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Metal Ion, Light, and Redox Responsive Interaction of Vesicles by a Supramolecular Switch

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Abstract: Chemical, photochemical and electrical stimuli are versatile possibilities to exert external control on self-assembled materials. Here, a trifunctional molecule that switches between an "adhesive" and a "non-adhesive" state in response to metal ions, or light, or oxidation is presented. To this end, an azobenzene–ferrocene conjugate with a flexible *N,N'*-bis(3-aminopropyl)ethylenediamine spacer was designed as a multistimuli-responsive guest molecule that can form inclusion complexes with β-cyclodextrin. In the absence of any stimulus the guest molecule induces reversible aggregation of host vesicles composed of amphiphilic β-cyclodextrin due to the formation of intervesicular inclusion complexes. In this case, the guest molecule operates as a noncovalent cross-linker for the host vesicles. In response to any of three external stimuli (metal ions, UV irradiation, or oxidation), the conformation of the guest molecule changes and its affinity for the host vesicles is strongly reduced, which results in the dissociation of intervesicular complexes. Upon elimination or reversal of the stimuli (sequestration of metal ion, visible irradiation, or reduction) the affinity of the guest molecules for the host vesicles is restored. The reversible cross-linking and aggregation of the cyclodextrin vesicles in dilute aqueous solution was confirmed by isothermal titration calorimetry (ITC), optical density measurements at 600 nm (OD_{600}), dynamic light scattering (DLS), ζ-potential measurements and cyclic voltammetry (CV). To the best of our knowledge, a dynamic supramolecular system based on a molecular switch that responds orthogonally to three different stimuli is unprecedented.

Introduction

Confinement is instrumental to all biological and physiological processes. Each living cell has a cell membrane composed of phospholipids, proteins and carbohydrates which gives rise to a closed interior separated from the extracellular environment. Recognition, adhesion and fusion of membranes are crucial to living cells since these mediate the transport of molecules between and within cells. The current boom in the field of biomimicry includes the development of complex supramolecular assemblies and soft materials which replicate and emulate biological structures and functions.[1] During the last decades, many studies have been performed to replicate cell–cell interaction by constructing artificial nanocontainers, such as liposomes, synthetic vesicles and polymersomes, which contribute to an understanding of biological processes and provide novel advances for future therapeutic applications.[2–7] Vesicles are ubiquitous in living systems and have drawn significant attention to the advancement of chemistry, biology, and material science. Fabrication of vesicles with desired size, structure, and properties facilitates the development of biomimetic systems, drug and gene delivery systems, light-harvesting systems, and microreactors.[8–15] The implantation of stimuli-responsive sites can lead to the preparation of vesicles which are receptive to light, redox, pH, temperature, or chemical stimuli.[16–18] Similarly, aggregation, adhesion and fusion of vesicles can be induced by change of pH, temperature, ionic strength, metal-ion complexation and specific molecular recognition. Several groups have investigated the interactions of vesicular systems induced by hydrogen bonding,[19–22] electrostatic attraction[23,24] and metal-ion coordination.[25–27] In the course of these investigations, it has become increasingly evident that the understanding and control of multivalent interactions at the surface of the vesicles is key to the elucidation and mimicking of recognition, adhesion and fusion of biological cell membranes. In this respect, vesicles and vesicle clusters which respond to external stimuli, such as light, redox or metal-ion binding are particularly attractive, since these stimuli can be delivered with high spatial and/or temporal resolution so that dynamic assemblies may occur that are not available in the absence of the stimulus.

In recent years we have investigated the formation of unilamellar vesicles of amphiphilic cyclodextrins (**CDV**) and the molecular recognition of guest molecules at the surface of such self-assembled nanoscale containers.[28–33] To this end, cyclodextrins (CD) are modified with long alkyl chains and short oligo(ethylene glycol) head groups (Figure 1). These macrocyclic amphiphiles form unilamellar bilayer vesicles in aqueous medium upon hydration of a thin film cast by evaporation

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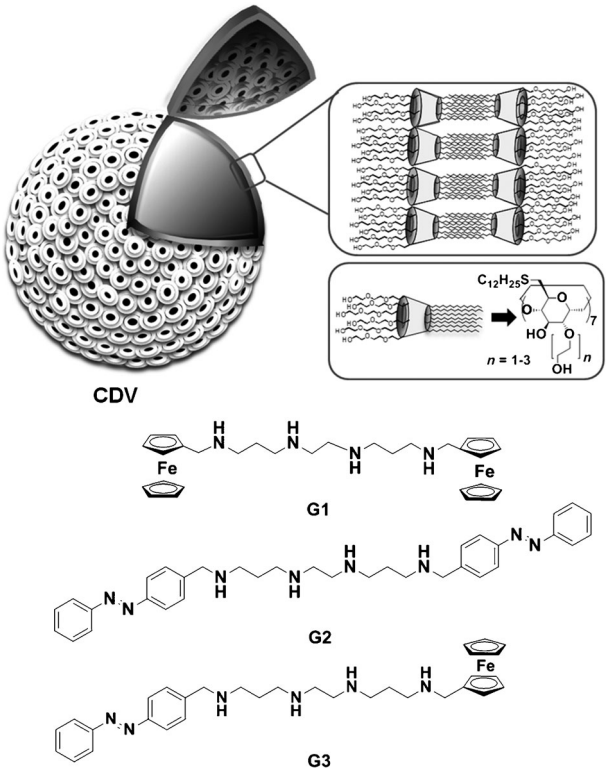

Figure 1. Schematic representation of a unilamellar β-cyclodextrin vesicle (**CDV**) and molecular structures of amphiphilic β-cyclodextrin and divalent guest molecules **G1**–**G3**.

from organic solution and extrusion through a polycarbonate membrane.[30] The cavities of the CD on the outer surface of the **CDV** are accessible to form inclusion complexes with hydrophobic guest molecules. The molecular recognition and interaction of vesicles is a simple and versatile model system to mimic recognition, adhesion and fusion of biological cell membranes.[4] We have recently demonstrated that the adhesion of **CDV** can be mediated by light-responsive and metal-responsive divalent guest molecules that act as noncovalent cross-linkers.[34,35] We have also described the self-assembly of supramolecular systems of **CDV** and azobenzene–spermine or azobenzene–carbohydrate conjugates, which can photoreversibly capture and release DNA or proteins.[36,37]

Herein, we report a dynamic supramolecular system in which aggregation and adhesion of bilayer vesicles is controlled by a multistimuli-responsive noncovalent cross-linker. We designed homofunctional as well as heterobifunctional azobenzene–ferrocene conjugates (**G1**–**G3**, Figure 1) with a *N,N′*-bis(3-aminopropyl)ethylenediamine spacer, which are used as a "supramolecular glue" to induce the aggregation and adhesion of host vesicles. The conformation and/or polarity of each guest **G1**–**G3** and hence its affinity for the **CDV** can be controlled by metal ion, irradiation and oxidation. Thus, the aggregation and disaggregation of **CDV** can be controlled by any of three external stimuli. The interaction of **CDV** and guest molecules **G1**–**G3** was investigated by isothermal titration calorimetry (ITC), optical density measurements at 600 nm (OD_{600}), dynamic light scattering (DLS), ζ-potential measurements and cyclic voltammetry (CV). To the best of our knowledge, a dynamic supramolecular system based on a molecular switch that responds orthogonally to three different stimuli is unprecedented.

Results and Discussion

Amphiphilic β-CD was synthesized as described previously.[30] **CDV** with an average diameter of about 100 nm were prepared by extrusion in a buffered aqueous solution. Guest molecules **G1**, **G2** and **G3** were prepared by the condensation of *N,N′*-bis(3-aminopropyl)ethylenediamine with ferrocenecarboxaldehyde, or 4-(phenyldiazenyl)benzaldehyde, or both, respectively, followed by the reduction of the imines with sodium borohydride. Details of the synthesis are described in the Supporting Information. The spectroscopic and analytic data for **G1**–**G3** are consistent with their molecular structure. NMR spectra are provided as Supporting Information.

Guest molecules **G1** and **G2** are homobifunctional noncovalent conjugates that carry two hydrophobic recognition units for β-CD, ferrocene and azobenzene, respectively, which are connected through the linear hydrophilic spacer *N,N′*-bis(3-aminopropyl)ethylenediamine. In contrast, guest **G3** is a heterobifunctional noncovalent linker which carries both azobenzene and ferrocene moieties as supramolecular binding sites separated by the same hydrophilic tetraamine spacer. The stimulus-responsive conformational changes of **G3** are illustrated in Figure 2. The ferrocene and azobenzene units on conjugates **G1**–**G3** are known to be good inclusion guests for β-CD.[37,38] The binding affinity of guest to **CDV** is somewhat weaker than the affinity for unmodified CDs in aqueous solution due to presence of oligo(ethyleneglycol) residues on the vesicle sur-

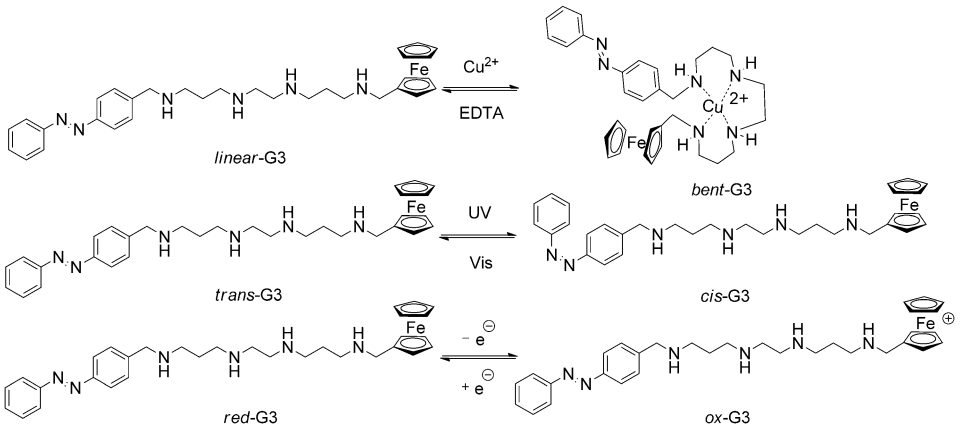

Figure 2. Stimuli-responsive conformational changes in the molecular structure of **G3**.

face.[30] Formation of host–guest complex of **G2** and **G3** with CDs should be photoresponsive because the apolar, rod-like *trans* isomer of azobenzene favors complexation with CD, whereas the polar, bent *cis* form does not.[34,36,37] The inclusion complex of **G1** and **G3** with CDs should be redox responsive due to the fact that the polar, oxidized form of ferrocene (i.e., the ferrocenium ion) has very low affinity to form inclusion complex with CD in comparison to the apolar, reduced form (i.e., ferrocene).[38] Finally, also the tetraamine linker in **G1–G3** is critical to the interaction with **CDV**: the *N,N′*-bis(3-aminopropyl)ethylenediamine spacer forms a highly stable tetradentate coordination complex with suitable metal ions ($K_a \sim 10^{17}\,M^{-1}$ for the Cu^{2+} complex of *N,N′*-dibenzylated-bis(3-aminopropyl)ethylenediamine and $K_a \sim 10^{20}\,M^{-1}$ for the Cu^{2+} complex of triethylenetetramine).[39] Due to the formation of the coordination complex, the conformation of the guest switches from linear (free linker in the absence of metal ions) to bent (complex in the presence of metal ions). The X-ray structures of such complexes are available in the literature and confirm the bent conformation of the ligand and a square-planar coordination of Cu^{2+}.[39] As a consequence, the average distance of azobenzene and ferrocene groups in **G1**–**G3** should be severely reduced upon coordination of Cu^{2+}, resulting in an *intra*vesicular rather than *inter*vesicular interaction with **CDV**.[35]

The interaction between host β-CD and guests **G1**, **G2** and **G3** was investigated by using ITC (Table 1 and Figures S1–S8 in the Supporting Information). We performed "reverse titrations" in which a 10 mM solution of β-CD host was titrated into a 0.5 mM solution of bifunctional guest. It can be seen from Table 1 that the thermodynamic parameters of the interaction are comparable for homobifunctional linkers **G1** and **G2** and similar to literature data for inclusion complexes of azobenzenes and ferrocenes with β-CD.[40] The complexation of β-CD to **G1** and **G2** is of stoichiometry 1:2 with nearly equal affinity for both ferrocene and azobenzene groups, respectively. In case of heterobifunctional linker **G3**, we calculated two different binding constants for stepwise binding phenomena (Scheme S1 in the Supporting Information). The azobenzene and ferrocene moieties have almost similar affinity toward β-CD and the groups are distant from each other so that they act as independent binding sites. It can be observed that the second binding constant (K_2) is only slightly higher than the first binding constant (K_1; Table 2). For comparison, we also carried out titrations for the complexation of β-CD with ferrocenecarboxylic acid and azobenzenecarboxylic acid and it can be seen that in those cases the stoichiometry is 1:1 and the association constants $K_a = 1.93 \times 10^3\,M^{-1}$ for ferrocenecarboxylic acid and $2.48 \times 10^3\,M^{-1}$ for azobenzenecarboxylic acid are in accordance with the literature.[40] Each titration was performed at pH 7.4 as well as pH 9.2 and the affinity of guest moieties towards β-CD is very similar at those pH values (Tables 1 and 2).

Table 1. Thermodynamic parameters for the interaction of homobifunctional guest molecules (**G1** and **G2**) and monomers (ferrocenecarboxylate and azobenzenecarboxylate) with β-CD.

Host	Guest	*n*	K_a [M^{-1}]	ΔH [kJ mol^{-1}]	ΔG [kJ mol^{-1}]
β-CD	**G1** (pH 7.4)	2	2291	−19.5	−17.8
β-CD	**G1** (pH 9.2)	2	2303	−22.3	−19.2
β-CD	**G2** (pH 7.4)	2	2876	−11.8	−19.7
β-CD	**G2** (pH 9.2)	2	2040	−7.1	−17.9
β-CD	ferrocenecarboxylate (pH 9.2)	1	1932	−7.6	−24.9
β-CD	azobenzenecarboxylate (pH 9.2)	1	2477	−5.1	−20.2

Table 2. Thermodynamic parameters for the interaction of heterobifunctional guest molecule **G3** with β-CD.

Host	Guest	*n*	K_1 [M^{-1}]	K_2 [M^{-1}]	ΔH_1 [kJ mol^{-1}]	ΔH_2 [kJ mol^{-1}]	ΔG_1 [kJ mol^{-1}]	ΔG_2 [kJ mol^{-1}]
β-CD	**G3** (pH 7.4)	2	812	1259	−7.2	−14.8	−16.6	−17.7
β-CD	**G3** (pH 9.2)	2	800	1369	−7.9	−10.4	−16.6	−17.9

Having verified the formation of inclusion complex between guest molecules **G1**, **G2** and **G3** and β-CD, we investigated the stimulus-responsive aggregation of **CDV** induced by each of the divalent guest molecules. We first turned our attention to the metal-ion responsive aggregation of **CDV**. All experiments were carried out in dilute aqueous solution at pH 9.2 to exclude the detrimental effect of protonation of **G1**–**G3**, which would reduce the affinity of **G1**–**G3** for metal coordination. The addition of as little as 15 μM of **G1**, **G2** or **G3** to a dilute solution of **CDV** (30 μM of amphiphilic β-CD) at pH 9.2 caused a time-dependent increase of OD_{600} from about 0.05 to approximately 0.5 (Figure 3A). The average particle size increased from about 100 nm to approximately 1000 nm according to DLS (data shown for **G3** in Figure 4D). The rate and extent of the vesicle aggregation depends on the concentration of guest molecule: fast and extensive aggregation occurred at a higher concentration of linker, whereas at low concentration the rate of aggregation was much slower (data shown for **G3** in Figure 3A, inset). The initial OD_{600} was quickly recovered by addition of excess host β-CD and α-CD (1 mM; Figure S9 in the Supporting Information). This result indicates that guest molecules **G1**–**G3** trigger the aggregation of **CDV** due to the selective formation of multivalent intervesicular host–guest complexes, which are readily disrupted in the presence of an excess of host in solution.

Interestingly, when 50 μM of Cu^{2+} is added to the aggregated complex of **CDV** and linkers (**G1**, **G2** and **G3**), both the original OD_{600} (ca. 0.05; Figure 3B) and the average vesicle diameter (ca.100 nm) were rapidly and efficiently recovered (data shown for **G3** in Figure 4D, blue trace). This observation can be explained by the fact that all cross-linkers form stable metal-coordination complexes with Cu^{2+} and rearrange from a linear to a bent conformation (Figure 2). We suggest that the bent conformational isomer of **G1**, **G2** and **G3** can only bind to the surface of one vesicle and intervesicular complexation is not feasible. For example, in the bent conformation of **G3** the azobenzene and the ferrocene groups are so close to each

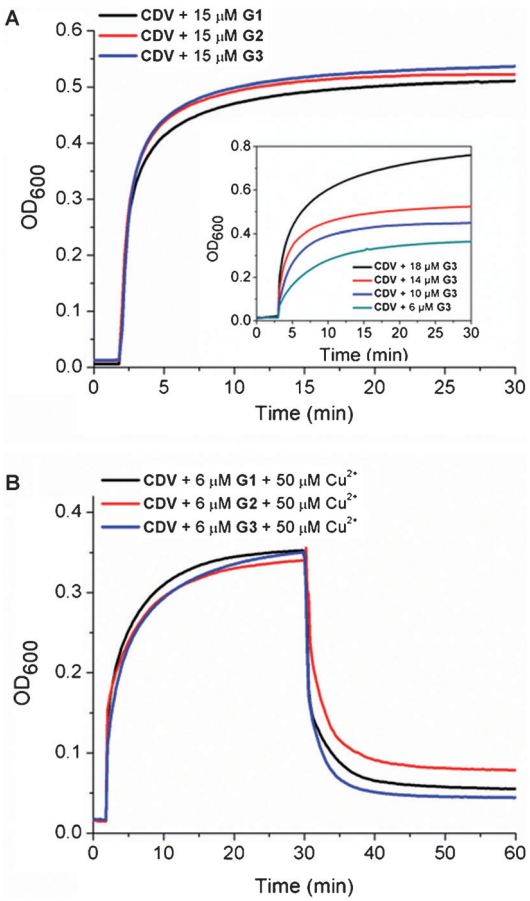

Figure 3. Metal-ion responsive aggregation of host vesicles of β-CD (**CDV**) by divalent guests **G1**, **G2** and **G3**. A) Time-dependent measurement of OD_{600}. Inset: concentration-dependent aggregation of **CDV** and **G3**. B) Dispersion of aggregates in the presence of Cu^{2+}. Typical conditions: [**CDV**] = 30 μM, [**G1**] = [**G2**] = [**G3**] = 6 to 18 μM, [Cu^{2+}] = 50 μM in 20 mM carbonate/bicarbonate buffer (pH 9.2). Guest was added after 2 min and Cu^{2+} was added after 30 min.

other that only one of them can form inclusion complex with **CDV**. The coordination-induced conformational change of the linker causes the dispersion of the vesicles. We note that these observations are fully consistent with our previous report on comparable noncovalent linkers with *tert*-butylbenzyl instead of azobenzene and ferrocene moieties.[35] In that study, we had also noticed that there is no significant fusion of vesicles while metal-ion coordination with guest at the vesicle surface.[35] However, the linker concentration plays a critical role in the metal responsive dispersion of vesicles. The addition of as much as 100 μM of Cu^{2+} to a higher concentration of **G1**–**G3** (12 μM) in the aggregated solution of **CDV** did not induce any substantial changes in either OD_{600} or average particle size (Figure S10 in the Supporting Information). This phenomenon is attributed to the fact that at a high concentration of guest molecule at the vesicle surface, a 2:1 intermolecular metal coordination takes place, which does not lead to the change in conformation of **G1**–**G3**. Furthermore, the metal-ion induced dispersion of vesicles can be reversed in the presence of strong metal chelator, such as EDTA. The addition of 200 μM EDTA to a solution of **CDV** in the presence of **G3**-coordinated Cu^{2+} resulted in reaggregation of the **CDV**, as can be seen from the increase in OD_{600} from about 0.05 to 0.3 (Figure 4A and B) and the average vesicle diameter from about 100 to 900 nm (Figure 4D; turquoise trace). These measurements show that EDTA scavenges the Cu^{2+} ion and allows the relaxation of **G3** from a bent conformation to a linear conformation, which induces the reaggregation of **CDV**.

In addition, ζ-potential measurements were performed to further investigate the interaction of **G3** with **CDV** in the presence of metal ions. As shown in Figure 4C, the ζ-potential of **CDV** is about −19.5 mV at pH 9.2. The negative surface potential arises due to the oligo(ethylene glycol) residues on the vesicle surface.[30] The surface potential increases from about −19.5 to −11.2 mV after addition of **G3**. The increase of surface potential is due to the presence of guest **G3** on the surface of vesicle aggregates. The subsequent addition of Cu^{2+} induces the disaggregation of vesicles and the resultant ζ-potential further increases to about −7.2 mV. This increment of surface potential indicates the metal-ion coordination with guest **G3** at the vesicle surface. The addition of excess EDTA decreases the ζ-potential from about −7.2 mV to approximately −11.5, which confirms the removal of Cu^{2+} from the vesicle surface.

Taken together, the results shown above provide compelling evidence for the metal-ion responsive aggregation of **CDV** by divalent guests **G1**–**G3**. In the absence of metal ion, the divalent guest operates as a noncovalent cross-linker that aggregates the **CDV**. In the presence of metal ion, the divalent guest folds into a tetradentate complex that cannot act as intervesicular cross-linker. Hence, the metal ion induces a conformational change that reversibly switches **G1**–**G3** from an "adhesive" to a "non-adhesive" state.

Next, we investigated the light-responsive interaction of **CDV** with **G2** and **G3**. The homobifunctional guest **G2** and heterobifunctional guest **G3** both have azobenzene groups that can form inclusion complexes with β-CD. The formation of the host–guest complex of azobenzene is light-responsive: only the *trans*-azobenzene is an appropriate guest for β-CD, the *cis*-azobenzene is not. Hence, **G3** can bind two molecules of β-CD when the azobenzene moiety is in the *trans* form, but only one molecule of β-CD when it is in the *cis* form (ferrocene still can bind to β-CD in *cis*-**G3** form). On the other hand, *trans*-**G2** forms an inclusion complex with two molecules of β-CD but its *cis* isomer does not bind to any of the β-CDs. As discussed above, the addition of divalent guests **G2** or **G3** to a dilute solution of **CDV** induces a rapid aggregation of **CDV** (Figure 3A). It is likely that both *trans*-**G3** and *trans*-**G2** act as supramolecular cross-linkers of **CDV** by forming 2:1 host–guest inclusion complexes with β-CD on the surface of two different vesicles, consistent with our earlier observations on divalent azobenzenes.[34] The photoisomerization of **G3** occurs readily and results in a photoreversible aggregation and dispersion of **CDV**, simply by irradiation at 350 nm (to isomerize *trans*-**G3** to *cis*-**G3**) followed by irradiation at 455 nm (to obtain *trans*-**G3** from *cis*-**G3**; Figure 5). Selected data for the photoresponsive aggregation of **CDV** mediated by **G2** are provided in the Supporting Information (Figure S11). Upon 40 min UV irradiation of a binary mixture of **CDV** and *trans*-**G3** decreases both the

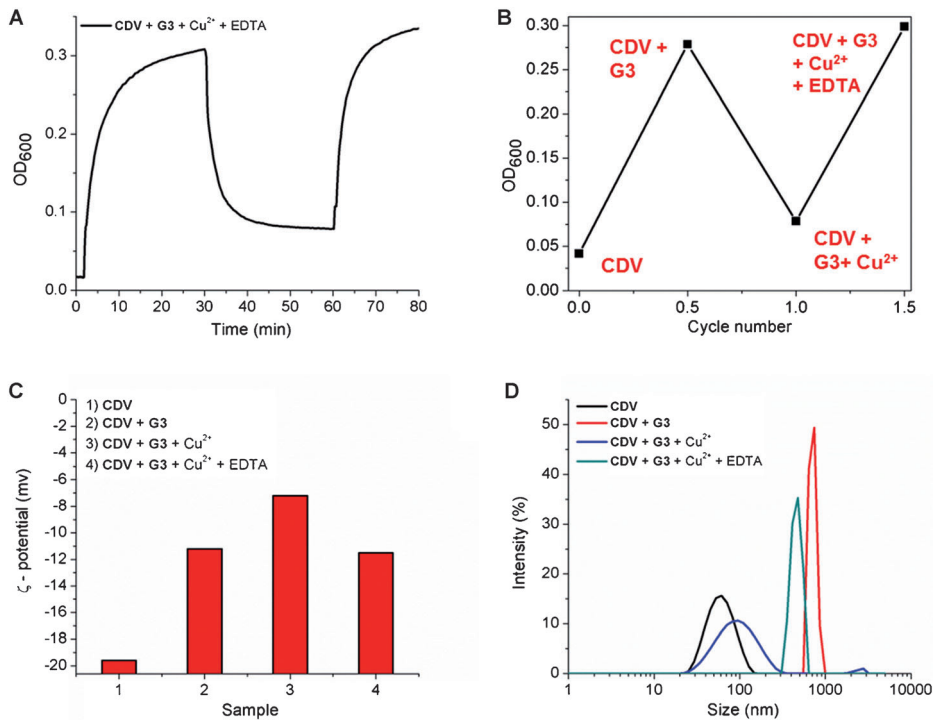

Figure 4. Metal-ion responsive aggregation of host vesicles of β-CD (**CDV**) by divalent guest **G3**. A) Aggregation, disaggregation and reaggregation of **CDV** induced by G3, Cu^{2+} and EDTA, respectively, monitored by OD_{600}. **G3** was added after 2 min, Cu^{2+} after 30 min and EDTA after 60 min. B) Reversible metal-ion responsive aggregation of **CDV**. C) The ζ-potential of CDV and CDV clusters. D) Size distribution according to DLS. Typical conditions: [**CDV**] = 30 μM, [**G3**] = 6 μM, [Cu^{2+}] = 50 μM (15 μM for ζ-potential measurement) and [Na_2EDTA] = 200 μM in 20 mM carbonate/bicarbonate buffer (pH 9.2).

OD_{600} from roughly 0.3 to 0.05 (Figure 5A, black trace) and average particle size from 900 nm to about 90 nm (Figure 5C, blue trace). UV irradiation (350 nm) induces photoisomerization of *trans*-**G3** to *cis*-**G3**, and the azobenzene moiety (not the ferrocene moiety) detaches from the **CDV** surface, since only *trans*-**G3** can bind to β-CD. As a result the divalent linker **G3** transforms into a monovalent guest molecule, and dispersion of **CDV** takes place. Upon subsequent visible-light irradiation of the dispersed **CDV** at 455 nm for 40 min (to obtain *trans*-**G3** from *cis*-**G3**), OD_{600} increases from about 0.05 to 0.3 (Figure 5A, red trace) and the average particle size increases from about 90 nm to approximately 850 nm (Figure 5C, green trace). The OD_{600} and DLS measurements clearly indicate that the complex of **CDV** and cross-linker *trans*-**G3** reassembles upon visible irradiation. The reversibility of photoinduced aggregation of vesicles is evident over three cycles, provided that the irradiation time is sufficient (40 min at both 350 and 455 nm) and the vesicle concentration is limited to 30 μM (Figure 5B).

The results shown in this section provide clear cut evidence for the light-responsive aggregation of **CDV** by divalent guests **G2** and **G3**. Alternating UV and visible irradiation induces an isomerization of the azobenzene that reversibly switches **G2** and **G3** from an "adhesive" *trans* state to a "non-adhesive" *cis* state.

Finally, we investigated the redox-responsive interaction of **CDV** with **G1** and **G3**. The homobifunctional guest **G1** and heterobifunctional guest **G3** both have ferrocene groups that can bind to β-CD. It is well known that ferrocene and β-CD are a redox-responsive molecular recognition pair: β-CD can form stable inclusion complexes with the reduced form (ferrocene) but not with the oxidized form (ferrocenium ion).[38,41] The redox behavior of **G1** and **G3** was investigated by CV in the absence and presence of β-CD (Figure 6). From these voltammograms, the potential of oxidation and reduction peaks (E_{pa} and E_{pc}, respectively) and peak current at oxidation and reduction (i_{pa} and i_{pc}, respectively) were determined and are listed in Table 3. In the absence of β-CD, both guest molecules **G1** and **G3** show a single redox wave in the potential range of −0.4 to

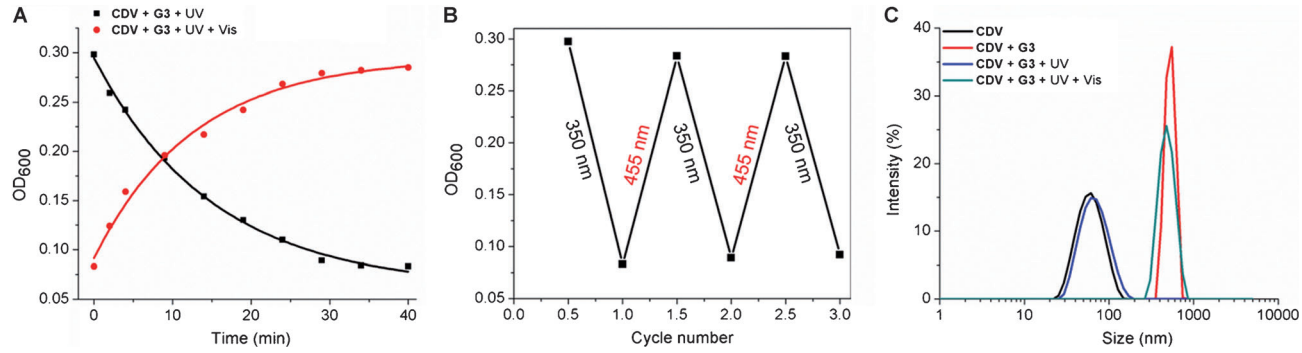

Figure 5. Light-responsive reversible aggregation and dispersion of host vesicles of β-CD (**CDV**) by divalent guest **G3**. A) Time-dependence of OD_{600}. B) Reversible switching of OD_{600} under alternate UV (350 nm) and visible (455 nm) light irradiation. C) Size distribution according to DLS. Typical conditions: [**CDV**] = 30 μM, [**G3**] = 6 μM in 20 mM phosphate buffer (pH 7.4).

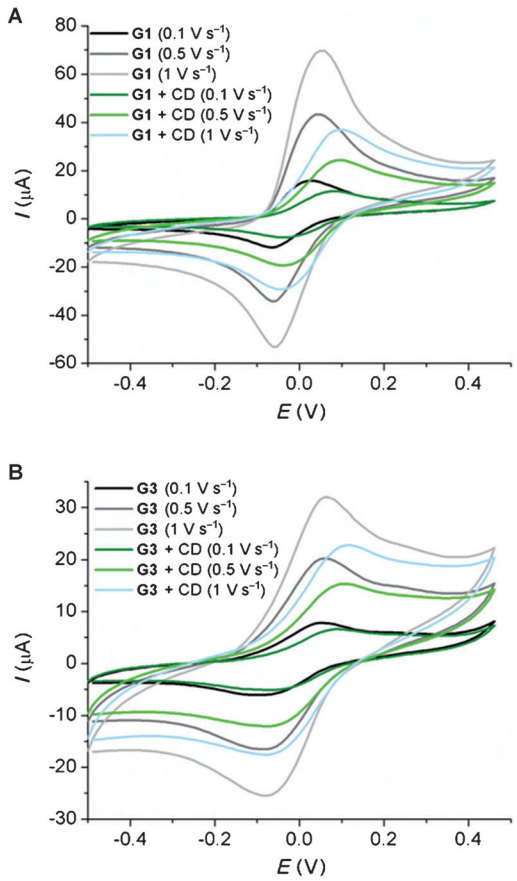

Figure 6. Cyclic voltammetry of guests **G1** and **G3** in the absence and presence of β-CD. A) *I* versus *E* voltammogram of **G1** in the absence (black, deep grey and light grey traces) and presence (deep green, light green, cyan traces) of β-CD. B) *I* versus *E* voltammogram of **G3** in the absence (black, deep grey and light grey traces) and presence (deep green, light green, cyan traces) of β-CD. Concentration: [**G1**] = [**G3**] = 1 mM, [β-CD] = 10 mM. Scan rates: 0.1, 0.5 and 1 V s^{-1}.

Table 3. Results of CV measurements for 1 mM **G1** and **G3** in the absence and presence of 10 mM β-CD at 0.1 V s^{-1} scan rate.

Guest	E_{pa} [V]	E_{pc} [V]	i_{pa} [μA]	i_{pc} [μA]
G1	0.026	−0.065	15.8	−11.8
G1 + β-CD	0.095	−0.042	11.3	−7.68
G3	0.049	−0.099	7.80	−6.07
G3 + β-CD	0.095	−0.065	6.59	−5.10

+0.4 V versus Ag/AgCl. These observation indicate that both ferrocene moieties in **G1** undergo the redox reaction independently, presumably because of the relatively long *N,N′*-bis(3-aminopropyl)ethylenediamine spacer. The values of E_{pa} and E_{pc} (for both **G1** and **G3**) increase in the presence of β-CD, indicating that oxidation of ferrocene moieties requires a higher potential in the presence of β-CD due to the stable inclusion complex formation. The values of i_{pa} and i_{pc} in the presence of β-CD are smaller than those in the absence, which indicates the fact that β-CD retards redox reaction on the electrode surface. The peak current values of **G1** are almost two-times higher than those of **G3** because of two ferrocene moieties in homobifunctional **G1**. We measured CV at three different scan rates and the above observations are essentially identical in all the experiments. In addition, since the differences between E_{pa} and E_{pc} are larger than 0.057, the redox reactions in all cases are not diffusion controlled under the conditions of the experiment.

As discussed above, the addition of divalent guests **G1** or **G3** to a dilute solution of **CDV** induces a rapid aggregation of **CDV** (Figure 3 A). The ferrocene groups can be readily oxidized chemically with sodium hypochlorite solution.[42] To investigate the redox reversibility of the **CDV** aggregation, a 30 μM solution of NaOCl was added to the aggregated mixture of **CDV** and ferrocene appended guests **G1** and **G3**. The OD_{600} decreases from about 0.45 to approximately 0.05 (Figure 7 A) and the average particle size decreases to about 100 nm from approximately 1000 nm (Figure 7 B). The in situ oxidation of ferrocene moieties to ferrocenium ions transforms the divalent homobifunctional guest **G1** to an inert and highly water soluble compound and divalent heterobifunctional guest **G3** to a monovalent guest for **CDV**. The in situ reduction of ferrocenium ion to ferrocene with excess potassium iodide resulted in renewed aggregation of the **CDV** (Figure S12 in the Supporting Informa-

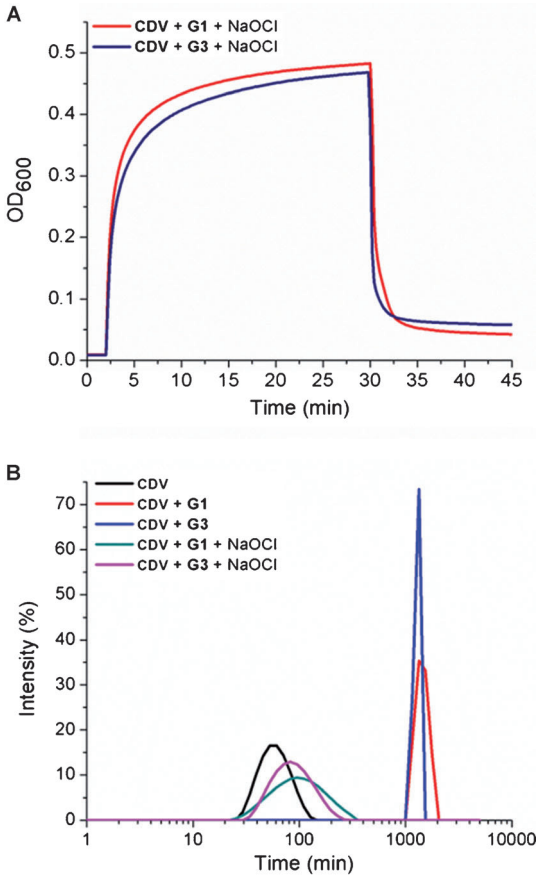

Figure 7. Redox-responsive reversible aggregation dispersion of vesicles of β-CD (**CDV**) by divalent guest **G3**. A) Aggregation and disaggregation of CDV monitored by OD_{600}. **G1** or **G3** was added after 2 min, NaOCl after 30 min. B) Size distribution according to DLS. Typical concentrations: [**CDV**] = 30 μM, [**G3**] = 15 μM, [NaOCl] = 30 μM (for **G3**) or 60 μM (for **G1**) in 20 mM phosphate buffer (pH 7.4).

tion). A complete reduction proved to be difficult due to the competing redox reaction of oxidizing and reducing agents. We conclude that the guest molecules **G1** and **G3** induce vesicle aggregation which can be fully disrupted by the addition of an oxidizing agent, and that in principle the aggregation can be restored by the addition of excess reducing agent.

Conclusion

A trifunctional molecule with a flexible *N,N'*-bis(3-aminopropyl)ethylenediamine spacer was used as a noncovalent cross-linker that induces aggregation and adhesion of host vesicles composed of amphiphilic β-CDs by the formation of host guest inclusion complexes at the surface of the vesicles. In response to external stimuli (metal ion, light and oxidation) the guest molecule changes its conformation and polarity and hence loses its affinity for the host vesicles, which strongly decreases intervesicular binding and causes dispersion of vesicle clusters. The reversible transition from the aggregated state to the dispersed state of vesicles was mediated effectively by addressing a single molecular switch selectively with metal ion, light or redox chemistry. To the best of our knowledge, a dynamic supramolecular system based on a molecular switch that responds orthogonally to three different stimuli is unprecedented. We anticipate that the proof-of-concept demonstrated here for the aggregation of nanoscale vesicles into microscale vesicle clusters can be easily adapted to the multistimulus responsive assembly of other materials, such as hydrogels, polymers, colloids and nanoparticles in solution as well as on surfaces.

Experimental Section

Materials

All chemicals used in this study were purchased from Acros Organics (Schwerte, Germany) or Sigma–Aldrich Chemie (Taufkirchen, Germany) and used without further purification. β-Cyclodextrin was kindly donated by Wacker Chemie (Burghausen, Germany). All solvents used in reactions and purification were dried according to conventional methods. All aqueous solutions were prepared in Milli-Q water.

Synthesis

The synthesis of **G1**, **G2** and **G3** are described in the Supporting Information. The spectroscopic and analytical data for **G1**–**G3** are consistent with their molecular structure. The synthesis of amphiphilic β-CD was performed as reported previously.[30] All reactions were carried out in oven-dried glassware and magnetically stirred under an inert gas atmosphere. Analytical TLC was performed on Merck silica gel 60 F_{254} plates. All compounds were visualized either by UV light or by dipping in basic permanganate solution. Column chromatography was carried out by using silica gel 60 (230–400 mesh). ^{1}H and ^{13}C NMR spectroscopic measurements were carried out by using Bruker ARX 300 MHz or Varian 500 MHz INOVA spectrometers. Chemical shifts were referenced to internal standards $CDCl_3$ ($\delta = 7.26$ ppm for ^{1}H and 77.0 ppm for ^{13}C) or TMS ($\delta = 0.00$ ppm for ^{1}H and ^{13}C). High-resolution mass spectrometry (HRMS) was performed by using a Bruker MicroTof instrument.

Preparation of vesicles

Unilamellar bilayer vesicles (**CDV**) were prepared by extrusion. Several milligrams of amphiphilic β-CD in 2–3 mL of chloroform were dried by slow rotary evaporation to yield a thin film in a 10 mL round-bottom flask. Residual solvent was removed under high vacuum. Aqueous buffer (10 mL; 20 mM phosphate buffer, pH 7.4 or 20 mM carbonate/bicarbonate buffer, pH 9.2) was added and stirred, overnight. The resulting suspension was repeatedly passed through a polycarbonate membrane with 100 nm pore size in a Liposofast manual extruder.

Isothermal titration calorimetry (ITC)

ITC was performed by using a Nano-isothermal titration calorimeter low volume equipped with a 200 μL gold cell (model SNL 10099; TA Instrument Waters, Lindon, Utah, USA). ITC measurements were performed in 20 mM phosphate buffer or carbonate/bicarbonate buffer (in 10% DMSO). A 10 mM solution of β-CD was titrated into a 0.5 mM solution of guest molecules. For each titration, 20 injections of 2.5 μL were performed with an interval of 300 s. The stirring rate was 300 rpm.

UV/Vis spectroscopy

Optical density measurements at 600 nm were carried out at in 1.5 mL disposable cuvettes with dimensions 12.5×12.5×45 mm and 10 mm path-length using an Uvikon 923 double-beam spectrophotometer (Kontron Instruments). Measurements were performed for 30 to 90 min with data points collected every 12 s. Freshly prepared vesicles and metal ion solutions were used for each measurement and the measurement procedure was as follows: for example, 1 mL solutions of **CDV** (30 μM in 20 mM phosphate buffer, pH 7.4 or 20 mM carbonate/bicarbonate buffer, pH 9.2) were taken in a semimicro disposable cuvette and OD_{600} was measured for 2 min. After 2 min, 7.5 μL of guest **G3** (2 mM solution in DMSO) was added to make the resultant concentration 15 μM (approximately 100% surface coverage of vesicles) in the cuvette (this addition was done with slight mixing within a single interval of 12 s) and the measurement was carried out for at least 30 min. After 30 min a few microliters of concentrated metal ion solution in Millipore water was added to the above solution for metal-ion responsive experiments. The same measurements were performed with the other samples by following the above procedure. Typical concentrations: [**CDV**] = 30 μM, [**G1**] = [**G2**] = [**G3**] = 5–50 μM, [$CuCl_2$] = 50–100 μM and [Na_2H_2EDTA] = 100–200 μM in corresponding buffer solution.

Dynamic light scattering (DLS) and ζ-potential measurements

DLS measurements were performed by using a Malvern Nano-ZS instrument (Malvern Instruments) with low-volume disposable cuvettes kept at 25 °C. The average size of **CDV** and mixtures of vesicles of **CDV** and guests **G1**–**G3** were measured after mixing the corresponding components. Immediately after alternate UV (350 nm, 30 min) and visible light (455 nm, 30 min) irradiations, the corresponding average size of the binary complex was measured. Typical concentrations: [**CDV**] = 30 μM, [**G1**] = [**G2**] = [**G3**] = 5–50 μM, [$CuCl_2$] = 50–100 μM and [Na_2H_2EDTA] = 100–200 μM in corresponding buffer solution. Similarly, ζ-potential measurements were performed using a Malvern Nano-ZS instrument (Malvern Instruments) in disposable folded capillary cells at 25 °C.

Irradiation experiments

Two different light sources were utilized for irradiation experiments. One source was a Rayonet photochemical reactor (The Southern New England Ultraviolet Company) equipped with 16 RPR-3500 lamps used to generate UV light (350 nm) to isomerize azobenzene moieties from *trans* to *cis*. The other source was a Philips Lumileds royal blue LUXEON K2 emitter (LXK2-PR14-Q00) used to generate visible light (455 nm) to isomerize azobenzene moieties from *cis* to *trans*. The irradiation time was 30 min in either case.

Cyclic voltammetry (CV)

CV measurements were performed in a glass cell. From the top of the cell the working electrode (glassy carbon), the reference electrode (Ag/AgCl) and the counter electrode (Pt) were placed inside and the cell was filled with a degassed solution of tetrabutylammonium tetrafluoroborate in acetonitrile (0.1 M). For potential generation and measurement an Autolab potentiostat (Eco Chemie, Netherlands) was used with the software GPES. Integration and detection of peak locations of the CV curves was done with the software supplied, all other calculations were done using Origin (Origin Labs, version 8 SR0). Typical concentrations: [β-CD] = 10 mM, [**G1**] = [**G3**] = 1 mM.

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