Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information for

Enzymatic Assembly of Adhesive Molecular Networks with Sequence-Dependent Mechanical Properties Inspired by Mussel Foot Proteins

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Materials and Methods

Gene Construction. *Escherichia coli* strain DH5α was used for molecular cloning. The synthetic DNA fragments (Sangon Co.) encoding GRKYYGRGD (TM3-GRGD), GYKKYYGRGD (TM5-1-GRGD) and KKYYYKYGRGD (TM5-2-GRGD) were inserted into the N-, C-termini and the middle of ELP within the pQE80l-*ELP* plasmid using restriction site pairs: Nhe1/Sal1, Sac1/Spe1, Xho1/Kpn1 for N-terminus, center, and C-terminus respectively. The GRGD cell-binding ligand was introduced to enhance cell adhesion.

DNA sequences of TM fragments with RGD sites. TM3: GGACGAAAGTATTATGGTCGTGGTGAT, **TM5-1:** GGTTACAAGAAGTATTATGGTCGTGGTGAT, **TM5-2:** AAGAAATACTATTATAAATATGGTCGTGGTGAT.

Protein Expression and Purification. *E. coli* BL21 (DE3) cells transformed with the desired construct were grown in LB at 37ºC until an OD_{600} of ~0.8. Then the protein expression was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG, 500 μM). After 4 h at 37ºC, cells were harvested. The cells were then lysed through sonication on ice at 150 W power, 0.5-sec interval with a ¾ inch disruptor horn for 3 min. The supernatant was obtained through centrifugation at 12000 g and collected for protein purification. The proteins were purified by HisTrap HP columns (GE Healthcare, Inc.) following the manufacturer's instructions. Protein purity was assessed by SDS-PAGE. The resulting proteins were dialyzed against MilliQ water at 4°C, flash frozen with liquid N_2 and lyophilized for 60 h. Lyophilized protein powders were stored at -80ºC before use.

MALDI-TOF Mass Spectroscopy. Lyophilized TM proteins were dissolved in MilliQ water to a concentration to 50 μM. The protein solutions were then analyzed with Bruker Daltonics ultraflex MALDI-TOF/TOF at the Biosciences Central Research Facility HKUST.

Reconstitution of Tyrosinase. *Streptomyces antibioticus* tyrosinase was produced using standard recombinant technology and *E. coli* expression system. The gene encoding *S. antibioticus* tyrosinase was purchased as gBlocks™ from Integrated DNA Technologies. This gene was cloned into pACYCDuet plasmid and expressed in *E. coli* BL21(DE3) in TB medium. The *E. coli* cells were grown at 37°C until OD_{600} reached 0.6-0.8. The protein expression was induced with 500 μM IPTG at 16°C for 20 h. Tyrosinase was purified with HisTrap HP columns (GE Healthcare Inc.) following the manufacturer's instructions. Tyrosinase was dialyzed against the Dialysis Buffer (50 mM sodium phosphate, 0.01 mM $CuSO_4$, pH 6.5) at 4 °C. The resulting protein solution was diluted to 1.0 mg/mL, frozen with liquid N_2 and then stored at -80°C before use.

Preparation of Hydrogels. Lyophilized protein powder was dissolved in PBS to make a 10 wt% solution. Tyrosinase (1 μg) was added to 60 μL of protein solution to induce gelation. The reaction mixture—a sitting drop with 8 mm in diameter—was placed on parafilm inside a petri dish. Wet Kimwipe was used to provide moisture within the petri dish. The gel was cured for 12 h to ensure complete reaction.

Dynamic Shear Rheology. Rheological measurements were performed on a TA Instruments ARES-RFS strain-controlled rheometer with a standard steel parallel-plate geometry (8-mm diameter). The gap was set at 0.9 mm. The mounted sample was sealed with silicone oil to prevent water evaporation.

Test modes included dynamic frequency- and strain-sweep tests. The frequency-sweep test was performed by holding the strain at 10% and varying the frequency from 100 to 0.1 rad/s. The strain-sweep test was performed by fixing the frequency at 10 rad/s and increasing the strain from 0.1 to 200%. All experiments were performed at room temperature.

Adhesion Tests. Adhesive strength was measured by using the lap shear adhesion test. The PMMA interface (25 mm × 25 mm) was glued by the mixture of 10 μL of protein solution (10 wt%) and tyrosinase (0.17 μg). The reaction was cured for 12 h inside a sealed container that was half filled with water to maintain 100% relative humidity. The Instron testing machine was set up with a 5 kN load cell and compressed air pressure clamps. The measurements were conducted at room temperature. The samples were extended at a rate of 10 mm/min until rupture point.

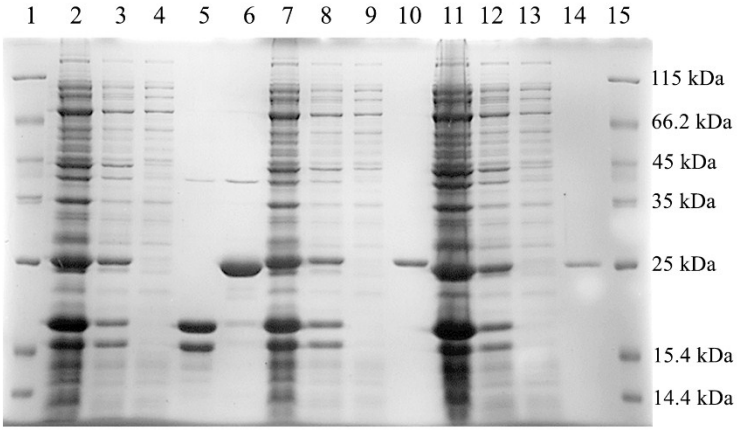
Cell Encapsulation and Confocal Microscopy. The protein powders was dissolved in DMEM (Sigma Aldrich) to a 10 wt% solution. A549 cells [American Tissue Cell Culture (ATCC); CCL-185] were first sub-cultured, harvested, and concentrated to a density of 10^6 cells/mL. A549 cells (~1000 cells) were resuspended within 50 μL of protein solution supplemented with tyrosinase. To accelerate the gelation, 2 μg of tyrosinase was added to 50 μL of protein solution. This cell suspension in protein solution was plated on a bottom coverslip petri dish. The petri dish was flipped every 30 s to ensure full suspension of cells within the gel during the gelation process; this process lasted 15 min. The gel was then immersed with DMEM, incubated at 37°C, 5% CO_2 for 24 h before viability assays and confocal imaging.

LIVE/DEAD™ Viability/Cytotoxicity Kit for mammalian cell (Invitrogen) was used as per the manufacturer's instructions to stain the encapsulated A549 cells. The stained cells were visualized on a Nikon confocal C2+ system with two LED laser sources at 488 and 561 nm. The emission wavelengths were set at 510 and 610 nm for Calcein and Ethidium homodimer-1, respectively. The laser power was at 1% for both excitation wavelengths and the sensor gain was adjusted to 40 and 45 for the emission at 510 and 610 nm, respectively. To reduce noise, each image was scanned 4 times and averaged. The 20× objective was used for z-stack scanning.

Supplementary Table and Figures

Table S1 The amino acid sequences of the full-length MFP-3 and -5. Truncated peptide motifs (TM3, TM5-1 and TM5-2) are shown in red, orange and purple, respectively.

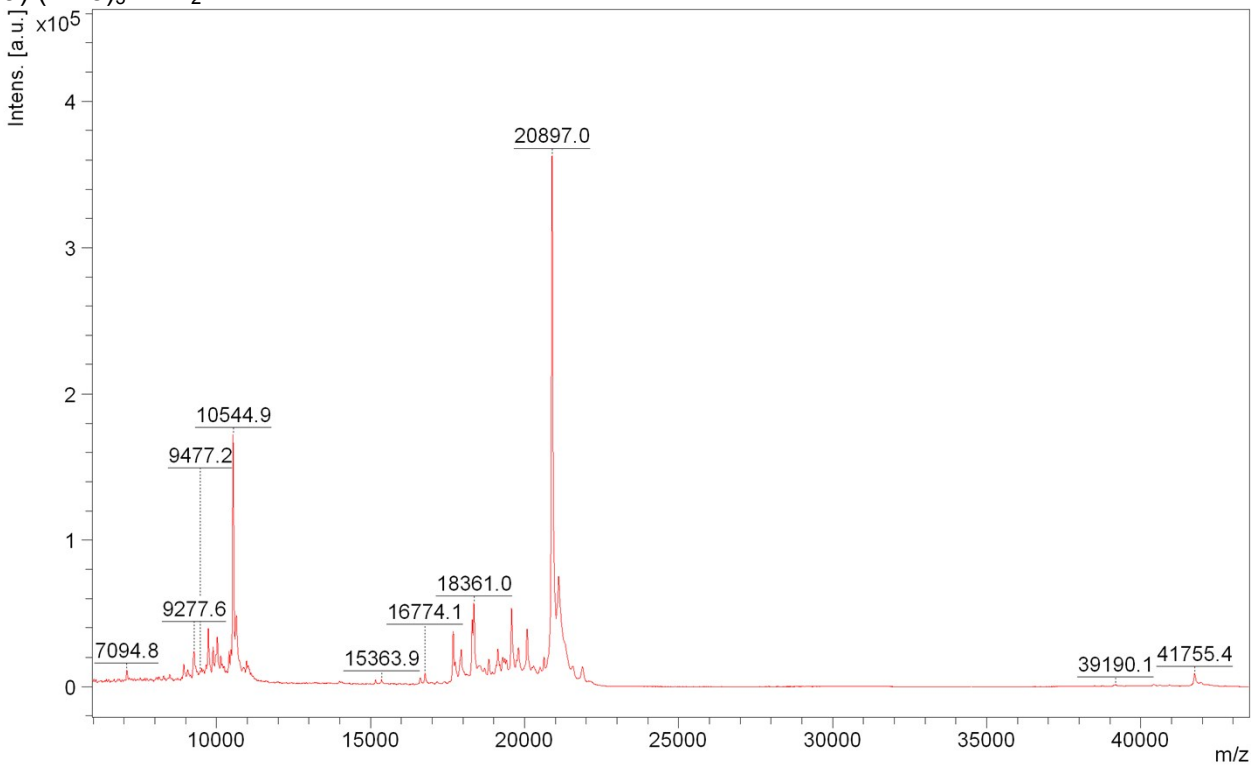
	Amino Acid sequence
Mfp-3	MNNFSVSVLEVLVLIGSFAVESDAAAYYGPNYGPPRRYGGYNGYNRYARGYGGNRGWNR GWNRGWR**GRKYY**
Mfp-5	MKLSCVVLVLFLVTLAACIDVGSGYDGYSDGYYPGSAYNYPSGSHGYHGHGYKGKYYGKG **KKYYYKY**KRTGKYKYLKKARKYHRK**GYKKYY**GGGSS



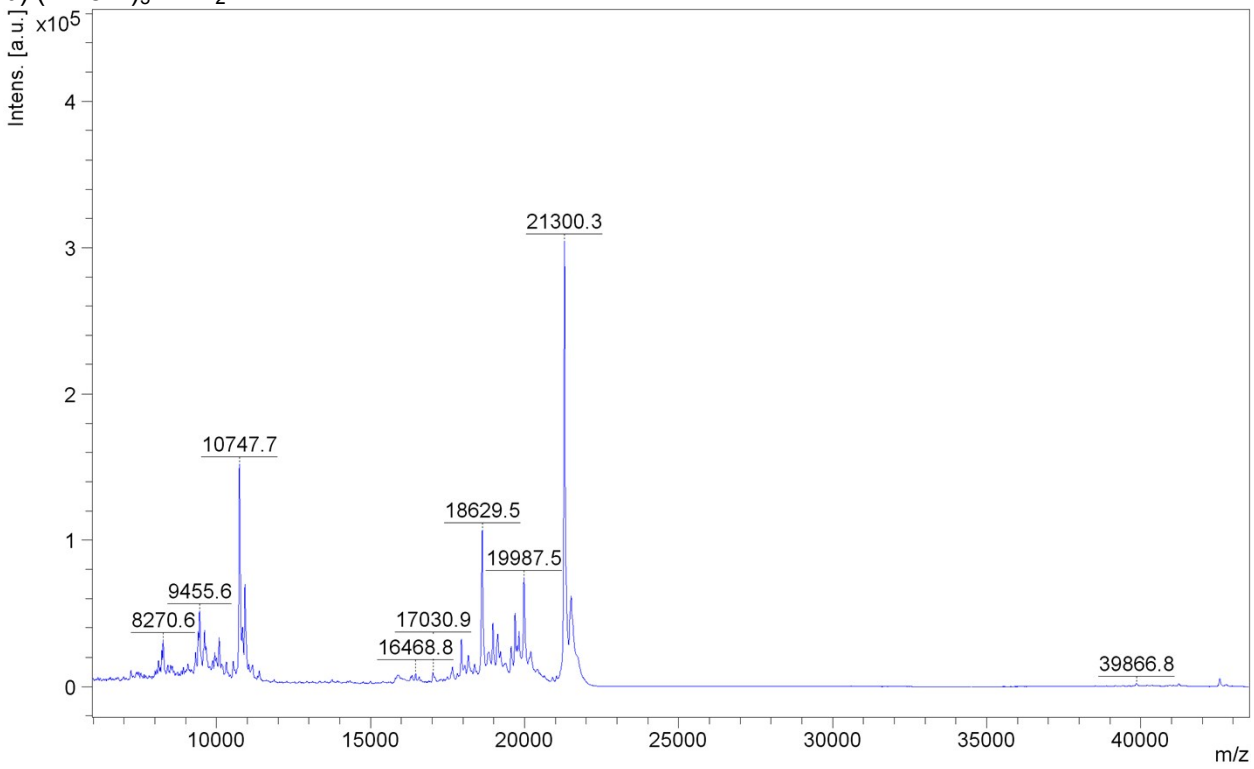
Lane	Protein	Content
1	Ladder	
2	$(TM5\text{-}1)_3$-ELP_2	Cell lysate
3		Supernatant
4		Flow through
5		Elute 1
6		Elute 2
7	$(TM5\text{-}2)_3$-ELP_2	Cell lysate
8		Supernatant
9		Flow through
10		Elute
11	$(TM3)_3$-ELP_2	Cell lysate
12		Supernatant
13		Flow through
14		Elute
15	Ladder	

Fig. S1 SDS-PAGE analysis.

a) $(TM3)_3$-ELP_2



b) $(TM5\text{-}1)_3$-ELP_2



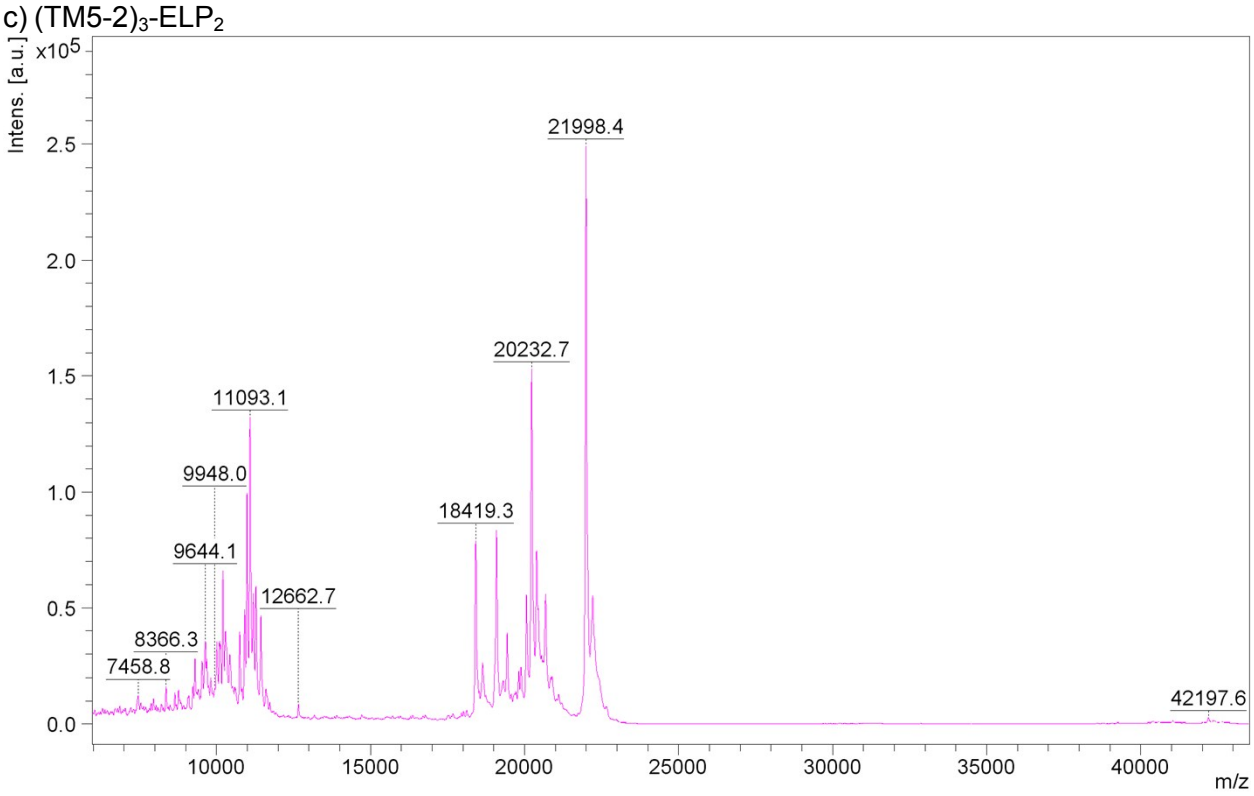


Fig. S2 MALDI-TOF mass spectra of $(TM3)_3$-ELP_2, $(TM5\text{-}1)_3$-ELP_2 and $(TM5\text{-}2)_3$-ELP_2. The theoretical molecular weights of $(TM3)_3$-ELP_2, $(TM5\text{-}1)_3$-ELP_2 and $(TM5\text{-}2)_3$-ELP_2 are 20885.74, 21291.23 and 21994.13 Da, respectively.