Electronic supplementary information

for

Self-Assembly of Virulent Amyloid-Derived Peptides into Nanoantibacterials

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1. General Materials

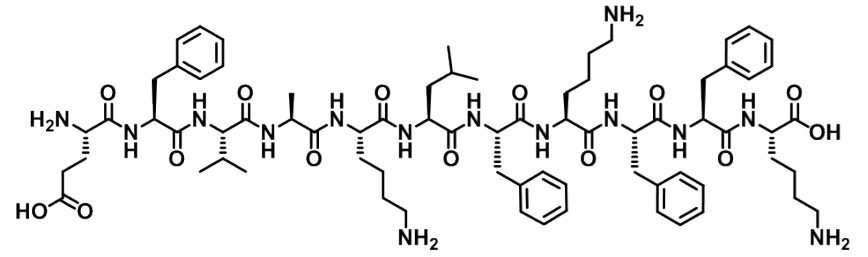
2-Chlorotrityl chloride resin (loading density: 0.5 mmol / g) was purchased from ApexBio Technology Co., Ltd. Fmoc-Glu(OtBu)-OH, Fmoc-Phe-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-Arg(Pbf)-OH, Boc-(2S, 4S)-4-amino1-Fmoc pyrroliddine-2-carboxylic acid, N, N'-diisopropylethylamine (DIEA), and hexafluorophosphate azabenzotriazole tetramethyl uronium (HBTU) used in solid phase peptide synthesis were purchased from Bide Pharmatech Co., Ltd. Trifluoroacetic acid (TFA) and triisopropylsilane were obtained from Meryer Chemical Technology Co. Ltd. Dichloromethane (CH_2Cl_2), methanol (CH_3OH), and N, N'-dimethylformamide (DMF) were supplied by Tianjin Concord Technology Co., Ltd. Piperidine was obtained from Sinopharm Chemical Reagent Co., Ltd. Thioflavin T (Th-T) was purchased from J&K Scientific Ltd. 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was obtained from Sigma-Aldrich. Acridine oange (AO, Fluk) and ethidium (EB, Fluk) were bought from Beijing Solarbio Science & Technology Co. Ltd. (Beijing, China). All reagents were analytical grade. Ultrapure water was produced by Arium Pro Ultrapure water systems (Sartorius, 18.2 MΩ). Dulbecco's modified Eagle's medium (DMEM), penicillin / streptomycin solution, trypsin, and fetalbovine serum (FBS) were purchased from Gibco (CA, America). *Staphylococcus aureus* (*S. aureus*) ATCC 6538, *Bacillus amyloliquefaciens* (*B. amyloliquefaciens*) ATCC 23842, *Escherichia coli* (*E. coli*) ATCC 8739, and *Streptococcus mutans* (*S. mutans*) CGMCC 1.2499 strains were provided by Department of Microbiology of Nankai University (Tianjin, China).

Human renal epithelial 293T cells and mouse fibroblast 3T3 cells were purchased from Shanghai Huzhen Bio. Tech. Co., Ltd.

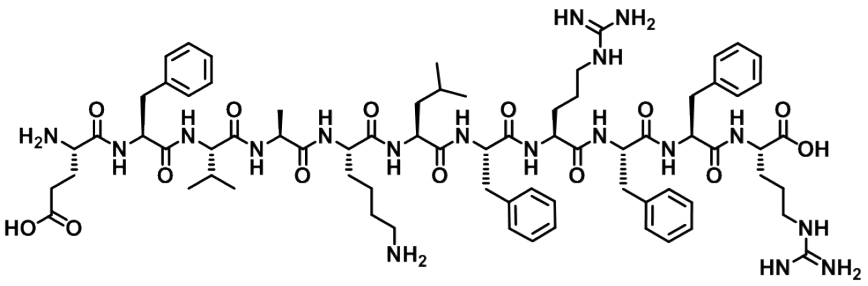
2. Peptide Synthesis

All the undecapeptides UP (EFVAKLFKFFK), UP-RR (EFVAKLFRFFR) and UP-RWR (EFVAKLFRWFR) were synthesized via conventional Fmoc solid-phase peptide synthesis (SPPS) method by utilizing the 2-chlorotrityl chloride resin. During the synthetic process, 0.25 mmol 2-chlorotrityl chloride resin was soaked in 15 mL anhydrous dichloromethane (CH_2Cl_2) for 20 min. Afterwards, a mixture of amino acids (4 eq. relative to resin) and DIEA (6 eq.) was dissolved in 20 mL anhydrous CH_2Cl_2 and added to the swelled resin. After shaking for one hour, 2 mL CH_3OH was added and shaked for another 30 min to quench active sites on the resin. The sealed resin was washed by CH_3OH, DMF, and CH_2Cl_2. For the rest amino acid coupling reactions, a mixture of amino acid / HBTU / DIEA (4: 3.95: 6 relative to the resin) was dissolved in DMF and subsequently added into the resin and shaked for at least one hour. 30 % piperidine in DMF solution was utilized as Fmoc deprotection reagent and the deprotection process took place for at least 30 min. The undecapeptide sequences were cleaved from the resin by utilizing a cocktail solution consisting of 95 % trifluoroacetic acid, 2.5 % triisopropyl silane, and 2.5 % H_2O for two hours. The trifluoroacetic acid in the peptide solution was removed by rotary evaporation, and the crude peptides were obtained by precipitating from cold diethyl ether. The crude peptide was further purified by preparative reversed phase high performance liquid chromatography (RP-HPLC)

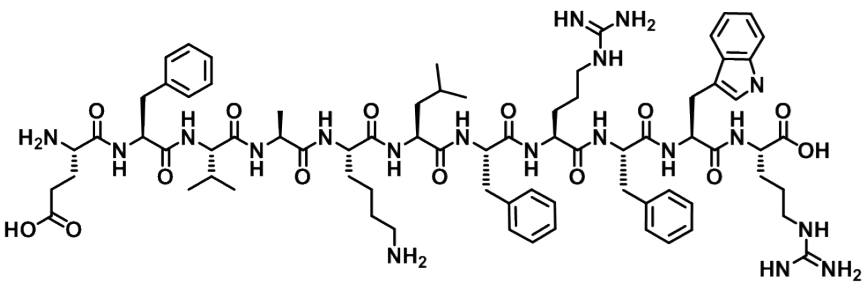
with the binary water / MeCN solvent system containing 0.1% TFA (vol %), in which H_2O and MeCN were set as solvent A and solvent B, respectively and the gradient was set in the range from "5 % solvent B + 95 % solvent A" to "95 % solvent B +5 % solvent A" with a flow rate of 10 mL / min. The structure and purities of the lyophilized UP, UP-RR and UP-RWR were characterized by mass spectrometry (Waters SQ Detector 2) and ultra performance liquid chromatography (UPLC, Waters Acquity system), respectively.



UP (EFVAKLFKFFK)



UP-RR (EFVAKLFRFFR)



UP-RWR (EFVAKLFRFWR)

Figure S1. Chemical structures of peptides UP, UP-RR, and UP-RWR.

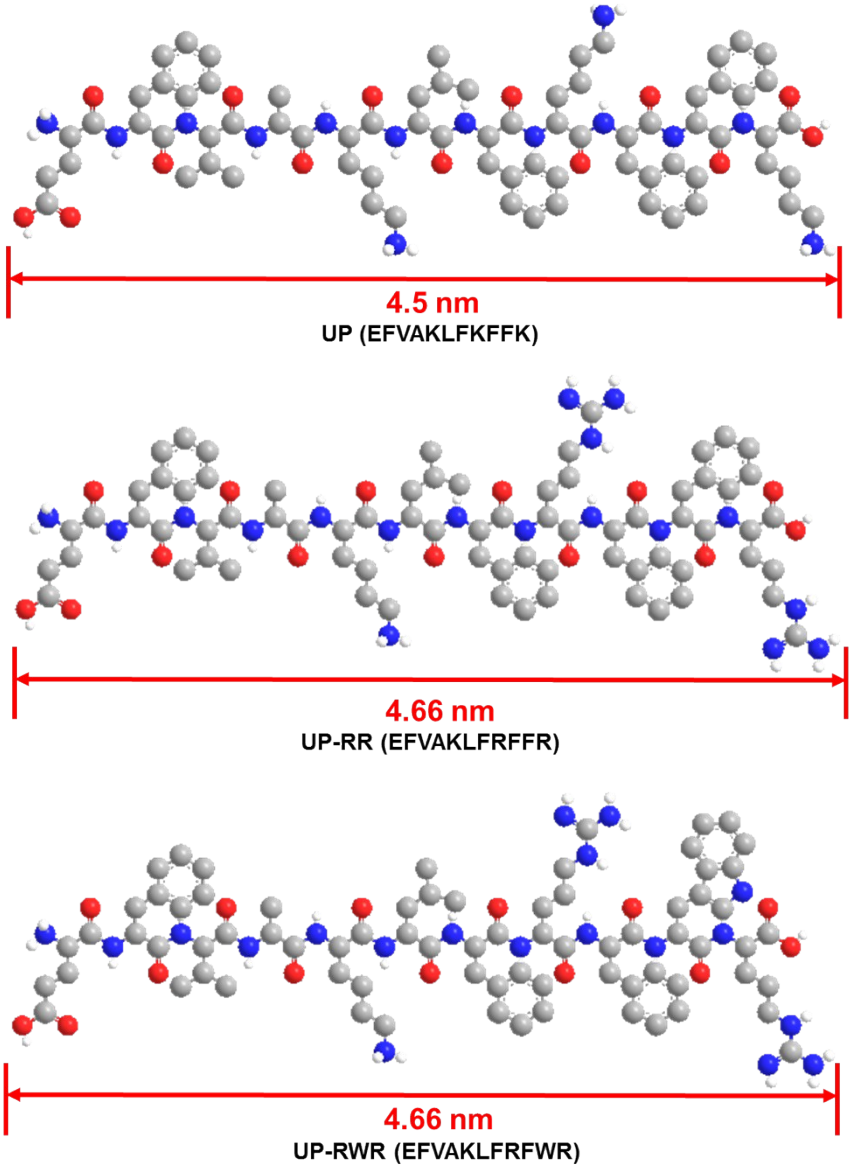


Figure S2. Structural models of peptides UP, UP-RR, and UP-RWR, as well as their length in a straight state.

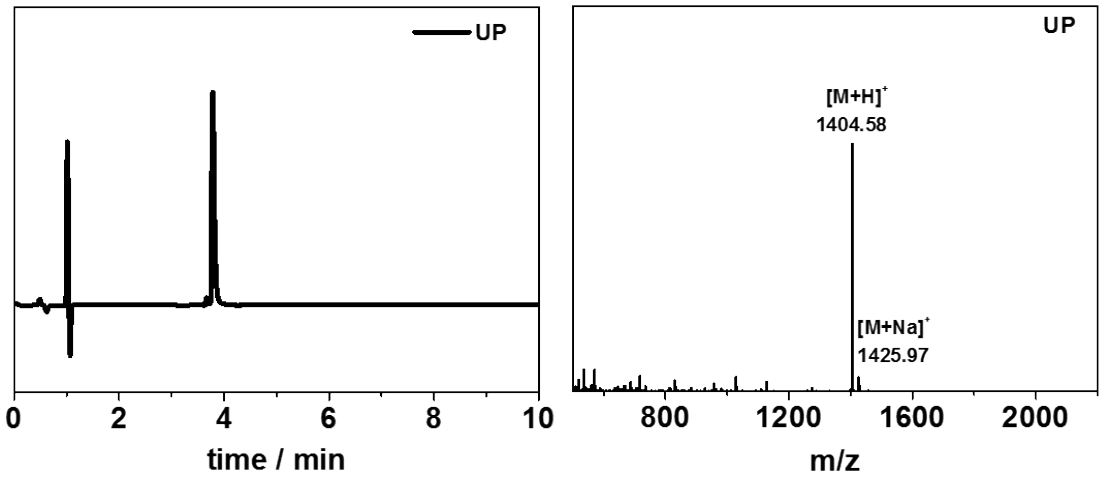


Figure S3. UPLC trace (left) and mass spectrum (right) of peptide UP.

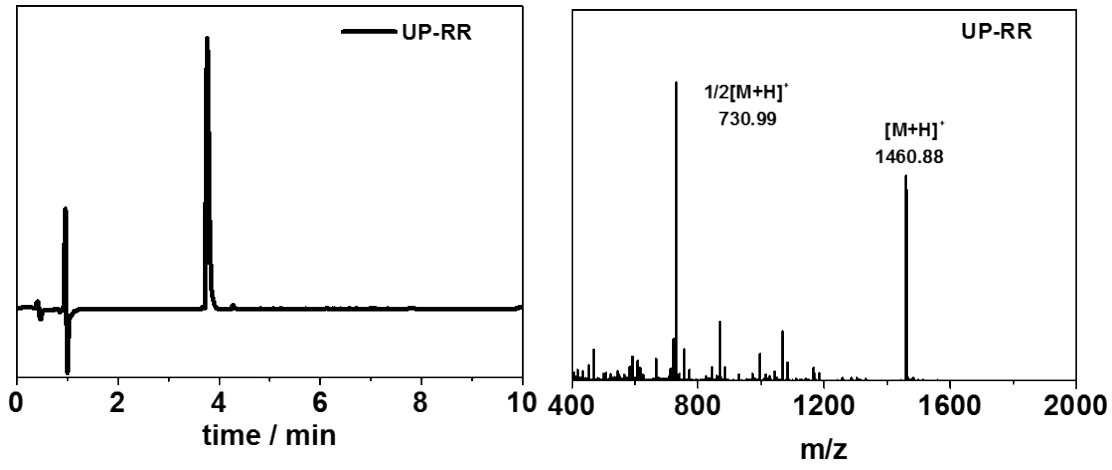


Figure S4. UPLC trace (left) and mass spectrum (right) of peptide UP-RR.

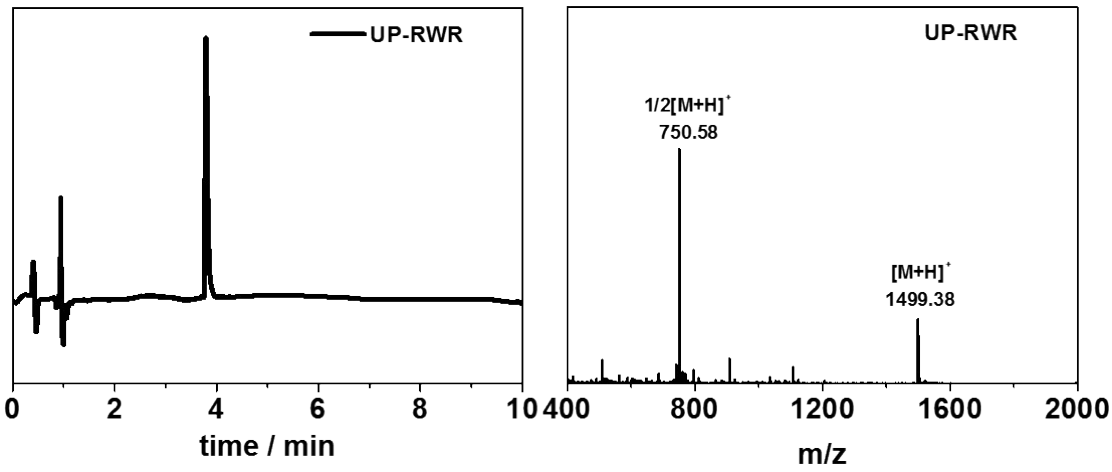


Figure S5. UPLC trace (left) and mass spectrum (right) of peptide UP-RWR.

3. Characterization of Self-Assembly of Undecapeptides

3.1 Critical Aggregating Concentration (CAC)

We estimated the critical aggregating concentration of undecapeptides by recording the maximal emission wavelength of Nile Red, on the basis of the shift of its emission wavelength arising from the change of microenvironment hydrophobicity. The annealed solution of undecapeptides UP, UP-RR, and UP-RWR (2 mM) was diluted by phosphate buffer solution (PBS, 10 mM, pH 7.4) to obtain a serial of peptide solutions (2 mL) with a concentration in the range from 0.1 mM to 100 mM. Subsequently, the Nile Red solution (100 μM, 2 μL) in ethanol was added to each diluted peptide solution and the eventual concentration of Nile Red in the solutions of each peptide was maintained as 100 nM. All the samples containing peptides and Nile Red were aged for 12 h prior to the measurement of fluorescence spectra using an Agilent Cary Eclipse fluorescence spectrophotometer. The fluorescence spectra were recorded by using an excitation wavelength of 550 nm and in a range of 600-700 nm. the excitation and emission slit widths were set as 10 nm and 20 nm, respectively. The wavelength of the maximal emission of Nile Red in the presence of different peptides was plotted as a function of peptide concentration to determine the CAC values of undecapeptides.

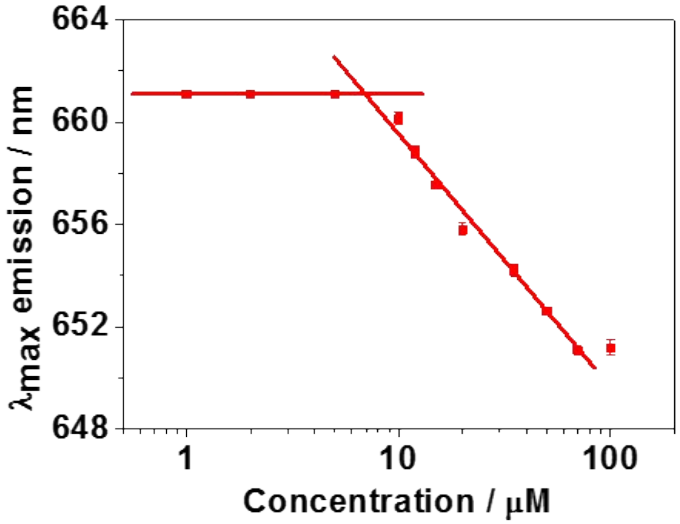


Figure S6. The wavelength of the maximal emission (λ_{max}) of Nile Red in the presence of peptide UP at a concentration in the range of 1.0 and 100 μM. Based on the CAC determination, UP starts to aggregate at a concentration of approximately 6.89 μM.

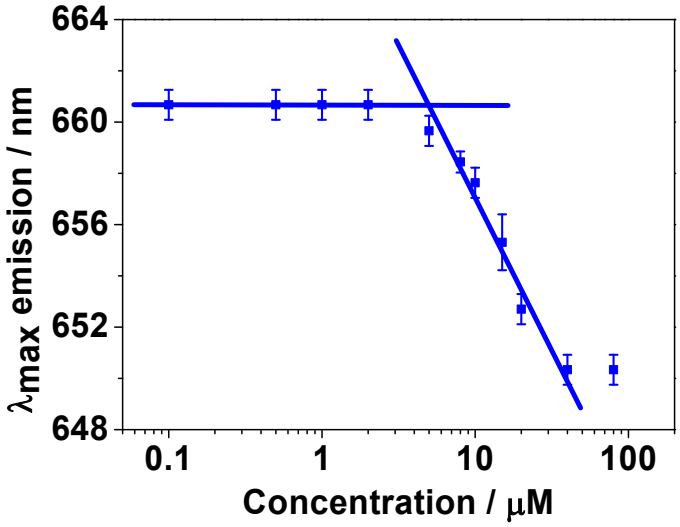


Figure S7. The wavelength of the maximal emission (λ_{max}) of Nile Red in the presence of peptide UP-RR at a concentration in the range of 0.1 and 80 μM. Based on the CAC determination, UP-RR starts to aggregate at a concentration of approximately 4.89 μM.

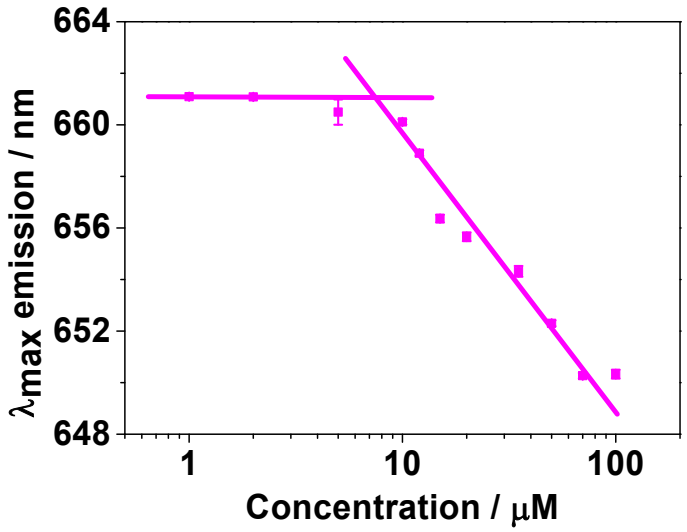


Figure S8. The wavelength of the maximal emission (λ_{max}) of Nile Red in the presence of peptide UP-RWR at a concentration in the range of 1.0 and 100 μM. Based on the CAC determination, UP-RWR starts to aggregate at a concentration of approximately 7.32 μM.

3.2 Circular Dichroism (CD) Spectroscopy

CD experiments of the annealed solutions of the peptides were carried out by using a Biologic MOS-450 CD spectrometer at ambient temperature using a 0.2 cm quartz cuvette or 0.1 mm quartz slides for the samples at a low (0.1-0.2 mM) or high (1.0-2.0 mM) concentration, respectively. The spectra were scanned in a wavelength range of 190 and 260 nm with an interval of 0.5 nm and a slit width of 2 nm. Prior to the measurements, the background signals of solution medium were subtracted.

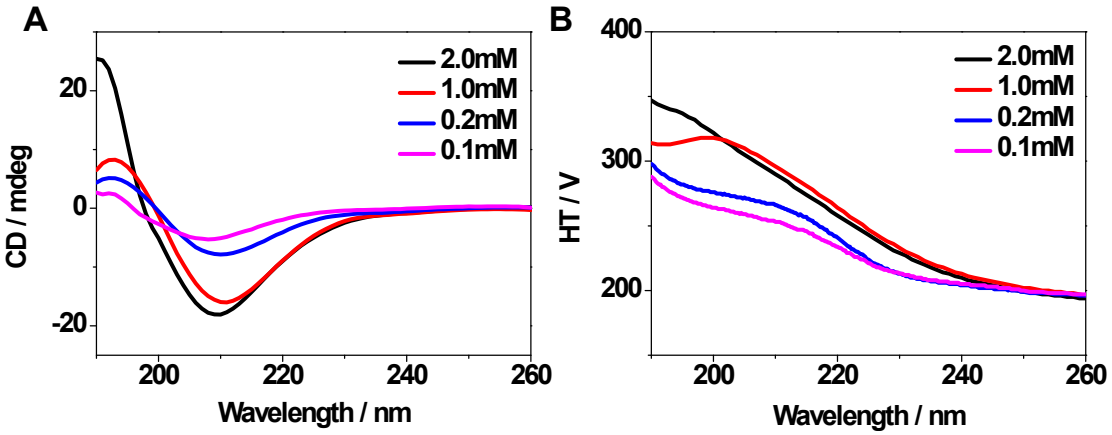


Figure S9. (A) Circular dichroism (CD) of peptides UP at different concentrations and (B) the corresponding high tension (HT) voltages.

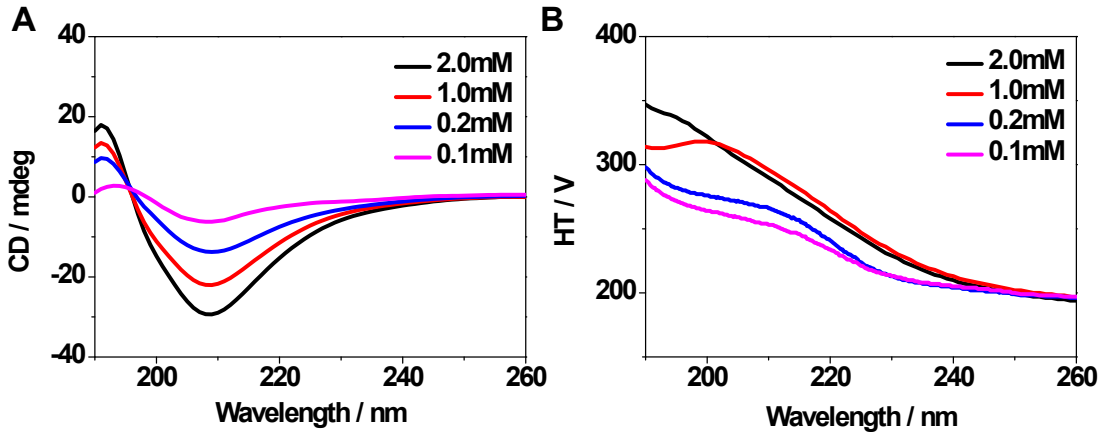


Figure S10. Circular dichroism (CD) of peptides UP-RR at different concentrations and (B) the corresponding high tension (HT) voltages.

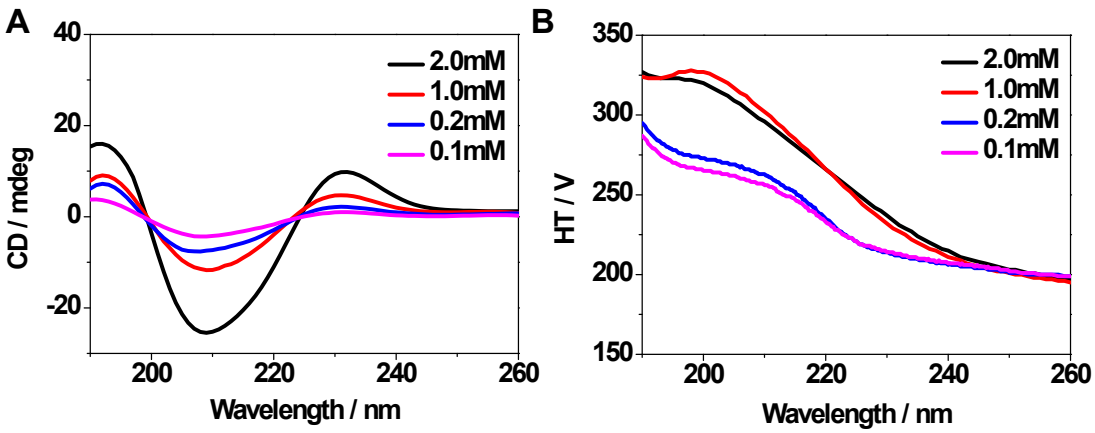


Figure S11. Circular dichroism (CD) of peptides UP-RWR at different concentrations and (B) the corresponding high tension (HT) voltages.

3.3 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra were recorded by using a Tensore II FTIR spectrometer (Brucker). The annealed solutions (2 mM) of undecapeptides were dropped on the quartz detector under atmospheric condition. At room temperature, the infrared spectrum signals were collected in the wavenumber range of 4000 and 400 cm^{-1} and the region covering amide I and II absorption features was shown.

3.4 Thioflavin T (Th-T) Binding Assay

Dye thioflavin T (Th-T) was used to detect the formation of β-sheet amyloid-like structures by undecapeptides through comparing the fluorescence intensity of Th-T in the absence or presence of undecapeptides. The concentration of Th-T and the undecapeptides in all samples was maintained as 50 μM and 2 mM, respectively. Fluorescence spectra were obtained using a HITACHI fluorescence spectrophotometer

F-4600 with an excitation wavelength of 421 nm and the excitation and emission slit widths of 2.5 and 10 nm in the range of 450 and 600 nm.

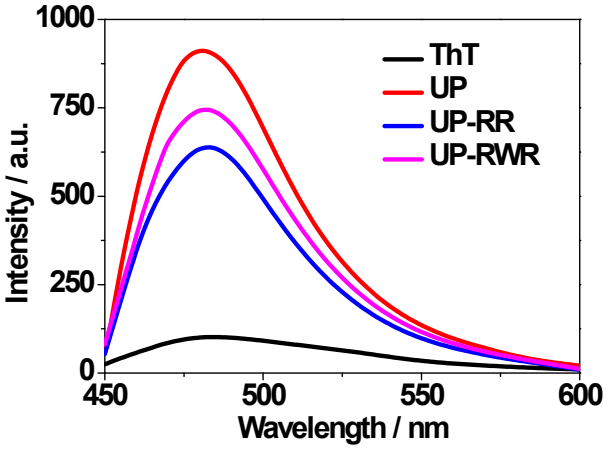


Figure S12. Fluorescence spectra of dye Th-T in the absence or presence of peptides UP, UP-RR, and UP-RWR.

3.5 Confocal Laser Scanning Microscopy (CLSM).

All the samples of the undecapeptides (UP, UP-RR, and UP-RWR) were prepared by adding 2 μL of Th-T solution (0.5 mM) to 48 μL of the annealed undecapeptide solutions (2 mM). The mixed solutions were aged for 24 h and dropped on the carrier glass pieces. After covering the drops with slides, CLSM images of the undecapeptides were recorded by using a Leica TCS SP8 confocal microscope.

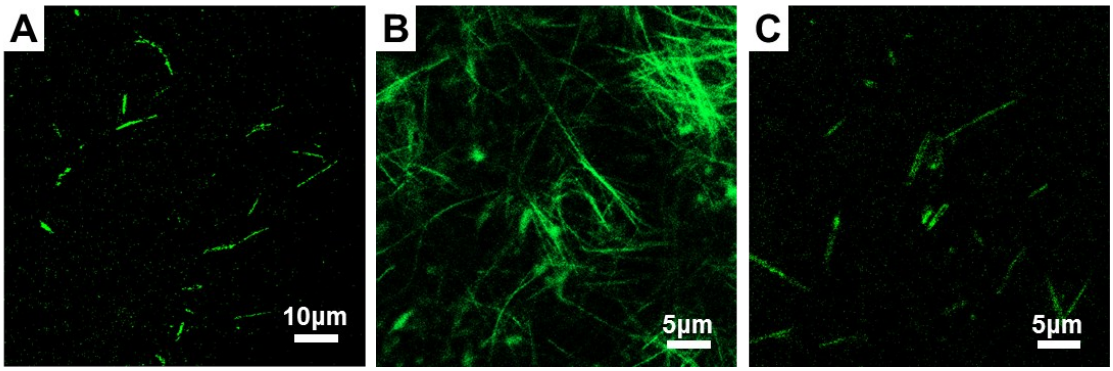


Figure S13. CLSM images of one-dimensional nanostructures formed by peptides UP (A), UP-RR (B), and UP-RWR (C), respectively, stained by Th-T dye.

3.6 Wide-Angle X-Ray Scattering (WAXS) Experiments

After lyophilizing the annealed solution (2 mM), the powders of undecapeptide samples were scanned under conventional conditions for 1 h. WAXS tests were performed on the Xeuss WAXS system (Xenocs, France) at the National Center for Nanoscience and Technology in Beijing.

3.7 Atom Force Microscopy (AFM).

All of the undecapeptides samples (10 μL, 100 μM) were prepared from the annealed solutions by pipetting and depositing on the cleaned mice surface. After 5 min, the surplus liquid on the mica surface was removed by filter paper, and the mica slides were dried under atmospheric environment and were used to perform AFM imaging on a Bruker ICON instrument under the tapping mode.

3.8 Transmission Electron Microscopy (TEM)

TEM images were taken by a Philips Tecnai G2 20 S-TWIN microscope at an accelerating voltage of 100 kV. All the undecapeptides samples (10 μL, 1 mM) were prepared by diluting the annealed solutions and pipetting onto the carbon-coated copper grid for 5 min. After removing the remained solution by filter paper, the grids were dries under the atmospheric environment. Subsequently 8 μL of 2 wt % uranyl acetate was dropped on the grid and stayed for 3 min to stain the sample. Afterwards, the extra staining solution was removed by the filter and the samples were put in a desiccator to be dried prior to measurement.

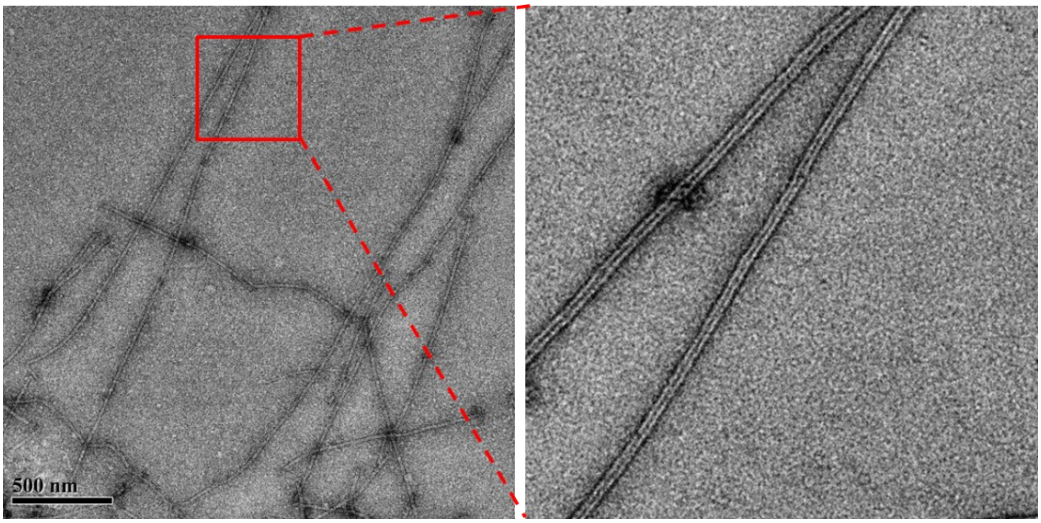


Figure S14. A representative TEM image of the nanotubes formed by peptide UP and a zoomed-in region for clearly showing the hollow nanotubes.

3.9 Diffusion of Peptides in Annealed or Fresh Solutions

To confirm the assembling effect on prolonging the half-life time of peptides, we carried out the in vitro studies of the diffusion of the mutant undecapeptides in either annealed or fresh solution, in which the peptides were suspected to be in a well or

inferior assembled state, respectively. Prior to the dialysis experiments, we prepared a standard curve by plotting the UV/vis absorption intensity at 256 or 280 nm for peptide UP-RR or UP-RWR, respectively, as a function of the concentration for the peptide in the range from 1 to 256 μM (Figure S16). During the dialysis diffusion experiments, 300 μL of the fresh or annealed peptide solution was added to a dialysis tube equipped with 2000-MW-cutoff membrane immersed in PBS buffer at 37 °C, under which only the monomeric peptides were expected to be extracted from the dialysis tube. We periodically monitored the absorption intensity of the annealed solution of peptides UP-RR and UP-RWR at 2, 4, 6, 8, 10, 12, 14, 16, 24, 30, 36, 48 h (Figure S17, A and C). Meanwhile, we also monitored the absorption intensity of the fresh solution of peptides UP-RR and UP-RWR at 0.5, 1, 1.5, 2.5, 4.0, 5.0, 8.0 h (Figure S17, B and D).

The released peptides from the dialysis tube with 2000-MW-cutoff membrane were quantified by comparing the UV/vis absorption intensity of the remained peptides in the tube with the standard absorption intensity curves. Eventually, the diffusion profiles of the peptides in the annealed or fresh solutions were plotted as a function of time (Figure S18). The results showed the significant increase of the diffusion time of the annealed peptides UP-RR and UP-RWR compared to the corresponding peptides in fresh solutions. Within 8 hours, the peptides UP-RR and UP-RWR in fresh solution diffused through the membrane with a percentage over 90%, whereas less than 30% peptides in the anneal solutions were extracted. These in vitro studies potentially indicate the prolonged half-life time of well-defined assembled peptides potentially arising from the slow-releasing effect.

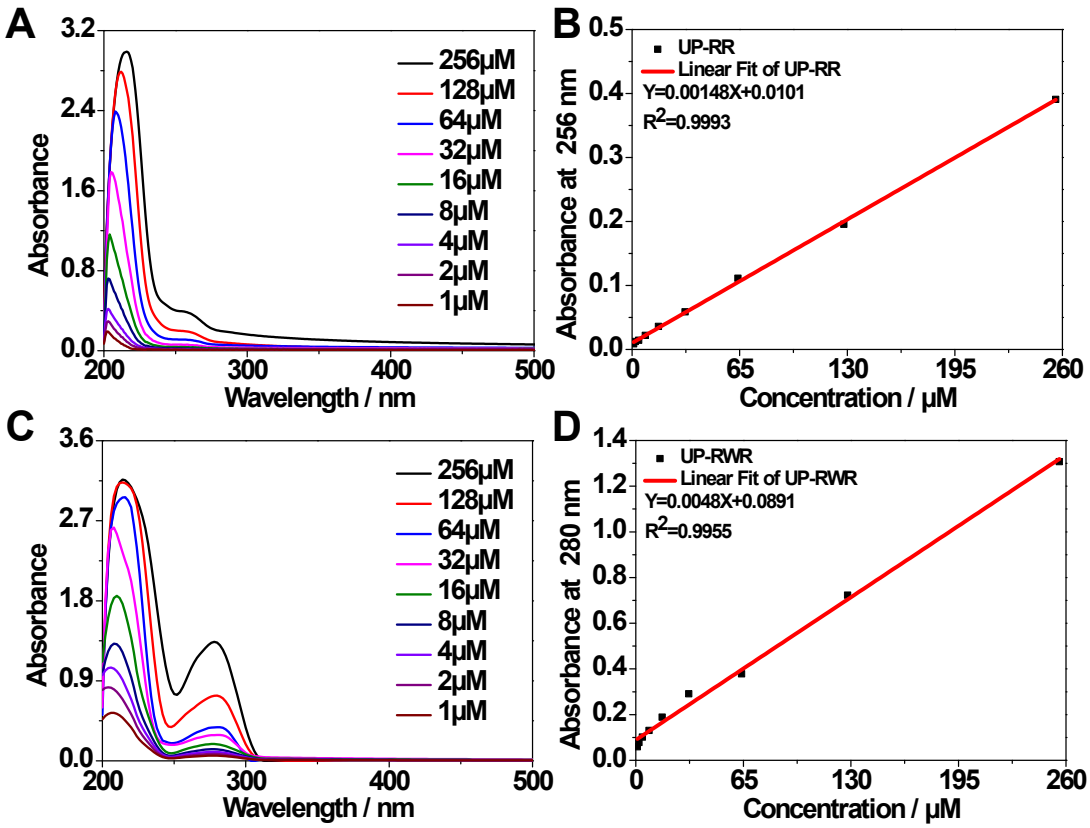


Figure S15. UV/vis absorption spectra of peptides UP-RR (A) and UP-RWR (C) at different concentrations. The standard UV/vis absorption curve of UP-RR (B) and UP-RWR (D) at 256 and 280 nm, respectively, as a function of its concentration in the range of 1 to 256 μM, which showed a linear absorption-concentration relationship.

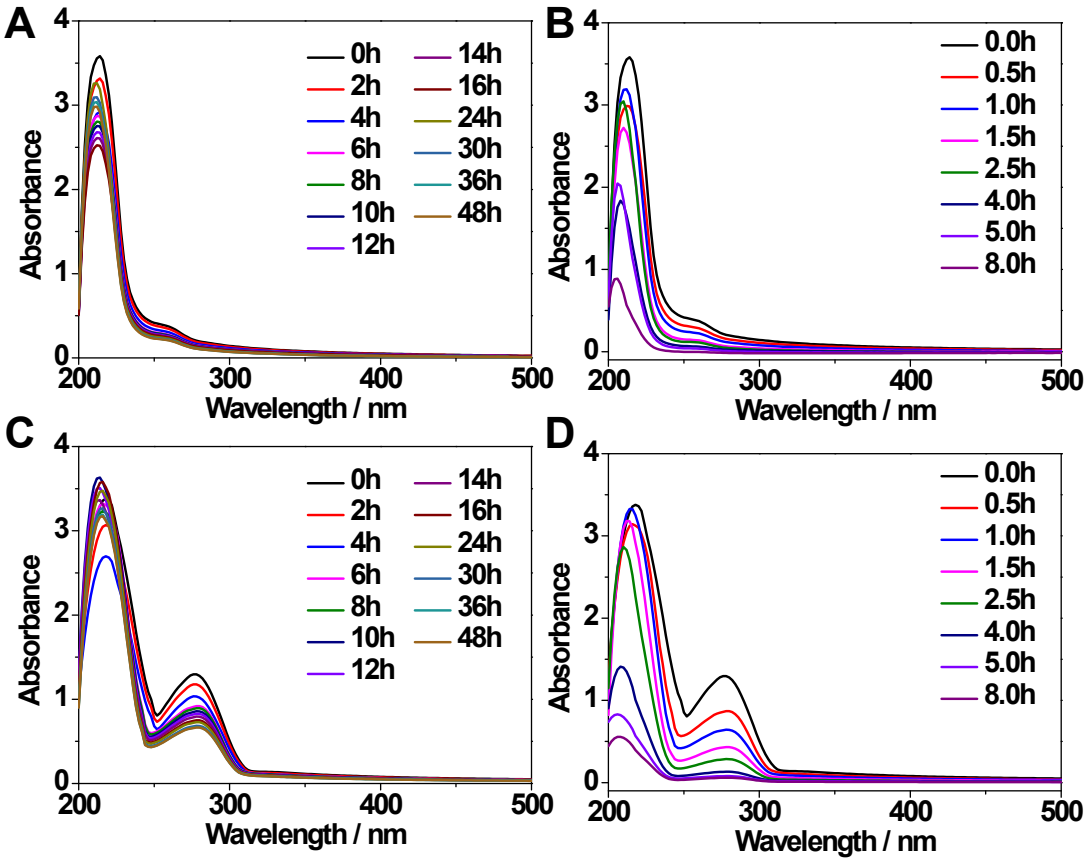


Figure S16. UV/vis absorption spectra of peptide UP-RR at a concentration of 256 μM in the annealed (A) or fresh (B) solution, or peptide UP-RWR at a concentration of 256 μM in the annealed (C) or fresh (D) solution, sealed in the dialysis tube immerged in PBS at 37 °C for different time points.

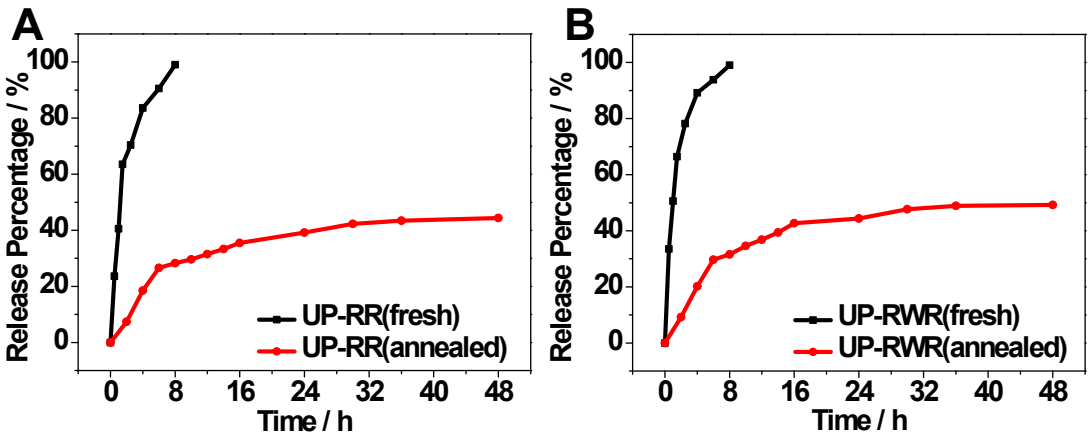


Figure S17. The diffusion profiles of peptides UP-RR (A) and UP-RWR (B) in annealed or fresh solution during the dialysis studies at different time points in PBS.

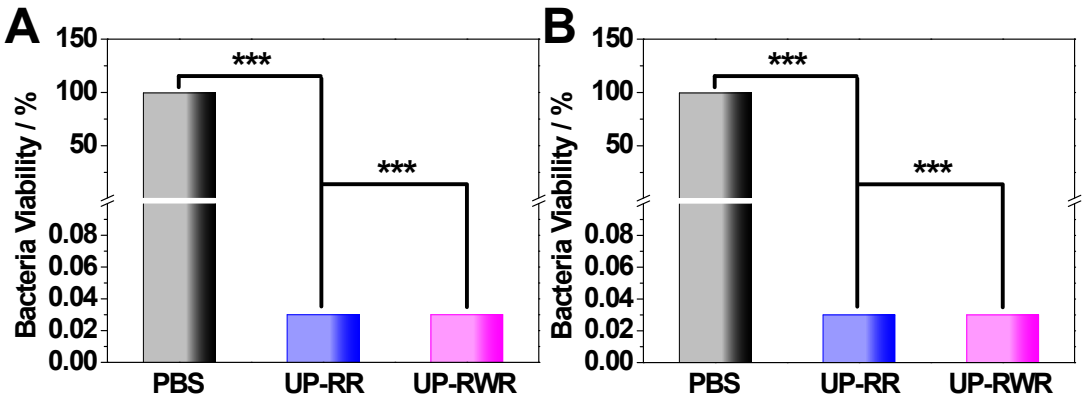


Figure S18. Bacteria viability of *E. coli* (A) and *S. aureus* (B) treated with PBS, peptide UP-RR at a concentration of 64 μM or peptide UP-RWR at a concentration of 128 μM. Difference analysis: ***p<0.001.

Table S1. Minimal inhibitory concentration and Minimal bactericidal concentration of peptides UP, UP-RR, and UP-RWR against Gram-negative bacterium *E.coil* and Gram-positive bacteria *S.aureus*, *B. amyloliquefaciens*, and *S. mutans*.

Peptide	Minimal inhibitory concentration (MIC) / Minimum bactericidal concentration (MBC) (μM)			
	E. coil [ATCC 8739]	*S. aureus* [ATCC 6538]	*B. amyloliquefaciens* [ATCC 23842]	*S. mutans* [CGMCC1.2499]
UP	>128 / > 128	>128 / > 128	128 ± 0.4 / > 128	64 ± 1.8 / > 128
UP-RR	32 ± 2.1 / 64 ± 0.4	32 ± 1.3 / 64 ± 0.1	16 ± 0.6 / 32 ± 0.3	32 ± 0.2 / 64 ± 0.4
UP-RWR	64 ± 0.4 / 128 ± 0.3	64 ± 1.5 / 128 ± 0.2	32 ± 0.7 / 64 ± 0.1	32 ± 0.2 / 64 ± 0.2