Engineering Protein Polymers of Ultrahigh Molecular Weight via Supramolecular Polymerization: Towards Mimicking the Giant Muscle Protein Titin

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Electronic Supplementary Information

Table S1. Protein amino acid sequences. Residues colored in red correspond to cysteine mutations in GL5CC as well as W34F mutation in I27. Sequences highlighted in grey are generated from the ligation of BamHI and BglII sticky ends.

Name	Protein sequence
I27-G_N	*LIEVEKPLYGVEVFVGETAHFEIELSEPDVHGQFKLKGQPLAASPDCEIIE DGKKHILILHNCQLGMTGEVSFQAANTKSAANLKVKEL* RS MDTYKLIL *I27* NGKTLKGETTTEAVDAATAEKVFKQYANDNGVGCGLG G_N (GL5CC 1-42)
G_C-I27	CGDGEWTYDDATKTFTVTE RS *LIEVEKPLYGVEVFVGETAHFEIELSE* G_C (GL5CC 43-61) *PDVHGQFKLKGQPLAASPDCEIIEDGKKHILILHNCQLGMTGEVSFQAA NTKSAANLKVKEL* *I27*
G_C-I27-G_N	CGDGEWTYDDATKTFTVTE RS *LIEVEKPLYGVEVFVGETAHFEIELSE PDVHGQFKLKGQPLAASPDCEIIEDGKKHILILHNCQLGMTGEVSFQAA NTKSAANLKVKEL* RS MDTYKLILNGKTLKGETTTEAVDAATAEKVF KQYANDNGVGCGLG

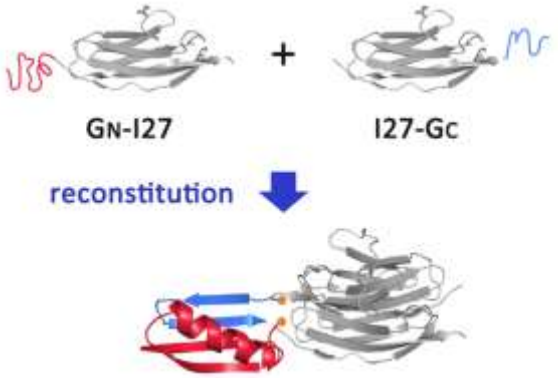

Figure S1. Placing GN at the N-terminus and GC at the C-terminus of two interacting protein partners can lead to steric hindrance when GN and GC undergo protein fragment reconstitution.

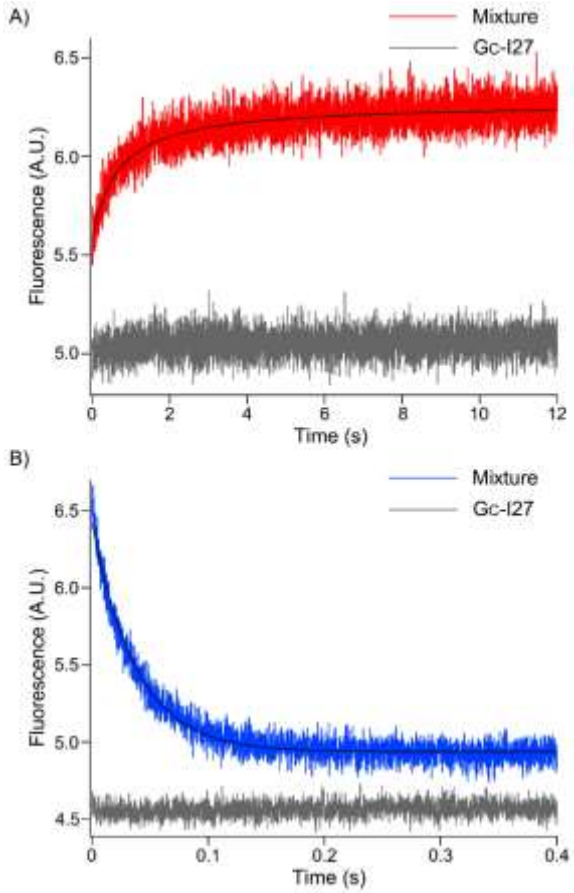

Figure S2. Stopped-Flow fluorimetry of protein fragment association and reconstitution complex denaturation. A) Association kinetics of mixing equal I27-G_N and G_C-I27 solution and adjusting the final concentration to 2×10^{-5} M was shown in red. The rate constant was $1.29\times10^{3} \pm 311$ M^{-1} s^{-1} fitting to the second-order rate law. B) The blue curve was unfolding kinetics curve of I27-(G_N-G_C)-I27 complex at 2×10^{-5} M treated with 4 M GdmCl and the single-exponential fitting gave a rate constant of 26.4 ± 0.125 s^{-1}. In both experiments, G_C-I27 curves colored gray had no obvious change.

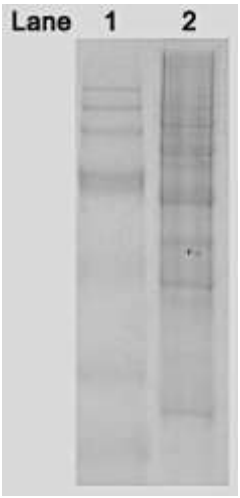

Figure S3. Native-PAGE of G_C-I27-G_N polymers reduced by DTT at 4 °C. Lane 1 was standard protein marker whose bands altered position in Native-PAGE; lane 2 showed the native state G_C-I27-G_N can still polymerize without disulfide bonds.

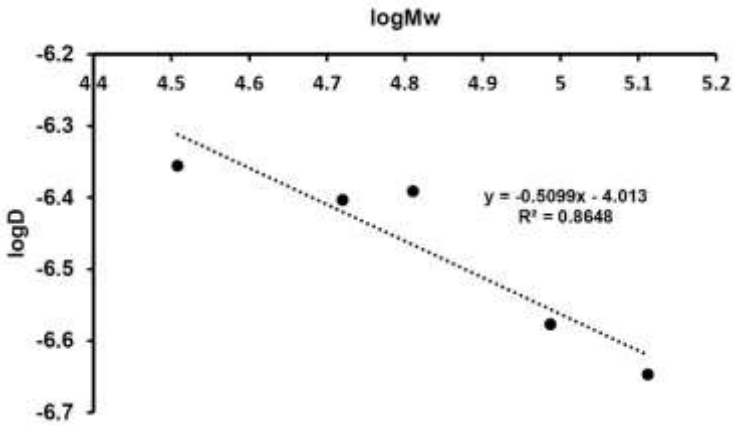

Figure S4. Plot of diffusion coefficient versus polyprotein molecular weight. In DLS experiments, we used Mark-Houwink-Sakurada (MHS) equation to measure the molecular weight:

$D = K \cdot Mw^{'}$ where K and ! are constants and depend on the macromolecule, solvent and temperature, D is the diffusion coefficient. Because the G_C-I27-G_N-based protein polymers are not globular proteins, we used purified polyproteins $(GR)_4$, $(NuG2)_8$, $(GR)_8$, $(GR)_{12}$ and $(GR)_{16}$, which showed a single band on SDS-PAGE (corresponding to a polydispersity index of 1) and have known molecular weights, to calculate K and α for such protein polymers. DLS experiments were carried out at 25 °C and all proteins were dissolved in water. Fitting the MHS equation to the experimental data yielded a $K = 9.71 \times 10^{-5}$, ! = -0.51. Using this method, we determined the diffusion-averaged molecular weight of the G_C-I27-G_N-based protein polymers Mw=458±16 kDa. All measurements were done in triplicate.

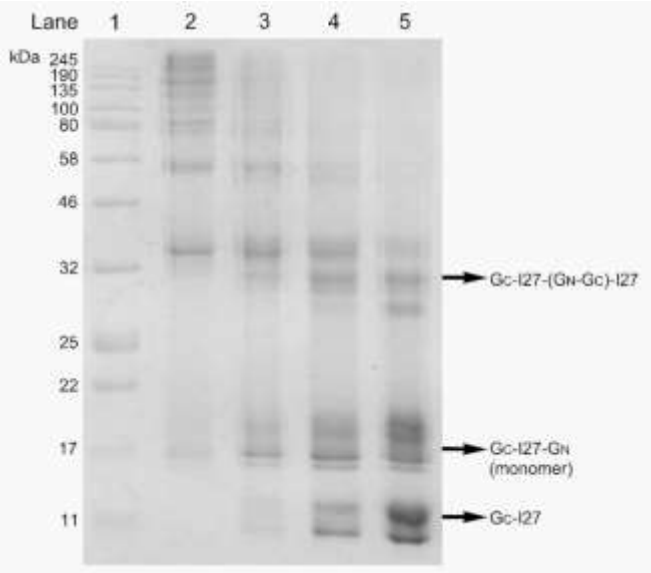

Figure S5. Competitive effect between G_C-I27-G_N and G_C-I27. The amount of higher order of G_C-I27-G_N polymers decreased with the increasing of G_C-I27 concentrations due to the competition of the monofunctional monomer. Lane 1: protein molecular weight marker; lane 2: oxidized G_C-I27-G_N protein polymers; lane 3-5: oxidized G_C-I27-G_N and G_C-I27 mixture samples, and the ratio of G_C-I27-G_N:G_C-I27 is 5:1, 2:1, 1:1, respectively.

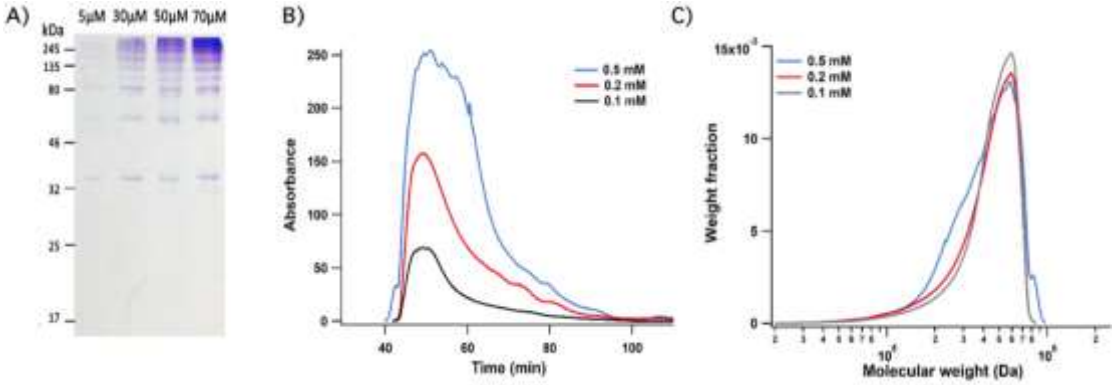

Figure S6. The effect of monomer concentration on the molecular weight of the engineered protein polymers. A) Non-reducing 12% SDS-PAGE analysis of the engineered protein polymers. Monomer concentration is indicated in each lane. B-C) SE-FPLC elution time plot (B) and molecular weight distribution (C) of polymerized G_C-I27-G_N-based protein polymers.

Table S2. The effect of monomer concentration on the molecular weight and molecular weight distribution of G_C-I27-G_N-based protein polymers

Protein conc. (mM)	Mn (kDa)	Mw (kDa)	PDI
0.5	441	513	1.16
0.2	447	505	1.13
0.1	464	496	1.07

Figure S7. A photograph of a native PAGE of G_C-I27-G_N-based protein polymers and depolymerized product after being incubated under reducing condition at 37 °C for 30 minutes.

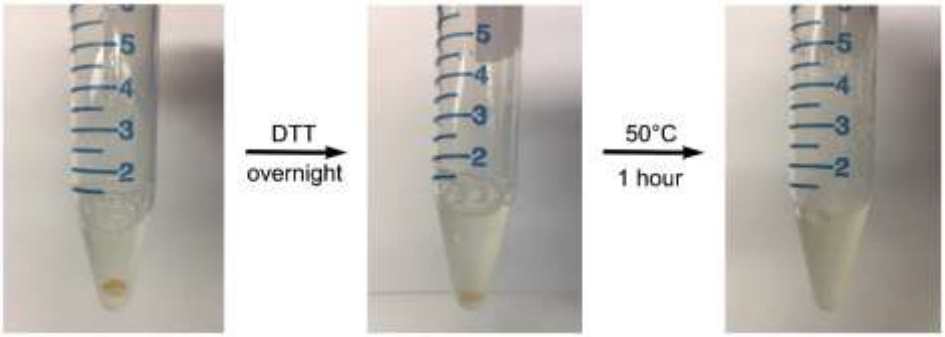

Figure S8. Protein hydrogels made of G_C-I27-G_N (2%) can undergo gel-sol transition under a reducing condition at elevated temperatures. Photographs show the protein hydrogels under different conditions. The yellowish solid are the hydrogel samples.