Molecular modelling of the second extracellular loop of G-protein coupled receptors and its implication on structure-based virtual screening

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GPCR extracellular loop (ecl) 2

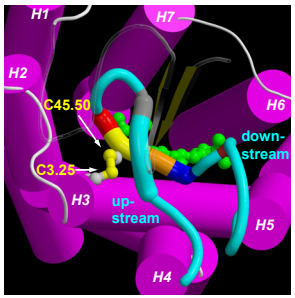
G-protein coupled receptors (GPCRs) share a common topology with an extracellular N terminus, a cytoplasmic C terminus, and 7 transmembrane helices (TMs) connected by 3 intracellular (icls) and 3 extracellular loops (ecls) (see **Fig. 1**). Although the ligand binding cavity is mostly delimited by the 7-TM domain [1], extracellular residues, especially those in ecl2, may participate in ligand binding in GPCRs [e.g. 2, 3, 4].

Aim

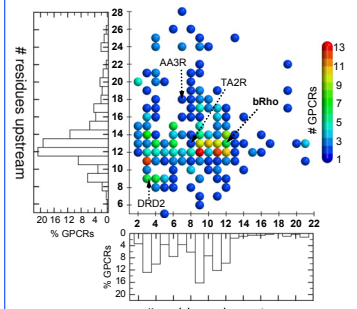
To evaluate the effect of different ecl2 modelling strategies on structure-based virtual screening for GPCR antagonists

Fig. 1: Ecl2 structure and sequence analysis

Ecl2 folds deep into the TM binding pocket of the bRho X-ray structure [5].



Upstream vs. downstream loop length of 365 human GPCR ecl2s.



Sequence alignment of ecl2s of bRho and three human GPCR test cases.

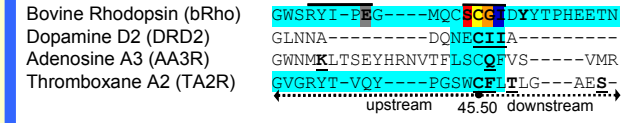
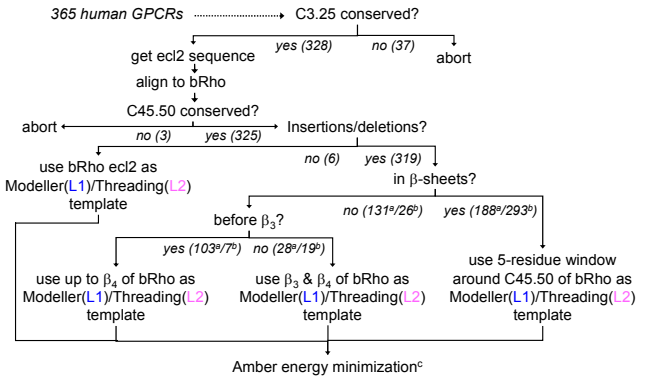


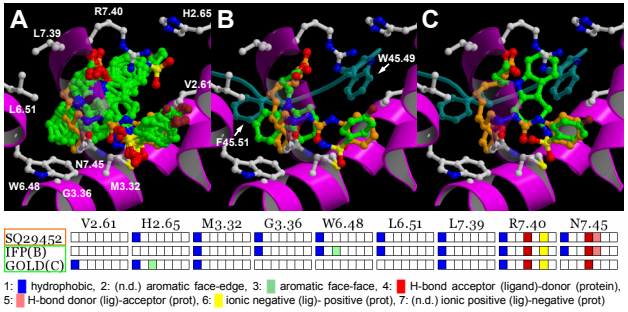
Fig 2.: Ecl2 modelling flow chart



a) strict classification criterion
b) mild classificaton criterion
c) in DRD2, AA3R & TA2R: in the presence of a reference antagonist docked under pharmacophore restraints (see **Fig. 3, 4**)

Fig 3.: Interaction Fingerprint (IFP) scoring

*Top ranked docking poses of ramatroban in the TM model of TA2R (A) according to Gold (B) and IFP (C) scoring [6], using the binding mode of SQ29452 (in line with SAR & mutagenesis data, **Fig. 2**) as a reference.*



1: hydrophobic, 2: (n.d.) aromatic face-edge, 3: aromatic face-face, 4: H-bond acceptor (ligand)-donor (protein), 5: H-bond donor (lig)-acceptor (prot), 6: ionic negative (lig)- positive (prot), 7: (n.d.) ionic positive (lig)-negative (prot)

Fig. 4: Effect of ecl2 on antagonist binding modes

*Top IFP-ranked (see **Fig. 3**) antagonist docking pose compared to reference antagonist binding mode in different DRD2 (up) and AA3R (down) models: Full model with all icls and ecls; TM model without any loops, high-throughput L1/L2 ecl2 models (see **Fig. 2**, **5**).*

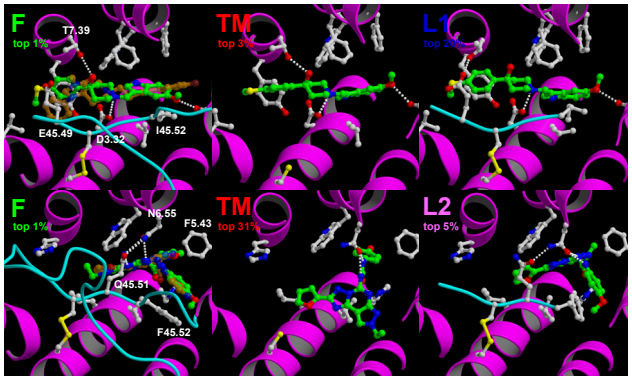
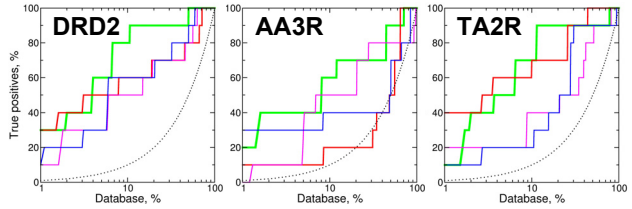


Fig. 5: Structure-based virtual screening (VS)

*VS of 10 receptor-specific antagonists + 990 random drug-like compounds against F, TM, L1 & L2 models (see **Fig. 2, 4**) using IFP scoring (**Fig. 3**).*



Conclusions

- bRho is an "all-round" template for modelling the ecl2 of GPCRs (**Fig. 1**, **2**)
- TM models (without any loops) of GPCRs can be suitable targets for VS by applying IFP scoring (**Fig. 3**, **4**, **5**)
- The effects of ecl2 on structure-based VS are GPCR specific (**Fig. 4**, **5**)

References: [1] Surgand (2006) *Proteins* 303:797; [2] Shi & Javitch (2002) *PNAS* 99: 5361; [3] Duong (2005) *NNNA* 24:1507; [4] Khasawneh(2006) *JBC* 281:26951; [5] Li (2004) *J Mol Biol* 343:1409; [6] Marcou & Rognan (2007) *JCIM* 47: 195.