pH Switchable Strand Orientation in Peptide Assemblies

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Supporting Information:

CD Spectroscopy. All experiments were performed on an Aviv model 202 circular dichroism spectrometer, equipped with a Microlab 500 series automated titration assembly. Sample concentrations were measured by UV absorbance of an acetamidobenzoate label at 270 nm (attached to a solvent exposed lysine). Wavelength data are the average of three scans from 250 to 200 nm in 1 nm steps. Thermal denaturation experiments at 222 nm were run from 0° to 90°C in two-degree steps, at a two-degrees/minute rate of increase with one-minute equilibration and data averaging at each temperature. T_m values were obtained from minima of the first derivative of θ vs. 1/T plots.[1] Guanidinium titrations were performed using the automated titration assembly. The signal at 222 nm was recorded for solutions of constant peptide concentration with guanidine hydrochloride concentrations varied from 0 to 3M. Data were collected for one minute at each step, with ten-minute equilibration times (solutions were stirred during equilibration but not data collection).

Analytical Ultracentrifugation. Sedimentation equilibrium experiments were performed using a Beckman XL-I analytical ultracentrifuge equipped with an An60-Ti rotor. Data were collected using 12 mm path length six-sector centerpieces at 270 nm. Samples were dialyzed against the reference buffer at 4ºC overnight. Data were collected at 38,000 and 48,000 r.p.m. at concentrations spanning 17-55 μM. Samples were equilibrated at 38,000 r.p.m. for 16 hours, followed by collection of two scans four hours apart. The speed was then increased to 48,000 r.p.m., followed by a 16 hour equilibration, and collection of two scans four hours apart, as before. Each scan consisted of 10 replicates at 0.002 cm radial steps. Solvent densities and partial molar volumes were calculated in the manner prescribed by Laue.[2] Data were analyzed using Origin and fit to ideal single-species models.

Ni-NTA Affinity Tag Experiments. A 0.5 mL sample of a 50% slurry of Ni-NTA agarose (Qiagen) in an Eppendorf tube was centrifuged for 30 s, followed by removal of the superantant. Peptide solution was added, and the tube was repeatedly inverted for 5 minutes. The sample was centrifuged (30 s) and the supernatant (flow-through fraction) was removed. The procedure was then repeated with 1 mL of buffer (wash fraction) and 1 mL of buffer containing 250 mM imidazole (elution fraction), except that the wash fraction was only agitated for 30 seconds. Solutions were analyzed by RP-HPLC.[3]

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(1) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry of Macromolecules, Pt. 3: The Behavior of Biological Macromolecules*; W. H. Freeman: New York, NY, 1980, p.1132.

(2) Laue, T. M.; Shah, B. D.; Ridgeway, T. M.; Pelletier, S. L. in *Analytical Ultracentrifugation in Biochemistry and Polymer Science*; Harding, S. E., Rowe, A. J., Horton, J. C., Eds.; The Royal Society of Chemistry: Cambridge, 1992; pp 90-125.

(3) Method patterned after that in: Brown, M. B.; Sauer, R. T. *Proc. Nat. Acad. Sci. U.S.A.*, **1999**, *96*, 1983-1988.

Disulfide Exhange Assay. Three derivatives were prepared by attaching a Gly-Gly-Cys sequence to the N-terminus of T_9K ($T_9K_{N\text{-}Cys}$), and separately to the N- and C-termini of $T_{16}E^a$ ($T_{16}E^a_{N\text{-}Cys}$, $T_{16}E^a_{C\text{-}Cys}$).

Disulfide-linked heterodimers between $T_9K_{N\text{-}Cys}$ and each of the other two derivatives were prepared by air oxidation. Undetermined amounts of the appropriate peptides were mixed in PBS buffer (10mM sodium phosphate, 150mM NaCl, pH = 9.4) in a 1.5 mL Eppendorf tube. Small holes were punched in the top of the tube to allow free air exchange. After 12 to 15 hours of exposure, the heterodimers were purified by reverse-phase HPLC: (C-18 column, solvent A: 1% CH_3CN in H_2O, 0.1% (v/v) CF_3CO_2H; solvent B: 10% H_2O in CH_3CN, 0.07% (v/v) CF_3CO_2H).

For each exchange experiment, a 2.0 mL solution containing $T_{23}K$ and an appropriate heterodimer (10 μM total peptide concentration, PBS as above, pH = 9.4) was sparged with argon for fifteen minutes in a 15 mL Falcon tube equipped with a small rubber septum and outlet needle. A specified amount of thiol containing peptide stock solution was quickly added to the original solution (13.3 μM final peptide concentration). A 0.5 ml aliquot was immediately taken for HPLC analysis (conditions as above). The tube was resealed and sparged with argon for 15 minutes. The argon needle was then brought well above the solvent level and a slow, steady stream was blown through the tube for approximately 20 minutes. This process was repeated after 8 hours.

Analytical Ultracentrifugation Details

Table S-1. Relative Molecular Masses from Analytical Ultracentrifugation[a]			
Sample		MW_{obs} 38 krpm	MW_{obs} 48 krpm
T_9K:$T_{16}E^a$:$T_{23}K^b$			
	17 μM	10 654	--
	33 μM	11 696	10 333
	55 μM	12 176	11 554
	average	11 282	
	calc. (trimer)	11 559	
T_9K:$T_{16}E^a$:$T_{23}E^c$			
	33 μM	11 633	11 158
	55 μM	12 064	12 200
	average	11 763	
	calc. (trimer)	11 568	

[a] All samples were in aqueous buffer (10 mM phosphate buffer, 150 mM NaCl, pH=7.0). [b] pH = 9.1. [c] pH = 5.1.

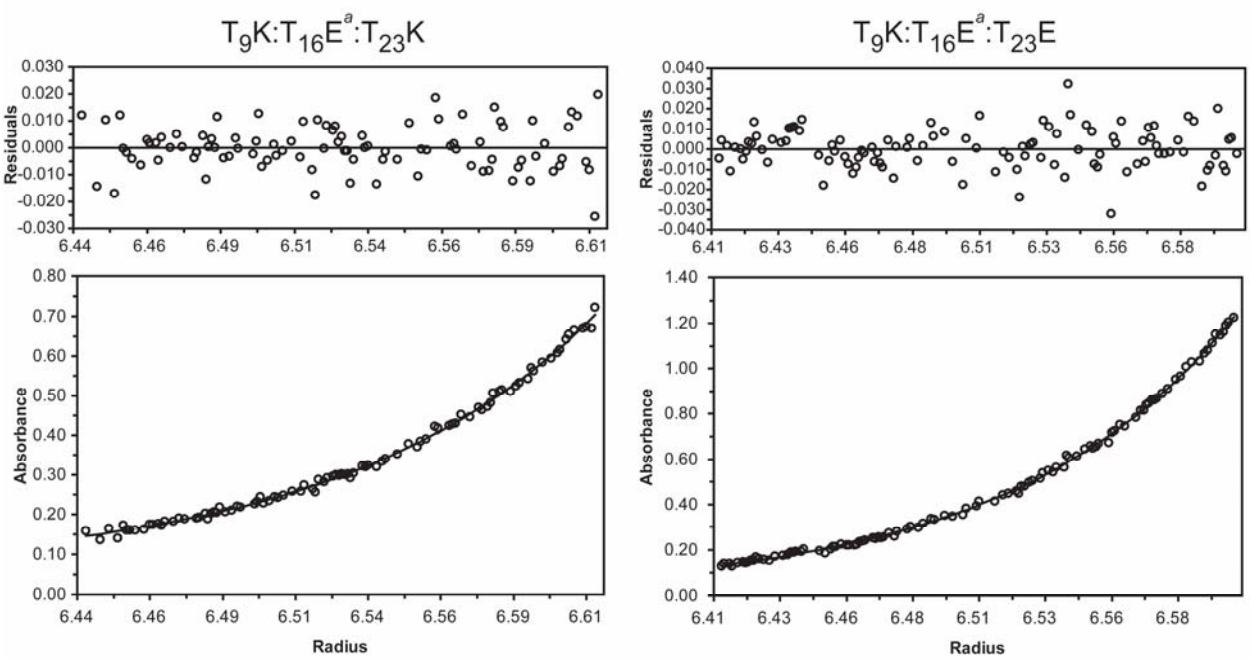

Figure S-1. Representative fits from ultracentrifugation. Data from 33 μM 38K runs.