Supplementary Information

Unbiased in-silico design of pH-sensitive tetrapeptides

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MD Simulations

MD simulations were performed on local workstations equipped with 12 CPUs and 1 NVIDIA Quadro RTX5000 GPU. For our models (≈7000 particles) the system provided a performance of ≈ 30 μs/day. The overall simulated time is 72,000×50 ns (3,600 μs), thus requiring ≈120 days of computation. All the systems are minimized for 5,000 steps using the steepest descent method and equilibrated for 50 ns under constant pressure and temperature (NPT). In the NPT simulations, the temperature is kept constant at 300 K using a velocity rescaling thermostat with a time constant of 1.5 ps.[1] The pressure coupling is maintained at 1 bar, employing the Berendsen barostat with a time constant of 3 ps and compressibility of 3×10^{-4} bar^{-1}.[2] A neighbor list is maintained within a radius of 1.2 nm using the Verlet scheme and is updated every 10 time-steps. Electrostatic interactions are calculated using the reaction field scheme with a cut-off of 1.2 nm and a relative dielectric constant of 15, while van der Waals interactions are treated with the potential-shift scheme with a cut-off at 1.2 nm. Each model is simulated for 5×10^5 steps with a time step of 25 fs, resulting in 12.5 ns of MD. Due to the scale factor of 4 in CG simulations, the effective simulation time results are 50 ns.[3,4]

Chemicals

Tetrapeptides DHHR, FHYH, and HHFF with N-terminal acetylation and C-terminal amidation (>99%) were purchased from ABclonal. Potassium phosphate monobasic (KH_2PO_4, 99%) and potassium phosphate dibasic (K_2HPO_4, ≥98%) were purchased from Sigma-Aldrich. Deionized (DI) water with a resistivity of 18.2 MΩ·cm was used in all experiments.

Preparation of 50mM 10× phosphate buffer

Potassium phosphate monobasic and potassium phosphate dibasic were dissolved in 20 mL DI water to prepare 50 mM 10× phosphate buffer with a pH of 5, 7, and 9. Specifically, 23.4 mg K_2HPO_4 and 1342.6 mg KH_2PO_4 for pH=5; 1005.2 mg K_2HPO_4 and 575.5 mg for pH=7; 1729.1 mg K_2HPO_4 and 9.9 mg K_2HPO_4 for pH=9. Using potassium hydroxide and hydrochloric acid to adjust the pH under the monitoring of a pH meter.

Tetrapeptides at different concentrations were fully dissolved in 0.9 mL DI water, sonicated with VWR Symphony 97043-936 Ultrasonic Cleaner for 10 minutes, and followed by adding 0.1 mL 10× phosphate buffer. Peptide solutions were standing at room temperature overnight. Self-assembly was determined by observing precipitations or gelation in vials. Concentration screening suggests that at pH=7 and 9, tetrapeptides FHYH and HHFF start self-assembly at the concentration of 2.5-5.0 mg/mL.

Scanning Electron Microscope (SEM)

Zeiss MERLIN HR-SEM was carried out to characterize morphologies of tetrapeptide gels and solutions. Peptide solutions and gels under different pH were left at room temperature for at least 24 hours, then lyophilized with a freeze dryer (FreeZone 4.5 Liter Benchtop Freeze Dry System, Labconc) and observed under SEM at an accelerated voltage of 1kV.

Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Tetrapeptide samples in water and buffer solutions were frozen at -80°C for an hour and then frozen and dried with a Labconco FreeZone overnight. FTIR analysis of powders was performed with PerkinElmer Spectrum 65 FT-IR Spectrometer, equipped with Universal ATR accessory Crystals Diamond/ZnSe (L1250241). For each sample, 32 accumulated scans were conducted with a resolution of 4 cm^{-1}, and the data range was 3500-1000 cm^{-1}. Amide region I 1705-1595 cm^{-1} was used for data analysis. To eliminate the influence of potassium phosphate salts, potassium phosphate buffer solutions were dried on mica substrates, and their Amide I region was recorded (Figure S2) and compared to that of tetrapeptides. Notably, the buffer salts did not exhibit strong absorbance at 1630 cm^{-1}, indicating that they did not contribute to the FTIR absorbance associated with the tetrapeptide beta-sheet. Moreover, the buffer salt containing a higher concentration of potassium phosphate dibasic (K_2HPO^4) displayed stronger absorption at 1650 cm^{-1}, which can be attributed to the H-O-H scissor bend resulting from water remaining during the drying process.[5]

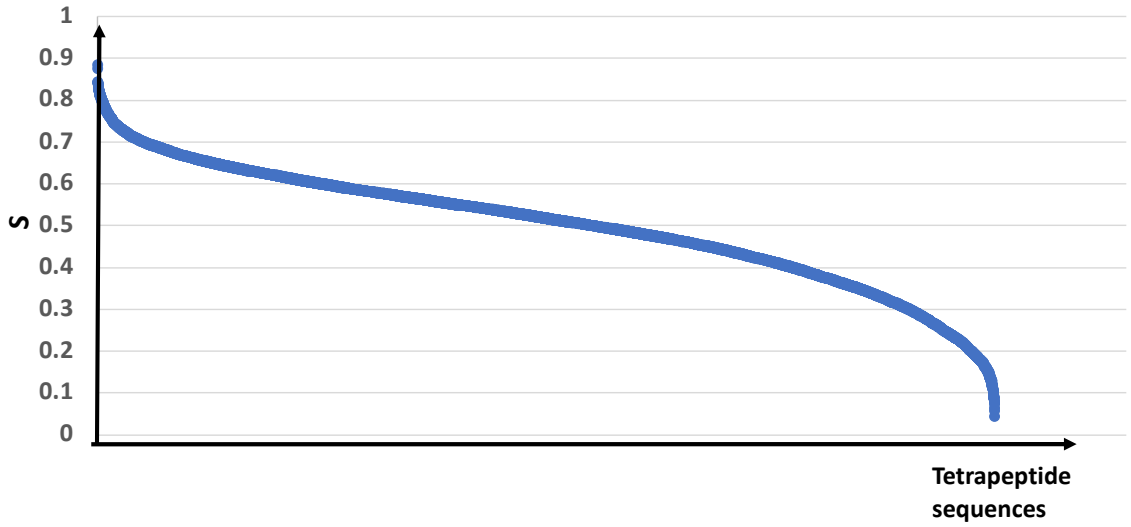

Figure S1. S protonated score

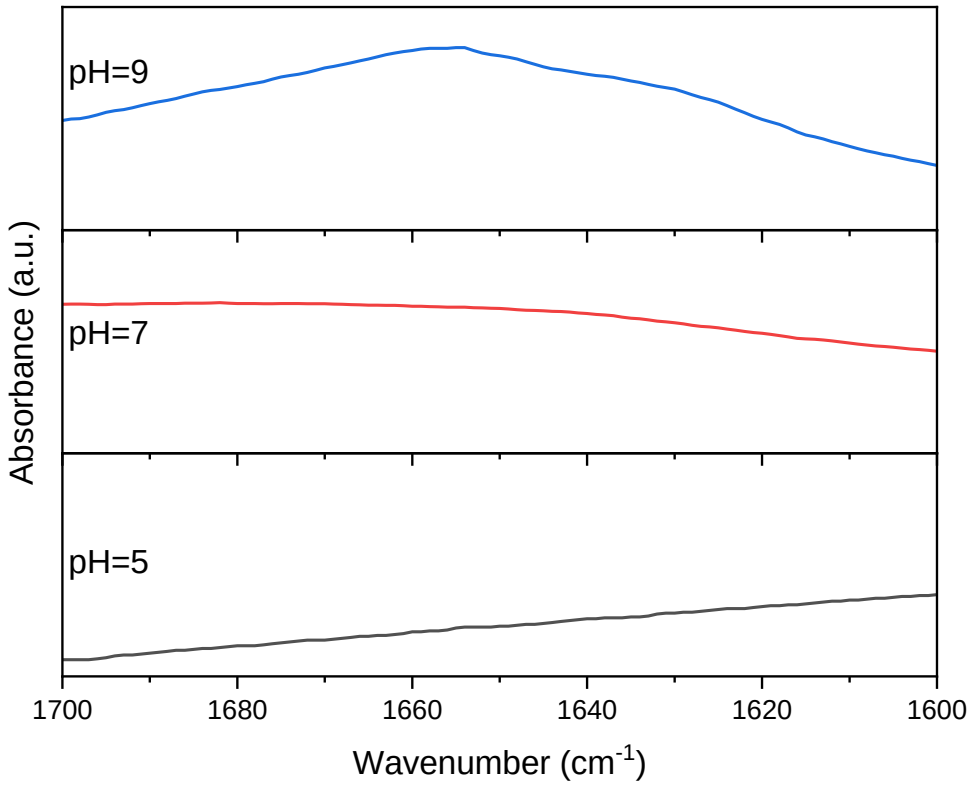

Figure S2. FTIR spectra of dried potassium phosphate buffer salt with different pH.

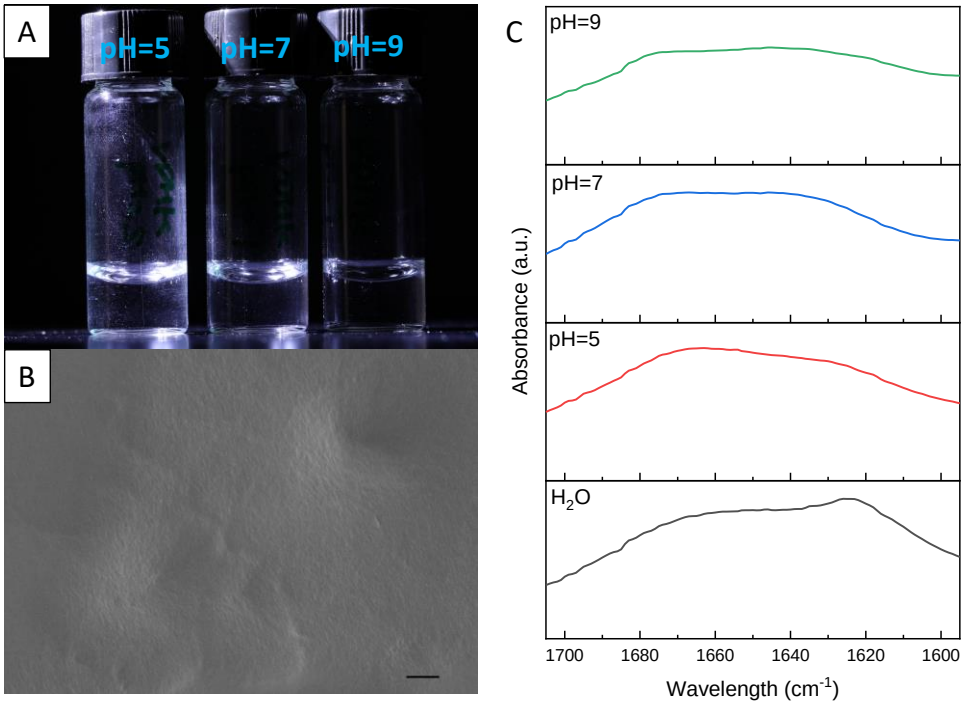

Figure S3. (A) Photo image of tetrapeptides VDHK in 50 mM phosphate buffer solutions. (B) Scanning Electron Microscope (SEM) images of VDHK in water, scale bar= 2 µm. (C) VDHK in water (black) and 50mM potassium phosphate buffer (pH=5, red; pH=7, blue; pH=9, green).

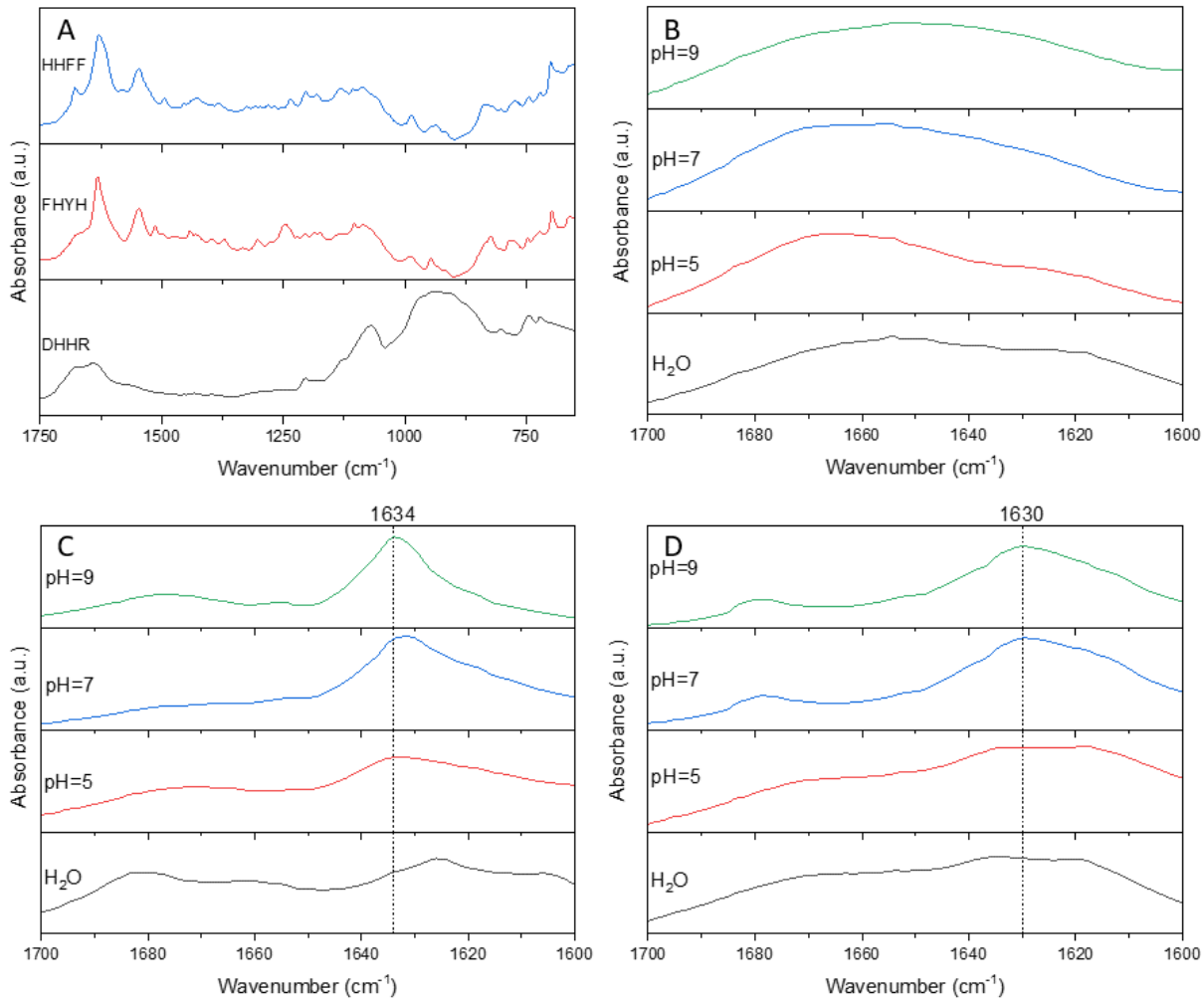

Figure S4 -FTIR spectra (A) of peptides DHHR, FHYH, and HHFF at the range of 1750-650 cm^{-1} treated with phosphate buffer at pH=7. The FTIR spectra show amide I region (1705-1595 cm^{-1}) of freeze dried tetrapeptides (B) DHHR, (C) FHYH, (D) HHFF in water solution (black) and 50mM potassium phosphate buffer (pH=5, red; pH=7, blue; pH=9, green).

References

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