Photoresponsive Systems

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Light-Responsive Capture and Release of DNA in a Ternary Supramolecular Complex**

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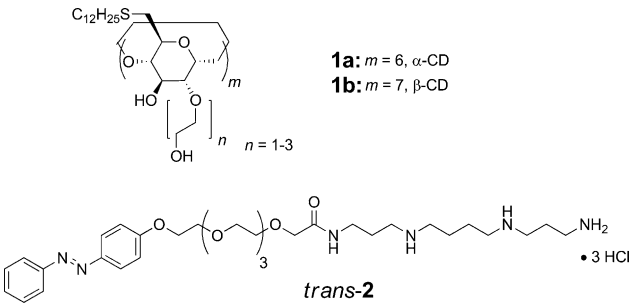
One of the main advances in supramolecular chemistry has been the progress from the general principles of molecular recognition and self-assembly to applications in medicine and material sciences. The correction of genetic defects and disorders by gene therapy is an important challenge in modern medicine. Cationic amphiphiles,[1] polymers,[2] and dendrimers[3] have been investigated for their propensity to transfect DNA and RNA in the form of nanoscale "lipoplexes" and "polyplexes". Several approaches have been proposed to develop transfection agents which also include a release mechanism, such as release triggered by a change in pH,[4] the reduction of disulfides,[5] and the light-induced dissociation of covalent bonds.[6]

The light-induced release of DNA or RNA from a carrier system is a most elegant way to combine efficiency, mild conditions, and the absence of any additives. Photolabile esters can easily be dissociated upon irradiation at 350 nm.[6,7] On the other hand, also noncovalent complexes can be light-sensitive. Azobenzenes constitute a well-known class of light-responsive compounds that can be reversibly isomerized from *trans* to *cis* form by irradiation at 350 nm and from *cis* to *trans* form by irradiation at 455 nm. The inclusion of azobenzene as a guest into a cyclodextrin host is light-responsive: the rodlike and apolar *trans* isomer forms a stable inclusion complex with α-cyclodextrin (α-CD) as well as with β-cyclodextrin (β-CD), while the bent and polar *cis* isomer does not fit in either CD. The light-controlled molecular recognition of CDs with azobenzenes has been used to develop light-responsive hydrogels,[8] molecular shuttles,[9] micelles and vesicles,[10] surfaces,[11] and drug-delivery vehicles.[12]

Herein, we report the self-assembly of a ternary supramolecular system based on a light-responsive azobenzene–cyclodextrin complex that can reversibly capture and release DNA. The key innovation of this noncovalent complex is a photoinduced transition from a high-affinity, multivalent binding mode to a low-affinity, monovalent binding mode. The ternary system is based on bilayer vesicles of amphiphilic CDs and the molecular recognition of guest molecules at the surface of such host vesicles.[13] The inclusion of functional guests at the surface of the CD vesicles offers a straightforward alternative to the cumbersome synthesis of CD polymers and cationic amphiphilic CDs that have shown potential in gene delivery.[14]

We have recently reported a binary supramolecular system in which the photoisomerization of a divalent azobenzene 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 upon irradiation with visible light (455 nm) and dissociation upon irradiation with UV light (350 nm).[15] Herein, we describe the light-responsive formation and dissociation of a ternary complex of vesicles composed of amphiphilic CD **1**, azobenzene–spermine conjugate **2**, and DNA.



Azobenzene–spermine conjugate **2** binds to CD vesicles through inclusion of the hydrophobic *trans*-azobenzene group and to negatively charged DNA through an electrostatic interaction of the positively charged spermine unit. The surface of the CD vesicles decorated with conjugate *trans*-**2** displays multiple protonated spermine groups and thus exhibits high-affinity multivalent DNA binding. A ternary complex ("supramolecular lipoplex") is formed between CD vesicles, conjugate *trans*-**2**, and DNA through multiple noncovalent interactions. Upon UV irradiation, *trans*-**2** isomerizes to *cis*-**2**, and conjugate *cis*-**2** detaches from the vesicle surface. As a consequence, the multivalent display of spermine groups is disrupted and the resulting low-affinity monovalent spermine units readily dissociate from the DNA. Hence, the ternary supramolecular complex disassembles and DNA is effectively released upon UV irradiation. Furthermore, upon visible-light irradiation, the conjugate *cis*-**2**

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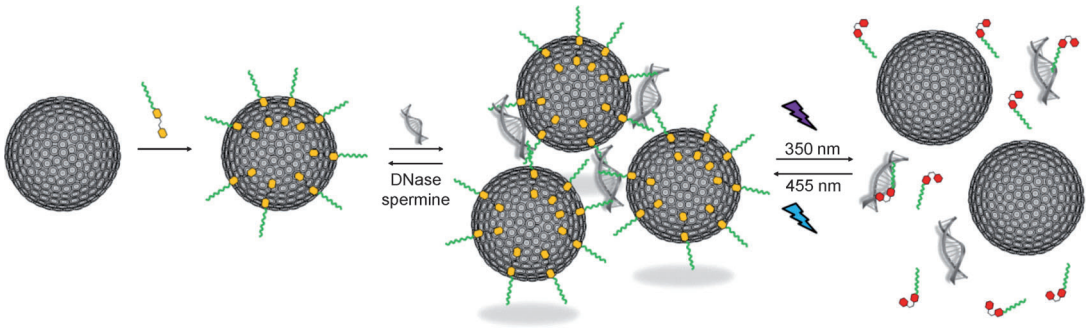

Figure 1. The light-responsive formation and dissociation of a ternary complex of vesicles of composed of CD (α-CD **1a** or β-CD **1b**), azobenzene–spermine conjugate *trans*-**2**, and DNA (50-mer single-stranded DNA (ssDNA) or salmon testes double-stranded DNA (dsDNA) consisting of 2000 base pairs).

isomerizes back to *trans*-**2** and the ternary complex is formed again. The light-responsive ternary complex is illustrated in Figure 1.

Amphiphilic α-CD **1a** and β-CD **1b** were synthesized as reported previously.[13b,c] Unilamellar CD bilayer vesicles with a diameter of 100–150 nm were prepared in buffer at pH 7.2 by extrusion.[13] Conjugate **2** was synthesized as reported in the Supporting Information. The reversible photoisomerization of conjugate **2** is shown in Figures S1 and S2 in the Supporting Information. The interaction of conjugate *trans*-**2** with α-CD and β-CD was measured by using isothermal titration calorimetry (ITC). It was found that *trans*-**2** forms inclusion complexes with α-CD ($K_a \approx 6 \times 10^3\,\text{M}^{-1}$) as well as with β-CD ($K_a \approx 4 \times 10^3\,\text{M}^{-1}$). The ITC data are summarized in Table S1 in the Supporting Information.

The ternary supramolecular system of α-CD **1a**, conjugate *trans*-**2**, and single-stranded DNA (ssDNA) was investigated in particular detail. The optical density (OD600) of a solution of vesicles of α-CD **1a** at a concentration of 30 μM is less than 0.05. When conjugate *trans*-**2** is added to vesicles of α-CD **1a**, there is no change in either the OD600 or the average vesicle diameter (Figure 2 Aand 3 A). However, the ζ-potential of the vesicles increases from roughly −26 mV in the absence of guest[13c] to about +11 mV upon addition of *trans*-**2** (Figure 3 C), indicating that the surface of the CD vesicles displays a high density of positively charged spermine groups. When 50-mer ssDNA is added to the binary mixture of vesicles of α-CD **1a** and conjugate *trans*-**2**, the OD600 increases from approximately 0.05 to 0.9 within 30 min (Figure 2 A). According to dynamic light-scattering (DLS) measurements, the average particle size increases to more than 1000 nm (Figure 3 A). These observations indicate that rapid aggregation of vesicles occurs in the ternary complex of vesicles of **1a**, conjugate *trans*-**2**, and ssDNA. In every other combination (i.e. absence of vesicles of **1a**, absence of conjugate **2**, and/or absence of ssDNA), no aggregation was observed (see Figure S4 in the Supporting Information). Furthermore, the ζ-potential of the ternary complex is approximately +0.4 mV (Figure 3 C). The near-neutral ζ-potential of the ternary complex indicates that positively charged spermine-decorated vesicles and negatively charged DNA aggregate upon charge neutralization. Indeed the rate and extent of formation of the ternary complex is dependent

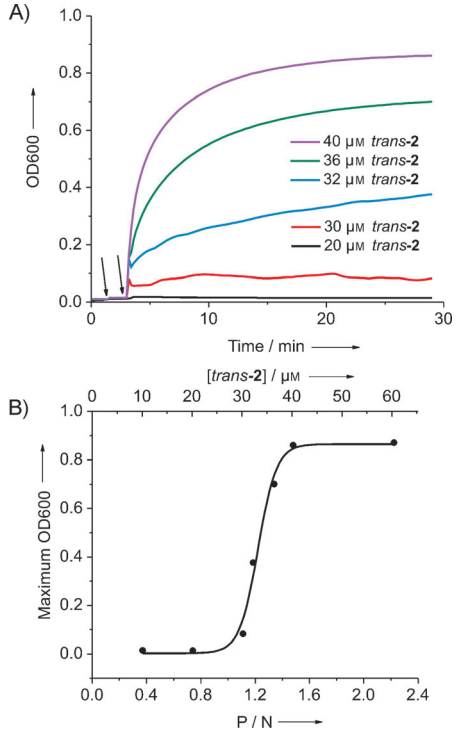

Figure 2. Formation of a ternary complex of vesicles of α-CD **1a**, conjugate *trans*-**2**, and 50-mer ssDNA. A) Time-dependent measurement of OD600. B) Maximum OD600 versus concentration of *trans*-**2** and P/N charge ratio. Concentrations: [**1a**] = 30 μM and [ssDNA] = 1.6 μM in aqueous buffer (2 mM HEPES and 10 μM EDTA at pH 7.2).

on the surface coverage of positively charged spermine moieties at the vesicle surface. If less conjugate *trans*-**2** is added to the vesicles of α-CD **1a**, the surface density of *trans*-**2** will be lower and hence the vesicles interact more slowly with ssDNA and it takes longer before the aggregation reaches a maximum OD600 (Figure 2 A). The maximum OD600 was plotted against the concentration of *trans*-**2** and the overall positive (P)/negative (N) charge ratio in the ternary system. The P/N ratio was calculated by assuming that there are 3 positive charges per *trans*-**2** and 50 negative charges for each 50-mer ssDNA. As shown in Figure 2 B, aggregation has a threshold P/N ratio close to 1.0.

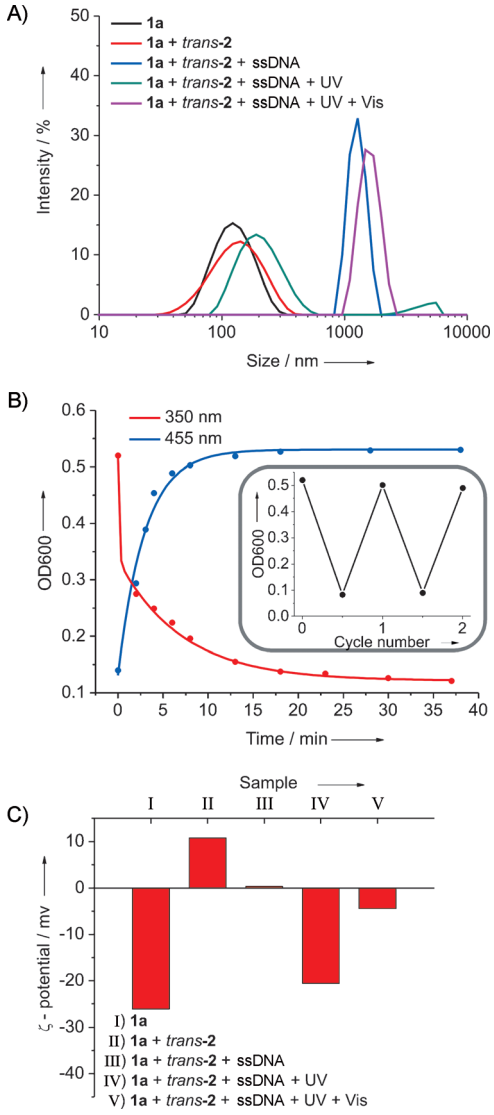

Figure 3. Light-responsive formation of a ternary complex of vesicles of α-CD **1a** (30 μM), conjugate *trans*-**2** (30 μM), and 50-mer ssDNA (0.7 μM). A) Light-responsive size distribution according to DLS. B) Time-dependent increase and decrease of OD600 under alternate irradiation with UV light (350 nm) and visible light (455 nm). The inset shows the reversible light-responsive formation of the ternary complex induced by conjugate **2**. C) ζ-Potential. All measurements were performed in aqueous buffer (2 mM HEPES and 10 μM EDTA at pH 7.2).

The critical role of charge neutralization was confirmed by the fact that the addition of excess spermine·4HCl (50 mM) leads to an immediate dissociation of the ternary complex: excess spermine screens the electrostatic interaction and in addition may act as a competitive monovalent binder to DNA (see Figure S5 in the Supporting Information). Also the addition of enzyme DNase I (30 U ml^{-1}) leads to slow dissociation of the ternary complex (see Figure S5 in the Supporting Information). This observation can be explained by the fact that the ternary complex dissociates upon enzymatic hydrolysis of the captured DNA.

Most interestingly, UV irradiation of the ternary complex of vesicles of α-CD **1a**, conjugate *trans*-**2**, and 50-mer ssDNA at 350 nm for 40 min decreases both the OD600 from roughly 0.52 to 0.12 and the average particle size from more than 1000 nm to about 200 nm (Figure 3A,B). The ζ-potential of the remaining particles decreases from approximately +0.4 mV to −20.6 mV (Figure 3C). UV irradiation induces the photoisomerization of *trans*-**2** to *cis*-**2** (see Figures S1 and S2) and the OD600, DLS and ζ-potential measurements consistently indicate that the ternary system dissociates into its components (CD vesicles, *cis*-**2**, and ssDNA) upon UV irradiation. As shown in Figure 3B, the dissociation of the ternary complex occurs within the first 20 min of UV irradiation. The negative surface potential of the ternary system after UV irradiation can be ascribed to the fact that the positively charged spermine groups of *cis*-**2** dissociate from the surface of the vesicles, since only *trans*-azobenzenes form inclusion complexes with α-CD. As a consequence, the spermine groups are no longer displayed in a high-affinity multivalent arrangement, and they readily dissociate from the DNA since monovalent binding is insignificant at these concentrations.[16] The negatively charged vesicles do not bind to negatively charged ssDNA because of electrostatic repulsion. We conclude that ssDNA is released from the vesicle surface upon UV irradiation of the ternary system.

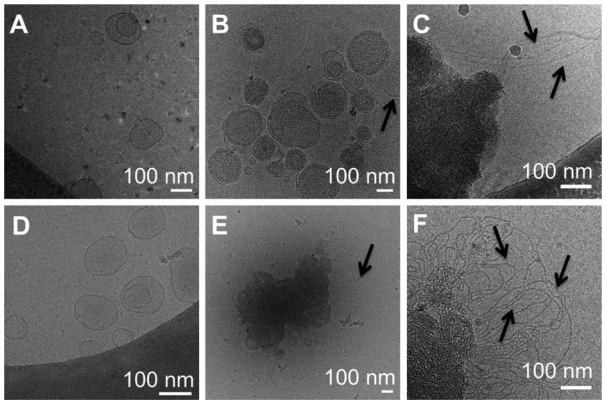

Figure 4. Cryo-TEM images. A) Vesicles of α-CD **1a.** B,C) Ternary complex of vesicles of **1a**, conjugate *trans*-**2**, and dsDNA. D) Vesicles of β-CD **1b.** E,F) Ternary complex of vesicles of **1b**, conjugate *trans*-**2**, and dsDNA. DNA strands are marked with black arrows.

Upon subsequent irradiation of the ternary system of vesicles of α-CD **1a**, conjugate *cis*-**2**, and ssDNA with visible light at 455 nm for 40 min (to convert *cis*-**2** to *trans*-**2**), both the OD600 increases from approximately 0.14 to 0.53 and the average particle size increases from roughly 200 nm to more than 1000 nm (Figure 3A,B). The ζ-potential increases from about −20.6 mV to −4.4 mV (Figure 3C). The OD600, DLS, and ζ-potential measurements consistently indicate that the ternary complex of vesicles of **1a**, conjugate *trans*-**2**, and ssDNA reassembles upon visible-light irradiation. In other words, the light-induced formation and dissociation of the ternary complex is reversible and thus allows the light-responsive capture and release of low-molecular-weight DNA. The reversibility of the light-induced formation of the ternary complex is quantitative over two cycles provided

that the irradiation time is sufficient (40 min at 350 nm and 40 min at 455 nm), the vesicle concentration is limited to 30 μM (so that the maximum OD600 is about 0.5), and the concentration of ssDNA is approximately 2.0 μM.

In the same way, experiments were performed with vesicles of β-CD **1b** (instead of α-CD **1a**). The inclusion complexes of *trans*-**2** with α-CD and β-CD are of comparable stability (see Table S1 in the Supporting Information), and the addition of conjugate *trans*-**2** and 50-mer ssDNA to vesicles of β-CD **1b** leads to the formation of a light-responsive ternary supramolecular complex (see Figure S6 in the Supporting Information). Also in this case, electrostatic complexation has a threshold P/N ratio close to 1.0. Light-induced formation and dissociation of the ternary complex is quantitatively reversible and thus allows the light-responsive capture and release of DNA.

Also the interaction of CD vesicles (α-CD **1a** as well as β-CD **1b**), conjugate *trans*-**2,** and double-stranded (ds) DNA consisting of 2000 base pairs (instead of 50-mer ssDNA) resulted in the formation of ternary complexes (see Figures S7 and S8 in the Supporting Information). The average particle size increases to more than 1000 nm. Complex formation has a threshold P/N ratio close to 1.0. The P/N ratio was estimated by assuming that there are 3 positive charges per *trans*-**2** and 4000 negative charges per dsDNA. The ζ-potential of the ternary complex is close to neutral. The formation of the complex was confirmed by using cryo-TEM (Figure 4). It is evident from Figure 4 A,D that CD vesicles are spherical and unilamellar in the absence of guest. However, after the sequential addition of *trans*-**2** and dsDNA, large aggregates of (multilamellar) vesicles and dsDNA are observed due to multivalent noncovalent interactions with conjugate *trans*-**2.** The vesicles form extensive areas of close contact surrounded by long strands of dsDNA (Figure 4 B,C,E,F). It appears that DNA functions as an electrostatic glue that induces the tight adhesion, flattening, and stacking of CD vesicles, resulting in dense, multilamellar complexes. Electrostatic complexation may result in significant dehydration of the bilayer surfaces.

Unlike the light-responsive ternary system described for ssDNA, UV irradiation of the ternary complex of CD vesicles (α-CD **1a** or β-CD **1b**), conjugate *trans*-**2**, and dsDNA does not have any significant effect on either the maximum OD600 (which remains roughly 0.40) or the average particle size (which remains more than 1000 nm). UV irradiation of the ternary complex decreases the ζ-potential from about −0.42 mV to −15.4 mV. The decrease of ζ-potential can be ascribed to the partial release of the positively charged spermine unit of *cis*-**2** from the vesicle surface. However, the combination of OD600, DLS, and ζ-potential measurements indicate that even after UV irradiation, a stable ternary complex persists. We propose that the ternary complex of vesicles, *trans*-**2**, and dsDNA is more robust owing to increased multivalency and increased kinetic stability as a consequence of the length of the dsDNA.[16] In other words, the formation of the ternary complex is not reversible and cannot be used to capture and release high-molecular weight-DNA.

In summary, a light-responsive ternary complex is formed by self-assembly of three components: vesicles of amphiphilic CDs, azobenzene–spermine conjugates *trans*-**2**, and 50-mer ssDNA. The photoisomerization of azobenzene leads to a light-responsive capture and release of ssDNA. The key innovation in this ternary system is a light-induced switch from a high-affinity multivalent display to a low-affinity monovalent binding mode. We envisage that the incorporation of selective target ligands at the surface of vesicles[13d,e] along with the light-responsive conjugates could make these systems versatile for the capture, delivery, and release of low-molecular-weight DNA and RNA to target cells for gene therapy.

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[16] Upon UV irradiation, a photostationary state with roughly 20% remaining *trans*-**2** is obtained (see the Supporting Information). It can be estimated that in a charge-neutral complex, ssDNA will only carry a few residual units of *trans*-**2** (i.e. too little for multivalent interaction), whereas dsDNA will carry many hundreds of units of residual *trans*-**2** (i.e. more than enough for multivalent interaction).

