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Nanotube Confinement Denatures Protein Helices

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The folding of proteins in confined spaces is a ubiquitous theme in biological and biomaterial applications, including folding in chaperones[1] and pores,[2] nanotube-based drug delivery,[3] and co-translational folding of nascent peptides in the ribosomal exit tunnel.[4] The role of confinement on peptide conformational equilibrium has thus gained much interest in recent years, and a natural first hypothesis to investigate is the role of confinement alone in protein conformational preferences. Indeed, the application of simplified polymer physics-based models has offered significant insight into the effects of confinement on polymer chains.[5,6] For example, simulations of simplified coarse-grained bead models suggest that β-hairpin and small protein structures are stabilized by moderate confinement, such as in spherical pores,[2] and destabilized in the limiting case of over-confinement.[2,7−9] These models do not explicitly consider water, however, thereby examining the hypothesis that the primary role of confinement is to sterically and entropically disfavor non-native protein conformations.[10]

Still, there exists a growing body of evidence to suggest that molecular water plays a role in the conformational preferences and assembly of biomolecular systems.[11−14] For example, it has been suggested that the addition of crowding agents[15] or chemical denaturants[16] destabilizes proteins by affecting the structure of water. Moreover, the character of water in confined environments is expected to differ significantly from that in bulk. As the level of confinement increases, the potential hydrogen bond (HB) network grows less extensive, becoming negligible for extremely confined regions such as narrow carbon nanotubes (CNTs),[17] and thus decreasing the entropy of the solvent. In molecular dynamics studies of narrow solvated nanotubes (~8.1 Å diameter), conduction of single-formation water networks was reported.[18] In contrast, relatively long-lived water clusters were observed in simulations of a hydrophobic polymer tube of diameter ~10 Å,[19] and hydration shells lining the inner surface of slightly larger tubes (13.6−16.3 Å) were observed in similar molecular dynamics studies, indicating that water does indeed assume organized structure in confinement larger than a critical radius on the nanometer scale.[17,20]

What role does water play in the stability of confined proteins? To address this question, we have simulated a well-characterized 23-residue helical peptide[21] inside six fully solvated single-walled CNTs with diameters ranging from ~15 to ~35 Å. For each tube modeled, 1000 independent molecular dynamics trajectories were started from the fully helical and extended states (Figures 1 and 2a). Simulations were performed on the Folding@Home distributed computing network as described previously[11] using the AMBER-99ϕ helix−coil force field[21] and the TIP3P water model[22] in the NVT ensemble at the approximate midpoint temperature of 305 K.[11] With individual trajectories on the 100−300 ns time scale and an aggregate time exceeding 2.5 ms, our extensive sampling allows us to extract equilibrium thermodynamic data.

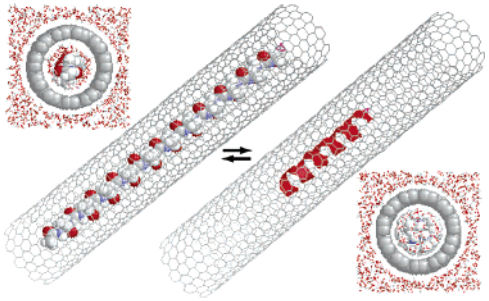

Figure 1. Starting structures for the smallest peptide−CNT system studied, with chirality (11,11). Axial views are shown to demonstrate the solvation employed in all simulations and the tight fit between the smallest tube and the ideal α-helical structure (far right). All CNTs studied include chirality (m, $n = m$), where $m = \{11, 13, 15, 19, 22, 26\}$, yielding tube diameters of 14.9, 17.6, 20.3, 25.8, 29.8, and 35.3 Å, respectively.

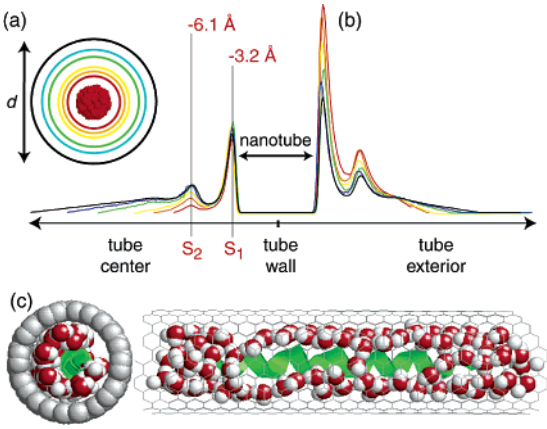

Figure 2. (a) Schematic of the tube diameters employed in this study relative to the all-atom van der Waals surface of the ideal α-helix. (b) Radial distributions of water oxygen atoms in the simulated nanotubes normalized to unity, following the coloring scheme shown in (a). S_1 and S_2 are the first and second hydration shells inside the CNTs. (c) Example of the water "sheet" that forms between the nanotube and peptide (green). The axial view demonstrates the tight packing of the water layer against the nanotube wall.

Confinement alters both the polymer physics of the helix and the nature of water, and it is important to consider both contributions. For the case of simple helices confined in nanotubes, polymer physics theory predicts that helix stability increases as tube diameter decreases.[23] In contrast, the mean helicity observed in our simulations is low for the narrowest CNT and increases monotonically with tube diameter, thus suggesting that polymer theory alone is insufficient to describe this phenomenon and that consideration of solvent properties is important in predicting the thermodynamics of proteins in confined spaces.

We therefore propose an alternate theory for the behavior of helices in confined spaces by accounting for the presence of molecular solvent. Put simply, the formation of a protein−water HB decreases the translational entropy of a water molecule, and

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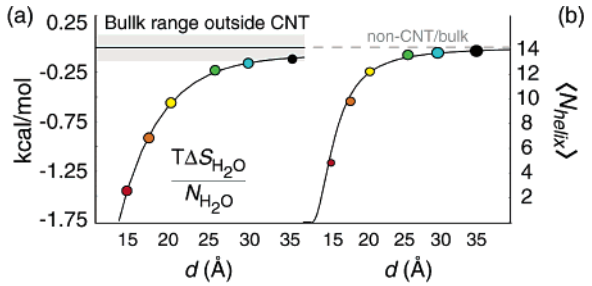

Figure 3. (a) Relative solvent entropy as a function of tube diameter, demonstrating the decrease of solvent entropy with decreasing confinement volume. (b) Mean helical content vs tube diameter from simulation (points) and theory (solid line). Standard deviations are smaller than the radius of the points plotted.

this entropy is directly related to tube diameter d. As the solvent entropy is altered, the relative stability of peptide conformational states should change accordingly.

To put this theory on more quantitative grounds, we consider the nature of confined water: the radial distributions of water inside the CNTs (Figure 2b) demonstrate a significant degree of solvent structure, indicating the presence of both first and second solvation shells ($S_1 = 3.2$ Å and $S_2 = 6.1$A). Figure 2c illustrates hydration of the internal wall for the smallest nanotube studied. Since the structured water within these solvation layers contributes little to the solvent entropy inside the tube and significantly limits the confined region available to the polymer, we define the effective diameter as $d_{\mathrm{eff}} = d - 2S_2$.

Within this effective confinement volume, molecular water is free to translate unless constrained by the formation of a protein−water HB, which causes a significant loss in solvent entropy, thereby stabilizing protein−protein HBs. The relative solvent entropy from our simulations reduces rapidly from bulk-like values as the vessel size decreases (Figure 3a). Thus, we find that the confinement-induced decrease in solvent entropy is directly responsible for the generally hydrophilic nature of confining spaces encountered by proteins. The decreased helical propensity of the peptide with decreasing vessel size, and thus decreasing solvent entropy, is analogous to the hydrophilic destabilization reported for this same peptide under "hydrophilic titration" computational experiments.[11]

We incorporate this vessel-dependent change in solvent entropy for molecular water, ΔS, by considering the formation of a protein−protein HB inside an infinite CNT of diameter d by considering the free-particle translation within the effective confinement volume, leading to $\Delta S(d) = -\mathrm{k} \ln [\pi(d_{\mathrm{eff}}/2)^2/A_{\mathrm{H_2O}}]$, where k is Boltzmann's constant and $A_{\mathrm{H_2O}}$ is the cross-sectional area of molecular water. When a protein−protein HB is formed during helix formation, this translational entropy is gained in tandem with a change in enthalpy, ϵ_{HB}, yielding the free energy difference $\Delta G(d) = \epsilon_{\mathrm{HB}} - T\Delta S(d)$. The entropic contribution to the free energy is dominant for large d_{eff}, becoming less so with decreasing size of the confining vessel, thereby stabilizing protein−water HBs relative to protein−protein HBs in smaller effective confinement volumes.

We employ a simple two-state model for protein−protein hydrogen bonds to describe peptide helicity, which yields $\langle N_{\mathrm{helix}}(d)\rangle = \langle N_{\mathrm{helix}}(\mathrm{bulk})\rangle/(1 + \exp[-\Delta G(d)/kT])$. For simplicity, we choose textbook values for the physical constants in this model: $\epsilon_{\mathrm{HB}} = -0.9$ kcal/mol is the change in enthalpy per residue upon helix formation,[24] $A_{\mathrm{H_2O}} = (1.4\ \text{Å})^2$, and $kT = 0.60573$ kcal/mol at 305 K. Taking $\langle N_{\mathrm{helix}}(\mathrm{bulk})\rangle \approx 14.2$ for the peptide in bulk water, which we note is based solely on the simulated model and methodology employed, we obtain a prediction of $\langle N_{\mathrm{helix}}(d)\rangle$ with no free parameters. As shown in Figure 3b, the agreement between this very simple theory and the relatively complex all-atom simulation results is excellent, demonstrating the dominant role of solvent entropy in determining polymer conformational preferences in confinement.

Our theory can further be used to explore the role of confinement in specific biological contexts. For example, the polypeptide exit tunnel in the ribosome is roughly 100 Å long with a mean diameter of ~15 Å.[4] While confinement alone predicts stabilization of helices in the exit tunnel,[23] the inclusion of solvation effects results in destabilization of helical structure: the wider regions of the solvated tunnel may allow for formation of helix nuclei, and the narrow diameter of the exit tunnel will decrease solvent entropy, which will act to hinder significant helix formation prior to exiting the ribosome.

On the basis of the physical arguments discussed above, we suggest that the role of solvent entropy in protein confinement is likely a general phenomenon, as hydrogen bonding and the hydrophobic effect are both driven by solvent entropy.[14] Thus, by greatly affecting solvent entropy, confinement directly alters many of the commonly held rules of protein stability.

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Supporting Information Available: Modeling details, molecular dynamics protocols, and analytic calculations of solvent entropy and helical content. This material is available free of charge via the Internet at http://pubs.acs.org.

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