

Supplementary Information

Transition from disordered aggregates to ordered lattices: Kinetic control of the assembly of a computationally designed peptide

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Sequence and calculated net charge of sequence at different pH values

The designed sequence is DQEIR QMAEW IKKMA QMIDK MAHRI DREA-NH₂. In this study, we use the N-terminus acetylated variant, the bold letters indicate the interior residues.

Table 1 Designed sequence information

Sequence code	∑AAs	pI	M _w [Da]
Ac-DQEIR QMAEWIKKMA QMIDK K MAHRIDREA -NH ₂	29	8.12	3614

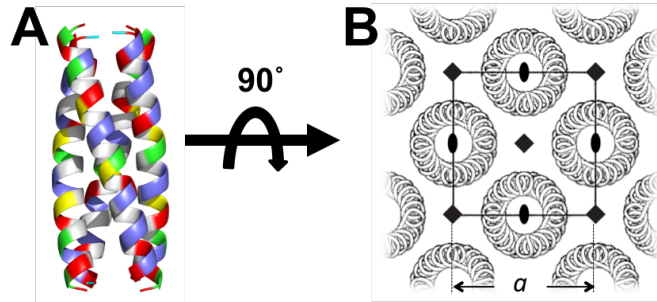


Figure S1. A: The peptide homotetrameric coiled-coil bundle building block with exterior residues coloured according to amino acid properties: blue indicates positively charged residues, red indicates negatively charged residues, green indicates polar residues, and yellow indicates hydrophobic residues. Interior residues are coloured grey. B: Rendering of one layer from a lattice with P422 symmetry, viewed from the top of the 2-D assembly.

The peptide is designed to assemble into a lattice with the homotetrameric bundle as building block, as shown in **Figure S1**. The pI of this sequence was calculated from a web-calculator <http://www.bachem.com/service-support/peptide-calculator/>. The pI estimation assumes the independence of the residues from each other. The approximate net charge of peptide at different pH values was obtained from the estimated net charge Z vs. pH titration curve, given as following:

$$Z = \sum_i N_i \frac{10^{pKa_i}}{10^{pH} + 10^{pKa_i}} - \sum_j N_j \frac{10^{pH}}{10^{pH} + 10^{pKa_j}}$$

In the algorithm, Z is the net charge and N is the number of amino acids. The first sum (i -index) is over positively charged groups: the N-terminus and the side-chains of arginine (pKa 12.48), lysine (pKa 10.53), and histidine (pKa 6.00). The second sum (j -index) is over negatively charged groups: the C-terminus and the side-chains of aspartic acid (pKa 3.65), glutamic acid (pKa 4.25). Amino acid residue pKa values are taken from *Lehninger Principles of Biochemistry* (D. L. Nelson, A. L. Lehninger and M. M. Cox, *Lehninger Principles of Biochemistry*, W. H. Freeman, New York, NY, 4th edition., 2005).

Table 2 Estimated net charge of each peptide bundle at different pH values

pH values	Calculated net charge of each peptide bundle (approximate values)
4.5	+12
7	+1
8	0
10	-2

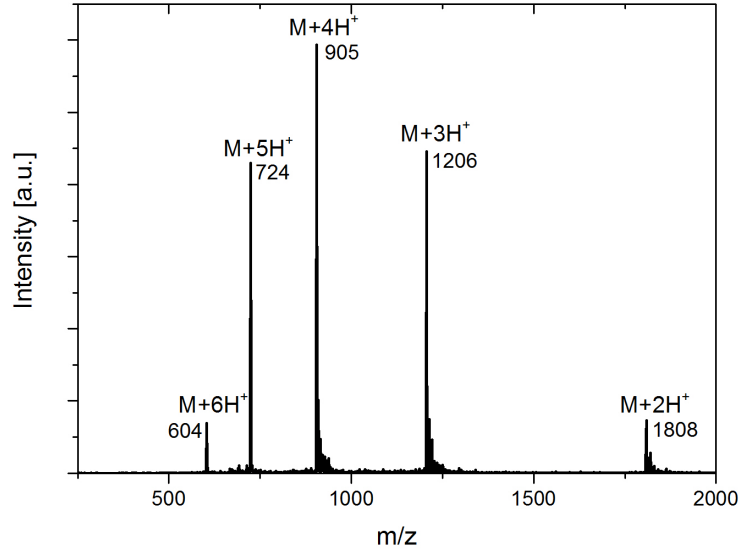


Figure S2. ESI-mass spectrometry for designed sequence. Designed molecular weight: [M] 3614 Da. The accordingly calculated mass is: [M+2H⁺] 3616 Da, [M+3H⁺] 3617 Da, [M+4H⁺] 3618 Da, [M+5H⁺] 3619 Da, [M+6H⁺] 3620 Da. The experimental measured mass is: [M+2H⁺] 3616 Da, [M+3H⁺] 3618 Da, [M+4H⁺] 3620 Da, [M+5H⁺] 3620 Da, [M+6H⁺] 3624 Da.

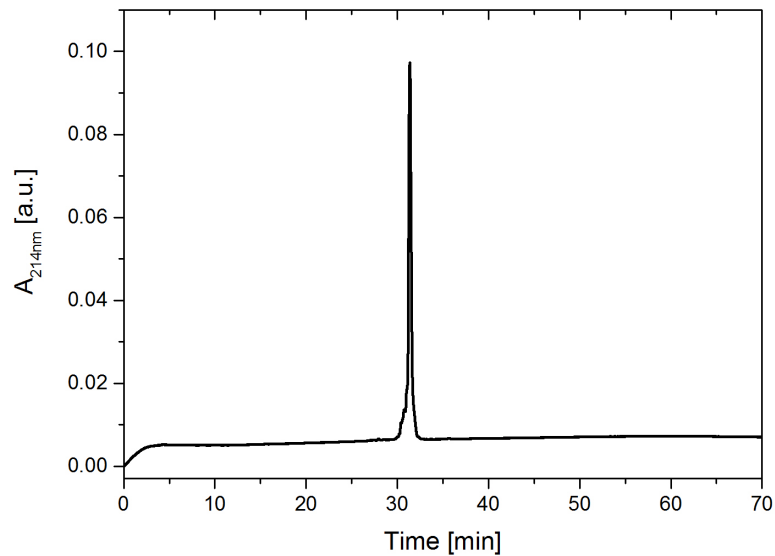


Figure S3. Analytical HPLC trace for designed sequence. The single peak indicates the high purity of the peptide products.

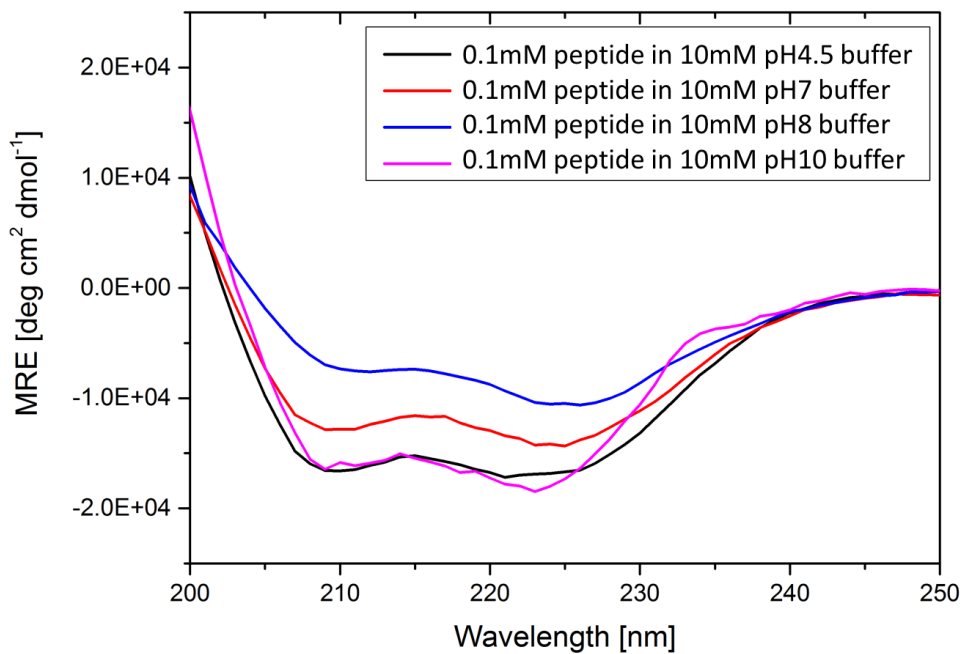


Figure S4. Circular dichroism spectroscopy data for designed sequence measured at 20°C in four different buffer conditions as shown in the figure legend. The ratios of $[MRE]_{222\text{nm}}/[MRE]_{208\text{nm}}$ are 1.06, 1.12, 1.62 and 1.16 for pH 4.5, 7, 8 and 10, respectively. The ratios at pH4.5 and pH10 are close to 1 indicating the stabilized coiled-coil formation. While for pH7 and pH8, due to the fast assembly process, peptides already started to precipitate, causing the loss of CD signal for this measurement. Formation of lattices is consistent with stable coil-coils at the pH 7 and pH 8 conditions.

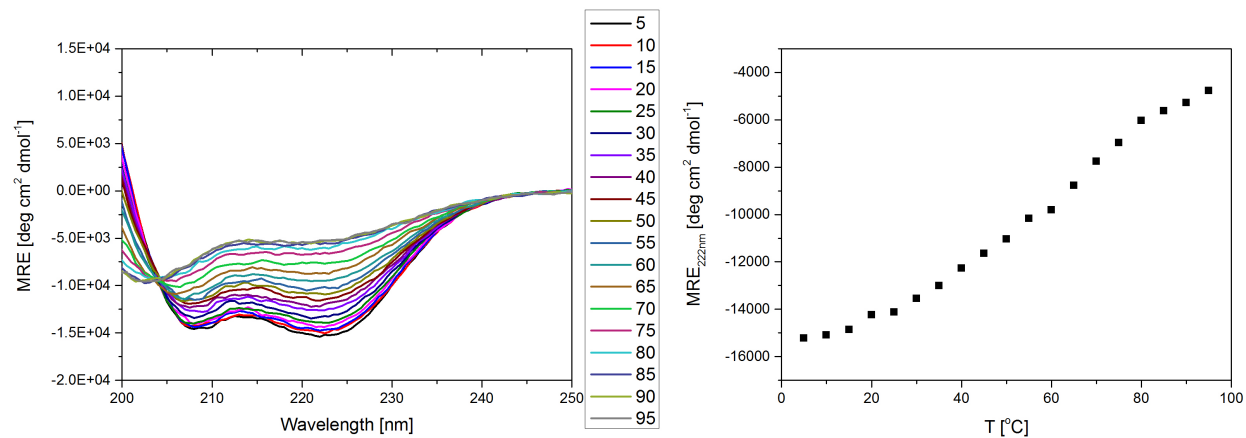


Figure S5. Temperature-dependent circular dichroism spectroscopy data for designed peptide sequence. 0.1mM peptide in 10mM pH7 phosphate buffer was measured upon heating. Left: wavelength scan at different temperatures on heating. The ratios of $[MRE]_{222nm}/[MRE]_{208nm}$ at 5°C and 20°C are 1.05 and 1.02, respectively, consistent with stable coiled-coil formation. Right: MRE at 222nm with increasing temperature, showing the melting temperature of approximately 60°C.

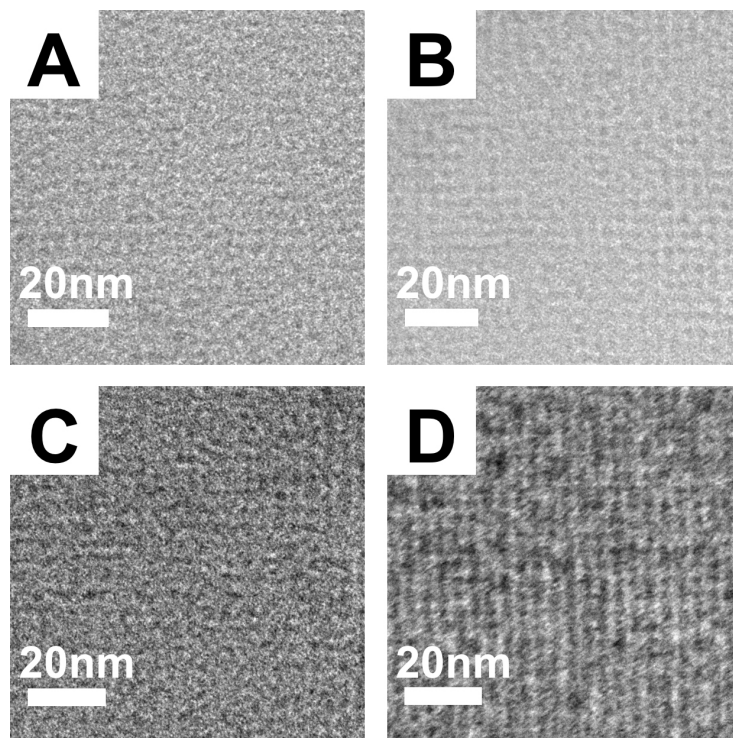


Figure S6. Dry state transmission electron microscopy high magnification images of designed peptides assembled at room temperature show the symmetry of lattices is the same. Images were taken with 2% wt. phosphotungstic acid negative staining to enhance the contrast. Scale bars are all in 20nm. A-B: Peptides assembled at RT pH8 for 1 week and 8 weeks, respectively. C-D: Peptides assembled at RT pH7 for 1 week and 8 weeks, respectively.

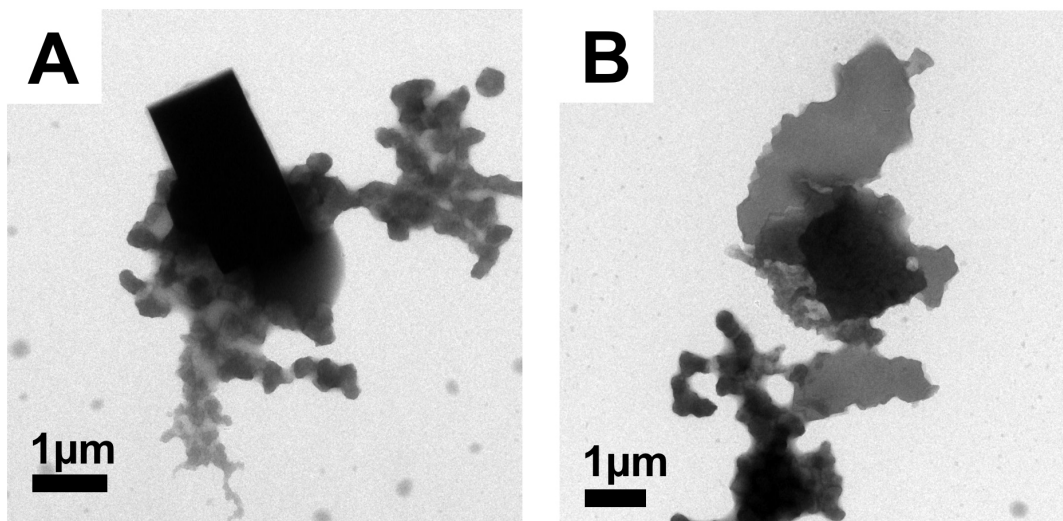


Figure S7. Dry TEM images of designed peptides assembled at 40°C. Scale bars are all in 1 μm . A: Peptides assembled at 40°C pH8 for 24 hours. B: Peptides assembled at 40°C pH7 for 24 hours. Compared to the assemblies formed at 50°C, irregular aggregates are observed around the peptide plates.

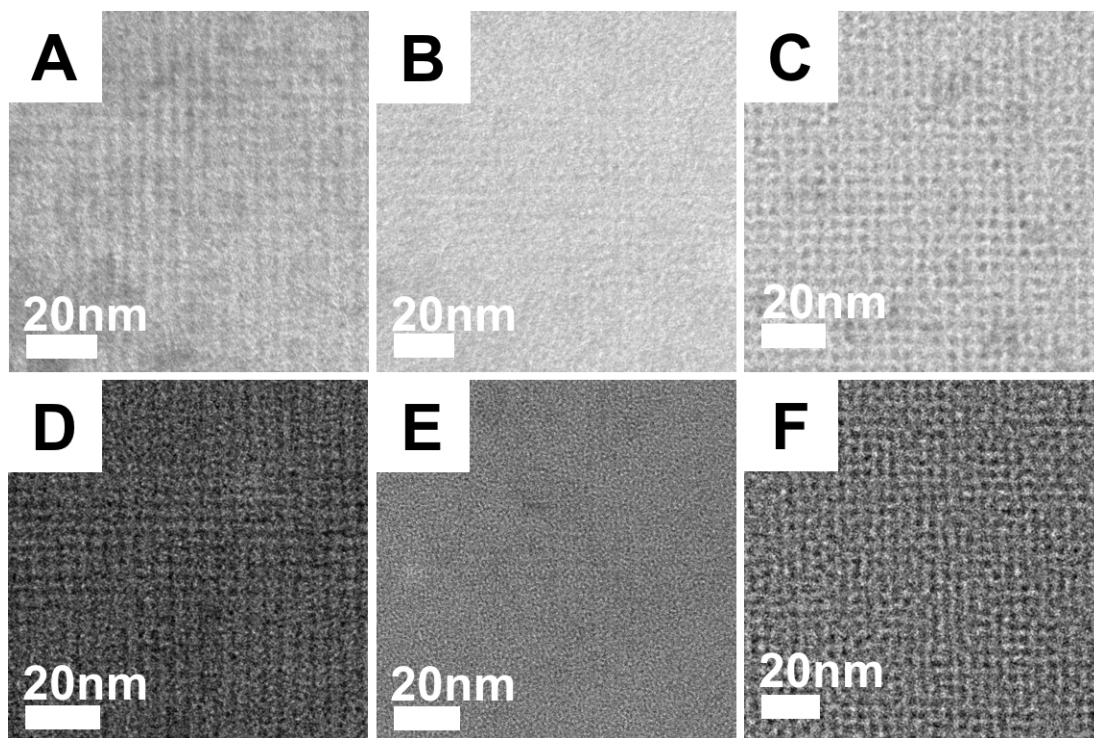


Figure S8. Dry TEM high magnification images of peptides assembled at 50°C and observed at different time point show the symmetry of lattices is the same. Images were taken with 2% wt. phosphotungstic acid negative staining to enhance the contrast. Scale bars are all in 20nm. A-C: Peptides assembled at 50°C pH8 for 15 min, 30 min and 6 hours, respectively. D-F: Peptides assembled at 50°C pH7 for 15 min, 30 min and 6 hours, respectively.

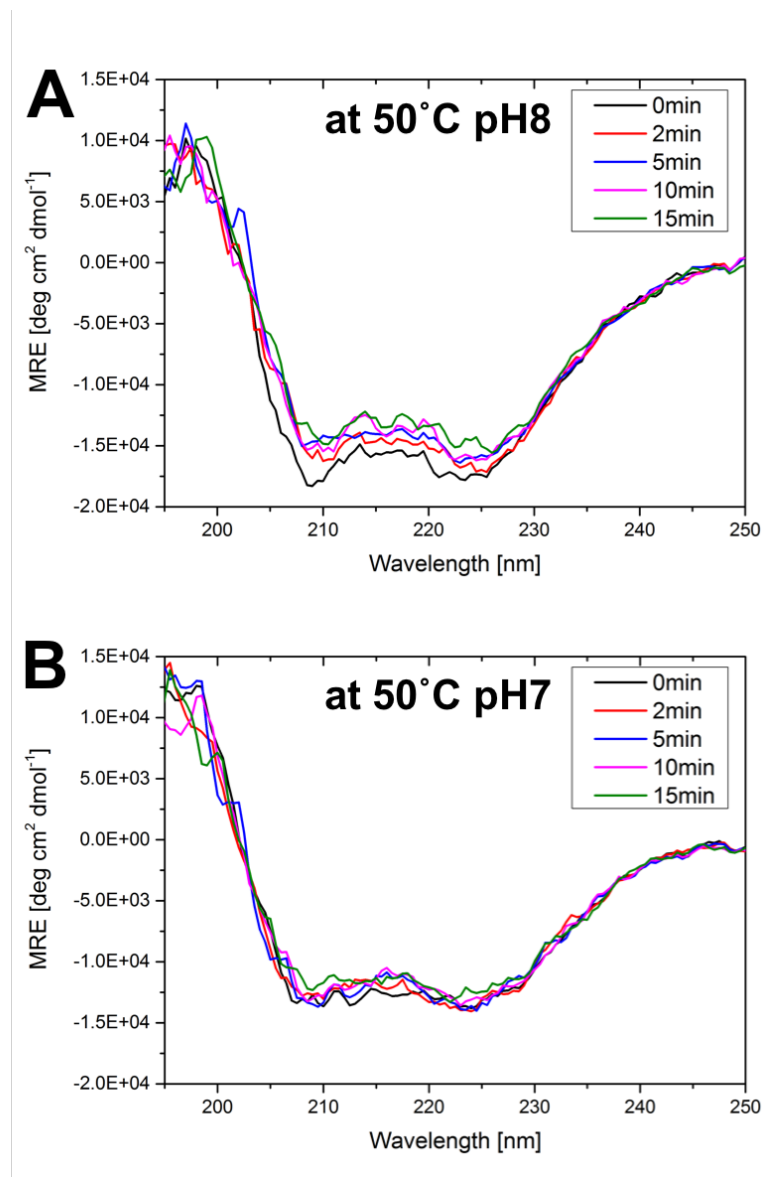


Figure S9. Circular dichroism spectroscopy data for sample quenched to 50 °C. Peptide solution was first denatured in an incubator at 95 °C, while CD chamber was pre-heated to 50 °C. Peptide solution was then moved from incubator directly to CD chamber with measurements started immediately with fast scanning rate 50nm/min. A: 0.1mM peptide at 50 °C pH8, CD spectra measured as peptide solution quenched from 95 °C to 50 °C. The ratio of $[MRE]_{222nm}/[MRE]_{208nm}$ at time 0 is 1.00, indicating the coiled-coil formation. The gradual loss of signal is because of larger assemblies precipitating from of solution. B: 0.1mM peptide at 50 °C pH7, CD spectra were measured as peptide solution quenched from 95 °C to 50 °C. The ratio of $[MRE]_{222nm}/[MRE]_{208nm}$ at time 0 is 0.98, indicating the coiled-coil formation.

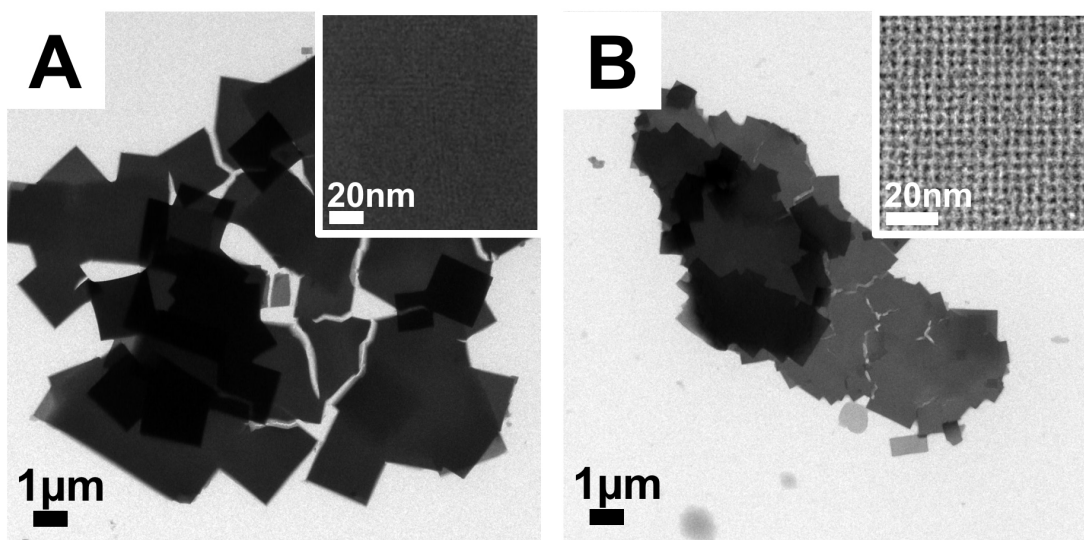


Figure S10. Dry state TEM images of peptide assemblies assembled at 50°C. High magnification images are taken with 2% wt. phosphotungstic acid negative staining to enhance the contrast. A: Peptides assembled at 50°C pH8 for 48 hours. B: Peptides assembled at 50°C pH7 for 48 hours.

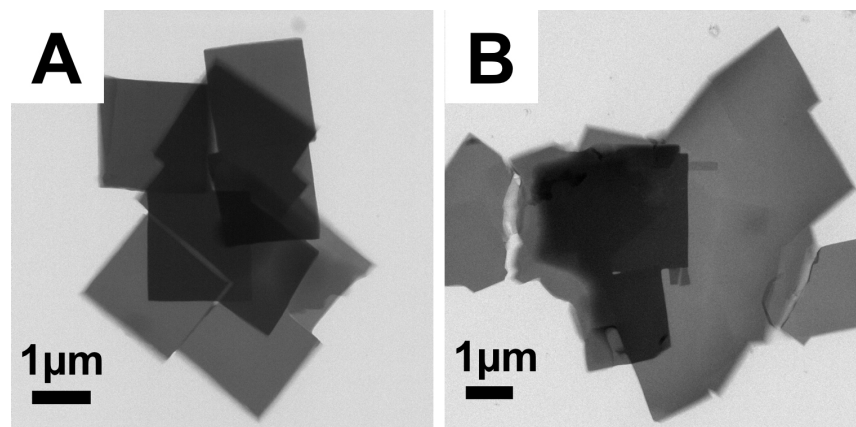


Figure S11. Transmission electron microscopy images of peptide assemblies, the scale bars are both in 1μm. A: Peptides assembled at 50°C pH 8 for 48 hours then incubated at room temperature for another 5 days. B: Peptides assembled at 50°C pH 7 for 48 hours then incubated at room temperature for another 5 days. The images are showing the large plates formed at elevated temperature are stable even at room temperature.

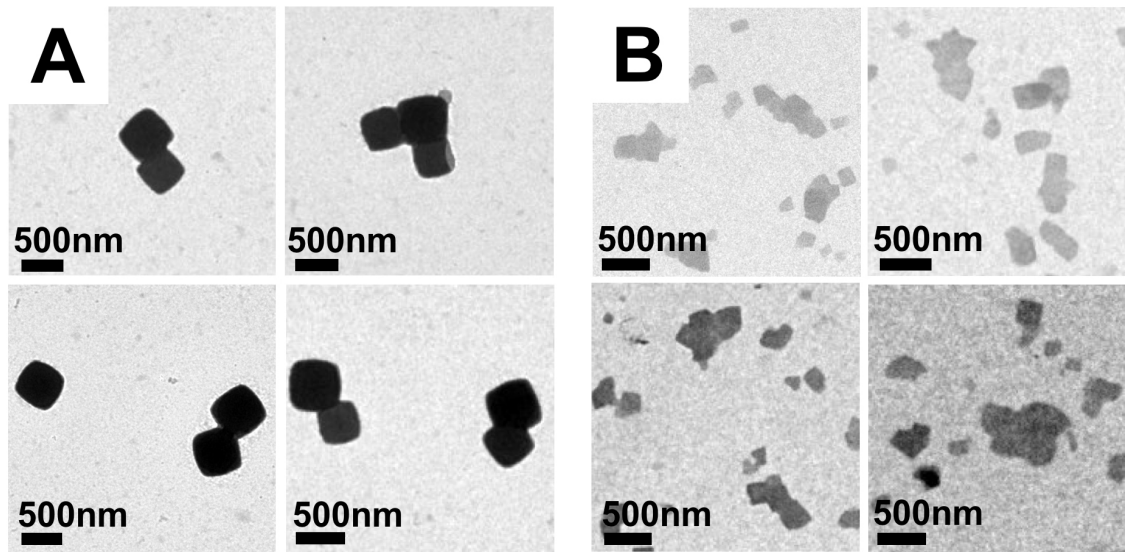


Figure S12. Dry state TEM images of peptides assembled at 50°C. All scale bars are all in 500nm. A: Peptides assembled at 50°C pH8 for 15 min. The observed platelets tend to have a square shape and are isolated. B: Peptides assembled at 50°C pH7 for 15 min. The observed platelets already started fusing and giving less regular shapes.