Supporting Information

Antimicrobial D-Peptide Hydrogels

Zhen Guo,[1, 2, 3] Yujiao Wang, [1, 2, 3] Tingyuan Tan, [1, 2, 3] Yuwen Ji, [1, 2, 3] Jun Hu[1, 2] and Yi Zhang[1, 2*]

[1]Key Laboratory of Interfacial Physics and Technology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China
[2]Zhangjiang Lab, Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201210, China
[3]University of Chinese Academy of Sciences, Beijing 100049, China

*: Corresponding author. Email: zhangyi@sinap.ac.cn

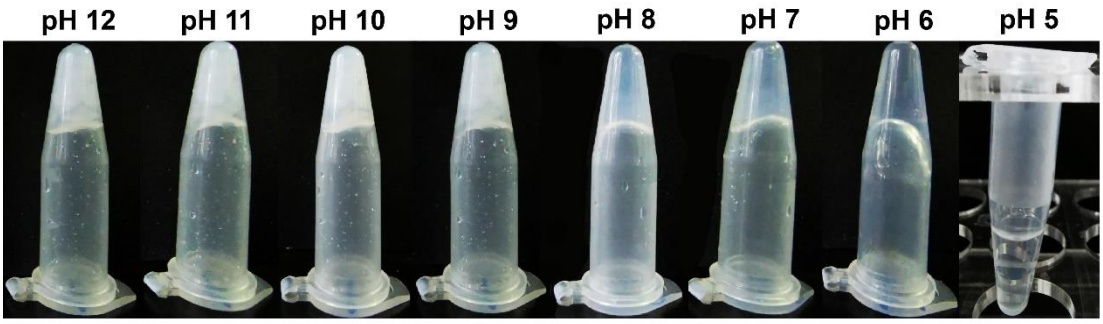

Fig. S1 Optical images of the gels and clear solutions formed by KKd-11 with a concentration of 10 mg/mL under different pH conditions.

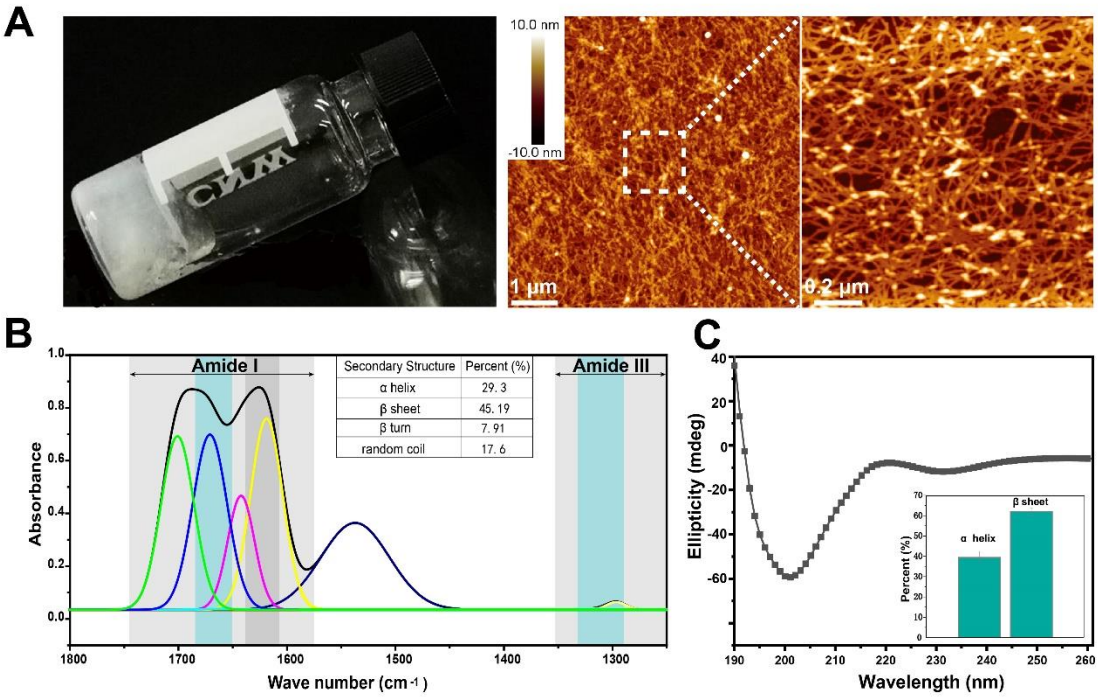

Fig. S2 (A) Optical image (left panel) of the KK-11 peptide hydrogel in a tilted tube and topographic AFM images (middle and right panels) of the surface of KK-11 peptide hydrogel. (B) FTIR and (C) CD spectra of KK-11 hydrogels. The KK-11 hydrogel was formed at a peptide concentration of 10 mg/mL under pH 10.

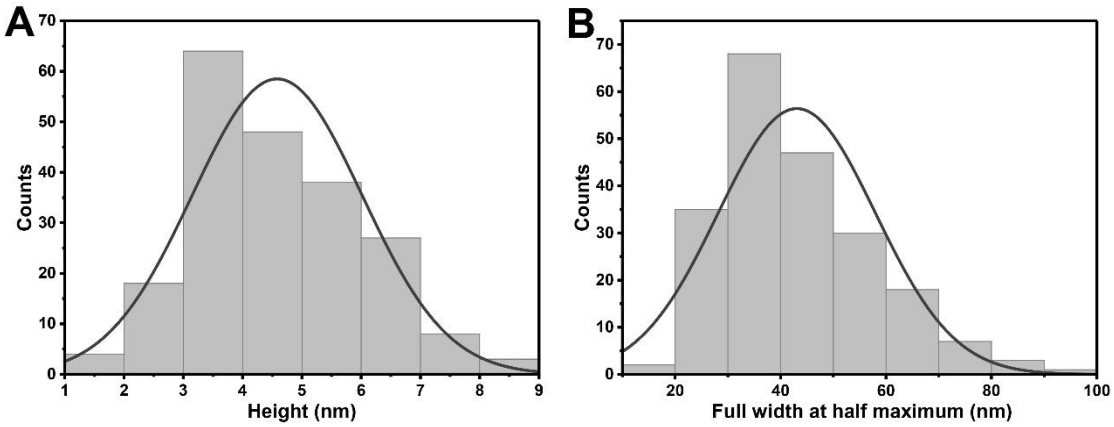

Fig. S3 Histograms of (A) height and (B) full width at half maximum (FWHM) of the peptide nanofibers on the KKd-11 hydrogel surface as measured by AFM. In total 200 fibrils randomly selected from different images were measured.

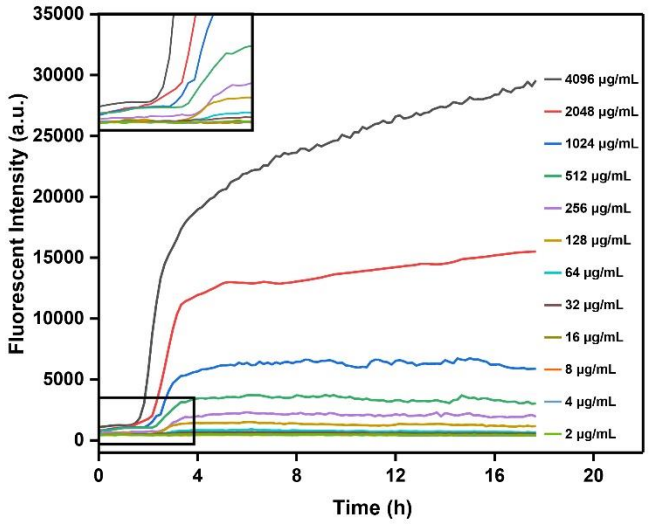

Fig. S4 Dynamic monitoring of ThT fluorescence intensity in the KKd-11 solutions with varied concentrations. ThT fluorescence measurements were performed at 37 °C using a Thermo Scientific Fluorskan Ascentsystem (Thermo Fisher Scientific, USA) in quiescence. The excitation and emission wavelengths were 440 nm and 485nm, respectively. Fluorescence was measured immediately after preparing a reaction mixture containing 10 μM ThT. At least 3 different samples were independently measured to obtain the average of the fluorescence intensity. The results indicated S-shaped fluorescence curves in the KKd-11 solutions with sufficient concentrations. We think that, at the early stage of peptide assembly (0~2 h), the ThT fluorescence intensity gently increased, which suggest the formation of a small number of nucleation seeds;

during the period of 2~4 h, the ThT fluorescence intensity increased sharply, indicating rapid assembly and fibril growth; after that, the curves evaluated to a plateau in which peptide assembly turned to be completed.

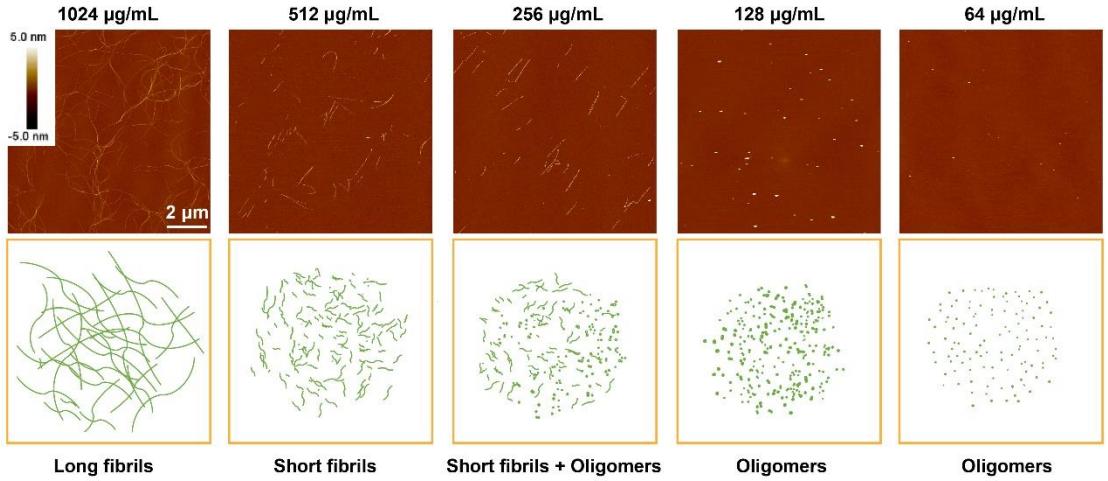

Fig. S5 (Top panels) Typical AFM topography images of the self-assembled nanostructures of KKd-11 peptide deposited on the freshly-cleaved mica substrates. To prepare the sample, a drop of 5μL peptide solution in a certain concentration was placed onto a mica substrate after incubation at 37 °C for 4h. The sample was allowed to adsorb for 2 min and then was dried with airflow. (Bottom panels) Schematic drawings indicating the assembled nanostructures of the KKd-11 peptides at different concentrations.

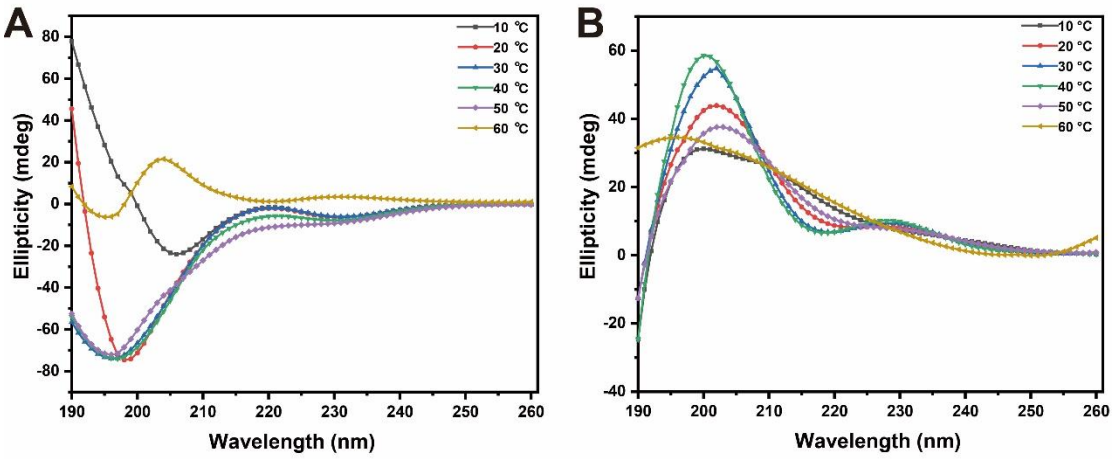

Fig. S6 CD spectra of gels formed by KK-11 (A) and KKd-11 (B) measured at different temperatures.

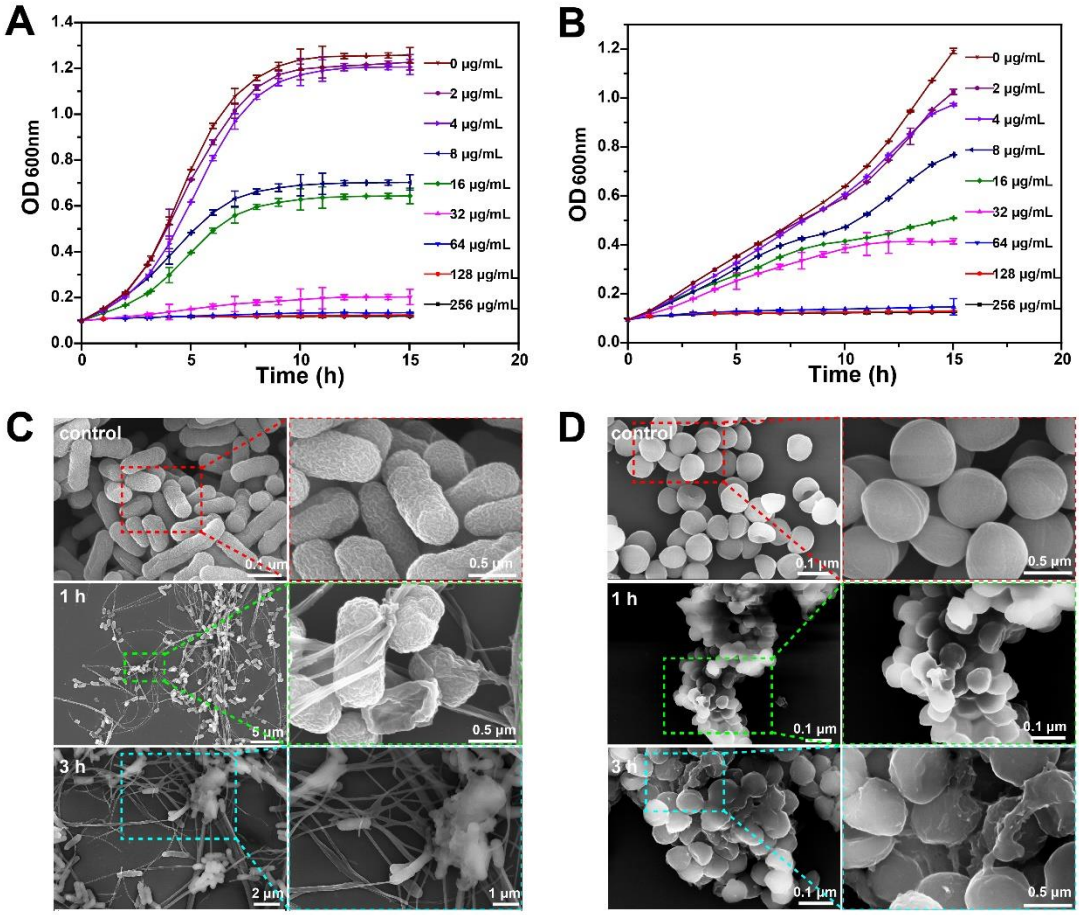

Fig. S7 (A, B) Growth inhibition profiles of the KK-11 against *E. coli.* (A) and *S. aureus* (B), respectively. (C, D) SEM micrographs of *E. coli* and *S. aureus* treated with 1 mg/mL KK-11.

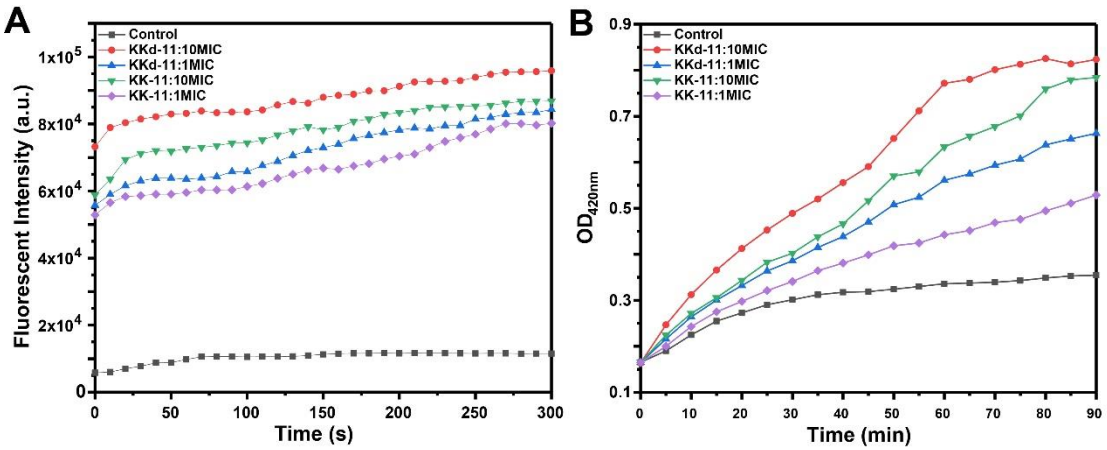

Fig. S8 Outer and inner membrane permeability assay. (A) The intake of NPN by *E. coli* in the presence of KKd-11 or KK-11. *E. coli* was cultured in Luria–Bertani (LB) medium at 37 ℃ to the mid-logarithmic stage and then washed and resuspended in HEPES buffer (5 mM HEPES, 5 mM glucose, pH 7.4) to an $OD_{600\ nm}$ of 0.5. NPN was dissolved in acetone and added to the cell suspension to a final concentration of 10 μM. The KKd-11 or KK-11 peptides were dissolved in

the HEPES buffer and mixed with bacteria 3 minutes before measurement. The control group used PBS instead of the peptides. The fluorescence spectra and emission intensities were measured with a spectrophotometer (Edinburgh Instruments FS920, UK) with excitation λ = 350 nm, emission λ = 420 nm, and $\Delta\lambda$ = 5 nm at 37°C. The fluorescence background of the mixture of peptide and NPN was subtracted from all the experimental groups. (B) The amount of ONPG intake ingested by *E. coli* incubated with KKd-11 or KK-11. *E. coli* were cultured in an LB medium containing 2% lactose to the mid-logarithmic stage. The addition of lactose was to induce the production of β-galactosidase by the cells. The cells were spun down at 1000 rpm for 1 min and then resuspended in PBS (pH 7.4). After washing three times, the cells were diluted to an $OD_{600\ nm}$ of 0.5 by PBS containing peptides. In the control group, the peptide was replaced with PBS. Then, the cells were mixed with ONPG, and the mixtures were added into 96-well plates, in which each well contained cells (100 μL) and ONPG (10 μL). The mixture was shaken for 5 s before measurement. $OD_{420\ nm}$ was recorded every 2 min for 1.5 h at 37 °C in a plate reader (VersaMax Microplate Reader).

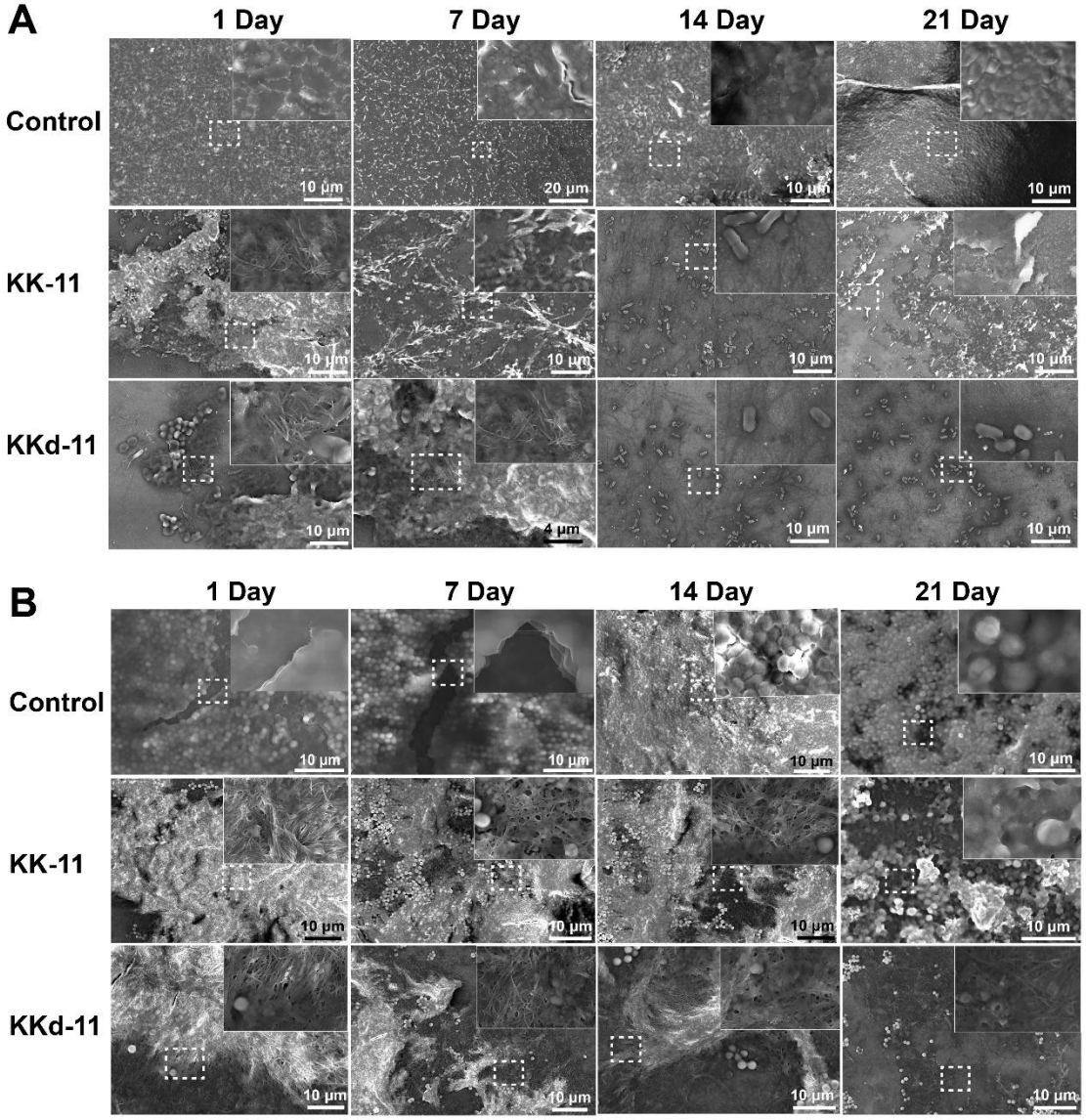

Fig. S9 SEM images of the biofilms formed on the surfaces of silicon wafers after incubation with the bacteria for certain days. (A) *E. coli. (B) S. aureus*.

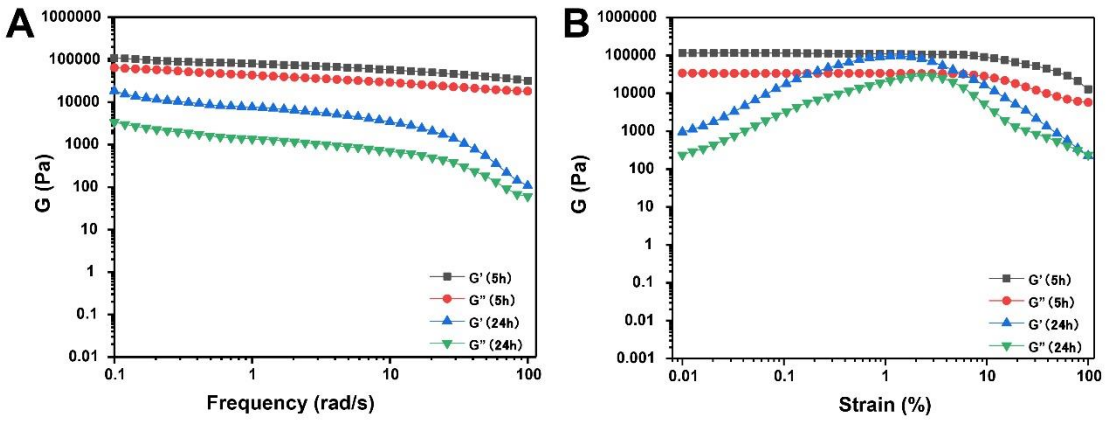

Fig. S10 Rheological properties of KKd-11 hydrogels (pH=10) after incubation with protease K

(concentration of 3.2 units/mL) at 37°C. Storage modulus (G') and loss modulus (G") during (A) frequency sweeping and (B) strain sweeping.