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Supplemental information

Xport-A functions as a chaperone by stabilizing the first five transmembrane domains of rhodopsin-1

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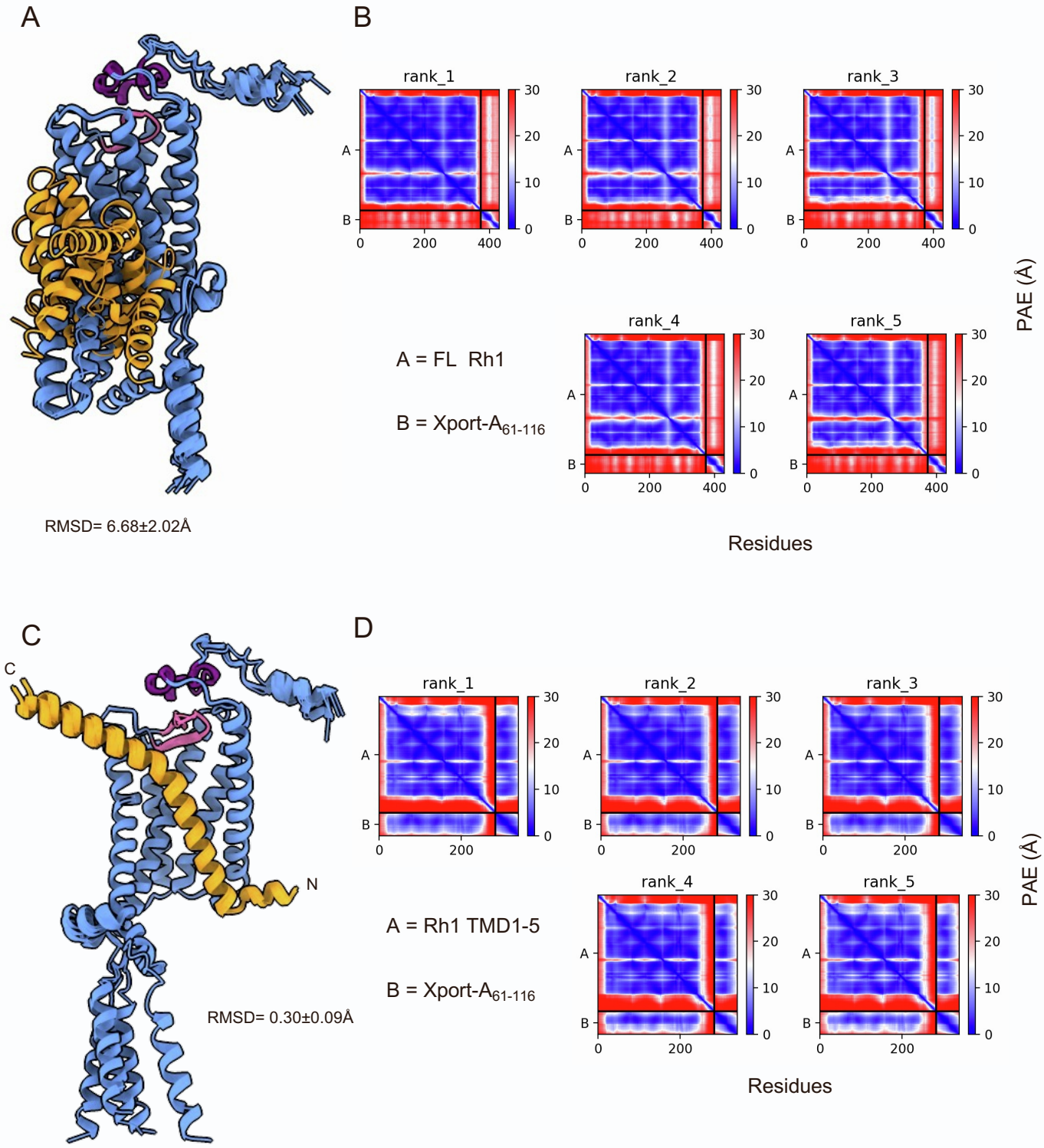

Figure S1. AF2 predicted complexes for Xport-A_{61-116} together with full length Rh1 or Rh1 TMD 1-5, related to Figure 2. (A) Overlay of the ribbon representations for the five top ranked AF2 models for Xport-A_{61-116} (orange) together with full length (FL) Rh1 (light blue). The beta-loop-beta and extracellular "crown" N-terminal regions are colored pink and purple, respectively. RMSD is the root mean square deviation, a quantitative measure of the average distance (in Å) between the atoms of the backbones of the superimposed predicted structures. (B) Predicted Aligned Errors (PAE) on the scale from 0 to 30 Å in a blue-white-red gradient. PAE is a metric of confidence in the relative position and orientation of the different chains of the model (i.e., A= FL Rh1, B=XportA_{61-116}). All models display high inter-chain PAE values (red) regarding Xport-A_{61-116}, indicative of poorly-defined relative positions and orientations within the predicted complexes. (C) Overlay of the ribbon representations for the five top ranked AF2 models for Xport-A_{61-116} (orange) together with Rh1 TMD1-5 (light blue). (D) Predicted Aligned Errors (PAE) on the scale from 0 to 30 Å in a blue-white-red gradient. PAE is a metric of confidence in the relative position and orientation of the different chains of the model (i.e., A=Rh1 TM1-5, B=XportA_{66-116}). All models display low inter-chain PAE values (blue), indicative of well-defined relative positions and orientations within the predicted complexes.

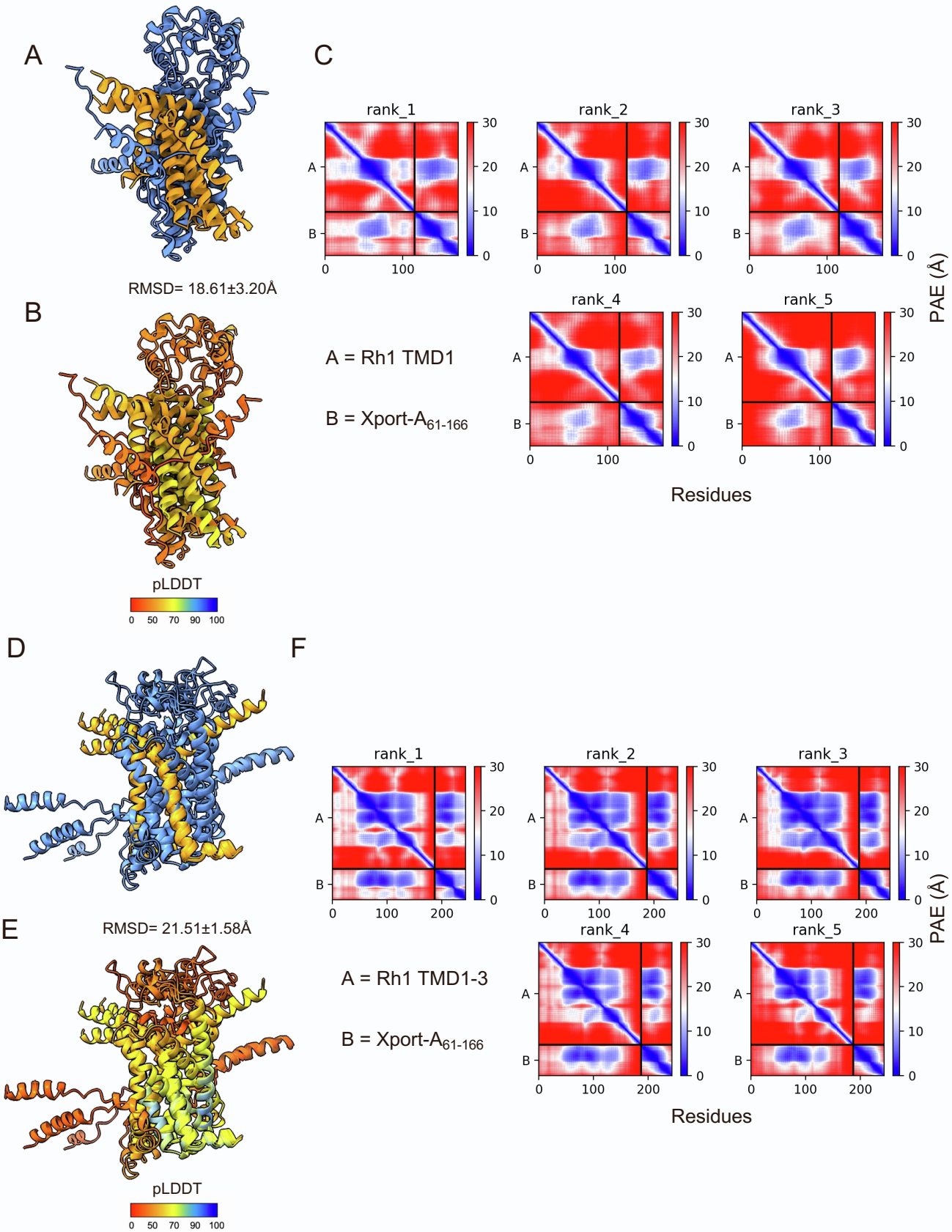

Figure S2. AF2 predicted complexes for Xport-A_{61-116} together with Rh1 TMD1 (A-C) or Rh1 TMD 1-3 (D-F), related to Figure 2. (A) Overlay of the ribbon representations for the five top ranked AF2 models for Xport-A_{61-116} (orange) together with Rh1 TMD1 (M1 to I74 + H333-A373 - light blue). (B) Overlay of the complexes coloured according to the pLDDT metric. All display low pLDDT values. (C) Predicted Aligned Errors (PAE) on a scale from 0 to 30 Å in a blue-white-red gradient. PAE is a metric of confidence in the relative position and orientation of the different chains of the model (i.e., A= Rh1 TMD1, B=XportA_{61-116}). (D) Ribbon representation of the five AF2-based models with Xport-A_{61-116} (orange) bound to Rh1 TMD1-3 ((M1 to L146 + H333-A373 - light blue). (E) Overlay of the complexes coloured according to the pLDDT metric. All display low pLDDT values. (F) PAE on a scale from 0 to 30 Å in a blue-white-red gradient. PAE is a metric of confidence in the relative position and orientation of the different chains of the model (i.e., A= Rh1 TMD1, B=XportA_{61-116}). The low inter-chain PAE values (blue), indicate consistent intermolecular contacts within the predicted complexes.

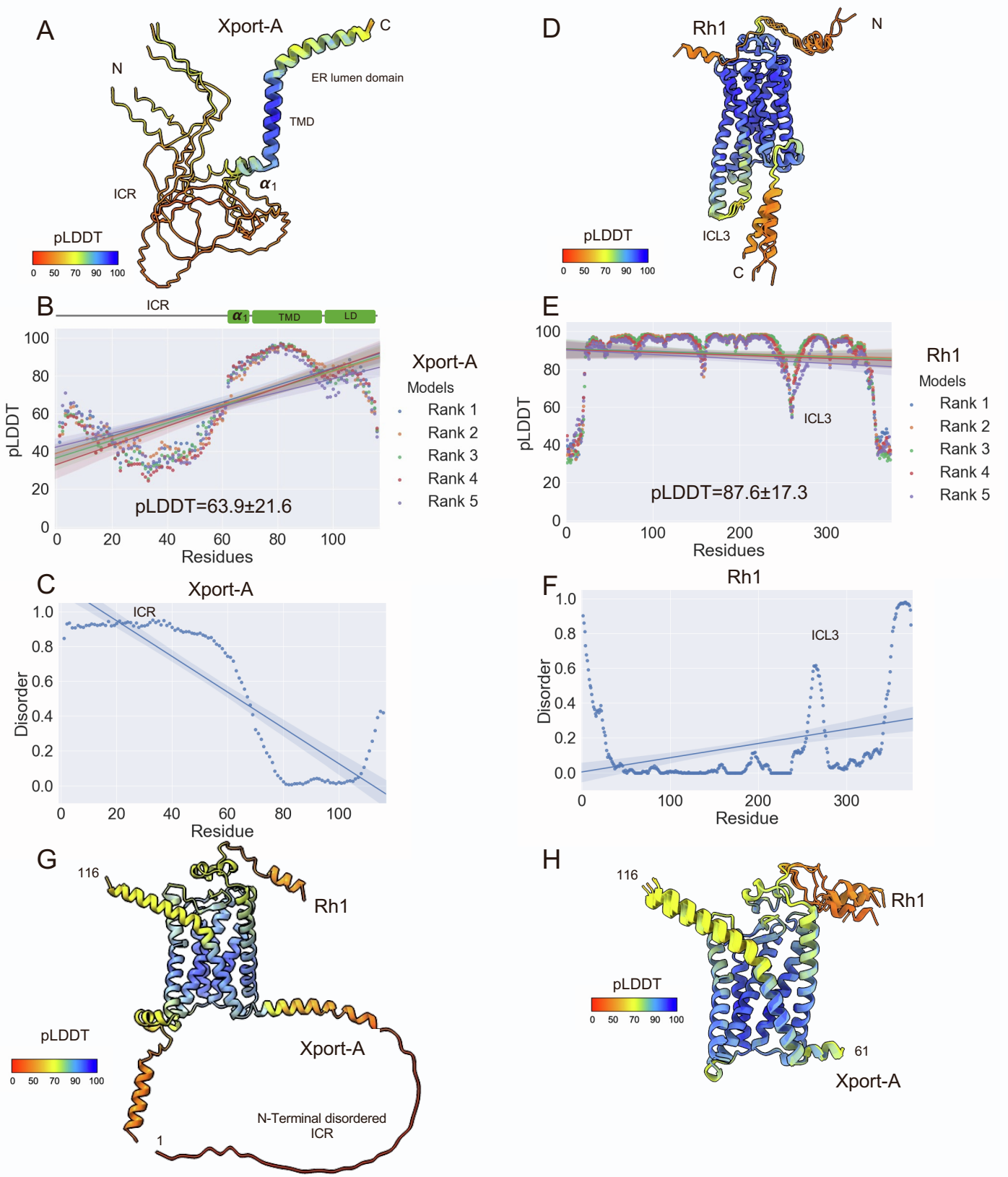

Figure S3. AF2 predicted structures for unbound full length Xport-A (A-C), Rh1 (D-F) and the complex between full length Xport-A and Rh1 TMD1-5 (G,H), related to Figure 2. (A) Overlay of the ribbon representations for the five top ranked AF-2 models for full length Xport-A (residues 1 to 116), color-coded on a scale from 0 (red) to 100 (blue) with the estimate of confidence for each amino acid residue (pLDDT - Local Distance Difference Test). (B) Profiles of pLDDT per residue for the top five ranked (color coded) AF2 models of Xport-A. All Xport-A models have high confidence in the C-terminal region, including the TMD and the ER luminal domain. Well-defined structural elements are highlighted on top of the plot. The N-terminal first 60 residues show low pLDDT. (C) Disordered profiles propensities (blue dots) as computed with Metapredict [S1]. Xport-A bears a disordered N-terminal cytosolic tail (ICR). (D) Rh1 models display a high pLDDT score, except in the N and C termini and the ICL3. (E) Profiles of pLDDT per residue for each model of Rh1 display a high pLDDT score, except in the N and C termini and the ICL3. (F) Disordered profiles propensities (blue dots) for Rh1 as computed with Metapredict. (G) AF2 structural prediction of full length Xport-A together with Rh1 TMD1-5 (M1 to V241 + H333-A373). (H) Overlay of AF2 structural predictions for Xport-$A_{61\text{-}116}$ Xport-A together with Rh1 TMD1-5 (M1 to V241). The complexes are represented with the pLDDT values color-coded on a scale from 0 - 100. Values of pLDDT > 90 (blue) are expected to be modeled with high accuracy.

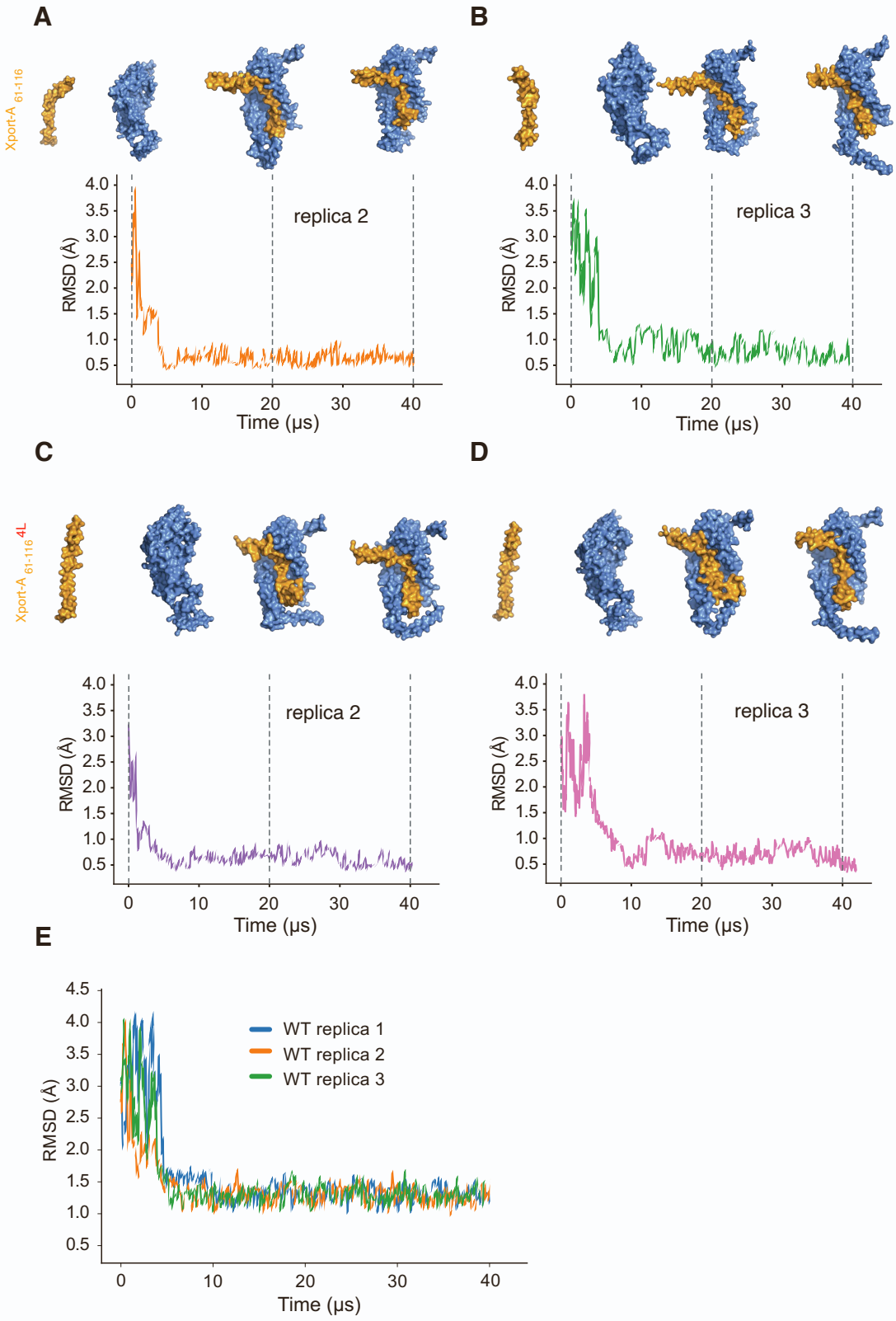

Figure S4. Molecular dynamics simulations of complexes between Rh-1 TMD 1-5 with Xport-A_{61-116} or Xport-A_{61-116} 4L, related to Figure 6. (A, B) RMSD for replicas 2 (A) and 3 (B) of the molecular dynamics simulation between Rh-1 TMD 1-5 and Xport-A_{61-116}. The reference frames are depicted above the respective time points at 0, 20 and 40 µs, in surface representation for coarse-grained Xport-A_{61-116} (orange) and Rh1 TMD 1-5 (light blue). (C, D) RMSD for replicas 2 (C) and 3 (D) of the molecular dynamics simulation between Rh-1 TMD 1-5 and Xport-A_{61-116} 4L. (E) RMSD of the molecular dynamics trajectories of the Rh-1 TMD 1-5 and Xport-A_{61-116} complexes (replica 1, in blue, replica 2 in orange and replica 3, in green), in comparison to the highest scoring AF2 model (rank 1).

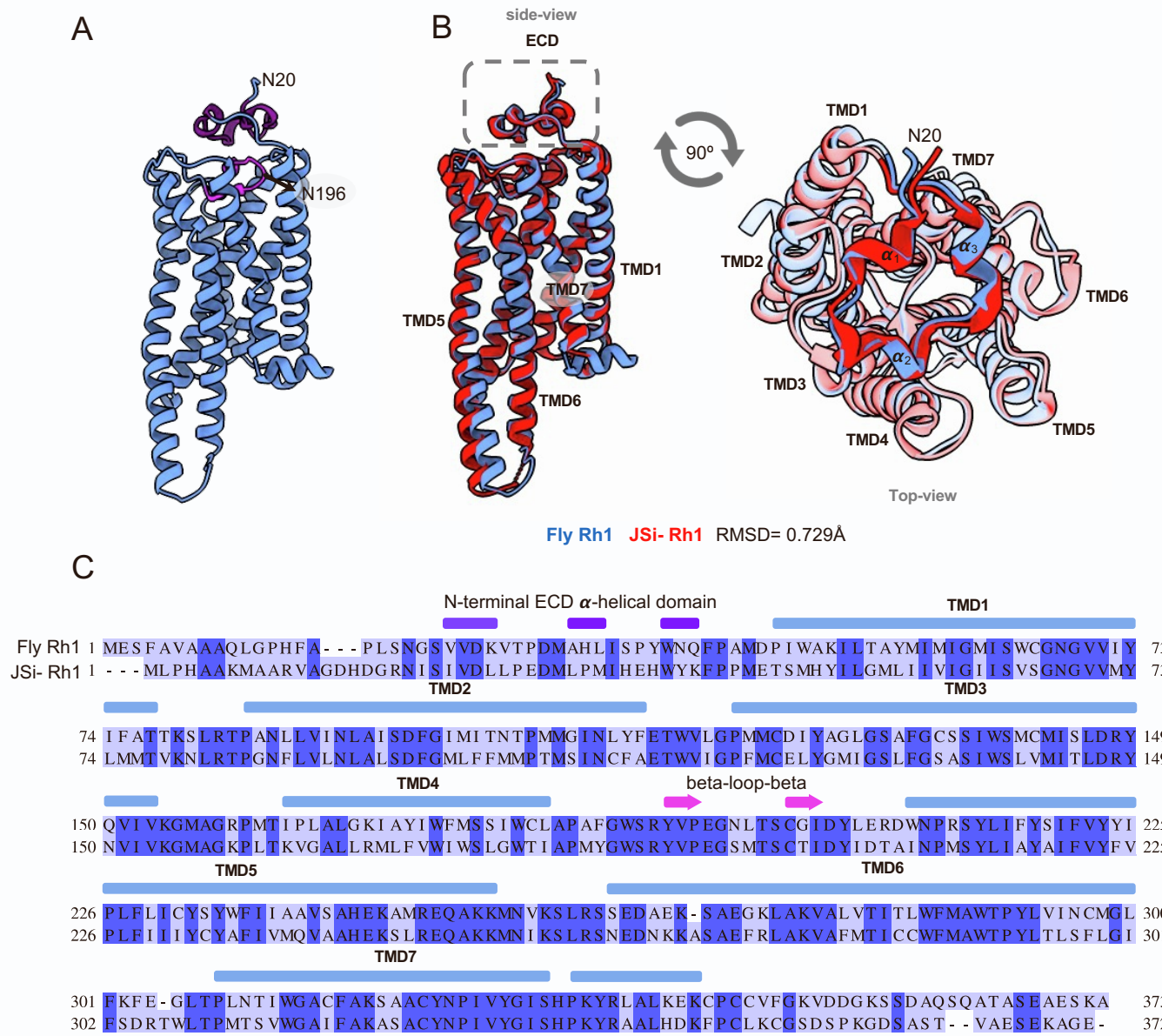

Figure S5. AF2 model for *Drosophila* Rh1 shares a similar fold with the crystal structure of jumping spider rhodopsin-1, related to Figure 7. (A) Ribbon representation of the AF2-based Rh1 full-length protein (blue) with the beta-loop-beta and extracellular "crown" N-terminal regions in pink and purple, respectively. The disordered N- and C- termini with low pLDDT value were omitted in this representation. (B) Overlay of *Drosophila* Rh1 AF2 predicted structure (blue) onto the crystal structure of jumping spider Rh1 (JSi-Rh1) in red**.** (C) Amino acid sequence alignment between Rh1 and JSi-Rh1. Conserved positions are shaded in lavender. Structural elements regions are delimited in the same colour code as in panel A. H36 and H38 are not conserved in *Drosophila* Rh1. N196 is not conserved in Jsi-Rh1.

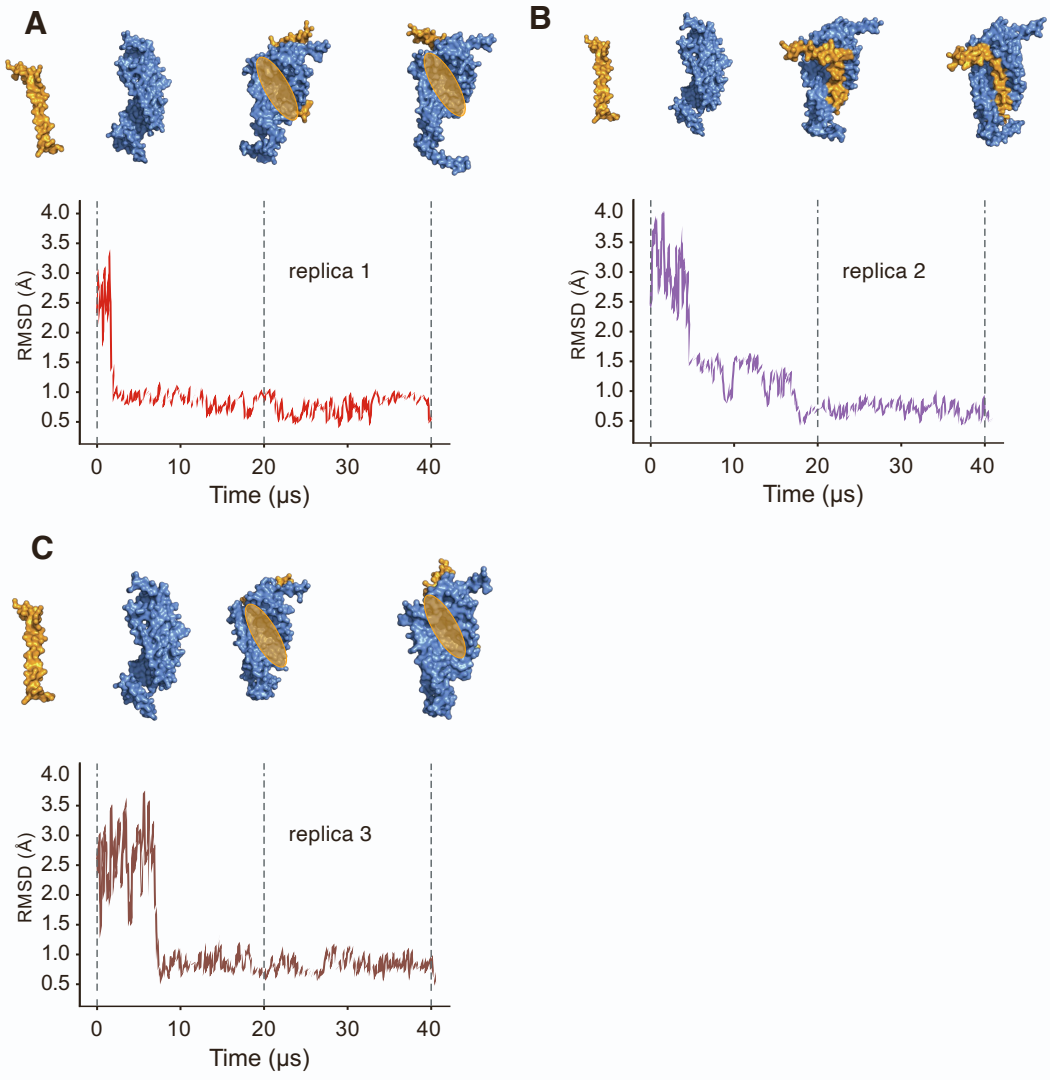

Figure S6. Molecular dynamics simulations of Rh-1 TMD 1-5 with Xport-A$_{61-116}$H95A Y101A, related to Figure 7. (A) (B) and (C) represent the RMSD of replicas 1, 2 and 3, in red, purple and brown, respectively. Xport-A$_{61-116}$ H95A Y101A is represented in orange and Rh1 TMD 1-5 in blue. The transparent ellipsoid in orange indicates the expected binding pocket for Xport-A WT as observed for the simulations in Figure 6A and S5A,B.

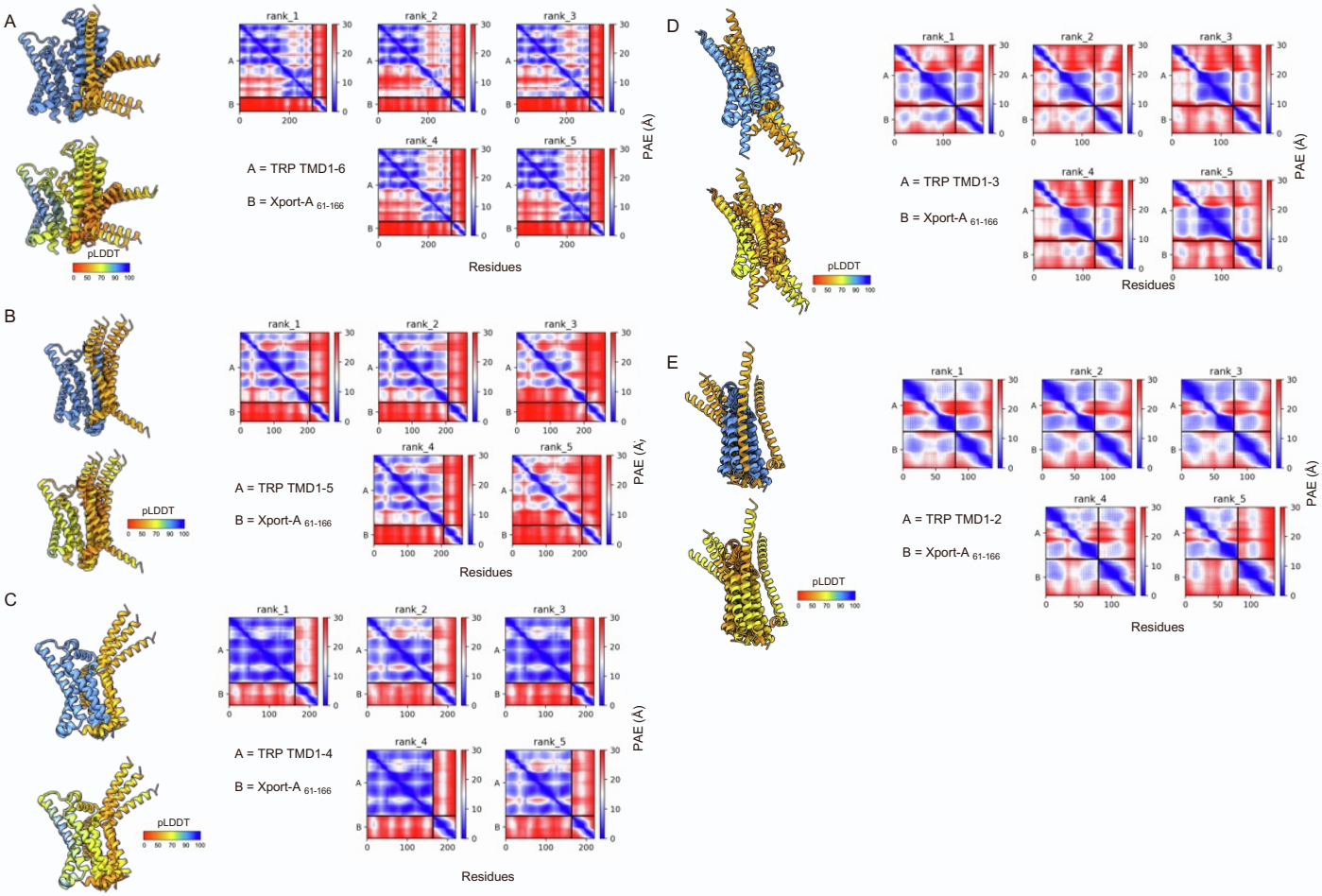

Figure S7. AF2 predictions for complexes of Xport-A$_{61-116}$ together with the TMDs of TRP, related to Figure 2. (A) TRP TMD1-6 (aa 367 – 658). (B) TRP TMD1-5 (aa 367 – 630). (C) TRP TMD1-4 (aa 367 – 562). (D) TRP TMD1-3 (aa 367 – 471). (E) TMD1-2 (aa 367 – 439). In each panel, in the top left, it is shown the ribbon representation of the five AF2-based models with Xport-A$_{61-116}$ in orange and TRP TMDs in light blue. In the bottom left are represented the respective overlays with the complexes colored according to the pLDDT metric. On the right of each panel is the PAE for each predicted complex, on a scale from 0 to 30 Å in a blue-white-red gradient. All models display high inter-chain PAE values (red-white), indicative of high uncertainty at the interface.

Supplementary References

S1. Emenecker R.J., Griffith D., Holehouse A.S. Metapredict: a fast, accurate, and easy-to-use predictor of consensus disorder and structure. Biophys J. 2021; 120: 4312-4319. doi: 10.1016/j.bpj.2021.08.039.