Supplemental Information

Structure of a Key Intermediate of the SMN Complex Reveals Gemin2's Crucial Function in snRNP Assembly

Rundong Zhang, Byung Ran So[1], Pilong Li[1], Jeongsik Yong, Tina Glisovic, Lili Wan and Gideon Dreyfuss*

Supplemental Figure Legends

Figure S1. Structure of the Sm proteins in the Gemin2-SMN$_{Ge2BD}$-Sm pentamer.

(A) Ribbon diagram of the Sm pentamer organization in our structure. The five Sm proteins are arranged in the clockwise order of SmD1, D2, F, E and G (colored in lime, lemon, pink, dark green and orange, respectively) to form 5/7 of doughnut shape with their canonical N-terminal helices facing out. The secondary structure of the Sm proteins, which includes N-terminal α-helix (α1) and five-stranded strongly bent anti-parallel β-sheet (β1- β5) with loops connecting each segments, is labeled on SmD1. The β5 of an Sm protein pairs with the β4 of its right-side neighbor, forming a continuous anti-parallel β-sheet. The amphipathic N-terminal helix of the left Sm protein lays on top of the β-sheet surface of its right-side neighbor, forming a hydrophobic interaction patch.

(B) Superimposition of the Cα backbones of SmD1/D2 in our structure with that in 2.5 Å resolution structure (Kambach et al., 1999). SmD1 and D2 in our structure are shown in lime and lemon, respectively. SmD1/D2 dimer structure (PDB code 1B34) is shown in grey. The Sm folds from the two structures are aligned,

showing rmsd of 0.39 Å and with most of the variations found in loops and termini. In our structure, more residues in SmD2's α1 and loop 4 are visible. Loop 2 and C-terminal tail of SmD2 as well as loop 4 of SmD1 adopt slightly different conformations, likely due to their contact with SmF/E/G as well as with Gemin2.

Figure S2. A narrower conformation of the Sm pentamer in our structure compared to that in the U1 snRNP structure.

(A) Superimposition of the Cα backbones of SmD1/D2 in our structure (blue) with the corresponding Cα backbones in the 4.4 Å resolution structure of U1 snRNP (gray) produces 0.78 Å rmsd. In this alignment, the rmsd values for SmF, E and G when comparing the two structures are 3.13, 5.90, and 8.07 Å, respectively. The colored sticks show the measurement of the distances between the corresponding Sm proteins in the overlays, which are used for the calculation of the rmsd values.

(B) Superimposition of the Cα backbones of SmF/E/G in our structure (blue) with the corresponding Cα backbones in the 4.4 Å resolution structure of U1 snRNP (gray) produces 1.16 Å rmsd. In this alignment, the rmsd values for SmD1 and D2 are 5.85 and 3.05 Å, respectively.

(C) Conserved residues, Asn37, Asn41 and Asn39 from SmD1, F and G, respectively, are marked with red balls in our structure and green balls in the U1 snRNP structure. In our structure, the distance between SmD1 Asn37 to SmG Asn39 is shorter (27.4 Å vs. 31.2 Å) and the angle formed at SmF Asn41 is smaller (64.9° vs. 76.2°). All measurements are based on the coordinates of the Cα backbones.

(D) Superimposition of the Cα backbones of SmD1/D2 in our structure (blue) with the corresponding Cα backbones in the 4.4 Å resolution structure of U1 snRNP (gray). The asterisk indicates the region of overlap between SmB/D3 as seen in the U1 snRNP structure (green) and SmG in our structure (blue). In panels A-D, residues 1-20 of SmD2 and 77-86 of SmF in the U1 snRNP structure are removed for clarity.

Figure S3. **Electron density maps of the interaction regions between Gemin2 and the Sm pentamer.**

SigmaA-weighted 2Fo-Fc electron density maps contoured at 1.5σ (panels A and B) and 1.2σ (panels C and D) are shown in gray mesh. The orientations and the color schemes of the structure view are the same as in Figure 4. Gemin2 and the Sm pentamer structures are shown in line representation. Water molecules are shown as red spheres. In panel D, the α1 of Gemin2 is removed for clarity and the map shows the N-terminal tail of Gemin2 contained within a thin slab of the pentamer.

Figure S4. Hydrophobic interactions between SMN and Gemin2.

Gemin2 is shown by surface representation with red surface indicating hydrophilic residues and light gray surface indicating hydrophobic residues. The main chain of SMN is shown by stick view in blue with the four spheres indicating major hydrophobic residues (Leu39, Tyr43, Val47 and Phe50) that interact with Gemin2's hydrophobic pocket.

Supplemental Experimental Procedures

GST-Gemin2 and mutant protein expression and purification

Cultures of 500 ml were grown at 37°C to an OD_{600} of 0.6 and then transferred to 16°C for 30 minutes before induction with 0.5-1 mM IPTG. The cells were cultured at 16°C for another 18 hours, harvested and snap frozen in liquid N_2. To purify GST-Gemin2, the frozen cell pellet was thawed in 5 ml lysis buffer (25 mM Tris-HCl, pH8.0, 250 mM NaCl, 0.1% NP-40 and protease inhibitors) and disrupted by sonication. Lysates were clarified by centrifugation, 0.2 μm filtration and adsorbed to a 2 ml glutathione-Sepharose resin bed. The resin was washed once with 15 ml lysis buffer, followed by five washes with 10 bed volume of wash buffer (25 mM Tris-HCL, pH8.0, 250 mM NaCl and protease inhibitors) and eluted in wash buffer containing 10 mM reduced glutathione. The eluted protein was dialysed against 1 liter of wash buffer and 20% (v/v) glycerol at 4°C overnight and flash frozen in liquid N_2.

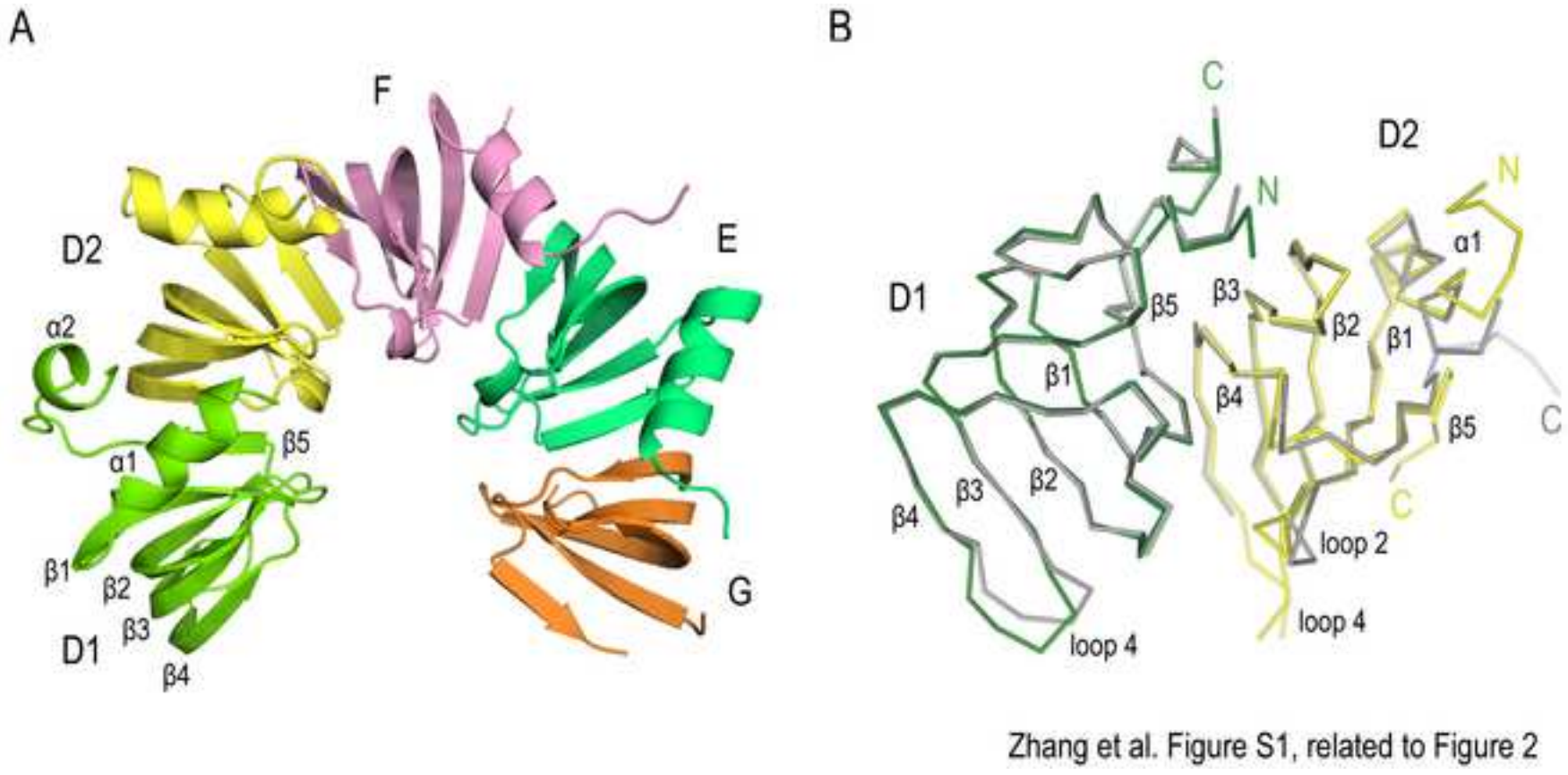
In vitro protein binding assays

1 μg GST-Gemin2 per binding reaction was immobilized on 25 μl glutathione-Sepharose beads in RSB-100 (20 mM Tris-HCl, pH8.0, 100 mM NaCl, 2.5 mM $MgCl_2$) containing 0.01% NP-40 on a rotator at 4°C. The beads were subsequently washed three times in 1 ml of RSB-100 containing 0.1% NP-40 and resuspended in 500 μl binding buffer (50 mM Tris, 200 mM NaCl, 0.2 mM EDTA, 0.05% NP-40, 2 mM DTT and protease inhibitors). 10 μl of the in vitro transcribed and translated [^{35}S]-Met labeled Myc-SMN or mutant proteins were added to the beads and the binding reaction was

incubated on a rotator at 4°C for one hour. The beads were then washed five times in 1 ml binding buffer. The bound proteins were eluted by boiling in 2x sample buffer, resolved by SDS-PAGE and detected by autoradiography.

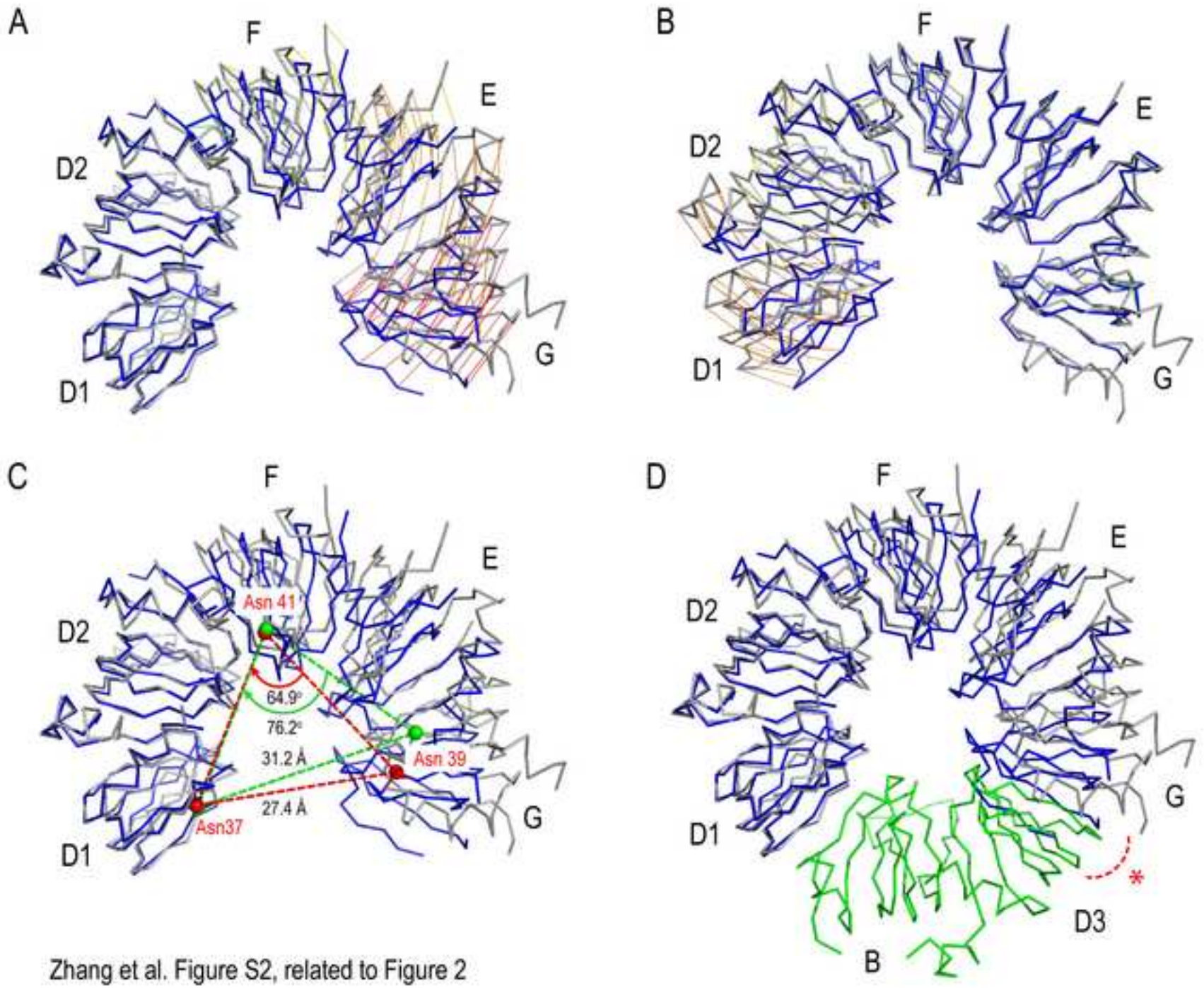
Binding experiments using recombinant Sm proteins, SMN and Gemin2 were performed in RSB-250 binding buffer (20 mM Tris-HCl, pH8.0, 250 mM NaCl, 2.5 mM $MgCl_2$) containing 0.02% Triton X-100. Approximately 3 μg of recombinant proteins were used for each binding assay. GST fusion proteins of either SMN or Gemin2 were first bound to glutathione beads, washed and then incubated with recombinant Sm proteins at 4°C for 2 hours and then washed 5 times with binding buffer. Bound proteins were then eluted by boiling in 2x sample buffer, resolved on 12% SDS-PAGE and visualized by SimplyBlue staining (Invitrogen).

Supplemental Figure



Zhang et al. Figure S1, related to Figure 2

Supplemental Figure



Zhang et al. Figure S2, related to Figure 2

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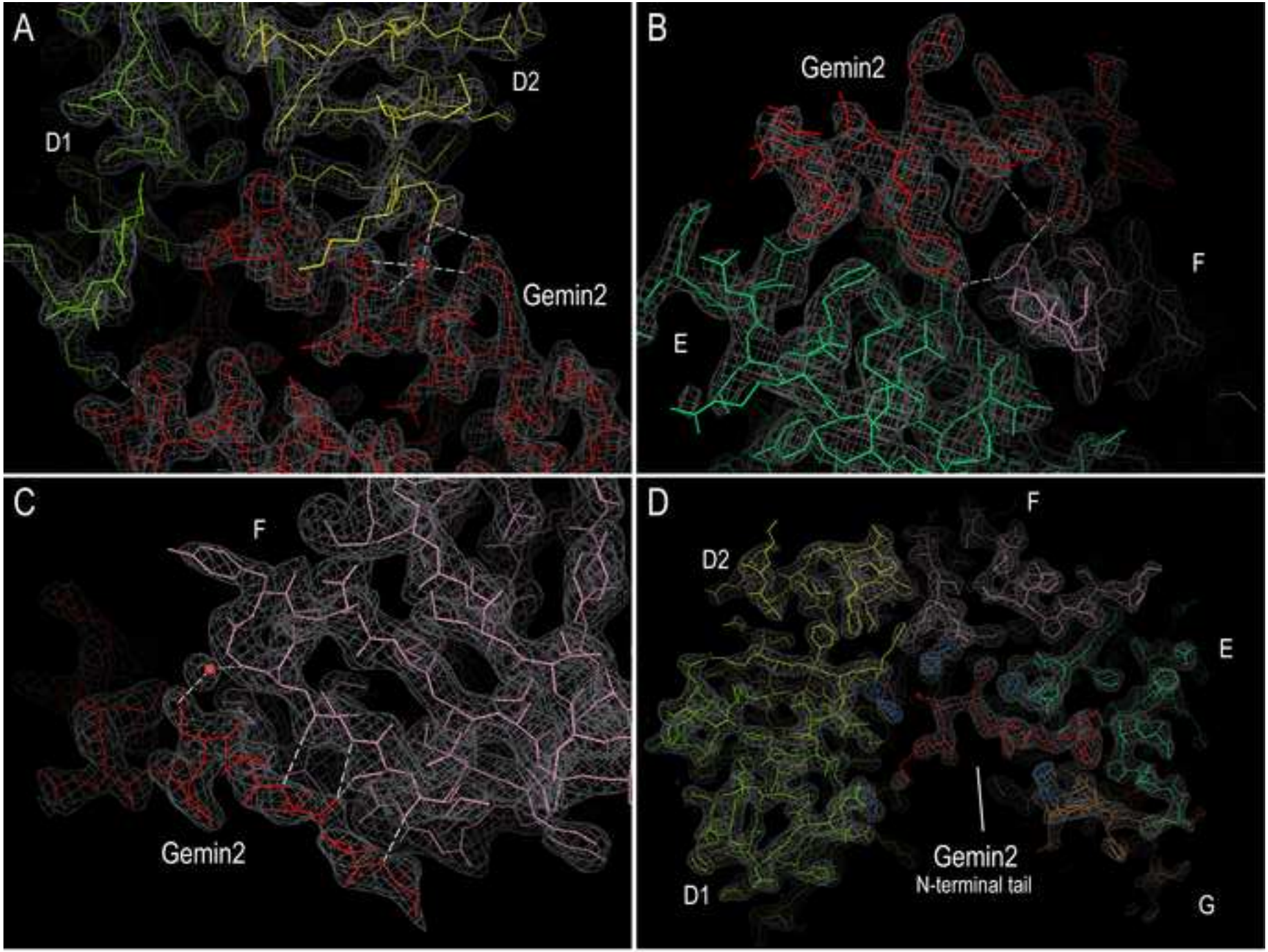
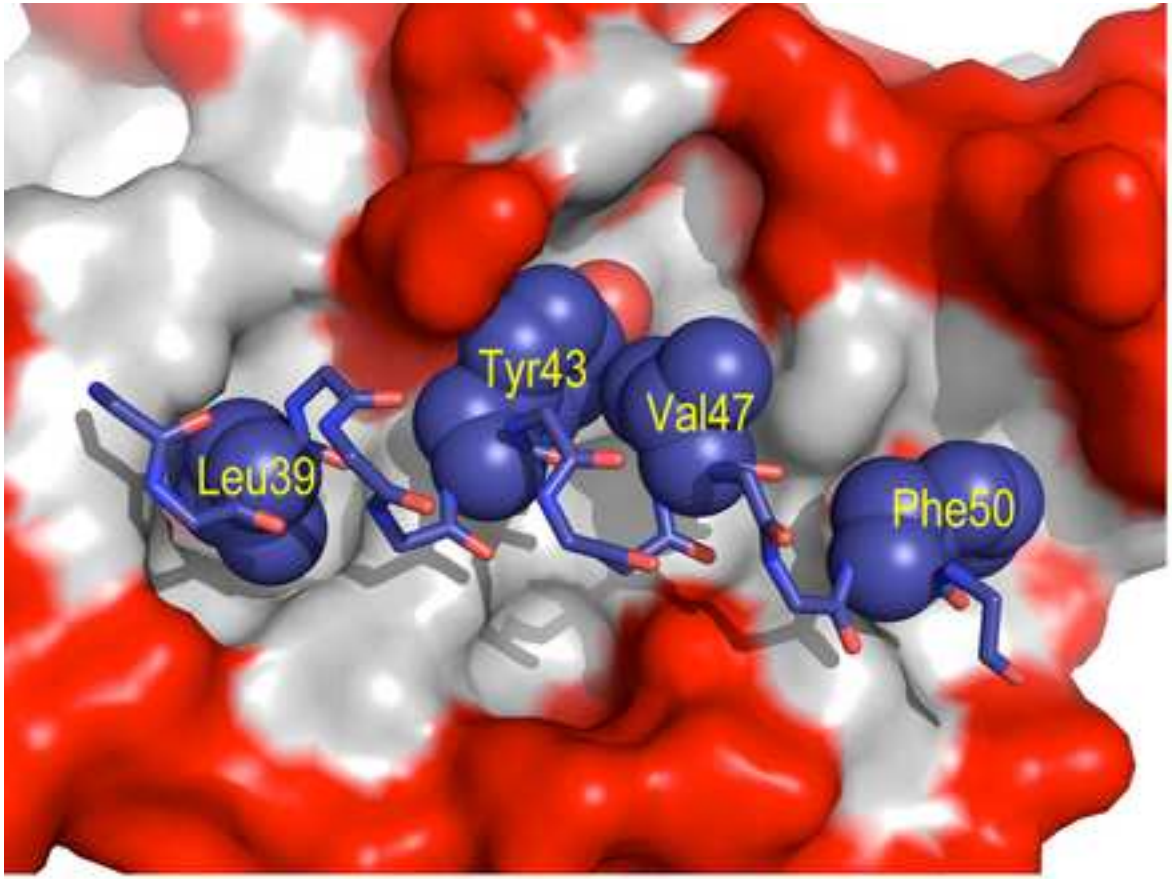


Zhang et al. Figure S3, related to Figure 4

Supplemental Figure



Zhang et al. Figure S4, related to Figure 6