7]-trans-37-3H3+. We used this highly pH responsive UV/vis active system to construct indicator displacement assays for acetylcholine, caffeine, nicotine, dopamine, l-adrenaline, ephedrine, and...
pseudoephedrine and were able to quantitate pseudoephedrine concentration in the over-the-counter nasal decongestant Sudafed in the 5−350 μM range.

Controlled Folding Processes

Our earlier experience with the binding of 27 with CB[7], CB[8], and CB[10] prompted us to use CB[n] complexation to control the conformation of longer triazene−arylene oligomer 38 and related structures.29 By virtue of its four exocyclic triazene−NHAr bonds (green), oligomer 38 populates 2^4 conformations of which 10 are unique (Scheme 6). We studied the interaction of 38 with CB[7], CB[8], and CB[10] by a combination of 1H NMR spectroscopy and X-ray crystallography. Based on symmetry arguments and the observed direction and magnitude of changes in chemical shift upon complexation, we established that two CB[7] bind to the terminal benzylammonium rings of 38 to give CB[7]·a,s,s,a−38−CB[7], whereas CB[8] forms the CB[8]·a,a,a,s−38, and CB[10] forms the CB[10]·a,a,a,a−38 complex. We envisioned that we could fold, unfold, and refold a single oligomer strand in response to chemical stimuli. Initially, 38 populates the full 10-component conformational ensemble. However, upon addition of CB[8], the CB[8]·a,a,a,s−38 conformation is selectively stabilized. Addition of 2 equiv of CB[7] and 26 results in ejection and unfolding of 38 followed by the refolding into the CB[7]·a,s,s,a−38−CB[7] conformation. The formation of the CB[8]·26 complex provides the thermodynamic driving force to promote this process. Interestingly, we observed that the addition of 1 equiv of CB[7] enhances the rate of dissociation of the CB[8]·a,a,a,a−38 by formation of ternary complex CB[8]·a,a,s,s−38−CB[7]. Finally, the addition of 2 equiv of 24 and CB[10]·CB[5] results in the expulsion and forced unfolding of 38 into free solution where it displaces CB[5] and folds to form CB[10]·a,a,a,a−38 driven by the free energy of formation of the CB[7]·24 complex. The work is reminiscent of chaperone proteins, which forcibly unfold misfolded proteins and provide an environment for the refolding.

In 2009, we isolated acyclic glycoluril decamer (±)−39 in racemic form.30 When dissolved in water, (±)−39 exhibits a poorly resolved 1H NMR spectrum indicative of a range of conformations around the central N−CH2−N bridge. Quite interestingly, addition of metal sulfate salts (100 mM Li+, Na+, K+, or Ca2+) triggers folding and heterochiral dimerization processes to yield (+)−39·(−)−39·6Na+ driven by strong ion−dipole interactions between three or four C=O groups and each of the Na+ ions. The X-ray crystal structure of (+)−39·(−)−39·6Na+ is shown in Scheme 7. It is possible to reverse this folding and heterochiral dimerization process by the addition of 31, which gives the (±)−39·31 complex; subsequent addition of CB[7] sequesters 31 as the CB[7]·31 complex and results in the reformation of the (+)−39·(−)−39·6Na+ complex. These processes amount to a metal ion responsive folding and assembly process that is reminiscent of RNA folding processes. CB[n] containers are privileged in that they display protein mimetic (e.g., tight and selective binding) and nucleic acid mimetic (e.g., metal ion responsive) processes.

Recently, we reported the preparation of CB[6] dimer 40 by the reaction of glycoluril hexamer with 1,2,4,5-tetraformylbenzene.31 Compound 40 (3 equiv) undergoes self-assembly with oligomer 41 (2 equiv) to yield supramolecular ladder 40·41 as shown in Scheme 8 as established by 1H NMR symmetry
considerations and diffusion coefficient measurements (DOSY). Complex 40,41 is comparable in weight (7389 amu) and dimension (40 × 29 × 11 Å3) to small proteins. Interestingly, an equimolar mixture of 40 and 41 yields the folded 40·41 assembly wherein the two terminal hexylene binding sites of 41 are complexed to fully satisfy both cavities of 40. The folded structure of 40·41 was assigned on the basis of complexation induced chemical shifts, symmetry considerations, DOSY, and electrospray ionization mass spectrometry measurements. When more 40 (0.5 equiv) is added, the assembly is transformed into 40·412, in a process that is responsive to host–guest stoichiometry.

Scheme 7. Folding and Assembly of (±)-39

Scheme 8. Formation of Supramolecular Ladder 40·412

Scheme 9. Control of Enzymatic Activity of BCA

Control over Enzymatic Catalysis

We envisioned that the extremely high binding constants commonly exhibited by CB[7]−guest complexes could be used to outcompete biological macromolecules for their targets and thereby exert stimuli responsive control over the cognate biological functions. First, we decided to exert control over the catalytic activity of bovine carbonic anhydrase (BCA).32 We prepared a series of compounds (42a–c) that we refer to as two-faced inhibitors that contain a benzenesulfonamide binding epitope, which is well-known to inhibit BCA via binding to the Zn-cofactor at the active site (Ka ≥ 107 M−1), and an ammonium ion binding face, which binds to CB[7] (Scheme 9). Compounds 42a–c contain adamantaneammonium, trimethylsilylmethylammonium, and hexylammonium binding epitopes, which possess vastly different Ka values (∼1012, 109, and 106 M−1, respectively) toward CB[7]. Experimentally, we found that addition of 42a–c to BCA turns off the catalytic ability of BCA as monitored by a standard UV/vis p-nitrophenylacetate hydrolysis assay. Addition of CB[7] sequesters 42a or 42b, but not 42c, as their CB[7] complexes and turns on the catalytic activity of BCA. The lower binding affinity of CB[7]·42c is insufficient to drive the reactivation of BCA. Similarly, we were unable to reactivate the BCA·42a or BCA·42b systems by addition of β-cyclodextrin because of the low Ka values of β-CD−guest complexes. Subsequent addition of 43 to the BCA + CB[7]·42b system competitively displaces two faced inhibitor 42b from its CB[7]·42b complex, which then turns off BCA by formation of BCA·42b. We could not regenerate activity of the BCA + CB[7]·42a system in this manner because the CB[7]·42a complex (Ka ≈ 1012 M−1) exhibits slow dissociation kinetics (koff ∼ 2 × 10−5 s−1; half-life = 9 h) on the time scale of the experiment. Alternate addition of CB[7] and 43 was able to control enzymatic catalysis of BCA over four on–off cycles. Very interestingly, we found that
CB[7] enhances the rate of dissociation of BCA-42a (11-fold) and BCA-42b (19-fold) due to the formation of a transient ternary complex (BCA-42·CB[7]). We independently confirmed that the CB[7]-42a and CB[7]-42b complexes are ∼100-fold weaker inhibitors ($K_a \approx 10^5$–$10^6$ M$^{-1}$) of BCA than 42a or 42b presumably due to steric interactions between the convex surface of CB[7] and the active site of BCA. The work suggests a path toward stimuli responsive control of protein function in biological systems.

**Control over in Vitro Biological Systems**

In collaboration with the Rotello group, we sought to use the high affinity and highly selective binding interactions of CB[7] to activate nanoparticle therapeutics intracellularly in a stimuli responsive manner. For this purpose, gold nanoparticles (2.5 ± 0.4 nm diameter) were decorated with hexadecanammonium ion terminated alkanethiolates. The terminal hexadecanammonium ions renders nanoparticles 44 cytotoxic and also serves as a recognition site for CB[7] (Figure 7). We envisioned that the CB[7] complexed nanoparticle 44-CB[7]$_n$ would cloak the cytotoxic hexadecanammonium ion units and reduce cytotoxicity toward MCF-7 cells. This prediction was borne out experimentally, and one reason for the reduced cytotoxicity was the fact that the 44-CB[7]$_n$ nanoparticles become entrapped in endosomes. Furthermore, we found that the addition of 24 to MCF-7 cells that had already been treated with 44-CB[7]$_n$ sequesters CB[7] from the nanoparticle surface driven by the free energy of formation of CB[7]-24. This process triggers the release of the naked nanoparticle 44 from the endosome and results in higher levels of cytotoxicity. The ability to intracellularly activate nanoparticle therapeutics triggered by 24 offers potential routes to dual (e.g., host and guest) targeted therapeutics with higher levels of site-specific activity.

Recently, we have been studying the preparation and molecular recognition properties of acyclic CB[n]-type molecular containers 45 and 46 (Figure 8a). Compounds 45 and 46 feature a central glycoluril tetramer, two terminal aromatic rings, and four SO$_3^-$ groups, which dramatically enhance their solubility in water. Compounds 45 and 46 are preorganized into a C-shaped conformation (Figure 8b) and are not significantly self-associated in water. Significantly, the acyclic nature of 45 and 46 and the flexibility of the glycoluril tetramer backbone allow these compounds to flex like a hand to accommodate a wide range of guest sizes. We found that 45 and 46 greatly enhance the water solubility (from 23-fold to 2750-fold) of a number of insoluble drugs (e.g., paclitaxel, melphalan, clopidogrel, amiodarone, and camptothecin, Figure 8c). Acyclic CB[n]-type container 45 does not display significant in vitro toxicity toward kidney (HepG2), liver (HEK 293), and human monocyte (THP-1) cells according to metabolic cell viability and adenylate kinase release cell death assays. The in vivo (Swiss Webster mice) maximum tolerated dose (MTD) studies with 45 indicated an MTD greater than 1.23 g kg$^{-1}$. Treatment of HeLa cells with paclitaxel solubilized with 45 results in enhanced cell killing relative to the paclitaxel alone. This study establishes proof-of-principle for use of

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**Figure 7.** Intracellular activation of nanoparticle therapeutics.

**Figure 8.** Acyclic CB[n] solubilizing containers.
acyclic CB[n]-type containers for drug solubilization and delivery. These in vitro studies rely on drug displacement by competitive binding of 45 toward endogenous compounds whereas the corresponding in vivo application of cyclodextrin-drug complexes also benefits from the dilution of the container-drug complex from above to below K_d.

Recently, the Isaacs group discovered the templated synthesis of methylene bridged glycoluril hexamer, which can be transformed into monofunctionalized CB[7] derivatives by reaction with glycoluril bis(cyclic ethers). We adapted this chemistry to prepare CB[7] derivative 47, which bears a covalently attached biotin ligand on its convex surface. Container 47 is capable of forming complexes with chemotherapeutic agents (oxaliplatin, camtothecin, tamoxifen, temezolomide, albendazole, or irinotecan). We monitored the internalization of the fluorescent complexes 47-48 into murine lymphocytic leukemia cells (L1210) and their derived cell line L1210FR, which overexpresses biotin receptors, by confocal microscopy and flow cytometry (Figure 9). These experiments indicated enhanced uptake of 47-48 by a receptor-mediated endocytosis pathway. Subsequently, we monitored the cell viability of L1210 and L1210FR cells after treatment with targeted 47 oxaliplatin versus untargeted CB[7] oxaliplatin. We observed an approximate 10-fold decrease in EC_50 for oxaliplatin relative to CB[7] oxaliplatin. This work allowed us to measure the K_d values for 45 and 46 toward neuromuscular blocking agents 49-51 and acetylcholine 52. The affinity of 46 toward rocuronium is 300-fold higher than that of sugammadex and is 19000-fold selective for rocuronium over acetylcholine 52, which prompted us to perform in vivo reversal experiments (Figure 10c). Experimentally, rats were anesthetized with isoflurane, and their neuromuscular function was blocked by administration of rocuronium (3.5 mg kg⁻¹). Subsequent treatment with 46 (30 mg kg⁻¹) resulted in a rapid reversal of neuromuscular block as evidenced by the dramatic decrease in time required for spontaneous breathing and a return to a train-of-four (TOF) ratio of 0.9. Related experiments conducted using 45 demonstrate the reversal of neuromuscular block induced by rocuronium and also cis-atracurium, which has not been demonstrated for sugammadex. Treatment with 45 (<150 mg kg⁻¹) does not result in changes in heart rate, mean arterial pressure, or blood chemistry (pH, pO_2, or pCO_2). Further development of 45 and particularly 46 as broad spectrum reversal agents for neuromuscular block is in progress.

**CONCLUDING REMARKS**

CB[n] and CB[n]-type receptors display remarkably high affinity toward their target guests in water as exemplified by the CB[7]:30 complex with K_d = 1.4 aM. Furthermore, CB[n]-type receptors are quite selective in that different hosts display quite different binding affinities. The a value for CB[7] toward rocuronium is 300-fold higher than that of sugammadex and is 19000-fold selective for rocuronium over acetylcholine, which has not been demonstrated for sugammadex. Treatment with 45 (<150 mg kg⁻¹) does not result in changes in heart rate, mean arterial pressure, or blood chemistry (pH, pO_2, or pCO_2). Further development of 45 and particularly 46 as broad spectrum reversal agents for neuromuscular block is in progress.

**Figure 9.** In vitro targeting of 47-oxaliplatin.

**Figure 10.** Binding of NMBAs by 45 and 46 in vitro and reversal of neuromuscular block in vivo in rats.

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different affinity toward a common guest and conversely that a given host is quite selective among a pool of guests. Accordingly, CB[n]–guest derived systems are inherently stimuli responsive (e.g., pH, photochemical, electrochemical, chemical). In this Account, we presented the progression of our work in this area beginning with self-selected H-bonded molecular clips and collections of H-bonded aggregates by self-association processes and continuing to the development of stimuli responsive systems based on the extremely high affinity (K up to 10^{17} M^{-1}) and selectivity displayed by CB[n]-type containers toward their guests, which provides the free energy driving force to reconfigure multicomponent systems. For example, we constructed systems that display size-dependent homotropic allostery, controlled folding—unfolding—refolding transitions of a triazine–arylene oligomer, pH triggered guest swapping, pH promoted azobenzene trans–cis isomerization, and control over enzymatic activity of BCA. In the final sections, we showed that CB[n]-type receptors could be used to express control over biological systems both in vitro and in vivo. For example, CB[7] can be used to cloak the toxicity of gold nanoparticles, which can be decloaked by addition of 24 inside MCF-7 cells, biotin-targeted CB[7] enhances the bioactivity of oxaliplatin toward L1210FR cells 10-fold, and acyclic CB[n]-type receptors 45 and 46 can enhance the solubility and bioactivity of drugs like paclitaxel. Finally, compound 46 binds to rocuronium in vitro and reverses neuromuscular block in vivo in rats. Accordingly, the future prospects for the use of CB[n]-type receptors as stimuli responsive components to trigger reconfiguration of a system between two or more distinct states for both technological and biological applications are extremely bright.

AUTHOR INFORMATION

Corresponding Author

*Phone: 301-405-1884. Fax: 301-314-9121. E-mail: LIsaacs@umd.edu.

Notes

The authors declare no competing financial interest.

Biography

Lyle Isaacs was born in New York City in 1969 and was educated at the Bronx High School of Science, University of Chicago (B.S. 1991), University of California (M.S. 1992), and Swiss Federal Institute of Technology (Zürich), where he received his doctoral degree under the guidance of Prof. Francois Diederich in 1995. After an NIH postdoctoral fellowship with Prof. George Whitesides at Harvard, he started his independent career at the University of Maryland in 1998 and is currently Professor of Chemistry. The Isaacs group uses its knowledge of the fundamental molecular recognition properties and mechanism of CB[n] formation to create new CB[n]-type receptors for biomimetic and drug delivery applications.

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