Electronic Supplementary Information

Cation-π interaction in DOPA-deficient mussel adhesive protein mfp-1

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1. **Recombinant mussel foot protein-1 (Rmfp-1) and its decapeptide preparation.** Rmfp-1 was prepared from *E. coli* as described previously.[4d] The purity of Rmfp-1 (～95 %) was confirmed using SDS-PAGE and amino acid analysis. To prepare the decapeptide, purified mfp-1 was digested with trypsin. Purified Rmfp-1 (10 mg·mL^{-1}) was dissolved in a 50 mM ammonium acetate buffer, 1 mM $CaCl_2$, pH 8.0 with trypsin (Sigma, 0.1 mg·mL^{-1}), and incubated at 37 °C for 24 h. After the trypsin digestion, the decapeptide was purified by reversed-phase HPLC (Aquapore RP-300 column 250 × 7.0 mm, Brownlee, Perkin-Elmer, USA) at a flow rate of 1.0 mL·min^{-1} and an elution gradient described in a previous report. The size of the decapeptide was confirmed by ESI-MS.
2. **Force measurement by surface forces apparatus (SFA).** The molecular forces of Rmfp-1 were measured using an SFA, as follows. Thin mica sheets (1–5 μm) were glued on two cylindrical silica disks (radius R = 2 cm). 100 μL of an Rmfp-1 or decapeptide solution (10 μg□mL^{-1} in 0.1 M acetic acid) was dropped on the mica surface and incubated for 30 min in a sealed chamber saturated with water vapor. The surfaces were then thoroughly rinsed with buffer solution and mounted onto the SFA chamber in a crossed-cylinder geometry, which locally

corresponds to a sphere of radius R approaching a flat surface based on the Derjaguin approximation.[14] The interaction forces were measured in both symmetric and asymmetric configurations (see Figure 3), and the separation distance between the surfaces was determined in situ and in real time by multiple beam interferometry using the SFA, with fringes of equal chromatic order (FECO).[16] The measured adhesion force F_{ad} is correlated to the adhesion energy per unit area W_{ad} as per the expression $F_{ad} = 1.5\pi RW_{ad}$ for soft deformable materials.[11,14] The use of chloride ions in the buffer solution was avoided to reduce the possible corrosion of the semi-reflecting silver layers under the mica substrates.

3. **Surface topography using atomic force microscopy (AFM).** 100 μL of Rmfp-1 or decapeptide solution (10 μg·mL^{-1} in 0.1 M acetic acid) was dropped on freshly cleaved mica surface and incubated for 30 min in a sealed chamber saturated with water vapor. The surfaces were thoroughly rinsed with the buffer solution (0.1 M acetic acid, pH 3.0) in order to remove unbound proteins. The surfaces were imaged using a Nanoscope III AFM (Veeco, Santa Barbara, CA, USA) using a silicon nitride probe (Olympus, Tokyo, Japan), with a spring constant of 0.35 N m^{-1}, in tapping mode at room temperature (~23°C) in dry condition.
4. **Quantum simulation method.** The strength of the cation-π interaction was measured by ab initio quantum mechanical calculation using Gaussian09.[13] For simplicity, only single lysine and single tyrosine were considered in the calculation. Initial configurations for the structure optimization were prepared from an all-atomic level NVT molecular dynamics simulation, with a length of 1.2 ns at 1 fs time steps after standard systematic optimization; heating, cooling, and NPT MD were performed to

adjust the molecular density of water. Four hundred configurations, in which two residues were closely interacting, were selected from the MD simulation and used as initial configurations for the quantum mechanical optimization, which was carried out with a 6-31+G(d) basis set and the RHF theory with an SMD solvation model.[17]

5. **Rmfp-1 conformation.** All atomic scale MD simulations were performed for 6.8 ns with a time step of 1 fs within the NVT ensemble using AMBER11 within a ff99SB force field in order to explore the molecular structure of Rmfp-1.[12] Salt (0.1 M) and water were treated implicitly. The initial conditions were decided after systematic heating from 0 K to 350 K and subsequent cooling to 300 K from a random configuration that AMBER generates from the PDB file. Unlike the randomly coiled polymer, the simulation results show anisotropic configurations that resembles elongated ellipsoids (Figure S1). This anisotropy may have originated from the proline-induced secondary structures (PPII) and the strong line charge density, 1.26 $e \cdot nm^{-1}$, as noted in the manuscript. It is noted that the configuration we determined resembles one of the candidate structures predicted by I-TASSER (Figure S2).For a more detailed analysis of the conformation, we calculated the axial lengths of the polymers from the simulations. We used principal component analysis to define the major axis. For a random coil, the axial lengths are almost the same, regardless of the polymer direction, because of the coil's isotropic shape. By contrast, the major axis length is about 19.1 nm ± 2.6 nm, while the radial length along the minor axis is only 3.1 nm ± 0.41 nm. The aspect ratio of the configuration is about 3.1. The major axis length is much larger than the radius of gyration of the random coil, i.e., 2.36 nm, or 8.3 nm by consideration of the excluded volume if we set the inter-amino acid

distance as 0.5 nm. It is noted that the charged proteins adsorbed on the oppositely charged mica can show different rigidity and conformations as compared to that in bulk solution, which typically makes the conformations of adsorbed polymer chains more elongated and in more parallel configurations, contributing to the strong cohesion measured between the Rmfp-1 surfaces.

6. **Film thickness D_c**. The confined film thicknesses of Rmfp-1 and the decapeptide-coated mica, denoted as D_c, were determined from their force-distance profiles where the confined separation between two mica substrates or the thickness of the confined protein layer does not appear to change with increasing normal compressive load. The D_c values of Rmfp-1 vs. mica and decapeptide vs. mica were 5.8 nm and 1.3 nm, respectively (Figure 2), corresponding to the confined thickness of an Rmfp-1 layer and a decapeptide layer. The D_c value for different force levels generally increased as the concentration of KNO_3 increased. For the symmetric case, D_c almost doubled. The D_c values for Rmfp-1 vs. Rmfp-1 and the decapeptide vs. decapeptide cases were 11.3 nm and 4.5 nm, respectively. In the text, we have added details for the estimation of the thicknesses based on conformation studies of the proteins.

   The D_c (the confined film thicknesses) values of Rmfp-1 vs. mica and the decapeptide vs. mica were ~5.8 nm and ~1.3 nm, respectively. Computer simulations show that Rmfp-1 adopts a cylinder-like conformation. The estimated length of the cylinder along the principal axis in Rmfp-1 is about 19.1±2.6nm, and the radius is about 3.1±0.4nm. Similarly, it was reported that the decapeptide length is about 2.7 nm and the radius is about 1 nm.[15a] Additionally, dynamic light scattering (DLS) experiment was performed, and the hydrodynamic diameter of Rmfp-1 in bulk

solution was ~9 nm. All these results indicate that the deposited Rmfp-1 or the decapeptide film (after rinsing with buffer) is close to a monolayer.

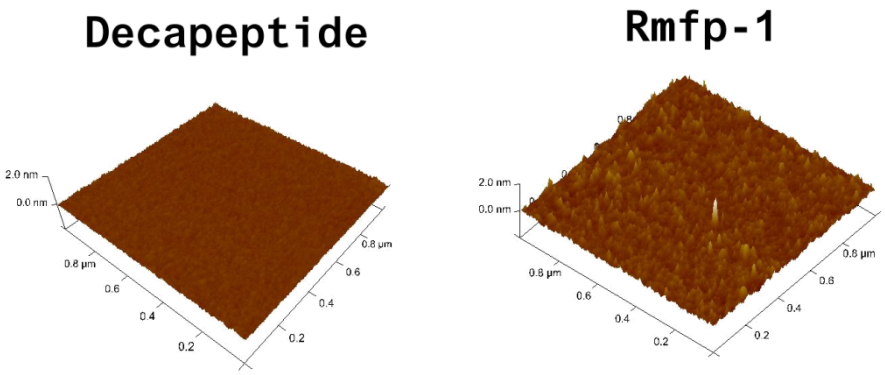

Figure s1. AFM tapping mode image of the decapeptide (left) and Rmfp-1 (right) films on freshly cleaved mica.

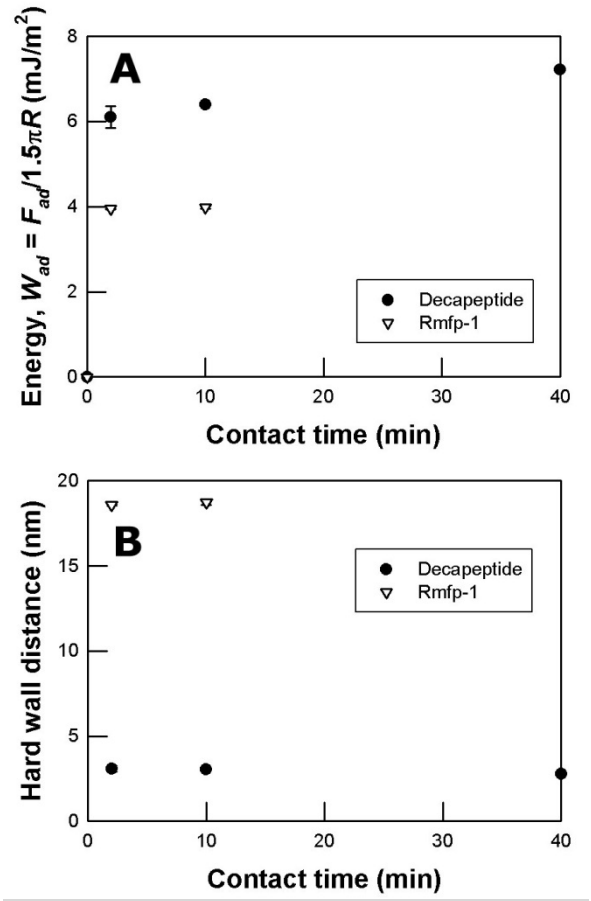

Fig. S2 (A) Normalized cohesion energy vs contact time and (B) hard wall distance between two decapeptide layers (closed circle) or Rmfp-1 layers (open triangle)

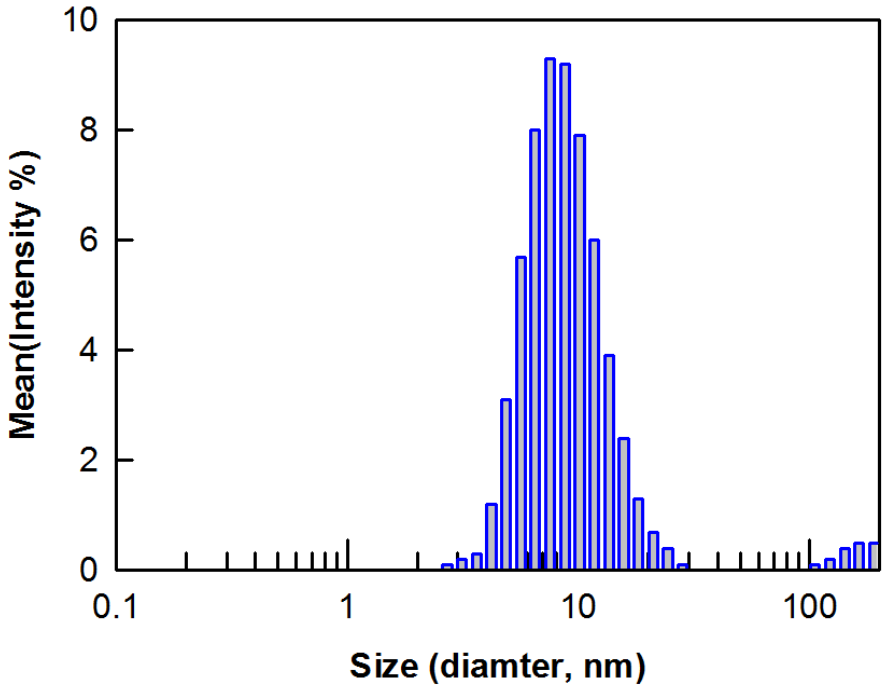

Figure S3. Hydrodynamic diameter distribution of Rmfp-1 in 0.1 M acetic acids (pH 3.0)

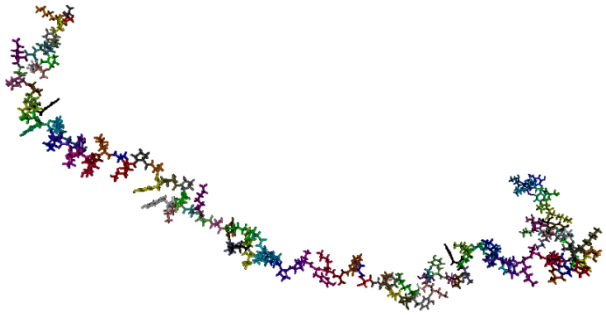

Fig. S4. Typical configuration of Rmfp1 calculated from the all-atomic level AMBER simulation.

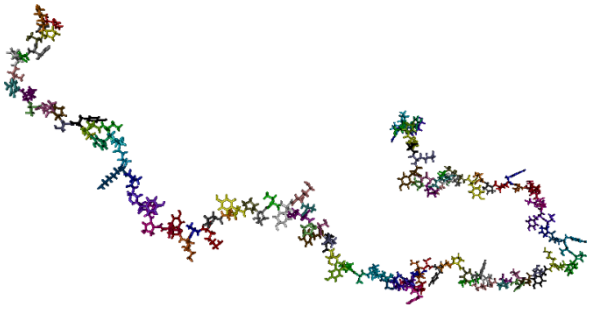

Fig. S5. Rmfp1 model configuration predicted by the I-TASSER