Expanded View Figures

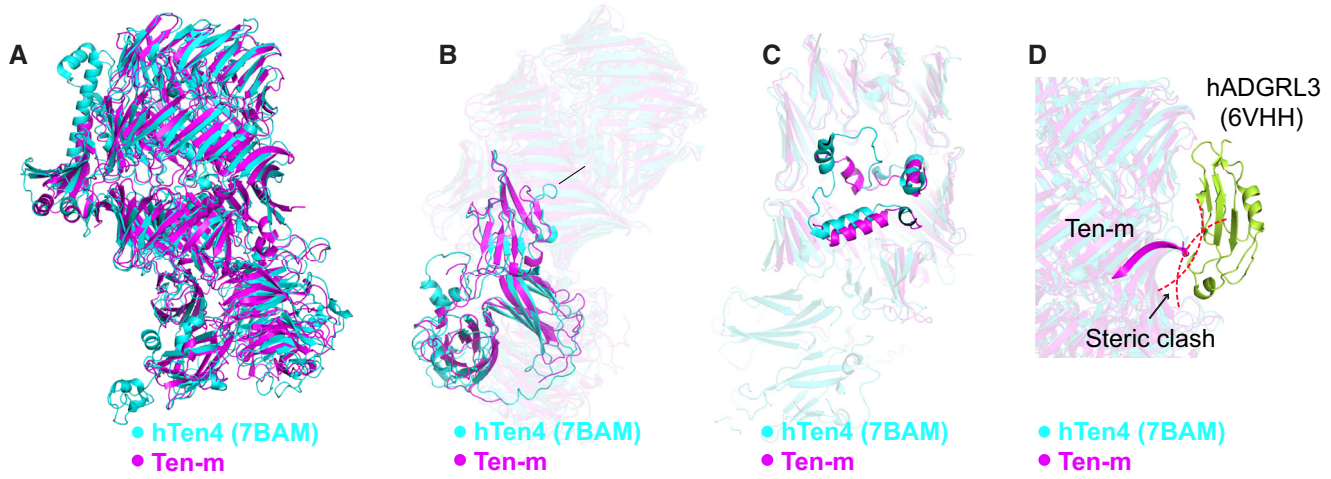
Figure EV1. Structural and sequence differences between TENs.

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).



E

	Ten-m	hTen4	ggTen2	hTen2	mTen3
Ten-m	0	5.9	5.8	5.7	5.1
hTen4	5.9	0	2.5	3.1	2.5
ggTen2	5.8	2.5	0	2.0	1.9
hTen2	5.7	3.1	2.0	0	2.4
mTen3	5.1	2.5	1.9	2.4	0

all-atom RMSD (Å)

F

	Ten-m	Ten-a	ceTen-1	zfTen2	ggTen2	mTen2	hTen2
Ten-m	100	50	27	34	35	35	35
Ten-a	50	100	29	40	40	41	41
ceTen-1	27	29	100	27	27	27	27
zfTen2	34	40	27	100	77	78	78
ggTen2	35	40	27	77	100	92	93
mTen2	35	41	27	78	92	100	98
hTen2	35	41	27	78	93	98	100

full length sequence identity (%)

G

	Ten-m	Ten-a	ceTen-1	zfTen2	ggTen2	mTen2	hTen2
Ten-m	100	57	42	49	51	53	51
Ten-a	57	100	39	50	44	45	44
ceTen-1	42	39	100	48	37	36	37
zfTen2	49	50	48	100	88	88	91
ggTen2	51	44	37	88	100	95	97
mTen2	53	45	36	88	95	100	96
hTen2	51	44	37	91	97	96	100

TTR sequence identity (%)

H

	Ten-m	Ten-a	ceTen-1	zfTen2	ggTen2	mTen2	hTen2
Ten-m	100	50	30	43	40	41	40
Ten-a	50	100	27	40	41	41	42
ceTen-1	30	27	100	33	31	31	30
zfTen2	43	40	33	100	77	77	77
ggTen2	40	41	31	77	100	91	93
mTen2	41	41	31	77	91	100	97
hTen2	40	42	30	77	93	97	100

Ig-like sequence identity (%)

I

	Ten-m	Ten-a	ceTen-1	zfTen2	ggTen2	mTen2	hTen2
Ten-m	100	50	33	41	42	43	43
Ten-a	50	100	31	39	40	40	40
ceTen-1	33	31	100	33	32	32	32
zfTen2	41	39	33	100	87	86	86
ggTen2	42	40	32	87	100	96	97
mTen2	43	40	32	86	96	100	98
hTen2	43	40	32	86	97	98	100

β-propeller sequence identity (%)

J

	Ten-m	Ten-a	ceTen-1	zfTen2	ggTen2	mTen2	hTen2
Ten-m	100	32	23	28	29	29	29
Ten-a	32	100	23	27	29	29	29
ceTen-1	23	23	100	23	22	23	23
zfTen2	28	27	23	100	78	79	79
ggTen2	29	29	22	78	100	95	95
mTen2	29	29	23	79	95	100	99
hTen2	29	29	23	79	95	99	100

β-barrel sequence identity (%)

K

	Ten-m	Ten-a	ceTen-1	zfTen2	ggTen2	mTen2	hTen2
Ten-m	100	28	22	28	24	27	27
Ten-a	28	100	20	33	30	28	28
ceTen-1	22	20	100	32	28	28	28
zfTen2	28	33	32	100	67	69	70
ggTen2	24	30	28	67	100	90	91
mTen2	27	28	28	69	90	100	99
hTen2	27	28	28	70	91	99	100

toxin-like sequence identity (%)

Figure EV1.

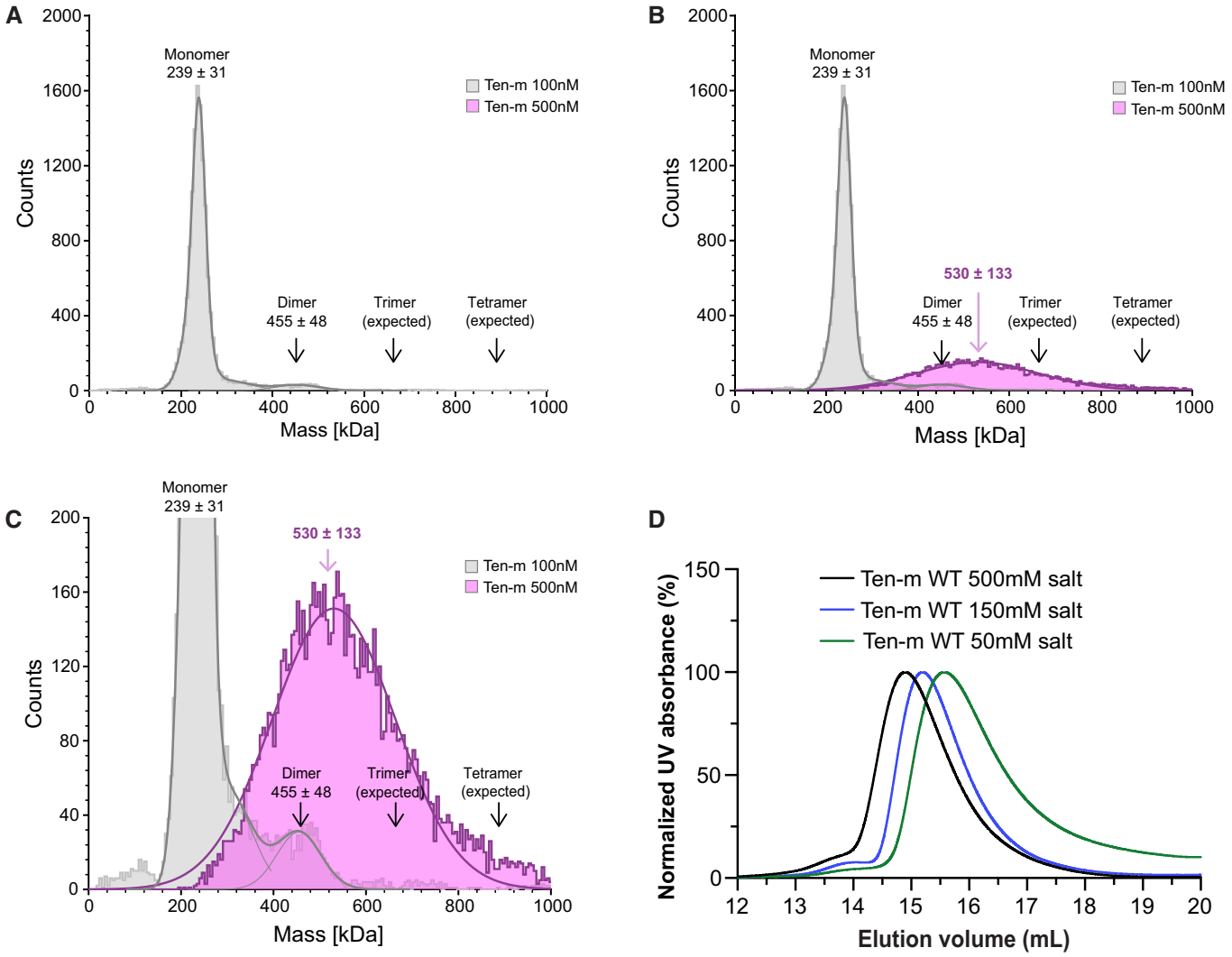


Figure EV2. Further biophysical studies on Ten-m self-association using mass photometry and size exclusion chromatography.

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, $N = 1$.

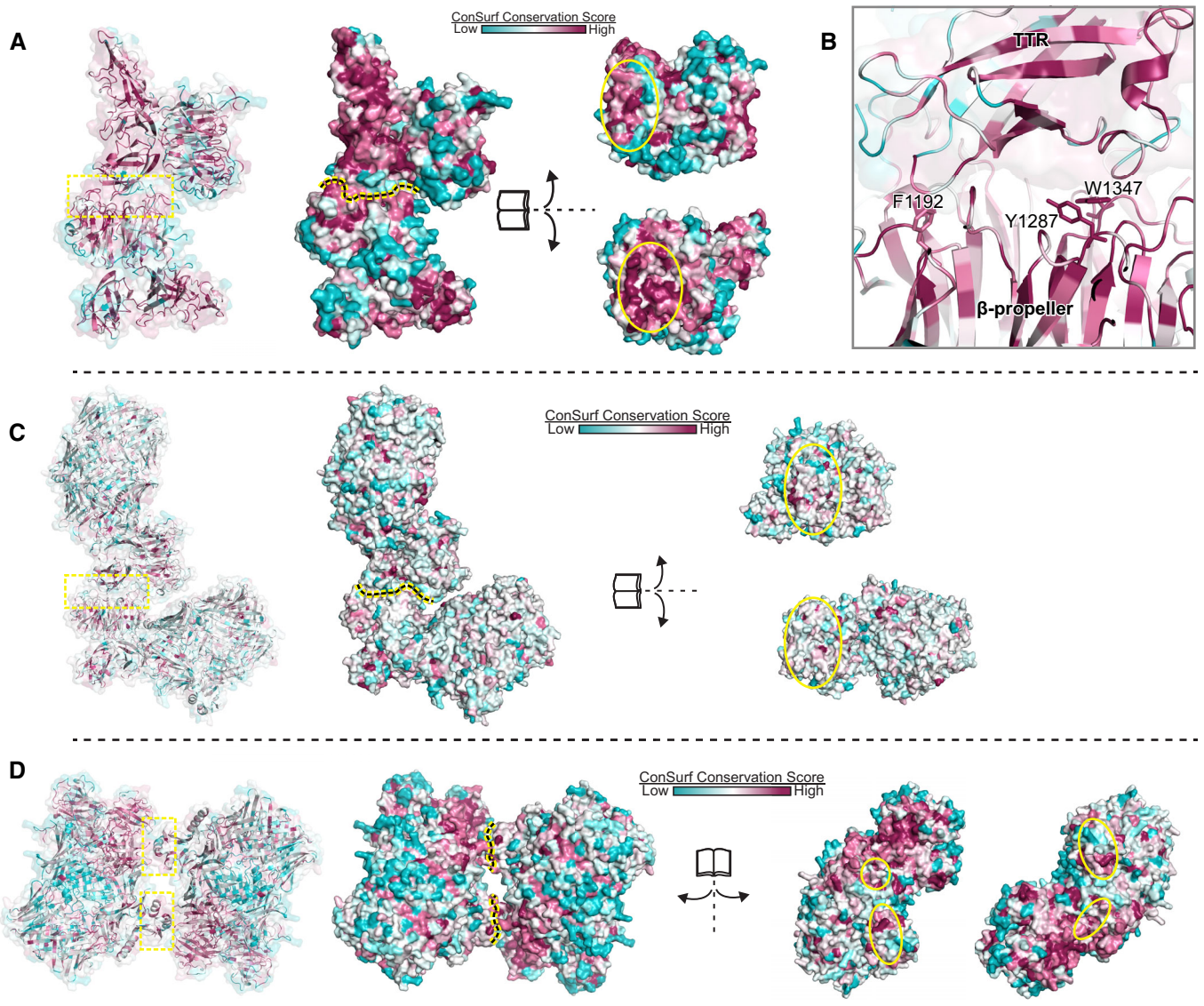


Figure EV3. Sequence conservation of the Ten-m self-association interface.

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.

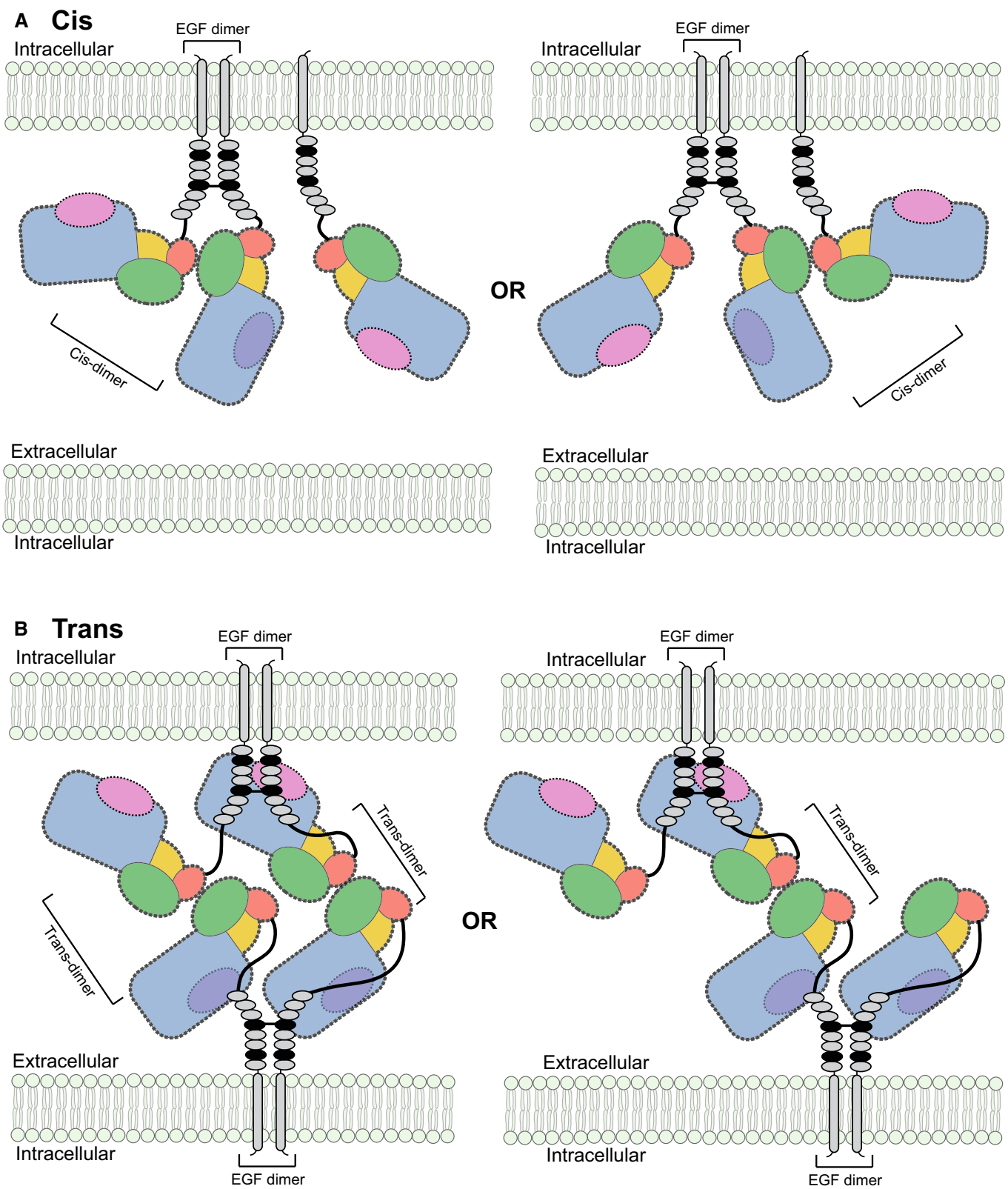


Figure EV4.

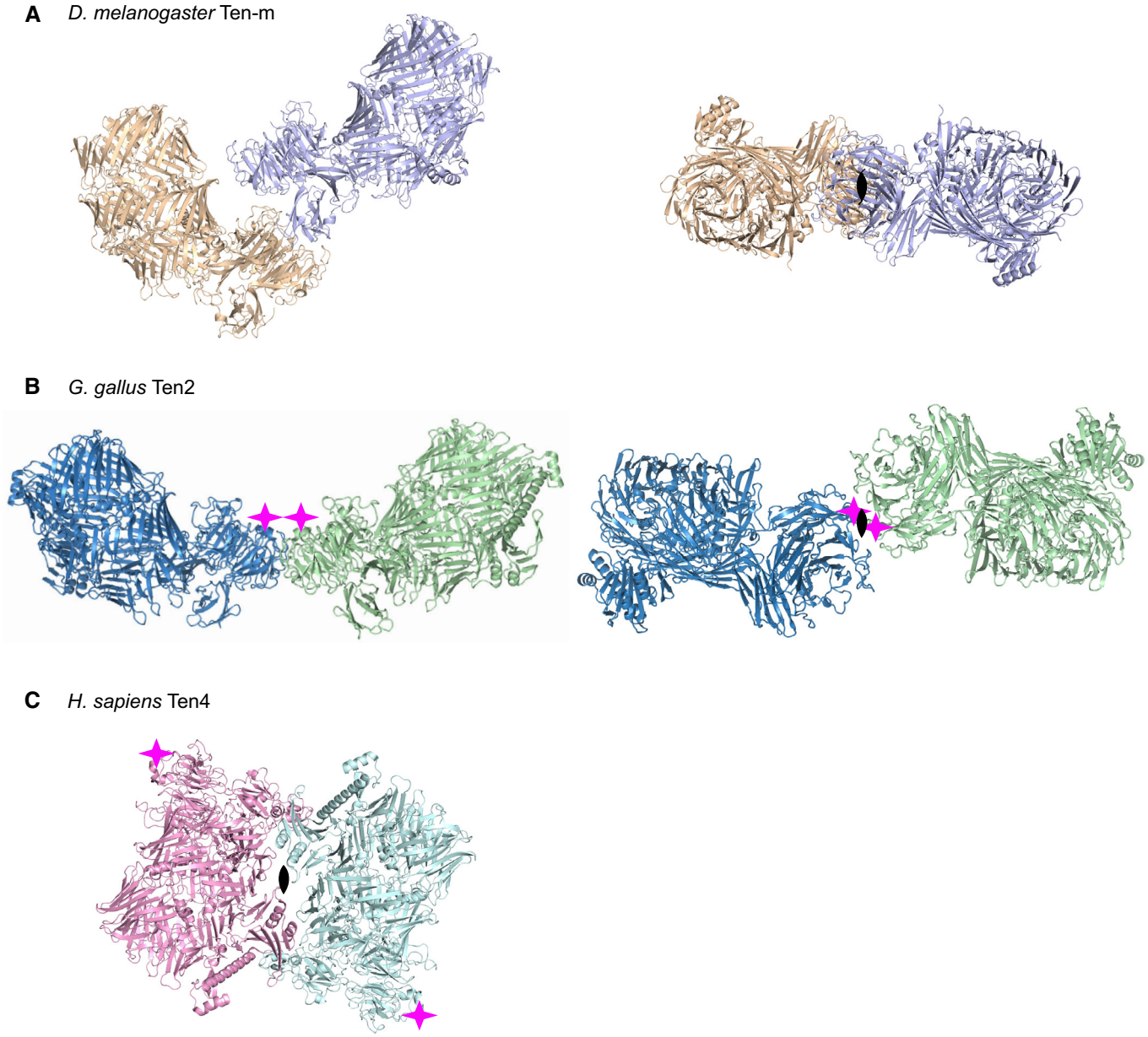


Figure EV5. Comparing available TEN oligomer structures.

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.