JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

pubs.acs.org/JACS

Photoresponsive Capture and Release of Lectins in Multilamellar Complexes

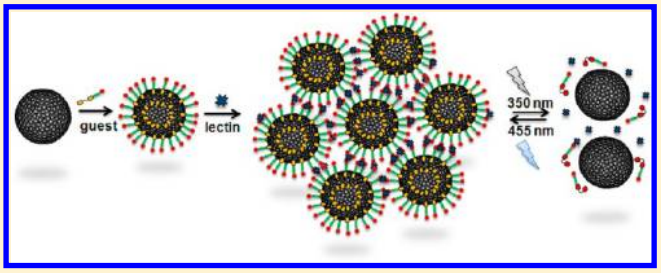
Avik Samanta,[†] Marc C. A. Stuart,[‡] and Bart Jan Ravoo*[,†]

[†]Organic Chemistry Institute and Graduate School of Chemistry, Westfälische Wilhelms-Universität Münster, Corrensstrasse 40, 48149 Münster, Germany

[‡]Biophysical Chemistry, Groningen Biomolecular Science and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Supporting Information

ABSTRACT: The development of triggered release systems for delivery of peptides and proteins is critical to the success of biological drug therapies. In this paper we describe a dynamic supramolecular system able to capture and release proteins in response to light. The ternary system self-assembles in a dilute aqueous solution of three components: vesicles of amphiphilic cyclodextrin host, noncovalent cross-linkers with an azobenzene and a carbohydrate moiety, and lectins. The cross-linkers form inclusion complexes with the host vesicles, provided the azobenzene is in the *trans* state. The formation of a ternary complex with lectins requires a high density of cross-linkers on the surface of vesicles. The key innovation in this system is a photoinduced switch from a multivalent, high-affinity state that captures protein to a monovalent, low-affinity state that releases protein. By using isothermal titration calorimetry, dynamic light scattering, UV/vis spectroscopy, and cryogenic transmission electron microscopy, we demonstrate that photoinduced capture and release of lectins in dense multilamellar complexes is highly efficient, highly selective, and fully reversible.



INTRODUCTION

Peptides and proteins can be powerful drugs to regulate, restore, or suppress a wide range of physiological processes.[1,2] Proteins can also serve as vaccines.[2] The breakthrough of peptide and protein drug therapy has been hampered by two major obstacles: (1) the large-scale preparation and isolation of complex peptides and proteins, and (2) the development of an effective delivery system for peptides and proteins, which are of course rapidly digested and metabolized *in vivo*.[3] Considerable progress has recently been made in the area of large-scale peptide and protein synthesis,[4] and also a number of promising approaches to versatile peptide and protein delivery systems—mostly based on polymer hydrogels[5] and polymer nanogels[6]—have meanwhile been reported. However, stimulus-responsive supramolecular systems can also play a key role in innovative delivery systems. Among others, Stoddart and co-workers reported light-responsive mesoporous silica nanoparticles that release small molecules,[7] Grzybowski and co-workers reported a redox-responsive nanosponge that can bind and release nanoparticles,[8] and Rotello and co-workers described a recognition-mediated system to release nanoparticles inside cells.[9]

In this paper we report a dynamic supramolecular system that is able to capture and release proteins in response to light. Two orthogonal molecular recognition motifs operate simultaneously in this system: the host−guest interaction of cyclodextrins (CDs) and azobenzenes, and the ligand−receptor interaction of carbohydrates and lectins. Both recognition motifs are inherently weak (i.e., binding constant for a single interaction $K_a \approx 10^3-10^4$ M^{-1}), so a multivalent display is essential for the formation of a stable complex in dilute aqueous solution. The key innovation in this system is a photoinduced switch from a multivalent, high-affinity state that captures protein to a monovalent, low-affinity state that releases protein.

The supramolecular system is based on vesicles composed of amphiphilic CDs (Figure 1).[10−12] Vesicles with an average diameter of 100−150 nm are obtained by extrusion of a solution of α-CD amphiphiles in water through a 100 nm polycarbonate membrane. An important advantage of CD vesicles in comparison with conventional liposomes is the fact that CD vesicles can selectively bind hydrophobic guest molecules (such as adamantanes, *tert*-butylbenzenes, and azobenzenes) and hence can be decorated with functional guest molecules such as carbohydrates, peptides, and proteins simply by mixing the host vesicles with the desired mixture of guest-appended biomolecules.[13−17]

Azobenzenes are particularly interesting photoresponsive guest molecules for CDs since they can be reversibly isomerized from *trans* to *cis* by irradiation at 350 nm and from *cis* to *trans* by irradiation at 455 nm. The rod-like, apolar *trans* isomer forms a stable inclusion complex with α-CD and β-CD, whereas

Received: October 15, 2012

   dx.doi.org/10.1021/ja3101837 | J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

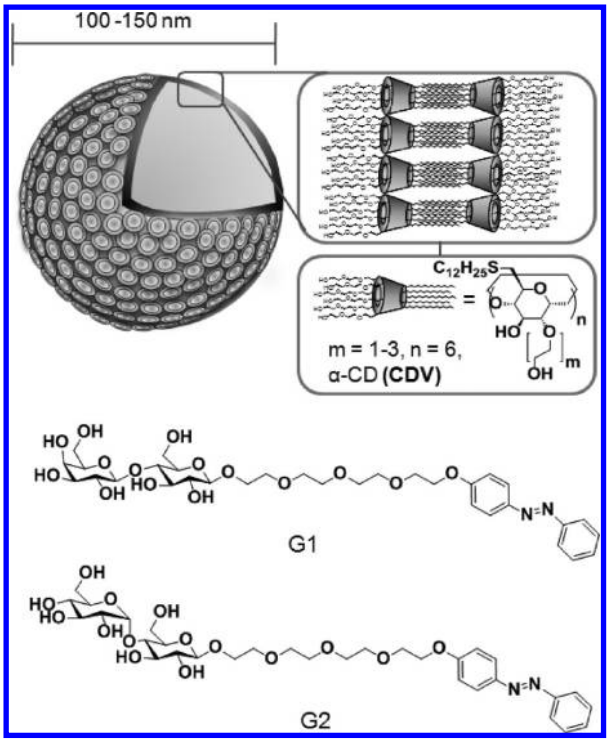


Figure 1. Schematic representation of a cyclodextrin bilayer vesicle and molecular structures of amphiphilic α-CD and azobenzene–carbohydrate cross-linkers.

the bent, polar *cis* isomer does not fit into the cavity of either CD. In recent years, the photoresponsive molecular recognition of CDs with azobenzenes has been used to develop light-responsive hydrogels,[18−21] molecular shuttles,[22,23] micelles and vesicles,[24,25] surfaces,[26,27] and mesoporous nanoparticles.[7] Our group has reported the photoreversible adhesion of CD vesicles using a divalent azobenzene cross-linker,[28] and we have also described the self-assembly of a supramolecular system of CD vesicles and an azobenzene–spermine conjugate which can photoreversibly capture and release DNA.[29]

Here we demonstrate that the surface of CD vesicles can be decorated with bifunctional azobenzene–carbohydrate conjugates **G1** and **G2**, which can bind to CD vesicles (**CDV**) through inclusion of the hydrophobic *trans*-azobenzene group. The high density of carbohydrate ligands on the vesicle surface leads to high-affinity binding of lectins [peanut agglutinin (**PNA**) in the case of azobenzene–lactose conjugate (**G1**) and concanavalin A (**ConA**) in the case of azobenzene–maltose conjugate (**G2**)] in a dense multilamellar complex. The most remarkable feature of this ternary complex is a fully reversible photoinduced transition from a high-affinity, multivalent state to a low-affinity, monovalent state, which effectively results in the light-controlled capture and release of protein. The supramolecular system operates in dilute aqueous solution under ambient conditions at physiological pH.

EXPERIMENTAL SECTION

Materials. All chemicals and lectins used in this study were purchased from Acros Organics (Schwerte, Germany) or Sigma-Aldrich Chemie (Taufkirchen, Germany) and were used without further purification. α-CD was kindly donated by Wacker Chemie (Burghausen, Germany). All solvents were dried according to conventional methods before use.

Syntheses and Analyses. The syntheses of **G1** and **G2** were carried out by coupling of trichloroimidates of the peracetylated disaccharides lactose and maltose with 2-(2-(2-(2-(4-(phenyldiazenyl)-phenoxy)ethoxy)ethoxy)ethoxy)ethanol. After deprotection with $NaOCH_3$, the target products were obtained. Details of the syntheses are reported in the Supporting Information. The spectroscopic and analytical data for **G1** and **G2** are consistent with their molecular structure. The synthesis of **CDV** was performed as described in the literature.[12] All reactions were carried out in oven-dried glassware under an inert gas atmosphere. Analytical thin-layer chromatography was performed on Merck silica gel 60 F254 plates. All compounds were visualized by dipping in basic permanganate solution. Column chromatography was carried out by using Kieselgel 60 (230–400 mesh). ^{1}H and ^{13}C NMR spectroscopic measurements were carried out by using a Bruker ARX 300 MHz or Varian 500 MHz INOVA spectrometer. 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 (d = 0.00 ppm for ^{1}H and ^{13}C). High-resolution mass spectrometry was performed by using a Bruker MicroTOF instrument.

Preparation of Cyclodextrin Vesicles. Unilamellar bilayer **CDV** were prepared by extrusion.[12] In brief, several milligrams of **CDV** in 1 mL of chloroform was dried by slow rotary evaporation to yield a thin film of **CDV** in a glass vial. Residual solvent was removed under high vacuum. Aqueous buffer (10 mL, 20 mM HEPES, 1.0 mM $MnCl_2$, 1.0 mM $CaCl_2$, and 0.15 M NaCl, pH 7.4) 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 III (model CSC 5300; Calorimetry Sciences Corp., London, UT). ITC measurements were performed in 20 mM HEPES buffer. A 10 mM solution of α-CD was titrated into a 1 mM solution of *trans*-**G1** or *trans*-**G2**. Twenty-five injections (10 μL) were performed with an interval of 400 s. The stirring rate was 300 rpm.

UV/Vis Spectroscopy. Optical density measurements were carried out 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 is far from the absorption of the azobenzene chromophore. Measurements were performed for 30–40 min, unless otherwise noted, with data points collected every 12 s. Freshly prepared vesicles and lectin solutions were used for each measurement, and the measurement procedure was as follows. A 1 mL solution of **CDV** (0.1 mM) in aqueous buffer (20 mM HEPES, 1.0 mM $MnCl_2$, 1.0 mM $CaCl_2$, and 0.15 M NaCl, pH 7.4) was injected in a cuvette, and OD600 was measured for 2 min. After 2 min, 20 μL of conjugate *trans*-**G1** or *trans*-**G2** (2.5 mM, prepared in Millipore water) was added to the solution in the cuvette to make the resultant conjugate concentration 50 μM (this addition was done with slight mixing within one interval of 12 s), and OD600 was measured for 2 min. After 2 min, 25 μL of **PNA** or **ConA** (1 mg mL^{-1} in Millipore water) was added to the above solution, and OD600 was measured for at least 30 min. The same measurements were performed with the other samples by following the above procedure. Typical concentrations: [**CDV**] = 0.1 mM, [*trans*-**G1**] = [*trans*-**G2**] = 0.05 mM, and [**PNA**] = [**ConA**] = 1 mg mL^{-1}.

Dynamic Light Scattering (DLS). DLS measurements were performed by using a Malvern Nano-ZS instrument (Malvern Instruments) with low-volume disposable cuvettes kept at 25 °C. The average sizes of the free **CDV**, the binary mixture of **CDV** and bifunctional conjugate *trans*-**G1** (or *trans*-**G2**), and the ternary complex of **CDV**, bifunctional conjugate *trans*-**G1** (or *trans*-**G2**), and **PNA** (or **ConA**) 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 ternary complex was measured. Typical concentrations: [**CDV**] = 0.1 mM, [*trans*-**G1**] = [*trans*-**G2**] = 0.05 mM, and [**PNA**] = [**ConA**] = 1 mg mL^{-1} in aqueous buffer (20 mM HEPES, 1.0 mM $MnCl_2$, 1.0 mM $CaCl_2$, and 0.15 M NaCl, pH 7.4).

dx.doi.org/10.1021/ja3101837 | J. Am. Chem. Soc. XXXX, XXX, XXX–XXX

Table 1. Thermodynamic Data for the Host−Guest Interaction of α-CD with *trans*-G1 and *trans*-G2

host	guest	K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})
α-CD	*trans*-**G1**	8.42×10^3	−15.8	−22.4	21.8
α-CD	*trans*-**G2**	8.43×10^3	−15.5	−24.5	30.0

Irradiation Experiments. Two different light sources were utilized for irradiation experiments. One source was a Rayonet photochemical reactor (The Southern New England Ultraviolet Co.) equipped with 16 RPR-3500 lamps used to generate UV light (350 nm) to isomerize azobenzene 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 from *cis* to *trans*.

Cryogenic Transmission Electron Microscopy (Cryo-TEM). Samples for cryo-TEM were prepared by deposition of a few microliters of free vesicles or a ternary complex (15 min after the addition of the corresponding components when OD600 of the ternary complex is ca. 1.0) on glow-discharged holey carbon-coated grids (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). After the excess liquid was blotted 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, CA) 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 (FEI, Eindhoven, The Netherlands) equipped with a LaB6 tip operated at 120 kV. Typical concentrations: [**CDV**] = 1 mM, [*trans*-**G1**] = [*trans*-**G2**] = 0.5 mM, and [**PNA**] = [**ConA**] = 3 mg mL^{-1} in 20 mM HEPES buffer (pH 7.4).

RESULTS AND DISCUSSION

Amphiphilic α-CD was synthesized as described.[12] Unilamellar bilayer **CDV** were prepared by extrusion in 20 mM HEPES buffer at pH 7.4. The diameter of the vesicles was around 100 nm according to DLS. The syntheses of **G1** and **G2** were carried out using trichloroimidate glycoside donors. Details of the syntheses are included in the Supporting Information. The spectroscopic and analytic data for **G1** and **G2** are consistent with their molecular structure.

The guest molecules **G1** and **G2** each carry two orthogonal molecular recognition sites. The azobenzene group forms an inclusion complex with α-CD and β-CD. The azobenzene unit on conjugate **G1** and **G2** is known to be a good inclusion guest for **CDV**,[28,29] even though the binding of guests to **CDV** is typically somewhat weaker than that to unmodified CDs in solution due to presence of the oligo(ethyleneglycol) residues on the vesicle surface.[12] The azobenzene−carbohydrate conjugates have a tetra(ethyleneglycol) spacer between the azobenzene and the carbohydrate. It is anticipated that this spacer enhances the tendency of **G1** and **G2** to form pseudorotaxane inclusion complexes with both CDs.[30,31] Formation of the host−guest complex of **G1** and **G2** with CDs should be photoresponsive because the rod-like and apolar *trans*-azobenzene isomer favors complexation with CD, whereas the bent and polar *cis*-azobenzene does not.

The interaction between host α-CD and guests *trans*-**G1** and *trans*-**G2** was measured by using ITC (see Table 1 as well as Figures S1 and S2 in the Supporting Information). We performed an "inverse titration" in which a 10 mM solution of α-CD host was titrated into a 1 mM solution of azobenzene guest. It can be seen from Table 1 that the thermodynamic parameters for the titration are very similar for *trans*-**G1** and *trans*-**G2** and characteristic for the formation of an inclusion complex of a hydrophobic guest molecule and α-CD. The association constants $K_a = 8.42 \times 10^3$ for *trans*-**G1** and 8.43×10^3 for *trans*-**G2** are typical for nonionic azobenzene derivatives and α-CD.[31] It can be seen from Figures S1 and S2 that the ratio of host to guest is slightly higher than 1:1, which indicates that an inclusion complex of α-CD and azobenzene with some secondary interaction (pseudorotaxane inclusion complex) of the α-CD and the tetra(ethyleneglycol) spacer is formed. The quality of the titration is somewhat affected by the amphiphilic character of **G1** and **G2**, which have the tendency to form micelles at higher concentrations in aqueous media. These findings are fully consistent with our earlier work on azobenzene−CD inclusion complexes on vesicles.[28,29]

Having verified the formation of an inclusion complex between conjugates *trans*-**G1** and *trans*-**G2** and α-CD, we investigated the characteristics of CD vesicles decorated with azobenzene−carbohydrate conjugates. To this end, a 0.05 mM solution of either *trans*-**G1** or *trans*-**G2** was added to a 0.1 mM solution of **CDV**. It can be assumed that *trans*-**G1** and *trans*-**G2** do not (or only very slowly) permeate the vesicle membrane due to the hydrophilicity of the carbohydrates. Hence, at these concentrations most of the CD cavities on the outside surface of the vesicles will be occupied with *trans*-**G1** (or *trans*-**G2**), so that the carbohydrates are displayed in high density at the vesicle surface, while relatively little carbohydrate is available in solution. We have previously coined the term "artificial glycocalix" for this self-assembled multivalent arrangement of carbohydrate ligands.[14] **CDV** decorated with **G1** or **G2** should respond strongly to the addition of lectins that bind to the carbohydrates. To investigate this phenomenon we selected two lectins: **PNA** and **ConA**. It is known that both **PNA** and **ConA** form tetramers at neutral pH, and each lectin has four carbohydrate binding sites.[33] **PNA** binds exclusively to β-galactose and lactose and their derivatives and shows no affinity toward other carbohydrates (such as α-glucose and maltose). The high-affinity, multivalent interaction of **PNA** with lactose **G1** at the surface of **CDV** should result in agglutination (aggregation) of the vesicles due to the formation of numerous noncovalent cross-links between the CD vesicles. On the other hand, **ConA** binds exclusively to α-glucose and α-mannose and their derivatives (including maltose) and shows no significant affinity toward other carbohydrates (such as galactose and lactose). Because **ConA** binding at the **CDV** surface is also mediated by a multivalent interaction, the addition of **ConA** to **CDV** decorated with maltose **G2** should also result in agglutination.

Optical density measurements at 600 nm (Figure 2A) indicate that indeed spontaneous aggregation of vesicles occurs in the ternary systems of **CDV**, conjugate *trans*-**G1**, and **PNA**. The OD600 of a solution of **CDV** at a concentration of 0.1 mM is less than 0.05. When *trans*-**G1** is added (after 2 min, denoted by first arrow in Figure 2A) to **CDV**, there is no change in OD600. When PNA is added (after 4 min, denoted by second arrow in Figure 2A) to the binary mixture of **CDV** and conjugate *trans*-**G1**, the OD600 increases from approximately 0.05 to 1.0 within 30 min. This observation indicates the spontaneous formation of a ternary complex of **CDV**, *trans*-**G1**,

dx.doi.org/10.1021/ja3101837 | J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

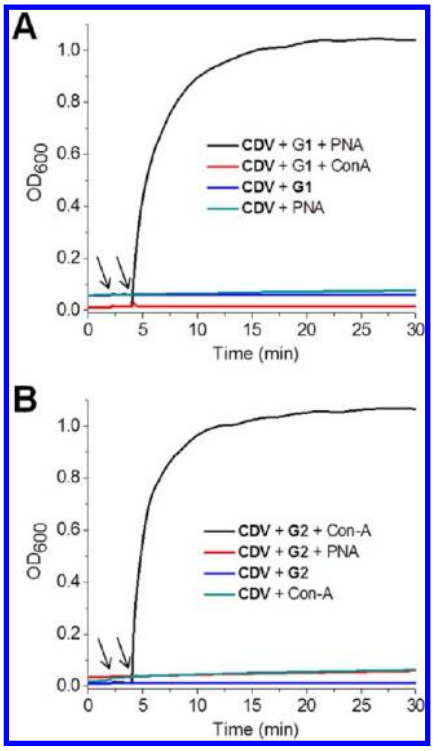


Figure 2. Formation of a ternary complex of CD vesicles, azobenzene–carbohydrate linkers, and lectins. Time-dependent optical density measurement at λ = 600 nm. Conditions: [**CDV**] = 0.1 mM, [*trans*-**G1**] = [*trans*-**G2**] = 0.05 mM, [**PNA**] = [**ConA**] = 0.1 mg mL^{-1}; 20 mM HEPES buffer (1.0 mM $MnCl_2$, 1.0 mM $CaCl_2$, 0.15 M NaCl, pH 7.4); T = 23 °C.

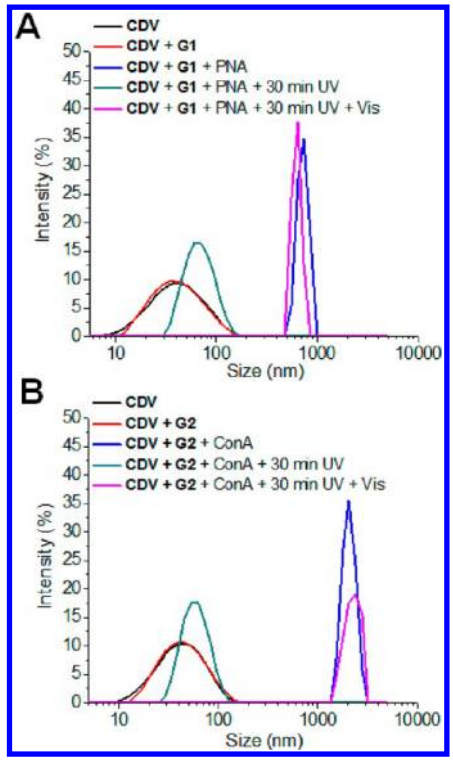


Figure 3. Formation of a ternary complex of CD vesicles, azobenzene–carbohydrate linkers, and lectins. Size distribution according to DLS. Conditions: [**CDV**] = 0.1 mM, [*trans*-**G1**] = [*trans*-**G2**] = 0.05 mM, [**PNA**] = [**ConA**] = 0.1 mg mL^{-1}; 20 mM HEPES buffer (1.0 mM $MnCl_2$, 1.0 mM $CaCl_2$, 0.15 M NaCl, pH 7.4); T = 23 °C.

and **PNA**. In every other case (**ConA** instead of **PNA**, no conjugate, no lectin), no aggregation is observed. This experiment demonstrates two vital points: complexation is the result of a high density of lactose **G1** on the vesicle surface, and **PNA** binds lactose-decorated vesicles only. Similarly, optical density measurements (Figure 2B) show that agglutination of the vesicles occurs exclusively in the ternary system of **CDV**, conjugate *trans*-**G2**, and **ConA**. In every other case (**PNA** instead of **ConA**, no conjugate, no lectin), no agglutination is observed. Furthermore, agglutination can be reversed by the addition of a large excess of glucose (see Figure S3 in the Supporting Information). These experiments demonstrate that in this case agglutination is the result of a high density of maltose **G2** on the vesicle surface and **ConA** binds lactose-decorated vesicles only. We note that the rate of agglutination is higher for **ConA** and **G2** than for **PNA** and **G1**, which is consistent with observations reported for a comparable supramolecular system.[15]

The results of the optical density measurement were corroborated by DLS measurements before and after formation of the ternary complex (Figure 3). According to DLS measurement the average diameter of the **CDV** is less than 100 nm. There is no change in the average particle size after addition of *trans*-**G1** or *trans*-**G2**. Upon addition of **PNA** to vesicles decorated with *trans*-**G1**, the average particle size increases from ca. 100 to ca. 900 nm (Figure 3A). Similarly, the average particle size increases from ca. 80 to more than 1000 nm after adding **ConA** to vesicles decorated with *trans*-**G2** (Figure 3B).

The formation of ternary complexes of **CDV** with azobenzene–carbohydrate conjugates (either *trans*-**G1** or *trans*-**G2**) and lectins (either **PNA** or **ConA**) was confirmed by cryo-TEM (see Figure 4). It is evident from Figure 4A that CD vesicles are spherical and unilamellar in the absence of guest and lectin. However, after sequential addition of guest and lectin, large aggregates of multilamellar vesicles and lectins are observed (Figure 4B,C). It appears the lectin–carbohydrate interaction and host–guest complexation function as a two-component supramolecular glue that induces tight adhesion, flattening, and stacking of CD vesicles, which results in dense, multilamellar complexes in which the lectins are buried between bilayers of CD amphiphiles. The particle size observed in cryo-TEM is consistent with the diameters obtained by DLS. We note that on the basis of the mixing ratio, the multilamellar complexes consist of approximately 26% protein, 65% CD, and 9% linker by weight.

Most significantly, UV irradiation of the ternary complex of **CDV**, conjugate *trans*-**G1**, and **PNA** at 350 nm for 20 min decreases both the OD600 from roughly 1.0 to 0.12 (Figure 5A, red trace) and the average particle size from 900 to about 100 nm (Figure 3A, cyan trace). UV irradiation (350 nm) induces the photoisomerization of *trans*-**G1** to *cis*-**G1**, and the azobenzene–lactose conjugates detach from the vesicle surface, since only *trans*-azobenzene forms an inclusion complex with α-CD. As a consequence, the carbohydrates are no longer displayed in a high-affinity multivalent arrangement, and they readily dissociate from lectins since monovalent binding is insignificant at this concentration. The OD600 and DLS measurements indicate that the ternary complex dissociates into its components, **CDV**, conjugate *trans*-**G1**, and **PNA** (Figures 3A and 5A). Upon subsequent visible light irradiation of the

dx.doi.org/10.1021/ja3101837 | J. Am. Chem. Soc. XXXX, XXX, XXX–XXX

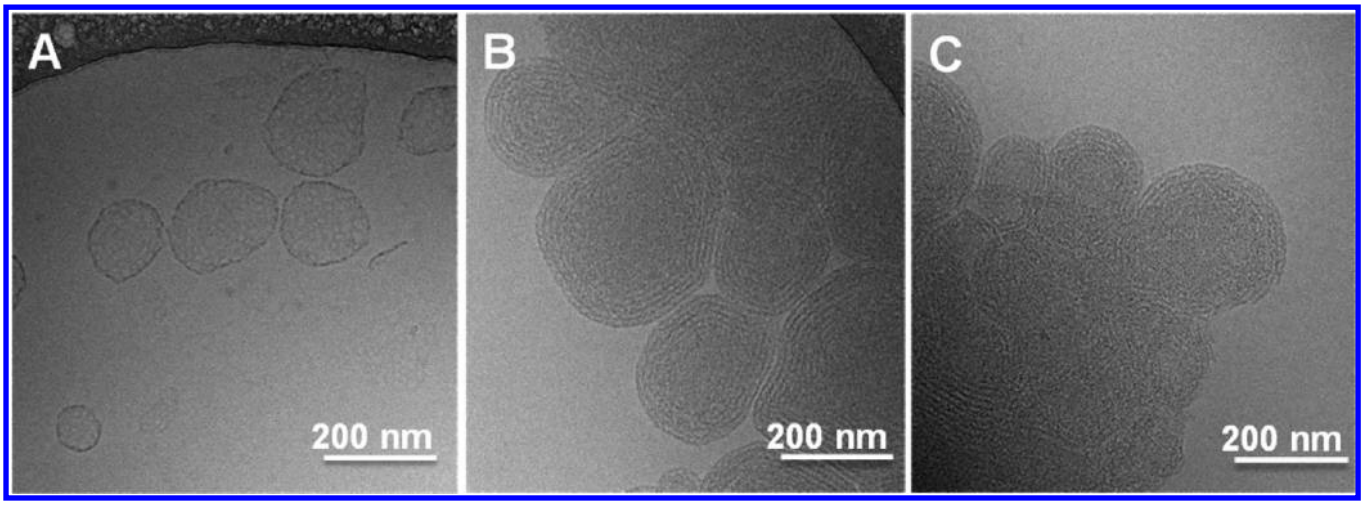


Figure 4. Cryo-TEM images. (A) Unilamellar **CDV**. (B) Multilamellar complexes of **CDV** in the presence of *trans*-**G1** and **PNA**. (C) Multilamellar complexes of **CDV** in the presence of *trans*-**G2** and **ConA**.

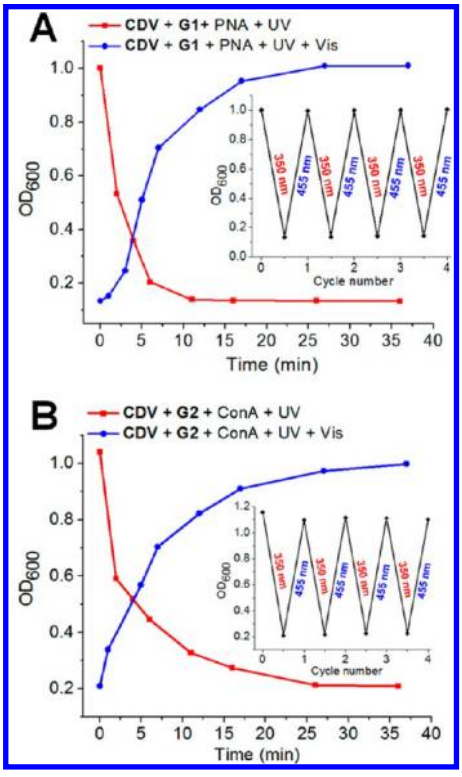


Figure 5. Photoresponsive assembly and disassembly of a ternary complex of CD vesicles, azobenzene-carbohydrate linkers, and lectins. Time-dependent increase and decrease of OD600 under alternate irradiation with UV light (350 nm) and visible light (455 nm). (A) **CDV** in the presence of *trans*-**G1** and **PNA**. (B) **CDV** in the presence of *trans*-**G2** and **ConA**. The inset shows the reversible light-responsive formation of the ternary complex.

ternary system at 455 nm for 30 min (to obtain *trans*-**G1** from *cis*-**G1**), both OD600 increases from ca. 0.12 to ca. 1.0 (Figure 5A, blue trace) and the average particle size increases from ca. 90 to ca. 1000 nm (Figure 3A, pink trace). The OD600 and DLS measurements consistently indicate that the ternary complex of **CDV**, conjugate *trans*-**G1**, and **PNA** reassembles upon visible light irradiation. Identical observations are made for the complex of **CDV**, conjugate *trans*-**G2**, and **ConA** upon UV irradiation (Figures 3B and 5B). The light-induced formation and dissociation of the ternary complex is reversible and thus allows the light-responsive capture and release of lectins. The reversibility of the light-induced formation of the ternary complex is quantitative over four cycles, provided the irradiation time is sufficient (40 min at 350 nm and 40 min at 455 nm) (Figure 5). In comparison, the thermal isomerization of *cis*-azobenzene to *trans*-azobenzene is very slow (see Figure S4 in the Supporting Information).

CONCLUSION

We have developed a photoresponsive ternary supramolecular system based on orthogonal host–guest complexation and carbohydrate–lectin interaction that can selectively capture and release lectins. OD600, DLS, and cryo-TEM measurements prove that a ternary complex was formed by sequential addition of azobenzene–carbohydrate conjugate and lectin to the cyclodextrin vesicle solution at physiological pH. The formation and dissociation of the ternary complex is based on the light-responsive interactions of azobenzene–carbohydrate conjugates with vesicles. Light-induced dissociation of the ternary complex leads to the release of lectins at this dilute concentration. The complexation is highly selective and fully reversible over four cycles. In the experiments shown here, the complex contained more than 25% of protein by weight, which is a significant loading efficiency for a drug delivery system. We envisage that the incorporation of selective ligands at the surface of the vesicles, along with light-responsive conjugates, could yield a versatile modular delivery system for the capture, transport, and triggered release of proteins to target cells.

ASSOCIATED CONTENT

Supporting Information

Synthesis, NMR spectra, and ITC data. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

b.j.ravoo@uni-muenster.de

Notes

The authors declare no competing financial interest.

dx.doi.org/10.1021/ja3101837 | *J. Am. Chem. Soc.* XXXX, XXX, XXX–XXX

■ ACKNOWLEDGMENTS

We are grateful to the Graduate School of Chemistry for financial support of A.S.

■ REFERENCES

(1) Vlieghe, P.; Lisowski, V.; Martinez, J.; Khrestchatisky, M. *Drug Discovery Today* **2010**, *15*, 40−56.
(2) Leader, B.; Baca, Q. J.; Golan, D. E. *Nature Rev. Drug Discovery* **2008**, *7*, 21−39.
(3) Latham, P. W. *Nat. Biotechnol.* **1999**, *17*, 755−757.
(4) Bray, B. L. *Nature Rev. Drug Discovery* **2003**, *2*, 587−593.
(5) Lee, K. Y.; Yuk, S. H. *Prog. Polym. Sci.* **2007**, *32*, 669−697.
(6) Nochi, T.; Yuki, Y.; Takahashi, H.; Sawada, S.; Mejima, M.; Kohda, T.; Harada, N.; Kong, I. G.; Sato, A.; Kataoka, N.; Tokuhara, D.; Kurokawa, S.; Takahashi, Y.; Tsukada, H.; Kozaki, S.; Akiyoshi, K.; Kiyono, H. *Nat. Mater.* **2010**, *9*, 572−578.
(7) Ferris, D. P.; Zhao, Y. L.; Khashab, N. M.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I. *J. Am. Chem. Soc.* **2009**, *131*, 1686−1688.
(8) Klajn, R.; Olson, M. A.; Wesson, P. J.; Fang, L.; Coskun, A.; Trabolsi, A.; Soh, S.; Stoddart, J. F.; Grzybowski, B. A. *Nature Chem.* **2009**, *1*, 733−738.
(9) Kim, C.; Agasti, S. S.; Zhu, Z.; Isaacs, L.; Rotello, V. M. *Nature Chem.* **2010**, *2*, 962−966.
(10) Ravoo, B. J.; Darcy, R. *Angew. Chem., Int. Ed.* **2000**, *39*, 4324−4326.
(11) Mazzaglia, A.; Donohue, R.; Ravoo, B. J.; Darcy, R. *Eur. J. Org. Chem.* **2001**, 1715−1721.
(12) Falvey, P.; Lim, C. W.; Darcy, R.; Revermann, T.; Karst, U.; Giesbers, M.; Marcelis, A. T. M.; Lazar, A.; Coleman, A. W.; Reinhoudt, D. N.; Ravoo, B. J. *Chem.—Eur. J.* **2005**, *11*, 1171−1180.
(13) Versluis, F.; Tomatsu, I.; Seda, K.; Fregonese, C.; Tepper, A. W. J. W.; Stuart, M. C. A.; Ravoo, B. J.; Koning, R. I.; Kros, A. *J. Am. Chem. Soc.* **2009**, *131*, 13186−13187.
(14) Voskuhl, J.; Stuart, M. C. A.; Ravoo, B. J. *Chem.—Eur. J.* **2010**, *16*, 2790−2796.
(15) Vico, R. V.; Voskuhl, J.; Ravoo, B. J. *Langmuir* **2011**, *27*, 1391−1397.
(16) Uhlenheuer, D. A.; Wasserberg, D.; Haase, C.; Nguyen, H. D.; Schenkel, J. H.; Huskens, J.; Ravoo, B. J.; Jonkheijm, P.; Brunsveld, L. *Chem.—Eur. J.* **2012**, *18*, 6788−6794.
(17) Kauscher, U.; Ravoo, B. J. *Beilstein J. Org. Chem.* **2012**, *8*, 1543−1551.
(18) Tomatsu, I.; Hashidzume, A.; Harada, A. *J. Am. Chem. Soc.* **2006**, *128*, 2226−2227.
(19) Liao, X.; Chen, G.; Liu, X.; Chen, W.; Chen, F.; Jiang, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 4409−4413.
(20) Yamaguchi, H.; Kobayashi, Y.; Kobayashi, R.; Takashima, Y.; Hashidzume, A.; Harada, A. *Nature Commun.* **2012**, *3*, 603.
(21) Harada, A.; Kobayashi, R.; Takashima, Y.; Hashidzume, A.; Yamaguchi, H. *Nature Chem.* **2011**, *3*, 34−37.
(22) Murakami, H.; Kawabuchi, A.; Kotoo, K.; Kunitake, M.; Nakashima, N. *J. Am. Chem. Soc.* **1997**, *119*, 7605−7606.
(23) Wenz, G.; Han, B. H.; Müller, A. *Chem. Rev.* **2006**, *106*, 782−817.
(24) Wang, Y. P.; Ma, N.; Wang, Z. Q.; Zhang, X. *Angew. Chem., Int. Ed.* **2007**, *46*, 2823−2826.
(25) Zou, J.; Guan, B.; Liao, X. J.; Jiang, M.; Tao, F. G. *Macromolecules* **2009**, *42*, 7465−7473.
(26) Yabe, A.; Kawabata, Y.; Niino, H.; Tanaka, M.; Ouchi, A.; Takahashi, H.; Tamura, S.; Tagaki, W.; Nakahara, H.; Fukuda, K. *Chem. Lett.* **1988**, *1*, 1−4.
(27) Wan, P. B.; Jiang, Y. G.; Wang, Y. P.; Wang, Z. Q.; Zhang, X. *Chem. Commun.* **2008**, 5710−5712.
(28) Nalluri, S. K. M.; Ravoo, B. J. *Angew. Chem., Int. Ed.* **2010**, *49*, 5371−5374.
(29) Nalluri, S. K. M.; Voskuhl, J.; Bultema, J. L.; Boekema, E. J.; Ravoo, B. J. *Angew. Chem., Int. Ed.* **2011**, *50*, 9747−9751.
(30) Nepogodiev, S. A.; Stoddart, J. F. *Chem. Rev.* **1998**, *98*, 1959−1976.
(31) Harada, A. *Acc. Chem. Res.* **2001**, *34*, 456−464.
(32) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875−1917.
(33) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637−674.