

Supplementary Information

De novo design of modular protein hydrogels with programmable intra- and extracellular viscoelasticity

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Supplementary Movie Legends

Supplementary movie 1: Fluorescence recovery after photobleaching (FRAP) imaging of the noncovalent C2-5-HA x C5-5-HB-GFP droplet in HeLa cell.

Supplementary movie 2: FRAP imaging of C5-5-HB-GFP alone in HeLa cell.

Supplementary movie 3: FRAP imaging of covalent C2-5-SC x C5-5-ST-GFP gel in HeLa cell.

Supplementary movie 4: FRAP imaging of C5-5-ST-GFP alone in HeLa cell.

Supplementary movie 5: FRAP imaging of covalent C2-1-SC x C5_{2H}-1-ST-GFP in HeLa cell.

Supplementary movie 6: FRAP imaging of covalent C2-1-SC x C5_{7H}-1-ST-GFP in HeLa cell.

Supplementary movie 7: FRAP imaging of covalent C2-1-SC x C5_{15H}-1-ST-GFP in HeLa cell.

Supplementary Figures

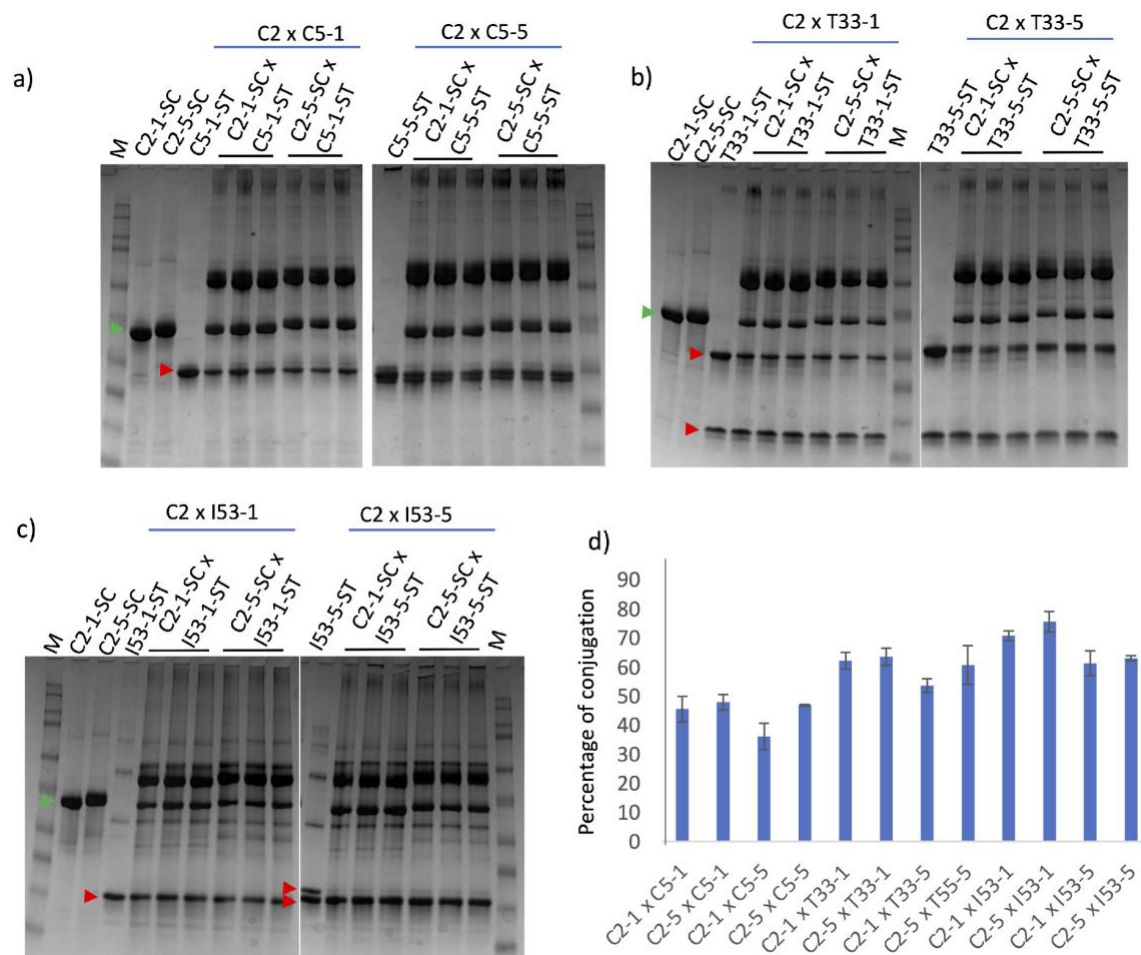


Figure S1. SDS-PAGE gel showing covalent crosslinking between SpyCatcher-modified C2 (green arrow) and SpyTagged higher-order oligomers (red arrow). After hydrogel formation between the respective constructs, samples were dissolved in denaturing (SDS) running buffer and boiled before running on a SDS-PAGE. Results show that upon covalent network formation through SpyLigation, the conjugated oligomers shift to higher position on the gel. a) C2 x C5; b) C2 x T33; and c) C2 x I53 hydrogel systems. Note that T33 and I53 oligomers are constituted of two components and hence two bands are observed in the gel (marked with red arrows). However, in I53-1-ST, both the components have identical sizes and the two bands are not distinguishable. Hydrogels of each formulation (*i.e.*, C2 x C5, C2 x T33, C2 x I53) were run in

triplicate (a, b, c). M: standard protein ladder. d) Quantitative estimation of hydrogel network formation (conjugation efficiency) between the components in each hydrogel. Briefly, the SDS-PAGE images in a, b, and c were subjected to image analysis to quantify the percentage conjugation efficiency. Percentage of unreacted C2-1 or C2-5 in each gel was estimated using ImageJ to determine the percentage conjugation. Results show that the conjugation efficiency varies between ~35% to ~75% among the different constructs.

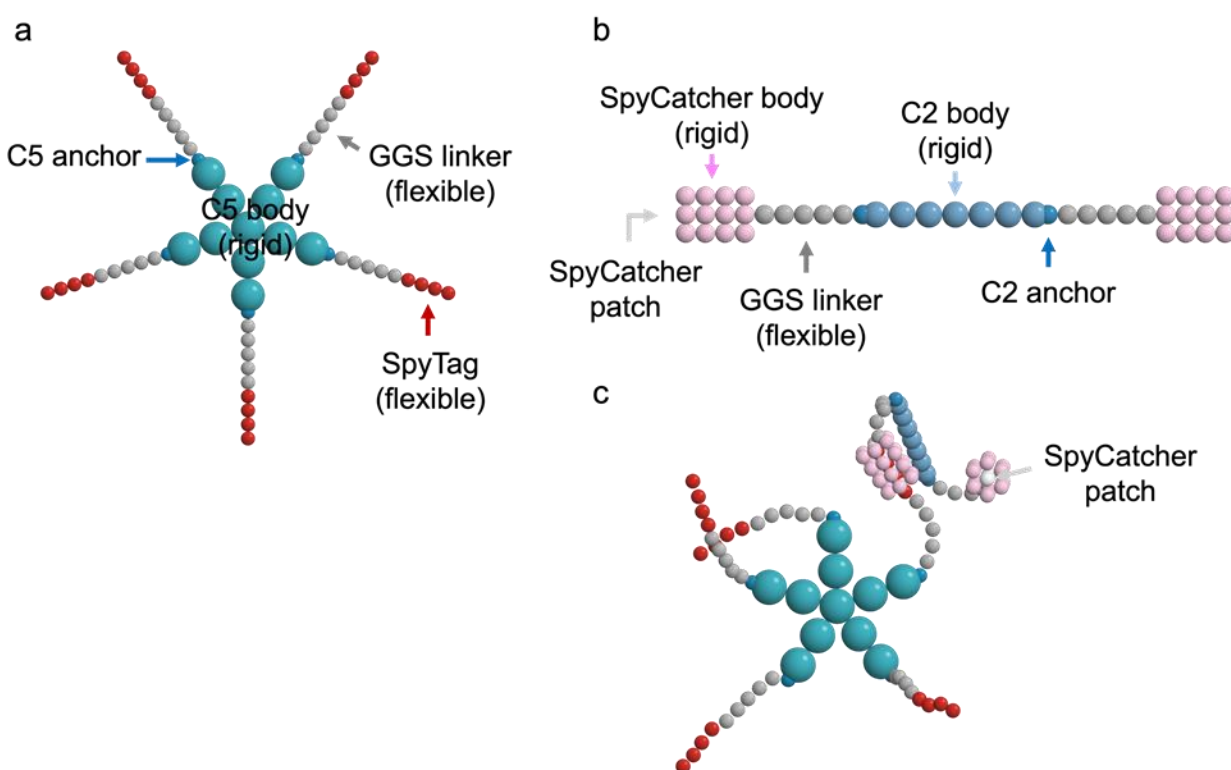


Figure S2. MD simulation model with representative examples C5-5-ST and C2-5-SC. (a) C5 consists of C5 body, C5 anchor, GGS linker, and SpyTag, and their coordinates are listed in Table S1. (b) C2 consists of C2 body, C2 anchor, GGS linker, SpyCatcher body, and SpyCatcher patch. (c) An attractive potential is applied between SpyTag and SpyCatcher patch so that the SpyTag can go into the SpyCatcher body.

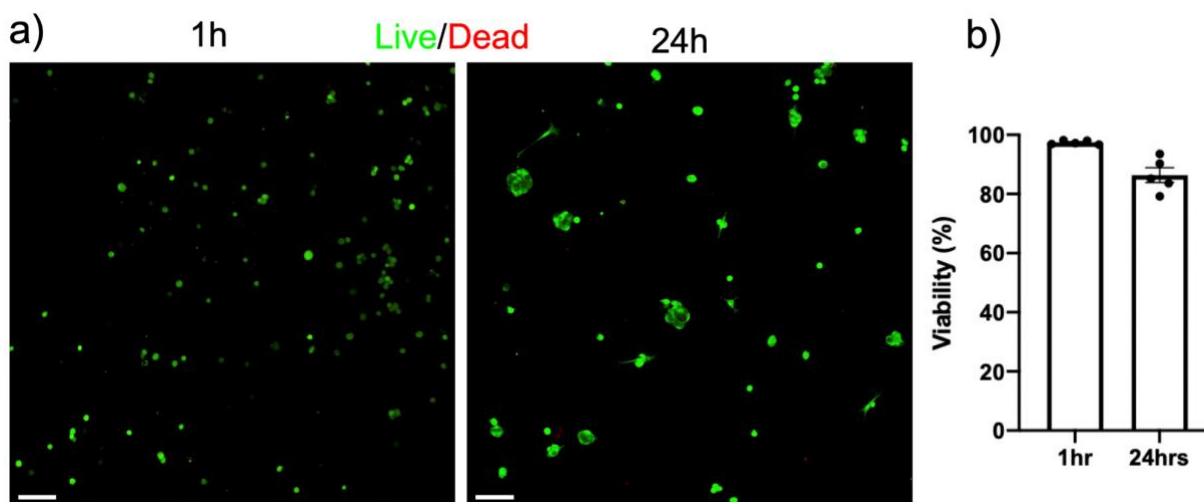


Figure S3: Cell encapsulation of 10T1/2 fibroblast cells in C2-5-SC x C5-5-ST gels. a) Cells were encapsulated and grown for 1 and 24h. Cell viability was assessed by LIVE/DEAD staining and confocal imaging. b) Percentage of cell viability was quantified by CellProfiler. Scale bar 20 μm .

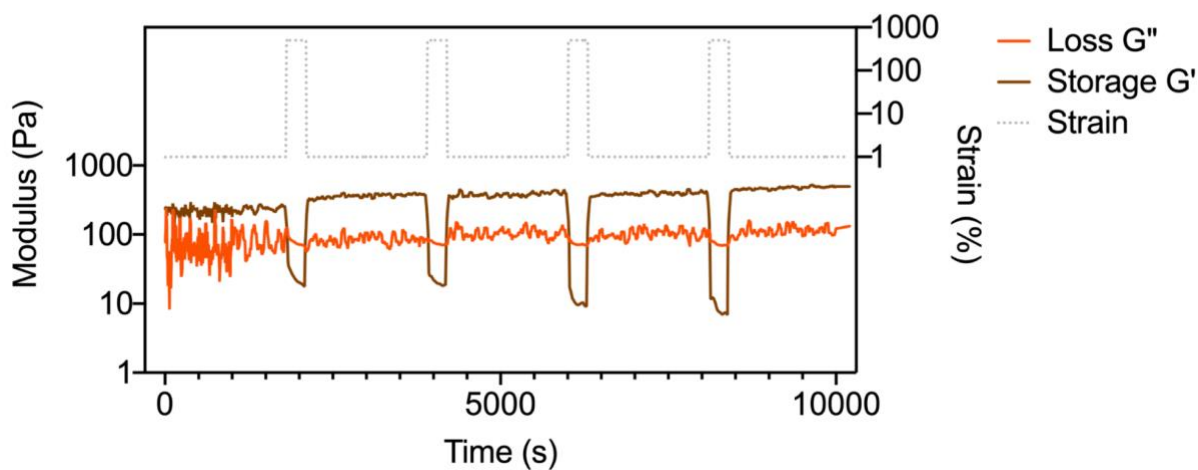


Figure S4. Self-healing properties of C2-5-HA x C5-5-HB noncovalent protein networks mediated by LHD101 heterodimer. The gel networks were capable of self-healing over multiple cycles of 500% strain.

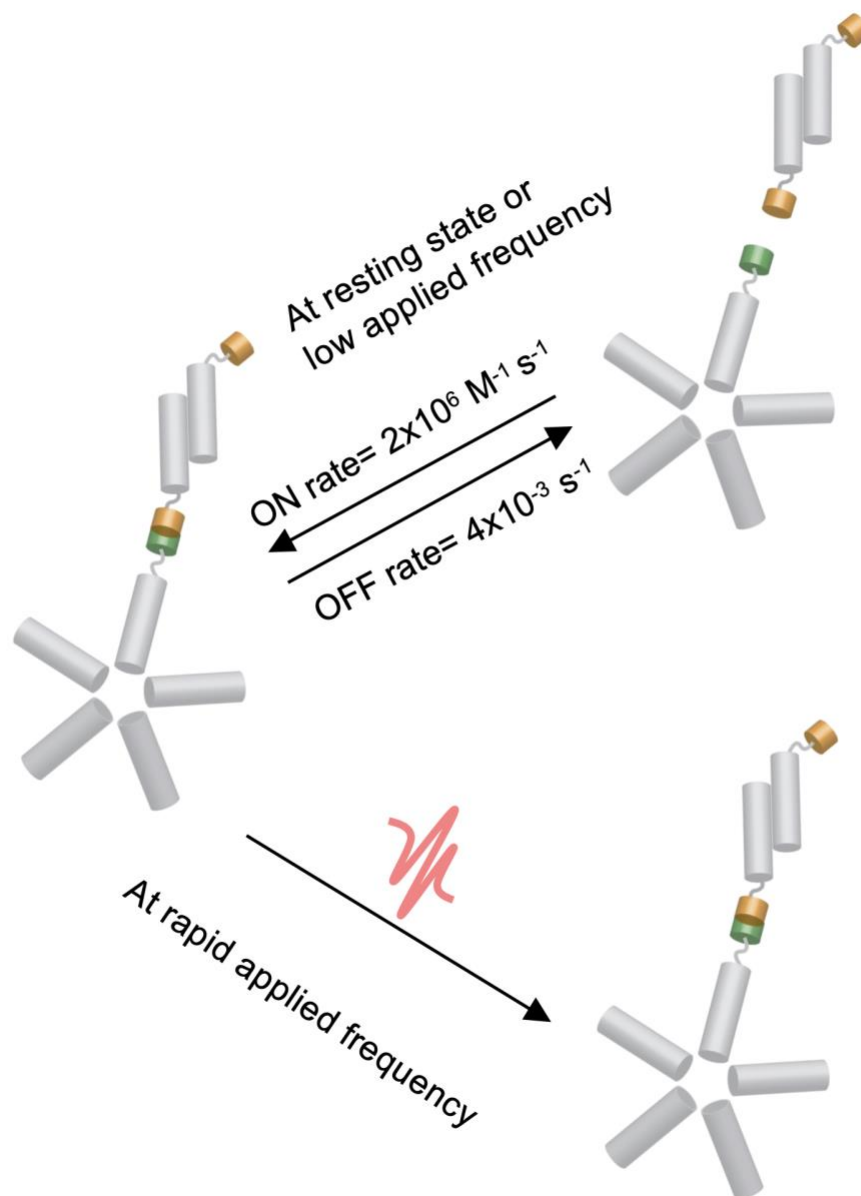


Figure S5. Model showing the noncovalent assembly between C2-5-HA and C5-5-HB at a resting state or at a state when the applied frequency is low vs when the applied frequency is rapid. At the resting state, due to the rapid ON/OFF rate of the heterodimer units, the material behaves like a liquid, whereas, if the assembly exerts a deformation frequency faster than the heterodimer LHD101A:B (HA and HB) ON/OFF rate then the intermolecular interactions get 'locked', and therefore behaves like a gel.

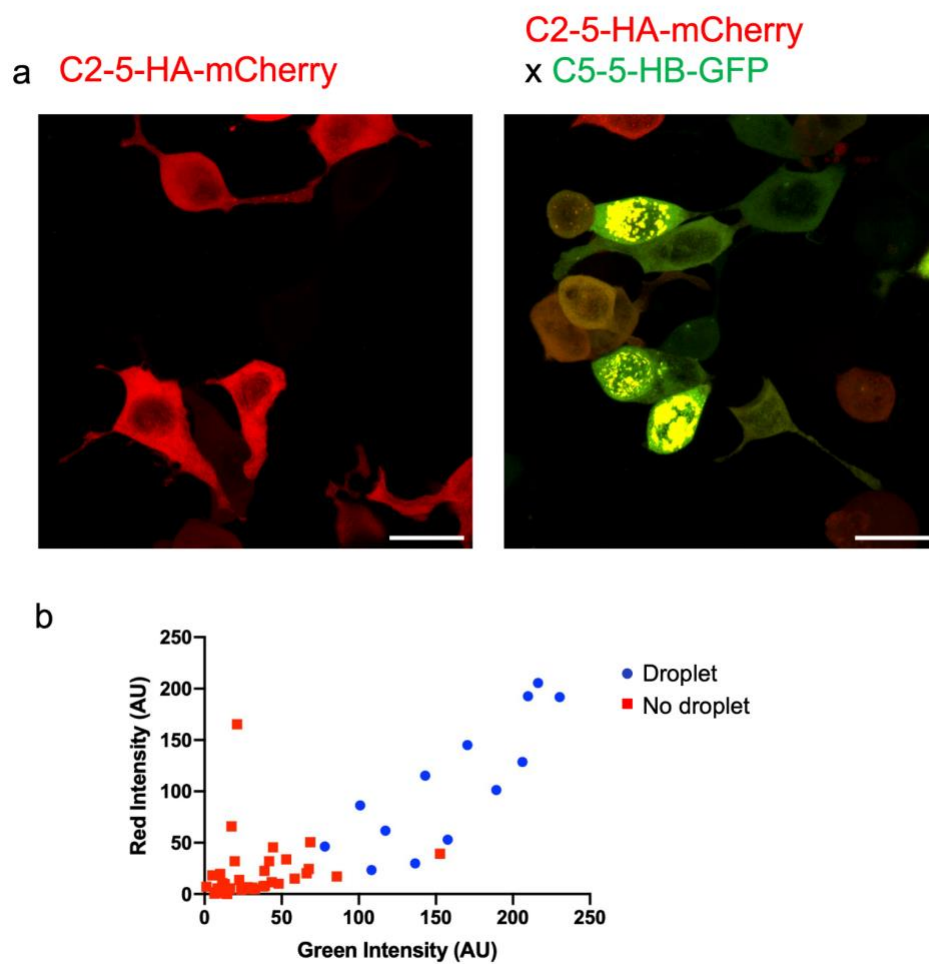


Figure S6. The phase transition from freely diffused constituents to droplet punctae. a) Expression of C2-5-HA-mCherry alone or the coexpression of C2-5-HA-mCherry and C2-5-HB-GFP. b) Droplet formation occurred only at a certain threshold expression of both the components, measured through red (mCherry) and green (GFP) fluorescent intensity.

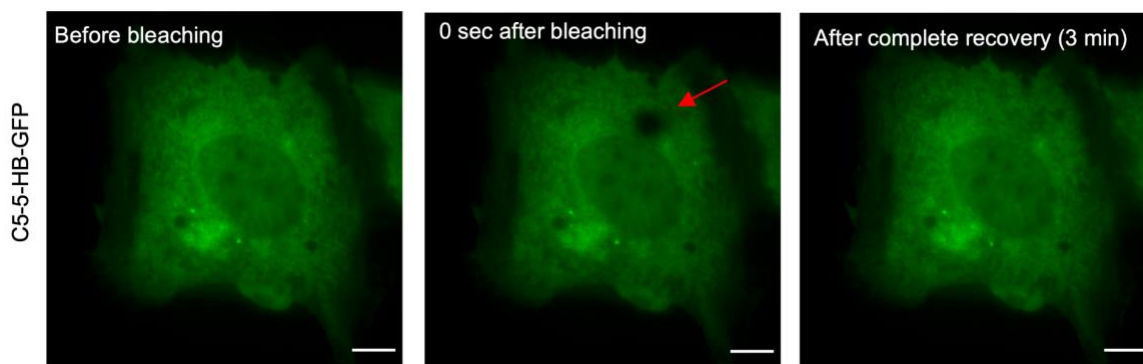


Figure S7. Fluorescence recovery after photobleaching (FRAP) experiment of the control C5-5-HB-GFP expressing cells demonstrated a rapid recovery in the absence of a droplet formation.

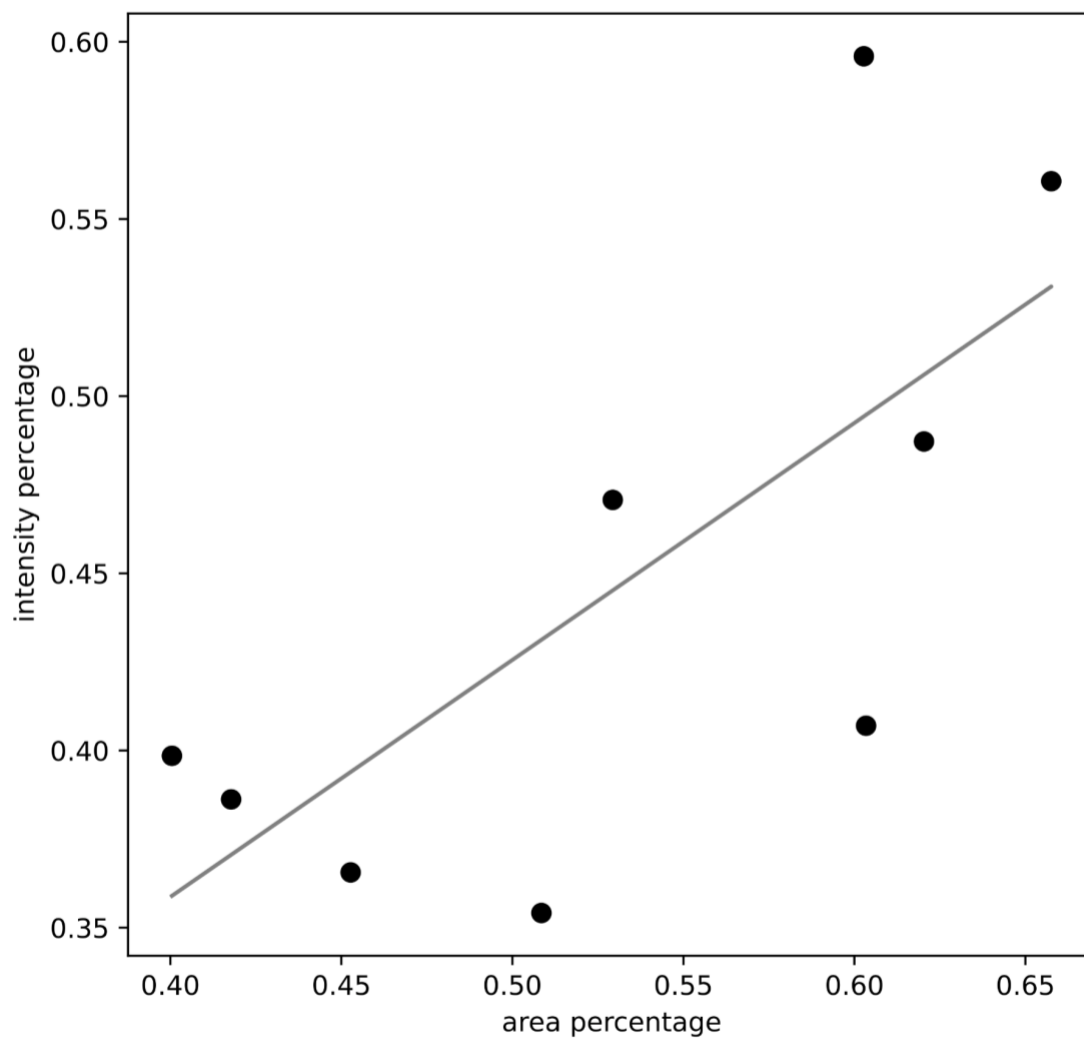


Figure S8. Measurement of bleached area versus fluorescence intensity recovery. The x-axis represents the area percentage, measured by taking the ratio of the droplet area pre and post bleach; whereas the y-axis represents the intensity percentage, measured by taking the ratio of full droplet intensity pre-bleach to the intensity post-recovery.

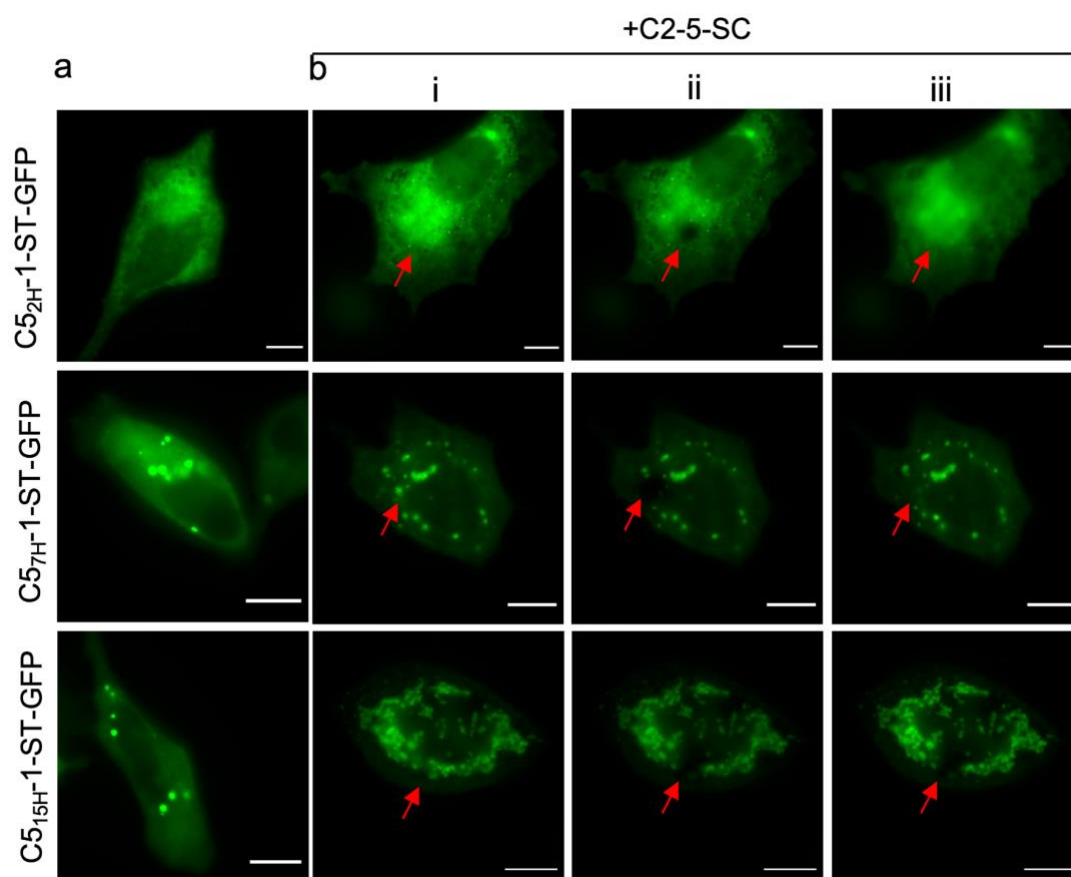


Figure S9: Fluorescence recovery after photobleaching (FRAP) experiment of the rigid arm series. a) Expression of C5_{2H}-1-ST-GFP, C5_{7H}-1-ST-GFP, and C5_{15H}-1-ST-GFP constructs alone, or with C2-5-SC (b). (i) Before photobleaching, (ii) right after photobleaching, and (iii) 3 minutes after photobleaching. Scale bar 10 μ m.

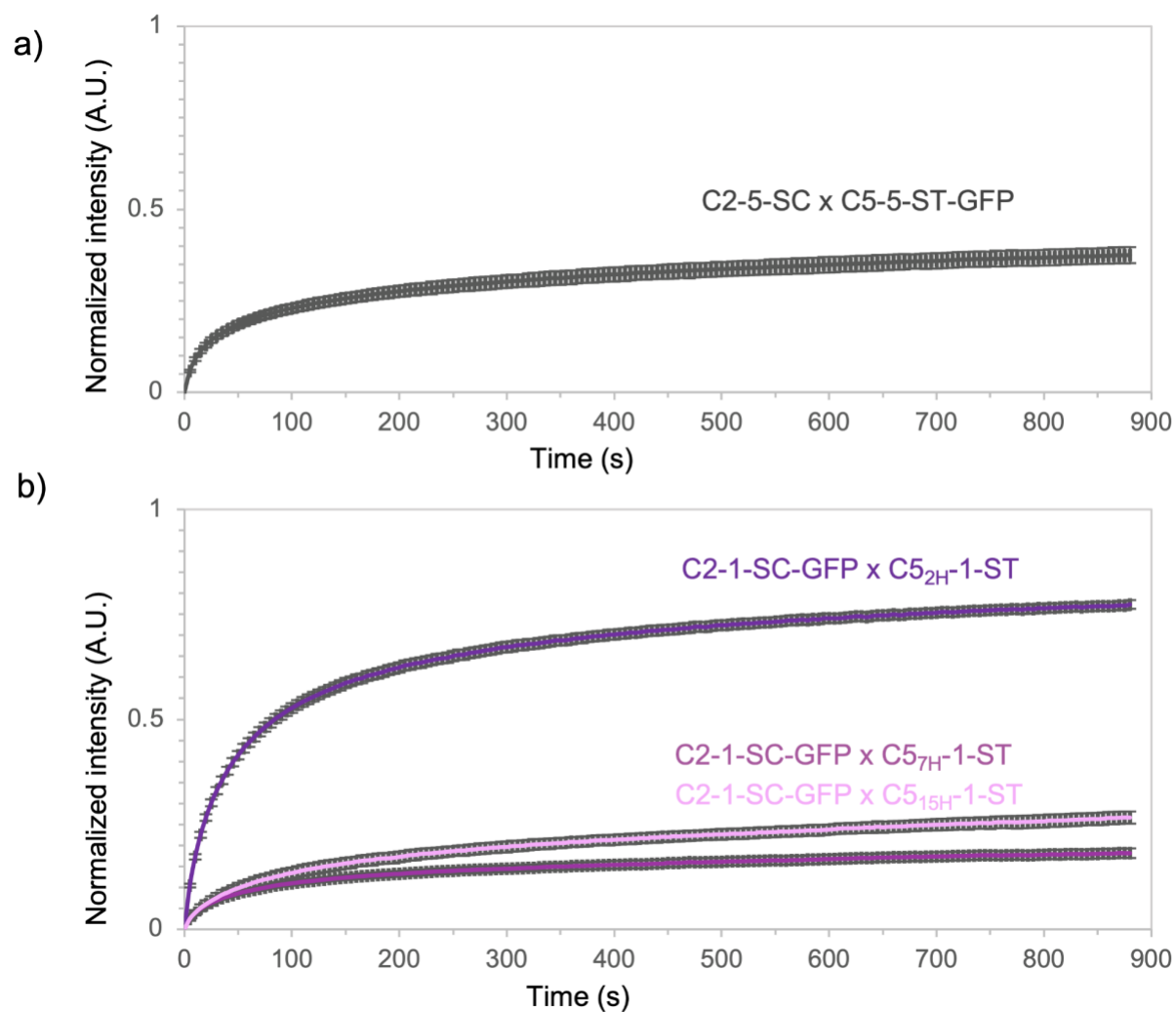


Figure S10: Fluorescence recovery after photobleaching (FRAP) experiment of bulk hydrogel formed by a flexible linker (a) and the rigid arm building block series (b). Time-dependent fluorescence recovery plot of covalent bulk hydrogel (*ex cellulo*) of (a) C2-5-SC x C5-5-ST-GFP; and (b) C2-1-SC-GFP x C5_{2H}-1-ST; C2-1-SC-GFP x C5_{7H}-1-ST; and C2-1-SC-GFP x C5_{15H}-1-ST.

Supplementary Tables

Table S1. Coordinates (XYZ) of components comprising C5-5-ST and C2-5-SC models. The radius of each bead of the C5 body is 1.2σ , and that of the C2 body is 0.67σ . The radius of all other beads is 0.5σ . The length of the GGS linker (*i.e.* the number of beads of the GGS linker) can be changed depending on the system.

C5-5				C2-5			
Type	x	y	z	Type	x	y	z
C5 body	0.000	-0.002	0.000	C2 anchor	-5.466	0.000	-0.059
C5 body	0.000	2.390	0.000	C2 anchor	3.934	0.000	-0.059
C5 body	0.000	4.782	0.000	C2 body	-4.795	0.000	-0.059
C5 body	2.275	0.738	0.000	C2 body	-3.452	0.000	-0.059
C5 body	4.550	1.477	0.000	C2 body	-2.109	0.000	-0.059
C5 body	1.406	-1.937	0.000	C2 body	-0.766	0.000	-0.059
C5 body	2.812	-3.872	0.000	C2 body	0.576	0.000	-0.059
C5 body	-1.406	-1.937	0.000	C2 body	1.919	0.000	-0.059
C5 body	-2.812	-3.872	0.000	C2 body	3.262	0.000	-0.059
C5 body	-2.275	0.738	0.000	GGS linker	-6.466	0.000	-0.059
C5 body	-4.550	1.477	0.000	GGS linker	-7.466	0.000	-0.059
C5 anchor	0.000	5.978	0.000	GGS linker	-8.466	0.000	-0.059
C5 anchor	5.687	1.846	0.000	GGS linker	-9.466	0.000	-0.059
C5 anchor	3.515	-4.840	0.000	GGS linker	-10.466	0.000	-0.059
C5 anchor	-3.515	-4.840	0.000	GGS linker	4.934	0.000	-0.059
C5 anchor	-5.687	1.846	0.000	GGS linker	5.934	0.000	-0.059

GGs linker	0.000	6.978	0.000	GGs linker	6.934	0.000	-0.059
GGs linker	0.000	7.978	0.000	GGs linker	7.934	0.000	-0.059
GGs linker	0.000	8.978	0.000	GGs linker	8.934	0.000	-0.059
GGs linker	0.000	9.978	0.000	SpyCatcher patch	-11.466	0.000	-0.059
GGs linker	0.000	10.978	0.000	SpyCatcher patch	-12.466	0.000	-0.059
GGs linker	6.638	2.155	0.000	SpyCatcher patch	-13.466	0.000	-0.059
GGs linker	7.589	2.464	0.000	SpyCatcher patch	-14.466	0.000	-0.059
GGs linker	8.540	2.773	0.000	SpyCatcher patch	12.934	0.000	-0.059
GGs linker	9.492	3.082	0.000	SpyCatcher patch	11.934	0.000	-0.059
GGs linker	10.443	3.391	0.000	SpyCatcher patch	10.934	0.000	-0.059
GGs linker	4.103	-5.649	0.000	SpyCatcher patch	9.934	0.000	-0.059
GGs linker	4.691	-6.458	0.000	SpyCatcher body	-11.466	0.000	1.041
GGs linker	5.278	-7.267	0.000	SpyCatcher body	-11.466	0.953	0.491
GGs linker	5.866	-8.076	0.000	SpyCatcher body	-11.466	0.953	-0.609
GGs linker	6.454	-8.885	0.000	SpyCatcher body	-11.466	-0.953	-0.609

GGs linker	-4.103	-5.649	0.000	SpyCatcher body	-11.466	-0.953	0.491
GGs linker	-4.691	-6.458	0.000	SpyCatcher body	-12.466	0.000	1.041
GGs linker	-5.278	-7.267	0.000	SpyCatcher body	-12.466	0.953	0.491
GGs linker	-5.866	-8.076	0.000	SpyCatcher body	-12.466	0.953	-0.609
GGs linker	-6.454	-8.885	0.000	SpyCatcher body	-12.466	-0.953	-0.609
GGs linker	-6.638	2.155	0.000	SpyCatcher body	-12.466	-0.953	0.491
GGs linker	-7.589	2.464	0.000	SpyCatcher body	-13.466	0.000	1.041
GGs linker	-8.540	2.773	0.000	SpyCatcher body	-13.466	0.953	0.491
GGs linker	-9.492	3.082	0.000	SpyCatcher body	-13.466	0.953	-0.609
GGs linker	-10.443	3.391	0.000	SpyCatcher body	-13.466	-0.953	-0.609
SpyTag	0.000	11.978	0.000	SpyCatcher body	-13.466	-0.953	0.491
SpyTag	0.000	12.978	0.000	SpyCatcher body	-14.466	0.000	1.041
SpyTag	0.000	13.978	0.000	SpyCatcher body	-14.466	0.953	0.491
SpyTag	0.000	14.978	0.000	SpyCatcher body	-14.466	0.953	-0.609
SpyTag	11.394	3.700	0.000	SpyCatcher body	-14.466	-0.953	-0.609
SpyTag	12.345	4.009	0.000	SpyCatcher body	-14.466	-0.953	0.491
SpyTag	13.296	4.318	0.000	SpyCatcher body	12.934	0.000	1.041

SpyTag	14.247	4.627	0.000	SpyCatcher body	12.934	0.953	0.491
SpyTag	7.042	-9.694	0.000	SpyCatcher body	12.934	0.953	-0.609
SpyTag	7.629	-10.503	0.000	SpyCatcher body	12.934	0.000	-1.159
SpyTag	8.217	-11.312	0.000	SpyCatcher body	12.934	-0.953	-0.609
SpyTag	8.805	-12.121	0.000	SpyCatcher body	12.934	-0.953	0.491
SpyTag	-7.042	-9.694	0.000	SpyCatcher body	11.934	0.000	1.041
SpyTag	-7.629	-10.503	0.000	SpyCatcher body	11.934	0.953	0.491
SpyTag	-8.217	-11.312	0.000	SpyCatcher body	11.934	0.953	-0.609
SpyTag	-8.805	-12.121	0.000	SpyCatcher body	11.934	0.000	-1.159
SpyTag	-11.394	3.700	0.000	SpyCatcher body	11.934	-0.953	-0.609
SpyTag	-12.345	4.009	0.000	SpyCatcher body	11.934	-0.953	0.491
SpyTag	-13.296	4.318	0.000	SpyCatcher body	10.934	0.000	1.041
SpyTag	-14.247	4.627	0.000	SpyCatcher body	10.934	0.953	0.491
				SpyCatcher body	10.934	0.953	-0.609
				SpyCatcher body	10.934	0.000	-1.159
				SpyCatcher body	10.934	-0.953	-0.609
				SpyCatcher body	10.934	-0.953	0.491
				SpyCatcher body	9.934	0.000	1.041
				SpyCatcher body	9.934	0.953	0.491
				SpyCatcher body	9.934	0.953	-0.609
				SpyCatcher body	9.934	0.000	-1.159

				SpyCatcher body	9.934	-0.953	-0.609
				SpyCatcher body	9.934	-0.953	0.491

Supplementary Protein Sequences

Amino acid sequence: **Yellow:** linker; **Red:** SpyCatcher; **Blue:** SpyTag; **Orange:** LHD101A (HA); **Purple:** LHD101B (HB); **Green:** Fluorescent protein

Covalent:

>C2-GGS-SpyCatcher (C2-1-SC)

MGTREEIIRELARSLAEQAELTARLERLLRELERLQREGSSDEDVRELLREIKELVREILKLIAEQILLI
AELLAAIRSEAAELALRAIREAIELCKRSTDEELCQLLLRLALLLMEALLLYPDSEAAKLALKAALAEAI
ELCKQSTDEELCEELVKLAQKLIELAKRYPDSEAAKLALKAALAEAIELCKQSTDEELCEELVKLAQKLI
LAKRYPDSEAAKRALKEAKELIEQCKESTDEDECRELVKRAEELIREAKE**GGSDSATHIKFSKRIDGKE**
LAGATMELRDSSGKTISTWISDGQVKDFYLMPGKYTFVETAAPDGYEIATAITFTVNEQGQVTVNGKATK
GGSWGLEHHHHHH

>C2-(GGS)₅-SpyCatcher (C2-5-SC)

MGTREEIIRELARSLAEQAELTARLERLLRELERLQREGSSDEDVRELLREIKELVREILKLIAEQILLI
AELLAAIRSEAAELALRAIREAIELCKRSTDEELCQLLLRLALLLMEALLLYPDSEAAKLALKAALAEAI
ELCKQSTDEELCEELVKLAQKLIELAKRYPDSEAAKLALKAALAEAIELCKQSTDEELCEELVKLAQKLI
LAKRYPDSEAAKRALKEAKELIEQCKESTDEDECRELVKRAEELIREAKE**GGSGSGSGSGSGSDSATH**
IKFSKRIDGKELAGATMELRDSSGKTISTWISDGQVKDFYLMPGKYTFVETAAPDGYEIATAITFTVNE
QQGQVTVNGKATK**G**GSWGLEHHHHHH

>C5-(GGS)₁-SpyTag (C5-1-ST)

MGHHHHHHGWSGAHIVMVDAYKPTK**GG**SNDKEKELKELLKRAEELAKSPDPEDLKEAVRLAEVVRERPG
SNLAKKALEIILRAAEELAKLPDPEALKEAVKAAEKVVREQPGSNLAKKAQEIILRAAEELAKLEDEEAL
KEAIKAAEKVIELEPGSELAKEAKRIIEKAAKMLADILRKEMEKIREETEEVKKEIEESKKRPQSESAN
LILIMQLLINQIRLLALQIRMLVLQLIL

>C5-(GGS)₅-SpyTag (C5-5-ST)

>I53-(GGG)₁-SpyTag_chainB (I53-1-ST_chainB)

MEEAELAYLLGELAYKLGEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALELDPN
 NAEAWYNLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGSAHIVMVDAYKPTKGSWG
 LEHHHHHH

>I53-(GGG)₅-SpyTag_chainB (I53-5-ST_chainB)

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 NAEAWYNLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGSGSGSGSGSGSAHIVM
 VDAYKPTKGSWGLEHHHHHH

>I53-(GGG)₁₀-SpyTag_chainB (I53-10-ST_chainB)

MEEAELAYLLGELAYKLGEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALELDPN
 NAEAWYNLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGSGSGSGSGSGSGSGG
 SGGSGSGSGSAHIVMVDAYKPTKGSWGLEHHHHHH

>C5_{2H}-(GGG)₁-SpyTag (C5_{2H}-1-ST)

MGHHHHHHGWSGAHIVMVDAYKPTKGGSTRRKQEMKRLKKEMEKIREETEEVKKEIEESKKRPQSES
 AKNLILIMQLLINQIRLLALQIRMLALQLQE

>C5_{7H}-(GGG)₁-SpyTag (C5_{7H}-1-ST)

MGHHHHHHGWSGAHIVMVDAYKPTKGGSDLQEVADRIVEQLKREGRSPPEARKEARRLIEEIKQSAGGD
 SELIEVAVRIVKFLEEAGMSPSEAAKVAVELIERIRRAAGDSELIEKAVRIVRRLERRGLSPA
 EAAKIAVAIIAAEVLRSREAEKIREETEEVKKEIEESKKRPQSES
 AKNLILIMQLLINQIRLLALQIQMLRLQLEL

>C5_{15H}-(GGG)₁-SpyTag (C5_{15H}-1-ST)

MGHHHHHHGWSGAHIVMVDAYKPTKGGSDLQEVADRIVEQLKREGRSPPEARKEARRLIEEIKQSAGGD
 SELIEVAVRIVKELEEQGRSPSEAAKEAVELIERIRRAAGDSELIEVAVRIVKELEEQGRSPSEAAKEA
 VELIERIRRAAGDSELIEVAVRIVKELEEQGRSPSEAAKEAVELIERIRRAAGDSELIEVAVRIVKEL
 EEQGRSPSEAAKEAVELIERIRRAAGDSELIEVAVRIVKFLEEAGMSPSEAAKVAVELIERIRRAAGD
 SELIEKAVRIVRRLERRGLSPA
 EAAKIAVAIIAAEVLRSREAEKIREETEEVKKEIEESKKRPQSES
 AKNLILIMQLLINQIRLLALQIQMLRLQLEL

Noncovalent:>C2-(GGG)₅-LHD101A (C2-5-HA)

MGTREEIIRELARSLAEQAELTARLERLLRELERLQREGSSDEDVRELLREIKELVREILKLI
 AEQILLIAELLAAIRSEAAELALRAIREAIELCKRSTDEELCQLLLRLALLLME
 LALLYPDSEAAKLALKA
 ALEAIELCKQSTDEELCEELVKLAQKLI
 ELAKRYPDSEAAKLALKA
 ALEAIELCKQSTDEELCEELVKLAQKLI

LAKRYPDSEEAKRALKEAKELIEQCKESTDEDECRELVKRAEELIREAKEGGSGGSGGSGGSGGSGRQEK
 VLKSIETVRKMGVTMETHRSGNEVKVVIKGLHIKQQRQLYRDVRETSKKQGVETEIEVEGDTVTIVVRE
 GSWGLEHHHHHH

>C5-(GGG)₅-LHD101B (C5-5-HB)

MGHHHHHHGWSGGRQEKVLKSIETVRKMGVTMETHRSGNEVKVVIKGLHESQQEQILLEDVLRTAEKQGV
 RVRIRFKGDTVTIVVREGGSGGSGGSGGSGGSGNDEKEKLKELLKRAEELAKSPDPEDLKEAVRLAEVVR
 ERPGSNLAKKALEIILRAAEELAKLPDPEALKEAVKAAEKVVREQPGSNLAKKAQEIILRAAEELAKLED
 EEALKEAIKAAEKVIELEPGSELAKEAKRIIEKAAKMLADILRKEMEKIREETEEVKKEIEESKKRPQSE
 SAKNLILIMQLLINQIRLLALQIRMLVLQLIL

GFP/mCherry fusion proteins:

>C5-(GGG)₅-SpyTag-GFP (C5-5-ST-GFP)

MSKGEEELFTGVVPIIVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLTYGVC
 FSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIIDFKEDGNIILGHK
 LEYNYNSHNVIIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKD
 PNEKRDHMLLEFVTAAGITLGMDELYKGGSGGSGGSGGSGGSAHIVMVDAYKPTKGGSGGSGGSGGSGG
 SNDEKEKLKELLKRAEELAKSPDPEDLKEAVRLAEVVRERPGSNLAKKALEIILRAAEELAKLPDPEAL
 KEAVKAAEKVVREQPGSNLAKKAQEIILRAAEELAKLEDEEALKEAIKAAEKVIELEPGSELAKEAKRII
 EKAAMMLADILRKEMEKIREETEEVKKEIEESKKRPQSESANLILIMQLLINQIRLLALQIRMLVLQLI
 L

>C5-(GGG)₅-LHD101B-GFP (C5-5-HB-GFP)

MSKGEEELFTGVVPIIVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLTYGVC
 FSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIIDFKEDGNIILGHK
 LEYNYNSHNVIIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKD
 PNEKRDHMLLEFVTAAGITLGMDELYKGGSGGSGGSGGSGGSGRQEKVLKSIETVRKMGVTMETHRSG
 NEVKVVIKGLHESQQEQILLEDVLRTAEKQGVVRIRIRFKGDTVTIVVREGGSGGSGGSGGSGGSGNDEKEKL
 KELLKRAEELAKSPDPEDLKEAVRLAEVVRERPGSNLAKKALEIILRAAEELAKLPDPEALKEAVKAAE
 KVVREQPGSNLAKKAQEIILRAAEELAKLEDEEALKEAIKAAEKVIELEPGSELAKEAKRIIEKAAKMLA
 DILRKEMEKIREETEEVKKEIEESKKRPQSESANLILIMQLLINQIRLLALQIRMLVLQLIL

>C2-GGS-SpyCatcher-mCherry (C2-1-SC-mCherry)

MGTREEIIRELARSLAEQAELTARLERLLRELERLQREGSSDEDVRELLREIKELVREILKLI AEQILLI
 AELLAAIRSEAAELALRAIREAIELCKRSTDEELCQLLLRLALLMELALLYPDSEAAKLALKAALAEAI
 ELCKQSTDEELCEELVKLAQKLI ELAKRYPDSEAAKLALKAALAEAI ELCKQSTDEELCEELVKLAQKLI
 LAKRYPDSEEAKRALKEAKELIEQCKESTDEDECRELVKRAEELIREAKEGGSDSATHIKFSKRDI DGKE
 LAGATMELRDSGKTISTWISDGQVKDFYLMPGKYTFVETAAPDGYEIAITAITFTVNEQGQVTVNGKATK

GGSGSGSGSGSGSVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTK
 GGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIY
 KVKLRGTNFPDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLDGGHYDAEVKTTYKAKKPVQLP
 GAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK

>C5_{2H}-GGG-SpyTag-GFP (C5_{2H}-1-ST-GFP)

MSKGEELFTGVVPIIIVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTTLTYGVQC
 FSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNIILGHK
 LEYNYNSHNVIYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKD
 PNEKRDHMLLEFVTAAGITLGMDELYKGGSGSGSGSGSGSAHIVMVDAYKPTKGGSTRRKQEMKRLK
 KEMEKIREETEEVKKEIEESKKRPQSESANKLILIMQLLINQIRLLALQIRMLALQLQE

>C5_{7H}-GGG-SpyTag-GFP (C5_{7H}-1-ST-GFP)

MSKGEELFTGVVPIIIVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTTLTYGVQC
 FSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNIILGHK
 LEYNYNSHNVIYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKD
 PNEKRDHMLLEFVTAAGITLGMDELYKGGSGSGSGSGSGSAHIVMVDAYKPTKGGSDLQEVADRIV
 EQLKREGRSPPEARKEARLIEEIKQSAGGSELIEVAVRIVKFLLEEAGMSPSEAAKVAVELIERIRRAA
 GGSELIEKAVRIVRRLERRGLSPAEEAKIAVAIIAAEVLSREAEEKIREETEEVKKEIEESKKRPQSESA
 KNLILIMQLLINQIRLLALQIQMLRLQLEL

>C5_{15H}-GGG-SpyTag-GFP (C5_{15H}-1-ST-GFP)

MSKGEELFTGVVPIIIVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTTLTYGVQC
 FSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKIDFKEDGNIILGHK
 LEYNYNSHNVIYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKD
 PNEKRDHMLLEFVTAAGITLGMDELYKGGSGSGSGSGSGSAHIVMVDAYKPTKGGSDLQEVADRIV
 EQLKREGRSPPEARKEARLIEEIKQSAGGSELIEVAVRIVKELEEQGRSPSEAAKEAVELIERIRRAA
 GGSELIEVAVRIVKELEEQGRSPSEAAKEAVELIERIRRAAGGSELIEVAVRIVKELEEQGRSPSEAA
 KEAVELIERIRRAAGGSELIEVAVRIVKELEEQGRSPSEAAKEAVELIERIRRAAGGSELIEVAVRIV
 KFLEEAGMSPSEAAKVAVELIERIRRAAGGSELIEKAVRIVRRLERRGLSPAEEAKIAVAIIAAEVLSR
 EAEKIREETEEVKKEIEESKKRPQSESANKLILIMQLLINQIRLLALQIQMLRLQLEL

>C2-(GGG)₅-LHD101A-mCherry (C2-5-HA-mCherry)

MGTREEIIRELARSLAEQAELTARLERLLRELERLQREGSSDEDVRELLREIKELVREILKLIQILLI
 AELLAAIRSEAAELALRAIREAIELCKRSTDEELCQLLLRLALLLMEALALYPDSEAAKLALKAALAEAI
 ELCKQSTDEELCEELVKLAQKLIELAKRYPDSEAAKLALKAALAEAIELCKQSTDEELCEELVKLAQKLI
 LAKRYPDSEAAKRALKEAKELIEQCKESTDEDECRELVKRAEELIREAKEGGSGSGSGSGSGGRQEK
 VLKSIETVRKMGVTMETHRSGNEVKVVIKGLHIKQQRQLYRDVRETSKKQGVETEIEVEGDTVTVVRE
 GSGSGSGSGSGSGSVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKG
 GPLPFAWDILSPQFMYGSKAYVKHPADIPDYLLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYK
 VKLRGTNFPDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLDGGHYDAEVKTTYKAKKPVQLPG
 AYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK

>C5-(GGG)₅-LHD101B-Lifeact-GFP (C5-5-HB-Lifeact-GFP) [Lifeact in cyan]

MGVADLIKKFESISKEEGDPPVATMVSKEELFTGVVPIVVELDGDVNGHKFSVSGEGEGDATYGKLTLLK
 FICTTGKLPVPWPTLVTTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFE
 GDTLVNRIELKIDFKEDGNIILGHKLEYNFNHNVYITADKQKNGIKANFKIRHNVEDGVSQVLADHYQQN
 TPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDMVLEFVTAAGITHGMDELYKGGSGSGSGSGSGSGS
 GRQEKVLKSI EETVRKMGVTMETHRSGNEVKVVIKGLHESQQEQLLLEDVLRTAEKQGVVRVIRFKGDTVTIV
 VREGGSGSGSGSGSGSGSNDEKEKLLKELLKRAEELAKSPDPEDLKEAVRLAEVVRERPGSNLAKKALEI
 ILRAAEELAKLPDPEALKEAVKAAEKVVREQPGSNLAKKAQEII LRAAEELAKLEDEEALKEAIKAAEKV
 IELEPGSELAKEAKRIIEKAAKMLADILRKEMEKIREEVEVKKEIEESKKRPQSESANLILIMQLLIN
 QIRLLALQIRMLVLQLIL

>C2-GGS-SpyCatcher-GFP (C2-1-SC-GFP) (bacterial expression)

MGTREEIIRELARSLAEQAELTARLERLLRELERLQREGSSDEDVRELLREIKELVREILKLI AEQILLI
 AELLLAAIRSEAAELALRAIREAIELCKRSTDEELCQLLLRLALLLMEALLLYPDSEAAKLALKALEAI
 ELCKQSTDEELCEELVKLAQKLI ELAKRYPDSEAAKLALKALEAIELCKQSTDEELCEELVKLAQKLI
 LAKRYPDSEAAKRALKEAKELIEQCKESTDEDECRELVKRAEELIREAKEGGSDSATHIKFSKRDI DGKE
 LAGATMELRDSGKTISTWISDGQVKDFYLMPGKYTFVETAAPDGYEIAATAITFTVNEQGQVTVNGKATK
 GGGSGSGSGSGSGSGSMKSGEELFTGVVPIVVELDGDVNGHKFSVRGEGEGDATNGKLTLLKFICTTGKLP
 VPWPTLVTTTLTYGVQCFAFYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIE
 LKIDFKEDGNIILGHKLEYNFNHNVYITADKQKNGIKANFKIRHNVEDGVSQVLADHYQQNTPIGDGPVL
 LPDNHYLSTQSVLSKDPNEKRDMVLEFVTAAGITHGMDELYKLEHHHHHH

>C5-(GGG)₅-SpyTag-GFP (C5-5-ST-GFP) (bacterial expression)

MSKGEELFTGVVPIVVELDGDVNGHKFSVRGEGEGDATNGKLTLLKFICTTGKLPVPWPTLVTTTLTYGVQC
 FARYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKIDFKEDGNIILGHK
 LEYNFNHNVYITADKQKNGIKANFKIRHNVEDGVSQVLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSK
 PNEKRDMVLEFVTAAGITHGMDELYKGGSGSGSGSGSGSGSAHIVMVDAYKPTKGGSGSGSGSGSGSG
 SNDEKEKLLKELLKRAEELAKSPDPEDLKEAVRLAEVVRERPGSNLAKKALEIILRAAEELAKLPDPEAL
 KEAVKAAEKVVREQPGSNLAKKAQEII LRAAEELAKLEDEEALKEAIKAAEKVIELEPGSELAKEAKRII
 EKAAKMLADILRKEMEKIREEVEVKKEIEESKKRPQSESANLILIMQLLINQIRLLALQIRMLVLQLI
 LLEHHHHHH