Water Transport

Diffusion of Water in a Nonporous Hydrophobic Crystal**

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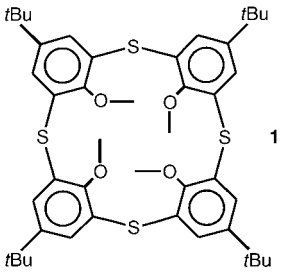
The transport of water molecules across cell membranes in biological systems is significantly enhanced by the presence of aquaporins,[1] proteins that associate as tetramers to form cylindrical transmembrane pores 2 to 3 nm long and about 0.3 nm wide at their narrowest point. Water travels through these channels at bulk diffusion rates,[2] presumably as single-file, hydrogen-bonded chains consisting, at any instant, of about seven to nine water molecules.[3] While interest in the transport properties of aquaporins has mainly focused on their selectivity for water over protons,[4] the influence of the channel size on water permeation has also received significant attention.[4f,5]

The shape, dimensions, and electrostatic profile of biological pores are considered to be the major determinants of their function. Intuitively, the simple notion of molecular transport through any matrix implies the presence of suitably sized channels defined and bounded by van der Waals surfaces. For example, molecular-scale hydrodynamics is only expected to occur if the narrowest site of the channel is at least wide enough to admit a single water molecule. Our own recent work with crystals composed of relatively small organic molecules has raised questions about the transport of small, mobile molecular species in solid media and we have shown that the conventional conception of crystal porosity may be flawed.[6] We have now extended our investigations of transport phenomena within seemingly nonporous crystals to include studies of water diffusion processes.

Crystals of pure 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetramethoxy-2,8,14,20-tetrathiacalix[4]arene (**1**) were prepared



by sublimation at 240 °C under reduced pressure. Visual examination of the crystals revealed two different morphologies: primarily parallelepipeds (**1a**), interspersed with a relatively small number of square plates (**1b**). The structures of both crystal forms were elucidated by single-crystal X-ray diffraction analysis and found to be polymorphic variants of one another. In both cases the calixarene assumes the 1,3-alternate conformation, and the molecule is approximately box-shaped, with estimated dimensions of $10 \times 10 \times 14$ Å^3. The space group of polymorph **1a** is $C2/c$ and the molecules are arranged in two distinct orientations, each with the principal molecular axis canted at an angle of 83° with respect to the other. However, in the context of the present study, the structure of **1a** is of limited interest. The space group of polymorph **1b** is $P\bar{4}2_1m$ and the structure possesses two crystallographically unique molecules of **1** (Figure 1). One of the molecules (**1b$_1$**) is situated on the intersection of the two mirror planes at (110) and (−110) while the other (**1b$_2$**) straddles a position of $\bar{4}$ site symmetry at 0,0,0. All of the calixarene molecules in the crystal are aligned with their principal molecular axes parallel to [001] and the extended structure consists of columns of **1b$_1$** and **1b$_2$** stacked end-to-end along this direction. Although **1b$_1$** and **1b$_2$** have the same overall conformation, remarkably subtle yet ultimately critical metric differences exist between them.

It is useful to briefly discuss the shape of the 1,3-alternate conformation of **1** in relation to its potential as a host molecule. In the context of host–guest chemistry, calix[4]-arenes are usually of more interest when they incorporate phenolic rather than methoxy groups at the lower rim. A cyclic hydrogen-bonded arrangement of hydroxy groups enforces a truncated cone conformation, thus giving rise to

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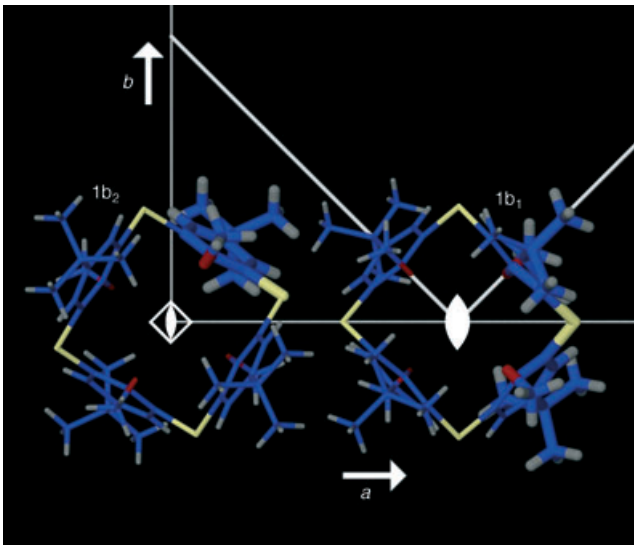

Figure 1. Perspective view of **1b** projected along [001]. The space group is $P\bar{4}2_1m$ (no. 113) with $a=b=19.494(2)$, $c=12.102(1)$ Å. Two crystallographically unique molecules are shown in capped-stick representation and the asymmetric unit in each case is indicated by thick bonds. As indicated, molecule $\mathbf{1b_1}$ resides on a 2*mm* axis while $\mathbf{1b_2}$ occupies a position of $\bar{4}$ site symmetry.

a molecular cleft large enough to accommodate small molecular moieties. Substitution of the phenolic protons usually causes distortion of the molecule to form the pinched cone, the 1,2-alternate, or the 1,3-alternate conformation. None of these substituted conformations result in the formation of a sizeable cleft, and the molecular host properties specifically associated with the cone conformation are generally forfeited.

Each pair of distal aromatic rings in the 1,3-alternate conformation of **1** forms a pincerlike arrangement. At each extremity of the molecule along its principal axis the "pincer" enfolds a small cleft with a narrow entrance guarded by two *para-tert*-butyl moieties. It is interesting to note that **1b** contains two crystallographically distinct molecules of **1**, with three of the four clefts unique. As a consequence of its $\bar{4}$ site symmetry, both clefts of $\mathbf{1b_2}$ are identical, and the dihedral angle between the distal rings is 26.0°. Two unique clefts are present in $\mathbf{1b_1}$ and the dihedral angles between their distal rings are 20.7° and 35.6°. This observation implies that one cleft is significantly larger than the other. The radius of the largest sphere capable of passing through the center of $\mathbf{1b_2}$ along its principal axis is 0.97 Å and the corresponding value for $\mathbf{1b_1}$ is 0.89 Å (note that the van der Waals radius of a hydrogen atom is often taken to be about 1.2 Å).

After collection of X-ray diffraction data, the crystal of **1b** was immersed in water for 8 h. Data were then recollected, and yielded structure **1c**. The space group of **1c** is also $P\bar{4}2_1m$ ($a=b=19.516(2)$, $c=12.096(1)$ Å) and structure solution reveals the same arrangement and relative conformations of the calixarene moietiess (correspondingly designated $\mathbf{1c_1}$ and $\mathbf{1c_2}$) as observed in **1b** (distal ring dihedral angles are 26.4° for $\mathbf{1c_2}$, and 20.2° and 35.0° for $\mathbf{1c_1}$). FT-IR analysis of carefully dried crystals, after similar treatment with water, indicated the presence of water molecules in **1c** and an overall calixarene:water ratio of 1:1 was determined by thermogravimetric analysis. In accordance with these results, least-squares refinement of **1c** yielded difference electron density peaks consistent with the presence of water oxygen atoms in the structure. Each of the two clefts of $\mathbf{1c_2}$ contains a water molecule at 50% site occupancy. The larger of the two clefts of $\mathbf{1c_1}$ contains one water molecule, while the smaller cleft remains unoccupied. A lower-symmetry structure of an inclusion complex of **1** with both water and dichloromethane has been previously published.[7]

The structure of **1b** is most likely dictated by complex packing considerations that do not favor a more symmetrical arrangement containing a single, unique conformation of **1**. Our experimental results show unequivocally that exposure of single crystals of **1b** to liquid water results in water molecules becoming lodged within some of the pincerlike clefts of the calixarenes. The water uptake is stoichiometric and the lattice structure remains otherwise unchanged. It appears that the three crystallographically different but similar molecular clefts exhibit remarkably subtle degrees of selectivity towards the guest water molecules. Figure 2 shows the two unique clefts of $\mathbf{1c_1}$ represented as Connolly[8] surfaces. The *tert*-butyl groups guarding the mouth of the smaller of the two clefts (Figure 2a) are rotated such that the cleft volume is maximized. Although the void is large enough to accommodate a water molecule, it remains unoccupied in **1c** (Figure 2c). The cleft at the other end of the calixarene

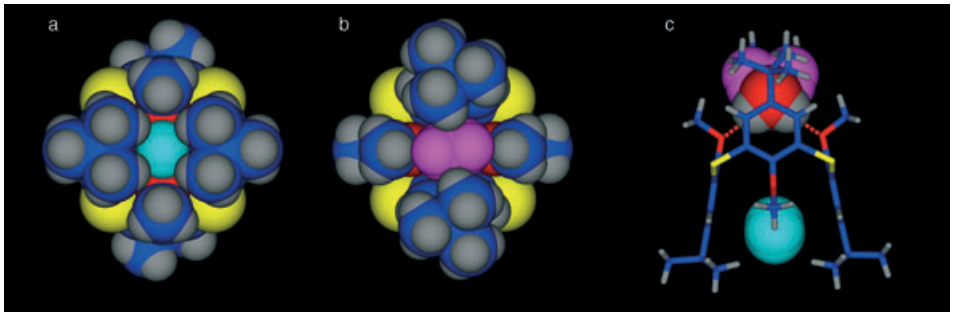

Figure 2. Molecule $\mathbf{1c_1}$ with its two clefts shown as Connolly surfaces. A probe radius of 1.4 Å was used to calculate the surfaces representing the voids. a) At one extremity of the host molecule, the unoccupied void (16.1 Å^3) is shown as a light blue surface. b) The fully occupied void (35.8 Å^3) at the other end of the molecule is shown as a purple surface. c) A side-view of **1** showing a section through the two cavity surfaces. The water molecule within the larger cavity is shown in van der Waals representation. Hydrogen bonds are indicated as dashed red lines.

(Figure 2b) is larger, as a result of the greater angle between its distal aromatic rings. The two *tert*-butyl groups at this end are each disordered over two distinct positions. Four combinations of positions of these groups relative to one another are possible, but only two are geometrically unique (the combination defining the maximal void space is shown in Figure 2). This cleft accommodates one water molecule which donates two hydrogen bonds (O···O = 2.998 Å) to the methoxy oxygen atoms at the base of the cleft (Figure 2c).

The two molecular clefts in $\mathbf{1c_2}$ (shown as Connolly surfaces in Figure 3) are equivalent in size and shape, and

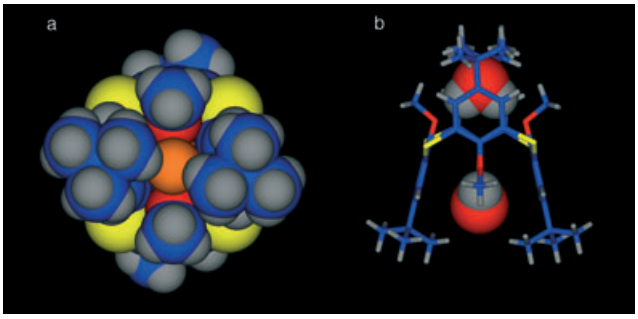

Figure 3. Connolly surfaces (orange) representing the two symmetry-related clefts (13.5 Å^3 each) in $\mathbf{1c_2}$. a) A view along the principal axis of the host molecule shows that the mouth of the cleft is guarded by two *tert*-butyl groups. b) A side-view of **1** showing two water molecules, each at 50% site occupancy, in space-filling representation.

each is barely large enough to accommodate a single molecule of water (ca. 19.7 Å^3) in a hydrogen-bonded environment (assuming that hydrogen bonding involves a small amount of van der Waals overlap of molecules). The *tert*-butyl groups do not appear to be disordered, and are positioned relative to one another so as to minimize the total void volume as well as the opening to the cleft. In the final crystallographic model, $\mathbf{1c_2}$ acts as host to only one water molecule that is disordered equally over the two possible sites. The water molecule donates two hydrogen bonds (O···O = 2.995 Å) to the calixarene methoxy oxygen atoms.

From our thermogravimetric and X-ray structural analyses we infer that any molecule of **1** only accommodates a maximum of one water molecule, even though there is enough space for two. Indeed, repeated soaking experiments lasting from eight hours to several days yielded consistent host:guest ratios of 1:1. The two distal *tert*-butyl groups at the mouth of each cleft are too close to one another to allow a water molecule to enter without conformational distortion of the calixarene. We believe that water uptake occurs through a complex mechanism involving rotation of the *tert*-butyl groups, with concomitant flexing of the host molecule whereby the distal aromatic rings lining the target cleft move apart. Such flexing most likely occurs as rotation about the C–S bonds of the thioether linkages. Thus the aromatic rings of the remaining cleft would be required to pinch closer together to compensate energetically for bond strain. Once a water molecule is admitted into the cleft, the host molecule can return to its original conformation. The water molecule now finds itself in a favorable environment where it is stabilized by two hydrogen bonds to symmetrically placed methoxy oxygen atoms. According to our assumption, the incorporation of a water molecule into one of the calixarene clefts inhibits uptake of water by the remaining cleft. The water molecule serves as a steric brace to the pinching motion that is required to open the opposite cleft of the host in order to capture a second water molecule. One of the voids of $\mathbf{1c_1}$ is significantly larger than the other and is also guarded by rotationally compliant *tert*-butyl groups. Hence, it is plausible that water uptake by this void is kinetically favored over that of the other, and the crystallographic model with a single water molecule at only one end of $\mathbf{1c_1}$ seems reasonable. Since both of the host cavities of $\mathbf{1c_2}$ are equivalent, we propose that a water site occupancy of 50% in each cavity implies that each molecule of **1** only captures one water molecule, but that the two cavities are equally favored.

Although we have discussed the possible mechanism by which water molecules enter the voids of isolated host molecules, careful scrutiny of the packing mode of the calixarene molecules reveals that the host framework is nonporous. This observation raises the intriguing question of how the water molecules diffuse through the lattice before becoming lodged within the calixarene cavities. Since structures **1b** and **1c** were determined using the same crystal, we can disregard the possibility of a water-inclusion process involving crystal dissolution and regrowth. Indeed, compound **1** is highly hydrophobic with no discernable water solubility, even in boiling water.

The calixarene molecules are stacked in columns parallel to [001]. Connolly surface plots (Figure 4a) reveal lattice voids (shown in green) embedded within bundles composed of four nearest-neighbor calixarene columns. These voids are isolated from one another by *tert*-butyl and methoxy methyl groups and do not merge to form channels. Assuming that the

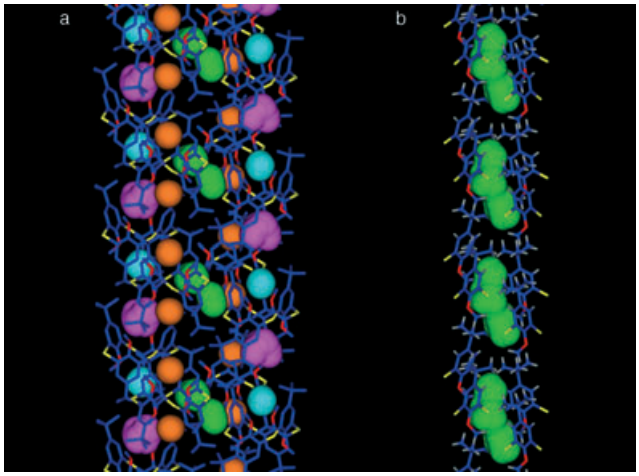

Figure 4. Four adjacent columns of **1** in **1c**, viewed perpendicular to [001]. a) The Connolly surfaces of the molecular clefts in **1** are shown in blue, purple, and orange (see Figures 2 and 3). The lattice voids between the calixarene atoms are shown in green (45.6 Å^3). Hydrogen atoms are omitted for clarity. b) The *tert*-butyl groups of **1** have been rotated to allow maximum extension of the lattice voids (91.4 Å^3) along [001]. Only the calixarene aromatic rings defining the lattice voids are shown.

tert-butyl groups can rotate in a concerted fashion during water uptake, it is possible to construct a model in which the lattice voids can be extended maximally towards one another along [001] (Figure 4b). However, there appears to be no set of orientations for the *tert*-butyl groups that allows the lattice voids to transform into channels. Therefore, no rational pathway can be traced whereby water molecules might be transported through the crystal lattice. Moreover, with the exception only of the location between the two methoxy oxygen atoms embedded within each calixarene cleft, the entire host framework presents a highly hydrophobic environment. In summary of the above, the molecular clefts of the host molecules constitute the only hydrophilic regions of the crystal structure, and there are no channels leading to these clefts.

The uptake of an individual water molecule can be simplified as a two-step process involving diffusion through the lattice, followed by complexation by the host molecule. While water molecules travel through the nonporous hydrophobic lattice with seemingly little concern for van der Waals surface constraints, they appear to be quite selective with regard to the size, shape, and electrostatic profile of their final resting places. Carbon nanotubes have been studied extensively as model systems for small hydrophobic channels by using molecular dynamics simulations.[4f–h,9] These studies indicate that water can be transported quite rapidly through such channels in single file. However, for diffusion to occur, it is generally understood that the channels must be of a suitable diameter (as defined by the van der Waals surfaces of the constituent atoms) to allow free passage of the water molecules. Indeed, this is taken to be a universal requirement for the diffusion of any substance through any matrix.[10]

The double-ended host molecule features two pincerlike clefts, each defining a void approximately the size of a single water molecule. Encapsulation of water at one extremity of the molecular receptor appears to inhibit guest inclusion at the other end. This observation is easily rationalized in terms of conformational flexibility of the host molecule during guest complexation, as well as by our comprehension of van der Waals interactions. However, conventional wisdom does not explain why water molecules display seemingly little regard for van der Waals constraints during the dynamic diffusion process: it is only when they finally come to rest that the water molecules appear to obey spatial restrictions imposed by van der Waals surface contacts. These findings have potentially important implications for both theoretical and experimental studies of water-transport phenomena, particularly across biological membranes.

Our results imply that the classical view of diffusion might be inappropriate when applied at the atomic scale. We have shown that water molecules have the ability to burrow through a rigid medium such as a crystal without the clear presence of suitable channels. It is our belief that the lattice and molecular voids in the structure of **1b** are essential to the diffusion process. Although these voids do not merge to form channels, they are situated relatively close to one another at the atomic scale. In such cases, it is perhaps possible for water molecules to exploit the voids as "stepping stones" to travel through the lattice. The water-transport mechanism would thus involve water molecules hopping between voids until a thermodynamically favorable location can be found. The implications of these findings are that, either crystals are not as rigid as generally presumed, or that van der Waals surfaces do not behave as classical barriers to small molecules in motion.

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[1] a) G. M. Preston, T. P. Carroll, W. B. Guggino, P. Agre, *Science* **1992**, *256*, 385–387; b) P. Agre, M. Bonhivers, M. J. Borgnia, *J. Biol. Chem.* **1998**, *273*, 14659–14662; c) M. J. Borgnia, S. Nielsen, A. Engel, P. Agre, *Annu. Rev. Biochem.* **1999**, *68*, 425–458; d) M. Yasui, T. H. Kwon, M. A. Knepper, S. Nielsen, P. Agre, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5808–5813.

[2] M. L. Zeidel, S. V. Ambudkar, B. L. Smith, P. Agre, *Biochemistry* **1992**, *31*, 7436–7440.

[3] a) M. Ø. Jensen, E. Tajkhorshid, K. Schulten, *Biophys. J.* **2003**, *85*, 2884–2899; b) A. Yarnell, *Chem. Eng. News* **2004**, *82*, 42.

[4] a) C. J. T. Grotthuss, *Ann. Chim.* **1806**, *LVIII*, 54–74; b) B. L de Groot, H. Grubmüller, *Science* **2001**, *294*, 2353–2357; c) E. Tajkhorshid, P. Nollert, M. Ø. Jensen, L. J. W. Miercke, J. O'Connell, R. M. Stroud, K. Schulten, *Science* **2002**, *296*, 525–530; d) B. L. de Groot, T. Frigato, V. Helms, H. Grubmüller, *J. Mol. Biol.* **2003**, *333*, 279–293; e) A. Burykin, A. Warshel, *Biophys. J.* **2003**, *85*, 3696–3706; f) G. Hummer, J. C. Rasaiah, J. P. Noworyta, *Nature* **2001**, *414*, 188–190; g) F. Zhu, K. Schultern, *Biophys. J.* **2003**, *85*, 236–244; h) A. Berezhkovskii, G. Hummer, *Phys. Rev. Lett.* **2002**, *89*, 64503/1–64503/4.

[5] a) J. Wang, Y. Zhu, J. Zhou, X.-H. Lu, *Phys. Chem. Chem. Phys.* **2004**, *6*, 829–835; b) M. S. P. Sansom, P. C. Biggin, *Nature* **2001**, *414*, 156–157; c) O. Beckstein, M. S. P. Sansom, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 7063–7068; d) H. Grubmüller, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 7421–7422.

[6] J. L. Atwood, L. J. Barbour, A. Jerga, B. L. Schottel, *Science* **2002**, *298*, 1000–1002.

[7] P. Lhoták, M. Himl, I. Stibor, J. Sýkora, H. Dvořáková, J. Lang, H. Petříčková, *Tetrahedron* **2003**, *59*, 7581–7585.

[8] a) M. L. Connolly, *Science* **1983**, *221*, 709–713; b) M. L. Connolly, *J. Am. Chem. Soc.* **1985**, *107*, 1118–1124.

[9] a) A. Kalra, S. Garde, G. Hummer, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 10175–10180; b) S. Joseph, R. J. Mashi, E. Jakobsson, N. R. Aluru, *Nano Lett.* **2003**, *3*, 1399–1403.

[10] T. K. Maji, K. Uemura, H.-C. Chang, R. Matsuda, S. Kitagawa, *Angew. Chem.* **2004**, *116*, 3331–3334; *Angew. Chem. Int. Ed.* **2004**, *43*, 3269–3272.

