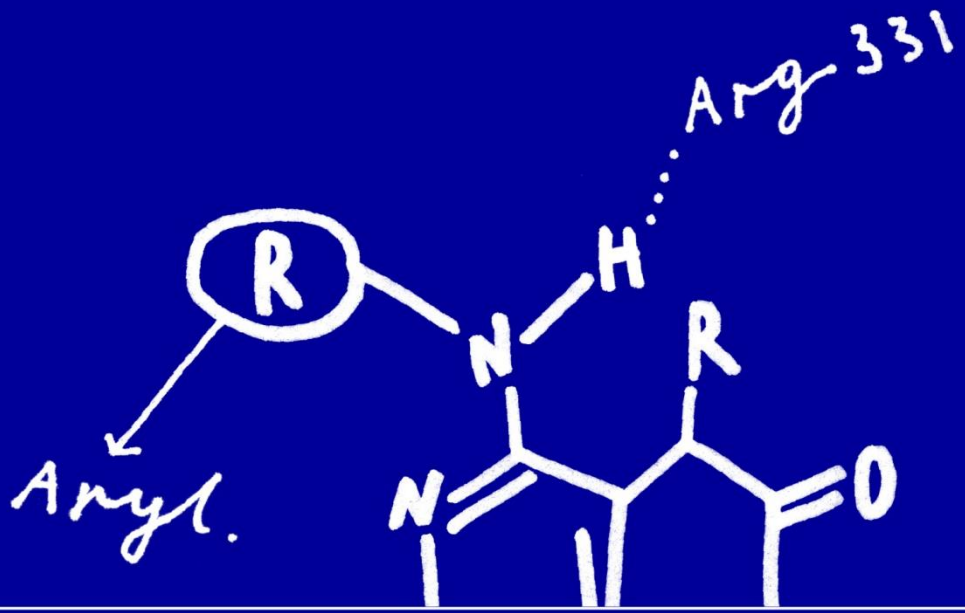
evotec

'RESEARCH NEVER STOPS'

Toward an Understanding of GPCR-ligand Interactions

Alexander Heifetz

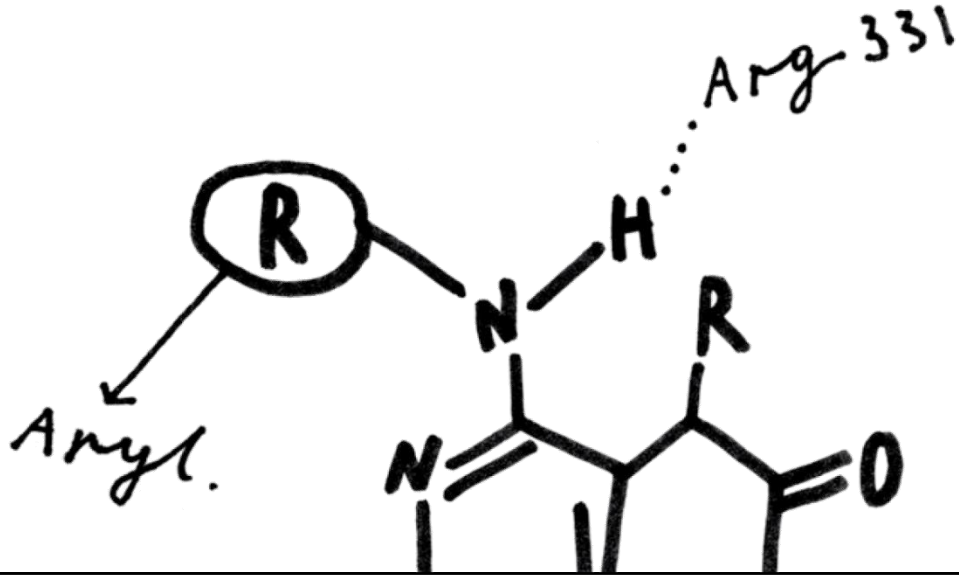


UK QSAR and ChemoInformatics Group Conference , Cambridge, UK October 6th, 2015

Agenda

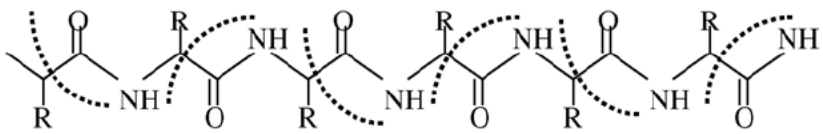
Fragment Molecular Orbitals (FMO) for GPCR exploration

HGMP-C4XD approach for GPCR drug discovery



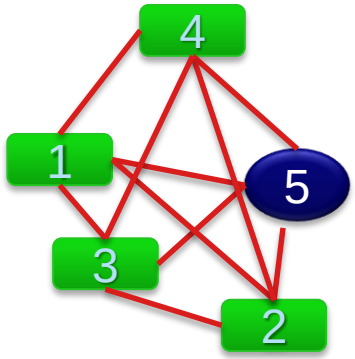
Fragment molecular orbital (FMO)

- "Visual inspection" and molecular mechanics cannot fully explain complex protein-ligand interactions



Fragmentation of peptide

- FMO is a quantum mechanical method that has been developed[1] for application to large (biological) systems
  - takes 24h on 36 CPUs per protein-ligand system



- FMO provides detailed analysis of GPCR-ligand interactions and their chemical nature
  - Calculate individual contribution of each residue and water molecule to binding enthalpy

$$\Delta E_{IJ}^{\mathrm{PIE/PCM}} = \Delta E_{IJ}^{\mathrm{ES}} + \Delta E_{IJ}^{\mathrm{EX}} + \Delta E_{IJ}^{\mathrm{CT+mix}} + \Delta E_{IJ}^{\mathrm{DI}} + \Delta E_{IJ}^{\mathrm{SOLV}}$$

PIE (Pair Interaction Energy)

- Exploration of these receptor-ligand interactions provide key insights for further SBDD

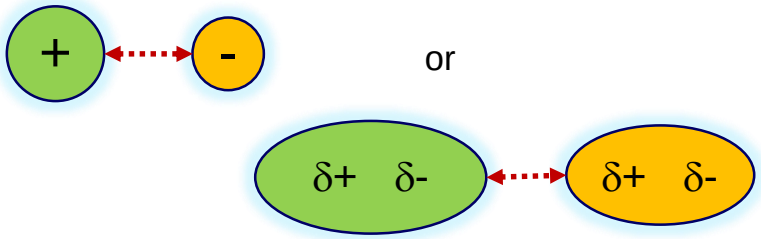
[1]Fedorov DG and Kitaura K. *J. Phys. Chem. A, Vol. 111, No. 30, 2007*

Pair interaction energy decomposition analysis (PIEDA)

Four energy terms obtained

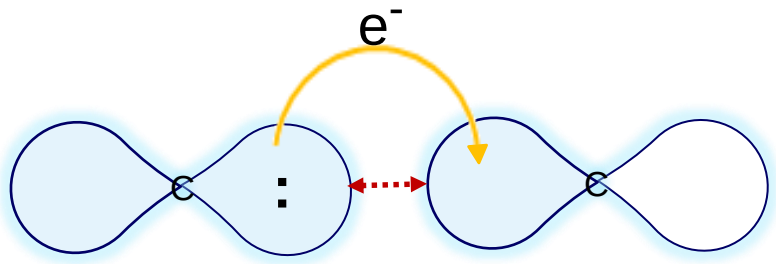
Electrostatic (PIE^{es})

Forces between point charges / (permanent and induced) dipoles/quadrupoles



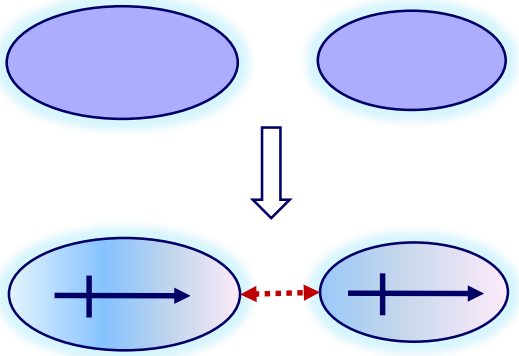
Charge transfer (PIE^{ct})

Interactions between occupied orbital of the donor and unoccupied orbital of the acceptor. Orbital energy gap and overlap are the important factors.



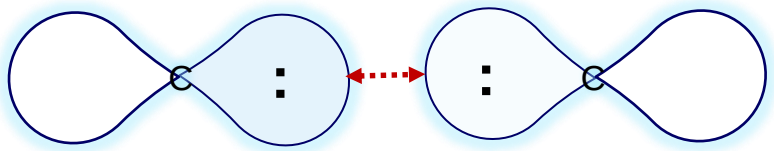
Dispersion (PIE^{dis})

Non-polar molecule can have dipolar moment for a short period of time due to the movement of electrons within the molecule; this induces weak dipole-dipole interaction



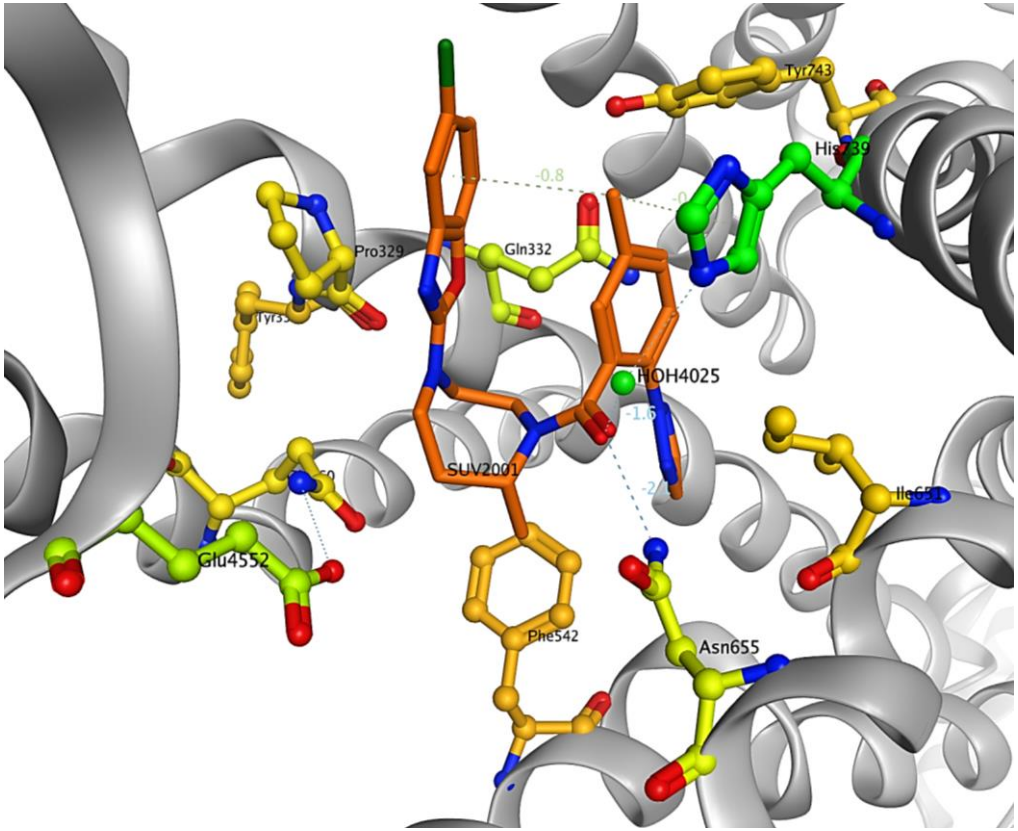
Exchange repulsion (PIE^{ex})

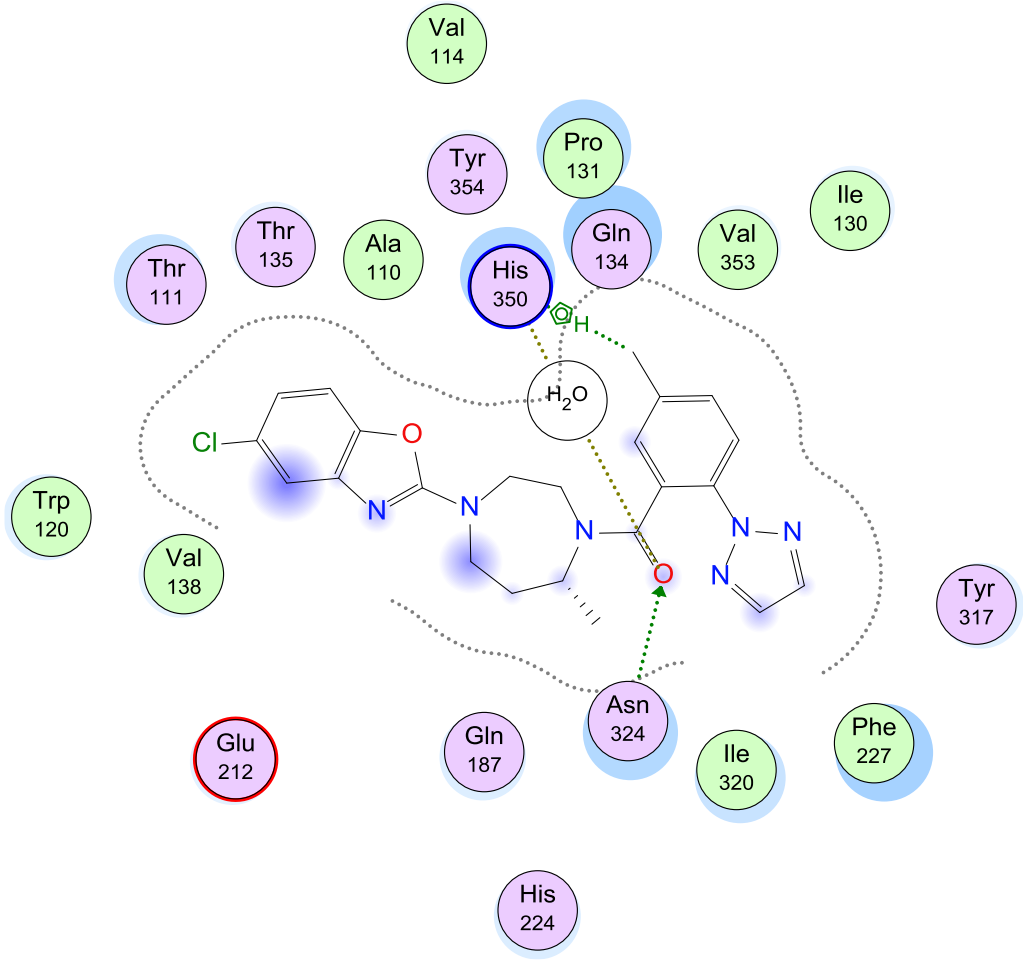
Forces between molecules placed close together and always repulsive. Mainly due to the overlap of two occupied orbitals.



FMO for hOX2-Suvorexant complex

PDB entry 4SoV[1]



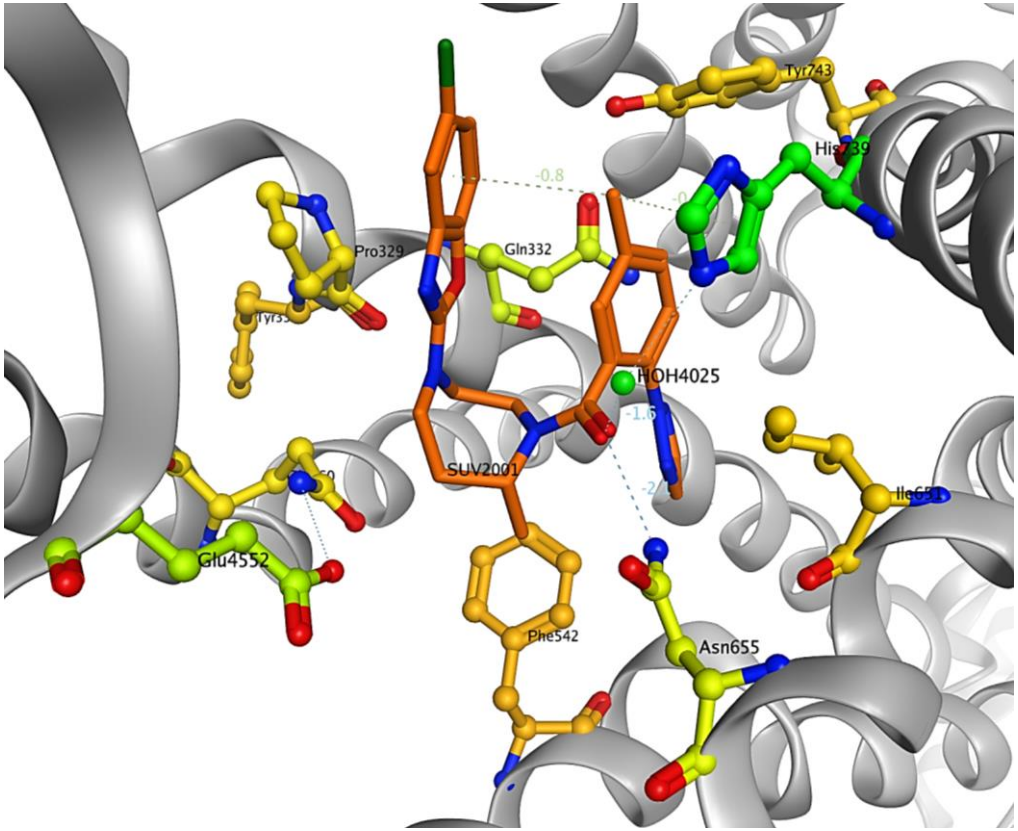


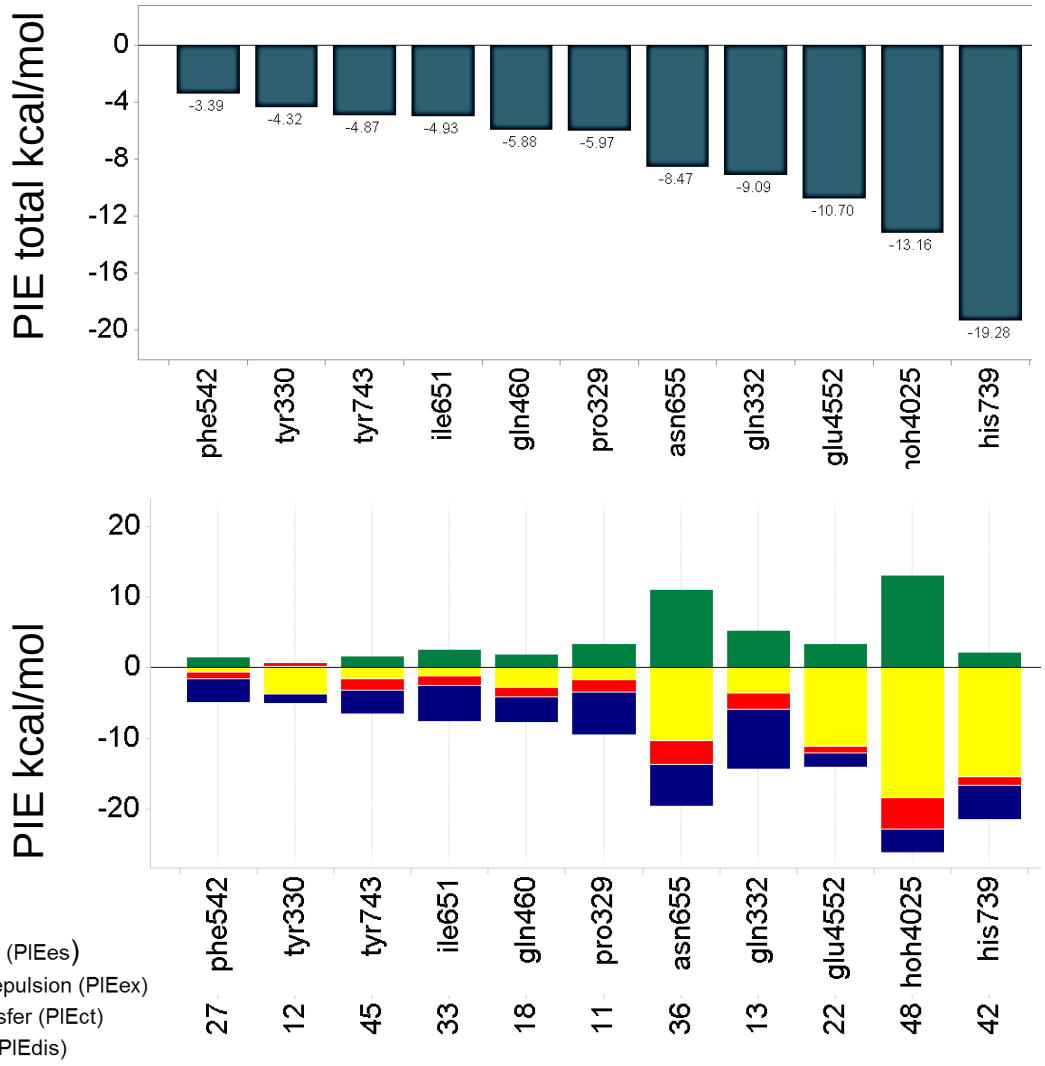
- Suvorexant (hOX1R Ki = 0.54 nM, hOX2R Ki = 0.35 nM)[2],

[1]Yin J. et. al.; Nature (2015) **519:** 247-250
[2]J. Comput Aided Mol Des (2014) 28:5–12

FMO for hOX2-Suvorexant

Filtered* PIE plots



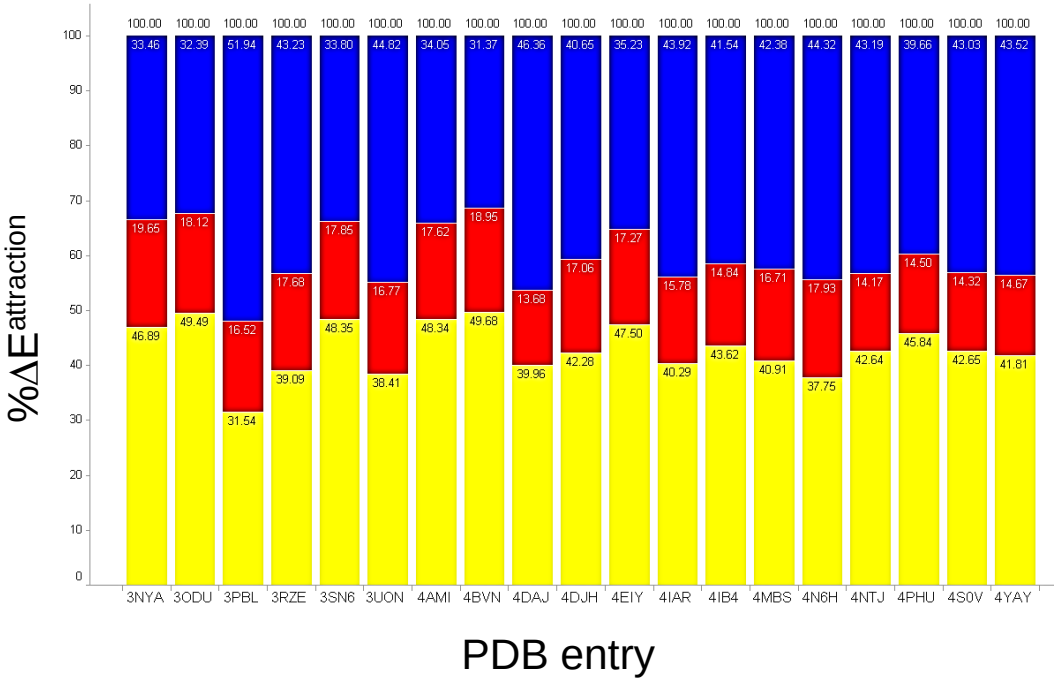


- Suvorexant (hOX1R Ki = 0.54 nM, hOX2R Ki = 0.35 nM, J Comput Aided Mol Des (2014) 28:5–12)

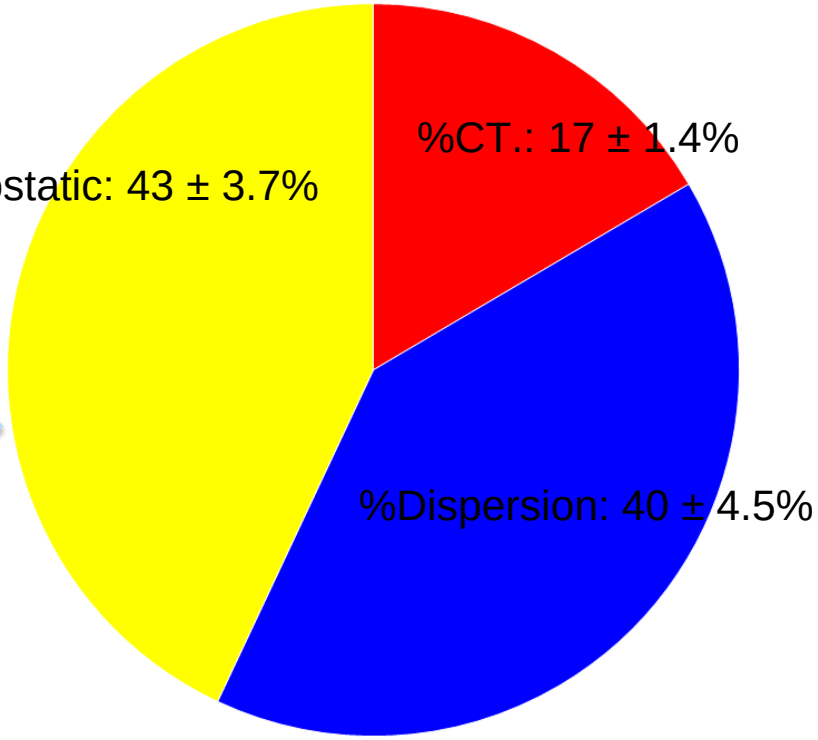
*FMO was calculated for all pocket residues in radius of ≤ 4.5Å from the Suvorexant. In above plots only filtered PIE energies are showed ≤ average PIEtotal (-2.0 kcal/mol)

Interaction signature

Analysis of 19 GPCR-ligand crystal structures



Average



- Interesting observations:
  - Hydrophobic interactions (Dispersion) has almost equal importance for ligand binding in GPCRs like Electrostatics
  - Typical interaction proportion between %Electrostatic, %Dispersion and %CT is: 1:1:0.5 accordingly

$$\Delta E^{attraction} = \Delta E^{\mathrm{ES}} + \Delta E^{\mathrm{CT+mix}} + \Delta E^{\mathrm{DI}}$$

FMO pros, cons and future plans

Pros:

- FMO provides important information on GPCR-ligand interactions and their chemical nature that cannot be explored using other non QM methods
- Structural insights provided by FMO are essential for further SBDD[1]

Cons:

- FMO calculations requires accurate (good resolution) structures, which are not available for every GPCR or GPCR-ligand complex

Future plans

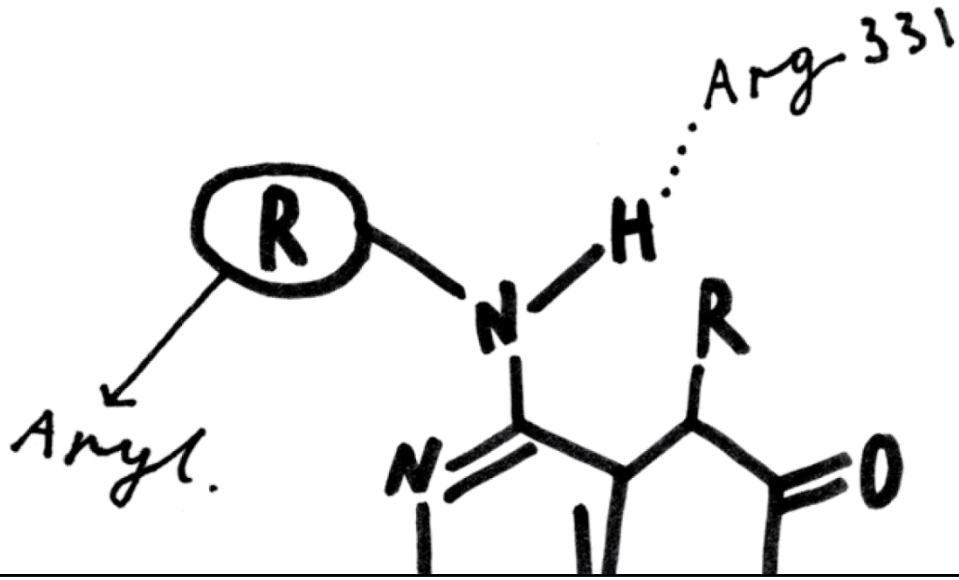
- To adapt the HGMP-FMO method[2] for optimisation of GPCR-ligand interaction geometries to a degree that will allow reliable FMO calculations
- To add solvation effect into FMO calculations by combining this method with the heterogeneous polarizable continuum model (het-PCM)[3]

[1]Mazanetz M. et. al.; J Cheminform. 2011 Jan 10;3(1)
[2]Fedorov DG. et. al.; Acc. Chem. Res. 2014, 47, 2846−2856
[3]Li H., Fedorov DG. et. al.; J Comput Chem. 2010 Mar;31(4):778-90

Agenda

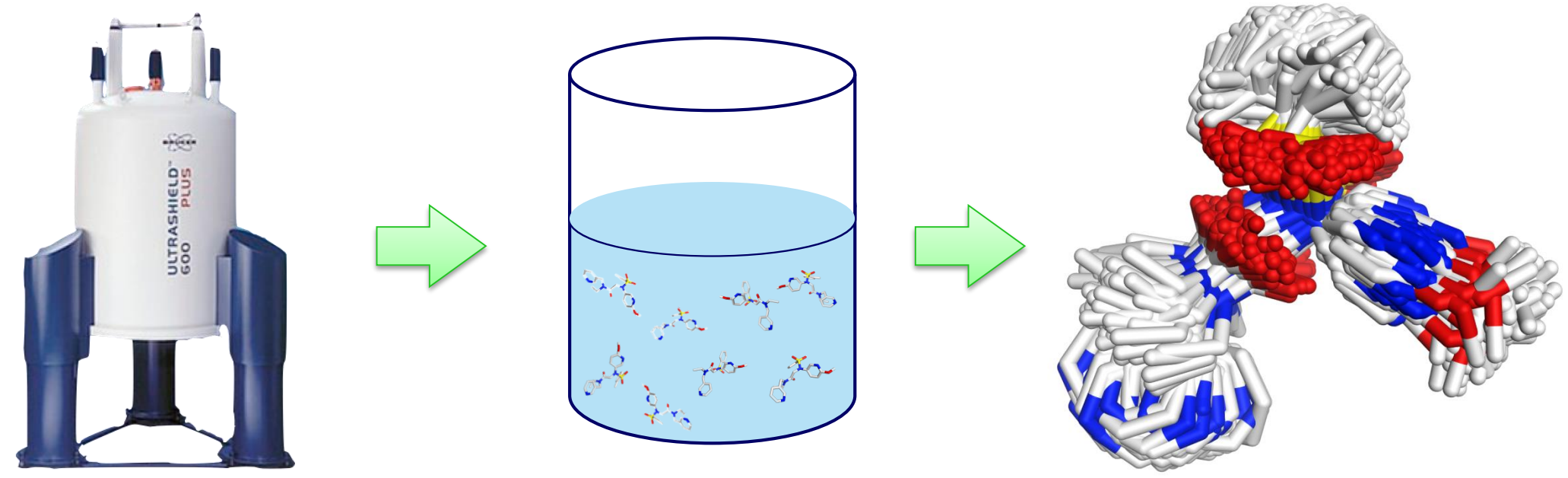
Fragment Molecular Orbitals (FMO) for GPCR exploration

HGMP-C4XD approach for GPCR drug discovery



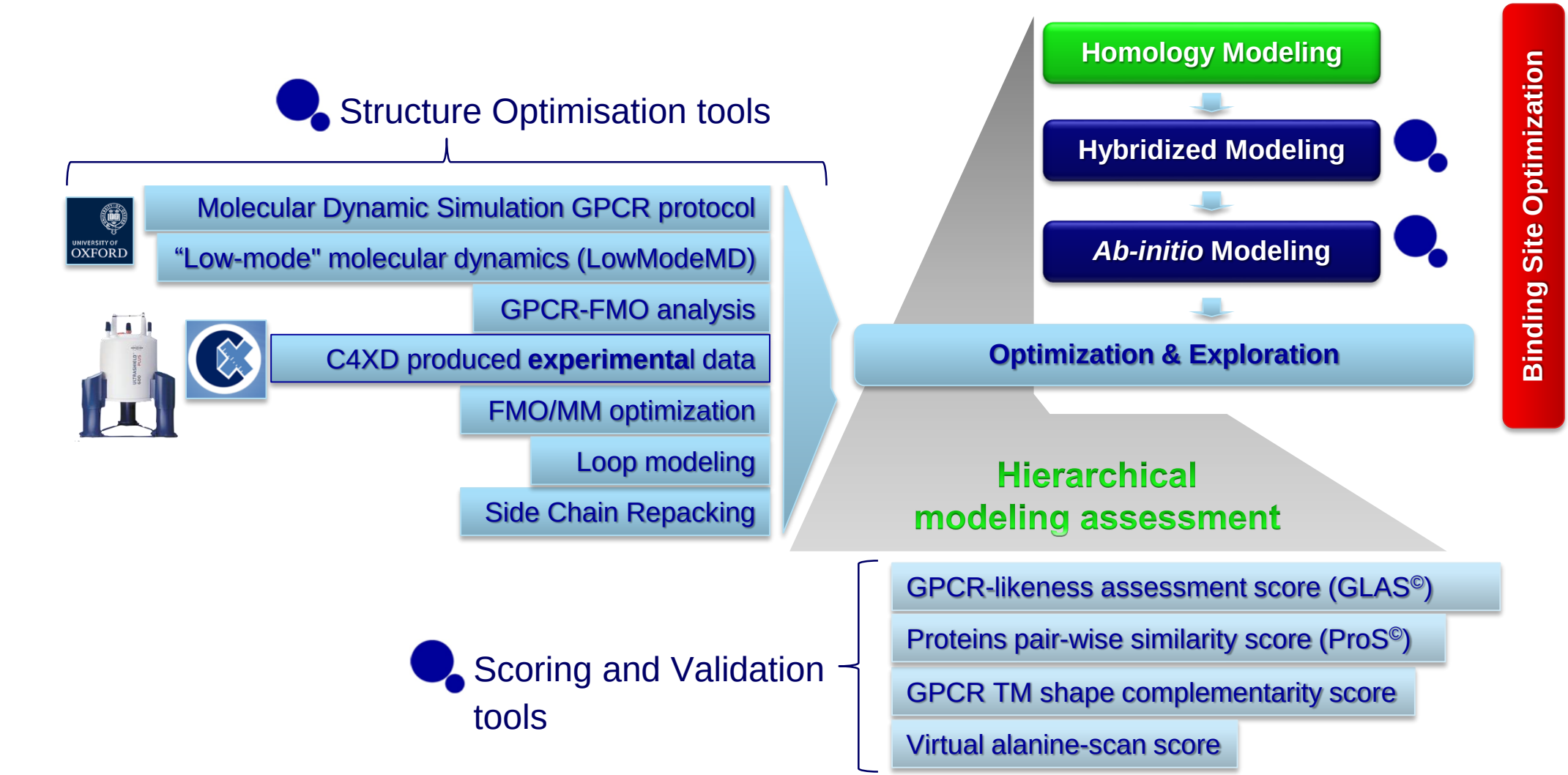
C4XD technology

- C4XD is an NMR-based technology to explore how molecules behave in physiological-relevant solution
- C4XD was developed by C4X Discovery Ltd[1]
- C4X Discovery Ltd[1] experimentally demonstrated that small molecules exist in relatively few conformations in solution and that one of those conformations closely resembles the bioactive form – but which one?
- Can the C4XD technology identify the bioactive conformation of GPCR ligands?



[1]Blundell CD. et. al.; Bioorg Med Chem. 2013 Sep 1;21(17):4976-87

Hierarchical GPCR modeling protocol (HGMP)[1-2]



[1]Heifetz A. and Biggin PC. et. al.; Naunyn Schmiedebergs Arch Pharmacol. 2015 Mar 14
[2]Storer I. and Heifetz A. and et. al.; J Med Chem. 2014 Jun 26;57(12):5258-69

Prediction of the GPCR-ligand binding pose

Challenges

- The binding sites of GPCRs adopt their topology* for ligand binding
  – this need to be addressed by GPCR modelling

- Most standard docking protocols keep the receptor (largely) rigid, and so do not address this flexibility

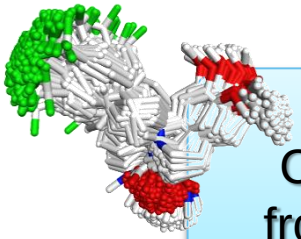
- Fully flexible (ligand and protein) docking can generate a lot of solutions that are difficult to separate

- By limiting the ligand to **experimentally determined** conformations and allowing receptor flexibility we are better able to address the ligand-induced GPCR flexibility

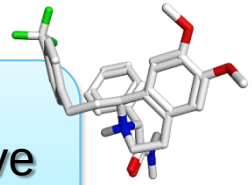
*Binding site topology includes – sidechains and backbone of residues, water molecules networking and binding site surface

Prediction of GPCR-ligand complex

Workflow of HGMP-C4XD protocol



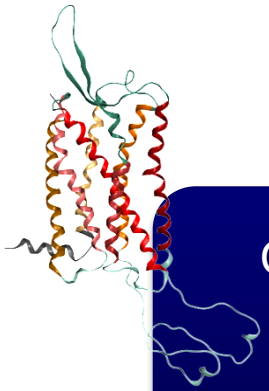
Conformations from C4XD data



Predicted bioactive conformation

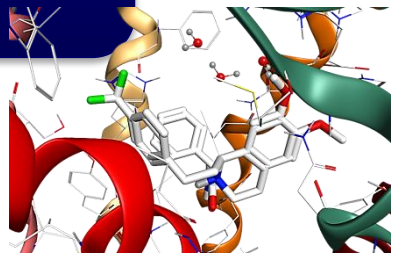
Rigid ligand, flexible protein docking (RLF)*

GPCR Ligand- or/and Structure- DD



GPCR Model produced by HGMP

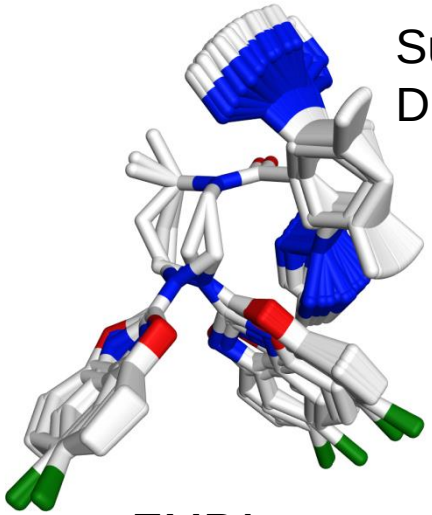
Optimised GPCR-ligand complex



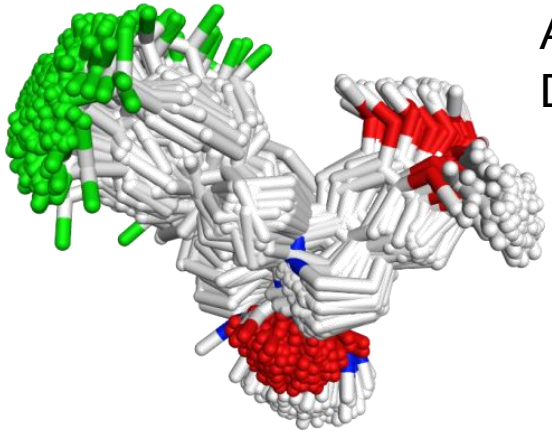
Rigid ligand, flexible protein docking protocol (RLF), implemented in the MOE v.2014.09 software package, treats the ligand as a rigid-body that allows it translational/rotational changes but keeps the dihederal angles fixed. The protein backbone and sidechains are flexible but the heavy atoms have distance restraints.

Designing OX-1 selective anatagonists

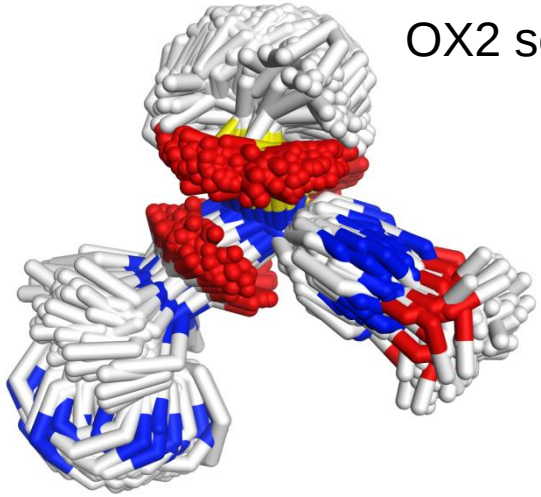
HGMP-C4XD



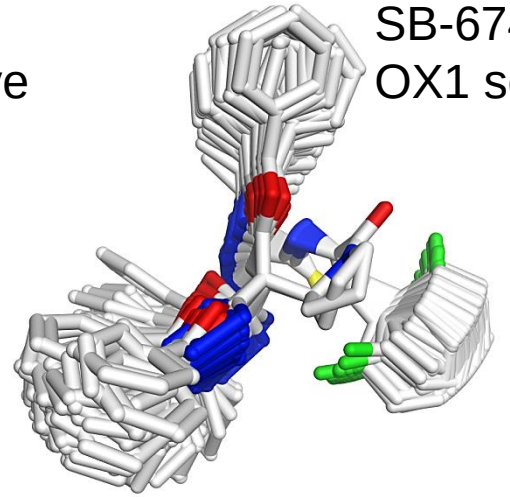
Suvorexant
Dual



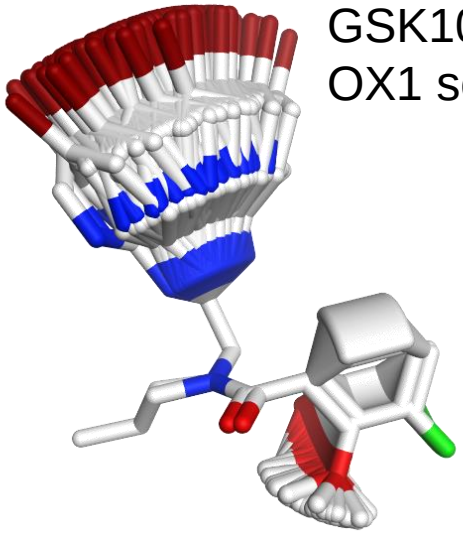
Almorexant
Dual



EMPA
OX2 selective



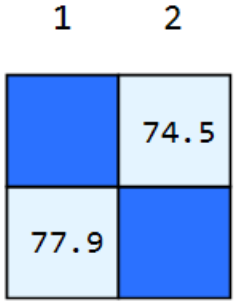
SB-674042
OX1 selective

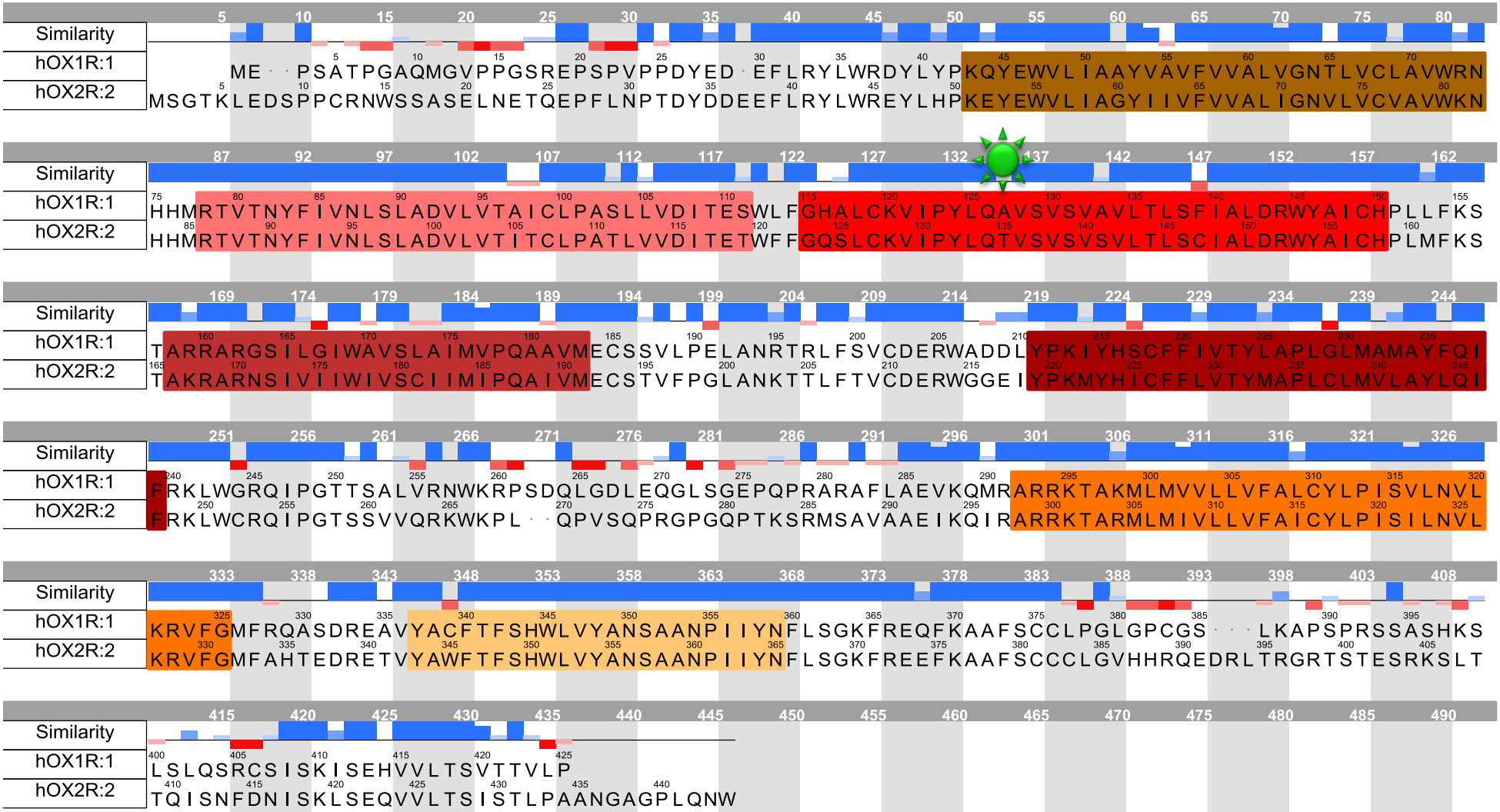


GSK1059865
OX1 selective

hOX1/hOX2 Sequence alignment

Similarity

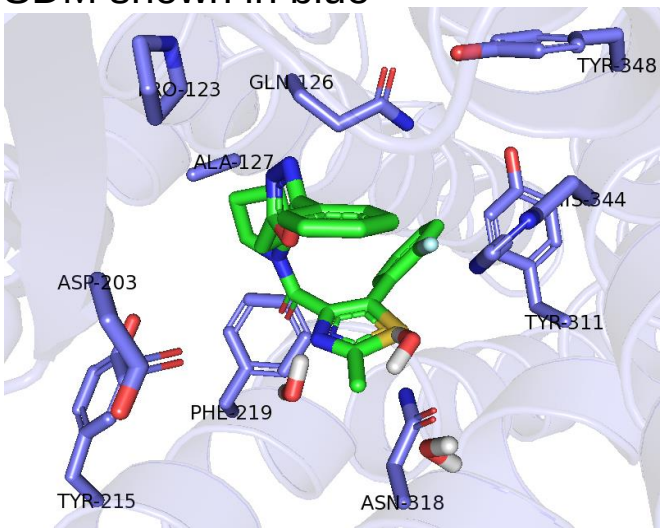


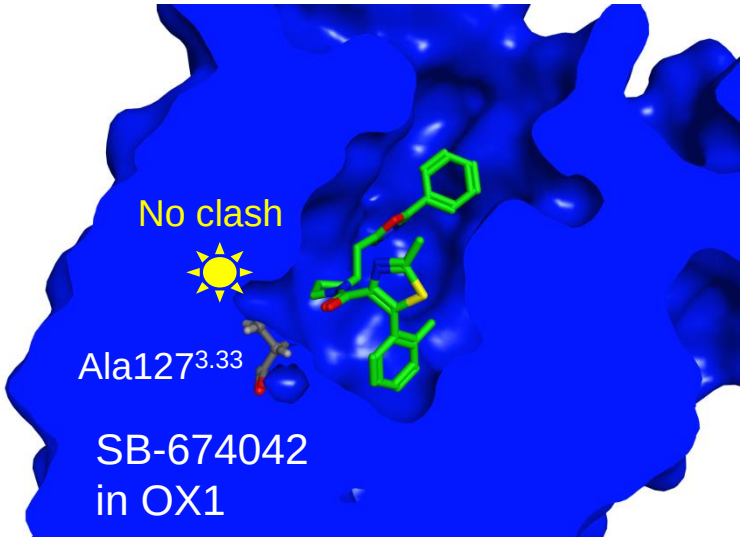


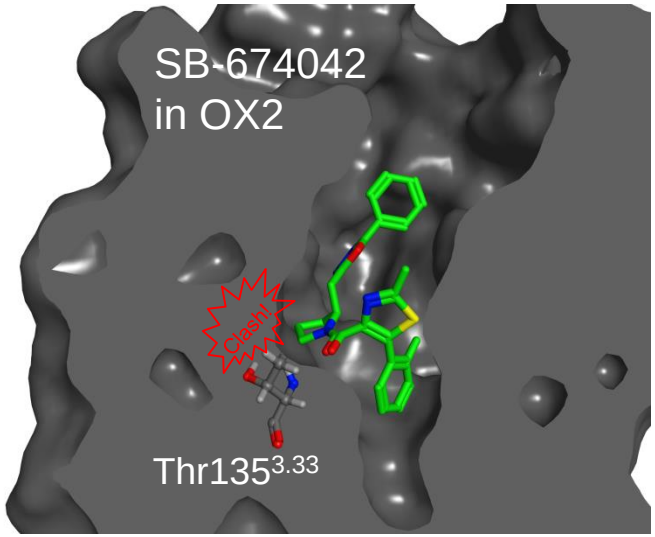
OX1 Selective Antagonists

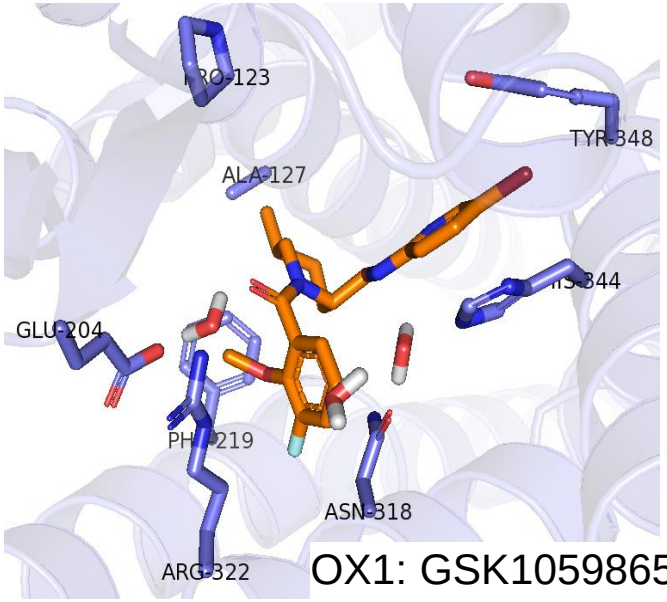
RLF docking poses

OX1: SB-674042
SDM shown in blue

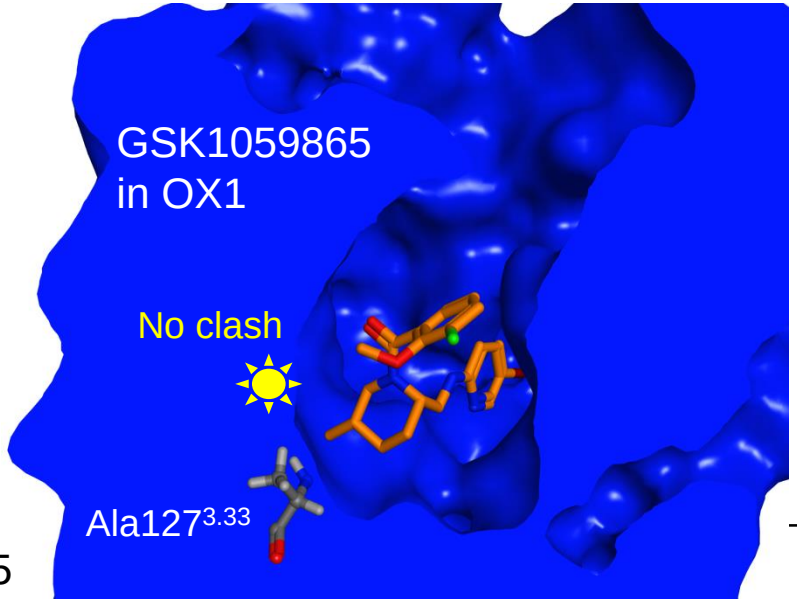


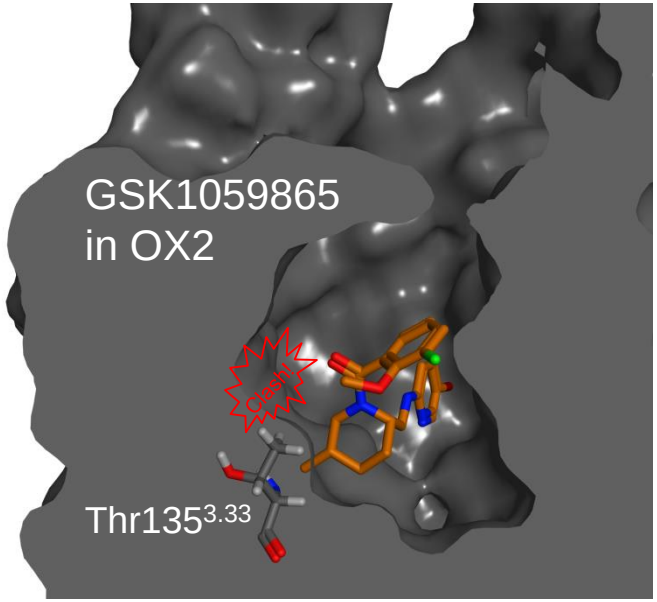






OX1: GSK1059865
SDM shown in blue

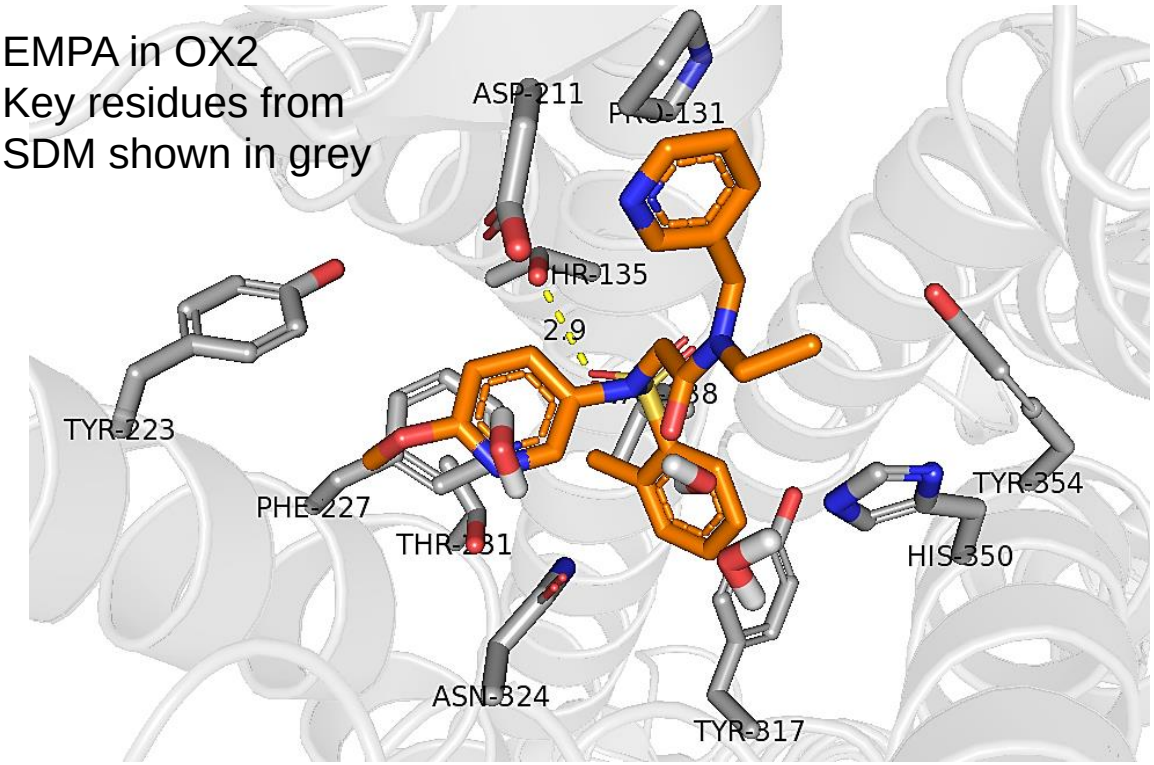




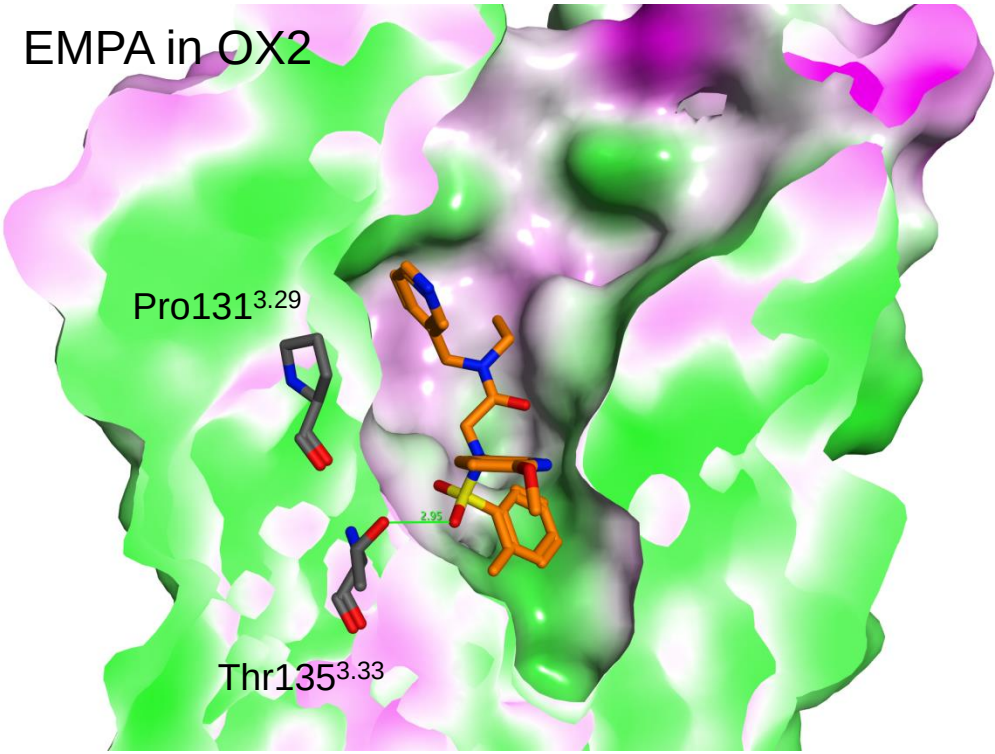
OX2 Selective Antagonist

EMPA

EMPA in OX2
Key residues from
SDM shown in grey



EMPA in OX2



- The docking pose of EMPA was validated by SDM data including the interaction with Thr135$^{3.33}$
- EMPA forms hydrogen bond with non-conserved Thr135$^{3.33}$, this interaction does not exist in OX1 – this can explain the OX2 selectivity of EMPA

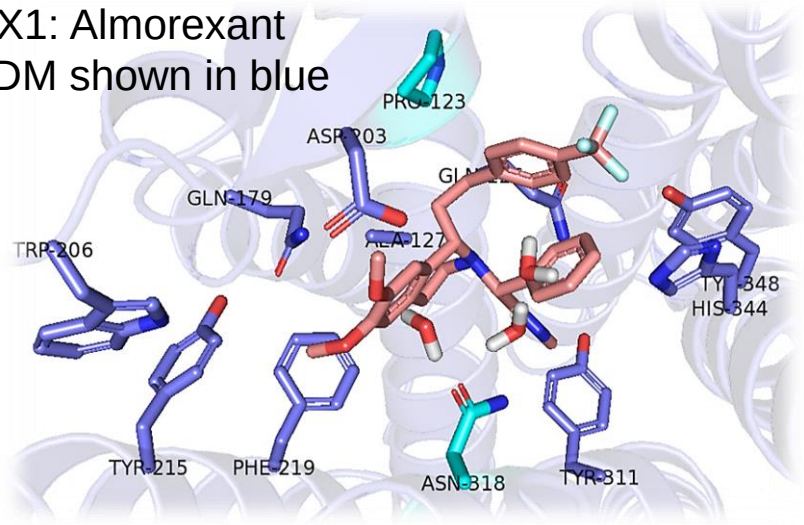
[1]Heifetz A. and Biggin PC. et. al.; Biochemistry. 2012 Apr 17;51(15):3178-97
[2]Malhebre P., et. al.; Mol Pharmacol. 2010 Jul;78(1):81-93

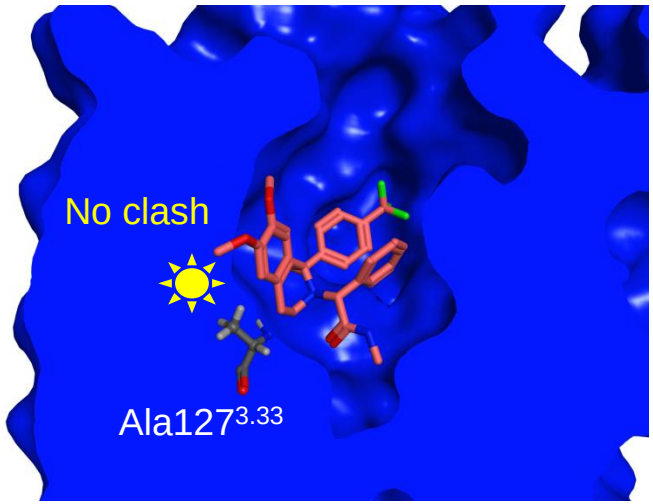
OX1/2 Dual Antagonists

Almorexant

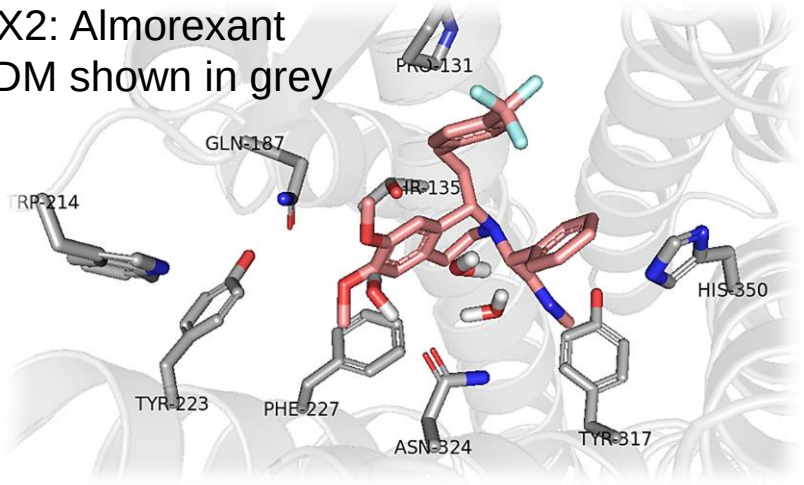
- The docking pose of Almorexant within OX1 and OX2 receptors
- The docking pose in both cases validated by site-directed mutagenesis data[1-2]
- No clashes observed between Almorexant with OX2 or OX1 receptors that can explain its duality

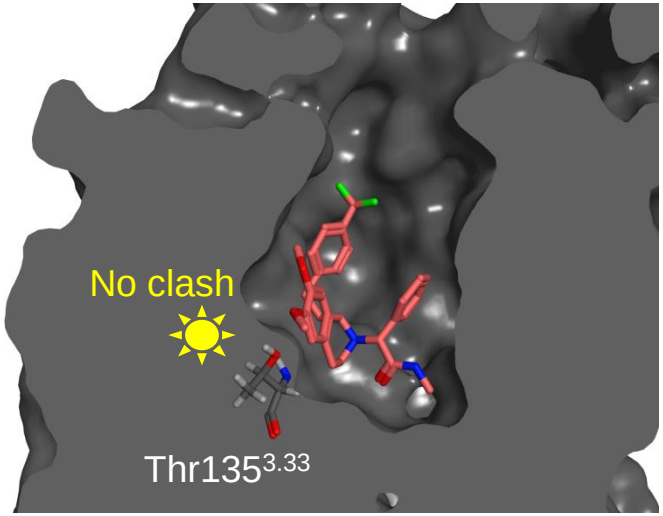
OX1: Almorexant
SDM shown in blue





OX2: Almorexant
SDM shown in grey



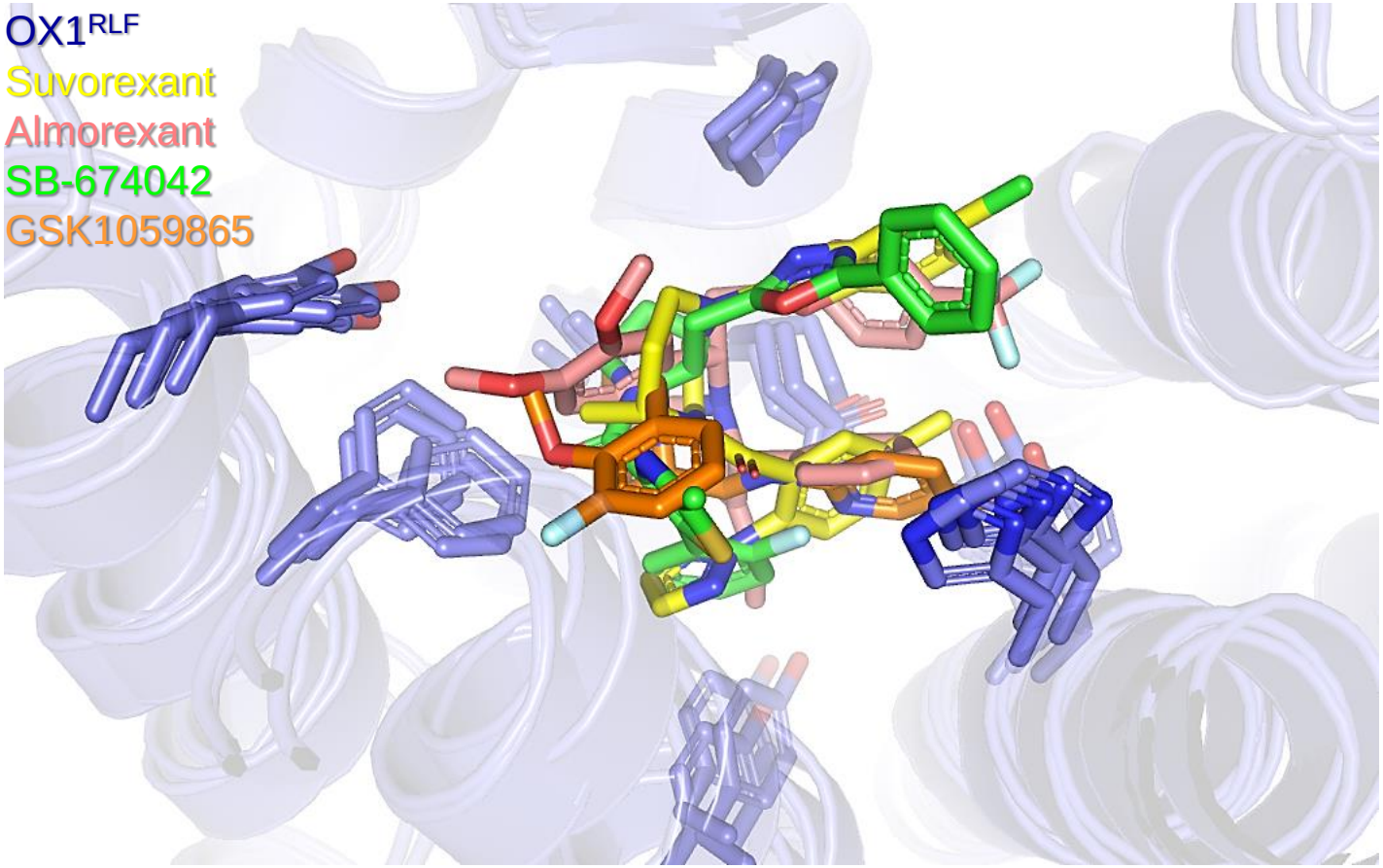


[1]Heifetz A. and Biggin PC. et. al.; Biochemistry. 2012 Apr 17;51(15):3178-97
[2]Malhebre P., et. al.; Mol Pharmacol. 2010 Jul;78(1):81-93

OX1 Antagonists

Addressing receptor flexibility

- RLF produced binding poses for key OX1 antagonists
- Different antagonists can access different areas of the OX1 pocket
  - The same phenomenon is observed in OX2
- Different ligand chemotypes induce different conformational changes in the OX1 binding site
- HGMP-C4XD (RLF) docking protocol addresses ligand-induced binding-site flexibility



HGMP-C4XD

Preliminary conclusions and working plan

- The combination of C4XD data and HGMP was very efficient in predicting the binding poses of 5 dual and selective OX1/2 antagonists
- The HGMP-C4XD protocol can isolate the bioactive conformation from the ensemble of ligand conformations in solvent
- The bioactive conformation was found amongst the most populated conformers in the solvent
- The structural insights produced by this approach are very useful in further SBDD of OX1 selective antagonists
- Further validation of the method is required/ongoing

*Binding site topology includes – sidechains and backbone of residues, water molecules networking and binding site surface

Acknowledgements

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Matteo Aldeghi

Dr. Philp C. Biggin

AIST Tsukuba Japan

Dr. Dmitri Fedorov

Evotec

Dr. Ewa Chudyk

Dr. Laura Gleave

Dr. Tim James

Dr. Tara Fryatt

Dr. Jonathan Bentley

Dr. Mike Bodkin

C4X Discovery

Dr. Thorsten Nowak

Dr. Barrie Martin

evotec

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Your contact:

Alexander Heifetz
Principal Scientist

+44.(0) 1235 83 8925
alexander.heifetz@evotec.com