Experimental and Computational Study of BODIPY Dye-Labeled Cavitand Dynamics

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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–ethylene linker, 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 diquinoxaline–diquinone cavitands.\(^6\) 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\(^8\) and 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.\(^10\) 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:\(^5\) cavitands 1a–c are present in the vase form (\(^1\)H NMR methine proton shifts at 5.61 and 5.69 ppm),\(^1,12\) while cavitand 2 adopts the kite form (methine protons at 3.69 and 4.35 ppm) in CDCl\(_3\) solution.

\(^{19}\)F 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 BF\(_2\) 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 BF\(_2\) units during the course of the cavitand syntheses, resulting in mixtures of fully labeled cavitands and cavitands lacking the BF\(_2\) unit on the acceptor dyes.\(^13\) Loss of the BF\(_2\) unit in BODIPY dyes has precedence and was observed in high acidity media\(^14\) or under strongly basic conditions,\(^15\) and the corresponding products have been shown to be nonfluorescent.\(^14\) 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\(^16\) and 5\(^17\) 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 (CH\(_3\))\(_2\)CO/CH\(_2\)Cl\(_2\) while compound 5 crystallized in the kite form from CDCl\(_3\). While in cavitand 4 the I-bearing 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 (\(\lambda_{\text{max}} = 529 \text{ nm}\)) and the acceptor dye moieties (\(\lambda_{\text{max}} = 619 \text{ nm}\)). The fluorescence spectra were recorded using an excitation wavelength of \(\lambda_{\text{exc}} = 490 \text{ nm}\); direct excitation of the acceptor is negligible at this wavelength.\(^18\) The emission maxima at \(\lambda_{\text{max}} = 542 \text{ nm} (I_D)\) stem from the donor and at \(\lambda_{\text{max}} = 630 \text{ nm} \) from the acceptor (\(I_A\)) dye moieties. The intensity scale is referenced relative to the intensity maximum \(I_D\) of donor-only substituted cavitand 3. The FRET efficiencies can be estimated according to eq 1.
hexyl chains, and hydrogen atoms are omitted for clarity. Crystals of 4 and 5 were obtained by evaporation from (CH3)2CO/CH2Cl2 and CDCl3, respectively. Solvent molecules, the crystals. Crystals of Figure 2.

Figure 1. Molecular structures of 46 (at 123 K) and 5 (at 100 K) in the crystals. Crystals of 4 and 5 were obtained by evaporation from (CH3)2CO/CH2Cl2 and CDCl3, respectively. Solvent molecules, n-hexyl chains, and hydrogen atoms are omitted for clarity.

![Molecular structures of 4 and 5](Image)

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![Figure 2. Absorption (top) and fluorescence emission (bottom, ε = 0.5 \times 10^{-7} M, λ_{exc} = 490 nm, I0 is the emission maximum of reference donor dye cavitand 3) spectra of cavitands 1a–d and 2 in CDCl3.](Image)

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\[ E = 1 - \frac{I_{DA}}{I_D} \]  

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 abovementioned 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 λ_{exc} = 470 nm for each sample containing cavitands 1a–d and 2 (Figure 3) at concentrations of ca. 10^{-6} M in CHCl3. 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 \( \tau_D \) of reference dye reduced by a factor of \( 1 - E \) as compared to the mean donor fluorescence lifetime \( \tau_D \) 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 \( \tau_D = 1.51 \) 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 \( \tau_{exc} = 582 \) 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...
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 BODIPY dye. Such multiexponential 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 $L_p = 18.4 \pm 2.2$ nm for CgenFF and $L_p = 27.0 \pm 2.4$ nm for GAFF were obtained. Comparison to the experimental value of $L_p = 13.8 \pm 1.5$ nm 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 $(d(B−B))$ 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:

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

(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 $54.7^\circ$ 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 \( t \), \( \langle \cdots \rangle \) 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 (\( \lambda_{\text{exc}} = 582 \text{ nm} \)); 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 \( I_{\parallel} \) and \( I_{\perp} \)

\[
I_{\parallel} = I_0(1 + 2r(t))e^{-t/\tau_{\parallel}} \\
I_{\perp} = I_0(1 + 2r(t))e^{-t/\tau_{\perp}}
\]

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 \( \lambda_{\text{exc}} = 582 \text{ nm} \). \( I_{VV} \) 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.
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 ($k^2$) and compared them with the measured decay curves. The decay curves were emulated using the Markov chain model presented in Figure 7 (top), which was shown to be the best approach for calculating fluorescence observables. Starting from the donor excited state (D*), the probabilities of FRET ($p_{\text{FRET}}$), donor emission ($p_D$), and acceptor emission ($p_A$) were calculated for every saved snapshot of the MD trajectory according to

$$p_{\text{FRET}} = \frac{(1 - e^{-(k_D + k_{\text{FRET}})\Delta t})}{k_D + k_{\text{FRET}}}$$

$$p_D = \frac{(1 - e^{-(k_D + k_{\text{FRET}})\Delta t})}{k_D + k_{\text{FRET}}}$$

$$p_A = 1 - e^{-k_A\Delta t}$$

with $\Delta t = 4$ ps being the time step at which coordinates were saved, $k_{\text{FRET}}$ the rate constant of energy transfer, $k_D = 1/\tau_D = 0.24$ ns$^{-1}$, and $k_A = 1/\tau_A = 0.19$ 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

$$k_{\text{FRET}} = k_D \left( \frac{R_0(k^2)}{R} \right)^6$$

with $R$ being the dye–dye distance ($d(B\cdots B)$, and $R_0(k^2)$ the dye–dye orientation-dependent Förster radius$^8$. $R_0(k^2)$ was calculated according to

$$R_0(k^2) = R_0(2/3) \sqrt{\frac{3}{2}} k^2$$

The Förster radius $R_0(2/3)$ for $k^2 = 2/3$ that is valid in case of freely rotating, isotropically averaged dyes was determined to be 4.91 nm. The orientation factor $k^2$ was calculated according to$^{26}$

$$k^2 = \left( \cos \theta_{\text{DA}} - 3 \cos \theta_D \cos \theta_A \right)^2$$

with the angles defined by $\theta_{\text{DA}} = \angle(\vec{D},\vec{A}), \theta_D = \angle(\vec{D},\vec{R})$, and $\theta_A = \angle(\vec{A},\vec{R})$. 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 D* and advanced by stepping through the trajectory. For every snapshot, transition probabilities according to eqs 7–9 were recalculated as functions of $R$ and $k^2$ (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

$$I(t) = I_0(1 - r(t)) e^{-t/\tau}$$

$\tau_A$ is the mean fluorescence lifetime of the acceptor dye and was determined to be $\tau_A = 5.24$ ns.$^{20}$ $I_0$ and $I_1$ were convolved with the IRF to yield $I_{1V} = \text{IRF} \otimes I_1$ and $I_{1V} = G \otimes \text{IRF} \otimes I_1$. 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 $r(t) = (I_\parallel - I_\perp) / (2I_\parallel + 2I_\perp)$. We expected $r(t)$ to decay with two decay times: one corresponding to the overall rotational diffusion of the whole cavitand ($\tau_M$), and the other corresponding to the rotational motion of the BODIPY dye relative to the cavitand ($\tau_{rot}$). Hence, the anisotropy decay was described according to$^{31}$

$$r(t) = \left( (r_0 - r_\infty) e^{-t/\tau_M} + r_\infty \right) e^{-t/\tau_{rot}}$$

where $r_0$ is the limiting anisotropy, which was fixed to 0.37,$^{22a}$ and $r_\infty$ is the residual anisotropy. Fitting the intensity decays (Figure 6, top) yielded $\tau_M = 1.64$ ns, $\tau_{rot} = 0.15$ 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$^{31}$

$$r(t) = r_0(P_{\vec{A}}(\vec{A}(t') \cdot \vec{A}(t' + t)))$$

Here, $P_\alpha(x) = (3x^2 - 1)/2$ 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 $\tau_M = 1.57$ ns, $\tau_{rot} = 0.16$ 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.
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 components in the experimental curve that we attribute to emulated and the measured curves stems from the fast decay should not be obscured by a possibly suboptimal orientation of FRET emulation.11i by Fo only be reproduced if a donor-only labeled fraction is taken into account 1a vase cavitands 1a 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.

$$
\langle E \rangle = \frac{1}{1 + \left( \frac{1}{\left( R_0/\sigma \right)^2} \int P(r)dr \right)^{-1}}
$$

(13)

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.

Figure 8. Measured and emulated donor fluorescence emission decay curves of vase cavitand 1a (with an implemented donor-only fraction of 18%) and kite cavitand 2.
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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(16) Published in ref 10.
(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.