Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for

Self-assembly of Cationic Multidomain Peptide Hydrogels: Supramolecular Nanostructure and Rheological Property Dictate Antimicrobial Activity

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Materials

Fmoc protected amino acids and 2-(6-Chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) were purchased from Novabiochem. Diisopropylethylamine (DIPEA), triisopropylsilane (Tis), piperidine, Mueller Hinton Broth (MHB), XTT sodium salt and menadione were purchased from Sigma-Aldrich. N,N-dimethylformamide (DMF), 10X phosphate buffered saline, acetic anhydride, trifluoroacetic acid (TFA), diethyl ether, agar powder and 0.5 ml syringes were purchased from Fisher Scientific. Bacterial live/dead viability kit was purchased from Life Technologies. Dialysis tube was purchased from Spectrum Laboratories. Confocal dishes were purchased from MatTek. All the materials were used as received.

Methods

Hydrogel formation

Peptide stock solution was prepared by dissolving desired amounts of lyophilized peptide powder in deionized water. The actual concentration of peptide solution was accurately determined by UV-spectroscopy and the peptide solution then was diluted to the desired concentration and used as a stock solution. Peptide hydrogel (2 wt%) was prepared by equal volumetric mixing of peptide stock solution (4 wt%) and 2X PBS in a 0.5 ml syringe. Peptide hydrogel (0.5 wt%) was prepared by equal volumetric mixing of peptide stock solution (1 wt%) and 2X PBS in a 0.5 ml syringe.

Fourier Transform Infrared (FTIR) Spectroscopy

A drop of peptide hydrogel (2 wt%) was dropped onto the ATR plate by syringe delivery. Afterwards, the droplet was allowed to be completely air dry. Spectra were obtained by using a FTIR spectrometer (Agilent technologies, Cary 630) with a resolution of 2 cm^{-1} in the range of 4000-400cm^{-1}. The data are the average of 128 scans. Deconvolution was resolved by using OMNIC software.

Circular Dichroism (CD)

CD spectra were acquired on a Jasco-J715 Spectropolarimeter using a quartz cell with 1 mm path length. Peptides (0.05 wt%) were transferred into the quartz cell by syringe and

then examined on the CD spectrometer. All the data were collected from 250 nm to 190 nm at room temperature for 10 scans with each scan rate set at 100 nm/min, a response time of 2 sec, and a bandwidth of 1 nm. mDeg of rotation was converted to molar residual ellipticity via the formula q=(mDeg*1000)/(c*n*l), where c is the concentration of the peptide solution expressed in mM, n is the number of amino acids in the peptide sequence and l is the path length of the cell used in mm.

Minimum Inhibition Concentration (MIC) Assay. The MIC of each peptide against different bacteria was measured using the broth micro-dilution method. A bacterial suspension was diluted to 10^5 CFU/mL in MHB medium. Peptides at varying concentrations (1600, 800, 400, 200, 100, 50, 25, and 12.5μM) were prepared by serial dilution and 10 μL of peptide solution was added to 90 μL of bacterial suspension of *S. aureus* in a 96-well microtiter plate. The UV absorbance at 600 nm of the bacterial suspensions was measured after 36 hrs. The MIC was reported as the minimum concentration of the peptides required to completely inhibit bacterial growth. In order to ensure an aseptic handling environment, a negative control of uninnoculated broth was included in each MIC test. Each MIC test was reproduced three times with three replicates per experiment.

XTT-menadione Assay

2 μl of bacterial suspension (10^8 CFU/ml) was innoculated onto an agar plate. 40 μl of hydrogel was delivered by syringe to cover the bacterial-loaded agar surface, followed by incubation for 16 hrs at 37°C. The hydrogel was then gently removed along with the underlying agar and transferred to 10 ml MHB medium (23 g/L) for resuspension of the bacteria. Freshly prepared XTT/deionized water solution (1mg/mL) and menadione/deionized water solution (0.4 mM) were mixed at a 5:1 volumetric ratio. 14.4 μl of the mixed solution then was added to 120 μl of the bacterial suspension, followed by incubation for 30 minutes at 37°C. 100 μl of the final mixture was then transferred to 96-well plate for absorbance measurement at 450 nm. Bacteria without peptide hydrogel treatment were used as a control. Each assay was performed in triplicates in two independent experiments.

Scanning electron microscopy (SEM)

A droplet of peptide hydrogel was delivered to the silicon wafer surface by syringe. Excess sample was wicked away with filter paper after incubation for 1 hr. Samples were then subjected to air-drying. Sample was sputter coated with a 5 nm thick gold layer for imaging using a JEOL 7400 high-resolution field emission electron microscope.

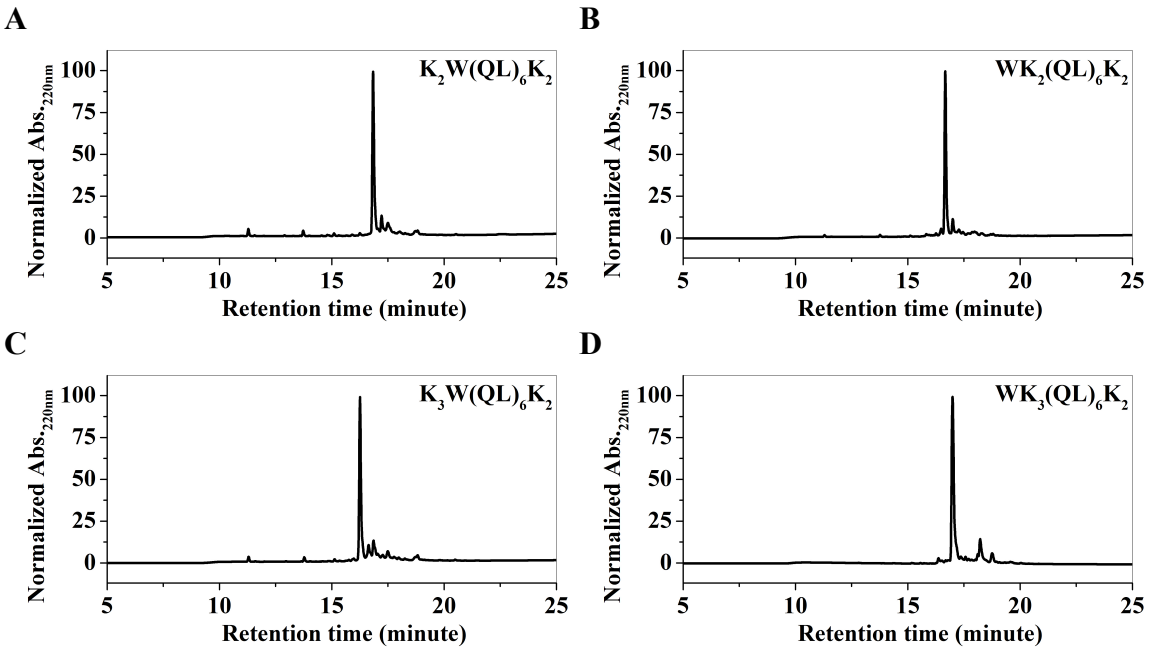

Figure S1. HPLC spectra of MDPs (A) $K_2W(QL)_6K_2$ (B) $WK_2(QL)_6K_2$ (C) $K_3W(QL)_6K_2$ and (D) $WK_3(QL)_6K_2$

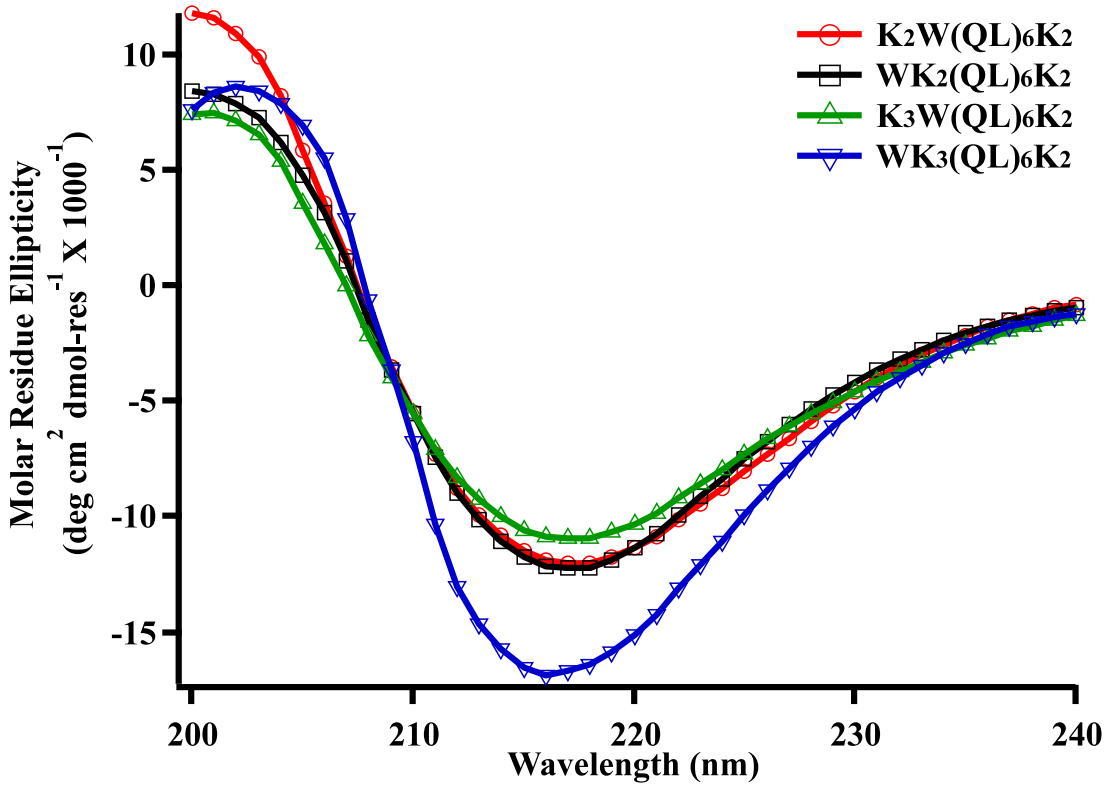

Figure S2. Circular dichroism spectra of peptide hydrogels (0.05 wt%) showing the β-sheet conformation as characterized by the negative absorption at 216 nm.

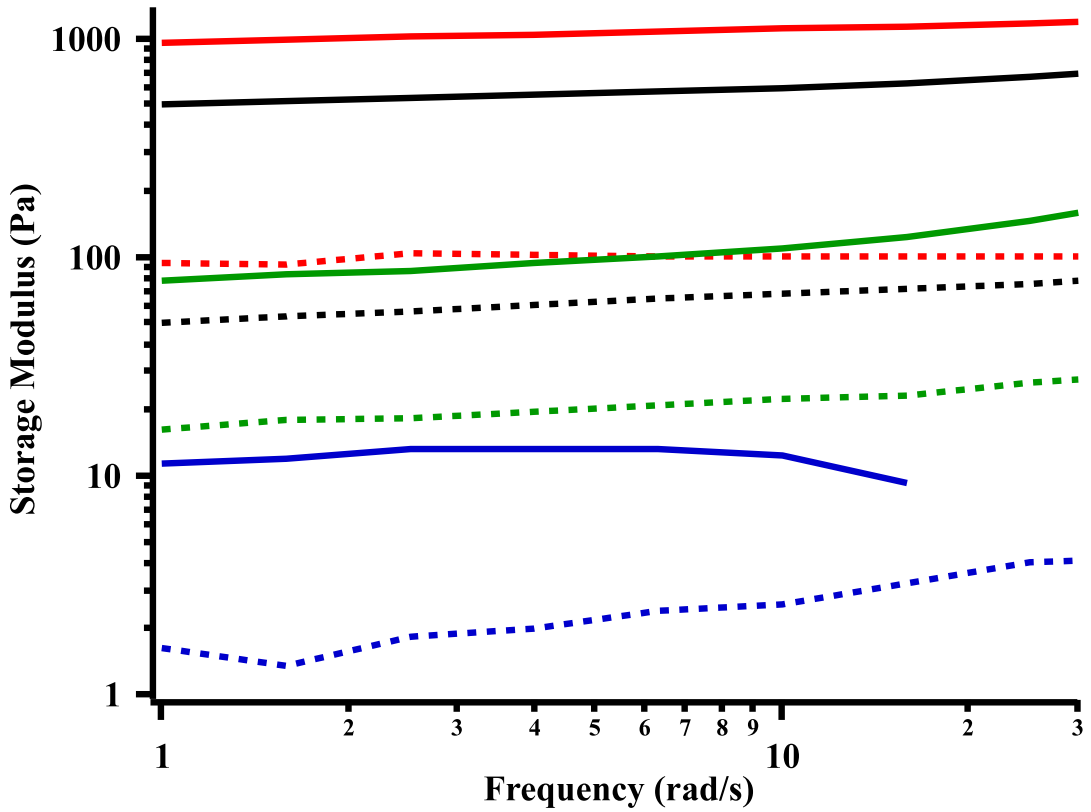

Figure S3. Rheological properties of peptide hydrogels (2 wt%) during frequency sweep, strain: 0.2%. Solid line: storage modulus; dashed line: loss modulus. $K_2W(QL)_6K_2$ in red, $WK_2(QL)_6K_2$ in black, $K_3W(QL)_6K_2$ in green, $WK_3(QL)_6K_2$ in blue.

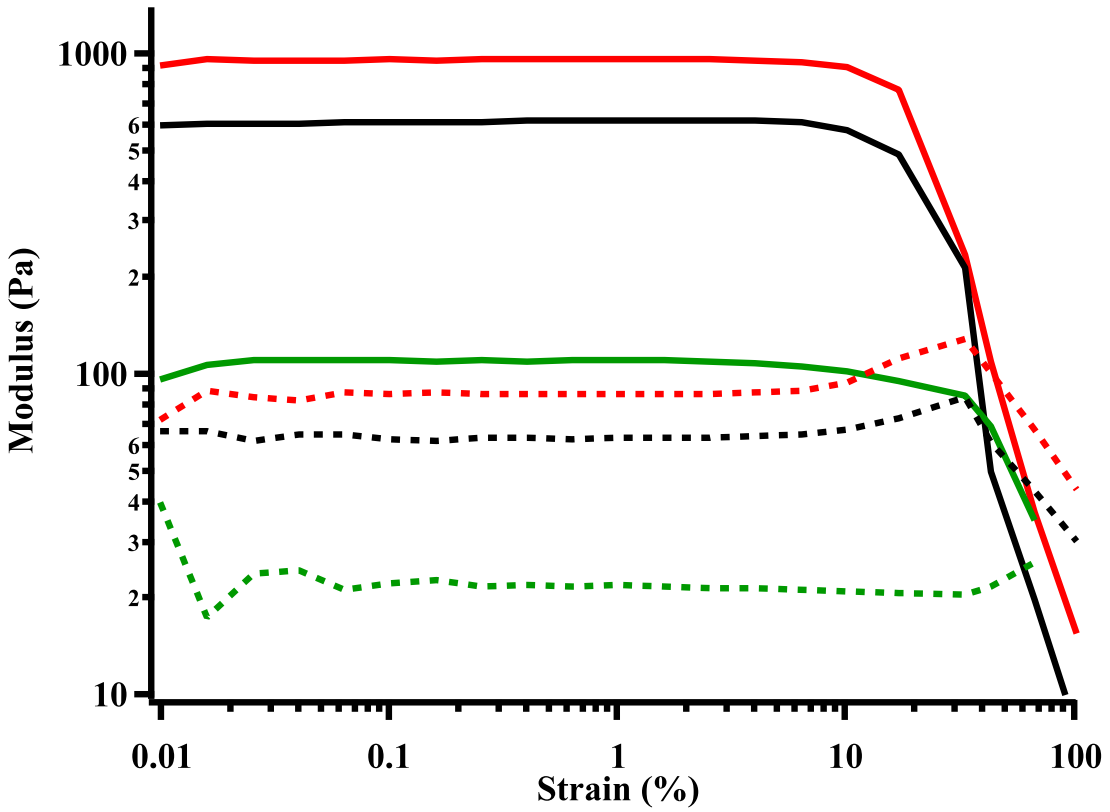

Figure S4. Rheological properties of peptide hydrogels (2 wt%) during strain sweep, frequency: 6 rad/s. Solid line: storage modulus; dashed line: loss modulus. $K_2W(QL)_6K_2$ in red, $WK_2(QL)_6K_2$ in black, $K_3W(QL)_6K_2$ in green.

Figure S5. SEM image of $K_2W(QL)_6K_2$ hydrogel (2 wt%).