The structural basis for ligand efficacy in the $\beta_1$-adrenoceptor

Chris Tate
4.4% of FDA approved drugs target the \( \beta_1 \) and \( \beta_2 \) adrenoreceptors

**\( \beta_1 \) receptor**
- Heart
- Beta blockers (antagonists)
  - e.g. bucindolol
  - carvedilol
- Various heart problems

**\( \beta_2 \) receptor**
- Lungs
- Bronchodilators (agonists)
  - e.g. salbutamol
  - formoterol
  - carmoterol
  - Asthma

**Sympathomimetics** (agonists)
- e.g. dobutamine
- Heart failure

**Antagonists: receptor inhibitors**

**Agonists: receptor activators**
Differences in activity between $\beta_1$ and $\beta_2$ adrenergic receptors

$\beta_2$AR shows higher basal (constitutive) activity

$\beta_2$AR shows greater efficacy (response to agonist stimulus) than $\beta_1$AR

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

But $\alpha$ helices of $\beta_1$ and $\beta_2$ are 67% identical, and there are only two differences within 8Å of the ligand binding pocket

Engelhardt et al. (2001) Mol Pharm 60, 712-717
Comparison of the ligand binding pockets of $\beta_1$ and $\beta_2$ adrenergic receptors

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

Cherezov et al. (2007) Science 318, 1258-1265
Comparison of the thermostabilities of the human and turkey $\beta_1$-adrenoceptors with the human $\beta_2$-adrenoceptor
\( \beta_1 \text{AR-m23} \) 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.

\( \beta_1 \text{AR-m23} \) is stable in short-chain detergents like octylglucoside, which facilitates the formation of well-ordered crystals in vapour diffusion experiments.

\[\text{OG: octylglucoside; NG: nonylglucoside; DM: decylmaltoside; DDM: dodecylmaltoside}\]

Thermostabilised βAR-m23 receptor couples to G proteins in a whole cell assay and shows no basal activity.
Thermostabilisation of the $\beta_1$-adrenergic receptor

Serrano-Vega et al. (2008) PNAS 105, 877-882
β1 data collection: t1043

Isotropic diffraction

Spacegroup P1

a = 55.5 Å, b = 86.8 Å, c = 95.50 Å
α = 67.60, β = 73.30, γ = 85.80
Crystallisation construct of the $\beta_1$ receptor

Warne et al. (2009) Protein Exp. Purif. 65, 204-213
Structure the thermostabilised avian $\beta_1$-adrenoceptor

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

β₁AR versus β₂AR (overall rmsd 0.6 Å)  

Agonist-bound conformations of A₂AR  
(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**

<table>
<thead>
<tr>
<th></th>
<th>A$_{2A}$AR-WT</th>
<th>A$_{2A}$AR-BRIL-ΔC</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td><img src="image1.png" alt="Graph A" /></td>
<td><img src="image2.png" alt="Graph A" /></td>
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<tr>
<td>NaNCl</td>
<td><img src="image3.png" alt="Graph A" /></td>
<td><img src="image4.png" alt="Graph A" /></td>
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<tr>
<td>Aniloride</td>
<td><img src="image5.png" alt="Graph A" /></td>
<td><img src="image6.png" alt="Graph A" /></td>
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<tr>
<td>Aniloride + NaNCl</td>
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<td><img src="image8.png" alt="Graph A" /></td>
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<tr>
<td>Choline chloride</td>
<td><img src="image9.png" alt="Graph A" /></td>
<td><img src="image10.png" alt="Graph A" /></td>
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**Agonist binding**

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<tr>
<td>Control</td>
<td><img src="image11.png" alt="Graph B" /></td>
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<tr>
<td>Aniloride</td>
<td><img src="image15.png" alt="Graph B" /></td>
<td><img src="image16.png" alt="Graph B" /></td>
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<tr>
<td>Aniloride + NaNCl</td>
<td><img src="image17.png" alt="Graph B" /></td>
<td><img src="image18.png" alt="Graph B" /></td>
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<tr>
<td>Choline chloride</td>
<td><img src="image19.png" alt="Graph B" /></td>
<td><img src="image20.png" alt="Graph B" /></td>
</tr>
</tbody>
</table>

**EC50**

~40-50 mM

**Inactive state**

**Active-like state**

**Legend**

- ![Image D](image21.png)
The ultra-stable $\beta_1$AR mutant JM50 contains 3 additional thermostabilising mutations, which gave 12°C further stability to $\beta_1$AR-m23.
The stability of $\beta_1$AR-JM50 in different detergents

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonylglucoside</td>
<td>34.5 ± 0.7</td>
</tr>
<tr>
<td>Octylglucoside</td>
<td>26.8 ± 0.2</td>
</tr>
<tr>
<td>Heptylthioglucoiside</td>
<td>22.5 ± 0.7</td>
</tr>
<tr>
<td>Fos choline 9</td>
<td>35.3 ± 0.5</td>
</tr>
<tr>
<td>SDS</td>
<td>33.0 ± 0.7</td>
</tr>
<tr>
<td>LDAO</td>
<td>31.6 ± 0.3</td>
</tr>
<tr>
<td>CYMAL3</td>
<td>33.8 ± 0.5</td>
</tr>
<tr>
<td>DHPC</td>
<td>37.4 ± 0.9</td>
</tr>
</tbody>
</table>
2.1 Å resolution structure of an ultra-stable β1AR 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 Ca, 2.4 Å
- Rmsd of Ca in the Na\(^+\) binding pocket, 0.3 Å
The intramembrane Na⁺ ion in β₁AR 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.

**Diagram:**
- **[cAMP] (nM)** vs **Log [isoprenaline] (M)**
  - WT vs D87A

**Agonist affinities are identical**

**Expression levels are identical**
Asp\textsuperscript{2.50} that co-ordinates Na\textsuperscript{+} in the R state makes 3 hydrogen bonds to side chains in the R\textsuperscript{*} state.

Structure of $\beta_2$AR in the activated state bound to Nb80 showing re-organisation of the Na\textsuperscript{+} binding pocket.
So what is the role of the intramembrane Na$^+$ in β$_1$AR?

**A: stabilisation of the ligand-free receptor**

The stability of ligand-free detergent solubilised β$_1$AR 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 \(\beta_1\)AR 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 β₁AR

<table>
<thead>
<tr>
<th>Ligand</th>
<th>PDB</th>
<th>Space group</th>
<th>Ligand type</th>
<th>Detergent or Lipidic Cubic Phase</th>
<th>Resolution Å</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Methyl cyanopindolol</td>
<td>P2₁</td>
<td>Inverse agonist</td>
<td>D</td>
<td>2.50</td>
<td></td>
<td>Sato et al. unpublished</td>
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<tr>
<td>Cyanopindolol</td>
<td>4bvn</td>
<td>P2₁22₁</td>
<td>Weak partial agonist</td>
<td>LCP</td>
<td>2.10</td>
<td>Miller-Gallacher et al. PlosOne (2014)</td>
</tr>
<tr>
<td>Nadolol</td>
<td>C2</td>
<td>Weak partial agonist</td>
<td>D</td>
<td>3.40</td>
<td></td>
<td>Li et al. unpublished</td>
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<tr>
<td>Timolol</td>
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<td>Weak partial agonist</td>
<td>LCP</td>
<td>3.40</td>
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<td>&quot;</td>
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<tr>
<td>Carazolol</td>
<td>2ycw</td>
<td>P2₁</td>
<td>Weak partial agonist</td>
<td>D</td>
<td>3.00</td>
<td>Moukhametzianov et al. PNAS (2011)</td>
</tr>
<tr>
<td>Cyanopindolol</td>
<td>2ycx 2ycy</td>
<td>P2₁</td>
<td>Weak partial agonist</td>
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<tr>
<td>Carvedilol</td>
<td>4amj</td>
<td>P2₁</td>
<td>Biased agonist</td>
<td>D</td>
<td>2.30</td>
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<tr>
<td>Dobutamine</td>
<td>2y00 2y01</td>
<td>P2₁</td>
<td>Partial agonist</td>
<td>D</td>
<td>2.70</td>
<td>Warne et al. Nature (2011)</td>
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<tr>
<td>Salbutamol</td>
<td>2y04</td>
<td>P2₁</td>
<td>Partial agonist</td>
<td>D</td>
<td>3.00</td>
<td>&quot;</td>
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<tr>
<td>Isoprenaline</td>
<td>2y03</td>
<td>P2₁</td>
<td>Full agonist</td>
<td>D</td>
<td>2.85</td>
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<td>Carmoterol</td>
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<td>P2₁</td>
<td>Full agonist</td>
<td>D</td>
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<tr>
<td>Quinolone fragment 20</td>
<td>3zpr</td>
<td>P2₁</td>
<td>?</td>
<td>D</td>
<td>2.70</td>
<td>&quot;</td>
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What are the structural differences in the $\beta_1$ receptor when an agonist binds compared to when an antagonist binds?

<table>
<thead>
<tr>
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<th>t$\beta$1</th>
<th>h$\beta$2</th>
<th>B-W</th>
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<tr>
<td>D121</td>
<td>D138</td>
<td></td>
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</tr>
<tr>
<td>S211</td>
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</tr>
<tr>
<td>N329</td>
<td>N312</td>
<td></td>
<td>7.39</td>
</tr>
<tr>
<td>W330</td>
<td>W313</td>
<td></td>
<td>7.40</td>
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</table>

Noradrenaline

Warne et al. (2011) Nature 469, 241-244
Determination of serine rotamer configurations in $\beta_1$AR structures

Ser$211^{5.43}$, $\beta_1$AR with carvedilol (2.3Å)

Ser$211^{5.43}$, $\beta_1$AR with cyanopindolol (2.1Å)
Assignment of water molecules in $\beta_1$AR structures, $\beta_1$AR with carvedilol (2.3Å)

$\beta_1$AR overview

Positive density features at the H5-H3/4 interface
Assignment of water molecules in $\beta_1$AR structures, $\beta_1$AR with carvedilol (2.3Å)

$\beta_1$AR overview

Positive density features at the H5-H3/4 interface

Three water molecules (+) fitted to density (B factors 21-46 Å²)
Assignment of water molecules in β₁AR structures, β₁AR with carvedilol (2.3Å)

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

hydrogen bonds (-----)
What are the structural differences in the $\beta_1$ 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 C$\alpha$ atoms of N329$_{7.39}$ and S211$_{5.42}$

hydrogen bonds (-----)

A minimal interface is observed between H5 and H3/H4 in crystal structures of the $\beta_1$ and $\beta_2$ARs with inverse agonists bound. The interactions at this interface differ between the two receptors because of the presence of Thr164$^{4.56}$ in the $\beta_2$AR instead of Val172$^{4.56}$ as in the $\beta_1$AR.

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.

β₁AR overview

β₁AR + full agonist

β₂AR + full agonist

Warne & Tate (2013) Biochem. Soc. Trans. 41, 159-165
The effect of the T164I polymorphism on the activity of the β₂ adrenergic receptor

Pharmacology of Thr 164 and Ile 164 isoforms


**Graphs**

A Reduced response to agonist in Ile 164 isoform

B Reduced cardiac output in Ile 164 isoform
Rotamer conformation changes of Ser215 occurs on agonist binding, but not when an antagonist binds.

- Cyanopindolol: Weak partial agonist
- Isoprenalin: Full agonist
Is the rotamer change of Ser\textsuperscript{5.46} really that important in determining efficacy?

Add methyl group: 7-methyl-cyanopindolol

Tomomi Sato & Jill Baker; unpublished
The structure of $\beta_1$AR 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

<table>
<thead>
<tr>
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<td>D121</td>
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<td>F325</td>
<td>Y308</td>
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7-methyl-cyanopindolol
Inverse agonist

Tomomi Sato & Jill Baker; unpublished
The $\beta_1$AR-Arg389 isoform is more active than the $\beta_1$AR-Gly389 isoform:

- increased adenylyl cyclase activity in cell lines
- greater contractility in cardiomyocytes
- and increased sensitivity to carvedilol

Mason et al (1999) JBC 274 12670-12674
The environment of R3558.56 in the crystal structure of the thermostabilized β1AR
The human $\beta_1$AR-Arg389 also features an unfavourable pairing of Lys85$^{1.59}$ and Arg389$^{8.56}$, this is absent in $\beta_1$AR-Gly389, more active isoform (model).

The destabilizing effect of Lys-Arg juxtaposition has been utilized to enhance constitutive activity in the human $\beta_1$AR.

Residue pairings at the H1/H8 interface in other $\beta$ARs:

<table>
<thead>
<tr>
<th></th>
<th>pos$^{1.59}$</th>
<th>pos$^{8.56}$</th>
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</thead>
<tbody>
<tr>
<td>$\beta_1$</td>
<td>K 85</td>
<td>R/G 389</td>
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<tr>
<td>$\beta_2$</td>
<td>K 60</td>
<td>E 338</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>W/K 64 *</td>
<td>R</td>
</tr>
</tbody>
</table>

*Another polymorphism that affects $\beta_3$ and has been associated with obesity.
Structures of agonist-bound GPCRs

**β₁AR**
- 2.6 - 3.0 Å resolution
- Isoprenaline, carmoterol
dobutamine, salbutamol
- Six mutations
- R-like state

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

**A₂AR**
- 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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β₁ adrenergic receptor
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