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Electronic Supplementary Information to: DNA-templated assembly of viral protein hydrogel

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Hydrogels are a promising class of biomaterial that can be easily tailored to produce a native extracellular matrix that exhibits desirable mechanical and chemical properties. Here we report the construction of a hydrogel via the assembly of Cucumber Mosaic Virus (CMV) capsid protein and Y-shaped and cross-shaped DNAs.

The sequences of Y-DNA and cross-DNA were purchased from SBS Genetech and purified by denaturing polyacrylamide gel electrophoresis.
Ya: 5'-GGATCCGCATGACATTCGCCGTAAT-3'
Yb: 5'-TTACGGCGAATGACCGAATCAGCCT-3'
Yc: 5'-GGCTGATTCGGTTCATGCGGATCCT-3'
+a: 5'-GCAAGCGGACTCTGGCT-3'
+b: 5'-GCCAGAGTGGAAGGCGT-3'
+c: 5'-CGTGTTCACCGCTTGCT-3'
+d: 5'-CGCCTTCCTGAACACGT-3'

The formation of Y DNA and "+"DNA was confirmed by electrophoresis in 1.7% agarose gel (**Fig. S1**).

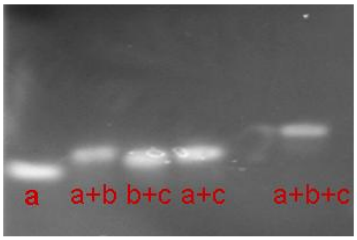

Fig. S1. Evaluation of Y-DNA formation. Lane 1 is oligonucleotide Ya; Lanes 2, 3 and 4 correspond to hybridized products of Ya and Yb , Yb and Yc, and Ya and Yc, respectively. Lane 5 is hybridized final products of Ya, Yb and Yc.

The 500-mer double-stranded DNA was amplified by polymerase chain reaction using the pBR plasmid template. The forward primer: 5′ -CCC TTA TGT TAC GTC CTG -3′ and the reverse primer: 5′ -TGG TGT AGA GCA TTA CGC -3′ . The amplification was achieved using 30 cycles at the following temperatures: 94°C for 0.5 min, 55°C for 0.5 min, and 72°C for 0.5 min. Finally, the reaction was maintained at 72°C for 2 min. The PCR products were purified using a DNA fragment purification kit. (pBR plasmid purchased from Takara, Japan; the primers were synthesized by Saibaisheng Biocompany; the DNA purification kit was purchased from Tiangen Biocompany.)

Fig.S2. Evaluation of Cross-DNA formation. Lane 1 is +(a+b+c+d); Lanes 2 and 3 are +(a+b) and +(b+c) respectively; Lane 4 is +(a+b+c) and Lane 5 is +(a+b+c+d).

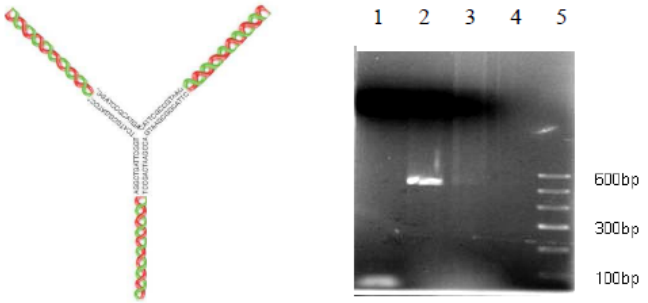

Fig. S3. Evaluation of the elongation of Y-DNA. Lane 1 is Y-DNA(Ya+b+c); Lane 2 is 500mer dsDNA from PCR amplification;

Lane 3 is the ligation product (smear) and Lane 5 is the DNA marker.

The ligation product was diluted into 0.1 nM and was evaporated onto a freshly cleaved mica surface. Ligated branch-DNA molecules were observed by tapping mode AFM using a commercial instrument.

Buffers used for CMV CP expression and purification are as follows: Solution buffer (500 ml): 50 mM Tris buffer pH 8.0, 25% Sucrose, 1 mM EDTA, 0.1% NaAzide, 10 mM DTT. Lysis buffer (500 ml): 50 mM Tris buffer pH 8.0, 1% Triton X-100, 0.1% sodium deoxycholate, 100 mM NaCl, 0.1% sodium azide, 10 mM DTT. Washing buffer: 50 mM Tris buffer pH 8.0 with 0.5% Triton X-100, 100 mM NaCl, 1 mM EDTA, 0.1% NaAzide, 1 mM DTT.

Full-length CP gene of 218 amino acid residues (GenBank ID AB008777) of CMV was PCR-amplified for protein expression by the forward primer: 5'-AACCATGGACAAATCTGAATCAACC-3' and the reverse primer: 5'-TTGGATCCTCAAACTGGGAGCACCCCAGACGTGGG-3'. The PCR products were gel-purified, restriction enzyme digested with NcoI and BamHI, and ligated with NcoI and BamHI restricted pET11d (Novagen, USA). *E. coli* BL21 (DE3) Rosseta (Novagen) was used as a host strain for the production of recombinant protein. Cultures were grown at 37°C by inoculating a 5 ml overnight culture into 2 L LB medium containing ampicillin (10 mg/L). Incubation was continued until the culture reached an OD600 of between 0.4 and 0.6, at which protein expression was induced by the addition of 1 mM isopropyl-β-d-thiogalactopyranoside (IPTG). After 4 h incubation, cells were harvested by centrifugation at 4000 g for 10 min. The cell pellets were then stored at -20°C.

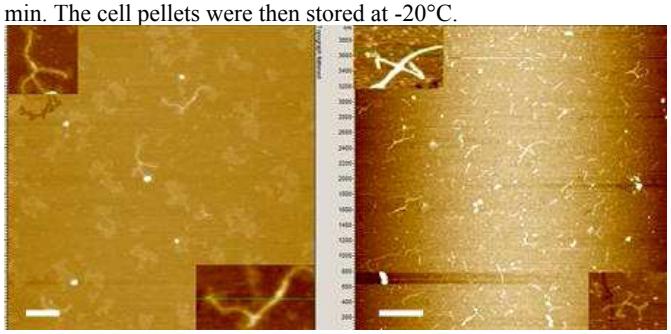

Fig. S4. Atomic force microscopy images of (left) Y-DNA, (right) "+"DNA, scale bar 200 nm.

All protein purification steps were performed on ice or at 4°C. The pellets (crude inclusion body) were re-suspended in solution buffer and then homogenized by sonication and lysis. The protein products were washed 3 times in washing buffer by centrifugation at 11,000 g for 20 min each time.

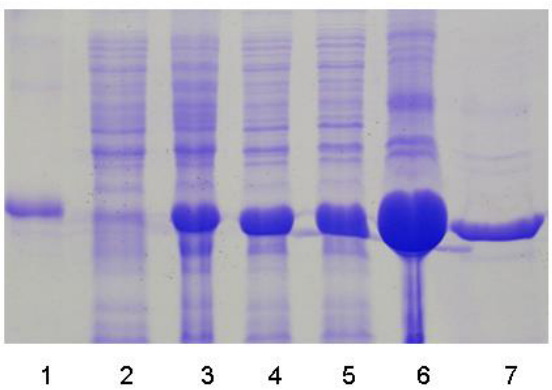

Fig. S5. Recombinant CMV CP induced-expression and inclusion body purification in *E. coli*. Lane 1, 29 kDa pre-stained protein marker; Lane 2, uninduced; Lane 3, IPTG induced total bacterial proteins; Lane 4, crude inclusion protein; Lanes 5 and 6 are the inclusion body protein after washing; Lane 7, the final washed inclusion body.

Buffers used for protein refolding are as follows: Refolding buffer (250 ml): 100 mM Tris buffer pH 8.0, 400 mM L-arginine-HCL, 2 mM EDTA, 0.5 mM oxidated glutathione, 5 mM reduced glutathione. Guanidine solution: 3 M guanidine-HCL pH4.2, 10 mM sodium acetate, 10 mM EDTA. Exchange buffer (500 ml): 20 mM Tris buffer, 50 mM NaCl, pH 8.0.

To obtain soluble and re-natured proteins, the CP inclusion bodies were desolved in a 2 M urea solution and guanidine solution was added. 2 ml of 100 mM PMSF, 100 µl of 2 mg/ml pepstatin and 100 µl of 2 mg/ml leupeptin were mixed with 200 ml refolding buffer. The protein solution was added into above prepared refolding buffer while circumgyrating at high speed. The refolding process was then carried out for 8 h at 4°C at a lower circumgyrating speed. To achieve higher concentrations, the refolded protein solution was ultra-filtered using Ultrafree-CL (10,000 MW) filters (Millipore). After washing 10 times with diluted exchanger buffer, the CP or CMV CP preparation was finally dissolved in exchange buffer to a final concentration of 50 µg/ml.

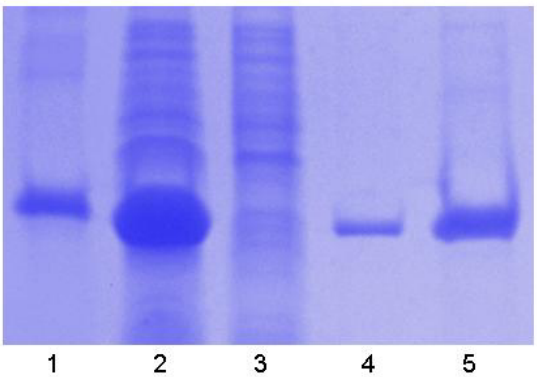

Fig. S6. Re-natured CMV CP Lane 1, 29Kda pre-stained protein marker; Lane 2, induced CP; Lane 3, uninduced CP; Lane 4, re-natured CP; Lane 5, higher concentration CP in exchanger buffer (final assembly buffer)

These are other images observed with ESEM that represent some of the stepped configuration stages of the gelation process.

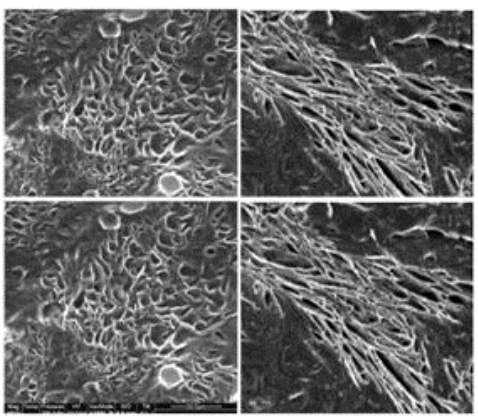

Fig. S7. Other ESEM images of the surface morphology of DNA-templated protein hydrogel.

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