Bilayer Vesicles of Amphiphilic Cyclodextrins: Host Membranes That Recognize Guest Molecules

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Abstract: A family of amphiphilic cyclodextrins (**6, 7**) has been prepared through 6-*S*-alkylation (alkyl = *n*-dodecyl and *n*-hexadecyl) of the primary side and 2-*O*-PEGylation of the secondary side of α-, β-, and γ-cyclodextrins (PEG = poly(ethylene glycol)). These cyclodextrins form nonionic bilayer vesicles in aqueous solution. The bilayer vesicles were characterized by transmission electron microscopy, dynamic light scattering, dye encapsulation, and capillary electrophoresis. The molecular packing of the amphiphilic cyclodextrins was investigated by using small-angle X-ray diffraction of bilayers deposited on glass and pressure–area isotherms obtained from Langmuir monolayers on the air–water interface. The bilayer thickness is dependent on the chain length, whereas the average molecular surface area scales with the cyclodextrin ring size. The alkyl chains of the cyclodextrins in the bilayer are deeply interdigitated. Molecular recognition of a hydrophobic anion (adamantane carboxylate) by the cyclodextrin vesicles was investigated by using capillary electrophoresis, thereby exploiting the increase in electrophoretic mobility that occurs when the hydrophobic anions bind to the nonionic cyclodextrin vesicles. It was found that in spite of the presence of oligo(ethylene glycol) substituents, the β-cyclodextrin vesicles retain their characteristic affinity for adamantane carboxylate (association constant $K_a = 7.1 \times 10^3\,M^{-1}$), whereas γ-cyclodextrin vesicles have less affinity ($K_a = 3.2 \times 10^3\,M^{-1}$), and α-cyclodextrin or non-cyclodextrin, nonionic vesicles have very little affinity ($K_a \approx 100\,M^{-1}$). Specific binding of the adamantane carboxylate to β-cyclodextrin vesicles was also evident in competition experiments with β-cyclodextrin in solution. Hence, the cyclodextrin vesicles can function as host bilayer membranes that recognize small guest molecules by specific noncovalent interaction.

Keywords: amphiphiles · cyclodextrins · membranes · molecular recognition · vesicles

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Introduction

Amphiphilic cyclodextrins are cyclic oligo(α-(1–4)-glucopyranosides) modified with hydrophobic and hydrophilic substituents that aggregate into a variety of lyotropic phases in water.[1–5] The hydrophobic groups drive hydrophobic aggregation of the amphiphiles, while the hydrophilic groups are required to guarantee sufficient water solubility. The type and stability of the lyotropic phases critically depend on the nature and number of hydrophobic and hydrophilic substituents, the balance between hydrophobic and hydrophilic groups, and factors such as temperature, concentration, and ionic strength. A particularly interesting example of aggregation of amphiphilic cyclodextrins in water is the formation of bilayer vesicles composed entirely of (modified) cyclodextrins. Cyclodextrin vesicles consist of bilayers of cyclodextrins, in which the hydrophobic "tails" are directed inward and the hydrophilic macrocycle "head groups" are facing water, thereby enclosing an aqueous interior (Figure 1 A and B). We and others have recently described vesicles composed entirely of nonionic,[6] anionic,[7] and cationic[8] amphi-

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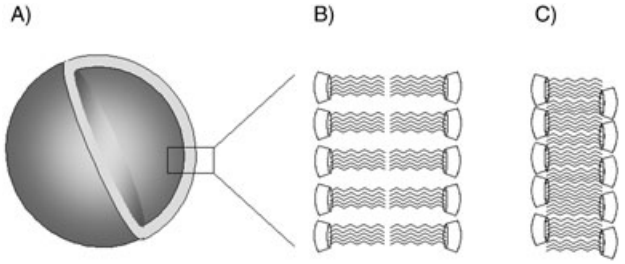


Figure 1. Cyclodextrin vesicles consist of bilayers of cyclodextrins (in which the hydrophobic 'tails' are directed inwards and hydrophilic macrocycle 'head groups' are facing water) enclosing an aqueous interior. A) Schematic representation of a unilamellar vesicle. B) Illustration of an extended, all-*trans* packing of the alkyl chains in a cyclodextrin bilayer. C) Illustration of an interdigitated packing of the alkyl chains in a cyclodextrin bilayer.

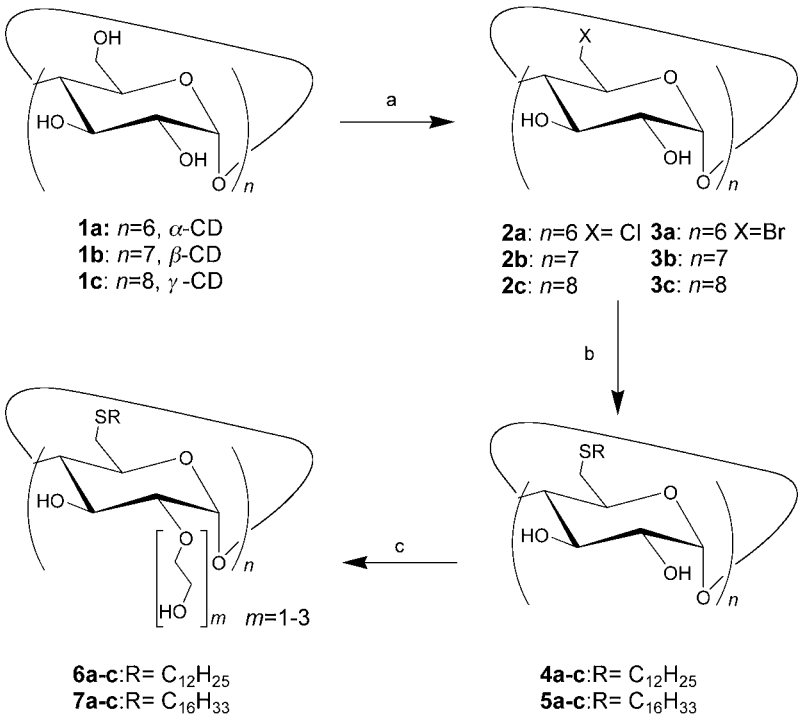
philic cyclodextrins. From these studies it is evident that a combination of hydrophobic alkyl substituents on one face of the cyclodextrin ring and hydrophilic (poly(ethylene glycol), sulfonate, ammonium, etc.)[6–8] substituents on the other side of the cyclodextrin ring is essential to obtain water-soluble amphiphiles. Several other cyclodextrin derivatives can be admixed with liposomes, but are not able to form stable vesicles by themselves.[9] Most of the studies cited above have been limited to β-cyclodextrins.

Cyclodextrin vesicles combine the properties of liposomes and macrocyclic host molecules in their potential to encapsulate water-soluble molecules in the aqueous interior, to absorb hydrophobic molecules into the bilayer membrane, and finally to recognize and bind specific types of guest molecules through inclusion in the cyclodextrin cavities at the surface of the vesicle. In a recent communication,[6c] we demonstrated that vesicles of nonionic β-cyclodextrin derivatives (**6b**) bind *tert*-butylbenzoate and adamantane carboxylate, and they have a high affinity for "guest polymers" (polyelectrolytes modified with adamantyl and *tert*-butylbenzyl groups) due to multivalent interactions between the cyclodextrin hosts in the bilayer and the hydrophobic guest substituents on the polymer. The recognition of small guest molecules by cyclodextrin hosts assembled in a bilayer membrane is a useful model of recognition of substrates or ligands by receptors on the surface of cell membranes. Molecular recognition and specific ion binding at model membrane surfaces is a topic of interest in supramolecular chemistry, with an increasing emphasis on multivalent interactions.[10] Many recognition processes at the cell surface in nature are also amplified in affinity and selectivity by multivalent interactions.[11]

Here we describe in detail a family of amphiphilic cyclodextrins that form stable nonionic vesicles in water. Cyclodextrins of various ring sizes (α-, β-, and γ-cyclodextrins, with six, seven, and eight glucose units, respectively) were modified with hydrophobic *n*-dodecyl or *n*-hexadecyl and hydrophilic oligo(ethylene glycol) substituents (see Scheme 1). The properties of vesicles of these cyclodextrins were studied, with an emphasis on the packing of the amphiphilic macrocycles in the vesicle bilayer. We also examined the inclusion of a small guest molecule (adamantane carboxylate) in the cyclodextrin cavities at the surface of the vesicles.

Results and Discussion

Synthesis: The preparation of the amphiphilic cyclodextrins is outlined in Scheme 1. The synthesis of 6-chloro-cyclodextrins **2a–c** and 6-bromo-cyclodextrins **3a–c** was carried out



Scheme 1. Synthesis of amphiphilic cyclodextrins. a) CH_3SO_2Cl, DMF, 65 °C, two days (to give chlorides **2a–2c**) or NBS, Ph_3P, DMF, 60 °C, 4 h (to give bromides **3a–3c**); b) *t*BuOK or NaH, RSH, DMF, 80 °C, 3–4 days; c) ethylene carbonate, K_2CO_3, *N*,*N*,*N*′,*N*′-tetramethylurea, 150 °C, 4 h. DMF = *N*,*N*-dimethylformamide, NBS = *N*-bromosuccinimide.

according to literature procedures.[12,13] Either of these precursors can be used to obtain the alkyl thioethers **4a–c** and **5a–c** by nucleophilic substitution, although compounds **2a–c** are less reactive than **3a–c** and usually require longer reaction times to afford complete substitution in a good yield. While β-cyclodextrins **4b** and **5b** have been described,[14–16] α- and γ-cyclodextrins **4a**, **4c**, **5a**, and **5c** are new. The most effective purification of these alkyl thioethers is a Soxhlet extraction with hexane to remove excess alkyl thiols. Amphiphilic cyclodextrins **6a–c** and **7a–c** were obtained in good yields by graft polymerization of ethylene carbonate in the presence of potassium carbonate. This reaction has been described in detail for the preparation of **6b** and **7b**.[17] Cyclodextrins **6a**, **6b**, **7a**, and **7c** are new derivatives, and their preparation (analogous to that of **6b** and **7b**)[17] and full characterization is given in the Experimental Section. We note that **6a–c** and **7a–c** are polydisperse products with an average degree of substitution of 2–3 units of ethylene oxide per glucose based on MALDI-TOF MS. Substitution occurs exclusively at C-2, not C-3.[17] Although the degree of polymerization tends to vary from batch to batch (and is rather high, with approximately 4 units of ethylene oxide per glucose for **7c**), we did not observe any significant differences in reactivity for the α-, β-, and γ-cyclodextrins.

Cyclodextrin vesicles: According to dynamic light scattering, cyclodextrin vesicles prepared by extrusion through a 0.1 μm polycarbonate membrane invariably have an average diameter of 140–160 nm, irrespective of the type of cyclodextrin (α-, β-, or γ-cyclodextrin) and the alkyl chain length (*n*-dodecyl or *n*-hexadecyl).[6c] Cyclodextrin vesicles prepared by sonication are usually smaller (80–100 nm).[6a,b,8] These observations were confirmed by using transmission electron microscopy.[6,8] Representative micrographs are shown in Figure 2. Vesicles of this size are expected to be mostly unilamellar, not multilamellar. The cyclodextrin vesicles are stable in aqueous solution for several days, although they tend to precipitate if kept longer or in the presence of salt (>0.1 M). The larger vesicles prepared by extrusion precipitate faster than the smaller vesicles prepared by sonication.

Vesicles of *n*-dodecyl tri(ethylene glycol) ether ($C_{12}EO_3$) were prepared to serve as reference vesicles with similar surface potential but without cyclodextrin host cavities. As reported in the literature,[18] the reference vesicles of $C_{12}EO_3$ are generally somewhat larger (180–200 nm) than the cyclodextrin vesicles and tend to precipitate after several hours.

Further evidence that cyclodextrins **6a–c** and **7a–c** form closed bilayer vesicles was obtained by encapsulation of the hydrophilic fluorescent dye carboxyfluorescein in the aqueous interior of the vesicles. Cyclodextrin vesicles were prepared in a solution of carboxyfluorescein at self-quenching concentration (see the Experimental Section for details). Encapsulated carboxyfluorescein was separated from the free dye by gel filtration on a column of Sephadex G25. The vesicles eluted at 5–8 mL, whereas the free dye eluted beyond 12 mL. The results are presented as the ratio of fluorescence intensity after (F_{TX}) and before (F_{init}) addition of

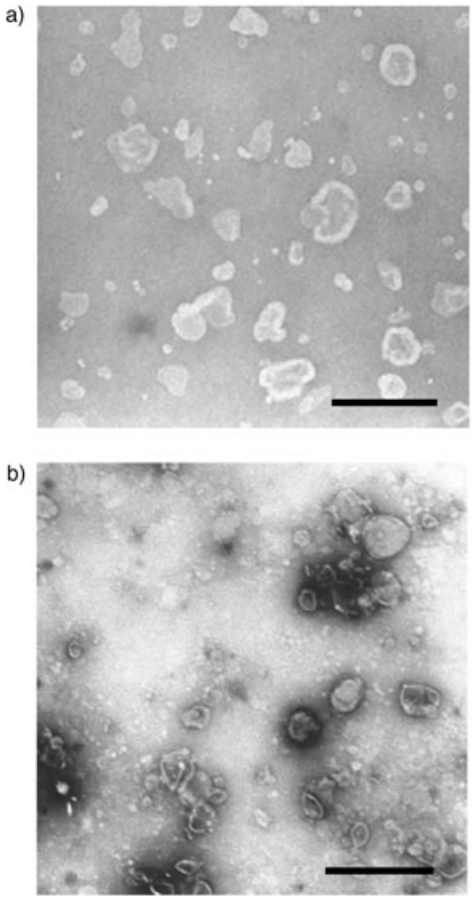


Figure 2. Transmission electron microscopy of α-cyclodextrin vesicles stained with uranyl acetate. a) Vesicles of **6a**. b) Vesicles of **7a**. Scale bar: 500 nm.

0.1% of Triton TX-100 (which solubilizes the cyclodextrin vesicles and causes release of their contents); this can be taken as a measure of the presence of entrapped carboxyfluorescein (Figure 3). Coincidence of entrapped dye with the elution of the vesicles confirms the existence of an aqueous interior. As anticipated, the amount of entrapped carboxyfluorescein in, for example, vesicles of **7b** correlates with the concentration of **7b** (Figure 3b).[6a] Encapsulation of carboxyfluorescein in vesicles of **6a–c** was generally less efficient, which may be due to the shorter alkyl chains in **6a–c** compared to those in **7a–c**. The amount of entrapped carboxyfluorescein is rather small considering the diameter of vesicles. Typically, the entrapment efficiency is in the order of 1 μmol carboxyfluorescein per mmol cyclodextrin, or 1:1000. For liposomes, the entrapment is usually about 1:100.[19]

Bilayer packing of cyclodextrin amphiphiles: A particularly important question is the packing of the amphiphilic cyclodextrin molecules in the bilayer. The molecular surface area of the α-, β-, or γ-cyclodextrin molecule (170 $Å^2$ for **1a**, 190 $Å^2$ for **1b**, and 240 $Å^2$ for **1c**) is significantly larger than

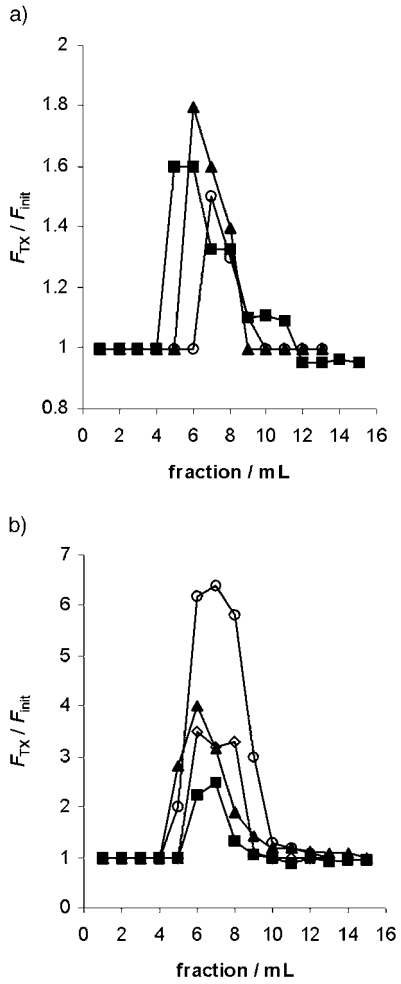


Figure 3. Encapsulation of carboxyfluorescein in cyclodextrin vesicles. Elution profiles of vesicles of cyclodextrins **6a–6c** (a) and cyclodextrins **7a–7b** (b) loaded with carboxyfluorescein on a 18×1 cm Sephadex G25 column. The relative fluorescence intensity scale F_{TX}/F_{init} indicates encapsulated carboxyfluorescein only, as explained in the text. a) ■: **6a** at 5 mg mL^{-1}; ○: **6b** at 5 mg mL^{-1}; ▲: **6c** at 5 mg mL^{-1}. b) ◇: **7a** at 5 mg mL^{-1}; ■: **7b** at 5 mg mL^{-1}; ▲: **7b** at 8 mg mL^{-1}; ○: **7b** at 20 mg mL^{-1}.

the combined area of six, seven, or eight all-*trans* alkyl chains, respectively (6×20 Å^2=120 Å^2, 7×20 Å^2=140 Å^2, and 8×20 Å^2=160 Å^2). Hence, in order to fill the 'void' under the macrocycle to obtain a nonleaky bilayer membrane, either the alkyl chains should interdigitate, tilt, or fold back, or the macrocycle should collapse. The first scenario is well known for many amphiphilic molecules in bilayers and monolayers and can be verified by measuring the thickness of the bilayers and the average orientation of the alkyl chains. The second scenario is known for extensively substituted cyclodextrins.[20] Collapse of the cavity would have important consequences for the inclusion properties of these cyclodextrin molecules and can be verified by measuring the molecular surface area of the molecules in a monolayer on the air–water interface.

Small-angle X-ray diffraction of an air-dried multilayer deposited on a glass cover slide shows a *d* spacing of approximately 42 Å for cyclodextrins **6a–c** and approximately 45 Å for cyclodextrins **7a–c** at 25 °C (Table 1 and Figure 4). Several higher order reflections are observed (in particular for **6a–c** and **7c**, but not for **7a** and **7b**),[21] which indicates a strong tendency for layering of these amphiphiles. The *d* spacing can be taken as a measure of the average bilayer

Table 1. First-order reflection angle (2θ), *d* spacing, and molecular surface area (A_0 at zero compression and A_C at maximum compression) of amphiphilic cyclodextrins.

Cyclodextrin	2θ [°]	*d* [Å]	A_0 [Å^2 mol^{-1}]	A_C [Å^2 mol^{-1}]
6a	2.0143	43.8	340	142
6b	2.1089	41.9	375	162
6c	2.0758	42.5	420	240
7a	1.9463	45.4	285	174
7b	1.9304	45.7	410	160
7c	1.9749	44.7	424	128

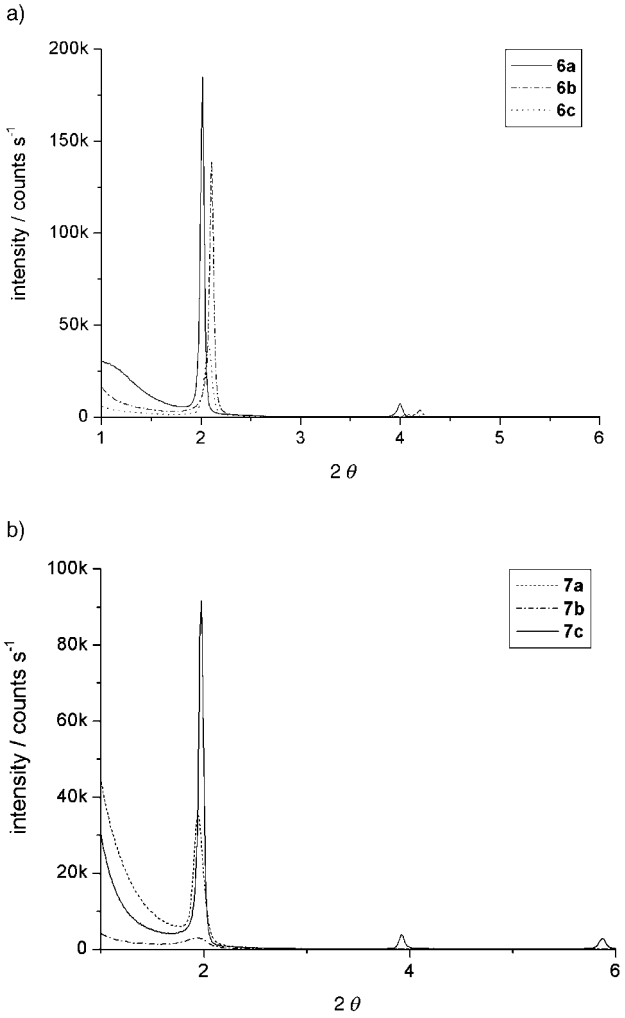


Figure 4. Small-angle X-ray diffraction pattern for cyclodextrin vesicles deposited and air-dried on glass cover slides. a) Diffraction pattern for *n*-dodecyl cyclodextrins **6a–6c**. b) Diffraction pattern for *n*-hexadecyl cyclodextrins **7a–7c**.

thickness. As expected, the *d* spacing depends on the length of the alkyl substituent (*n*-dodecyl versus *n*-hexadecyl) but is independent of the cyclodextrin ring size. From Corey–Pauling–Koltun (CPK) models we estimate a molecular length of approximately 31 Å for **6a–c** and approximately 37 Å for **7a–c** (assuming all-*trans* alkyl substituents at C6 and di(ethylene glycol) at C-2 of the cyclodextrin), which would suggest a bilayer thickness of $2\times31=62$ Å and $2\times37=74$ Å, respectively. Since the experimental bilayer thickness is much smaller than the predicted thickness for extended cyclodextrin molecules, the alkyl chains of the molecules must be deeply interdigitated, tilted, or back-folded, or exist in a combination of these three possibilities.

Brewster angle microscopy indicates that cyclodextrins **6a–c** and **7a–c** form homogeneous monolayers on the air–water interface (Figure 5). Characteristic pressure–area isotherms for each cyclodextrin were obtained upon gradual compression of these monolayers. The collapse of the monolayers occurs at 40–50 mN m^{-1}, which is a normal value for monolayers of cyclodextrins with long ($>C_{10}$) hydrophobic groups at the primary face.[14–16] The area per molecule at zero compression (A_0) and also at maximum compression (A_C) scales with the cyclodextrin ring size (Table 1), but it is similar for cyclodextrins with *n*-dodecyl and *n*-hexadecyl substituents.[22] The area per molecule at zero compression (A_0) is *larger* than the area for the cyclodextrins without ethylene glycol substitution. For the analogues of **4a–c** and **5a–c** described in the literature,[14–16] the molecular area is hardly different from unmodified cyclodextrin (170 Å^2 for **1a**, 210 Å^2 for **1b**, and 240 Å^2 for **1c**). However, the presence of ethylene glycol residues clearly increases the molecular surface area. In particular, the A_0 value is sensitive to the degree of substitution with poly(ethylene glycol), which somewhat obscures the clear trend of molecular area with cyclodextrin ring size and may explain the differences observed between cyclodextrins with *n*-dodecyl and *n*-hexadecyl substituents. A notable exception to the observed trend is the small value of A_C for **7c**, which may arise either from collapse of the cavity of the more flexible γ-cyclodextrin or from extrusion of the amphiphile into the subphase as vesicles upon compression of the monolayer. Cyclodextrin **7c** has a higher degree of hydrophilic substitution than any of the other cyclodextrins studied.

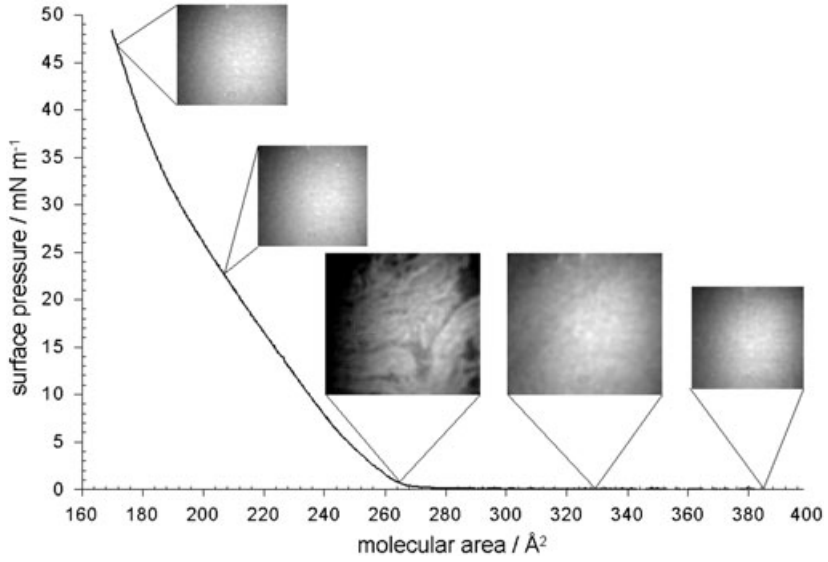


Figure 5. Pressure–area isotherm for a Langmuir monolayer of β-cyclodextrin **7b** spread on the air–water interface, including Brewster angle micrographs.

In summary, according to the pressure–area isotherms, the molecular areas of **6a–c** and **7a–c** are significantly increased due to the presence of the ethylene glycol substituents. Since we find larger, not smaller, molecular areas as a result of ethylene glycol substitution, there is no reason to assume that the cyclodextrin cavities have collapsed. Since the diameter of an alkyl chain is approximately 20 Å^2, the alkyl groups in **6a** and **7a** occupy 6×20 Å$^2=120$ Å^2 when they are in an all-*trans* conformation (hence 7×20 Å$^2=140$ Å^2 for **6b** and **7b** and 8×20 Å$^2=160$ Å^2 for **6c** and **7c**). So, the ethylene glycol substituted cyclodextrin "head group" has a molecular surface area A_0 that is almost twice the area of the seven alkyl chains together, and to ensure optimal packing with minimal voids the alkyl chains must either interdigitate, tilt, or back-fold, as discussed above. By using CPK models it can be estimated that for fully interdigitated alkyl chains (that is, the terminal methyl groups of one bilayer leaflet reside near the first methylene of the opposite bilayer leaflet) the thickness of the bilayer would be approximately 44 Å for **6a–c** and approximately 50 Å for **7a–c**. These values are close to the experimental values from X-ray diffraction. Given a 1:2 "mismatch" of head-group area and alkyl-chain volume, such a bilayer thickness can also be achieved by a chain tilt of 60°, which seems an unrealistically high tilt angle.[23] Alternatively, one could imagine that many alkyl chains fold back instead of extending all-*trans*, but this is unlikely due to the high entropy penalty. Hence, the most likely molecular packing is deep interdigitation of extended alkyl chains (with possibly a modest chain tilt and some back-folding), as observed for asymmetric phospholipids[24] and also for amphiphilic calixarenes.[25] This interdigitated mode of bilayer packing obviously restricts the mobility of the alkyl chains in the bilayer and would nicely explain the lower enthalpy for the cyclodextrin amphiphile compared to a phospholipid in an L_β–L_α phase transition.[6a,24] The result is a relatively

thin but dense bilayer, with hydrophobic alkyl chains extending inwards and hydrophilic cyclodextrins decorated with oligo(ethylene glycol) pointing outwards (Figure 1 C).

Molecular recognition of adamantane carboxylate by cyclodextrin vesicles: Given that all amphiphilic cyclodextrins described above form bilayer vesicles in water, is it possible to bind small guest molecules in the cyclodextrin cavities at the surface of these vesicles? Adamantane carboxylate—like all adamantanes—is known to be a good guest for inclusion into β-cyclodextrin **1b** (association constant $K_a = 3.2 \times 10^4\,\text{M}^{-1}$ at pH 7.2 and 25 °C), while it has much weaker interaction with γ-cyclodextrin **1c** ($K_a = 5.0 \times 10^3\,\text{M}^{-1}$) and even less affinity for α-cyclodextrin **1a** ($K_a = 2.3 \times 10^2\,\text{M}^{-1}$).[26] The inclusion interaction of this typical guest with the cyclodextrin host vesicles was investigated by using capillary electrophoresis. This technique exploits the difference in electrophoretic mobility between the free host and the host–guest complex (or the free guest and the host–guest complex) to quantify host–guest interactions.[27] In our hands, capillary electrophoresis has proven particularly useful for quantifying the interaction between β-cyclodextrin and anionic guests,[28] as well as between β-cyclodextrin vesicles and anionic guests[6c] in dilute aqueous solution. Here, the electrophoretic mobility of the host vesicles of **6a–c** was measured in the presence of an increasing concentration of adamantane carboxylate in the background electrolyte (Figure 6). There is some precedent

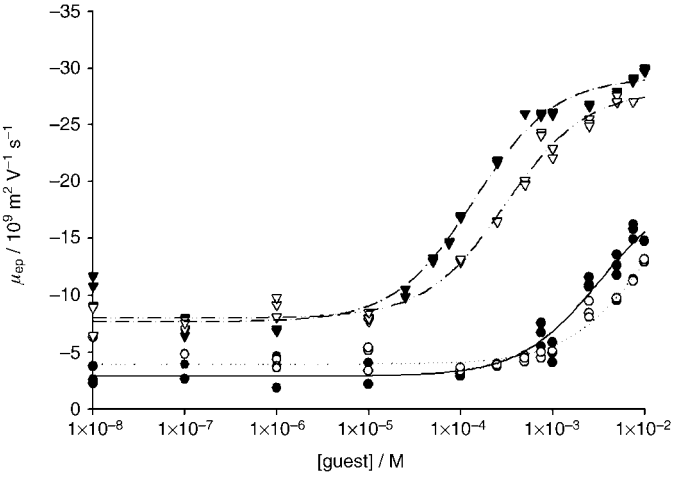


Figure 6. Electrophoretic mobility (μ_{ep}) of vesicles in the presence of the adamantane carboxylate guest. ○: α-Cyclodextrin vesicles (**6a**); ▼: β-cyclodextrin vesicles (**6b**); ▽: γ-cyclodextrin vesicles (**6c**); ●: $C_{12}EO_3$ reference vesicles.

for the investigation of liposomes by using capillary electrophoresis.[29]

All cyclodextrin vesicles, as well as the $C_{12}EO_3$ reference vesicles, invariably have significant negative electrophoretic mobility ($\mu_{ep} \approx -8 \times 10^{-9}\,\text{m}^2\text{V}^{-1}\text{s}^{-1}$) at neutral pH values in dilute buffer solution. The negative electrophoretic mobility most likely results from a preferential absorption of hydroxy anions at the interface between the PEGylated cyclodextrin vesicles and bulk aqueous solution.[30] For an electrophoretic mobility of $-8 \times 10^{-9}\,\text{m}^2\text{V}^{-1}\text{s}^{-1}$ and a vesicle diameter of 160 nm, the Smoluchovski equation predicts a Zeta potential of −11 mV. In the presence of excess adamantane carboxylate, vesicles of **6b** and **6c** have an electrophoretic mobility of approximately $-30 \times 10^{-9}\,\text{m}^2\text{V}^{-1}\text{s}^{-1}$, which would imply a Zeta potential of approximately −42 mV.

The increase of electrophoretic mobility of the host vesicles in the presence of an increasing concentration of the guest was analyzed in terms of the formation of a 1:1 inclusion complex of **6a–c** and adamantane carboxylate, characterized by the K_a value. The results are summarized in Table 2. As anticipated, vesicles of the β-cyclodextrin amphiphile **6b** have the highest binding constant with $K_a = 7.1 \times 10^3\,\text{M}^{-1}$. This is significantly lower than for β-cyclodextrin **1b**. The difference might be attributed to some hindrance of inclusion into the cavity of **6b** due to the presence of oligo(ethylene glycol) residues or a degree of anticooperativity due to the increasing presence of anionic guests on the vesicle surface. However, the Scatchard plot has a linear slope and an abscissa intercept very close to 1.0, a fact indicating the presence of identical and independent binding sites on the vesicle surface. We can therefore ascribe the inferior binding constant for **6b** (as compared to **1b**) to some steric hindrance and some reduction in the hydrophobicity of the host by the oligo(ethylene glycol) residues. This is consistent with the observation that nonamphiphilic PEGylated cyclodextrins are also poorer hosts than native cyclodextrins.[31]

Table 2. Binding constants, K_a, of adamantane carboxylate to vesicles in 10 mM phosphate buffer (pH 7.5) at 25 °C.

Vesicle	K_a [M^{-1}]
6a	96 ± 40
6b	$7.1 \pm 0.6 \times 10^3$
6c	$3.2 \pm 0.3 \times 10^3$
$C_{12}EO_3$	$3.1 \pm 0.6 \times 10^2$

In comparison to vesicles of **6b**, vesicles of **6c** have considerably less affinity for adamantane carboxylate ($K_a = 3.0 \times 10^3\,\text{M}^{-1}$), a result reflecting the lower tendency of a γ-cyclodextrin cavity relative to that of a β-cyclodextrin to form inclusion complexes with this guest. Also, one expects steric hindrance and reduction in the hydrophobicity of the host by the oligo(ethylene glycol) residues. For host **6c** we do not exclude the formation of 1:2 host–guest complexes, particularly at high guest concentrations. However, the Scatchard plot again has a linear slope and an abscissa intercept very close to 1.0.

The association constant of vesicles of **6a** with adamantane carboxylate ($K_a = 96\,\text{M}^{-1}$) is very small compared to that of **6b** and **6c**; this reflects the fact that the cavity of α-cyclodextrin is too narrow to be a good host for adamantane guests. In fact, the affinity of **6a** is comparable to that of the reference vesicles of $C_{12}EO_3$, which lack any specific host cavities. The increase in electrophoretic mobility of vesicles

of **6a** and $C_{12}EO_3$ in the presence of high concentrations of adamantane carboxylate (>1 mM) most likely results from partitioning of the hydrophobic anion from aqueous solution into the hydrophobic bilayer.

To confirm the specific and reversible binding of adamantane carboxylate to vesicles of **6b**, competition experiments were carried out in the presence of β-cyclodextrin **1b**. To this end, the electrophoretic mobility of vesicles of **6b** was determined in the presence of a given concentration (0.5, 1.0, and 5.0 mM) of **1b** and various concentrations of adamantane carboxylate in the capillary (Figure 7). The association constant of adamantane carboxylate to cyclodextrin **6b** was calculated by using the concentration of free adamantane carboxylate calculated from the total concentration after subtraction of adamantane carboxylate complexed with **1b**, as calculated from the binding constant ($K_a = 3.2 \times 10^4\,M^{-1}$). As can be readily deduced from Figure 7 and Table 3, a similar binding constant was obtained from each competition experiment. These results demonstrate that capillary electrophoresis provides reliable quantitative information about these dynamic equilibria.

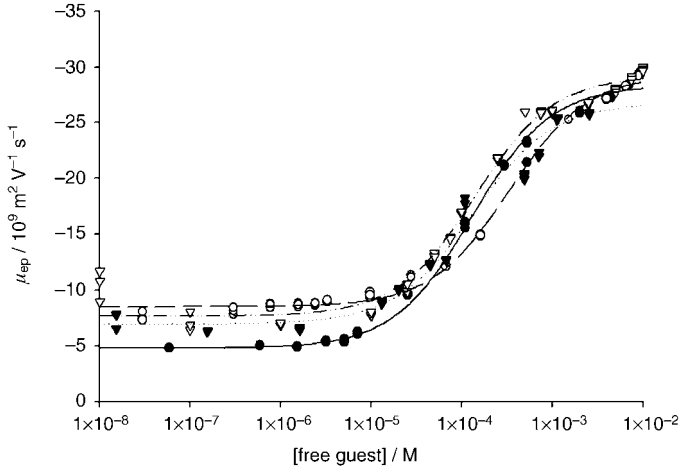


Figure 7. Electrophoretic mobility (μ_{ep}) of β-cyclodextrin vesicles (**6b**) in the presence of the adamantane carboxylate guest and competing β-cyclodextrin host (**1b**). ▽: 0 mM **1b**; ●: 0.5 mM **1b**; ○: 1 mM **1b**; ▼: 5 mM **1b**.

Table 3. Binding constants, K_a, of adamantane carboxylate to β-cyclodextrin vesicles (**6b**) obtained from competition experiments in the presence of β-cyclodextrin (**1b**).

β-Cyclodextrin [mM]	K_a [M^{-1}]
0	$7.1 \pm 0.6 \times 10^3$
0.5	$7.7 \pm 0.5 \times 10^3$
1.0	$3.0 \pm 0.2 \times 10^3$
5.0	$7.4 \pm 0.9 \times 10^3$

Conclusion

Bilayer vesicles formed by nonionic amphiphilic cyclodextrins function as host membranes that bind suitable guest molecules by hydrophobic inclusion at their surface. Capillary electrophoresis provides quantitatively reliable information about these dynamic interactions at the membrane surface. The cyclodextrin cavities function as independent host sites and their characteristic affinity and selectivity for a given guest molecule is not affected when they are confined to a hydrophobic bilayer membrane. The recognition of small guest molecules by cyclodextrin hosts assembled in a bilayer membrane is a useful model of recognition of substrates and ligands by receptors on the surface of cell membranes. At present we are investigating the multivalent interaction of oligomeric guest molecules with host molecules at the vesicle surface. Critical parameters will be the density and mobility of the host in the membrane, the number and flexibility of binding moieties on the guest, and the presence of monovalent competitors. We aim to exploit these specific interactions to bind molecules to vesicles, vesicles to vesicles, and vesicles to surfaces.

Experimental Section

Synthesis: All commercial reagents were used without further purification. Cyclodextrins **1a–c** were dried in vacuum at 80 °C for at least 5 h. *n*-Dodecyl tri(ethylene glycol) ether ($C_{12}EO_3$; containing some *n*-tetradecyl tri(ethylene glycol) ether ($C_{14}EO_3$)) was kindly donated by Servo Sasol (Delden, The Netherlands; the trade name is Serdox NES3). Chlorocyclodextrins **2a–c** and bromocyclodextrins **3a–c** were prepared according to literature procedures.[12,13] β-Cyclodextrins **4b**, **5b**, **6b**, and **7b** have been described previously.[15,17]

General procedure for the preparation of cyclodextrin alkyl thioethers 4a–c and 5a–c:[17] *n*-Alkylthiol (3 equiv for each halogen of cyclodextrin) was dissolved in DMF, and NaH or *t*BuOK (3 equiv) was added. The mixture was stirred at room temperature for 1 h. The appropriate cyclodextrin **2a–c** or **3a–c** was added and the mixture was stirred at 60–80 °C for 3–5 days. The reaction was monitored by TLC (EtOAc/iPrOH/NH_4OH/H_2O 7:7:5:2, $R_f = 0.8$ for **2a–c** and **3a–c**, $R_f = 0$ for **4a–c** and **5a–c**). The reaction mixture was then cooled to room temperature before being added to water. The resulting white precipitate was filtered off and washed with water, methanol, and hexane. The solid was stirred in boiling hexane for 1 h to remove excess thiol, filtered, and dried under high vacuum at 60 °C for 5 h. In some cases, residual thiol was removed by Soxhlet extraction in hexane.

Hexakis(6-dodecylthio)-α-cyclodextrin 4a: Compound **2a** (3.0 g) was treated with dodecanethiol to yield **4a** (4.5 g, 80 %). Alternatively, **3a** (6.0 g) was treated with dodecanethiol to yield **4a** (6.1 g, 64 %). ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 5.90$ (s, 6H, OH-2); 5.84 (s, 6H, OH-3); 4.89 (d, 6H, $J_{1,2} = 3.1$ Hz, H-1); 3.77 (m, 6H, H-3); 3.6 (m, 6H, H-5); 3.05–3.32 (m, 12H, H-2, H-4); 2.7–2.85 (m, 12H, H-6); 2.57 (t, SCH_2); 1.53 (m, SCH_2CH_2); 1.27 (br m, 108H, CH_2); 0.87 (d, 21H, CH_3) ppm; ^{13}C NMR (75 MHz, [D_6]DMSO): $\delta = 105.9$ (C-1), 88.7 (C-4), 76.6, 76.2, 75.5 (C-3, C-2, C-5), 37.4–22.0 (dodecyl), 26.0 (C-6), 14.0 (CH_3) ppm; elemental analysis calcd (%) for $C_{108}H_{204}O_{24}S_6$ (2077.3): C 62.39, H 9.89, S 9.25; found: C 60.55, H 9.55, S 8.99; MALDI MS: *m*/*z*: 2099 $[M+Na]^+$, 2115 $[M+K]^+$.

Octakis(6-dodecylthio)-γ-cyclodextrin 4c: Compound **2c** (2.0 g) was treated with dodecanethiol to yield **4c** (3.5 g, 91 %). Alternatively, **3c** (0.75 g) was treated with dodecanethiol to yield **4c** (1.2 g, 75 %). ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 5.90$ (s, 8H, OH-2); 5.84 (s, 8H,

OH-3); 4.89 (d, 8H, $J_{1,2}=3.1$ Hz, H-1); 3.77 (m, 8H, H-3); 3.6 (m, 8H, H-5); 3.05–3.32 (m, 16H, H-2, H-4); 2.7–2.85 (m, 16, H-6); 2.57 (t, 16H, SCH_2); 1.53 (m, 16H, SCH_2CH_2); 1.27 (brm, 144H, CH_2); 0.87 (d, 24H, CH_3) ppm; ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=105.9$ (C-1), 88.7 (C-4), 76.6, 76.2, 75.5 (C-3, C-2, C-5), 37.4–22.0 (dodecyl), 26.0 (C-6), 14.0 (CH_3) ppm; elemental analysis calcd (%) for $C_{144}H_{272}O_{32}S_8$ (2769.7): C 62.39, H 9.89, S 9.25; found: C 63.51, H 9.70, S 9.18; MALDI MS: *m*/*z*: 2792 $[M+Na]^+$, 2808 $[M+K]^+$.

Hexakis(6-hexadecylthio)-α-cyclodextrin 5a: Compound **2a** (1.0 g) was treated with hexadecanethiol to yield **5a** (1.8 g, 74%). Alternatively, **3a** (6.5 g) was treated with hexadecanethiol to yield **5a** (6.8 g, 58%). ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=5.90$ (s, 6H, OH-2); 5.84 (s, 6H, OH-3); 4.89 (d, 6H, $J_{1,2}=3.1$ Hz, H-1); 3.77 (m, 6H, H-3); 3.6 (m, 6H, H-5); 3.05–3.32 (m, 12H, H-2, H-4); 2.7–2.85 (m, 14H, H-6); 2.57 (t, 12H, SCH_2); 1.53 (m, 12H, SCH_2CH_2); 1.27 (brs, 156H, $13\times CH_2$); 0.87 (d, 18H, CH_3) ppm; ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=102.9$ (C-1), 86.7 (C-4), 74.8, 74.1, 73.5 (C-3, C-2, C-5), 60.2 (C-6), 37.4–22.0 (hexadecyl), 26.0 (C-6), 13.9 (CH_3) ppm; elemental analysis calcd (%) for $C_{132}H_{252}O_{24}S_6$ (2413.7): C 65.63, H 10.51, S 7.96; found: C 63.83, H 9.70, S 7.48; MALDI MS: *m*/*z*: 2437 $[M+Na]^+$, 2451 $[M+K]^+$.

Octakis(6-hexadecylthio)-γ-cyclodextrin 5c: Compound **2c** (0.3 g) was treated with hexadecanethiol to yield **5c** (0.28 g, 43%). Alternatively, **3c** (0.75 g) was treated with hexadecanethiol to yield **5c** (1.0 g, 76%). ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=5.90$ (s, 8H, OH-2); 5.84 (s, 8H, OH-3); 4.89 (d, 8H, $J_{1,2}=3.1$ Hz, H-1); 3.77 (m, 8H, H-3); 3.6 (m, 8H, H-5); 3.05–3.32 (m, 16H, H-2, H-4); 2.7–2.85 (m, 16H, H-6); 2.57 (t, 16H, SCH_2); 1.53 (m, 16H, SCH_2CH_2); 1.27 (brm, 208H, CH_2); 0.87 (d, 24H, CH_3) ppm; ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=105.9$ (C-1), 88.7 (C-4), 76.6, 76.2, 75.5 (C-3, C-2, C-5), 37.4–22.0 (hexadecyl), 26.0 (C-6), 14.0 (CH_3) ppm; elemental analysis calcd (%) for $C_{176}H_{336}O_{32}S_8$ (3218.2): C 65.63, H 10.51, S 7.96; found: C 64.81, H 10.21, S 8.09; MALDI MS: *m*/*z*: 3255 $[M+K]^+$.

General procedure for the preparation of amphiphilic cyclodextrins 6a–c and 7a–c:[17] The appropriate alkyl thioether **4a–c** or **5a–c** (0.5–5.0 g), K_2CO_3 (10% by weight of cyclodextrin), and ethylene carbonate (50 equiv) were mixed in *N*,*N*,*N*′,*N*′-tetramethylurea (5–15 mL). The mixture was stirred at 150°C for 4 h. The reaction was monitored by TLC ($CHCl_3$/MeOH/H_2O 50:10:1, $R_f=0$ for **4a–c** and **5a–c**, $R_f=0.6$ for **6a–c** and **7a–c**). The solvent was evaporated under high vacuum at 70°C in a bulb-to-bulb destillation unit. In large scale reactions (>1 g), the crude product was stirred overnight in a solution of NaOMe (0.1 M) in methanol.[17] The crude product was purified by size-exclusion chromatography (Sephadex LH-20, methanol). For further purification, **6c** was crystallized in methanol and **7c** was eluted over a silica-gel column with a mixture of chloroform and methanol (9:1).

Hexakis [6-dodecylthio-2-oligo(ethylene oxide)]-α-cyclodextrin 6a: Compound **4a** (5.0 g) was treated with ethylene carbonate to yield **6a** (4.9 g, 93%). ^{1}H NMR (300 MHz, [D]$CHCl_3$): $\delta=5.05$ (brs, 6, H-1); 4.0–3.4 (m, H-3, H-5, H-2, H-4, OCH_2CH_2O); 3.00 (m, 12, H-6); 2.60 (m, 12H, SCH_2); 1.60 (m, 12H, CH_2); 1.27 (brs, 108H, CH_2); 0.89 (t, 18H, CH_3) ppm; ^{13}C NMR (75 MHz, [D]$CHCl_3$): $\delta=100.7$ (C-1), 81.0 (C-2, C-4), 71.0–72.0 (C-3, C-5, CH_2O), 61.2 (CH_2OH), 33.4 (C-6), 33.4 (CH_2S), 31.7 (CH_2), 29.5 $(CH_2)_n$, 29.2 (CH_2), 28.8 (CH_2), 22.4 (CH_2), 13.9 (CH_3) ppm; elemental analysis calcd (%) for $C_{132}H_{254}O_{36}S_6$ (2607.6): C 60.75, H 9.81, S 7.37; found: C 59.45, H 9.75, S 7.42; MALDI MS: *m*/*z* (%): 2851 (24) $[M_{17EO}+Na]^+$, 2807 (47) $[M_{16EO}+Na]^+$, 2762 (73) $[M_{15EO}+Na]^+$, 2719 (100) $[M_{14EO}+Na]^+$, 2674 (100) $[M_{13EO}+Na]^+$, 2630 (71) $[M_{12EO}+Na]^+$, 2586 (36) $[M_{11EO}+Na]^+$.

Octakis [6-dodecylthio-2-oligo(ethylene oxide)]-γ-cyclodextrin 6c: Compound **4c** (100 mg) was treated with ethylene carbonate to yield **6c** (90 mg, 69%). ^{1}H NMR (300 MHz, [D]$CHCl_3$): $\delta=5.05$ (brs, 8H, H-1); 4.0–3.4 (m, H-3, H-5, H-2, H-4, OCH_2CH_2O); 3.00 (m, 16H, H-6); 2.60 (m, 16H, SCH_2); 1.60 (m, 16H, CH_2); 1.27 (brs, 144H, CH_2); 0.89 (t, 24H, CH_3) ppm; ^{13}C NMR (75 MHz, [D]$CHCl_3$): $\delta=100.7$ (C-1), 81.0 (C-2, C-4), 71.0–72.0 (C-3, C-5, CH_2O), 61.2 (CH_2OH), 33.4 (C-6), 33.4 (CH_2S), 31.7 (CH_2), 29.5 $(CH_2)_n$, 29.2 (CH_2), 28.8 (CH_2), 22.4 (CH_2), 13.9 (CH_3) ppm; elemental analysis calcd (%) for $C_{176}H_{336}O_{48}S_8$ (3474.2): C 60.80, H 9.74, S 7.38; found: C 61.74, H 9.68, S 8.11; MALDI MS: *m*/*z* (%): 3764 (39) $[M_{22EO}+Na]^+$, 3720 (62) $[M_{21EO}+Na]^+$, 3676 (87) $[M_{20EO}+Na]^+$, 3632 (100) $[M_{19EO}+Na]^+$, 3588 (92) $[M_{18EO}+Na]^+$, 3544 (67) $[M_{17EO}+Na]^+$, 3500 (29) $[M_{16EO}+Na]^+$.

Hexakis [6-hexadecylthio-2-oligo(ethylene oxide)]-α-cyclodextrin 7a: Compound **5a** (4.0 g) was treated with ethylene carbonate to yield **7a** (4.3 g, 89%). ^{1}H NMR (300 MHz, [D]$CHCl_3$): $\delta=5.05$ (brs, 6H, H-1); 4.0–3.3 (m, H-3, H-5, H-2, H-4, OCH_2CH_2O); 3.00 (m, 12H, H-6); 2.60 (m, 12H, SCH_2); 1.57 (m, 12H, CH_2); 1.30 (brs, 156H, CH_2); 0.88 (t, 18H, CH_3) ppm; ^{13}C NMR (75 MHz, [D]$CHCl_3$): $\delta=100.9$ (C-1), 81.2 (C-2, C-4), 71.0–72.5 (C-3, C-5, CH_2O), 61.5 (CH_2OH), 34.1 (C-6), 33.7 (CH_2S), 32.0 (CH_2), 29.8 $(CH_2)_n$, 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 22.7 (CH_2), 14.1 (CH_3) ppm; elemental analysis calcd (%) for $C_{156}H_{300}O_{36}S_6$ (2941.4): C 63.64, H 10.27, S 6.53; found: C 61.22, H 9.75, S 6.10; MALDI MS: *m*/*z* (%): 3070 (24) $[M_{15EO}]^+$, 3027 (55) $[M_{14EO}]^+$, 2983 (86) $[M_{13EO}]^+$, 2940 (100) $[M_{12EO}]^+$, 2896 (90) $[M_{11EO}]^+$, 2853 (55) $[M_{10EO}]^+$, 2808 (26) $[M_{9EO}]^+$.

Octakis [6-hexadecylthio-2-oligo(ethylene oxide)]-γ -cyclodextrin 7c: Compound **5c** (0.75 g) was treated with ethylene carbonate to yield **7c** (0.75 g, 85%). ^{1}H NMR (300 MHz, [D]$CHCl_3$): $\delta=5.05$ (brs, 8H, H-1); 4.0–3.3 (m, H-3, H-5, H-2, H-4, OCH_2CH_2O); 3.00 (m, 16H, H-6); 2.60 (m, 16H, SCH_2); 1.57 (m, 14H, CH_2); 1.30 (brm, 208H, CH_2); 0.88 (t, 24H, CH_3) ppm; ^{13}C NMR (75 MHz, [D]$CHCl_3$): $\delta=100.9$ (C-1), 81.2 (C-2, C-4), 71.0–72.5 (C-3, C-5, CH_2O), 61.5 (CH_2OH), 34.1 (C-6), 33.7 (CH_2S), 32.0 (CH_2), 29.8 $(CH_2)_n$, 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 22.7 (CH_2), 14.1 (CH_3) ppm; elemental analysis calcd (%) for $C_{208}H_{400}O_{48}S_8$ (3922.7): C 63.64, H 10.27, S 6.53; found: C 63.82, H 10.01, S 6.48; MALDI MS: *m*/*z* (%): 4756 (67) $[M_{35EO}+Na]^+$, 4741 (75) $[M_{34EO}+Na]^+$, 4696 (92) $[M_{33EO}+Na]^+$, 4652 (92) $[M_{32EO}+Na]^+$, 4607 (82) $[M_{31EO}+Na]^+$, 4568 (77) $[M_{30EO}+Na]^+$, 4524 (65) $[M_{29EO}+Na]^+$.

Vesicle preparation: Vesicles of **6a–c** and **7a–c** were prepared by sonication or extrusion. Typically, amphiphilic cyclodextrin (several mg) in chloroform (approximately 1 mL) was dried by rotary evaporation to yield a thin film in a glass vial. Residual solvent was removed under high vacuum. Water or buffer (1–5 mL, 10 mM phosphate or 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), pH 7.2) was added and the sample solution was kept for 1 h at room temperature (**6a–c**) or at 50°C (**7a–c**). The resulting suspension was sonicated in a Bransonic 1510 sonication bath for 1 h to give small unilamellar vesicles. Alternatively, the suspension was repeatedly passed through a polycarbonate membrane with 0.1 μm pore size in a LiposoFast extruder. Vesicles of **6a–c** were sonicated or extruded at room temperature, whereas vesicles of **7a–c** (with a melting temperature, T_m, estimated to be around 48°C)[6a] were sonicated or extruded at 50°C. Reference vesicles were prepared from $C_{12}EO_3$ (10 mg) that was dissolved in chloroform (1 mL) and then dried by rotary evaporation for 3 h to give a thin film. Water (2.5 mL) was added and the sample was sonicated below 20°C for 30 min.

Transmission electron microscopy (TEM): Samples for TEM were prepared on 200 mesh formvar-carbon-coated copper grids. A drop of cyclodextrin solution (approximately 0.1 mg mL^{-1}) was left on the grid for 2 min then gently blotted with filter paper. The samples were stained with a drop of 2% (w/w) uranyl acetate, left for 5 min, and blotted again. The samples were investigated in a JEOL 2000 transmission electron microscope operating at 80 kV.

Dynamic light scattering: Dynamic light-scattering measurements were carried out at room temperature by using Malvern instrumentation. The amphiphile concentration was approximately 0.2 mg mL^{-1}. The solutions were filtered through 0.45 μm Gelman Acrodisk syringe filters prior to light-scattering measurements. Size distributions were obtained from a CONTIN analysis of the scattering data.

Dye encapsulation: A 10 mM solution of carboxyfluorescein was prepared in 10 mM HEPES buffer solution (pH 7.2). Cyclodextrin vesicles were prepared by dissolving the appropriate cyclodextrin **6a–c** or **7a–c** in chloroform and then evaporating the solvent to form a thin film. The carboxyfluorescein solution (1 mL) was added and the cyclodextrin film was hydrated for 1 h. Next, the sample was shaken vigorously and sonicated for 1 h at 50°C. Cyclodextrin concentrations ranging from 0.5–20 mg mL^{-1} were evaluated, although little entrapment of dye molecules

was obtained below concentrations of 1.5 mg mL^{-1}. Above concentrations of 1 mg mL^{-1}, **7c** and carboxyfluorescein precipitated and no encapsulation could be measured. The solution of encapsulated guest molecules (250 μL) was loaded onto a Sephadex G-25 size-exclusion column (18× 1 cm, void volume = 4 mL) with HEPES (10 mM) solution as the eluent. Fractions of 1 mL were collected. These samples were made up to 2.5 mL in transparent perspex cuvettes. A Perkin–Elmer LB50 fluorescence spectrometer was used to measure the fluorescence of the fractions (λ_{ex} = 485 nm and λ_{em} = 520 nm). Vesicles were detected by light scattering at $\lambda_{ex} = \lambda_{em}$ = 400 nm. The fluorescence of each fraction was measured immediately and after storage for 2 days at room temperature, over which time there was less than 10% difference. Finally, the fluorescence of each fraction was reexamined upon addition of the detergent Triton X-100, which lyses the vesicles.

X-ray diffraction: The X-ray diffraction measurements were performed on a Panalytical X'Pert Pro diffractometer (Panalytical, Almelo, The Netherlands) by using nickel-filtered $Cu_{K\alpha}$ radiation (tube operating at 40 kV and 40 mA). The data were collected by using an automatic divergence slit (5 mm irradiated length) and a 0.2 mm receiving slit. The data were collected from 1–10° (2θ) with a step size of 0.005° (2θ) and a counting time of 1.0 s per step. The samples were prepared by depositing a droplet of cyclodextrin vesicle solution (approximately 2 mg mL^{-1}) on a microscope cover slide and letting this dry in a flow of warm air until a thin film of cyclodextrins remained on the cover slide. In the X-ray diffractogram, first-, second-, and third-order Bragg peaks were observed, except with cyclodextrins **7a** and **7b**, for which only first-order peaks were observed. From the positions of the first-order peaks, the bilayer thickness was calculated by using Bragg's law.

Langmuir monolayers: Spreading solutions were prepared by dissolving a known quantity (approximately 5 mg) of the cyclodextrin in chloroform (5 mL). Pressure–area measurements were carried out in a Teflon trough of 400 mL. Cyclodextrin solutions were deposited in an appropriate volume (approximately 12 μL) with a micropipette at the air–water interface. 30 min was allowed for solvent evaporation and equilibration. Pressure–area isotherms were measured at 20 °C on a Langmuir type balance (Nima Technology). Compressions were performed continuously at a rate of 20 cm^2 min^{-1} from 510–50 cm^2. Each sample was run at least twice to ensure reproducibility of results. Brewster angle microscopy was carried out by using an NFT Mini Brewster angle microscope on the Nima film balance. Compression rates were the same as for pressure–area measurements. The image size is 4×6 mm and resolution is $<$20 μm.

Capillary electrophoresis (CE): Capillary electrophoresis was carried out as described previously.[6c,28] Measurements were carried out on a 57 cm (48.5 cm from inlet to detector) fused silica capillary (75 μm internal diameter; Polymicro Technologies) with a separation voltage of 25 kV, by using an Agilent HP 3D CE system. The capillary was conditioned with 1 N NaOH (5 min), water (1 min), and 10 mM phosphate buffer (1 min) before each series of measurements and running buffer (1 min) before each measurement. The running buffer was prepared with a varying concentration of adamantane carboxylate in 10 mM phosphate buffer adjusted to pH 7.5. The analyte sample (0.2 mg mL^{-1} in 5 mM phosphate buffer) was introduced with 34.5 mbar injection for 5 s and detected with a diode array detector at 200 nm. Measurements were repeated two or three times for each concentration. For competition experiments, the elution time of the vesicles was determined in the presence of a known concentration (0.5, 1.0, or 5.0 mM) of β-cyclodextrin **1b** and various concentrations of adamantane carboxylate in the capillary. The electrophoretic mobility, μ_{ep}, of the vesicles was determined from the elution time according to Equation (1), where l and L denote the effective length (in m) of the capillary from injector to detector and the total length (in m), respectively, V is the voltage (in V), and t_{eof} and t represent the elution times (in s) of the electroosmotic flow (detected by a negative peak) and the sample, respectively.

$$\mu_{ep} = l\,L\,V^{-1}(1/t - 1/t_{eof}) \quad (1)$$

Binding contants, K_a, were calculated from a nonlinear regression of the change of electrophoretic mobility of the vesicles as a function of the adamantane carboxylate concentration, with the assumption that the concentration of complexed guest is always small relative to the total guest concentration.[28]

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