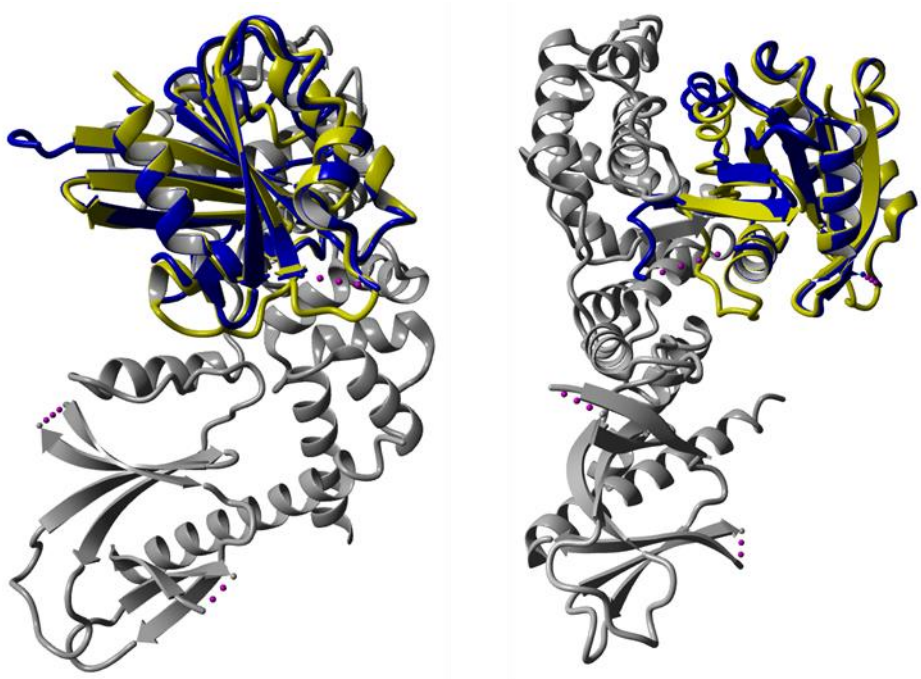

Figure S1. Alignment of ARF6 (yellow) ARF1 (blue) in the Sec7 (grey) -ARF1 complex (6FAE.pdb) in two projections, RMSD = 0.96 Å. Red dots marked the unresolved by X-ray loops.

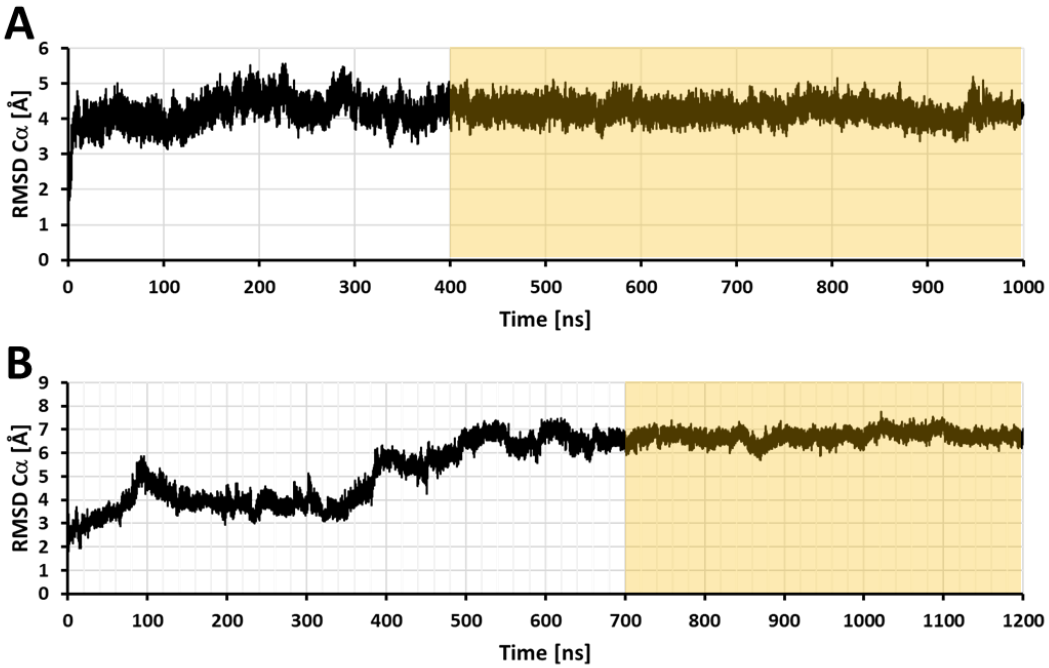

Figure S2. RMSD Cα atoms convergence control of AMBER20 cMD simulation production trajectories (SPT). **A.** The graph of RMSD Cα corresponds to the apoCM-$IQ_{wildtype}$ complex containing 1000000 frames. RMSD was measured to the reference structure - the first frame of SPT. The last yellow highlighted SPT fragment (600000 frames) of the RMSD graph corresponds to the equilibrated structure of the apoCM-$IQ_{wildtype}$ complex. The average RMSD value on this fragment of SPT is 4.22 Å with STDEV = 0.22 Å. This SPT fragment was used for the following conformational cluster analysis of the apoCM-$IQ_{wildtype}$ complex. **B.** The graph of RMSD Cα corresponds to the CM-4Ca2+-$IQ_{wildtype}$ complex containing 1200000 frames. RMSD was measured to the reference structure - the first frame of SPT. The last yellow highlighted SPT fragment (500000 frames) of the RMSD graph corresponds to the equilibrated structure of the CM-4Ca2+-$IQ_{wildtype}$ complex. The average RMSD value on this fragment of SPT is 6.73 Å with STDEV = 0.22 Å. This SPT fragment was used for the following conformational cluster analysis of the CM-4Ca2+-$IQ_{wildtype}$ complex..

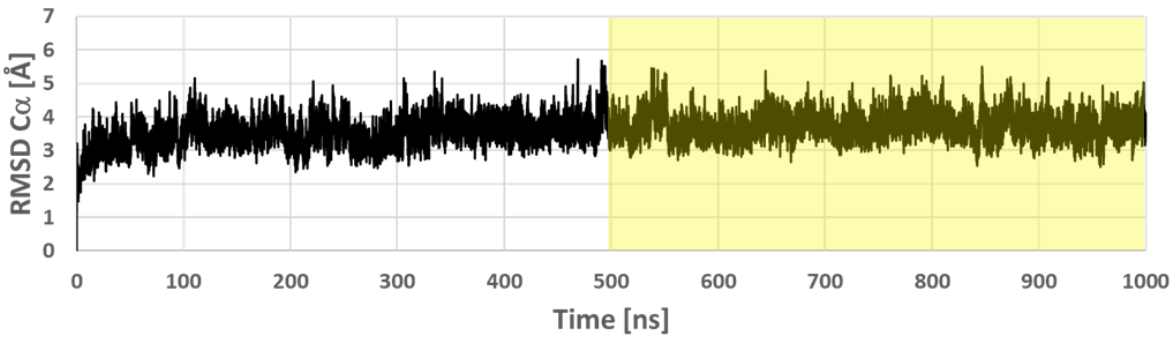

Figure S3. The graph of RMSD Cα atoms convergence control of AMBER20 aMD simulation production trajectory (SPT) of Sec7 domain of IQSEC2 containing 1000000 frames. RMSD was measured to the reference structure - the first frame of SPT. The last yellow highlighted SPT fragment (500000 frames) of the RMSD graph corresponds to the equilibrated structure of SEC7. The average RMSD value on this fragment of SPT is 3.74 Å with STDEV = 0.38 Å . This SPT fragment was used for the following conformational cluster analysis of the Sec7.

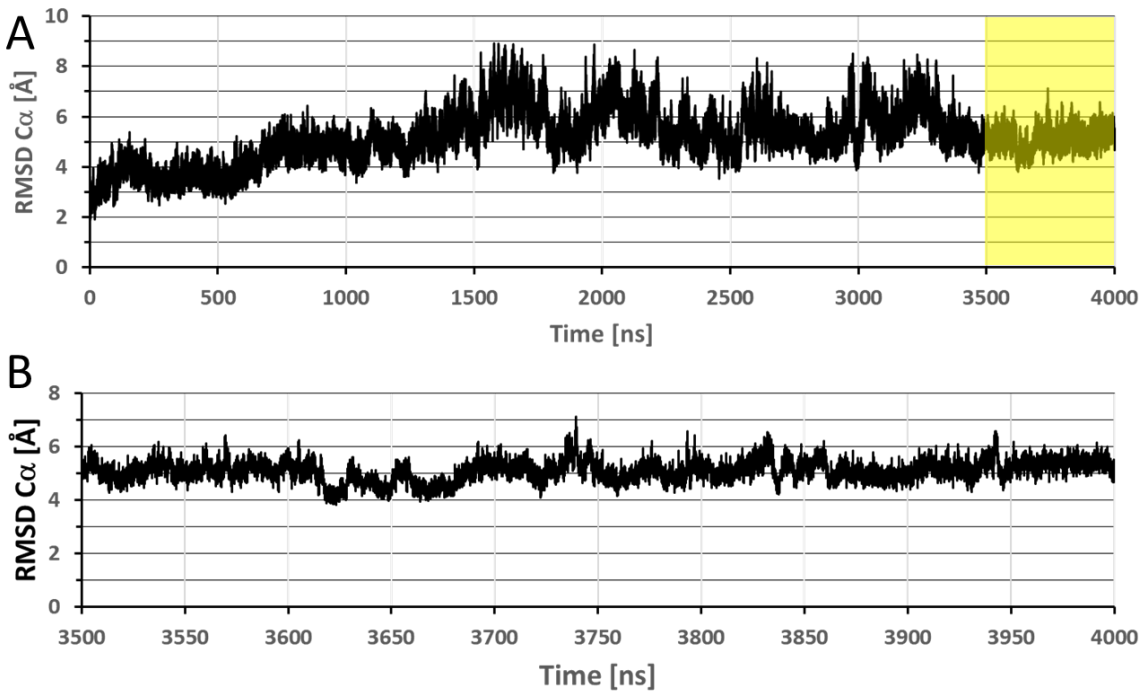

Figure S4. RMSD Cα atoms convergence control of AMBER20 aMD simulation production trajectory (SPT). **A.** The graph of RMSD Cα atoms corresponds to the Sec7-ARF6 complex containing 4000000 frames. RMSD was measured to the reference structure - the first frame of SPT. The last yellow highlighted SPT fragment (500000 frames) of the RMSD graph corresponds to the equilibrated structure of the SEC7-ARF6 complex. **B.** For better visualization the separated end yellow highlighted fragment (500000 frames) of the total RMSD graph is presented. The average RMSD value on this fragment of SPT is 5.10 Å with STDEV = 0.39 Å. This SPT fragment was used for the following conformational cluster analysis of the Sec7-ARF6 complex.

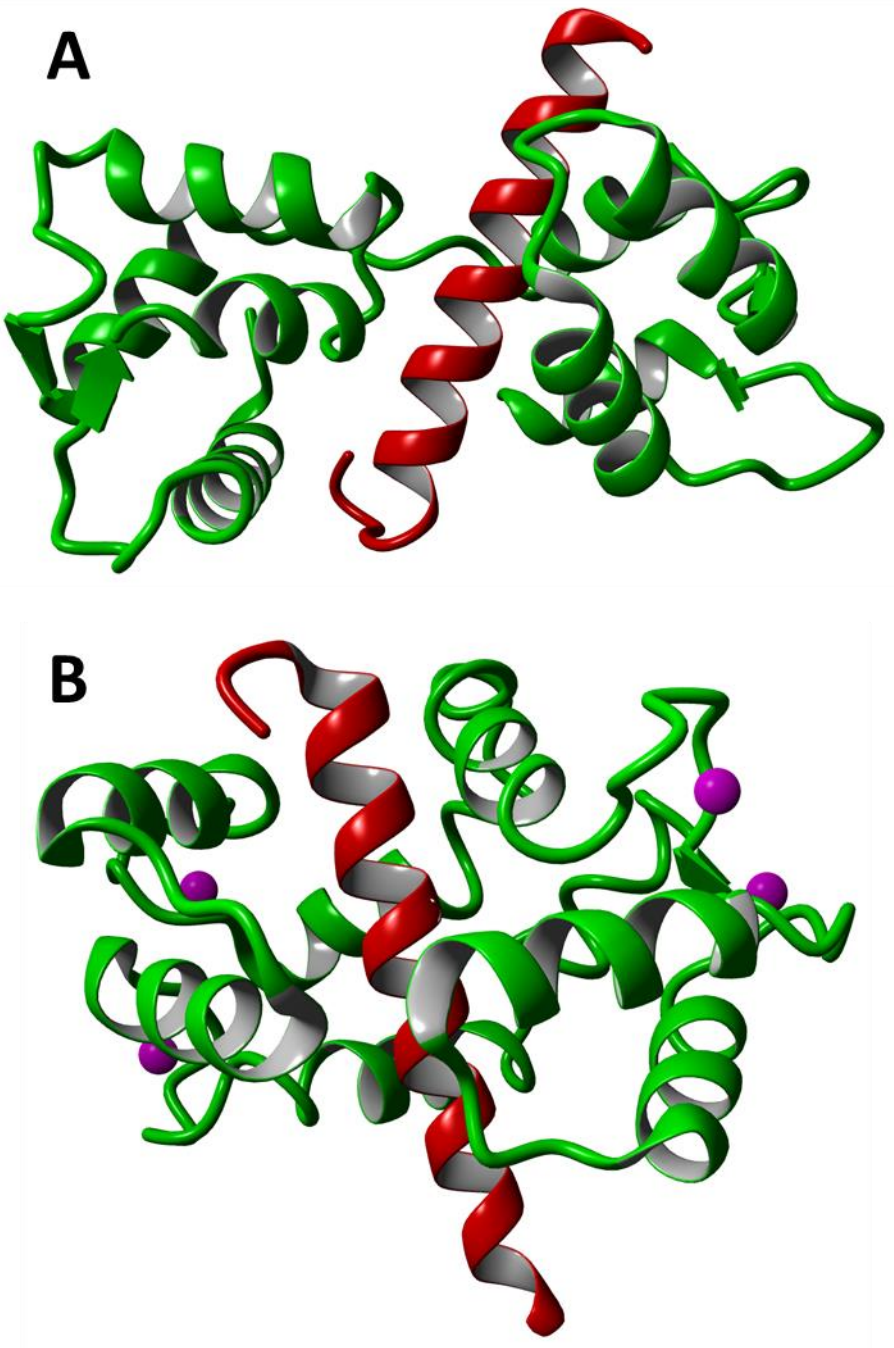

Figure S5. A. apoCM-$IQ_{wildtype}$ (residues 343-370) complex. **B.** CM-4Ca2+-$IQ_{wildtype}$ complex. CM - green, $IQ_{wildtype}$ -red, Ca2+ cations presented in a ball style are magenta colored.

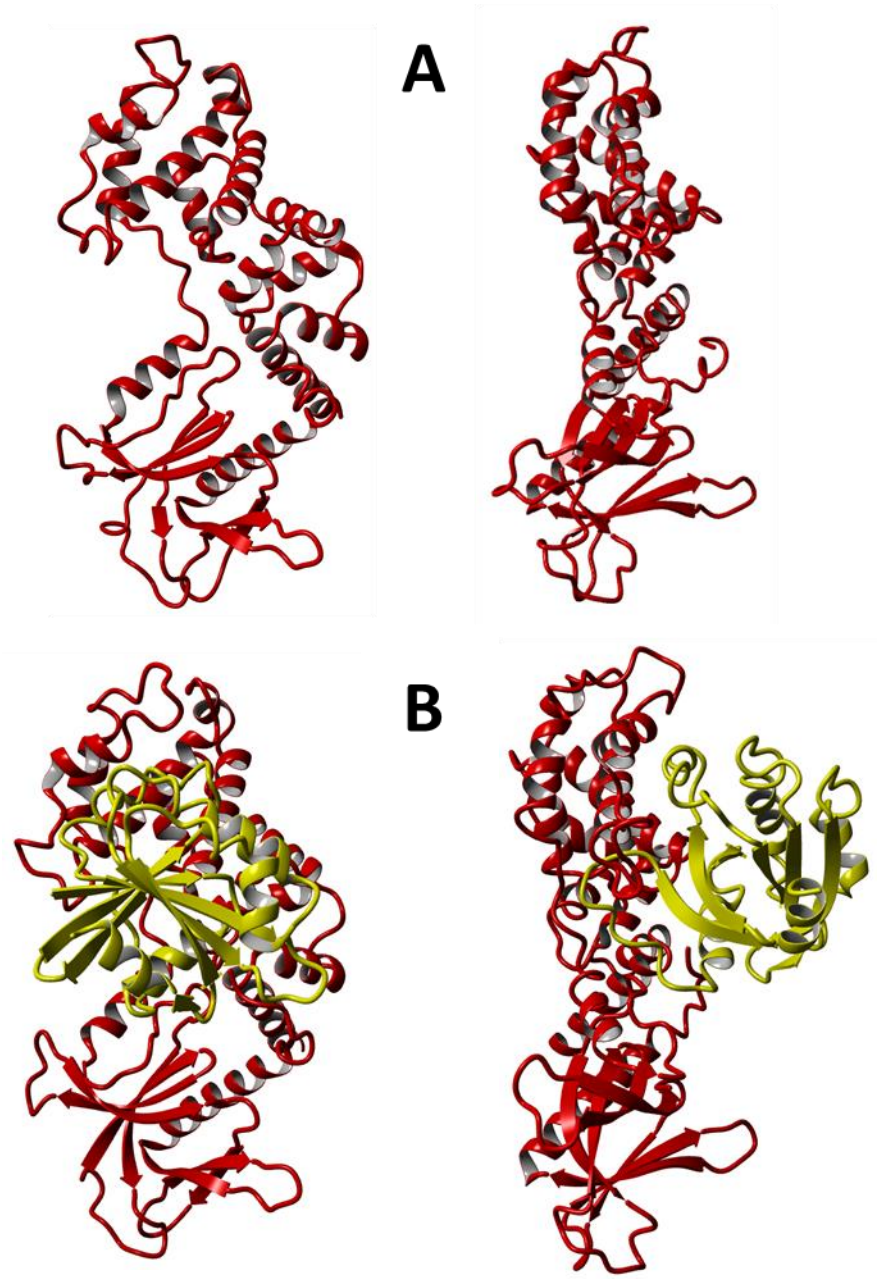

Figure S6. Two projections on the conformational cluster centroids of Sec7 and Sec7-ARF6 complex selected on the equilibrated fragments of the corresponding production trajectories of molecular dynamics simulations. **A.** Sec7. **B.** Sec7-ARF6 complex. Sec7-red and ARF6 – yellow.

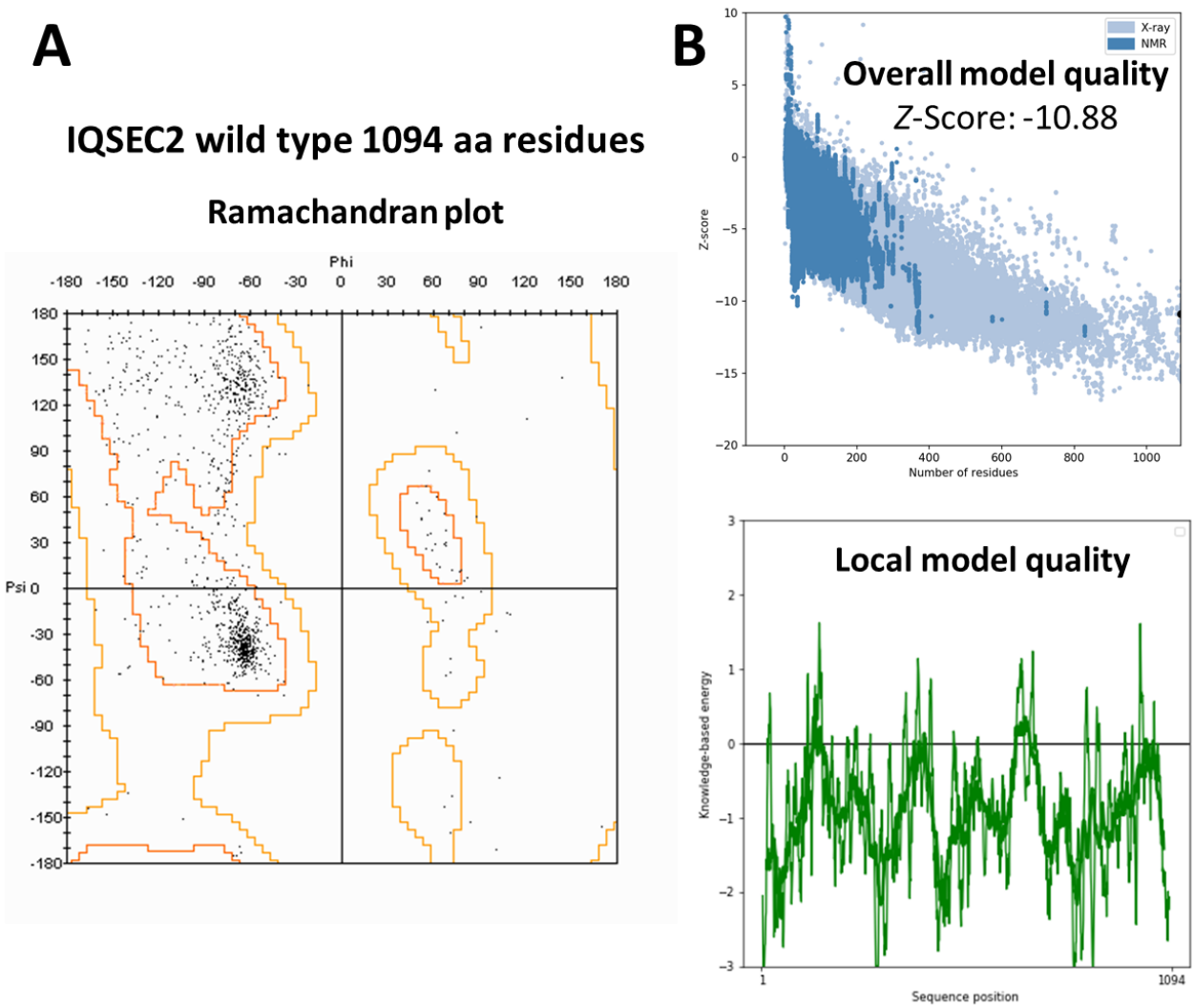

Figure S7. Structure validation of the IQSEC2 wild type (1094 aa residues) built by YASARA Structure molecular modeling as two joined fragments predicted by RaptorX server (1-760 residues) and equilibrated by aMD simulation of SEC7 domain (749-1094 residues). Geometries of the generated IQSEC2 wild type (1-1094 residues) was fully optimized in frames of ff14SB force field by YASARA Structure software in periodic simulation cell filled with explicit water molecules and Na+ and Cl- ions in physiological concentration. **A.** Ramachandran plot built by Stride WEB server.[1] **B**. Model verification by ProSA WEB server.[2] The *z*-score indicates overall model quality. Local model quality analyzed by plotting energies as a function of amino acid sequence position.

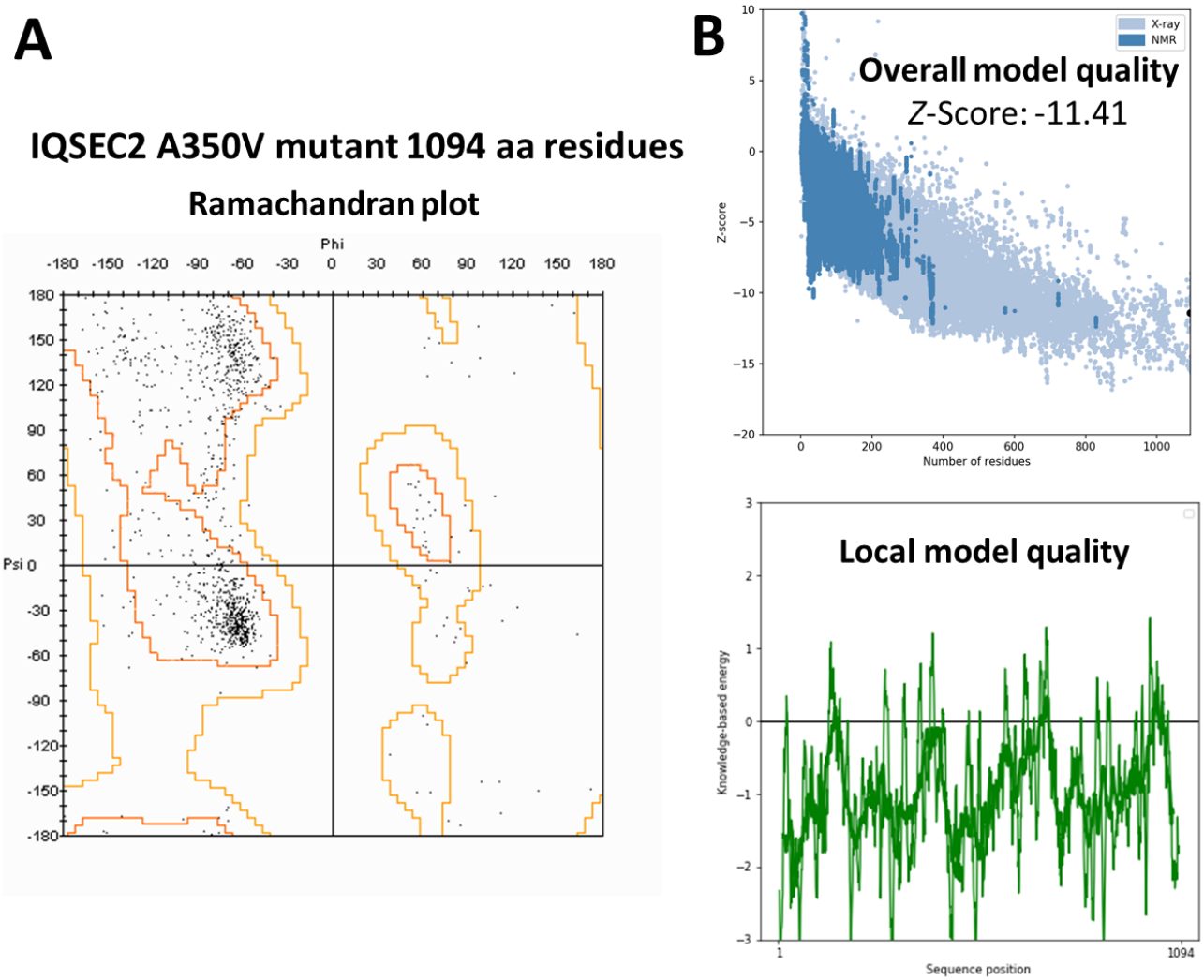

Figure S8. Structure validation of the IQSEC2 A350V mutant (1094 aa residues) built by YASARA Structure molecular modeling as two joined fragments predicted by RaptorX server (1-760 residues) and equilibrated by aMD simulation of SEC7 domain (749-1094 residues). Geometries of the generated IQSEC2 A350V mutant (1-1094 residues) was fully optimized in frames of ff14SB force field by YASARA Structure software in periodic simulation cell filled with explicit water molecules and Na+ and Cl- ions in physiological concentration. **A.** Ramachandran plot built by Stride WEB server.[1] **B**. Model verification by ProSA WEB server.[2] The *z*-score indicates overall model quality. Local model quality analyzed by plotting energies as a function of amino acid sequence position.

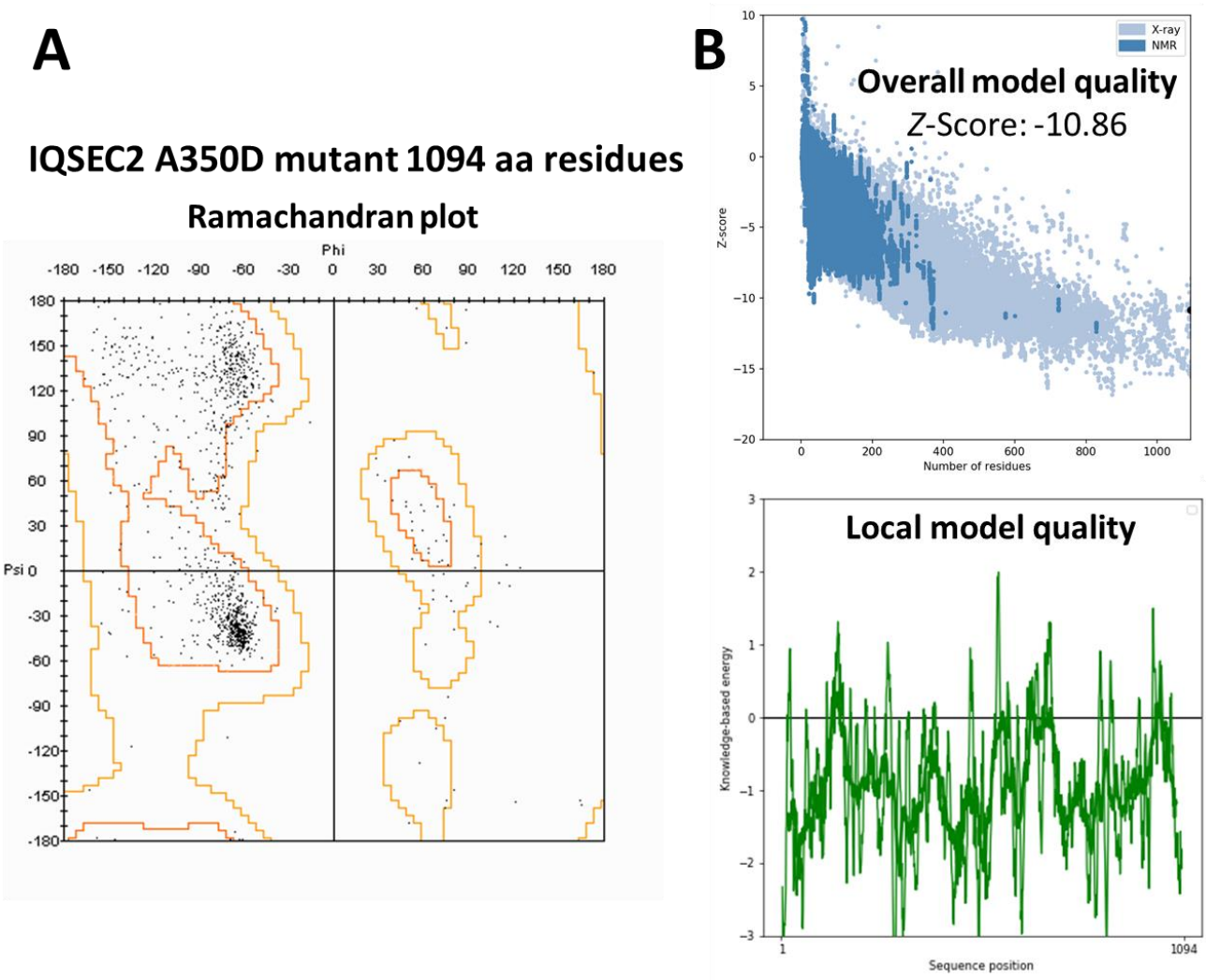

Figure S9. Structure validation of the IQSEC2 A350D mutant (1094 aa residues) built by YASARA Structure molecular modeling as two joined fragments predicted by RaptorX server (1-760 residues) and equilibrated by aMD simulation of SEC7 domain (749-1094 residues). Geometries of the generated IQSEC2 A350D mutant (1-1094 residues) was fully optimized in frames of ff14SB force field by YASARA Structure software in periodic simulation cell filled with explicit water molecules and Na+ and Cl- ions in physiological concentration. **A.** Ramachandran plot built by Stride WEB server.[1] **B**. Model verification by ProSA WEB server.[2] The *z*-score indicates overall model quality. Local model quality analyzed by plotting energies as a function of amino acid sequence position.

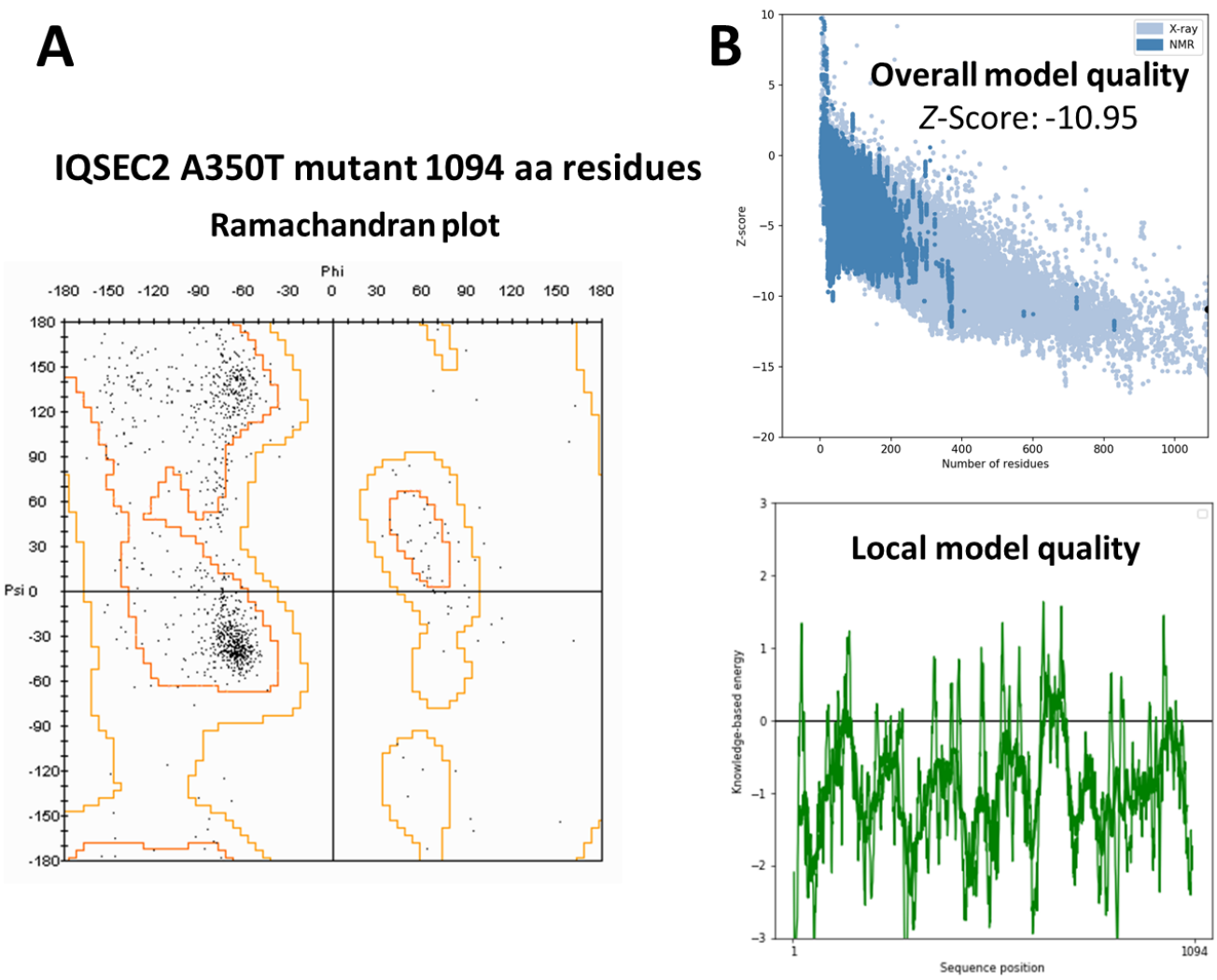

Figure S10. Structure validation of the IQSEC2 A350T mutant (1094 aa residues) built by YASARA Structure molecular modeling as two joined fragments predicted by RaptorX server (1-760 residues) and equilibrated by aMD simulation of SEC7 domain (749-1094 residues). Geometries of the generated IQSEC2 A350T mutant (1-1094 residues) was fully optimized in frames of ff14SB force field by YASARA Structure software in periodic simulation cell filled with explicit water molecules and Na+ and Cl- ions in physiological concentration. **A.** Ramachandran plot built by Stride WEB server.[1] **B**. Model verification by ProSA WEB server.[2] The *z*-score indicates overall model quality. Local model quality analyzed by plotting energies as a function of amino acid sequence position.

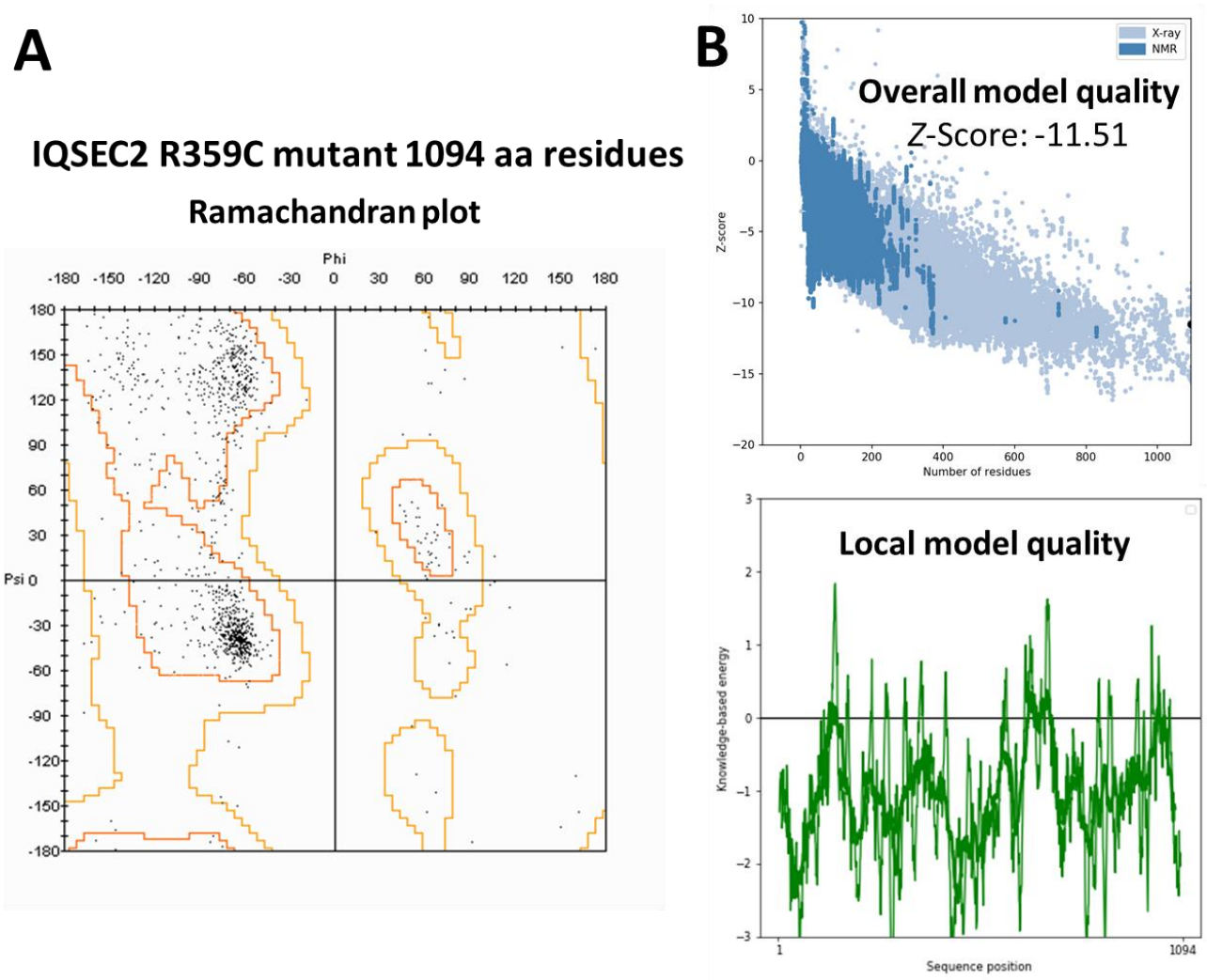

Figure S11. Structure validation of the IQSEC2 R359C mutant (1094 aa residues) built by YASARA Structure molecular modeling as two joined fragments predicted by RaptorX server (1-760 residues) and equilibrated by aMD simulation of SEC7 domain (749-1094 residues). Geometries of the generated IQSEC2 R359C mutant (1-1094 residues) was fully optimized in frames of ff14SB force field by YASARA Structure software in periodic simulation cell filled with explicit water molecules and Na+ and Cl- ions in physiological concentration. **A.** Ramachandran plot built by Stride WEB server.[1] **B**. Model verification by ProSA WEB server.[2] The *z*-score indicates overall model quality. Local model quality analyzed by plotting energies as a function of amino acid sequence position.

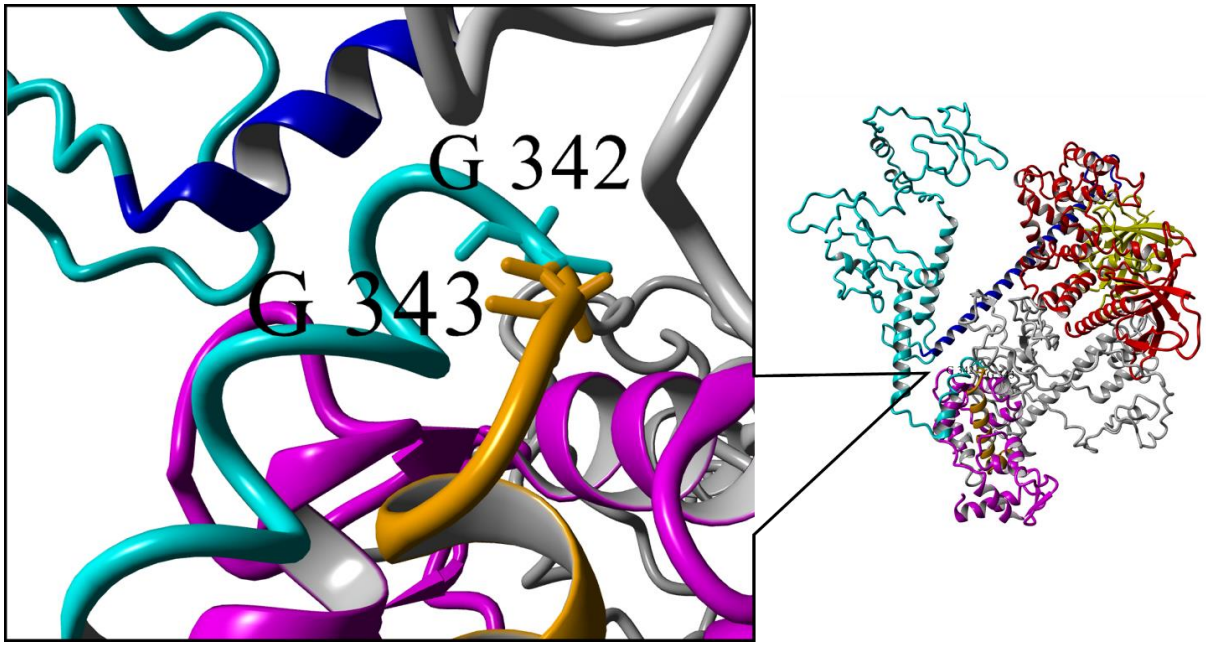

Figure S12. $IQSEC2_{wildtype}$ complex with apo-M (magenta) and ARF6 (yellow). The left panel is a magnified view of the G342-G343 hinge, whereby binding of the IQ fragment (343-370 residues, orange) to apoCM changes the G343 Φ torsion angle from 72° in free $IQSEC2_{wildtype}$ to 177.5° in the $IQSEC2_{wildtype}$ apoCM complex. As a result, the 1-342 domain (1-70 residues in blue, 71-342 residues in cyan) undergoes a large-scale conformational movement leading to inhibition by the N-end fragment (1-70 residues) of ARF6 binding to SEC7 (red). The right panel is a general view, where the remaining fragments are grey colored.

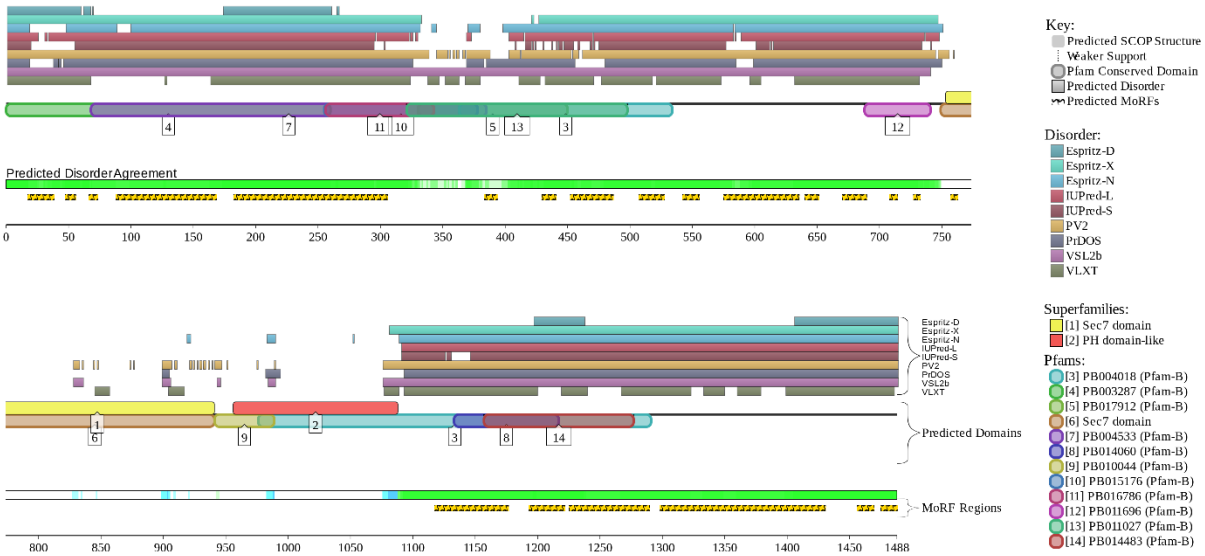

Figure S13. Functional disorder profile of human IQSEC2 (UniProt ID: IQSEC2) generated by the D^2P^2 platform (http://d2p2.pro/), which is a database of predicted disorder for a large library of proteins from completely sequenced genomes. D^2P^2 database generates disorder profile based on the outputs of several commonly used disorder predictors, such as IUPred, PONDR® VLXT, PrDOS, PONDR® VSL2, PV2, and ESpritz, which are shown as 9 colored bars representing the location of disordered regions. The middle of the D^2P^2 plot contains a green-white bar showing the predicted disorder agreement between nine disorder predictors, with darker green parts corresponding to disordered regions with stronger consensus. Above the disorder consensus bar are two lines with colored and numbered bars that show the positions of the predicted (mostly structured) SCOP domains using the SUPERFAMILY predictor. Yellow zigzagged bars in the bottom of the plot show the location of the predicted disorder-based binding sites (MoRF regions) identified by the ANCHOR algorithm.

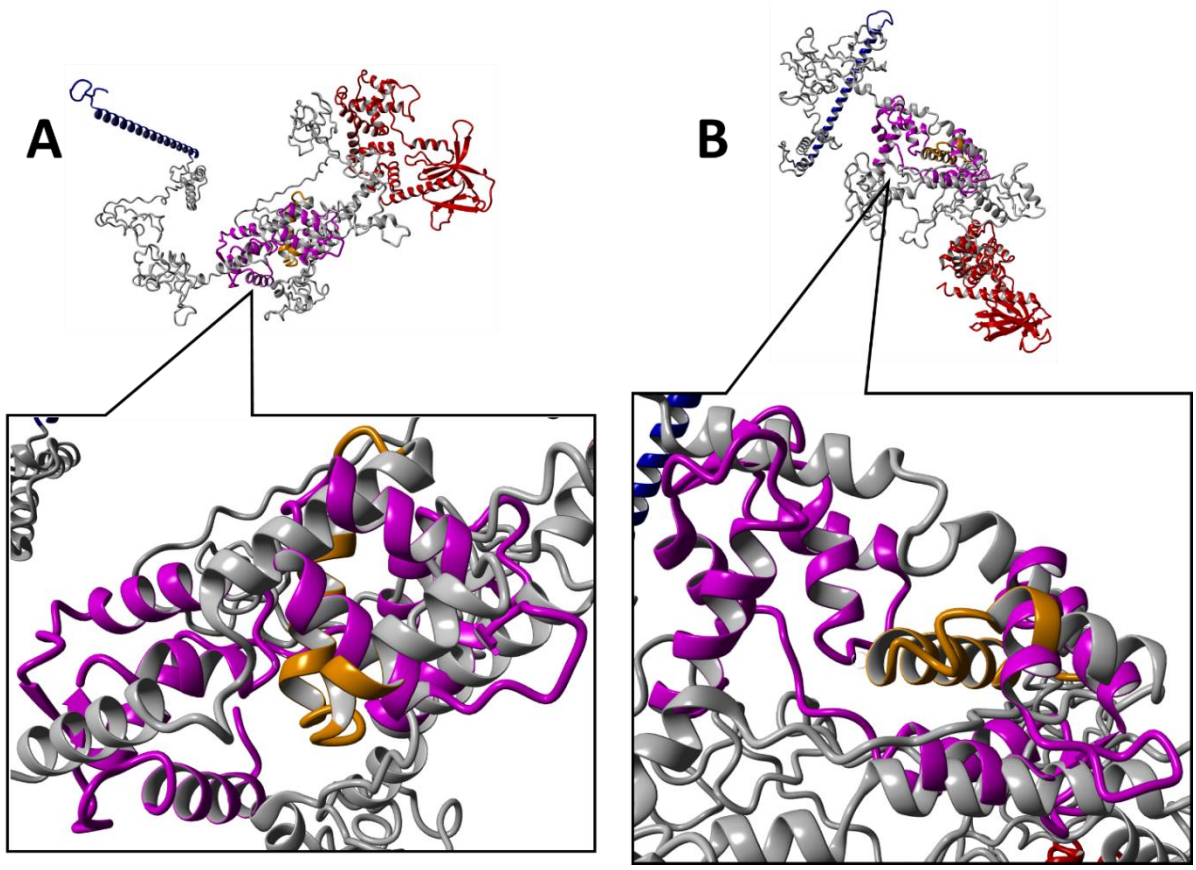

Figure S14. IQSEC2 A350V complex with apoCM demonstrating unremovable conformational clashes between protein backbones of IQSEC2 and apo-CM. **A and B.** Two projections. Colors: apoCM – magenta; IQ (343-370 residues) – orange; Sec7 (749-1094) – red; N-end helical domain (1-70) – blue; the remaining IQSEC2 fragments -grey.

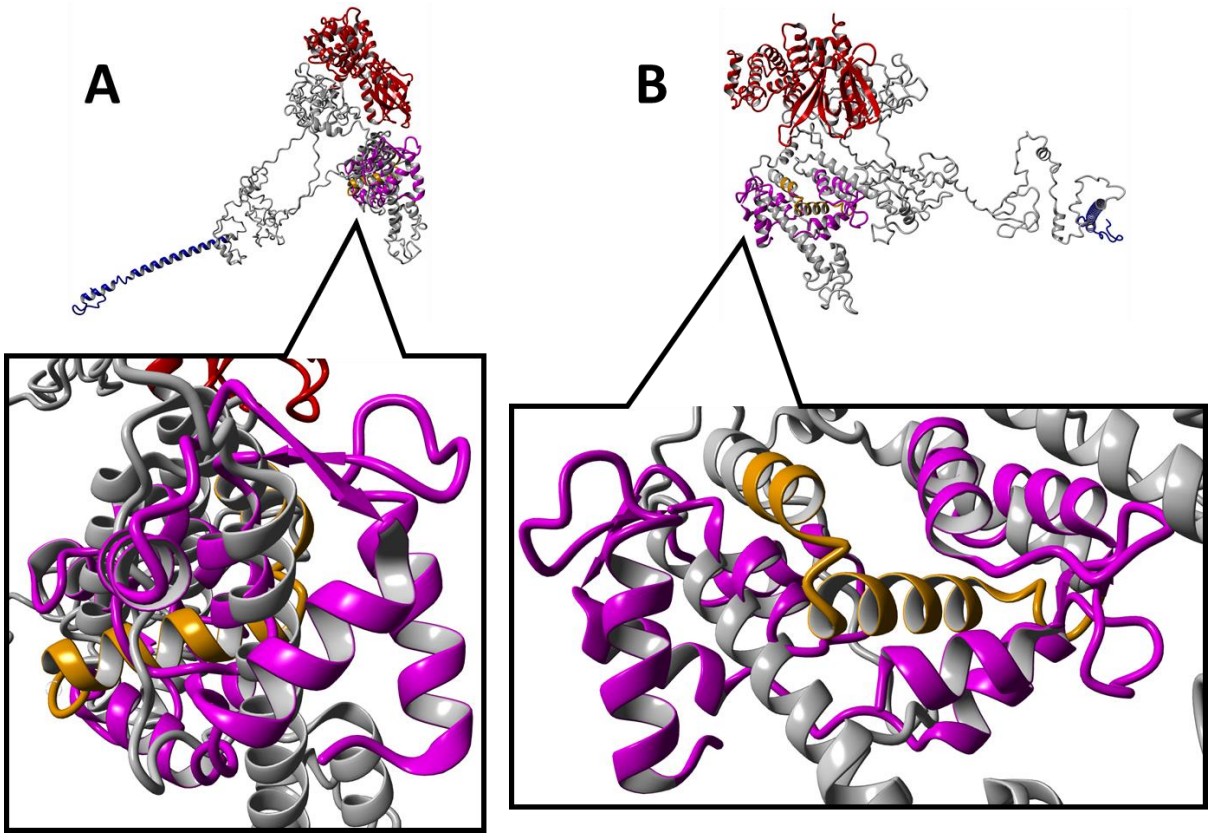

Figure S15. IQSEC2 A350T complex with apoCM demonstrating unremovable conformational clashes between protein backbones of IQSEC2 and apoCM. **A and B.** Two projections. Colors: apoCM – magenta; IQ (343-370 residues) – orange; Sec7 (749-1094) – red; N-end helical domain (1-70) – blue; the remaining IQSEC2 fragments - grey.

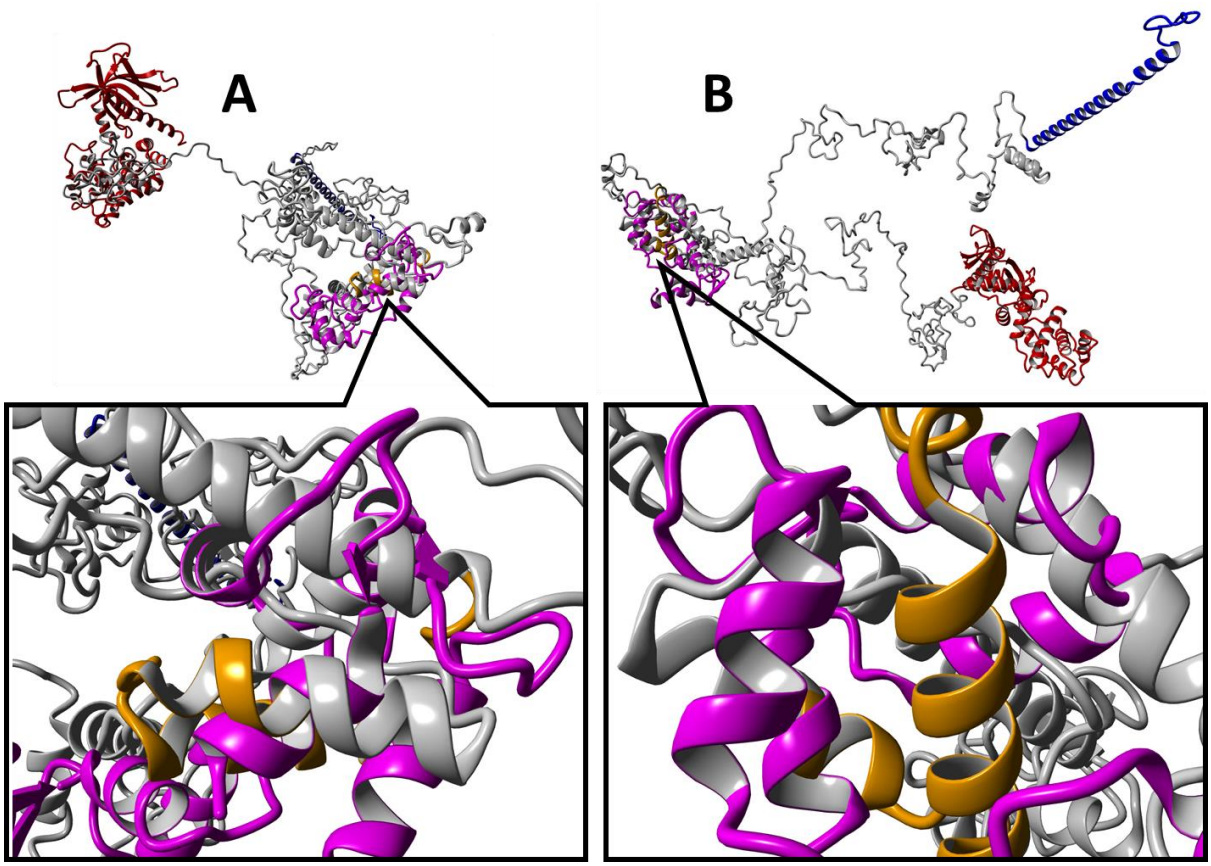

Figure S16. IQSEC2 A350D complex with apoCM demonstrating unremovable conformational clashes between protein backbones of IQSEC2 and apoCM. **A and B.** Two projections. Colors: apoCM – magenta; IQ (343-370 residues) – orange; Sec7 (749-1094) – red; N-end helical domain (1-70) – blue; the remaining IQSEC2 fragments - grey.

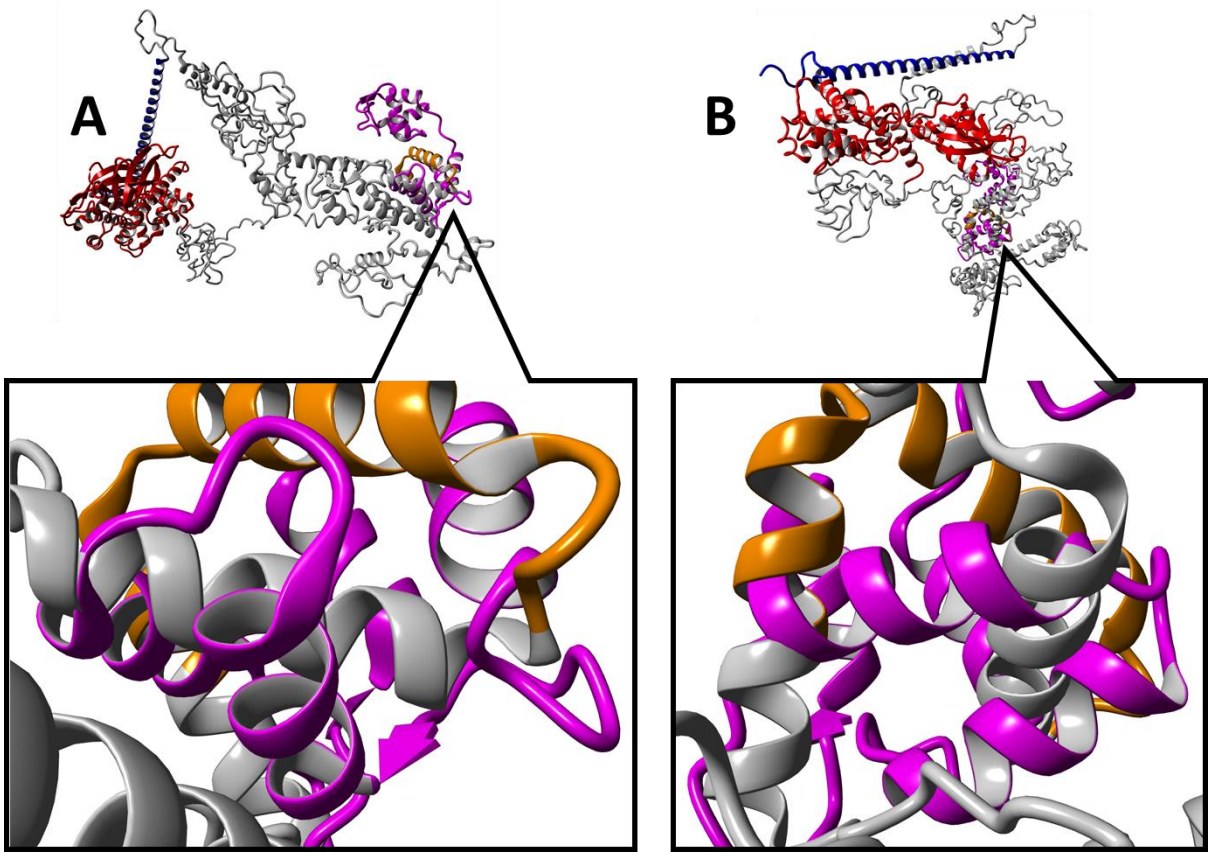

Figure S17. IQSEC2 R359C complex with apoCM demonstrating unremovable conformational clashes between protein backbones of IQSEC2 and apoCM. **A and B.** Two projections. Colors: apoCM – magenta; IQ (343-370 residues) – orange; Sec7 (749-1094) – red; N-end helical domain (1-70) – blue; the remaining IQSEC2 fragments - grey.

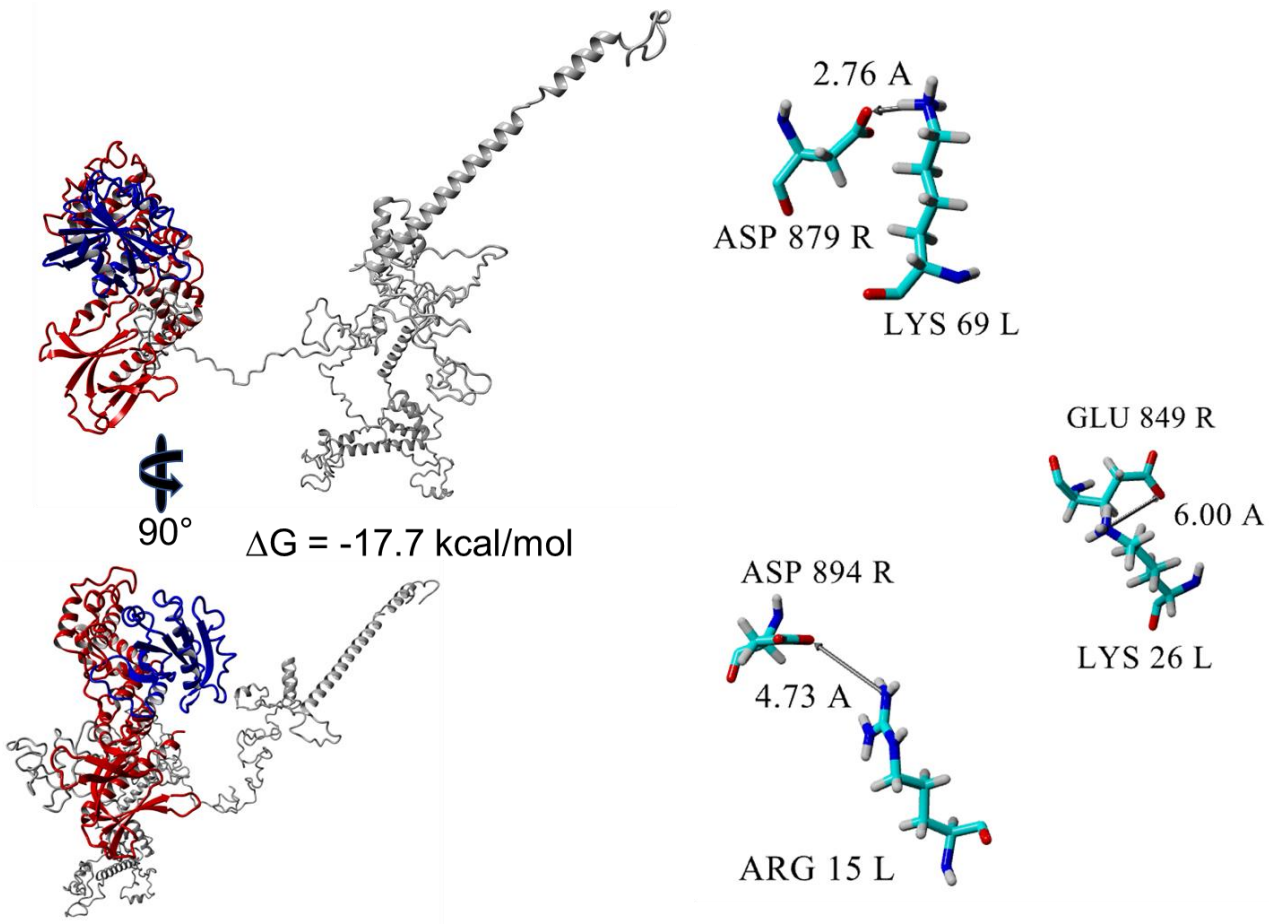

Figure S18. 3D structure in two projections of ARF6-Sec 7 complex with IQSEC2 A350D mutant, initially generated by Raptor X (see details in Section 3.4). Free energy of binding ΔG is calculated by the FoldX server. The three amino acid pairs that dominate the stability of the ARF6-IQSEC2 wild type complex are presented in the right side of figure. Interactions between corresponding functional groups of amino acid pairs are connected by arrows and labeled by distance values in Å. Color scheme: ARF6 – blue, Sec7 – red, the remainder of IQSEC2 is grey. **L** and **R** refer to ARF6 (**L**igand) and Sec 7 (**R**eceptor) respectively. Residues dominating stability of the ARF6-Sec7 complexes are element colored.

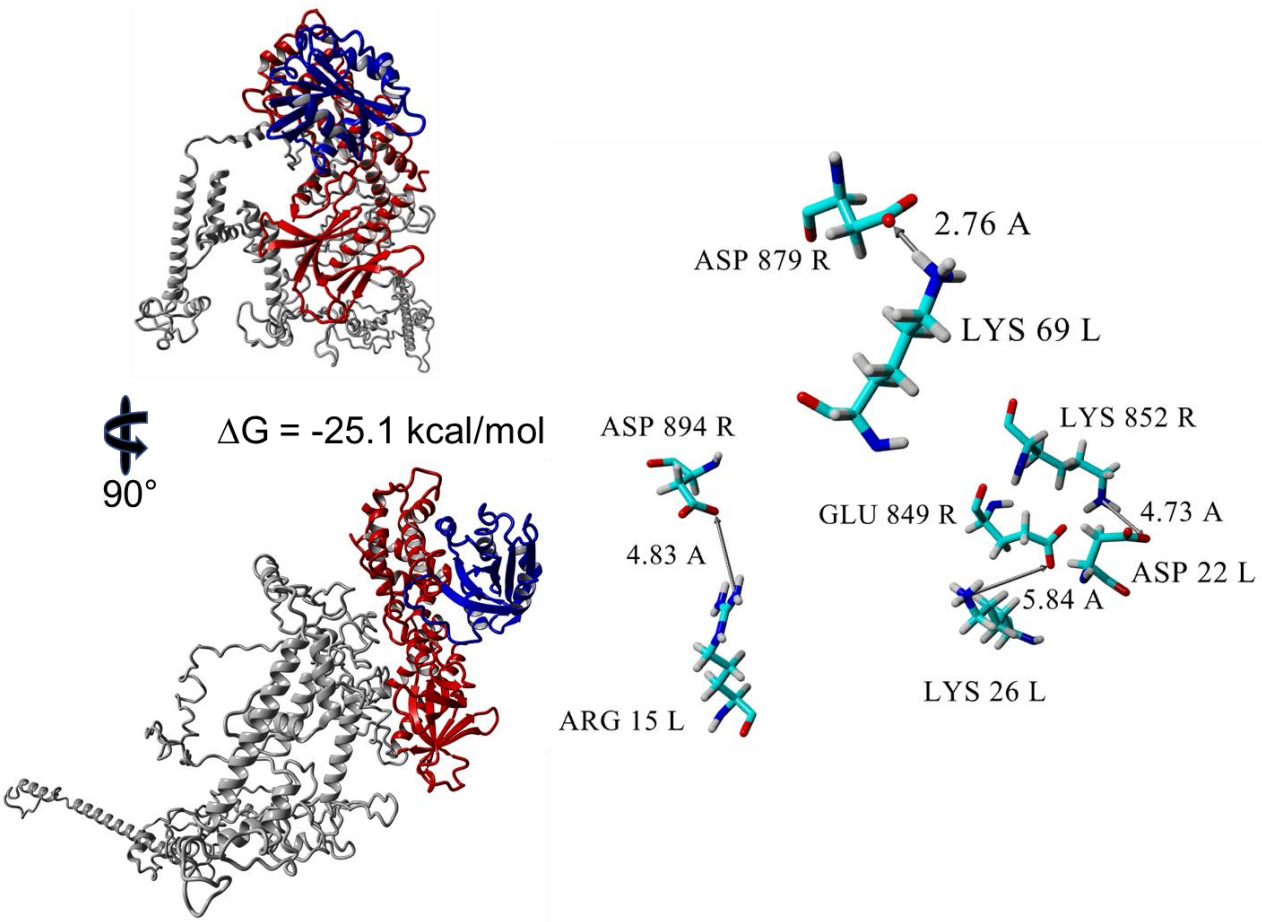

Figure S19. 3D structure in two projections of ARF6-Sec 7 complex with IQSEC2 A350T mutant, initially generated by Raptor X (see details in Section 3.4). Free energy of binding ΔG is calculated by the FoldX server. Free energy of binding ΔG is calculated by the FoldX server. The four amino acid pairs that dominate the stability of the ARF6-IQSEC2 wild type complex are presented on the right side of figure. Interactions between corresponding functional groups of amino acid pairs are connected by arrows and labeled by distance values in Å. Color scheme: ARF6 – blue, Sec7 – red, the remainder of IQSEC2 is grey. **L** and **R** refer to ARF6 (**L**igand) and Sec 7 (**R**eceptor) respectively. Residues dominating stability of the ARF6-Sec7 complexes are element colored.

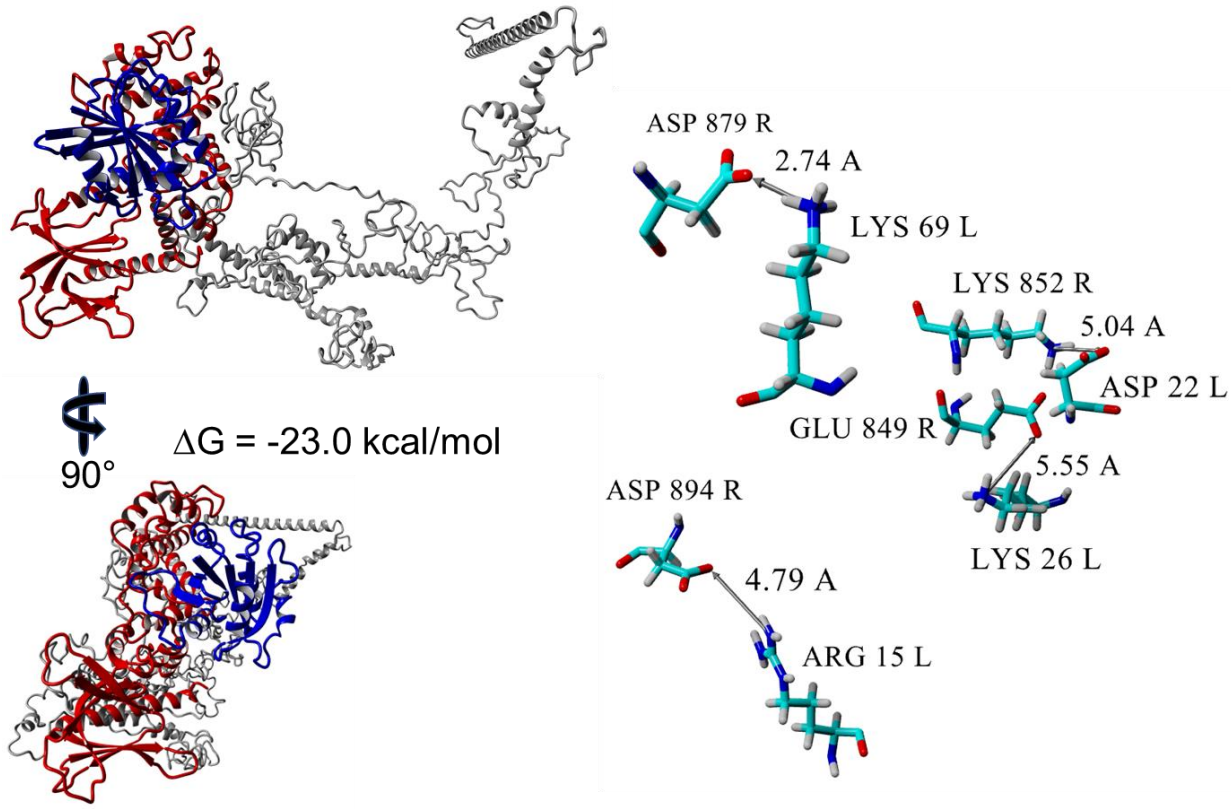

Figure S20. 3D structure in two projections of ARF6-Sec 7 complex with IQSEC2 A350V mutant, initially generated by Raptor X (see details in Section 3.4). Free energy of binding ΔG is calculated by the FoldX server. The four amino acid pairs that dominate the stability of the ARF6-IQSEC2 wild type complex are presented on the right side of figure. Interactions between corresponding functional groups of amino acid pairs are connected by arrows and labeled by distance values in Å. Color scheme: ARF6 – blue, Sec7 – red, the remainder of IQSEC2 is grey. **L** and **R** refer to ARF6 (**L**igand) and Sec 7 (**R**eceptor) respectively. Residues dominating stability of the ARF6-Sec7 complexes are element colored.

POISSON BOLTZMANN: Calculations performed using 200 complex frames and internal PBSA solver in mmpbsa_py_energy.
All units are reported in **kcal/mole.**

Table S1. Complex of apocalmodulin with IQ 343-370 residues.

Differences (Complex - Receptor - Ligand):

Energy Component	Average	Std. Dev.	Std. Err. of Mean
VDWAALS	-161.3402	7.6148	0.5384
EEL	-2893.9434	52.8717	3.7386
EPB	2874.9460	46.4814	3.2867
ENPOLAR	-127.9099	3.4275	0.2424
EDISPER	219.6218	4.2628	0.3014
DELTA G gas	-3055.2836	52.7287	3.7285
DELTA G solv	2966.6579	46.6759	3.3005
DELTA TOTAL	**-88.6256**	**15.0944**	**1.0673**

Table S2. Complex of calmodulin chelating 4 Ca(2+)cations with IQ 343-370 residues.

Differences (Complex - Receptor - Ligand):

Energy Component	Average	Std. Dev.	Std. Err. of Mean
VDWAALS	-142.3290	6.2384	0.4411
EEL	-2292.6283	62.9239	4.4494
EPB	2310.6285	60.0826	4.2485
ENPOLAR	-115.5905	2.9086	0.2057
EDISPER	203.6443	3.6992	0.2616
DELTA G gas	-2434.9573	62.6672	4.4312
DELTA G solv	2398.6822	60.7667	4.2969
DELTA TOTAL	**-36.2750**	**12.1422**	**0.8586**

REFERENCES

1. Stride Web Interface: http://webclu.bio.wzw.tum.de/cgi-bin/stride/stridecgi.py
2. ProSA Web Interface: https://prosa.services.came.sbg.ac.at/prosa.php