How ligands achieve biased signaling at opioid receptors

Joe Paggi, Ron Dror lab, Stanford University
G protein coupled receptors (GPCR) signal through multiple transducers.
Biased signaling

G protein biased agonist

Arrestin biased agonist

Always relative to a reference agonist, I’ll call these “balanced”
There is ongoing debate about the importance of bias signaling, as opposed to partial agonism.
Biased agonists favor “biased” receptor conformations

A variety of biophysical experiments support that biased agonists favor distinct receptor conformations.

1. What are these “biased” conformations?

2. How do agonists favor different conformations?
Experimentally determined structures provide an incomplete picture

- Solving an active state structure requires a transducer to be bound
- The conformation of the transducer binding site is largely determined by the transducer

μOR bound to arrestin-biased ligand
μOR bound to G protein-biased ligand
How we study biased signaling using MD simulations

1. Simulate receptor with no transducer bound.

2. Identify conformations of the transducer coupling interface. Assess their potential to bind with G protein or arrestin.

3. Run simulations with agonists with a variety of bias profile and observe which receptor conformations they favor.

4. Confirm hypotheses by designing novel agonists or receptor mutations.
Angiotensin receptor (AT$_1$R) transitions between two active intracellular conformations in simulation

Alternative conformation disfavors G-protein binding but can couple to arrestin

Arrestin-biased ligands favor alternative conformation, G protein–biased ligands disfavor it.
Do these results transfer to opioid receptors?

Insights into distinct signaling profiles of the µOR activated by diverse agonists

Qianhui Qu, Weijiao Huang, Deniz Aydin, Joseph M. Paggi, Alpay B. Seven, Haoqing Wang, Soumen Chakraborty, Tao Che, Jeffrey F. DiBerto, Michael J. Robertson, Asuka Inoue, Carl-Mikael Suomivuori, Bryan L. Roth, Susruta Majumdar, Ron O. Dror & Brian K. Kobilka & Georgios Skiniotis

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Molecular mechanism of biased signaling at the kappa opioid receptor

Amal El Daibani, Joseph M. Paggi, Kuglae Kim, Yianni D. Laloudakis, Petr Popov, Sarah M. Bernhard, Brian E. Krumm, Reid H. J. Olsen, Jeffrey Diperto, F. Ivy Carroll, Vsevolod Katritch, Bernhard Wünsch, Ron O. Dror & Tao Che

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Agonists studied at µOR

Mitragynine pseudoindoxyl (MP)  
DAMGO  
Lofentanil

G protein biased  
Balanced  
Arrestin biased

Please don’t take kratom because of this talk, it probably won’t kill you, but it isn’t good for you…
We observe the canonical and alternative states at $\mu$OR. Occupancy of states explains bias profile of agonists.

MP: G protein biased
DAMGO: balanced
Lofentanil: Arrestin biased

Statistical testing: Ran multiple independent simulations (6 for $\mu$OR, 10 for kOR), compute average value for each simulation, check for significance using t-test or Wilcoxon rank test.
Agonists studied at kOR

- Nalfurafine is approved in Japan since 2009 for use as an antipruritic, only one!
- Unlike other kOR agonists, Nalfurafine does not induce dysphoria at therapeutic doses
- Believed to be at least in part due to G protein bias

The balanced agonist already maxes out canonical state. How can you get G protein bias?

- U50,488: Balanced
- WMS-X600: Arrestin biased
- Nalfurafine: G protein biased

**Diagram:**

- **Frequency**
- **Intracellular TM7 rotation**
  - Alternative
  - Canonical
At kOR, we observe a third receptor conformation: the “occluded state”
The occluded state presents an electrostatic barrier to arrestin coupling
Intracellular TM7 rotation

Occluded / Canonical

Alternative-like / Canonical

Frequency

U50,488: Balanced

T2.39–D8.47 (Å)

Canonical

Alternative
Intracellular TM7 rotation

Nalfurafine: G protein biased
U50,488: Balanced
WMS,X600: Arrestin biased

Occluded / Canonical

Alternative-like / Canonical

Side view
Intracellular view
G protein

Arrestin

Receptors

Canonical active

AT1R, μOR, kOR

Alternative

AT1R, μOR, kOR

Occluded

kOR

...?
1. What are these “biased” conformations?

2. How do agonists favor different conformations?
   - What are the differences in protein–ligand interactions?
   - How are these differences transmitted through the receptor?
Before we get lost in the trees…

• I’m only going to share a subset of the results here
  • See our papers for more details!
  • We validate much of our proposed mechanism with mutagenesis experiments

1. Multiple layers of abstraction, things get confusing if you stay too low
  • Transducer site conformations
  • Allosteric pathways
  • Direct protein–ligand interactions

2. MD is a powerful tool
  • Subtle differences in ligands can have large and hard to predict impacts on binding pocket conformations
  • Even for major differences between ligands, the implications on protein dynamics are not clear from structures alone
Most importantly, a rigid body rotation connects the binding pocket to the transducer binding site.
Tyrosine pathway

Agonist

Transducer

Canonical

Alternative
Lofentanil stabilizes a polar network holding Y7.43 inwards
MP disrupts this polar network

Representative MD frames
Nalfurafine has a similar effect as MP, but through a different mechanism.
Tryptophan pathway
Vertical displacement of tryptophan explains arrestin-bias of WMS-X600

• Mutating this tryptophan to an alanine removes bias between WMS-X600 and U50,488
• Nalfurafine becomes even more G protein biased. Opportunity!

Initial docked poses  MD frame

Nalfurafine: G protein biased
U50,488: Balanced
WMS-X600: Arrestin biased

*Nalfurafine not significantly different than others
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