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ARTICLE

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Entrapment of a Water Wire in a Hydrophobic Peptide Channel with an Aromatic Lining

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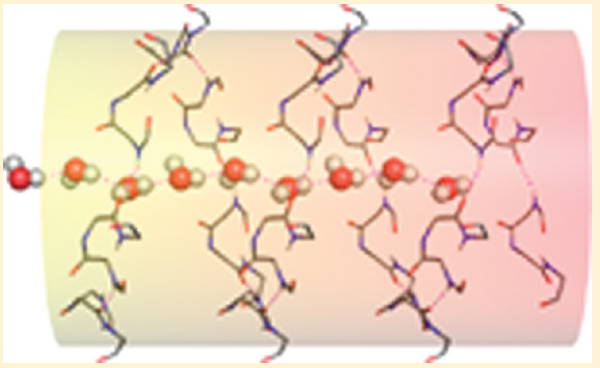
Supporting Information

ABSTRACT: A one-dimensional water wire has been characterized by X-ray diffraction in single crystals of the tripeptide Ac-Phe-Pro-Trp-OMe. Crystals in the hexagonal space group $P6_5$ reveal a central hydrophobic channel lined by aromatic residues which entraps an approximately linear array of hydrogen bonded water molecules. The absence of any significant van der Waals contact with the channel walls suggests that the dominant interaction between the "water wire" and "peptide nanotube" is electrostatic in origin. An energy difference of 16 kJmol^{-1} is estimated for the distinct orientations of the water wire dipole with respect to the macrodipole of the peptide nanotube. The structural model suggests that Grotthuss type proton conduction may, through constricted hydrophobic channels, be facilitated by concerted, rotational reorientation of water molecules.



INTRODUCTION

Single-file arrangements of water molecules encapsulated within a constricting channel have been considered as attractive models for the transport of protons across biological membranes and carbon nanotubes.[1] In such arrangements each water molecule can make only two hydrogen bonds, one in which it functions as an acceptor and the other in which it serves as the donor. Such a one-dimensional array ("water wires") provides an idealized framework for Grotthuss type proton conduction.[2] Molecular dynamics simulations have provided insights into the proton conduction facilitated by water wires in hydrophobic channels and carbon nanotubes.[1b−e,3] In the biological context aqueous channels in protein environments are often formed by interactions of hydrophobic groups with side chain and/or backbone atoms.[4] Short stretches of single-file water molecules have been characterized in aquaporin crystal structures, in which one face of the lining which interacts with water is hydrophobic, whereas the other is polar and participates in hydrogen bonding interactions with water.[5] The entrapment of water wires in purely hydrophobic environments within the framework of peptidic structures is a matter of considerable interest.[6]

Microporous crystal structures formed by hydrophobic unprotected dipeptides have attracted considerable recent interest in view of potential applications anticipated for organic nanotubes.[7] The work of Gorbitz has established the presence of porous channels containing multiple water molecules in dipeptide structures with aromatic side chains.[8] A new class of tubular peptide structures ("peptide nanotubes") have been shown to encapsulate one-dimensional water arrays ("water wires") inside narrow channels (diameter ∼7.5 Å) with completely hydrophobic walls. Crystal structures of linear pentapeptides in hexagonal space groups $P6_1/P6_5$ have been shown to form hydrophobic channels in which the encapsulated water molecules do not have any strong interactions with the walls of the channels which are lined by aliphatic hydrocarbon groups.[9] We describe in this report the structural characterization of water wires with distinct structural properties encapsulated in a hydrophobic channel which is lined exclusively with aromatic groups. The synthetic tripeptide Ac-Phe-Pro-Trp-OMe (**1**) crystallizes in the space group $P6_5$ forming porous crystals in which the internalized water molecules are held together only by hydrogen bonds to nearest neighbors. Electrostatic forces between the macrodipoles of the peptide channels and the water wire dipoles may be invoked to rationalize the observation of an ordered water array in the absence of explicit hydrogen bonding between the trapped water molecules and the walls of the peptide nanotube. The sequence of peptide **1** in which a proline residue is sandwiched between two aromatic residues was designed as part of a program to evaluate the influence of aromatic side chains in stabilizing *cis* Xxx-Pro peptide bonds. Indeed, the molecular conformation of peptide **1** in crystals described herein reveals a Phe-Pro *cis* peptide unit, resulting in the characterization of a type VI β-turn structure. The serendipitous discovery of a "water wire" in the crystal structure of **1** provides insights into possible models for proton conduction through single-file water arrays.

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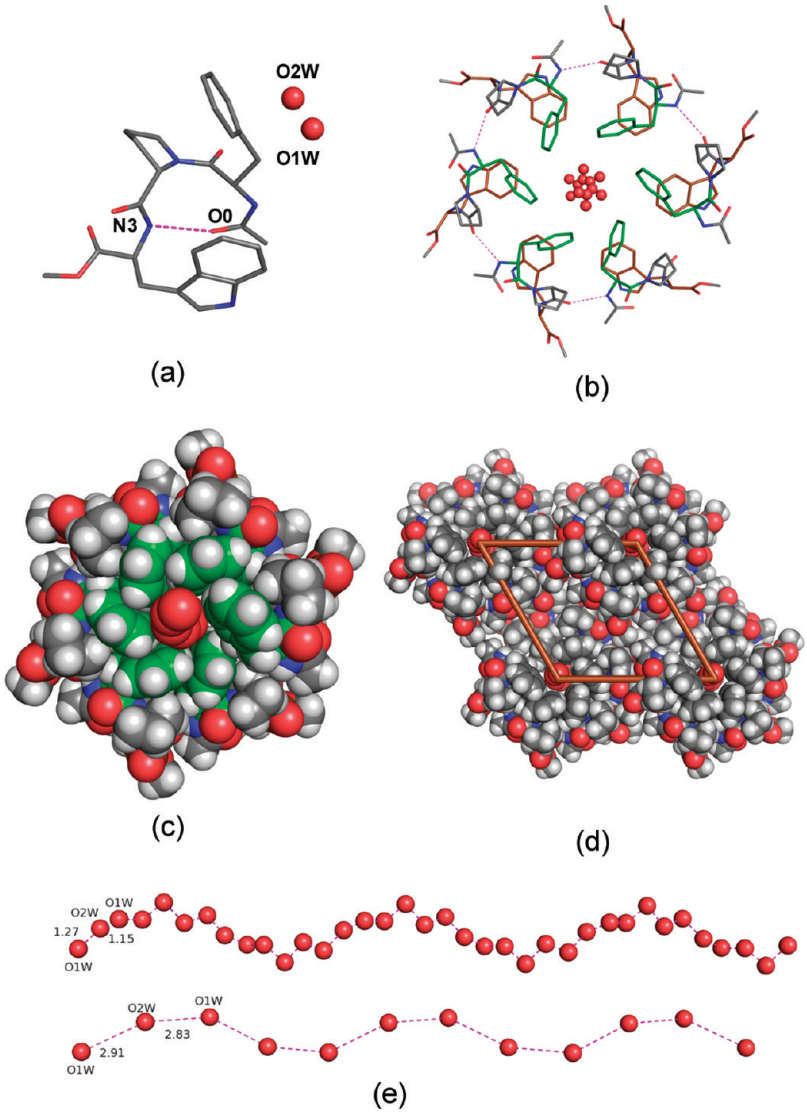

Figure 1. (a) Molecular conformation of the peptide Ac-Phe-Pro-Trp-OMe (Form **1**). (b) Assembly of the peptide molecules around the crystallographic *c* axis (6-fold). The inner lining of the tube wall consists of Phe residues (green) (c) Space-filling model of the self-assembly along the 6-fold axis. The internal diameter of the tube is ~5.9 Å. (d) Lateral arrangements of individual peptide nanotubes in the unit cell gives rise to a honeycomb pattern. (e) The single-file one-dimensional water molecules along the 6-fold axis (top) and the generated water wire using stereochemical considerations (bottom). O···O separations are indicated.

RESULTS

The neutral peptide **1** crystallized in the hexagonal space group $P6_5$ with the cell dimensions $a = b = 21.5813$ (1) Å and $c = 10.1229$ (1) Å. The molecule adopts a typeVIa β-turn ($\varphi_{Phe} = -58.2°$, $\psi_{Phe} = 137.7°$, $\omega_{Phe} = 9.2°$ and $\varphi_{Pro} = -101.3°$, $\psi_{Pro} = 23.8°$, $\omega_{Pro} = 178.6°$) conformation stabilized by a 4 → 1 hydrogen bond between the acetyl CO and Trp NH groups (N···O = 2.905 Å, H···O = 2.135 Å, ∠NH···O = 148.82°).[10] The Phe-Pro peptide bond adopts a *cis* conformation. The crystal structure solution and refinement revealed a cocrystallized water molecule disordered over two positions (O1w and O2w) with the site occupation factors in the ratio 0.42:0.58 (B-factors: B(O1w) = 15.96 Å^2 and B(O2w) = 20.94 Å^2). Figure 1 shows a view of the molecular conformation and assembly of the tripeptide **1** in crystals. One intermolecular hydrogen bond between Phe NH and Pro CO (N(x,y,z)···O($y,-x+y,z+1/6$) = 3.024 Å, H(x,y,z)···O($y,-x+y,z+1/6$) = 2.233 Å, ∠NH···O = 152.79°) connects the adjacent peptide molecules, which wind around the 6-fold axis, resulting in the formation of a hydrophobic tube with an internal diameter of ~5.9 Å. Single tubular peptide columns are then assembled in parallel fashion in crystals, with a single hydrogen bond between the Trp indole NH group and the Trp CO group of a symmetry related molecule (N(x,y,z)···O($-x,1-y,z-1/2$) = 3.115 Å, H(x,y,z)···O($-x,1-y,z-1/2$) = 2.434 Å, ∠NH···O = 136.51°). The cocrystallized water molecules occupy the hydrophobic pores. The crystal structure reveals a chain of water molecules and separations of 1.27 Å and 1.15 Å between the adjacent oxygen atom positions. The structurally and chemically sensible entrapped water wire is readily generated by removing alternate oxygen atoms in a single channel and retaining those oxygen atoms which satisfy the hydrogen bonding parameters, yielding an O···O separations of 2.91 Å and 2.83 Å (Figure 1).[9] The oxygen atom of the water molecule does not occupy a special

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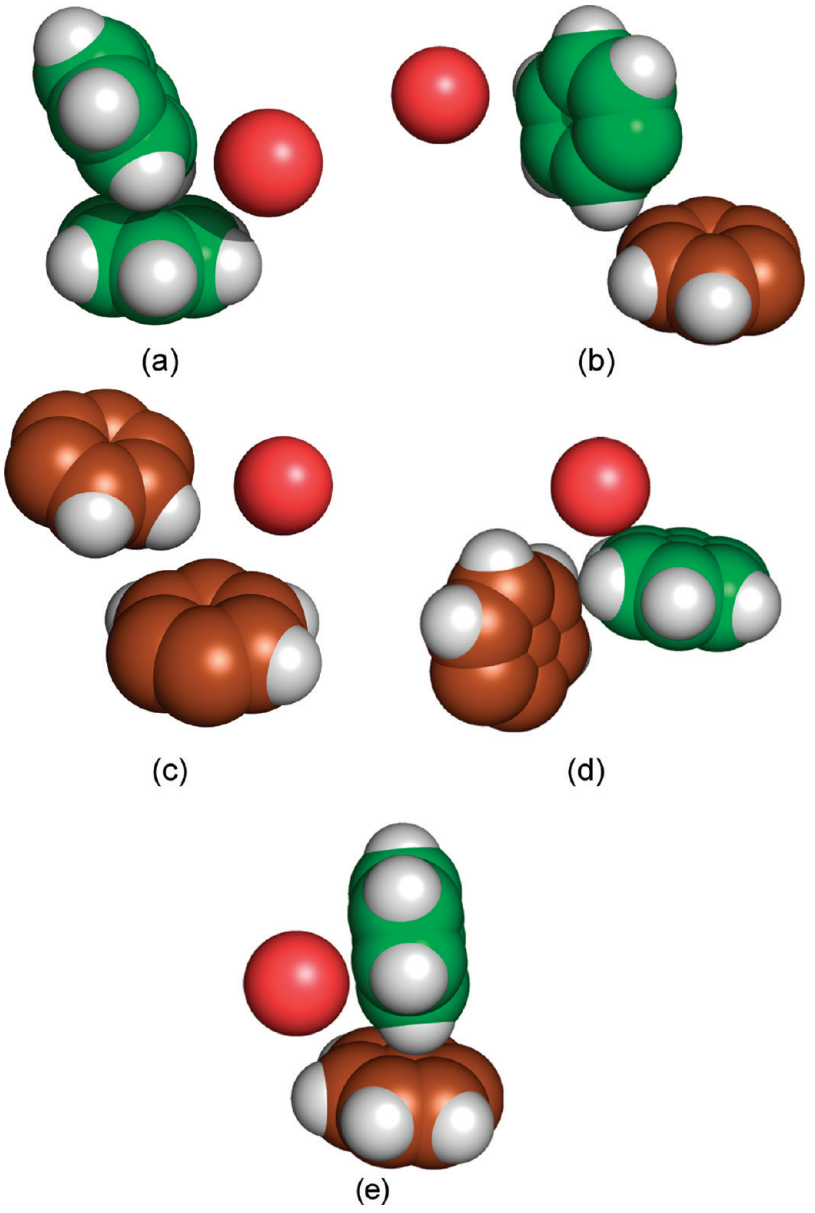

Figure 2. Aromatic−aromatic interactions in the tripeptide Ac-Phe-Pro-Trp-OMe, Form **1**. (a) Phe(x,y,z)···Phe($y,-x+y,z+1/6$), (b) Phe(x,y,z)···Trp(x,y,z), (c) Trp(x,y,z)···Trp($y,-x+y,z+1/6$), Interaction parameters are given in the text. (d) Phe(x,y,z)···Trp($x-y,x,z+5/6$) R_{cen} = 5.765 Å, R_{clo} = 3.631 Å, γ = 83.51°. (e) Phe(x,y,z)···Trp($x,y,1+z$) R_{cen} = 5.026 Å, R_{clo} = 3.912 Å, γ = 79.50°. One water molecule is also shown.

position, and the occupancy of the disordered sites sums to 1. The water molecules in the water wire spiral around the crystallographic c axis with minimal or weak interactions with the channel walls. The water wire forms a 3-fold helix whose long axis is coaxial with the crystallographic c axis of the nanotube (channel axis) but appears to be displaced laterally resulting in the observation of nonuniform displacement of the water molecules with respect to the c axis. The water oxygen atoms are spatially displaced with respect to the c axis with the displacement being in the range 0.15−1.25 Å along the c axis. The observed hydrogen bonded structure of the entrapped water wire is in good agreement with that anticipated in a molecular dynamics study of liquid water confined in nanotubes.[11] The channel lining is entirely composed of the aromatic groups of the Phe residues. Aromatic−aromatic interactions are conspicuously present in the crystal structure.[12] The intramolecular Phe···Trp(Phe(x,y,z)···Trp(x,y,z) (R_{cen} = 5.781 Å, R_{clo} = 3.755 Å, γ = 79.50°) and the Phe···Phe(Phe(x,y,z)···Phe($y,-x+y,z+1/6$) (R_{cen} = 4.669 Å, R_{clo} = 3.601 Å, γ = 57.42°) interaction between the adjacent molecules lining the channel may be categorized as T-shaped, while the indole rings which line the exterior of the tube are positioned in a parallel displaced orientation (Trp(x,y,z)···Trp($y,-x+y,z+1/6$) (R_{cen} = 6.359 Å, R_{clo} = 4.256 Å, γ = 23.34°), where R_{cen} is the centroid-to-centroid distance and R_{clo} is the shortest distance between two carbon atoms (Figure 2). The disorder present in the water oxygen atom positions leads to the observation of a continuous electron density throughout the hydrophobic channel (Figure 3).

In an attempt to resolve the positional disorder observed for water oxygen atoms in the crystal structure of Form **1**, X-ray diffraction data was recollected on the same crystal after a period of two years at room temperature. The cell parameters for Form

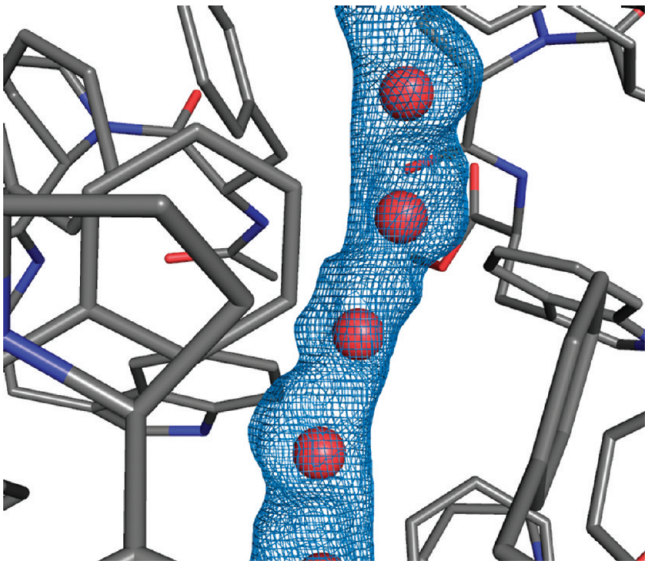

Figure 3. Electron density carved around the water molecules encapsulated in the hydrophobic channels of Ac-Phe-Pro-Trp-OMe. The peptide atoms are shown as sticks. The water molecules are shown as spheres.

2 are $a = b = 21.5674\ (3)$ Å and $c = 10.1035\ (1)$ Å. The structure solution revealed the presence of a peptide molecule in the asymmetric unit with a cocrystallized water molecule. The cocrystallized water molecule (oxygen atom) was refined with full occupancy and the molecule did not reveal any disorder. The B-factor after the anisotropic refinement was significantly larger than peptide atoms ($B = 31.65$ Å^2) and the refinement using SHELXL-97 did not reveal "split peaks or positions".[13] Least-squares refinement carried out with the site occupancy factor of the oxygen atom being allowed to refine with (occupancy = 1/2, 1/3, 1/5) and without constraints (occupancy = 1) suggested that the refinement parameters are the best (convergence of shift/e.s.d to 0) when refined with full occupancy. The water wire in Form **1** forms a helical structure with a 3-fold axis of symmetry, whereas in Form **2** the wire has a 2-fold axis. This leads to the observation of two distinct O···O distances in one and one O···O distance in the latter. The long intervening period between the determination of structures of Forms **1** and **2** has presumably resulted in a loss of water from crystal with a concomitant lengthening of the O···O distance in the water wire. Attempts to obtain crystals without entrapped water by prolonged desiccation or heating were unsuccessful, suggesting that the energetics of interactions between the porous peptide matrix and the entrapped water wire are appreciably stabilized.

Thermogravimetric analysis (TGA) experiments were carried out on a powder (microcrystalline) sample of the peptide. Figure 4 shows the TGA profile obtained for the peptide Ac-Phe-Pro-Trp-OMe (**1**). It is evident that a weight loss of ∼3−4% is observed in the temperature range 20−76 °C, with the midpoint at 50 °C. The melting point of the peptide microcrystals is in the range of 78.5−86.3 °C. Clearly, the observed weight loss prior to the melting point may be correlated to the loss of entrapped water. The crystal structure of **1** yields a peptide water stoichiometry of 1:1. The loss of water in the hydrophobic channels would correspond to ∼2% weight loss, which is consistent with TGA observations. The difference between the expected and observed weight loss may be attributable to the peripherally adsorbed water molecules in the powder peptide samples.

In the case of Form **2** also the water oxygen is seen to be positioned slightly displaced from the crystallographic 6-fold axis, with the displacement in the range 0.39−0.84 Å along the *c* axis. Evidently, the water oxygens in Forms **1** and **2** correspond to two distinct structures of the array of water molecules ("water wires") entrapped in the hydrophobic channel. The crystal for Form **2** was also cooled to 260 K and a fresh diffraction data set was collected. However, there was no change in the structure of the encapsulated water wire. Interestingly, the cooled crystal upon standing yielded diffraction data which refined to the Form **1** structure. This is presumably a consequence of humidity effects which result in a transformation to a form which contains a greater amount of water.

X-ray diffraction does not permit the location of hydrogen atoms for the water molecules in cases where problems occur in collecting the higher angle (Bragg reflections) data precisely and accurately. The data sets of Forms **1** and **2** reveal that R_{int} and R_{sigma} are slightly better for Form **2** than Form **1**, presumably because the data was collected with a high intensity Cu Kα radiation (rotating anode). The least-squares refinement did not reveal any peaks which could be modeled as hydrogens of the water oxygens. Chemically and stereochemically acceptable structural models may be generated by positioning one hydrogen atom between two adjacent oxygen atoms with the formation of a hydrogen bond.[9] The second hydrogen atom may then be arbitrarily fixed in a position where the shortest distance is obtained to the walls of the channel (Form **1**: H(O1w)···H = 2.45 Å and H(O2w)···H = 2.58 Å, Form **2**: H(O1w)···H = 2.81 Å). The interactions of the water molecules in the single-file water wire are stronger in the case of Form **1** as opposed to Form **2**. The shortest distances between the oxygen atom of the water molecule and the channel lining composed of phenylalanine residues (centroid of the six-member ring) are for Form **1** O1w···C = 5.14 Å and H1w···C = 4.85 Å and O2w···C = 4.80 Å and H2w···C = 4.19 Å and for form **2** O1w···C = 5.24 Å and H1w···C = 4.25 Å, suggesting that OH···π type interaction between the water molecules and the channel walls are not significant. The stoichiometry of the unit cell is 6 peptide/4 water molecules in Form **1** and 6 peptide/3 water molecules in Form **2**. In principle rotation of individual water molecules about the crystallographic 6-fold axis (equivalent to rotation about the helix axis of the wire) can result in significant entropic contributions to the overall free energy. This feature is illustrated in Figure 5.

DISCUSSION

Continuous channels have been previously reported in crystals of peptides containing aromatic residues. In the case of a protected heterochiral dipeptide, Boc-(S,S)$c_3$diPhe-(R,R)$c_3$diPhe-NH^{i}Pr, an empty pore of 7 Å diameter has been reported, with the aromatic rings oriented on the outer wall of the channel.[14] In the unprotected dipeptides, Phe-Phe and Phe-Trp, large polar channels are observed in crystals in which multiple water molecules have been located. The aromatic rings line the outer surface of the channel.[15] Aromatic dipeptide nanotubes have been recently exploited in nanofabrication.[16] An interesting recent example illustrates the use of steroidal *bis*-phenylureas in forming nanoporous crystals in which the internal walls of the hydrophobic channel are lined by aromatic surfaces, with the

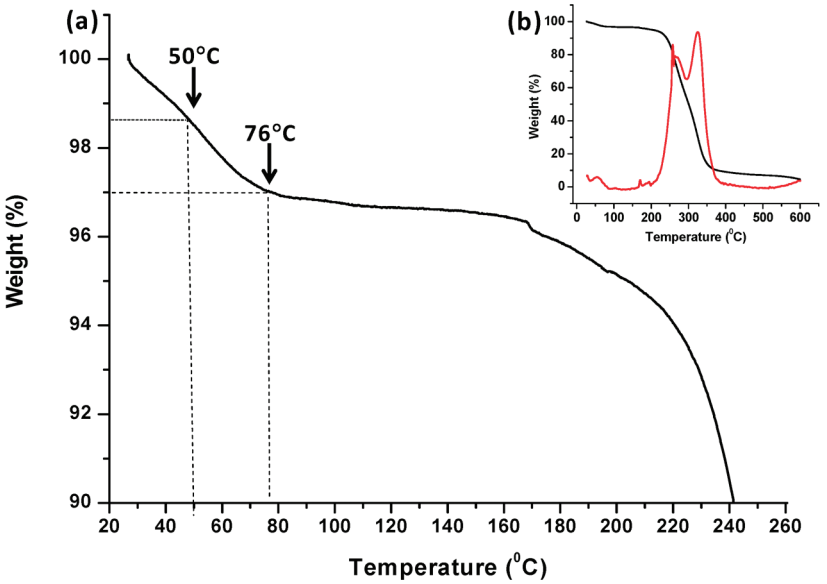

Figure 4. Thermogravimetric analysis (TGA) of the Peptide Ac-Phe-Pro-Trp-OMe (**1**). Black line shows the weight loss of the peptide. (a) Approximately 3−4% weight loss is observed in the temperature range 20−76 °C, corresponding to the loss of water from the hydrophobic channels. (b) Inset shows the TGA profile of the peptide over the range 26−500 °C with a decomposition transition observed at 257 and 324 °C (the first derivative curve is also shown in red color).

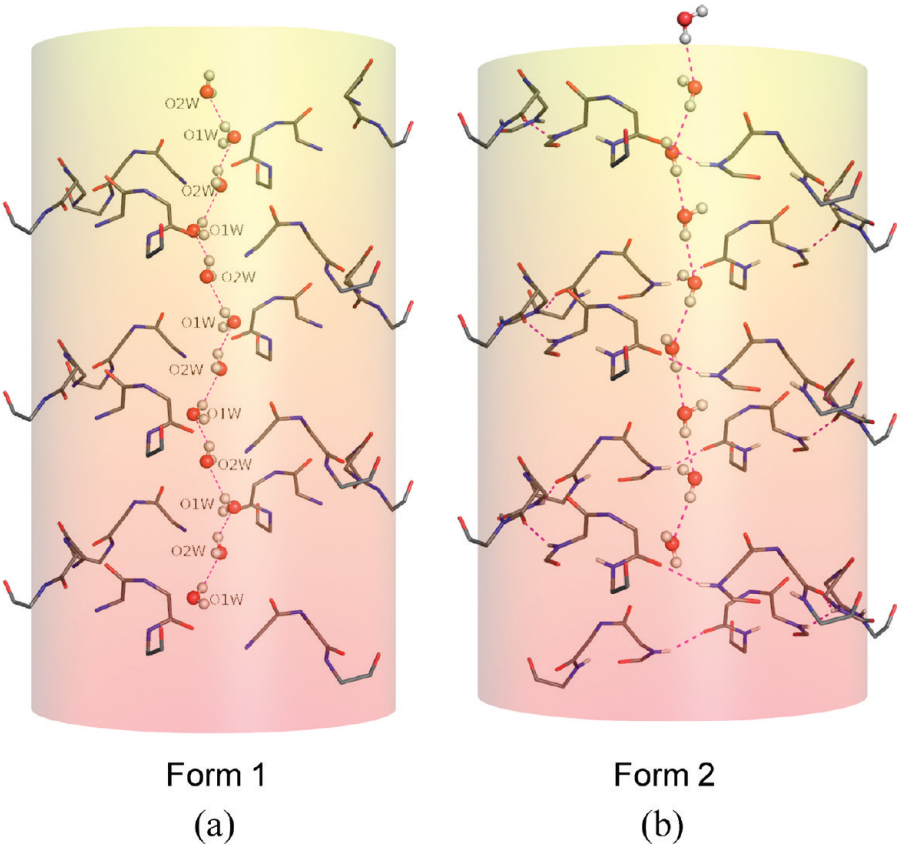

Figure 5. Model of water wire spanning the hydrophobic channels formed by Ac-Phe-Pro-Trp-OMe Forms (a) **1** and (b) **2**. The peptide backbone atoms have been shown as sticks. The hydrogen bonds are shown as dashed lines (magenta). The water molecules are shown as ball-and-stick. The peptide nanotube has been schematically shown as a cylinder. Molecules in three unit cells (∼30.31 Å) along the crystallographic *c* axis are shown.

pore diameters ranging from 5.5 to 6.6 Å. The channel encloses a water wire with modeled O···O distance of 3.15 Å.[17]

The presence of a weak van der Waals interaction of the water wire with the atoms of the channel walls in Form **1** and the

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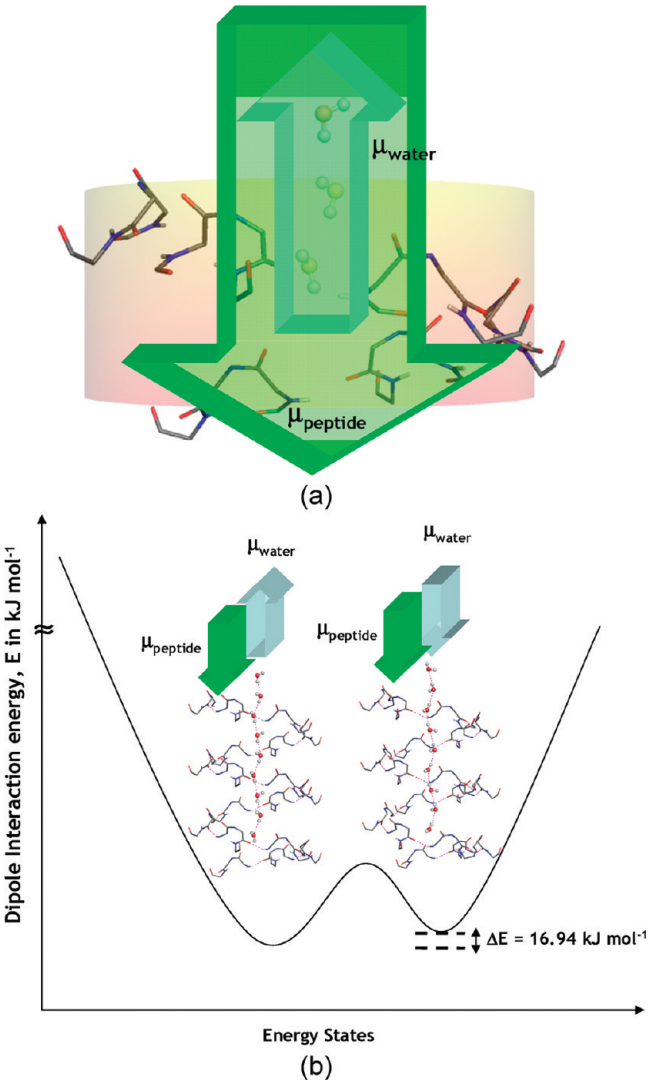

Figure 6. Electrostatic interactions in form **2** of Ac-Phe-Pro-Trp-OMe. (a) Orientation of the resultant peptide bond dipoles and water dipoles in the unit cell of form **2**. (b) Dipole interaction energy (classical) evaluated for two possible water dipole arrangements/configurations, parallel and antiparallel with respect to peptide macrodipole, inside the peptide nanotube of form **2**. Peptide dipole is shown as green arrow and water dipole as gray arrow.

reduction in the strength of the interaction in Form **2**, as a consequence of the diameter of the peptide nanotube (~5.9 Å), suggests that optimal positioning of the water wire must be achieved through electrostatic interactions. Significant dipole moments are associated with the backbone peptide bonds, suggesting that an appreciable component of the macrodipole may be generated along the 6-fold axis. Two energetically distinct states of the water wires may then be envisaged, corresponding to parallel and antiparallel orientations of the water and peptide bond dipoles along the 6-fold axis (Figure 6).[18] An estimate of 16 kJ mol^{-1} is obtained for the difference between the antiparallel and parallel orientations of the peptide and water dipoles, with the antiparallel orientation being preferred. The energetics of interactions of the two possible orientations of the collective water dipoles with homogeneous electric fields has been estimated for water wires confined to the pores in carbon nanotubes.[19] The indexing of the morphological crystal faces of the needle-shaped crystals of Form **2** reveal that the crystallographic *c* axis (6-fold axis) is parallel to the long dimension of the crystal (0.13 mm), suggesting that the crystal growth along the tube axis is significantly more rapid than in the directions perpendicular to the *c* axis. The "sidedness" of such crystals implicit in the electrostatic considerations discussed above may influence the macroscopic material properties of such peptides (Figure 7). Coupling between electric fields and the orientation of water molecules inside constricted hydrophobic channels has been suggested to be of relevance in the mechanism of proton pumping through cytochrome oxidase.[20]

The inherent "electrostatic sidedness" of the tubular peptide columns suggests that a preferred direction of proton (H^+) transport may be readily envisaged. The Grotthuss mechanism would require water molecules to flip.[2] A 2-fold rotation, in a direction perpendicular to the *c* axis (6-fold axis), will result in an interchange of the hydrogen bonded donor and acceptor positions of an individual water molecule. Concerted rotation of the water molecules along the water wire would then result in facile proton conduction across the dimensions of the channel. Reorientation returns the water wire to its original state (see the Supporting Information).

Water and proton (H^+) transport across lipid bilayers are very important processes in cellular physiology.[4,5,21] The mechanisms by which solvated protons and neutral water wire molecules cross lipid bilayers are distinct.[22] A Grotthuss chain mechanism, in which one-dimensional water chains span hydrophobic channels, has been invoked to rationalize the rapid rates of proton conduction.[23] Water transport is mediated by the transmembrane proteins aquaporins which bind a one-dimensional water wire within the confines of an amphipathic channel.[24] The characterization of water wires sequestered in model hydrophobic channels and carbon nanotubes has also attracted considerable theoretical attention.[23] Atomic level structural models have not yet been determined for water wires in membrane protein channels. The direct visualization of water wires in hydrophobic channels formed by apolar peptides in single crystals provides a unique opportunity for developing atomic level models for water wires and proton transport, which may be of relevance in view of the increasing interest in the development of nanofluidic structures for applications in medicine and engineering.[25]

EXPERIMENTAL SECTION

Peptide Synthesis. The peptide Ac-Phe-Pro-Trp-OMe (**1**) was synthesized by conventional solution-phase methods strategy. The final peptide was purified by reverse-phase medium pressure liquid chromatography (MPLC, C_{18}, 40−60 μ), followed by high performance liquid chromatography (HPLC, C_{18}, 10 μ, 7.8 mm × 250 mm) using methanol−water gradients. The purified peptide was characterized by electrospray ionization mass spectrometry (ESI-MS) on Bruker Daltonics Esquire-3000 instrument: M_{cal} = 504 Da, $[M+H]^+$ = 505.2 Da, $[M+Na]^+$ = 527.2 Da.

X-ray Diffraction Analysis. Crystals of form **1** ($C_{28}H_{32}N_4O_5$) were grown by slow evaporation from a solution of organic solvents (methanol and water). A single crystal of size 0.13 × 0.04 × 0.02 mm was used for the diffraction data collection. X-ray intensity data on Form **1** was collected on a Bruker AXS SMART APEX diffractometer (sealed tube) using Mo Kα (λ = 0.71073 Å) radiation, ω-scans, for a total of 25 650 measured reflections. Space group $P6_5$, $a = b$ = 21.5813(1) Å,

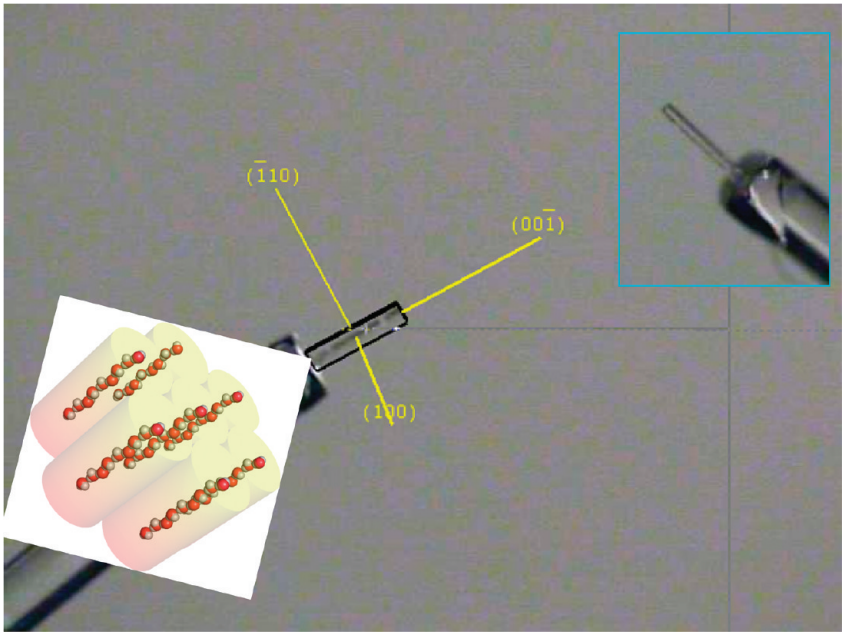

Figure 7. Indexing of the crystal faces of form **2** of Ac-Phe-Pro-Trp-OMe. Crystallographic *c* axis is seen to be collinear with the long dimension of the crystal (0.13 mm). Crystallographic *ab* planes are found to be coplanar to the width of the crystal. Inset shows the original crystal photograph and also a schematic representation of the alignment of peptide nanotubes of form **2**, shown as cylinders, with the entrapped water wire along the long dimension.

c = 10.1229(1) Å, V = 4083.1(7) Å^3, Z = 6 for chemical formula $C_{28}H_{32}N_4O_5.H_2O$, with one molecule per asymmetric unit, ρ_{calc} = 1.27 g cm^{-3}, R_{int} = 0.30, R_{sigma} = 0.15. The structure was obtained by direct methods using SHELXS-97.[26] One water molecule showing disorder over two positions was located from a difference Fourier map. Refinement was carried out against F^2 with full-matrix least-squares methods using SHELXL-97.[13] All non-hydrogen atoms were refined anisotropically and the hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they were bonded. The final R value was 0.0796 (wR_2 = 0.1480) for 1091 observed reflections ($F_0 \geq 4\sigma|F_0|$)(SHEL = 0.9) and 343 variables, where the data-to-parameter ratio is 3.18:1.0 and goodness-of-fit (S) = 0.996. The largest difference peak and hole were +0.29 and −0.24 e Å^{-3}. The X-ray diffraction data set of Form **2** was collected on the same crystal, after a period of two years, on a Bruker AXS SMART APEX II ULTRA diffractometer (rotating anode) using Cu Kα (λ = 0.71073 Å) radiation, $\omega + \varphi$ scans, for a total of 15877 measured reflections. Space group $P6_5$, $a = b$ = 21.5674(3) Å, c = 10.1035(2) Å, V = 4070.0(1) Å^3, Z = 6 for chemical formula $C_{28}H_{32}N_4O_5.H_2O$, with one molecule per asymmetric unit, ρ_{calc} = 1.25 g cm^{-3}, R_{int} = 0.09, R_{sigma} = 0.08. The structure was obtained by direct methods using SHELXS-97.[26] One water molecule was located from a difference Fourier map. Refinement was carried out against F^2 with full-matrix least-squares methods using SHELXL-97.[13] All non-hydrogen atoms were refined anisotropically and the hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they were bonded. The final R value was 0.0640 (wR_2 = 0.1550) for 1566 observed reflections ($F_0 \geq 4\sigma|F_0|$) and 344 variables, where the data-to-parameter ratio is 4.55:1.0 and goodness-of-fit (S) = 0.862. The largest difference peak and hole were +0.20 and −0.23 eÅ^{-3}. A low temperature diffraction data set (T = 260 K) collected on Form **2** crystals, immediately after the room temperature data collection, revealed identical structure and conformational parameters. CCDC reference numbers for Forms **1** and **2** are 803659 and 776385.

■ ASSOCIATED CONTENT

Supporting Information. X-ray crystallographic characterization of peptide Ac-Phe-Pro-Trp-OMe . The tables for crystal and diffraction parameters, main-chain and side-chain torsion angles, and hydrogen bonds for Forms **1** and **2** (room and low temperature) are provided. Proposed Grotthuss mechanism, presented as an animation, showing the transport of proton along the one-dimensional water wire observed in the structure of Ac-Phe-Pro-Trp-OMe (Form **2**). The water molecules are shown as ball-and-stick and the green colored ball represents the proton. The transport of proton is accompanied by the concerted reorientation of the water hydrogens and the hydrogen bonding along the water wire. This material is available free of charge via the Internet at http://pubs.acs.org.

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