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Water Plays a Diverse Role in a Hydrogen-Bonded, Hexameric Supramolecular Assembly†

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The interactions between water and a hexameric resorcin[4]arene assembly formed in wet chloroform are examined by molecular dynamics simulations of the diffusion coefficients. It is found that the water diffusion coefficients provide a route to understanding the degree of water association with the assembly. The simulated diffusion coefficients are in excellent agreement with prior measurements and the diffusion data are well described by a simple adsorption model. This analysis demonstrates that a significant number of waters are encapsulated within the assembly or hydrogen-bonded to its exterior, consistent with and elucidated by a direct examination of the water molecules in the simulations.

Self-assembled molecular capsules have attracted significant attention in recent years for their potential use in applications ranging from catalysis to drug delivery.[1–10] Since Cram and Collet reported the first covalent molecular container more than three decades ago,[11–15] a diverse range of covalent and non-covalent capsules has been synthesized and characterized. Among these, the hexameric resorcin[4]arene assembly, shown in Fig. 1, has received intense scrutiny.[16–20]

The assembly is composed of six resorcin[4]arene monomers, **1**, and eight water molecules, yielding an approximately spherical cavity with a diameter of ~ 1.4 nm.[16] Rebek and coworkers showed that in water-saturated $CDCl_3$ solution, resorcinarene self-assembles into the hydrogen-bonded hexameric assembly in the presence of appropriate guest molecules.[21,22] It was subsequently demonstrated that the assembly can form in the absence of guest molecules by instead encapsulating solvent molecules.[18,23–27] While detailed experiments have characterized the thermodynamics for the encapsulation of a diverse set of guests,[28,29] little is known about the molecular-level mechanisms of guest exchange.

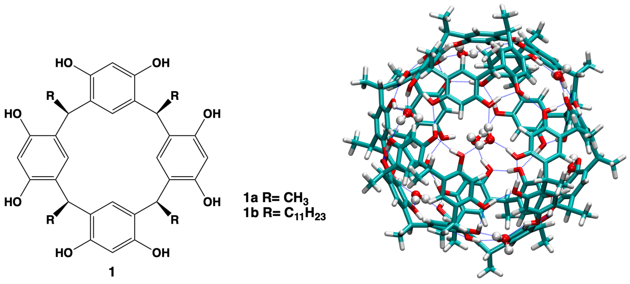

Fig. 1 Resorcin[4]arene monomer structure (left) and the hexameric resorcin[4]arene assembly (right). Color code: carbon (cyan), oxygen (red), hydrogen (white); waters are shown as ball-and-stick structures and hydrogen bonds indicated by blue lines.

The eight water molecules that are part of the assembly structure, Fig. 1, sit at the vertices where three resorcinarene monomers meet and each engages in two or three hydrogen bonds. While it is known that the assembly will not form in the absence of water[16,25,30] (*e.g.*, dry chloroform), the precise role of water in assembly formation and guest exchange have not been elucidated. There is thus a significant impetus to better understand how water participates in and interacts with the resorcinarene assembly.

To investigate the role of water molecules, Avram and Cohen measured the diffusion coefficients of both water and the assembly for different **1b**:H_2O ratios using NMR.[24] They observed a single water peak in the ^1H NMR spectra at all **1b**:H_2O ratios, indicating fast exchange (on the NMR timescale) between different water populations. Because of the rapid exchange, these NMR measurements provide a diffusion coefficient that is a weighted average of those from the different water populations, *i.e.*, wa-

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ters interacting with the assembly and those free in the solution. They concluded that: "No evidence was found for a large fraction of encapsulated water molecules."[24] On the other hand, it has been suggested that encapsulated waters may be present when chloroform solvent molecules are the guests.[31]

In this work, we use molecular dynamics (MD) simulations to provide new insights into the interactions between the excess water molecules and the resorcinarene hexameric assembly. To connect with the measurements of Avram and Cohen,[24] which provide detailed insight into the water behavior, we focus on water diffusion. Specifically, we report simulated water diffusion coefficients for different **1a**:H_2O ratios in chloroform solvent, compare to the measured values,[24] and show that both simulation and experimental results can be explained by a two-parameter adsorption isotherm model.

The MD simulation details are provided in the ESI. Briefly, each simulation consisted of one hexameric assembly (six prearranged resorcin[4]arene **1a** monomers) a fixed number of water molecules ranging from 8 to 50, and chloroform solvent, six molecules of which are encapsulated in the assembly. The diffusion coefficients of both the water molecules and the assembly (using the center-of-mass position of each) were calculated from the long-time slope of the mean squared displacement; details are given in the ESI. The diffusion coefficients of both water and the assembly obtained in this way are tabulated in Table S1.

We first carried out simulations with minimal waters, *i.e.*, a **1a**:H_2O ratio of 6:8 so that only "structural" waters are present. The resulting assembly diffusion coefficient was found to be $D_a = 0.26 \pm 0.02 \times 10^{-5}$ cm^2/s. This is in very good agreement with the experiments of Avram and Cohen, which yielded $D_a = 0.22 \pm 0.01 \times 10^{-5}$ cm^2/s for a **1b**:H_2O ratio of 6:7.2.[24] Note that our model assembly, with R = CH_3, is not identical to the experimental system, for which R = $C_{11}H_{23}$, so it is expected the simulations would exhibit slightly faster diffusion for the assembly compared to the measurements.

We next evaluated the diffusion coefficient for both water and the hexameric assembly for varying water content in the chloroform solution. The results at several values of the **1a**:H_2O ratio from 6:8 up to 6:50 are plotted in Fig. 2 and given in Table S1. The values obtained in the NMR experiments of Avram and Cohen[24] for several **1b**:H_2O ratios are shown in Fig. 2 for comparison. The agreement between the simulations and measurements is excellent. The diffusion coefficient calculated for the assembly changes little with water content. In contrast, the water diffusion coefficient, D_w, increases sharply with water content at small values of the **1a**:H_2O ratio before saturating. At the highest water content, D_w is approximately 16 times that of the assembly.

The rapid rise followed by a saturation in D_w as the water content increases is qualitatively consistent with the standard, "two-state" picture noted above, which assumes that water molecules present are divided between the eight waters involved in the assembly structure and those free in solution. This assumption can be mathematically expressed as

$$D_{w,2st} = \frac{8D_a + (N_{tot} - 8)D_{w,free}}{N_{tot}}, \tag{1}$$

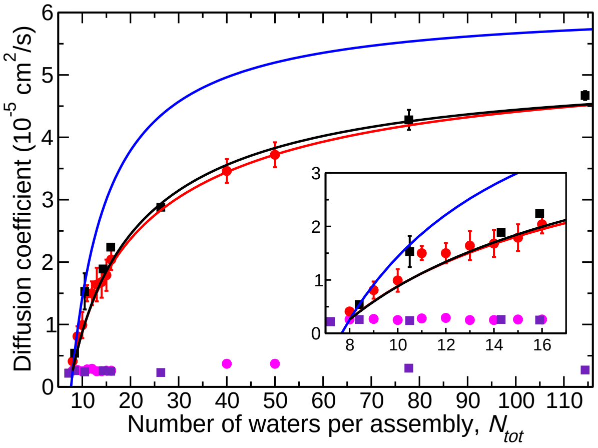

Fig. 2 Diffusion coefficients of water (present simulations: red circles and prior experiments:[24] black squares) and of the assembly (present simulations: magenta circles and prior experiments:[24] violet squares) as a function of the number of waters per hexameric assembly. The two-state prediction, $D_{w,2st}$ from Eq. 1, (blue line) is also shown along with the fit assuming a variable number of bound waters, Eq. 2, for the simulation (red line) and experimental[24] (black line) data. The inset represents a close-up view at low water content ($N_{tot} \leq 16$).

where N_{tot} is the total number of waters in solution per 6 resorcin[4]arene monomers, D_a and $D_{w,free}$ are the assembly and free water diffusion coefficients, and $D_{w,2st}$ is the population-averaged water diffusion coefficient in the two-state model. The value of $D_{w,free}$ can be determined by evaluating the water diffusion coefficient in water-saturated chloroform with no resorcinarene present. Thus, Eq. 1, shown as the blue line in Fig. 2, represents a prediction for the water diffusion coefficient free of any fitting parameters.

This two-state model $D_{w,2st}$ is compared to the experimental and simulation data in Fig. 2. While the qualitative behavior is indeed in agreement with the data, there is considerable quantitative disagreement. In particular, the two-state model prediction overestimates the actual D_w values for $N_{tot} > 11$. At high water content ($N_{tot} \approx 100$) it approaches the $D_{w,free}$ value, which is more than 25% greater than the actual values obtained in the NMR experiments.[24] These results show that such a two-state model is not consistent with either these prior measurements or the present simulations.

The above analysis strongly indicates that a significant number of waters, in addition to the structural ones, are bound to the assembly, either encapsulated within it or attached *via* hydrogen bonding to the exterior. If it is assumed that all the bound water molecules have the same diffusion coefficient as the hexameric assembly, the observed D_w can be fit by

$$D_w = \frac{N_{bound}D_a + (N_{tot} - N_{bound})D_{w,free}}{N_{tot}}, \tag{2}$$

Here, N_{bound} is the number of bound water molecules (including the 8 structural waters), which is itself a function of the total

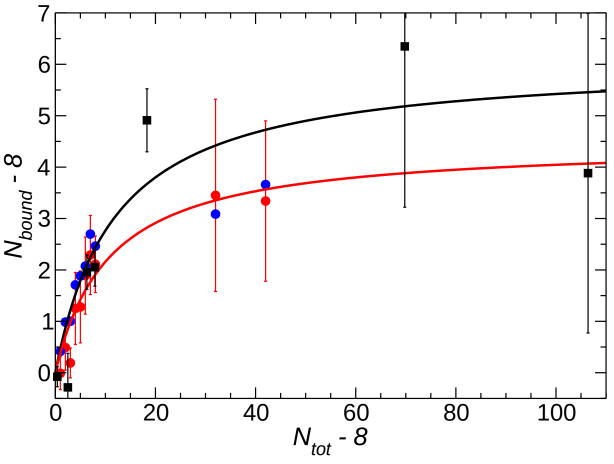

Fig. 3 Number of bound water molecules, N_{bound}, versus the total number of waters, N_{tot}, per assembly in the solution calculated from D_w obtained by NMR measurements[24] (black squares) and MD simulations (red circles) are shown along with the adsorption isotherm fit, Eq. 3, to the experimental (black line) and simulation (red line) data. N_{bound} values calculated from the relative probability distribution (blue circles) are also shown.

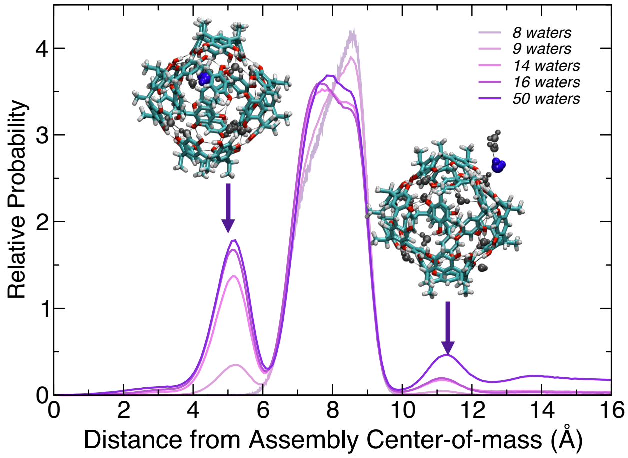

Fig. 4 Water probability distribution as a function of distance from the assembly center-of-mass for different number of waters, N_{tot}, per assembly. Structures illustrate waters (shown in blue) that are encapsulated (left) and hydrogen bonded on the assembly exterior (right).

number of waters present, N_{tot}. This expression is used to fit the measured[24] and simulated D_w, with N_{bound} as the adjustable parameter, as a function of water content. The results are shown in Fig. 2. This model provides an excellent description of both sets of data, providing strong evidence that water is encapsulated within and/or adsorbed to the assembly.

The number of waters bound to the assembly, N_{bound}, obtained by fitting the data in Fig. 2, are plotted as a function of N_{tot} in Fig. 3 and given in Table S2. Mathematically, N_{bound} can be obtained by rearranging Eq. 2 as

$$N_{bound} = \frac{D_w - D_{w,free}}{D_a - D_{w,free}} N_{tot}, \tag{3}$$

which can be used to quantify the number of assembly-bound waters from diffusion coefficients from either NMR measurements or simulations. The results in Fig. 3 show a sharp rise as waters beyond those in the assembly structure are added, followed by a plateau in N_{bound} for $N_{tot} > 45$. The data are suggestive of an adsorption isotherm model,

$$N_{bound} = \frac{K_{eq} N_{tot}}{1 + K_{eq} N_{tot}} N_{max}, \tag{4}$$

where K_{eq} is the adsorption equilibrium constant and N_{max} is the maximum number of waters that can be bound. The N_{bound} data in Fig. 3 obtained from both the measured and simulated water diffusion coefficients are both well described by this simple adsorption model. The fit gives N_{max} = 4.5 and 6.0 for the simulation and NMR data,[24] respectively. These indicate that the number of waters associated with the assembly at the highest water content is 50-75% larger than the number that are incorporated in the structure itself. Note that this result is derived from the water diffusion coefficients alone. The adsorption equilibrium constants are found to be 0.093 and 0.084 corresponding to a free energy of adsorption of $\Delta G_{ads} = -1.4$ to -1.5 kcal/mol for the simulation and experimental results, respectively.

The simulations also offer a direct way to investigate the presence and nature of H_2O molecules bound to the assembly. To examine the distribution of water molecules in the system, we calculated the water relative probability as a function of the distance from the assembly center-of-mass. This distribution is shown for several **1a**:H_2O ratios in Fig. 4. When only 8 waters are present all are part of the assembly structure and thus this distribution is instructive as a reference. It shows a single broad peak between $\sim 7-9$ Å, with the distribution skewed toward larger distances. The width of the peak is representative of the fluctuations of water locations *via* assembly deformations (the waters are not leaving the structural locations).

When a small number of additional waters are present, two other peaks appear in the probability distribution. The first is located between $4-6$ Å from the assembly center, closer than the structural waters and thus corresponding to waters encapsulated within the assembly. The second is found at $10-12$ Å, representing water molecules that are interacting with the exterior of the assembly *via* hydrogen bonding. Visual inspection of the trajectories reveals that the exterior waters associate with the assembly almost exclusively through hydrogen bonding with the structural waters and not the –OH moieties of the resorcinarene monomers.

Note that the probability distributions in Fig. 4 are normalized so that their integral is equal to the number of waters present; the area under each peak thus represents the average number of water molecules in that location. This gives a direct measure of the total number of bound waters, N_{bound}, by integrating to 16 Å and these results are compared to those obtained from the adsorption isotherm model in Fig. 3. The agreement between the two estimates is excellent, providing strong support for the use of the water diffusion coefficient as a probe of water association with the resorcinarene assembly.

It is interesting to examine where the waters reside. As the total number of waters in solution is increased, there is a rapid rise in the population of encapsulated waters saturating around 2-2.5 water molecules for a **1a**:H_2O ratio of 6:16. A slower increase in the exterior associated waters is observed with, on average, < 1 water molecule hydrogen-bonded to the exterior, even for 50 waters per assembly.

A third feature appears in the water probability distribution at high water content, as illustrated for the case of the **1a**:H_2O ratio of 6:50 shown in Fig. 4. Namely, the peak corresponding to directly hydrogen-bonded waters does not return to zero around 12.5 Å, as it does for lower water content, but is instead is replaced by a tail in the distribution that extends from 16 to 20 Å. This peak is indicative of water molecules that are part of a hydrogen-bonded chain tethered to the assembly. These chains are transient but can involve as many as 17 waters based on examination of the trajectories (see Fig. S1), but lead to an average of just over 1 additional water bound to the assembly.

In summary, we have presented the results of MD simulations of a hydrogen-bonded hexameric resorcin[4]arene assembly incorporating eight water molecules in wet chloroform. The simulations yield water diffusion coefficients in excellent agreement with prior measurements from NMR[24] and, more importantly, demonstrate that these can be used to extract quantitative information about the association of water molecules in solution with the assembly. In contrast to previous interpretations, the present results indicate that waters are encapsulated within the assembly, attached to the exterior of the assembly through hydrogen bonds, and, at high water content, present in extended hydrogen-bonded chains dangling from the assembly. This water binding can be quantitatively described using a two-parameter adsorption isotherm model. It remains to understand how the water behavior is affected by the number and properties of the guest molecules.[32] The improved understanding of the role of water in these assemblies will assist in elucidating the mechanisms of guest exchange that is important in applications.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

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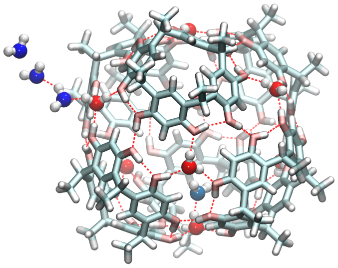

Diffusion coefficients reveal that water molecules are encapsulated within, or attached to the exterior of, a hexameric resorcin[4]arene assembly.