Supporting Information for:

Biomimetic Fabrication of Genetically-Engineered Collagen Peptide-Assembled Freestanding Films Reinforced by Quantum Dot Joints

Zengyan Wei, Yoshiaki Maeda and Hiroshi Matsui*

Department of Chemistry and Biochemistry, City University of New York – Hunter College, New York, NY 10065

* to whom correspondence should be sent. E-mail: hmatsui@hunter.cuny.edu

Figure S1. (a) Optical image of the holy Cu grid. (b) AFM image and height profiles (between arrows) of the holy Cu grid.

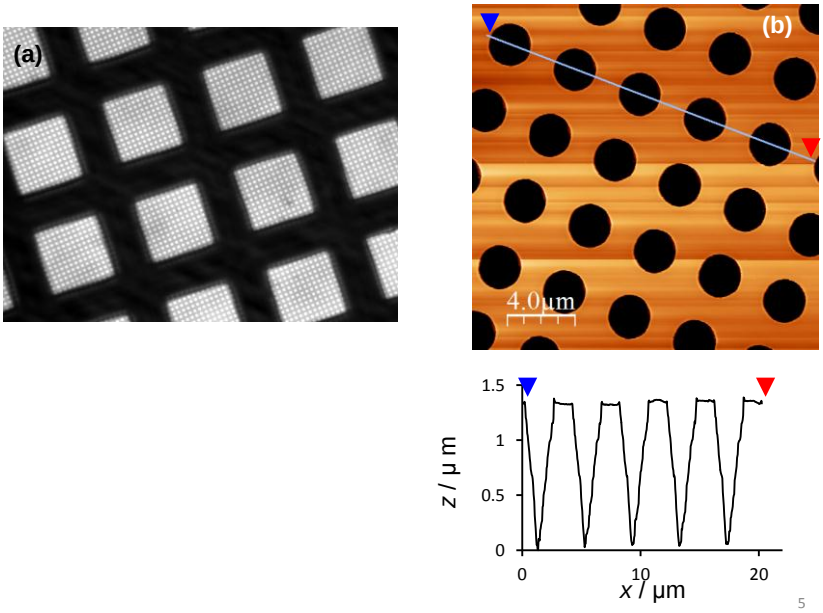


Figure S2. (a) Optical image of the peptide based freestanding film. The boundary section of the film was marked with arrows. (b) AFM image and height profiles (between arrows) of the film. (c) TEM image of the film at low magnification.

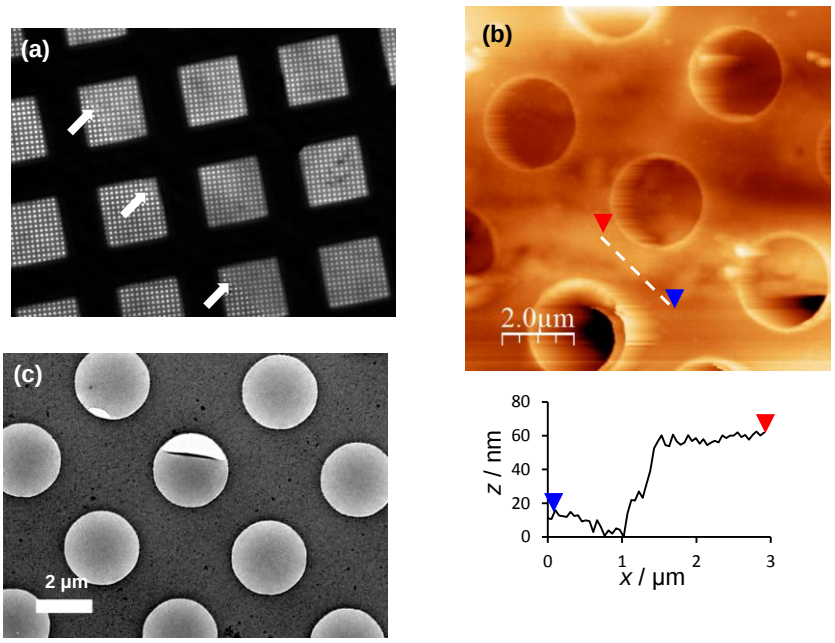


Figure S3. (a) Force plot on mica for the cantilever sensitivity measurement. (b) Force (F)-displacement (δ) curve on 70 different films (scatter) and the curve fitting (red line) to Eq. 1.

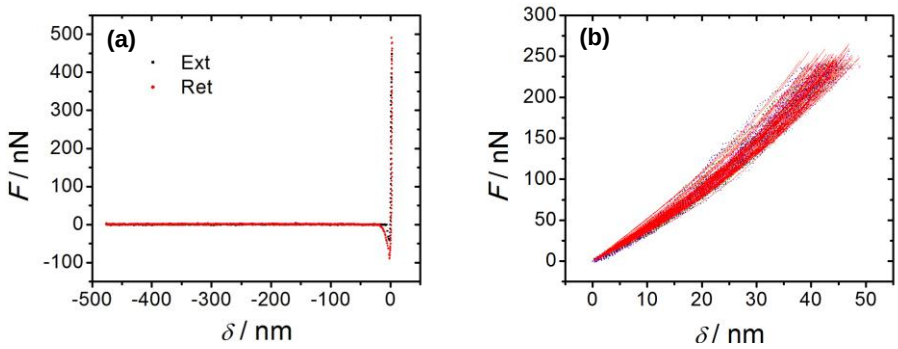
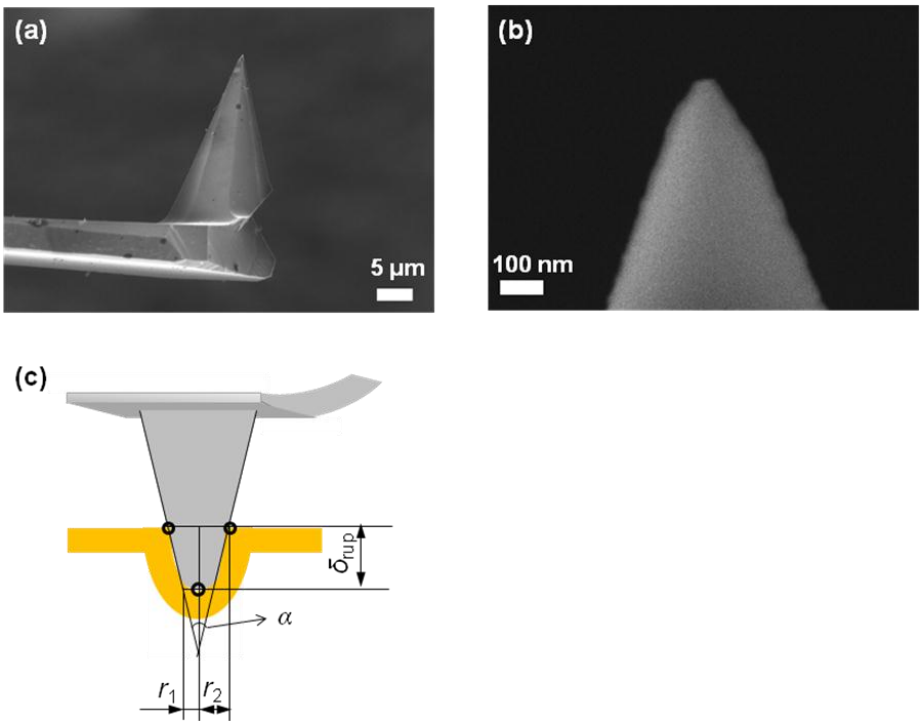


Figure S4. Scanning electron microscopy (SEM) images of the AFM probe after nanoindentation in (a) low magnification (b) high magnification. (c) Schematic diagram of a film indented with AFM probe on the basis of Qin's model.[4c] The curvature radius of the tip (r_1) is ~15 nm. The tip angle between diagonal ridges (α) is ~ 45°. Therefore, $A = \pi r_2^2 = \pi(r_1 + \delta_{rup} \times \tan(\alpha/2))^2 = 4.2\times10^3$ nm^2.



Production of recombinant collagen as fusion protein for biotinylation

The synthesis and biotinylation of B877B peptide followed the method shown in our previous work[17]. The recombinant collagen-like fragment (B877B) was generated using the original F877 bacteria expression construct with the insertion of a 15 amino acid sequence of Biotin Acceptor Peptide (BAP, GLNDIFEAQKIEWHE) at both N- and C-terminus (Scheme 1). At first, *Bam*HI site between collagen gene and foldon gene was deleted using point mutation technique. Following the insertion of the 15 amino acids of BAPs is achieved by PCR using the PrimeSTAR HS DNA Polymerase (Takara Bio Inc) with the mutated pET32-F877 plasmid as the template and the following forward and reverse primers (F 5'-GGATCCGGTCTGAACGACATCTTTGAAGCGCAGAAAATTGAGTGGCACGAAGGTCCTCCTGGACCACCTGGG-3', R 5'-GAATTCTTATTATTCGTGCCACTCAATTTTCTGCGCTTCAAAGATGTCGTTCAGACCCAGGAAGGTAGACAGCAG-3', BAP genes are underlined). The fusion gene was sub-cloned into a pCR4 blunt TOPO (Invitrogen, Carlsbad, CA) plasmid and the sequence was verified by DNA sequencing (ABI PRISM 3100-Avant Genetic Analyzer, Applied and Biosystems, Carlsbad, CA). The plasmid was digested with *Bam*HI and *Eco*RI, and the fragment containing the fusion gene was cloned into *Bam*HI and *Eco*RI-digested pET32, designated pET32B877B.

The constructed pET32B877B was transformed into chemically competent *E. coli* BL21 (DE3) harboring pBirAcm (Avidity, LLC, CO, USA) for co-expression of *E. coli* biotin ligase. The transformed cells were grown in TPP media (20g L^{-1} typtone 15g L^{-1} yeast extract, 8g L^{-1} NaCl, 4g L^{-1} Na_2HPO_4, 2g L^{-1} KH_2PO_4) with 1% glucose, 25μg mL^{-1} ampicillin 10μg mL^{-1} chloramphenicol and 50μM d-biotin to optical density OD_{600} of 0.8. Expression of both B877B and biotin ligase was induced by adding 0.5mM isopropyl-*b*-D-thiogalactoside (IPTG) followed by incubation at 25°C overnight. Cell pellets were harvested by centrifugation, resuspended in Tris buffer (50 mM Tris-HCl pH 8.0 and 300 mM NaCl). Cell suspensions were incubated for 60 min at 4°C in the presence of 1mg mL^{-1} lysozyme (Sigma, St. Louis, MO, USA), 1μg mL^{-1} DNase I (Roche Molecular Biochemicals, Indianapolis, IN, USA), 1mg mL^{-1} PMSF, (Roche Molecular Biochemicals) and broken by sonication. Following the centrifugation, the protein in the supernatant was purified using nickel affinity chromatography according to the native purification protocol provided by manufacturer (Ni-NTA Superflow; Qiagen, Valencia, CA, USA). The Ni-NTA column was washed with buffer containing 10-50 mM imidazole and eluted with buffer containing 500 mM imidazole. The 6x His and thioredoxin tag was cleaved using thrombin (Sigma) overnight at 4°C. The expression level and purity of the sample was assayed qualitatively by Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) (Fig.S5).The final protein was purified by RP-HPLC (Beckman Coulter) with C18 column (Vydac) with a 20-70% acetonitrile-water with 0.1 % TFA (Fig.S6). Peptide elution peak was collected, lyophilized, stored at -80°C, which was stable for months. Circular dichroism (CD) spectrum of B877B at 25°C indicates that the genetically-engineered peptides maintain the triple helical conformation with a small positive peak at ~225nm and a deep negative peak at ~ 197nm. (Fig. S7).

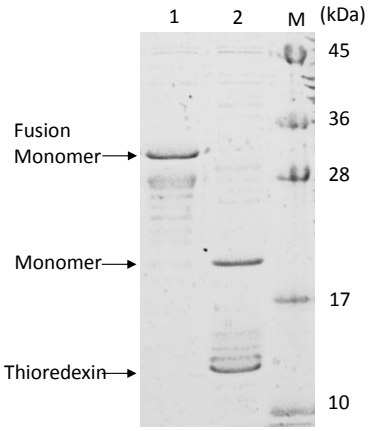


Figure S5. SDS-PAGE analysis of the expression of B877B. Lane 1: Purified protein by Ni-NTA affinity column, Lane 2: After thrombin cleavage. Lane M: Protein molecular weight marker

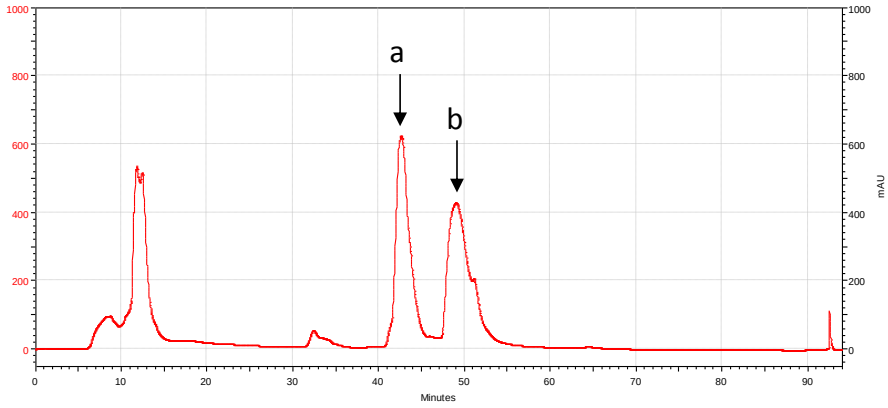


Figure S6. High-Performance Liquid Chromatography (HPLC) of B877B (absorbance at 280 nm). The peak for the pure protein appears at an absorbance of 280nm.The arrows 'a' and 'b" correspond to the peaks of pure protein and thioredoxin after thrombin cleavage, respectively.

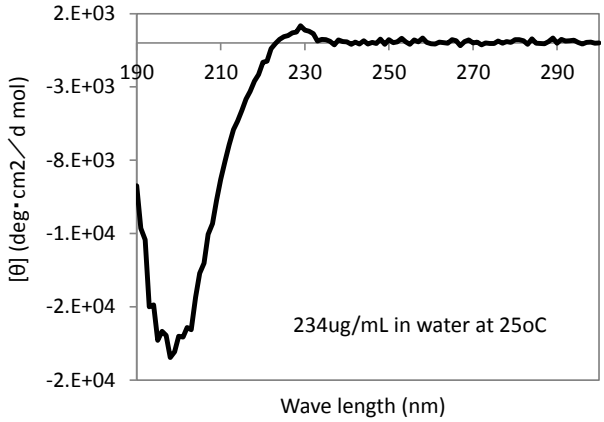


Figure S7. CD spectrum of B877B. The spectrum is normalized to molar ellipticity

Recombinant protein B877B was expressed in the presence or absence of excess biotin (50μM) and coexprssed biotin ligase. After purification with Ni-NTA column and HPLC, protein dissolved in the mixture of water acetonitrile was analyzed with Agilent 6520 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS (Agilent Technologies). The number of biotin attached on the single B877B triple helix was determined from the molecular mass. With increasing the biotin number, B877B increase its molecular weight from 47013Da (Biotin = 0) to 48370Da (Biotin = 6) (Fig. S8). Abundance ratio of differently biotinylated B877B was calculated from the height of the each peak which shows the molecular weight of biotinylated B877B.

Although TOF-MS analysis can reveal the number of biotin on the B877B triple helix, the biotinylated position is not determinable. Both ends of B877B triple helix have three biotinylation sites respectively. Therefore, at least four biotin moieties need to be displayed on the triple helix to ensure that both ends are biotinylated. As results, TOF-MS analysis revealed that adding the excess amount of biotin in growth media is not enough to display four biotins and full biotinylation of B877B can be accomplished by the co-expression of biotin ligase in *E. coli* during ligation (Fig. S9).

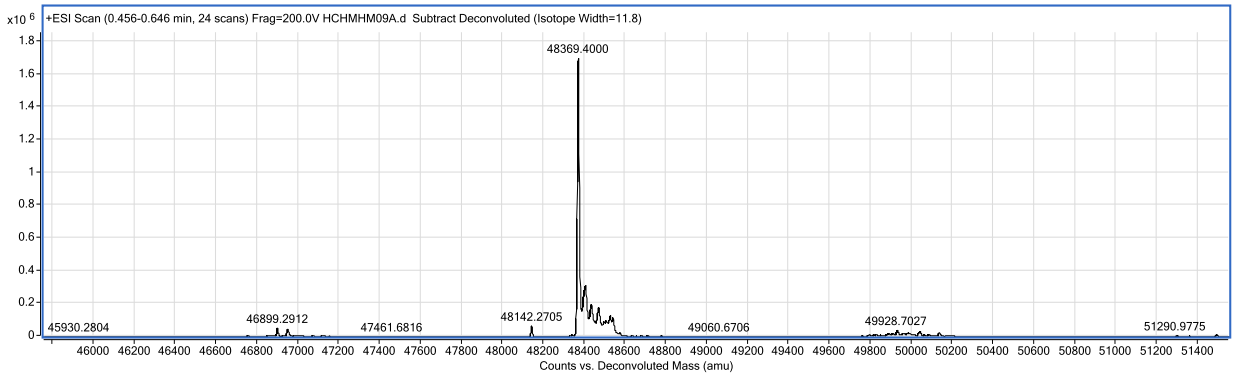


Figure S8. MS of B877B expressed in *E. coli* BL21(DE3) which co-expressed biotin ligase and was cultured in TPP media including 50μM biotin. Typical peak corresponds to B877B trimer which is fully biotinlated at 6 sites.

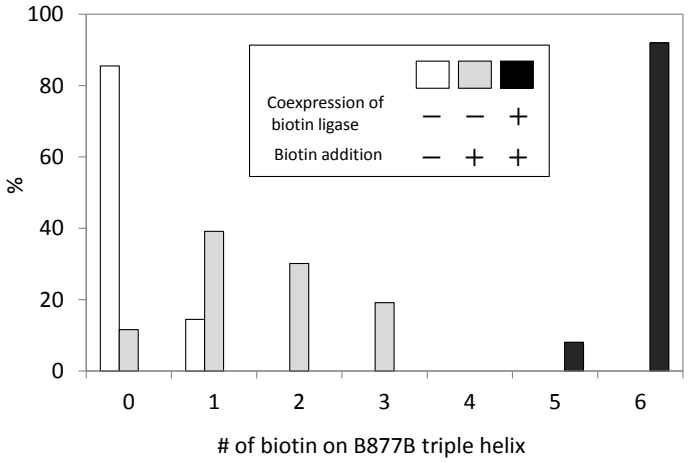


Figure S9. Abundance ratio of differently biotinylated B877B expressed in different condition