Supporting Information

From 4-Arm Star Proteins to Diverse Stimuli-Responsive Molecular Networks Enabled by Orthogonal Genetically Encoded Click Chemistries

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Table S1. Amino acid sequences of proteins used in this study.

Name	**Molecular Weight (Da)**	**Protein sequence**
BPB	47244	MKGSSHHHHHHVDIPTTENLYFQGAMVDTLSGLSSEQGQSGD MTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDG QVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGK ATKGDAHIDGPQGIWGQLDGHGVGVPGVGVPGVGVPGEGVP GVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPG VGVPGEGVPGVGVPGVGELIDKLGDIEFIKVNKPGTSVPGVGVP GVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPG VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLDIPTTENLYF QGAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELA GATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGY EVATAITFTVNEQGQVTVNGKATKGDAHIDGPQGIWGQLEWK K
BQB	59482 (pACYCDuet-1 construct), 58497 (pQE80l construct)	pACYCDuet-1 construct: MGSSHHHHHHSQDPHHHHHVDIPTTENLYFQGAMVDTLSGLS SEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGK TISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQ GQVTVNGKATKGDAHIDGPQGIWGQLDGHGVGVPGVGVPGV GVPGEGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVG VPGVGVPGVGVPGEGVPGVGVPGVGELACKPLRGAVFSLQKQ HPDYPDIYGAIDQNGTYQNVRTGEDGKLTFKNLSDGKYRLFENS EPAGYKPVQNKPIVAFQIVNGEVRDVTSIVPQDIPATYEFTNGKH YITNEPIPPKMHTSVPGVGVPGVGVPGEGVPGVGVPGVGVPGV GVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVG VPGVGVPGGLLDIPTTENLYFQGAMVDTLSGLSSEQGQSGDMT IEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQV KDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKAT

		KGDAHIDGPQGIWGQLEWKK pQE80l construct: MKGSSHHHHHHVDIPTTENLYFQGAMVDTLSGLSSEQGQSGD MTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDG QVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGK ATKGDAHIDGPQGIWGQLDGHGVGVPGVGVPGVGVPGEGVP GVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPG VGVPGEGVPGVGVPGVGELACKPLRGAVFSLQKQHPDYPDIYG AIDQNGTYQNVRTGEDGKLTFKNLSDGKYRLFENSEPAGYKPVQ NKPIVAFQIVNGEVRDVTSIVPQDIPATYEFTNGKHYITNEPIPPK MHTSVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVP GEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPG GLLDIPTTENLYFQGAMVDTLSGLSSEQGQSGDMTIEEDSATHIK FSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGK YTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHIDGP QGIWGQLEWKK
AA	19708	MKGSSHHHHHHVDAHIVMVDAYKPTKLDGHGVGVPGVGVPG VGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGV GVPGVGVPGVGVPGEGVPGVGVPGVGELGRGDTSVPGVGVP GVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPG VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLDAHIVMVD AYKPTKLEWKK
A-SNAC-A	19927	MKGSSHHHHHHVDAHIVMVDAYKPTKLDGHGVGVPGVGVPG VGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGV GVPGVGVPGVGVPGEGVPGVGVPGVGELGSHHWTSVPGVGV PGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVP GVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLDAHIVMV DAYKPTKLEWKK
AEC	32182	MKGSSHHHHHHVDAHIVMVDAYKPTKLDGHGVGVPGVGVPG VGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGV GVPGVGVPGVGVPGEGVPGVGVPGVGELPEDLGTGLLEALLRG DLAGAEALFRRGLRFWGPEGVLEHLLLPVLREVGEAWHRGEIGV AEEHLASTFLRARLQELLDLAGFPPGPPVLVTTPPGERHEIGAML AAYHLRRKGVPALYLGPDTPLPDLRALARRLGAGAVVLSAVLSEP LRALPDGALKDLAPRVFLGGQGAGPEEARRLGAEYMEDLKGLAE ALWLPRGPEKEAI

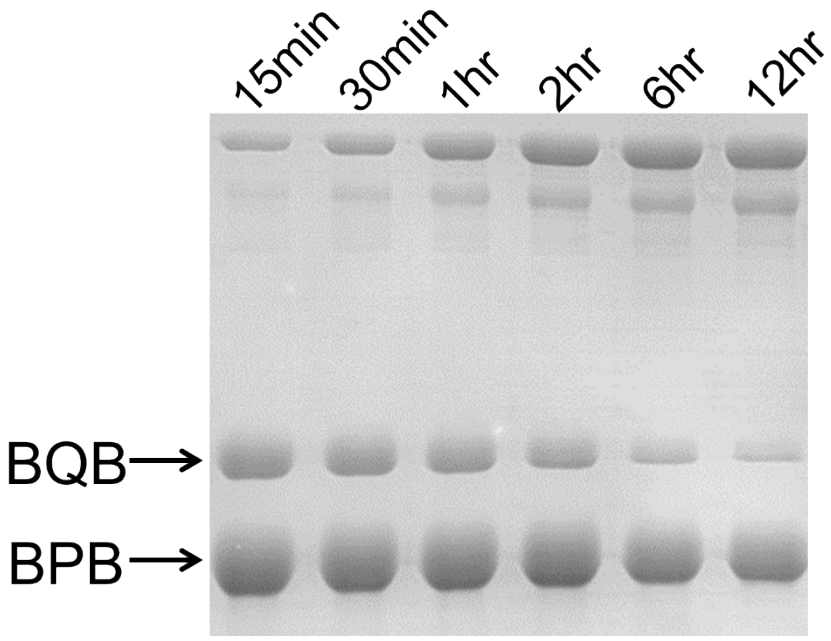

Figure S1. SDS-PAGE analysis of reconstitution of purified BPB and BQB.

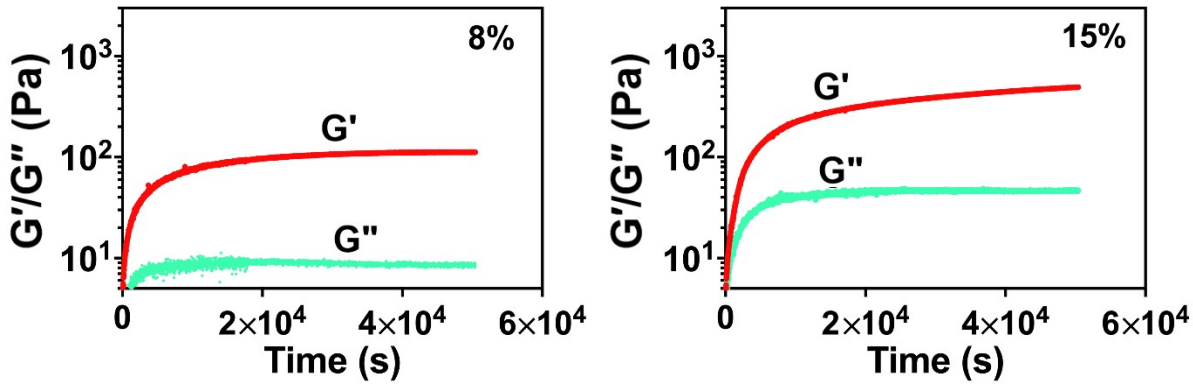

Figure S2. Time-sweep tests of Snoop-Spy hydrogels (8 and 15 wt %). The shear frequency and strain were fixed at 1 rad/s and 10%, respectively.

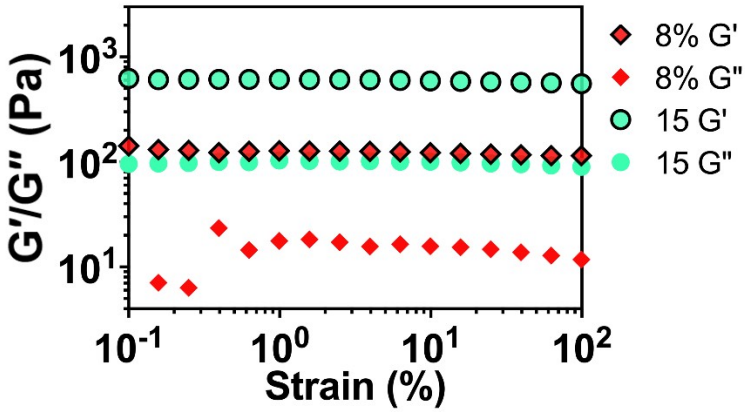

Figure S3. Strain-sweep tests of Snoop-Spy hydrogels (8 and 15 wt %). The shear frequency was fixed at 10 rad/s.

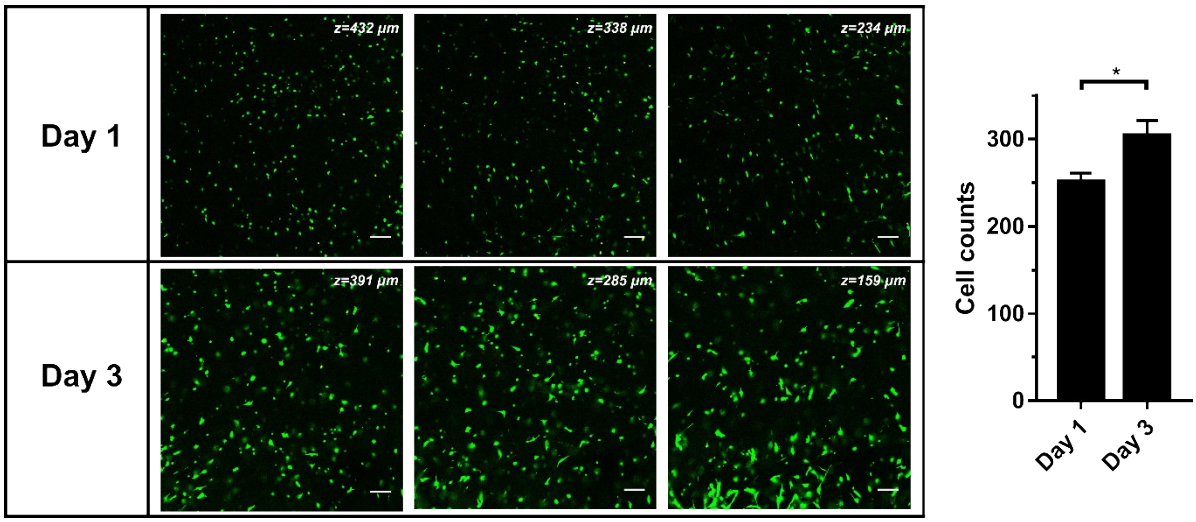

Figure S4. Additional representative z slice images of encapsulated 3T3 fibroblasts (Live (green)/Dead (red) staining) by Snoop-Spy hydrogels. Data are shown as mean ± SD (n = 3). *, $p \leq 0.05$. Scale bar: 100 µm.

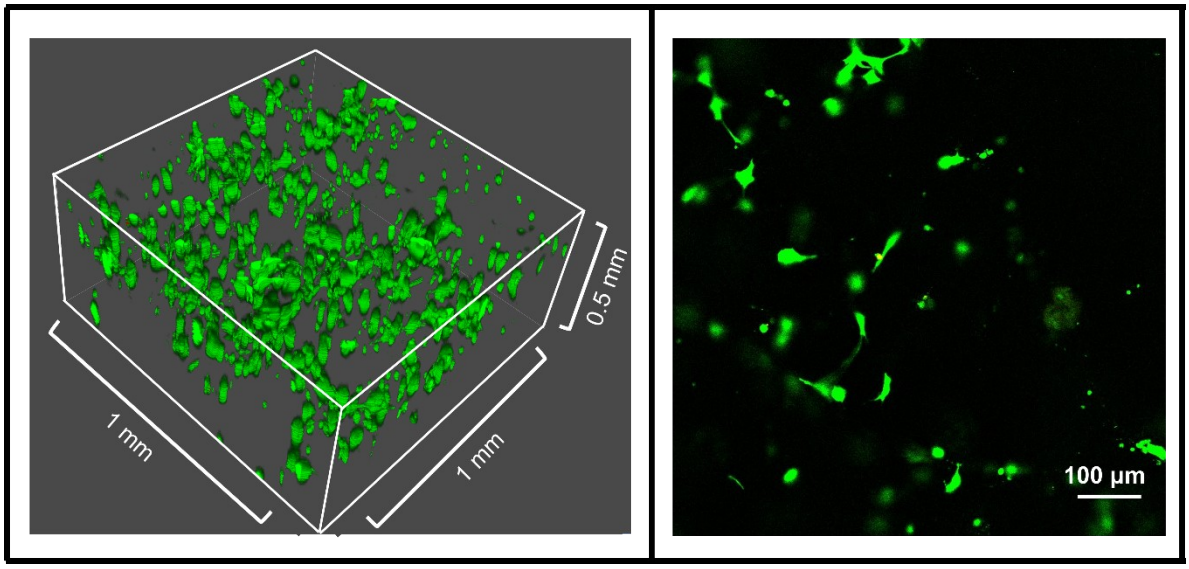

Figure S5. Encapsulation of 3T3 fibroblasts by Snoop-Spy Hydrogels for 5 days. Live (green)/Dead (red) staining was performed to assess cell viability. Representative 3D rendering and *z* slice are shown.

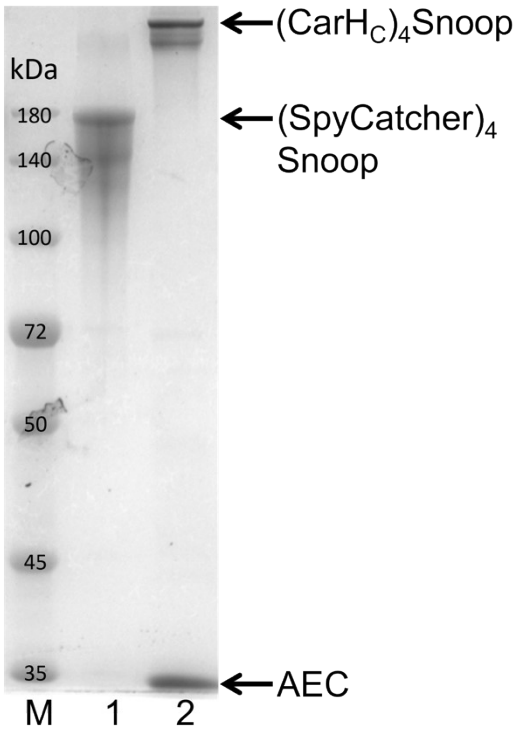

Figure S6. SDS-PAGE analysis of 4-arm star proteins including (SpyCatcher)$_4$Snoop and (CarH$_C$)$_4$Snoop. (CarH$_C$)$_4$Snoop was obtained as a crude product from the reaction of (SpyCatcher)$_4$Snoop + SpyTag-ELP-CarH$_C$ at a 1:5 molar ratio. AEC, SpyTag-ELP-CarH$_C$.

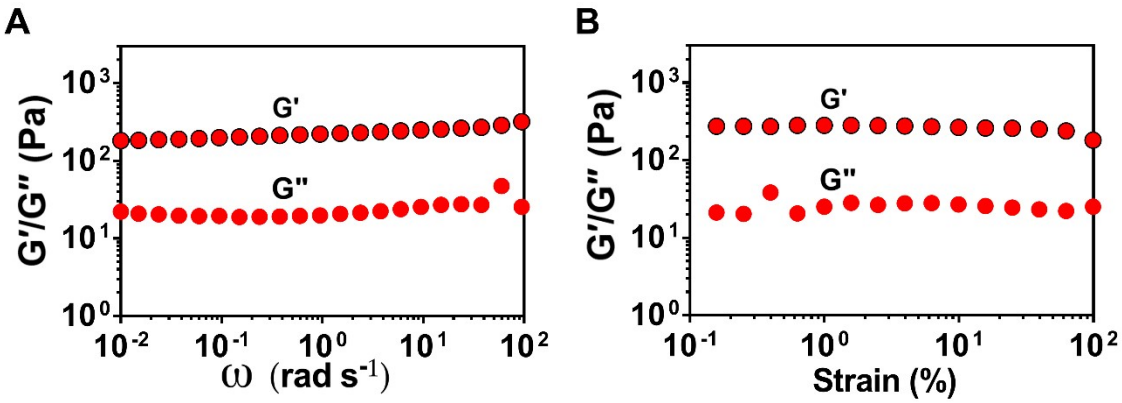

Figure S7. Frequency- and strain-sweep tests of $(CarH_C)_4$Snoop hydrogels (7 wt %). The shear frequency and strain were fixed at 10 rad/s and 10%, respectively.

Video S1. Light-induced release of cells from $(CarH_C)_4$Snoop hydrogel (20X speed).