Experimental and Computational Study of BODIPY Dye-Labeled Cavitand Dynamics

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Supporting Information

ABSTRACT: Understanding the distance distribution and dynamics between moieties attached to the walls of a resorcin[4]arene cavitand, which is switchable between an expanded kite and a contracted vase form, might enable the use of this molecular system for the study of fundamental distance-dependent interactions. Toward this goal, a combined experimental and molecular dynamics (MD) simulation study on donor/acceptor borondipyrromethene (BODIPY) dye-labeled cavitands present in the vase and kite forms was performed. Direct comparison between anisotropy decays calculated from MD simulations with experimental fluorescence anisotropy data showed excellent agreement, indicating that the simulations provide an accurate representation of the dynamics of the system. Distance distributions between the BODIPY dyes were established by comparing time-resolved Förster resonance energy transfer experiments and MD simulations. Fluorescence intensity decay curves emulated on the basis of the MD trajectories showed good agreement with the experimental data, suggesting that the simulations present an accurate picture of the distance distributions and dynamics in this molecular system and provide an important tool for understanding the behavior of extended molecular systems and designing future applications.

INTRODUCTION

Resorcin[4]arene cavitands are a fascinating switching platform because of their ability to adopt two spatially well-defined conformations: an expanded kite and a contracted vase. Switching the conformational and binding properties of cavitands has been achieved with a variety of stimuli, such as changes in temperature, pH, metal ion concentration, light irradiation, solvent, and redox state. Besides employing conformational switching of resorcin[4]arene cavitands as a means to change their binding properties, the cavitand system could eventually be used as a platform to investigate fundamental interactions between objects attached to the cavitand’s walls with respect to their variable distance. The development of such molecular machines able to controllably perform mechanical motions involving large spatial rearrangements is a long-standing goal. The cavitand system could be used for this purpose if the distance distribution and dynamics between objects connected to the cavitand can be precisely determined in both the vase and kite conformations.

We had set out toward this goal by preparing a donor–acceptor borondipyrromethene (BODIPY) dye-substituted cavitand 1a (Chart 1), present in the vase form, for Förster resonance energy transfer (FRET) studies. Surprisingly, an unexpectedly low FRET efficiency was observed already in the vase form (ca. 66%), although an efficiency close to 100% was expected assuming a close dye–dye distance of ca. 1 nm. Such distance would prevail if the cavitand arms were oriented parallel to one another, i.e., the average opening angle of the cavitand walls would be 0°. The low FRET efficiency was attributed to either dynamic behavior of the cavitand or an unfavorable orientation of the transition dipole moments of the dyes. To gain more insights into this problem, cavitand 1a was resynthesized together with the analogous cavitands with shorter phenylene–ethynylene linkers, 1b and 1c. As the FRET efficiencies increased toward cavitands with shorter linkers (in the series 1a, 1b, 1c), it was concluded that the two arms of the cavitands are separated by a certain average opening angle and are not aligned parallel. Thereby, an average opening angle of 16° was inferred to explain the observed FRET efficiencies.

In this work, we sought to gain detailed insights into the distance distribution and dynamics of the cavitand system in both the vase and the kite conformations. Toward this goal, we prepared BODIPY dye-labeled quinone-based cavitand 2 (Chart 1) that is present in the kite form (designed based on...
what we learned about conformational properties of diquinone–diquinoxaline cavitands). In addition, we expanded the BODIPY dye-labeled cavitand vase series 1a–c by cavitand 1d featuring phenylene–ethynylene linkers of different lengths. Together, systems 1a–d and 2 embody the two conformational extremes of cavitands and are therefore ideally suited for the projected investigation. We investigated the BODIPY dye-labeled cavitands in a combined experimental and theoretical study consisting of time-resolved fluorescence spectroscopy and molecular dynamics (MD) simulations. BODIPY donor dye-substituted cavitand 3 shown in Chart 1 served as a reference compound for fluorescence studies. MD simulations complemented the experimental results and yielded theoretical dye–dye distance distributions. Emulation of fluorescence decay curves based on the distance distributions obtained by MD simulations allowed direct comparison of experimental and theoretical results. This work not only provides insights into the conformational dynamics of cavitands but also serves as a case study for the interplay between time-resolved fluorescence spectroscopy and MD simulations—a combination of methods that is often used to investigate the dynamics of biological macromolecules—applied to a relatively simple artificial small molecule system.

**RESULTS AND DISCUSSION**

**Synthesis and Characterization.** The synthesis of cavitands 1a–c and 3 had been recently reported. The synthesis and characterization of the newly prepared cavitands 1d and 2 is described in section 1 of the Supporting Information.

Cavitands 1a–d and 2 differ in that 1a–d possess two quinoxaline walls, while 2 is equipped with two quinone walls. This small structural difference has a dramatic effect on cavitand conformation: cavitands 1a–c are present in the vase form (1H NMR methine proton shifts at 5.61 and 5.69 ppm), while cavitand 2 adopts the kite form (methine protons at 3.69 and 4.35 ppm) in CDCl₃ solution. ¹⁹F NMR spectroscopy employing a pulse sequence with a 30° flip angle enabled determination of the BODIPY dye donor/acceptor ratios in cavitands 1a–d and 2 based on the integral ratios of the respective BF₂ units. In cavitand 1a this ratio was 1.00/0.82, corresponding to a donor-only fraction of 18%. In cavitand 1b the donor-only fraction was 11%. On the other hand, in cavitands 1c, 1d, and 2, the donor-only fractions were below the sensitivity limit of the NMR measurements (~2%). A possible explanation for higher ratios between donor/acceptor F atoms could be partial loss of the BF₂ units of the acceptor dyes during the course of cavitand syntheses, resulting in mixtures of fully labeled cavitands and cavitands lacking the BF₂ unit on the acceptor dyes. Loss of the BF₂ unit in BODIPY dyes has precedence and was observed in high acidity media or under strongly basic conditions, and the corresponding products have been shown to be nonfluorescent. Nevertheless, this finding is unexpected and has important implications for fluorescence studies, since the emission from this fraction of donor-only molecules needs to be taken into account for analysis of the fluorescence emission data.

**X-ray Analysis.** The X-ray structures of the precursors of cavitands 1a–d and 2 and diiodocavitands 4 and 5 are shown in Figure 1. The solid-state conformational properties of the diiodocavitands are reflected by their solution-state properties: compound 4 crystallized in the vase form from (CH₃)₂CO/CH₂Cl₂ while compound 5 crystallized in the kite form from CDCl₃. While in cavitand 4 the 1-bearin carbon atoms are placed at a distance of 0.86 nm to one another, in cavitand 5 the corresponding distance is 2.39 nm, which is almost 3 times larger.

**Absorption and Steady-State Fluorescence Spectroscopy.** The absorption and steady-state fluorescence spectra of cavitands 1a–d and 2 are depicted in Figure 2 (top and bottom, respectively). The absorption spectra exhibit two main absorption bands corresponding to the donor dye moieties (λ_{max} = 529 nm) and the acceptor dye moieties (λ_{max} = 619 nm). The fluorescence spectra were recorded using an excitation wavelength of λ_{exc} = 490 nm; direct excitation of the acceptor is negligible at this wavelength. The emission maxima at λ_{max} = 542 nm (I_{DA}) stem from the donor and at λ_{max} = 630 nm from the acceptor (I_{DB}) dye moieties. The intensity scale is referenced relative to the intensity maximum I₀ of donor-only substituted cavitand 3. The FRET efficiencies can be estimated according to eq 1
Higher FRET efficiencies are expected for shorter dye–dye distances. Consequently, cavitand 2, which is present in the kite conformation, exhibits the lowest FRET efficiency (64%) among the five cavitands. On the other hand, vase cavitands 1a–d exhibit $E$ values ranging from 84% to 97% with smaller values for cavitands with longer arms. While these efficiencies are higher than in cavitand 2, suggesting shorter dye–dye distances, they are not consistent with the assumption of parallel oriented cavitand walls found in X-ray structures, in which case FRET efficiencies of over 99% would be expected. This trend was originally explained with the presence of a cavitand opening angle of ca. 16°. In light of the above-mentioned finding based on NMR that samples of cavitands with longer arms possess significant donor-only labeled fractions (18% and 11% for cavitands 1a and 1b, respectively), the increasing donor fluorescence intensity for cavitands with longer arms could also be explained by residual fluorescence stemming from these contributions. This interpretation is supported by fluorescence lifetime measurements, where the contribution of doubly and singly labeled molecules can be resolved more easily.

**Time-Resolved Fluorescence Spectroscopy.** We recorded donor and acceptor fluorescence decay curves using time-correlated single-photon counting after pulsed excitation of the donor dye at $\lambda_{\text{exc}} = 470$ nm for each sample containing cavitands 1a–d and 2 (Figure 3) at concentrations of ca. $10^{-6}$ M in CHCl$_3$. For a pure sample of donor–acceptor-labeled species with a single fixed distance, one expects to measure single-exponential donor fluorescence decays with a mean fluorescence lifetime $\tau_D$ reduced by a factor of $(1 - E)$ as compared to the mean donor fluorescence lifetime $\tau_0$ obtained in the absence of the acceptor. Further, the acceptor decay curve should exhibit an initial rise (with rate constant 1/$\tau_A$) due to the FRET-induced population of the acceptor excited state and a subsequent single-exponential decay with 1/$\tau_A$, where $\tau_A$ is the mean fluorescence lifetime of the acceptor dye. This behavior is clearly observed for the kite cavitand 2. The corresponding donor decay curve was fitted with a single-exponential decay convolved with the instrument response function (IRF), yielding $\tau_{DA} = 1.51$ ns. This value results in a mean FRET efficiency of 64% (using $\tau_D = 4.21$ ns of reference cavitand 3 that is lacking the acceptor dye), which is in excellent agreement with the steady-state fluorescence spectroscopy data. Note, however, that obtaining accurate distance information from this value requires the distance distribution and dynamics of the system to be taken into account.

The acceptor decay curves of vase cavitands 1a–d are virtually identical and identical to the decay curve of the acceptor in 1a directly excited with $\tau_{\text{exc}} = 582$ nm. Their steep initial rise, which occurs within the response time of the instrument, shows that the FRET efficiencies in cavitands 1a–d are near 100% and donor and acceptor dyes thus in very close proximity. The fluorescence emission of the donor is thus expected to be very weak, and the corresponding donor fluorescence lifetimes are very short. Note, however, that monitoring the sensitized acceptor emission has the advantage that only signal from molecules containing both a donor and an intact acceptor fluorophore is detected. In contrast, the fluorescence emission from the donor contains the signal of molecules lacking an active acceptor, whose presence we

$$E = 1 - \frac{I_{DA}}{I_D}$$

**Figure 1.** Molecular structures of 4 (at 123 K) and 5 (at 100 K) in the crystals. Crystals of 4 and 5 were obtained by evaporation from (CH$_3$)$_2$CO/CH$_2$Cl$_2$ and CDCl$_3$, respectively. Solvent molecules, $n$-hexyl chains, and hydrogen atoms are omitted for clarity.

**Figure 2.** Absorption (top) and fluorescence emission (bottom, $c = 0.5 \times 10^{-7}$ M, $\lambda_{\text{exc}} = 490$ nm, $I_D$ is the emission maximum of reference donor dye cavitand 3) spectra of cavitands 1a–d and 2 in CDCl$_3$. 

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quantified using NMR spectroscopy (see Synthesis and Characterization). The residual donor fluorescence intensity decays of cavitands 1a–d (Figure 3b) are thus dominated by the emission from these donor-only molecules. As a result, they exhibit decay components up to the lifetime of the isolated donor fluorophore in the range of 4 ns but also additional shorter lifetime components, presumably because of collisional quenching with the acceptor moiety lacking the BF2 unit. Such multieponential behavior is typical of BODIPY dye dimers or BODIPY dyes in confined environments (in proteins, lipids, micelles, or glasses).22

**MD Simulations.** To obtain a detailed molecular picture of the dye–dye distance distributions and dynamics underlying the fluorescence results, we performed MD simulations with explicit chloroform solvent for cavitands 1a–d and 2. For direct comparison to the experimental data and benchmarking of the MD results, we calculated observable properties such as fluorescence intensity and fluorescence anisotropy decay curves from the MD trajectories. Reports on MD simulations of supramolecular systems in explicit solvents are rare.23 One reason for this is that early force fields were designed to simulate biomolecules in aqueous media. Recently, all-atom general force fields and tools for automatic parameter assignment have been developed, as well as structure and topology files for various common organic solvents.26 One goal of our study was therefore to utilize these developments and to investigate the applicability of MD simulations to molecular and supramolecular chemistry. The most common general force fields employed in contemporary MD literature are the General AMBER Force Field (GAFF) and the CHARMM General Force Field (CGenFF).24 For comparison, we included both force fields in our study.27

The most critical aspect in modeling cavitands 1a–d and 2 are the phenylene–ethynylene linkers; small errors in their force field parameters would propagate to larger errors in BODIPY dye–dye distances. A common parameter in describing the stiffness of linker units is the persistence length \( L_p \) whereby larger \( L_p \) values represent stiffer linkers. The persistence length of the phenylene–ethynylene unit had been recently experimentally determined on the basis of pulse electron paramagnetic resonance (EPR) spectroscopy on spin-labeled test systems.28 We subjected these phenylene–ethynylene-containing systems (see the Supporting Information, section 6) to MD simulations with CGenFF and GAFF (explicit chloroform, 100 ns each) to investigate how well the two force fields reproduce the experimentally determined \( L_p \) value. Values of \( L_p = 18.4 \pm 2.2 \text{ nm} \) for CGenFF and \( L_p = 27.0 \pm 2.4 \text{ nm} \) for GAFF were obtained. Comparison to the experimental value of \( L_p = 13.8 \pm 1.5 \text{ nm} \)28 suggests that both force fields slightly overestimate the rigidity of the oligo (phenylene–ethynylene) linker, but especially the results from CGenFF provide reasonably good agreement.

The cavitands 1a–d and 2 were subjected to MD simulations with the force fields CGenFF and GAFF (explicit chloroform, 500 ns each). The resulting BODIPY dye–dye distance histograms are illustrated in Figure 4. For the vase cavitands 1a–d, both force fields yield histograms composed of sharp maxima at ca. 0.5 and 1.0 nm and broader distributions spanning from 0.5 to 1.9 nm in the case of the smallest cavitand 1c and from 0.5 to 3.0 nm in the case of the largest cavitand 1a. While the sharp maxima can be ascribed to arrangements where the BODIPY dyes are in direct contact, the broader distributions are due to motions of separated dyes.

On the other hand, the kite cavitand 2 yields single-distribution histograms spanning from 3.0 to 5.0 nm. The mean dye–dye distances \( \langle d(B--B) \rangle \) obtained with the two force fields differ only marginally. The MD simulations slightly underestimate the average opening angle of cavitand 2, as evidenced by comparing the C370–C420 distance (Figure 1) in the X-ray structure of cavitand 5 (2.39 nm) with the corresponding average C–C distance stemming from the CGenFF simulation of cavitand 2 (2.33 nm). If this discrepancy of 0.06 nm is propagated toward the BODIPY dyes, a small deviation of ca. 0.1 nm for the average simulated dye–dye distance can be expected.

Notably, there is no overlap between the dye–dye distance distributions of vase cavitand 1c and kite cavitand 2, which have the same linker length. Thus, switching between both cavitand conformations can entirely change the distance distribution profile of moieties attached to the cavitand walls.

**Distance Autocorrelation from MD Data.** To determine the time scale on which the distance dynamics of the BODIPY dye arms relative to each other takes place, we calculated the time-correlation functions

\[
g(t) = \frac{\langle R(t + \tau) R(t) \rangle}{\langle R(t) \rangle^2} 
\]

Figure 3. Fluorescence decay curves of the acceptor (top) and donor (bottom) moieties of cavitands 1a–d and 2. Measurements were performed under magic angle configuration, i.e., with the emission polarizer set to 54.7° with respect to the excitation polarization. IRF = Instrument response function.
from the CGenFF-simulated interdye distance data of cavitands 1a and 2 (Figure 5); \( R(t) \) is the dye–dye distance \( d(B\cdots B) \) at time \( t \), \( \langle \ldots \rangle \) denotes the time average over \( t \), and \( \tau \) is the lag time. The results show that the interdye distance dynamics of both cavitands occur on a subnanosecond time scale, with the dynamics of vase cavitand 1a being slower than those of kite cavitand 2 by almost an order of magnitude. This finding is presumably due to a slow down of the distance dynamics in cavitand 1a caused by dye–dye contacts in the closed conformation.

**Fluorescence Anisotropy.** A stringent way of testing the accuracy of the time scales of dynamics in the simulations, including the viscosity of the solvent, is direct comparison to fluorescence anisotropy data. Anisotropy decays report on rotational diffusion times of the entire cavitands as well as on segmental rotation times of the BODIPY dye arms.\(^{30}\) We measured the anisotropy decay of the acceptor dye of cavitand 1a and compared it to the expected anisotropy decay as calculated from the corresponding MD simulations.

Fluorescence anisotropy decays were obtained by measuring the fluorescence intensity decays \( I_{VV}(t) \) and \( I_{VH}(t) \) observed after pulsed excitation of the acceptor dye (\( \lambda_{exc} = 582 \text{ nm} \)); in both measurements the plane of the linear polarized excitation light was vertically oriented and the emission polarizer was set vertically for \( I_{VV} \) and horizontally for \( I_{VH} \) (Figure 6, top). We fitted the two curves globally with model curves \( I_{||} \) and \( I_{\perp} \):

\[
I_{||} = I_0(1 + 2r(t))e^{-t/\tau_m}
\]

Figure 4. Histograms of distances between the B atoms of the donor and acceptor BODIPY dyes of cavitands 1a–d and 2, simulated with CGenFF (black) and GAFF (red).

Figure 5. Autocorrelation functions of the interdye distances derived from CGenFF-MD simulations of cavitands 1a and 2.

Figure 6. (Top) Acceptor fluorescence intensity decay curves of cavitand 1a measured after acceptor excitation with \( \lambda_{exc} = 582 \text{ nm} \). \( I_{VV} \) was recorded with an emission polarization filter set to 0° and \( I_{VH} \) to 90° with respect to excitation polarization. (Bottom) Fluorescence anisotropy decay curves constructed from CGenFF-MD trajectory of cavitand 1a using eq 6, fitted by the model described by eq 5, and the curve resulting from measured data inserted in eq 5.
\[ I_\perp = I_0(1 - r(t))e^{-t/r_\alpha} \]  

(4)

\( r_\alpha \) is the mean fluorescence lifetime of the acceptor dye and was determined to be \( r_\alpha = 5.24 \text{ ns} \).\(^{30}\) \( I_0 \) and \( I_\perp \) were convolved with the IRF to yield \( I_{\text{irr}} = \text{IRF} \otimes I_\perp \) and \( I_{\text{irr}} = G \cdot \text{IRF} \otimes I_\perp \). The factor \( G \) describes the relative difference in detection efficiencies of vertical and horizontal polarized photons of the instrumentation.\(^{30}\) For our instrument, we determined \( G = 1.1 \). The fluorescence anisotropy decay is defined as \( r(t) = (I_\perp - I_\|)/(I_\parallel + 2I_\perp) \). We expected \( r(t) \) to decay with two decay times: one corresponding to the overall rotational diffusion of the whole cavitand \( \langle r_\text{rot} \rangle \), and the other corresponding to the rotational motion of the BODIPY dye relative to the cavitand \( \langle r_\text{rot} \rangle \). Hence, the anisotropy decay was described according to\(^{31}\)

\[ r(t) = (r_\alpha - r_\infty)e^{-t/r_\alpha} + r_\infty e^{-t/r_\text{rot}} \]  

(5)

where \( r_\alpha \) is the limiting anisotropy, which was fixed to 0.37,\(^{22a}\)\(^{22a}\) and \( r_\infty \) is the residual anisotropy. Fitting the intensity decays (Figure 6, top) yielded \( r_\text{rot} = 1.64 \text{ ns} \), \( r_\text{rot} = 0.15 \text{ ns} \), and \( r_\infty = 0.07 \).

For direct comparison, we determined \( r(t) \) also from the MD simulation of cavitand 1a. The time trajectory of the normalized orientation vector \( \vec{A} (t) \) of the acceptor dye was used to obtain the anisotropy decay according to\(^{31}\)

\[ r(t) = r_{\alpha}(P_{\alpha}(A(t') \vec{A}(t' + t)))e^{-t/r_\alpha} \]  

(6)

Here, \( P_\alpha(x) \approx (3x^2 - 1)/2 \) is the second Legendre polynomial and vector \( \vec{A} \) is defined as indicated in Figure 7. We fitted the resulting anisotropy decay by eq 5 and obtained \( r_\text{rot} = 1.57 \text{ ns} \), \( r_\text{rot} = 0.16 \text{ ns} \), and \( r_\infty = 0.08 \) (Figure 6, bottom). The excellent agreement between these values and the ones obtained from experimental anisotropy decay measurements provides good evidence that the MD simulations accurately capture the dynamics of the cavitands, including the effect of solvent and viscosity. The MD simulations should thus also be able to provide accurate insights in the combined effects of distance distributions and dynamics on the experimentally observed fluorescence intensity decays.

Fluorescence Decay Curves from MD Data. To enable a direct comparison of the simulations to the experimental data, we emulated donor fluorescence decay curves that would be expected on the basis of the CGenFF-simulated MD trajectories by taking into account the dye–dye distance and orientation time series \( (\vec{k}^2) \) and compared them with the measured decay curves. The decay curves were emulated using the Markov chain\(^{32}\) model presented in Figure 7 (top), which was shown to be the best approach for calculating fluorescence observables.\(^{11b–k}\) Starting from the donor excited state \( (\text{D}^*\text{A}) \), the probabilities of FRET \( (p_{\text{FRET}}) \), donor emission \( (p_\text{D}) \), and acceptor emission \( (p_\text{A}) \) were calculated for every saved snapshot of the MD trajectory according to

\[ p_{\text{FRET}} = (1 - e^{-(k_i + k_{\text{FRET}})\Delta t}) \frac{k_{\text{FRET}}}{k_i + k_{\text{FRET}}} \]  

(7)

\[ p_\text{D} = (1 - e^{-(k_i + k_{\text{FRET}})\Delta t}) \frac{k_i}{k_i + k_{\text{FRET}}} \]  

(8)

\[ p_\text{A} = 1 - e^{-k_\text{a}\Delta t} \]  

(9)

with \( \Delta t = 4 \text{ ps} \) being the time step at which coordinates were saved, \( k_{\text{FRET}} \) the rate constant of energy transfer, \( k_i = 1/\tau_i = 0.24 \text{ ns}^{-1} \), and \( k_\text{a} = 1/\tau_\text{a} = 0.19 \text{ ns}^{-1} \) the respective fluorescence rate constants of the donor and acceptor dyes. The rate constant of energy transfer \( k_{\text{FRET}} \) was calculated from

\[ k_{\text{FRET}} = \frac{k_i}{R_0^{(k_2^2)}} \]  

(10)

with \( R \) being the dye–dye distance \( d(B\cdots B) \), and \( R_0^{(k_2^2)} \) the dye–dye orientation-dependent Förster radius.\(^{5} \) \( R_0^{(k_2^2)} \) was calculated according to

\[ R_0^{(k_2^2)} = R_0^{(2/3)} \sqrt[3]{\frac{\pi}{2}} \]  

(11)

The Förster radius \( R_0^{(2/3)} \) for \( k_2^2 = 2/3 \) that is valid in case of freely rotating, isotropically averaged dyes was determined to be 4.91 nm. The orientation factor \( k_2^2 \) was calculated according to

\[ k_2^2 = (\cos \theta_{\text{DA}} - 3 \cos \theta_\text{D} \cos \theta_\text{A})^2 \]  

(12)

with the angles defined by \( \theta_{\text{DA}} = \angle(\vec{D},\vec{A}) \), \( \theta_\text{D} = \angle(\vec{D},\vec{R}) \), and \( \theta_\text{A} = \angle(\vec{A},\vec{R}) \). The vectors \( \vec{A} \) and \( \vec{D} \) represent the emission transition dipole moment of the donor and the absorption transition dipole moment of the acceptor dye, respectively. \( \vec{R} \) is the connection vector between the boron atoms of the dyes (Figure 7, bottom).

A total of 2500 different snapshots picked along regular intervals of 0.2 ns served as starting points for the Markov model. The model was started at state \( \text{D}^*\text{A} \) and advanced by stepping through the trajectory. For every snapshot, transition probabilities according to eqs 7–9 were recalculated as functions of \( R \) and \( k_2^2 \) (eqs 10–12). This process was stopped if photon emission from either the donor or the acceptor occurred or if the end of the trajectory was reached. The donor/acceptor photon counts at their respective emission times were summed up. To collect a statistically significant number of photon emission events, each of the 2500 runs was repeated 48 000 times to inject overall 120 millions of photons into each FRET emulation. The resulting decay curves were...
convolved with the respective IRFs to allow a direct comparison with experimental data.

The measured and emulated donor fluorescence emission decay curves of vase cavitand 1a and kite cavitand 2 based on CGenFF trajectories are presented in Figure 8. When performing the emulation of the donor decay in cavitand 1a without taking a donor-only-labeled fraction into account, an extremely rapid decay is observed that basically parallels the IRF. The experimentally observed donor decay curve can only be reproduced if a donor-only labeled fraction is taken into account. Thus, 100% FRET efficiency is indeed expected for vase cavitands 1a–d due to the close dye–dye distance and should not be obscured by a possibly suboptimal orientation of transition dipole moments. The deviation between the emulated and the measured curves stems from the fast decay component in the experimental curve that we attribute to dynamic collisional quenching between the fluorophores. The effect of quenching at low dye–dye distances is not described by Förster theory and is therefore not taken into account in the FRET emulation. In contrast, the emulated donor decay curve of kite cavitand 2 is close to the experimental result, albeit with a slightly shorter average lifetime (1.01 ns) than the measured curve (1.51 ns), suggesting that the MD simulation underestimates the average dye–dye distance to some extent, most probably because of a slight deviation in the kite opening angle. Indeed, emulation of the donor fluorescence intensity decay curve on the basis of a simulated dye–dye distance distribution that is shifted by +0.1 nm yielded excellent overlap with the measured curve (Figure 8). This deviation in the average distance of ~2% is in the same range as the difference between the results from the two force fields used (Figure 4) and illustrates the accuracy of MD simulations in reflecting the structural properties of molecular systems. With the information from the simulations, we can also test the accuracy of simple averaging regimes commonly used for analysis of FRET in dynamic systems.

Since for cavitand 2 both the rotational correlation times of the dyes (Figure 6) and the distance dynamics between them (Figure 5) are much shorter than the fluorescence lifetimes, the system should be well approximated by the dynamic averaging regime, for which

$$\langle E \rangle = \frac{1}{1 + \left( \int (R_d/\sigma)^6 P(r)dr \right)^{-1}}$$

Using the distance distribution of cavitand 2 from the simulations shifted by 0.1 nm as described above, we obtain an average transfer efficiency, \( \langle E \rangle \), of 68%. The fair agreement with the experimental value of 64% indicates that dynamic averaging is a reasonable approximation.

**CONCLUSIONS**

The distance distribution and dynamics between moieties attached to resorcin[4]arene cavitand walls in both the kite and the vase conformations have been studied by a combination of experimental and theoretical methods on the basis of BODIPY dye-substituted vase cavitands 1a–d and kite cavitand 2. In kite cavitand 2, featuring one phenylene–ethynylene linker unit per dye, the dye moieties adopt an average distance of ca. 4.2 nm according to MD simulations (CGenFF). Time-resolved fluorescence spectroscopy revealed a FRET efficiency of 64%. In the case of vase cavitands 1a–d, steady-state and time-resolved fluorescence spectroscopy showed increasing amounts of donor fluorescence for cavitands with longer arms. In early studies, this trend was explained by increasing dye–dye distances for cavitands with longer arms, which would be consistent with the presence of a nonzero cavitand opening angle. In the current study, we found according to MD simulations (CGenFF) that while the average dye–dye distance does indeed increase from 0.8 nm in the shortest cavitand 1c to 1.2 nm in the longest cavitand 1a, these distances are still in a range that should yield FRET efficiencies of 100%. Instead, the increasing donor fluorescence for cavitands with longer arms could be explained by the previously undetected presence of cavitand fractions with inactive acceptor components lacking the BF2 unit. Emulation of the fluorescence intensity decay curve of cavitand 1a from simulated MD trajectories confirmed that the experimentally observed decay curve can only be reproduced when a donor-only-labeled cavitand fraction is taken into account.

The dynamics of vase cavitand 1a was investigated by fluorescence anisotropy measurements. Evaluation of both measured and simulated fluorescence anisotropy decay curves showed that the rotation time of a BODIPY dye arm in cavitand 1a is ca. 0.15 ns. Autocorrelation analysis of the dye–dye distance time series revealed that the distance dynamics of the BODIPY dye arms takes place on the subnanosecond time scale. Most importantly, the fluorescence lifetime decays calculated based on the MD simulations of cavitand 2 are in excellent agreement with the experimental data, showing that the simulations present an accurate picture of the distance distribution and dynamics in this molecular system. With these results, the cavitand system can now be used as a platform to investigate fundamental, distance-dependent interactions between objects attached to the cavitand’s walls.

In the case of extended molecular and supramolecular systems with pronounced flexibility and therefore broad intramolecular distance distributions, the combination of time-resolved FRET with simulations is essential to quantify the underlying dynamics because the experimental observables depend on both the shape of the distance distribution and the time scale of the dynamics. In this work, we have in-depth structurally characterized the two conformational states of resorcin[4]arene cavitands, which opens the possibility to utilize the cavitand system for studying intraspace interactions.
ranging from 1 to 7 nm (adjustable via the linker length). Furthermore, the good agreement between MD simulations and experimental data should further encourage application of MD simulations to molecular chemical systems. Progress in force-field development now allows such simulations to be performed with high accuracy. MD simulations could serve as a testing tool for envisioned concepts and thereby guide the synthetic chemist toward successful implementation of his/her ideas. Although the dyes used in this study are not suitable for single-molecule fluorescence detection, an approach analogous to the one demonstrated here could be employed for single-molecule studies. Such experiments could further enhance the resolution of structural and dynamic heterogeneities in molecular systems.

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Notes

The authors declare no competing financial interest.

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(13) A more detailed discussion on the instability of the acceptor dye is provided in section 1.4 of the Supporting Information.
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(17) For details, see section 2 of the Supporting Information.
(18) There are no indications that electron transfer, neither between the two dyes nor between the dyes and the quinone walls, significantly affects the transfer dynamics. For a more detailed discussion, see section 3.2 of the Supporting Information.
(20) For details, see Figure S5 in section 3.1 of the Supporting Information.

(21) An analysis of the decay curves is provided in section 3.4 of the Supporting Information.


(27) For details, see section 4 of the Supporting Information.


(33) See Figure S9 in section 5 of the Supporting Information.

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Supporting Information

Experimental and Computational Study of BODIPY Dye-Labeled Cavitand Dynamics

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1 Synthesis Section

1.1 Synthetic Schemes

Figure S1. Synthesis of BODIPY dye-substituted cavitands 1d and 2. a) [Pd(PPh₃)₄], Cul, i-Pr²NEt, THF, 35 °C, 2 d; 37%. b) Cs₂CO₃, THF, 70 °C, 24 h; 77%. c) [Pd(PPh₃)₄], Cul, i-Pr²NEt, THF, 25 °C, 3 d; 17%. d) [Pd(PPh₃)₄], Cul, i-Pr₂NEt, THF, 35 °C, 3 d; 11%.
1.2 Materials and General Methods

All chemicals were purchased as reagent grade and used without further purification. When stated, solvents were degassed by bubbling Ar through the solution for 30 min. Flash chromatography (FC) was performed using SiO$_2$-60 (230–400 mesh ASTM, 0.040–0.063 mm; Fluka) or SiO$_2$-F60 (0.040–0.063 mm, 60 Å, Silicycle). Preparative recycling gel permeation chromatography (GPC) was run on a Japan Analytical Industries LC-9101 preparative recycling HPLC apparatus using HPLC-grade CHCl$_3$ as the mobile phase. Melting points were measured on a Büchi B-540 melting-point apparatus in open capillaries. $^1$H NMR, $^{13}$C NMR, and $^{19}$F NMR spectra were recorded on a Bruker DRX 400 or Bruker AV 400 spectrometer at 298 K. Residual solvent peaks were used as internal references. ATR Infrared spectra (IR) were recorded on a Varian 800 FT-IR spectrometer. Selected absorption bands are reported in wavenumbers (cm$^{-1}$). Mass spectrometry was performed by the MS-service at ETH Zürich. High-resolution electron impact mass spectra were measured on a Waters Micromass AutoSpec Ultima spectrometer. High-resolution matrix-assisted laser-desorption-ionization mass spectra were measured on a Varion Ionspec Ultima MALDI-FTICR mass spectrometer using 3-hydroxypyridine-2-carboxylic acid (3-HPA) as matrix or on Bruker Daltonics Ultraflex II MALDI-TOF mass spectrometer using (2-[(2E)-3-(4-t-butylphenyl)-2-methylprop-2-enylidene]malononitril) (DCTB) as matrix. High-resolution electro-spray-ionisation mass spectra were measured on a Bruker Daltonics maXis spectrometer. UV/Vis spectroscopy was carried out with a Varian Cary 500 Scan spectrophotometer. Steady state fluorescence spectroscopy was carried out with an Instruments S. A. Fluorolog-3 spectrofluorimeter. Both UV/Vis and fluorescence experiments were carried out in standard 3.5 mL quartz cells (4 optical windows for UV/Vis, 2 optical windows for fluorescence) with 10 mm path length.
1.3 Synthetic Procedures

Compounds $6^1, 7^{1-2}, 8^3, 9^{1-2}, 10^{1-2}$, and $12^{1-2}$ were prepared according to literature procedures.

Cavitand 1d

$[\text{Pd}(\text{PPh}_3)_4]$ (19 mg, 0.016 mmol) and CuI (3 mg, 0.016 mmol) were added to a degassed solution of $6$ (76 mg, 0.121 mmol), $7$ (0.17 g, 0.081 mmol), and DIPEA (0.28 mL, 1.6 mmol) in THF (5 mL) and CHCl$_3$ (5 mL). The mixture was stirred for 2 d at 35 °C, after which the solvent was evaporated. FC (SiO$_2$; CH$_2$Cl$_2$ → CH$_2$Cl$_2$/EtOAc 98:2) and recycling GPC (Jaigel-2H; CHCl$_3$) afforded 1d (78 mg, 37 %) as a blue solid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 0.90 – 1.05 (m, 18H), 1.27 – 1.64 (m, 50H), 2.16 – 2.41 (m, 19H), 2.51 – 2.63 (m, 10H), 2.83 – 2.99 (m, 4H), 5.63 (t, $J$ = 8.1, 2H), 5.71 (t, $J$ = 8.1, 2H), 7.26 – 7.64 (m, 26H), 7.70 (d, $J$ = 8.1, 1H), 7.75 (d, $J$ = 8.1, 2H), 7.85 – 7.95 (m, 4H), 8.26 (s, 2H), 8.26 (s, 2H), 8.82 ppm (d, $J$= 8.0, 2H); $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ = 11.96, 12.33, 12.56, 14.04, 14.60, 17.11, 18.05, 18.06, 18.08, 18.10, 20.49, 22.65, 27.93, 27.96, 29.33, 29.35, 30.53, 31.85, 31.86, 32.16, 32.70, 34.20, 34.30, 89.55, 90.20, 90.25, 90.37, 90.43, 90.97, 118.79, 122.99, 123.24, 123.51, 123.77, 125.02, 125.11, 127.32, 128.05, 128.39, 128.73, 129.02, 129.09, 129.14, 129.36, 129.44, 130.57, 131.21, 131.34, 131.66, 131.72, 131.81, 132.17, 132.29, 132.34, 132.36, 132.43, 133.03, 133.75, 135.70, 135.84, 136.12, 136.13, 136.34, 136.54, 136.86, 137.40, 137.51, 138.14, 138.76, 139.12, 139.82, 140.69, 141.48, 150.95, 152.04, 152.25, 153.09, 154.16, 158.85, 158.87, 161.43 ppm; $^{19}$F NMR
(376 MHz, CDCl₃): δ = −145.63 (q, J = 31.7, 2F), −134.96 (q, J = 33.2, 2F); IR (ATR): ν̃ = 2926 (w), 2857 (w), 1741 (m), 1525 (m), 1480 (m), 1442 (w), 1411 (m), 1362 (m), 1326 (s), 1275 (m), 1231 (m), 1191 (s), 1157 (s), 1114 (m), 1082 (s), 978 (m), 898 (m), 837 (m), 792 (w), 761 (s), 709 (m), 688 (m), 626 (w); UV/Vis (CHCl₃): λ_{max} (ε) = 530 (69000), 620 nm (110000); HR-MALDI-MS (3-HPA): m/z (%): 2581.1280 (100, [M−F]⁺, calcd for C₁₆₄H₁₄₄B₂F₃N₁₄O₁₂⁺: 2581.1260).

Cavitand 5

Cs₂CO₃ (2.17 g, 6.67 mmol) was added to a solution of tetrol 8 (1.8 g, 1.6 mmol) and iodoimide 9 (1.49 g, 3.34 mmol) in THF (100 mL). The mixture was stirred for 24 h at 70 °C, filtered over silica, and the solvent was evaporated. FC (SiO₂; CH₂Cl₂ → CH₂Cl₂/EtOAc 95:5) afforded 5 (2.3 g, 77%) as a yellow solid. Rₖ = 0.48 (SiO₂; CH₂Cl₂/EtOAc 98:2); m.p. > 320 °C (decomp); ¹H NMR (400 MHz, CDCl₃): δ = 0.79 – 0.96 (m, 12H), 1.21 – 1.41 (m, 32H), 2.01 – 2.15 (m, 14H), 2.17 (s, 6H), 3.68 (t, J = 7.5, 2H), 4.37 (t, J = 7.5, 2H), 6.15 – 7.23 (m, 4H), 7.42 (br s, 4H), 7.57 (s, 2H), 7.60 (s, 2H), 7.65 – 7.80 (m, 4H), 8.02 – 8.26 ppm (m, 4H); ¹³C NMR (101 MHz, CDCl₃): δ = 13.97, 13.99, 17.74, 17.80, 22.52, 22.58, 26.96, 27.05, 29.04, 29.12, 31.49, 31.60, 31.66, 35.55, 37.70, 96.21, 126.76, 128.88, 130.30, 134.35, 137.60, 137.69, 138.84, 139.05, 151.77, 161.78 ppm; IR (ATR): ν̃ = 2926 (w), 2856 (w), 1795 (w), 1741 (m), 1682 (w), 1605 (w), 1578 (w), 1481 (w), 1351 (s), 1319 (m), 1248 (s), 1197 (s), 1157 (m), 1103 (m), 958 (m), 897 (w), 825 (w), 794 (w), 749 (w), 717 (m), 683 cm⁻¹ (w); HR-MALDI-MS (3-HPA): m/z (%) 1884.4403 (100, [M+H]⁺, calcd for C₁₀₀H₈₉I₂O₁₆N₆⁺: 1884.4458).
Cavitand 11

[Pd(PPh$_3$)$_4$] (46 mg, 0.040 mmol) and CuI (8 mg, 0.04 mmol) were added to a degassed solution of 9 (161 mg, 0.398 mmol), 5 (0.75 g, 0.40 mmol), and DIPEA (1.4 mL, 8.2 mmol) in THF (30 mL). The mixture was stirred for 3 d at 25 °C, after which the solvent was evaporated. FC (SiO$_2$; CH$_2$Cl$_2$ → CH$_2$Cl$_2$/EtOAc 97:3) and recycling GPC (Novogrom 100; CH$_2$Cl$_2$) afforded 11 (148 mg, 17 %) as a red solid. $R_f = 0.57$ (SiO$_2$; CH$_2$Cl$_2$/EtOAc 97:3); m.p. > 320 °C (decomp);

$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 0.84 – 0.93$ (m, 12H), 1.02 (t, $J = 7.5$, 6H), 1.19 – 1.47 (m, 38H), 2.02 – 2.15 (m, 11H), 6.12 – 7.13 (m, 4H), 2.18 (s, 6H), 2.24 (s, 3H), 2.34 (q, $J = 7.5$, 4H), 2.57 (s, 6H), 3.61 – 3.78 (m, 2H), 4.37 (t, $J = 7.4$, 2H), 7.32 – 7.36 (m, 2H), 7.37 – 7.54 (m, 6H), 7.57 (s, 1H), 7.60 (s, 1H), 7.71 – 7.77 (m, 4H), 8.07 – 8.22 ppm (m, 4H);

$^{13}$C NMR (101 MHz, CDCl$_3$): $\delta = 11.96, 12.56, 13.97, 13.99, 14.63, 17.10, 17.74, 17.80, 18.08, 18.14, 22.52, 22.59, 26.96, 27.05, 29.05, 29.13, 31.50, 31.60, 31.67, 35.55, 37.70, 53.44, 89.67, 89.74, 96.21, 123.60, 124.65, 126.76, 128.57, 128.87, 129.20, 130.30, 130.56, 131.66, 131.78, 132.34, 132.96, 134.31, 136.07, 137.11, 137.31, 137.59, 137.67, 138.25, 138.83, 139.03, 139.21, 151.74, 154.05, 161.76, 161.92 ppm; $^{19}$F NMR (377 MHz, CDCl$_3$): $\delta = -145.77$ ppm (q, $J = 33.0$, 2F); UV/Vis (CHCl$_3$): $\lambda_{\text{max}}$ ($\varepsilon$) = 530 (59000); HR-MALDI-MS (3-HPA): $m/z$ (%): 2139.7494 (100, $[M-F]^+$, calcd for C$_{125}$H$_{114}$BFIn$_8$O$_{16}$: 2139.7475).
Cavitand 2

[Pd(PPh₃)₄] (11 mg, 0.093 mmol) and Cul (1.7 mg, 0.0093 mmol) were added to a degassed solution of 12 (36 mg, 0.069 mmol), 11 (0.10 g, 0.046 mmol), and DIPEA (0.16 mL, 0.93 mmol) in THF (10 mL). The mixture was stirred for 3 d at 35 °C, after which the solvent was evaporated. FC (SiO₂; CH₂Cl₂ → CH₂Cl₂/EtOAc 97:3) and recycling GPC (Novogrom 100; CH₂Cl₂) afforded 2 (13 mg, 11%) as a red solid. 

Rf = 0.57 (SiO₂; CH₂Cl₂/EtOAc 97:3);

¹H NMR (400 MHz, CDCl₃): δ = 0.89 (q, J = 6.7, 12H), 1.02 (t, J = 7.5, 6H), 1.19 – 1.43 (m, 38H), 1.45 (s, 6H), 2.11 (s, 8H), 2.18 (d, J = 3.4, 6H), 2.25 (d, J = 3.4, 6H), 2.35 (q, J = 7.6, 4H), 2.55 – 2.63 (m, 10H), 2.88 – 2.96 (m, 4H), 3.71 (t, J = 7.2, 2H), 4.38 (t, J = 7.6, 2H), 6.07 – 7.11 (m, 4H), 7.26 – 7.38 (m, 8H), 7.38 – 7.53 (m, 10H), 7.65 – 7.80 (m, 8H), 8.02 – 8.28 (m, 4H), 8.78 – 8.91 ppm (m, 2H);

¹³C NMR (101 MHz, CDCl₃): δ = 11.96, 12.41, 12.56, 13.98, 14.00, 14.63, 17.10, 18.09, 18.15, 20.49, 22.53, 22.59, 26.97, 27.06, 29.05, 29.14, 30.54, 31.51, 31.61, 35.57, 37.72, 53.44, 89.67, 89.74, 89.94, 123.60, 123.86, 124.63, 124.65, 126.77, 127.35, 128.08, 128.38, 128.58, 128.66, 129.04, 129.20, 129.25, 129.46, 130.31, 130.56, 131.68, 131.79, 132.17, 132.34, 132.43, 132.96, 133.75, 135.93, 136.08, 136.41, 137.11, 137.13, 137.33, 137.34, 138.25, 138.82, 140.70, 150.92, 151.75, 154.06, 161.93 ppm; 

¹⁹F NMR (377 MHz, CDCl₃): δ = −145.77 (q, J = 31.8, 2F), −134.97 ppm (q, J = 33.1, 2F); 

IR (ATR): ν̃ = 2927 (w); 2857 (w), 1741 (m), 1680 (w), 1607 (w), 1579 (w), 1543 (w), 1480 (m), 1406 (m), 1336 (m), 1263 (m), 1188 (s), 1159 (m), 1084 (m), 959 (m), 897 (w), 796 (w), 774 (w), 715 (m), 648 cm⁻¹ (w); 

UV/Vis (CHCl₃): λmax (ε) = 530 (70000), 620 nm (114000); HR-MALDI-MS (3-HPA): m/z (%): 2537.0707 (100, [M–F]⁺, calcd for C₁₆₀H₁₄₀B₂F₃N₁₀O₁₆⁺: 2537.0621).
1.4 Quantification of Donor-Only Fractions in The Cavitand Samples

Cavitand 1a had been initially published in 2005, and an unexpectedly high donor fluorescence intensity had been observed in its steady-state fluorescence spectrum. The compound was resynthesized in 2010, together with compound 1b and 1c. While the newly synthesized 1a sample exhibited lower donor fluorescence intensity than the originally published one, it was still too high for assuming a close dye-dye distance.

However, the time-resolved fluorescence spectroscopy results of the current work supported the hypothesis of close dye-dye distances in all cavitands 1a-d. Therefore, we suspicioned that donor-only fractions in the cavitand samples could explain the high donor fluorescence intensities in the steady state fluorescence spectra.

We assumed that the reason for the presence of donor-only fractions is the loss of the BF$_2$ unit of the acceptor dye during the course of cavitand synthesis, presumably during a TMS-promoted deprotection step of a cavitand dye arm precursor. The larger instability of the acceptor dye compared to the donor dye had been noticed in the SI of reference [2]. Loss of the BF$_2$ unit in BODIPY dyes has precedence and was observed in high acidity media, or under strongly basic conditions. Hints for the partial lack of the BF$_2$ unit were found in MALDI-MS spectra in the form of [M–BF$_2$]$^+$ signals (which, however, might also form during the ionization in the mass spectrometer), while no evidence could be drawn from $^1$H, $^{13}$C, and $^{19}$F NMR spectra published in reference [2] for cavitands 1a–c.

Therefore, we obtained new $^{19}$F NMR spectra using special conditions (pulse sequence with a 30° flip angle) that allowed quantitative integration of the BF$_2$ signals, corresponding to the donor and acceptor moieties (see Figure S2). These spectra revealed that cavitands 1a and 1b indeed posses significant donor-only portions (18% and 11%, respectively), while cavitands 1c, 1d, and 2 show very small donor-only portions that are smaller than the NMR integration error of up to 2%. Due to the small molecular mass difference between the fractions equipped with and lacking the BF$_2$ unit, separation with gel permeation chromatography (GPC) was not possible. Neither was silica gel chromatography possible due to negligible polarity differences.
Figure S2. $^{19}$F NMR spectra (298 K, 376 MHz) of cavitands 1a–d and 2 in CDCl$_3$ employing a 30° flip angle.
2 X-Ray Data of Cavitand 5

Crystal data for cavitand 5 were deposited with the Cambridge Crystallographic Data Base with CCDC number 953763 (CDCl₃, kite), and can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

A clear yellow prism-like specimen of C₁₁₅H₁₀₃Cl₄₅I₂N₆O₁₆ (formula weight: 3674.08), approximate dimensions 0.140 mm × 0.240 mm × 0.300 mm, was used for the X-ray crystallographic analysis at 100(2) K. The X-ray intensity data were measured on a Bruker Kappa Apex-II Duo system equipped with a graphite monochromator (λ = 0.71073 Å).

The integration of the data using a monoclinic unit cell yielded a total of 239167 reflections to a maximum θ angle of 27.55° (0.77 Å resolution), of which 67783 were independent (average redundancy 3.528, completeness = 98.9%, Rint = 7.89%, Rsig = 11.35%) and 33738 (49.77%) were greater than 2σ(F²). The index range was: −35 ≤ h ≤ 36, −37 ≤ k ≤ 27, −49 ≤ l ≤ 49. The final cell constants of a = 27.741(2) Å, b = 29.059(3) Å, c = 38.382(3) Å, β = 106.309(4)°, V = 29696.(4) Å³, are based upon the refinement of the XYZ-centroids of 9104 reflections above 20σ(I²) with 4.422° < 2θ < 53.93°. Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.796. The absorption coefficient is 1.293 mm⁻¹.

The structure was solved by direct methods and refined using the OLEX2 and Bruker SHELXTL Software Package, using the space group P 1 21/n 1, with Z = 8 for the formula unit C₁₁₅H₁₀₃Cl₄₅I₂N₆O₁₆. The final anisotropic full-matrix least-squares refinement on F² with 2049 variables converged at R1 = 22.48% (wR2 = 54.52%), for the observed data and R1 = 32.80% (wR2 = 58.43%) for all data. The goodness-of-fit was 3.173. The largest peak in the final difference electron density synthesis was 8.089 e Å⁻³ and the largest hole was −5.517 e Å⁻³ with an RMS deviation of 0.419 e Å⁻³. On the basis of the final model, the calculated density was 1.644 g cm⁻³ and F(000), 14672 e.
Figure S3. Asymmetric unit of the crystal structure of cavitand 5 (kite, from CDCl$_3$) measured at 100 K. Thermal ellipsoids are shown at the 50% probability level.
Figure S4. Molecular structures of 5 in the crystal including atom numbers (kite, from CDCl₃) measured at 100 K. Thermal ellipsoids are shown at the 50% probability level.
3 Fluorescence Section

3.1 Reference Donor and Acceptor BODIPY Dye Fluorescence Lifetimes

Fluorescence lifetime decays were measured at the University of Zurich using a custom-built instrument described previously. Picosecond light pulses from a white light source (SC-450-4, 20 MHz, Fianium, Southampton, UK) were used for excitation. The excitation wavelengths were selected by HQ470/40 (Chroma) and z582/15 (Semrock) bandpass filters. The binning width of the recorded histograms is 4 ps.

Cavitands 3 and 1a were employed to measure the mean fluorescence lifetimes $\tau_D$ and $\tau_A$. The donor dye decay curve of cavitand 3 after donor excitation with $\lambda_{\text{exc}} = 470$ nm, and the acceptor dye decay curve of cavitand 1a after acceptor excitation with $\lambda_{\text{exc}} = 582$ nm are presented below. The curves were fitted with monoexponential decay functions convolved with the instrument response functions (IRF), yielding the mean fluorescence lifetimes $\tau_D = 4.21$ ns and $\tau_A = 5.24$ ns.

![Fluorescence decay curves](image)

Figure S5. Fluorescence decay curves of reference donor cavitand 3 (left) excited with $\lambda_{\text{exc}} = 470$ nm, and selectively excited acceptor of cavitand 1a (right) with $\lambda_{\text{exc}} = 582$ nm. Measurements were performed under magic angle configuration i.e. emission polarizer set to 54.7° with respect to excitation polarization. Monoexponential decay fitting yielded mean fluorescence lifetimes $\tau_D = 4.21$ ns for the donor BODIPY dye and $\tau_A = 5.24$ ns for the acceptor BODIPY dye.
3.2 Discussion on Potential Electron Transfer

While electron-poor quinones can act as electron acceptors, in case of our quinone moieties the acceptor property is diminished due to electron-donation from the O-atoms of the cavitand backbone; the first redox potential of the quinone moiety was recorded at $-1.1$ V.\textsuperscript{8} In addition, owing to the stiffness of the linker, direct contact between the quinone walls and the dyes through space is essentially impossible, and electron transfer through the linker arm is very unlikely. Electron transfer from the quinone moieties to the excited dyes would also be accompanied by broad, red-shifted charge-transfer bands in the fluorescence spectra of the cavitands. The absence of such bands is another argument against the occurrence of electron transfer in these molecules. Finally, Figure S6 below shows quantum yields (QY) of relevant single BODIPY dyes 6–8 and cavitands 1a–d (Details on the determination of the quantum yields are reported in Section 3.4 of the Supporting Information). The QYs are independent of the length of the linker, which also strongly argues against electron transfer to the quinone walls. In summary, electron transfer from the chromophoric group to the quinone groups does not have to be taken into account for our analysis.

Concerning potential photoinduced electron transfer between the two BODIPY dyes, this energetics can be estimated with the Rehm-Weller-equation, which takes into account the redox potentials of the donor and acceptor dyes. The redox potentials of the BODIPY dyes used in this study were previously measured (see Figure S6 below). Further required parameters are the excited state energy of the $S_0\rightarrow S_1$ transition of the donor ($E_{00} = 2.33$ eV, calculated from the wavelength of the absorption maximum of the donor dye, $\lambda_{\text{max}} = 529$ nm) and a work term of 0.3 eV (empirical coulombic factor for non-polar solvents). Using these data, we obtain a Gibbs free energy of $\Delta G = 1.2$ kcal mol\textsuperscript{-1} for the photoinduced electron transfer from the excited donor to the acceptor dye. On the other hand, we obtain $\Delta G = 3.9$ kcal mol\textsuperscript{-1} for the photoinduced electron transfer from the acceptor to the excited donor. Both processes are thus endergonic. Not only is electron transfer between the donor and acceptor BODIPY dyes in the closed cavitands unlikely due to thermodynamic reasons, electron transfer has even more stringent requirements for the distance between the two dyes (i.e. if electron transfer does take place, Förster transfer will be extremely efficient also). Possible electron transfer would therefore not have any bearing on the central conclusion regarding the vase cavitands, which is that the opening angle is close to
zero. In the kite cavitand, the distance between donor and acceptor is clearly too large for electron transfer to occur.

![Chemical structures](image)

**Donors:**
- 6: \(QY(\text{CHCl}_3) = 0.78\) in CHCl\(_3\)
- J. Org. Chem. 1999, 64, 7813-7819
- \(E_{1/2}^{\text{ox}} = 0.64\) V in CH\(_2\)Cl\(_2\) vs. Ferrocene
- \(E_{1/2}^{\text{red}} = -1.62\) V in CH\(_2\)Cl\(_2\) vs. Ferrocene
- ChemPhysChem, 2013, 14, 3348

**Acceptors:**
- 7: \(QY(\text{CHCl}_3) = 0.69\) in CHCl\(_3\)
- this study
- \(E_{1/2}^{\text{ox}} = 0.64\) V in CH\(_2\)Cl\(_2\) vs. Ferrrocene
- \(E_{1/2}^{\text{red}} = -1.62\) V in CH\(_2\)Cl\(_2\) vs. Ferrrocene

**Cavitands:**
1a: \(QY(\text{CHCl}_3) = 0.49\) in CHCl\(_3\)
1b: \(QY(\text{CHCl}_3) = 0.44\) in CHCl\(_3\)
1c: \(QY(\text{CHCl}_3) = 0.44\) in CHCl\(_3\)
1d: \(QY(\text{CHCl}_3) = 0.44\) in CHCl\(_3\)

Figure S6. Quantum yields and redox potentials of relevant BODIPY dyes 6–8 and cavitands 1a–d.

### 3.3 Determination of Quantum Yields and Förster Radius \(R_0\)

Measurements of quantum yields (QY) and determination of \(R_0\) were carried out in the Beckman Institute Laser Resource Center (California Institute of Technology) and were supported by the Arnold and Mabel Beckman Foundation. All QY’s were measured at concentrations of <1 µM in chloroform at room temperature and in ambient air. Solid samples were stored in the dark and refrigerated, solutions were prepared directly before taking data. Maximum peak absorptions did not exceed 0.25 to avoid detrimental self-absorption effects. UV/Vis absorption spectra were collected with a Cary 50 UV/Vis spectrophotometer in 1 cm pathlength quartz cuvettes. The acceptor extinction coefficient was determined in the following way: Four small amounts of acceptor (9, Figure S8) were weighed out and dissolved in known volumes of chloroform. The optical spectra of these solutions of known concentrations were measured and the average extinction coefficient of 9 was calculated using Beer's law. Emission spectra were recorded on a Jobin Yvon Spec Fluorolog-3-11. Samples were excited with 532-nm light, which was provided by a xenon arc lamp equipped with a monochromator for wavelength selection. Right angle emission was diffracted with a monochromator and detected with a Hamamatsu R928P photomultiplier tube with photon counting.
QY data of samples 1a–d and 7 were collected using the comparative method of Williams et al.\textsuperscript{9} This method involves the use of a well-characterized standard sample (here: anthracene in ethanol) with a known QY value. The measured QY data are summarized in Figure S7.

![Figure S7](image)

Figure S7. Measured integrated fluorescence intensity vs. absorbance (circles). The solid lines are linear fits with the slope $m$ and the intercept 0.

QY's were derived by comparison to a known value in the following way: The slopes $m$ of the unknown substances ($x$) and standard (std) were measured as described above. The QY of an unknown substance ($x$) is given by the equation below, in which $n$ denotes the index of refraction of the solvent.

$$QY_x = QY_{std} \cdot \left( \frac{m_x}{m_{std}} \right) \cdot \left( \frac{n_{x}^{2}}{n_{std}^{2}} \right)$$

The Förster radius $R_0$ was determined using the equations below and the measured acceptor extinction spectrum (experimental details see above).

**Donor:** \( R_0^D = \frac{9QY_D (\ln 10) \kappa^2 J}{128 \pi^5 n_A^4 N_A} \)

- $\kappa^2 = 2/3$ random orientation of donor molecules in solution
- $n = 1.4460$ refractive index of chloroform
- $N_A$ : Avogadro's number

$$J = \int f_D(\lambda)\epsilon_A(\lambda)\lambda^4 \, d\lambda$$

- $f_D$ : normalized donor fluorescence spectrum
- $\epsilon_A$ : acceptor molar extinction spectrum
- $\lambda$ : wavelength

The acceptor (9) extinction and normalized donor (7) emission spectra are depicted in Figure S8.
Figure S8. Acceptor extinction (9, red) and normalized donor emission (7, blue) spectra.

A Förster radius $R_0 = 49.1$ Å was obtained from steady-state absorption and emission spectra, and the equation above was used; $\kappa^2$ was assumed to be $2/3$, which is a good approximation for a random orientation of donor and acceptor molecules in solution.

### 3.4 Analysis of Decay Curves

The fluorescence decay curves of the donor dyes in cavitands 1a–d show multiexponential behavior whose description requires at least two decay times. The results of biexponential fits are shown in the Table below.

Table S1. Biexponential fits of the fluorescence decay curves of the donor moieties of cavitands 1a–d

<table>
<thead>
<tr>
<th>Cavitand</th>
<th>$\tau_1$ / ns</th>
<th>$\tau_2$ / ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>0.3</td>
<td>3.8</td>
</tr>
<tr>
<td>1b</td>
<td>0.5</td>
<td>3.9</td>
</tr>
<tr>
<td>1c</td>
<td>0.5</td>
<td>3.7</td>
</tr>
<tr>
<td>1d</td>
<td>0.8</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Fitted with IRF-convolution
4 MD Setup

4.1 Preparation of GAFF Input Files
Cavitands and linker molecules were assembled from HF/6-31G(d)-optimized fragments. Atom charges were calculated with the restrained electrostatic potential (RESP) fitting method of the molecular electrostatic potential (MEP), performed on the RED Server (development version 2012; allows to input up to 350 atoms) with the charge model RESP-A1A (HF/6-31G(d)//HF/6-31G(d) – Connolly surface algorithm used in MEP computation – 2 stage RESP fit qwt = 0.0005/0.001), using the Gaussian 09 software. Bonded and non-bonded parameters were assigned with the program ACPYPE, which interfaces with the program ANTECHAMBER. Bonded and non-bonded parameters involving the boron atoms were taken from parameters derived for CHARMM, and ported to GROMACS by means of unit conversion. Chloroform parameters (GAFF) and liquid structure coordinates were obtained from virtualchemistry.org.

4.2 Preparation of CGenFF Input Files
Cavitands and linker molecules were parameterized for CGenFF (version 2b7) via ParamChem Web service (www.paramchem.org, version 0.9.6). Bonded and non-bonded parameters involving the boron atoms were taken from literature. Chloroform parameters (OPLS) and liquid structure coordinates were obtained from virtualchemistry.org.

4.3 MD Run Parameters.
Cavitands or linker molecules, respectively, were solvated in a box of pre-equilibrated chloroform molecules with a minimal distance of 13 Å between any atom of the solute and the periodic boundary. As the guest-exchange rate for 2,6-dimethylphenyl-substituted cavitands is slow on the NMR timescale, we expected that chloroform molecules would not diffuse into the cavity on the MD simulation timescale by itself. Therefore, one chloroform molecule was manually placed into the cavities of cavitands 1a–d. It was observed that this chloroform molecule remained in the cavity throughout the whole simulation. The simulation time was 500 ns for cavitands and 100 ns for linker molecules, with a time step of 1 fs (such a short time step is required due to the high vibrational frequency of triple bonds present in linker units). Cavitand coordinates were saved every 4 ps and linker molecule coordinates every 5 ps. Long-
range electrostatics and van der Waals interactions were treated with a simple cut-off scheme using PME and a cut-off at 10 Å.\textsuperscript{19} The temperature was kept constant at 298 K by applying the Velocity Rescaling algorithm.\textsuperscript{19} The system pressure was kept constant at 1 atm with the Parinello Rahman Barostat.\textsuperscript{19} The LINCS algorithm was used to keep all bonds involving hydrogen atoms constrained.\textsuperscript{19} All molecular dynamics simulations were performed with a Message Passing Interface (MPI) version of GROMACS v. 4.5.5.\textsuperscript{20} Simulations were performed on the Brutus super computer,\textsuperscript{21} employing 16 cores per simulation. The total simulation time accumulated over all simulations was 5 µs for the cavitand and 0.8 µs for the linker molecules. Atom coordinates were extracted from each snapshot via the g\_traj routine of GROMACS, and subsequent calculations were performed with the Python programming language version 2.7.
5 Fluorescence Decay Curves from MD Data

Figure S9. Donor fluorescence emission decay curve of vase cavitand 1a with an implemented donor-only fraction of 0% emulated on the basis of a CGenFF-simulation trajectory: an extremely rapid decay is observed that basically parallels the IRF, if a donor-only fraction is not taken into account for the emulation.
6 Linker Study

We subjected molecules 13a–d\textsuperscript{22} (Figure S10, O' was replaced by H) to MD simulations with CGenFF and GAFF (explicit chloroform, 100 ns each).

![Figure S10](image)

The obtained $d(C1\cdots C2)$ distance distribution histograms are illustrated in Figure S11. The histograms obtained with CGenFF are broader than the ones obtained with GAFF, indicating that GAFF parameters result in stiffer phenylene-ethynylene linkers than CGenFF parameters. Quantification of $L_p$ values from MD data can be achieved by applying the Worm-Like Chain (WLC) model, which is often used to describe the behavior of semi-flexible polymers.\textsuperscript{23}

According to the WLC model, the mean square end-to-end distance $\langle R^2 \rangle = \langle d(C1\cdots C2) \rangle$ of a chain can be described by Equation:\textsuperscript{22a}

\begin{equation}
\langle R^2 \rangle = 2c_lL_p \left( 1 - \frac{L_p}{c_1} \left( 1 - e^{-\frac{c_1}{L_p}} \right) \right)
\end{equation}

where $c_l$ is the contour length – the $\langle d(C1\cdots C2) \rangle$ distance of the chain in its stretched, linear form. The contour lengths of systems 13a–d are composed of one phenyl unit with the length $a$, and $(n + 1)$ phenylene-ethynylene units with the length $b$ (Figure S10). Thus, the compound-specific $c_l$ values of 13a–d can be described according to:

\begin{equation}
c_l(n) = a + (n + 1)b
\end{equation}

The parameters $a$ and $b$ are force field-specific and were therefore used as fit parameters together with $L_p$. Substituting Equation (2) in (1) yields:
\[
\langle R^2 \rangle(n) = 2(a + (n + 1)b)L_p \left( 1 - \frac{L_p}{a + (n+1)b} \left( 1 - e^{-\frac{a + (n+1)b}{L_p}} \right) \right)
\]  

Equation (3) was used to fit the \( \langle R^2 \rangle(n) \) values obtained by CGenFF and GAFF, with \( a, b, \) and \( L_p \) as fit parameters. The resulting plots and fits are illustrated in Figure S12.

Figure S11. Histograms of distances \( \langle d(C1 \cdots C2) \rangle \) in test compounds 13a–d obtained from MD simulations with GAFF (top) and CGenFF (bottom). \( \langle R^2 \rangle = \langle d(C1 \cdots C2) \rangle \).
Figure S12. Plots of the mean square end-to-end distances \( \langle R^2 \rangle \) against the number of phenylene-ethynylene linker units \( n \) in test compounds 13a–d simulated with CGenFF (left) and GAFF (right). The plots were fitted to Equation (3) to determine the contour lengths \( L_p \).

\[
\begin{align*}
\text{CGenFF:} & \quad a = 0.2814 \pm 0.0069 \text{ nm} \\
& \quad b = 0.6947 \pm 0.0043 \text{ nm} \\
& \quad L_p = 18.4 \pm 2.2 \text{ nm} \\
\text{GAFF:} & \quad a = 0.2789 \pm 0.0034 \text{ nm} \\
& \quad b = 0.6898 \pm 0.0021 \text{ nm} \\
& \quad L_p = 27.0 \pm 2.4 \text{ nm}
\end{align*}
\]
7 NMR Spectra of the Products

Figure S13. $^1$H NMR (top, 400 MHz) and $^{13}$C NMR (bottom, 100 MHz) spectra of compound 5 in CDCl$_3$ at 298 K.
Figure S14. $^1$H NMR (top, 400 MHz) and $^{13}$C NMR (bottom, 100 MHz) spectra of compound 11 in CDCl$_3$ at 298 K.
Figure S15. $^{19}$F NMR (376 MHz) spectrum of compound 11 in CDCl$_3$ at 298 K.
Figure S16. $^1$H NMR (top, 400 MHz) and $^{13}$C NMR (bottom, 100 MHz) spectra of compound 2 in CDCl$_3$ at 298 K.
Figure S17. $^{19}\text{F}$ NMR (376 MHz) spectrum of compound 2 in CDCl$_3$ at 298 K.
Figure S18. $^1$H NMR (top, 400 MHz) and $^{13}$C NMR (bottom, 100 MHz) spectra of compound 1d in CDCl$_3$ at 298 K.
Figure S.19. $^{19}$F NMR (376 MHz) spectrum of compound 1d in CDCl$_3$ at 298 K.
8 References

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