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Photoresponsive Molecular Recognition and Adhesion of Vesicles in a Competitive Ternary Supramolecular System

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Abstract: A competitive photoresponsive supramolecular system is formed in a dilute aqueous solution of three components: vesicles of amphiphilic α-cyclodextrin host **1a**, divalent *p*-methylphenyl guest **2** or divalent *p*-methylbenzamide guest **3**, and photoresponsive azobenzene monovalent guest **5**. Guests **2** and **3** form weak inclusion complexes with **1a** ($K_a \approx 10^2\,M^{-1}$), whereas azobenzene guest **5** forms a strong inclusion complex ($K_a \approx 10^4\,M^{-1}$), provided it is in the *trans* state. The aggregation and adhesion of vesicles of host **1a** is mediated by guest **2** (or **3**) due to the formation of multiple intervesicular noncovalent links, as confirmed by using isothermal titration calorimetry (ITC), optical density measurements at 600 nm (OD600), dynamic light scattering (DLS), and cryogenic transmission electron microscopy (cryo-TEM). The addition of excess monovalent guest *trans*-**5** to vesicles of **1a** aggregated by divalent guest **2** (or **3**) causes the dispersion of vesicles of **1a** because *trans*-**5** displaces **2** (as well as **3**) from the vesicle surface. Upon UV irradiation of a dilute ternary mixture of vesicles of **1a**, guest **2** (or **3**), and competitor *trans*-**5**, compound *trans*-**5** isomerizes to *cis*-**5**, and renewed aggregation of vesicles of **1a** by guest **2** (or **3**) occurs because **2** (as well as **3**) displaces *cis*-**5** from the vesicle surface. Subsequent visible irradiation causes the redispersion of vesicles of **1a** because *cis*-**5** reisomerizes into *trans*-**5**, which again displaces guest **2** (or **3**) from the vesicle surface. In this way, the competitive photoresponsive aggregation and dispersion of vesicles can be repeated for several cycles.

Keywords: cyclodextrins · host–guest systems · molecular recognition · photoresponsive systems · vesicles

Introduction

One of the main challenges in nanotechnology is the preparation of well-defined, self-assembled supramolecular materials with dynamic and adaptive properties that emulate biological systems. A number of reports have demonstrated that sophisticated and stimuli-responsive materials and surfaces can be assembled by a careful combination of orthogonal noncovalent interactions.[1–8] The photoisomerization of azobenzene has been the molecular basis for a range of photosensitive supramolecular materials,[9–25] including photoresponsive vesicles.[26–30] Azobenzenes constitute a well-known class of photoresponsive compounds that can be reversibly isomerized from *trans* to *cis* by irradiation at 350 nm and from *cis* to *trans* by irradiation at 455 nm. Also, the inclusion of azobenzene as a guest into a cyclodextrin (CD) host is photoresponsive: the rod-like and apolar *trans* isomer forms a stable inclusion complex with α- (α-CD) and β-cyclodextrin (β-CD), whereas the bent and polar *cis* isomer does not fit into either CD. The photocontrolled molecular recognition of CDs with azobenzenes has been used to develop photoresponsive host molecules,[31,32] hydrogels,[33–40] molecular shuttles,[41,42] micelles and vesicles,[43–46] ion channels,[47] surfaces,[48,49] and drug-delivery vehicles.[50]

In recent years we have explored the formation of vesicles of amphiphilic CDs and the molecular recognition of guest molecules at the surface of such host vesicles.[51–60] The molecular recognition and interaction of bilayer vesicles is a versatile model system for the recognition, adhesion, and fusion of biological cell membranes.[61] We have recently described a straightforward binary supramolecular system in which the photoisomerization of a divalent noncovalent diazobenzene linker can be used as a trigger to induce, as well as reverse, the molecular recognition and adhesion of CD vesicles in two directions: adhesion by visible light and dispersal by UV light.[62] If the supramolecular linker is in the thermally stable *trans* state, it induces aggregation and adhesion of CD vesicles and if the linker is in the *meta*-stable *cis* state, it does not induce any significant aggregation.

Herein, we describe a supramolecular system in which aggregation and adhesion of bilayer vesicles is controlled by a photoresponsive competitor. This system operates in dilute

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aqueous solution under ambient conditions. In this ternary supramolecular system, a photoresponsive molecule is used as a competitive switch that either permits or inhibits the adhesion of vesicles induced by a weak, noncovalent, linker molecule. The ternary photoresponsive supramolecular system is based on the host–guest interactions of vesicles of amphiphilic CD **1a** with bis(*p*-methylphenyl) derivative **2** or bis(*p*-methylbenzamide) derivative **3** and azobenzene derivative **5**. The ternary photoresponsive system is illustrated in Figure 1.

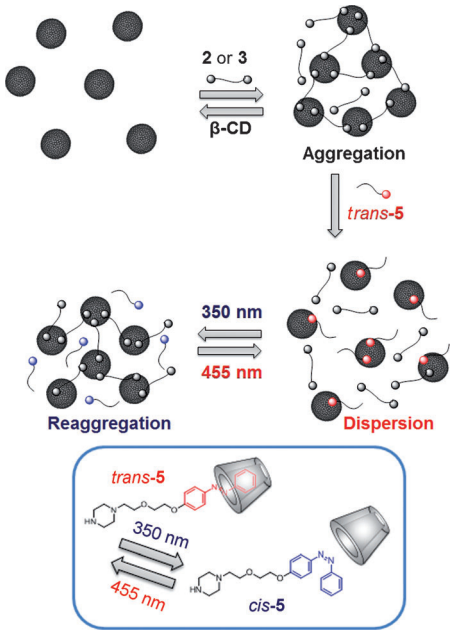
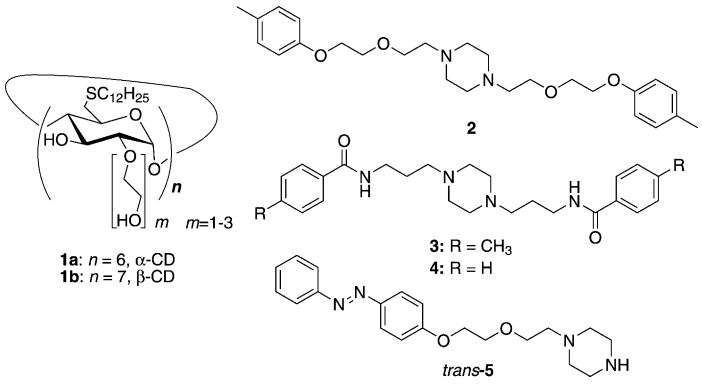


Figure 1. Schematic representation of a photoresponsive supramolecular system in which aggregation and adhesion of CD vesicles is mediated by noncovalent linker **2** or **3** in the presence of a photoresponsive competitive guest **5**. The photoresponsive interactions of guest **5** with CD are shown in the inset.



As a consequence of the involvement of a photoresponsive competitor (monovalent guest **5**), the aggregation and adhesion of CD vesicles induced by the weak, noncovalent linker (guest **2** or **3**) is photoresponsive in two directions: adhesion by UV light and dispersal by visible light. If the competitor is in the thermally stable *trans* state, it inhibits vesicle aggregation and if the competitor is in the *metastable* *cis* state, it enables a rapid aggregation and adhesion of vesicles. Therefore, this ternary competitive photoresponsive system is complementary to the earlier binary photoresponsive system.[62] The interaction of CD vesicles composed of **1a** and **1b**, and guests **2**, **3**, **4**, and **5** was investigated by optical density measurements at 600 nm (OD600), dynamic light scattering (DLS), and cryogenic transmission electron microscopy (cryo-TEM).

Results and Discussion

Amphiphilic α-CD **1a** and β-CD **1b** were synthesized as described previously.[51–54] Unilamellar CD bilayer vesicles with a diameter of about 100 nm were prepared by extrusion in buffer at pH 7.4.[54] Guest molecules **2**, **3**, **4**, and **5** were synthesized as reported in the Supporting Information. The analytical and spectroscopic data for **2**, **3**, **4**, and **5** are consistent with their molecular structure. Guest molecule **2** is a homobifunctional, noncovalent linker that carries two identical supramolecular hydrophobic binding sites: *p*-methylphenyl groups connected through a hydrophilic piperazine moiety substituted with diethylene glycol spacers at each nitrogen atom. Each *p*-methylphenyl group of **2** forms weak inclusion complexes ($K_a \approx 100\,M^{-1}$) with α- and β-CD.[63] However, it is anticipated that the diethylene glycol spacers enhance the tendency of **2** to form pseudorotaxane inclusion complexes with both CDs.[64,65] Guest molecules **3** and **4** are homobifunctional, noncovalent linkers that carry two identical supramolecular hydrophobic binding sites, *p*-methylbenzamide groups and benzamide groups, respectively, connected through a hydrophilic 1,4-bis(3-amidopropyl)piperazine linker with an amido linkage. Each *p*-methylbenzamide group of **3** and each benzamide group of **4** form weak inclusion complexes ($K_a \approx 100\,M^{-1}$) with α- and β-CD.[63] Also in this case, it is anticipated that the propylene spacers enhance the tendency of **3** and **4** to form pseudorotaxane inclusion complexes with both CDs.[64,65]

In contrast, guest **5** is a monovalent guest that carries only one supramolecular hydrophobic binding site: an azobenzene group that forms an inclusion complex with α- and β-CD. The formation of the host–guest complex of **5** with CDs should be photoresponsive: only the *trans*-azobenzene is a suitable guest for α- and β-CD, the *cis*-azobenzene is not. The reversible *trans–cis* isomerization of **5** under UV and visible-light irradiation is shown in Figure S1 in the Supporting Information. The interaction between hosts α- or β-CD, respectively, and guest *trans*-**5** was measured by using isothermal titration calorimetry (ITC). Since *trans*-**5** is amphiphilic, it will probably form micelles above its critical micelle concentration, which may affect the ITC measurement. To avoid this artifact, we performed "inverse titrations" in which a 10 mM solution of α- or β-CD host was titrated into a 1.0 mM solution of *trans*-**5** (Figure 2). At this concentra-

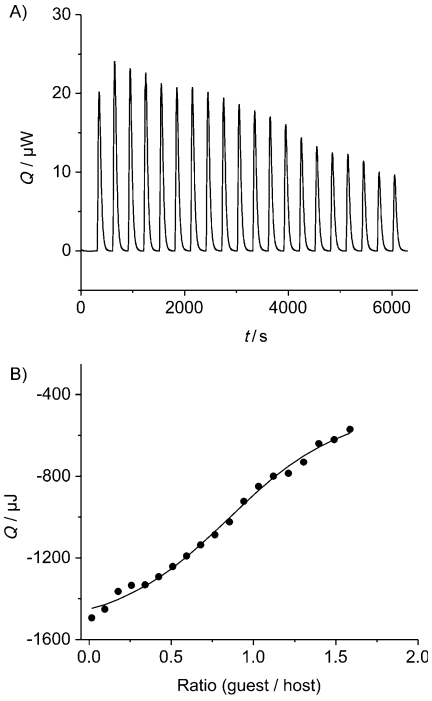


Figure 2. ITC data corresponding to the host–guest interaction of α-CD with *trans*-**5**. A 10 mM solution of α-CD was titrated into a 1.0 mM solution of *trans*-**5**. A) Injection peaks (raw data vs. time) and B) integration of the injection peaks (heat vs. guest/host ratio) are shown. In the ITC fit (——), the first data point (●) is omitted.

Table 1. Thermodynamic data for the host–guest interaction of α- and β-CD with *trans*-**5**.

Host	Guest	K_a [M^{-1}]	n	ΔH [kJ mol^{-1}]	ΔS [J K^{-1} mol^{-1}]	ΔG [kJ mol^{-1}]
α-CD	*trans*-**5**	5.40×10^3	1	−12.06	30.97	−21.29
β-CD	*trans*-**5**	8.04×10^3	1	−6.04	54.48	−22.28

guest **5**. The thermodynamic parameters are fully consistent with literature data on cyclodextrin–azobenzene inclusion complexes.[43] It is also clear from the titration data that no secondary heat effect due to (de)micellization is observed.

The interaction of CD vesicles composed of **1a** and **1b** with divalent guests **2**, **3**, and **4** and monovalent guest **5** was investigated by using OD600 measurements, DLS, and cryo-TEM. The OD600 of a solution of vesicles of α-CD **1a** at a concentration of 30 μM is lower than 0.05. When divalent guest **2** (40 μM) is added (after 3 min) to vesicles of α-CD **1a** (30 μM) at pH 7.4, OD600 increases from about 0.05 to about 0.4 within 30 min (Figure 3A). According to DLS, the average particle size increases from about 100 nm to more than 1000 nm (Figure 3C). The aggregation and adhesion of vesicles of **1a** mediated by guest **2** was confirmed by using cryo-TEM (Figure 4). It is clear from Figure 4A that vesicles of **1a** are unilamellar and spherically shaped in the absence of any guest. However, upon addition of guest **2**, large

tion, the solution of *trans*-**5** is clear and does not contain any aggregates.

It can be seen in Table 1 that the thermodynamic parameters for the interaction of *trans*-**5** with α-CD (Figure 2) and *trans*-**5** with β-CD (Figure S2 and S3 in the Supporting Information) are not identical. The *trans*-azobenzene group of guest **5** binds slightly more strongly to β-CD ($K_a = 8.04\times10^3\,M^{-1}$) than to α-CD ($K_a = 5.40\times10^3\,M^{-1}$) for a 1:1 inclusion complex, possibly due to secondary interactions with the diethylene glycol spacer and the piperazine ring of guest **5**. The cavity of β-CD (6.0–6.5 Å) is slightly larger than the cavity of α-CD (4.7–5.3 Å)[66] and it is likely that β-CD has a higher tendency to form a pseudorotaxane inclusion complex with

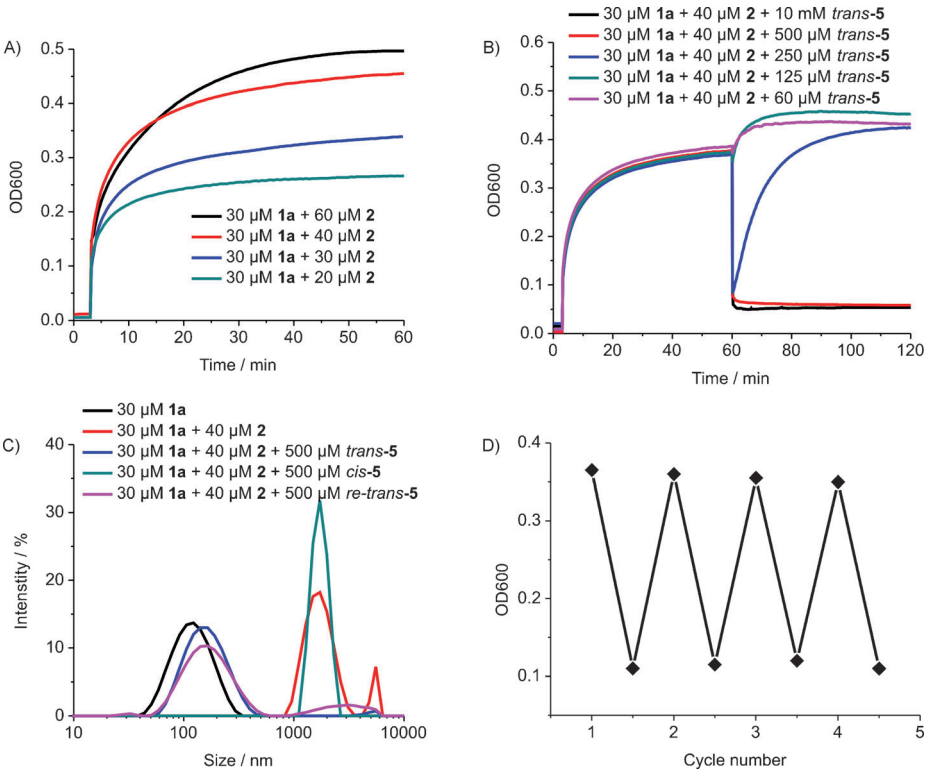


Figure 3. Competitive photoresponsive aggregation of host vesicles of α-CD **1a** by divalent guest **2** in the presence of a competitive monovalent guest **5**. A) Time-dependent measurement of OD600. B) Concentration-dependent displacement of guest **2** by competitor *trans*-**5** from the vesicle surface. C) Size distribution according to DLS. D) Competitive photoresponsive aggregation induced by divalent guest **2** in the presence of monovalent guest **5**.

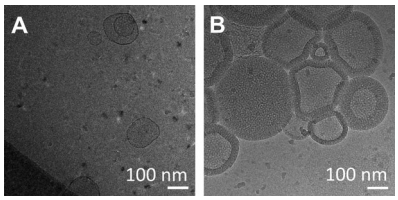


Figure 4. Cryo-TEM images of A) vesicles of α-CD **1a**, and B) aggregation of vesicles of α-CD **1a** mediated by divalent guest **2**. The dark line at the bottom left corner in A) is the edge of the holey carbon film.

aggregates of (multilamellar) vesicles are observed (Figure 4B). The vesicles form extensive areas of close contact. We take these microscopic observations as direct evidence for adhesion of the vesicles induced by guest **2**.

The OD600, DLS, and cryo-TEM data indicate that guest **2** induces rapid aggregation and adhesion of vesicles of **1a**. It should be emphasized that the rate of vesicle aggregation is determined by the limited mobility of the vesicles, not by the formation of inclusion complexes. The rate and extent of vesicle aggregation is concentration dependent: if less guest **2** (20 μM instead of 40 μM) is added to the CD vesicles, it takes longer before a maximum OD600 is reached and if more guest **2** (60 μM instead of 40 μM) is added to the CD vesicles, it reaches a maximum OD600 within a shorter time (Figure 3A). In analogy to our recent findings,[62] it can be assumed that inclusion complexation between vesicles of host α-CD **1a** and guest **2** is responsible for the formation of multiple intervesicular noncovalent links, which leads to the aggregation of vesicles of **1a**. It should be noted that, in view of the rather low binding constant of host α-CD **1a** and guest **2** ($K_a \approx 100\,M^{-1}$), a substantial fraction of guest **2** is likely to be in solution rather than bound at the surface of the vesicles. Nevertheless, our data clearly show that the surface coverage of vesicles of **1a** with guest **2** is sufficient to establish a significant number of intervesicular noncovalent links, even in dilute aqueous solution.

This interpretation is confirmed by the fact that the addition (after 60 min) of an excess of host β-CD (10 mM) leads to immediate dispersion of the aggregated vesicles of **1a** in the presence of guest **2** (Figure S4 in the Supporting Information). Moreover, the addition (after 60 min) of an excess of competitive monovalent guest *trans*-**5** (10 mM) also leads to immediate dispersion of the aggregated vesicles of **1a** in the presence of guest **2** (Figure 3B). The effect of excess guest at different concentrations (from 60 μM to 10 mM) on the aggregation of the binary mixture of vesicles of **1a** (30 μM) and guest **2** (40 μM) was also investigated. The addition of 500 μM of an excess competitive guest *trans*-**5** decreases OD600 from about 0.4 to 0.05, in other words, it disperses the aggregated vesicles of **1a** despite the presence of divalent guest **2** (Figure 3B) and the average vesicle diameter of about 150 nm is obtained again (Figure 3C). The addition of 250 μM of *trans*-**5** results in rapid dispersion of the vesicles, followed by slow reaggregation. The addition of 125 or 60 μM of *trans*-**5** results in a slight increase of vesicle aggregation, possibly as a consequence of secondary interactions of surface-bound *trans*-**5** in the intervesicular contact areas. From our data, it can be concluded that, at high excess (>10-fold) of competitive guest **5**, guest **2** is completely displaced from the vesicle surface, whereas at low excess (<3-fold) of competitive guest **5**, guest **2** is not displaced to a significant extent. The intermediate concentration range (around 250 μM of guest **5**) reveals the underlying interplay of rapid, diffusion-controlled dynamics of the inclusion complexes at the surface of the vesicles versus slow adhesion of vesicles by a limited number of intervesicular links, that is, although most links are broken initially, at least some will re-form over time, since guest **2** is not completely displaced from the vesicle surface. These findings consistently show that the aggregation and adhesion of vesicles of **1a** is mediated by the formation of intervesicular inclusion complexes, since excess competitive binders (host β-CD as well as guest *trans*-**5**) in solution disrupt inclusion complexes at the vesicle surface.

Most interestingly, UV irradiation of the ternary mixture of vesicles of α-CD **1a** (30 μM), guest **2** (40 μM), and competitive guest *trans*-**5** (500 μM) at 350 nm for 20 min raises OD600 from about 0.05 to about 0.37 and the average particle size from around 150 nm to more than 1000 nm (Figure 3C and D). UV irradiation at 350 nm induces the photoisomerization of *trans*-**5** to *cis*-**5** (Figure S1 in the Supporting Information). These observations can be taken as further evidence that the photoinduced aggregation and dispersion of vesicles is due to photoresponsive interactions of competitive guest **5**, since only *trans*-azobenzenes (but not *cis*-azobenzenes) form inclusion complexes with α-CD. Upon subsequent visible irradiation of the ternary mixture of aggregated vesicles of **1a** (30 μM), guest **2** (40 μM), and guest *cis*-**5** (500 μM) at 455 nm for 30 min (to obtain *trans*-**5** from *cis*-**5**), both OD600 decreases from around 0.37 to around 0.11 and the average particle size decreases from more than 1000 nm to about 150 nm (Figure 3C and D). In other words, the aggregation of vesicles of **1a** is reversible in the presence of the competitive photoresponsive guest **5**. The reversibility of the competitive photoinduced aggregation is essentially complete over 5 cycles provided that the irradiation time is sufficient (20 min at 350 nm and 30 min at 455 nm), the vesicle concentration is limited to 30 μM (so that the maximum OD600 is less than 0.5), and the concentration of competitive guest **5** is no more than 500 μM. It likely that photoisomerization is hindered or even inhibited if the OD600 of the vesicle solution is higher than 0.5 and/or the concentration of 5 is more than 500 μM.

The addition of divalent guest **3** (instead of **2**) to vesicles of α-CD **1a** leads to very similar results. When **3** is added to vesicles of **1a** at pH 7.4, OD600 increases from around 0.05 to around 0.3 within 30 min (Figure 5A). According to DLS, the average particle size increases from about 150 nm to more than 1000 nm (Figure 5B). These observations indicate that—similar to guest **2**—guest **3** induces rapid aggregation and adhesion of vesicles of **1a**. Also in this case, the rate and extent of vesicle aggregation is concentration dependent. Moreover, the addition of excess host β-CD (10 mM)

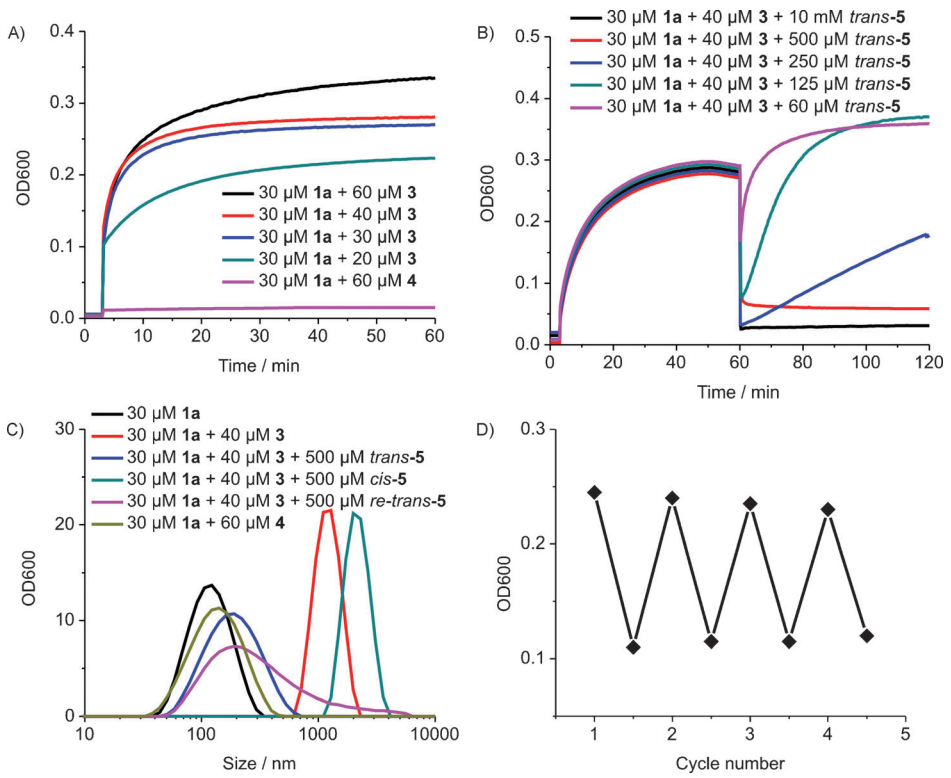


Figure 5. Competitive photoresponsive aggregation of host vesicles of α-CD **1a** by divalent guest **3** in the presence of a competitive monovalent guest **5**. A) Time-dependent measurement of OD600. B) Concentration-dependent displacement of guest **3** by competitor *trans*-**5** from the vesicle surface. C) Size distribution according to DLS. D) Competitive photoresponsive aggregation induced by divalent guest **3** in the presence of monovalent guest **5**.

or excess guest *trans*-**5** (10 mM) leads to immediate dispersion of the aggregation and adhesion of vesicles of **1a** mediated by guest **3** (Figure 5B and Figure S4 in the Supporting Information).[66] The effect of excess guest at different concentrations (from 60 μM to 10 mM) on the aggregation of the binary mixture of vesicles of **1a** (30 μM) and guest **3** (40 μM) was also investigated. In short, the same observations were made as described above for guest **2**: at high excess (>10-fold) of competitive guest **5**, guest **3** is completely displaced from the vesicle surface, in the intermediate concentration range guest **3** is only partially displaced, and at low excess (<3-fold) of competitive guest **5**, guest **3** is not displaced to a significant extent. These results show that the aggregation and adhesion of vesicles of **1a** by guest **3** is also mediated by the formation of intervesicular inclusion complexes. Upon UV irradiation of the ternary mixture of vesicles of **1a** (30 μM), guest **3** (40 μM), and competitive guest *trans*-**5** (500 μM) at 350 nm for about 20 min (to obtain *cis*-**5** from *trans*-**5**), both OD600 increases from about 0.05 to about 0.25 and the average particle size increases from about 150 nm to more than 1000 nm (Figure 5C and D). Upon subsequent visible irradiation of a mixture of aggregated vesicles of **1a**, guest **3**, and a competitive guest *cis*-**5** at 455 nm for about 30 min (to obtain *trans*-**5** from *cis*-**5**), OD600 decreases from about 0.25 to about 0.11 and the average particle size decreases from more than 1000 to about 200 nm (Figure 4C and D). The reversibility of the competitive photoinduced aggregation is essentially complete over five cycles.

In contrast, it was found that divalent guest **4** (instead of **2** or **3**) did not induce any significant aggregation of vesicles of α-CD **1a** (Figure 5A and C). It is likely that guest **4** does not induce any intervesicular interaction because the inclusion complex of α-CD with the benzamide group of guest **4** is too weak ($K_a < 100\ M^{-1}$)[63] to establish a significant number of intervesicular links in dilute aqueous solution.

The interaction of guests **2**, **3**, and **4** with vesicles of β-CD **1b** (instead of α-CD **1a**) revealed an important difference as a result of specific molecular recognition. It was found that, when guest **2**, **3**, or **4** (120 μM) was added (after 3 min) to vesicles of **1b** (30 μM) at pH 7.4, OD600 (ca. 0.05) and the average vesicle diameter (ca. 100 nm) were constant (Figure S5 in the Supporting Information). These measurements indicated that guests **2**, **3**, and **4** did not induce any significant aggregation of vesicles of β-CD **1b**. This observation can be explained from the fact that the β-CD cavity of **1b** is too large to form a stable inclusion complex with the *p*-methylphenyl groups of guest **2** or the *p*-methylbenzamide groups of guest **3** or the benzamide groups of guest **4**. Unlike unmodified β-CD,[64,65] the amphiphilic CDs at the vesicle surface do not easily form pseudorotaxane inclusion complexes.

Conclusion

We have developed a ternary photoresponsive supramolecular system in which the aggregation and adhesion of vesicles was simply achieved through competitive host–guest interactions. OD600 and DLS measurements showed that the strength of the interactions at the surface of vesicles of α-CD **1a** was in the sequence *trans*-**5**–**1a** > guest **2** or **3**–**1a** > *cis*-**5**–**1a**. None of the divalent guests interacted with β-CD **1b**. The aggregation and adhesion of vesicles of **1a** was mediated by guest **2** or **3** and inhibited by the addition of competitive guest *trans*-**5** because *trans*-**5** displaced guest **2** or **3** from the vesicle surface. The reversible photoisomerization of guest **5** made the aggregation and adhesion of CD

vesicles induced by weak noncovalent linkers (guest **2** or **3**) photoresponsive in two directions: adhesion by UV light and dispersal by visible light. If the competitor was in the thermally stable *trans* state, it inhibited vesicle aggregation and if the competitor was in the *meta*-stable *cis* state, it enabled rapid aggregation and adhesion of vesicles. This competitive photoresponsive system may find applications in smart materials based on self-assembly of nanoscale building blocks. The ultimate aim of this research is a supramolecular approach towards semisynthetic tissue engineering, that is, the development of adaptive materials on the basis of self-organizing compartments.

Experimental Section

Chemicals: All chemicals used in this study were purchased from Acros Organics (Schwerte, Germany) or Sigma-Aldrich (Taufkirchen, Germany) and used without further purification, unless otherwise noted. CDs were kindly donated by Wacker Chemie (Burghausen, Germany). All solvents were dried according to conventional methods before use. All aqueous solutions were prepared in Milli-Q water. Two different light sources were utilized for photochemical 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 azobenzenes 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 azobenzenes from *cis* to *trans*.

Synthesis: Amphiphilic α-CD **1a** and β-CD **1b** were synthesized as described previously.[51–54] The precursor (*E*)-1-{4-[2-(2-chloroethoxy)ethoxy]phenyl}-2-phenyldiazene was synthesized as described previously.[62] Guest molecules **2**, **3**, **4**, and **5** were synthesized as reported in the Supporting Information. The analytical and spectroscopic data for **2**, **3**, **4**, and **5** are consistent with their molecular structure. All reactions were carried out in oven-dried glassware and stirred magnetically under an inert-gas atmosphere. Analytical TLC was performed on Merck silica gel 60 F_{254} plates. All compounds were visualized 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 300MHz or 400MHz spectrometers. Chemical shifts were referenced to internal standards: $CDCl_3$ (δ = 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C) or TMS (δ = 0.00 ppm for ^{1}H and ^{13}C). High-resolution mass spectrometry (HRMS) was performed by using a MicroTof spectrometer (Bruker).

Methods: Unilamellar CD bilayer vesicles of α-CD **1a** and β-CD **1b** were prepared by extrusion, as described previously.[54] In short, several milligrams of **1a** (or **1b**) dissolved in chloroform (≈1 mL) were dried by slow rotary evaporation to yield a thin film in a glass vial. Residual solvent was removed under high vacuum. Buffer (10–20 mL; 10 mM phosphate buffer) 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 measurements were performed by using a Nano-Isothermal Titration Calorimeter III (model CSC 5300; Calorimetry Sciences Corporation, London, Utah, USA). ITC measurements were performed in Milli-Q water. A 10 mM solution of α- or β-CD host was titrated into a 1 mM solution of guest **5**. Twenty injections (10 μL) were performed with an interval of 300 s. The stirring rate was 300 rpm.

UV/Vis spectroscopy: Optical density measurements were carried out at in 1.5 mL disposable cuvettes with dimensions 12.5×12.5×45 mm and 10 mm path length using a Uvikon 923 double-beam spectrophotometer. The optical density was measured at λ = 600 nm (OD600), which was far from absorption of the azobenzene chromophore. Measurements were performed for 60 min, unless otherwise noted, with data points collected every 12 s. Freshly prepared vesicles were used for each measurement. Typical concentrations were [**1a**] = [**1b**] = 30 μM, [**2**] = [**3**] = [**4**] = 40 μM, [**5**] = 500 μM, and [α-CD] = [β-CD] = 10 mM in 10 mM phosphate buffer (pH 7.40).

Dynamic light scattering: 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 the aggregated vesicles of **1a** (or **1b**) was measured after 60 min after the addition of the guest **2** (or **3** or **4**) to host vesicles of **1a** (or **1b**). The average size of the dispersed vesicles was measured again after 60 min after the addition of the guest **5** to the above solution. Immediately after alternate UV and visible-light irradiation, the corresponding average size of the reaggregated and dispersed vesicles was measured. Typical concentrations: [**1a**] = [**1b**] = 30 μM, [**2**] = [**3**] = [**4**] = 40 μM and [**5**] = 500 μM and in 10 mM phosphate buffer (pH 7.40).

Cryogenic TEM: Samples for cryo-TEM were prepared by deposition of a few μL of vesicle solution on glow-discharged holey carbon-coated grids (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). After blotting the excess liquid at 100 % humidity and 22 °C, the grids were vitrified in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands). The vitrified specimens were mounted in a liquid-nitrogen-cooled Gatan 626 cryo-holder (Gatan Inc., Pleasanton, USA) and inserted in the electron microscope. Low-dose images were recorded with a Gatan 4K slow-scan CCD camera (Pleasanton, CA) on a Philips CM 120 electron microscope (Eindhoven, The Netherlands) equipped with a LaB6 tip operated at 120 kV. Typical concentrations were [**1a**] = 1 mM and [**2**] = 0.4 mM in 10 mM phosphate buffer (pH 7.40).

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