
MRC
Laboratory of
Molecular Biology
The structural basis for ligand efficacy in the β_1-adrenoceptor
Chris Tate
The new LMB

4.4% of FDA approved drugs target the β_1 and β_2 adrenoceptors

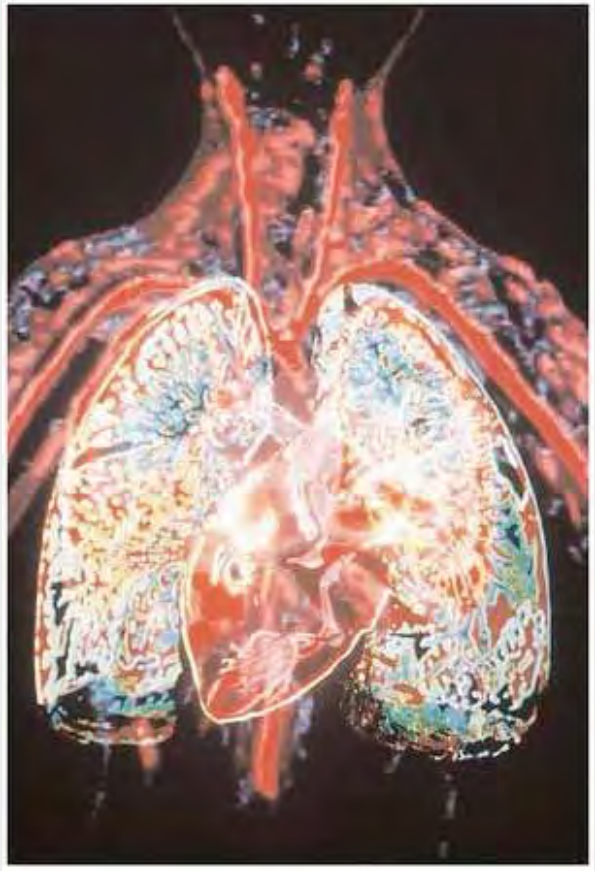
β_1 receptor Heart

Beta blockers
(antagonists)
e.g. bucindolol
carvedilol

various heart problems

Sympathomimetics
(agonists)
e.g. dobutamine

heart failure



β_2 receptor Lungs

Bronchodilators
(agonists)
e.g. salbutamol
formoterol
carmoterol

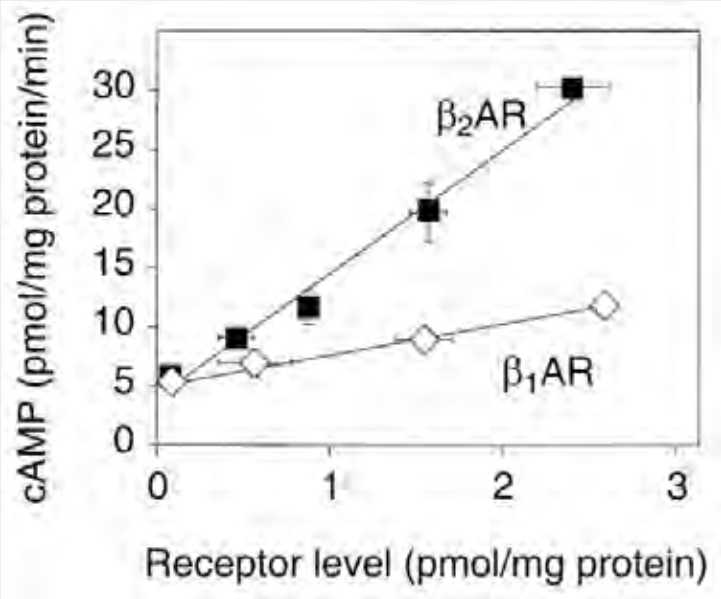
asthma

Antagonists: receptor inhibitors

Agonists: receptor activators

Differences in activity between β_1 and β_2 adrenergic receptors

β_2AR shows higher basal (constitutive) activity



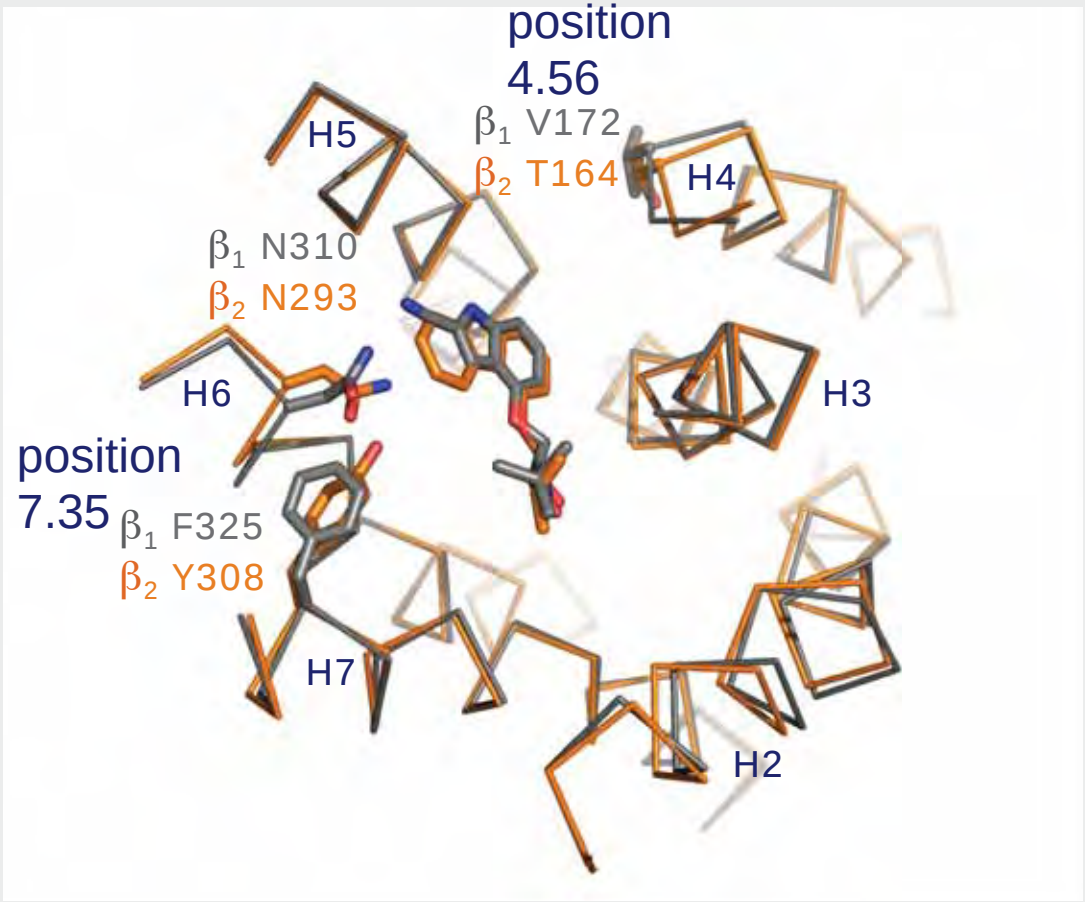
β_2AR shows greater efficacy (response to agonist stimulus) than β_1AR

There are also important differences in ligand selectivity between the two receptors

Engelhardt et al. (2001) Mol Pharm **60**, 712-717

But tm helices of β_1 and β_2 are 67% identical, and there are only two differences within 8Å of the ligand binding pocket

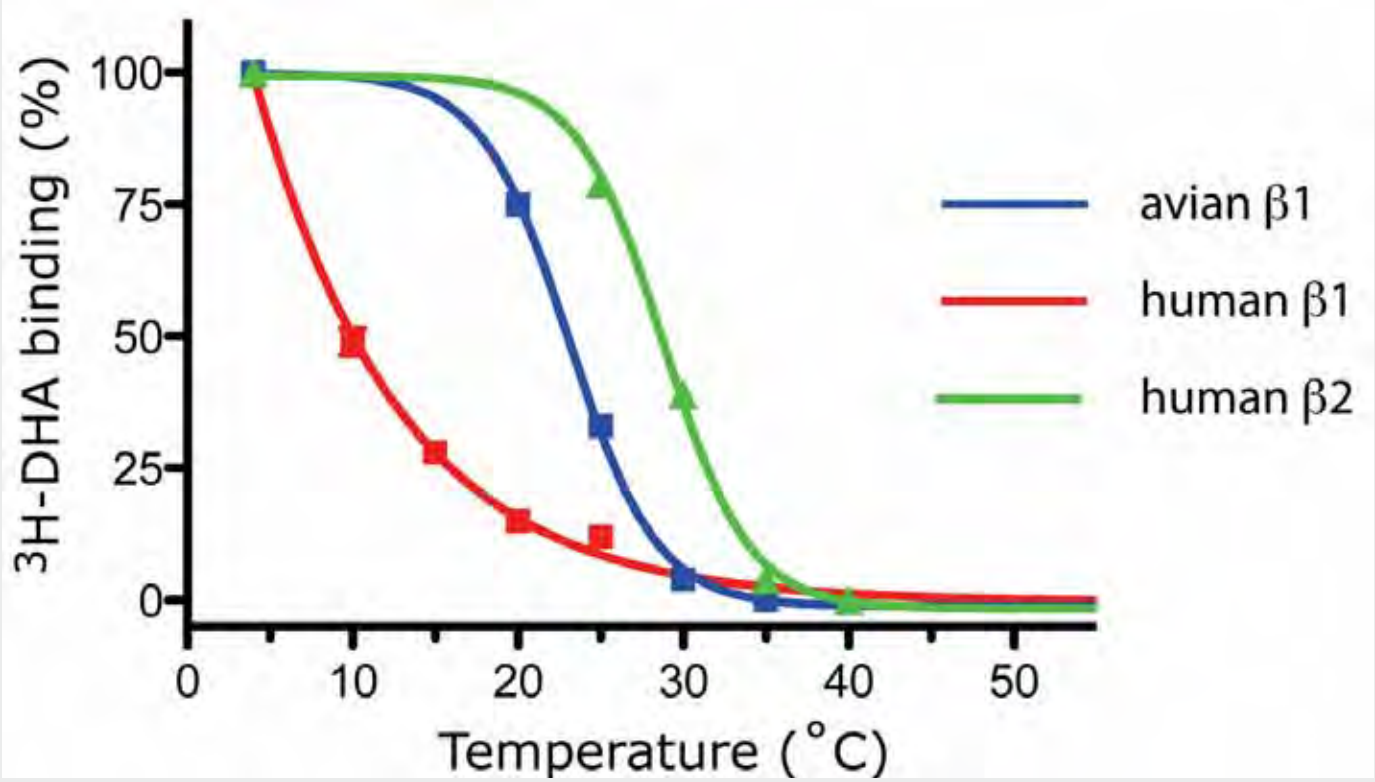
Comparison of the ligand binding pockets of β_1 and β_2 adrenergic receptors



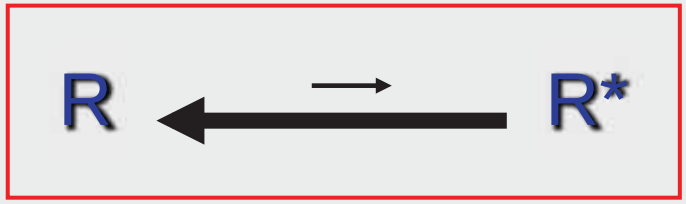
There are only two amino acid substitutions within 8Å of the ligand binding site

Cherezov *et al.* (2007) Science **318,** 1258-1265
Warne *et al.* (2008) Nature **454**, 486-451

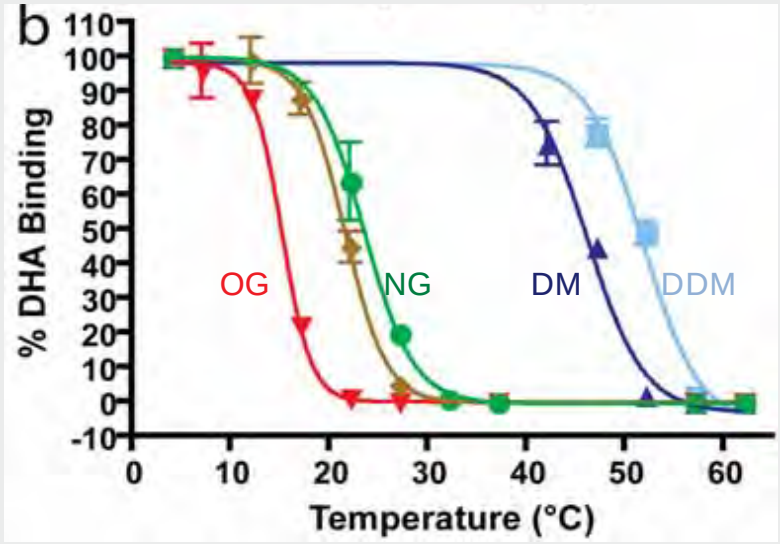
Comparison of the thermostabilities of the human and turkey β_1-adrenoceptors with the human β_2-adrenoceptor



β_1AR-m23 is a thermostabilised mutant ideal for crystallography



The six thermostabilising mutations have affected the global conformation of the receptor so that it is predominantly in an inactive (R) state

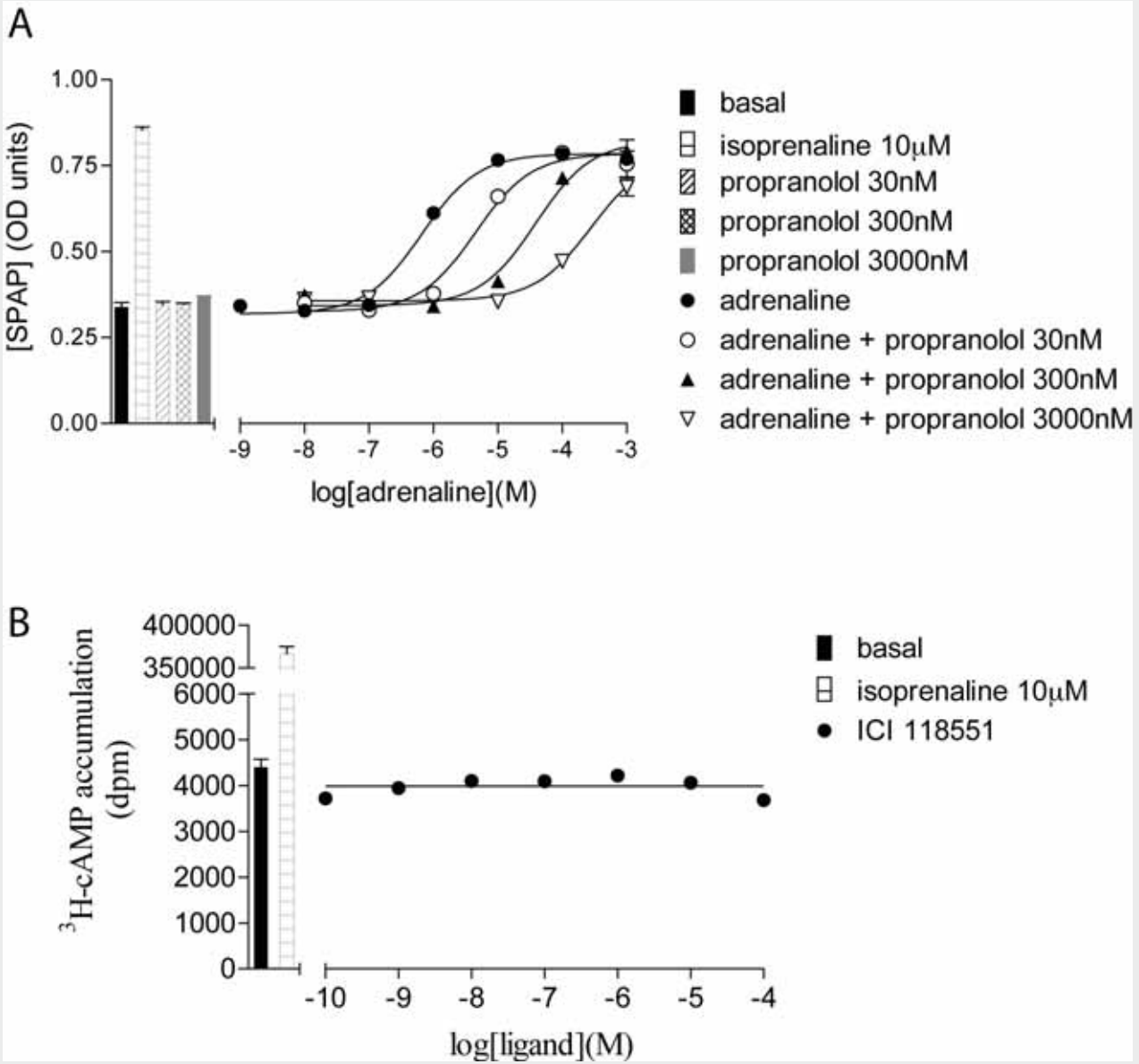


OG: octylglucoside; NG:nonylglucoside;
DM: decylmaltoside; DDM: dodecylmaltoside

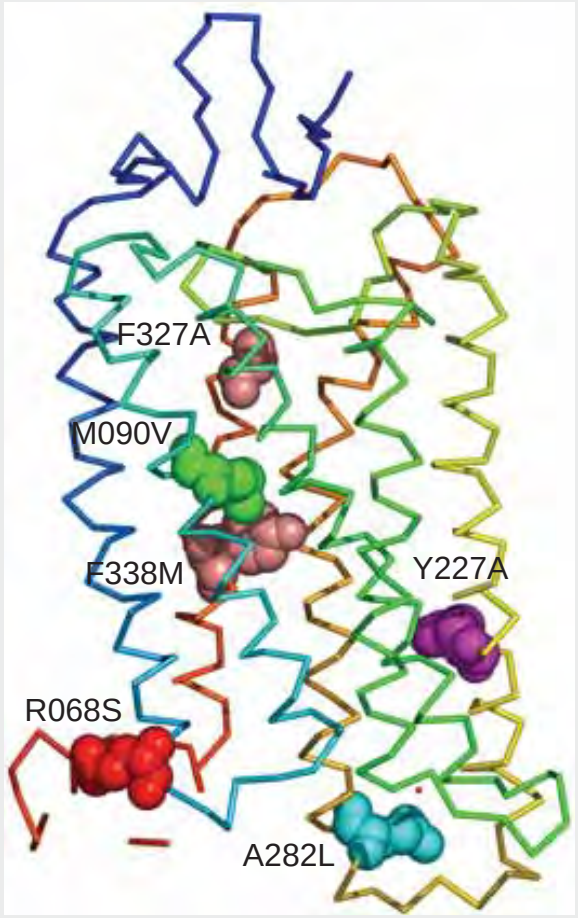
β_1AR-m23 is stable in short-chain detergents like octylglucoside, which facilitates the formation of well-ordered crystals in vapour diffusion experiments

Serrano-Vega et al., (2008) PNAS **105**, 877-882

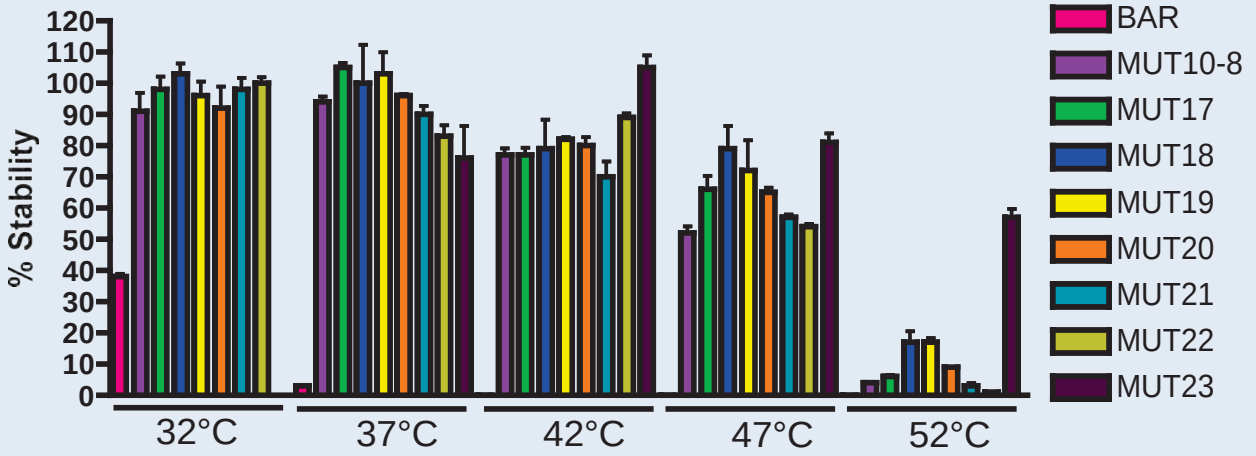
Thermostabilised βAR-m23 receptor couples to G proteins in a whole cell assay and shows no basal activty

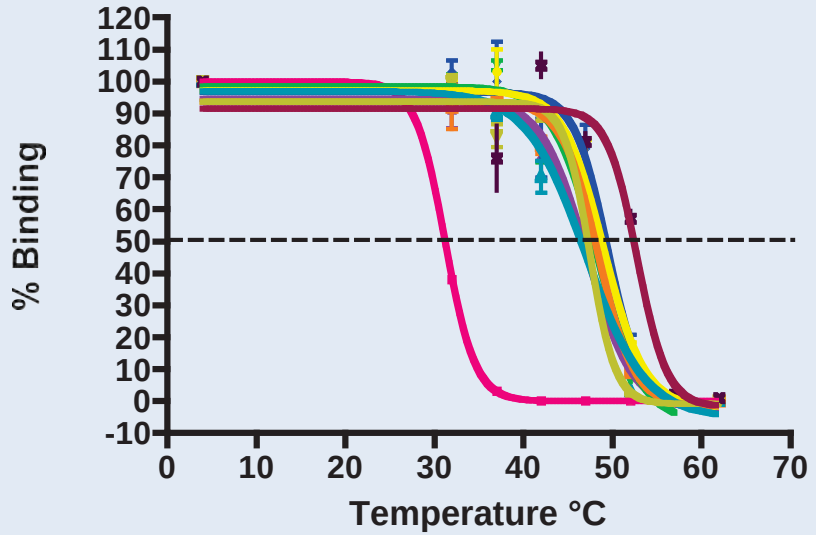


Thermostabilisation of the β_1-adrenergic receptor



β_1AR-m23

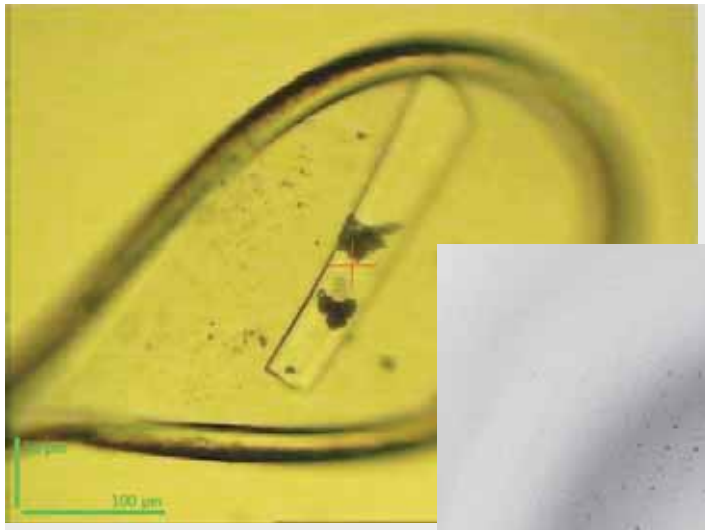


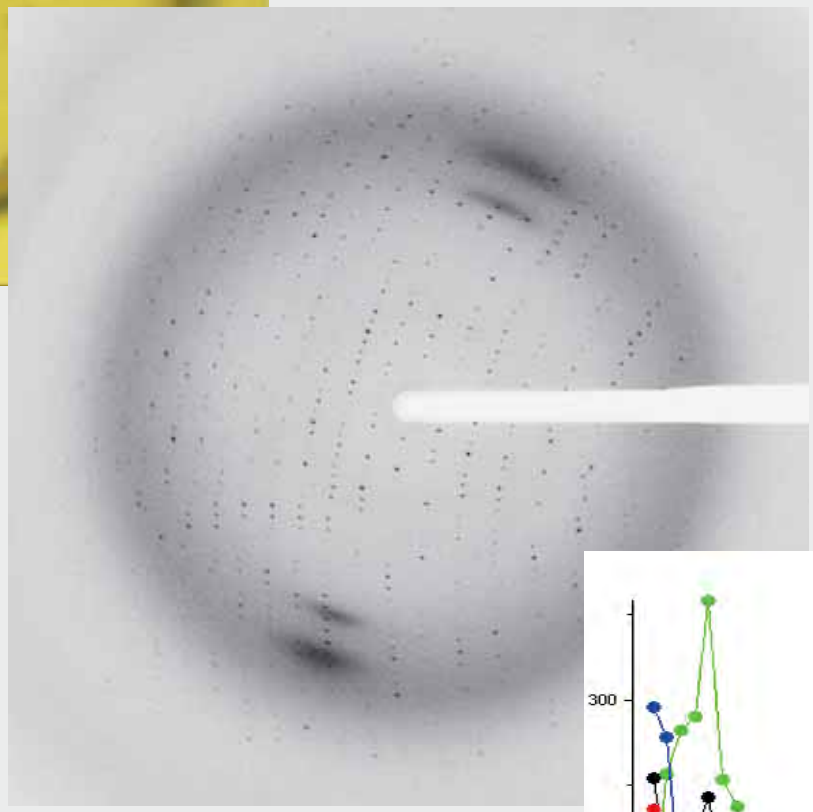


T_m	ΔT_m
31	
47	⇒16
48	⇒17
50	⇒19
49	⇒18
49	⇒18
48	⇒17
47	⇒16
52	⇒21

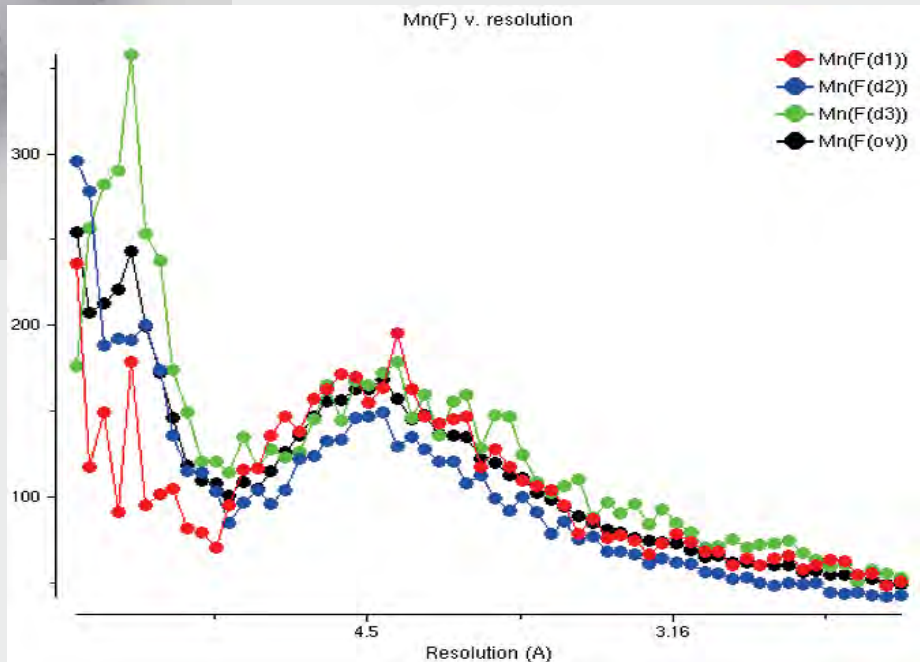
Serrano-Vega et al. (2008) PNAS **105**, 877-882

β1 data collection: t1043





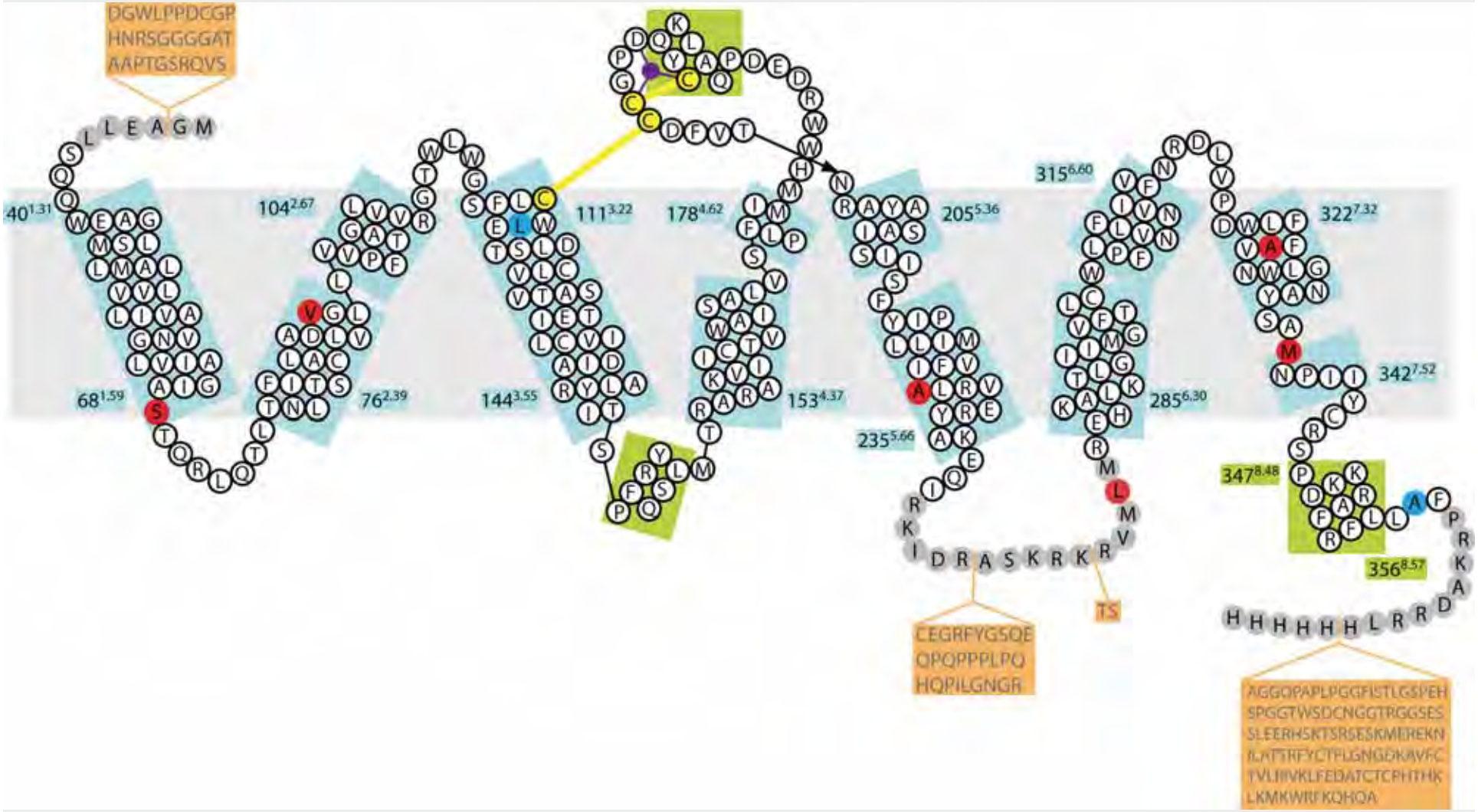
Isotropic diffraction



Spacegroup P1

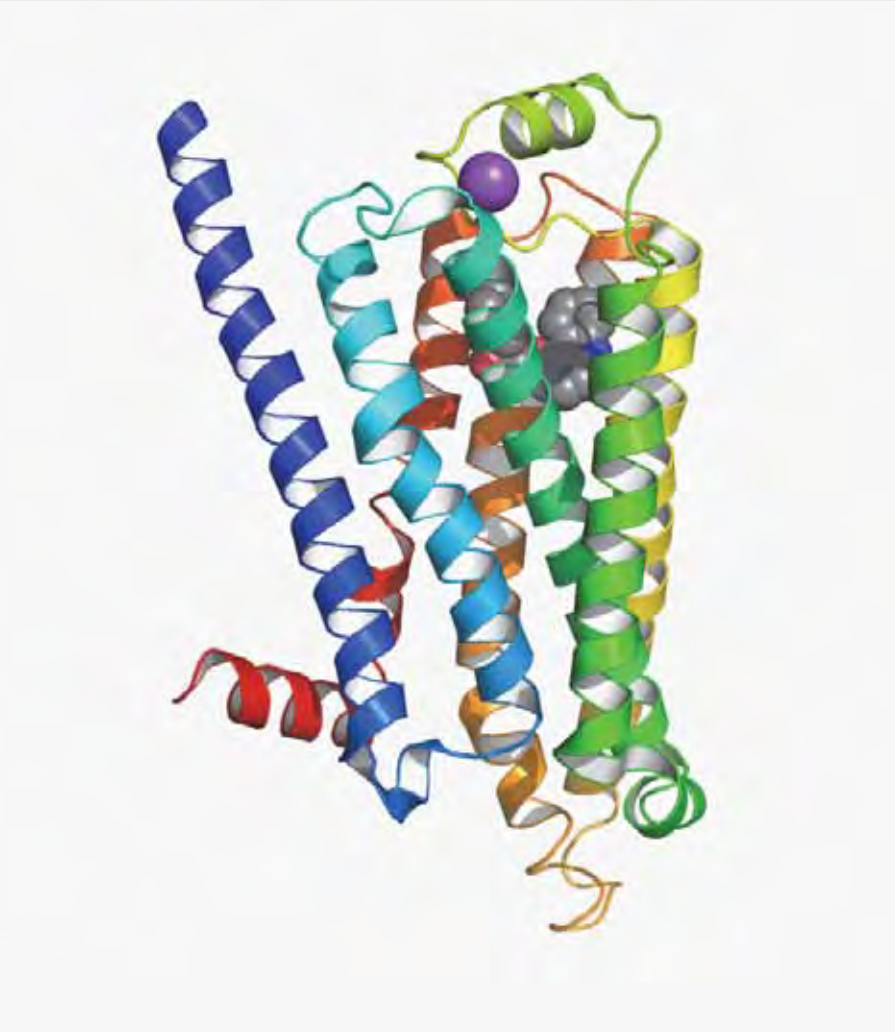
a=55.5Å, b=86.8Å, c=95.50Å
α=67.60, β=73.30, γ=85.80

Crystallisation construct of the β_1 receptor



Warne *et al*. (2009) Protein Exp. Purif. **65**, 204-213

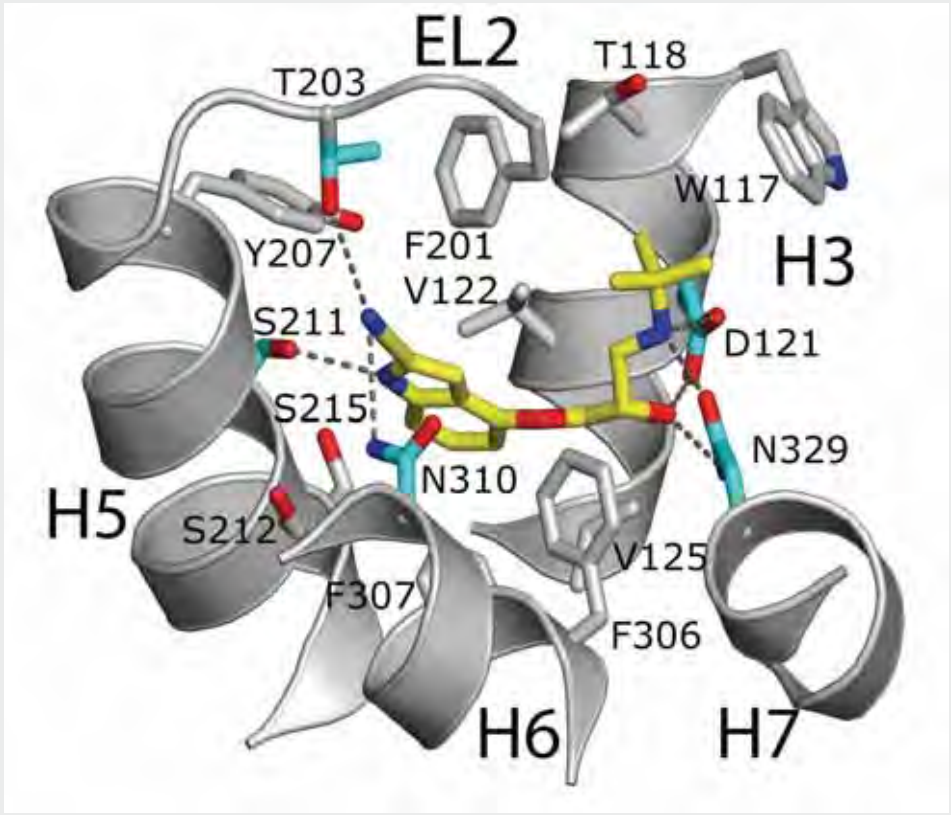
Structure the thermostabilised avian β_1-adrenoceptor

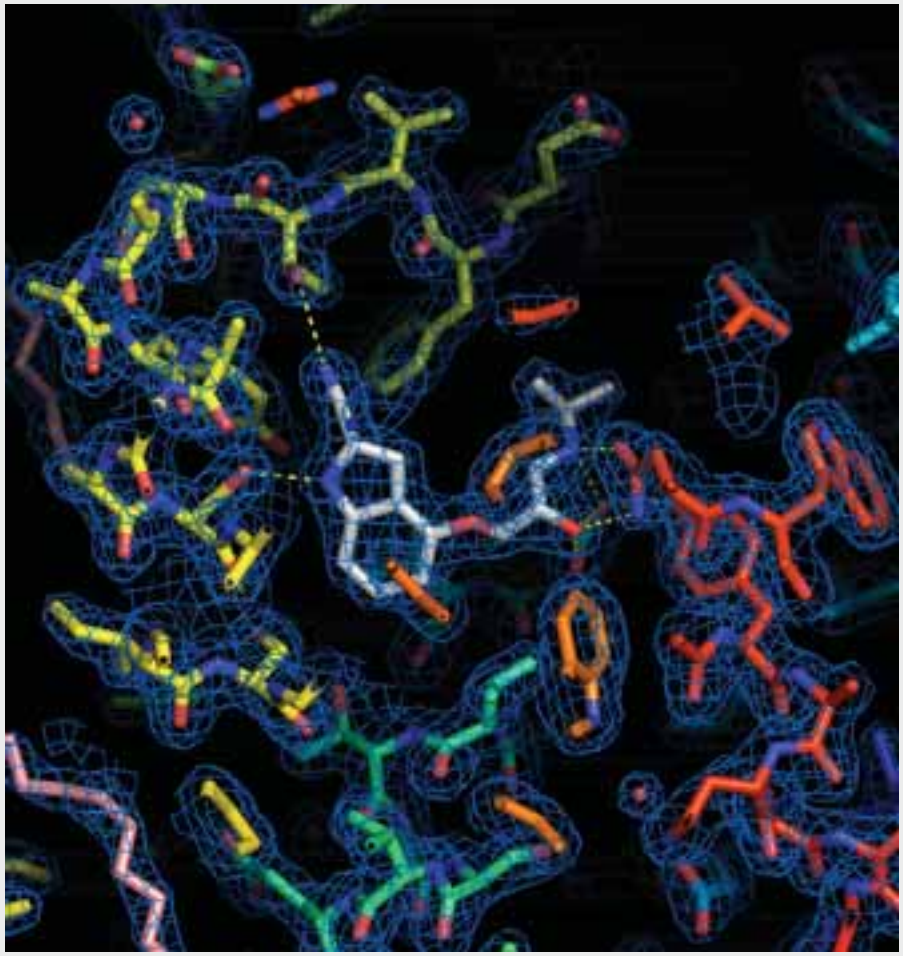


Tony Warne

Warne *et al*. (2008) Nature **454**, 486-491
Moukhametzianov *et al* (2011) PNAS. **108**, 8228-8232

Cyanopindolol binding site



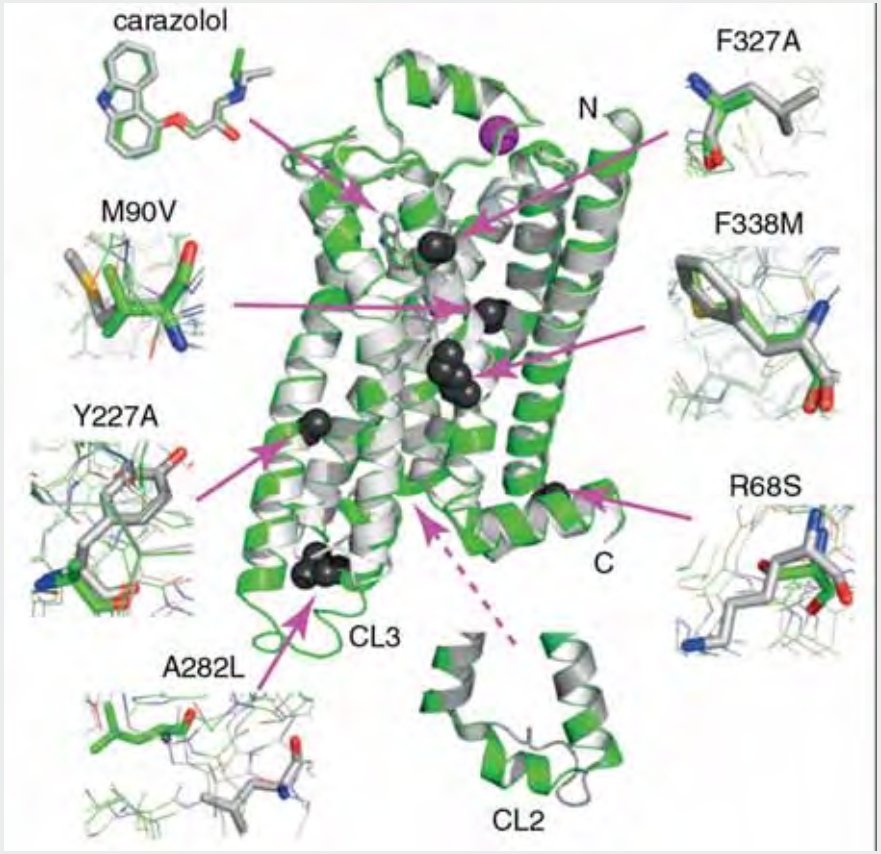


FAQs 7

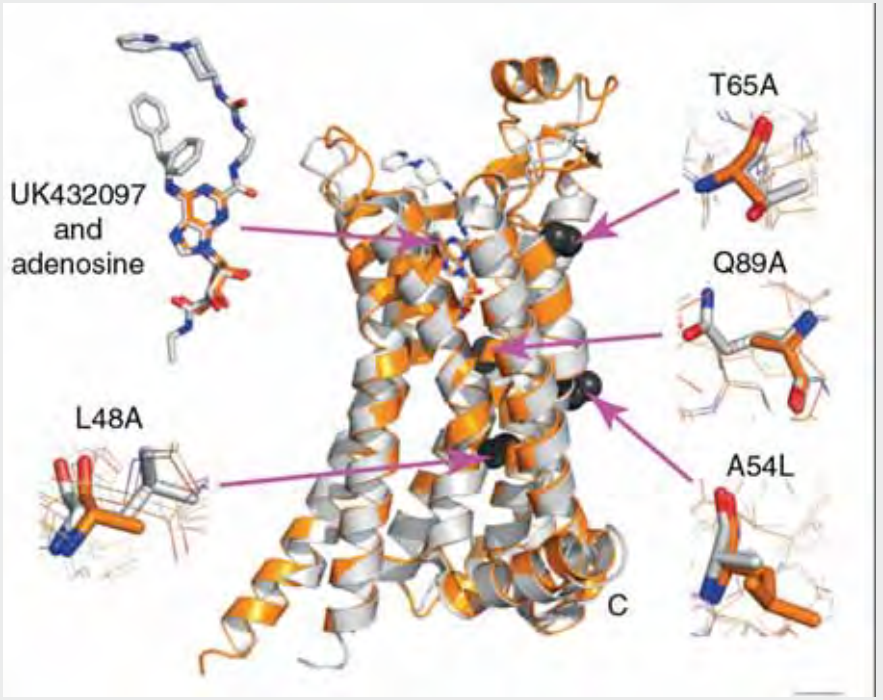
Q: Are the structures of thermostabilised receptors and those fused to T4 lysozyme the same?

A: Yes, in the binding pocket, but there may be differences in the loop regions due to perturbations caused either by T4 lysozyme or crystal packing interactions

β_1AR versus β_2AR (overall rmsd 0.6 Å)



Agonist-bound conformations of A_{2A}R (overall rmsd 0.6 Å)

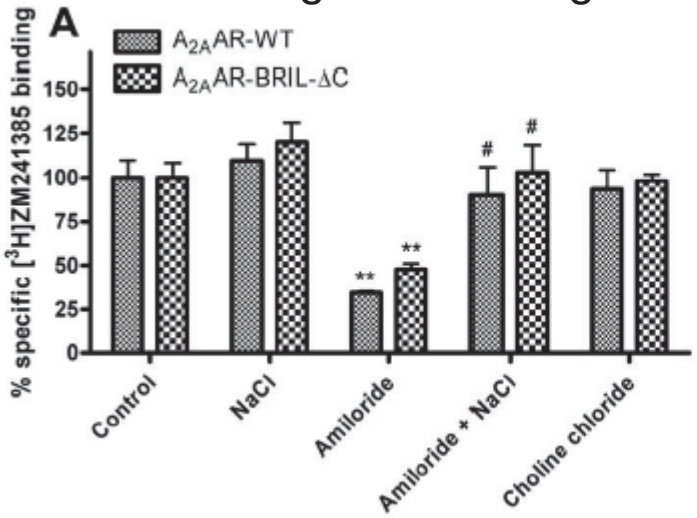


Tate (2012) Trends Biochem. Sci. **37**, 343-352

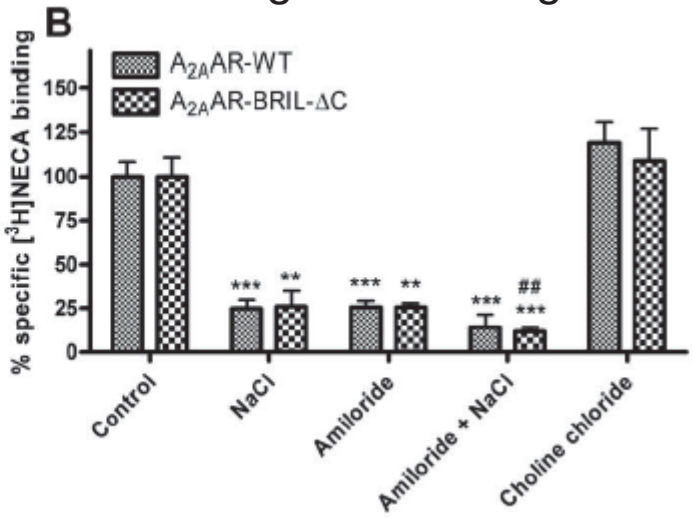
Structural basis for allosteric regulation of $A_{2A}R$ by Na^+ ions

Liu *et al* (2011) Science **337**, 232-236

Antagonist binding



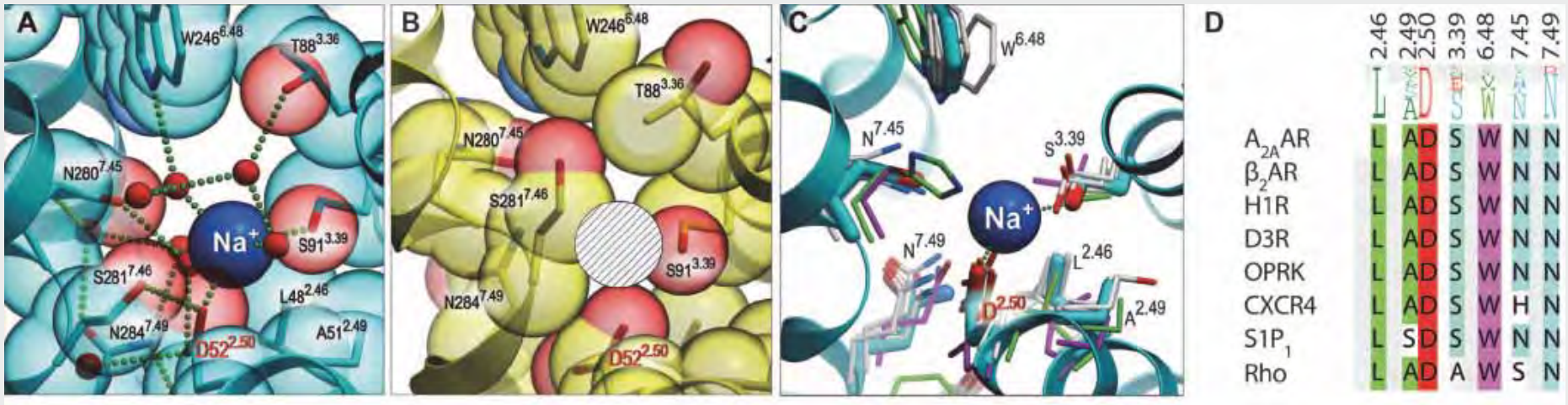
Agonist binding



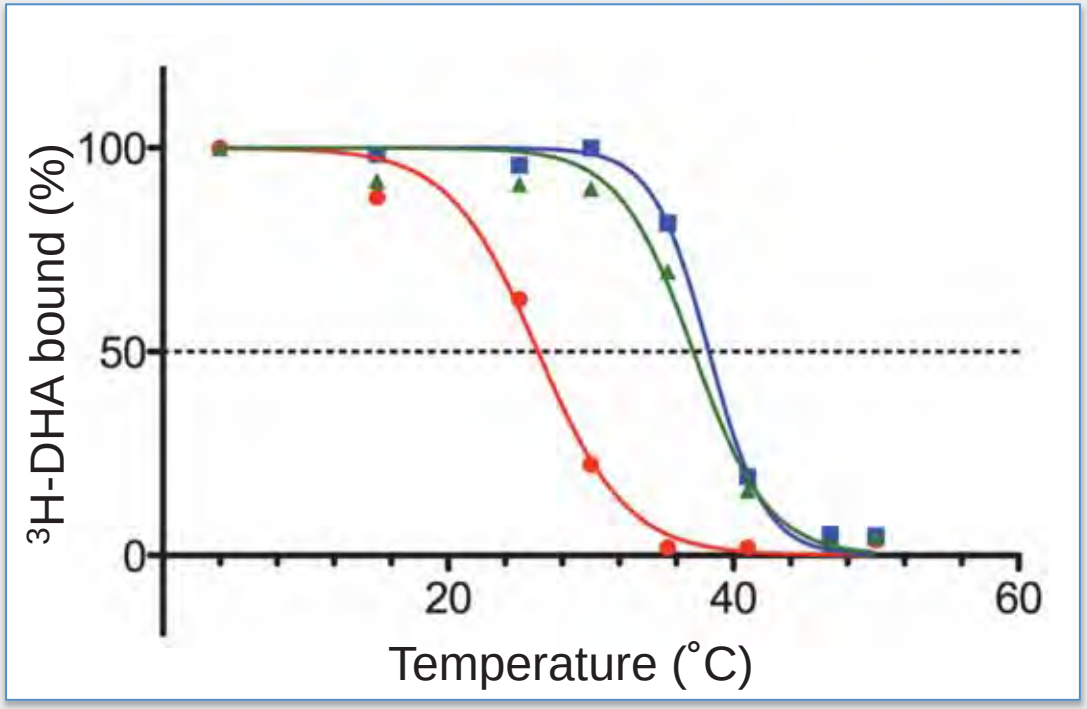
EC50
~40-50 mM

Inactive state

Active-like state



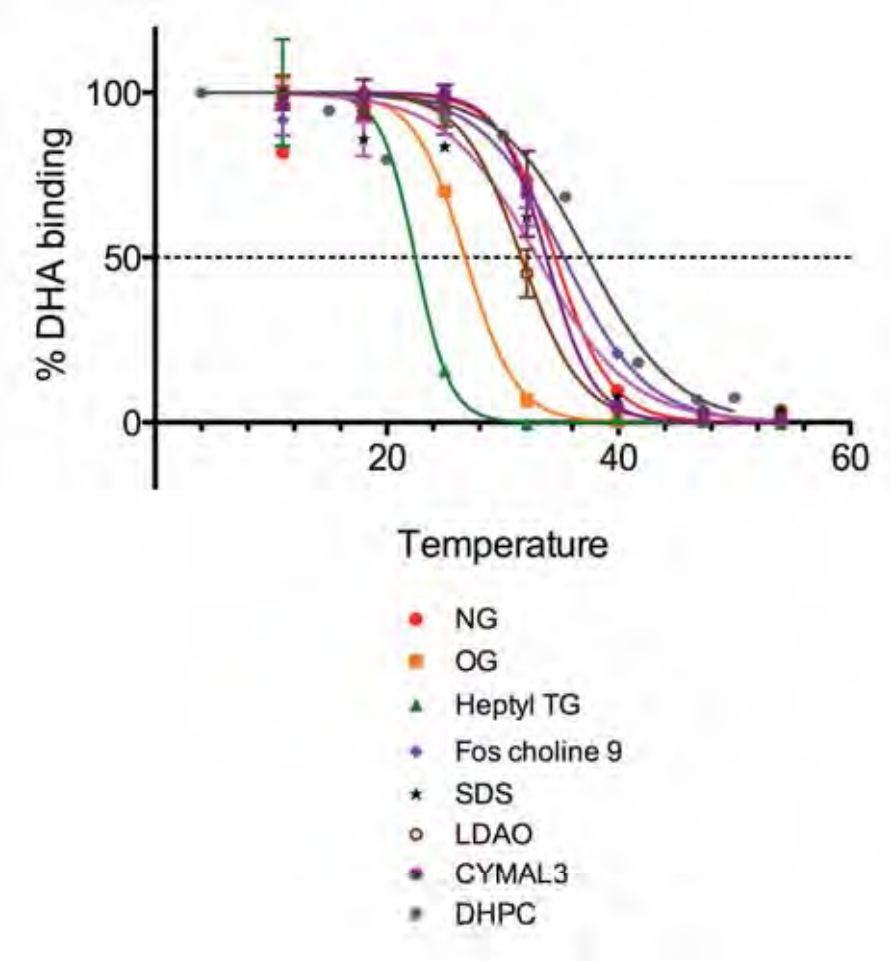
The ultra-stable β_1AR mutant JM50 contains 3 additional thermostabilising mutations, which gave 12˚C further stability to β_1AR-m23



Jenny
Miller-Gallacher

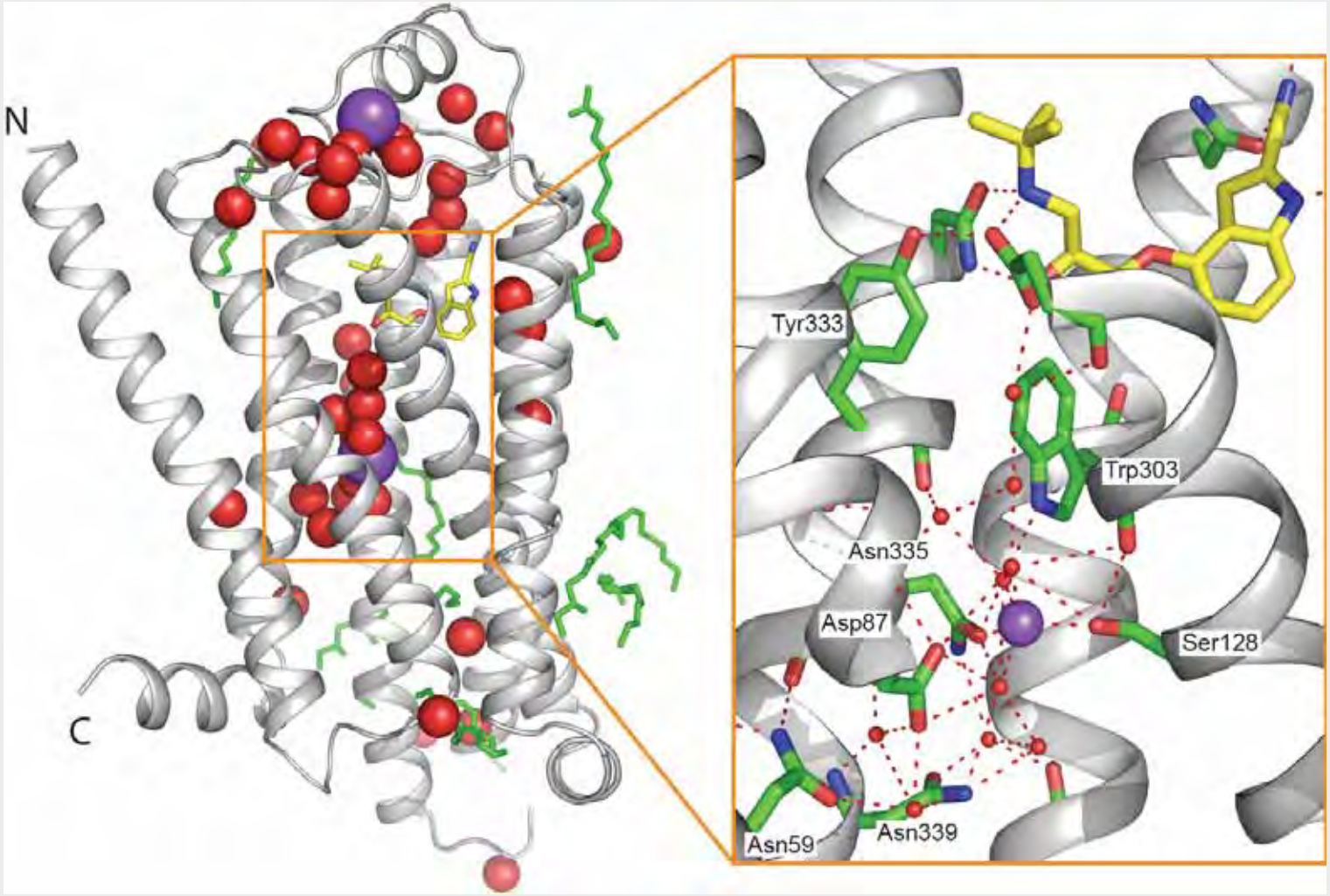
Miller & Tate (2011) J. Mol. Biol. **413**, 628-638

The stability of β_1AR-JM50 in different detergents



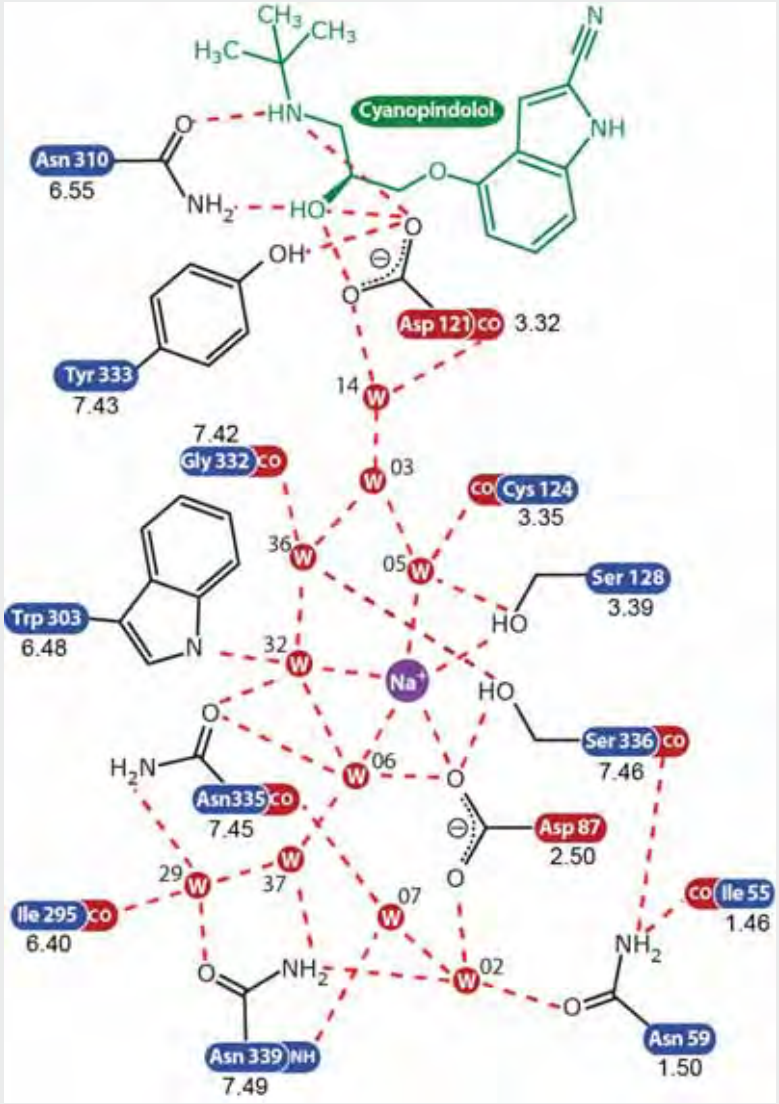
Detergent	Tm (ºC)
Nonylglucoside	34.5 ± 0.7
Octylglucoside	26.8 ± 0.2
Heptylthioglucoside	22.5 ± 0.7
Fos choline 9	35.3 ± 0.5
SDS	33.0 ± 0.7
LDAO	31.6 ± 0.3
CYMAL3	33.8 ± 0.5
DHPC	37.4 ± 0.9

2.1 Å resolution structure of an ultra-stable β_1AR mutant crystallised in LCP reveals an intramembrane Na^+ binding site

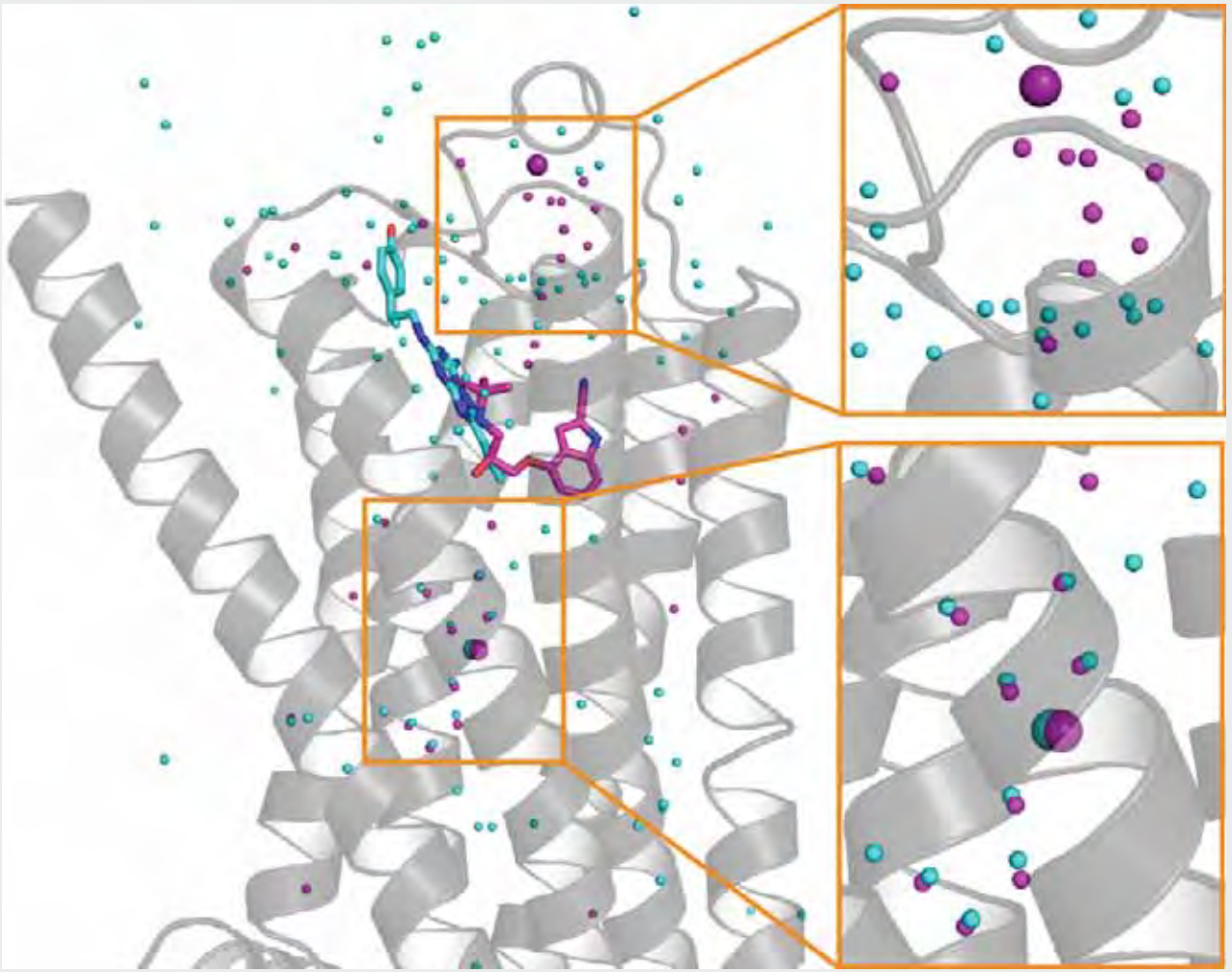


Miller-Gallacher et al. (2014) PLoS One **9**, e92727

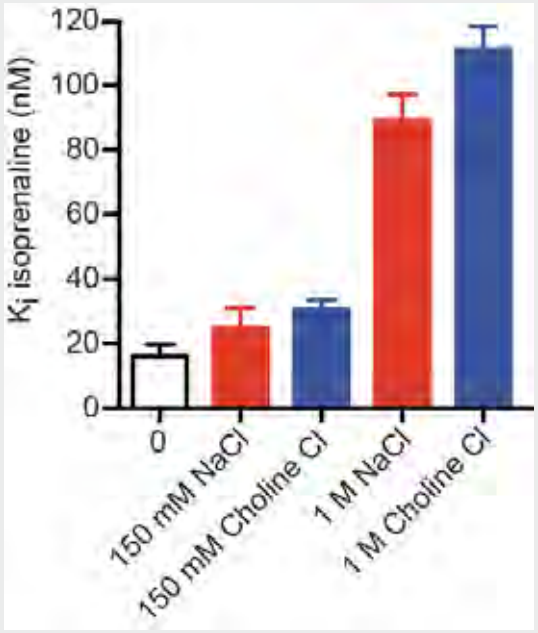
The intramembrane Na^+ is part of an extended hydrogen bond network from the ligand to the DRY motif



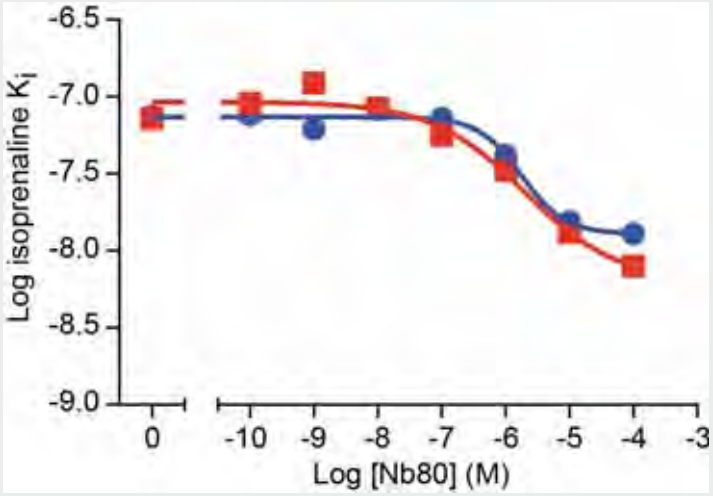
Remarkable conservation of the Na^+ binding pocket and positions of water molecules between β1AR and $A_{2A}R$: overall rmsd of Cα, 2.4 Å rmsd of Cα in the Na^+ binding pocket, 0.3 Å

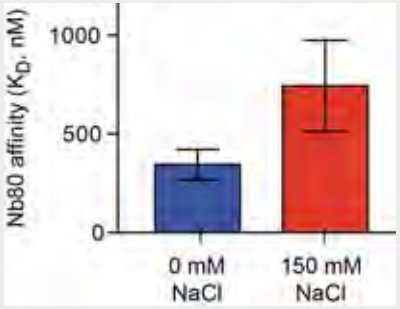


The intramembrane Na^+ ion in β_1AR does not affect receptor activation



Agonist binding is unaffected by Na^+ concentration

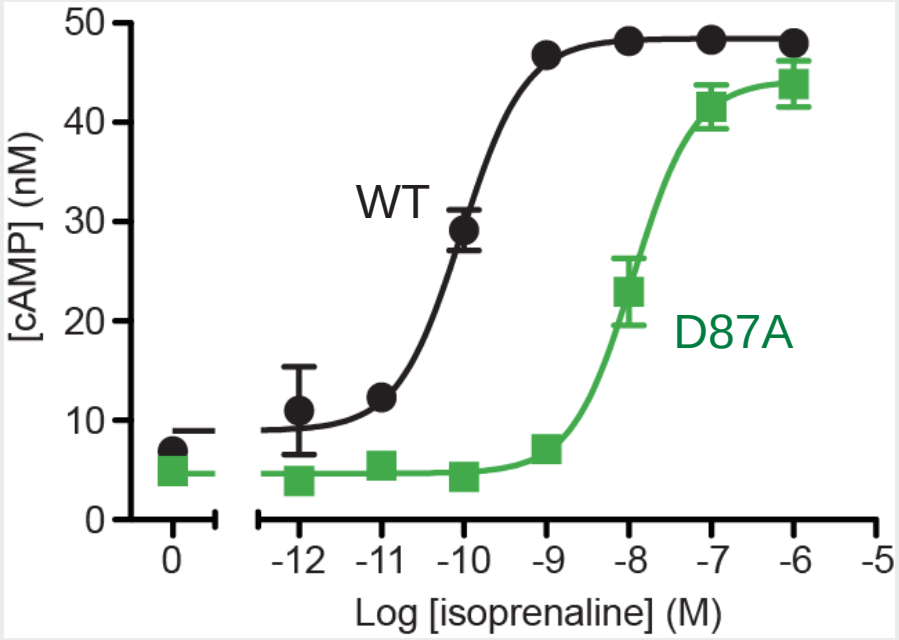


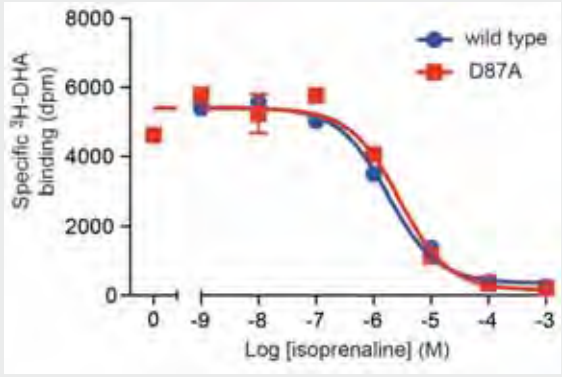


The affinity of the G protein mimetic Nb80 and its efficacy in increasing agonist affinity is unaffected by Na^+ concentration

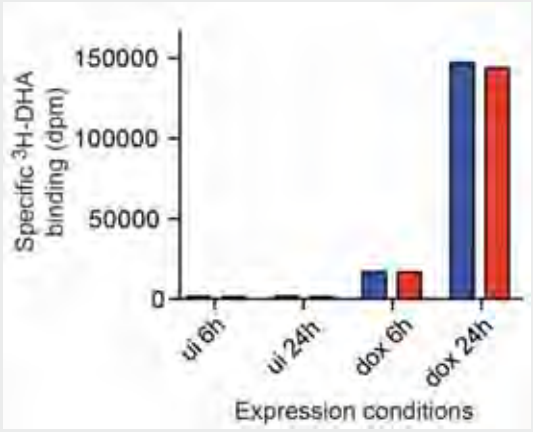
Rony Nehmé

Agonist activation of D87A$^{2.50}$ is impaired and basal activity is lowered in stable cell lines



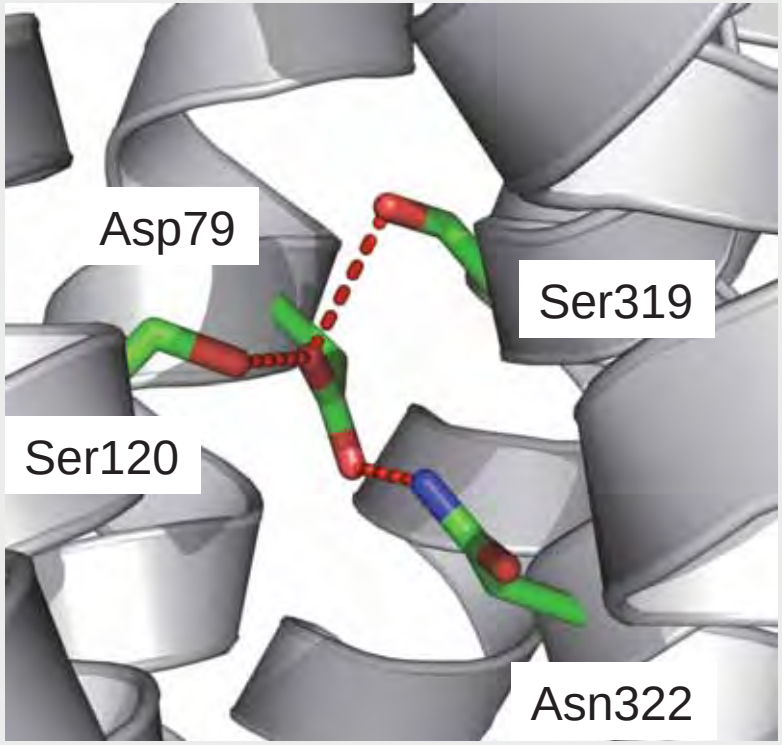


Agonist affinities are identical



Expression levels are identical

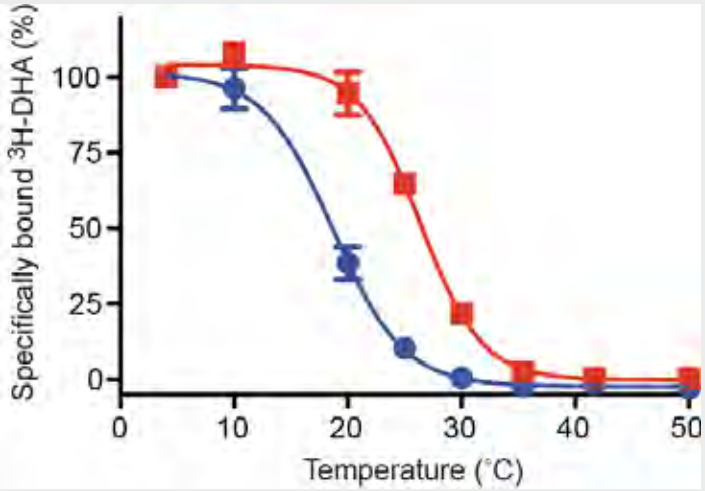
$Asp^{2.50}$ that co-ordinates Na^+ in the R state makes 3 hydrogen bonds to side chains in the R* state

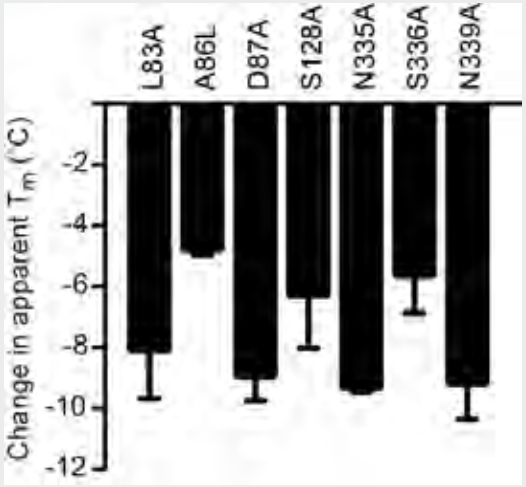


Structure of β_2AR in the activated state bound to Nb80 showing re-organisation of the Na^+ binding pocket

So what is the role of the intramembrane Na^+ in β_1AR?

A: stabilisation of the ligand-free receptor

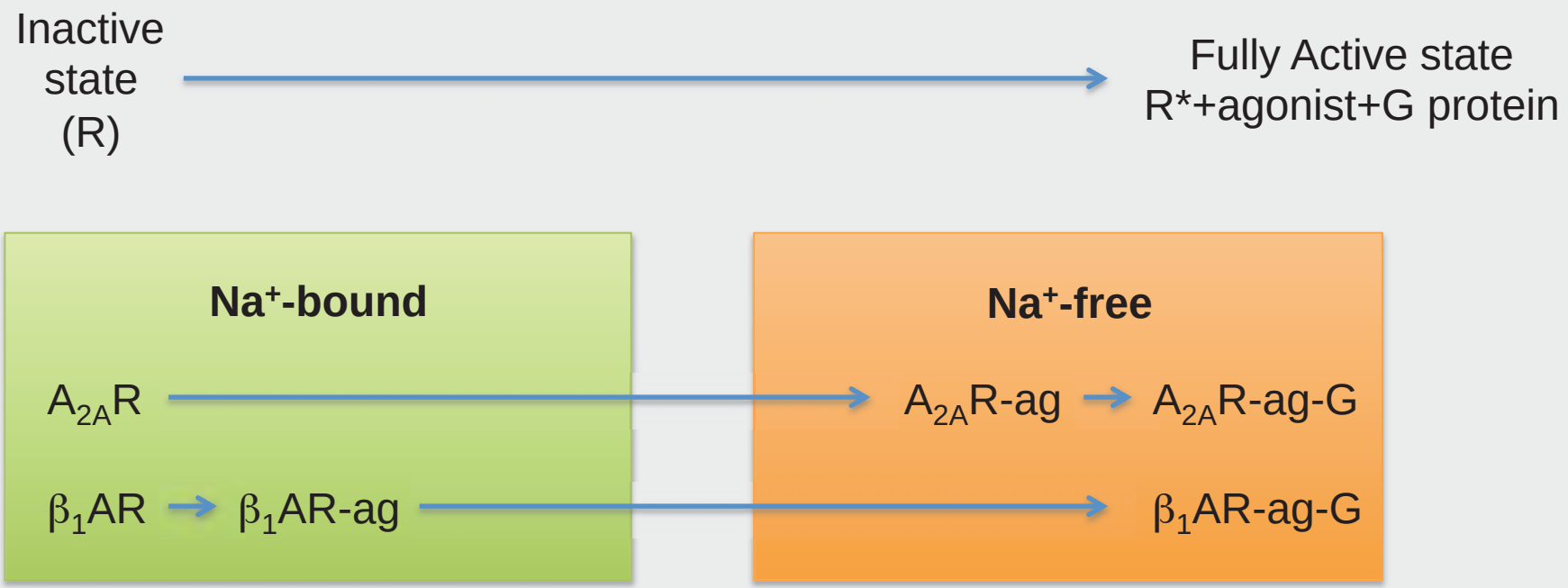




The stability of ligand-free detergent solubilised β_1AR is decreased by 7.5 ˚C in Na^+-free buffer compared to 150 mM NaCl

Mutation of residues lining the Na^+ binding pocket all decrease the stability of the ligand-free detergent-solubilised receptor

Na^+ is an allosteric antagonist of $A_{2A}R$ and not of β_1AR because of the different energy landscapes of the receptors

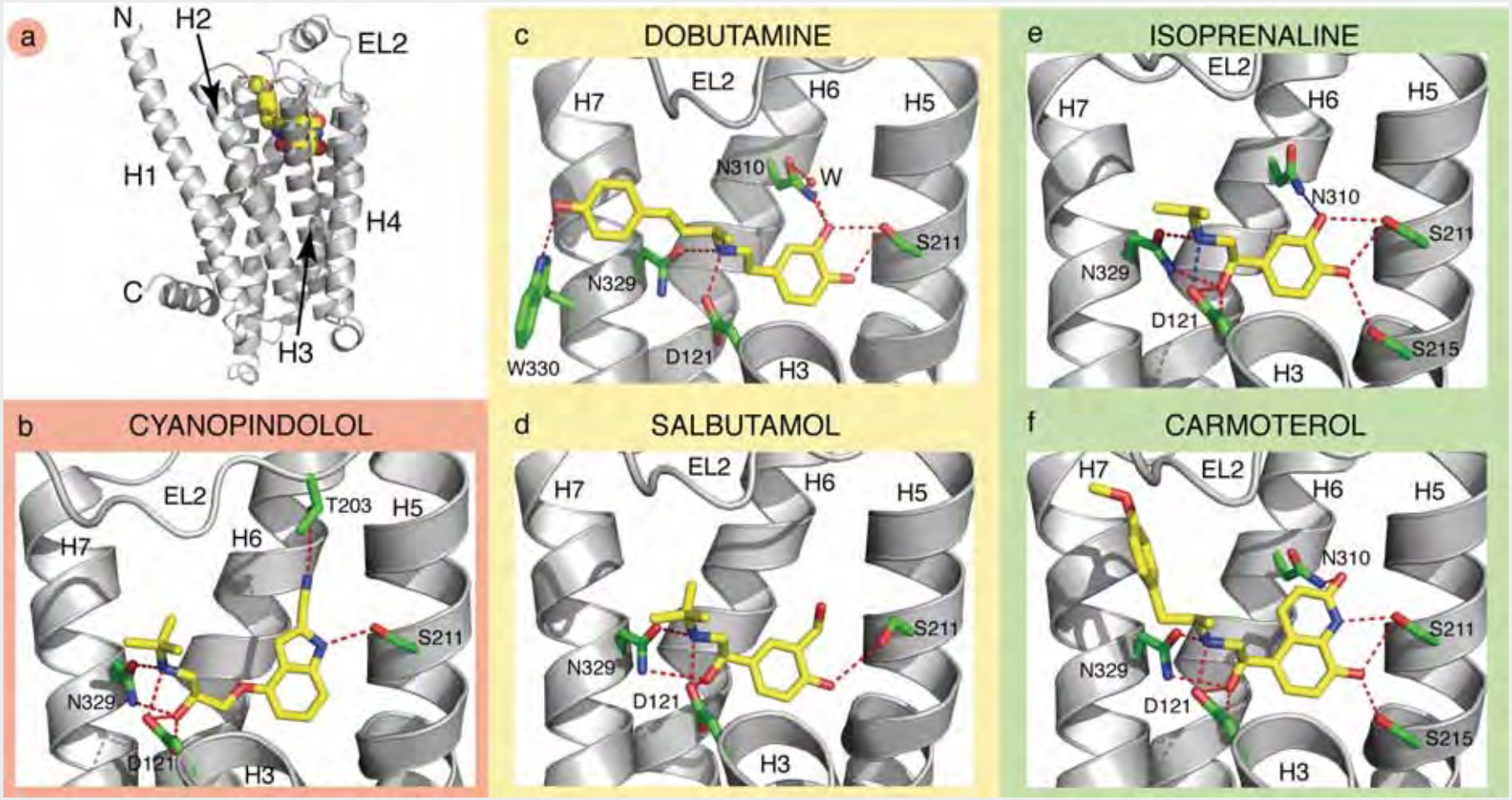


The Na+ and water create a ‘soft’ interface between 5 transmembrane helices (H2, H3, H6 and H7) that is sufficient to stabilise the ligand free structure, but is of sufficiently low energy to be easily disrupted on agonist binding to increase the probability of the R to R* transition.

Crystal structures determined of thermostabilised β_1AR

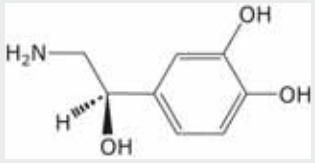
Ligand	PDB	Space group	Ligand type	Detergent or Lipidic Cubic Phase	Resolution Å	Reference
7-Methyl cyanopindolol		$P2_1$	Inverse agonist	D	2.50	Sato *et al*. unpublished
Cyanopindolol	2vt4	P1	Weak partial agonist	D	2.70	Warne *et al*. Nature (2008)
Cyanopindolol	4bvn	$P2_122_1$	Weak partial agonist	LCP	2.10	Miller-Gallacher *et al*. PlosOne (2014)
Nadolol		C2	Weak partial agonist	D	3.40	Li *et al*. unpublished
Timolol		C2	Weak partial agonist	LCP	3.40	"
Carazolol	2ycw	$P2_1$	Weak partial agonist	D	3.00	Moukhametzianov *et al*. PNAS (2011)
Cyanopindolol	2ycx 2ycy	$P2_1$	Weak partial agonist	D	3.15 3.25	"
Bucindolol	4ami	$P2_1$	Biased agonist	D	3.20	Warne *et al.* Structure (2012)
Carvedilol	4amj	$P2_1$	Biased agonist	D	2.30	"
Dobutamine	2y00 2y01	$P2_1$	Partial agonist	D	2.70	Warne *et al*. Nature (2011)
Salbutamol	2y04	$P2_1$	Partial agonist	D	3.00	"
Isoprenaline	2y03	$P2_1$	Full agonist	D	2.85	"
Carmoterol	2y02	$P2_1$	Full agonist	D	2.60	"
Indole fragment 19	3zpq	$P2_1$	?	D	2.80	Christopher *et al.* J Med Chem (2013)
Quinolone fragment 20	3zpr	$P2_1$	?	D	2.70	"

What are the structural differences in the β_1 receptor when an agonist binds compared to when an antagonist binds?



tβ1	hβ2	B-W
D121	D138	3.32
S211	S203	5.42
S215	S207	5.46
N310	N293	6.55
N329	N312	7.39
W330	W313	7.40

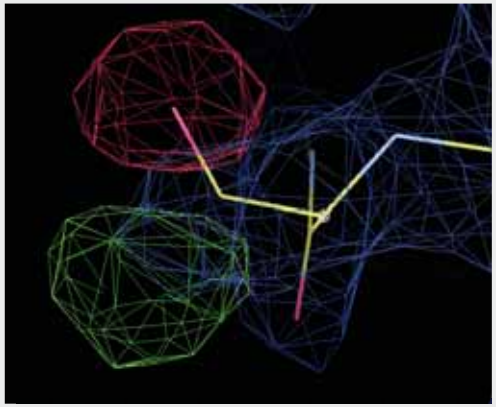
Noradrenaline



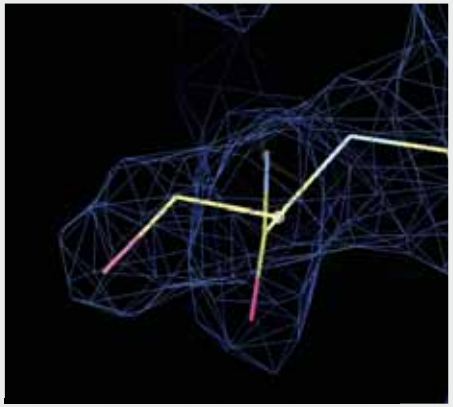
Warne *et al*. (2011) Nature **469**, 241-244

Determination of serine rotamer configurations in β_1AR structures

Ser211$^{5.43}$, β_1AR with carvedilol (2.3Å)

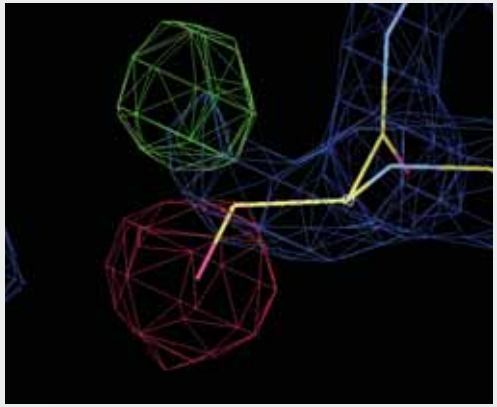


gauche+

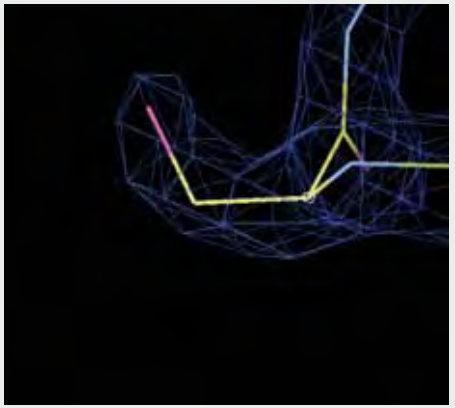


trans *(best fit)*

Ser211$^{5.43}$, β_1AR with cyanopindolol (2.1Å)



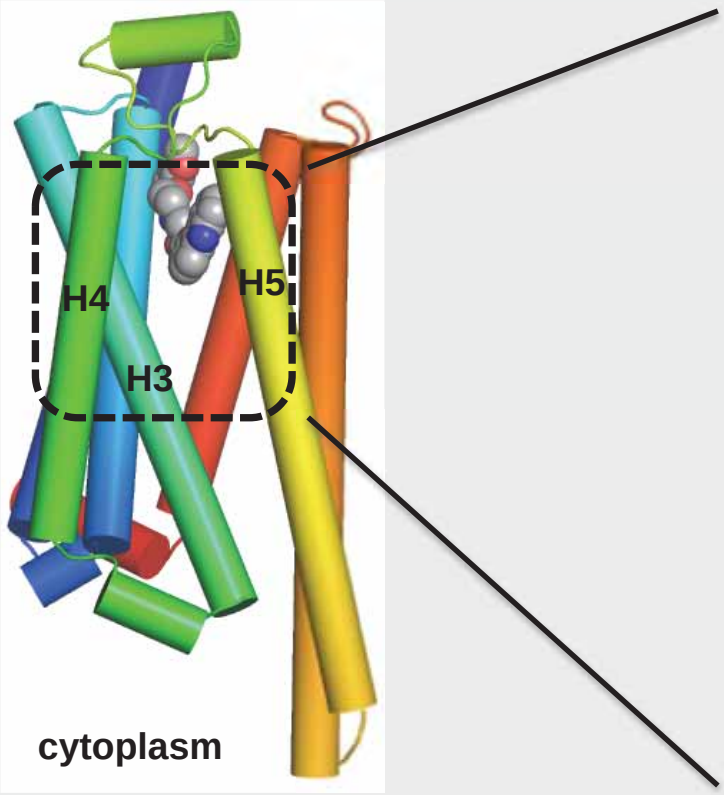
trans



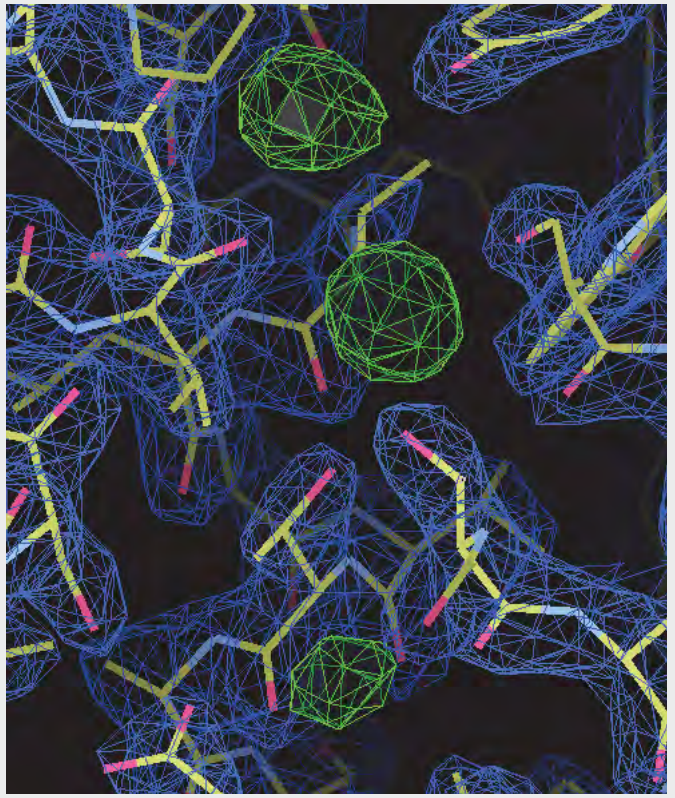
gauche+ *(best fit)*

Assignment of water molecules in β_1AR structures, β_1AR with carvedilol (2.3Å)

β_1AR overview

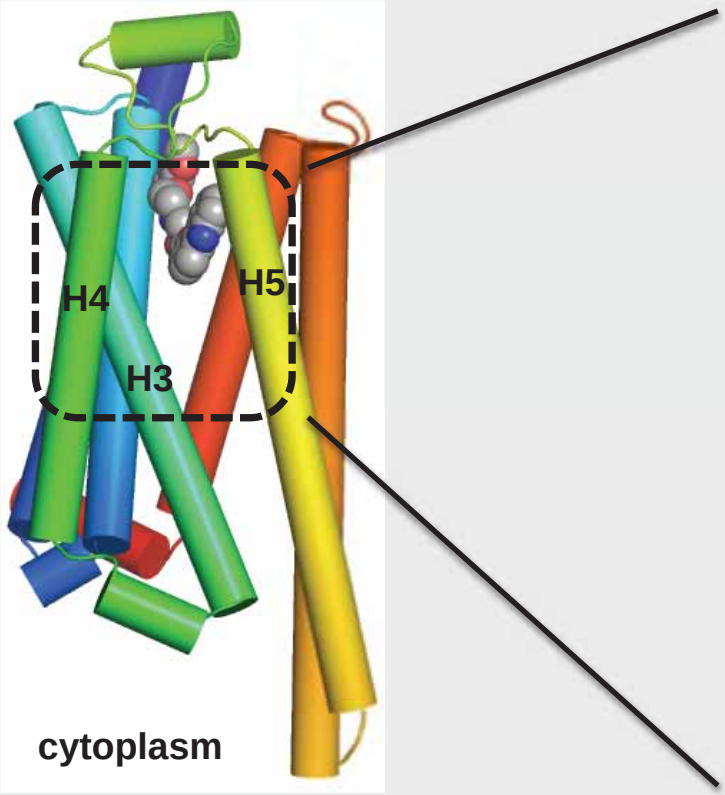


Positive density features at the H5-H3/4 interface

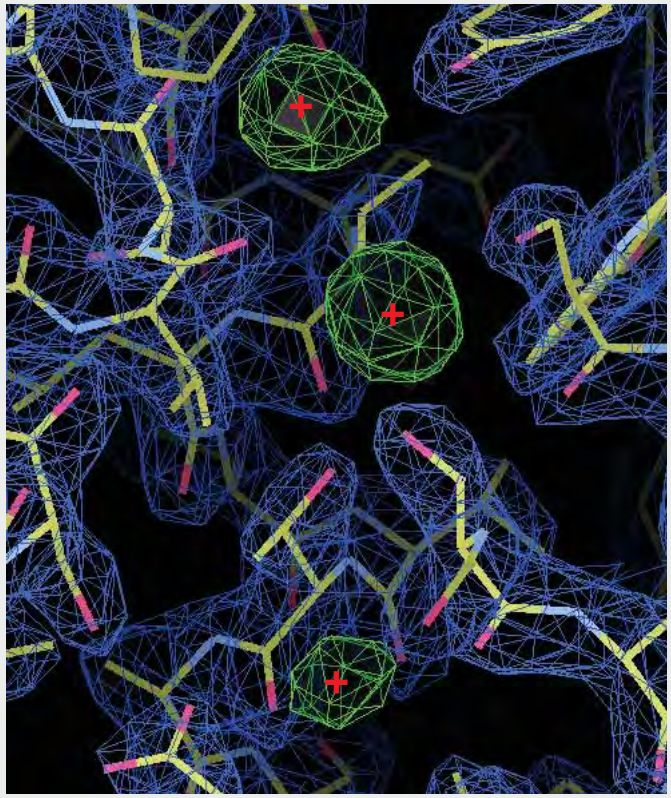


Assignment of water molecules in β_1AR structures, β_1AR with carvedilol (2.3Å)

β_1AR overview



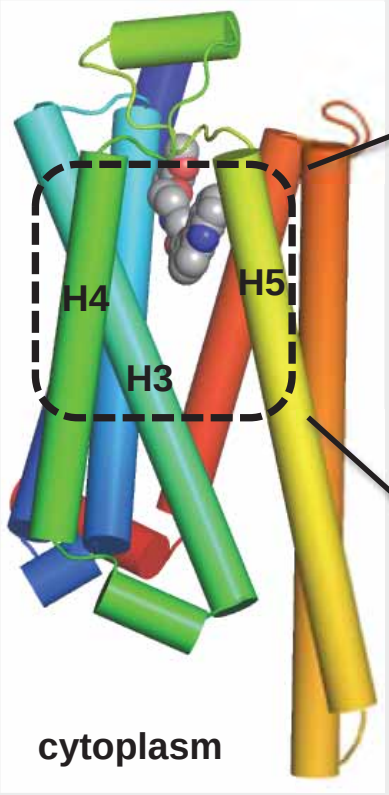
Positive density features at the H5-H3/4 interface



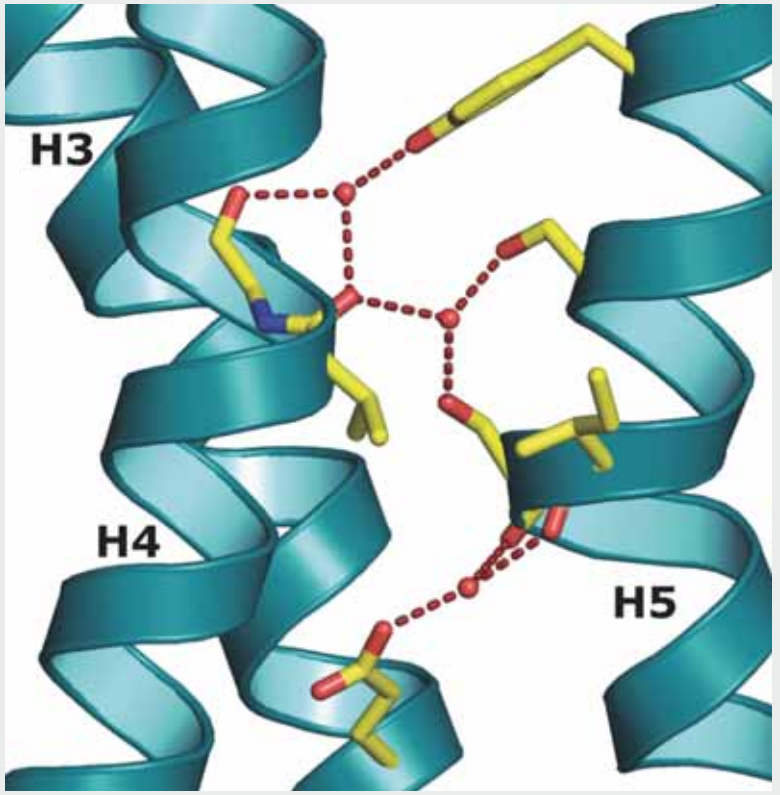
Three water molecules (+) fitted to density (B factors 21-46 Å^2)

Assignment of water molecules in β_1AR structures, β_1AR with carvedilol (2.3Å)

β_1AR overview



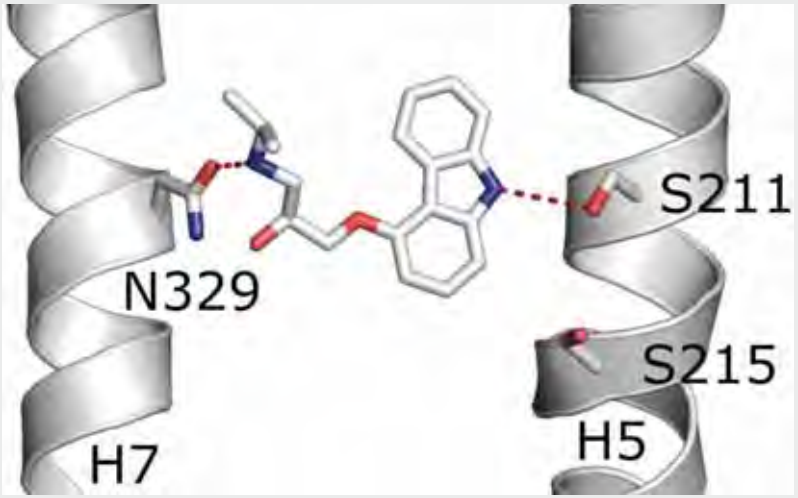
polar interactions at the H5-H3/4 interface mediated by water molecules



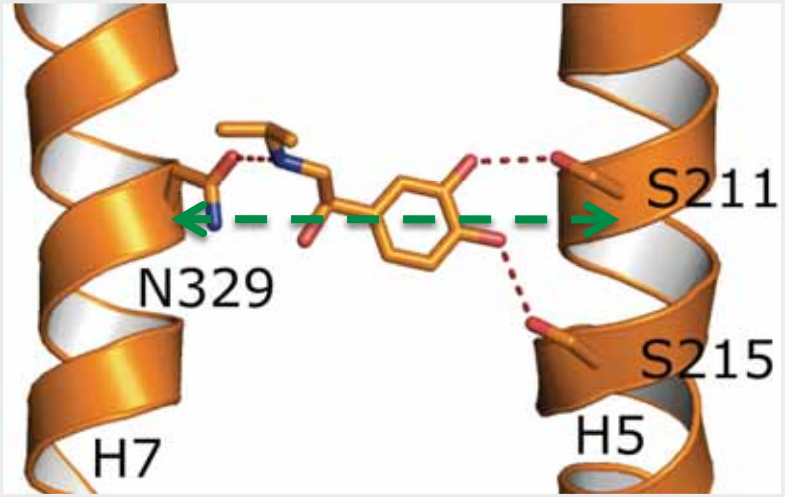
hydrogen bonds (-----)

What are the structural differences in the β_1 receptor when a full agonist binds compared to when an inverse agonist binds?

Carazolol, Very weak partial agonist



Isoprenaline, full agonist



Rotamer changes of S211$^{5.42}$ and S215$^{5.46}$

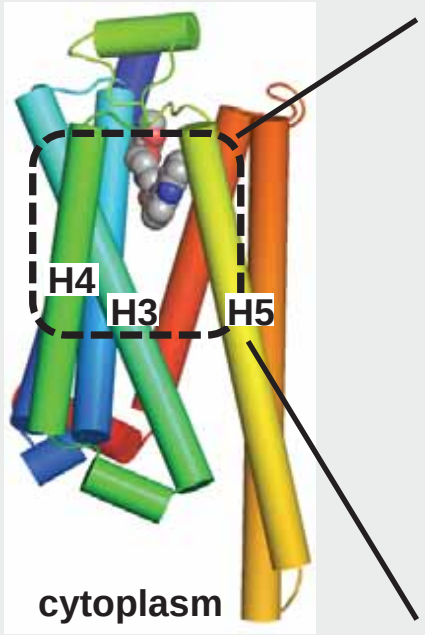
1.0 Å difference in distance between the Cα atoms of N329$^{7.39}$ and S211$^{5.42}$

hydrogen bonds (-----)

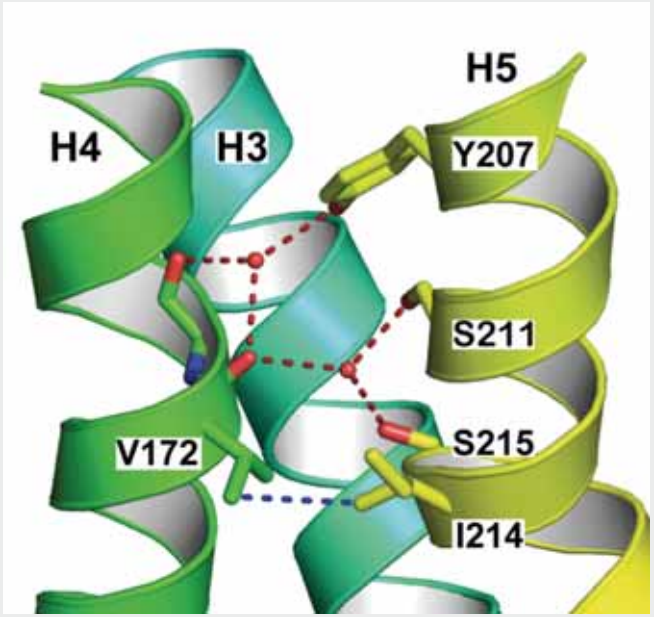
Moukhametzianov *et al* (2011) PNAS **108**, 8228-8232; Warne *et al* (2011) Nature **469**, 241-244

A minimal interface is observed between H5 and H3/H4 in crystal structures of the β_1 and β_2ARs with inverse agonists bound

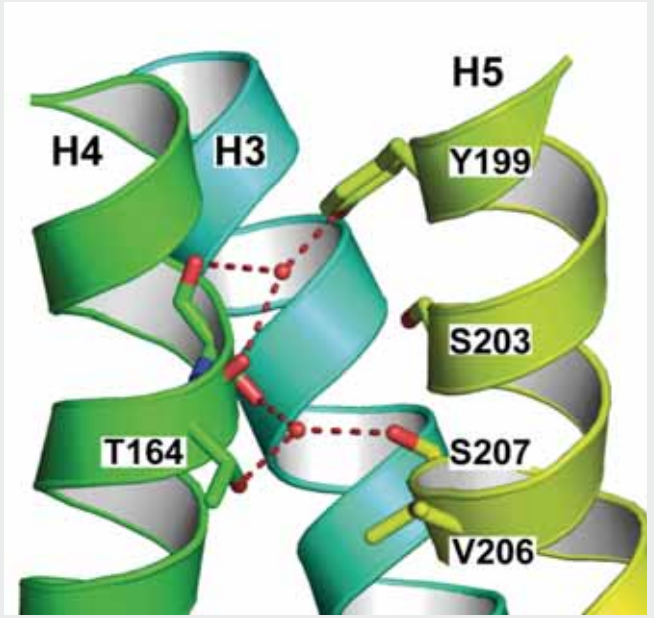
β_1AR overview



β_1AR



β_2AR



hydrogen bonds (-----), van der Waals (——)

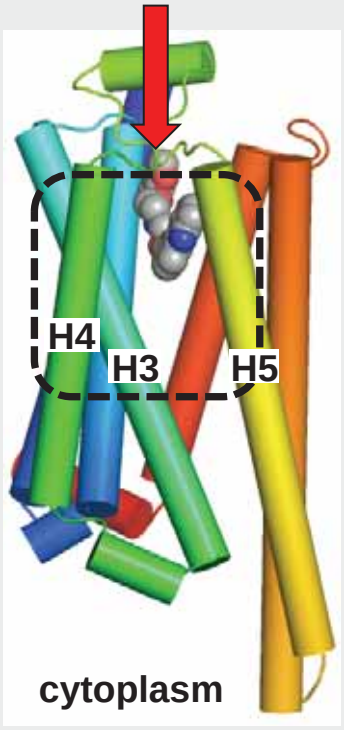
The interactions at this interface differ between the two receptors because of the presence of Thr164$^{4.56}$ in the β_2AR instead of Val172$^{4.56}$ as in the β_1AR

Warne *et al* (2012) Structure **20**, 841-9

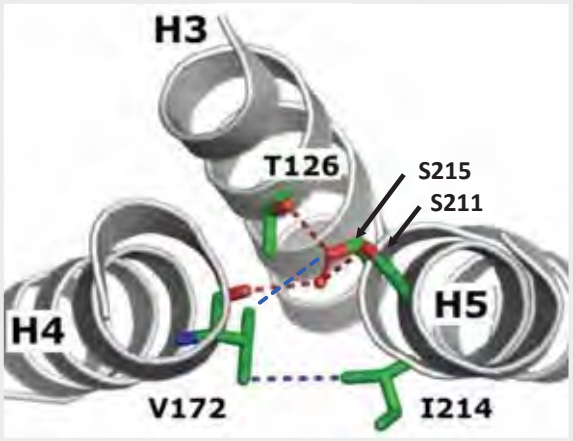
Hanson *et al* (2008) Structure **16**, 897-905

Serine rotamer changes occurring on the binding of full agonists decrease interactions to helix 5 and helix 3

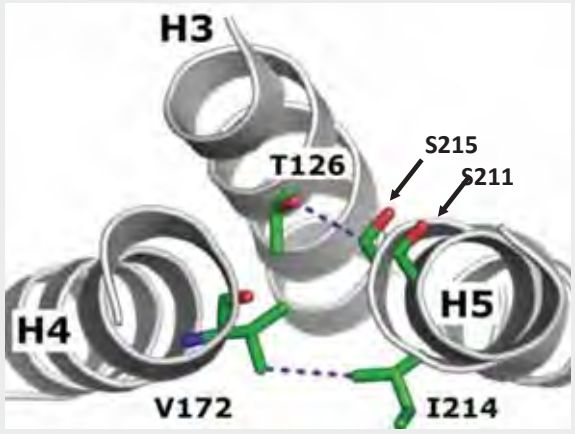
β_1AR overview



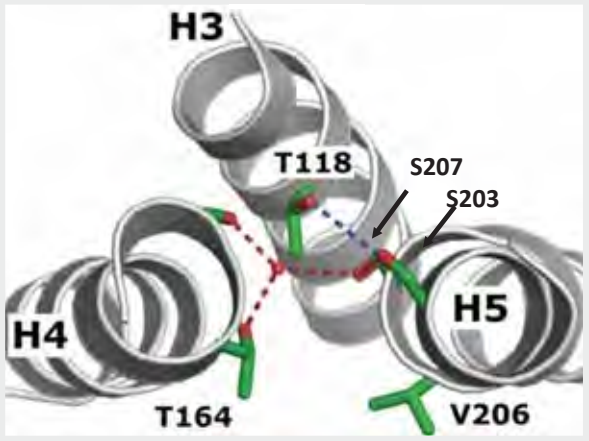
β_1AR



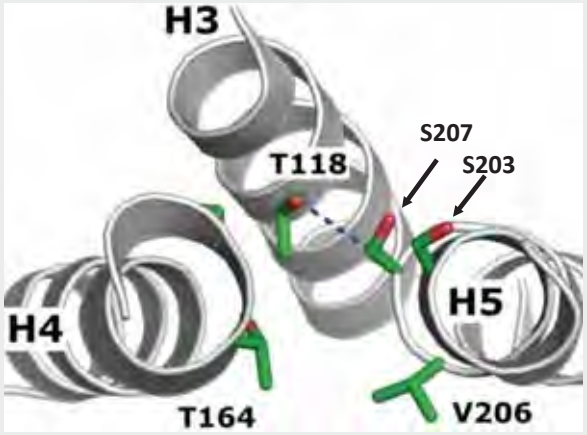
+ full agonist



β_2AR



+ full agonist

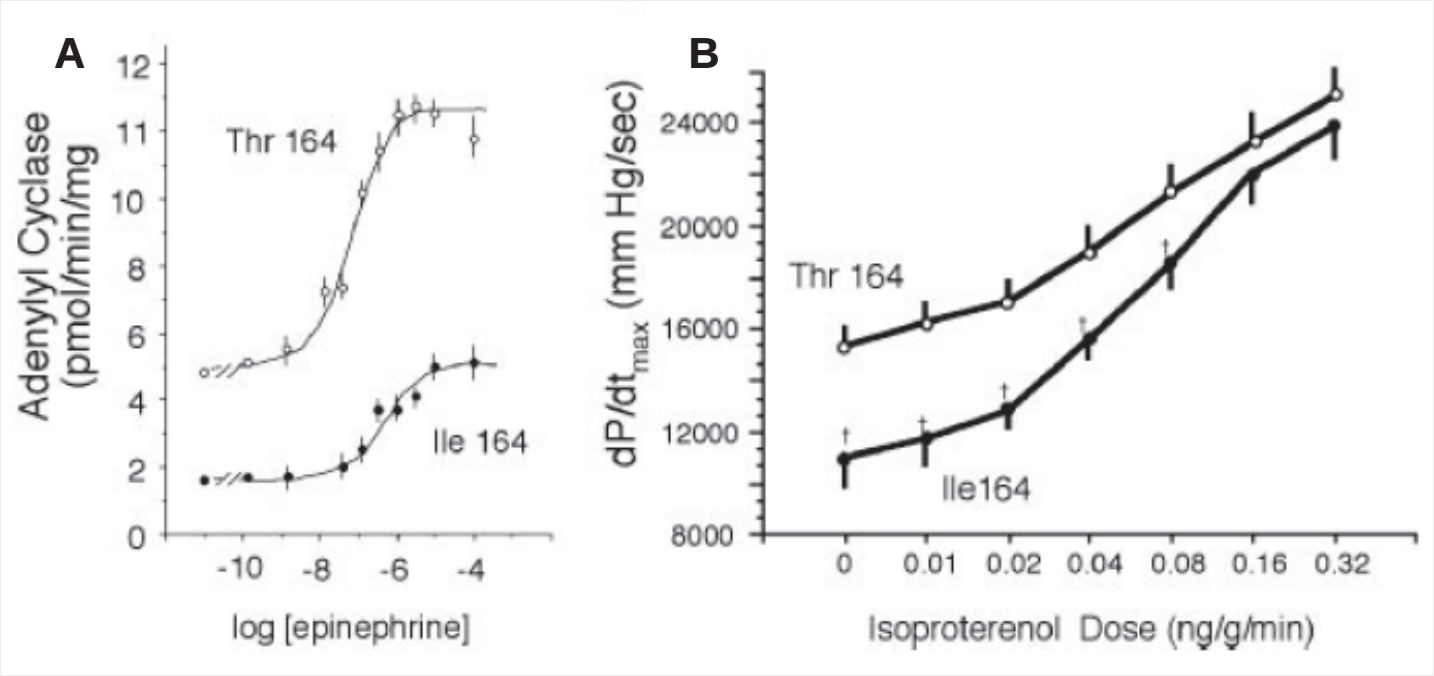


Warne & Tate (2013) Biochem. Soc. Trans. **41**, 159-165

The effect of the T164I polymorphism on the activity of the β_2 adrenergic receptor

Pharmacology of Thr 164 and Ile 164 isoforms

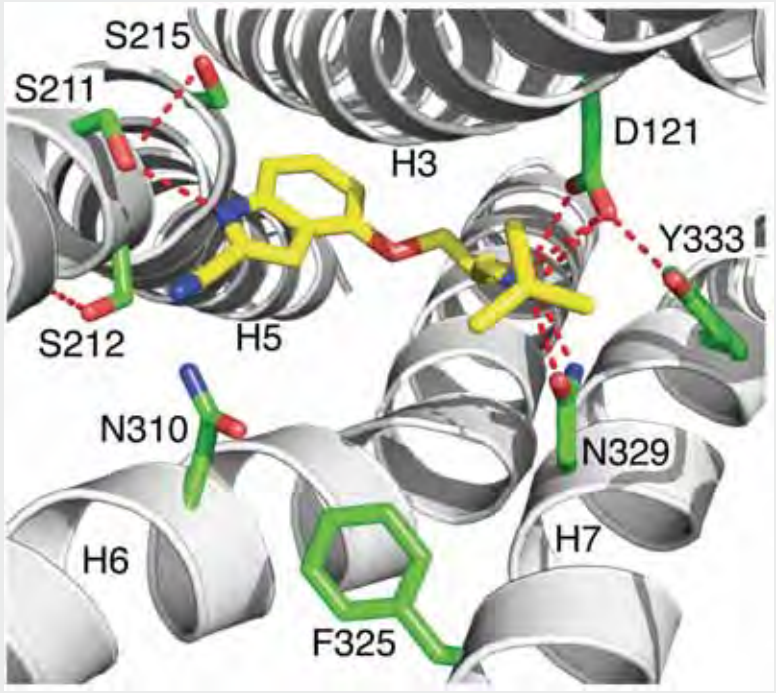
Small *et al.* (2003) Annu. Rev. Pharmacol. Toxicol. **43**, 381–411



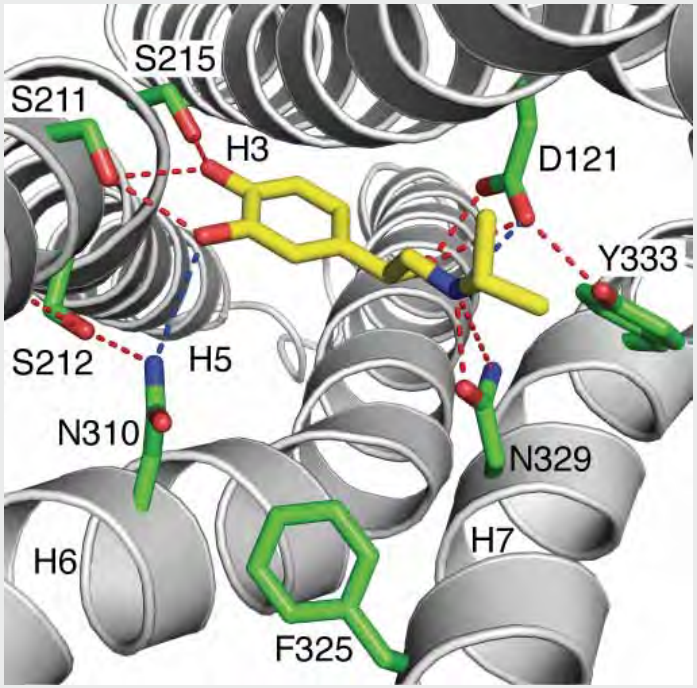
A Reduced response to agonist in Ile 164 isoform

B Reduced cardiac output in Ile 164 isoform

Rotamer conformation changes of Ser215$^{5.46}$ occurs on agonist binding, but not when an antagonist binds



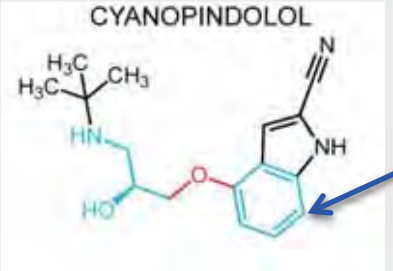
Cyanopindolol
Weak partial agonist



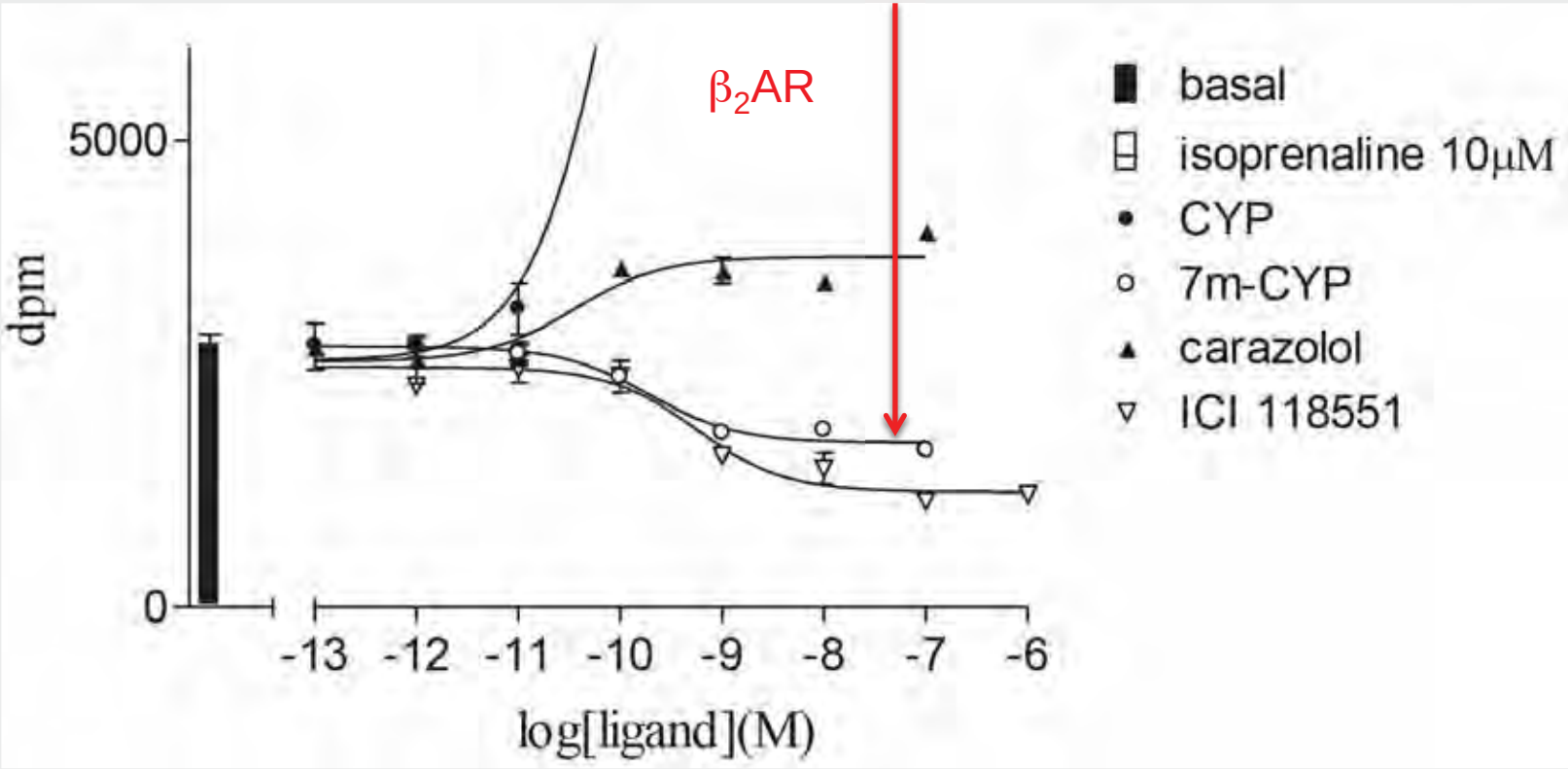
Isoprenaline
Full agonist

tβ1	hβ2	B-W	tβ1	hβ2	B-W
D121	D138	3.32	F325	Y308	7.35
S211	S203	5.42	N329	N312	7.39
S212	S204	5.43	W330	W313	7.40
S215	S207	5.46	Y333	Y316	7.43
N310	N293	6.55			

Is the rotamer change of Ser$^{5.46}$ really that important in determining efficacy?

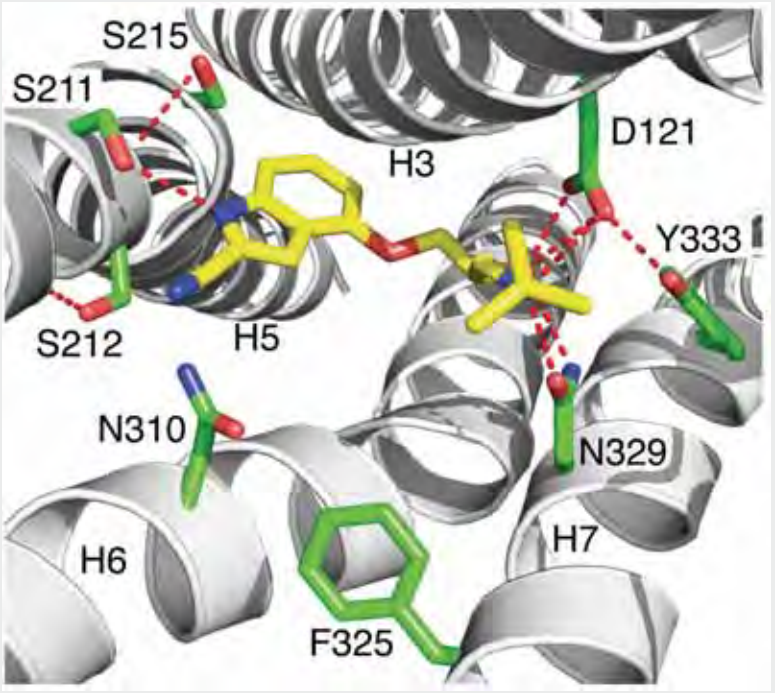


Add methyl group:
7-methyl-cyanopindolol

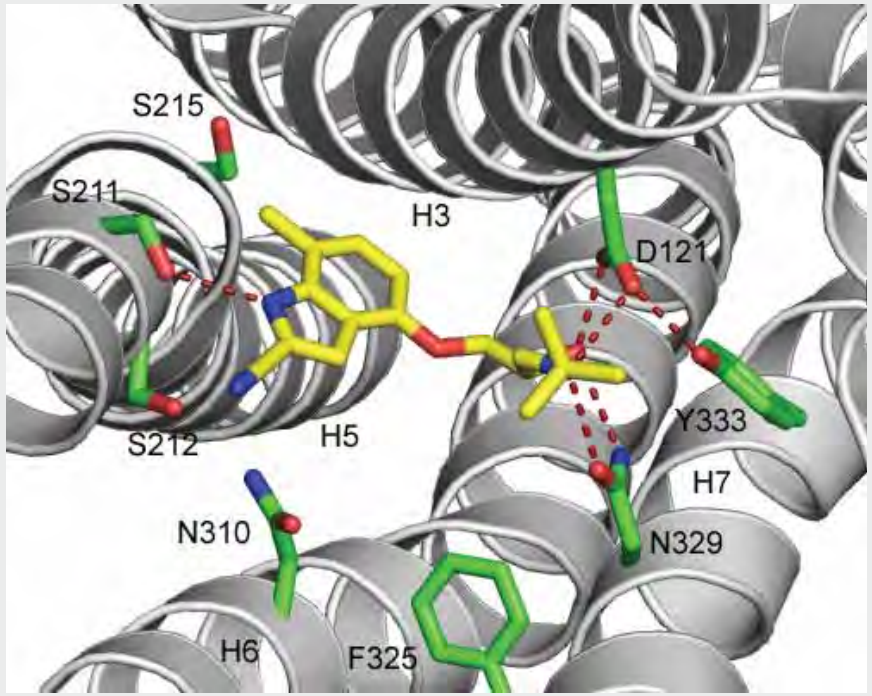


Tomomi Sato & Jill Baker; unpublished

The structure of β_1AR bound to 7MeCyp shows a 0.5 Å expansion of the ligand binding pocket and confirms the rotamer of Ser215$^{5.46}$



Cyanopindolol
Weak partial agonist



7-methyl-cyanopindolol
Inverse agonist

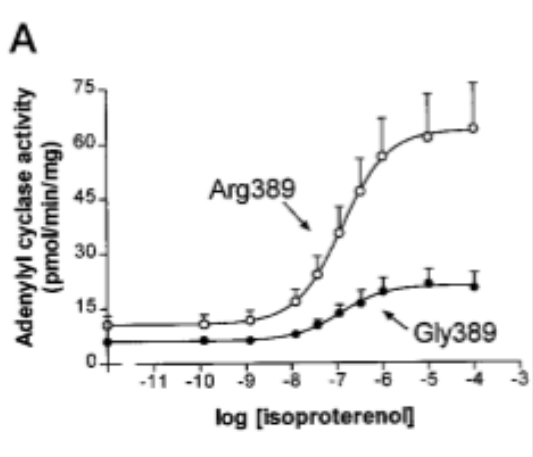
tβ1	hβ2	B-W	tβ1	hβ2	B-W
D121	D138	3.32	F325	Y308	7.35
S211	S203	5.42	N329	N312	7.39
S212	S204	5.43	W330	W313	7.40
S215	S207	5.46	Y333	Y316	7.43
N310	N293	6.55			

Tomomi Sato & Jill Baker; unpublished

A structural explanation for the differences in activity between the human β_1AR-Arg389 and β_1AR-Gly389 isoforms

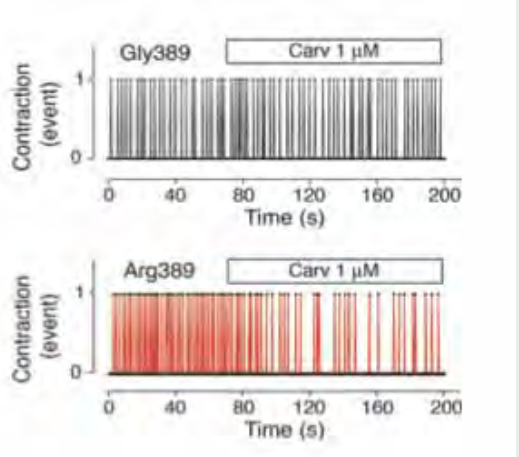
The β_1AR-Arg389 isoform is more active than the β_1AR-Gly389 isoform:

increased adenylyl cyclase activity in cell lines



Mason *et al* (1999) JBC **274** 12670-12674

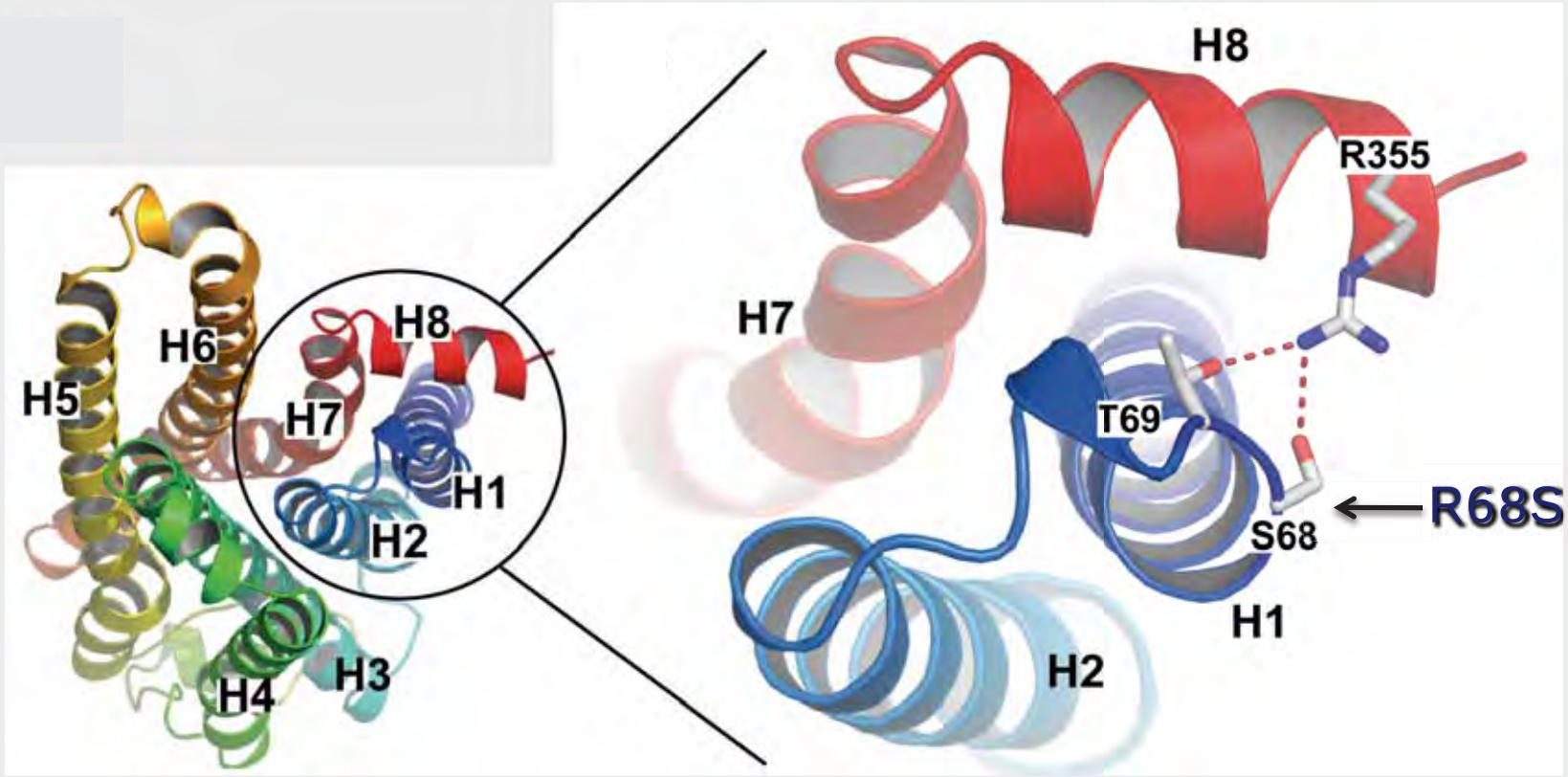
greater contractility in cardiomyocytes



and increased sensitivity to carvedilol

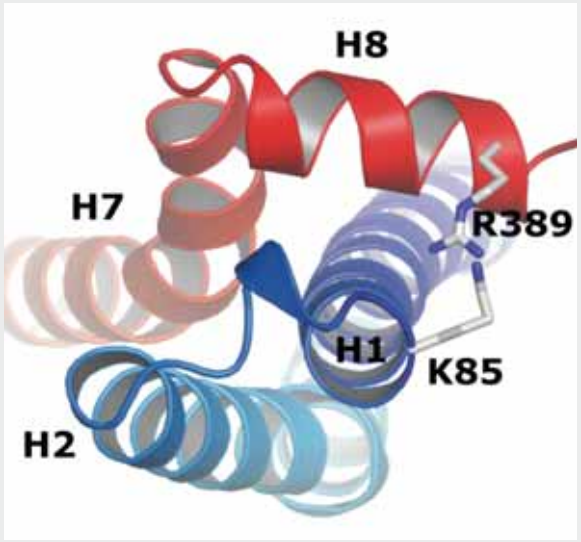
Rochais et al (2007) J Clin Invest **117** 229-35

The environment of R355$^{8.56}$ in the crystal structure of the thermostabilized β_1AR

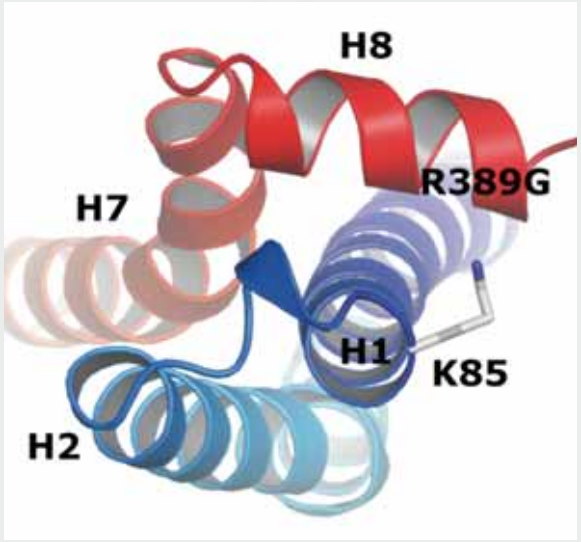


The human β_1AR-Arg389 also features an unfavourable pairing of Lys85$^{1.59}$ and Arg389$^{8.56}$, this is absent in β_1AR-Gly389

β_1AR-Arg389, more active isoform (model)



β_1AR-Gly389, less active isoform (model)



Residue pairings at the H1/H8 interface in other βARs

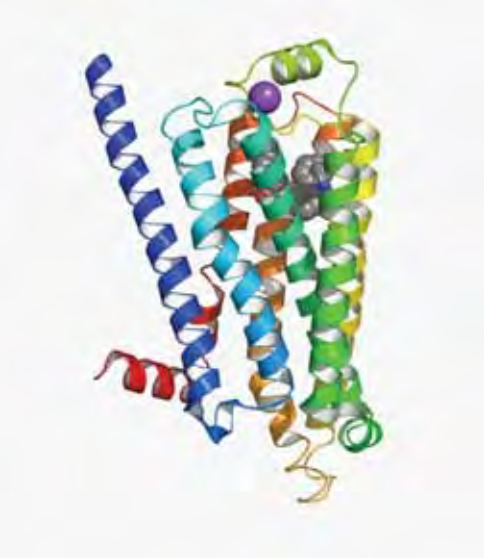
	pos$^{1.59}$	pos$^{8.56}$
β_1	K 85	R/G 389
β_2	K 60	E 338
β_3	W/K 64 *	R

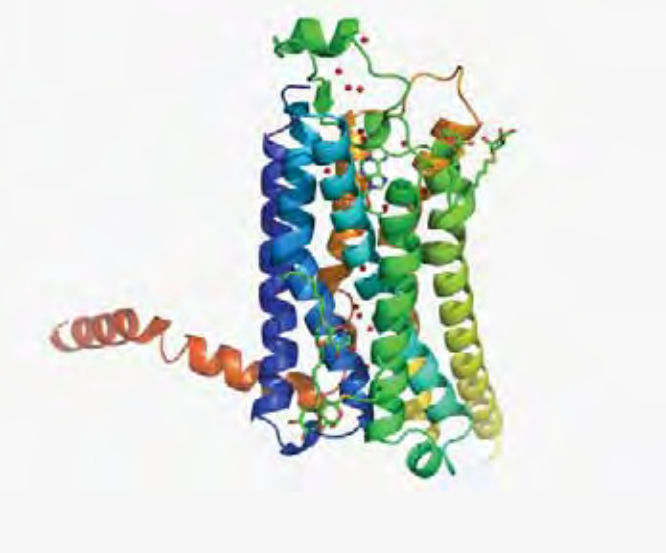
*Another polymorphism that affects β_3 and has been associated with obesity

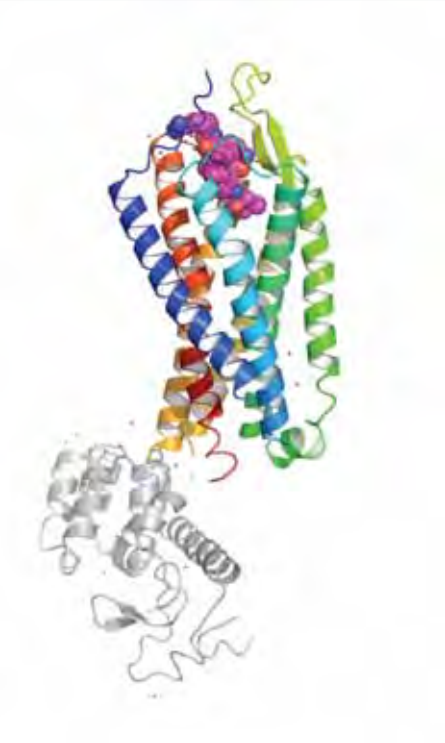
The destabilizing effect of Lys-Arg juxtaposition has been utilized to enhance constitutive activity in the human β_1AR

McLean et al (2002) Mol Pharmacol **62**, 747-55

Structures of agonist-bound GPCRs







β_1AR

- 2.6 - 3.0 Å resolution
- Isoprenaline, carmoterol dobutamine, salbutamol
- Six mutations
- R-like state

Warne *et al*. (2011)
Nature **469**, 241-244

A_{2A}R

- 2.6 - 3.0 Å resolution
- NECA, adenosine
- Four mutations
- R*-like state

Lebon *et al.* (2011)
Nature **474**, 521

NTSR1

- 2.8 Å resolution
- Neurotensin 8-13
- Six mutations
- T4 lysozyme fusion
- R*-like state

White *et al.* (2012)
Nature **490**, 508-513

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β_1 adrenergic receptor
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*Jessica Li

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