

Expanded View Figures

Figure EV1. Structural and sequence differences between TENs.

- A–C Structural superimposition of Ten-m (purple) and hTen4 (cyan, used as a stand in for vertebrate TEN structures as a whole) reveal that the overall structure of the TEN superfold is conserved, yet there are differences in specific regions. Inside the barrel, (B): the Ig-like domain takes a different track demarcated by arrows and (C): the portion of the toxin-like domain inside the barrel also follows a different track.
- D A β -strand in the β -barrel sterically clashes with the putative binding site of ADGRLs, based on the structure of the hTen2-hADGRL3 complex (PDB 6VHH).
- E The all-atom RMSD between representative TEN structures is shown in a table.
- F–K The sequence identity of different TEN homologs is compared using the full-length sequence, (F) and broken up individually by domain (G–K).

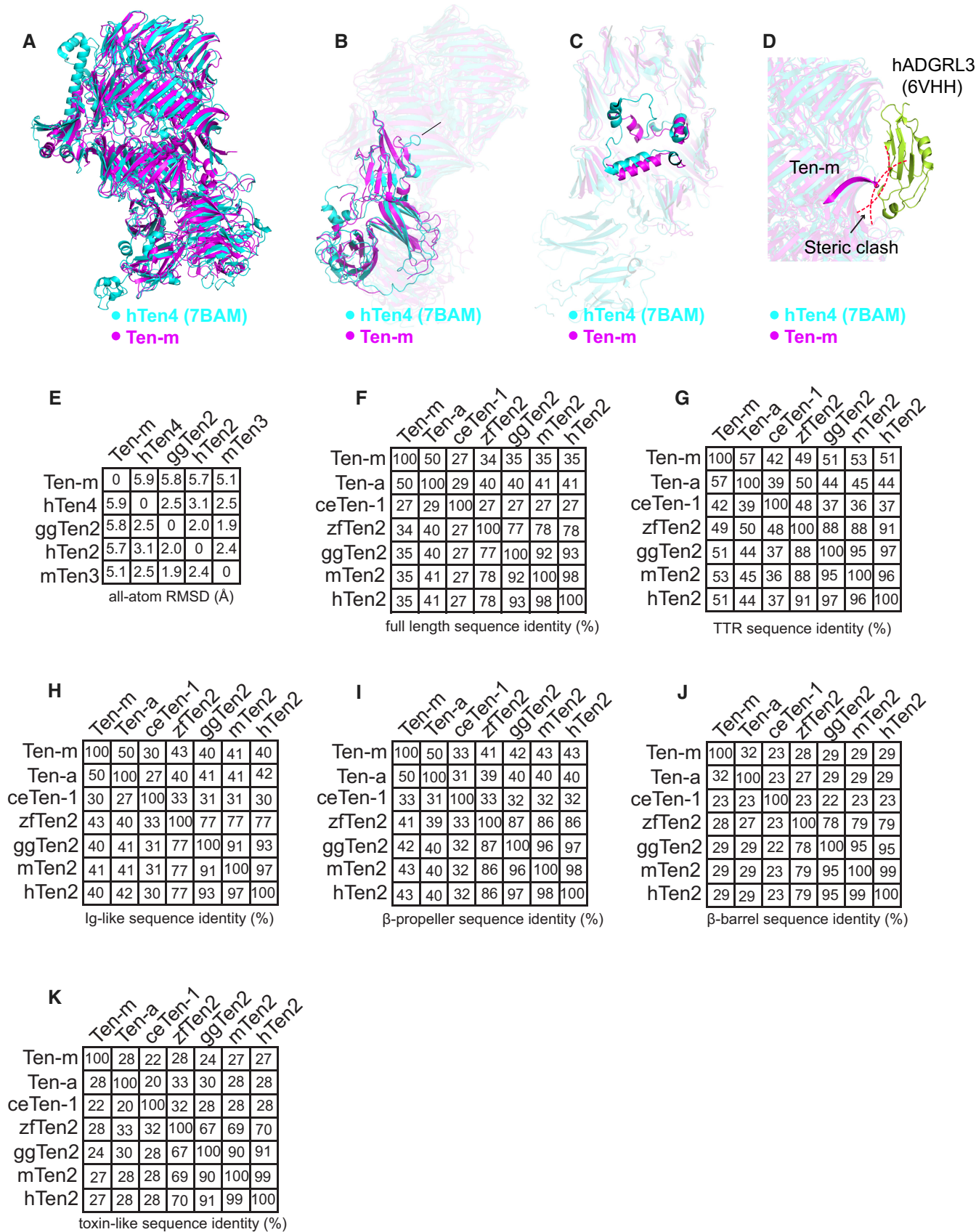


Figure EV1.

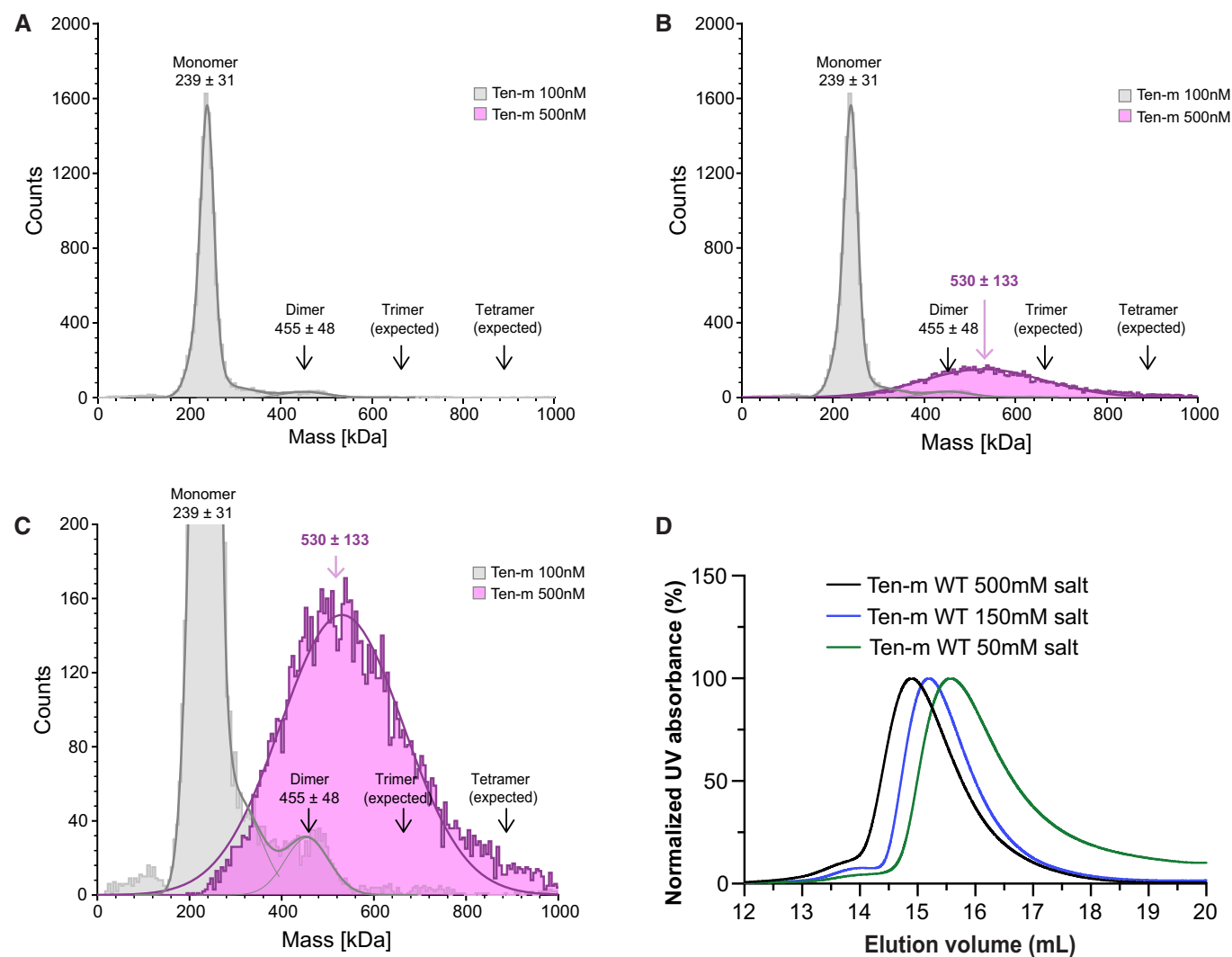


Figure EV2. Further biophysical studies on Ten-m self-association using mass photometry and size exclusion chromatography.

A–C Mass photometry shows an increase in oligomer proportion at higher concentrations. Mass photometry curves, molecular counts vs. mass, shown with 100 nM final Ten-m concentration in gray and 500 nM final concentration in pink. Various zooms in of the Y-axis are shown to show detail on the high concentration plot. The expected molecular mass of the monomer is ~ 222 kDa, dimer is ~ 444 kDa, trimer is ~ 666 kDa, and tetramer is ~ 888 kDa, and they are highlighted using arrows. Counts are observed consistent with the presence of higher order species.

D SEC chromatograms with A_{280} versus elution volume plotted show that increasing salt (NaCl) concentrations shift the protein toward an earlier elution volume. Mass photometry and SEC experiments were performed once, $N = 1$.

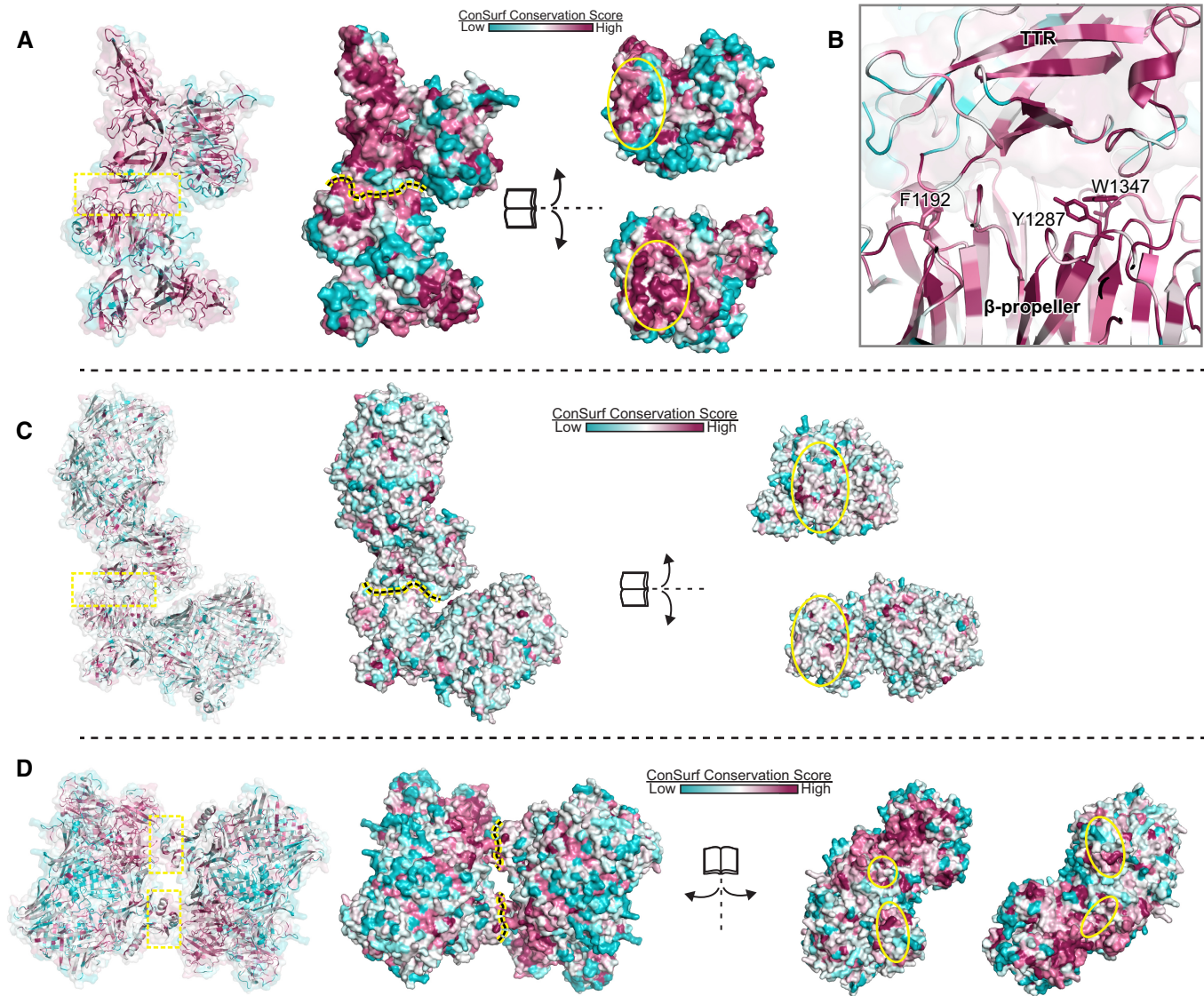


Figure EV3. Sequence conservation of the Ten-m self-association interface.

The ConSurf server was used to assess surface conservations of Ten-m and make comparisons with other TENs. The conservation score is plotted onto a surface representation of each structure. Purple indicates high conservation, cyan indicates low conservation, and white indicates intermediate conservation.

- A The ConSurf server was used to generate a sequence alignment of Ten-m using homologs with greater than 35% sequence identity. The conservation score is plotted onto a surface representation of the self-association interface with the TTR, Ig-like, and β -propeller shown. Yellow dotted lines and ovals enclose the Ten-m surface used for self-association and show that it is largely composed of conserved residues.
- B A close-up view shows that the residues which break the binding interface when mutated, including F1192, Y1287, and W1347 are strongly conserved.
- C A sequence alignment of Ten-m using homologs across animals was plotted onto the Ten-m self-association interface.
- D The ConSurf server is used to generate a sequence alignment of Ten-m using homologs with greater than 35% sequence identity. The conservation score is plotted onto a surface representation of Ten-m arranged in the Ten4 β -barrel/toxin-like dimer. Yellow dotted lines and ovals enclose the Ten4 dimer surface and show that it is not strongly conserved in arthropods.

Figure EV4. The EGF-dimer leads to many possible scenarios for Ten-m self-association.

Many possibilities are present and they can also mix and match each other.

- A If Ten-m self-associates in *cis*, there are two reasonable possibilities for this: the interface could form between two superfolds part of the same EGF-dimer (left), or between two superfolds protruding from different EGF dimers (right).
- B If the interface forms in *trans*, a “parallel” situation could occur (left) where a set of superfolds protruding from the same EGF dimer is occupied by another completed set, or an asymmetric situation (right) where the one superfold of each opposing EGF dimer is occupied in the Ten-m self-association interface, and the other two superfolds are free to form similar *trans* association with other EGF dimers, or *cis* self-associated Ten-m molecules.

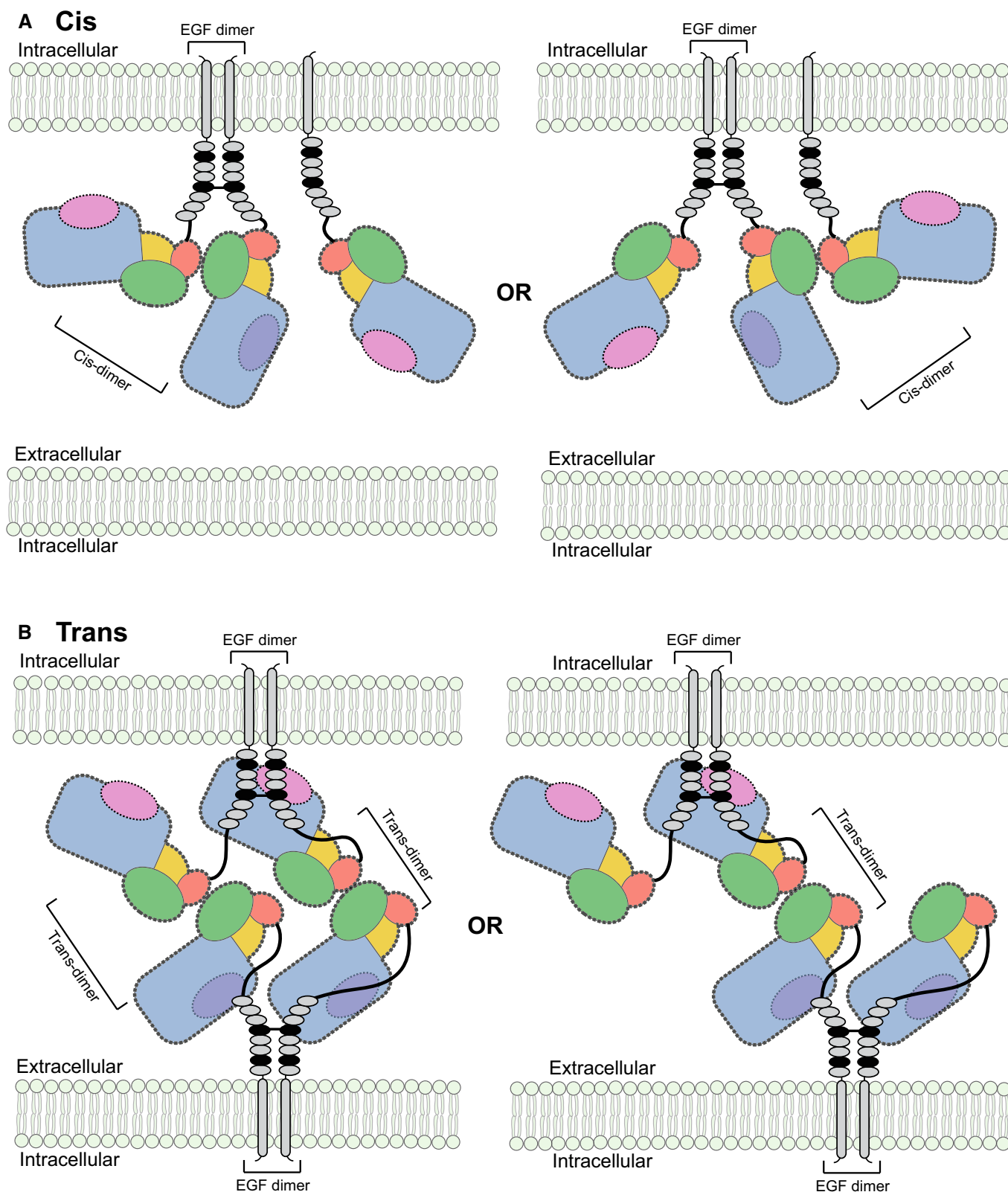
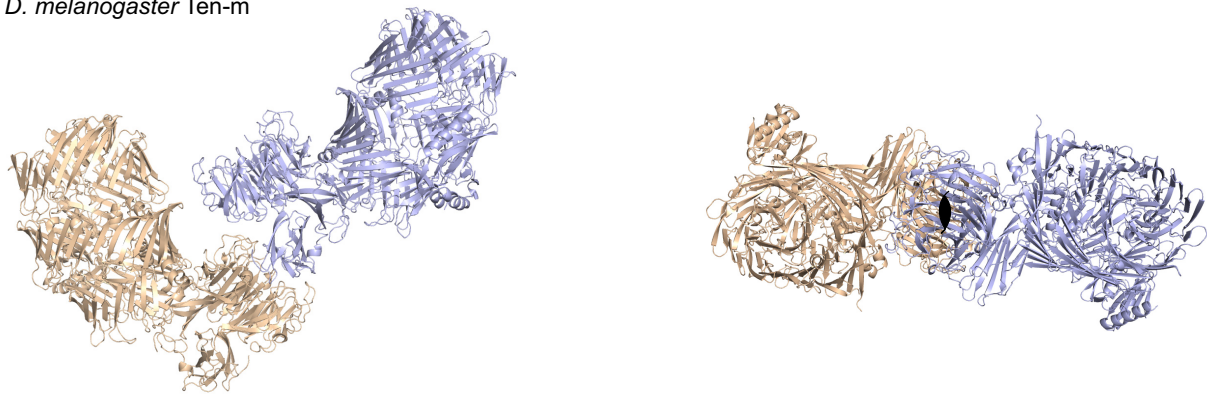
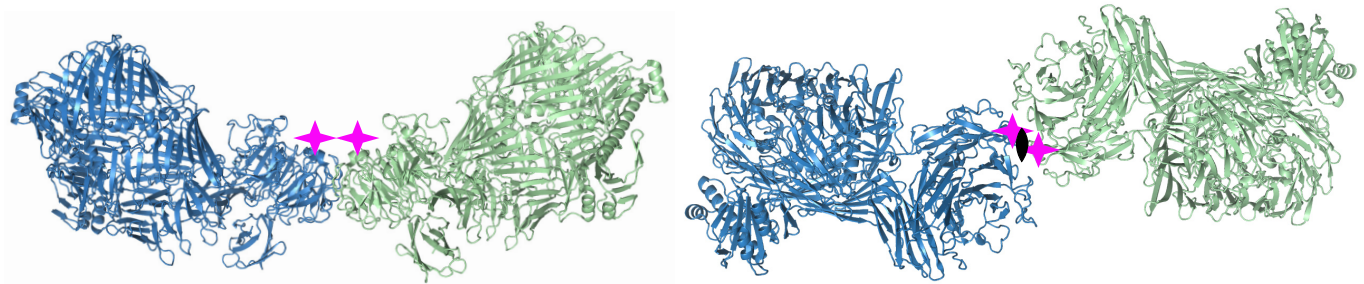
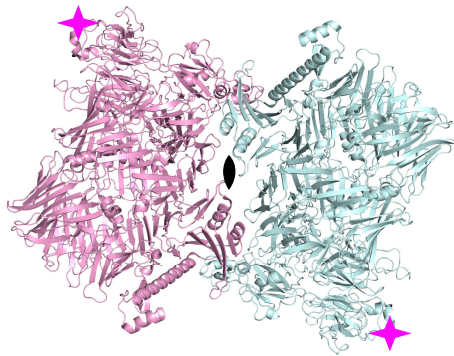


Figure EV4.

A *D. melanogaster* Ten-m**B** *G. gallus* Ten2**C** *H. sapiens* Ten4**Figure EV5. Comparing available TEN oligomer structures.**

PyMol was used to generate cartoon representations of TEN oligomerization.

A The Ten-m self-association interface (this manuscript, PDB 8FIA).

B The ggTen2 dimer (PDB 6FB3). Individual monomers are shown in contrasting colors. The vertebrate-specific splice site in the β -propeller domain is shown as a pink star.

C the hTen4 dimer (PDB 7BAM). Symmetry operators which generate each presented interface are shown, with a 2_1 screw axis for Ten-m self-association, and twofold axes for ggTen2 and hTen4 dimers.