Supporting Information

Benzylic bromide induced peptide cross-linking for nanofibers assembly

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Materials and supplies

1,2-Bis (bromomethyl) benzene (OB), 1,3-Bis (bromomethyl) benzene (MB), and 1,4-Bis (bromomethyl) benzene (PB) were purchased from Fuyun Biological Technology (Wuxi, China). Disodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate decahydrate, and ammonium bicarbonate were purchased from Sinopharm Chemical Reagent (Shanghai, China). Aqueous solutions were prepared with ultrapure water.

Peptide synthesis and characterization

All peptides were artificially synthesized using the solid phase FMOC synthesis method by GL Biochem (Shanghai, China). High Performance Liquid Chromatography (HPLC) was used to purify the peptides and their purity was above 90% (Fig. S1). Their mass spectrometry showed peaks of 2382 and 2385, which were consistent with theoretical mass (Fig. S2). Peptides were dialyzed in ultrapure water, aliquoted, and lyophilized at -80°C. After drying, the peptides were re-dissolved in 10 mM phosphate buffer.

Atomic force microscope (AFM)

About 5 μL sample solution was dropped slowly and uniformly on the surface of the mica sheet. The mica sheet was gently rinsed with water and dried with flowing air after 15 minutes of absorption. Then, the sample was scanned multiple times using the MultiMode 8 tapping mode of the Bruker atomic force microscope at a scanning speed of 1.0 Hz, and the NanoScope was used for analysis.

Dynamic light scattering (DLS)

DLS measurements were carried out using a Zetasizer Nano ZS (Malvern Instruments, UK). Data was collected using He-Ne laser at 25°C at an angle of 175° and Zetasizer Software was used for data analysis.

Quadrupole Time of Flight (Q-TOF)

The Q-TOF mass spectrometry experiment was performed using the Xevo G2 Q-TOF of waters company for detection. All Q-TOF mass spectrometry experiments were acquired in TOF mode, using an ESI ionization source with a positive charge. The peptide sample solution was detected by Q-TOF before and after it was assembled, then the data were deconvolved and exported by MassLynx V4.1.

Bonding schematic

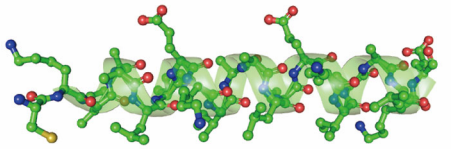
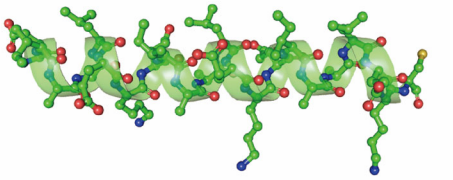
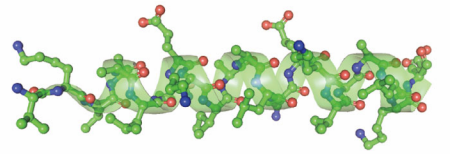
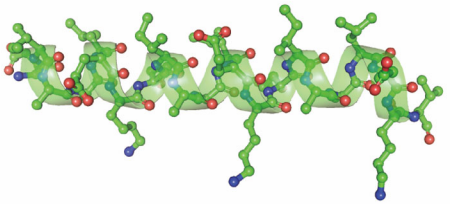
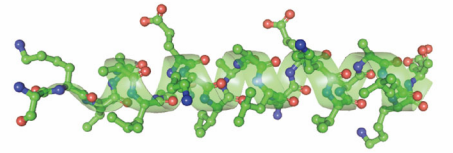
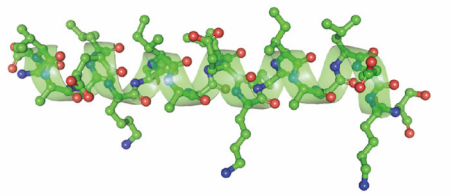
In PyMOL, the PDB structure (1u0i) was first imported through the PDB database, a cysteine was added at the N-terminus of E3 and C-terminus of K3 through the PyMOL builder module. Then, the disulfide bond and the thioether bond were added through the builder module.

Cell cytotoxicity

The assembled peptide solution was diluted at a concentration of 1, 10, and 40 μg/mL, and filtered with a 0.22 μm filter. An equal amount of 200 μL of the solution was placed into a 96-well tissue culture plate and absorbed at 37°C for 24 h. The plate was washed 3 times with 10 mM phosphate buffer and blocked with 5% bovine serum albumin at

37°C for 2 h. 5×10^3 NIH/3T3 mouse cells were seeded in 96-well plates and cultured in a 37°C, 5% constant temperature incubator. At 24 h, 48 h, and 72 h, the cell cytotoxicity was tested with the CCK-8 kit assay. Cells without peptides and natural type I collagen were used as negative and positive controls, and all the experiments were performed in three replicates.

Table S1. The sequence and the structure formula of the peptides.

Peptide name	**Sequence**	**Structure formula**
CE3	CKIAALKEKIAALKEKIAALKE	
K3C	EIAALEKEIAALEKEIAALEKC	
VE3	VKIAALKEKIAALKEKIAALKE	
K3V	EIAALEKEIAALEKEIAALEKV	
SE3	SKIAALKEKIAALKEKIAALKE	
K3S	EIAALEKEIAALEKEIAALEKS	

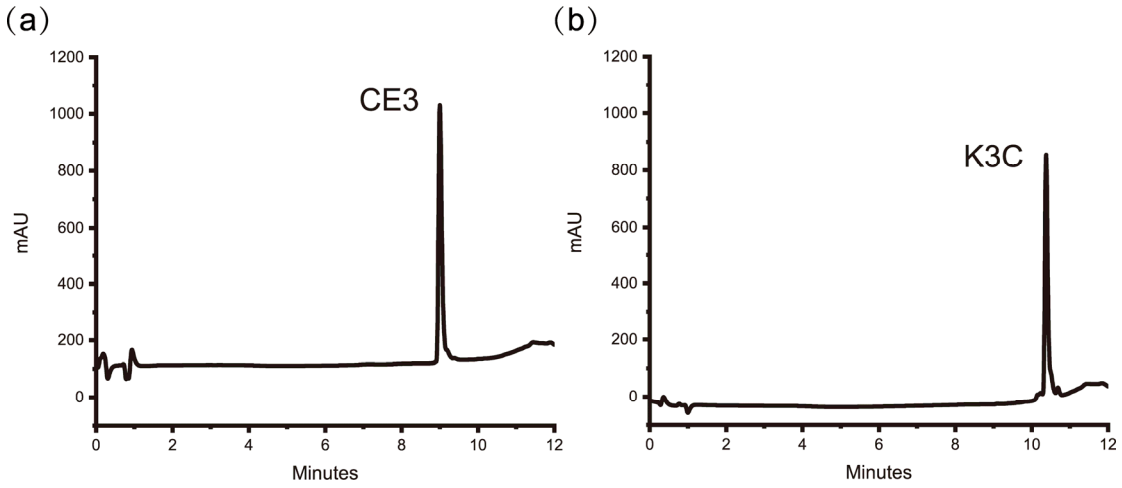


Figure S1. HPLC chromatograms of purified peptides (1 mM). (a) CE3. (b) K3C.

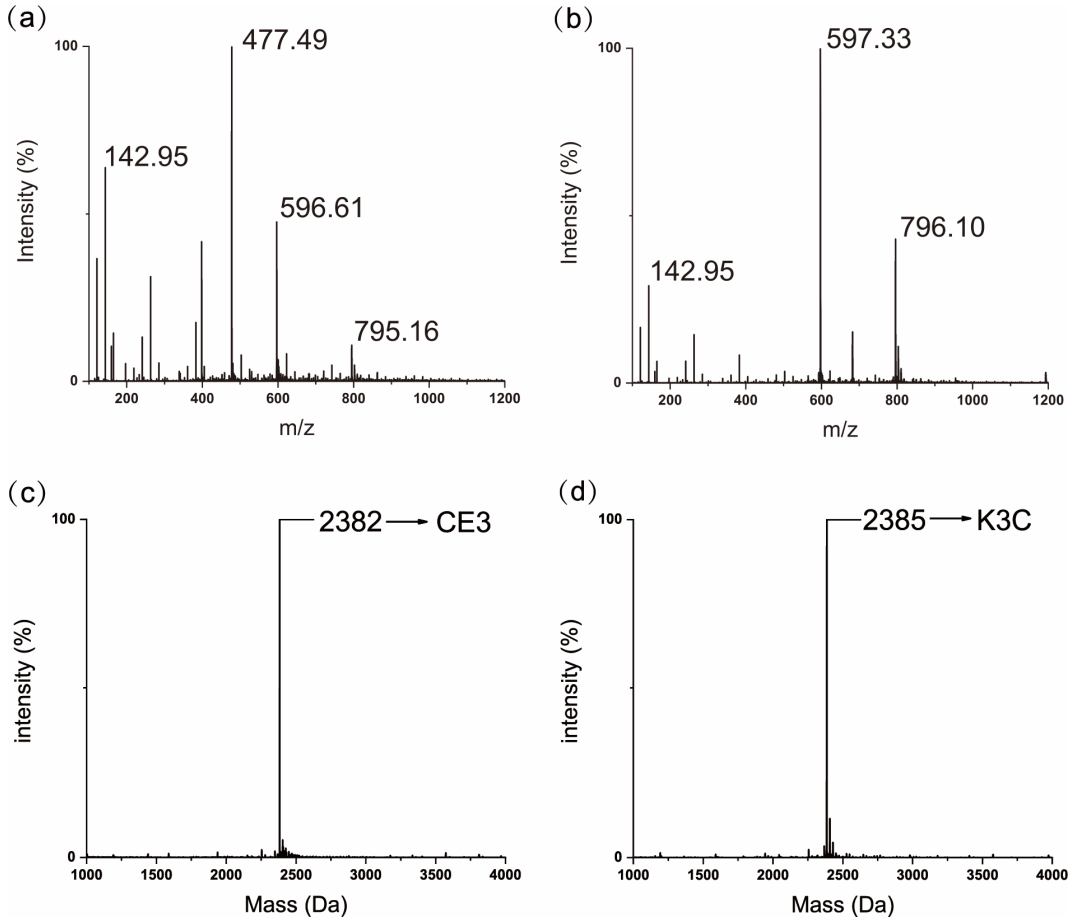


Figure S2. Q-TOF mass spectrometry analysis of purified peptides (1mM). Original data (a) CE3 and (b) K3C. Deconvolutional data (c) CE3 and (d) K3C.

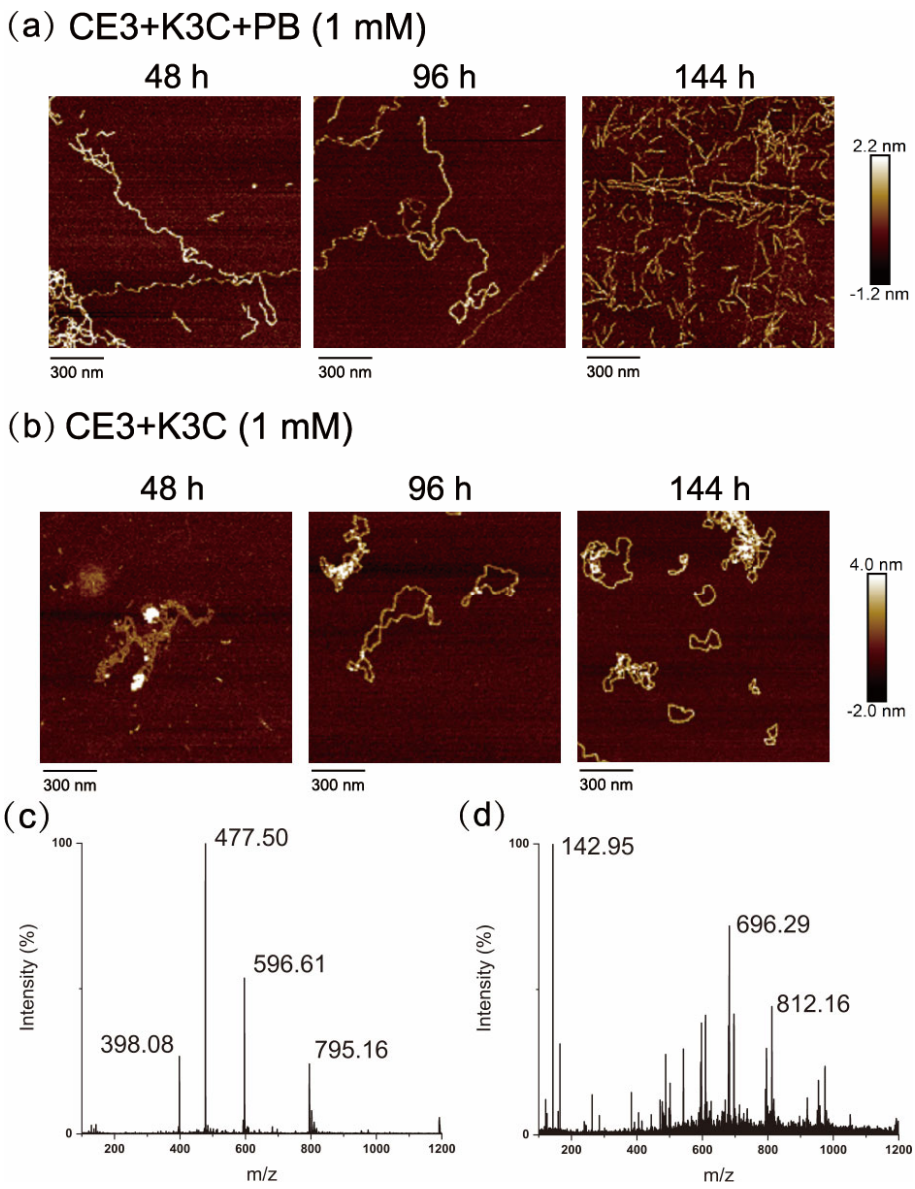


Figure S3. (a) AFM enlarged images of the CE3+K3C+PB (1mM) incubation system at 37°C after 48, 96, and 144 h. (b) AFM enlarged images of CE3+K3C (1mM) after 48, 96, and 144 h of incubation at 37°C. Original Q-TOF mass spectrometry of the CE3+K3C+PB incubation system at (c) 0 h and (d) 10 days.

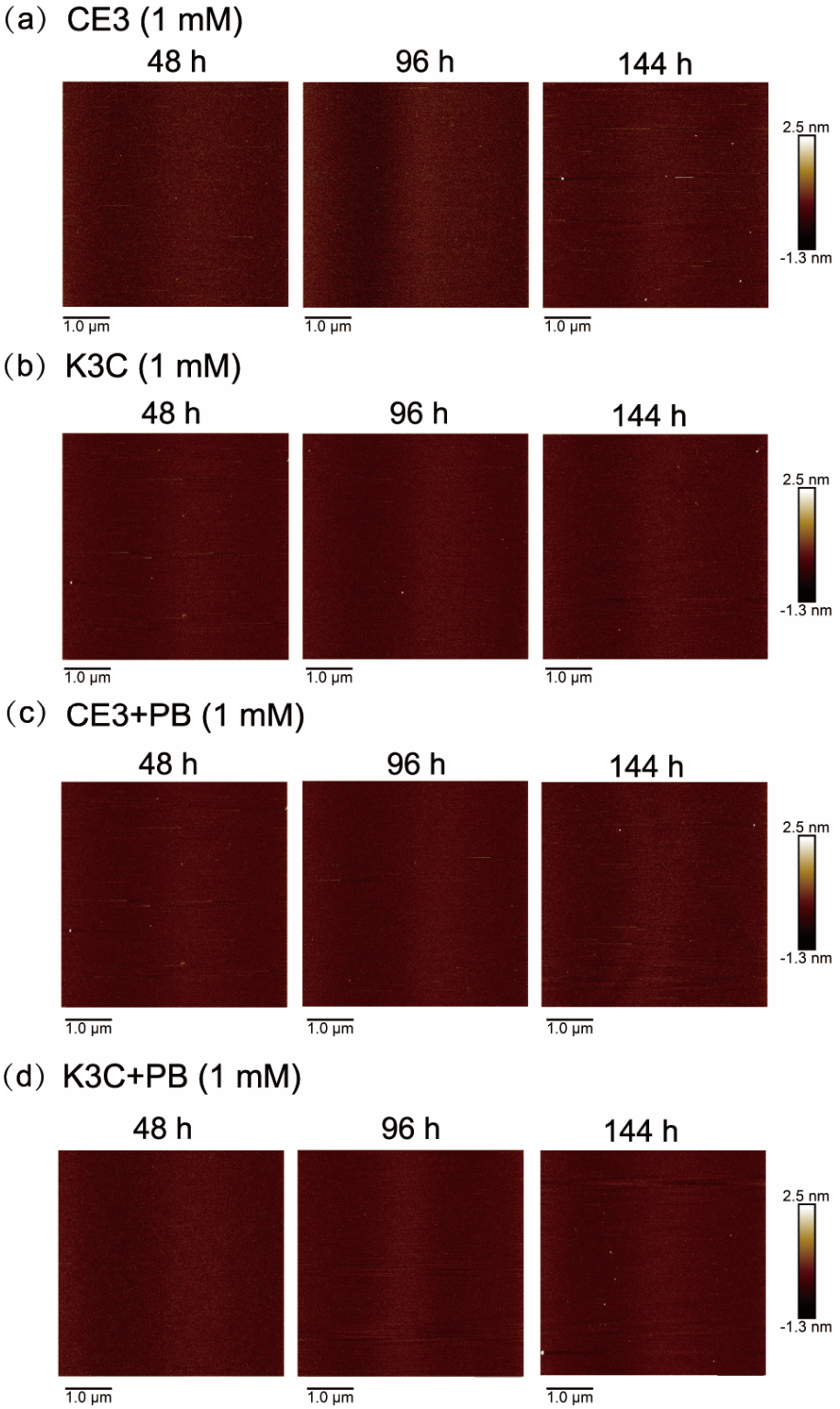


Figure S4. AFM images of one-component and two-component systems after 48, 96, and 144 h of incubation at 37°C. (a) CE3. (b) K3C. (c) CE3+PB. (d) K3C+PB.

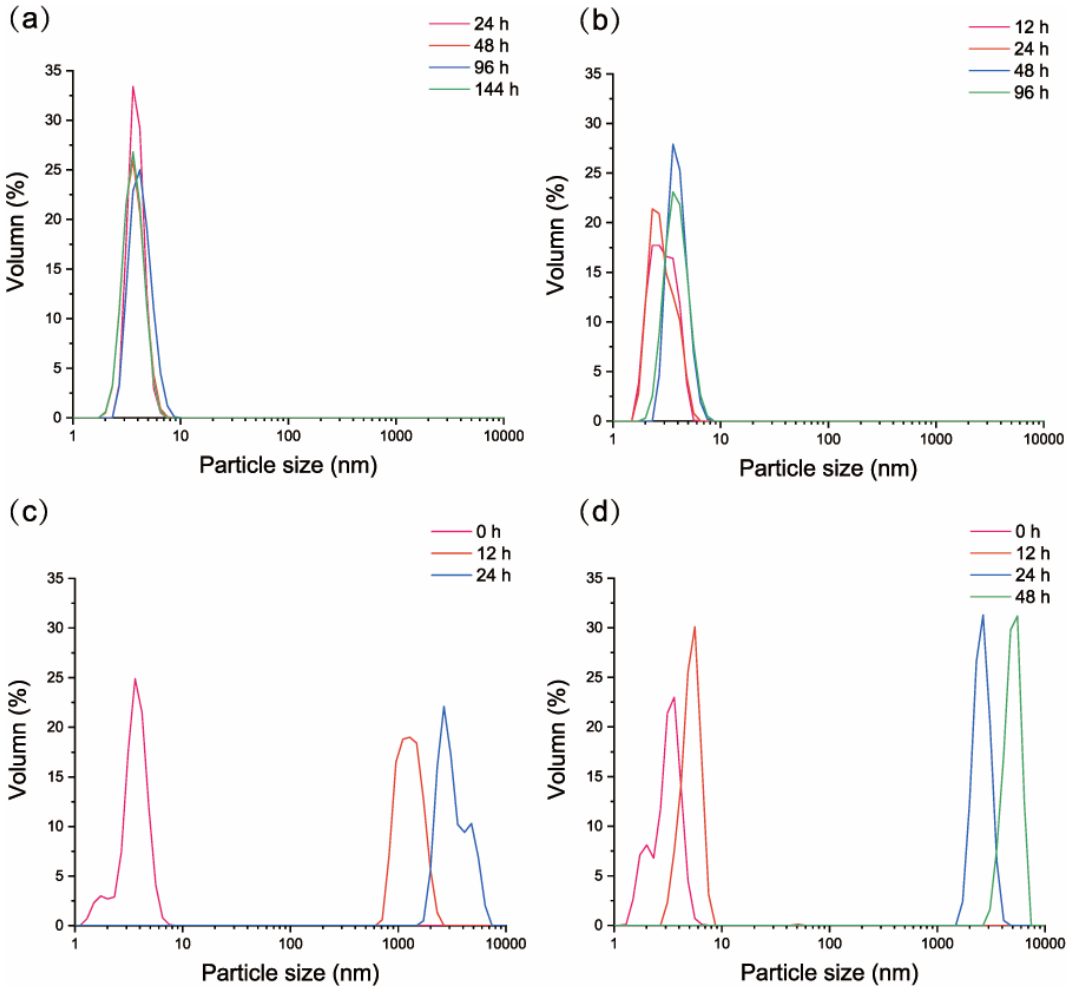


Figure S5. Particle size distribution in different concentrations of CE3+K3C+PB mixture detected by DLS at different hours of incubation at 37°C. (a) 0.2mM. (b) 0.4mM. (c) 0.8mM. (d) 1 mM.

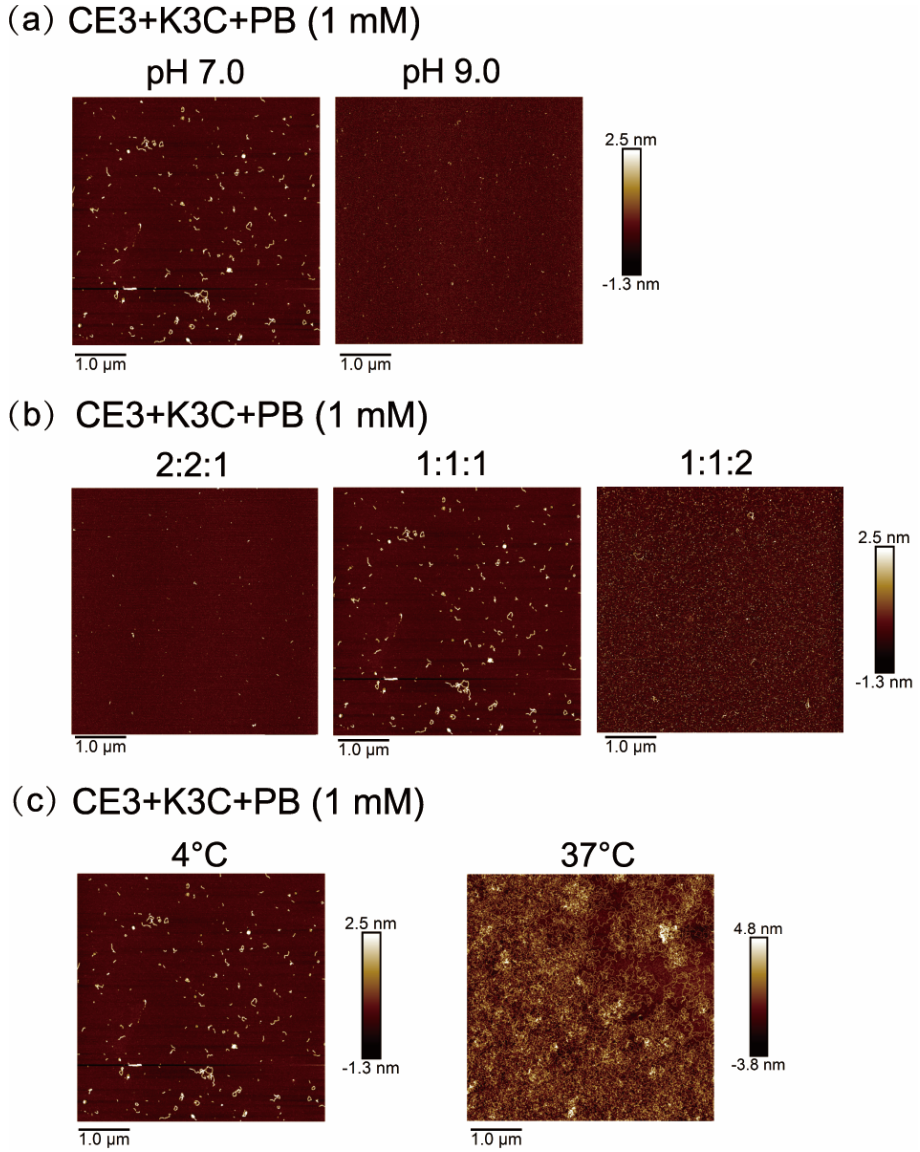


Figure S6. AFM images under different cross-linking reaction conditions with CE3+K3C+PB (1mM). The starting condition was annealed from 100°C before incubation at 4°C for 48 hours at pH 7.0 with a ratio of 1:1:1. (a) The samples were prepared at pH 7.0 or pH 9.0. (b) The ratios of CE3:K3C:PB were 2:2:1, 1:1:1, and 1:1:2, respectively with a consistent CE3 and K3C concentration at 1 mM. (c) The samples were annealed or incubated at 37 °C for 48 hours.

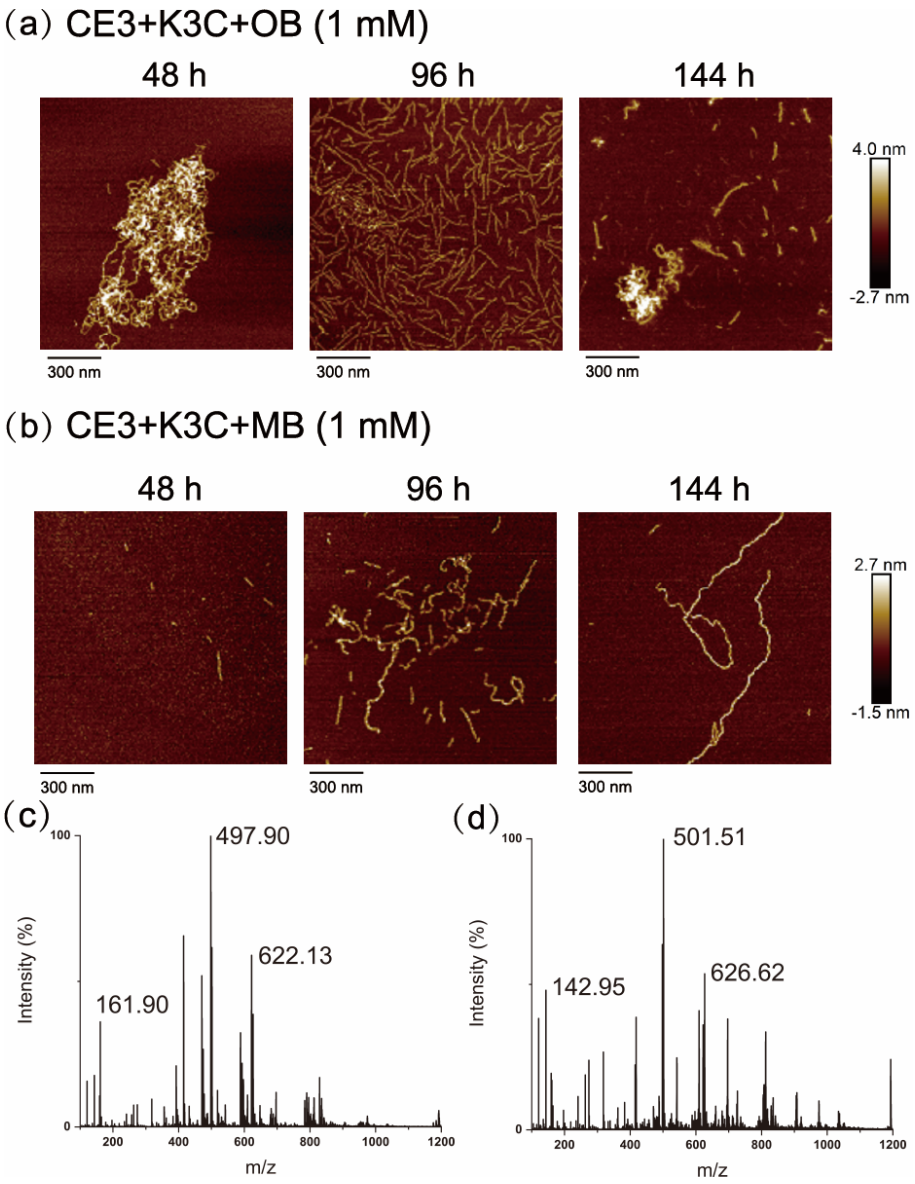


Figure S7. (a) AFM enlarged images of the CE3+K3C+OB (1mM) incubation system at 37°C after 48, 96, and 144 h. (b) AFM enlarged images of CE3+K3C+MB (1mM) after 48, 96, and 144 h of incubation at 37°C. Original Q-TOF mass spectrometry of (c) CE3+K3C+OB and (d) CE3+K3C+MB at 10 days.

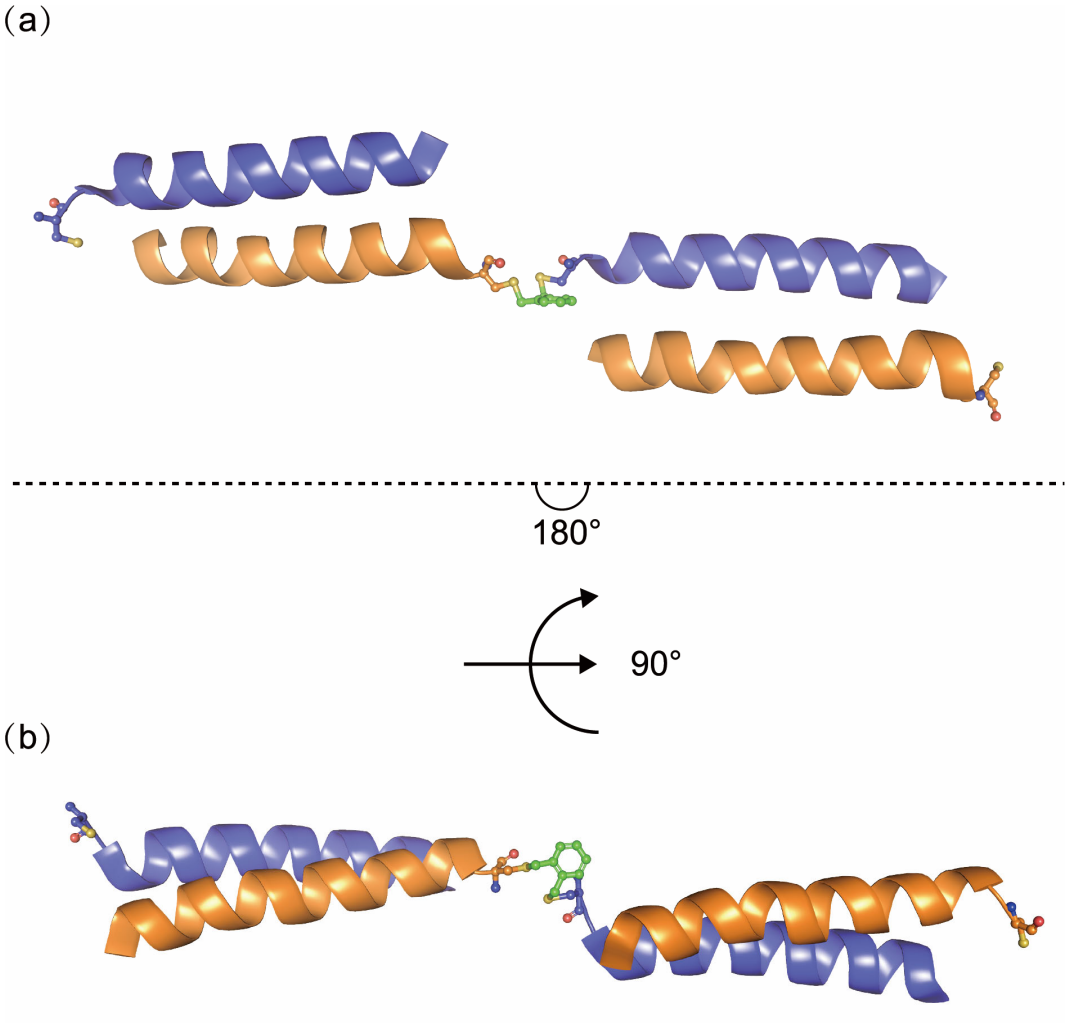


Figure S8. Schematic diagram of simulated cross-linking of two CE3/K3C coiled-coils with OB.

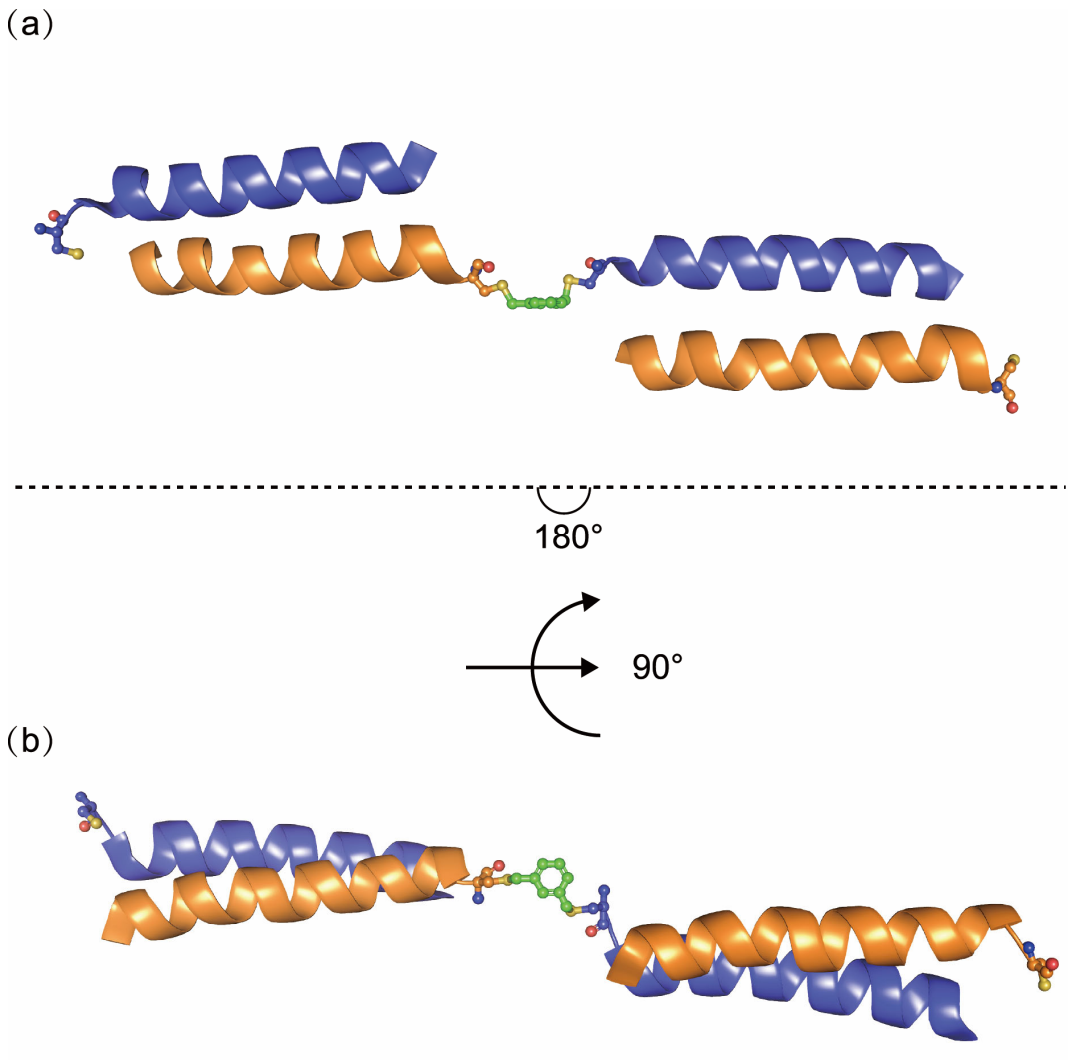


Figure S9. Schematic diagram of simulated cross-linking of two CE3/K3C coiled-coils with MB.

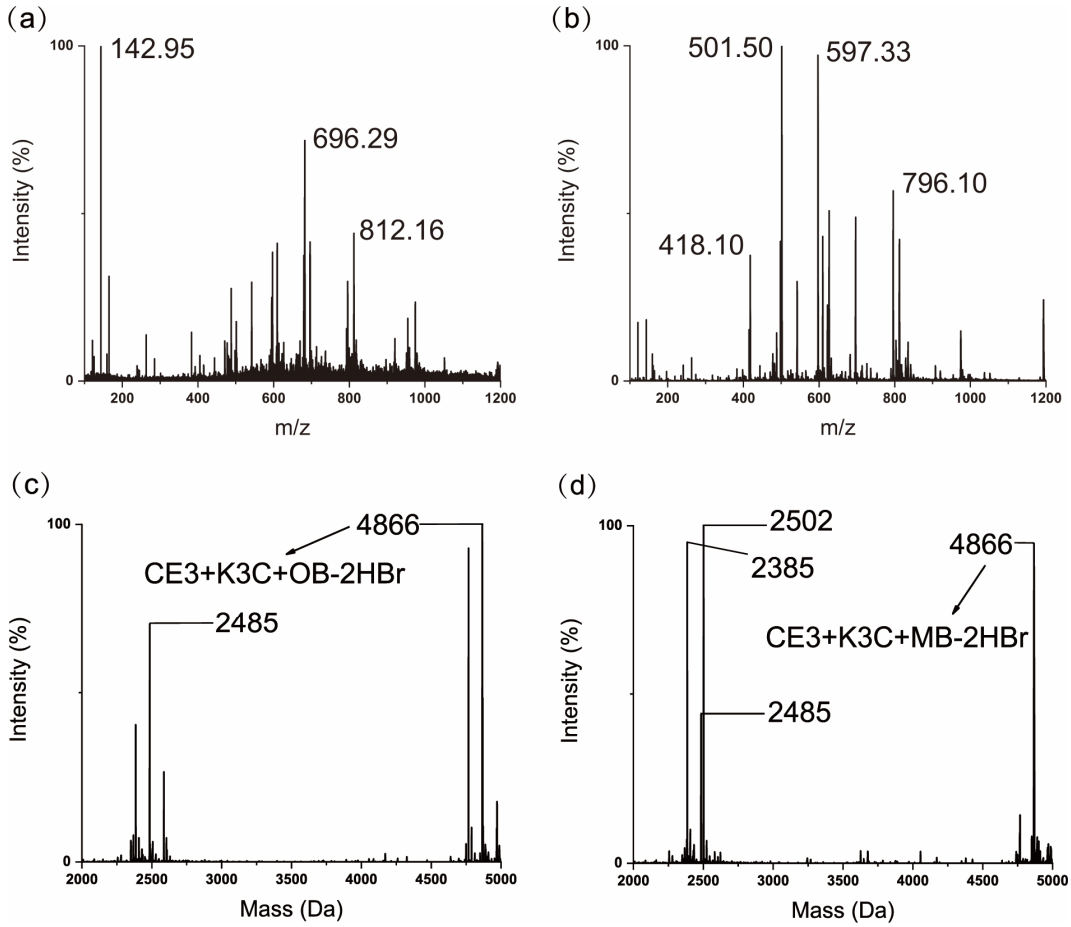


Figure S10. Q-TOF mass spectrometry analysis of CE3+K3C+OB system and CE3+K3C+MB system at 12 h. Original data (a) CE3+K3C+OB system and (b) CE3+K3C+MB system. Deconvolutional data (a) CE3+K3C+OB system and (b) CE3+K3C+MB system.

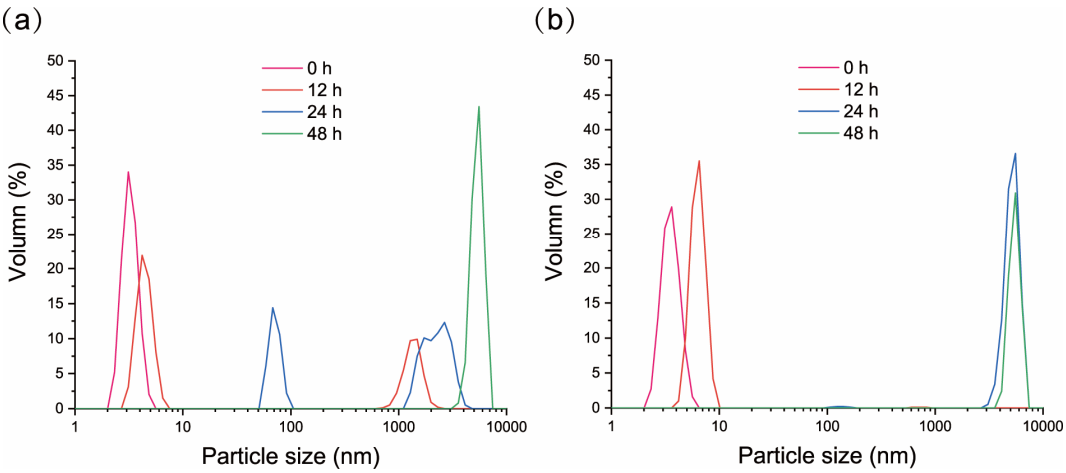


Figure S11. Particle size distribution of (a) CE3+K3C+OB and (b) CE3+K3C+MB mixture at 1mM detected with DLS at different hours of incubation at 37°C.

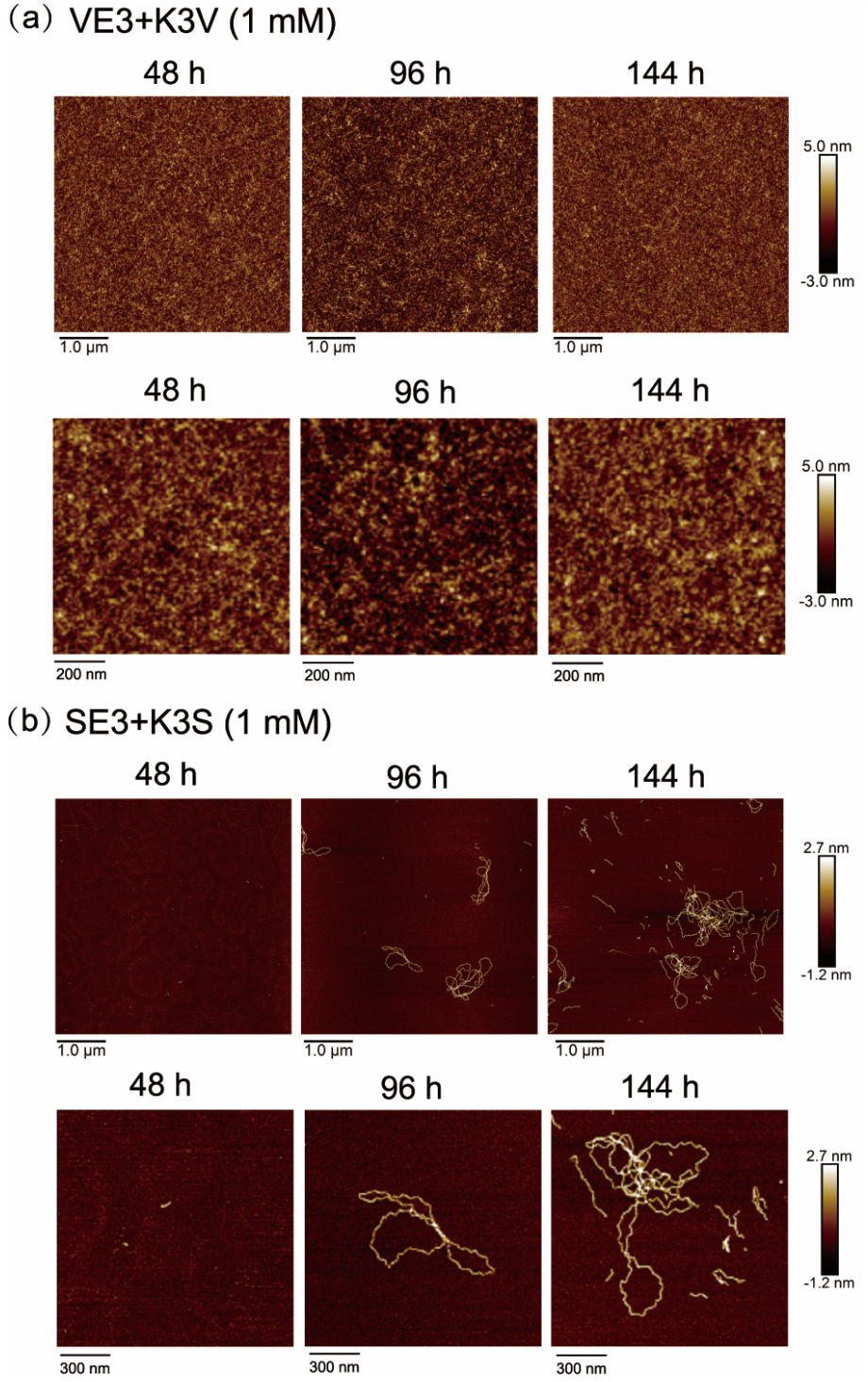


Figure S12. AFM original images (top) and enlarged images (bottom) of (a) VE3+K3V (1mM) and (b) SE3+K3S (1mM) at 48, 96, and 144 h of incubation at 37°C.