Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information for

Calcium-Responsive Hydrogels Enabled by Inducible Protein-Protein Interactions

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Materials and Methods

Gene Construction. The two gene constructs, *SpyTag-ELP-CaM-ELP-SpyTag* (*A-CaM-A*) and *SpyCatcher-ELP-M13-ELP-SpyCatcher* (*BMB*) were created by modifying the existing *SpyTag-ELP-SpyTag-ELP-SpyTag* (*AAA*) and *SpyCatcher-ELP-SpyCatcher* (*BB*). The *A-CaM-A* gene was created by replacing the middle SpyTag between SacI and SpeI restriction sites in *AAA* with the CaM gene derived from pcDNA-*4mtD3cpv*, a gift from the Roger Tsien group. The *BMB* gene was created by inserting the synthetic *M13* gene (Genewiz®) into *BB* using the SacI and SpeI restriction sites.

Recombinant Protein Expression and Purification. *E. coli* strain BL21(DE3) harboring the desirable plasmid, pQE80l::*A-CaM-A* or pQE80l::*BMB*, was grown at 37 °C, 220 rpm in LB medium till the optical density at 600 nm reached 0.6 ~0.8. AAfter 4 h, cells were harvested by centrifugation at 4,200 ×g, 4°C for 15 min. Cell pellets were frozen at -80°C for 2 hours before use. After thaw at room temperature, the pellets were resuspended in lysis buffer [300 mM NaCl, 10 mM Tris-HCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), pH 8.0]. Cell lysates were obtained using a sonication homogenizer and clarified by centrifugation at 18,000 ×g, 4°C for 30 min. The protein of interest was purified on HisTrap Ni-NTA columns (GE Healthcare, Inc.) following the manufacturer's recommendations. Protein purity was assessed by SDS-PAGE. After the Ni-NTA chromatography, the protein was dialyzed against Milli-Q water (4.5 L × 5) at 4°C, then centrifuged at 5,200 × g for 40 min to remove precipitants. The resulting protein solution was snap-frozen in liquid nitrogen, stored at -80°C overnight and lyophilized. The resulting protein powders were stored at -80°C before use.

Synthesis of Protein Hydrogels. Lyophilized proteins were dissolved in Tris-buffer (300 mM NaCl, 10 mM Tris-HCl, pH 8.0) supplemented with varied amounts of $CaCl_2$ (5, 25 or 50 mM) or in cell culture medium supplemented with 5 mM $CaCl_2$. The A-CaM-A and BMB protein solutions (10 wt %) were mixed at an equimolar ratio to initiate gelation at room temperature.

Dynamic Shear Rheology. Rheological measurements were performed on a TA Instruments ARES-RFS strain-controlled rheometer with a standard steel parallel-plate geometry. Test modes included dynamic time-, strain- and frequency-sweep tests. The gelation mixture was placed on a 25-mm plate, covered by an 8-mm plate to form an 8mm disk of gel with a thickness of ~0.9 mm. The gel was sealed with silicone oil to prevent water evaporation. Time-sweep tests were performed at room temperature with strain held constant at 5% and frequency at 10 rad/s. The storage modulus (G') and loss modulus (G") were recorded over a period of 40,000 s. Strain-sweep tests were performed at a fixed frequency of 10 rad/s at room temperature, with the strain amplitude varied from 0.1% to 100% to determine the linear viscoelastic region. Frequency-sweep tests were performed at a fixed strain of 10% at room temperature, by varying frequency from 100 to 0.01 rad/s.

Erosion Tests. The A-CaM-A and BMB proteins were dissolved in Tris buffer supplemented with varied amounts of $CaCl_2$ (0, 5, 25 or 50 mM), or DMEM with 10% FCS supplemented with $CaCl_2$ (0 or 5 mM), to make 10 wt% solutions. The two solutions (80 µl in total) were mixed in 1.5-ml Eppendorf tubes. After overnight gelation, 500 µl Tris-buffer (300 mM NaCl, 10 mM Tris-HCl, pH 8.0) was added to immerse the gels. The tubes were fixed on a tube rotator which rotates at 20 rpm or let stand still. The amounts of protein released from the gels were quantified based on the absorbance at 280 nm of the supernatants. A NanoDrop™ 2000c spectrometer (Thermo Scientific®) was used. Erosion tests were performed in triplicate.

Encapsulation of 3T3 Fibroblasts. NIH/3T3 fibroblasts were grown at 37 °C with 5% CO_2 in high glucose Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% (vol/vol) fetal calf serum (FCS) (Gibco) and 1% (vol/vol) penicillin-streptomycin (Sangon Bio-tech). Cells were passaged every 3-4 days. At ~75% confluence, cells were detached with 200 µL trypsin (0.25%) solution (Sangon Biotech) followed by addition of 1.5 ml medium to terminate the digestion. Cells were mixed thoroughly by pipetting. Cells were counted using a Coulter counter. Approximately 10,000 cells (per 20 µL of hydrogel sample) were pelleted at 400 × g for 4 min. Pelleted cells were resuspended in the BMB (10 wt %) protein solution in DMEM medium, and then were placed on a cell culture dish with a coverslip bottom. The A-CaM-A solution in DMEM medium (10 wt %) was added to initiate gelation. The hydrogel was cured at 37 °C for 1 h, followed by addition of 2 mL DMEM to cover the gel. Cells were incubated at 37 °C with 5% CO_2. After 24 h, the cells were assessed by a standard LIVE/DEAD staining assay (Invitrogen). After removal of DMEM, the cell culture was washed with 2 mL PBS for three times and then was treated with the PBS solution containing 4 µM ethidium homodimer and 2 µM calcein AM. After 20 min at room temperature, the cells were imaged using a Laser Scanning Confocal Microscope (Zeiss, LSM710).

Encapsulation of human mesenchymal stromal cells (hMSCs). Human mesenchymal stromal cells (hMSCs) were cultured at 37 °C with 5% CO_2 in minimum essential medium (MEM) alpha (Gibco), supplemented with 10% (vol/vol) fetal bovine serum (FBS) (Gibco), 2 mM L-glutamine and 1% (vol/vol) penicillin/streptomycin. Cells were passaged every 5-6 days using 0.25% trypsin (Sangon Biotech) solution. The encapsulation procedure was similar to that for 3T3 fibroblasts.

Supplementary Figures

(A) SpyTag-ELP-CaM-ELP-SpyTag (A-CaM-A)

MKGSSHHHHHHVDAHIVMVDAYKPTKLDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGV
PGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGELMHDQLTEEQIAEFKEAFSLLDKDGDGTITT
KELGTALRSLGQNPTEAELQDMINEVDADGNGTIYFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGYISA
AELRHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEFVQMMTAKTSVPGVGVPGVGVPGEGVPGVGVPG
VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLDAHIVMVDAYK
PTKLEWKK

(B) SpyCatcher-ELP-M13-ELP-SpyCatcher (BMB)

MKGSSHHHHHHVDIPTTENLYFQGAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMEL
RDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKAT***KGD***AHIDGPQGI
WGQLDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPG
VGVPGEGVPGVGVPGVGELRRWQKTGHAVRAFGRLKKISSSGALTSVPGVGVPGVGVPGEGVPGVGVPGV
GVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLDIPTTENLYFQGA
MVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGK
YTFVETAAPDGYEVATAITFTVNEQGQVTVNGKAT***KGD***AHIDGPQGIWGQLEWKK

Figure S1. Amino acid sequences of recombinant proteins used in this study. (A) SpyTag-ELP-CaM-ELP-SpyTag (A-CaM-A); (B) SpyCatcher-ELP-M13-ELP-SpyCatcher (BMB). 6xHisTag, SpyTag, CaM, SpyCatcher, and M13 domain are colored as dark blue, red, blue, orange, and light green, respectively. ELP domains are highlighted with grey background. KGD and MMP-cleavage sites are underlined.

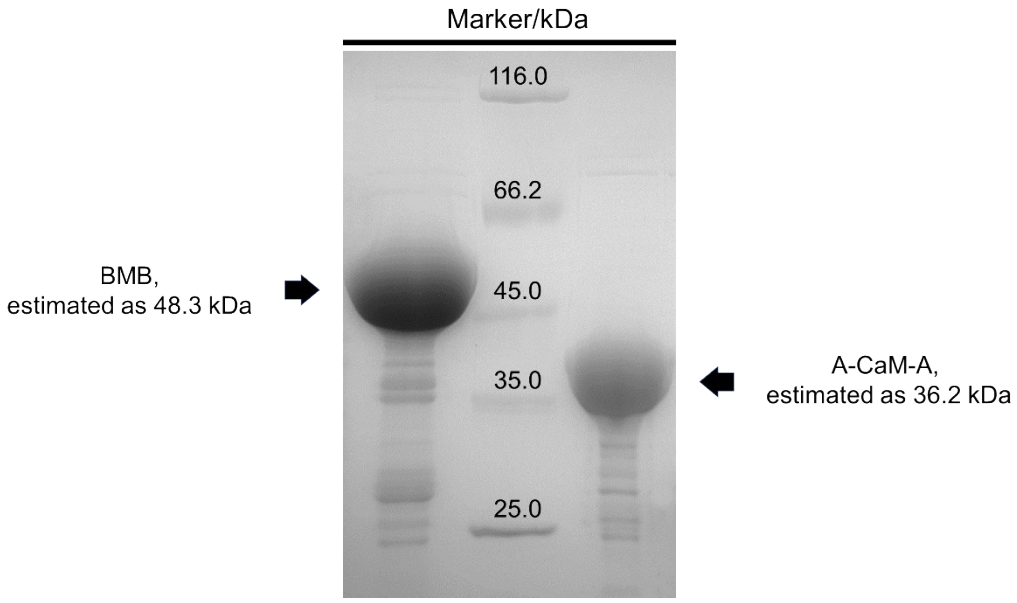

Figure S2. SDS-PAGE analysis of recombinant A-CaM-A and BMB after Ni-NTA purification and dialysis against ddH_2O.

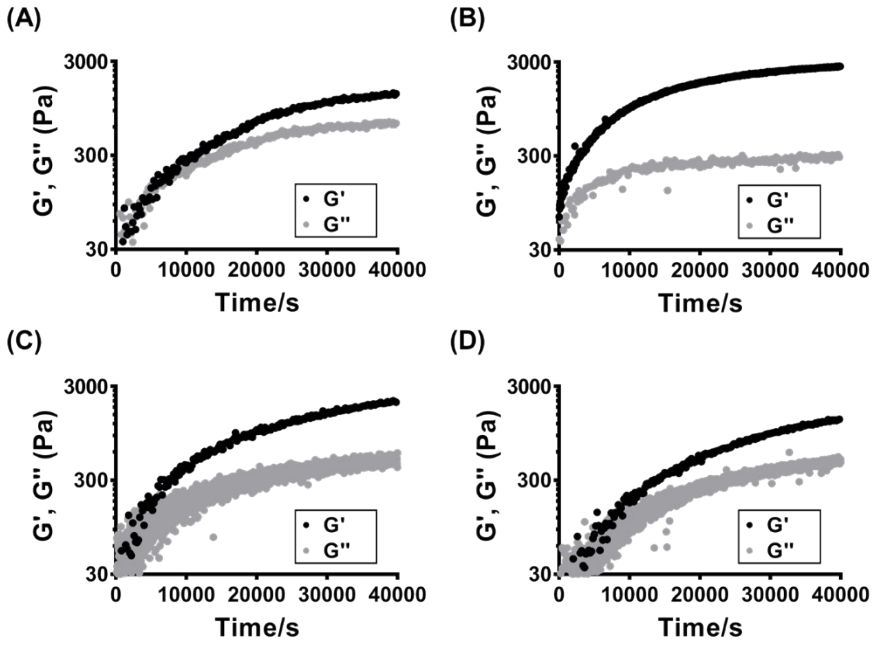

Figure S3. Evolution of storage modulus G' and loss modulus G" of the reaction mixture of A-CaM-A+BMB at an equimolar ratio as functions of time (A) in the absence of Ca^{2+}, (B) with 5 mM Ca^{2+}, (C) with 25 mM Ca^{2+}, and (D) with 50 mM Ca^{2+}.

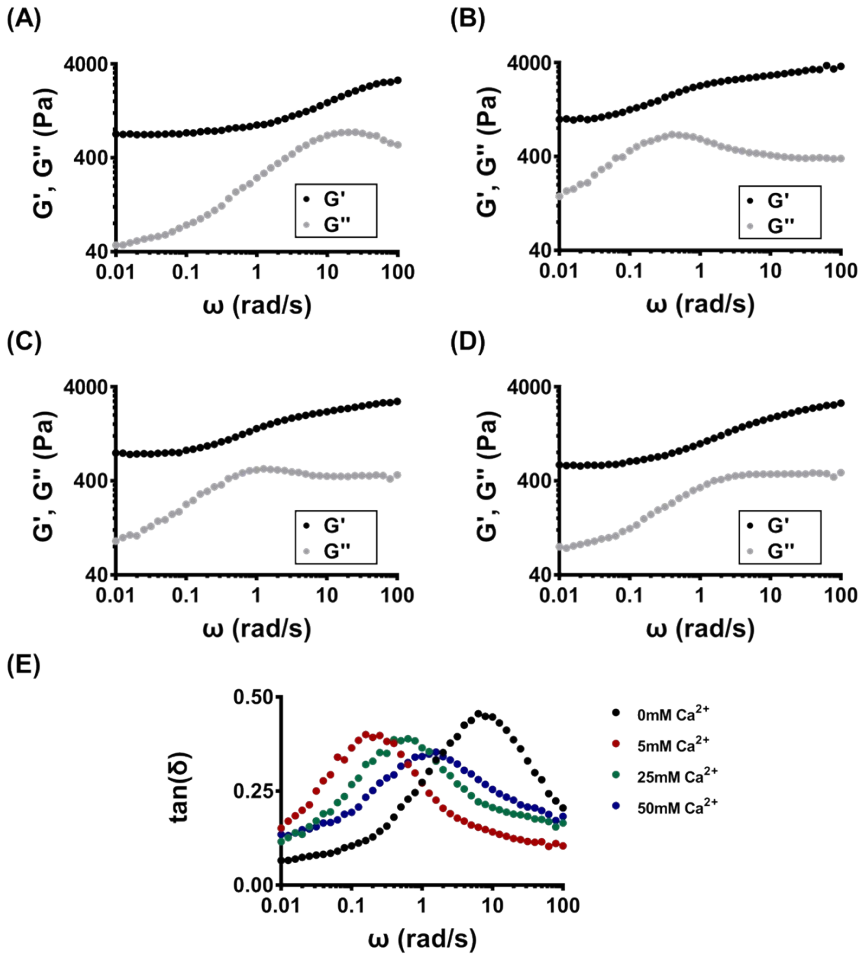

Figure S4. Frequency-sweep tests on the hydrogels comprising of A-CaM-A+BMB (A) in the absence of Ca^{2+}, (B) with 5 mM Ca^{2+}, (C) with 25 mM Ca^{2+}, and (D) with 50 mM Ca^{2+} at 5% shear strain. (E) tan(δ) (G''/G') of the hydrogels comprising of A-CaM-A+BMB in the presence of varied amounts of Ca^{2+}.

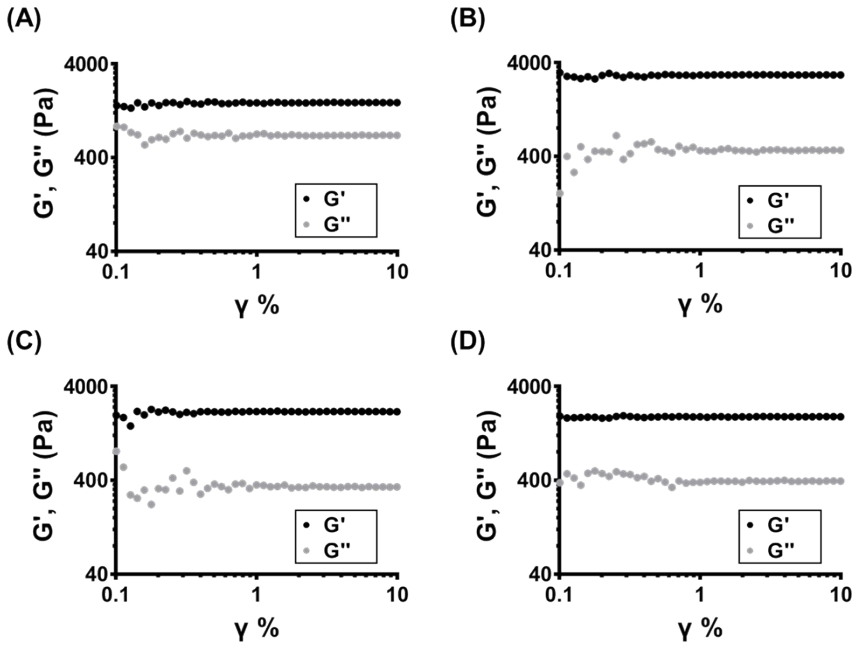

Figure S5. Strain-sweep tests on the hydrogels comprising of A-CaM-A+BMB (A) in the absence of Ca^{2+}; (B) with 5 mM Ca^{2+}; (C) with 25 mM Ca^{2+}; (D) with 50 mM Ca^{2+} at a fixed shear frequency of 10 rad/s.

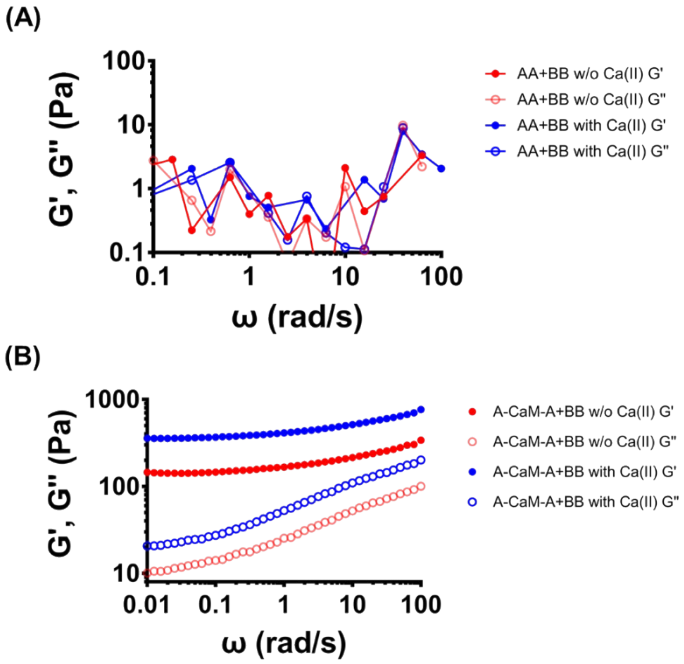

Figure S6. Frequency-sweep tests on the hydrogels (10 wt %) comprising (A) SpyTag-ELP-SpyTag (AA) + SpyCatcher-ELP-SpyCatcher (BB) and (B) A-CaM-A+BB in the absence and presence of Ca^{2+} (5 mM).

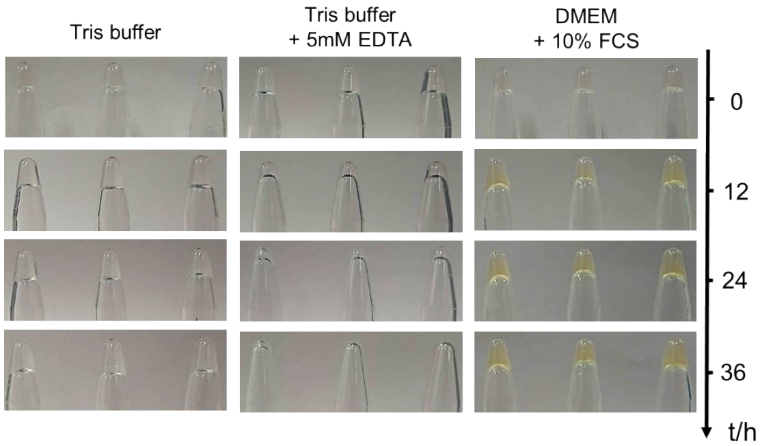

Figure S7. Erosion tests on the A-CaM-A+BMB hydrogels in Tris buffers, with and without EDTA, and in DMEM with 10% (vol/vol) fetal calf serum (FCS).