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Structure and Dynamics of Self-Assembling Peptide Nanotubes and the Channel-Mediated Water Organization and Self-Diffusion. A Molecular Dynamics Study

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Abstract: A 0.76 ns molecular dynamics simulation has been performed on a self-assembled peptide nanotube in water. The peptide structure is composed of 10 β-sheet-like hydrogen-bonded stacks of the flat ring-shaped cyclic D,L octapeptide subunit cyclo[-(Gln-D-Ala-Glu-D-Ala-)$_4$]. The synthesis and self-assembly of such nanotubes and their ability to form remarkably transport-efficient transmembrane ion channels and pore structures have been reported previously. During the simulation, the tubular construct retains its structural integrity with only slight distortion at the outer ends. Water molecules in the tube tend to be organized in alternating zones of one water near the plane of the backbone C_α atoms and two waters near the plane midway between two adjacent peptide subunits—the idealized 1–2 water structure. On average, 32.8 water molecules are found in the tube during the data-gathering phase of the simulation. Analysis of the motion of water molecules in the tube gives a diffusion constant of 0.44×10^{-5} cm^2 s^{-1}, which is approximately one-sixth of the self-diffusion constant of bulk water and much faster than either molecular dynamics or experimental measurements of the diffusion constant of water in the structurally related gramicidin A transmembrane channel. A detailed examination of the tube-water trajectories shows that water molecules can pass by one another in contrast to the single-file diffusion occurring in gramicidin A. We suggest that water diffusion can be understood as a series of "hops" between zones which can cause transient local deviations from the ideal number of water molecules in a zone. A slight excess of the average water population over the "ideal" number of water molecules expected from the alternating 1–2 structure suggests that diffusion in the tubes may be enhanced in a manner roughly analogous to the enhanced conductivity of n-type semiconductors due to doping.

Introduction

Self-assembling and self-organizing molecular processes have emerged as powerful techniques in the *de novo* design of topologically predetermined protein-like structures.[1] A particularly striking example has been the recent design of an open-ended hollow tubular structure based on the self-assembly of flat ring-shaped cyclic peptide subunits (Figure 1). Appropriately designed cyclic peptide subunits made up of an even number of alternating D- and L-amino acids have been shown to stack and assemble, under suitable conditions, to produce uniformly shaped and contiguously hydrogen-bonded β-sheet-like cylindrical structures.[2,3] The conformational requirements of the self-assembling peptide subunits restrict the position of amino acid side chains to the outside surface structure of the tubular ensemble, thus producing an open-ended hydrophilic cylindrical core structure lined primarily with the hydrogen-bonded backbone amide functionalities oriented parallel to the tube axis. The ability to tailor the outside surface property of the tubular constructs by choosing appropriate amino acid side chain functionalities has enabled the design of transmembrane ion channels and pore structures with transport efficiencies often rivaling those of naturally occurring counterparts.

The ion transport rates of the self-assembled peptide channels are greater than those of the very efficient and widely-studied gramicidin A (GA) channels. This finding is particularly interesting since the self-assembled tubular constructs and GA have related hydrophilic core structures. *A priori*, the greater transport efficiencies of the self-assembled tubular constructs may be attributed to the larger pore size(s) and/or the mode(s) by which the interior core structure interacts with and organizes the solvent (water) and other guest molecules. In general, the structure of the channel water and the mechanism(s) by which water molecules move through a transmembrane structure are among the most important factors governing channel-mediated transport phenomenon. The three-dimensional structures of several peptide nanotubes have been recently characterized by electron diffraction analysis and/or high-resolution X-ray crystallography, making this particular class of tubular structures well-suited for computational analyses.[2,4] In this study, we have set out not only to investigate the structural stability and dynamics of the tubular structure itself but also to examine the structure, dynamics, and self-diffusion of the water molecules residing within the hydrophilic cylindrical pore structure. A

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[1] (a) Ghadiri, M. R.; Case, M. A. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1594–1597. (b) Ghadiri, M. R.; Soares, C. J.; Choi, C. *J. Am. Chem. Soc.* **1992**, *114*, 4000–4002. (c) Ghadiri, M. R.; Soares, C. J.; Choi, C. *J. Am. Chem. Soc.* **1992**, *114*, 825–831. (d) Robertson, D. E.; Farid, R. S.; Moser, C. C.; Urbauer, J. L.; Mulholland, S. E.; Pidikiti, R.; Lear, J. D.; Wand, A. J.; DeGrado, W. F.; Dutton, P. L. *Nature* **1994**, *368*, 425–432. (e) Harbury, P. B.; Zhang, T.; Kim, P. S.; Alber, T. *Science* **1993**, *262*, 1401–1405. (f) Chin, T.-M.; Berndt, K. D.; Yang, N.-C. C. *J. Am. Chem. Soc.* **1992**, *114*, 2279–2280. (g) DeGrado, W. F.; Wasserman, Z. R.; Lear, J. D. *Science* **1989**, *243*, 622–628.

[2] (a) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993**, *366*, 324–327. (b) Khazanovich, N.; Granja, J. R.; McRee, D. E.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994**, *116*, 6011–6012. Also see: De Santis, P.; Morosetti, S.; Rizzo, R. *Macromolecules* **1974**, *7*, 52–58.

[3] (a) Ghadiri, M. R.; Granja, J. R.; Buehler, L. K. *Nature* **1994**, *369*, 301–304. (b) Granja, J. R.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994**, *116*, 10785–10786.

[4] (a) Ghadiri, M. R.; Kobayashi, K.; Granja, J. R.; Chadha, R. K.; McRee, D. E. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 93–95. (b) Kobayashi, K.; Granja, J. R.; Ghadiri, M. R. *Ibid.* **1995**, *34*, 95–98.

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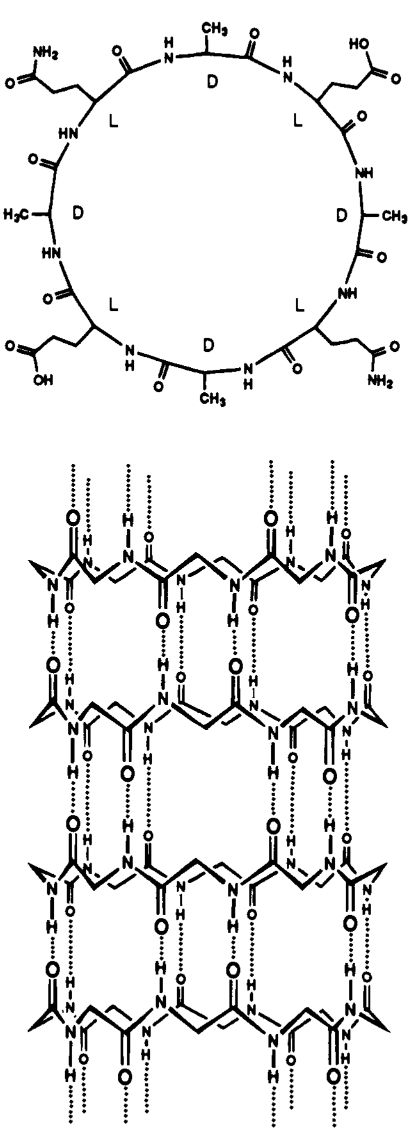

Figure 1. Chemical structure of the peptide subunit (top) and peptide subunits in a self-assembled tubular configuration (bottom) are shown. The figure serves to illustrate the antiparallel stacking and extensive network of intermolecular hydrogen-bonding interactions (for clarity only the backbone structure is represented).

tubular ensemble made up of 10 peptide subunits was chosen for this study because it is long enough (43 Å) to simulate its transmembrane channel counterpart[3] and provide meaningful statistics regarding the channel structure, channel-water stoichiometry, and water structure and dynamics. Here we present the results of a 0.76 ns molecular dynamics simulation of a fully hydrated peptide nanotube structure.

Methods

Initial Atomic Model. The peptide tube structure is a β-sheet-like hydrogen-bonded stack of 10 cyclic octapeptide subunits cyclo[-(Gln-D-Ala-Glu-D-Ala-)$_4$]. The starting tube coordinates were constructed according to the model proposed by Ghadiri *et al.*,[2a] modified by the X-ray crystallographic coordinates of a related peptide ensemble.[4a] The glutamic acid side chains were modeled in the protonated state because the assembly of the cyclic peptides into tubes occurs at low pH. The total charge state of the tube is thus neutral. The resulting tube structure is 10 Å in diameter, and the center-to-center distance between neighboring octapeptides is 4.73 Å (both measurements are from C_α atoms). The tube axis is parallel to the *Z*-coordinate axis. For convenience we designate the 10 peptides by the Roman numerals I–X in order of descending *Z* coordinate. The distance between the planes of C_α atoms of peptide subunits I and X is approximately 43 Å.

Computational Details and Simulation Procedure. The all-atom version of the CHARMM22 empirical potential energy function[5] and the TIP3P water model were used for all calculations.[6] The nonbonded interactions (electrostatics and van der Waals) were calculated using a distance cutoff of 12.0 Å. Electrostatic terms were calculated with a dielectric constant of 1.0, and the electrostatic cutoff was implemented by potential shifting. The van der Waals cutoff was implemented with a smooth switching function over 1.0 Å. Minimizations were done by the conjugate gradient method.[7] In the molecular dynamics calculations, the equations of motion were integrated by the Verlet algorithm and bonds were kept rigid using the SHAKE algorithm.[8] Conventional periodic boundary conditions were used. The nonbonded pair list was updated after every 20 steps of dynamics or minimization. The length of the time step in dynamics was 2 fs.[9] The analysis of the geometric features of the tube were mainly performed with the SIMLYS program package.[10] Computations were performed on Hewlett-Packard 9000 series processors contained within a Convex Meta Cluster computer using the CHARMM22 computer program.[5]

The initial atomic model of the tube was subjected to 200 steps of energy minimization without the presence of water to relieve strain in the model. The resulting tube structure (Figure 2) was placed in a 32.65 × 32.65 × 75.1 Å rectangular box of equilibrated TIP3P water, and any water molecules whose oxygen atoms were closer than 2.8 Å to a heavy protein atom were deleted as overlapping waters. The lengths of the box edges were then readjusted according to an assumed specific volume for the peptide of 0.73 cm^3/g and for water of 1.0 cm^3/g. The resulting system consisted of 1060 tube atoms and 2237 water molecules in a 32.3 × 32.3 × 73.8 Å rectangular box with the long side parallel to the tube axis. The positions of the water molecules were then optimized by 200 steps of conjugate gradient minimization during which the positions of the tube atoms were held fixed.

The molecular dynamics simulation was performed under NVT conditions (constant temperature and constant volume). The initial atomic velocities were taken from a Maxwell–Boltzmann distribution at 250 K. During the simulation the system was coupled to a temperature bath[11] at 298 K with a coupling constant of 0.4 ps. This brought the simulations to the 298 K target level after 5 ps of equilibration. The simulation was performed over 760 ps, and coordinates were stored every 0.1 ps for analysis. In order to compare the calculated features of the water inside the tube with bulk water, a separate MD simulation of water molecules was performed at a density of 1.0 g/cm^3 over 160 ps using the same empirical potential function and molecular dynamics protocol.

Results

Stability and Dynamics of the Self-Assembled Peptide Tube. The peptide backbone atoms remain close to the starting structure even after 0.76 ns of dynamics run (Figures 2 and 3). While the backbone atom rms deviation reaches a steady state mostly confined to the range between 0.4 and 0.6 Å after approximately 100 ps, the all-atom rms deviation is still slowly increasing at the end of the simulation because of the considerable rotational freedom of the side chains. Except for the outer peptide subunits I and X, the average intersubunit distances lie between 4.75 and 4.80 Å (Table 1), in good agreement with the Fourier-transform infrared spectroscopy, electron diffraction, and X-ray crystallographic analyses (4.73 Å).[2–4] The octapeptides on either end of the tube have larger rms deviations than

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(5) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187–217.

(6) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D. *J. Chem. Phys.* **1983**, *79*, 926–935.

(7) Fletcher, R.; Reevs, C. M. *Comput. J.* **1964**, *7*, 149–154.

(8) Ryckaert, J.-P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327–341.

(9) (a) van Gunsteren, W. F.; Berendsen, H. J. C. *Mol. Phys.* **1977**, *34*, 1311–1327. (b) van Gunsteren, W. F.; Karplus, M. *Macromolecules* **1982**, *15*, 1528–1544.

(10) Krüger, P.; Szameit, X. *Comput. Phys. Commun.* **1992**, *72*, 265–268.

(11) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684–3690.

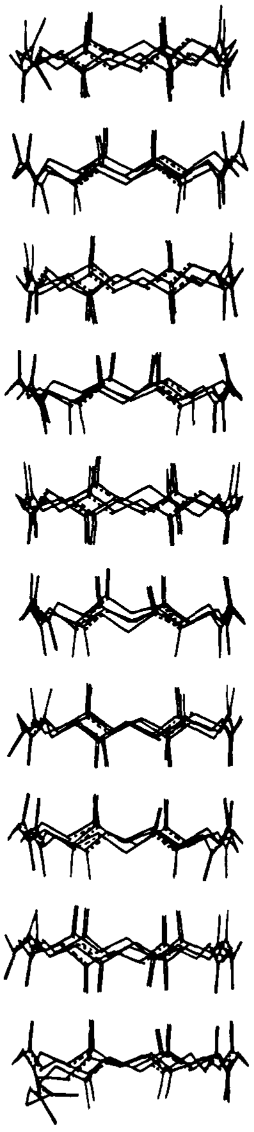

Figure 2. Superposition of the initial energy-minimized model structure and the final structure after 760 ps of dynamics simulation (only backbone heavy atoms are shown).

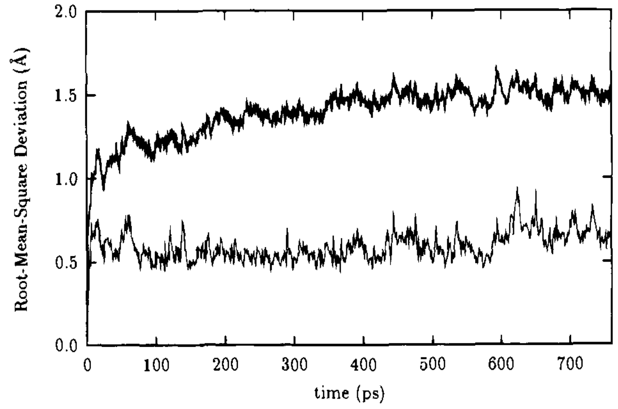

Figure 3. Root-mean-square (rms) deviation of all atoms (upper, thick line) and all backbone atoms (lower, thin line) from the energy-minimized starting structure for the whole simulation. Deviations are calculated from frames taken at 1.0 ps intervals. A rigid-body fit of each frame's structure of either all atoms or all backbone atoms on the initial starting structure is made prior to the rms calculation.

Table 1. Distances (Å) between Stacked Octapeptides Averaged over Different Time Periods of the Dynamics Simulation

peptide–peptide	time segment 360–460	460–560	560–660	660–760
I–II	4.77	4.77	4.78	4.77
II–III	4.75	4.76	4.75	4.75
III–IV	4.78	4.79	4.77	4.76
IV–V	4.75	4.76	4.76	4.75
V–VI	4.78	4.79	4.77	4.77
VI–VII	4.76	4.77	4.77	4.76
VII–VIII	4.78	4.78	4.80	4.77
VIII–IX	4.76	4.75	4.76	4.74
IX–X	4.78	4.94	5.03	5.18

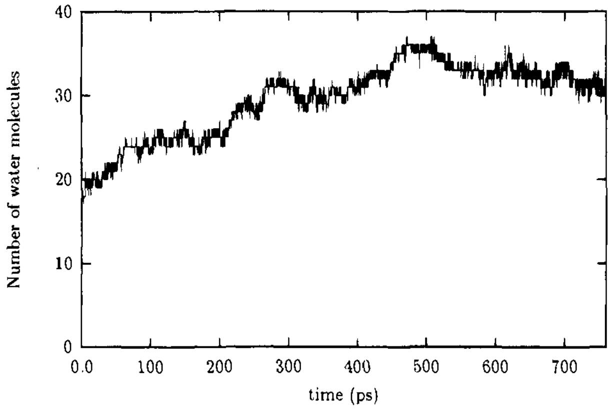

Figure 4. Number of water molecules in the tube *versus* time. The number of water molecules in the tube (initially 18) increased steadily during the long (360 ps) equilibration phase of the dynamics calculation. In the subsequent 400 ps data-gathering phase, the average number of waters in the 10-peptide tube was 32.8.

the others (plots not shown), and a slight tendency for one of the end peptides (X) to dissociate from the tube is visible in Figure 2 and evident in a slight increase in the IX–X intersubunit distance (Table 1), as well as in the loss of two of the eight interpeptide hydrogen bonds by the end of the simulation. Apart from the behavior of the end groups, the tube length shows remarkably little fluctuation during the simulation. Over the last 400 ps, the average distance between the α-carbon planes of peptides II and IX (the second ones in from the ends) is 33.35 Å and the rms deviation of the length from this average is only 0.17 Å. The backbone amide groups remain very nearly parallel to the tube axis as would be expected for favorable peptide–peptide hydrogen bonding and have very little tendency to incline into or out of the tube—the amide NH-bond-tube-axis angle is 0.2 ± 15.2°. This is in contrast to the 15–33° outward inclination of the amide NH observed in a molecular dynamics simulation of gramicidin A.[12] In general, the tube structure remains stable during the simulation with only small backbone deviations from the starting structure and thus provides a consistent basis for subsequent analysis of the internal water's structure and dynamic behavior.

Structure of the Tube Water. The number of waters in the tube structure has been determined by counting the water-oxygen atoms lying between the plane formed by the C_α atoms of the peptide subunits I and X. It increases considerably during the dynamics simulation (Figure 4). The initial structure produced by the superposition-and-deletion procedure described in the Methods placed 18 water molecules in the tube. But the number of waters in the tube increases steadily during the first 360 ps and reaches a range of 29–35 waters over the last 400 ps except for brief excursion up to 37 waters at the 480 ps mark. Because of the slow filling of the tube, we have taken the first 360 ps as an equilibration phase, and all subsequent analysis is based on the last 400 ps of simulation. The average number of waters inside the 10-peptide tube over this 400 ps period is 32.8.

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[12] Chiu, S.-W.; Subramaniam, S.; Jakobsen, E.; McCammon, J. A. *Biophys. J.* **1989**, *56*, 253–261.

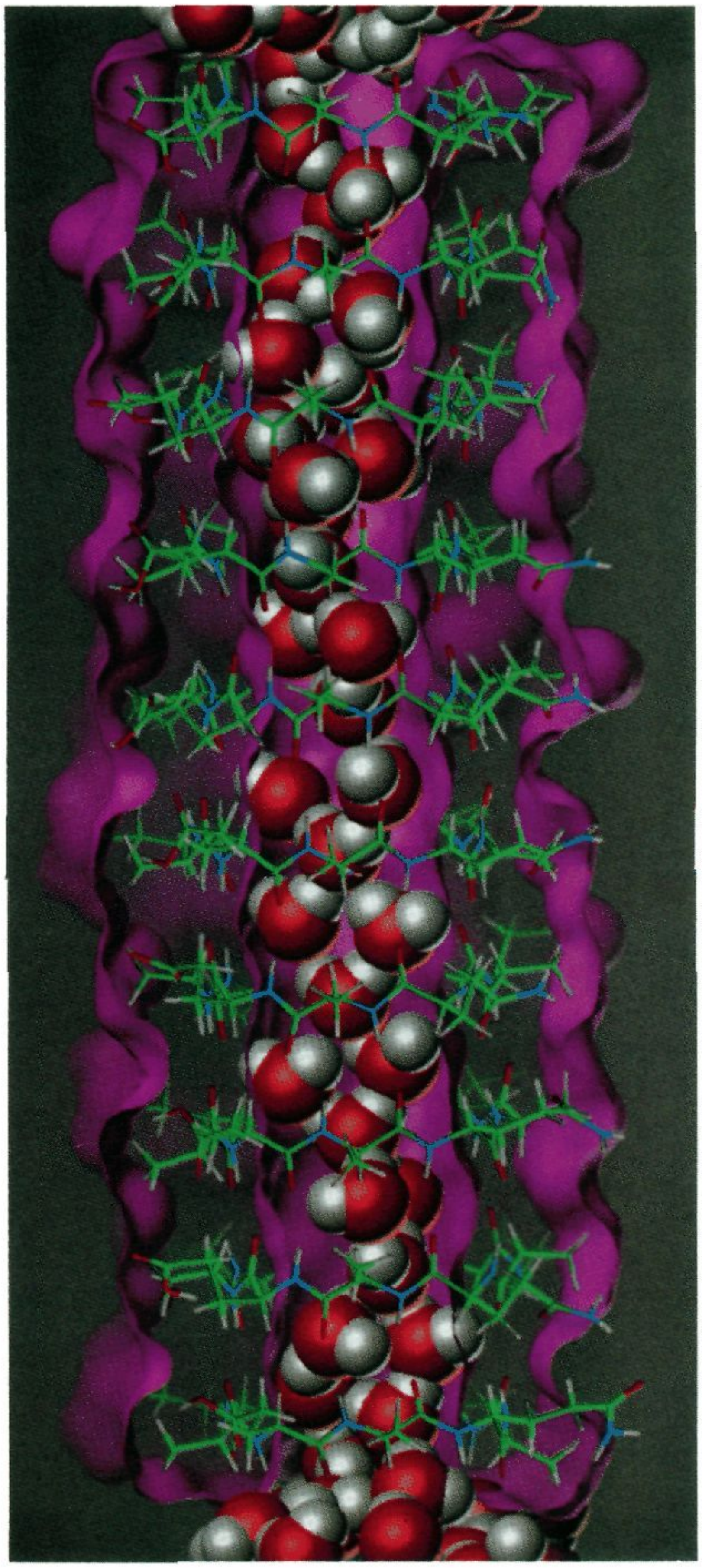

Figure 5. Section through the tube showing the structure of internal water. Half of the tube peptides are sliced away, and the tube molecular surface is shown in purple. All internal waters are shown. In the lower part of the figure water is organized into layers of one in the α-plane zone and two in the midplane zone. Water in the upper part of the figure is less ordered. Color graphics by Mike Pique, The Scripps Research Institute.

The arrangement of water molecules inside the tube is illustrated by Figure 5, which shows a snapshot from the trajectory. There is a tendency for water to be stacked in alternating layers of one and two water molecules. In a region near the plane of the peptide C_α atoms, which we designate as the *α-plane zone*, there is typically a single water molecule located on the tube axis. In the regions between two α-plane zones (the *midplane zones*), there are typically two waters in off-axis positions. The less populated α-plane zones are adjacent to a more hydrophobic part of the tube wall (the carbon backbone) while the more populated midplane zones are adjacent to the more hydrophilic parts of the tube wall.

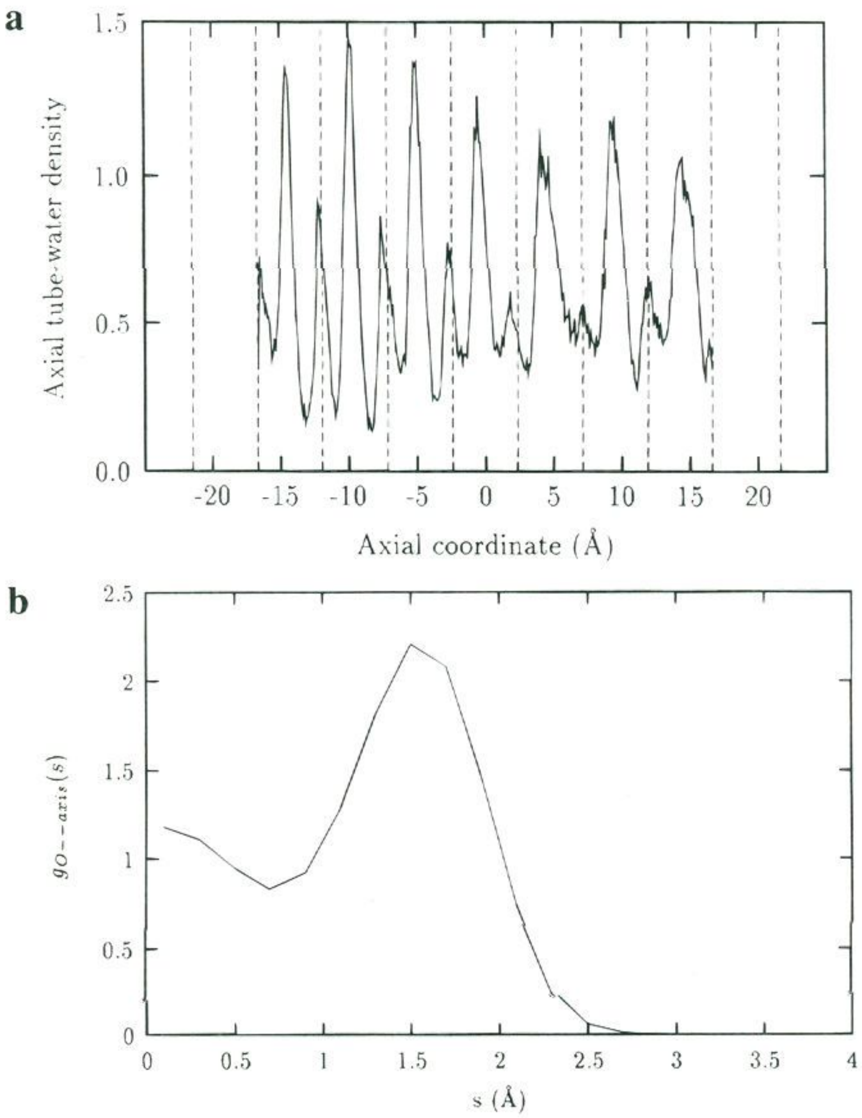

Figure 6. (a) Axial distribution of water oxygen atoms in the tube over the last 400 ps of the simulation as a function of the oxygen Z coordinate. Only water molecules lying between the planes formed by the C_α atoms of peptides II and IX and within 3.0 Å of the tube axis have been included (see Figure 6b regarding this cutoff). A total of 4000 trajectory frames with coordinates superimposed by least-squares fit to the starting peptide structure have been used. The curve has been normalized by the number of water molecules per Å of tube for an ideal tube of 3.4 Å radius (see text) and bulk number density. Vertical lines show the average positions of the C_α atoms during the 400 ps interval. The rms deviation of C_α positions from these averages is less than 0.5 Å for all peptides. The pattern of small peaks in the C_α positions and larger peaks midway between them is consistent with the 1−2 layer structure noted in the text. (b) Cylindrical distribution of the water−oxygen about the tube axis defined as $g_{\text{O-axis}}(s) \propto \langle \Delta N_c(s)\rangle / s\delta s$, where $\langle \Delta N_c(s)\rangle$ is the average number of water molecules in a cylindrical shell of thickness δs and radius s about the axis of the tube (units are arbitrary). Only water molecules lying between the planes formed by the C_α atoms of peptides II and IX have been included. The on-axis and off-axis maxima are consistent with the 1−2 layer structure noted in Figure 5.

Parts a and b of Figure 6 show the axial and radial distributions, respectively, for water inside the tubes. The axial distribution has strong peaks in the midplane zones and weaker peaks in the α-plane zone which is consistent with the 1−2 layered water structure noted above. Two maxima are found for the distribution of water oxygen atoms as a function of distance from the tube axis, one on the tube axis and one 1.5 Å from the axis (Figure 6b). The ratio between the peak heights, approximately 1.18/2.21, is consistent with the 1−2 layered structure. The distribution falls off rapidly as the wall of the tube is reached—at 2 Å, the distribution is at one-half its maximum value. By adding a water radius of 1.4 Å to this value, one obtains an estimated effective tube radius of 3.4 Å. Ten peptide rings stacked at 4.77 Å spacing will be 42.9 Å high from the first C_α plane to the last, from which a tube volume of 1560 Å^3 is estimated. Since there are an average of 32.8 waters in the space between the first and last C_α planes,

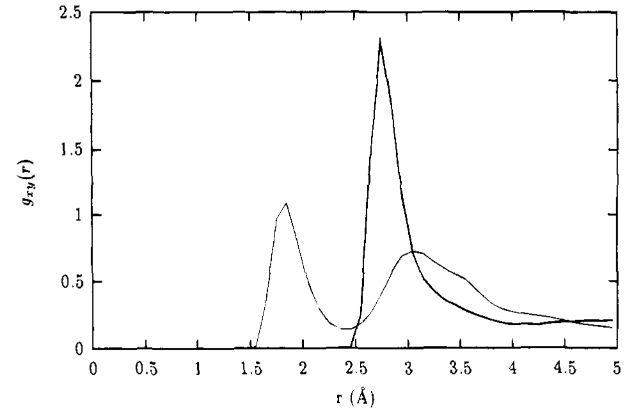

Figure 7. Radial pair distribution functions $g_{OO}(r)$ (thick line) and $g_{OH}(r)$ (thin line) for water molecules inside the tube. The radial pair distribution function is defined as $g_{xy}(r) \propto \langle \Delta N(r)\rangle / 4\pi r^2 \delta r$, where $\langle \Delta N(r)\rangle$ is the average number of atoms of type x around atoms of type y in a shell of thickness δr with an radius r. The proportionality constant is determined by the normalization method of Brooks *et al.*[25]

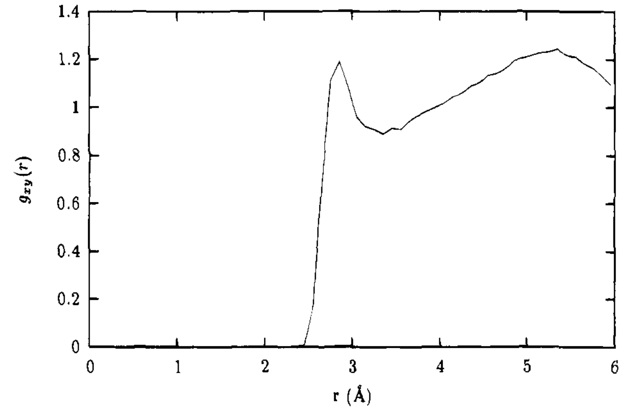

Figure 8. Radial pair distribution function between the carbonyl oxygens and internal water oxygen calculated for the last 400 ps of the MD simulation (methods as in Figure 7).

the average volume per water molecule is 47.6 Å^3. This is consistent with the range 39–64 Å^3 estimated for gramicidin A.[13] The comparable value for bulk water is approximately 30 Å^3. Simple cylindrical estimates of tube radius and volume must be used with caution since it appears from Figure 5 that the tube has small periodic variations in its radius rather than having straight cylindrical sides.

The water O$\cdots$O radial pair distribution function, computed for water inside the tube, shows a peak at 2.75 Å in agreement with TIP3P bulk simulations and our control simulation of water (Figure 7). Integration of the first peak up to a limit of 3.5 Å gives an average of 3.4 water molecules in the first solvation shell which is much lower than the value of 5.2 obtained in Jorgensen's original work[6] or 5.6 in our control simulation. The O$\cdots$H distribution functions for the water molecules inside the tube show a shape very similar to that of those obtained for the TIP3P bulk-water control simulation, with a first peak at 1.85 Å and a second, smaller one at 3.05 Å. Integration of the first peak up to 2.5 Å gives the value of 1.45, which can be interpreted as the average number of water–water hydrogen bonds per water molecule in the tube. Again, this is much less than the bulk value of 3.4 obtained by Jorgensen[6] or 3.5 from our control simulation. In short, water in the tube has pairwise interactions similar to those of bulk water, except that each water has fewer neighboring waters, apparently due to the steric constraint imposed by the tube.

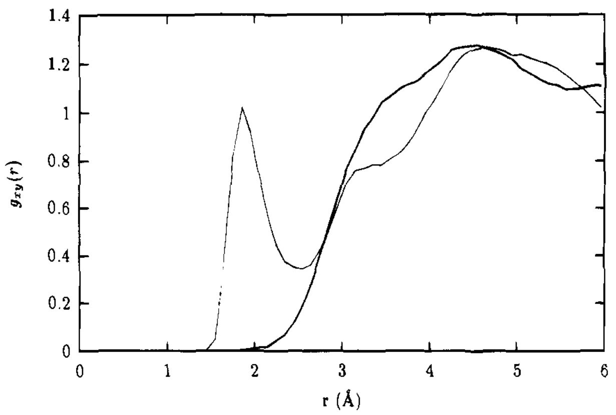

Figure 9. Radial pair distribution function between the carbonyl oxygens and internal water hydrogens (thin line) and between amide hydrogens and water oxygen (thick line) calculated for the last 400 ps of the MD simulation.

Distribution functions pertaining to the interactions of water with hydrophilic components of the peptide tube have also been calculated (Figure 8). The radial pair distribution function between peptide carbonyl-oxygens and the oxygens of waters inside the tube displays a sharp peak at 2.85 Å followed by a broad maximum around 5 Å (on the order of the tube diameter). Integration of the first peak up to the same length used for the calculation of the O$\cdots$O radial pair population (3.5 Å) gives a value of 0.60 water molecules per peptide carbonyl. The carbonyl oxygen–water hydrogen radial pair distribution shows a pronounced peak at 1.85 Å (Figure 9). Integrating this peak up to the same limit used for the determination of hydrogen bonds of channel water (2.5 Å) gives a population of 0.32 nearby water hydrogens per carbonyl oxygen. However, the average carbonyl carbon–carbonyl oxygen–water hydrogen angle deviates from linearity by 66°. The broad shape of the amide hydrogen–water oxygen distribution between the the N-H groups and channel water suggests almost no specific interactions between the N-H moieties and water. The absence of any tendency of the amide groups to tilt into the tube combined with the relative weakness and/or off-linear angle of amide water interactions suggests that water does not compete significantly with the peptide–peptide hydrogen-bonding system. The peptide side chains block the access of water outside the tube to the backbone amides. The coordination numbers found from the water O$\cdots$O distribution and the carbonyl oxygen–water oxygen distribution can be rationalized on the basis of the idealized 1–2 layered structure. A water molecule in the α-zone will have two neighbors in the midplane zone above it and two below for a total of four. A water molecule in the midplane zone will have one neighber in the same midplane zone and one neighber each in the α-plane zones above and below for a total of three. There are twice as many midplane waters as α-plane waters, so a weighted average gives 3.33 neighbors per water, which compares well to the integrated coordination number of 3.4. There are eight carbonyls in each midplane, and they occur in pairs close enough together so that a single water molecule can lie in the first solvation shell of two carbonyl oxygens at once. Therefore, the two water molecules in the midplane can lie in the first shell of only four of the eight carbonyls at any one time. One then predicts a coordination number of 0.5, which compares well with the integrated value of 0.6.

Dynamics of the Tube Water. Information about the mobility of water inside the peptide channel structure can be obtained by examining the time-dependent evolution of the Z coordinates of individual water molecules (Figure 10). The

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(13) Jordan, P. *Biophys. J.* **1990**, *58*, 1133–1156.

(14) Mills, R. *J. Phys. Chem.* **1973**, *77*, 685–688.

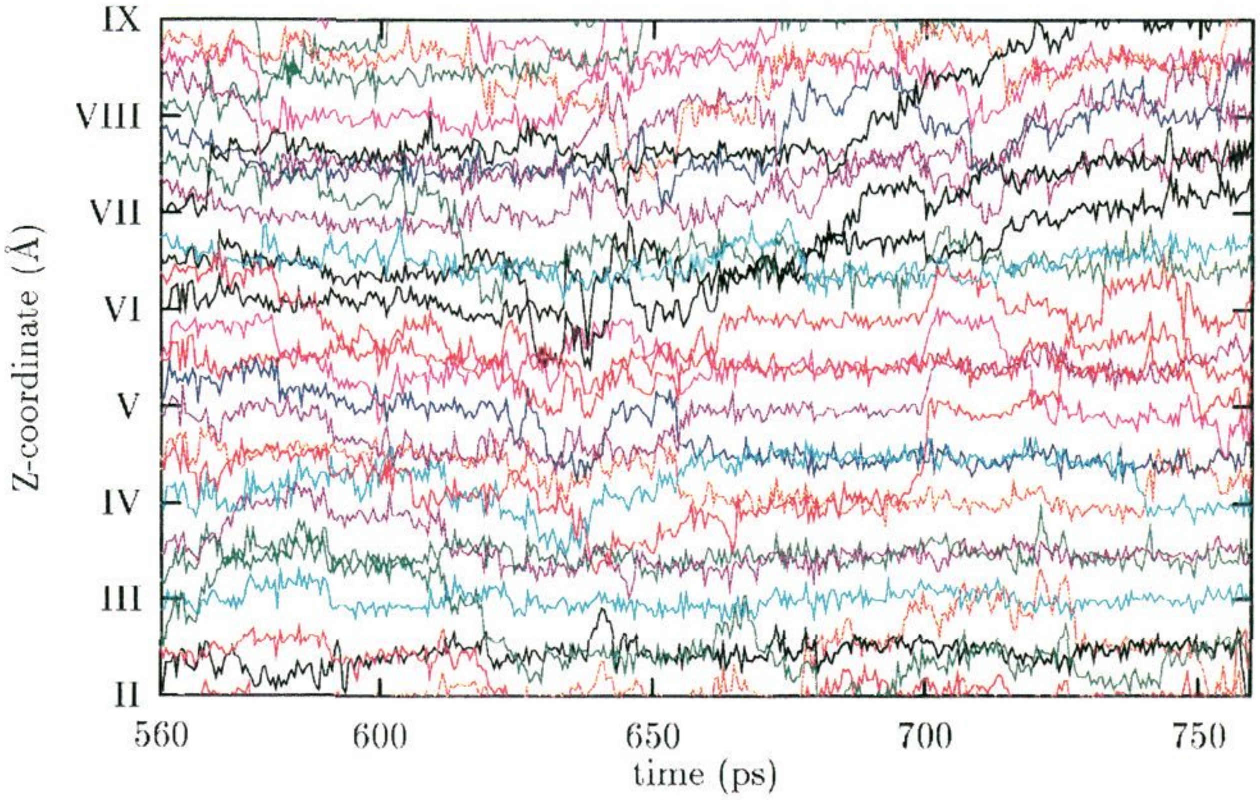

Figure 10. *Z*-coordinate of selected water molecules as a function of time. An average stacking distance of 4.77 Å between peptide subunits was used.

alternating layers of one and two water molecules described above in connection with Figures 5 and 6 are clearly visible in Figure 10. There are times when "defects" (or deviations from the ideal 1−2 structure) are observed which are connected to the hopping of water molecules from one zone to an adjacent one. These defects may be caused by small gaps or irregularities in the tube structure during the dynamics. The diffusive mechanism of the peptide channel is not restrained to single-file motion, as in gramicidin, but includes crossing events.

The self-diffusion coefficient of the water along the tube axis is related to the mean square displacement in the long time limit by

$$\langle (x(t + \Delta t) - x(t))^2 \rangle /2 = D\Delta t \tag{1}$$

where the x are the time-dependent positions of a particular water molecule and the angle brackets indicate averaging over both particles and reference times, t. In implementing eq 1 for tube waters, only those waters that remained inside the tube throughout the simulation are included in the statistics. The resulting self-diffusion coefficient is then the average of the diffusion coefficients of the full-time tube water molecules. A more complex alternative method that started and stopped individual timers as waters entered and left the tube gave similar long-term averages but less regular short-term statistics. The linear (diffusive) development of $\langle (x(t + \Delta t) - x(t))^2 \rangle /2 = D\Delta t$ with time can be seen in Figure 11, whose slope is related to the tube-water self-diffusion coefficient by eq 1. The values 0.41×10^{-5}, 0.48×10^{-5}, and 0.42×10^{-5} cm^2 s^{-1} are obtained for D from the time windows 360−560, 460−660, and 560−760 ps, respectively, with the average value of 0.44×10^{-5} cm^2 s^{-1}. In contrast, the water self-diffusion coefficient from the control simulation on bulk water was $(2.8 \pm 0.02) \times 10^{-5}$ cm^2 s^{-1} and the experimental value for bulk water is 2.3×10^{-5} cm^2 s^{-1} at $T = 298$ K (ref 14).

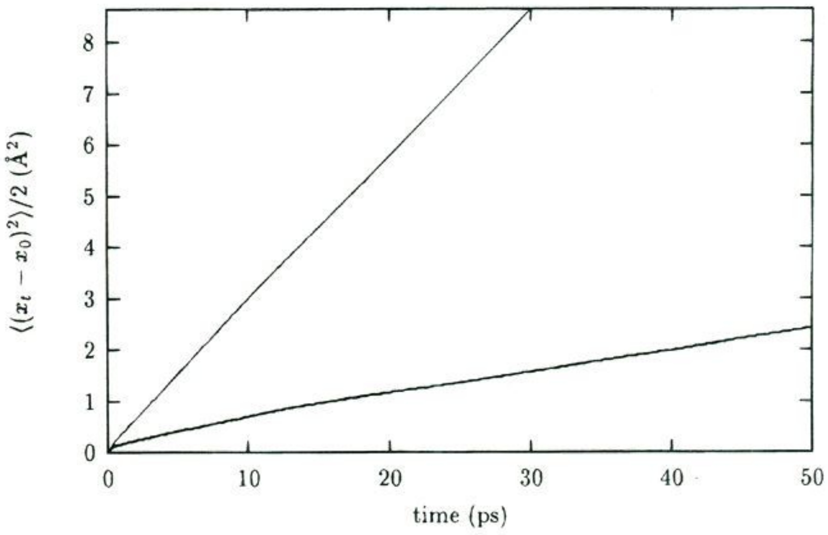

Figure 11. One-half mean square deviation of water oxygen atoms from their initial positions along the tube axis during the 550−750 ps time window: mean-square displacement is calculated by considering only water molecules which stay inside the tube during the analyzed time segment (thick line); control simulation of bulk water (thin line).

Discussion

The fact that the backbone rms deviation remains between 0.5 and 1.0 Å throughout the 760 ps simulation with very little tendency to increase over time (Figure 3) is evidence that the peptide tube is quite stable in the limited context of sub-nanosecond dynamics with the CHARMM22/TIP3P parameter model. The question of whether parameter models of this general kind and time scale give accurate indications of the stability of hydrogen-bonded peptide structures has been explored by Tirado-Rives and Jorgensen,[15] who simulated an analogue of the S-peptide of ribonuclease A, using an all atom force field (OPLS[16,17] rather than CHARMM) and TIP3P water. Their simulations carried out over a time scale of 300−500 ps correctly predicted that the peptide remained helical at 278 K and became disordered at 358 K, and their stable, low-temperature simulation had backbone rms fluctuations of ≈1.0 Å and considerable disruption of the α-helical hydrogen-bonding pattern. The present tube simulation shows significantly smaller rms fluctuations (Figure 3) and remains stable over a longer simulation. The stability of the hydrogen-bonded peptide tube ensemble in the simulation is consistent with experiments showing the remarkable cooperative nature of the peptide self-assembly process.[2−4] A potential-of-mean-force calculation of the self-assembling process would be of interest in this context.

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(15) Tirado-Rives, J.; Jorgensen, W. L. *Biochemistry* **1991**, *30*, 3864−3871.

(16) Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657.

(17) Jorgensen, W. L.; Severance, D. E. *J. Am. Chem. Soc.* **1990**, *112*, 4768.

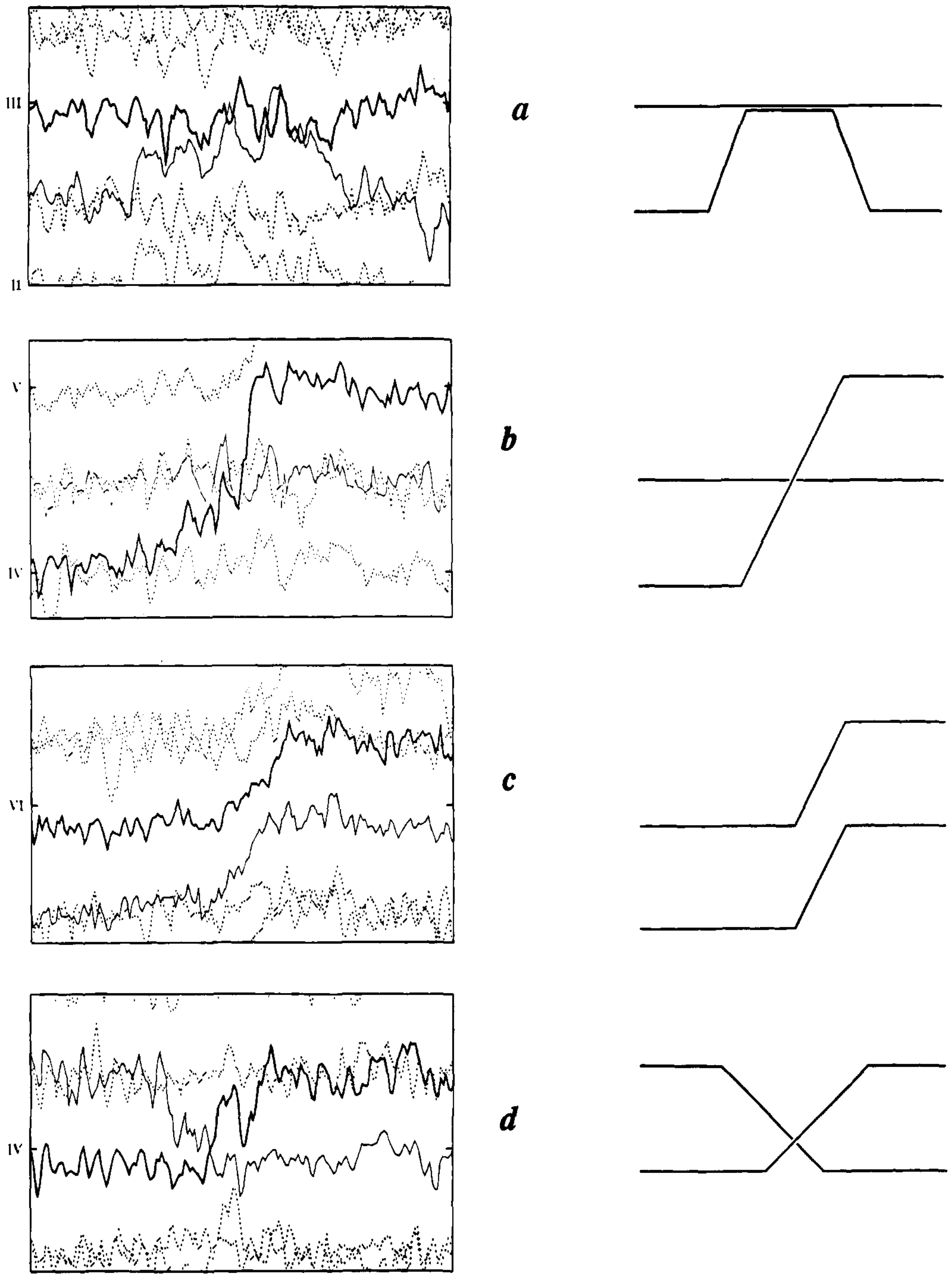

Figure 12. Schematic representation (right panels) of four types of orchestrated water movements inside the peptide channel which were observed in the course of the dynamics simulations (left panels are actual expanded views of selected regions from Figure 10) and are defined as (*a*) bouncing, (*b*) crossing, (*c*) dragging, or (*d*) exchanging motions.

There has not yet been an experimental measurement of the diffusion constant of water in the self-assembling transmembrane peptide channels for comparison with the diffusion coefficient calculated from the present dynamics simulation. However, there have been a number of experimental studies and simulations of water in gramicidin A, a transmembrane channel that shares some features with the present peptide tubes: an alternating D,L-amino acid structure and an internal channel lined with hydrogen-bonded backbone amide functionalities. The calculated diffusion coefficient of water in gramicidin A determined by molecular dynamics calculation is 0.025 × 10^{-5} $cm^2\ s^{-1}$ (ref 18), which is not far from the range suggested by experimental studies.[19-21] According to the present simulation, the diffusion constant of water in the peptide tube is 0.44 × 10^{-5} $cm^2\ s^{-1}$, which is 18 times faster than the value calculated from the gramicidin molecular dynamics simulation and significantly faster than the experimental range for gramicidin A.[19-21] The faster diffusion of water through the peptide nanotubes as compared to gramicidin is interesting in the context of the experimental results showing that transmembrane channels formed by octapeptide subunits similar to those studied here conduct ions 3−5 times faster than gramicidin A.[3a]

An obvious factor in the faster diffusion of water in the

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(18) Roux, B. In *Computer Simulation in Molecular Biology*; Goodefellow, J., Ed.; VHC: Weinheim, 1995.

(19) Based on experimental data from the following: Rosenberg, P. A.; Finkelstein, A. *J. Gen. Physiol.* **1978**, *72*, 341−350. Chiu *et al.* (ref 21) obtained the diffusion constant value of 2.8 × 10^{-7} $cm^2\ s^{-1}$.

(20) Dani, J. A.; Levitt, D. G. *Biophys. J.* **1981**, *35*, 501−502.

(21) Chiu, S.-W.; Jakobsson, E.; Subramaniam, S.; McCammon, J. A. *Biophys. J.* **1991**, *60*, 273−285.

peptide tubes compared to gramicidin is the larger effective radius of the peptide tube, 3.4 Å compared to the 2 Å radius of the gramicidin A channel.[22] Roux and Karplus have[23] suggested that end effects may significantly slow water diffusion in gramicidin on the basis of a comparison of between simulations of an infinite periodic gramicidin-like structure and a finite gramicidin dimer. But another factor in the fast diffusion rate appears to be related to the structure of water in the tubes—alternating layers of α-plane zones typically having one water and midplane zones having two (i.e., α-1; mid-2)—and the dynamics of water movement between these zones. This structure makes it possible for water molecules to move past each other in contrast to the apparently single-file structure and dynamics of water in gramicidin.[21,24] Deviations from this 1−2 structure can occur in that there may be transiently one more or one or two fewer water molecules in a zone than the "canonical" number, and these deviations may also be important for transport.

Inspection of the water *Z*-coordinate plots (Figure 10) and graphics snapshots from the trajectory suggest that the water motion could be understood in terms of elementary steps consisting of the movements of water molecules from one zone to an adjacent one. For example, the movement of a water from a midplane zone having two waters to an α-plane zone having one water so as to produce a noncanonical layer population, (mid-2; α-1) → (mid-1; α-2), might have one characteristic rate, while the noncanonical to noncanonical transition (mid-3; α-1) → (mid-2; α-2) might have another characteristic rate. Combinations of these elementary moves can lead to larger motions such as waters crossing past one another as shown in Figure 12. We do not have sufficient statistics to calculate rates for all the elementary motions that might occur, but if longer simulations could give such rates, it may be possible to understand diffusion qualitatively from a relatively simple "zone-hopping" model.

There is an intriguing discrepancy between the canonical and actual number of waters in the tube during the simulation. Canonically, there should be one water in each of the 10 α-plane zones (the use of the C_α plane cutoffs reduces the effective number of in-plane zones to nine for the purposes of averaging) and two waters in each of the nine midplane zones. This gives 27 as the canonical number of waters in the tube. However, the simulation gives an average of 32.8 waters in the tube with a considerable range of fluctuations (Figure 4). We speculate that the slight excess may be conducive to diffusion for the following reason: If a tube's equilibrium water population and structure were exactly canonical, the initiation of diffusion would require a zone hop from a state in which both zones have a canonical population to a state in which both zones have a noncanonical population, for example, (mid-2; α-1) → (mid-1; α-2). This may make the initiation of diffusion energetically unfavorable. But if the equilibrium population is slightly larger than the canonical, structures containing defects will be among the lower energy structures and diffusion can proceed by steps that may not require as large an energy increase, such as (mid-3; α-1) → (mid-2; α-2). The situation is roughly analogous to the increase in the conductivity of an n-type semiconductor due to its doping with atoms having a larger number of valence electrons than the intrinsic semiconductor material.

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(22) Urry, D. W.; Goodall, M.; Glickson, J. D.; Mayers, D. F. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1907−1911.

(23) Roux, B.; Karplus M. *J. Phys. Chem.* **1991**, *95*, 4856−4868.

(24) Roux, B.; Karplus M. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 731−761.

(25) Brooks, C. L.; Karplus, M. *J. Mol. Biol.* **1989**, *208*, 159−181.