Supporting information

Self-assembled nanopatch with peptide-organic multilayers and mechanical property

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Supporting information list:

Experimental Section

Sample Preparation. The synthetic peptide (H2N-KKKFAFAFAFAKKK-COOH) (The purity >95%, CASLO Laboratory ApS) and 4,4'-bipyridyl (*4Bpy*) molecule (99%,Sigma-Aldrich) were purchased. The HPLC and mass spectra of the synthetic peptide was presented in Figure S1. The mixture of peptide and *4Bpy* was incubated for 7 days in the

pH 7 aqueous solution with the concentration of 100 μM, and the ratio of peptide to *4Bpy* is 1:1 at 37 ^{0}C. The sample was used in STM, AFM and TEM characterizations.

***STM* experiments.** The *STM* experiments were carried out with a Nanoscope IIIa system (Veeco Metrology, USA) working at ambient conditions. Mechanically formed Pt/Ir (80%/20%) tips were used in the *STM* experiments. A 5 μL drop of peptide sample was added on freshly cleaved highly ordered pyrolytic graphite (HOPG) and dried in air. Sample was rinsed by applying some droplets of distilled water, followed by blotting. All *STM* images were obtained in constant current mode, on freshly cleaved HOPG and the tunneling conditions are shown in the corresponding figure captions.

***AFM* investigations.** A 5 μL drop of peptide sample was added on freshly cleaved mica and dried in air. Sample was rinsed by applying some droplets of distilled water, followed by blotting. The *AFM* investigation was performed on a Nanoscope V *AFM* (Bruker, USA). The quantitative nanomechanical mapping mode was operated under ambient conditions, which could control the applied force during the measurement. Commercial silicon tips with a nominal spring constant of 5 N/m were used in the experiment and the substrate used in *AFM* experiment is freshly cleaved mica. The following formula derived for a spherical tip indenting a semi-infinite planar sample was used to estimate a local reduced elastic modulus.

$$F_{\text{interaction}} = (4/3)E^{*}\sqrt{R}\,(d\text{-}d_0)^{3/2} + F_{\text{adh}} \quad (1)$$

where $F_{\text{interaction}}$ is the tip-sample force, E^{*} the reduced elastic modulus of the tip and the sample, R the tip radius, d_0 the surface rest position, $d - d_0$ the depth of indentation and

F_{adh} the constant adhesion force during the contact. The topography, elastic modulus and force maps were processed using SPIP^{TM} software.

Transmission electrion microscopy. *TEM* images were captured on Philips CM20 at 200 kV acceleration voltage. For the sample preparation, carbon films suspended on copper grids were cleaned by glow discharge with PELCO easiGlow^{tm} (Ted Pella, Redding, CA, USA) to make them more hydrophilic. A 5 μL drop of peptide sample was added on the carbon film and dried in air. Grids were rinsed by applying some droplets of distilled water, followed by blotting. Finally, samples were negatively stained by applying a 1% UA solution for 1 min, followed by blotting and drying in air.

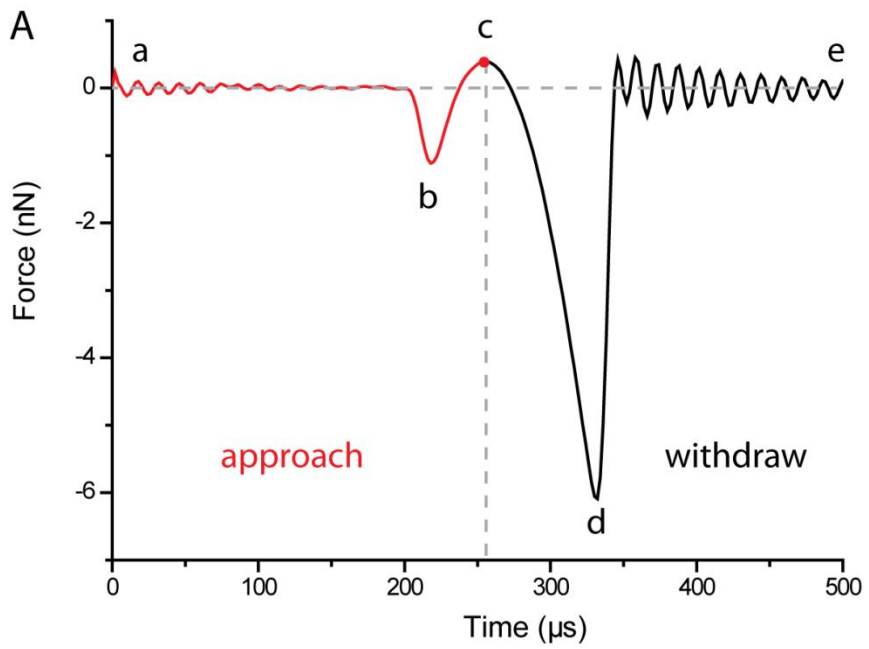
Fourier transform infrared spectroscpy. *FT-IR* spectra were recorded on Spetrum Two with UATR (Single Reflection Diamond) accessory (PerkinElmer, Waltham, MA, USA). Spectra were obtained from 32 scans at 4 cm^{-1} resolution.

Density functional calculation (*DFT*). The first-principles *DFT* calculations for *4Bpy* and *KFAK* peptide were performed with the *DMol* module in Materials Studio. The generalized gradient approximation with the PW91 functional was used to describe the exchange-correlation effects[36]. In *DMol*, the physical wave function is expanded on an accurate numerical basis set and fast convergent 3D integration is used to calculate the matrix elements occurring in the Ritz variational method[37]. In this study, a double numerical basis set with polarization functions (*DNP*) was used. For all of the calculations, the convergence in energy and force was set to 10^{-5} Ha and $2*10^{-3}$ Ha/Å. The interaction energy, E_{inter}, is defined by: $E_{inter} = E_{complex} - (E_{KFAK} + E_{molecule})$ with $E_{complex}$ is the energy of the system after introducing the small molecule into the *KFAK*,

while the E_{KFAK} and E_{molecule} are the energies of individual *KFAK* peptide and small molecule *4Bpy*, respectively.

Quantitative nanomechanical mapping

The quantitative nanomechanical mapping is force based spectroscopy with force control utilized in quantitative mechanical property measurements and submolecular resolution imaging of biological samples. The experiments were performed under the ambient conditions (Multimode SPM and Nanoscope V controller, Veeco Instruments). The cantilever is a commercial probe. The cantilever is for nanoscale material property mapping, and calibrated by ramp and thermal tuning before mapping. We imaged samples in Veeco 8 quantitative nanomechanical mapping mode AFM under ambient conditions, which is named peakforce QNM mode with precise force control. Peak Force Tapping mode oscillates, but far below the cantilever resonant frequency, the vertical motion of the cantilever using the (main) Z piezo element and relies on peak force for feedback. Peak interaction force and nanoscale material property information is collected for each individual tap. The **Force vs. Time** display, shown in the following figure. The initial contact of the probe with the sample (b), peak force (c) and adhesion (d) points are labeled.



The effective elastic modulus was derived from these waveforms using our previously described mathematical procedure. The interactions between the tip and sample are determined by the long-range electrostatic and van der Waals forces, and short-range mechanical restoration forces. The following formula derived for a spherical tip indenting a semi-infinite planar sample was used to estimate a local reduced elastic modulus.

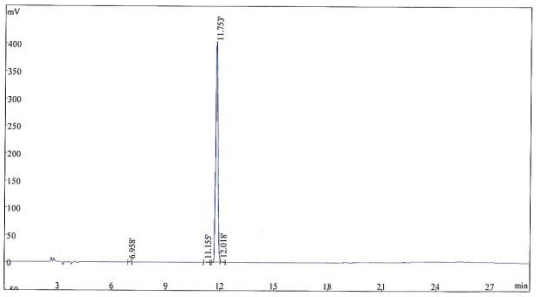
$$F_{\text{interaction}} = (4/3)E^{*}\sqrt{R}\,(d\text{-}d_0)^{3/2} + F_{\text{adh}} \quad (1)$$

where $F_{\text{interaction}}$ is the tip-sample force, E^{*} the reduced elastic modulus of the tip and the sample, R the tip radius, d_0 the surface rest position, $d - d_0$ the depth of indentation and F_{adh} the constant adhesion force during the contact. The topography, elastic modulus and force maps were processed using SPIP^{TM} software.

HPLC and Mass spectra of peptide NH2-KKKFAFAFAFAKKK-COOH

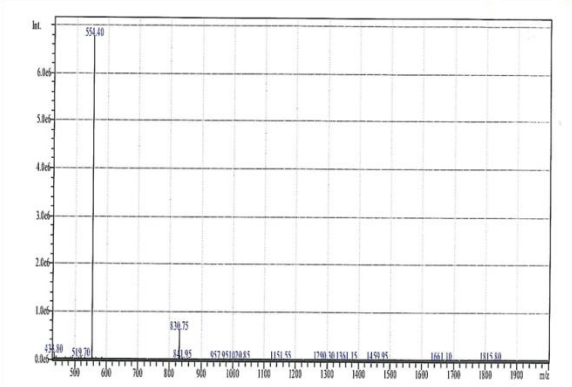
HPLC Certificate

Lot No	P280910-03-01		
Column	4.6mm x 250mm, SinoChrom ODS-BP 5um		
Solvent A	0.1%Trifluoroacetic in 100% Acetonirile		
Solvent B	0.1%Trifluoroacetic in 100% Water		
Gradient		A	B
	0.01min	19%	81%
	25.0min	44%	56%
	25.1min	100%	0%
	30.0min	Stop	
Flow rate	1.0ml/min		
Wavelength	220nm		
Volume	10ul		



Rank	Time	Conc.	Area	Height
1	0.053	0.01635	577	156
2	6.958	0.3215	11357	1972
3	11.155	0.4045	14286	1699
4	11.753	98.95	3494755	410682
5	12.018	0.3081	10881	2861
Total		100	3531856	417370

Mass Spectrometry Certificate

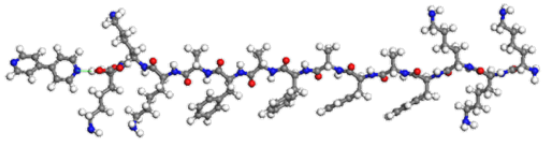


Lot No.: P280910-03-01
Method: ESI
Main Peak: 554.40
MW [M+3H+]:1663.2
MW:1660.2
Theoretical MW: 1660.10
Match: Approved
Z=3

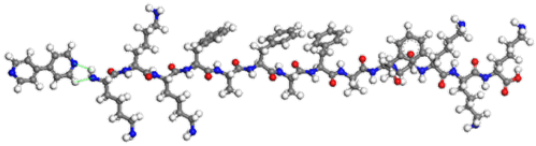
Figure S1. The HPLC and Mass spectra of the synthetic peptide NH2-KKKFAFAFAFAKKK-COOH.

Calculation bonding energy of peptide termini and 4Bpy molecules.

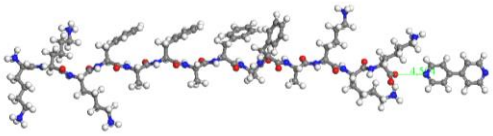
a.



b.



c.



d.

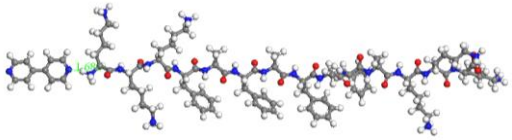


Figure S2. a. The interaction model of 4Bpy and the carboxylic terminus of peptide without charge, the binding energy of which is determined to be -0.80 eV. The interaction distance of the nitrogen atom of 4Bpy and hydrogen atom of C terminus is calculated to be 1.72 Å. b. The interaction model of 4Bpy and Nitrogen terminus of peptide without charge, the binding energy of which is -0.24 eV. The interaction distance of nitrogen atom of 4Bpy and hydrogen atom of N terminus of peptide is carried out to be 2.41 Å. c) The model of C-terminus of peptide with charge interacting with 4Bpy molecule, and the binding energy is -0.22 eV, and the distance between of nitrogen atom of 4Bpy and oxygen of peptide is carried out to be 4.5 Å. d) The model of N terminus with charge of

peptide interacting with 4Bpy molecule, and the binding energy is calculated to be -0.55 eV, and the distance between the nitrogen atom of 4Bpy molecule and hydrogen atom of N terminus of peptide was carried to be 1.68 Å .

There is the balance that

-COOH↔ -COO- + H+,

-NH2 ↔ -NH3+ + OH-

In both cases of peptides termini with charge or no charge, 4Bpy molecules could interact with the termini of peptides in different ways (Figure. S2a-d).

STM images of peptide assembly and hybrids of peptide and 4Bpy molecules

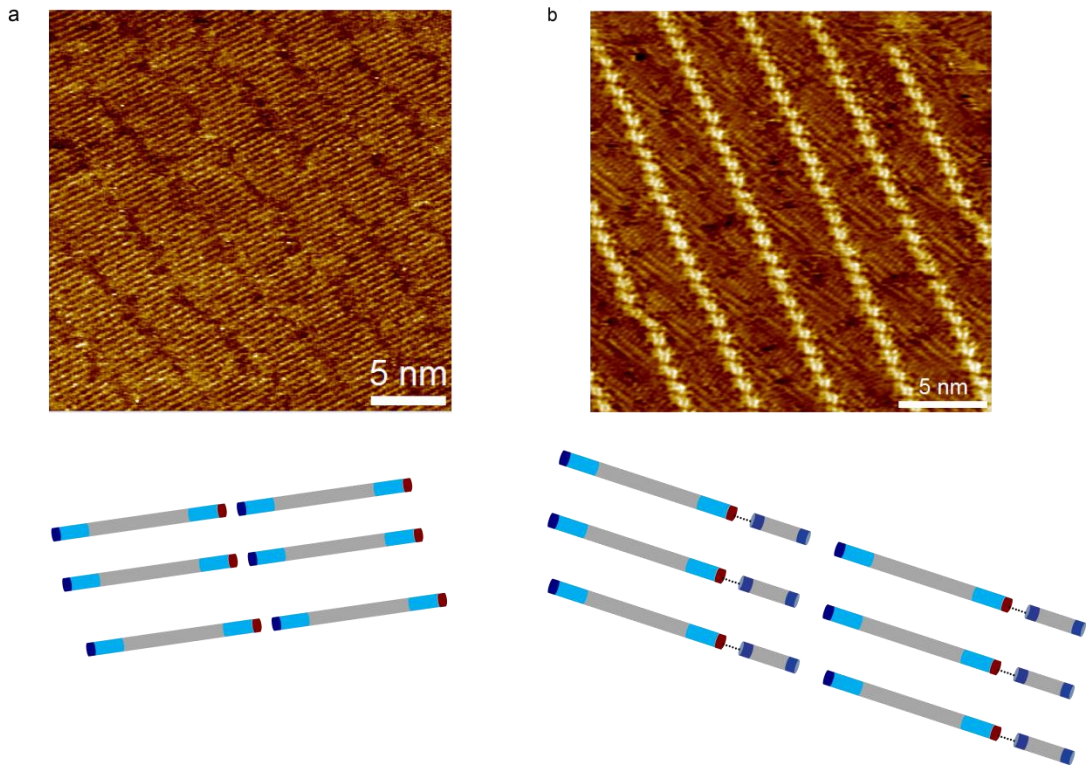


Figure S3. a) STM image of peptide assembly. b) STM image of hybrids of peptide and 4Bpy molecules. Tentative models of peptide assembly and co-assembly of peptide and 4Bpy molecule are in the lower panel of a and b.

It is very obvious that 4Bpy could modulate the peptide assembly from STM images above. Peptides are capable of self assembly into nanostripe via the intermolecular hydrogen bonding and hydrophobic interaction of peptides. 4Bpy as linker tunes peptide assembling into lamellae structures. After molecular tuning, the new building block is formed. The angle between peptide chain and the axis of assembly stripe dramatically change after molecular tuning peptide assembly compared to the angle before tuning. This also proves the tuning effect on peptide assemblies.

FT-IR spectra of small molecule.

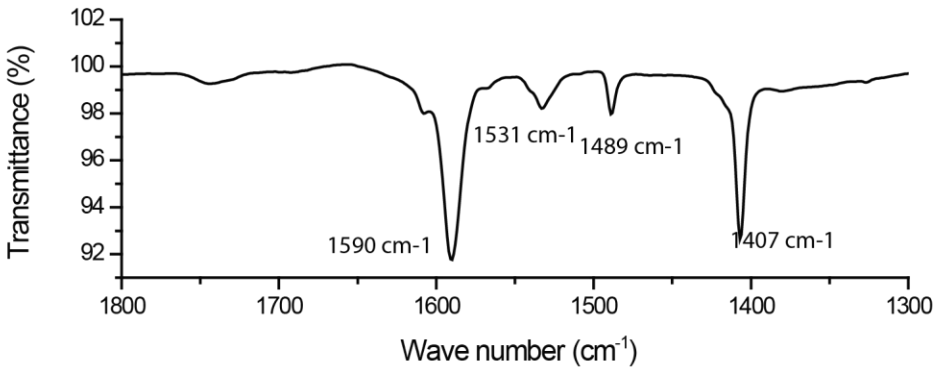
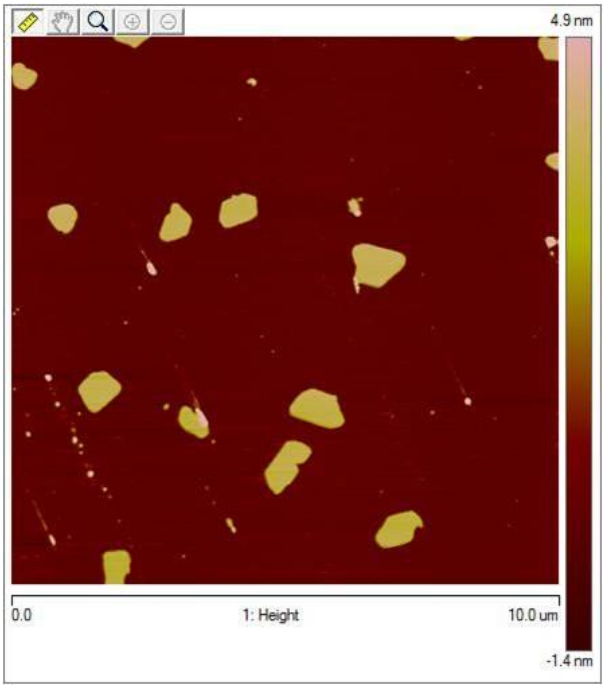


Figure S4. The FT-IR spectra of 4Bpy molecule.

The peaks of 1590 cm^{-1}, 1531 cm^{-1}, 1489 cm^{-1}, and 1407 cm^{-1} are representing the feature of 4Bpy molecule. In the spectra of hybrids of peptide and 4Bpy, the peak at 1532 cm^{-1} representing the feature of 4Bby suggests that the molecules exist in the hybrid, but could not affect the secondary structure of peptide.

AFM and TEM images of nanopatch structure in large area.



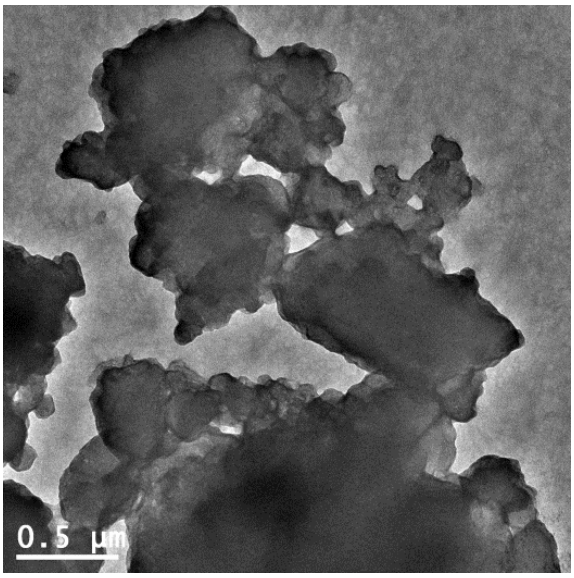


Figure S5**.** The 2D large area topography images of peptide patch structures tuned by 4Bpy molecules characterized by AFM and TEM. The scanning size of the AFM image is 10 µM x 10 µM.

2D larger area STM image of hybrids of peptide and 4Bpy molecules

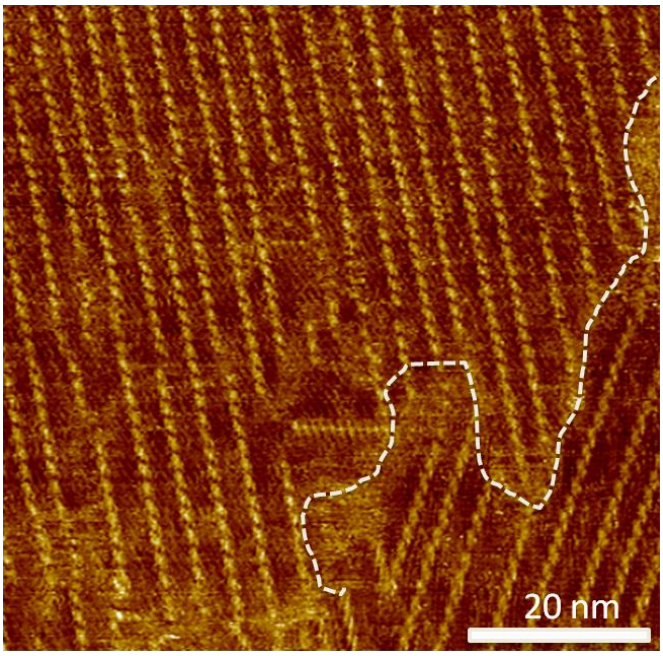


Figure S6. The 2D large area STM image of the hybrids of the peptides and 4Bpy molecules. The boundary of two domains is presented in the STM image.Tunnelling conditions: 709 mV and 495 pA, 709 mV and 495 pA.

It is obviously observed that the co-assemblies of peptide and 4Bpy molecules could form different domains. Some 4Bpy molecules are capable of forming separating domain. It is implied that the interaction of the boundary of two domains is likely to be weak, and the domain could be easily peeled.