Supporting Information for:

Diverse Supramolecular Nanofiber Networks Assembled by Functional Low-Complexity Domains

Bolin An[†,‡,§,#], Xinyu Wang[†,‡,¶,#], Mengkui Cui[†,‡,§], Xinrui Gui[±], Xiuhai Mao[†], Yan Liu[//,+], Ke Li[†,‡,¶], Cenfeng Chu[//,+], Jiahua Pu[†,‡], Susu Ren[†,‡,§], Yanyi Wang[†,‡], Guisheng Zhong[//,+], Timothy K. Lu[&], Cong Liu[±] and Chao Zhong[†,*]

[†]*School of Physical Science and Technology, ShanghaiTech University, Shanghai 201210, China;*

[‡]*University of Chinese Academy of Sciences, Beijing 100049, China;*

[¶]*Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai*

200050, China;

[§]*Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China;*

[±]*Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Science, Shanghai 200032, China;*

[//]*iHuman Institute, ShanghaiTech University, Shanghai, 201210. China;*

[+]*School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China;*

[&]*Department of Electrical Engineering and Computer Science and Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139-4307, USA*

[#] These authors contributed equally to this work

* Corresponding author. Email: zhongchao@shanghaitech.edu.cn

Supplementary Information:

Materials and Methods

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Materials and Methods

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1. Amino-acid sequences and genetic designs used in this study

1.1 Amino-acid sequences

Amino-acid sequence of the FUS LC domain:

ASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGYGQ
SSYSSYGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQP
APSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQSYNPPQGYG
QQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQDQSGGGGS
GGYGQQDRG

Amino-acid sequence of the EGFP domain:

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTG
KLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDD
GNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMAD
KQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLS
KDPNEKRDHMVLLEFVTAAGITLGMDELYKAG

Amino-acid sequence of the mCherry domain:

MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLK
VTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNF
EDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERM
YPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDIT
SHNEDYTIVEQYERAEGRHSTGGMDELYKAG

Amino-acid sequence of the mMaple3 domain:

MVSKGEETIMSVIKPDMKIKLRMEGNVNGHAFVIEGEGSGKPFEGIQTIDLEV
KEGAPLPFAYDILTTAFHYGNRVFTKYPRKIPDYFKQSFPEGYSWERSMTYED
GGICNATNDITMEEDSFINKIHFKGTNFPPNGPVMQKRTVGWEVSTEKMYVR
DGVLKGDVKMKLLLKGGSHYRCDFRTTYKVKQKAVKLPKAHFVDHRIEILS
HDKDYNKVKLYEHAVARNSTDSMDELYK

Amino-acid sequence of the PAtagRFP domain:

MSELIKENMHMKLYMEGTVNNHHFKCTSEGEGKPYEGTQTMRIKVVEGGPL
PFAFDILATSFMYGSSTFINHTQGIPDFWKQSFPEGFTWERVTTYEDGGVLTA
TQDTSLQDGCLIYNVKIRGVNFPSNGPVMKKKTLGWEPSTEKLKPADGGLEG
RVDMALKLVGGGHLICNFKTTYRSKKPAKNLKMPGVYYVDRRLEIIKEADK
ETYWEQHEVAVARYSDLPSKLGHKLN

Amino-acid sequence of the Spycatcher domain:

MVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSG
KTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVN
GKATKGDAHID

Amino-acid sequence of the CsgA domain:

MGVVPQYGGGGNHGGGGNNSGPNSELNIYQYGGGNSALALQTDARNSDLTI
TQHGGGNGADVGQGSDDSSIDLTQRGFGNSATLDQWNGKNSEMTVKQFGG
GNGAAVDQTASNSSVNVTQVGFGNNATAHQYLE

Amino-acid sequence of the Spytag domain:

AHIVMVDAYKPTK

Linker sequence:
GGGGS or GGGS

1.2 Genetic designs used in this study

a) FUS LC construct:
(HisTag-TEVsite-FUS LC)

MSYYHHHHHHDYDIPTTENLYFQGAMDPASNDYTQQATQSYGAYPTQPGQG
YSQQSSQPYGQQSYSGYSQSTDTSGYGQSSYSSYGQSQNTGYGTQSTPQGYG
STGGYGSSQSSQSSYGQQSSYPGYGQQPAPSSTSGSYGSSSQSSSYGQPQSGSY
SQQPSYGGQQQSYGQQQSYNPPQGYGQQNQYNSSSGGGGGGGGGGNYGQD
QSSMSSGGGSGGGYGNQDQSGGGGSGGYGQQDRG

b) EGFP-FUS LC construct:
(HisTag-TEVsite-EGFP-FUS LC)

MSYYHHHHHHDYDIPTTENLYFQGAMVSKGEELFTGVVPILVELDGDVNGH
KFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHM
KQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFK
EDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDEL
YKAGTMDPASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQS
TDTSGYGQSSYSSYGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSS
YPGYGQQPAPSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQSY
NPPQGYGQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQD
QSGGGGSGGYGQQDRG

c) mCherry-FUS LC construct:
(HisTag-TEVsite-mCherry-FUS LC)

MSYYHHHHHHDYDIPTTENLYFQGAMVSKGEEDNMAIIKEFMRFKVHMEGS
VNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYV
KHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLR
GTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDA
EVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMD

ELYKAGTMDPASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYS
QSTDTSGYGQSSYSSYGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQ
SSYPGYGQQPAPSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQ
SYNPPQGYGQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQ
DQSGGGGSGGYGQQDRG

d) mMaple3-FUS LC construct:(HisTag-TEVsite-mMaple3-GGGGS-FUS LC)

MSYYHHHHHHDYDIPTTENLYFQGAMVSKGEETIMSVIKPDMKIKLRMEGN
VNGHAFVIEGEGSGKPFEGIQTIDLEVKEGAPLPFAYDILTTAFHYGNRVFTKY
PRKIPDYFKQSFPEGYSWERSMTYEDGGICNATNDITMEEDSFINKIHFKGTNF
PPNGPVMQKRTVGWEVSTEKMYVRDGVLKGDVKMKLLLKGGSHYRCDFRT
TYKVKQKAVKLPKAHFVDHRIEILSHDKDYNKVKLYEHAVARNSTDSMDELY
KGGGGSGSASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQST
DTSGYGQSSYSSYGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSY
PGYGQQPAPSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQSYN
PPQGYGQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQDQS
GGGGSGGYGQQDRG

e) PAtagRFP-FUS LC construct: (HisTag-TEVsite-PatagRFP-FUS LC)

MSYYHHHHHHDYDIPTTENLYFQGAMSELIKENMHMKLYMEGTVNNHHFK
CTSEGEGKPYEGTQTMRIKVVEGGPLPFAFDILATSFMYGSSTFINHTQGIPDF
WKQSFPEGFTWERVTTYEDGGVLTATQDTSLQDGCLIYNVKIRGVNFPSNGPV
MKKKTLGWEPSTEKLKPADGGLEGRVDMALKLVGGGHLICNFKTTYRSKKP
AKNLKMPGVYYVDRRLEIIKEADKETYWEQHEVAVARYSDLPSKLGHKLNT
MDPASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGY
GQSSYSSYGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQ
QPAPSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQSYNPPQGY
GQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQDQSGGGG
SGGYGQQDRG

f) EGFP-FUS LC-Spycatcher construct:
(HisTag-TEVsite-EGFP-FUS LC-GGGGSGGGGS-Spycatcher)

MSYYHHHHHHDYDIPTTENLYFQGAMVSKGEELFTGVVPILVELDGDVNGH
KFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHM
KQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFK
EDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDEL
YKAGTMDPASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQS
TDTSGYGQSSYSSYGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSS
YPGYGQQPAPSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQSY
NPPQGYGQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQD
QSGGGGSGGYGQQDRGGGGGSGGGGSMVDTLSGLSSEQGQSGDMTIEEDSA
THIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVE

TAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHID

g) CsgA-Spytag construct: (CsgA-GGGSGGGS-Spytag-Histag)

MGVVPQYGGGGNHGGGGNNSGPNSELNIYQYGGGNSALALQTDARNSDLTI
TQHGGGNGADVGQGSDDSSIDLTQRGFGNSATLDQWNGKNSEMTVKQFGG
GNGAAVDQTASNSSVNVTQVGFGNNATAHQYGGGSGGGSAHIVMVDAYKPT
KLEHHHHHH

h) CsgA-Histag construct: (CsgA- Histag)

MGVVPQYGGGGNHGGGGNNSGPNSELNIYQYGGGNSALALQTDARNSDLTI
TQHGGGNGADVGQGSDDSSIDLTQRGFGNSATLDQWNGKNSEMTVKQFGG
GNGAAVDQTASNSSVNVTQVGFGNNATAHQYHHHHHH

2. Detailed procedures for plasmids construction

The original FUS LC, EGFP-FUS LC and mCherry-FUS LC plasmids were thankfully gifted from Dr. McKnight's research group. The other two plasmids, mMaple3-FUC LC and PAtagRFP-FUS LC, were constructed through excising and replacing the EGFP domain at Nco1 site in the original plasmid with the mMaple3 or PAtagRFP sequence. As for EGFP-FUS LC-Spycatcher, the original plasmid EGFP-FUS LC was cleaved at Xho1 sites and then the synthetic sequence of Spycatcher was added through one-step Gibson assembly. In addition, CsgA-Spytag plasmid was also constructed into a commercial pET22b plasmid through one-step Gibson assembly method. CsgA-His plasmid was adopted from a previous paper.[1] The genes of mMaple3, PAtagRFP, Spycatcher and CsgA-Spytag were separately amplified by PCR with appropriate overhangs for Gibson assembly. The cleaved vector and correspondent PCR products were mixed together with Gibson Assembly Master 2×Mix (New England Biolabs, Ipswich, MA) at 50°C for 1 h and then were transformed into DH5α *E. coli* cells. PCRs were carried out with primer oligos

(Supplementary Table 1) from Genewiz (SuZhou Branch, China). A Bio-Rad S1000 Thermal Cycler with Dual 48/48 Fast Reaction Modules (Bio-Rad) was used to perform PCRs, ligations and Gibson Assembly. Gel extractions were carried out with QIAquick Gel Extraction Kits (Qiagen). All constructs were sequence verified by Genewiz (SuZhou). All plasmid maps are described in Supplementary Fig. 1 and Fig. 2, and the sequencing results for all genes are presented in Figure S3-9.

3. Gene sequences for all genetic constructs

a) EGFP-FUS LC construct: (HisTag-TEVsite-EGFP-FUS LC)

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGACCG
AAAACCTGTATTTTCAGGGCGCCATGGTGAGCAAGGGCGAGGAGCTGTTC
ACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCA
CAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGC
TGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCA
CCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCG
ACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTAC
GTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCG
CGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGA
AGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAG
TACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAAC
GGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGT
GCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCG
TGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCAAACTGAGCAAA
GACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC
CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGCGGGTACCATGGA
TCCGGCCTCAAACGATTATACCCAACAAGCAACCCAAAGCTATGGGGCCTA
CCCCACCCAGCCCGGGCAGGGCTATTCCCAGCAGAGCAGTCAGCCCTACG
GACAGCAGAGTTACAGTGGTTATAGCCAGTCCACGGACACTTCAGGCTATG
GCCAGAGCAGCTATTCTTCTTATGGCCAGAGCCAGAACACAGGCTATGGAA
CTCAGTCAACTCCCCAGGGATATGGCTCGACTGGCGGCTATGGCAGTAGCC
AGAGCTCCCAATCGTCTTACGGGCAGCAGTCCTCCTATCCTGGCTATGGCC
AGCAGCCAGCTCCCAGCAGCACCTCGGGAAGTTACGGTAGCAGTTCTCAG
AGCAGCAGCTATGGGCAGCCCCAGAGTGGGAGCTACAGCCAGCAGCCTAG
CTATGGTGGACAGCAGCAAAGCTATGGACAGCAGCAAAGCTATAATCCCCC
TCAGGGCTATGGACAGCAGAACCAGTACAACAGCAGCAGTGGTGGTGGAG
GTGGAGGTGGAGGTGGAGGTAACTATGGCCAAGATCAATCCTCCATGAGTA

GTGGTGGTGGCAGTGGTGGCGGTTATGGCAATCAAGACCAGAGTGGTGGA
GGTGGCAGCGGTGGCTATGGACAGCAGGACCGTGGATAG

b) mCherry-FUS LC construct: (HisTag-TEVsite-mCherry-FUS LC)

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGACCG
AAAACCTGTATTTTCAGGGCGCCATGGTGAGCAAGGGCGAGGAGGATAAC
ATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCC
GTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTA
CGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTG
CCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCT
ACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCG
AGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTG
ACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGT
GAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGA
AGACGATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGC
GCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCC
ACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAG
CTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAAC
GAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTC
CACCGGCGGCATGGACGAGCTGTACAAGGCGGGTACCATGGATCCGGCCT
CAAACGATTATACCCAACAAGCAACCCAAAGCTATGGGGCCTACCCCACCC
AGCCCGGGCAGGGCTATTCCCAGCAGAGCAGTCAGCCCTACGGACAGCAG
AGTTACAGTGGTTATAGCCAGTCCACGGACACTTCAGGCTATGGCCAGAGC
AGCTATTCTTCTTATGGCCAGAGCCAGAACACAGGCTATGGAACTCAGTCA
ACTCCCCAGGGATATGGCTCGACTGGCGGCTATGGCAGTAGCCAGAGCTCC
CAATCGTCTTACGGGCAGCAGTCCTCCTATCCTGGCTATGGCCAGCAGCCA
GCTCCCAGCAGCACCTCGGGAAGTTACGGTAGCAGTTCTCAGAGCAGCAG
CTATGGGCAGCCCCAGAGTGGGAGCTACAGCCAGCAGCCTAGCTATGGTG
GACAGCAGCAAAGCTATGGACAGCAGCAAAGCTATAATCCCCCTCAGGGCT
ATGGACAGCAGAACCAGTACAACAGCAGCAGTGGTGGTGGAGGTGGAGG
TGGAGGTGGAGGTAACTATGGCCAAGATCAATCCTCCATGAGTAGTGGTGG
TGGCAGTGGTGGCGGTTATGGCAATCAAGACCAGAGTGGTGGAGGTGGCA
GCGGTGGCTATGGACAGCAGGACCGTGGATAG

c) mMaple3-FUS LC construct: (HisTag-TEVsite-mMaple3-GGGGS-FUS LC)

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGACCG
AAAACCTGTATTTTCAGGGCGCCATGGGCATGGTGAGCAAAGGCGAGGAG
ACAATCATGTCCGTGATCAAGCCCGACATGAAGATCAAACTGAGGATGGAG
GGCAACGTGAACGGCCACGCCTTCGTGATCGAGGGCGAAGGAAGCGGCA

AGCCCTTCGAGGGCATCCAGACCATCGATCTGGAGGTCAAGGAGGGCGCT
CCCCTCCCTTTCGCCTATGACATCCTGACCACCGCCTTCCACTACGGCAATA
GGGTGTTCACCAAGTATCCCAGGAAGATCCCCGACTACTTCAAGCAGAGCT
TCCCTGAGGGCTACAGCTGGGAGAGGAGCATGACATACGAGGACGGCGGC
ATCTGCAACGCCACCAACGACATCACAATGGAGGAGGACAGCTTCATCAA
CAAGATCCACTTCAAAGGCACAAACTTCCCCCCCAATGGCCCCGTGATGCA
GAAGAGGACCGTGGGCTGGGAGGTGAGCACCGAGAAGATGTACGTGAGG
GACGGCGTCCTGAAGGGCGACGTGAAGATGAAGCTCCTGCTCAAGGGCGG
CAGCCACTACAGGTGCGACTTTAGGACCACCTATAAGGTGAAGCAGAAGG
CTGTGAAGCTGCCCAAGGCCCACTTCGTCGACCATAGGATCGAGATCCTGT
CCCACGACAAGGACTACAACAAGGTCAAGCTGTACGAGCACGCCGTCGCT
AGGAACAGCACCGACAGCATGGACGAACTCTATAAGGGTGGCGGTGGCTC
GGGATCCGCCTCAAACGATTATACCCAACAAGCAACCCAAAGCTATGGGGC
CTACCCCACCCAGCCCGGGCAGGGCTATTCCCAGCAGAGCAGTCAGCCCTA
CGGACAGCAGAGTTACAGTGGTTATAGCCAGTCCACGGACACTTCAGGATA
TGGCCAGAGCAGCTATTCTTCTTATGGCCAGAGCCAGAACACAGGCTATGG
AACTCAGTCAACTCCCCAGGGATATGGCTCGACTGGCGGCTATGGCAGTAG
CCAGAGCTCCCAATCGTCTTACGGGCAGCAGTCCTCCTACCCTGGCTATGG
CCAGCAGCCAGCTCCCAGCAGCACCTCGGGAAGTTACGGTAGCAGTTCTC
AGAGCAGCAGCTATGGGCAGCCCCAGAGTGGGAGCTACAGCCAGCAGCCT
AGCTATGGTGGACAGCAGCAAAGCTATGGACAGCAGCAAAGCTATAATCCC
CCTCAGGGCTATGGACAGCAGAACCAGTACAACAGCAGCAGTGGTGGTGG
AGGTGGAGGTGGAGGTGGAGGTAACTATGGCCAAGATCAATCCTCCATGA
GTAGTGGTGGTGGCAGTGGTGGCGGTTATGGCAATCAAGACCAGAGTGGT
GGAGGTGGCAGCGGTGGCTATGGACAGCAGGACCGTGGATAG

d) PAtagRFP-FUS LC construct: (HisTag-TEVsite-PAtagRFP-FUS LC)

ATATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGACC
GAAAACCTGTATTTTCAGGGCGCCATGAGCGAGCTGATTAAGGAGAACATG
CACATGAAGCTGTACATGGAGGGCACCGTGAACAACCACCACTTCAAGTG
CACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAA
TCAAGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTA
CCAGCTTCATGTACGGCAGCAGCACCTTCATCAACCACACCCAGGGCATCC
CCGACTTCTGGAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTC
ACCACATACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCT
CCAGGACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCC
CATCCAACGGCCCTGTGATGAAGAAGAAAACACTCGGCTGGGAACCGAGC
ACCGAGAAACTGAAACCCGCTGACGGCGGCCTGGAAGGCAGGGTCGACA
TGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGATCTGCAACTTCAAGACC
ACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTAC
TATGTGGACCGCAGACTGGAAATCATCAAGGAGGCCGACAAAGAGACCTA
CTGGGAGCAGCACGAGGTGGCTGTGGCCAGATACTCCGACCTCCCTAGCA
AACTGGGGCACAAGCTTAATACCATGGATCCGGCCTCAAACGATTATACCC

AACAAGCAACCCAAAGCTATGGGGCCTACCCCACCCAGCCCGGGCAGGGC
TATTCCCAGCAGAGCAGTCAGCCCTACGGACAGCAGAGTTACAGTGGTTAT
AGCCAGTCCACGGACACTTCAGGATATGGCCAGAGCAGCTATTCTTCTTAT
GGCCAGAGCCAGAACACAGGCTATGGAACTCAGTCAACTCCCCAGGGATA
TGGCTCGACTGGCGGCTATGGCAGTAGCCAGAGCTCCCAATCGTCTTACGG
GCAGCAGTCCTCCTACCCTGGCTATGGCCAGCAGCCAGCTCCCAGCAGCAC
CTCGGGAAGTTACGGTAGCAGTTCTCAGAGCAGCAGCTATGGGCAGCCCC
AGAGTGGGAGCTACAGCCAGCAGCCTAGCTATGGTGGACAGCAGCAAAGC
TATGGACAGCAGCAAAGCTATAATCCCCCTCAGGGCTATGGACAGCAGAAC
CAGTACAACAGCAGCAGTGGTGGTGGAGGTGGAGGTGGAGGTGGAGGTA
ACTATGGCCAAGATCAATCCTCCATGAGTAGTGGTGGTGGCAGTGGTGGCG
GTTATGGCAATCAAGACCAGAGTGGTGGAGGTGGCAGCGGTGGCTATGGA
CAGCAGGACCGTGGATAG

e) EGFP-FUS LC-Spycatcher construct: (HisTag-TEVsite-EGFP-FUS LC-GGGGSGGGGS-Spycatcher)

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGACCG
AAAACCTGTATTTTCAGGGCGCCATGGTGAGCAAGGGCGAGGAGCTGTTC
ACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCA
CAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGC
TGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCA
CCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCG
ACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTAC
GTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCG
CGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGA
AGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAG
TACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAAC
GGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGT
GCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCG
TGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCAAACTGAGCAAA
GACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC
CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGCGGGTACCATGGA
TCCGGCCTCAAACGATTATACCCAACAAGCAACCCAAAGCTATGGGGCCTA
CCCCACCCAGCCCGGGCAGGGCTATTCCCAGCAGAGCAGTCAGCCCTACG
GACAGCAGAGTTACAGTGGTTATAGCCAGTCCACGGACACTTCAGGCTATG
GCCAGAGCAGCTATTCTTCTTATGGCCAGAGCCAGAACACAGGCTATGGAA
CTCAGTCAACTCCCCAGGGATATGGCTCGACTGGCGGCTATGGCAGTAGCC
AGAGCTCCCAATCGTCTTACGGGCAGCAGTCCTCCTATCCTGGCTATGGCC
AGCAGCCAGCTCCCAGCAGCACCTCGGGAAGTTACGGTAGCAGTTCTCAG
AGCAGCAGCTATGGGCAGCCCCAGAGTGGGAGCTACAGCCAGCAGCCTAG
CTATGGTGGACAGCAGCAAAGCTATGGACAGCAGCAAAGCTATAATCCCCC
TCAGGGCTATGGACAGCAGAACCAGTACAACAGCAGCAGTGGTGGTGGAG

GTGGAGGTGGAGGTGGAGGTAACTATGGCCAAGATCAATCCTCCATGAGTA
GTGGTGGTGGCAGTGGTGGCGGTTATGGCAATCAAGACCAGAGTGGTGGA
GGTGGCAGCGGTGGCTATGGACAGCAGGACCGTGGAGGTGGCGGTGGCA
GTGGCGGTGGCGGTTCGATGGTTGATACCTTATCAGGTTTATCAAGTGAGC
AAGGTCAGTCCGGTGATATGACAATTGAAGAAGATAGTGCTACCCATATTAA
ATTCTCAAAACGTGATGAGGACGGCAAAGAGTTAGCTGGTGCAACTATGG
AGTTGCGTGATTCATCTGGTAAAACTATTAGTACATGGATTTCAGATGGACA
AGTGAAAGATTTCTACCTGTATCCAGGAAAATATACATTTGTCGAAACCGCA
GCACCAGACGGTTATGAGGTAGCAACTGCTATTACCTTTACAGTTAATGAGC
AAGGTCAGGTTACTGTAAATGGCAAAGCAACTAAAGGTGACGCTCATATTG
ACTAA

f) CsgA-Spytag construct: (CsgA-GGGSGGGS-Spytag-Histag)

ATGGGTGTTGTTCCTCAGTACGGCGGCGGCGGTAACCACGGTGGTGGCGGT
AATAATAGCGGCCCAAATTCTGAGCTGAACATTTACCAGTACGGTGGCGGT
AACTCTGCACTTGCTCTGCAAACTGATGCCCGTAACTCTGACTTGACTATTA
CCCAGCATGGCGGCGGTAATGGTGCAGATGTTGGTCAGGGCTCAGATGACA
GCTCAATCGATCTGACCCAACGTGGCTTCGGTAACAGCGCTACTCTTGATC
AGTGGAACGGCAAAAATTCTGAAATGACGGTTAAACAGTTCGGTGGTGGC
AACGGTGCTGCAGTTGACCAGACTGCATCTAACTCCTCCGTCAACGTGACT
CAGGTTGGCTTTGGTAACAACGCGACCGCTCATCAGTACGGCGGGGGCTC
CGGCGGGGGCTCCGCGCACATCGTTATGGTCGATGCATATAAACCCACCAA
ACTCGAGCACCACCACCACCACCACTGA

g) CsgA-Histag construct: (CsgA-Histag)

ATGGGTGTTGTTCCTCAGTACGGCGGCGGCGGTAACCACGGTGGTGGCGGT
AATAATAGCGGCCCAAATTCTGAGCTGAACATTTACCAGTACGGTGGCGGT
AACTCTGCACTTGCTCTGCAAACTGATGCCCGTAACTCTGACTTGACTATTA
CCCAGCATGGCGGCGGTAATGGTGCAGATGTTGGTCAGGGCTCAGATGACA
GCTCAATCGATCTGACCCAACGTGGCTTCGGTAACAGCGCTACTCTTGATC
AGTGGAACGGCAAAAATTCTGAAATGACGGTTAAACAGTTCGGTGGTGGC
AACGGTGCTGCAGTTGACCAGACTGCATCTAACTCCTCCGTCAACGTGACT
CAGGTTGGCTTTGGTAACAACGCGACCGCTCATCAGTACCATCACCATCAC
CATCACTAA

4. Detailed procedures for protein expression, purification and His-tag cleavage by TEV protease

4.1 Procedures for protein expression and purification of FUS LC fusion proteins:

The recombinant plasmid, pET-22b/FUS LC, pET-22b/EGFP-FUS LC, pET-22b/mMaple3-FUS LC, pET-22b/PAtagRFP-FUS LC and pET-22b/EGFP-FUS LC-Spycatcher were individually transformed in BL21 *E. coli*. The expression and purification of recombinant FUS LC fusion proteins were carried out following a previous method.[2, 3] Specifically, the strains were grown to OD_{600} 0.5~1.0 in 1 L LB broth containing 50 mg/mL ampicillin at 37 °C. Protein expression was induced with 0.5 mM IPTG at 16 °C for 12 h. Cells were collected by centrifugation at 5000 g, and the collected cell pellets were stored at −80 °C. 5 gram cell pellets were resuspended and lysed by 50 mL lysis buffer (50 mM Tris-HCl, 500 mM NaCl, 20 mM β-mercaptoethanol (BME), pH 8.0). Lysozyme (with a final concentration of 0.2 mg/mL) and 0.1 mM PMSF were also added in the solution to inhibit protein degradation by enzymes. Lysates were then incubated on ice for 30 min, followed by sonication for 30 min. The insoluble portions of the lysates were removed through centrifugation at 30000g for 1 hour, and the separated supernatants were incubated with 6 mL Ni-NTA resin (Clontech) for 30 min at room temperature. Resin beads were then centrifuged at 200 g, and washed with 300 mL of washing buffer (50 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, 20 mM BME, pH 8.0). The proteins were eluted from the gravity column with 20-30 mL elute buffer (50 mM Tris-HCl, 500 mM NaCl, 400 mM imidazole, 20 mM BME, pH 8.0) and stored at 4 °C for later use.

4.2 N-terminus His-tag cleavage by TEV Protease:

20 mL freshly purified His-EGFP-FUS LC-Spycatcher monomers (40 μM) was treated by addition of 5 mL purified TEV protease (20 μM) and incubated for 2 hours at room temperature. The 25 mL mixed solution was then dialyzed against 2 L deionized water for 30 min for three times to remove imidazole. The process was carefully and quickly carried out to prevent fibers formation. 4 mL Ni-NTA resin solution was then added in the solution to remove those proteins containing uncleaved his-tag. The buffer solution was then filtered through a gravity column to remove the Ni-NTA resin and the eluted solution thus collected mainly contained EGFP-FUS-Spycatcher monomers and was stored in 4 °C fridge for later usage.

4.3 Procedures for Protein expression, purification of CsgA fusion proteins:

The recombinant plasmid, pET-11d/CsgA (described in a previous paper[1]) or pET-22b/CsgA-Spytag, were individually transformed into BL21 *E. coli*. Bacterial were grown to OD_{600} 0.5~1.0 in 1 L LB broth which contain 50 mg/mL ampicillin at 37 °C. Protein expression was induced upon addition of 0.5 mM IPTG at 37 °C for 1h. The cell pellets were collected after centrifugation at 4000 g. 5 grams of cell pellets were then lysed with 50 mL lysis buffer (8 M guanidine hydrochloride (GdnHCl), 300 mM NaCl, 50 mM K_2HPO_4/KH_2PO_4, pH 7.2) for 24 h at room temperature. The insoluble portion of the lysates was then removed by centrifugation at 30000 g for 30 min, and the supernatants were incubated with 6 mL Ni-NTA resin (Clontech) for another 30 min at room temperature. Beads that bound CsgA-His fusion proteins were then collected by centrifugation at 200 g and then washed with 100 mL washing buffer (50 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, pH 8.0). The bound proteins were then eluted from the resin in a gravity column with 10 mL elution buffer (50 mM Tris-HCl, 500 mM NaCl, 400 mM imidazole, pH 8.0) and stored at 4 °C for later use.

4.4 Detailed protocols for SDS/PAGE:

Samples of eluents were used for SDS/PAGE. Specifically, 90 μL samples were mixed with 30 μL loading sample buffer (NuPAGE, LDS sample buffer (4×) in 200 μL microtubes and boiled at 95°C for 30 min. The samples, together with a protein ladder (NOVEX Sharp Pre-stained Protein Standard), were then loaded into NuPAGE® 4-12% Bis-Tris gels. Electrophoresis was run at 165 volts for 45 min using Nupage MES SDS running buffer (NOVEX) following a standard SDS/PAGE procedure. The gels were then stained with Comassie Blue for 60 min and were immersed in 30 mL destaining solution (10% acetic acid / 40% Methanol / 50% DI water) for 3 h for three times. Afterwards, the gels were incubated in copious amounts of deionized water overnight and imaged using a Bio-Rad ChemiDoc MP system. The SDS/PAGE Data was presented in Supplementary Figure 10.

5. Synthesis of various Co-NTA decorated nano-objects (Co-NTA-Au NPs/ CdSeS@ZnS QDs/CdS NRs) and Ni-NTA decorated DNA origami:

Nano-objects were synthesized following a modified version of the protocol based upon previous literatures.[4-6] $HAuCl_4 \cdot 3H_2O$ was reduced with borane t-butylamine complex under N_2 atmosphere to produce Au NPs.

Cd $(OAc)_2$ reacted with S and Se at 150 °C to form CdSeS core, followed by addition of $Zn(OAc)_2$ and S and heated at 270 °C to form the ZnS shell to produce the CdSeS@ZnS QDs.

CdO and hexamethyldisilathiane reacted at 250 °C to give the seed solution. Then CdO and S were used to grow CdS NRs at 350 °C.

All products were stored in hexane. Au NPs, CdSeS@ZnS and CdS NRs were then carried out with a ligand exchange procedure using the following protocols.

Ligand exchange protocol for Au NPs: Au NPs (1 mL) in hexane was added to 2 mg/mL HS-NTA in PBS solution (10 mL). The mixed solution was stirred overnight at room temperature, then the aqueous layer was collected and filtered with 0.2 μm Acrodisc Syringe Filters (Pall). 800 μL of 50 mM $CoCl_2$ solution was added to 10 mL of HS-NTA Au NPs in PBS solution. Then the solution was vortexed, centrifuged at 5000 rpm for 3 min and filtered with 0.2 μm Acrodisc Syringe Filters (Pall). The samples thus prepared were stored at 4 °C for later use.

Ligand exchange for CdSeS@ZnS QDs and CdS NRs: 1 mL ethanol was added to 1 mL nano-objects (NOs) in hexane. The mixed solution was centrifuged and redispersed in CH_2Cl_2 (15 mL). 1 mL HS-NTA (2 mg/mL, pH 12) in methanol was added drop wise to the CH_2Cl_2 solution. The mixed solution was stirred for 5 min. 10 mL of PBS solution was then added to the mixed solution above. The aqueous layer was collected using a separation funnel and then filtered with 0.2 μm Acrodisc Syringe Filters (Pall). 200 μL of 50 mM $CoCl_2$ solution was added to 10 mL of HS-NTA NOs in PBS solution. Then the solution was vortexed, centrifuged at 5000 rpm for 3 min and filtered with 0.2 μm Acrodisc Syringe Filters (Pall). The samples thus prepared were stored at 4 °C for later use.

Spytag-decorated Au NPs: 1 mg synthesized Cys_2-Spytag peptide (QiangYao Biotechnology, Shanghai) was dissolved in 10 mL PBS buffer which contained 0.3 mL Au NPs solution and stirred acutely for 8 h. Then the aqueous layer was collected and filtered with 0.2 μm Acrodisc Syringe Filters (Pall). The samples thus prepared were stored at 4 °C for later use.

Conjugation of Maleimido-C3-NTA and Thiol-modified capture DNA strands: The capture DNA strands with protected thiol groups ("Thio") on their 3' ends used in this experiment were purchased in HPLC purified form from Jieli DNA Technologies, Inc. DTT treatment was performed immediately before conjugation to remove the C3 protection group to generate a thiol moiety. Maleimido-C3-NTA was purchased from Dojindo Molecular Technologies. For the conjugation, 1mM Maleimido-C3-NTA was added to 2 μM DNA solution in a 10 mM HEPES (PH 7.3, thermal) and 50 mM NaCl (thermal) buffer solution. The reaction was maintained overnight at room temperature. After the reaction, the excess Maleimido-C3-NTA was removed from the solution by Microcon centrifugal filtration devices (3 kD MWCO filters, Millipore, Bedford, MA).

Preparation of Ni-NTA decorated DNA origami structures: To assemble the triangular shaped DNA origami, 3 nM single stranded M13mp18 DNA (NEB, 7,249 nt long) was mixed in 1xHEPES-Mg^{2+} (20 mM HEPEs and 12.5 mM Magnesium acetate, pH 8.0) with staple strands (unpurified) and capture strands (Jieli, sequences detailed later) in a 1:10:20 molar ratio, following the design outlined by Rothemund.[7] To generate the binding sites for the His-tag modified protein on the origami, a number of selected staple strands were extended at the 5'-end by A15 sequence to hybridize with NTA-modified capture DNA. The resulting solution was cooled from 95 ^{o}C to 4 ^{o}C to form the DNA origami structure. Then 200 μL prepared origami (5 nmol/L) with capture DNA was incubated with 5 μM Ni^{2+} solution for 1 hour. The sample was subsequently purified by Microcon centrifugal filtration devices (100 kD MWCO filters, Millipore, Bedford, MA) to remove excess Ni^{2+} and resulted in Ni-NTA decorated DNA origami in the end.

6. Detailed procedures for preparation of various self-assembled supramolecular fibers

Preparation of single-component nanofibers: Purified protein monomers in eluted buffer (50 mM Tris-HCl, 500 mM NaCl, 400 mM imidazole, 20 mM BME, pH 8.0) were concentrated to a final molar concentration of 40 μM (~2 mg/mL). Protein solution was dialyzed against the gelation buffer (20 mM Tris-HCl, 200 mM NaCl, 20 mM BME, 0.5 mM Ethylenediaminetetraacetic acid (EDTA), pH 8.0) to remove excessive imidazole. Solution thus collected would contain self-assembled nanofibers after one day and was stored at 4 °C for later use.

Preparation of random copolymer-like supramolecular fibers: Two freshly purified monomers, for example, EGFP-FUS LC and mCherry-FUS LC, were mixed at a fixed molar concentration ratio (3:1 or 1:1 or 1:3) with a total monomer protein concentration of 40 μM. The mixed solution was then dialyzed against the gelation buffer (20 mM Tris-HCl, 200 mM NaCl, 20 mM BME, 0.5 mM EDTA, pH 8.0) overnight to remove imidazole. Dense protein nanofibers networks composed of the two proteins were obtained from the as-collected solution after a day. As for the random nanofibers comprising EGFP-FUS LC-Spycatcher and mCherry-FUS LC, the EGFP-FUS LC-Spycatcher monomers were first treated in the presence of TEV protease and were then mixed with mCherrry-FUS LC monomers at a molar concentration ratio 1:1, dense nanofibers networks would form in solution after dialysis. Solution after dialysis containing desired fibrous structures was stored at 4 °C for later use.

Preparation of multiblock copolymer-like supramolecular fibers: Solution containing single-component EGFP-FUS LC, mCherry-FUS LC or EGFP-FUS LC-Spycatcher nanofibers was used as seed solution, then three-fold molar of mCherry-FUS LC or EGFP-FUS LC monomers was added into the seed solution and dialyzed against the gelation buffer (20 mM Tris-HCl, 200 mM NaCl, 20 mM BME, 0.5 mM EDTA, pH 8.0) overnight. To prepare multiblock copolymer-like fibers comprising multiple blocks, three-fold molar of mCherry or EGFP fused FUS LC

monomers were added into the solution containing preformed block fibers. The mixed solution was then dialyzed against the gelation buffer overnight and the collected solution would contain desired fibrous structures and was stored at 4 °C for later use.

Preparation of self-sorted supramolecular fibers: To prepare self-sorted supramolecular fibers based on CsgA-His or CsgA-Spytag-His and FUS LC fusion proteins, the equal amount of protein monomer solutions with certain molar concentration (recombinant FUS LC was 80 μM and CsgA fusion protein was 8 μM) were mixed together and incubated at room temperature for 12 hours and then dialyzed against distilled water for 2 hours to remove the excessive imidazole. The solution thus collected would contain two independent fibrous networks and was stored at 4 °C for later use.

7. Detailed procedures for templated assembly of ligand-decorated nano-objects with tailor designed supramolcular fibrous structures

For templated assembly of ligand-decorated nano-objects (Co-NTA-Au NP/ Co-NTA-CdSeS@ZnS QDs/ Co-NTA-CdS nanorods) with supramolecular fiber structures, 1 mL PBS buffer containing 10 μL desired fibrous structures (40 μM) were mixed with 10 μL 1 wt.% nano-objects solution. The mixture reacted for 30 min at room temperature, and then the mixed solution was deposited on a mica surface for 30 min or TEM grid for 10 min to prepare AFM or TEM samples respectively.

For templated assembly of Spytag-Cys_2-Au NPs with EGFP- FUS LC-Spycatcher fibers, 1 mL PBS buffer containing 10 μL mature fibers solution (40 μM) react with 50 μL 1 wt.% Cys_2-Spytag-Au NPs for at least 2 h at room temperature and then to prepare AFM or TEM samples.

For templated assembly of nano-objects with random suparmolecular nanofibers composed of EGFP-FUS LC-Spycatcher/His-mCherry-FUS LC, 1 mL PBS solution containing 50 μL 1 wt.% of Spytag-Cys_2-Au NPs and 10 μL 1 wt.% of Co-NTA-CdS nanorods was added to 10 μL solution containing the random supramolecular copolymer-like nanofibers. The mixture then reacted for 2 h at room temperature.

For templated assembly of nano-objects with block supramolecular copolymer-like structures composed of EGFP-FUS LC-Spycatcher and His-mCherry-FUS LC or self-sorted supramolecular fiber networks based upon EGFP-FUS LC-Spycatcher and CsgA -His, 1 mL PBS buffer containing nano-objects (50 μL 1 wt.% Spytag-Cys_2-Au NPs alone or 10 μL 1 wt.% Co-NTA-CdSeS@ZnS QDs and 50 μL Spytag-Cys_2-Au NPs together) was added to 10 μL desired fibers solution. The mixture then reacted for 2 h at room temperature.

For templated assembly of Ni-NTA decorated DNA origami with suparmolecular nanofibers composed of His-mCherry-FUS LC. 1 mL HEPEs-Mg^{2+} buffer (20 mM HEPEs, 12.5 mM Magnesium acetate, pH 8.0) containing 10 μL mature fibers were react with 3 μL ligand-decorated DNA origami (5 nmol/L) for 30 min at 4 °C.

8. Circular Dichroism (CD) spectra of solutions containing self-assembled nanofibers

Far-UV CD spectra for solutions containing FUS LC fusion proteins were recorded using an Applied Photophysics chirascan spectrometer (Great Britain). Spectra were recorded as an average of 5 to 10 scans from 185 to 240 nm with a scan rate 0.5 step with a response time of 1 s at 25 °C. Solution containing 40 μM EGFP-FUS LC or 40 μM mCherry-FUS LC in Tris-HCl (pH 8.0) after aging for two

weeks was first dialyzed against deionized water overnight and then used for analysis. Each sample (in 100 μL) was tested three times, and the averaged spectrum was recorded.

9. X-Ray Fiber Diffraction of self-assembled structures based upon various FUS LC fusion proteins

FUS LC mature fibers formed by different protein samples was pelleted by centrifugation at 10000 g, followed by washing with copious distilled water for several times to remove remaining salts. Fiber pellets were then suspended in 5 μL distilled water. 2 μL suspension was pipetted between two fire-polished glass rods and dried for a couple of hours. The diffraction data was collected by an X-ray machine equipped with a Rigaku Micromax-007 X-ray generator and an R-Axis IV++ area detector.

10. Thioflavin T (ThT) assay

Following a previous method,[1] 200 μL solution containing the freshly purified CsgA-His or FUS LC or mixed solution containing both protein monomers with appropriate concentrations (40 μM for FUS LC and 4 μM for CsgA-His) was added into 96-well black plates. ThT buffer was then added and reached with a final concentration of 20 μM. The signals were measured every 5 min after shaking 5 sec by a BioTek Synergy H1 Microplate Reader using BioTek GEN5 software set to 438 nm excitation and 495 nm emission with a 475-nm cutoff. The ThT fluorescence was normalized by $(F_i-F_0)/(F_{max}-F_0)$. Here F_i stood for ThT intensity(fluorescence arbitrary unit) of samples, while F_0 was the ThT background intensity and F_{max} was the

maximum ThT intensity of samples at the experiments.[8]

11. Determination of the stoichiometry in complex nanofibers with Fluorescence Spectrometer

To determine the stoichiometry in the complex nanofiber structures, the ratio of fluorescent intensity of EGFP and mCherry in all samples were applied for comparisons. The fluorescent intensity of 300 μL 1μM EGFP-FUS LC and mCherry-FUS LC nanofibers solutions were used as internal references, referred to G_s and M_s respectively. Then the fluorescent intensity of EGFP and mCherry for all samples (random copolymer-like and multiblock nanofiber systems) were measured individually, and their corresponding intensity of the individual components in complex nanofibers is defined as G_n and M_n, respectively. The ratios of the individual components (EGFP-FUS LC and mCherry-FUS LC) in the complex nanofibers for each sample was calculated with the following formula: $(G_n/G_s):(M_n/M_s)$.

For fluorescence intensity measurement, 300 μL diluted recombinant protein monomers or nanofiber solutions were placed in fluorescence cuvette (Tansoole). The spectra were collected using HORIBA FL-3 with excitation of 395 nm light for EGFP-FUS LC and 520 nm light for mCherry-FUS LC. Each sample was measured for at least three times and the average intensity was accordingly calculated.

12. Fluorescence Microscopy (FM), Confocal Fluorescence Microscopy (CFM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM) imaging

12.1 Fluorescence Microscopy (FM) and Confocal Fluorescence Microscopy (CFM)

5 μL solution containing desired nanofibers was dropped on a piece of clean glass slide (CITOGLAS, 1.0 ± 0.2 mm in thickness) and then covered by a piece of cover glass (CITOGLAS, 0.13-0.16 mm, microscope cover glass). Fluorescence imaging of nanofibers was performed on a Zeiss Z2 fluorescence microscopy equipped with Axiocam 506 camera (Zeiss). The 488 nm laser was used for excitation of EGFP and mMaple3, while a 545 nm laser was applied to excite mCherry and PAtagRFP. The detection wavelength for EGFP and mMaple3 is 500-550 nm and 570-640 nm for mCherry and PAtagRFP, respectively.

The 3D confocal images of recombinant FUS LC nanofibers were obtained with a Leica TCS SP8 confocal microscopy (Germany) and the corresponding 3D movies and images were created using Leica application suite X software.

12.2 Transmission Electron Microscopy (TEM)

All TEM images were collected on a Tecnai G2 F20 TEM operated at 120 kV accelerating voltage. Detailed procedures for sample preparation were described below:

Sample preparation for nanofibers-templated assembly of nano-objects for TEM imaging: 10 μL PBS buffer solution containing nanofibers with templated assembly of ligand-decorated nano-objects (prepared as session 7 described) was directly deposited on a piece of carbon-film-supported 400 mesh nickel grid (Ted Pella, Inc.) for 10 min. Excessive solution was then quickly wicked away with pieces of filter paper and washed by 10 μL 20 mM imidazole solution (50 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, 20 mM BME, pH 8.0) three times. Samples were then negatively stained with 10 μL 2 wt % uranyl acetate right away.

12.3 Atomic Force Microscopy (AFM) imaging

Tapping mode (TM) AFM was performed on an Asylum MFP-3D AFM (Asylum Research, Santa Barbara, CA, USA) using micro cantilevers (k = 26.1 N/m, ν~ 300 kHz).

Sample preparation for self-assembled nanofibers for AFM imaging: 200 μL PBS solution containing desired nanofibers was incubated on a mica surface for 30 min. The excessive solution was then washed away by 100-200 mL deionized water. The mica surface was dried through a constant flow of nitrogen gas and then immediately collected for AFM imaging.

Sample preparation for nanofibers-templated assembly of nano-objects for AFM imaging: 200 μL PBS buffer solution containing nanofibers with assembled ligand-decorated nano-objects, as session 7 described, was deposited on a mica surface for 30 min. Then the mica was quickly washed away by 50 mL 20 mM imidazole solution and 100-200 mL deionized water. The mica surface was then dried through a constant flow of nitrogen gas and immediately collected for AFM imaging.

For the AFM imaging of nanofibers-templated assembly of Ni-NTA-origami, 1 mL HEPEs-Mg^{2+} buffer (20 mM HEPEs, 12.5 mM Magnesium acetate, pH 8.0) containing 10 μL mature fibers were react with 3 μL ligand-decorated DNA origami (5 nmol/L) for 30 min at 4 °C. Then the mixed solution was deposited on a mica for at least 30 min at 4 °C. The excessive solution was washed away gently by 50 mL 20 mM imidazole solution (50 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, 20 mM BME, pH 8.0) and 50 mL deionized water. The mica surface was then dried through a constant flow of nitrogen gas and immediately collected for AFM imaging.

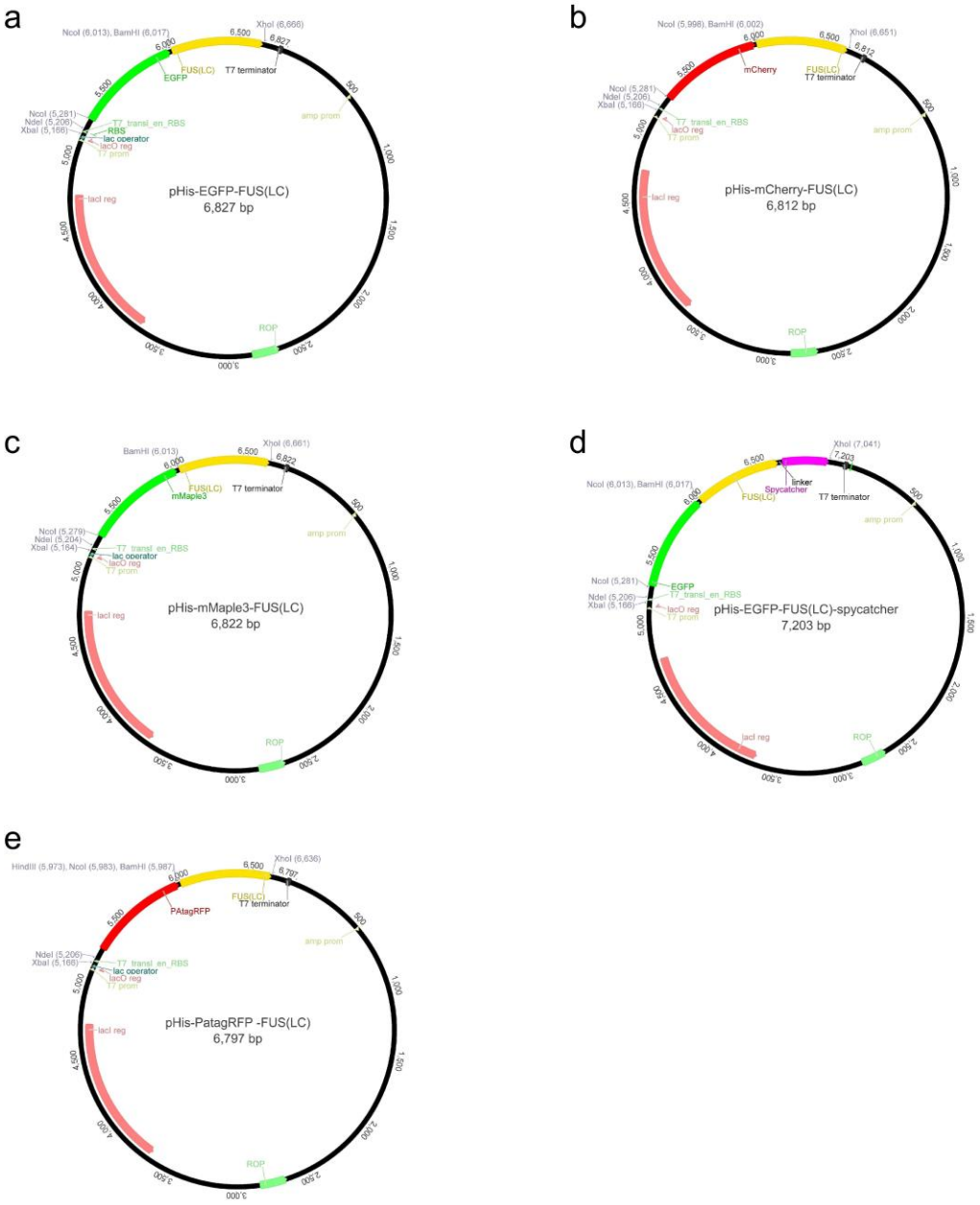


Figure S1. Plasmid maps for expressing recombinant FUS LC protein genes. (a) pET22b containing EGFP-FUS LC. (b) pET22b containing mCherry-FUS LC. (c) pET22b containing mMaple3-FUS LC. (d) pET22b containing EGFP-FUS LC-Spycatcher. (e) pET22b containing PAtagRFP-FUS LC.

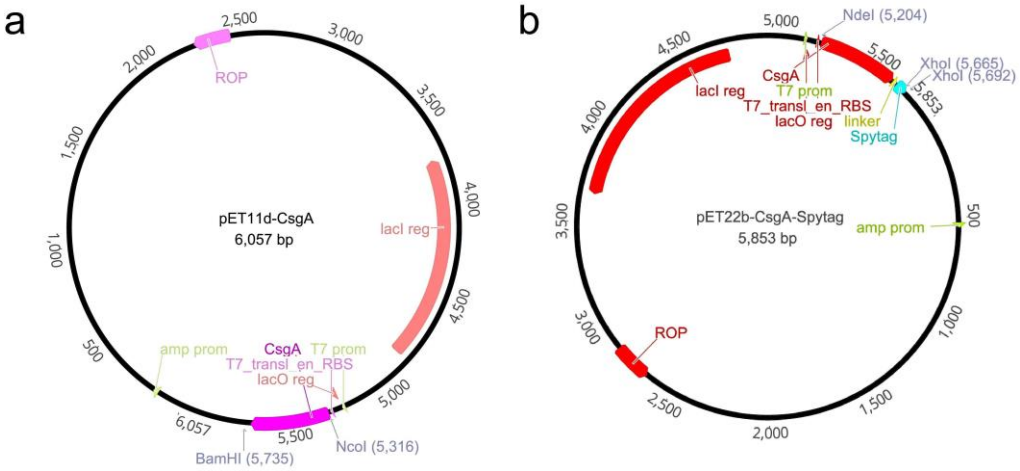


Figure S2. Plasmid maps for expressing recombinant CsgA protein genes. (a) pET11d containing CsgA gene. (b) pET22b containing CsgA-Spytag.

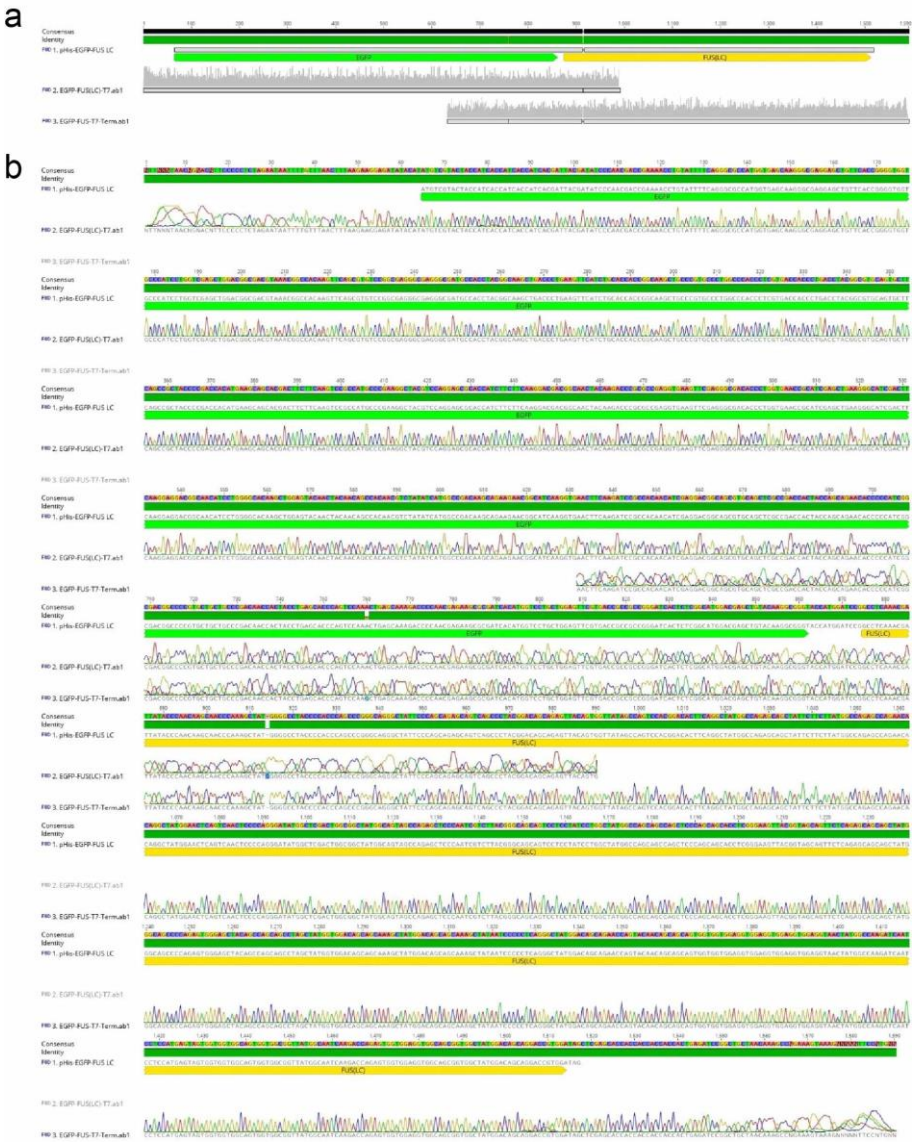


Figure S3. Sequencing results of the plasmid expressing EGFP-FUS LC. (a) The zoomed-out sequencing results show an overview of the sequenced DNA. (b) The zoomed-in sequencing results show the underlying DNA sequences. The consensus sequence obtained from integrating the two sequencing reactions is the sequence above the reference sequence (green and thick yellow line).

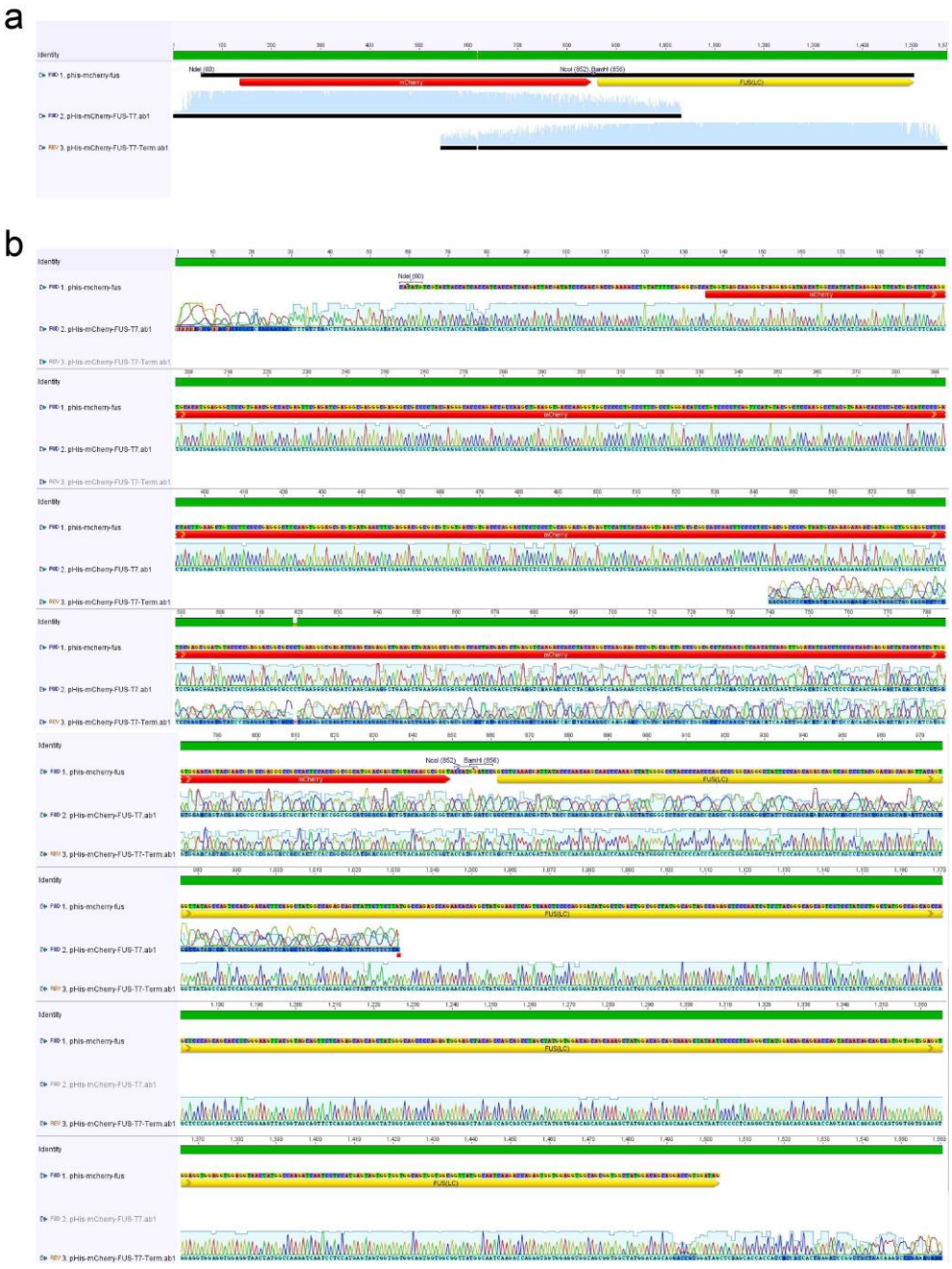


Figure S4. Sequencing results of the plasmid expressing mCherry-FUS LC. (a) The zoomed-out sequencing results show an overview of the sequenced DNA. (b) The zoomed-in sequencing results show the underlying DNA sequences. The consensus sequence obtained from integrating the two sequencing reactions is the sequence above the reference sequence (red and thick yellow line).

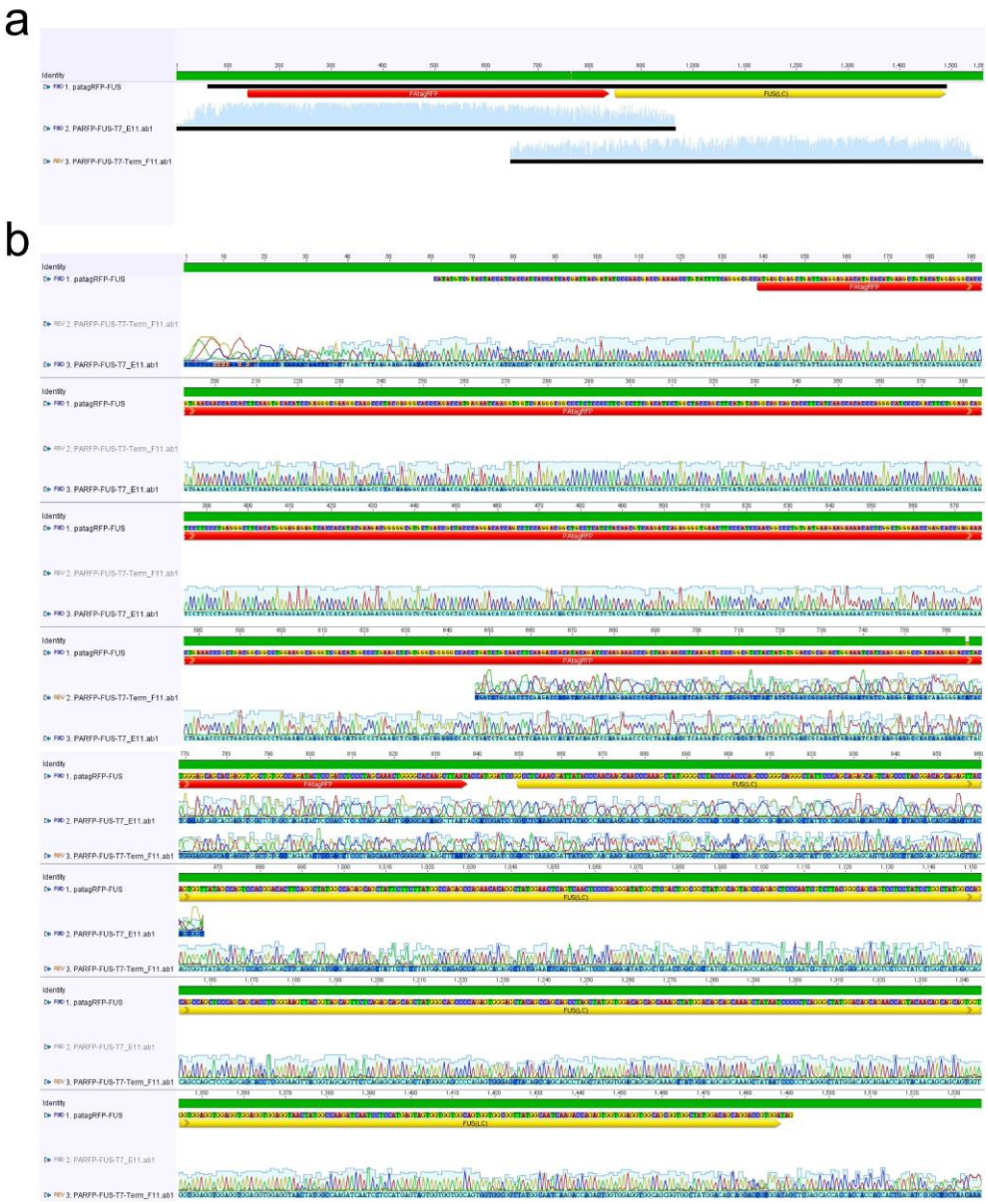


Figure S5. Sequencing results of the plasmid expressing PAtagRFP-FUS LC. (a) The zoomed-out sequencing results show an overview of the sequenced DNA. (b) The zoomed-in sequencing results show the underlying DNA sequences. The consensus sequence obtained from integrating the two sequencing reactions is the sequence above the reference sequence (red and thick yellow line).

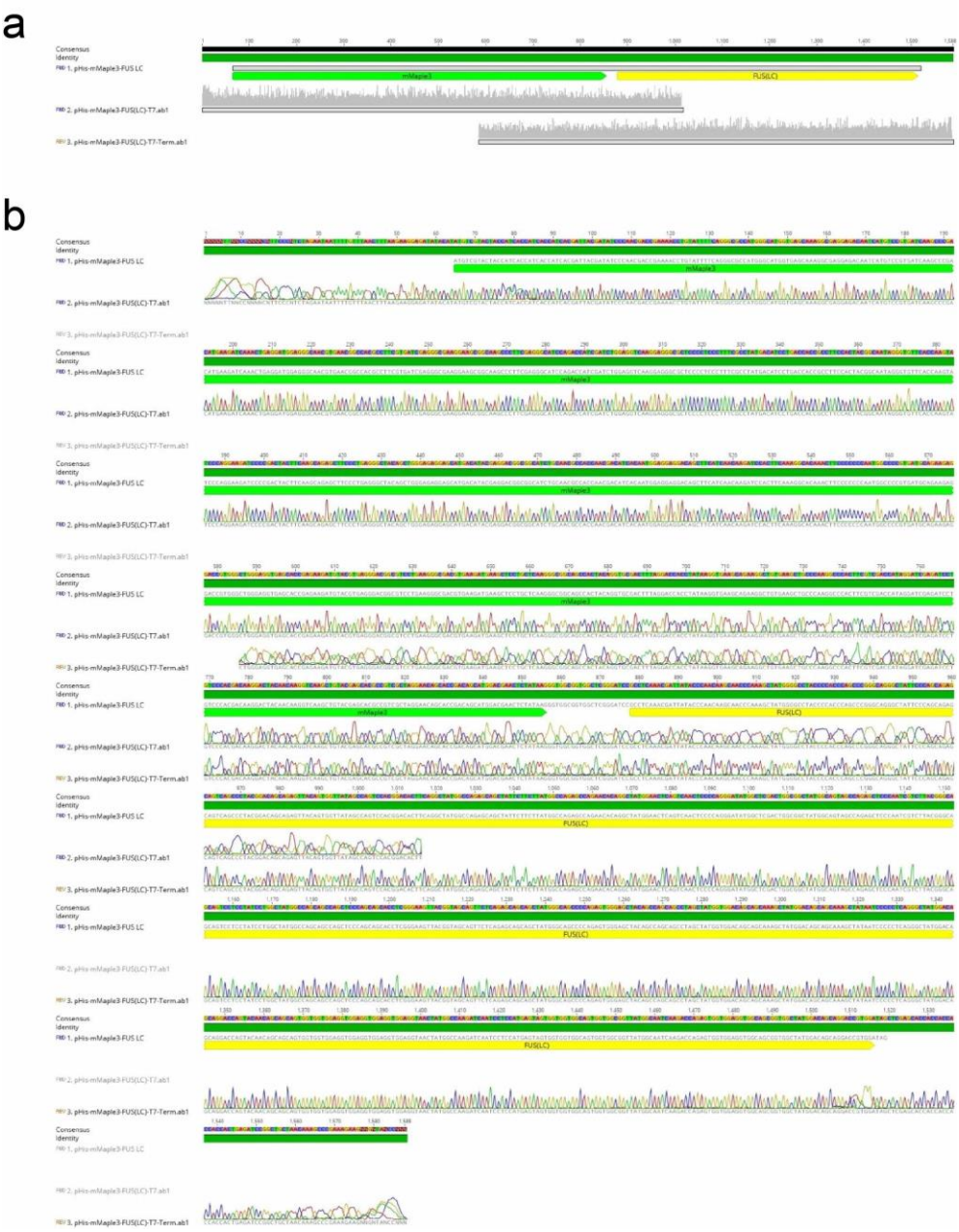
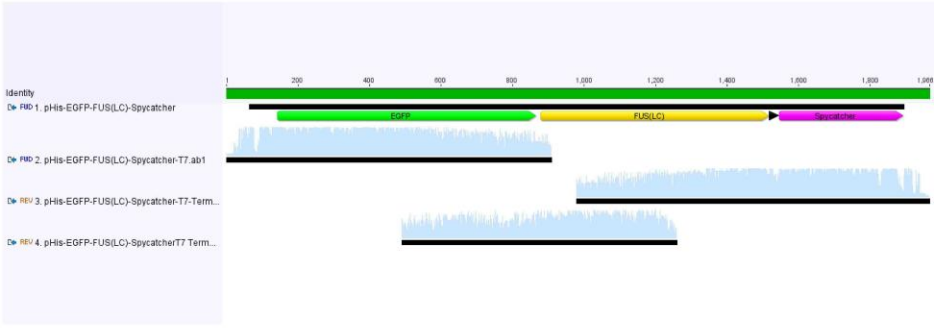


Figure S6. Sequencing results of the plasmid expressing mMaple3-FUS LC. (a) The zoomed-out sequencing results show an overview of the sequenced DNA. (b) The zoomed-in sequencing results show the underlying DNA sequences. The consensus sequence obtained from integrating the two sequencing reactions is the sequence above the reference sequence (green and thick yellow line).

a



b

Figure S7. Sequencing results of the plasmid expressing EGFP-FUS LC-Spycatcher.

(a) The zoomed-out sequencing results show an overview of the sequenced DNA. (b) The zoomed-in sequencing results show the underlying DNA sequences. The consensus sequence obtained from integrating the two sequencing reactions is the sequence above the reference sequence (green, thick yellow and pink line).

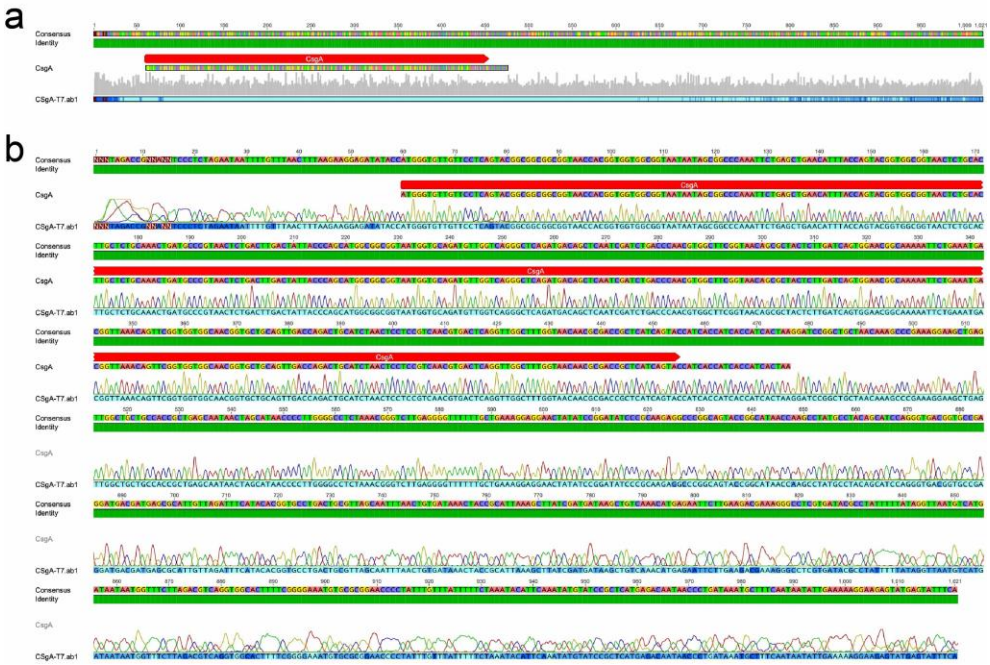


Figure S8. Sequencing results of the plasmid expressing CsgA. (a) The zoomed-out sequencing results show an overview of the sequenced DNA. (b) The zoomed-in sequencing results show the underlying DNA sequences. The consensus sequence obtained from the sequencing reaction is the sequence above the reference sequence (red line).

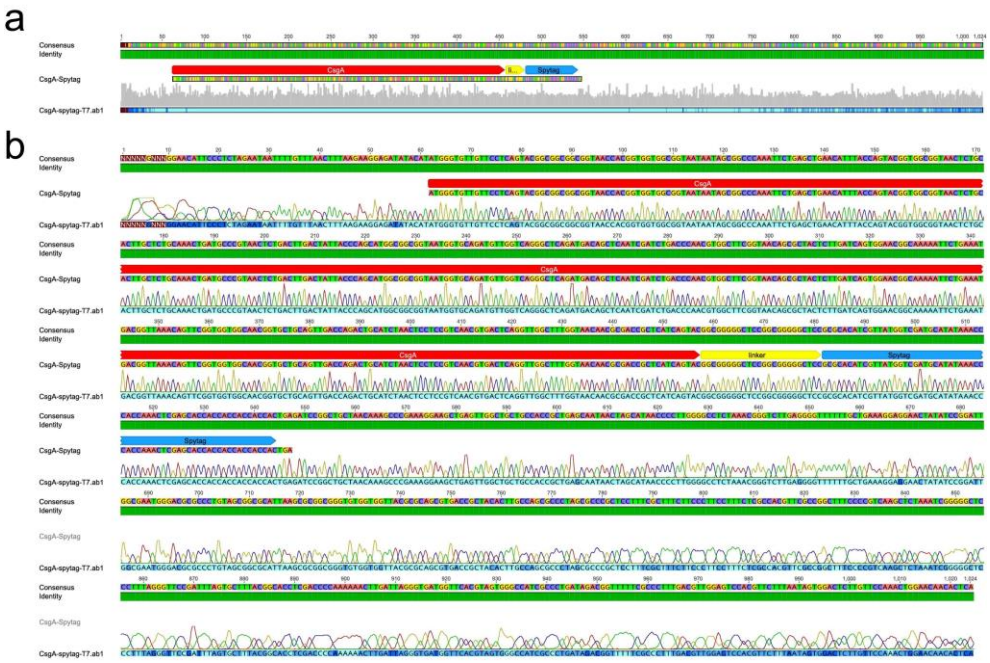


Figure S9. Sequencing results of the plasmid expressing CsgA-Spytag. (a) The zoomed-out sequencing results show an overview of the sequenced DNA. (b) The zoomed-in sequencing results show the underlying DNA sequences. The consensus sequence obtained from the sequencing reaction is the sequence above the reference sequence (red and blue line).

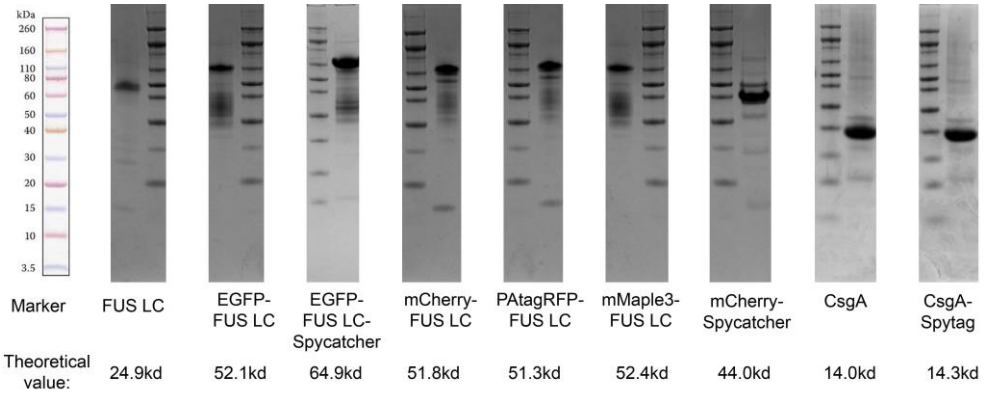


Figure S10. SDS/PAGE results of recombinant proteins reported in this paper.

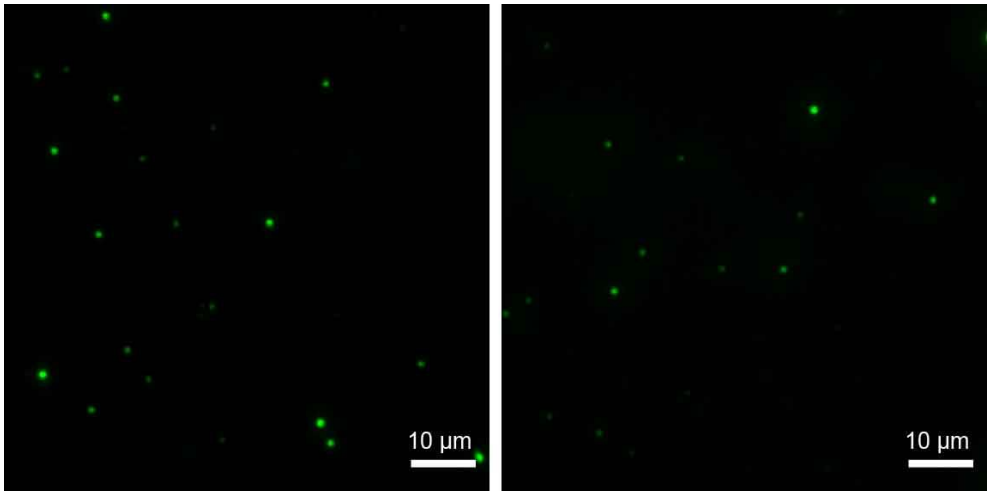


Figure S11. Detection of liquid-like EGFP-FUS LC droplets with fluorescent microscopy. The green dots in the pictures refer to the liquid-like droplets, which formed during early-stage evolution. The solution concentration of EGFP-FUS LC applied for observation was 40 μM.

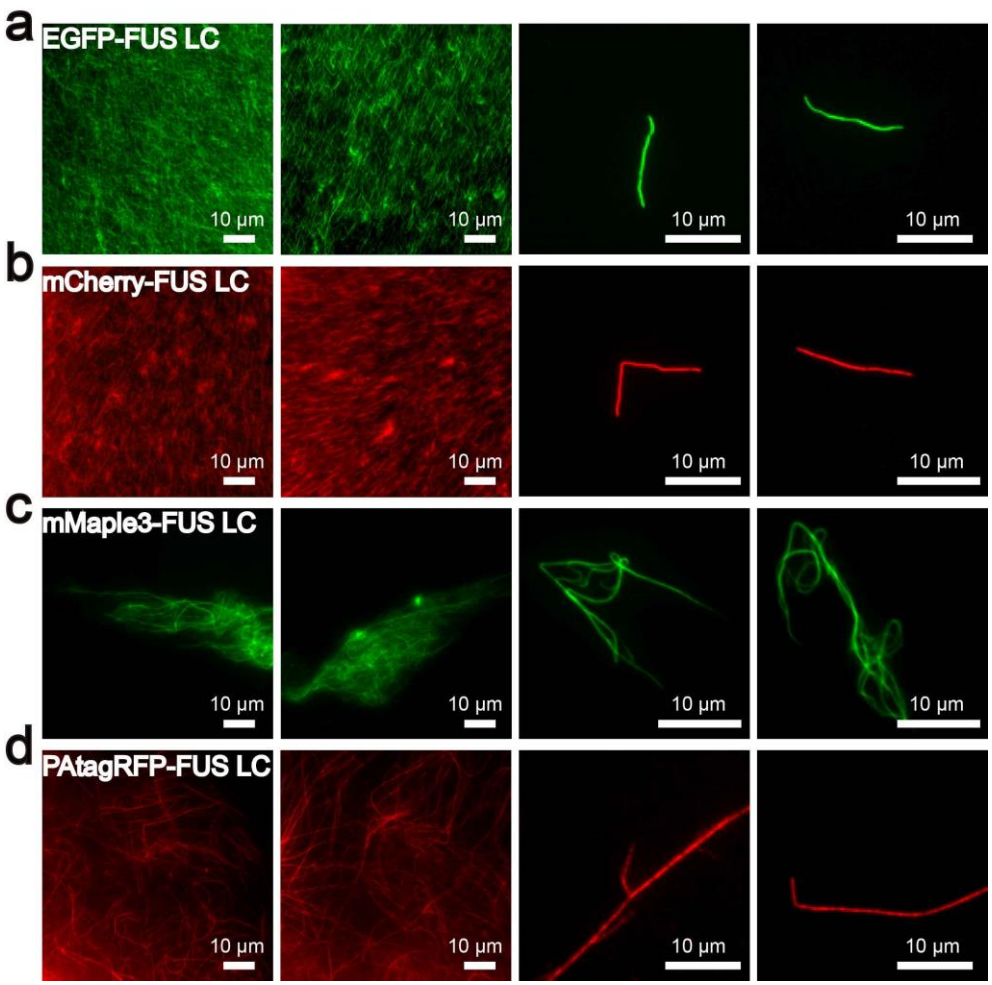


Figure S12. Fluorescent images of single-component recombinant FUS LC nanofibers. (a) The dense nanofiber networks and single fiber of EGFP-FUS LC. (b) The dense nanofiber networks and single fiber of mCherry-FUS LC. (c) The dense nanofiber networks of mMaple3-FUS LC. (d) The dense nanofiber networks and single fiber of PAtagRFP-FUS LC. Green fluorescence was excited by 488 nm channel, while red fluorescence was excited at 545 nm channel. The concentration applied for observation of dense nanofiber neworks and single fiber was 40 μM and 0.04 μM (1000-fold dilution), respectively.

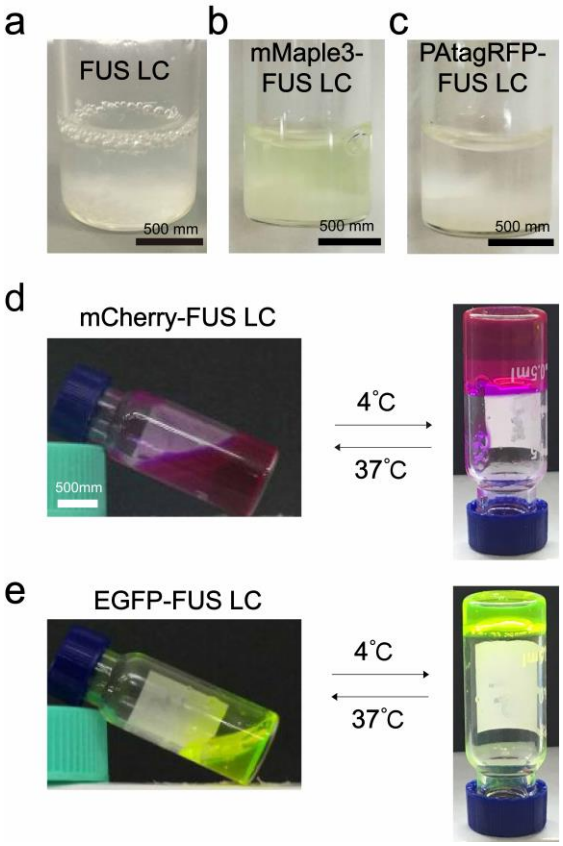


Figure S13. The reversible solution-to-hydrogel phase transition of FUS LC fusion proteins. (a) FUS LC solutions containing nanofiber aggregates at a concentration of 40 μM; (b) mMaple3-FUS LC solution containing protein aggregates at a concentration of 40 μM; (c) PAtagRFP-FUS LC solution containing protein aggregates at a concentration of 40 μM. Note: (a) (b)and (c) suggest that FUS LC, mMaple3-FUS LC and PAtagRFP-FUS LC fibers tend to precipitate from solution even at low concentration (40 μM). (d) mCherry-FUS LC solution (800 μM) containing dynamic nanofiber networks can reversibly form hydrogels upon cooling at 4 °C and returned to solution state at 37 °C. (e) EGFP-FUS LC solution (800 μM) containing dynamic nanofiber networks can quickly turn into hydrogels at 4 °C and return to solution state at 37 °C.

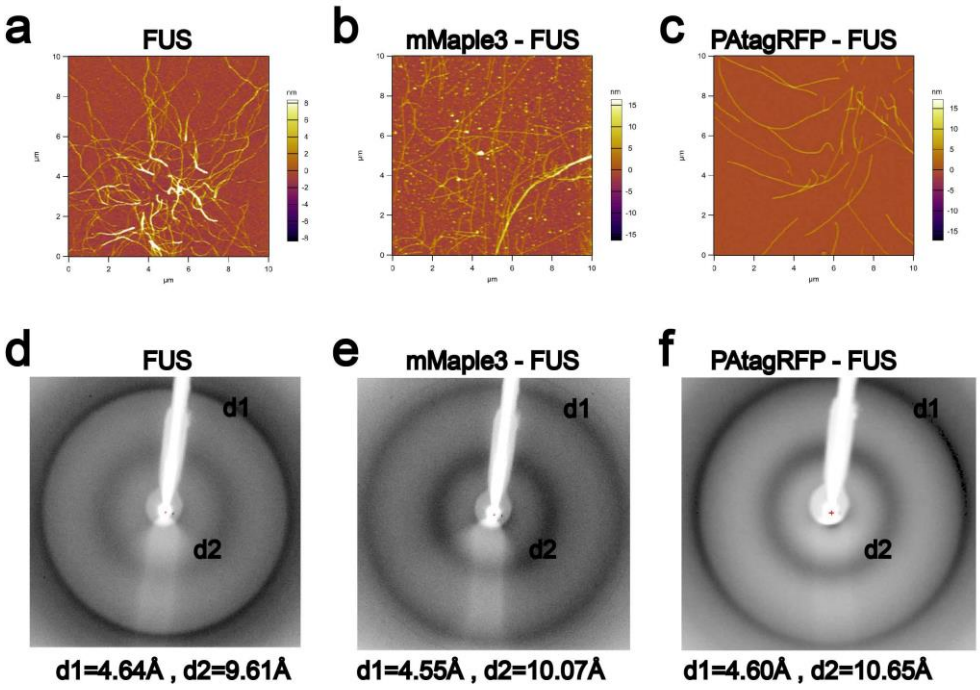


Figure S14. AFM morphology and X-ray fiber diffraction of FUS LC fusion proteins. (a-c) AFM height images of recombinant FUS LC nanofibers. FUS LC nanofibers (a), mMaple3-FUS LC nanfibers (b) and PAtagRFP-FUS LC nanofibers (c). (d-f) Diffraction pattern of nanofibers assembled from FUS LC fusion proteins, in which meridional reflection is ~4.55-4.64Å, and the equatorial reflection for FUS LC, mMaple3-FUS LC and PAtagRFP-FUS LC is 9.61Å, 10.07Å and 10.65Å, respectively.

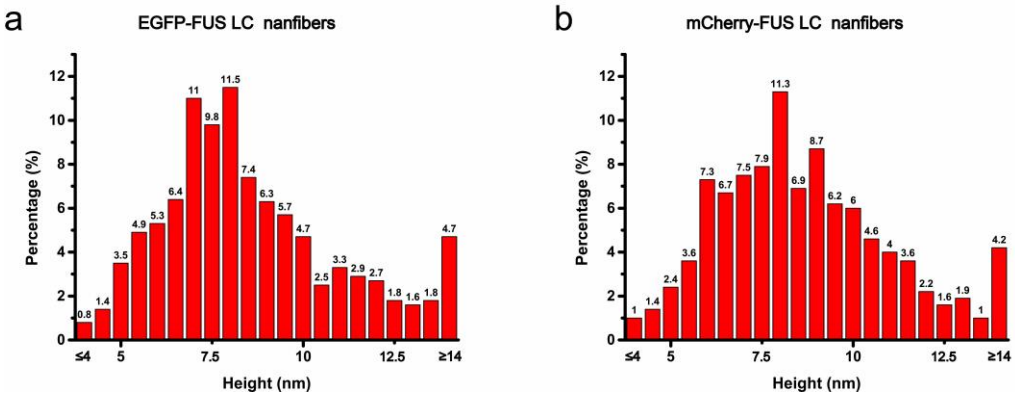


Figure S15. The diameter distributions of EGFP-FUS LC (a) and mCherry-FUS LC (b)

nanofibers based on AFM height methodology. 500 nanofibers for each sample was counted. The average diameter of EGFP-FUS LC and mCherry-FUS LC nanofibers is 8.5 ± 2.4 nm and 8.8 ± 2.3 nm, respectively.

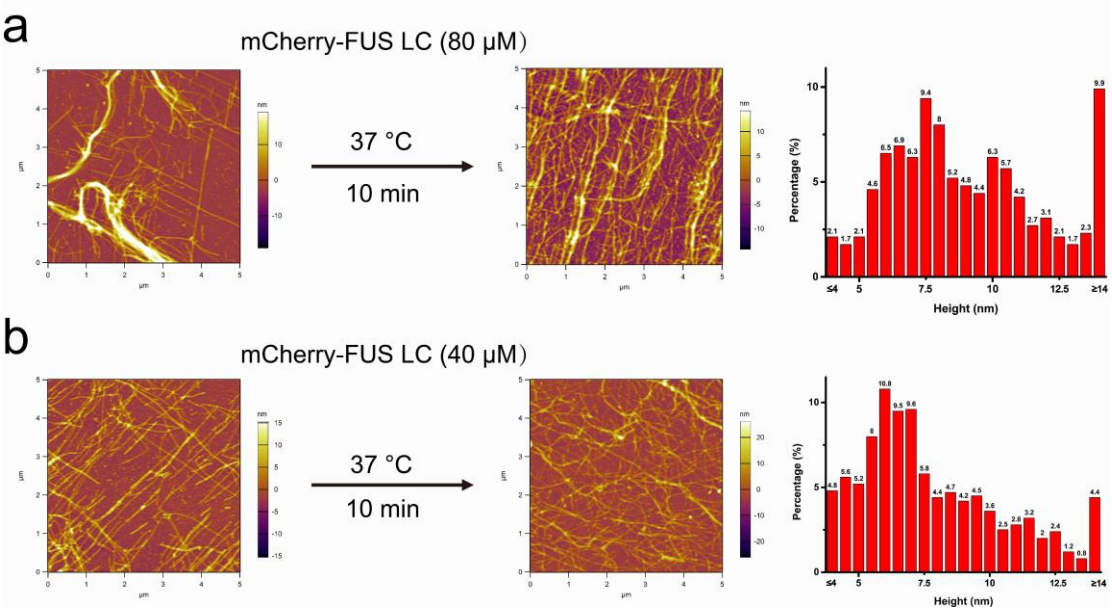


Figure S16. AFM images showing the mCherry-FUS LC nanofibers at different concentrations and their height distributions after incubation at 37 °C for 10 min. 500 nanofibers for each sample was counted. The high concentration of mCherry-FUS LC monomers resulted in many fiber bundles after aging for a week at 4 °C, the fiber bundles disassembled and reformed into thicker nanofibers after incubating at 37 °C for 10 min with the average height of 9.0 ± 2.8 nm (a). At lower protein concentration (40 μM), the average diameter of the resultant nanofibers at 4 °C was 8.8 ± 2.3 nm (Figure S15b), and slightly decreased to 7.8 ± 2.7 nm after incubation 37 °C for 10 min (Figure S16b). The increase in temperature seemed to disassemble the nanofibers, thus would reduce the average diameter of nanofibers.

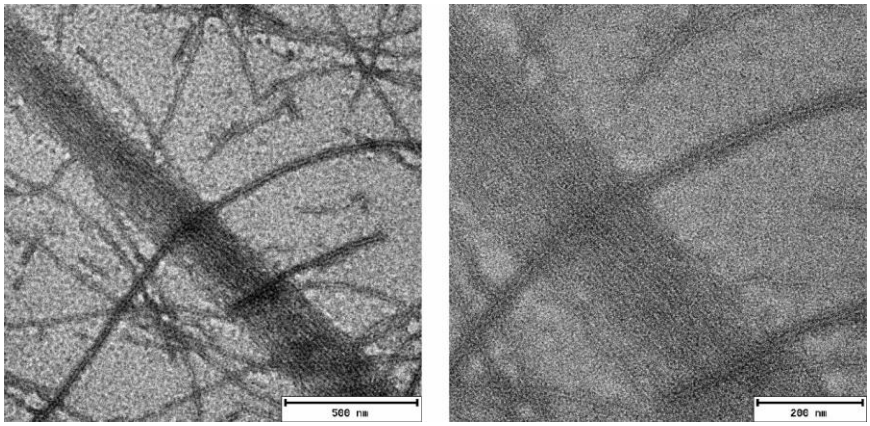


Figure S17. TEM images of mCherry-FUS LC nanofiber bundles assembled from high concentrations (80 μM) of protein monomers at 4 °C after incubation for a week.

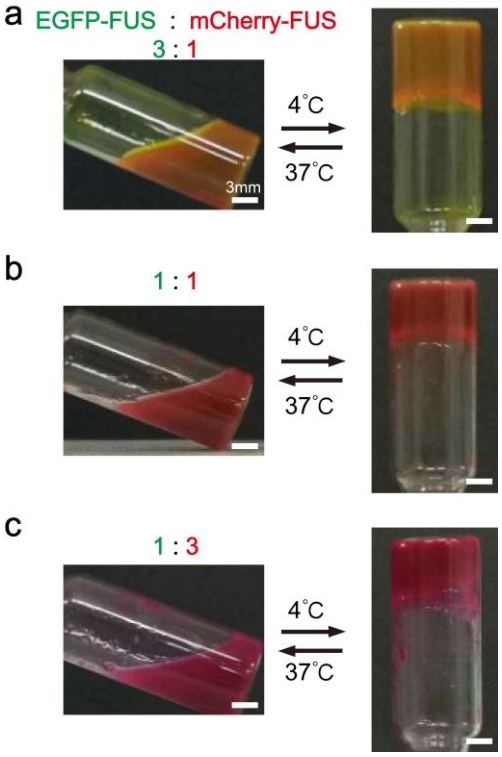


Figure S18. The reversible solution-to-hydrogel phase transition of random copolymer-like nanofibers composed of EGFP-FUS LC and mCherry-FUS LC proteins with different molar ratios. (a) Random copolymer-like hydrogel assembled from mixed protein monomers with a molar ratio of EGFP-FUS LC monomers to

mCherry-FUS LC monomers 3:1. (b) The molar ratio of EGFP-FUS LC monomers to mCherry-FUS LC monomers 1:1. (c) The molar ratio of EGFP-FUS LC monomers to mCherry-FUS LC monomers 1:3. All samples could reversibly form hydrogels upon cooling at 4 °C and returned to solution state at 37 °C. Note: The total protein concentration was 800 μM.

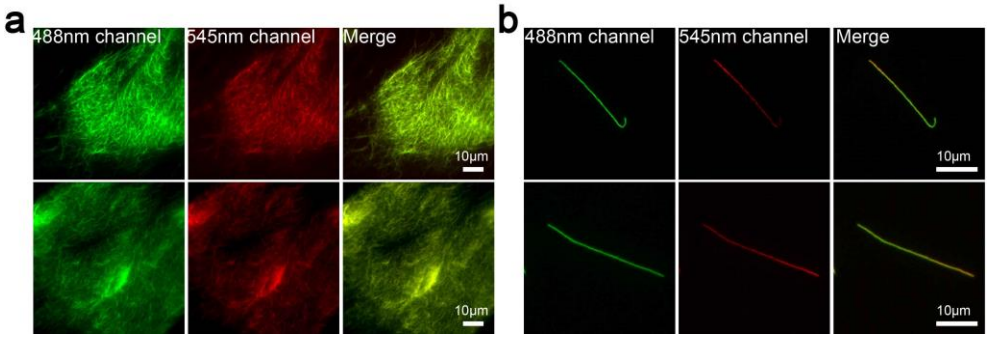


Figure S19. Fluorescent images of random copolymer-like nanofibers assembled from mixed protein monomers with a molar ratio of EGFP-FUS LC monomers to mCherry-FUS LC monomers 3:1. (a) Dense fibers networks obtained at a final protein concentration of 40 μM. (b) Selective single fiber acquired at a final protein concentration of 0.04 μM (1000-fold dilution). Green and red fluorescence was excited by 488 nm channel and 545 nm channel, respectively. Note: The samples were incubated for at least one week to obtain the desired fibers for fluorescent imaging.

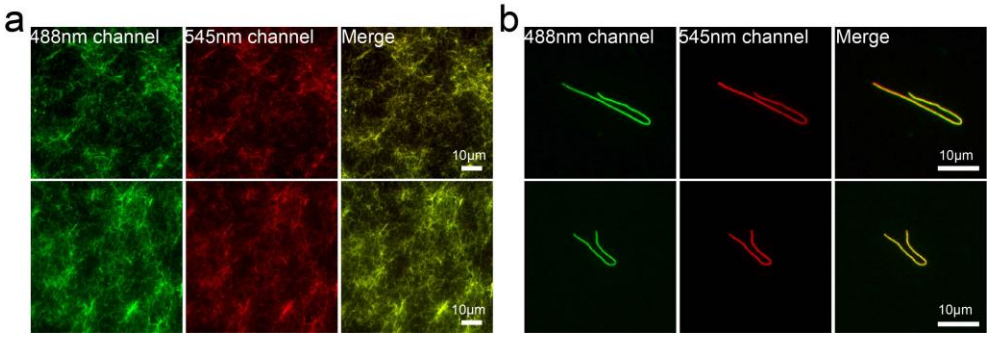


Figure S20. Fluorescent images of random copolymer-like nanofibers assembled from mixed protein monomers with a molar ratio of EGFP-FUS LC monomers to mCherry-FUS LC monomers 1:1. (a) Dense fibers networks obtained at a final protein concentration of 40 μM. (b) Selective single fiber acquired at a final protein concentration of 0.04 μM (1000-fold dilution). Green and red fluorescence was excited by 488 nm channel and 545 nm channel, respectively. Note: The samples were incubated for at least one week to obtain the desired fibers for fluorescent imaging.

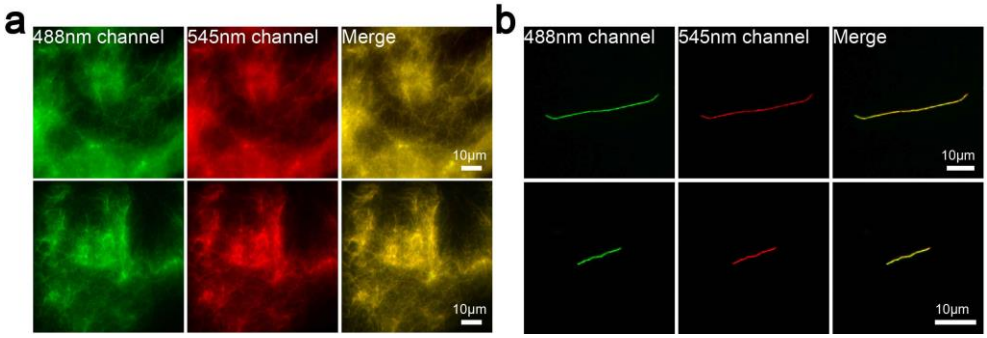


Figure S21. Fluorescent images of random copolymer-like nanofibers assembled from mixed protein monomers with a molar ratio of EGFP-FUS LC monomers to mCherry-FUS LC monomers 1:3. (a) Dense fibers networks obtained at a final protein concentration of 40 μM. (b) Selective single fiber acquired at a final protein concentration of 0.04 μM (1000-fold dilution). Green and red fluorescence was excited by 488 nm channel and 545 nm channel, respectively. Note: The samples were incubated for at least one week to obtain the desired fibers for fluorescent imaging.

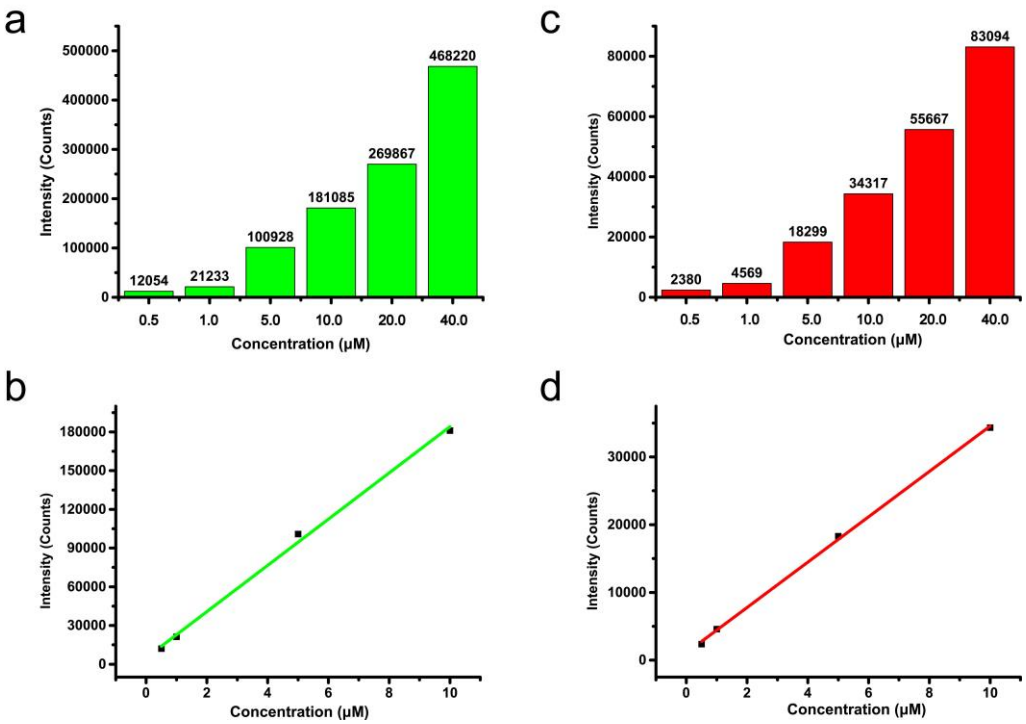


Figure S22. The relationship between fluorescent intensity and protein concentration of EGFP-FUS LC and mCherry-FUS LC proteins. (a) The fluorescent intensity of EGFP-FUS LC solutions at different concentrations. (b) The linear relationship between fluorescent intensity and protein concentration of EGFP-FUS LC solutions measured at low concentrations (≤10 μM). (c) The fluorescent intensity of mCherry-FUS LC solutions at different concentrations. (d) The linear relationship between fluorescent intensity and protein concentration of mCherry-FUS LC solutions measured at low concentrations (≤10 μM). 300 μL sample solution of different concentrations was measured for three times. The linear relationship between fluorescent intensity and protein concentration of both proteins suggests the validity of applying relative fluorescent intensity in determining the stoichiometry of the complex nanofibers (Figure S23 and S34).

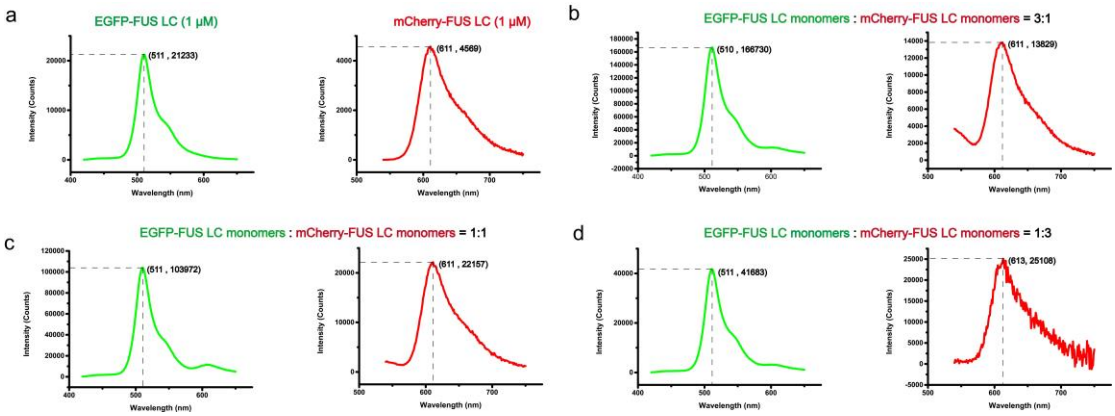


Figure S23. The fluorescent intensity of EGFP-FUS LC, mCherry-FUS LC and their random copolymer-like nanofibers. (a) The fluorescent intensity of 300 μL 1μM EGFP-FUS LC and mCherry-FUS LC nanofiber solutions as internal references. (b) The fluorescent intensity of 300 μL random (EGFP-FUS LC:mCherry-FUS LC = 3:1) copolymer-like nanofiber solution, the ratio of fluorescent intensity of EGFP and mCherry determined was 2.6:1, using (a) as references. (c) The fluorescent intensity of 300 μL random (EGFP-FUS LC:mCherry-FUS LC = 1:1) copolymer-like nanofiber solution, the ratio of fluorescent intensity of EGFP and mCherry in the nanofibers determined was 1.0:1. (d) The fluorescent intensity of 300 μL random copolymer-like nanofiber solutions (EGFP-FUS LC:mCherry-FUS LC = 1:3), the ratio of fluorescent intensity of EGFP and mCherry in the nanofibers determined was 1:2.8. Note: Determination of the stoichiometry of complex nanofibers was based on the relative fluorescence intensity ratio of individual components.

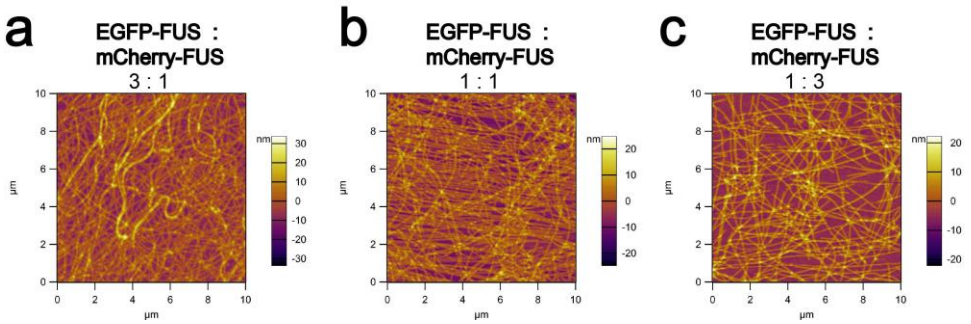


Figure S24. AFM height images of random supramolecular copolymer-like nanofibers assembled from mixed protein monomers with different molar ratios. (a) Nanofibers obtained at a molar ratio of EGFP-FUS LC to mCherry-FUS LC monomer 3:1. (b) Nanofibers obtained at a molar ratio of EGFP-FUS LC to mCherry-FUS LC monomer 1:1. (c) Nanofibers obtained at a molar ratio of EGFP-FUS LC to mCherry-FUS LC monomer 1:3. The final protein concentration for all the three cases (a-c) was 40 μM.

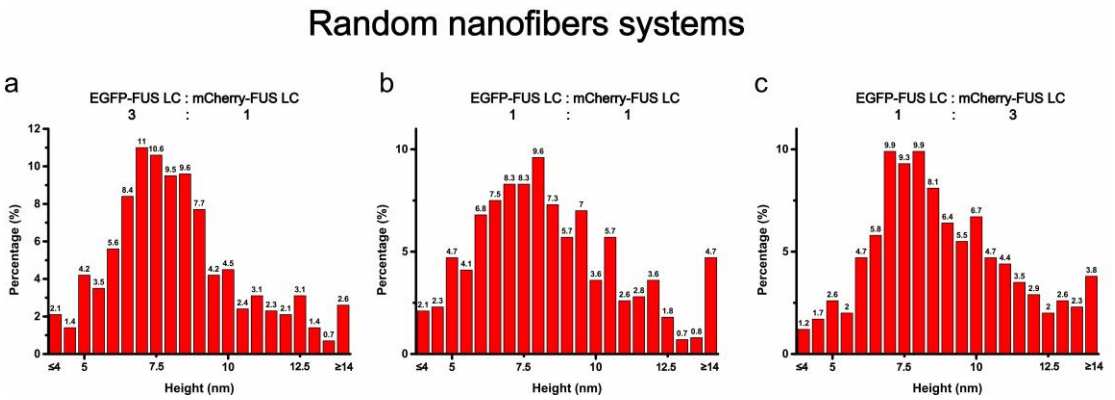


Figure S25. The height distributions of random copolymer-like nanofiber systems composed of EGFP-FUS LC and mCherry-FUS LC monomers with different ratios (3:1, 1:1 and 1:3). From left to right, the average heights of the samples are 8.3 ± 2.3, 8.4 ± 2.5 and 8.8 ± 2.4 nm, respectively. The data was based on statistic analysis of 500 counted nanofibers.

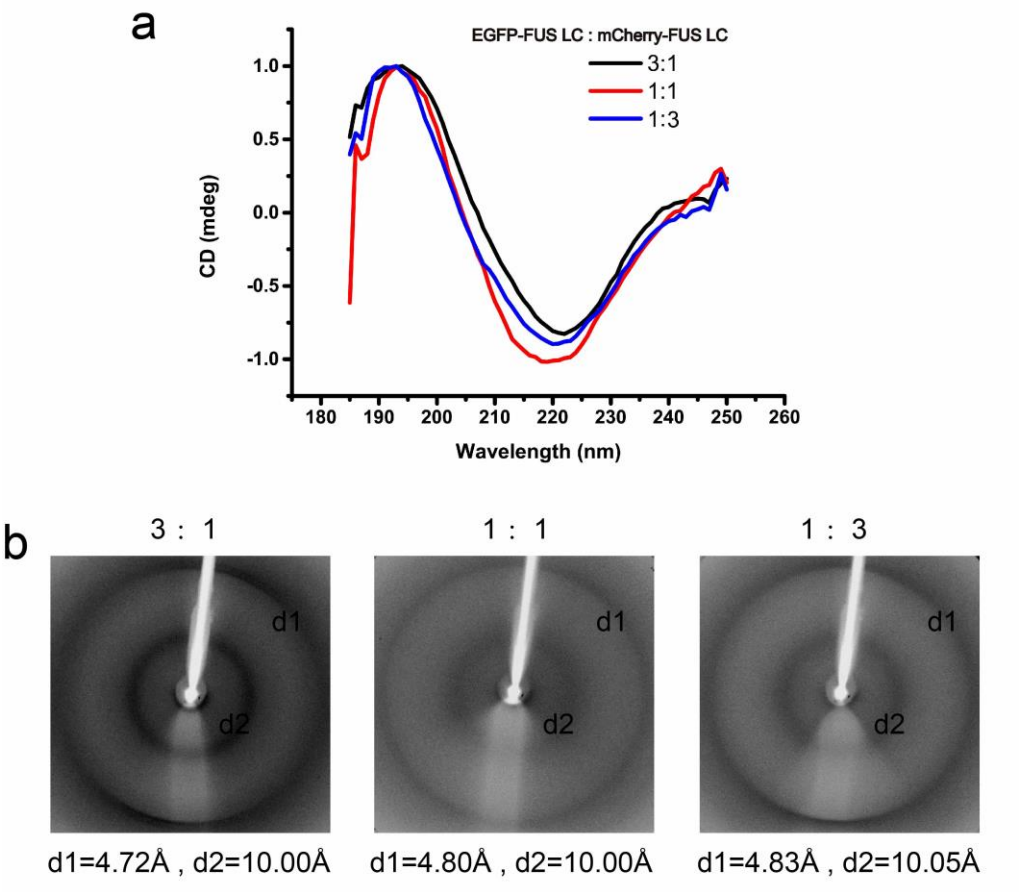


Figure S26. The CD and X-ray fiber diffraction of random copolymer-like nanofibers composed of EGFP-FUS LC and mCherry-FUS LC monomers with different ratios (3:1, 1:1 and 1:3). Both of the CD spectra (a) and XRD fiber diffraction (b) confirmed the strong β-sheet secondary structures in these random copolymer-like nanofibers.

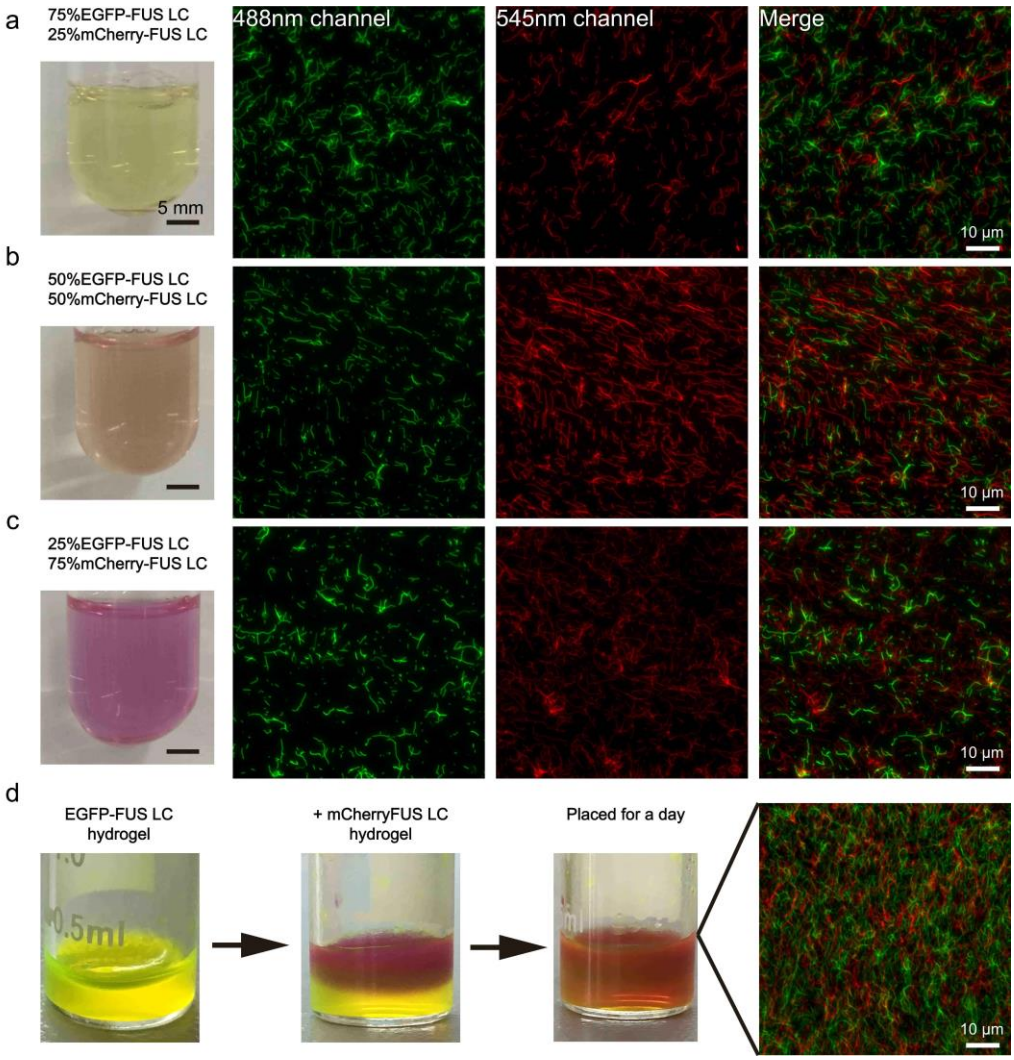


Figure S27. Digital and fluorescent images for samples prepared by mixing two protein solutions containing preformed EGFP-FUS LC and mCherry-FUS LC fibers at varied molar ratios. (a) Solution (40 μM) prepared by mixing EGFP-FUS LC fiber solution with mCherry-FUS LC nanofiber solution at a molar ratio 3:1. (b) Solution (40 μM) prepared by mixing EGFP-FUS LC nanofiber solution with mCherry-FUS LC nanofiber solution at a molar ratio 1:1. (c) Solution (40 μM) prepared by mixing EGFP-FUS LC nanofiber solution with mCherry-FUS LC nanofibers at a molar ratio 1:3. (d) Diffusion phenomenon between EGFP-FUS LC and mCherry-FUS LC hydrogel showing that the nanofiber networks can penetrate between each other. Green and red fluorescence was excited by 488 nm and 545 nm channel, respectively.

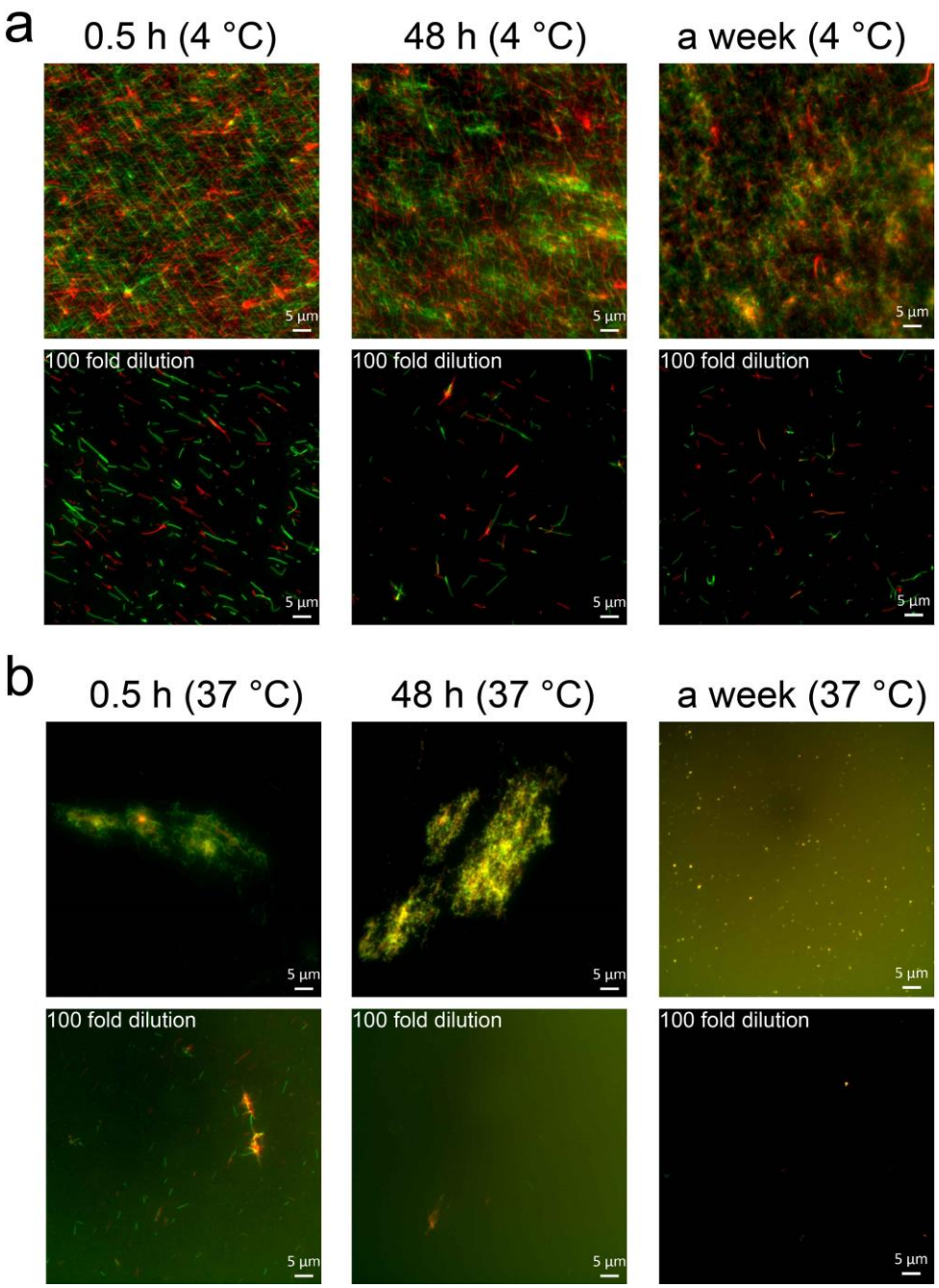


Figure S28. The fluorescent images of mixed pre-assembled EGFP-FUS LC and mCherry-FUS LC nanofibers when incubated at 4 °C (a) and 37 °C (b) during different time.

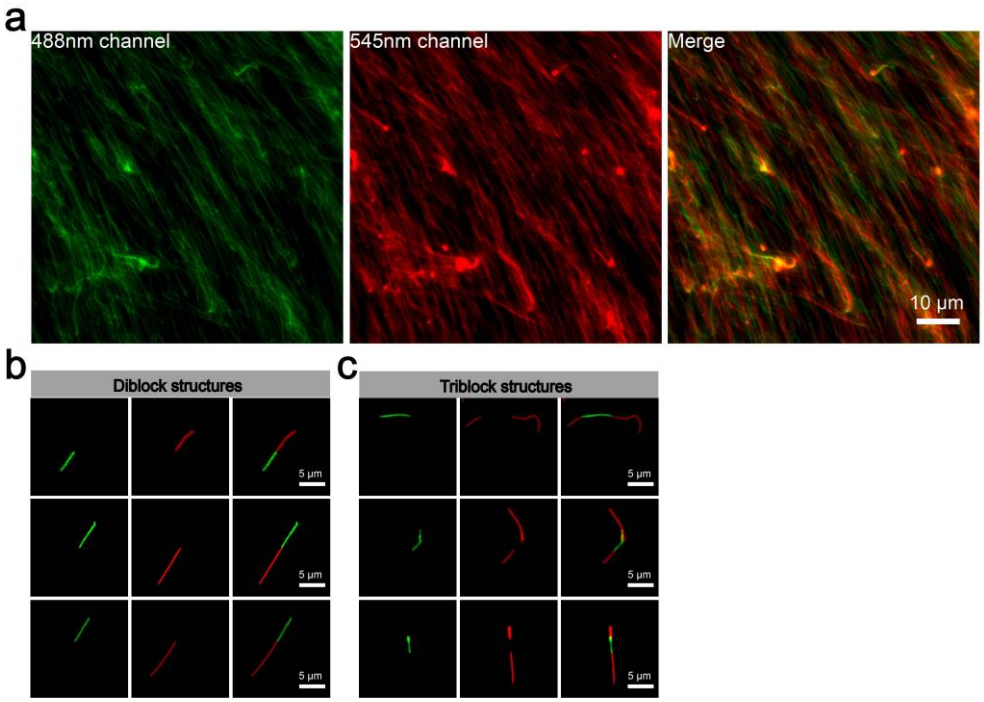


Figure S29. Fluorescent images of block copolymer-like supramolecular structures formed by unidirectional or bidirectional growth of mCherry-FUS LC monomers on EGFP-FUS LC fiber seeds (Stage 1 self-assembly). (a) Dense block copolymer-like fiber networks obtained at a final protein concentration of 40 μM; (b) Selective diblock fiber structures acquired at a final protein concentration of 0.04 μM (1000-fold dilution); (c) Selective triblock fiber structures acquired at a final protein concentration of 0.04 μM (1000-fold dilution). Note: The block copolymer-like supramolecular structures were prepared by addition of EGFP-FUS LC fibers seeds into mCherry-FUS LC monomers, with the molar ratio of monomers to fiber seeds 3:1. The total protein concentration applied was 40 μM.

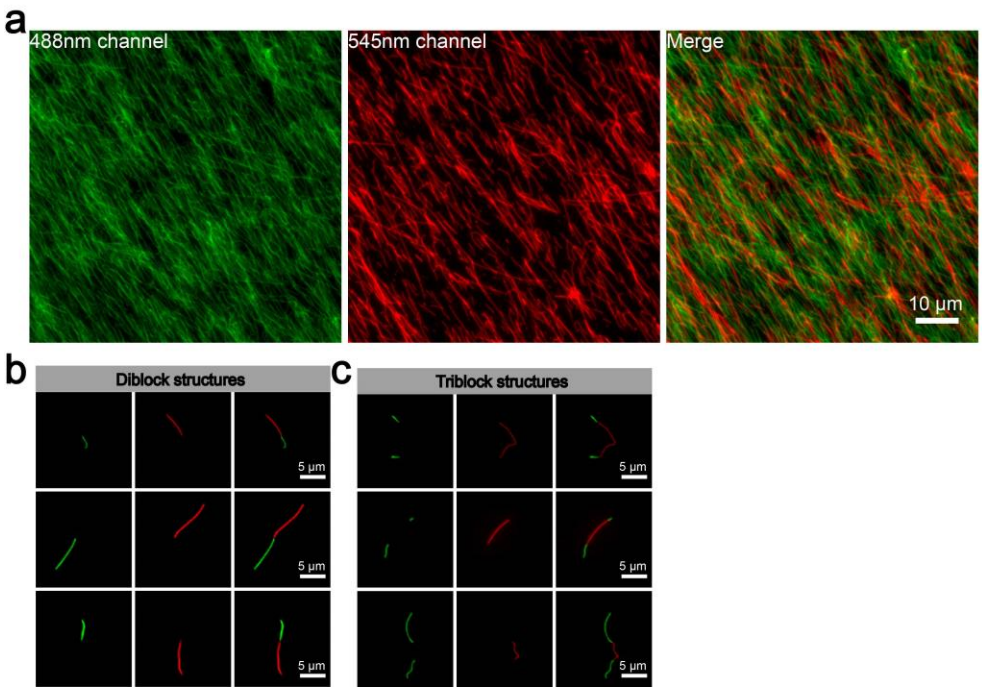


Figure S30. Fluorescent images of block copolymer-like supramolecular structures formed by unidirectional or bidirectional growth of EGFP-FUS LC monomers on mCherry-FUS LC fiber seeds. (a) Dense block copolymer-like fiber networks based upon mCherry-FUS LC fibers seeded growth of EGFP-FUS LC monomers, with the molar ratio of monomers to seeds 3:1, and total concentration was 40 μM. (b) Selective diblock fiber structures. (c) Selective triblock fiber structures. The single fiber images were acquired based upon a 1000-fold diluted solution.

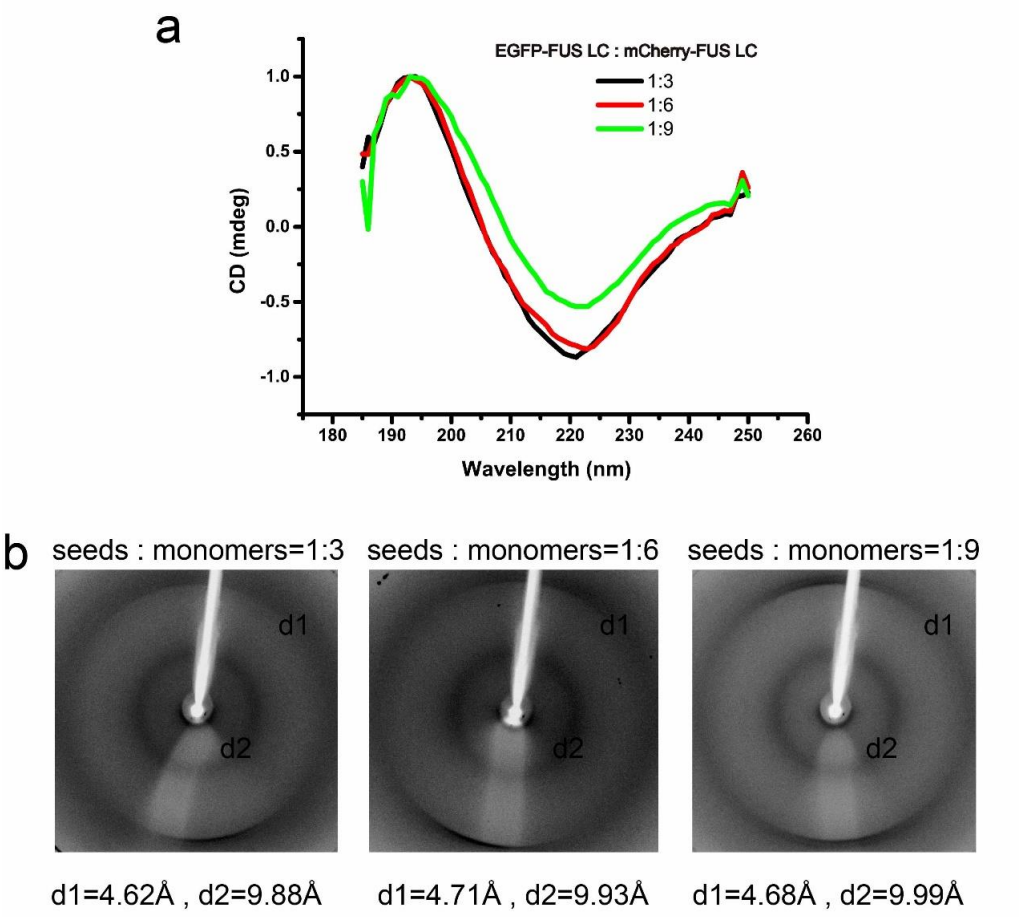


Figure S31. The CD and X-ray fiber diffraction of multiblock nanofibers prepared by addition of EGFP-FUS LC seeds to mCherry-FUS LC monomers at different ratios (1:3,1:6,1:9). Both of the CD spectra (a) and XRD diffraction (b) indicated the strong β-sheet secondary structures in these multiblock nanostructures.

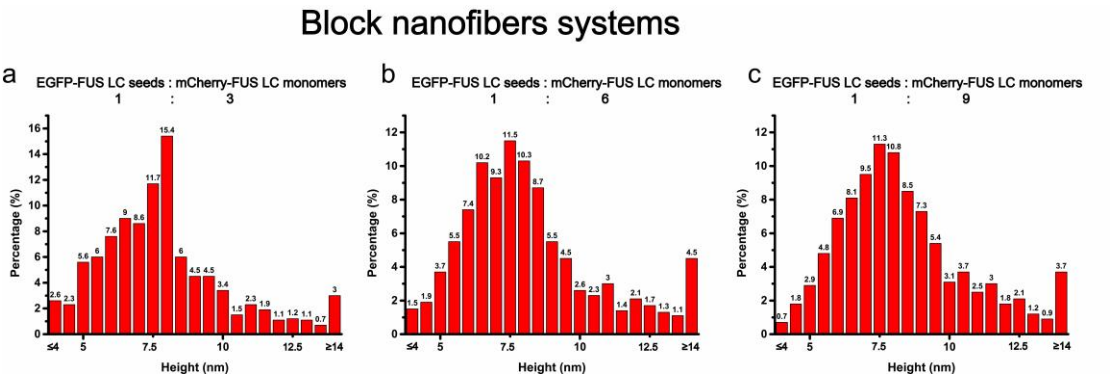


Figure S32. The height distributions of multiblock nanofiber systems prepared by addition of EGFP-FUS LC seeds to mCherry-FUS LC monomers at different ratios (1:3,1:6,1:9). From left to right, the average heights of the samples are 7.8 ± 2.2, 8.1 ± 2.4 and 8.3 ± 2.3 nm, respectively. The data was based on statistic analysis of 500 counted nanofibers.

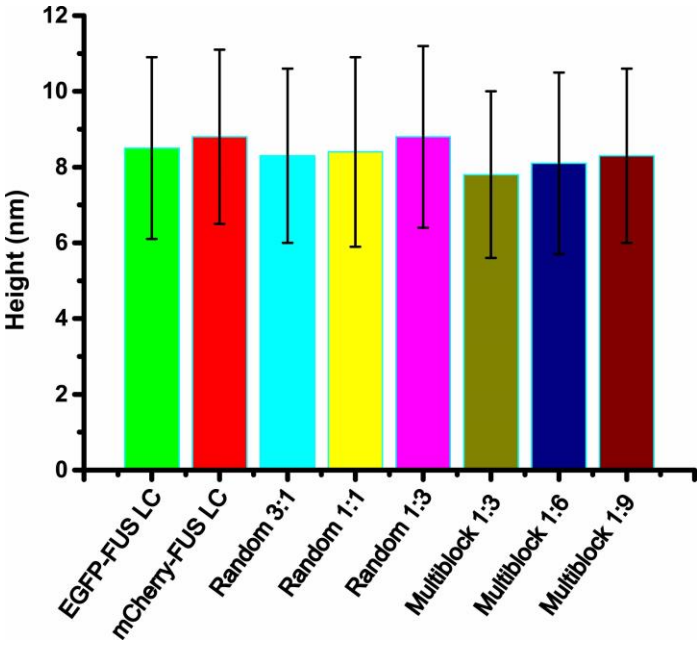


Figure S33. The average diameters of EGFP-FUS LC nanofibers, mCherry-FUS LC nanofibers, random copolymer-like nanofiber systems (EGFP-FUS LC monomers:mCherry-FUS LC monomers = 3:1,1:1and1:3), and multiblock nanofiber systems (EGFP-FUS LC seeds:mCherry-FUS LC = 1:3,1:6,1:9). The data was based on statistic analysis of 500 counted nanofibers for each sample.

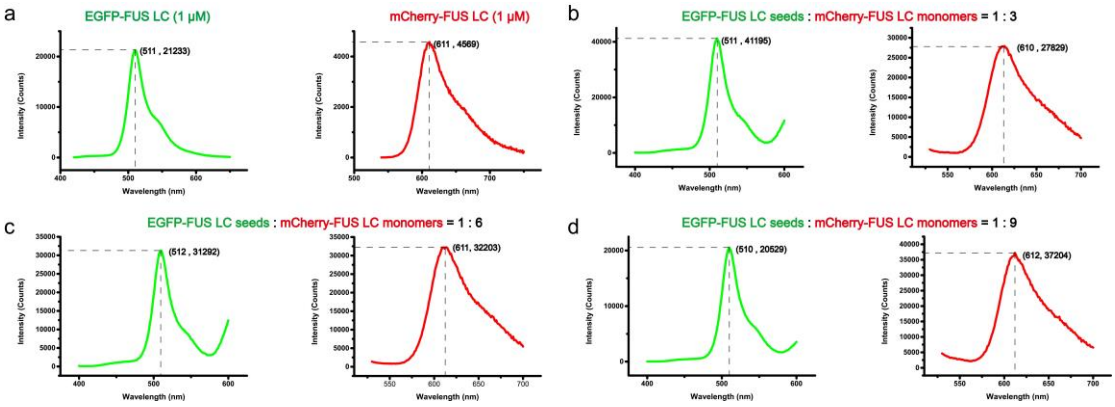


Figure S34. The fluorescent intensity of EGFP-FUS LC, mCherry-FUS LC and their multiblock nanofibers. (a) The fluorescent intensity of 300 μL 1μM EGFP-FUS LC and mCherry-FUS LC nanofiber solutions as internal references. (b) The fluorescent intensity of 300 μL multiblock nanofiber solution (EGFP-FUS LC seeds:mCherry-FUS LC monomers=1:3), the ratio of fluorescent intensity of EGFP and mCherry in the nanofibers determined was 1:3.1, using (a) as references. (c) The fluorescent intensity of 300 μL multiblock nanofiber solution (EGFP-FUS LC seeds:mCherry-FUS LC monomers = 1:6), the ratio of fluorescent intensity of EGFP and mCherry determined was 1:4.8. (d) The fluorescent intensity of 300 μL multiblock nanofiber solution (EGFP-FUS LC seeds:mCherry-FUS LC monomers = 1:9), the ratio of fluorescent intensity of EGFP and mCherry was 1:8.4. Note: Determination of the stoichiometry of complex nanofibers was based on the relative fluorescence intensity ratio of the individual components in the nanofibers.

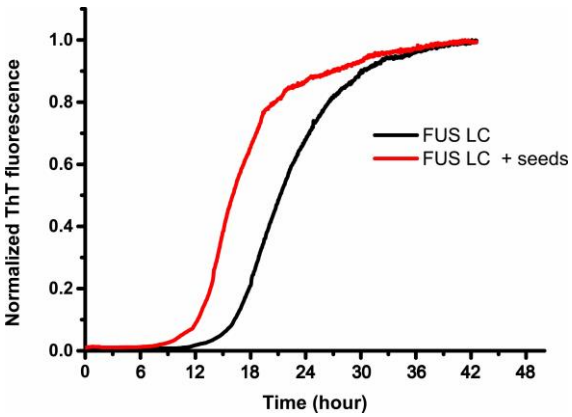


Figure S35. ThT fluorescence assay showing the assembly kinetics for FUS LC monomer solution (40 μM) in the presence/absence of FUS LC nanofiber seeds.

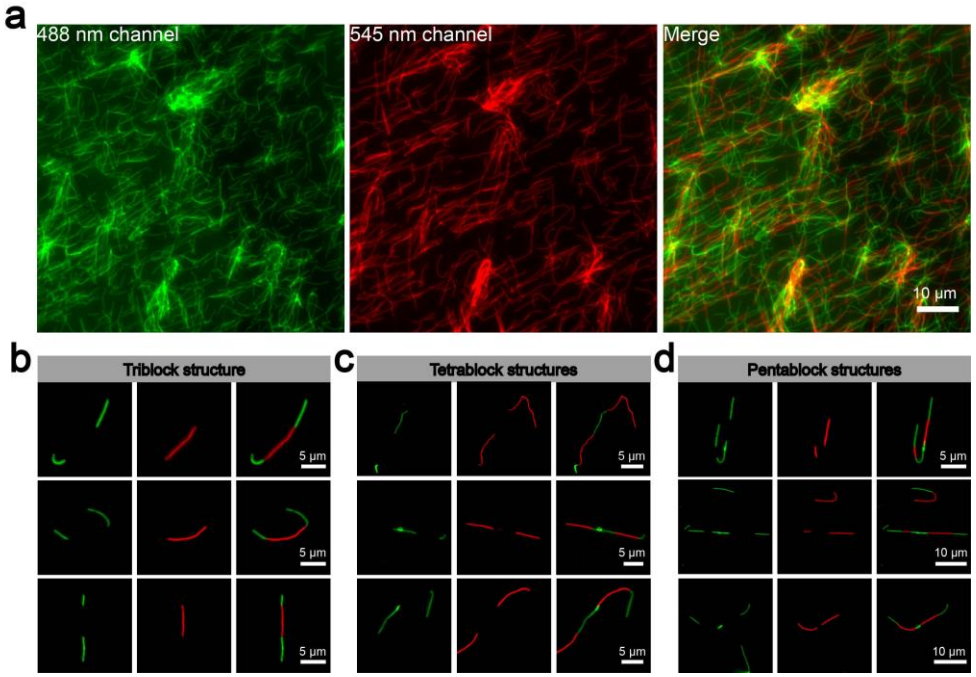


Figure S36. Fluorescent images of multiblock supramolecular structures formed by growth of EGFP-FUS-LC monomers on preformed diblock fibers from Stage 1 (Stage 2 self-assembly). (a) Dense block copolymer-like fiber networks obtained at a molar ratio of monomers to seeds 3:1. The total protein concentration was 40 μM. (b-d) Selective triblock fiber structures (b), Selective tetrablock fiber structures (c) and selective pentablock fiber structures (d). Imaging of the single fiber structures in (b-d) was acquired with a diluted solution at a concentration 0.04 μM (1000-fold dilution).

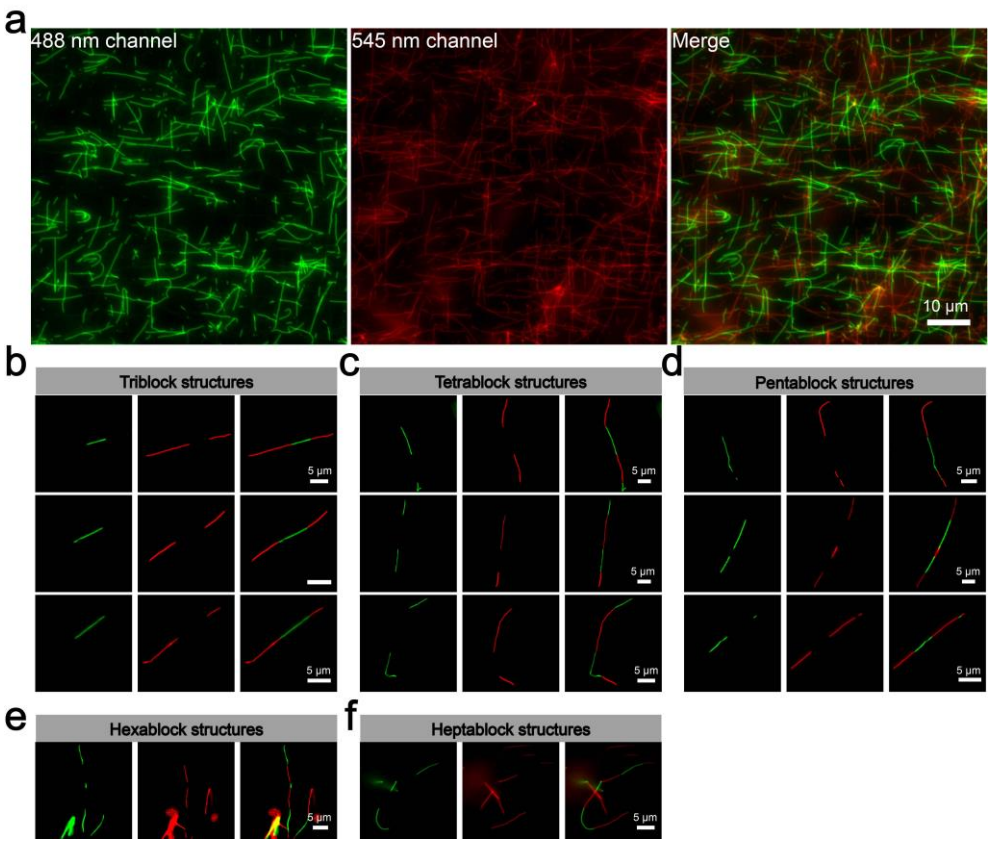


Figure S37. Fluorescent images of multiblock supramolecular structures formed by growth of EGFP-FUS-LC monomers on preformed diblock fibers from Stage 2 (Stage 3 self-assembly). (a) Dense block copolymer-like fiber networks obtained at a molar ratio of monomers to seeds 3:1. The total protein concentration was 40 μM. (b-f) Selective triblock fiber structures (b), Tetrablock fiber structures (c), Pentablock fiber structures (d) Hexablock fiber structures (e) and Heptablock fiber structures (f). Imaging of the single fiber structures in (b-f) was acquired with a diluted solution at a concentration 0.04 μM (1000-fold dilution).

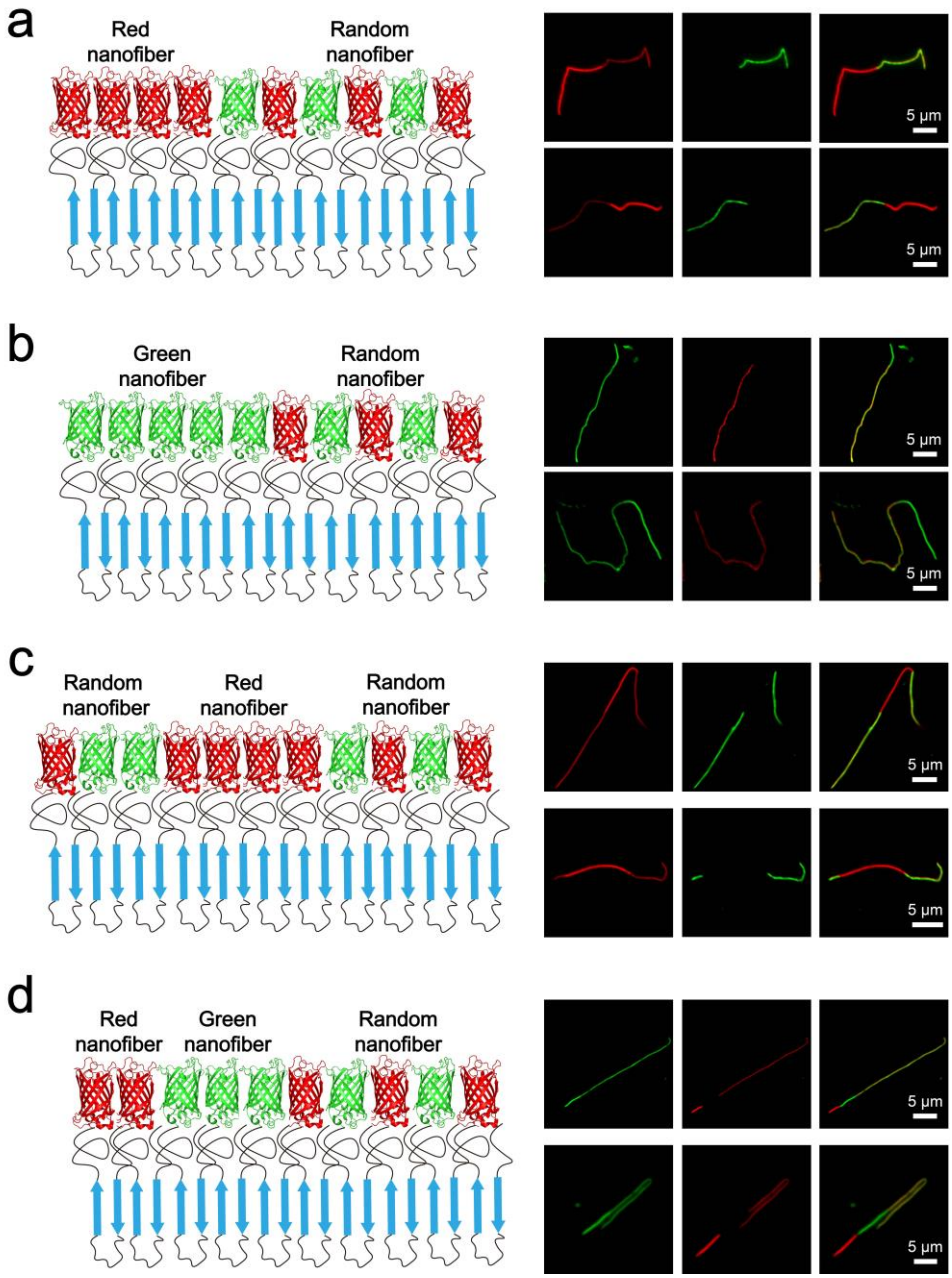


Figure S38. Fluorescent images of hybrid block copolymer-like supramolecular structure. (a) Selective diblock copolymer-like fibers formed by growth of mixed equal amount of mCherry-FUS LC and EGFP-FUS LC monomers on mCherry-FUS LC fiber seeds. (b) Selective diblock copolymer-like fibers formed by growth of mixed equal amount of mCherry-FUS LC and EGFP-FUS LC monomers on EGFP-FUS LC fiber seeds. (c) Selective triblock fiber structures formed by bidirectional growth of mixed EGFP-FUS LC and mCherry-FUS LC monomers on mCherry-FUS LC fiber seeds. (d) Selective triblock fiber structures formed by

unidirectional growth of mixed EGFP-FUS LC and mCherry-FUS LC monomers on preformed (mCherry-FUS LC)-co-(EGFP-FUS LC) diblock fibers. Solution concentration applied here was 40 μM for all solutions. The molar ratio of monomers to fiber seeds applied in all cases was 3:1.

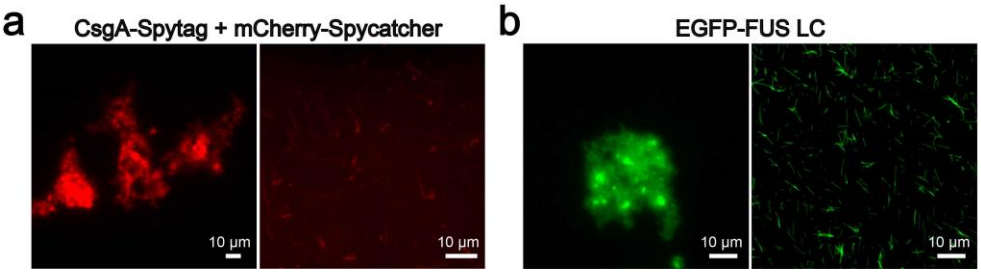


Figure S39. Fluorescent images of CsgA-Spytag and EGFP-FUS LC nanofibers. (a) Fluorescence images of aggregated and separated CsgA-Spytag fibers, red fluorescence was rendered by incubation of CsgA-Spytag fiber network in the presence of mCherry-Spycatcher solution based on the covalent reaction between Spytag and Spycatcher domains. (b) Fluorescence images of aggregated and separated EGFP-FUS LC nanofibers.

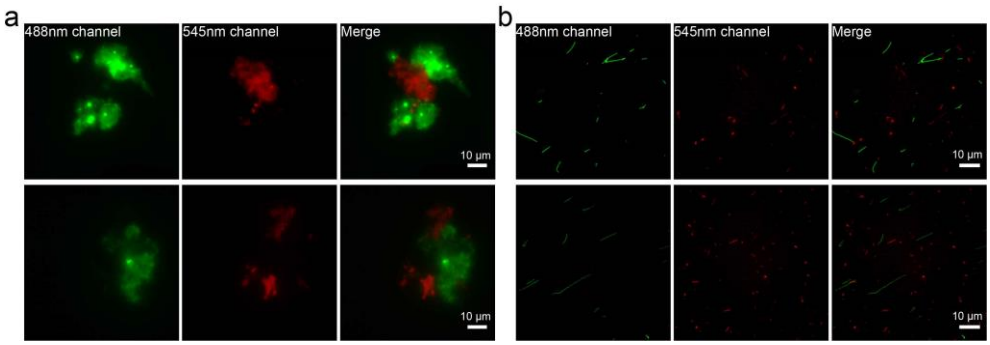


Figure S40. Fluorescent images of self-sorted supramolecular fiber networks based upon CsgA-Spytag and EGFP-FUS LC nanofibers. (a) Dense self-sorted nanofiber networks. (b) Diluted nanofiber sample based on a solution with 100-fold dilution.

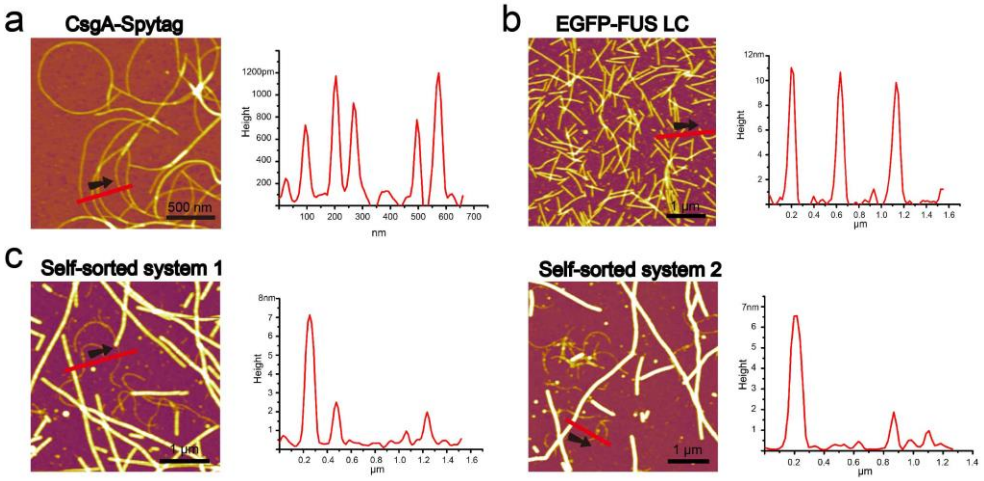


Figure S41. AFM morphology and height plots for CsgA-Spytag, EGFP-FUS LC and self-sorted supramolecular nanofibers formed by the two proteins. (a) AFM height image of CsgA-Spytag nanofibers and the corresponding height plot. (b) AFM height image of EGFP-FUS LC nanofibers and the corresponding height plot. (c) AFM height images of self-sorted structures based upon CsgA-Spytag and EGFP-FUS LC and the corresponding height plots.

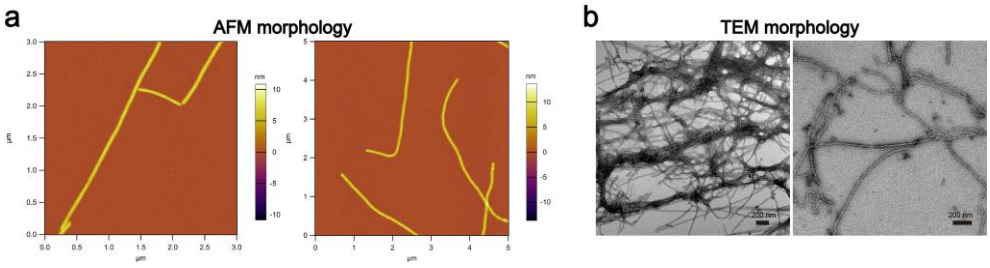


Figure S42. AFM and TEM images of His-mCherry-FUS LC nanofibers. (a) AFM height images. (b) TEM images.

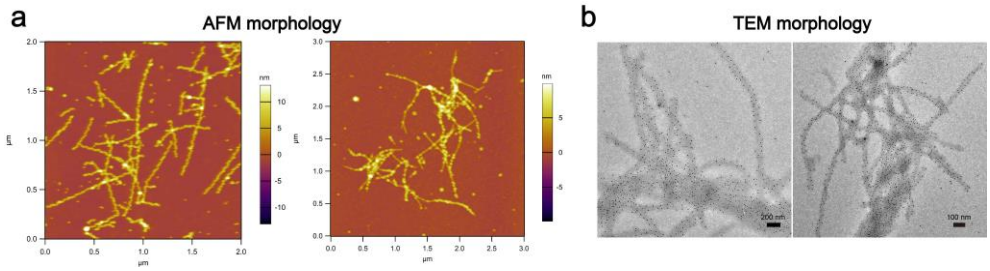


Figure S43. AFM and TEM images for His-mCherry-FUS LC nanofibers templated assembly of Co-NTA-Au NPs. (a) AFM images. (b) TEM images.

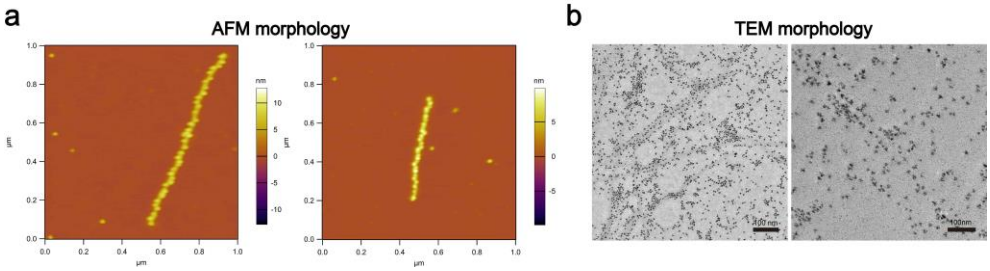


Figure S44. AFM and TEM images for His-mCherry-FUS LC nanofibers templated assembly of Co-NTA-CdSeS@ZnS QDs. (a) AFM height images. (b) TEM images.

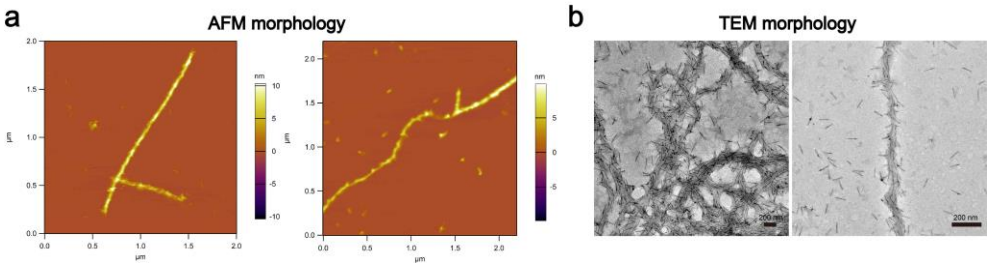


Figure S45. AFM and TEM images for His-mCherry-FUS LC nanofibers templated assembly of Co-NTA-CdS NRs. (a) AFM height images. (b) TEM images.

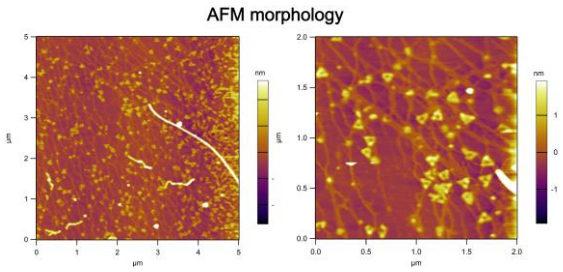


Figure S46. AFM height images for His-mCherry-FUS LC nanofibers templated assembly of Ni-NTA-DNA Origami.

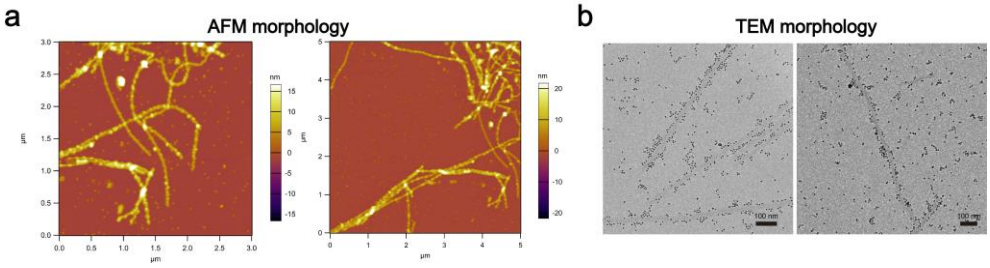


Figure S47. AFM and TEM images for EGFP-FUS LC- Spycatcher nanofibers templated assembly of Spytag-Cys_2-Au NPs. (a) AFM height images. (b) TEM images.

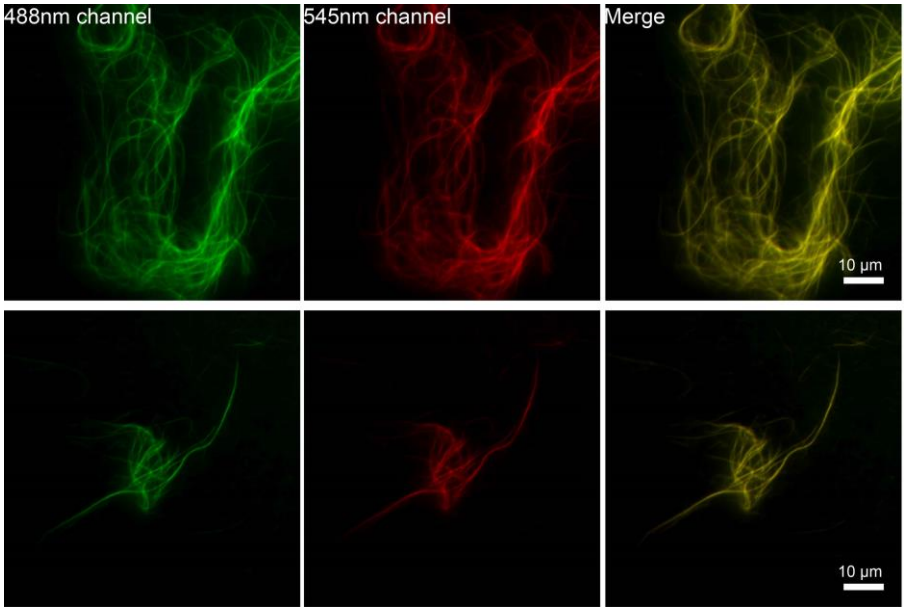


Figure S48. Fluorescent images of random copolymer-like nanofibers assembled from mixed protein monomers with a molar ratio of EGFP-FUS LC-Spycatcher monomers to His-mCherry-FUS LC monomers 1:1. Dense fibers networks obtained at a final protein concentration of 40 μM. Green and red fluorescence was excited by 488 nm channel and 545 nm channel, respectively. Note: The samples were incubated for at least one week to obtain the desired fiber for fluorescent imaging.

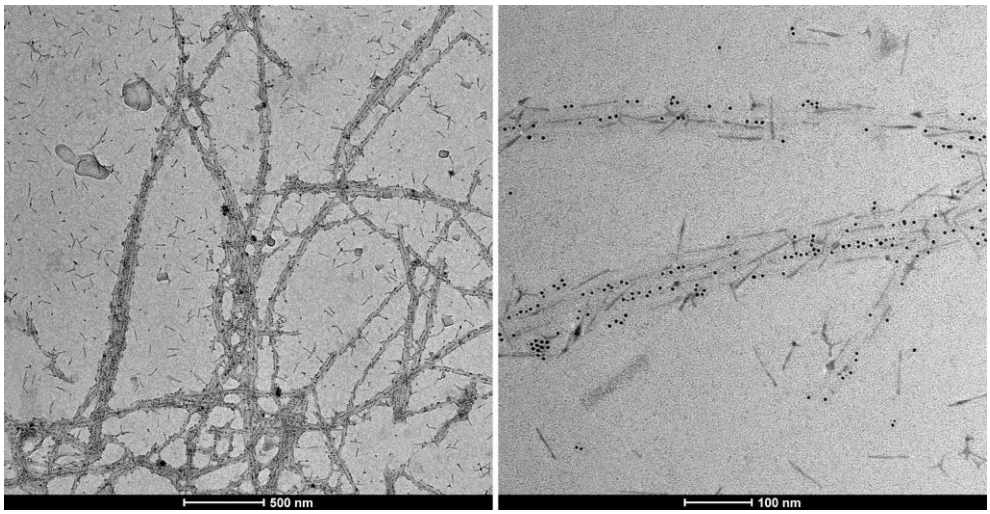


Figure S49. Random copolymer-like fibers templated assembly of Spytag-Cys_2-Au NPs and Co-NTA-CdS nanorods. The random copolymer-like fiber networks obtained at a final protein concentration of 40 μM were composed of equal amounts of

EGFP-FUS LC-Spycatcher and His-mCherry-FUS LC monomers.

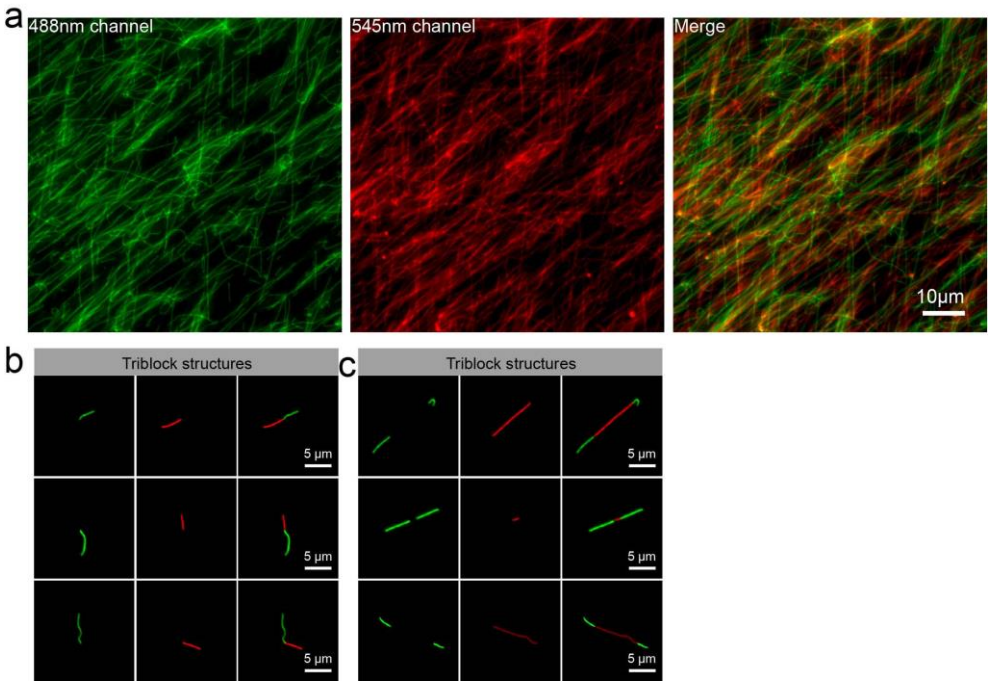


Figure S50. Fluorescent images of block supramolecular fiber structures formed by growth of EGFP-FUS LC-Spycatcher monomers on His-mCherry-FUS LC fiber seeds. (a) Dense block copolymer-like fiber networks. (b) Selective diblock fiber structures based upon unidirectional growth of monomers. (c) Selective triblock fiber structures based upon bidirectional growth of monomers. The molar ratio of monomers to seeds was 3:1 and the total solution concentration was 40 μM.

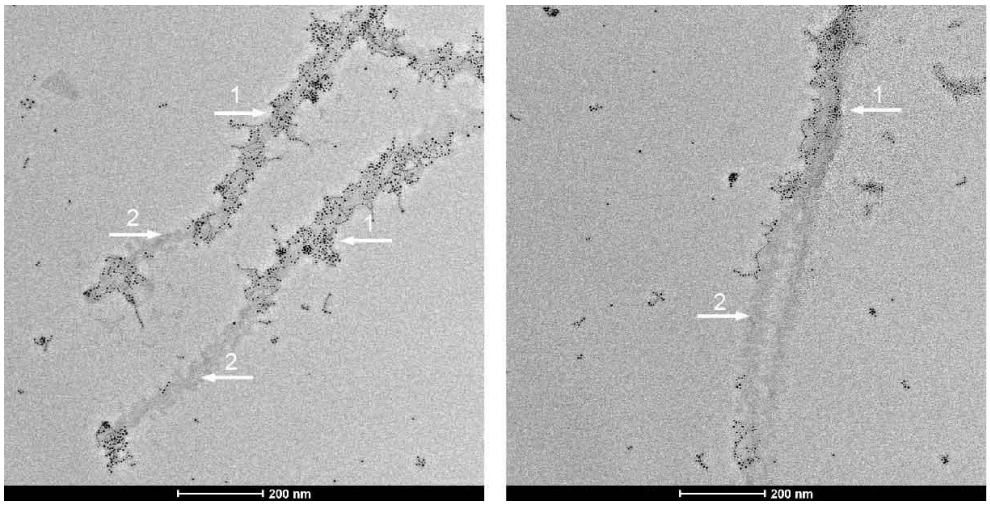


Figure S51. Multiblock copolymer-like supramolecular fibers for templated assembly of Spytag-Cys_2-Au NPs. Fibers were obtained by growth of EGFP-FUS LC-Spycatcher monomers on His-mCherry-FUS LC fiber seeds. The molar ratio of His-mCherry-FUS LC fiber seeds to EGFP-FUS LC-Spycatcher monomers (40 μM) was 1:3.

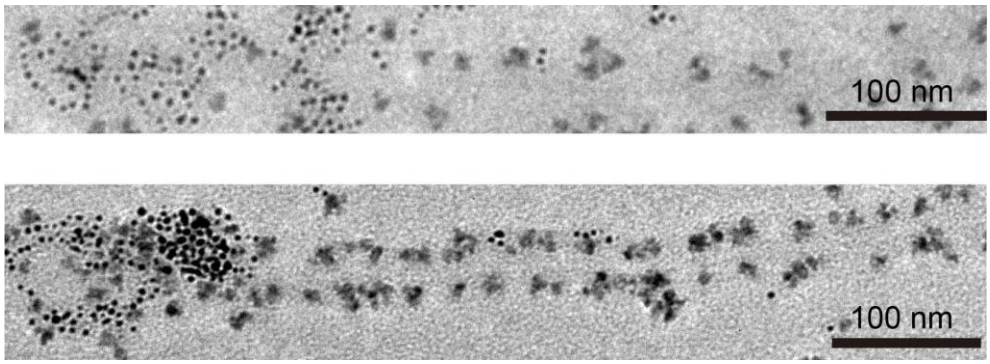


Figure S52. Multiblock copolymer-like supramolecular fibers for templated assembly of Spytag-Cys_2-Au NPs and Co-NTA- CdSeS@ZnS QDs together. Fibers were obtained by growth of EGFP-FUS LC-Spycatcher monomers on His-mCherry-FUS LC fiber seeds. The amount of His-mCherry-FUS LC fiber seeds solution to EGFP-FUS LC-Spycatcher monomers (40 μM) solution was 1:3.

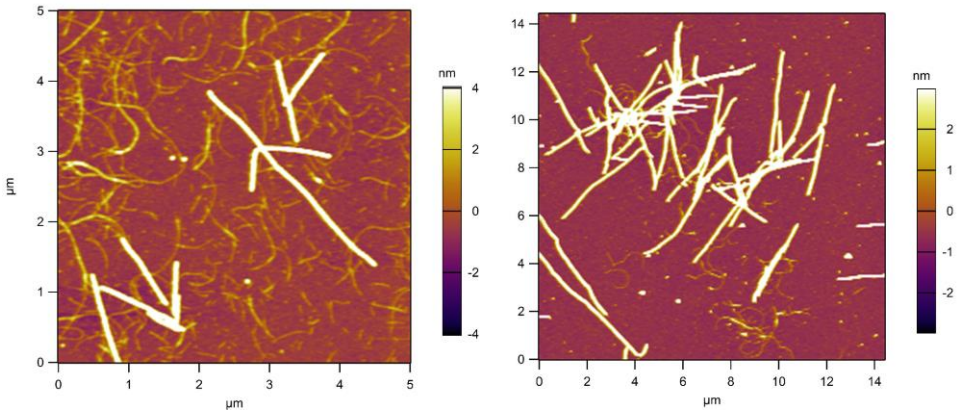


Figure S53. AFM images of self-sorted nanofibers composed of EGFP-FUS LC-Spycatcher (40 μM) and CsgA-His (4 μM). The straight fibers stand for FUS LC fibers, while the curved fibers refer to CsgA-His nanofibers.

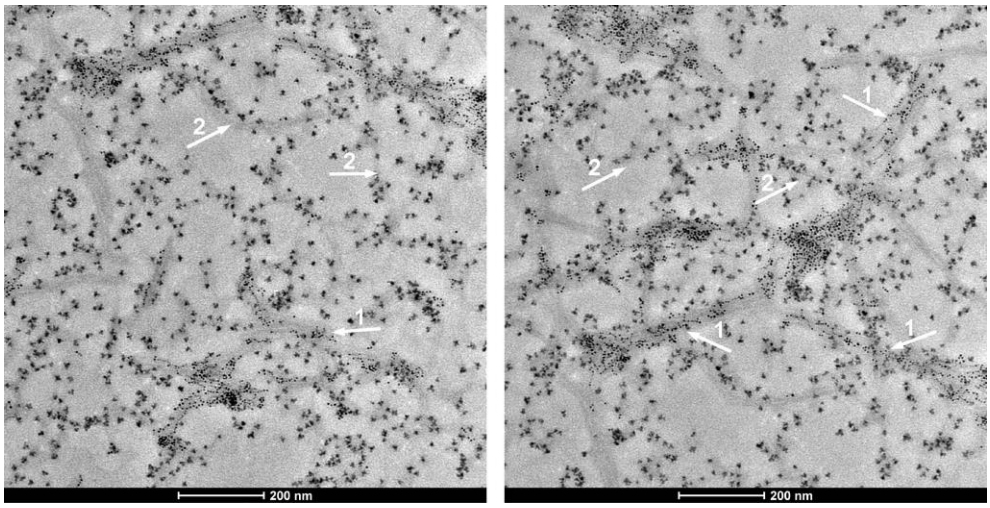


Figure S54. Self-sorted supramolecular fibers for templated assembly of Spytag-Cys_2-Au NPs and Co-NTA-CdSeS@ZnS QDs. Fibers were obtained by growth of EGFP-FUS LC-Spycatcher monomers (40 μM) and CsgA-His monomers (4 μM) together. The arrows 1 and 2 showed in the pictures represented Spytag-Cys_2-Au NPs and Co-NTA-CdSeS@ZnS QDs assembled on EGFP-FUS LC-Spycatcher and CsgA-His nanofibers, respectively.

Supplementary Table 1. Primers for constructing the genetic fusions in this study.

Primer sequences	**Template**	**Construct**
Forward: TATCCCAACGACCGAAAACCTGTATTTTCAGGGC GCCATGGGCATGGTGAGCAAAGGC	mMaple3	mMaple3 -FUS LC
Reverse: AGCTTTGGGTTGCTTGTTGGGTATAATCGTTTGAG GCGGATCCCGAGCCACCGCCACCCTTATAGAGTT CGTCCATGC		
Forward: TATCCCAACGACCGAAAACCTGTATTTTCAGGGC GCCATGAGCGAGCTGATTAAGGA	PAtagRFP	PAtagRFP -FUS LC
Reverse: CTTTGGGTTGCTTGTTGGGTATAATCGTTTGAGGC CGGATCCATGGTATTAAGCTTGTGCCCCAGTT		
Forwards: GGCAGCGGTGGCTATGGACAGCAGGACCGTGGA GGTGGCGGTGGCAGTGGCGGTGGCGGTTCGATG GTTGATACCTTATCAGG	Spycatcher	EGFP-FUS LC -Spycatcher
Reverse: GTTAGCAGCCGGATCTCAGTGGTGGTGGTGGTGG TGCTCGAGTTAGTCAATATGAGCGTCACC		

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