# Interconnective Host−Guest Complexation of β-Cyclodextrin−Calix[4]arene Couples

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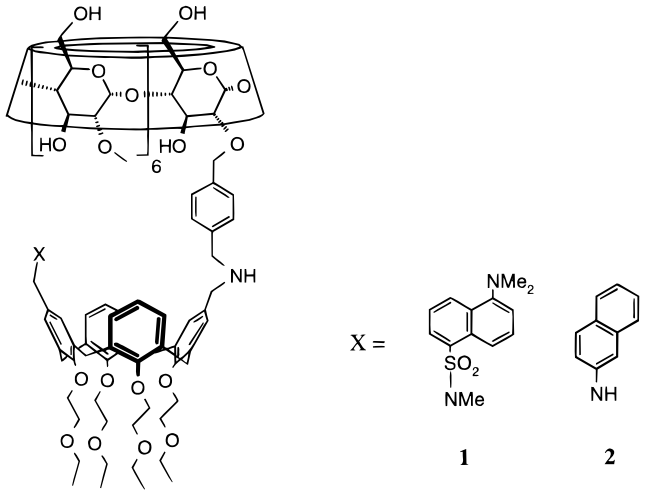
**Abstract:** The two β-cyclodextrin−calix[4]arene couples **1** and **2** were prepared as sensing molecules for the detection of organic analytes in water. Compounds **1** and **2** are amphiphilic in nature and form aggregates in aqueous solution. Compound **1** forms vesicles both in the absence and in the presence of guest species, and its fluorescence intensity does not change. Compound **2** forms fibers, which change into vesicles upon guest addition. This behavior is accompanied by a reduction in fluorescence intensity. The aggregates were visualized by transmission electron microscopy using both the freeze fracture technique and the uranyl staining method. Langmuir monolayer experiments show that intermolecular interactions lead to a preorganization of **2**, whereas molecules of **1** behave independently analogous to conventional amphiphiles. Fluorescence anisotropy decay measurements give evidence for rapid internal dye motion in the aggregates of both compounds **1** and **2**. In addition, a slower decay process of low amplitude is observed for both compounds, indicating free rotational motion of single molecules of **1** but the absence of rotational motion of individual molecules within the aggregates of **2**. This difference indicates the intermolecular complexation of the fluorophores in the aggregates of **2**. The fluorescence lifetimes of aqueous solutions of **2** reveal that the reduction in fluorescence intensity is based on static quenching by the amino group present in the spacer of **2**. Our results show the presence of vesicular bilayers of independent amphiphiles for **1**, and for **2** the formation of assemblies of molecular threads which are composed by interconnective, linear host−guest complexation.

## Introduction

Cyclodextrins are cyclic oligomers of glucose, the most common of which are α-, β-, and γ-cyclodextrin with six, seven, or eight glucose moieties, respectively.[1,2] These compounds are water soluble and have the shape of a truncated cone with a hydrophobic cavity, which makes them suitable as natural host compounds for organic guest molecules. A wide variety of possible applications based on this property has been investigated, like the development of sensing molecules. In this case a reporter group, for example a dye, is attached to a cyclodextrin.[3−8] The mechanism of sensing is based on the optical change of the dye when it is in competition with an organic analyte for accommodation in the relatively apolar cyclodextrin cavity.

**Chart 1**




Recently, we have synthesized the β-cyclodextrin−calix[4]-arene couples 1 and **2** (Chart 1) as fluorescent host species for the detection of organic analytes.[9] The calix[4]arene was introduced to orient cyclodextrin and fluorophore for intramo-

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lecular inclusion and to enlarge the hydrophobic surface for binding analytes in an aqueous environment. Although **1** and **2** differ only in the attached fluorophores, the 2-aminonaphthyl and the dansyl groups, respectively, they exhibit very different behavior upon the addition of organic analytes in aqueous solutions. Compound **2** responds, as intended, with a distinct reduction of the fluorescence intensity whereas compound **1** does not show any change in its fluorescence behavior. The insensitivity of **1** toward organic guest molecules has been attributed to a very strong inclusion of the dansyl fluorophore into the cyclodextrin cavity that is blocking the complexation of a competing organic analyte.

The $\beta$-cyclodextrin−calix[4]arene couples **1** and **2** are amphiphilic in nature, the cyclodextrin moiety being the hydrophilic part and the calixarene with the fluorophore being the hydrophobic part. In this paper we report the unprecedented aggregation behavior of **1** and **2** in aqueous solution. For **2** this results in a unique sensing mechanism upon addition of guest species. The aggregates formed by **1** and **2** have been characterized by light scattering, transmission electron microscopy, surface pressure/surface area isotherms, and fluorescence spectroscopy. On the basis of these results, molecular structures for the aggregates of **1** and **2** are proposed.

## Experimental Section

**Light Scattering.** QELS (quasi elastic light scattering) was performed with an ALV (Langen, Germany) goniometer at an angle of 90°, with an Adlas Model DPY 305 II, 50 mW, CD diode-pumped YAG laser, wavelength 532 nm (Adlas, Lübeck, Germany; now taken over by Coherent), and an ALV 5000 multiple $\tau$ digital correlator. The correlation function was transformed into a diameter distribution with an Inverse Laplace Transalgorthm program.[10] Sample concentrations were in the range of 0.5 mg/mL in doubly distilled water.

**Electron Microscopy.** For negative staining a drop of an aqueous dispersion of the amphiphile (0.5 mg/mL) was brought onto a Formvar-coated copper grid. After 1 min the excess of dispersion was blotted off and the sample was stained with a 2% (w/w) uranyl acetate solution (1 min). Freeze-fractured samples were prepared by bringing a drop of the dispersion onto a golden microscope grid (150 mesh), placed between two copper plates. These were injected into liquid propane and subsequently transferred to a sample holder kept under liquid nitrogen. The sample holder was placed in a Balzers Freeze Etching System BAF 400 D at $10^{-7}$ Torr and brought to −105 °C. After fracturing, the samples were etched for 1 min ($\Delta T = 20$ °C), shaded with Pt (layer thickness 2 nm), and covered with carbon (layer thickness 20 nm). Replicas were allowed to warm to room temperature, treated with 20% chromic acid for 16 h, and rinsed with water. After preparation the grids were allowed to dry and studied under a Philips TEM 201 microscope (60 kV).

**Monolayer Experiments.** Isotherms were recorded at 20.0 ± 0.1 °C using a double barrier R&K trough of dimensions 6 × 25 cm with a compression speed of 4.4 $cm^2 \cdot min^{-1}$. On the subphase 15 $\mu$L of a chloroform solution (0.5 mg/mL) of the amphiphile was spread and allowed to evaporate for 10 min. The recorded surface pressures were reproducible with an experimental error of ca. 3%. No changes in the isotherms were observed when longer (up to 30 min) evaporation times were applied.

**Fluorescence Measurements.** For fluorescence spectroscopy, solutions (1.4 mmol/L) of **1** and **2** were prepared with pure water (Millipore Q2) and a phosphate buffer (pH 7, $I = 0.02$). 1-Adamantanol was added as a solution in methanol (30 mmol/L). The effect of methanol was found to be negligible. Fluorescence decay and fluorescence anisotropy decay measurements were performed using a picosecond laser and time-correlated single photon counting setup described previously.[11] The excitation wavelength was 340 nm. For compound **2** an emission wavelength of 410 nm (line filter from Baird Atomic) and for dansyl compound **1** an emission wavelength of 522 nm (line filter from Schott, Mainz, Germany) were chosen. The instrumental response function was obtained with a reference compound (POPOP = 1,4-bis(5-phenyloxazol-2-yl)benzene in ethanol, $t = 1.35$ ns). Two time spacings of the multichannel analyzer were chosen: 12 and 50 ps per channel. The decay curves were collected in 1000 channels. The experimental data were analyzed using the global analysis program obtained from Globals Unlimited (Urbana, Illinois) and described by Beechem et al.[12] Since the short correlation time was reproducibly retrieved in both experimental time scales, the results obtained at the longer time scale (50 ps) are mentioned in the text. It should be noted that the longer fluorescence lifetimes and correlation times are more accurately recovered in the experiments at the longer time scale. Addition of guest to **1** or **2** does not result in change in absorption, implying that the efficiency of light excitation in the experiments is not altered.

## Results

Aqueous solutions of compounds **1** and **2** are strongly surface active and produce foams even at concentrations of a few micromoles per liter. This observation was an indication that possibly aggregates are formed and that the observed response in fluorescence intensity of compound **2** upon guest addition may be related to this phenomenon. Regarding the structural similarity**,** one would expect a similar aggregation pattern for the fluorescent $\beta$-cyclodextrin−calix[4]arene couples **1** and **2**. For the wavelength of the maximum fluorescence emission of compounds **1** and **2** in aqueous solution a hypsochromic shift was found compared to the emission of the native fluorophores in water.[9] From these shifts it was concluded that the fluorophores are both located in the cyclodextrin cavities. This result leaves open two possibilities for the molecular structure of the aggregates; i.e., the fluorophore can be included *intra*molecularly or *inter*molecularly in a cyclodextrin cavity. The first case results in aggregates composed of an assembly of individual molecules (Chart 2A). In the second case the amphiphiles are interconnected by inclusion of the fluorophore of a molecule in the cyclodextrin cavity of an adjacent one (Chart 2B).

**Characterization of the Aggregates.** Light scattering experiments (QELS) of aqueous solutions of compound **1** showed the presence of aggregates with an average diameter of 120 nm and a sharp size distribution. Aggregates of similar size were found for other cyclodextrin[13−16] or calix[4]arene[17] amphiphiles. In contrast, the experiments with compound **2** yielded two values, 3.6 and 85 nm, for the diameter of the aggregates occurring in a relative ratio of 1:3 and a relatively sharp size distribution. As light scattering experiments do not provide information on the structure of the observed aggregates, these results simply indicate that there is a difference in the aggregation behavior of **1** and **2**.

For visualization by transmission electron microscopy (TEM), the aggregates of **1** and **2** were stained with uranyl acetate. Compounds **1** and **2** possess 2-ethoxyethoxy groups at the lower

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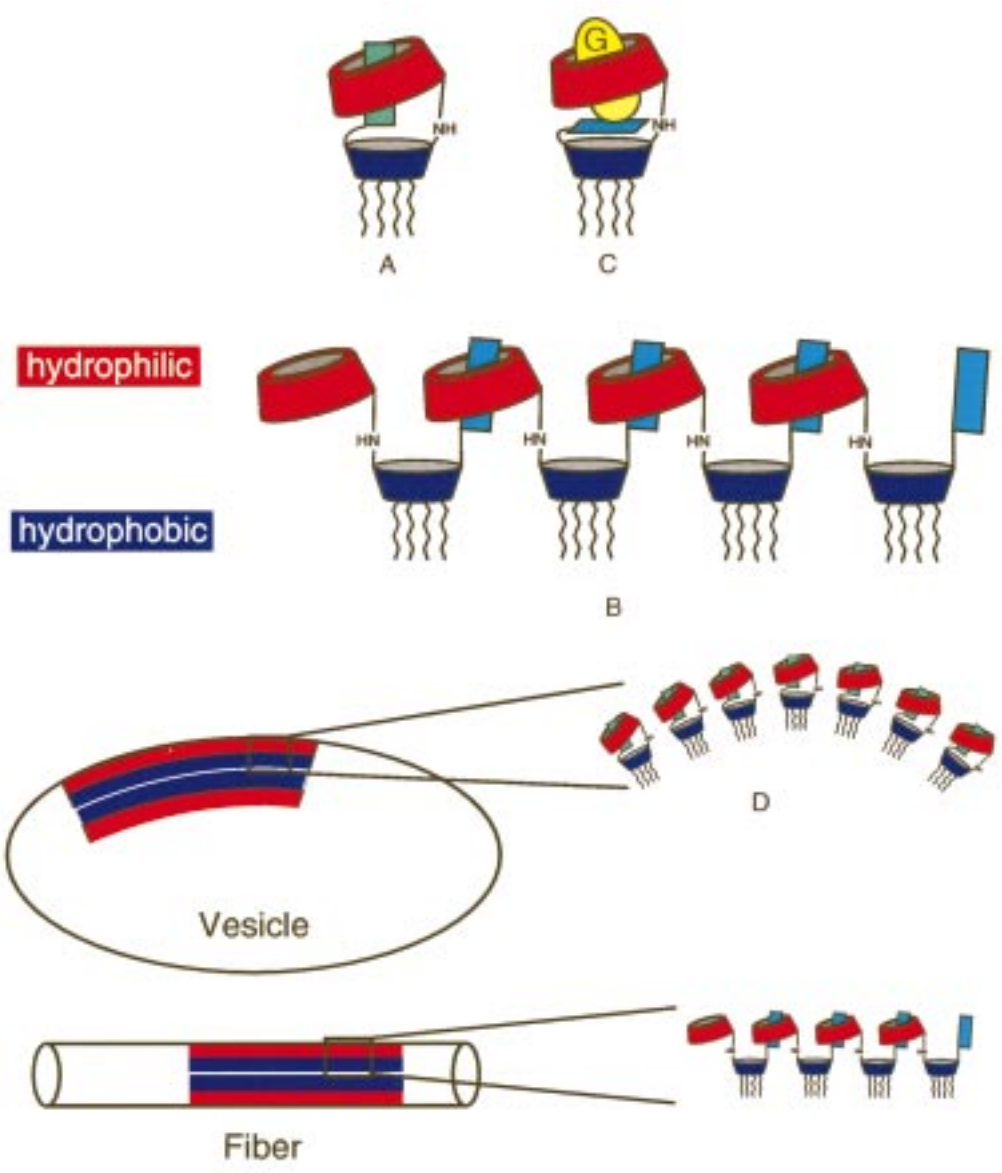
**Chart 2**




rim of the calixarene moiety, which are known to complex metal ions.[18,19] However, the possibility that the staining agent has influenced the observed mode of aggregation could be excluded by applying also the freeze fracture technique as a reference method which gave similar results. For the aggregates of compound **1**, the obtained micrographs clearly show vesicle structures with diameters in the range of 50−100 nm (Figure 1A), which is in accordance with the values obtained with the dynamic light scattering experiments. Surprisingly, for compound **2** only linear, fiberlike structures were observed (Figure 1B) with diameters on the order of 50 nm and lengths up to several micrometers.

To investigate possible morphological changes upon the addition of organic guest molecules, 1-adamantanol was added. In an earlier study it turned out that compound **2** responds very strongly to the addition of 1-adamantanol, resulting in a large reduction of the fluorescence intensity of **2**.[9] As can be seen in Figure 1C, the fiberlike structures have been converted into vesicles after addition of 1-adamantanol, having diameters similar to those found for **1**. The linear arrangement of the vesicles in the middle of Figure 1C suggests that it originates from the former fiber-type structure. In contrast, the microscopic appearance of **1** did not change upon the addition of 1-adamantanol.

**Preparation of Monolayers.** To gain more insight into the molecular structure of the aggregates, we measured Langmuir isotherms of monolayers of **1** and **2** at the air/water interface. The results are shown in Figure 2. For both compounds a molecular area of approximately 200 Å$^2$ was found by extrapolation of the isotherms to zero pressure. This area is close to the molecular area of a $\beta$-cyclodextrin oriented perpendicular to the interface (∼180 Å$^2$).[20] The isotherm for the monolayer of compound **1** corresponds to the behavior of a conventional amphiphile showing a liquid-expanded/liquid-compressed coexistence region with a significant increase in surface pressure, starting already from a surface area of >300 Å$^2$ per molecule. The monolayer of **1** behaves as if it was composed of independent, individual molecules, with their fluorophores being

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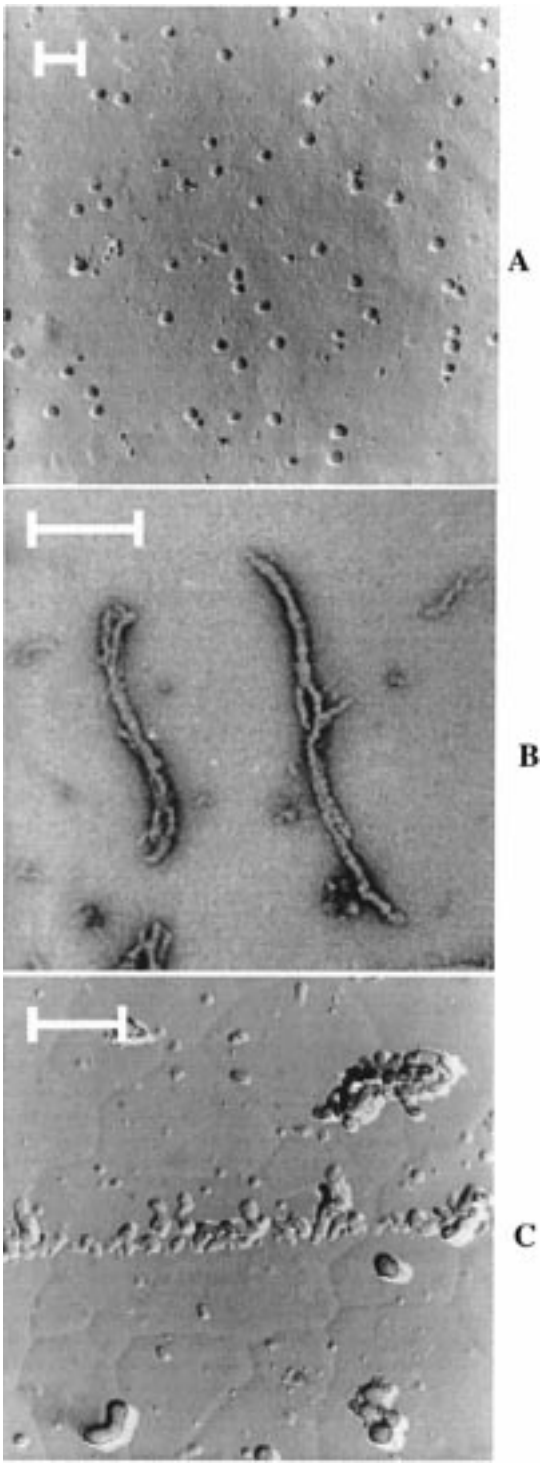


**Figure 1.** Micrographs made by transmission electron microscopy. Each bar represents 200 nm. Concentrations were each 0.4 mg/mL in doubly distilled water. (A) Compound **1** (freeze fracture), (B) compound **2** (uranyl staining), (C) compound **2** + 1 mmol/L 1-adamantanol (freeze fracture).

complexed intramolecularly. The absence of a clear collapse is interpreted as a gradual dissolution or multilayer formation upon compression below the limiting molecular area. This may be caused by the absence of long aliphatic tails, which prevent such a behavior in conventional surfactants.

The monolayer of compound **2** can be compressed to a molecular area of 200 Å$^2$ until a significant increase in surface pressure occurs, leading to the conclusion that the intermolecular interaction in **2** in the dilute state is much stronger than in **1**.

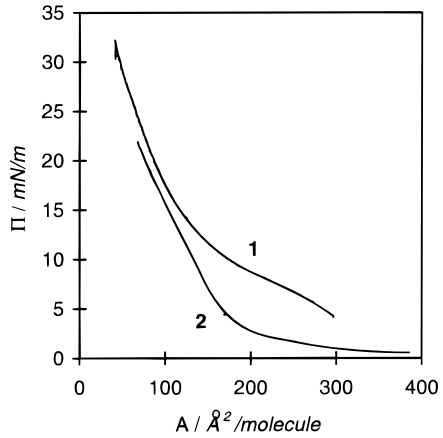


**Figure 2.** Surface area/surface pressure isotherms of monolayers of **1** and **2** at the air/water interface.

**Table 1.** Relative Fluorescence Intensity $\Delta I/I$, Average Fluorescence Lifetime $\langle\tau\rangle$, Fluorescence Lifetimes $\tau_i$, and Normalized Amplitudes $\alpha_i$ of Aqueous Solutions of **1** and **2** in Phosphate Buffer (pH 7, $I = 0.02$), Each 1.5 $\mu$mol/L[a]

| sample composition | $\Delta I/I_0$ | $\langle\tau\rangle$/ns | $\tau_1$/ns ($\alpha_1$) | $\tau_2$/ns ($\alpha_2$) | $\tau_3$/ns ($\alpha_3$) |
|---|---|---|---|---|---|
| **1** | 1 | 13.1 | 5.95 (0.43) | 9.44 (0.19) | 23.1 (0.38) |
| **1** + G | 0.98 | 12.1 | 5.44 (0.45) | 10.9 (0.29) | 24.9 (0.26) |
| **1** + G + US | 0.97 | 11.7 | 5.31 (0.44) | 9.80 (0.29) | 24.6 (0.27) |
| **2** | 1 | 12.4 | 0.14 (0.14) | 0.20 (0.23) | 17.3 (0.63) |
| **2** + G | 0.64 | 13.5 | 0.02 (0.04) | 0.20 (0.29) | 17.2 (0.66) |
| **2** + G + US | 0.51 | 13.8 | 0.01 (0.02) | 0.18 (0.26) | 16.9 (0.71) |

[a] US = solution was ultrasonicated for 1 h. G = 1-adamantanol was added (600 mmol/L).

This is an indication for the existence of preorganized monolayers of **2**, which can be caused by the complexation of each fluorophore by a cyclodextrin cavity of a neighboring molecule.

**Fluorescence Measurements.** Ueno and others have prepared cyclodextrins with an appended fluorophore at one of the primary or secondary hydroxyl groups as sensing molecules.[4−8] In two of these cases the mechanism of fluorescence quenching upon guest addition was studied by means of fluorescence lifetime measurements and found to be of dynamic nature.[4,5] The addition of guest species partly expels the fluorophore from the cyclodextrin cavity, which consequently is more exposed to water than without the guest species present. Water quenches fluorescence emission by radiationless decay, i.e., by dynamic quenching, and upon guest addition a decrease of the average fluorescence lifetime with concomitant biexponential decay was measured.

Upon guest addition to aqueous solutions of compound **1**, no decrease in the average fluorescence lifetime was found within the experimental error ($\langle\tau\rangle = 12.3 \pm 0.6$ ns; see Table 1).[21] This result was expected as the fluorescence intensity did not change in these experiments, i.e., $\Delta I/I_0 = 1$.

Surprisingly, also in the case of aqueous solutions of compound **2**, where the fluorescence intensity is reduced to a value of $\Delta I/I_0 = 0.51$ upon guest addition, the average lifetime of the excited state was constant within the experimental error ($\langle\tau\rangle = 13.2 \pm 0.6$ ns).[22] A reduction of fluorescence intensity without a concomitant change in lifetimes and preexponential factors is evidence for a *static quenching mechanism*, with the quencher being complexed or covalently bound to the fluorescent molecule.[23] The quenching moiety present in **2** is the

(21) The fluorescence decay of each sample was fitted to a triexponential decay curve. For clarity, the average lifetime of each set was calculated: $\langle\tau\rangle = \sum_{i=1-3}\alpha_i\tau_i$.

dibenzylic amine, which connects the cyclodextrin with the calixarene moiety. Amines are known to quench fluorescence emission by a photoinduced-electron-transfer (PET) mechanism.[24] Consequently, fluorescence quenching in **2** upon guest addition occurs by an enhanced PET as a result of a reduced distance between the fluorophore and the amine compared to that in **2** alone. This is supported by the lower sensitivity of **2** toward guest species at lower pH. At pH 9 compound **2** responds to the presence of 0.1 mmol/L norethindrone with a fluorescence intensity reduction of 51%, while the reduction is only 25% at pH 7. The higher fraction of nonprotonated amine at higher pH enables a more effective quenching of the fluorophore and consequently gives a higher response upon guest addition.

To evaluate the mobility of the fluorophores in the aggregates of **1** and **2**, the time-dependent fluorescence depolarization was measured. The biexponential fit of the depolarization data yielded two rotational correlation times $\phi_1$ and $\phi_2$ with preexponential factors $\beta_1$ and $\beta_2$ for the different modes of rotational diffusion of the fluorophores.

For compound **1**, the predominant contribution to the depolarization was found to be $\phi_1 \approx 1$ ns ($\beta_1 = 0.29$). This rapid rotational correlation time corresponds to the internal rotation of the fluorophore itself, irrespective of its incorporation into a larger aggregate. The large preexponential factor of $\beta_1 = 0.29$ implies that this rotation is almost unrestricted. For the second, smaller contribution ($\beta_2 = 0.015$) a value of $\phi_2 \approx 60$ ns could be resolved. This value is attributed to the rotational diffusion of a species with a radius of about 50 Å, which matches the rotation of a $\beta$-cyclodextrin−calix[4]arene molecule as a whole.[25]

Also for compound **2** a rotational correlation time $\phi_1 \approx 1$ ns ($\beta_1 = 0.20$) was found, representing the internal rotation of the fluorophore itself. The second, longer rotational correlation time $\phi_2$, having a smaller contribution ($\beta_2 = 0.008$), could not be resolved ($\phi_2 > 100$ ns) and has to be regarded as residual anisotropy originating from rotational motion of very large aggregates. The absence of a rotational correlation time $\phi_2$ of ∼60 ns, which was found for compound **1**, affirms that in the aggregates of **2** the rotation of single $\beta$-cyclodextrin−calix[4]-arene molecules is not possible, i.e., that the single molecules are immobilized in an interlocked structure. This locking is most likely due to intermolecular complexation of the fluorophores in neighboring cyclodextrin cavities.

The addition of guest molecules (1-adamantanol, 600 $\mu$mol/L) to aqueous solutions of **2** followed by ultrasonication caused a clear change in the slow fluorescence anisotropy decay curve (Figure 3), yielding a decrease in $\phi_2$ from the nonresolvable value >100 ns to a value of ∼80 ns.[26] This observation indicates that the inclusion of guest species in the cyclodextrin cavity induces a transition from the initial interlocked structure to a structure where the single molecules can rotate freely. For compound **1** no changes in rotational correlation times were found upon guest addition.[27]

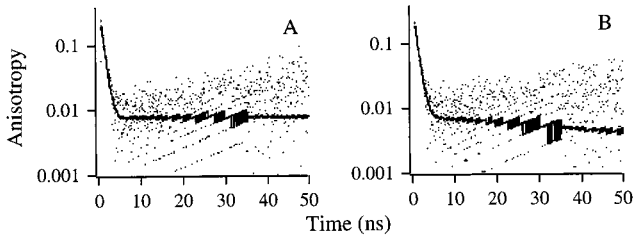


**Figure 3.** Experimental (dots) and fitted (solid lines) fluorescence anisotropy decay curves of (A) **2** alone and (B) **2** after addition of 1-adamantanol (600 $\mu$M/L) and ultrasonication (1 h). The initial fast decay is the same in both cases, but the slowly decaying part is clearly different, yielding an apparent immobilization in the case of **2** alone and a distinct slow decay for **2** after the addition of guest and ultrasonication. The fitted curves show some irregularities, which is due to the limited resolution in digitization. Note that the contribution of the slow decay is less than 0.01.

## Discussion

The TEM pictures show that **1** forms vesicles in aqueous solution. The driving force for aggregation is the amphiphilic nature of **1**. Previous experiments indicated that the dansyl fluorophore is strongly complexed in the cyclodextrin cavity and cannot be expelled easily by external guest molecules.[9] The Langmuir isotherms of monolayers of **1** on the air/water interface showed a behavior that is commonly found for independent amphiphiles. Additionally, the rotational correlation times of aggregates of **1** indicate a free rotation of each fluorescent $\beta$-cyclodextrin−calix[4]arene couple within the vesicle and the absence of intermolecular interactions.[28] Consequently, these results are in accordance with vesicles of compound **1** in which amphiphiles of structure A (Chart 2) are placed next to each other to form a vesicular bilayer (Chart 2D).

The structure of the aggregates of compound **2** is more complicated. The TEM micrographs show that solutions of **2** alone contain fiberlike structures. The complicated light scattering pattern and the residual anisotropy found for the aggregates of **2** point toward nonspherical structures and corroborate the microscopic observations. Monolayers of **2** at the air/water interface strongly indicate preorientation caused by intermolecular interactions between individual amphiphiles. The absence of rotational diffusion for the single $\beta$-cyclodextrin−calix[4]arene couples as deduced from the slower process of the fluorescence anisotropy decay provides further, strong evidence that single amphiphiles interact within the aggregates.

(22) The decrease in fluorescence intensity upon guest addition was larger after the sample was ultrasonicated after the addition of the guest species (see Table 1). This phenomenon is not surprising, as ultrasound is known to lower kinetic barriers in transformation processes of aggregates. See for example: Fuhrhop, J.-H.; Köning, J. Membranes and Molecular Assemblies: The Synkinetic Approach. In *Monographs in Supramolecular Chemistry*; Stoddard, J. F., Ed.; The Royal Society of Chemistry, London, 1994; p 87.

(23) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983.

(24) For a review of applications see: de Silva, A. P.; Nimal Gunaratne, H. Q.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.

(25) For spherical rotation the volume of the rotating unit $V$ is related via the Stokes−Einstein equation to the measured rotation correlation time $\phi$, where $\eta$ is the viscosity of the solution, $k$ is the Boltzmann constant, and $T$ is the absolute temperature: $\phi = \eta V/kT$. In our case the viscosity of plain water was taken as an approximation for the viscosity of the very dilute solutions of **1** and **2**.

(26) The long component of the fluorescence anisotropy decay could be resolved only for experiments where the solution of **2** was treated with ultrasound after the addition of guest species. Without ultrasound the mentioned changes were also in the offing but remained mathematically undetermined. A global error analysis showed that the difference in rotation correlation times of $\phi_2 = 60$ and 80 ns is not significant.

(27) It must be emphasized that the anisotropies presented in Figure 3 are reconstructions of the individual decay curves (parallel and perpendicularly polarized). The real analysis is done in which both polarized components are analyzed simultaneously in a global manner. Both polarized decay curves have maximum intensities which extend over 4−5 decades. In other words, the resolution in anisotropy decay experiments is better than 0.01. A rigorous error analysis shows that the longer correlation time is clearly different in both examples notwithstanding the fact that the amplitudes are small.

(28) For a study about other vesicles formed by amphiphilic cyclodextrins see: Gulik, A.; Delacroix, H.; Wouessidjewe, D.; Skiba, M. *Langmuir* **1998**, *14*, 1050.

Consequently, the fiberlike structures might be composed of molecular threads consisting of noncovalently polymerized molecules of **2**, with the fluorophores intermolecularly complexed by neighboring cyclodextrin cavities (Chart 2B,E).

The addition of organic guests displaces the naphthyl fluorophore of the amphiphile **2** from the cyclodextrin cavity with a concomitant reduction in fluorescence intensity. This fluorescence quenching is of static nature, which leads to the conclusion that the fluorophore is now closer to the quenching amine spacer. Considering the tendency of hydrophobic groups to minimize their overall surface that is exposed to water, the naphthyl group outside the cyclodextrin cavity is most probably stacked over the calix[4]arene and in this way is closer to the quenching amino group (Chart 2C; G = guest molecule). As structure C is not able to interact intermolecularly, the fibers break down and the amphiphilic molecules form vesicles, as observed by TEM. The static quenching upon addition of organic guest species is an unprecedented sensing mechanism and offers new possibilities for the tuning of the desired response.

The amphiphilicity of cyclodextrin derivatives has been the subject of a number of publications.[13−16] Cyclodextrin amphiphiles bearing rather small hydrophobic groups were found to form micelles at low concentrations already.[29] However, the aggregation tendency of cyclodextrin derivatives used for sensing purposes has not been taken into consideration yet. The behavior of **1** and **2** indicates that the sensing mechanism can be influenced by the amphiphilic character and concomitant aggregation of the species.

The spontaneous threading of cyclodextrins onto polyethers,[30] polyamines,[31] or polyurethanes[32] resulting in rotaxanes is a well-known process. Tubelike aggregates of $\beta$- and $\gamma$-cyclodextrin with diphenylhexatriene (DPH) were also reported in the literature.[33] Nevertheless, self-assembled nanostructures which are stable in aqueous solution and consist of only one species, like the supramolecular arrangements of **2**, were not reported up to now.[34]

The stability is primarily caused by strong hydrophobic interactions that cause the single amphiphiles to aggregate. The secondary, shape-determining interaction is the *inter*- or *intra*molecular complexation of the fluorophores, resulting in vesicle formation for **1** and yielding molecular threads in the case of **2**.[35] This approach using two complementary forces for the arrangement of supramolecular structures can also be found in nature, e.g., in the folding of DNA or peptides, which is based on hydrophobic effects and hydrogen bonding.

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