A

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MSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTT 50
GKLPVPWPTLVTTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISF 100
KDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGNILGHKLEYNFNSHNV 150
YITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHY 200
LSTQTVLSKDPNEKRDHMVLLEFVTAAGITHGMDELYK
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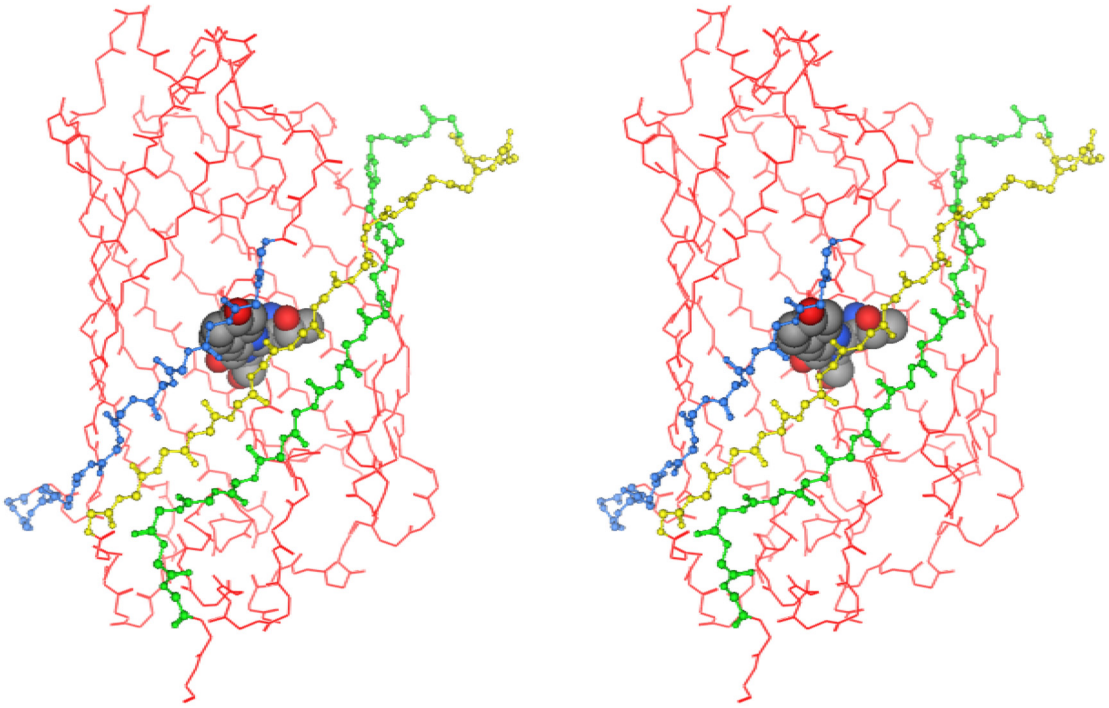
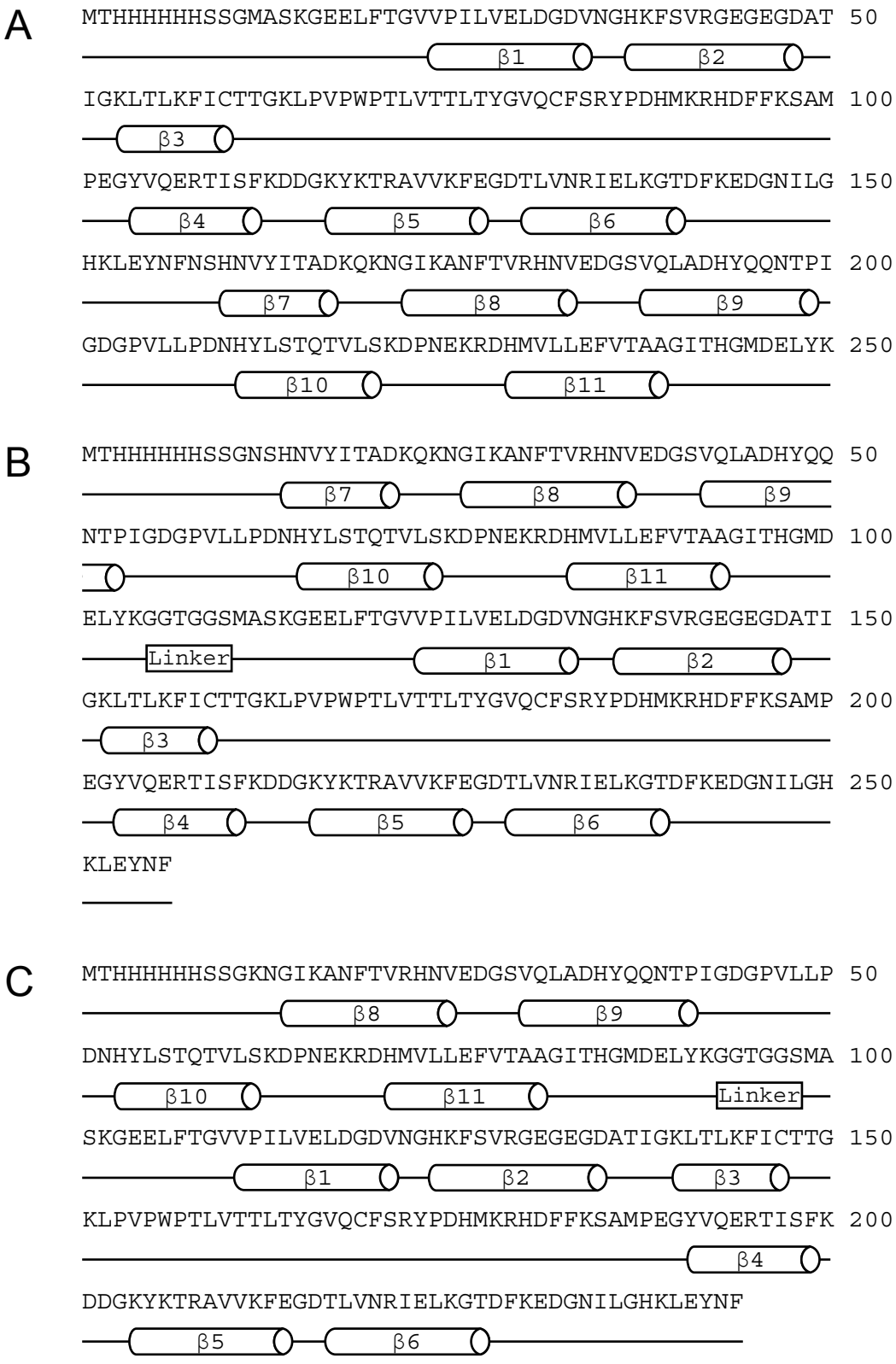
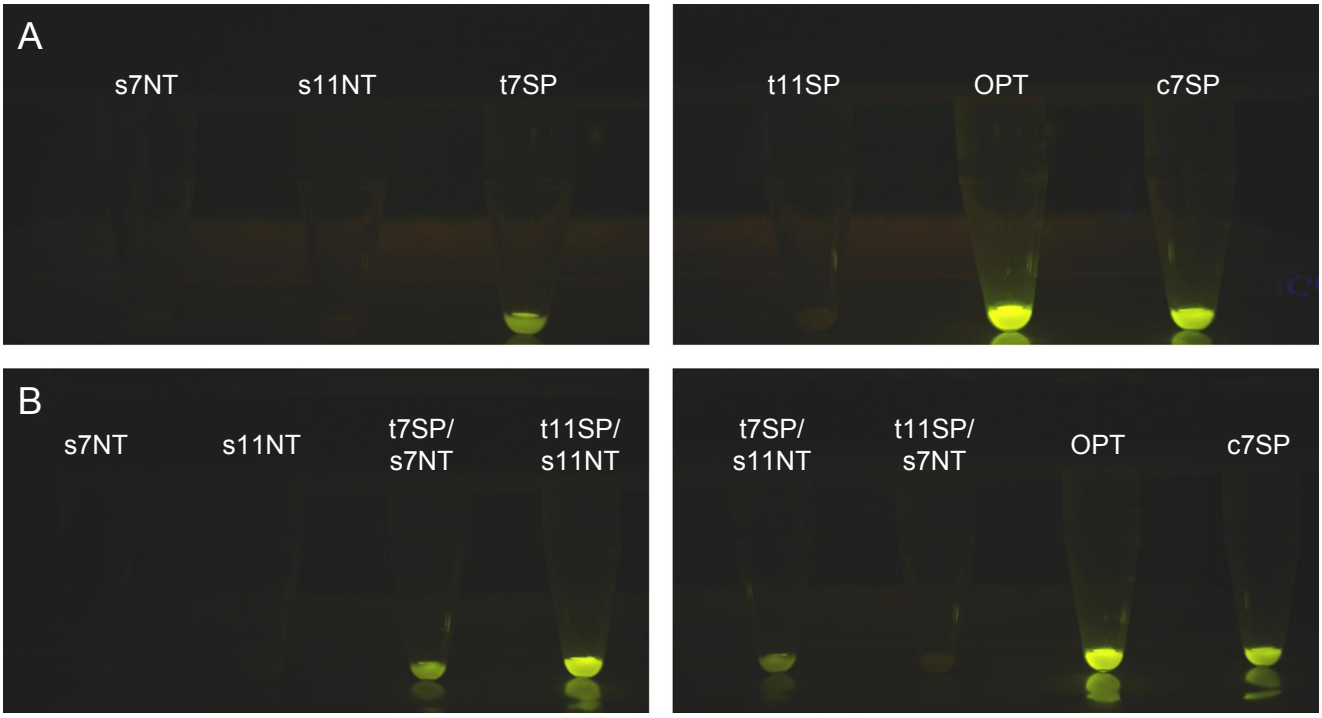
B



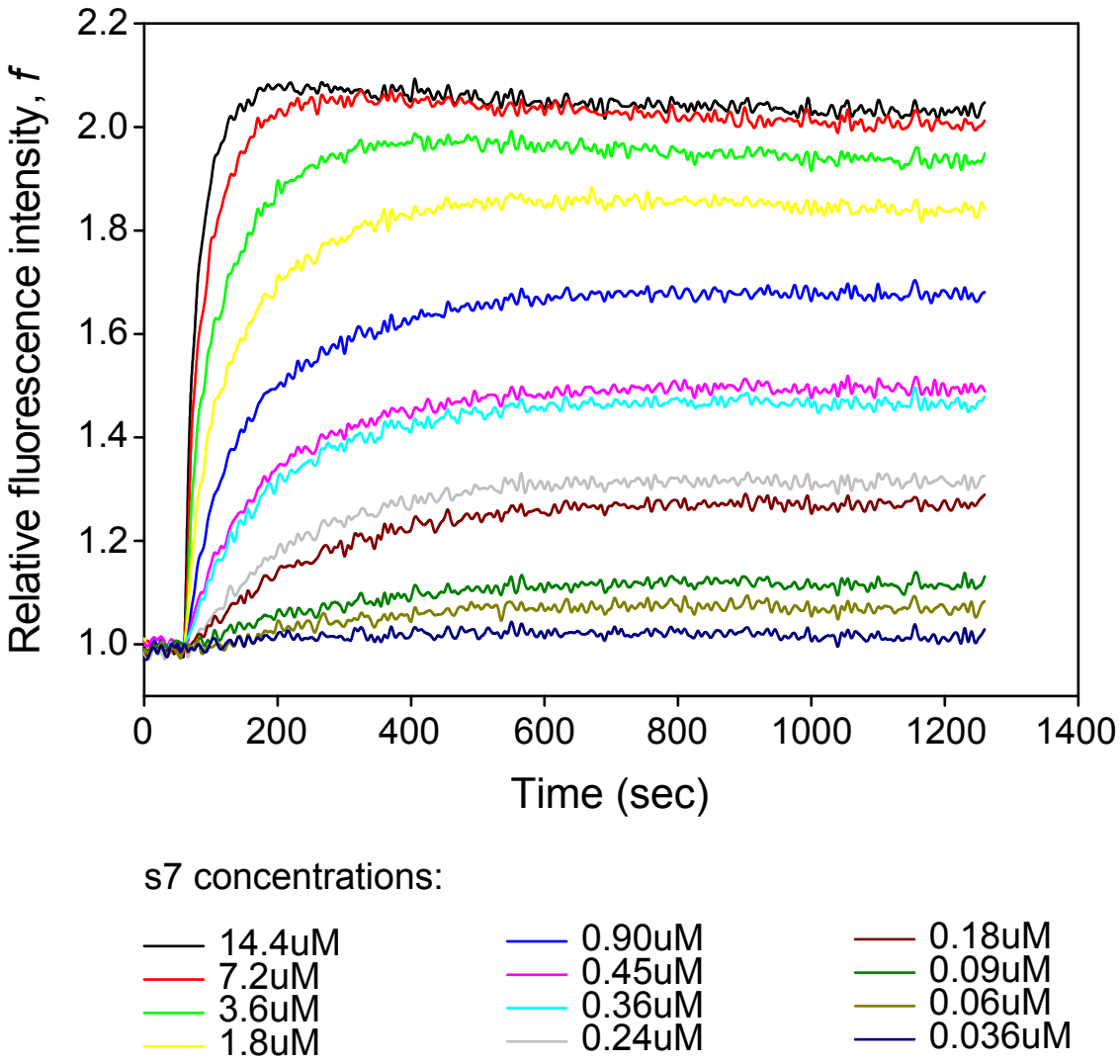
Supplementary figure 1. Exploring "leave-one-out" design of Superfolder GFP OPT.
(A) Primary sequence of Superfolder GFP OPT originated from *in vitro* evolution (Cabantous, S. *et al.* 2005). Segments of β strand 7 (blue), 10 (yellow) and 11 (green) were omitted individually to generate t7SP, t10SP and t11SP leave-one-out constructs respectively. Residues both omitted from GFP internal segments in t10SP and t11SP were indicated in red. (B) Corresponding locations of removed segments in three-dimensional structure. Leave-out sequences of strand 7 (blue), 10 (yellow) and 11 (green) were mapped to the known structure (PDB:2B3P). Only backbone atoms were shown in the figure.



Supplementary figure 2. Arrangements of β strands in primary sequences of GFP variants. Protein sequences of eleven β strands (β1~β11) in OPT (A) were determined according to the pairwise sequence alignment of OPT and superfolder GFP with known structure (PDB:2B3P) and were indicated as cylinders. Corresponding locations of β strands and linker sequences in c7SP (B) and t7SP/t7SPm (C) were also shown.



Supplementary figure 3. Expression of recombinant proteins.
Identified constructs were transformed and expressed in BL21(DE3) as described. Fluorescence was observed under blue light transilluminator with maximum light output between 400 and 500 nm. (A) Expression of single constructs (B) *In vivo* complementation – coexpression of t7SP/s7NT, t11SP/ s11NT, t7SP/s11NT, and t11SP/s7NT.



Supplementary figure 4. Concentration-dependent kinetics of *in vitro* complementation. Examples of s7 concentration-dependent kinetics of *in vitro* complementation with 0.1μM t7SPm under native conditions were shown here. The fluorescence emitted at 508nm was recorded every 5 seconds for 20 minutes under 485nm excitation at room temperature upon the complementation and the normalized fluorescence (F/F_0) was plotted as the relative fluorescence intensity *f*. After the complementation, the fluorescence recovery curves for 14.4, 7.2, 3.6, and 1.8μM s7 were best fitted to triple exponential equations with six parameters using SigmaPlot software. As shown in table 3, s7 concentration-dependent changes of amplitudes were observed, indicating the s7 ligand more tightly bound to the I_2 folding intermediate.