Supporting Information

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SI Materials and Methods

Peptide Preparation. The R2/wt fragment used in this study is located between the first and second imperfect repeats in the microtubule binding region of tau, a major functional region that binds to and regulates microtubule growth. We have also blocked the C and N termini of R2/wt by amidating and acetylating the ends to minimize spurious results from unneutralized and uncapped termini, and also to provide a more comparable system to full-length tau (1).

Replica Exchange Molecular Dynamics. All simulations were performed with GROMACS 4.6.1 (2) on the Stampede supercomputing cluster at the University of Texas at Austin on the Texas Advanced Computing Cluster. R2/wt topologies were derived from the all-atom Optimized Potentials for Liquid Simulation force fields (3), and three-site transferrable intermolecular potential rigid water (4) was used as solvent, with the addition of chloride ions to neutralize the positive charge of the peptide(s). Systems were first simulated under an NPT (constant number of atoms, pressure, and temperature) ensemble at 300 K and 1 bar of isotropic pressure, using the Berendsen weak-coupling thermostat and barostat (5) to obtain equilibrium box dimensions at a compressibility of 4.5 × 10⁻⁵ bar⁻¹. We then switched to a canonical NVT (constant number of atoms, volume, and temperature) ensemble using a Nosé–Hoover thermostat (6), which uses a modified Hamiltonian that is coupled to an external heat bath, to obtain proper thermodynamic ensembles. Peptide bonds were constrained using the LINCS (linear constraint solver) algorithm (7), and water bonds were constrained with the SETTLE algorithm (8), both of which use Lagrange Multipliers to constrain chemical bonds in the presence of a symplectic integrator.

To enhance our sampling frequency, we used replica-exchange molecular dynamics (9) over the range of 290–500 K for monomers (62 replicas) and 290–350 K for dimers (24 replicas), with an average exchange probability of 25% between replicas, which was attempted every 3 ps. Monomers were simulated for 200 ns, where the last 100 ns was used for analysis, whereas dimers were simulated for 245 ns, where the last 145 ns was used for analysis.

For urea and TMAO, we used the models developed by Weerasinghe and Smith (10) and Larini and Shea (11), in concentrations of 5 M for urea and 2 M for TMAO. These concentrations are typical in similar studies (12).

Molecular Dynamics Analysis. The GROMACS tools “g_hbond,” “g_traj,” “g_gyration,” and “g_cluster” were used to measure intramolecular peptide bonds, peptide end-to-end distance (Rₑₑ), peptide radius of gyration (Rₑₐₑ), and peptide clustering analyses, respectively, at room temperature. Hydrogen bonds were identified by O–H distances of 2.5 Å or smaller, and an OHN angle of 30 degrees or less. Rₑₑ was measured from the acetylated cap’s center of mass to the amidated cap’s center of mass. Peptide conformations were clustered together using the Daura algorithm (13), which compares protein backbone orientations (excluding termini) and groups them together based on root mean square deviations, which differ by no more than 1.4 Å for monomers and 2.6 Å for dimers. Peptide dimers were created by randomly orienting permuted monomers together and checking that they remained dimerized for at least 20 ns.

Thioflavin Assays. Thioflavin T (ThT) was purchased from Anaspec, and a stock solution was prepared in ethanol (Arcos Organics). R2/wt peptide aggregation mixtures were prepared as above, except that ThT was added to a final concentration of 2 μM. The ThT signal was monitored over time (450 excitation/525 emission) using a Wallac 1420 plate reader spectrofluorometer (PerkinElmer). Experiments were repeated at least three times.

TEM. For TEM, samples of aggregation mixtures were fixed in glutaraldehyde (8% stock from Ted Pella; final concentration in fixed samples was 1.6%). Fixed samples were placed on 300 mesh formvar/carbon-coated copper grids (Electron Microscopy Sciences), rinsed with deionized water, and then negatively stained with 2% uranyl acetate (Ted Pella). Grids were imaged on a JEOL 1230 TEM connected to an ORCA camera, using AMT Image Capture Software (Version 5.24).

Fig. S1. Clustering of the most dominant conformations found for monomer and dimer systems in various environments. Percentages indicate the relative time spent in each conformation during the production run of each system. Polar residues are highlighted for clarity (green, lysine; red, aspartic acid). Numbers at the top of the figure correspond to the dominance of each cluster below it.
Fig. S2. A representative snapshot of the R2/wt tau monomer in the presence of urea (Left) and TMAO (Right).
Fig. S3. Distribution of osmolytes at various distances around the backbone of R2/wt monomers for the most dominant peptide conformations. Urea tends to cluster around positively charged side chains (see, in particular, the Middle panel), whereas TMAO does not show this trend.

Fig. S4. Radial distribution function, g(r), of water, urea, and TMAO relative to the surface of R2/wt. On average, urea lies about 3 Å from the peptide surface, whereas TMAO lies closer to 4 Å. Note that there are more than twice as many urea bound to the peptide within 5 Å as there are bound TMAO (consistent with Table 1). Urea also dehydrates R2/wt to a much greater extent than TMAO.
Radial distribution function, g(r), of urea and TMAO in bulk water relative to their centers of mass. Although both osmolytes are net neutral, urea has a larger dipole moment compared with TMAO, and tends to be more highly associated with other urea (via carbonyl oxygen to NH₂ electrostatic interactions) rather than with water. Conversely, TMAO is more likely to interact with adjacent water molecules rather than with neighboring osmolytes. Although urea attracts water more tightly than TMAO, we see a broader hydration shell around TMAO. Upon binding to R2/wt, urea dehydrates much of the water present at the surface due to the dominance of urea–urea and urea–peptide interactions, whereas TMAO rearranges water at the peptide surface after it moderately dehydrates some of the amino acids.

Relative orientation of peptide backbones during tau dimerization in (A) water and (B) water + TMAO. Although dimers in water have strong antiparallel orientation [cos(θ) = –1], systems containing TMAO appear to have no preferred orientations as dimers exist over multiple relative orientations.

Distribution of water and osmolytes at various distances around the backbone of R2/wt dimers for the most dominant peptide conformations.