

Pseudo-Symmetric Assembly of Protodomains as a Common Denominator in the Evolution of Polytopic Helical Membrane Proteins

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SUPPLEMENTARY INFORMATION

MFS	5EQI	https://d55qc.app.goo.gl/wcE7nXES2yhE4CQr6
TRIC	5WUF 3KCU	https://d55qc.app.goo.gl/XUkPY7iPmR8LVJ8c9 https://d55qc.app.goo.gl/srgkH59q3ADhRSTS8
AQP1	3NE2	https://d55qc.app.goo.gl/pN9hZKMbiS4VML5H6
SemiSWEET Apo vs. Ligand	4QNC 4QND	https://d55qc.app.goo.gl/i9vWJfzXEcl8q2A69
SWEET vs. SemiSWEET	5CTH 4QND	https://d55qc.app.goo.gl/9NfEK6KmideLHYt1A Use keyboard "a" letter or "Alternate" command to visualize alternatively the 2 aligned structures of SWEET (PDB:5CTH) vs. 3TMH monomer A of SemiSWEET (PDB:4QND) (aligned only on 3TMH protodomain 1) Use "a" alternate command to alternate between structures
PnuC	4QTN	https://d55qc.app.goo.gl/S1atQgdGptdt7bCs6
GPCR C	4OR2	https://d55qc.app.goo.gl/ZFChMCsBRER3uSbD7
GPCR A α	5G53	https://d55qc.app.goo.gl/igoFR7sh9hZdjK1L9
Rhodopsin	1GZM	https://d55qc.app.goo.gl/KtAe6nkkSJ7fm3aHA
Rhodopsin active-inactive	6CMO 1GZM	https://d55qc.app.goo.gl/KLJg7G6Zx3g9hju4A
pLGIC AcetylCholine Receptor	6CNK	https://icn3d.page.link/Knzy

Table S1 - 3D visualization links - using iCn3D

	TM1	TM2	2b	TM3	3TMH	RMS (Å)
sequence length aligned (5CTH)	20	18		25	63	
SWEET 123 567 (# res. identical)	3	5		5	13	
%Id	15%	28%		20%	21%	1.36
vs. SemiSWEET (4QNC/4QND)	0/6(*)	3		4	7/13	
%Id	0/30%	17%		16%	11/21%	1.98
length aligned (4QTN)	13	42		23	78	
PnuC 123 vs 567	1	6		4	11	
%Id	8%	14%		17%	14%	1.26
length aligned (5WUF)	23	23		16	62	
TriC 123 vs 456	8	10		4	22	
%Id	35%	43%		25%	35%	1.53
length aligned (4FC4)	21	18	13	23	75	
FocA 123 vs 456	4	3	1	8	16	
%Id	19%	17%	8%	35%	21%	1.37
length aligned (5DYE)	24	25	10	19	78	
Aquaporin 123 vs 456	4	5	4	4	17	
%Id	17%	20%	40%	21%	22%	1.92
length aligned (3C02)	24	25	14	19	82	
Aquaglyceroporin 123 vs 456	5	5	3	4	17	
%Id	21%	20%	21%	21%	21%	1.92
length aligned (4FC4-1 / 5DYE-2)	21	17	12	23	73	
FocA vs. Aquaporin	3	4	0	8	15	
%Id	14%	24%	0%	35%	21%	2.94
length aligned (5EQI)	18	18		18	54	
MFS 123 vs 456 (protos1-2)	4	3		2	9	
%Id	22%	17%		11%	17%	1.70
vs 789 (protos1-3)	2/4(**)	1/6(**)		4		
%Id	11/22%(**)	6/33%(**)		22%	13/26%	1.88/2.97(**)
vs 10-11-12 (protos1-4)	3	1		2		
%Id	17%	6%		11%	11%	2.32
Average	19%	21%	17%	22%	20%	1.81
Min	8%	6%	0%	11%	11%	1.26
Max	35%	43%	40%	35%	35%	2.94

Table S2 - Protodomain alignment statistics for SWEET, PnuC, TriC, FoCA, Aquaporin, and MFS

(*) a shift of 3 in sequence will match SPLA sequence in SWEET proto2, part of the FxSP motif in TM1/5 conserved in SWEET protos

(**) A 4 protodomains optimized multiple structure alignment. A shift in sequence vs optimized structure alignment would for example increase the sequence match from 1 to 6 in TM2 for an overall RMS change from 1.88 Å to 2.97 Å

GPCRs	TM1/5	TM2/6	TM3/7	3TMH	RMS (A)	Symmetry	TM1/4	TM2/5	TM3/6	3TMH	RMS (A)	Symmetry
length aligned	21	29	27	77			21	29	27			
1F88 123 vs 567 (# res identical)	4	4	7	15		Rhodopsin	2	4	2			
%Id	19%	14%	26%	19%	3.24	SYM	10%	14%	7%	10%	7.73	NO SYM
length aligned	28	21	21	70			28	21	21			
4OR2 123 vs 567	5	0	4	9		Class C	3	1	0			
%Id	18%	0%	19%	13%	3.36	SYM	11%	5%	0%	5%	4.89	NO SYM
length aligned	22	21	24	67			22	21	24			
4MBS 123 vs 567	6	0	3	9		Class A	3	4	1			
%Id	27%	0%	13%	13%	2.62	SYM	14%	19%	4%	12%	6.78	NO SYM
length aligned	28	29	28	85								
4GRV 123 vs 567	6	2	7	15		Class A						
%Id	21%	7%	25%	18%	3.31	SYM						
length aligned	22	25	20	67								
3RZE 123 vs 567	4	0	4	8		Class A						
%Id	18%	0%	20%	12%	3.38	SYM						
length aligned	24	24	18	66								
3OE9 123 vs 567	5	3	1	9		Class A						
%Id	21%	13%	6%	14%	3.38	SYM						
length aligned	24	26	20	70			16	20	20	56		
4JKV 123 vs 567	6	2	2	10		Class F	2	3	3	8		
%Id	25%	8%	10%	14%	2.39	SYM	13%	15%	15%	14%	5.23	NO SYM
length aligned	25	22	22	69			16	28	21	65		
5EE7 123 vs 567	2	2	3	7		Class B	1	3	1	5		
%Id	8%	9%	14%	10%	2.56	SYM	6%	11%	5%	7%	6.49	NO SYM
length aligned	23	16	25	64								
4EIY 123 vs 567	1	2	4	7		Na ⁺ binding						
%Id	4%	13%	16%	11%	2.68	SYM						
length aligned	17	24	17	58								
1UAZ ABC vs EFG	3	6	0	9		Bacteriorhodopsin						
%Id	18%	25%	0%	16%	2.56	SYM						
Average (GPCRs)	18%	7%	16%	14%	2.99		11%	13%	4%	9%	6.47	
Min	4%	0%	6%	10%	2.39	SYM	10%	5%	0%	0%	5%	4.89
Max	27%	14%	26%	19%	3.38		14%	19%	0%	7%	12%	7.73

Table S3 - Protodomain alignment statistics for GPCRs (and Bacteriorhodopsin). [Left] Sequence Identity and RMSD of the two 3TMH TM123/TM567 protodomains matching symmetrically and individual matching transmembrane helices. [Right] Protodomain 1 TM123 vs 3TMH TM456 for comparison (not related symmetrically).

Odds Ratio	Motif1: S3.39		Motif1: D2.50	
	Motif2: NS7.46		Motif2: FxxxW6.48	
GPCR-Class	Aα	Aγ	Aα	Aγ
Humans	76.8	12.3	24.6	-
Mammals	29.0	21.3	9.6	2.6
Vertebrates	52.4	16.9	3.1	2.6

Table S4: Odds-Ratio of three pairs of co-evolutionary related residues/motifs, calculated using the GPCR-SAS server [<http://lmc.uab.cat/gpcrsas/>], Tamayo et al. (2018) *PLoS One*, **13**(7):e0199843] for available GPCRs from humans, mammals, and vertebrates. Numbers following the motifs are the BW numbers of the only residue or last residue in the motif.

Pairwise protodomain alignments

SWEET 1/2/3	5CTH_B	ag l ag N I F A L A L F L S P V T T F K R I L K a k s t ~ ~ e r f d G L P Y L F S L L N C L I C L W Y G L p w v a d g r L L V A T V N G I G A V F Q L A Y I C L F I F Y A d s
SWEET 5/6/7	5CTH_B	v g a v s M A S L I S M F A S P L A V M G V V I R s e s v ~ ~ e f m p F Y L S L S T F L M S A S F A L Y G L l l ~ ~ ~ r d F F I Y F P N G L G L I L G A M Q L A L Y A Y Y S s n
SemiSWEET	4QND_A	I e p I m L V M G L I S P L A T M P Q L Y K L Y V h s e h a g l s L T T W L L Y S F I A L L W T I Y G I y h ~ ~ ~ k n P T I W V G N C L G F L M Y V A M V G I I A H T g g
Pnuc 1/2/3	4QTN_A	v w L L X F L G I Q A V V F V f n ~ ~ ~ ~ p d S W L A S V A A V T G I L C V V F V G K G K I S N Y L F G L I S V S L Y A Y V S Y T F k ~ ~ ~ ~ Y G E X X L N L I V Y V P V Q F V G F A X W R K h x:
Pnuc 5/6/7	4QTN_A	I v V A A S V V G T S V Y I E w l h h l g s a l P T L D G V T V V V S I V A Q V L x I L R Y R E Q W A L W I V V N I L T I S L W A V A w f k n g e t s L P L L L X Y V ~ X Y L C N S V Y G Y I N W T K I v l
TRIC 1/2/3	SWUF_A	~ x n d F L F Y L D I F G V I V F A L S G A L X A G R Y Q L D P F G V V V V L A S V T A V G G G T I R D V I L Q T P V F W V E K P Y Y L Y V I L A T A I L T I V L i r q p k r i p
TRIC 4/5/6	SWUF_A	i p k r F L L I A D A L G L A L F A V L G T Q K A L Y L G A P I P V A V V L G T I T G I A G G X I R D V L C N V I P X I L R E E I Y A L A A X L G G S L F I I L h g l n w n d t
Foca 1/2/3	4FC4_A	I g F W V S S A M A G A Y V G L G I I L I F T L g n l l d p s v r p L V M G A T F G I A L T L V I I A G s e L F T G H T M F L T L G v k a g t i s ~ ~ h q q w a i L P Q T W L G N L V G S V ~ F V A L L Y S W G G g s
Foca 4/5/6	4FC4_A	t v L F F K G A L C N W L V C L A I W M A I R T e ~ ~ ~ ~ ~ g T A K F L A I W C L L A F I A S G y e H S V A N M T L F A L S w f g h h s d a y t l a g i g h n L L W V T L G N T L S G V v F M G L G Y W Y A T p k
Aqp1 1/2/3	5DYE_A	c s v a F L K A V F A E F L A T L I F V F F G L G S A L k w p s a l p t i I Q I A L A F G L A I G T L A Q A L G P V S G G H I N P A I T L A L L V g n q i s I I r a F F Y V A A Q L V G A I A G A G I L Y g v a p
Aqp1 4/5/6	5DYE_A	n n t t Q G Q A M V V E L I L T F Q L A L C I F A S T D s r r t ~ ~ e p v g S P A L S I G L S V T L G H L V G I Y F T G C S M N P A R S F G P A V v n r f s p a h W V F W V G P I V G A V L A A I L Y F Y I l f
Foca 1/2/3	4FC4_A	I g F W V S S A M A G A Y V G L G I I L I F T L g n l l d p s v r p L V M G A T F G I A L T L V I I A G s ~ ~ ~ ~ ~ e L F T G H T M F L T L G v k a g t i s h q q w a i L P Q T W L G N L V G S V F A L L Y S W G G g s
Aqp 4/5/6	5DYE_A	g q A M V V E L I L T F Q L A L C I F A S T D s r r t ~ ~ ~ e p v g S P A L S I G L S V T L G H L V G I y f t g c s N P A R S F G P A V V M n r f ~ ~ ~ ~ ~ s p a H W V F W V G P I V G A V L A A I L Y F Y L l f p
GApq1/2/3	3C02_A	y k S Y V R E F I G E F L G T F V L M F L G E G A T A n f t t g l s g d w Y K L C L G W G L A V F F G I L V S A K L S G A H L N L A V S I G L S S I n k f d ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ l k k I P V Y F F A Q L L G A F V G T S T V Y ~ G L y h
GApq4/5/6	3C02_A	s i S L T G A F F N E L I L T G I L L L V I L V V V D e n i c g ~ ~ k f h i L K L S S V V G L I I L C I G I T F G G G N T G F A L N P S R D L G S R F L s l i a y g k d t f t k d n f y F W V P L V A P C V G S V V F C Q F Y D k v i c p
MFS 1/2/3	SEQI_A	I L A V G G A V L G S L q ~ ~ F G Y N T G V i n a p q k v i e e f y n q t w v h r y g e s i l p t l t l w s l s V A I F S V G G M I G S F S V G L F V n r f ~ ~ ~ ~ g r r N S M L M N N L L A F V S A V L M G F S k l
MFS 4/5/6	SEQI_A	f e M L I L G R F I I G V y ~ ~ C G L T T G F v p y v g e v s ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ p t a l r g a l G T L H Q L G I V V G I l l I A Q V F G l d s i g n k d l w p L L L S I I F I P A L L Q C I V L P F C p e
SEQI_A	SEQL_A	p i L I A V V L Q L S Q Q l s g I N A V F Y Y s t s i f e k a ~ g v q q p v y A T I G S G I V N T A F T V V S L F V v e r a ~ ~ ~ ~ ~ ~ g r r T L H L I G L A G M A G C A I L M T I A l a
	SEQL_A	s y L S I V A I F G F V A f ~ ~ F E V G P G P i p w f i v a e l f s ~ g p Y V F I I F T V L L V L F F I F T Y F K v p

Figure S1 - Pairwise alignment of protodomains of: SWEET protodomains 1.36 Å RMS / 21 % Id (5CTH) vs. **SemiSWEET 1.98 Å RMS / 16% Id (4QND), **PnuC**: 1.26 Å RMS / 13% Id (4QTN), **TriC**: 1.53 Å RMS / 34% Id (5WUF), **FocA**: 1.83 Å RMS / 20% Id (4FC4), **Aqp1**: 1.36 Å RMS / 24% Id (5DYE), An example of surprising sequence/structure homology between **FocA (4FC4) protodomain 1** vs. **Aqp (5DYE) protodomain 2**: 2.94 Å RMS / 34% Id and 18 %Identity . **MFS** 1.70 Å RMS / 17% Id on first 2 protodomains and 1.70 Å RMS / 17% Id on protodomains 3&4 (5EQI) - after optimisation of protodomain boundaries for best structural match.**

Protodomain alignments (pairwise, familywise and across two families FocA and Aquaporin)

FocA 1/2ab/3	4FC4_A	I g FWVSSAMAGAYVGLGII LIFTgnlld~~~psvrpLVMGATFGIALTLVIIAGseLFTGHTMFLTLGvkag tis~~~~~hgq waiLPQTWLGNLVGSV~FVALLYSWGGg s l
FocA 4/5ab/6	4FC4_A	t vLFFKGALCNWLVC LAI WMAIRte~~~~~gTAKFLAIWCCLALAFIASGYeHSVANMTLFA LSwfghhsday~~~~~t lagighnLLWVTLGNTLSGVvFMGLGYWYATpk s
FocA 1/2ab/3	3KLZ_A	y kSFLLAISAGIQIGIAFVFYT VVttgahd pygvtkLLGGLAFSLGLILV VITGgeLFTSSV LILVAKasgk i sw~~~~~ke lvrnWTVVYFGNLCGS I~ILVFIMLATRqf
FocA 4/5ab/6	3KLZ_A	l qAFALGLMCN I LVCLAVWMTFSars~~~~~lTDKVMVLILPVAMFVSSGf eHClIANMFQVPMAigikyfapesfwa tgani aqyadlnfvnf vnnLIPVTLGNIVGGG~VFVG MWY WLiy l
FocA 1/2ab/3	3KCV_A	l kTFYLAITAGVFISIAFVFYIATTgtgt pfg akLVGGICFSLGLILC VVCGadLFTSTV LIVVAKasgr i tw~~~~~gqlaknWLNVYFGNLVGAL~LFVLLMWLSGey
FocA 4/5ab/6	3KCV_A	l eAVCLGILANLMVCLAVWMSYSGr s~~~~~IMDKAFIMVLPVAMFVASGfeHSIANMF MIPMGivirdfaspe fwtavgsapenfshltv nfitdnLIPV TIGNI IGGG~LLV GLTYWVi y l
Aqp 1/2ab/3	5I32_A	I a SRLAYLAEFISTLLFV FAGVGSAIayak l tsda~~~~~al dtpg l vIAVCHG FALFVAVAIGANI SGHHVNPAVTFGLAVggqi~~~~~t vi TG VFYWI AQLLGSTAACFLKKYVTgg l av
Aqp 1/4/5ab/6	5I32_A	I g SIEGVVME I I ITFALVYTVYATAAdpkkg~~~~~slgtiAPLAIGLIVGANI LAAGP FSGGSMNPARSFGPAVAag~~~~~dfsGHWVYVWVGP LIGGGLAGLIYGNVF gss e
Aqp 1/2ab/3	3NE2_A	t LAKRFTAEVVGTFLVFFGPGAAVitl i angadkpnefnigigalggldwfaIGMAFALAI AAVIYSLGRISGAHINPAV TIALWSi grf~~~~~ppgEVV PYI VAOQFIGAALGSSLFLFACVgpaaa
Aqp 1/4/5ab/6	3NE2_A	l gYQQAI LT E AIGTFLMLVIMGVAVVdera~~~~~ppgFAGLVIGLT VGGI ITTIGNITGSSLNPARTFGPYLgdsl g inlwqYFP I VGP IVGAVAAA WLYNLYLake
Aqp 1/2ab/3	5DYE_A	vaFLKAVFAEFLATL I FVFFGCLGSALKwp s~~~~~lpti l QI ALAFGLAIGTLAQALGPGVSGHINPAITALLVNg nqi~~~~~s l RAFFYVVAQQLVGAIAGAGI LYGVAp l n ar
Aqp 1/4/5ab/6	5DYE_A	t tQQQAMVVELI LT FQLALCI FASTD srrt~~~~~epvgSPALSIGLSVTLGHLVG I YFTGCSMNPARSFGPAVv nr~~~~~fspAHWVFWVGP I V GAVLA AILYFYLLfpns l
FocA 1/2ab/3	4FC4_A	I g FWVSSAMAGAYVGLGII LIFTgnlld~~~psvrpLVMGATFGIALTLVIIAGs~~~~~el FGTGHTMFLTLGvkag tis~~~~~hgq waiLPQTWLGNLVGSV~FVALLYSWGGg s l
FocA 4/5ab/6	4FC4_A	t vLFFKGALCNWLVC LAI WMAIRte~~~~~gTAKFLAIWCCLALAFIASGYeHSVANMTLFA LSwfghhsday~~~~~t lagighnLLWVTLGNTLSGVvFMGLGYWYATpk s
FocA 1/2ab/3	3KLZ_A	y kSFLLAISAGIQIGIAFVFYT VVttgahd pygvtkLLGGLAFSLGLILV VITGgeLFTSSV LILVAKasgk i sw~~~~~ke lvrnWTVVYFGNLCGS I~ILVFIMLATRqf
FocA 4/5ab/6	3KLZ_A	l qAFALGLMCN I LVCLAVWMTFSars~~~~~lTDKVMVLILPVAMFVSSGf eHClIANMFQVPMAigikyfapesfwa tgani aqyadlnfvnf vnnLIPVTLGNIVGGG~VFVG MWY WLiy l
FocA 1/2ab/3	3KCV_A	l kTFYLAITAGVFISIAFVFYIATTgtgt pfg akLVGGICFSLGLILC VVCGadLFTSTV LIVVAKasgr i tw~~~~~gqlaknWLNVYFGNLVGAL~LFVLLMWLSGey
FocA 4/5ab/6	3KCV_A	l eAVCLGILANLMVCLAVWMSYSGr s~~~~~IMDKAFIMVLPVAMFVASGfeHSIANMF MIPMGivirdfaspe fwtavgsapenfshltv nfitdnLIPV TIGNI IGGG~LLV GLTYWVi y l
Aqp 1/2ab/3	5I32_A	I rAYLAEFISTLLFV FAGVGSAIayak l tsda~~~~~al dtpg l vIAVCHG FALFVAVAIGANI SGHHVNPAVTFGLAVggqi~~~~~t vi TG VFYWI AQLLGSTAACFLKKYVTgg l av
Aqp 1/2ab/3	5I32_A	I eGVVME I I ITFALVYTVYATAAdpkkg~~~~~slgtiAPLAIGLIVGANI LAAGP FSGGSMNPARSFGPAVAag~~~~~dfsGHWVYVWVGP LIGGGLAGLIYGNVF gss e
Aqp 1/4/5ab/6	3NE2_A	akRFTAEVVGTFLVFFGPGAAVitl i angadkpnefnigigalggldwfaIGMAFALAI AAVIYSLGRISGAHINPAV TIALWSi Grf~~~~~grgVV PYI VAOFIGAA-LGSLLFLACVgp
Aqp 1/2ab/3	3NE2_A	g QAI LT E AIGTFLMLVIMGVAVVdera~~~~~ppgFAGLVIGLT VGGI ITTIGNITGSSLNPARTFGPYLgdsl g inlwqYFP I VGP IVGAVAAA WLYNLYLake
Aqp 1/4/5ab/6	5DYE_A	IKAVFAEFLATL I FVFFGCLGSALKwp s~~~~~lpti l QI ALAFGLAIGTLAQALGPGVSGHINPAITALLVNg nqi~~~~~s l RAFFYVVAQQLVGAIAGAGI LYGVAp l n ar
Aqp 1/2ab/3	5DYE_A	g QAMVVELI LT FQLALCI FASTD srrt~~~~~epvgSPALSIGLSVTLGHLVG I YFTGCSMNPARSFGPAVv nr~~~~~spAHWVFWVGP I V GAVLA AILYFYLLfp
Aqp 4/5ab/6	3C02_A	V F E FIGEFLGTFLVLMFLGEGATAnfttg~~~~~l sgdwyK1CLGWGLAVFGI LVSaki s gah INLA V S I GLSSINK fd~~~~~lkk I PVYFFAQQLLGAF~VG STVYGLYng
Aqp 4/5ab/6	3C02_A	t gAFFNEILTG I LLV LIVVVDen icg~~~~~kfh I K LSSVVG L I LCIGITFggntgfa INPSRDLGSRFLSI l a y gkd t~~~~~ft kdnfyFWVPLVACPGSV~VFCQFYDKV1cp

Figure S2 - Multiple alignment of FocA and Aquaporin protodomains. FocA (PDB: 4FC4, 3KLZ, 3KCV), AQP (PDB: 3NE2, 5I32, 5DYE, 3C02)

Protodomains RMSD optimized within the FocA family 1.88 Å (4FC4), 1.17 Å/2.11 Å (3KLZ), 1.55 Å/2.06 Å (3KCV), highlighting for each pair of protodomains in a structure the conserved residues in RED. Some residues are conserved at the domain level, and at the family level. Foca show a partial “internal” conservation between TM3 and TM6 at the family level, with especially a [G]NxxG[G] motif, while within each individual representative, internal” homology is significant. A larger sequence alignment of the Pfam01226 family confirms that motif.

Protodomains RMSD optimized within the Aquaporin family 1.37 Å (5I32), 0.80 Å/1.20 Å (3NE2), , 0.78 Å/1.58 Å (5DYE), highlighting for each pair of protodomains in a structure the conserved residues in RED. Some residues are conserved at the domain level, and at the family level. Aquaporins has retained a higher internal homology between protodomains than FocA, especially with its NPA motif in TM2b and TM5b at the family level.

Protodomains RMSD across FocA and Aquaporin families. The first protodomain of **FocA vs. other FocA protodomains** 1.88 Å (4FC4), 1.18 Å/2.05 Å (3KLZ), 1.54 Å/2.00 Å (3KCV) **vs. AQP protodomains** 2.17 Å/2.82 Å (5I32), 2.25 Å/2.43 Å (3NE2), 2.33 Å/2.94 Å (5DYE), 2.21 Å/2.71 Å (3C02). While the structure match is very good between any representative of these two families FocA and Aquaporin, the sequence match is rather poor overall, yet the FocA TM3 motif region shows some overlapping homology with AQP [G/A]xxx[G/S][G/A/S] motif. See Figure S10 for sequence vs. structure similarities.

Pairwise GPCR protodomain alignments

Class:TM

A:1/2/3 A:5/6/7	IF88_A q F S M L A A Y M F ~ L L I M L G F P I N F L t l y v t v q ~ ~ ~ ~ ~ h k k l r t P L N Y I L L N L A V A D L F M V F G G F T T L Y T S L H g y f v F G P T G C N L E G F F A T L G G E i a i W S L V V L A I E R IF88_A n N E S F V I Y M F v V H F I I P L I V I F F c y g q l v f t v k e a a a q q q e s a t t q k a e k E V T R M V I I M V I A F L I C W L P Y A G V A F Y I F T H q g s d F G P I F M T I P A F F A K T S A V ~ ~ ~ Y N P V I Y I M M n
A:5/6/7 A:1/2/3	2X72_A t n N E S F V I Y M F v V H F I I P L I V I F F C Y g q l v f t v k e a a a q q q e s a t t q k a e k e v t r m V I I M V I A F L I C W L P Y A G V a f y i f t h q g s c F G P I F M T I P A F F A K t s a v y n p v i y i m m n k q f 2X72_A w q F S M L A A Y M F ~ L L I M L G F P I N F L t l y v t v q h k k ~ ~ ~ ~ ~ l r t p i n y i l L N L A V A D L F M V F G G F T T l y t s l h g y f v F G P T G C N L Q G F F A l g g e i a l w s l v v l a i e r
A:5/6/7 A:1/2/3	6CMO_R p w q F S M L A A Y M F L L I V L G F ~ P I N F L T L Y V T V Q h k k ~ ~ ~ ~ ~ i r t P L N Y I L L N L A V A D L F M V L G G F T S T L Y T S L H g y f v F G P T G C N L Q G F F A t l g g e i a l w s l v v l a i e r 6CMO_R e v n N E S F V I Y M F v V H F T I p m I I I F F C Y G Q L V F t v k e a a a q q q e s a t t q k a e k E V T R M V I I Y V I A F L I C W V P Y A S V A F Y I F T H q g s c F G P I F M T I P A F F A K S A A I Y N P V I Y I M M n k q f
BR:A/B/C BR:E/F/G	IUAZ_A a r y t W W L F S T I C M I V V L Y F L A t s l r a a a k e r g p e v A S T F N T L T A L V L V L W T A Y P I L W I I G t e g a g v v ~ ~ g l g i e t L L F M V L D V T A K V G F G F I I l r s IUAZ_A p e t l W L G I G T L L M L I G T F Y F I v k g w g ~ ~ ~ v t d k e A R E Y Y S I T I L V P G I A S A A Y L S M F F G i g l t e v q v g s e l d i y Y A R Y A D W L F T T P L L L D I a l l
A:1/2/3 A:5/6/7	ZZYI_A y y S L G I F I G ~ I C C I I G C G G N G I V I Y L F T k t k s l ~ ~ ~ ~ ~ ~ ~ ~ ~ q t p a N M F I I N L A F S D F T F S L V N G F p l t i s c f l k k w i F G F A A C K V Y G F I G G I F G F M S I M T M A M I S i d ZZYI_A t r S N I L C M F I L G F F G P I L I I F F C Y F N I V s v s n h e k e a a a k r l n a k e l r k a q a g a n e r l A K I S I V I V S Q F L L S W S P Y A V v a l l a q ~ f g p l e w V T P Y A A Q L P V M F A K A S A I H N P M I Y S V S H p k
B:1/2/3 B:5/6/7	SEE7_A f w W I L R F P V F L A I L I N F F I F V R I V Q L L v a k l r a r q h h t d y a f R L A K S T L T L I P L L G V H F V V F A F v t d e h a ~ ~ ~ ~ ~ ~ ~ ~ ~ q g t l R S A K L F F D L A L S S F Q ~ G L L V A V L Y C F I n l SEE7_A f q V M Y T V G Y S L S A A L L L A L I L G G L S k l ~ ~ ~ ~ ~ ~ ~ ~ ~ h c T A N A I H A N L F L S F V L K A S A V L F I d g l l r t r y s q k i e d d l s v s t w l s d g a v a A C R V A A V F M Q Y G I V A n Y C W L L V E G L Y l h i
SEE7_A	p v F L A I L I N F F I F V R I V Q L L V A K L R A R q h ~ ~ h t d Y A F R L A K S T L T L I P L L G V H F V V F A F v t d e h ~ ~ ~ ~ ~ a q g t l R S A K L F F D L A L S S F Q ~ G L L V A V L Y C F I n l
SEE7_A	f q V M Y T V G Y S L S A A L L L A L I L G G L S k l h c t a n a I H A N L F L S F V L K A S A V L F I d g l l r t r y s q k i e d d l s v s t w l S D G A V A A C R V A A V F Q Y G I V A N Y C W l l
C:1/2/3 C:5/6/7	4OR2_A s n i e s I I A I A F S C L G I L V T L F V T L I F V L Y R D T P v v k s s S R E L C Y I I L A G I F L G Y V C P F T L I A k p t t t s c y l q R L L V G L S S A M C Y S A L V T K T N R I a r i l 4OR2_A c n t s n L G V V A P L G Y N G L L I M S C T Y Y A F K T R N V P a n ~ f n E A K Y I A F T M Y T C I I W L A F V P I Y F g s n y k ~ i t t C F A V S L S V T V A L G C M F T P K M Y i i i a k
F:1/2/3 F:5/6/7	4IKV_A h q d M H S Y I A A F g ~ A V T G L C L T L F T L A T F V A d w r ~ ~ ~ ~ ~ ~ ~ ~ ~ n s n R Y P A V I L F Y V N A C F F V G S I G W L A Q F M d g a r r e i v c r a d g ~ ~ ~ ~ ~ ~ ~ ~ ~ r l g e p t s n e l S C V I I F v I V Y Y A L M A G V V W F V V I t 4IKV_A g y k N Y R Y R A G F v I A P I G L V L I V G G Y F L I R g v t l f s i k s n h p g l l s e k a a s k i n e t L R L G I F G F L A F G F V L I T F S C H F Y D F F n q a e w e r s f r d y v l c q a n v t i g l p t k q p i p d c e i k n p S L L V E K i ~ N L F A M F G T G I A M S T w v
A:1/2/3 A:5/6/7	4MQT_A e v v f i V L V A G S L S L V T I I G N I I L V M V S I K V N r h l q t v ~ ~ ~ ~ ~ ~ ~ ~ ~ n n y f i F S L A C A D L I I G V F S M N L y t l y t v i g y w p l g p v v c d i W L A L D Y V V S N A S V M N L L I I s f d r 4MQT_A f f s n A A V T F G T A I A A F Y L P V I I M T V L Y W H I s r a k s r i k k d k k e p v a n q d p v s t r k p p p s r e k k v t r t i l A I L L A F I I T W A P Y N V M v l i n t f c a ~ ~ ~ p c i p n t v W T I G Y W L C Y I N S T I N P A C Y a l c n
A:1/2/3 A:5/6/7	4MQT_A e v v f i V L V A G S L S L V T I I G N I I L V M V S I K V N r h l q t v n ~ ~ ~ ~ ~ ~ ~ ~ ~ n y f i f s i A C A D L I I G V F S M N I y t l y t v i g y w p l g p v v c d i w l A L D Y V V S N A S V M N L L I I s f d r 4MQT_A n a a v T F G T A I A A F Y L P V I I M T V L Y W H I s r a k s r i k k d k k e p v a n q d p v s t r k p p p s r e k k v t r t i l a i l L A F I I T W A P Y N V M v l i n t f c a p c ~ i p n t v w t i g y W L C Y I N S T I N P A C Y A L C n a t f k
A:1/2/3 A:5/6/7	5U09_A q q l a i A V L S L T L G T F T V L E N L L V L C V I L H S r s l ~ ~ r c r P S Y H F I G S L A V A D L L G S V I F V Y S F I D F H V F h r k ~ d s R N V F L K L G G V T A S F T A S V G S L F L A a i d r y 5U09_A f p h i d E T Y L M F W I G V T S V L L F I V Y A Y M Y I l w k a ... r m d i R L A K T L V L I I V V L I I C W G P L L A I M V Y D V F G k m n k l i K T V F A F C S M L C L L N S T V N P I I Y A L R s k d l r
A:1/2/3 A:5/6/7	4U15_A v v f I A F L T G F L A L V ~ T I I G N I L V I V A F K V n k q ~ ~ l k T V N N Y F L L s l ~ a c A D L I I G V i s m n I f t t y i i m n r w a l g n I a c D L W L S I D Y V a s N A S V M N L L V I s f d r y 4U15_A f l s E P T I T F G T A I A a F Y M P V T I M T I L Y W R i y k e ... k k a A Q T L S A I I l a f i i T W T P Y N I m v l v n t f ~ ~ ~ ~ ~ c d s c i p k T Y W N L G Y W L c ~ Y I N S T V N P V C y a l c n
A:5/6/7 A:1/2/3	4MBS_A y q f w k N F Q T L K I V I L G L V L P L L V M V I c y s g i l k r d v r l i F T I M I V Y F L F W A P Y N I V L L N t f q e f f g l n n c s s s N R L D Q A M Q V T E T L G M T H ~ C C I N P I I y a f v g 4MBS_A a a r l I P P L Y S L V F I F G F V G N M L V I I l i n y k r ~ ~ l k s m t D I Y L L N L A I S D L F F L L T V P F W a h y a a a ~ ~ q w d f g N T M C Q L L T G L Y F I G F F S g I F F I I L L t i d r y
A:5/6/7 A:1/2/3	4IB4_A t c v l l k e r f G D F M L F G S L A A F F T P L A I M I V T Y F l t i h a r a s k V L G I V F F L F L L M W C P F F I t n i t l v l ~ ~ ~ ~ ~ c d s c n q T T L Q M L L E I F V V I G Y V S S G V N P L V Y T L F n k t 4IB4_A e e q g n k l h w A A L L I M V I I P T I G G N T L V I L A V S l e k k ~ ~ l q y A T N Y F L M S L A V A D L L V G L f v m p i a l l t i m f e a m w p l p L V L C P A W L F L D V L F S T A I W H L C A I S V D r y i

A:1/2/3	<i>4GRV_A</i>	y s KVLVTAIYLALFVVGTVGNSVTLFTLARKks l ~ ~ q s LQSTVHYHLGLSALS DLLL LAMPVELYN f i w v h ~ ~ h p w a f GDAGCR g YYFLR DACTY ATALNVASLS Vary <td>A:5/6/7</td> <td><i>4GRV_A</i></td> <td>t v KVVIQVNTFMSFLPMLVISILN TVIANKl t v m .. s v q ALRHGV LVARAVIAFVV CWL PYHVRRLM Fc y i s d e q w t t f l FD F Y H Y ~ FYMLTNALAYASSAINPILYNL v s</td>	A:5/6/7	<i>4GRV_A</i>	t v KVVIQVNTFMSFLPMLVISILN TVIANKl t v m .. s v q ALRHGV LVARAVIAFVV CWL PYHVRRLM Fc y i s d e q w t t f l FD F Y H Y ~ FYMLTNALAYASSAINPILYNL v s
A:1/2/3	<i>3RZE_A</i>	d v t WFKVMTAIINFYLptTLLMLWFYAKiyk a l LKTVI IIVLSVFIACWAPLF I L L L Dvg c k v ~ ~ ~ k t c d i L F R A E Y F L V L A V L N S G T N P I I Y t l t n k <td>A:5/6/7</td> <td><i>3RZE_A</i></td> <td>p l v VVLSTICLTVGL~NLLVLYAVRser ~ .. f HRPMYYF I G N L A L S D L L A G V A Y T A N L I I s g a t t y k l t p a QWFLREGSMFVALSASVFSLLAI a i e r y</td>	A:5/6/7	<i>3RZE_A</i>	p l v VVLSTICLTVGL~NLLVLYAVRser ~ .. f HRPMYYF I G N L A L S D L L A G V A Y T A N L I I s g a t t y k l t p a QWFLREGSMFVALSASVFSLLAI a i e r y
AA:1/2/3	<i>3V2W_A</i>	y i LFCTTVFTLLL S I V I LYCR I YSL v r t r n n v a l LKTVI IIVLSVFIACWAPLF I L L L Dvg c k v k t c d i l f r a e y f l v l a v l n s g t n p i i y t l t n k emr <td>AA:5/6/7</td> <td><i>3V2W_A</i></td> <td>t s VV F I L I C C F I I L E N I F V L L T I W K T k f h ~ ~ r p m Y Y F I G N L A L S D L L A G V A Y T A N L L L S g a t t y k ~ ~ l t p a q WFLREGSMFVALSASVFSLLI a i a i e r y</td>	AA:5/6/7	<i>3V2W_A</i>	t s VV F I L I C C F I I L E N I F V L L T I W K T k f h ~ ~ r p m Y Y F I G N L A L S D L L A G V A Y T A N L L L S g a t t y k ~ ~ l t p a q WFLREGSMFVALSASVFSLLI a i a i e r y
A:1/2/3	<i>3OE9_A</i>	n d l W V V V F Q F Q H I M V G L I L P G I V I L S C Y c i i i r k a l K P T V I L I L A F F A C W L P Y Y I G I S I d s f i l l e i i k q g c e f e n t v H K W I S I T E A L A ~ F F H C C L N p i l y a f g i <td>A:5/6/7</td> <td><i>3OE9_A</i></td> <td>n k i F L P T I Y S I I F L T G I V G N G L V I L V M G y q k k ~ l r s M T D K ~ Y R L H L S V A D L L F V I T L P F w a v d a v a n ~ ~ ~ w y f g n f l C K A V H V I Y T V N I Y S S V V I L a f i l d r y</td>	A:5/6/7	<i>3OE9_A</i>	n k i F L P T I Y S I I F L T G I V G N G L V I L V M G y q k k ~ l r s M T D K ~ Y R L H L S V A D L L F V I T L P F w a v d a v a n ~ ~ ~ w y f g n f l C K A V H V I Y T V N I Y S S V V I L a f i l d r y
A:5/6/7	<i>4N6H_A</i>	s p s W Y W D T V T K I C V F L F a f V V P I I I T V C y g l m l l r l r s v r l l s g s k e k d r s l r r i t r M V L V V V G A F V V C W A P I H I F V I V w t l v d ~ ~ ~ i d r r D P L V V A A L H L C I A L G Y A N S S L N P V L Y A f d <td>A:1/2/3</td> <td><i>4N6H_A</i></td> <td>I a I A I A I T A L Y S A V C A V g ~ L L G N V L V M F G i v r y t ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ k m k t A T N I Y I F N L A L A D A L A T S T L P F q s a k y l m e t w p f G E L L C K A V L S I D Y Y N M F T S I F T L T M M S v d r</td>	A:1/2/3	<i>4N6H_A</i>	I a I A I A I T A L Y S A V C A V g ~ L L G N V L V M F G i v r y t ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ k m k t A T N I Y I F N L A L A D A L A T S T L P F q s a k y l m e t w p f G E L L C K A V L S I D Y Y N M F T S I F T L T M M S v d r
A:1/2/3	<i>4EIY_A</i>	v y i T V E L ~ A I A V ~ L A I L G N V L V C W A V W L N s n l q ~ n v t N Y F V V S L A A A D I A V G V L A i p f a i t i s t g f c a a c h g C L F I A C F V L V L T Q S S I F S I L A I A I D r y <td>A:5/6/7</td> <td><i>4EIY_A</i></td> <td>v p M N Y M V y F N F F a C V L V P L L L M L G V Y L R I f l a a .. a k s l A I I V G L F A L C W L P L H I I n c f t f f c ~ ~ ~ ~ p d c s h a p L W L M Y L A I V L S H T N S V V n P F I Y A R i</td>	A:5/6/7	<i>4EIY_A</i>	v p M N Y M V y F N F F a C V L V P L L L M L G V Y L R I f l a a .. a k s l A I I V G L F A L C W L P L H I I n c f t f f c ~ ~ ~ ~ p d c s h a p L W L M Y L A I V L S H T N S V V n P F I Y A R i
A:1/2/3	<i>4EIY_A</i>	v y i T V E L ~ A I A V ~ L A I L G N V L V C W A V W L N s n l q ~ n v t n Y F V V S L A A A D I A V G V L A i p f a i t i s t g f c a a c h g C L F I A C F V L V L T Q S S I F S I L A I A I D r y <td>A:5/6/7</td> <td><i>4EIY_A</i></td> <td>v p M N Y M V y F N F F a C V L V P L L L M L G V Y L R I f l a a .. v h a a K S L A I I V G L F A L C W L P L h i i n c f t f f c p d c s h a p L W L M Y L A I V L S H T N S V V N ~ P F I Y A R i</td>	A:5/6/7	<i>4EIY_A</i>	v p M N Y M V y F N F F a C V L V P L L L M L G V Y L R I f l a a .. v h a a K S L A I I V G L F A L C W L P L h i i n c f t f f c p d c s h a p L W L M Y L A I V L S H T N S V V N ~ P F I Y A R i

Figure S3.A - Protodomains pairwise alignment (TM123 vs. TM567) of GPCRs. (Class A) Rhodopsin - 1F88: 3.24 Å; (A) Rhodopsin active 2X72: 2.59 Å; Rhodopsin active 6CMO: 3.26 Å; (B) 5EE7: 2.56 Å, alternate 3.11 Å, (C) 4OR2: 3.36 Å, (F) 4JKV: 2.39 Å; (A) active 4MQT: 2.73 Å - human M2 muscarinic acetylcholine receptor; (A) 4U15: 2.6 Å M3 muscarinic receptor bound to tiotropium; (A) 4MBS: 2.62 Å CCR5 chemokine receptor-HIV entry inhibitor maraviroc complex; (A) 4IB4: 2.71 Å; (A) 4GRV: 3.31 Å; (A) 3RZE: 3.01 Å; (A) 3V2W: 2.73 Å (FD), alternate 2.68 Å (WD); (A) 3OE9: 3.38 Å; (A) 4N6H: 2.87 Å human delta-opioid receptor (delta-OR) bound to Sodium (orange) and Naltrindole (green); (A) 4EIY: 2.68 Å (WD), alternate 2.89 Å (FD). Conserved residues are colored in red. Ligand binding/proximity (less than 4 Å) colored in green; Sodium binding residues colored in orange. (FD) means the alignment matches F6.44 with D2.50. (WD) matches W6.48 with D2.50. In most cases both alignments can be performed, as a rigid body translation along the symmetry axis of one protodomain vs. the other, in some cases TMH2/6 translation alone. Examples 3V2W and 4EIY are two cases where RMSD is low and similar for both alignments. In the case of Active vs. Inactive Rhodopsin, and alignment of protodomains gets them all within the same RMSD range: 6CMO protodomain 1 vs. 1F88 protodomain 1 : RMSD=1.78 Å; protodomain 2 RMSD=2.88 Å. **The associated identity scores are reported in Table S3 and discussed in the text.**

<i>2RHI_A</i>	g GIVMSLIVLAI1VFGNVLVITAIAKFerl~~~qtVTNY FITSLACADLVMGLavvPFGaalhik~~~~~wt fgnfWCE FWTSIDVLCLVTASIE TLCVIAVDRYFAITspfkq~~~
<i>3UON_A</i>	f iVLVAGSLSLVTI1IGNILVMVISIKVNrhil~~~qtVNYYFLSLACADLIIGVfs NLYtlytvig~~~~~wplgpvVCDLWLALDYVVSNASVMNLLIIISFDRYFCVTKpltyp~~~
<i>4MQS_A</i>	f iVLVAGSLSLVTI1IGNILVMVISIKVNrhil~~~qtVNYYFLSLACADLIIGVfs NLYtlytvig~~~~~wplgpvVCDLWLALDYVVSNASVMNLLIIISFDRYFCVTKpltyp~~~
<i>3SN6_R</i>	g GIVMSLIVLAI1VFGNVLVITAIAKFerl~~~qtVTNY FITSLACADLVMGLavvPFGaalhik~~~~~twt fgnfWCE FWTSIDVLCLVTASIE TLCVIAVDRYFAITspfkq~~~
<i>SG53_A</i>	v i1TVE LAIAVLA1LGNVLCWAVWLNsni~~~qnVTNY FVVS LAAAD1AVGVla iPFQsakyl e~~~~~fc aachGCLFIACFVLVLTQSSIFSLLAIADRYIAIRiplryn~~~
<i>4NH6_A</i>	a iTALYSACVAGLLGNVLMFGIVRYth~~~ktATNIYI FNLA DALA DSTATS~IPFQsakyl e~~~~~twpfgelLCKAVLSDYVNMFSTIFTLTMMMSVDRYIAVChpvkal~~~
<i>5IU4_A</i>	v i1TVE LAIAVLA1LGNVLCWAVWLNsni~~~qnVTNY FVVS LAAAD1AVGVla iPFQsakyl e~~~~~fc aachGCLFIACFVLVLTQSSIFSLLAIADRYIAIRiplryn~~~
<i>4PHU_A</i>	l sFGLYVAAFALGFPLNLVLAIRGATAHari~~~rltPSAVYALNLGCS DLLTVs~IPLKavealasg~~~~~awplpasLCPVFAVAHFAPLYAGGGFLAALSARYLGAAfp lgyq~~~
<i>4ZB8_A</i>	v i1AAYVAVFVVALVGNTLVCLAVWRNhh~~~rtVTNY FVNL SLADVLVTAI cIPAS1lvdite~~~~~swlfgbaLCKV1PYLQAVSVSVAVLTLSFIALDRWYAICChplf~~~
<i>4SOV_A</i>	v i1AGYI1VFFVVALIGNLVCVAVWKNh~~~rtVTNY FVNL SLADVLVTAI cIPAT1lvdite~~~~~twf gqgsLCKV1PYLQAVSVSVAVLTLSFIALDRWYAICChplf~~~
<i>5DSG_A</i>	f iATVTGSLSLVTVGNI1VMLS1KVNrgl~~~qtVNYYFLSLACADLIIGAfs NLYyyi kgg~~~~~ywpfgavVCDLWLALDYVVSNASVMNLLIIISFDRYFCVTKpltyp~~~
<i>5CXV_A</i>	f i1TTGLLSLATVTGNLLVLSFKVNe l~~~ktVNYYFLSLACADLIIGTfs NLYtlyi kgg~~~~~hwalg1ACDLWLALDYVASQASVMNLLIIISFDRYFSVTrplsy~~~
<i>3V2Y_A</i>	l tSVVF11CCF11LEN1FVLLTIWKTkkf~~~hrPMYYFIGNLALS DLLAGVa~yTAN111sgat~~~~~tyk1tpaQWFRLREGSMFVALSASVFSLLAIADRYI1TMlk klnh~~~
<i>5U09_A</i>	a iAVL5LTGFTVLENLLVLCVILHSrsl~~~rcrPSYHFIGSLAVADLLGSVi~FVSYFsd fhv f~~~~~hrkdsrnVFLKLGGSVFTASVGSFLAADI DRYI1Hrplayk~~~
<i>5GLH_A</i>	i nTVSCLVFLVGL1GNSTLLY1YKNC~~~rnGPNI1IASLALGDLLHIVia1PiNvyk1ae~~~~~dwpfgaeMCKLVPF1QKASVGITVLSLCALSIDDRYRAVASwsrik~~~
<i>2KS8_A</i>	I wAAAYT11VVT5SVVGNYVVMWI1LAHkr~~~rtVTNY FVLNLAFAEASMAAfntVNVft yavn~~~~ewyyglfYCKFHNF FP1IAAVFASIYSMTAVAFDRYMAI1hp1qp~~~
<i>3W7Z_A</i>	f vPSVYTGVFVVSPLPN1MA1VVFILk kv~~~kkPAV VYMLH LATA DLFVSV~IPFKIsyyfsgs~~~~~dwqfgseLCRFVTAAFYCNMYAS1LLNTVI1SDRFLAVVyp qsl~~~
<i>4IAR_A</i>	l iVMLLALITLATL1SNAFV1ATVYRTrk1~~~htPANYL1ASLAVTDLVLS11 PIST ytvg~~~~~rwtlgavVCDFWLSSD1TCCTAS1WHLCV1ADRYWAITdaveys~~~
<i>SUEN_A</i>	a yIGIEVLI1ALVSPGNVLV1WAVKVNqal~~~rdATFCF1VSLAVADVAGAIviPLA1linig~~~~~pqyf hTCLMVACPVL1LTQSS1LALLAIADRYLRVK1plryk~~~
<i>4ZUD_A</i>	i PTLYS11FVVG1FGFNGNSLV1VIVIYFY k1~~~ktVASVFLNLLALADLC1LLT~IPLWavyta ey~~~~~rwpfgnyLCK1ASASVSFNLYASVFLTLCLSIDDRYLA1Vhp ksr~~~
<i>4K5Y_A</i>	v a1I1NLYLGHCI1SLVALLVAFVFLRArsi~~~rcLRNI1IANLIAAF1LRNAT~wFVVqlt spe~~~~~vhqsnvgWCRLVTAAYNYFHTVNFWMFGE GCYLHTA1V1tnife I
<i>3RZE_A</i>	p iVVLST1CLVTVGLNLLVLYAVRSErk1~~~htVGNLYIVSLSVADLIVGAvv PMN1lyll s~~~~kws1grplCLFWLSMDYVASTAS1FSVFLC1IDDRYRSVQqp1r yl~~~
<i>3PBL_A</i>	y yALSYCAL1IA1VFGNGLVCMAVLKera l~~~qtTTNYL1VVS LAVADLLVAT1v PWVvylevtgg~~~~~vwnfrs1iCCDFVTLDBVMCTAS1WNLCA1SDR YTA VV pvhqhg~~~
<i>4DJH_A</i>	i iTAVYSVVFVGVGNGNSLVMFVI1RYtk~~~ktATNIYI FNLA DALA DLT11~PFQstvyl n~~~~~swpfgdLCK1IVS1D YVNMFSTIFTLTMMMSVDRYIAVChpvkal~~~
<i>5TVN_A</i>	w a1LL1LMV1IPT1GGNTL1V1LA VSLEKK1~~~qyATNYFLMSLAVADLLVGLfv P1A1lt1 fea~~~~~wp1plvLCPAWLFLDVLF STAS1WHLC1ASVDRYIA1Kkp1qan~~~
<i>5T1A_A</i>	i 1IPPLS1V1FGFVGNGMLV1VLLINCK1~~~kc LTD1Y1LLNL1ASD1LL1T~IPLWaha sane~~~~~wvfgnaMCKLFTGLHYIGF GG1F11LLT1DRYLA1Vhav fal~~~
<i>5VEW_A</i>	f i1Y1YTGVYALSFSAV1AS11LGFrhl~~~hc TRNY1IHLN1FAS11RL1c~vFKda alk gsg~~~~~dgl1syqds1ACRLVFLLXQCYVA1NYYWLLVEGVYLYTLLaf nife Ir~~~~~
<i>SEE7_A</i>	s fQVMYTGVY1SLSA1LL1AL1LGGLsk1~~~hc TANA1HNL1FSL1VKA~vLF1dg11try sqkiedd1svst1sldgavaACRVAAVFMQYGI1VAN YCWLLVEGLYLNLLG1nife Ir~~~~~
<i>4XVY_A</i>	y iPAVY11LVF11GFLGNSVA1WMFVFH kp~~~wsG1S1VYMFNL1ALADFLYV1l pAll1yyfnkt~~~~~dwi1fgd1MCKLQRF1FHVNL1YGS11FL1TC1SAHRYSGVYplks1~~~
<i>SUNF_A</i>	a iP1LY11FV1FGFLVNI1VVV1FLCCKQkgp~~~kkVSS1I1FLN1AVAD1LL1AT~IP1W1t ysyry~~~~~dwl1fgpvMCKVFGSFL1TNMFA1S1FF1TCMSVDRYQS1V1pf1s q~~~
<i>4MBS_A</i>	i 1PPPLS1V1FGFVGNGMLV1L1INYkr1~~~ks MTD1Y1LLNL1ASD1LL1T~IPFVWahyaaq~~~~~wdf1gntMCQLLTGLYF1GFFSG1FF11LLT1DRYLA1Vhav fal~~~
<i>SLWE_A</i>	f i1PPPLY1WLV1F1VGALGNS1V1LVY1WYCara~~~ktATDM1L1N1A1AD1LLFLV1lpFWA1aaadq~~~~~wkf1gtfMCKV1VNSM1YKMN1F1SCV1L1MC1CVDRYIA1Aqa raht~~~
<i>SNDD_A</i>	f i1PIVY11VFFV1ALPSNGMALWVFLFR1kk~~~kaPAV1YMAN1LA1AD1LLSV1w~fPLK1ayhihg n~~~~~nw1ygea1CNV1L1GFFYANM1YCS11FLTCLSVQR1WE1Vnp ghs~~~
<i>3ODU_A</i>	f i1PT1S11FLTG1VGNGLV1L1VMGYQkk1~~~rsMTDKYR1LHLSVAD1L1FV1t~LPFw1dava~~~~~nwyf1gqnlLCKAVH11Y1V1NLYSSV1W1AF1SLDRYLA1Vhatsq~~~
<i>4Z35_A</i>	l vMGL1ITC1F1ML1AN1LLVM1A1YVNrrf~~~hfPI1Y1LM1A1AA1DF1FAGLa~yFYL fntgpn~~~~~tr11tvstWLLRQGL1D1TS1TAS1VAN1LL1A1ERH1T1Vr qlht~~~
<i>AN4W_A</i>	d HSY1AAFGAVTGLC1L1T1LATF1VADwrn~~~snrYPAV1FLYVNACF1VG1S1g1AQF dgarreivc radg~t r1geptsnet1SCV11F1V1VY1ALMAGV1VWFFV1LTY1AWH1TSF1Ka1g t~~~
<i>4PKZ_A</i>	i f1PL1Y1TV1FLFVG1L1TNG1AM11FFQ1irs~~~ksN111FLKNTV1S1D1LL1M11tpF111sdak1g~~~~~tgp11rtfVCGVTS1V1F1FTM11S1S1FLGL1T1DRYQK1T1R1pfkts~~~
<i>1C1M_A</i>	1 AAYMF1LL1MLGF1P1FL1Y1V1T1VQHkk1~~~rtPLN11LLN1A1AD1L1M1F1MVF1ggf1TT1lyt1lg~~~~~yfvfgptGCNLEGFFATLGGE1ALWS1LV1A1ERYVVV1C1p snf~~~
<i>4OR2_A</i>	i eS111A1AF1S1CLG11L1V1T1F1V1Y1rd1p1vvkss S1RE1CY11LAG11FLG1Y1c~pFT11akp~~~~~tts1CYLQRL1GFL1G1SPAMS1S1LV1T1KYRA1R1L1Agas1kk1ct~~~~~
<i>4009_A</i>	paPIAAVVFAC1GLL1AT1FLF1V1Y1Y1rd1p1vvkss S1RE1CY11LAG11FLG1Y1c~pFT11akp~~~~~kq11CYLQRL1GFL1G1SPAMS1S1LV1T1KYRA1R1L1Agas1kk1ct~~~~~
<i>4Z9G_A</i>	va1I1NLYLGHCI1SLVALLVAFVFLRArsi~~~rcLRNI1IANLIAAF1LRNAT~wFVVqlt spe~~~~~vhqsnvgWCRLVTAAYNYFHTVNFWMFGE GCYLHTA1V1tnife I

TM1

TM2

TM3

TM4	TM5
<p>2RH1_A ~s l t knKARVII LMVWIVSGLTSFLPIQ hwyra thqeainc~~~~~ya eetccdf tnqaYAIASSIVSFYVPLVIMVFVYSR Vfpeakrql nife</p> <p>3UON_A ~vkrttkMAGMMIAAAWVLSFILWAPAIlfwqfivgvrtved~~~~~gecyiqffsnaaVTGTAIAAFYL PVIIMTVLYWHISrasksrinife</p> <p>4MQS_A ~vkrttkMAGMMIAAAWVLSFILWAPAIlfwqfivgvrtved~~~~~gecyiqffsnaaVTGTAIAAFYL PVIIMTVLYWHISrasksrkdk</p> <p>3SN6_R ~s l t knKARVII LMVWIVSGLTSFLPIQ hwyra thqeainc~~~~~ya eetccdf tnqaYAIASSIVSFYVPLVIMVFVYSR Vfpeakrqlqkide</p> <p>5GS3_A ~glvtgtRAKGIIAICWVLSFAIGLTPMLgwnncgqpkegkahsqgc~~~gegqvacfedvvp nyMVYFNFFACVLVPLLMLGVYLRIFlaarrqlkqe</p> <p>4N6H_A ~dfrtpaAKALINICIWVLASGVGVPIMV avtrprdgavvc ~~~~~lqfp spswywdtvTKICVFLFAFVVPIIITVCYGLMLIrirsrvlls~</p> <p>5IU4_A ~glvtgtRAAGIIAICWVLSFAIGLTPMLgwnncgqpkegkahsqgc~~~gegqvacfedvvp nyMVYFNFFACVLVPLLMLGVYLRIFaaarrqladle</p> <p>4PHU_A ~afrrpcYSGVCAAIWAVLVLCHLG LVFGIeapggwl dhsnts lgi nt p vngspvc leawdpasagpARFSLSLLFLP LAITAFCFVGCLralargsnife</p> <p>4ZJ8_A ~kstarRARGSI LGIWA VSLA M VPQAA ec svlp lan rtrl~~~~~fsvcderwaddl pkiYHSCFFIVTYLA PLG L MAMAY FQI Frklwgrqg idc</p> <p>4SOV_A ~kstakRARN SIVI I WIVS CII MIP QAIv ec stvfp glank t l~~~~~ftvcderwggei ypk YHICFFLV TYMAPLC LMV LAYLQI Frklwcrqg idc</p> <p>5DSC_A ~arrttkMAGLMIAAAWVLSFVLWAPAIlfwqf vvgkrt vpd~~~~~nqcfi qfls npa VTGTAIAAFYL P VVIMTVLYIHISlasrsrvnife</p> <p>5CXV_A ~akrtprRA ALMIGLA WLVSFVLWAPAIlfwqylvgertvl a~~~~~gacyiqflsqpi i TFGTAMA AFYL P VTMCTLYWR IYretenrnife</p> <p>3V2Y_A ~gsnnfRLFLLI SACWVISL LGGLP IM gwn c isal ss~~~~~cstvlp lyhkHYI LFCTTFT L L SIVI LYCRI Yslvrtrnife</p> <p>5U09_A ~riv trpKAVVA FCLMW TIAVIAVLP LLgwnce k lqsy~~~~~csdi fphide TYLMFWIGVTSV L L FIVYAYMYI Lwkagidcsfn</p> <p>5GLH_A ~givvpkWTAVEIVL VVSVVSLAVPEA I gfdi t dykgsyl ric~~~l lhpvqkta f qfyata KDWLWFSFYFCPLA ITAFFYTLMTce Irknipe</p> <p>2KS8_A ~~rlsatATKVVICVIVWVALLLA FPQGYsstt psrvvc ~~~~~iewpehpnk iye k YHICVTVL IYFLPLL VIGYAYTVVG i tlwaseipgd</p> <p>3VW7_A ~swrtlgRASFTCLAIWALAIAGVVP L Lk e qt iqvpgl gitte~~~~~hdvl setl leggyayYFSAFSAVFFFVPLIISTVCYVSI Irc lssanife</p> <p>4IAR_A ~akrtpkRAAVMIA LVWVFSISI S L P P F wrqakae eevs~~~~~ecvntdhi IYTVYSTVGA FYFPT L L L I A LYGR IY yearsriadle</p> <p>5UEN_A ~vvtp RAAVAIAGCW ILSFVVG LTPM Fgwnnl s averawa aags ~~~gepikce fekvis eyMVYFNFFVWVLPPLLMVLIY LEV Fylirkqladle</p> <p>4ZUD_A ~lrrt I VAKVTCI I WLLAGLASLPAI lhr nvfientn itvc~~~~~afhyesqnstl p i g L G L T K N I L G F L P F L L I I L T S Y T L I W kalkkaye i~~</p> <p>4K5Y_A ~wdaydrI RAWMFICIGWGVPFP I IVAWA I gklyydnek~~~~~cwagkrpgvYT D YI QGPMA L V L I N F I L FN I Vril tk ras~</p> <p>3RZE_A ~kyrtktRASATI LGAWFLSFLWVPI I LGwhnf q qtsv r re~~~~~dkcetd fdydvt wFKVMTA I I N F Y L P T L L M L W F Y A K I Y kavr qhc nife</p> <p>3PBL_A ~tgqsscrRVALMITAVWVLAFAVSCPLL Fg fnttgdp t~~~~~vcsisnpd F V I Y S S V V S F Y L P F G V T V L V Y A R I Y v v l k q r r k n i</p> <p>4DJH_A ~dfrtplKAKIINICIWL LSSSVGISAIVlggtkvredvdvi e c~~~~~slqfpdddy swwdl f MKICVFI FAFVIPV L I I VCYTLM I l rksvrlsg</p> <p>5TVN_A ~qynsraTAFIKITVVWLI SIGIAIPVPIkgietdvdnpnn~~~~~i tcv l tkerfgd FMLFGSLAAFFPLAIMIVTYFTL I halqkkaadle</p> <p>STIA_A ~kart tvtFGVVT S V I T W L V A V F A S L P N I I f t k x q k e d s v~~~~~yv c gpyfprg WNNFHTIMRN I L G L V L P L L I M V I C Y s g i s r a k s r i</p> <p>SVEW_A ~dayseqwI F RLYVAIGWGVP LL F V V P W G I v k y l y e d e g~~~~~cwt rnsn NYWL I I RPLI F ACIVN FLI F V R V I c ivvsklkan~</p> <p>SEEZ_A ~daypersFFSLYLGIGWGAPALFVVPWAVvk l fenvq~~~~~cwt sndn G FWWI L RFPVFLAILINFFI F V R V I V q l l v a k l r a r~</p> <p>4XNV_A ~g r l k k k N A I C I S V L V W L I V V V A I S P I L F ysgt g v r k n k t i t c~~~~~ydttsdeylrsyfi YSMCTTVAMFCVPLV L I L G C Y G L I V r aliyk kkyt</p> <p>SUNF_A ~~rrnpwQASYI VPLVWC MACLSSLP TFYfrdvrtieylgvna c~~~~~i a fppekyaqwsag i ALMKN I L G F I I P L I F I A T C Y F G I R k h l l k t n s y~~</p> <p>4MBS_A ~kart tvtFGVVT S V I T W W V A V F A S L P N I I f t r s q k e g l h y t c s~~~~~shf p s q y q fwk n F Q T L K I V I L G L V L P L L V M V I C Y S G I I k k t l r kkyt</p> <p>SLWE_A ~wrek r l I YSKMVCFTI WVLAAALCIPE I Lysqikees gai ct v~~~~~y p s d e s t k l k s a v l a k V I L G F F L P F V V M A C C Y T I I I H T L i q a k k~~~~~</p> <p>5NDD_A ~~rkkanIAIGISLAI WLL L L V T I P L Y V v k q t i f p a l q i t t c~~~~~hdvlpeql l vgd fnyflslaigvflfpa l t a s y V L M I r a l a d l e d n w e</p> <p>3ODU_A ~rprk l l AEK V V V Y V G V W I P A L L T I P D F I fanv seaddr i~~~~~cd r fy pnd l w v v V F Q F Q H I M V G L I L P G I V I L S C Y C I I i sk l sh g s n i</p> <p>4Z35_A ~~r snr R V V V V I V V I W T M A I V M G A I P S V gwn c i c d i e n~~~~~csn apl ysdSYLVFWAI F N L V T F V V M V V L Y A H I F gy v a d l e d n w e</p> <p>4N4W_A ~~qplsgKTSYFHLLTWSLPFVLTVAI L A v a q v d g d s v sg~~~~~i c f v g y k n y r Y R A G F V L A P I G L V L I V G G Y F L I R G V t l fsiksnhp</p> <p>4PXZ_A ~npkn l I GAKI LS V VI WAFMFL L SLPN M I I t n r q p r d k n v k k c~~~~~sflksef g l v w h e i V N Y I C Q V I F W I N F L I V I V C Y T L I T k e l y r s y v r t a</p> <p>IGZM_A ~~rfgenHAI MGVAFTWVMALACA APP LV gwsry i peg qcscg~~~~~idyyt phee t n n e s F V I Y M F V V H F I I P L I V I F F C Y G Q L V ftvkeaaaqqq</p> <p>4OR2_A ~prf sawAQVII ASILIS V Q L T L V T L I I epp pilsy p~~~~~i kev y l i c n t s n L G V V A P L G Y N G L L I M S C T Y Y A F K T R n v p~~~~~</p> <p>4009_A ~prf saxAQLVIAFILICIQ LG I I V A L F I eppdi hdps~~~~~irev y l i c n t n L G V V A P L G Y N G L L I L A C T F Y A F K T R n v p~~~~~</p> <p>4Z9G_A ~ayltdrI RAWMFICIGWGVPFP I IVAWA I gklyydnek~~~~~cwagkrpgvYT D YI QGPMA L V L I N F I L F N I V r i l tk ras~</p>	



Figure S3.B - GPCR domains TM1-TM7 multiple alignment - (mostly Class A). Red = conserved, Green = ligand binding

A:1/2/3 A:5/6/7 BR:A/B/C BR:E/F/G	1GZM_A e pwQFSMLAAYMFLIMLG~FPINFLtlyvtvqhk~~~~~klrtplnyiLLNLAVADLFMVFGGFTTLYTSLHgyfvf~~~~~gptgCNLEGFFATLGGEIALWSLV 1GZM_A eetNNE SFV1YMFVVHF1iplIV1FFcyqlvftvkeaaaqqqesattqkaekevtr VIIMVIAFLICWLPHYAGVAFYIFTHQgsdf~~~~~gpfMTI PAFFAKTSAVYNPVIyi 1UAZ_A etIWLGIGTLLMLIGTFYF~IVKGWG vtd~~~~~keAREYYSITILVPGIASAAYL SMFFF Giglt evqvgse IdiyyaRYADWLTTPLLLDLALLA 1UAZ_A ry tWWLFSTICMIVVLYFI~ATSLRAaaker~~~~~gpevASTFNTLTALVLVLTAYPILWIGtegagvvg~~~lgietlI FMVLDVTAKVGFGBILLRSR
A:1/2/3 A:5/6/7 A:A/B/C A:E/F/G	2ZIY_A yySLGIFIG~ICGIIGCGGNGIVIYLFTktksl~~~~~qtpaNMFIIINLA FSDFTFSLVNGFpl tiscflkkwiFGFAACKVYGFIGGIFGFMSIMTMAMISid 2ZIY_A trSNILCMFILGFFGPILLIFFCYFNIV svsnheke aa akrlnakelrkaqaganae rIAKISIVIVSQFLLSWSPYAVvallaq~fgplewVTPYAAQLPVMFAKASAIHNPMLYSVSHpk 1GZM_A fsMLAAYMF~LLIMLGFPINFLTLYVTvqhkkI~~~~~rtpIN YIILLNLAVADLFMVFGGFTTlyts~lhgyfvFGPTGCNLEGFFATLGGEIALWSLVLAie 1GZM_A neSFV1YMFvVHF1iplIV1FFCYGQLVftvkeaaaqqqesattqkaekevTRMVIIIMVIAFLICWLPHYAGvafyif~thqgsdFGPIFMTI PAFFAKTSAVYNPVIYIMMNkq
A :1-7 BR:A-G	1GZM_A wqfs LAAYMFLLIMLGFPINFLtlyvtvqhkkrlrtplnyi lAVADLFMVFGGFTTLYTslhgyfv~~~~~fgptgcnLEGFFATLGGEIALWSLvvlaieri yvvckp snfrfgenhai gVAFTWVMA LACAAPPlygvwsryipeg 1UAZ_A lwlgiGTLLMLIGTFYFIVKGWG vtdke~~~~~areyYSITILVPGIASAAYL SMFFF giglt evqvgse IdiyyaryADWLTTPLLLDLALLAk~~~~~DRVSIGTLVGVDAL ivtg lgvals~ 1GZM_A ryipeg qcscgldyypheetnneSFV1YMFVVHF1iplIV1ffcyqlvftvkeaaaqqqesattqkaekevtrMVIIMVIAFLICWLPHYAGvafyifth~~~~~qgsdfgpiFMTI PAFFAKTSAVYNPVIyi nkqfrnc vttlcgkn 1UAZ_A vgals~~~~~htplarytwlFSTICMIVVLYFLATSraaa~~~~~kergpEVASTFNTLTALVLVLTAYPILWIGtegagvvglietlI FMVLDVTAKVGFGBILLrsraigdteapepsagaesa

Figure S3.C - 1- Protodomains alignment of Rhodopsin (TM123 vs. TM567) vs. Bacteriorhodopsin (BR) (TM ABC vs. EFG). (Class A) Bovine Rhodopsin (1GZM) protodomain 1 vs. protodomain 2: 3.11 Å; vs. Halobacterium salinarum bacteriorhodopsin (1UAZ) protodomain 1 2.58 Å, protodomain 2 3.69 Å. The sequence symmetry pattern is unique to each, yet a Methionine Identity match in TM1 and TM5 with the TM5 Methionine in contact with retinal in addition to the Shiff base linkage in TM7 xxAK. Similarly to Rhodopsin, BR shows a T in TM3 residue in a symmetrically equivalent position to K in TM7, as if that pair had been inherited from an ancestor in a divergent scenario, or coevolved in a similar way in a pseudo-symmetric coevolution scenario. The TM3/TM7 pseudo symmetric match in structure and partially in sequence is common to both with some of the key binding residues, but most noticeable key binding positions. The orientation of the trans-retinal is different, due to a larger number of aromatic residues, especially W residues at various positions, shifted in position. Most noticeable the (Fxxx)WxxY motif Rhodopsin TM6, binding retinal in both is shifted along the TM6 helix with an . **2- Protodomains alignment (TM123 vs. TM567) of Bovine Rhodopsin vs. Squid Rhodopsin (BR) TM ABC vs. EFG.** (RMSD 3.22/1.28/3.22A resp.) Here the (FFA)K/T motif in TM7/TM3 has evolved dissymmetrically as (MFA)K vs. (FIG)G in the rhodopsin family itself, where trans-retinal ligand and the full rhodopsin structures are superimposable within 1.43A over 259 residues. Both have the protodomain “canonical” symmetry related pair match D in TM3 vs FxxxW in TM7, and most of the same retinal binding residues (highlighted in green) **3- Domain alignment** for reference Bovine Rhodopsin (1GZM) vs. Halobacterium salinarum bacteriorhodopsin (1UAZ) RMSD = 3.13A while Bovine and Quid Rhodopsins will match with an overall RMSD = 1.43A, and possess all the canonical GPCR Class A conserved residue.

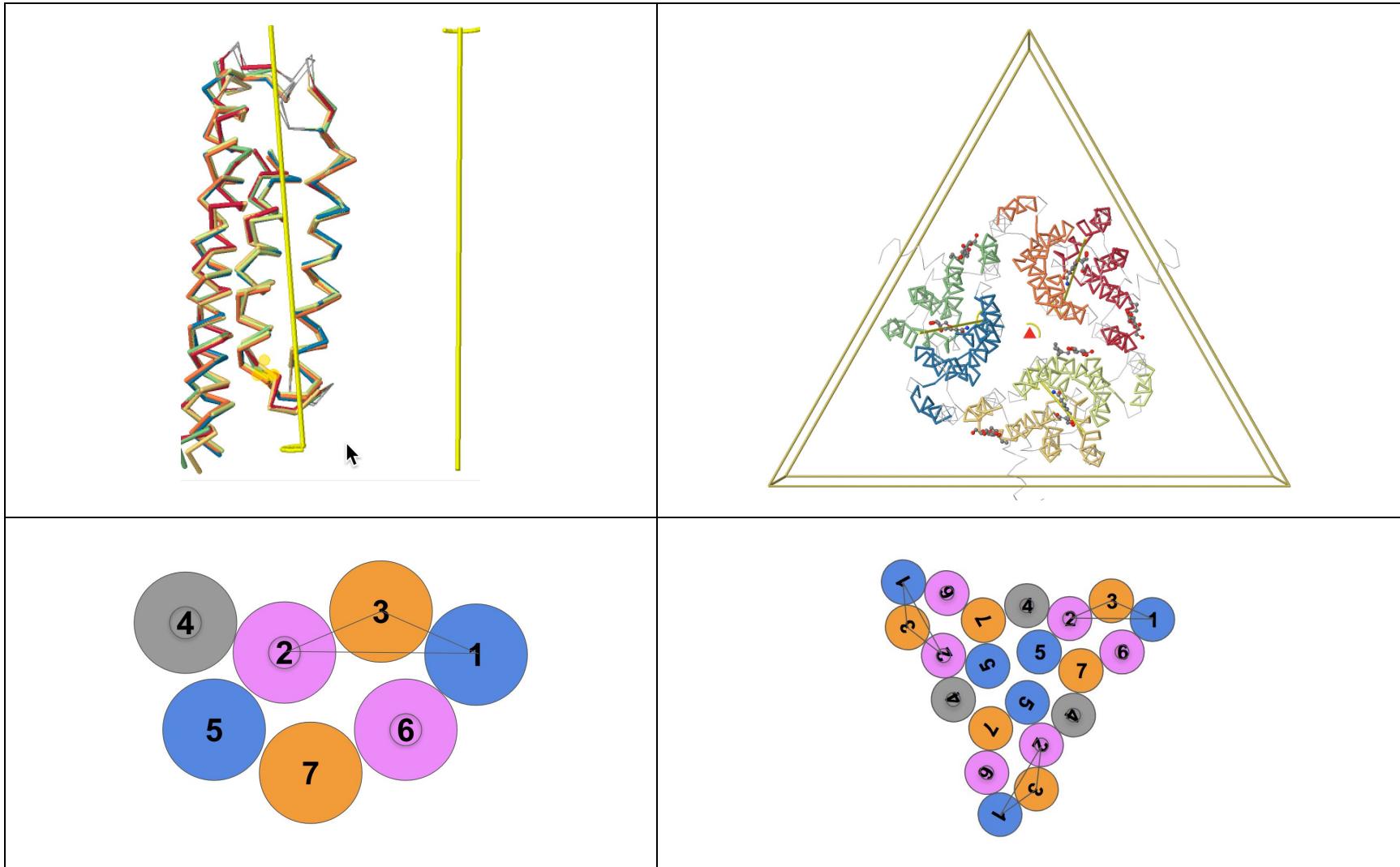


Figure S4 - Protodomains, Domain Pseudo-symmetry and Quaternary symmetry - SWEET Protodomain topology parallel C2 132c/s21a

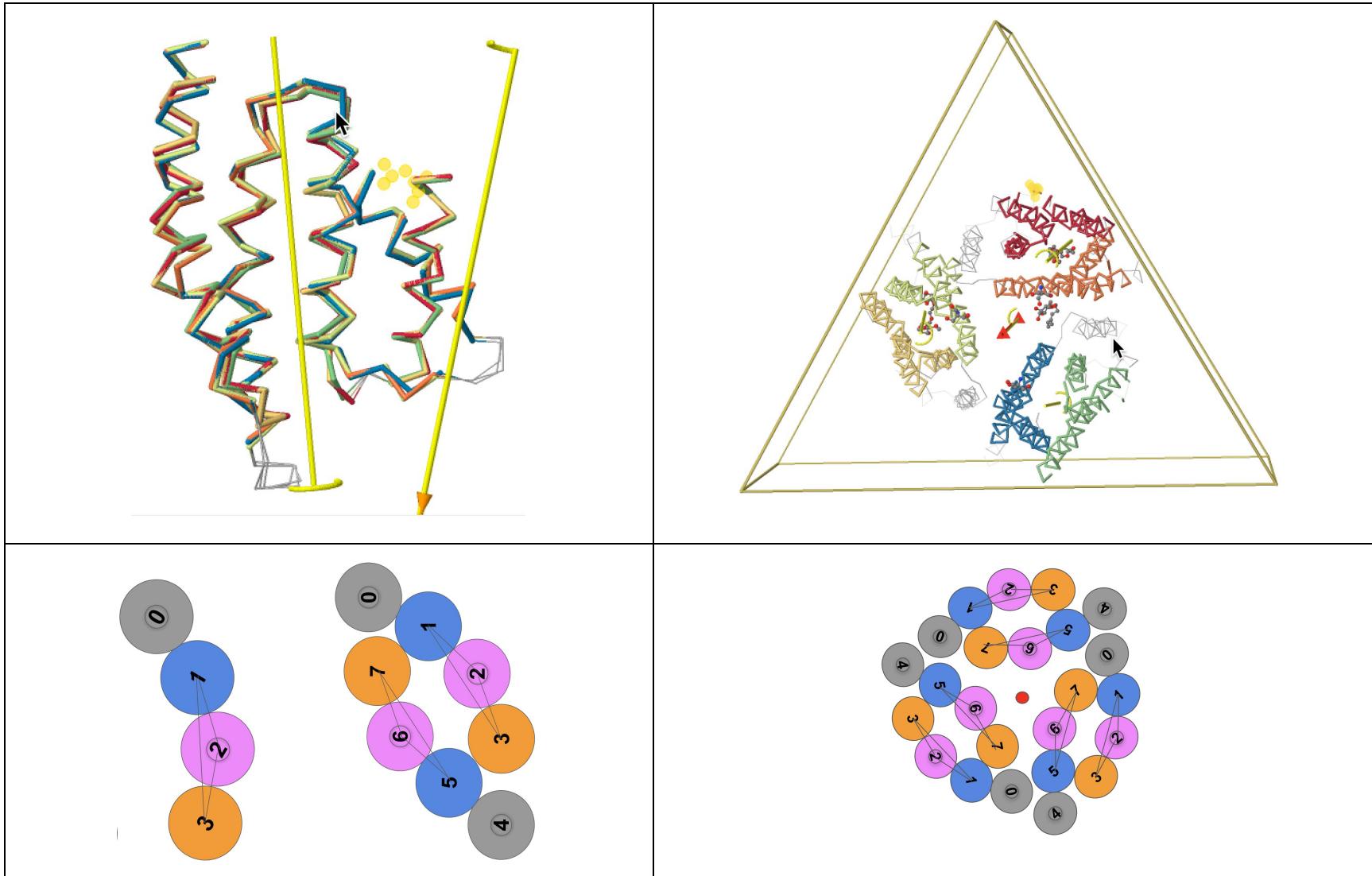


Figure S5 - Protodomains, Domain Pseudo-symmetry and Quaternary symmetry - PnuC Protodomain topology parallel C2 123c/s31p

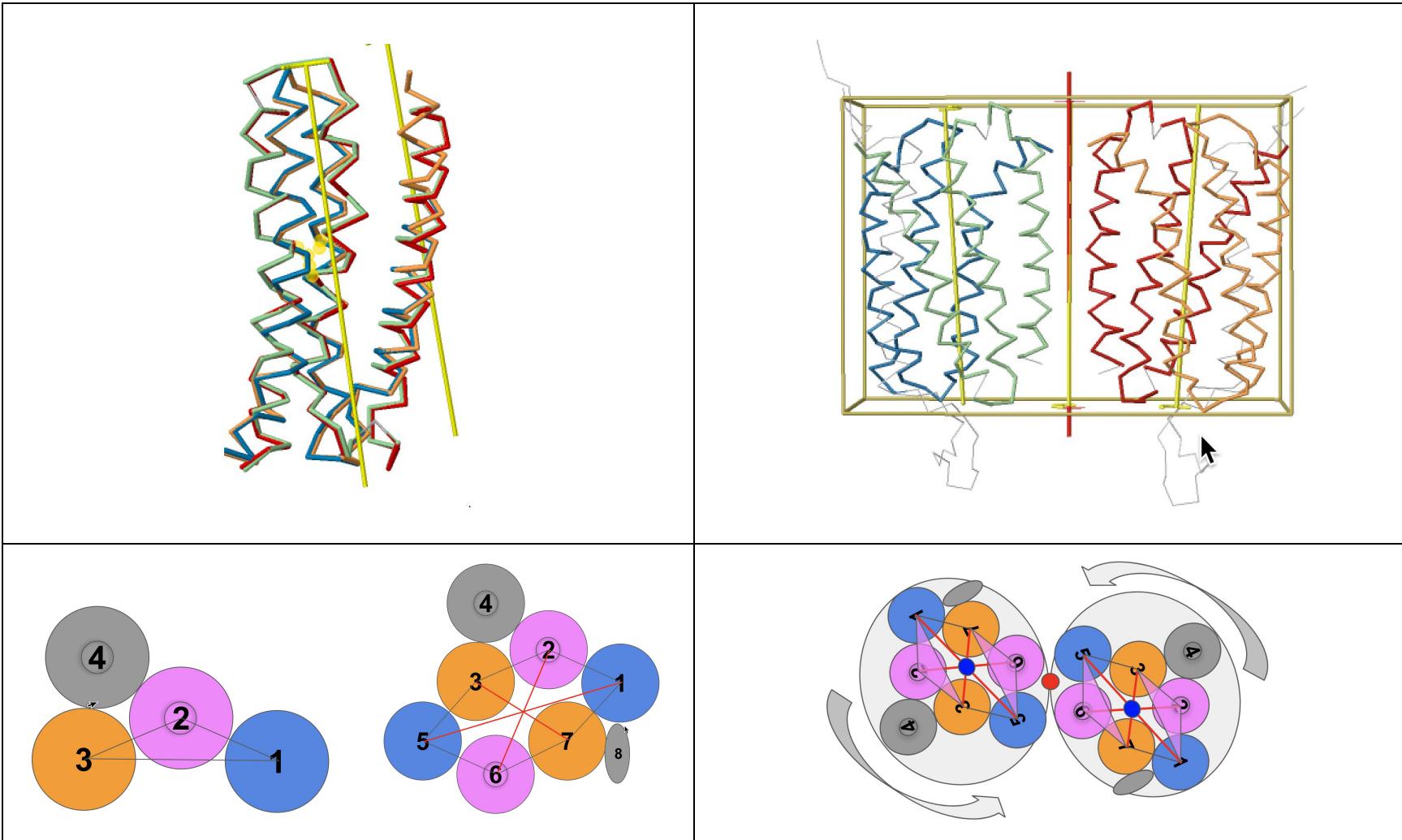


Figure S6 - Protodomains, Domain Pseudo-symmetry and Quaternary symmetry - GPCR Protodomain topology parallel C2 123cc/s31p.

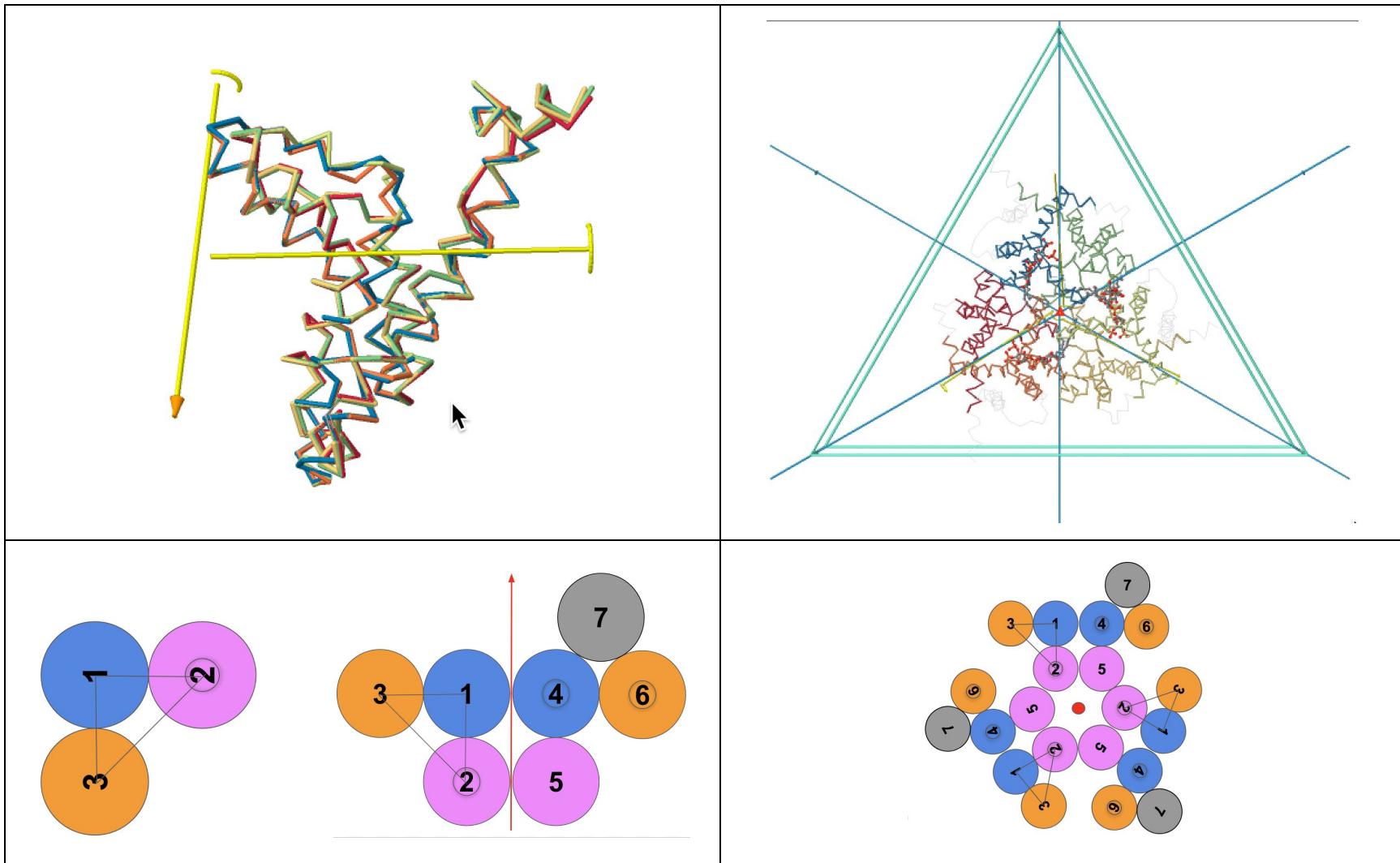


Figure S7 - Protodomains, Domain Pseudo-symmetry and Quaternary symmetry - TRIC Protodomain topology inverted 312c/s11a22a

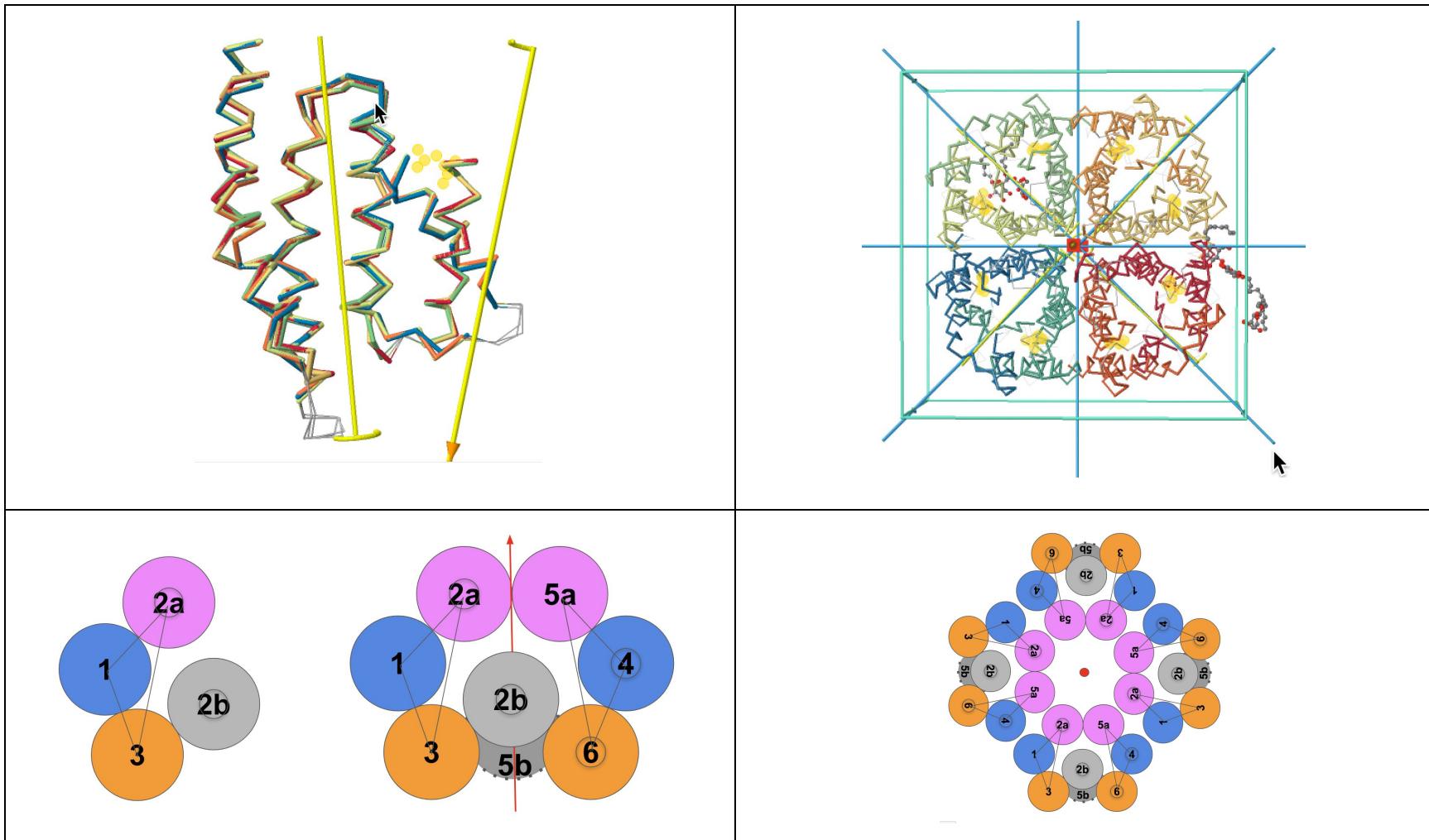


Figure S8 - Protodomains, Domain Pseudo-symmetry and Quaternary symmetry - Aquaporin Protodomain topology inverted C2 312cc/a22a [forms a tetramer quaternary structure, FocA uses the same protodomain and forms a pentamer]. This is the only example with an asymmetric (a) 22 interface, of a very peculiar and idiosyncratic nature: helix 2 splits in 2 forming 2a and 2b to with a helix 2a-2a antiparallel interface from each protodomain on one side and 2b-2b on the other where the latter stack on top of each other one going up the other down - [iCn3D 3D visualization](#)

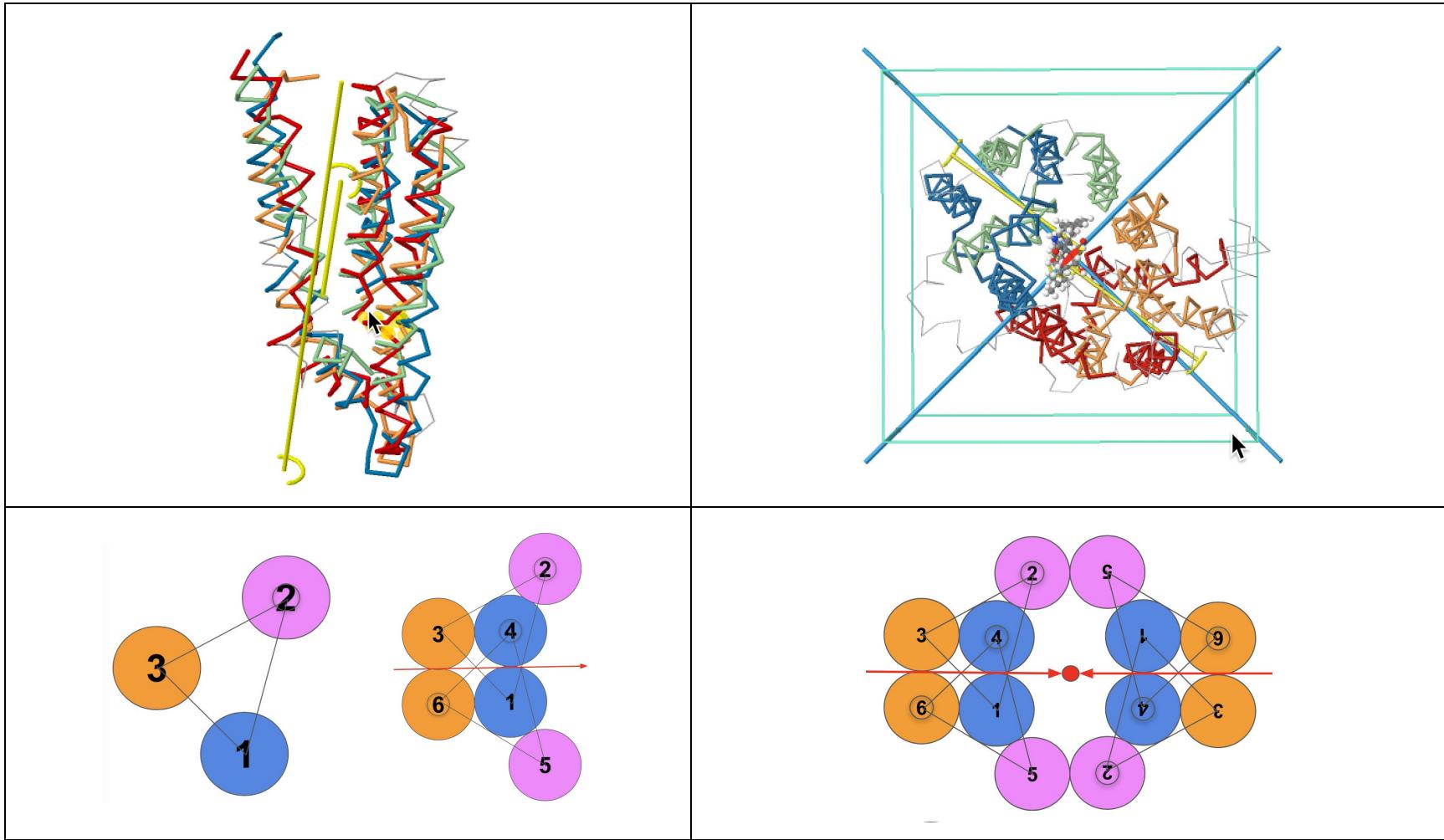


Figure S9 - Protodomains, Domain Pseudo-symmetry and Quaternary symmetry - MFS Protodomain topology inverted C2 interdigitated 132cc/s11a33a
MFS - Lower left) Inverted Interdigitated Protodomains forming a C2 symmetric domain. It possesses two locally symmetric interfaces TM1-TM1 and TM3-TM3 (s11a33a) **Lower right)** C2 symmetric domain packing through a double (symmetry related) TM2-TM2 interface (2^*s22a) formed by the second helix of each of the 4 protodomains. The 2-domains/4-protodomains MFS protein has an overall D2 symmetry. (PDB: [5EQI](#)) since the internal symmetry axes of each domain colinearize and are orthogonal to the central axis (perpendicular to the membrane planes).

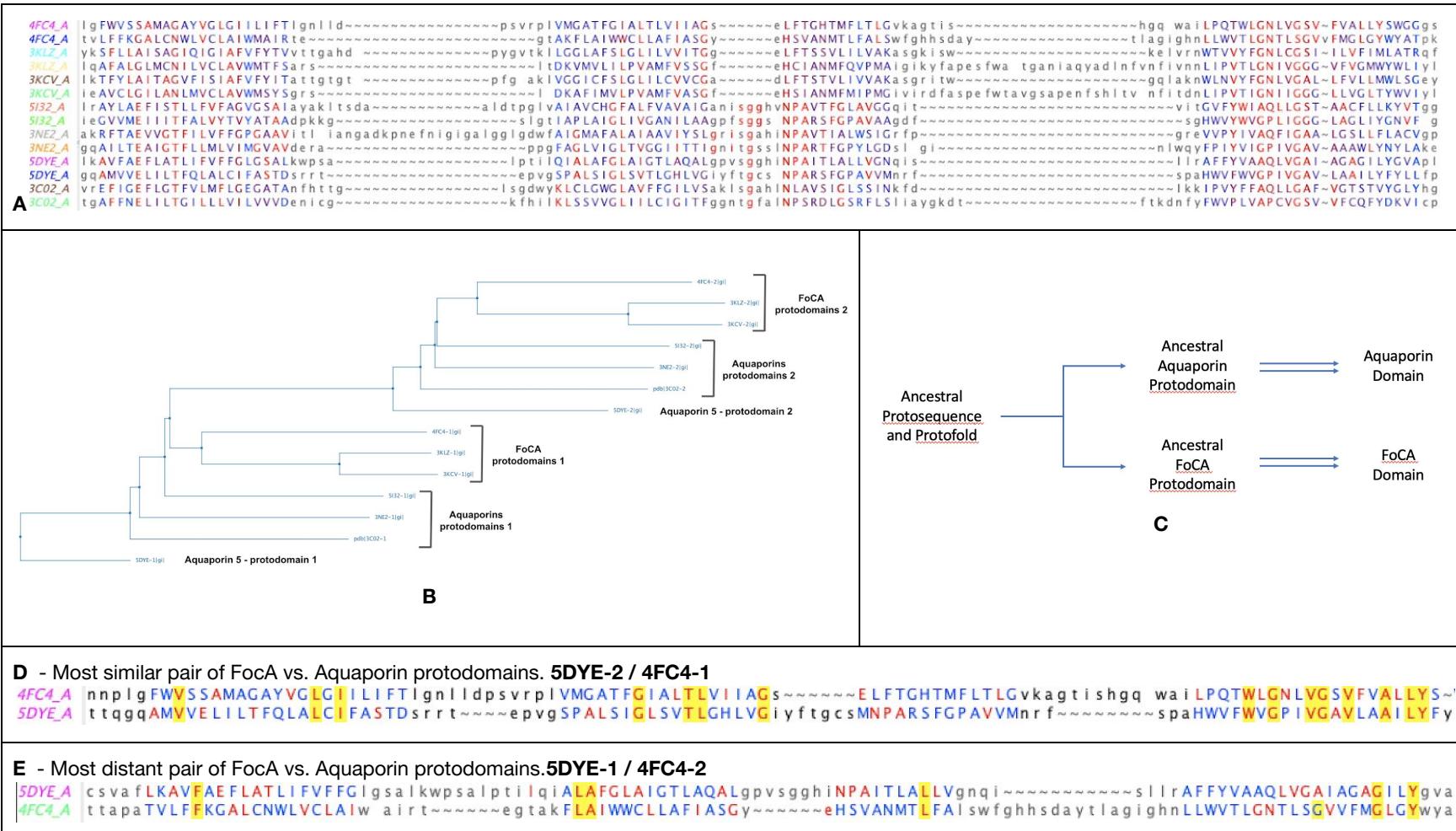


Figure S10: FocA vs Aquaporin A) Structure based multiple sequence alignment of protodomains of FoCA (4FC4, 3KLZ, 3KCV) and Aquaporins (5DYE, 5I32, 3NE2, 3C02) - RMS relative to the 4FC4-1: 1.88 Å (4FC4-2), 1.18 Å/2.05 Å (3KLZ), 1.54 Å/2.00 Å (3KCV), 2.17 Å/2.82 Å (5I32), 2.25 Å/2.43 Å (3NE2), 2.33 Å/2.94 Å (5DYE), 2.21 Å/2.71 Å (3C02) with a common G/AxxxG motif in TM3 **B)** Computed evolutionary tree (using Jalview/Neighbor-Joining/BLOSUM62) from the alignment **C)** Proposed parallel evolutionary mechanism (see text) **D)** 4FC4-1 (FocA's first protodomain) vs. 5DYE-2 (Aquaporin's second protodomain) optimized alignment, with surprising sequence similarity in the middle of a hypothetical common evolutionary tree TM1. **The sequence Identity is 21% between protodomains (35% in TM3)** (see Table S2 for details) **E)** 4FC4-2 (FocA's second protodomain) vs. 5DYE-1 (Aquaporin's first protodomain) at the extremes of a hypothetical common evolutionary tree in C) -optimized pairwise structural alignment (RMS of 2.16 Å) with a sequence Identity is 11% between protodomains (TM3 involving the G/AxxxG/A motif). The color red is used for residues conserved between protodomains within a domain, as well as conserved across domains. Note: The alignment of hundreds of sequences for Aquaporin and FocA families do not unveil more common or different patterns than in our set. We consider our dataset as representative]

Foca	TM1	TM2 a	TM2 b	TM3	Protodomain TM1-TM2-TM3			TM4	TM5 a	TM5 b	TM6	Protodomain TM4-TM5-TM6
	EC	28	38	-	36	34		39	34	39	33	36
IC	40	52	41	43	44			44	42	-	52	46
Full	34	44	41	40	40			42	38	39	43	41

PnuC	TM0	TM1	TM2	TM3	Protodomain TM1-TM2-TM3			TM4	TM5	TM6	TM7	Protodomain TM5-TM6-TM7
	EC	28	50	48	34	44		32	46	46	33	42
IC	28	43	58	46	49			32	57	61	31	50
Full	28	46	51	39	46			32	50	51	32	44

Tric	TM1	TM2	TM3	Protodomain TM1-TM2-TM3			TM4	TM5	TM6	TM7	Protodomain TM4-TM5-TM6
	EC	38	57	40	45		41	47	36	28	41
IC	41	48	68	52			40	42	44	27	42
Full	40	53	54	49			41	44	39	28	41

MFS	TM1	TM2	TM3	TM4	TM5	TM6	TM7	TM8	TM9	TM10	TM11	TM12
	EC	24	25	28	35	29	31	25	18	23	21	24
IC	22	41	35	34	28	30	22	26	21	16	21	23
Full	23	33	31	35	29	30	24	22	22	18	23	22
	Proto 1		29	Proto 2		31	Proto 3		23	Proto 4		21

Figure S11: Sequence Divergence of TMH Families - Sequence similarity score (see Methods section for details) of the aligned EC half, IC half, and Full TM sequences for each of the TMs **TMH families Aquaporin, Foca, PnuC, Tric, and MFS**. Protodomain 1 and 2 scores are given along with those for EC-facing and IC-facing halves. Higher numbers mean high sequence similarity (or higher conservation), where a maximum score of 100 would mean identical sequences or two sequences with similar residues at each position in the sequence alignment. Protodomains specific to each protein family are discussed in the main text and shown in **Figure 1** and Supplementary Figures S1 through S9. The list of proteins and PDB ids used for each family is provided in the Supplement File SF1. We use here a common 3TMH protodomain decomposition, including for PnuC (in the main text we used 4TMH)

6CMO_R: chimera protein of Soluble cytochrome b562 and Rhodopsin
1GZM_A: RHODOPSIN

10	20	30	40	50	60	
.....*.....*.....*.....*.....*.....*.....						
6CMO_R	142	Cgtcgpnfyvp FSN A t gvvrs p feypqy YLAEPWQFS mlaaymfllivlgfpinf l tyv	201			
1GZM_A	3	Ngtcgpnfyvp FSN K t gvvrs p feapqy YLAEPWQFS mlaaymfllimlgfpinf l tyv	62			
70	80	90	100	110	120	
.....*.....*.....*.....*.....*.....*.....						
6CMO_R	202	tvqhkkrltplny ILLNLAVADLFMV LGGFT STLYTS lhgyfvfgptgcnlq GFFATLGG	261			
1GZM_A	63	tvqhkkrltplny ILLNLAVADLFMV FGGFT TTLYS lhgyfvfgptgcnl eGFFATLGG	122			
130	140	150	160	170	180	
.....*.....*.....*.....*.....*.....*.....						
6CMO_R	262	EIALWSLVVLAIERYVV vckpmmsnfrfgenhaimgvaftw v malacaapplagwsryipe	321			
1GZM_A	123	EIALWSLVVLAIERYVV vckpmmsnfrfgenhaimgvaftw v malacaapplvgwsryipe	182			
190	200	210	220	230	240	
.....*.....*.....*.....*.....*.....*.....						
6CMO_R	322	glqcscgidyytlkpevnnesfv i ymfvvhft IPMI I IFFCYGQLVFTVKE aaaqqqesa	381			
1GZM_A	183	gmqcscgidyytpehetnnesfv i ymfvvhfi IPLIV I FFCYGQLVFTVKE aaaqqqesa	242			
250	260	270	280	290	300	
.....*.....*.....*.....*.....*.....*.....						
6CMO_R	382	ttqkaeketrvm <i>vi</i> yviaflicwvpyasvafyifthqgs CF gpifm TIPAFFAK saiiy	441			
1GZM_A	243	ttqkaeketrvm <i>vi</i> imviaflicwlpyagvafyifthqgs D Fgpifm TIPAFFAK tsavy	302			
.....*.....						
6CMO_R	442	npviyimm N	450			
1GZM_A	303	npviyimm N	311			

Figure S12 - VAST+ Invariant substructure alignment between the active (6CMO, human) vs, inactive (1GZM bovine) conformations of Rhodopsin - RMSD = 1.70 Å for 106 residues aligned (86% identity) . 3D visualization link: [iCn3D](#)