



## Decoding the role of solvent in ligand-protein binding kinetics

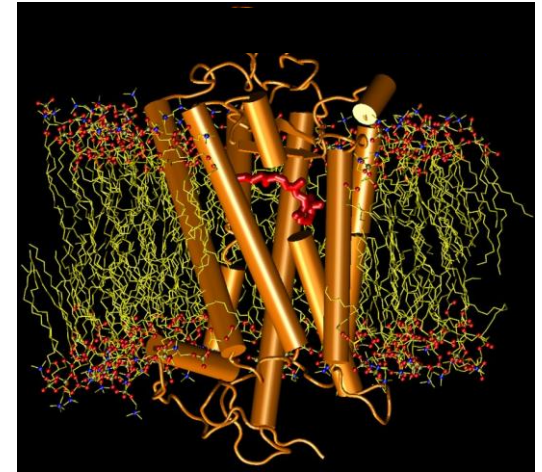
**Andrea Bortolato**

***UK-QSAR, Autumn Meeting***

***CCDC Cambridge, 30<sup>th</sup> September 2014***

# G Protein-Coupled Receptors (GPCRs) Super Family

- Most important family of drug targets in industry
- 800 GPCRs including ~400 olfactory
- 225 with known ligands, 150 'orphan' receptors
- Compelling biology across wide range of diseases
- Many valuable yet challenging targets still untapped



## Many Top-Selling Drugs Hit GPCRs

**Plavix**  
Clopidogrel 75 mg

**ADVAIR**  
Formoterol Fumarate / Salmeterol Xinafole

**OXYCONTIN<sup>®</sup> XL**  
(OXYCODONE HCl CONTROLLED-RELEASE) TABLETS

**ABILIFY<sup>®</sup>**  
ARIPRAZOLE

**BREO<sup>™</sup> ELLIPTA<sup>™</sup>**

**ANORO<sup>™</sup>**  
ELLIPTA<sup>™</sup>

**Opsumit**  
macitentan tablets 10 mg

**ONCE-A-DAY SINGULAIR**  
(MONTELUKAST SODIUM)

**Seroquel<sup>®</sup>**

**ZYPREXA**  
IntraMuscular  
Clonazepam for Injection

**SPIRIVA<sup>®</sup>**  
(tiotropium)

**Signifor<sup>®</sup>**  
pasireotide

**Erivedge<sup>®</sup>**  
(vismodegib) capsule

**ZIOPTAN<sup>™</sup>**  
(tafluprost ophthalmic solution)

**Diovan<sup>®</sup>**  
valsartan capsules

**Ventolin HFA**  
(albuterol sulfate)  
inhalation aerosol

**Suboxone<sup>®</sup>**  
(buprenorphine HCl/naloxone HCl dihydrate)

**GEODON<sup>®</sup>**  
(ziprasidone HCl) Capsules

**BELVIQ<sup>®</sup>**  
(lorcaserin HCl)

**Gattex<sup>®</sup>**  
(teduglutide [rDNA origin]) for Injection

**Tracleer**  
BOSENTAN TABLET

**Detrol<sup>®</sup> LA**  
lutheredine tartrate  
extended-release capsules

**Benicä<sup>®</sup>r**  
(olmesartan medoxomil)

**Byetta<sup>®</sup>**  
exenatide injection

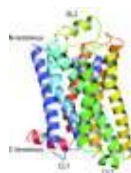
**Sensipar<sup>®</sup>**  
(cinacalcet) Tablets

**Myrbetriq<sup>®</sup>**  
(mirabegron)  
extended-release tablets

**Tudorza<sup>™</sup> Pressair<sup>™</sup>**  
(acclidinium bromide inhalation powder)

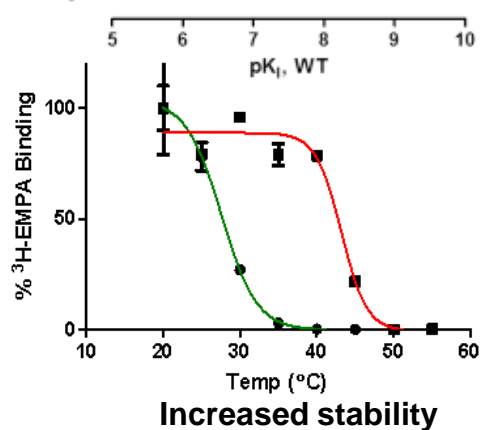
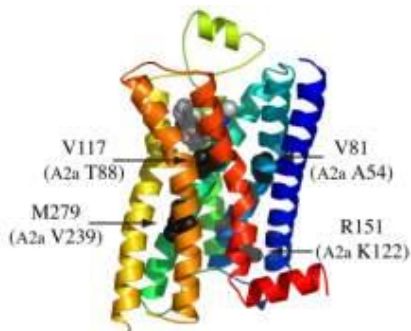
# Heptares Therapeutics

- Exceptional pipeline of new medicines targeting G protein-coupled receptors
- Multiple Phase 1 & Phase 2 clinical programs reporting data during 2014-16
- Indications: Alzheimer's, Schizophrenia, Diabetes, ADHD, Chronic Migraine
- Leading structure- and fragment-based GPCR discovery platform
- Proprietary StaR<sup>®</sup> technology enables small molecule & biologics discovery
- Deals include: AstraZeneca, MedImmune, Cubist, MorphoSys, Takeda
- Experienced team in UK (>70 staff) and USA / Boston

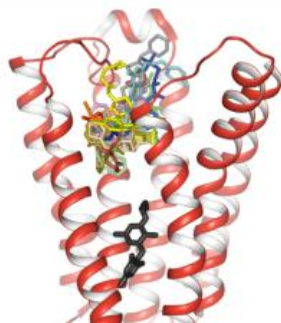


# Heptares Technology Platform

## Stabilised Receptor (StaR<sup>®</sup>)

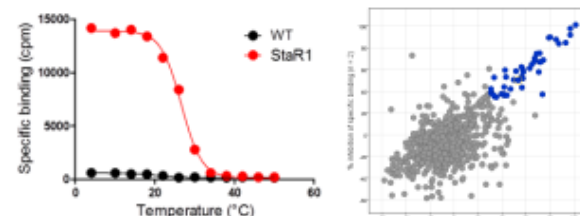


## GPCR Structure Determination



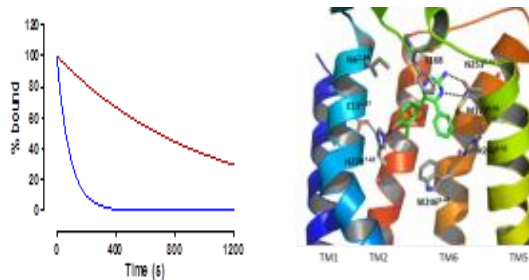
Landmark structure reveals new drug binding site in Family B GPCRs

## GPCR Fragment-Based Design



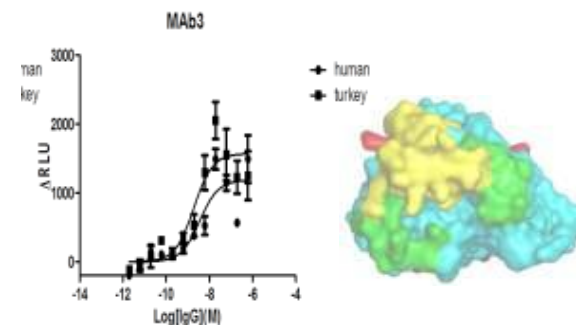
FBDD of mGlu modulator; sub-nanomolar affinity with superior PK

## Controlling Receptor Kinetics



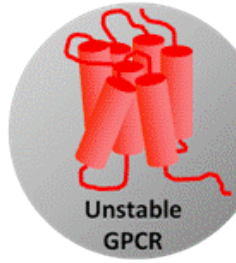
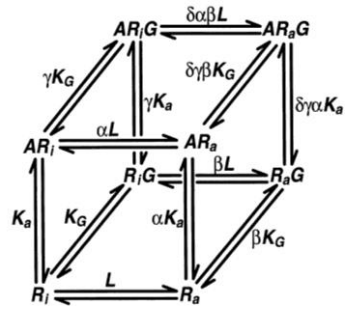
Drug kinetics by StaR<sup>®</sup> using SPR related to X-ray crystal structures

## Antigens for mAb Discovery



Functional or blocking mAbs generated using StaR<sup>®</sup> antigens

# StaR<sup>®</sup> approach delivers stabilised GPCRs

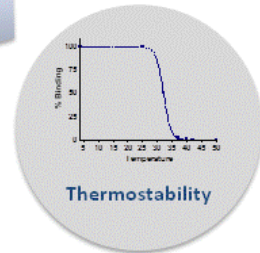


AATCAGC  
GTCG  
Mutagenesis

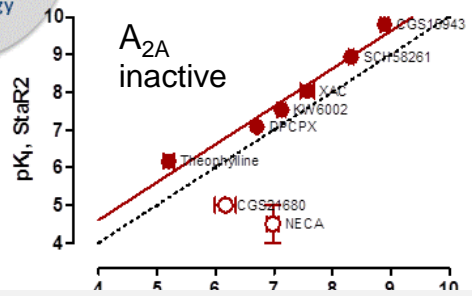
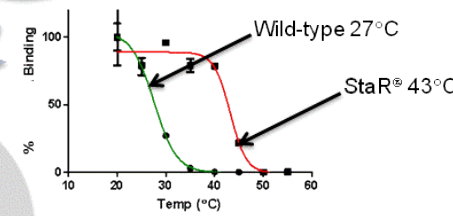
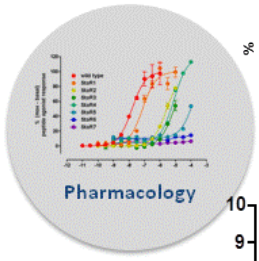
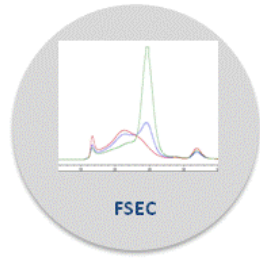
1000+ SDMs  
Multiple side chains



Iterative process for making StaRs

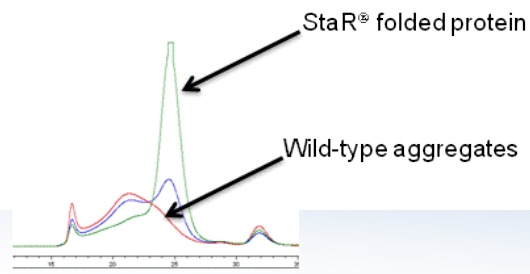


Screening  
Biacore kinetics  
Crystallisation  
Antibody Generation



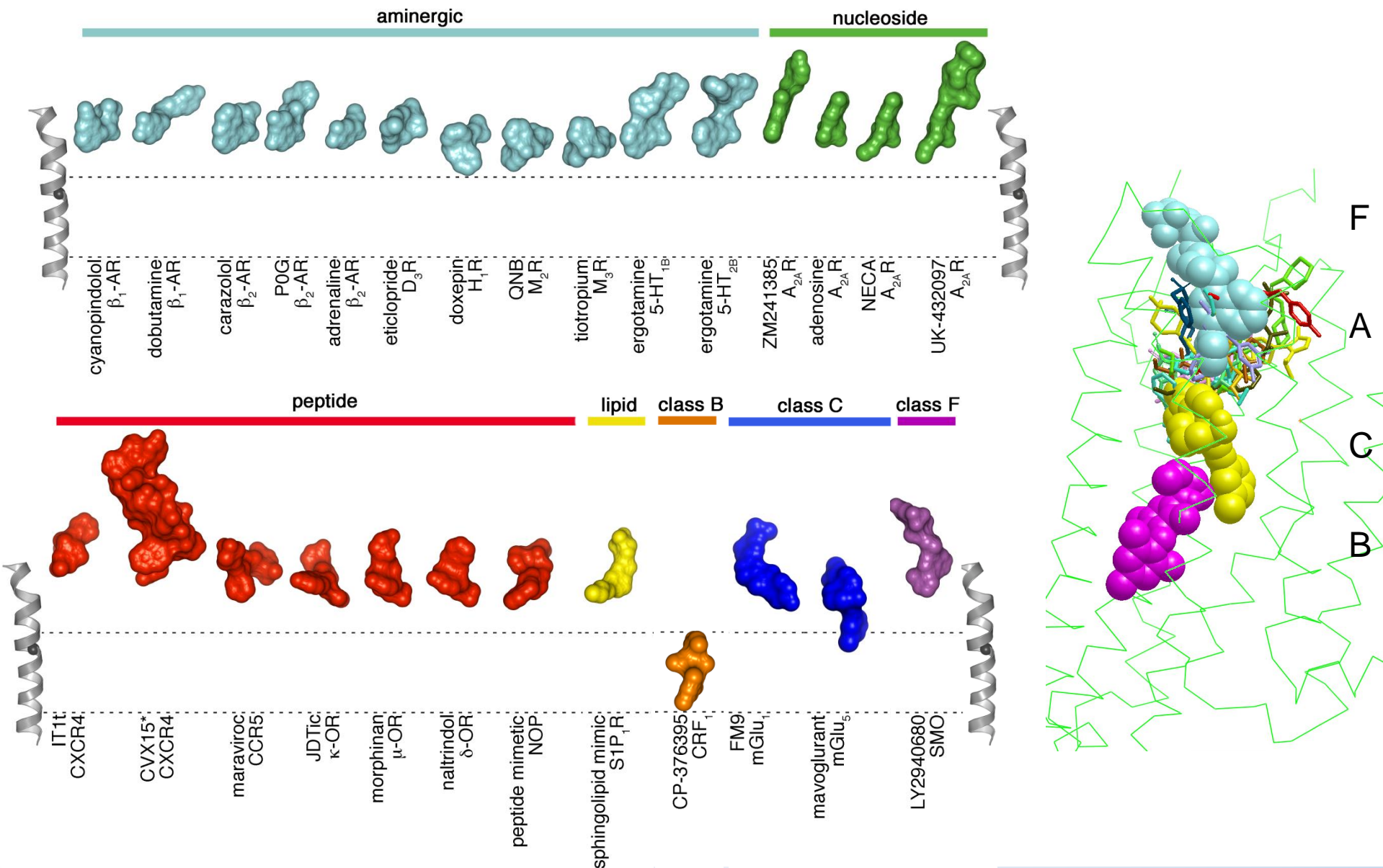
StaR<sup>®</sup>

- folded protein
- crystals

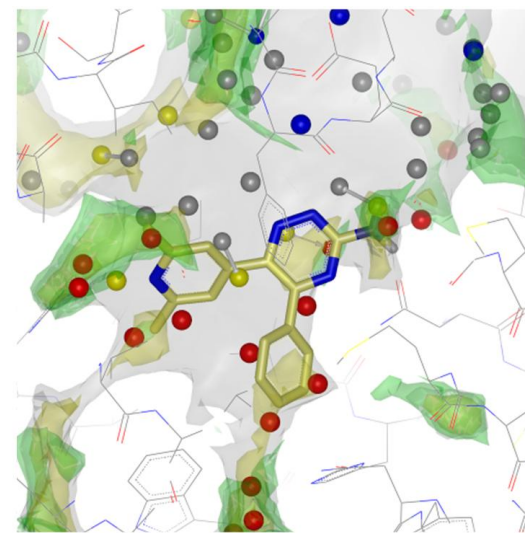
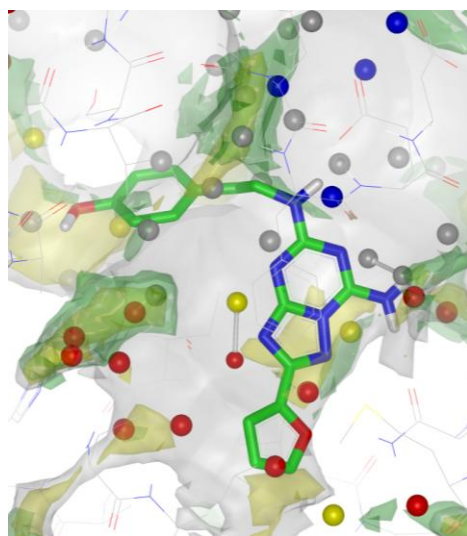
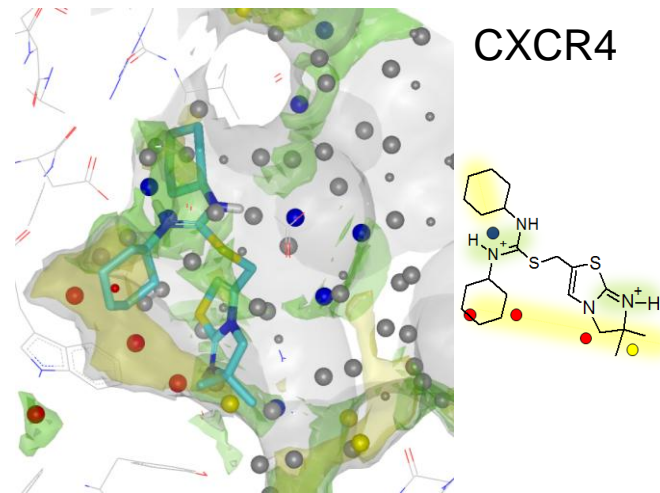
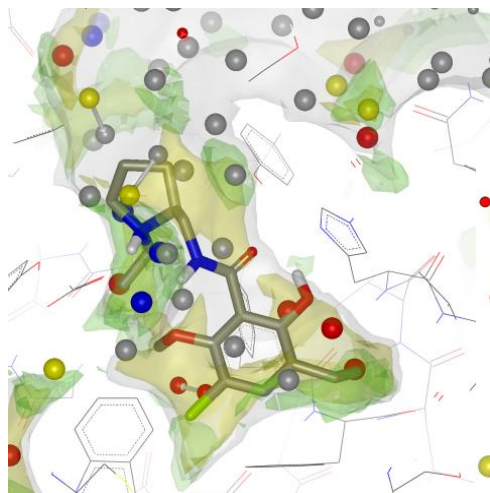
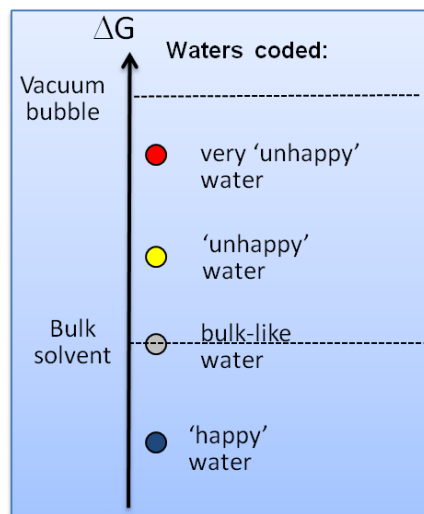
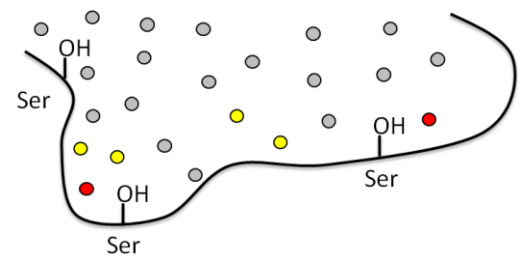


Pharmacology Correlates with StaR Conformation

# New X-Ray Structures Highlight the Complexity of GPCRs



# GPCR Structures → Druggability → SBDD



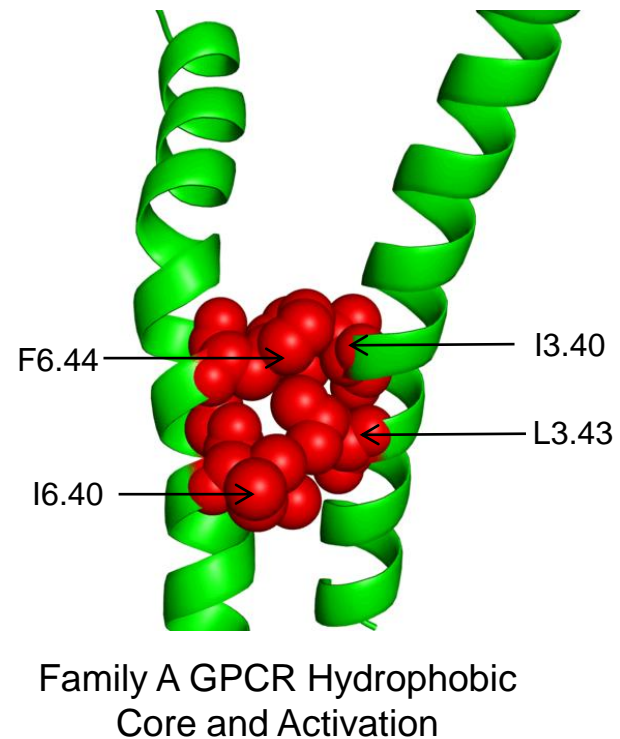
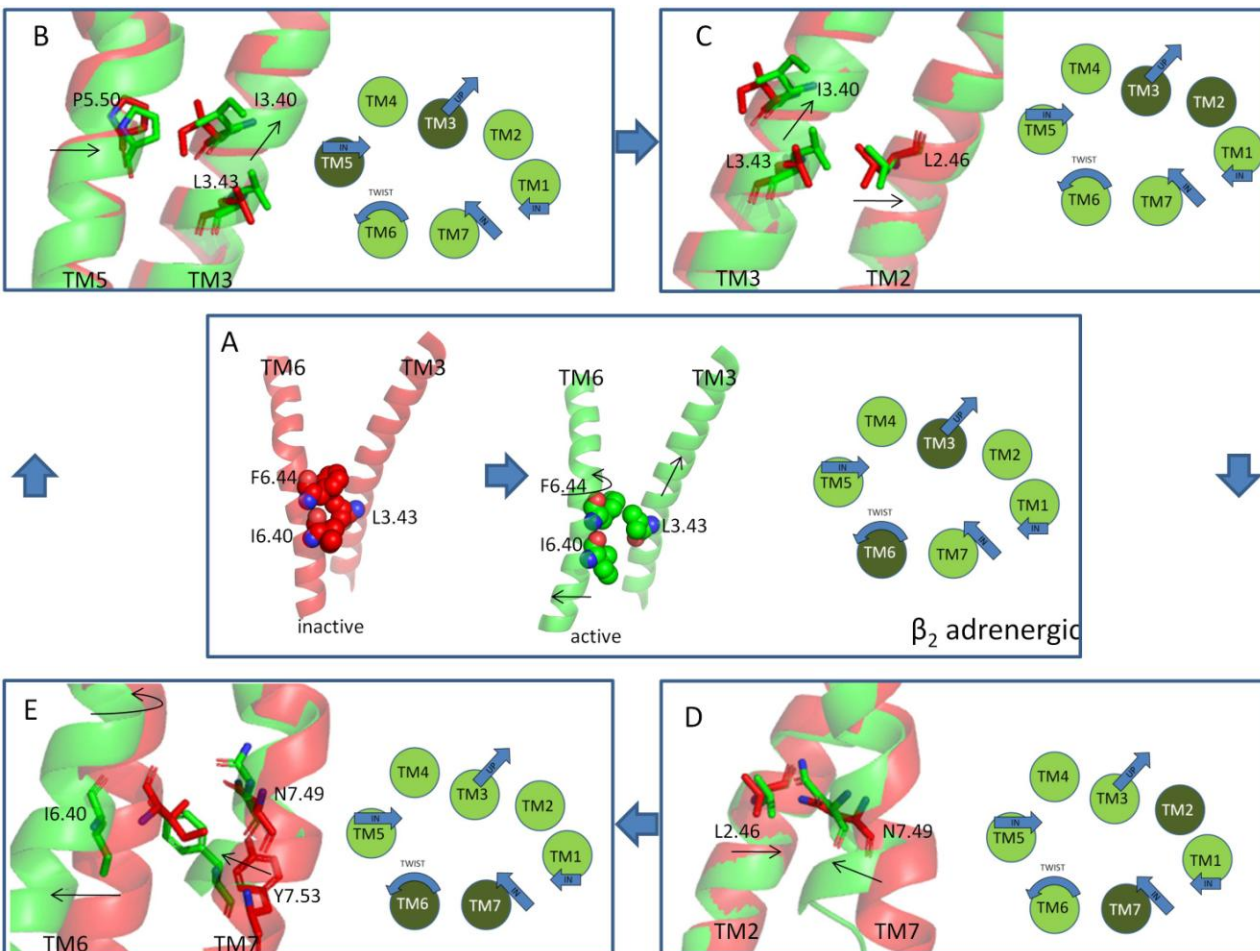
**GRID C1= hot-spot (hydrophobic)**

**GRID OH2 hot-spot (hydrophilic)**

**GRID C3 pocket shape**

New insights from structural biology into the druggability of G protein-coupled receptors. Mason, Bortolato, Congreve, Marshall. Trends Pharmacol Sci. 2012;33(5):249-60.

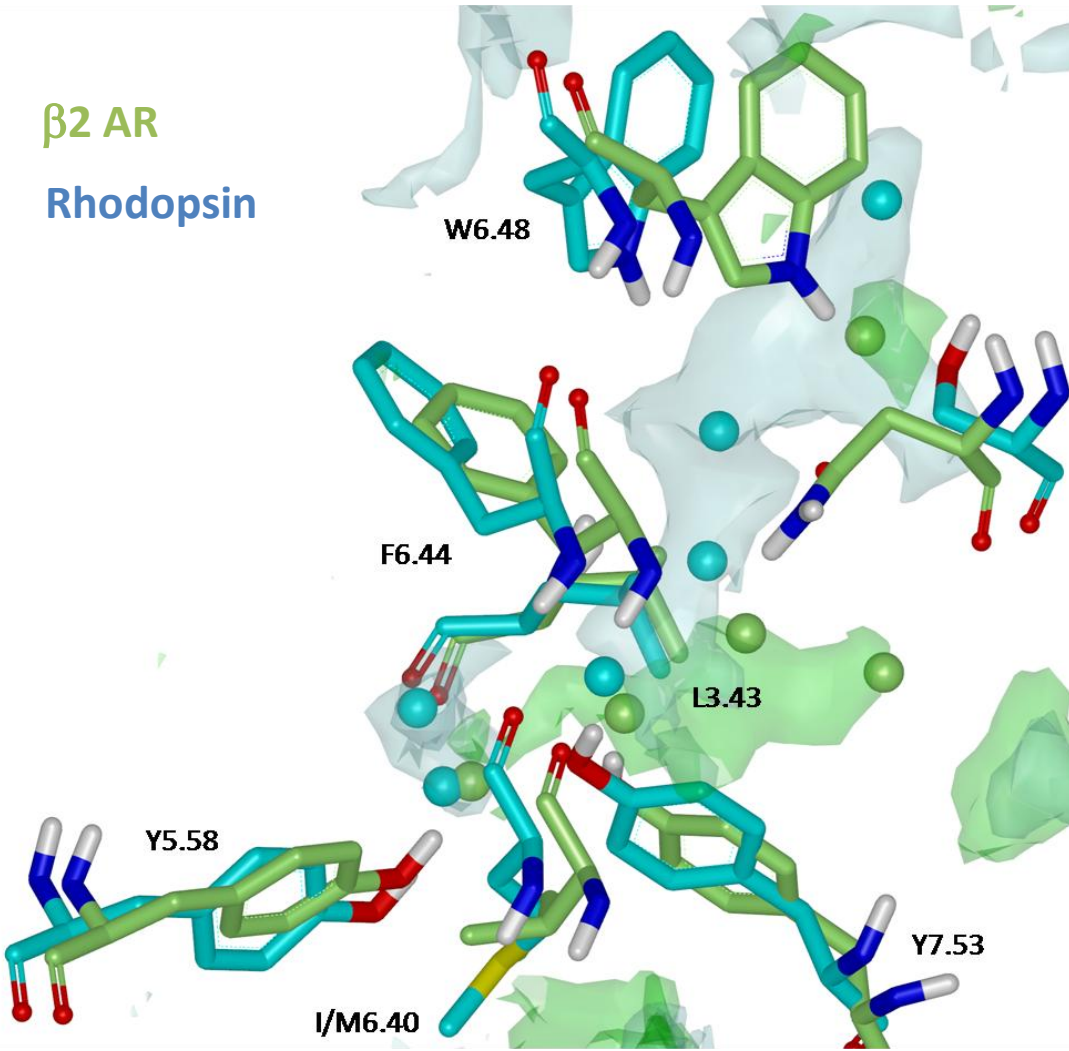
# GPCR Structures → Dynamics → Function



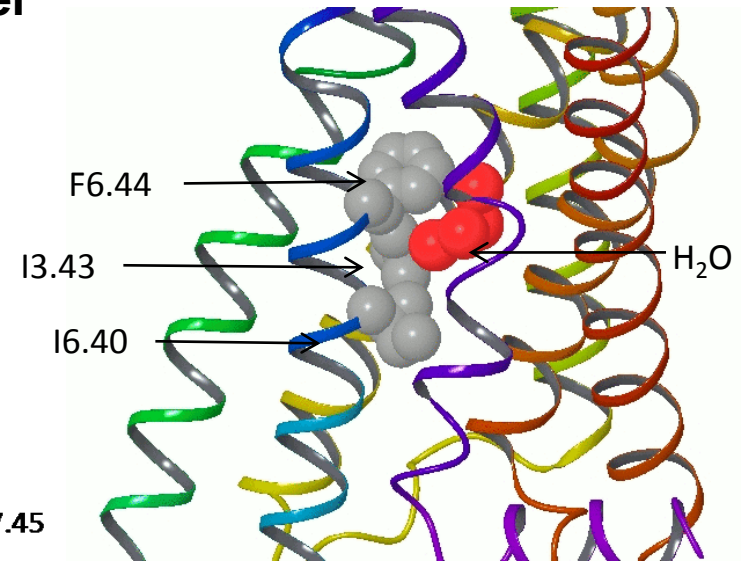


# Family A Hydrophobic Core Affects Water Channel

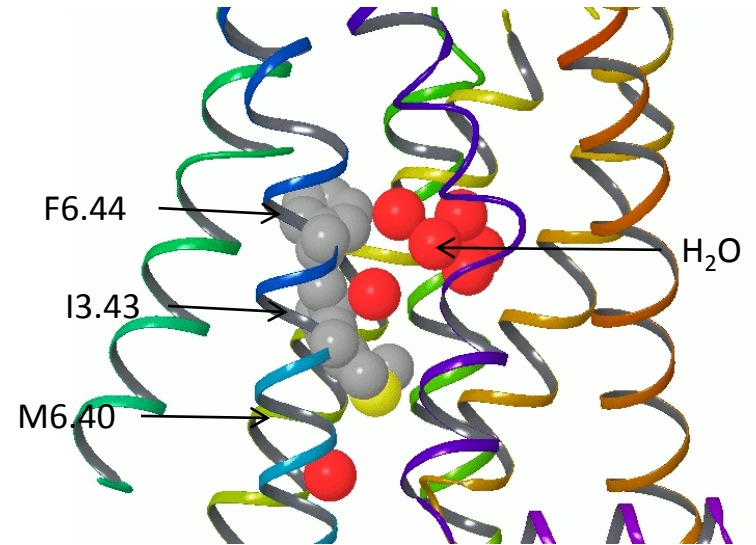
$\beta$ 2 AR  
Rhodopsin



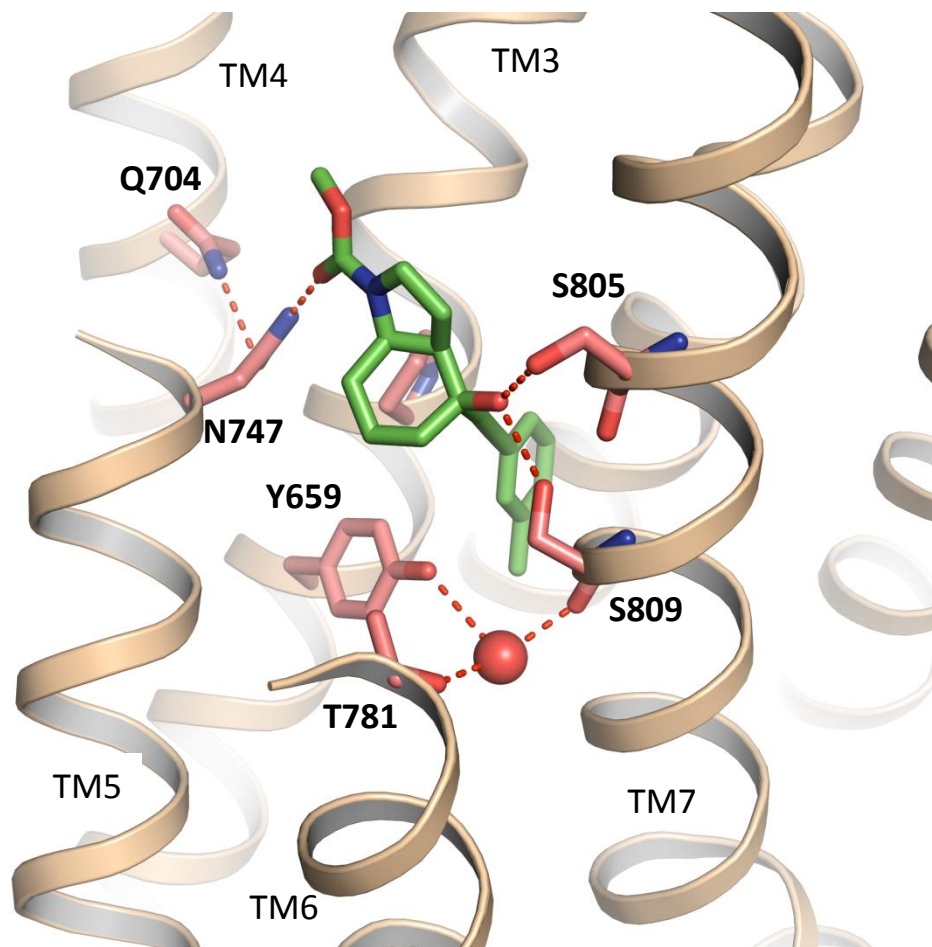
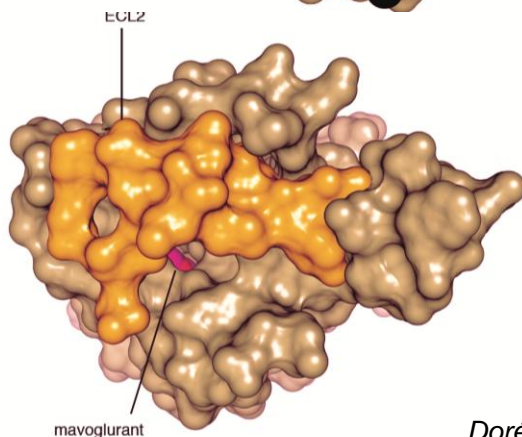
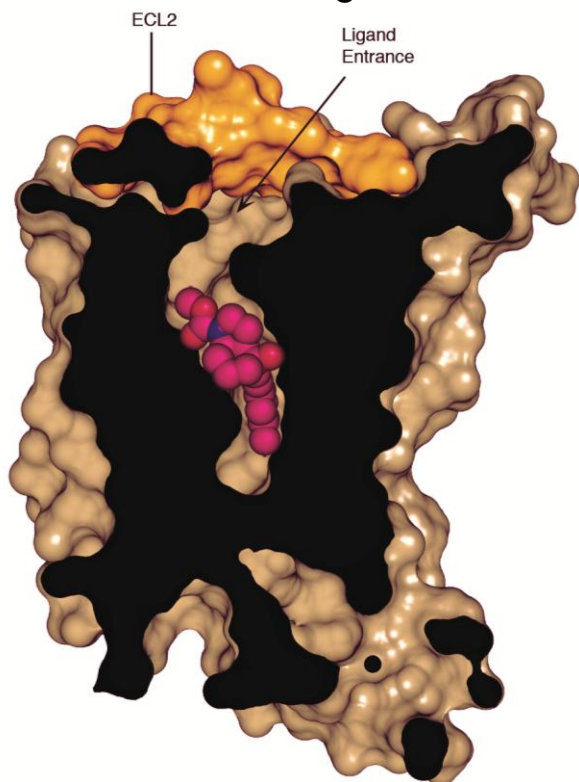
$\beta$ 2 AR



Rhodopsin



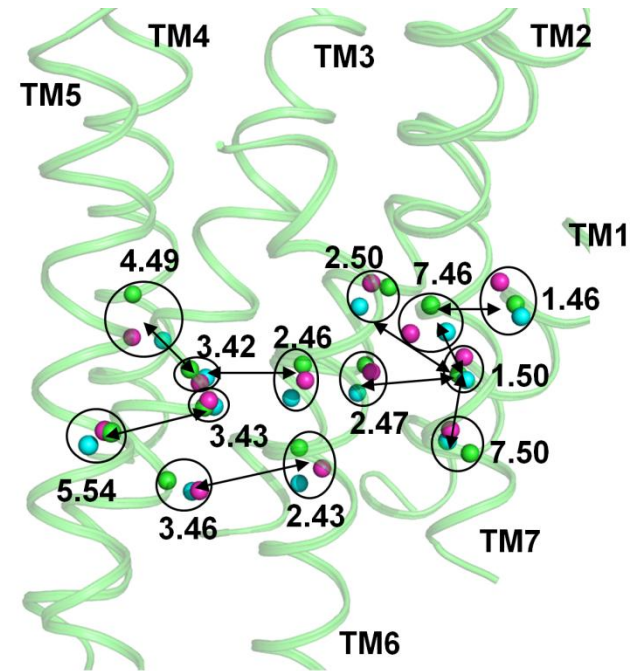
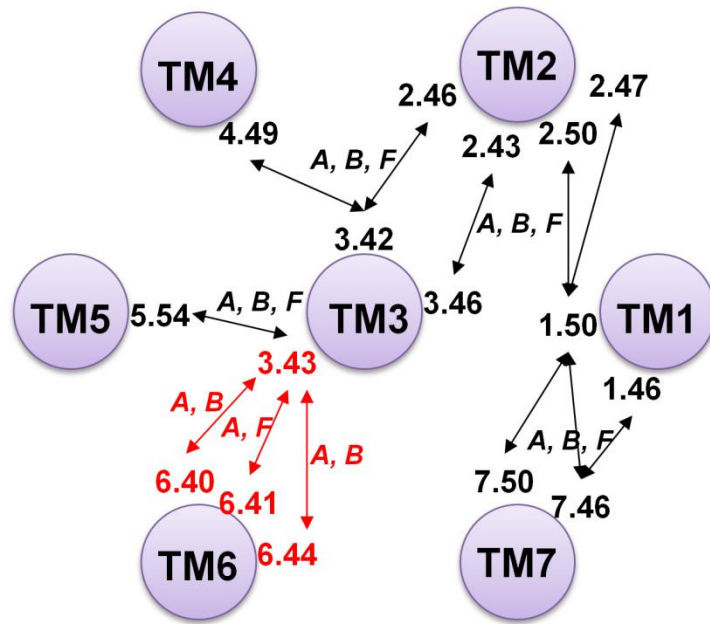
# mGlu<sub>5</sub> bound to mavoglurant at 2.6Å resolution



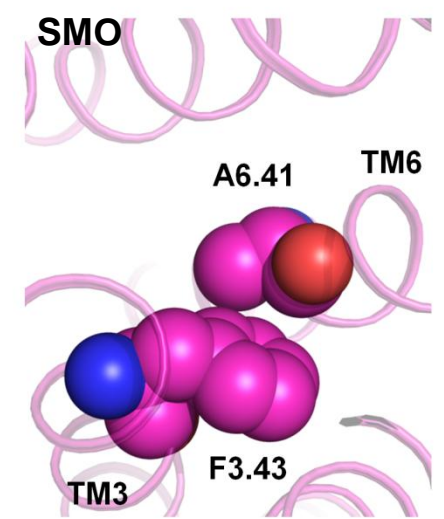
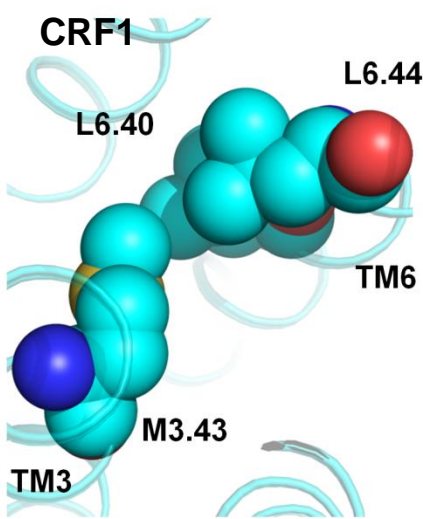
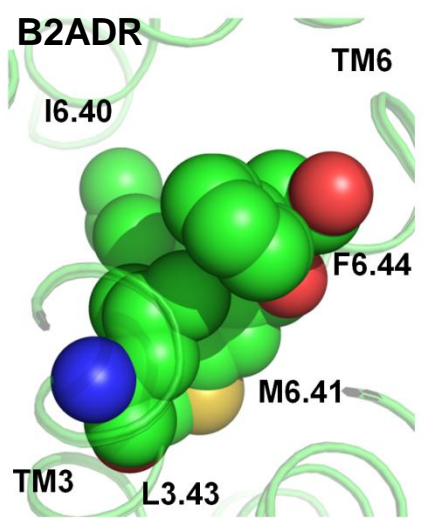
Vary 3-methyl : Methoxy > Chloro > Fluoro  
(DFB) NAM > neutral binder > PAM

Doré AS et al. Nature. 2014;511(7511):557-62.

# Extending To Family B and F GPCRs

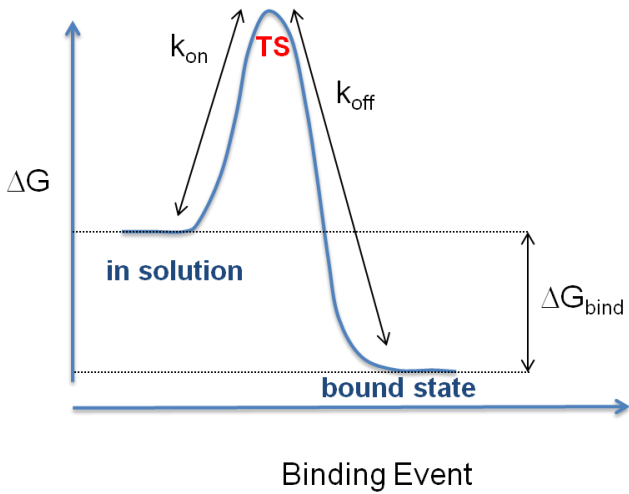


Hydrophobic Core common feature across subfamilies



Bortolato et al. *BJP* 2014, 171(13):3132-45.

# Understanding Ligand Binding Kinetics



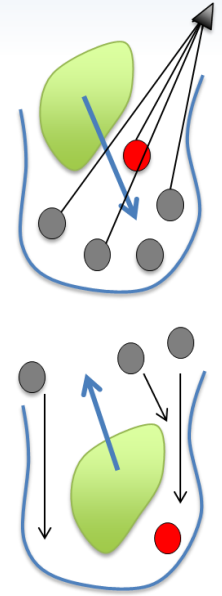
Transition state key for kinetics evaluation:

$k_{on}$  TS:

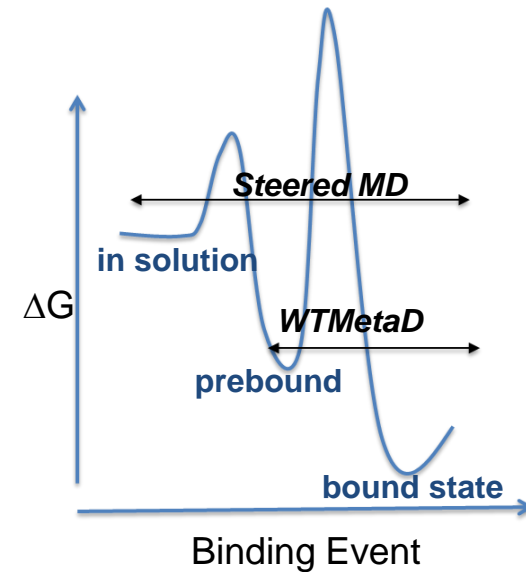
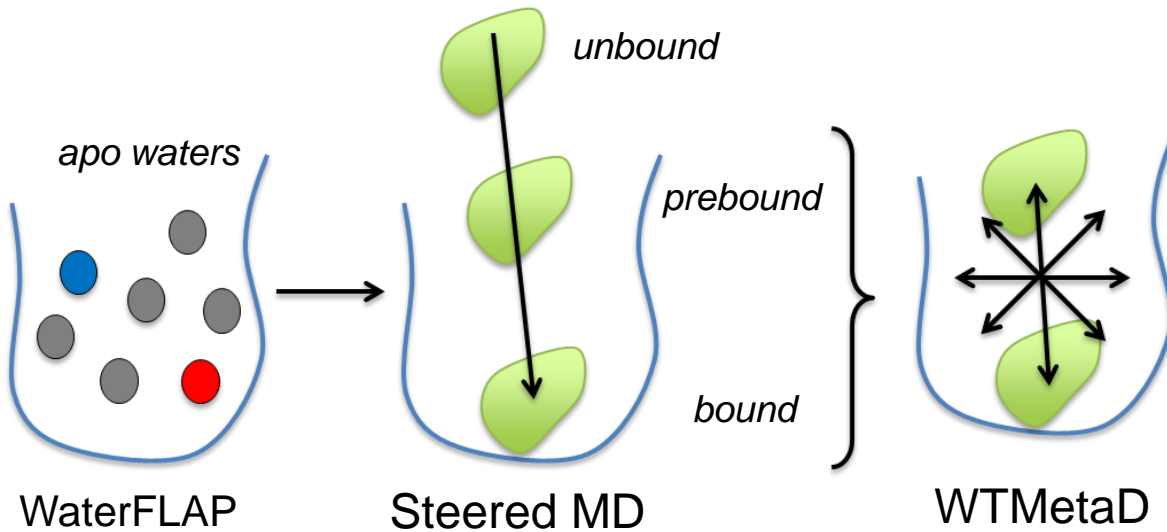
- Desolvation of ligand
- Desolvation of the pocket

$k_{off}$  TS:

- Breaking of the protein-ligand interactions
- Solvation of ligand
- Solvation of the pocket

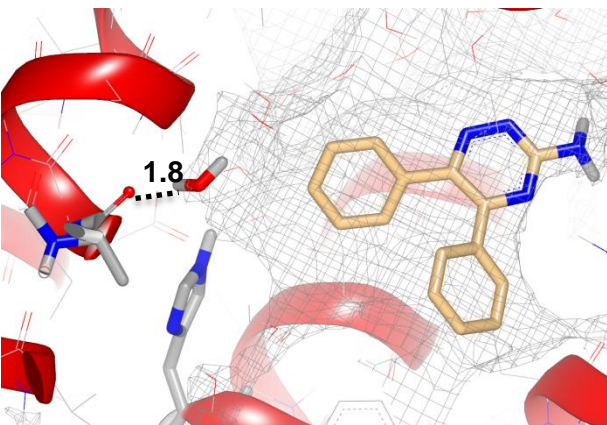


Computation protocol using one python script:



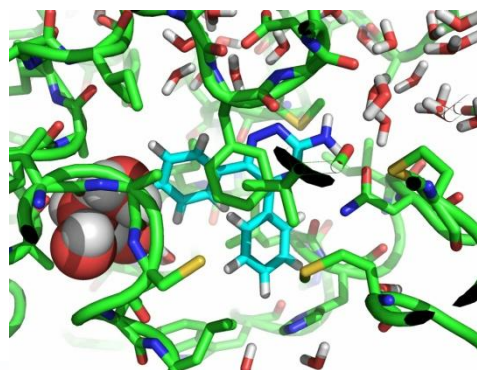
# Water Network Perturbation

$pK_i=6.9$   
 $\tau \sim 0s$

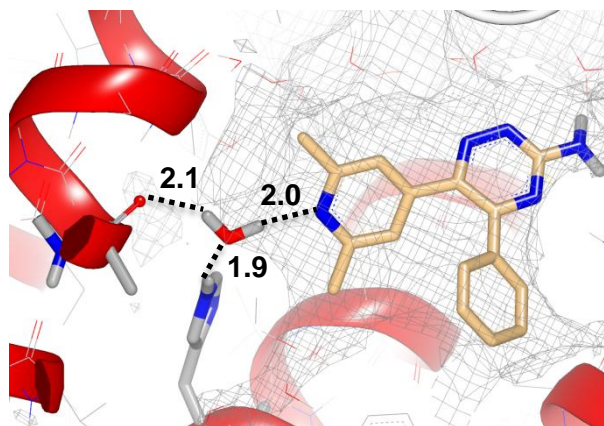


OH2 energy = -10.1 kcal/mol  
CRY energy = -2.9 kcal/mol

OH2/CRY energy = -4.3 kcal/mol



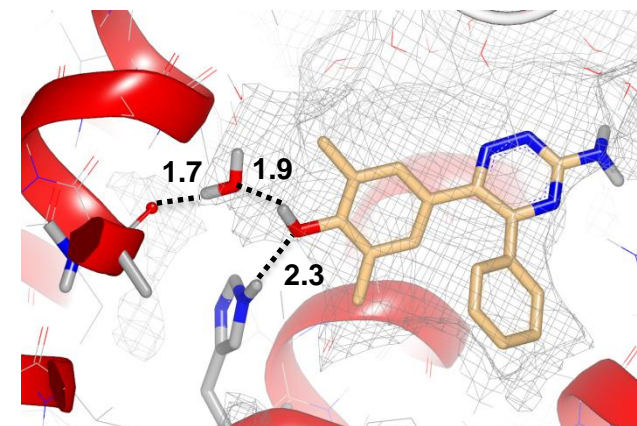
$pK_i=8.1$   
 $\tau = 87s$



OH2 energy = -12.8 kcal/mol  
CRY energy = -2.0 kcal/mol

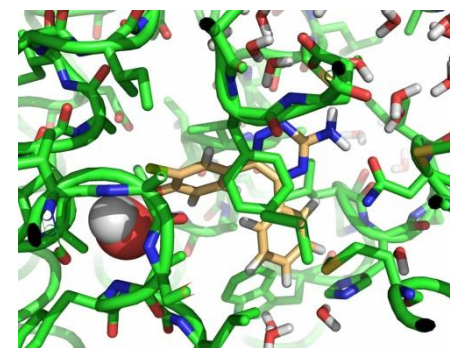
OH2/CRY energy = -8.8 kcal/mol

$pK_i=8.4$   
 $\tau = 735s$



OH2 energy = -12.3 kcal/mol  
CRY energy = -1.5 kcal/mol

OH2/CRY energy = -9.3 kcal/mol



Bortolato et al. JCIM 2013, 1700-13

**Please**

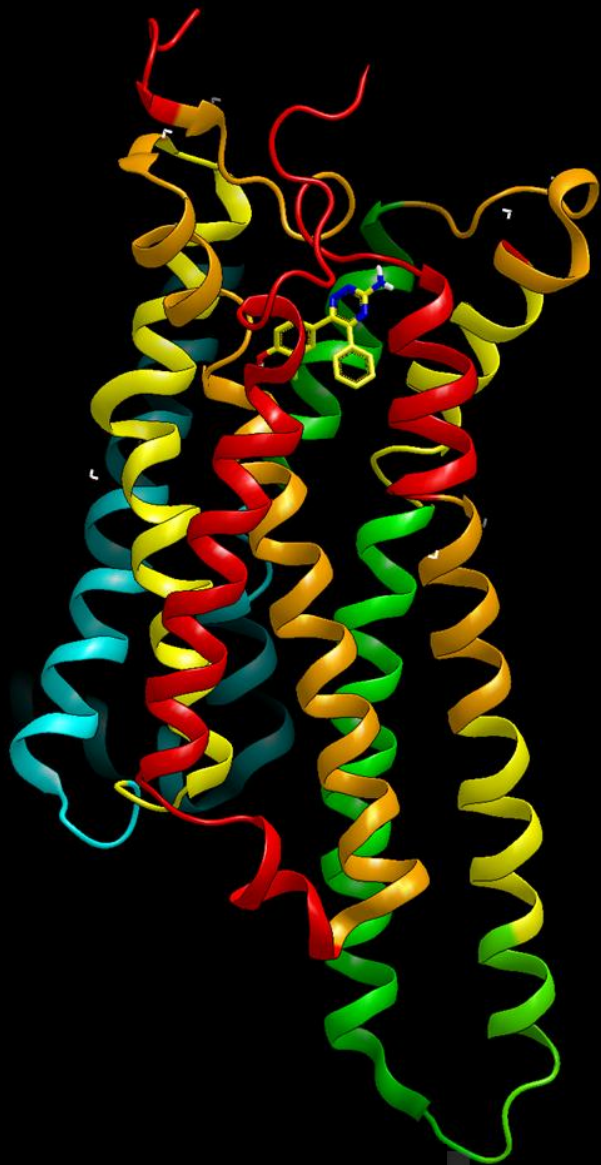
**put on**

**your**

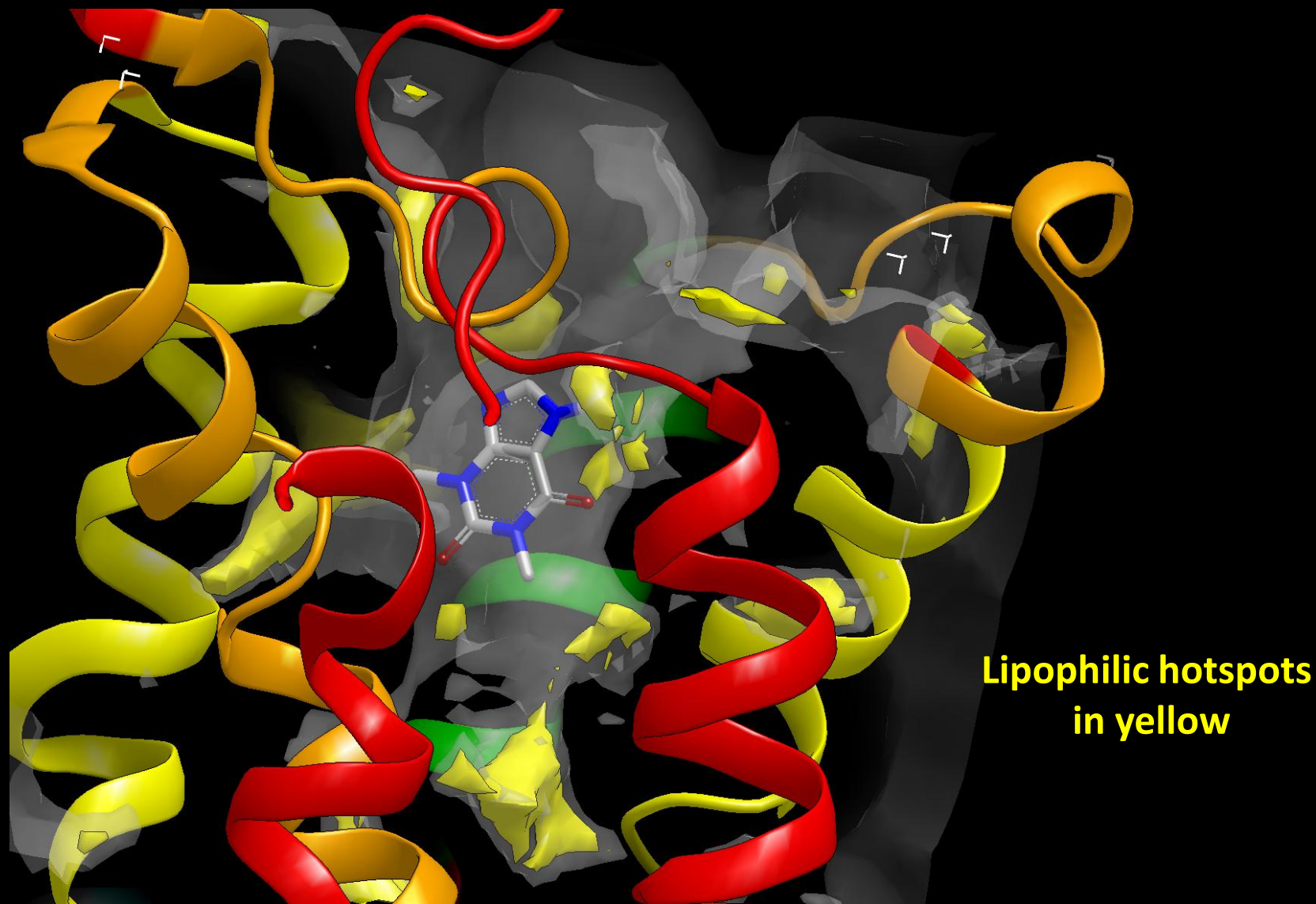
**ChromaDepth glasses**

**now**

**Please fold with writing inside & leave  
on stage at end - Thank you!**

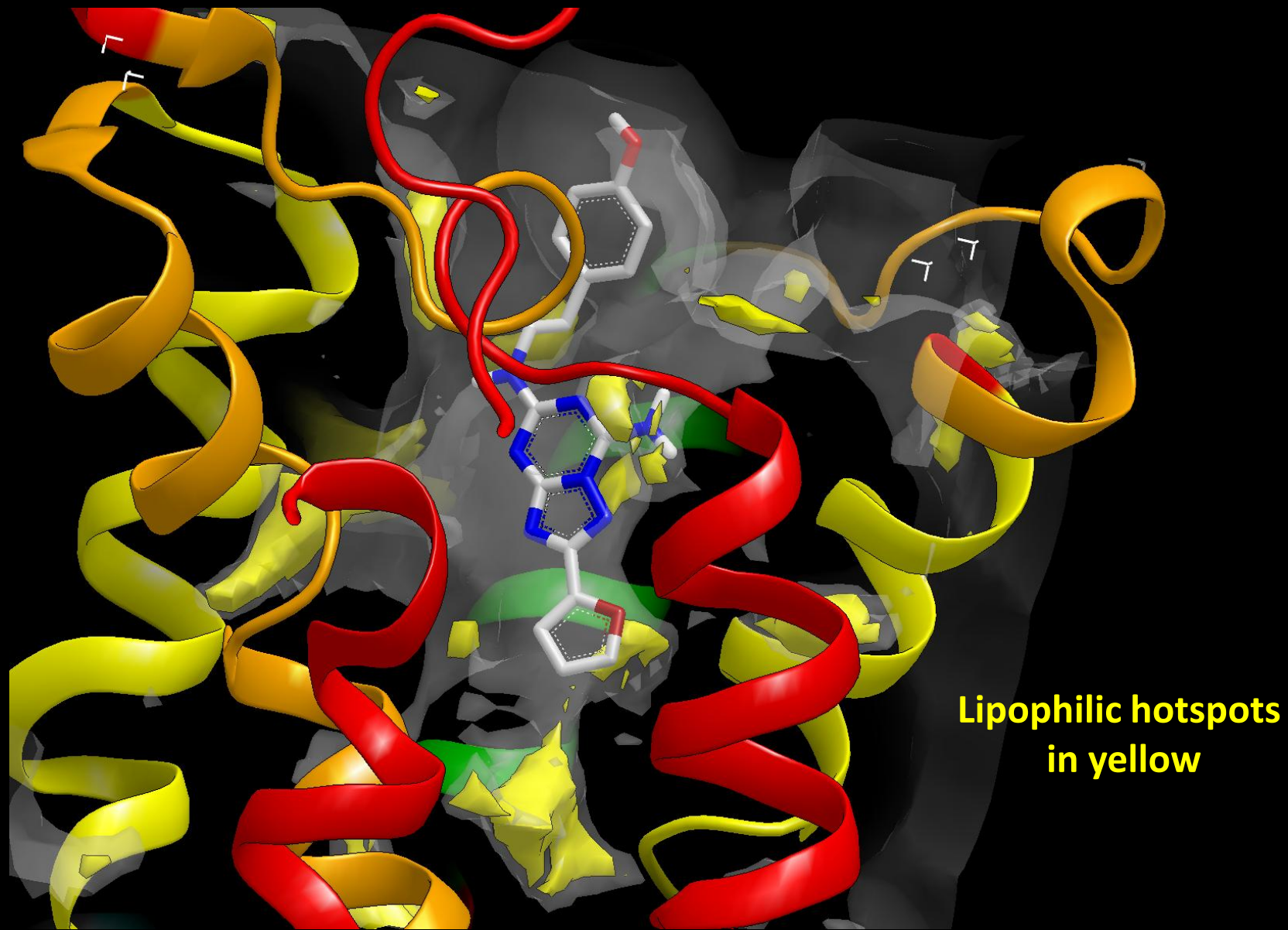


**Binding of the Heptares  
triazine antagonist to the A<sub>2A</sub>  
receptor**

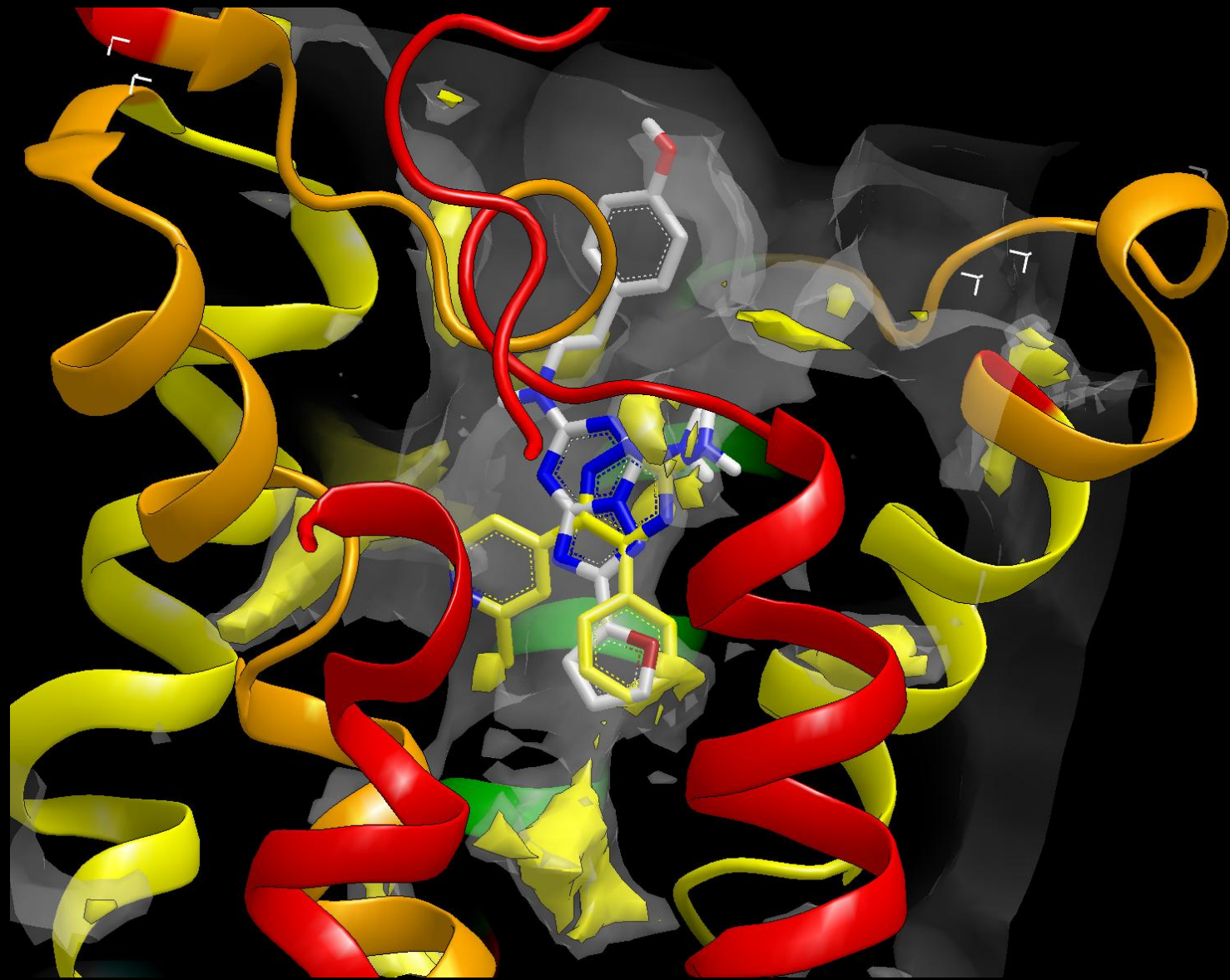


The caffeine binding pocket in the  $A_{2A}$  receptor  
A Neutral antagonist

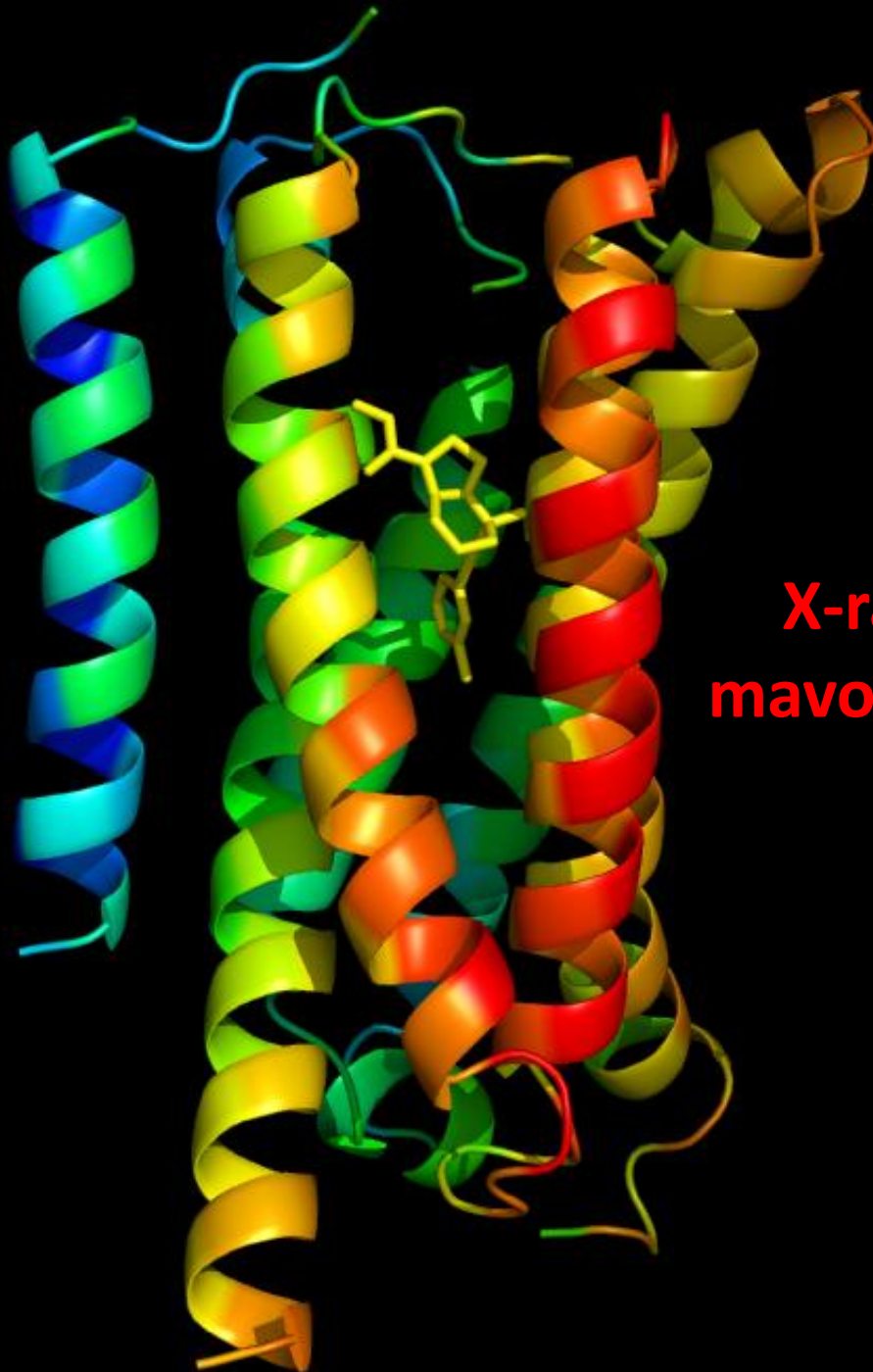




**A<sub>2A</sub> receptor bound to the inverse agonist - ZM241385**



**Overlay of ligands bound to the A<sub>2A</sub> receptor**

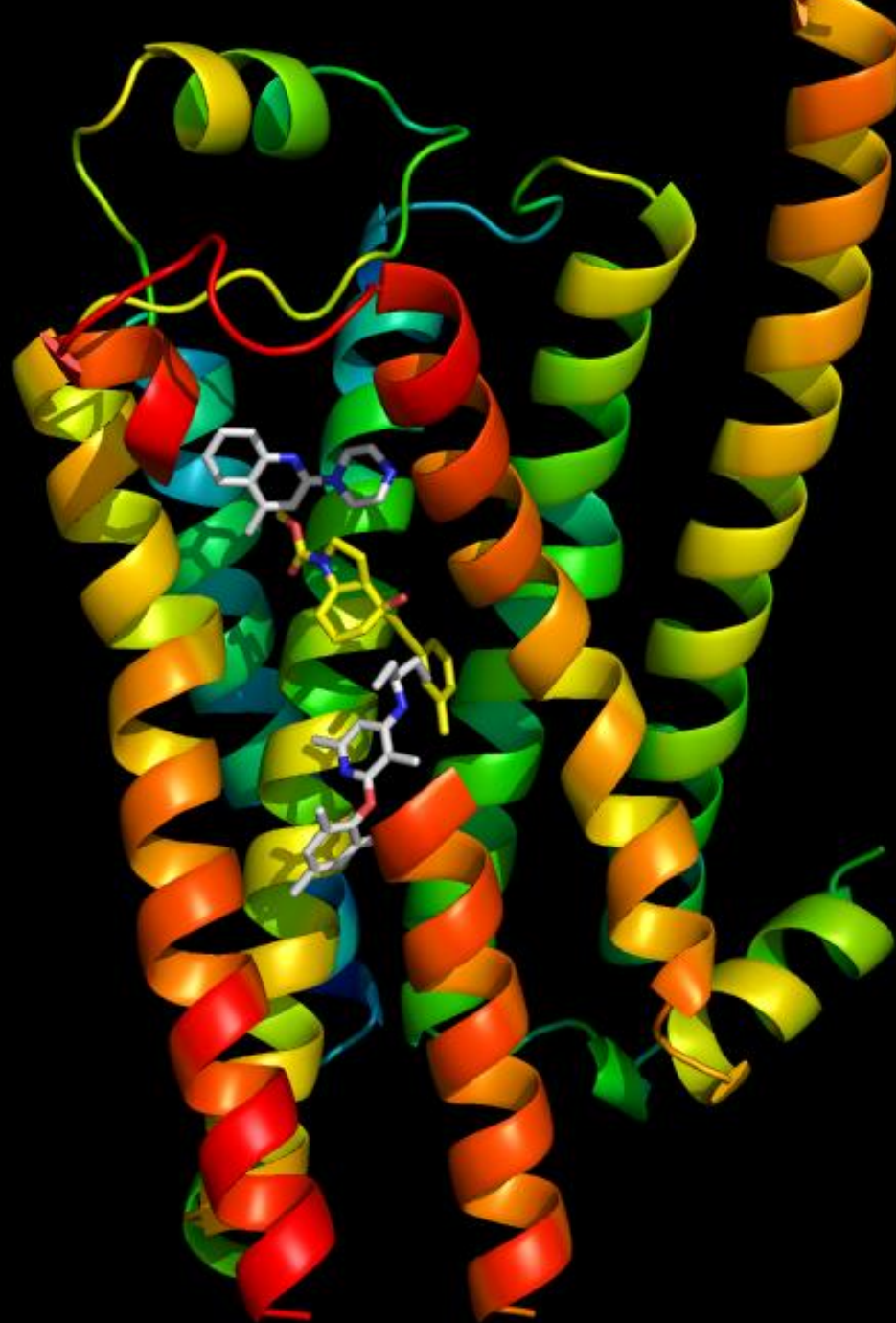


**X-ray structure of  
mavoglurant bound to  
mGlu5**

**Family A**  
-β1 AR fragment

**Family C**  
-mGlu5/mavoglurant

**Family B**  
-CRF1 antag



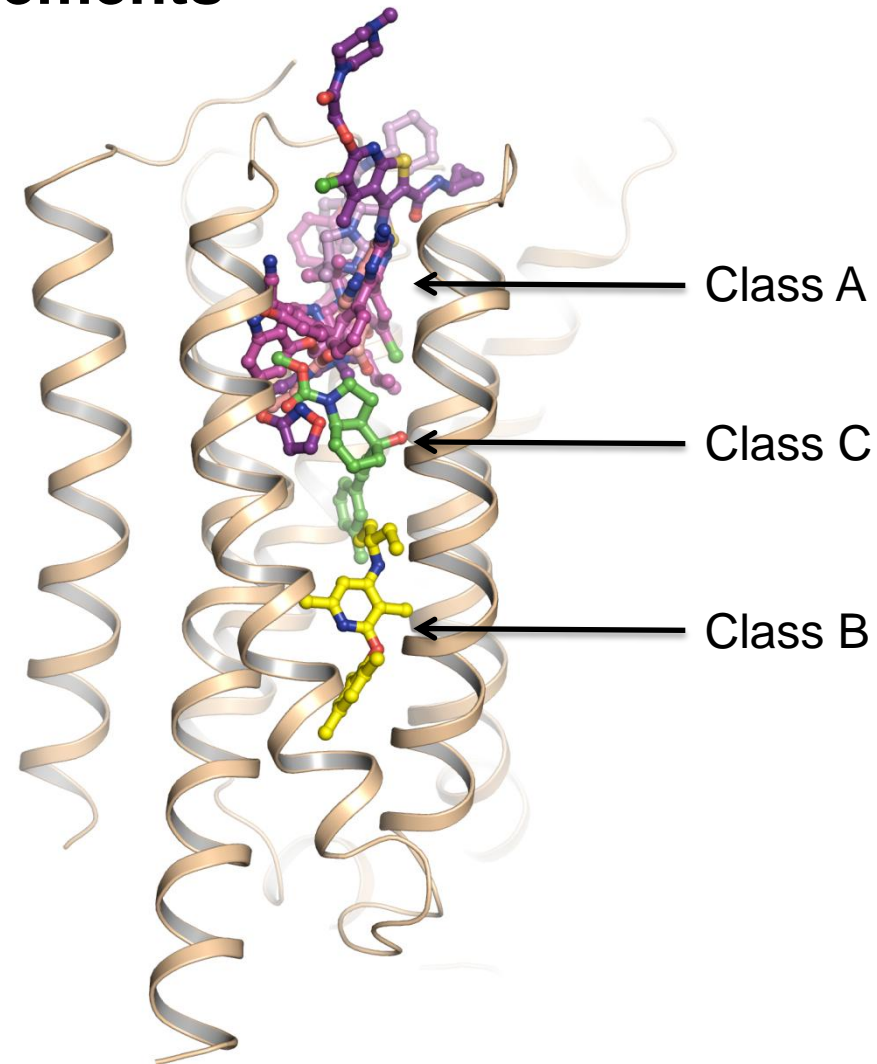
# Acknowledgements

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Cedric Fiez-Vandal  
James Errey

*Everyone at Heptares:*  
**Protein Engineering and Expression  
Chemistry  
Pharmacology  
Structural Biology and Biophysics  
Protein Biochemistry**

**Malcolm Weir (CEO)**

**Fiona Marshall (CSO)**



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