Supporting Information

Rational Design of a Reversible pH Responsive Switch for Peptide Self-Assembly

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Materials and methods

Peptide Synthesis and Purification. Peptide **TZ1H** was synthesized on a Symphony Quartet SPPS peptide synthesizer (Peptide Technologies, Inc., Tucson, AZ) via Fmoc chemistry. RP-HPLC purification was performed using the Waters 600 purification system (Waters Corp., Milford, MA) with a Waters 996 photodiode array and a reverse phase C-18 column (Atlantis dC18, 10 μm, 19x256mm). The molar mass of **TZ1H** was confirmed by MALDI-TOF mass spectrometry on a Voyager-DE STR instrument (Perseptive Biosystems, Framingham, MA). Peptide concentration was obtained in 6M guanidinium chloride solution utilizing the UV absorbance at 280 nm of the single tyrosine residue in **TZH1** where c = Molecular Weight x A_{280} / 1280.

Circular Dichroism. CD spectra obtained from JASCO J-810 CD spectropolarimeter (Jasco Inc., Easton, MD) equipped with a PFD-425S Peltier temperature control unit, using a 1 mm quartz cell. Peptide solutions were prepared in 10 mM sodium phosphate buffer. The pH was adjusted with 3N HCl or 5N NaOH solutions. Total sample volume in CD measurements was 300 μL. For pH-dependent CD studies, spectra were acquired at 4 ^{o}C or 25 ^{o}C using 1 nm intervals, 1 nm bandwidth, 20 nm min^{-1}, and 8 second response time. Spectra were baseline corrected before converting to mean residue ellipticities. Fractional helix content was calculated from MRE using equation $F_{helix} = [\theta]_{obs} - [\theta]_{coil} / [\theta]_{helix} - [\theta]_{coil}$' where $[\theta]_{helix}$ and $[\theta]_{coil}$ in MRE are described as (-42,500*(1-3/(# residues))) and +645, respectively. CD melting curves were generated by monitoring the absorbance at 222 nm of a stirred solution of **TZ1H** (15 μM, 10 mM sodium phosphate buffer pH 8.3) in a 1 cm quartz cuvette. Spectroscopic data were acquired within the temperature range from 20 °C to 100 °C with equilibration for 5 min at each temperature. The value of the T_m was determined from the first derivative of the CD melting curve. For determination of the pH-dependent reversibility of the helix-coil transition, solutions of peptide **TZ1H** were prepared at the indicated concentrations in 10 mM sodium phosphate buffer and the pHwas adjusted with 3N HCl or 5N NaOH to the appropriate value. CD spectra were recorded at 4 °C and 25 °C as described above.

TEM. Peptide **TZ1H** was dissolved to a concentration 1 mg/ml in 10 mM NaH_2PO_4 buffer at the appropriate pH. Annealed specimens were prepared from aqueous solutions of the peptide via thermal denaturation at 100 °C in either a water bath or an automated thermal cycler, followed by slow cooling of the specimen to the annealing temperature, usually 4 °C or 25 °C, over a period of at least 3h to afford the thermodynamic product. Annealed and unannealed peptide solutions (10 μL) were placed on a formvar/carbon-coated grid and let stand for 3 minutes before excess solution was wicked away with a triangle of filter paper. Samples were negatively stained with pH-appropriate stains. For pH 8.2, a 1:1 mixture of near-neutral stains, Nano methylamine vanadate (pH 8.0) and Nano methylamine tungstate (pH 6.8), were used (NanoProbes, Yaphank, NY). Stain (3 μL) was applied to the grid and incubated for 1 minute before being wicked away with a triangle of filter paper. This procedure was repeated before storing under vacuum overnight. For pH 4.0 samples, 1.5% uranyl acetate (pH 4.0) applied to the peptide sample on the grid for 2 minutes. Both annealed and unannealed specimens of **TZ1H** afforded fibrils at pH 8.2, while neither formed fibrils at pH 4.0. Slight differences were detected between the annealed and unannealed specimens in that the former usually afforded better developed fibrils and fiber bundles in terms of length and extent of lateral association. Image measurements performed using the software ImageJ, version 1.36b, freely available from U. S. National Institutes of Health, Bethesda, Maryland, USA (http://rsb.info.nih.gov/ij/). Briefly, the spatial scale was set to a known distance in the image and the distance in pixels. The segmented line tool was used to trace the diameter of fibrils or fiber segments that displaced well defined edges. Fifteen independent diameter measurements were made from fibers spanning every quadrant of the image. Errors in the distance measurements are reported as standard deviations.

Microrheology. Concentrated suspensions of 1.0 μm diameter fluorescent probes (Molecular Probes, Eugene, OR) were mixed with the buffered solutions of **TZ1H** (100-200 μM, 10 mM sodium phosphate buffer at pH 4.0 or 8.0) to achieve the correct tracer density. The samples were then monitored with epifluorescence on an inverted Leica DM-IRB microscope with a 63x long working distance objective (NA = 0.70). Digital movies were recorded directly onto the hard disk of a PC with a Peltier-cooled video camera (Cohu 4920; 640x480 pixels, 30 frames per second) and specialized image acquisition software (OpenBox). The particle positions in all images of the movie were determined using IDL image analysis software (Research Systems Inc., version 6.0) and pieced together to identify particle trajectories. From the particle trajectories, the mean squared displacement (MSD) was calculated as a function of lag time by averaging over all tracer particles within the viewing window and the generalized Stokes-Einstein relation was used to calculate the dynamic moduli G' and G" as a function of angular frequency.

Figure S1. MALDI-TOF mass spectrum of the purified peptide fraction from RP-HPLC of the peptide synthesis product. Expected mass of **TZ1H** is 4905.6 Da and the observed peak was 4904.15 +/- 1 Da.

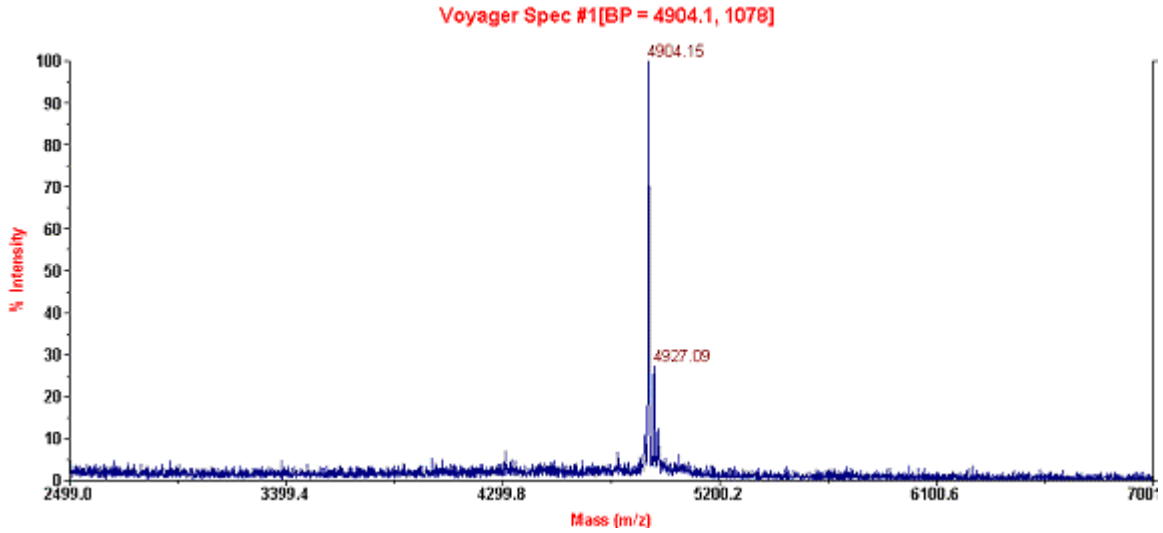

Figure S2. Concentration dependence of the circular dichroism spectra of **TZ1H** in 10 mM NaH_2PO_4 buffer, pH 8.3 at 25 °C. Lower arrows indicate the positions of the characteristic α-helical minima at 208 nm and 222 nm. The upper arrow indicates the effect of increased self-assembly due to higher concentration on the absorption at 208 nm, which results in attenuation of the signal and a shift to longer wavelengths.

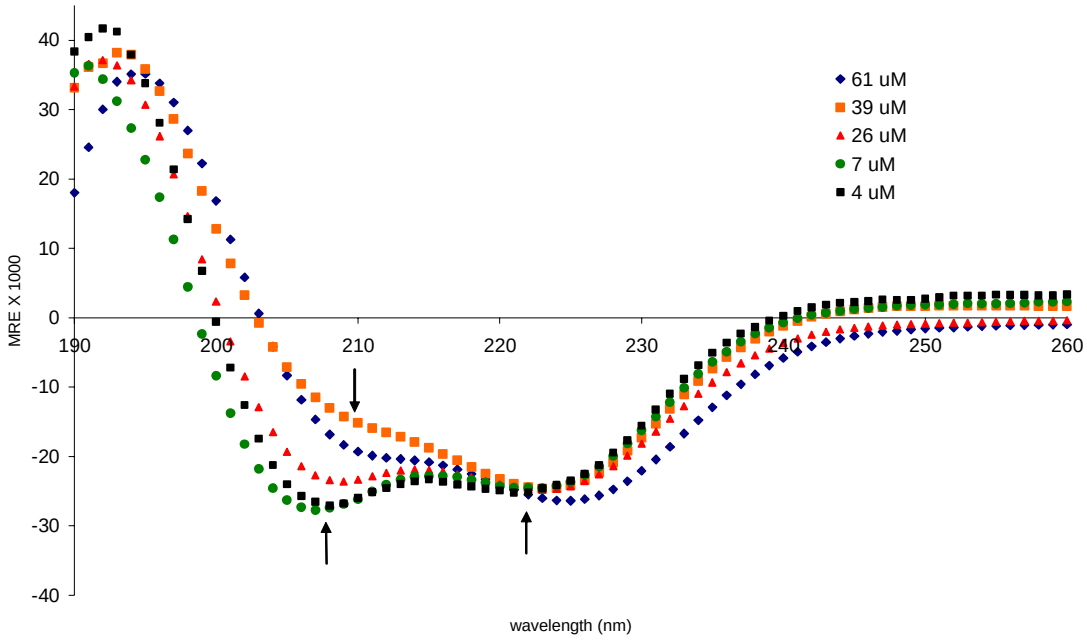

Figure S3. Circular dichroism spectroscopic determination of the reversibility of the pH-dependent conformational transition of peptide **TZ1H**. A. CD spectra for the coil-helix-coil transition of **TZ1H** (35 μM, 10 mM sodium phosphate buffer) at 25 °C. B. CD spectra for the helix-coil-helix transition of **TZ1H** (30 μM, 10 mM sodium phosphate buffer) at 25 °C.

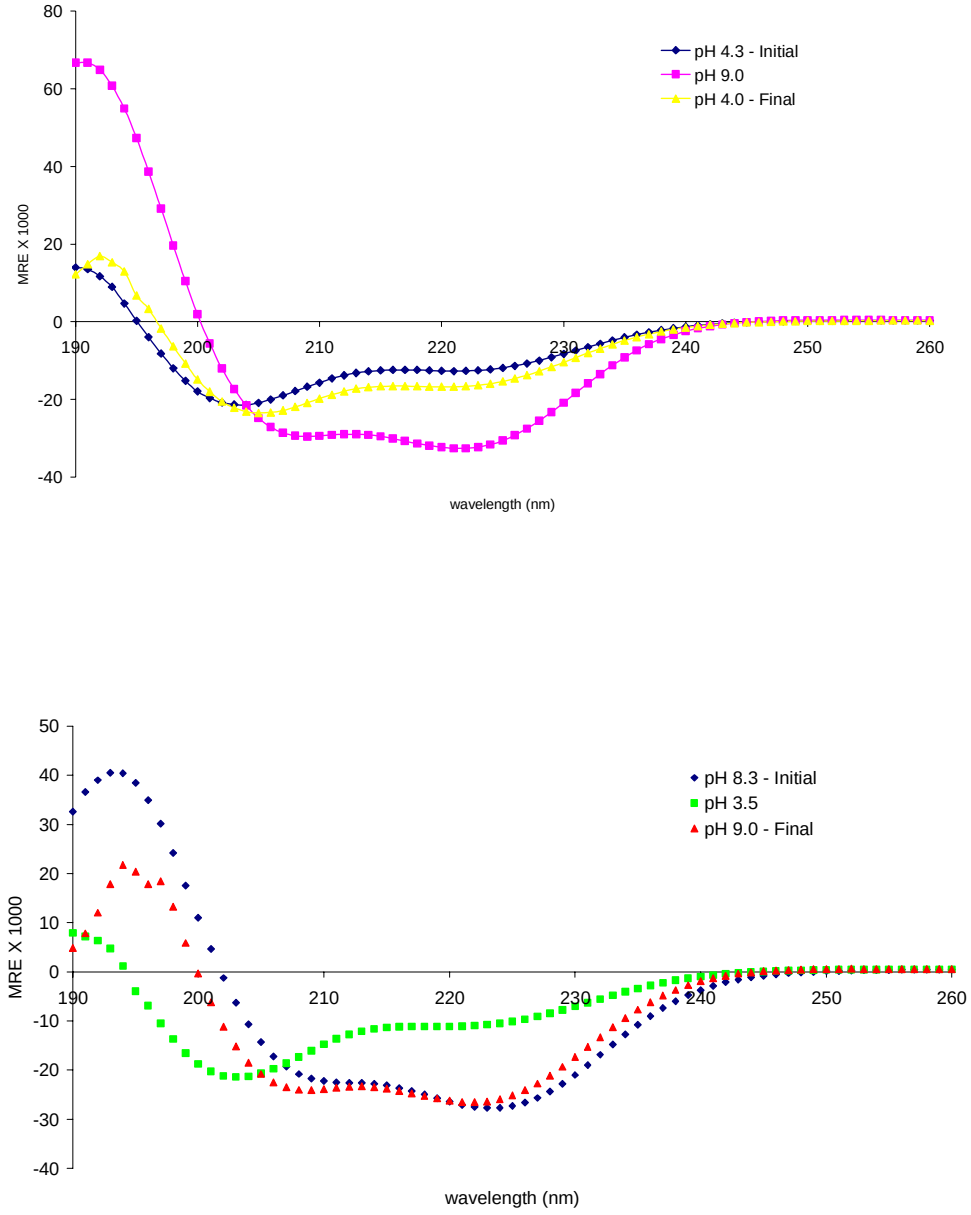

Figure S4. CD melting curve for **TZ1H** (15 μM, 10 mM sodium phosphate buffer pH 8.3). The T_m for the helical assembly was determined to be approximately 63 °C under the experimental conditions.

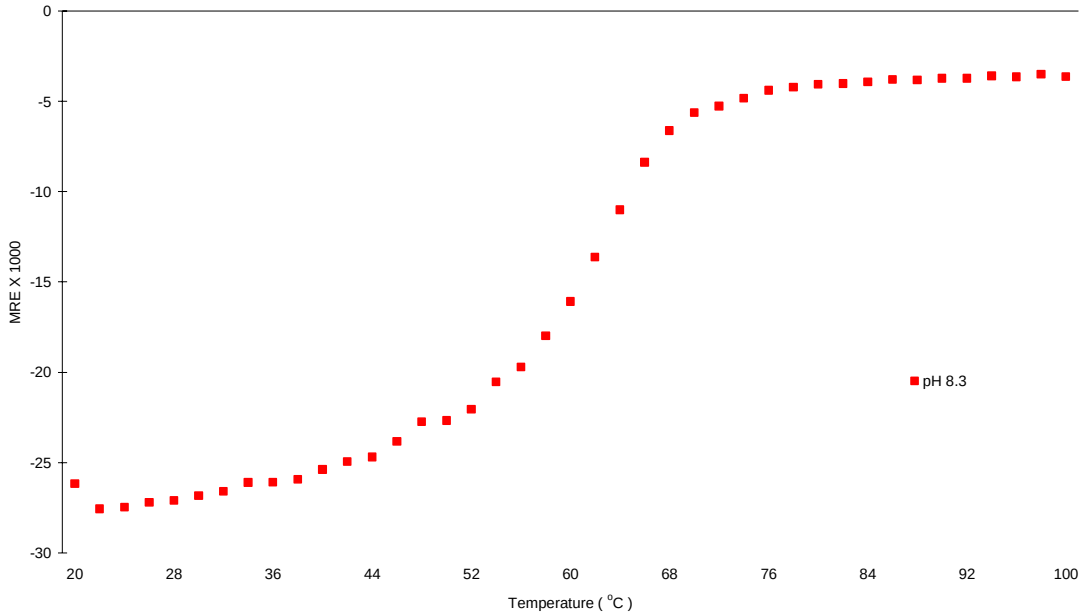

Figure S5. Storage and loss moduli, G' and G", for **TZ1H** solutions (200 μM, 10 mM sodium phosphate buffer) at pH 4.0 and 8.0. At pH 8.0, the solutions of the **TZ1H** peptide display an elastic storage modulus, *G´(ω)*, higher than the viscous loss modulus, *G˝(ω)*, for all frequencies probed. In contrast, the viscous modulus of the **TZ1H** solution at pH 4.0 closely parallels that of water, while the elastic modulus is below the detection limit for microrheology.