Photoresponsive Systems

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Light-Responsive Molecular Recognition and Adhesion of Vesicles**

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One of the main challenges in chemistry is the preparation of well-defined self-assembled supramolecular architectures with dynamic and adaptive properties that emulate biological systems. Numerous reports have demonstrated that sophisticated stimuli-responsive materials and surfaces can be assembled by the careful combination of orthogonal interactions.[1] In this context, the molecular recognition and interaction of bilayer vesicles is a versatile model system for the recognition, adhesion, and fusion of biological cell membranes.[2,3] 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. In this Communication, the photoisomerization of a bifunctional noncovalent linker molecule is used as a trigger to induce as well as reverse the molecular recognition and adhesion of vesicles. To our knowledge, this supramolecular photoresponsive system is unprecedented.

The present light-responsive supramolecular system is based on the host–guest interaction of azobenzenes with vesicles made up of amphiphilic cyclodextrins (CDs).[3] Azobenzenes constitute a well-known class of light-responsive 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. The photoisomerization of azobenzene is the molecular basis for a range of light-sensitive supramolecular materials,[4] including photoresponsive vesicles.[5,6] Also the inclusion of azobenzene as a guest into a CD host is light-responsive: the rodlike *trans*-isomer forms a stable inclusion complex with α-cyclodextrin (α-CD) as well as with β-cyclodextrin (β-CD), while the bent *cis* isomer does not fit in either CD. The light-responsive inclusion of azobenzenes in CDs has been exploited to make light-responsive hydrogels,[7] micelles and vesicles,[8] ion channels,[9] surfaces,[10] and drug-delivery vehicles.[11]

In this work we investigate the light-responsive interaction of vesicles composed of amphiphilic CDs **1a** and **1b** with bifunctional guest molecules **2** and **3** (Figure 1 A). Amphiphilic α-CD **1a** and β-CD **1b** were synthesized as described previously.[3b,d] Unilamellar CD bilayer vesicles with a diameter of ca. 100 nm were prepared in buffer at pH 7.4 by extrusion.[3] Guest molecules **2** and **3** were synthesized as reported in the Supporting Information. The analytical and spectroscopic data for **2** and **3** are consistent with their molecular structure. Guest molecule **2** is a homobifunctional noncovalent linker that carries two identical supramolecular binding sites: an azobenzene group that forms inclusion complexes with α-CD and β-CD. The formation of the host–guest complex of **2** should be light-responsive: only the *trans*-azobenzene is a suitable guest for α-CD and β-CD, the *cis*-azobenzene is not. Hence, **2** can bind two molecules of α-CD or β-CD when it is in the *trans* form, but none in the *cis* form (Figure 1 B). Guest molecule **3** is a heterobifunctional noncovalent linker that carries two different supramolecular binding sites: an azobenzene group that forms inclusion complexes with α-CD and β-CD, and a *tert*-butylbenzene group that forms inclusion complexes with β-CD (but not with α-CD, because it is too small to host *tert*-butylbenzene). The formation of the host–guest complex of **3** should be partially light-responsive: only the *trans*-azobenzene is a suitable guest for the CDs, the *cis*-azobenzene is not. Hence, **3** can bind two molecules of CD when it is in the *trans* form, but only one (β-CD) when it is in the *cis* form.

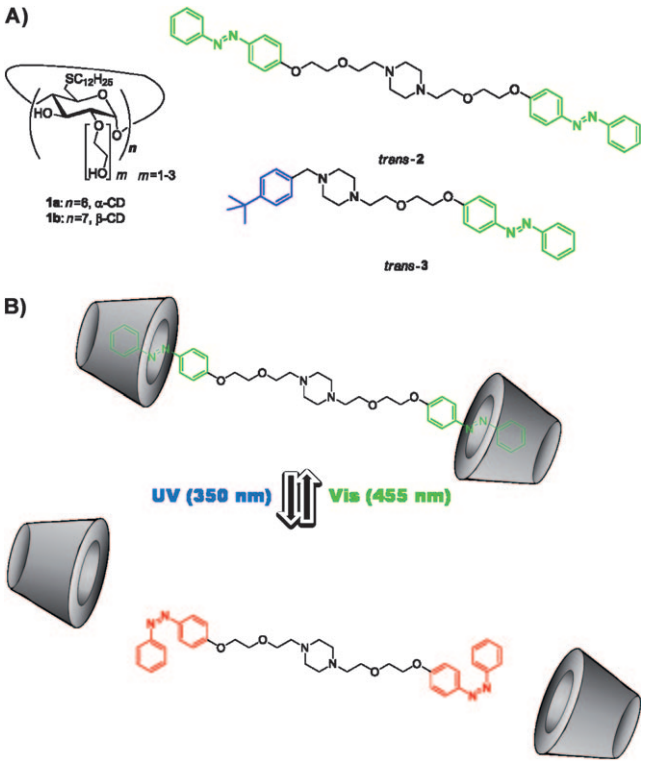

Figure 1. A) Structures of hosts **1a** and **1b** and guests **2** and **3**. B) Light-responsive inclusion of **2** in α-CD and β-CD.

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The interaction of CD vesicles composed of **1a** and **1b**, respectively, with homobifunctional guest **2** and heterobifunctional guest **3** was investigated by using optical density measurements at 600 nm (OD600)[12] and dynamic light scattering (DLS). At a CD concentration of 30 μM, OD600 is lower than 0.05. When *trans*-**2** or *trans*-**3** (15 μM) is added to vesicles of β-CD **1b** (30 μM), OD600 increases from ca. 0.05 to ca. 0.5 within 30 min (Figure 2A). According to DLS, the average particle size increases to more than 1000 nm (Figure 2B). These observations indicate that *trans*-**2** as well as *trans*-**3** induce a rapid aggregation and adhesion of vesicles of **1b**. We assume that both *trans*-**2** and *trans*-**3** form host–guest inclusion complexes at the surface of the CD vesicles and that the vesicles adhere owing to the formation of multiple intervesicular noncovalent links ("supramolecular glue"). This hypothesis is confirmed by the fact that the aggregation and adhesion of vesicles of **1b** in the presence of either *trans*-**2** or *trans*-**3** is immediately reversed by the addition of an excess (10 mM) of α-CD or β-CD: the excess host in solution dissociates the host–guest complexes at the vesicle surface (see the Supporting Information).

The rate and extent of vesicle aggregation is concentration dependent: if less *trans*-**2** or *trans*-**3** (10 μM instead of 15 μM) is added to the CD vesicles, it takes longer before a maximum OD600 is reached. Strikingly, when *cis*-**2** or *cis*-**3** (15 μM, obtained from *trans*-**2** or *trans*-**3** by 4 min irradiation at 350 nm) is added to the vesicles of **1b** (30 μM), both OD600 (ca. 0.05) and the average vesicle diameter (ca. 100 nm) are constant. These observations indicate that *cis*-**2** and *cis*-**3** do not induce any significant aggregation of vesicles of **1b**. We take this observation as further evidence that the aggregation and adhesion of vesicles of **1b** is mediated by the formation of host–guest complexes, since only *trans*-azobenzenes (but not *cis*-azobenzenes) form inclusion complexes with β-CD.

The remarkable difference between the *trans* and *cis* isomers of guests **2** and **3** would suggest that photoresponsive molecular recognition and adhesion of vesicles of **1b** can be achieved by the in situ photoisomerization of **2** (or **3**). As shown in Figures 2C and D, such a photoisomerization occurs readily and results in a reversible photoinduced aggregation and dispersion of the vesicles, simply by irradiation at 350 nm (to obtain *cis* from *trans*) followed by irradiation at 455 nm (to obtain *trans* from *cis*). The reversibility of the photoinduced aggregation is essentially complete over five cycles, provided the irradiation time is sufficient (20 min at 350 nm and 30 min at 455 nm) and the vesicle concentration is limited to 30 μM, so that the maximum OD600 is no more than 0.5.[13]

The interaction of guests **2** and **3** with vesicles of α-CD **1a** (instead of β-CD **1b**) revealed a number of important differences as a result of specific molecular recognition. When *trans*-**2** (15 μM) is added to vesicles of **1a** (30 μM), OD600 increases from ca. 0.05 to ca. 0.5 within 30 min (Figure 3A). According to DLS, the average particle size increases to more than 1000 nm (Figure 3B). These observations indicate that *trans*-**2** induces a rapid aggregation and adhesion of vesicles of **1a** as well as of vesicles of **1b**. However, it was found that *trans*-**3** does not induce any significant aggregation of vesicles of **1a**. We assume that *trans*-**2** and also *trans*-**3** bind at the surface of the vesicles of **1a** owing to formation of a host–guest complex of the *trans*-azobenzene group of **2** and **3**. Whereas vesicles of **1a** aggregate and adhere in the presence of *trans*-**2** because of the formation of intervesicular noncovalent links, *trans*-**3** does not induce any significant intervesicular interaction because its *tert*-butylbenzyl group is too large to form a host–guest inclusion complex with **1a**.[14] Also in this case, the rate and extent of vesicle aggregation is concentration dependent. Furthermore, when *cis*-**2** (15 μM,

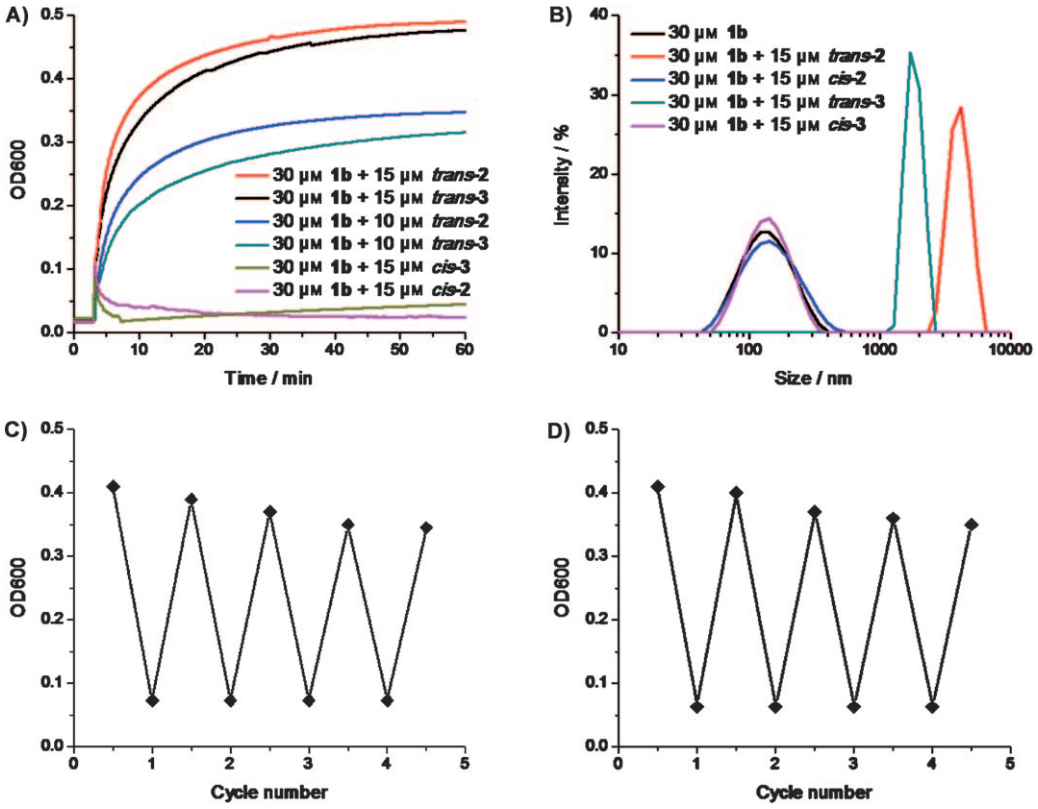

Figure 2. Light-responsive aggregation of host vesicles of **1b** by guests **2** and **3**. A) Time-dependent measurement of OD600. B) Size distribution according to DLS. C) Light-responsive aggregation induced by guest **2**. D) Light-responsive aggregation induced by guest **3**.

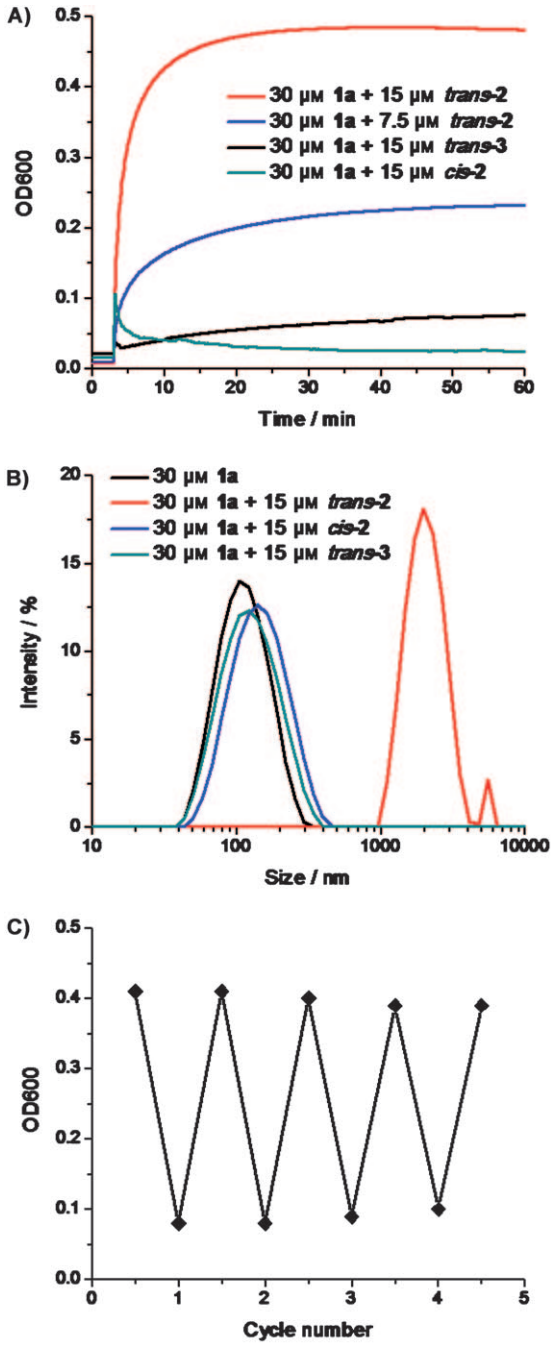

Figure 3. Light-responsive aggregation of host vesicles of **1a** by guest **2**. A) Time-dependent measurement of OD600. B) Size distribution according to DLS. C) Light-responsive aggregation.

obtained from *trans*-**2** by 4 min irradiation at 350 nm) is added to vesicles of **1a** (30 μM), both OD600 (ca. 0.05) and the average vesicle diameter (ca. 100 nm) are constant. These observations indicate that *cis*-**2** does not induce any significant aggregation of vesicles of **1a**, as is also observed for vesicles of **1b**. As described for vesicles of **1b**, a photoresponsive molecular recognition and adhesion of vesicles of **1a** can be achieved by the in situ photoisomerization of **2** (Figure 3C). The reversibility of the photoinduced aggregation is essentially complete over five cycles. The experiments described above reveal a remarkable selectivity in molecular recognition of vesicles of **1a** and **1b**: not only is the recognition and adhesion of the vesicles photoresponsive in two directions (adhesion by visible light, and dispersal by UV light), but also guest **3** is selective for vesicles of **1b** (instead of **1a**), since it cannot form a divalent complex with α-CD. The aggregation of vesicles of **1a** and **1b** in the presence of *trans*-**2** can also be observed on a microscopic scale (Figure 4).

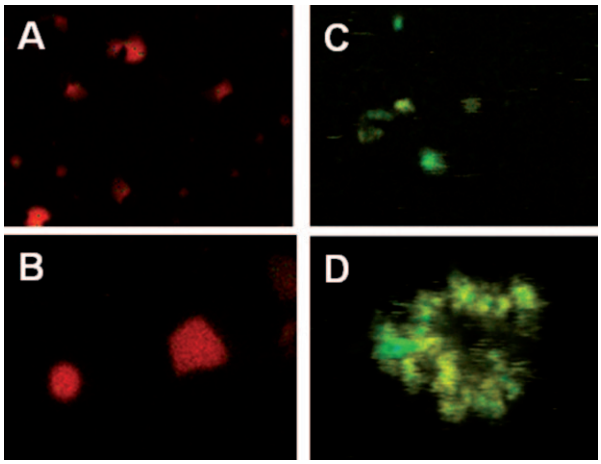

Figure 4. Confocal microscopy images of A) rhodamine-labeled vesicles of **1b**, B) aggregation of vesicles of **1b** in the presence of *trans*-**2**, C) vesicles of **1a** labeled with NBD-Chol, and D) aggregation of vesicles of **1a** in the presence of *trans*-**2**. Dimensions of each panel: 18×14 μm.

In summary, we have developed a photoresponsive supramolecular system in which the aggregation and adhesion of bilayer vesicles is mediated by a highly selective and fully reversible photoresponsive molecular recognition process. We expect that this work will contribute to the development of adaptive materials and macroscopic systems on the basis of self-organizing compartments.

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[12] We measured the optical density at 600 nm (OD600) instead of 400 nm (OD400). OD400 is a more common parameter to characterize a vesicle solution, but the azobenzene absorbance interferes at this wavelength.

[13] The photoisomerization is hindered or even inhibited if the OD600 of the vesicle solution is higher than 0.5.

[14] The hydrophobic *tert*-butylbenzene group could possibly fold back and insert into the bilayer membrane. This nonspecific hydrophobic effect is much weaker than inclusion into a β-CD cavity.

