

Supplementary Materials for

Low-complexity domain of U1-70K modulates phase separation and aggregation through distinctive basic-acidic motifs

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Predictions of disordered domains

Disorder of human full-length U1-70K (437 amino acids) was predicted using the following services (results shown in Fig. S1):

1. SEG (<http://mendel.imp.ac.at/METHODS/seg.server.html>)
2. PONDR (<http://www.pondr.com/>)
3. DISOPRED (<http://bioinf.cs.ucl.ac.uk/disopred/>)

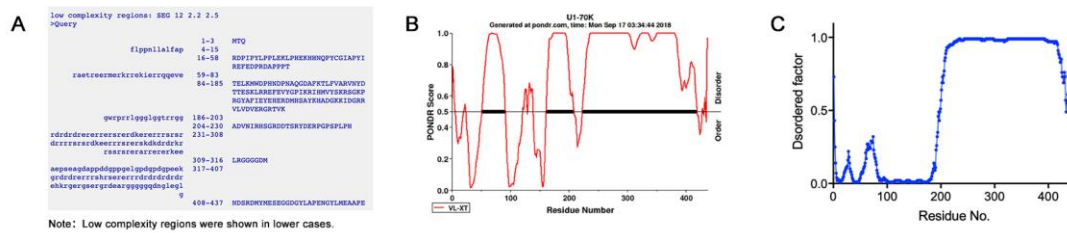


Fig. S1. Disordered motif of U1-70K predicted by different models. (A) Disordered motif predicted by SEG. (B) Disordered motif predicted by PONDR with VL-XT predictor. (C) Disordered motif predicted by DISOPRED3 method.

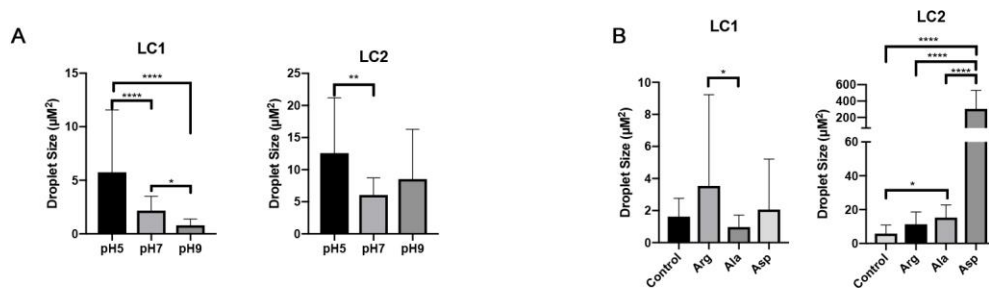


Fig. S2. Statistics of average sizes of droplets of LC1 and LC2. (A) under various of pH (B) with different amino acids added. Ordinary one-way ANOVA test, $n > 15$, depending on the densities of droplets, * $p < 0.1234$, ** $p < 0.0332$, **** $p < 0.0001$.

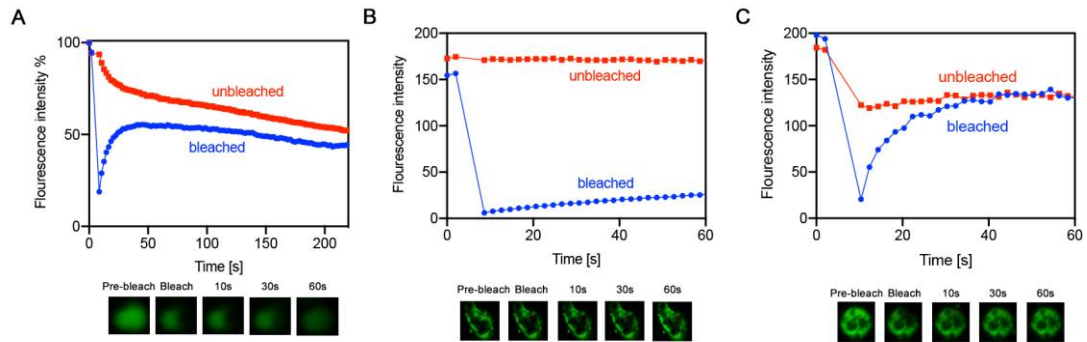


Fig. S3. FRAP of the irregular droplets. (A) 200 μM LC2 under pH 9. (B) 60 μM of LC1 was mixed with 300 μM Asp. (C) 60 μM of LC1 was mixed with 300 μM Arg.

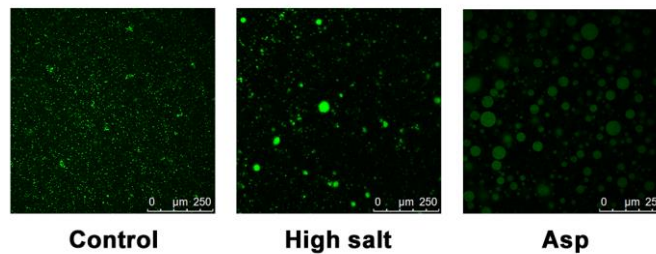


Fig. S4. Images showing effects of high salt (500 mM NaCl) to LC2 LLPS. The result was compared with LC2 with Asp added.

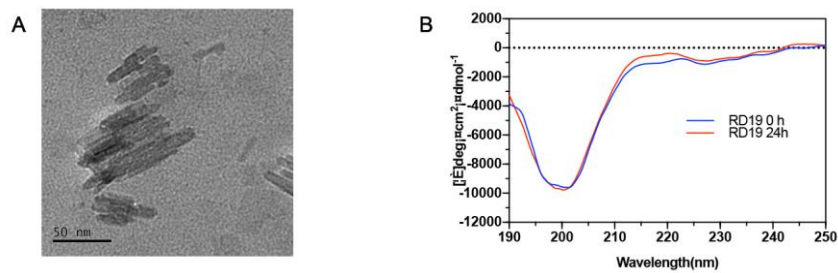


Fig. S5. Characterization of aggregation and secondary structure of RD19. (A) TEM of 30 μM RD19 incubated at 37 °C for 3 days. (B) CD spectrum of RD19 incubated for 0 and 24 h.

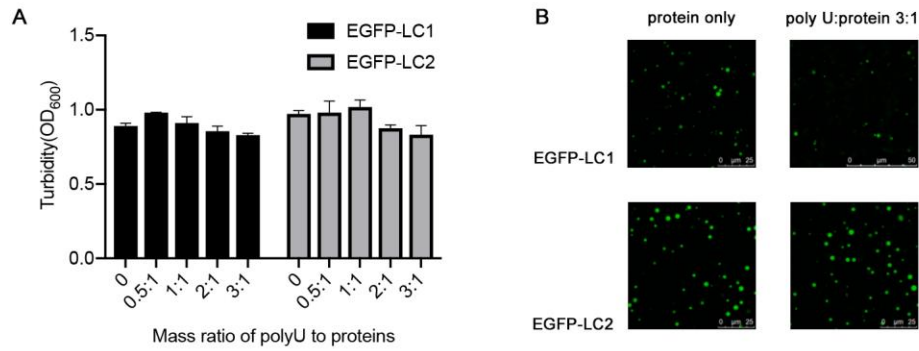


Fig. S6. The effects of RNA to the LLPS of LC1 and LC2. (A) The turbidity changes when adding different ratio of polyU to EGFP-LC1 or -LC2. (B) Images of droplets formation with or without 3 equivalents of polyU. (n=3)

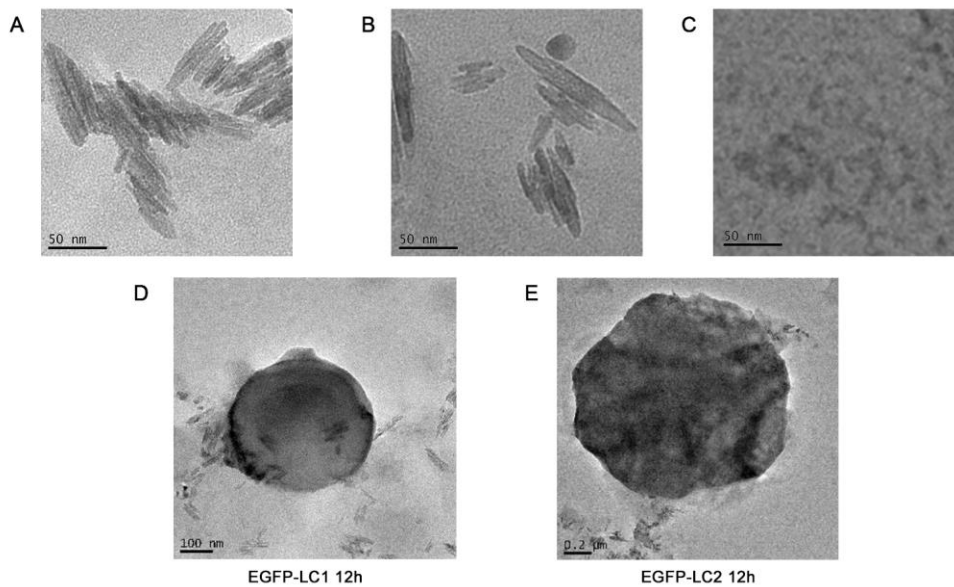


Fig. S7. TEM images of aggregates formed by LC1 and LC2 in solution and in droplets. (A) 10 μ M LC1, (B) 60 μ M LC2, (C) 60 μ M EGFP was incubated at 37 $^{\circ}$ C for 3 days. The phase separation samples of (D) EGFP-LC1 and (E) EGFP-LC2 were incubated at 37 $^{\circ}$ C for 12 h, showing aggregates growing inside of droplets.

Table S1. Densities of droplets of LC1 and LC2 under various pH values or with different amino acids added.

| Droplet count per mm ² | LC1 | LC2 |
|-----------------------------------|------|------|
| pH 5 | 2625 | 3000 |
| pH 7 | 4750 | 3750 |
| pH 9 | 9875 | 1875 |
| Control | 6125 | 2625 |
| Arg | 1500 | 2875 |
| Ala | 4500 | 1875 |
| Asp | 5750 | 500 |

Table S2. Primers used for gene construction.

| Primer name | Primer sequence |
|-------------------|---|
| U1-BamH231f | 5' CGGGATCCAGGGACCGGGACCGGGACCGTGAGCGGGAG 3' |
| U1-Xho308rstop | 5' CCGCTCGAGTCACTCCTCCTTGCCTCCCGCTCCCGCCGGG 3' |
| U1-BamH317f | 5' CGGGATCCGCGGAGCCCTCCGAGGCGGGTGACGCGCCC 3' |
| U1-Xho407rstop | 5' CCGCTCGAGTCAGCCCAGACCCTCCAGCCCGTTGTCCTGGCC 3' |
| U1-Nhe231f | 5'CTAGCTAGCATGAGGGACCGGGACCGGGACCGTGAGCGGGAGC GCAGA 3' |
| U1-Sac308rmostop | 5' TCCCCGCGGCTCCTCCTTGCCTCCCGCTCCCGCCGG 3' |
| U1-Nhe317f | 5' CTAGCTAGCATGGCGGAGCCCTCCGAGGCGGGTGACGCG 3' |
| U1-Sac407rmostop | 5' TCCCCGCGGGCCCAGACCCTCCAGCCCGTTGTCCTGGCC 3' |
| U1-Nhe1f | CTAGCTAGCATGACCCAGTTCCTGCCGCCAACCTTCTG |
| U1-Sac1314rmostop | GGACCGCGGCTCCGGCGCAGCCTCCATCAAATACCC |

Table S3. Parameters of LC1, LC2, and LC predicted by CIDAR.

| ID | Length | κ | FCR | NCPR | Hydropathy | Disorder promoting |
|-----|--------|----------|-------|--------|------------|--------------------|
| LC1 | 78 | 0.086 | 0.859 | 0.269 | 0.896 | 1.000 |
| LC2 | 92 | 0.095 | 0.543 | -0.043 | 2.127 | 0.946 |
| LC | 178 | 0.095 | 0.669 | 0.096 | 1.672 | 0.961 |

Note: κ - charge patterning parameter, discussed in the help section; FCR- fraction of charged residues; NCPR- net charge per residue