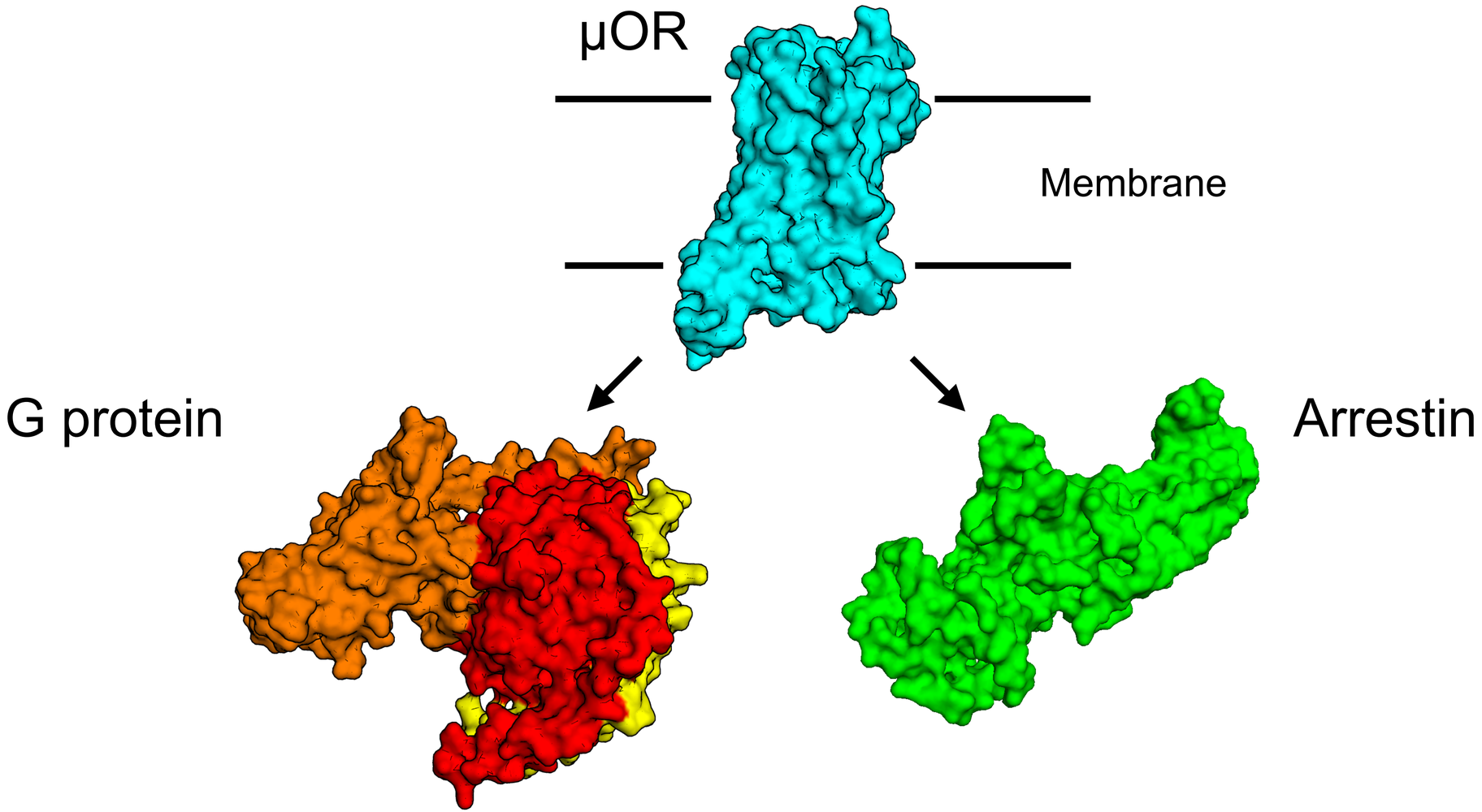
How ligands achieve biased signaling at opioid receptors

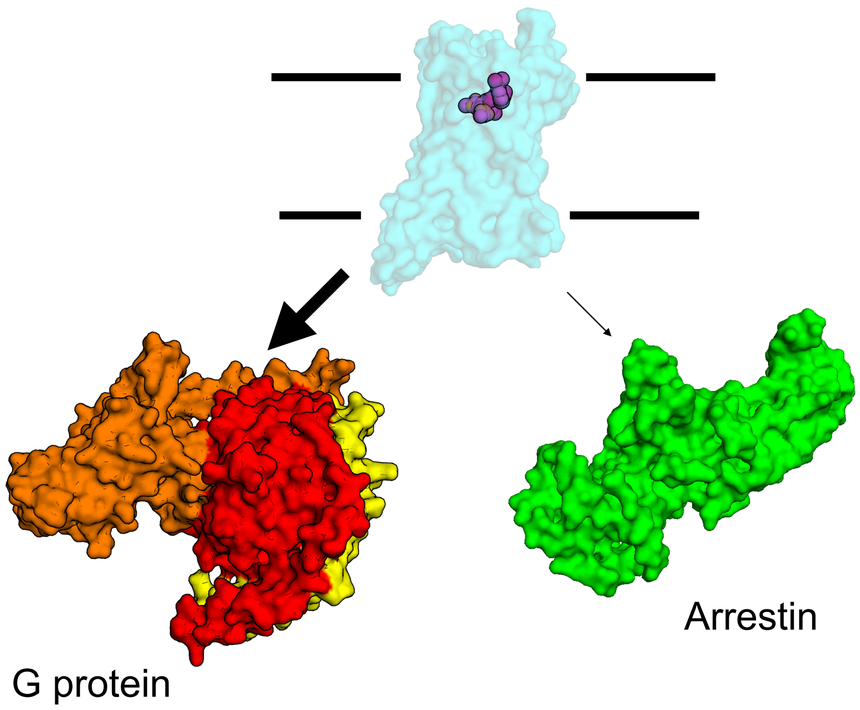
Joe Paggi, Ron Dror lab, Stanford University

G protein coupled receptors (GPCRs) signal through multiple transducers

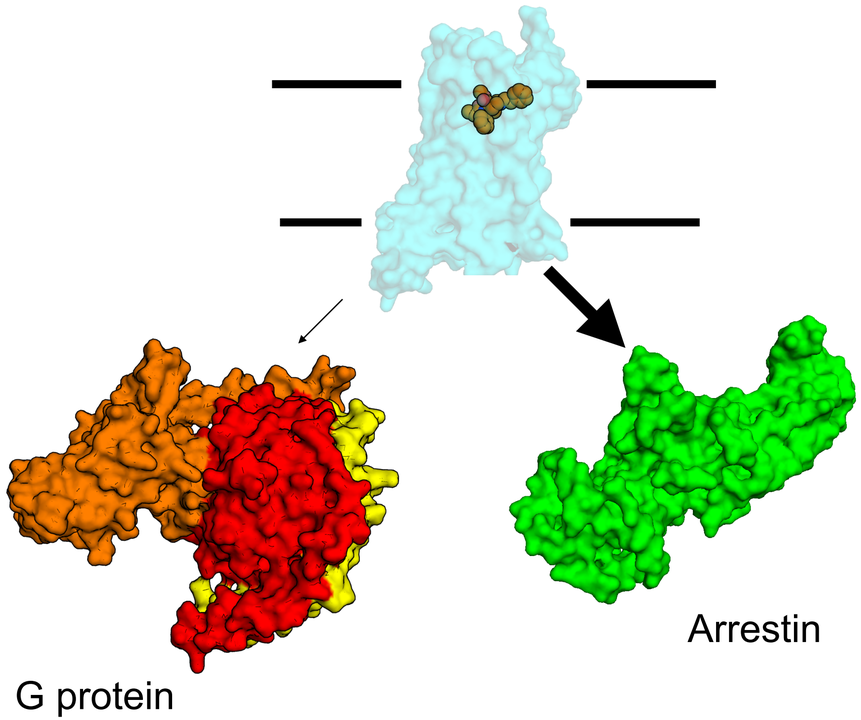


Biased signaling

G protein biased agonist

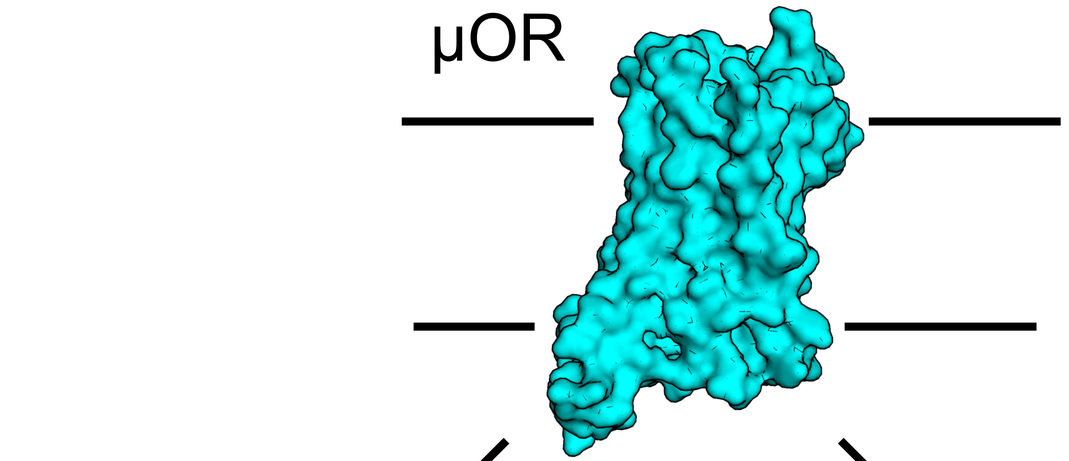


Arrestin biased agonist



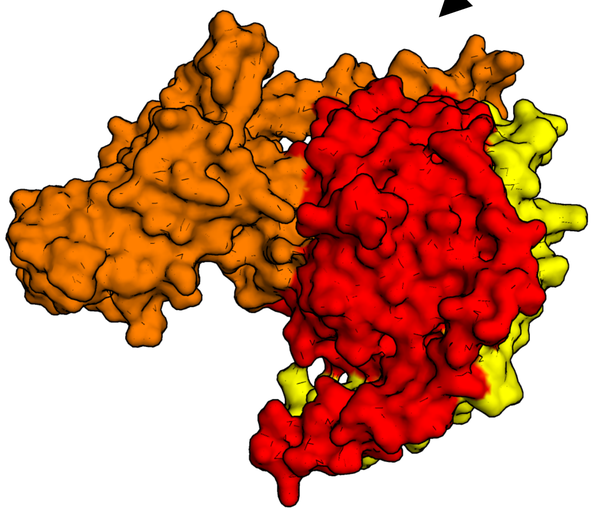
Always **relative** to a reference agonist, I'll call these "balanced"

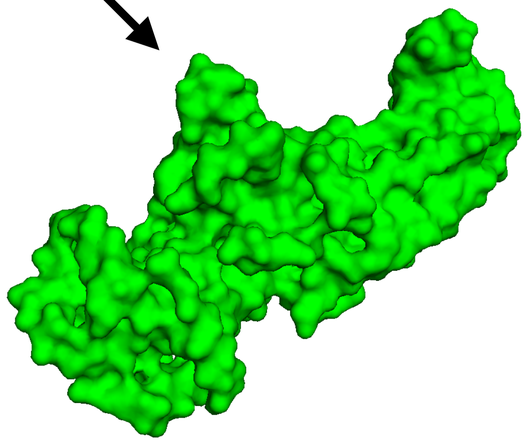
Biased signaling promises safer drugs



G protein

Analgesia





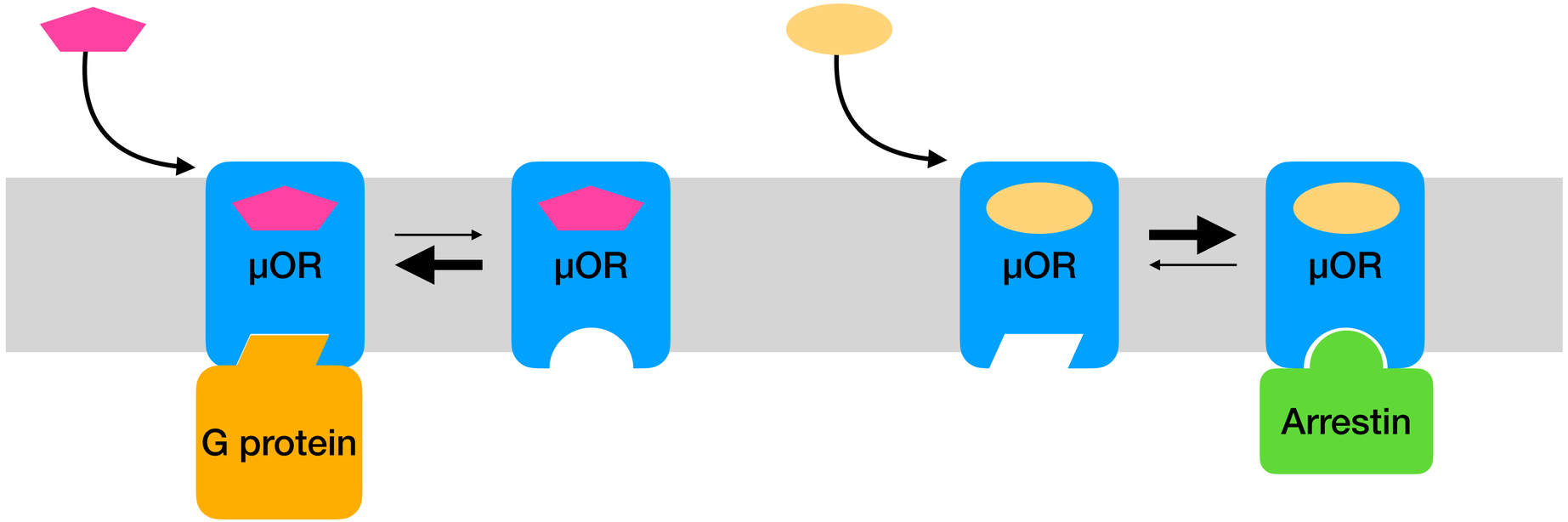
Arrestin

Respiratory depression
Constipation
Tolerance
Addiction

Stahl & Bohn, *Biochemistry,* 2022

There is ongoing debate about the importance of bias signaling, as opposed to partial agonism.

Biased agonists favor “biased” receptor conformations

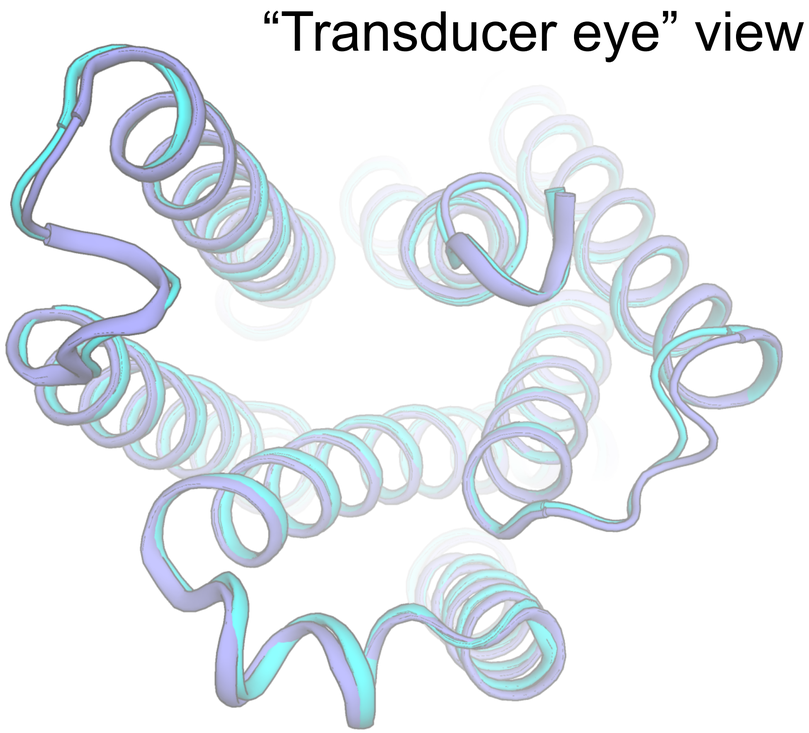


A variety of biophysical experiments support that biased agonists favor distinct receptor conformations.

- Liu et al, *Science*, 2012. Wingler et al, *Cell*, 2019. Cong et al, *Molecular Cell*, 2021.

1. What are these “biased” conformations?
2. How do agonists favor different conformations?

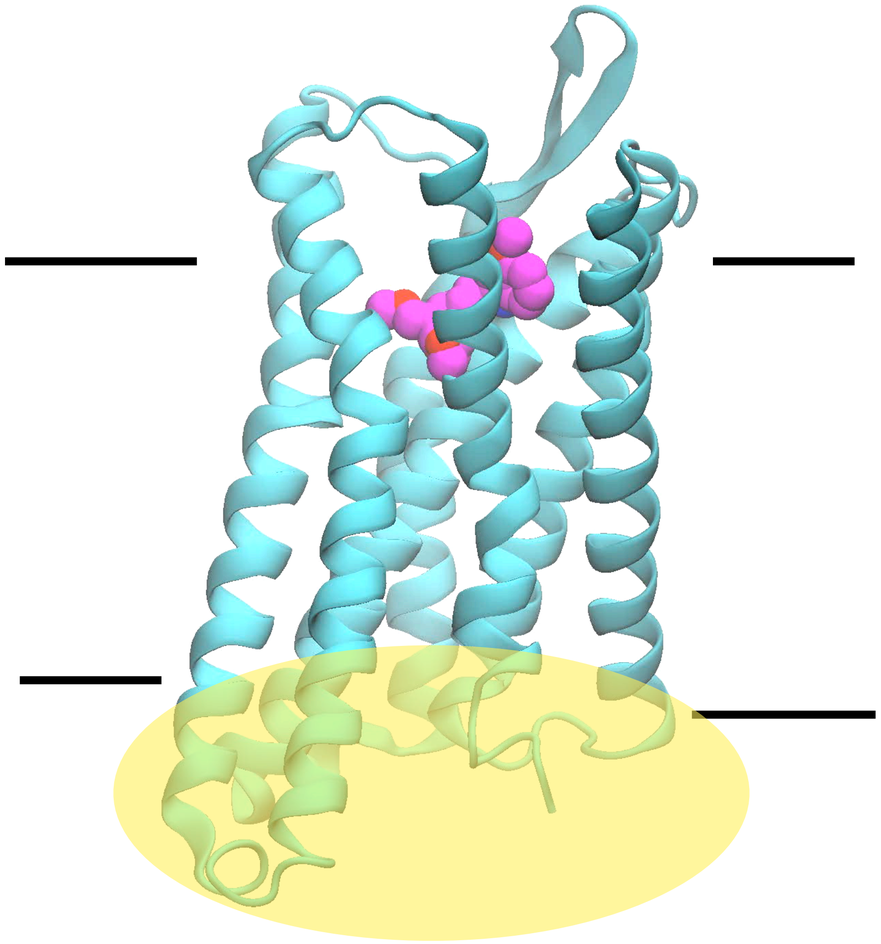
Experimentally determined structures provide an incomplete picture



μOR bound to arrestin-biased ligand
μOR bound to G protein-biased ligand

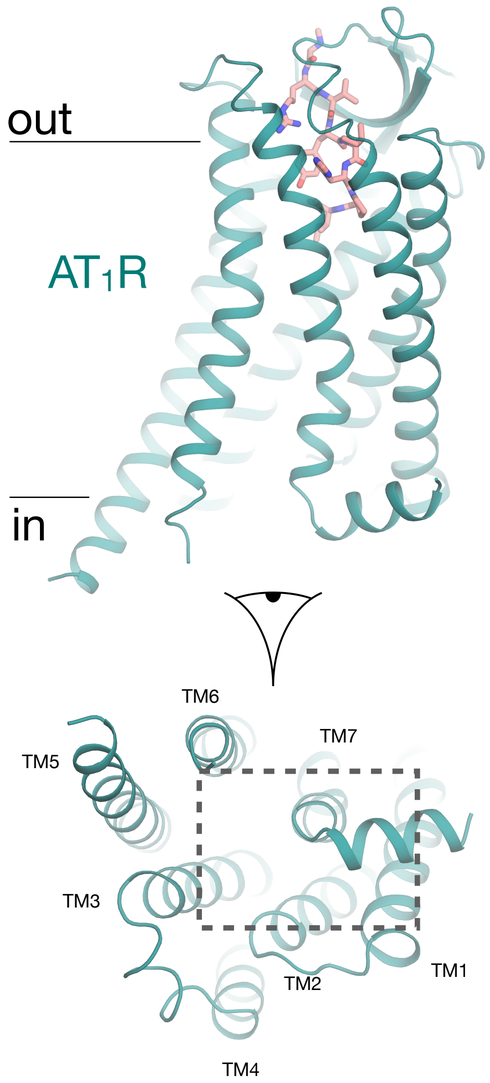
- Solving an active state structure **requires** a transducer to be bound
- The conformation of the transducer binding site is largely determined by the transducer

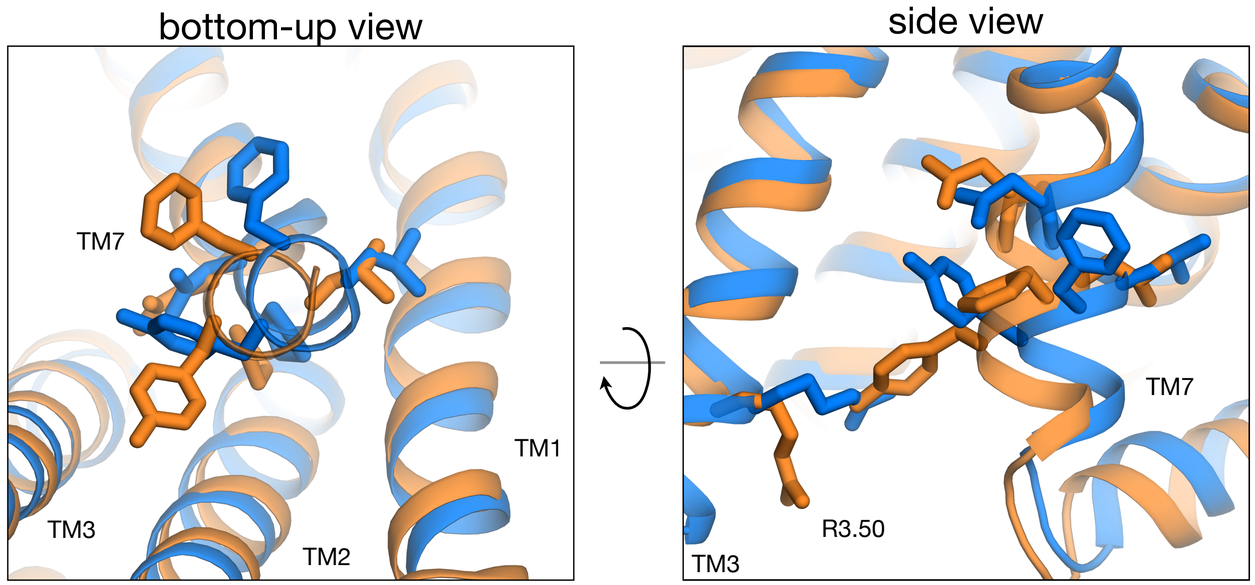
How we study biased signaling using MD simulations



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_1R) transitions between two active intracellular conformations in simulation



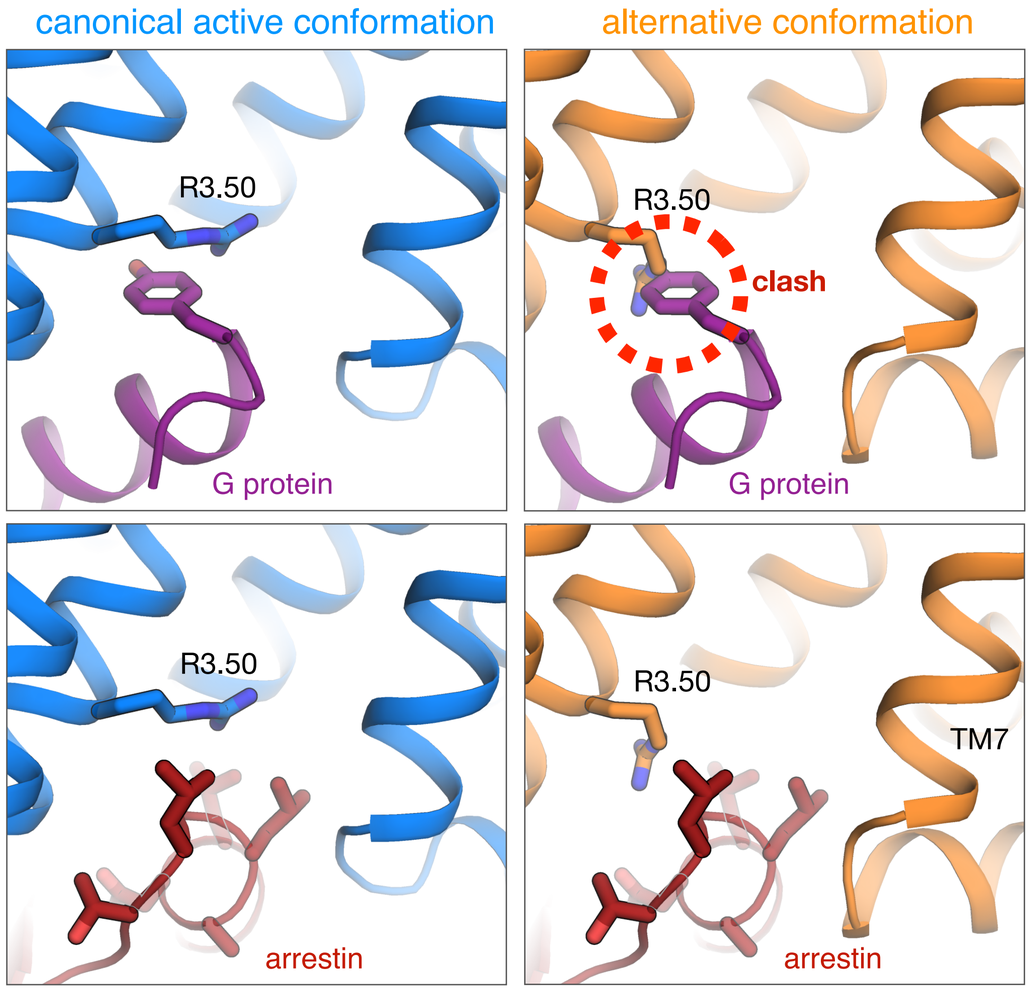


canonical active conformation

alternative conformation

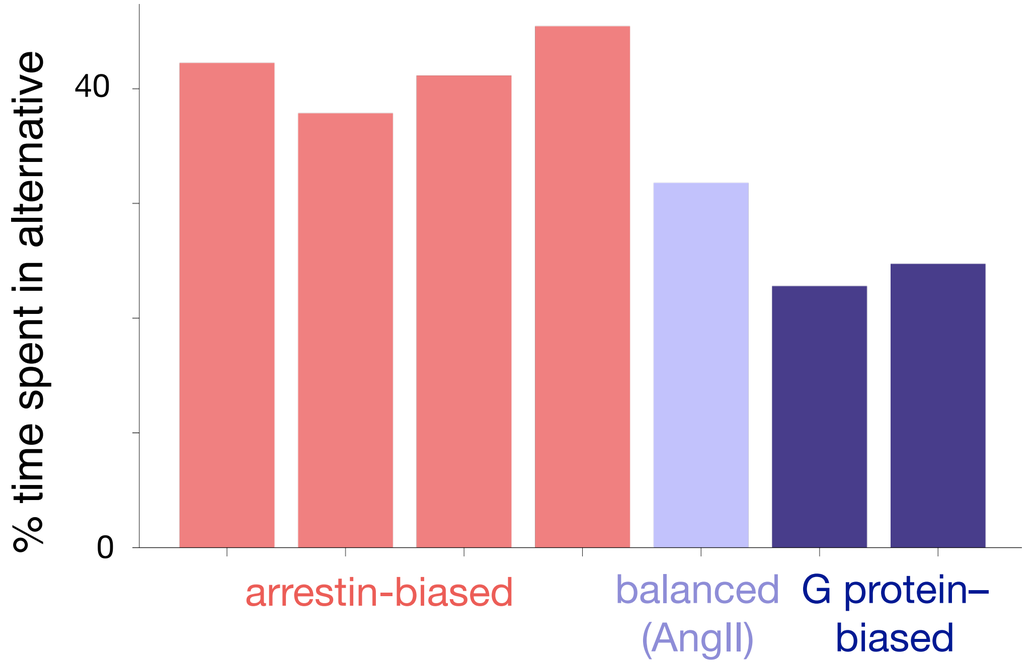
Suomivuori & Latorraca et al, *Science*, 2020

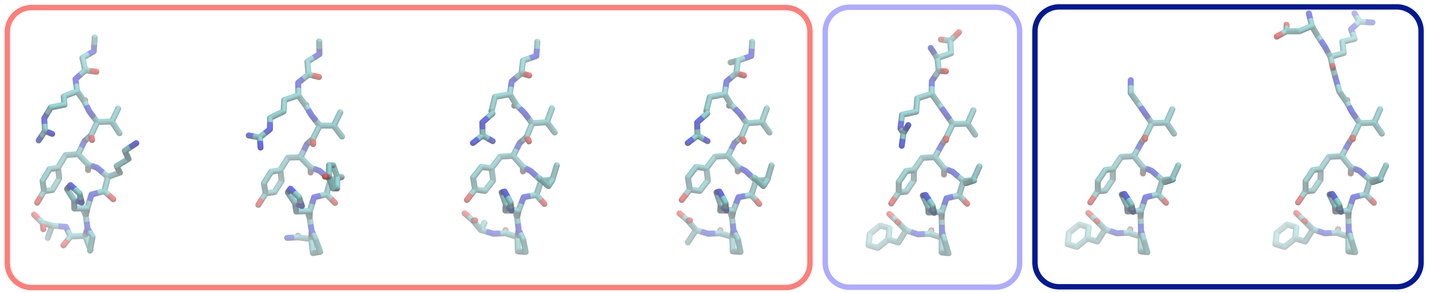
Alternative conformation disfavors G-protein binding but can couple to arrestin



Suomivuori & Latorraca et al, *Science*, 2020

Arrestin-biased ligands favor alternative conformation, G protein–biased ligands disfavor it





Suomivuori & Latorraca et al, *Science*, 2020

Do these results transfer to opioid receptors?

Deniz Aydin

Article | Published: 21 November 2022

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 ✉

Nature Chemical Biology (2022) | Cite this article

Yianni Laloudakis

Article | Open Access | Published: 11 March 2023

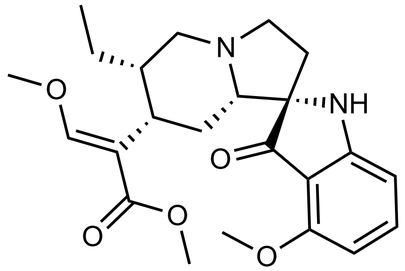
Molecular mechanism of biased signaling at the kappa opioid receptor

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

Nature Communications **14**, Article number: 1338 (2023) | Cite this article

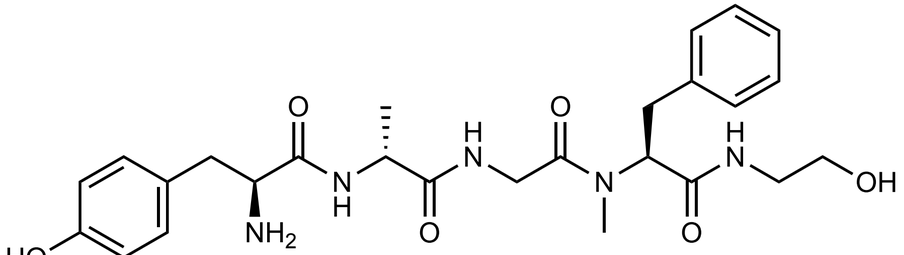
Agonists studied at μOR

Mitragynine pseudoindoxyl (MP)



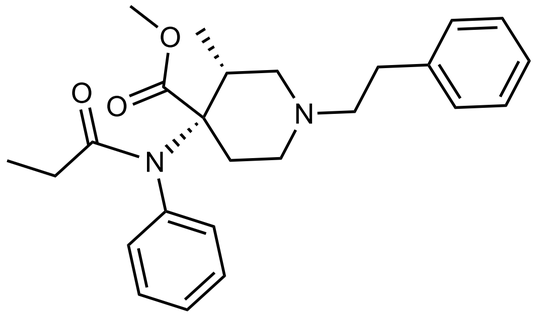
G protein biased

DAMGO



Balanced

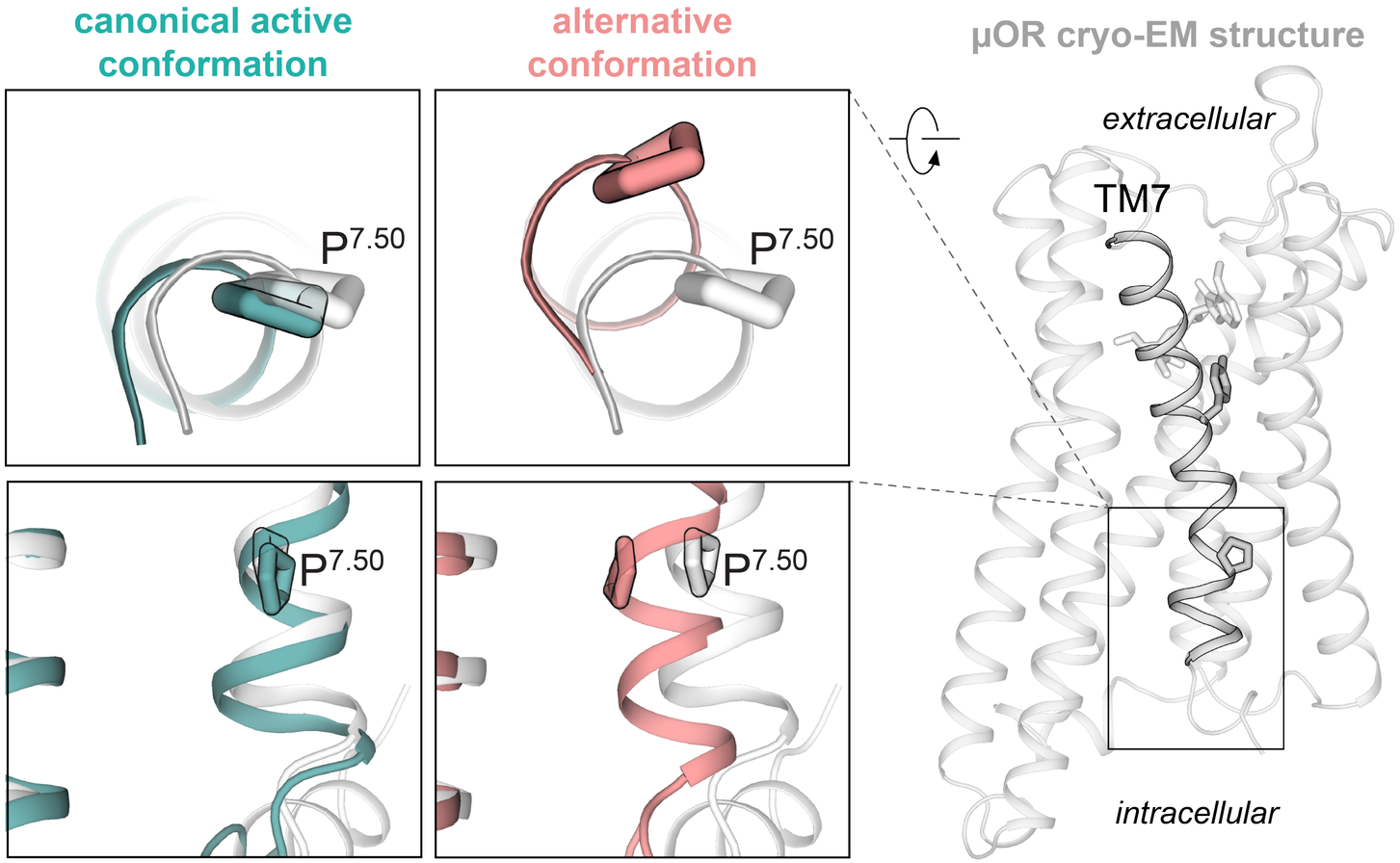
Lofentanil



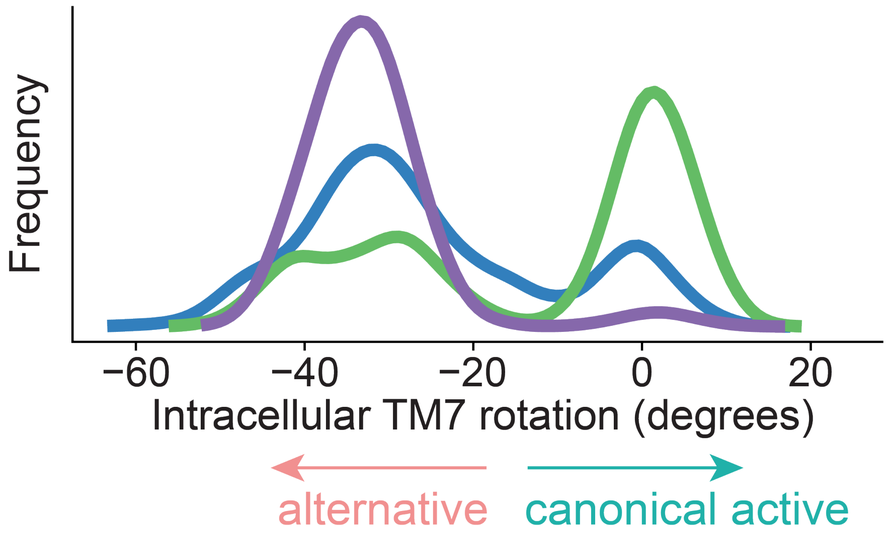
Arrestin biased

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 μOR
Occupancy of states explains bias profile of agonists



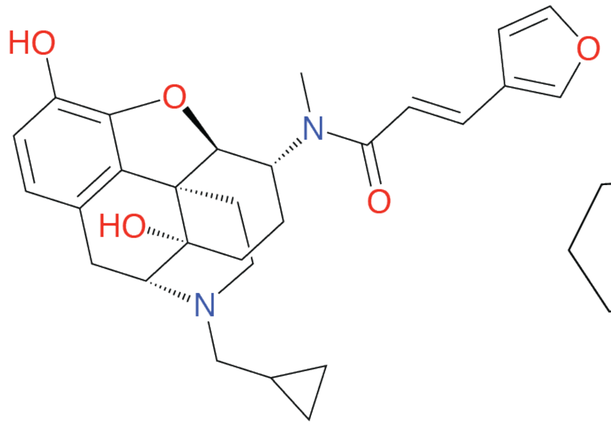
MP: G protein biased
DAMGO: balanced
Lofentanil: Arrestin biased



Statistical testing: Ran multiple independent simulations (6 for μOR, 10 for kOR), compute average value for each simulation, check for significance using t-test or Wilcoxon rank test.

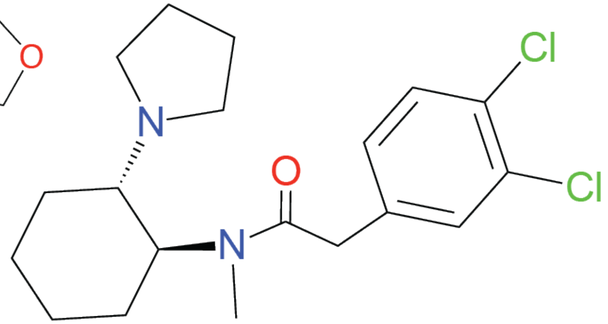
Agonists studied at kOR

Nalfurafine



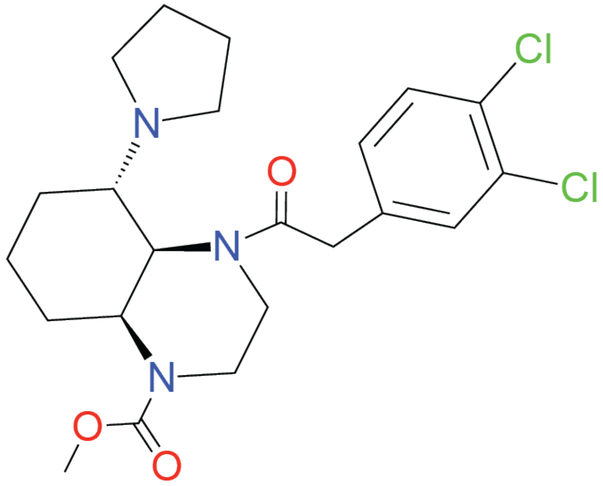
G protein biased

U50,488



Balanced

WMS-X600

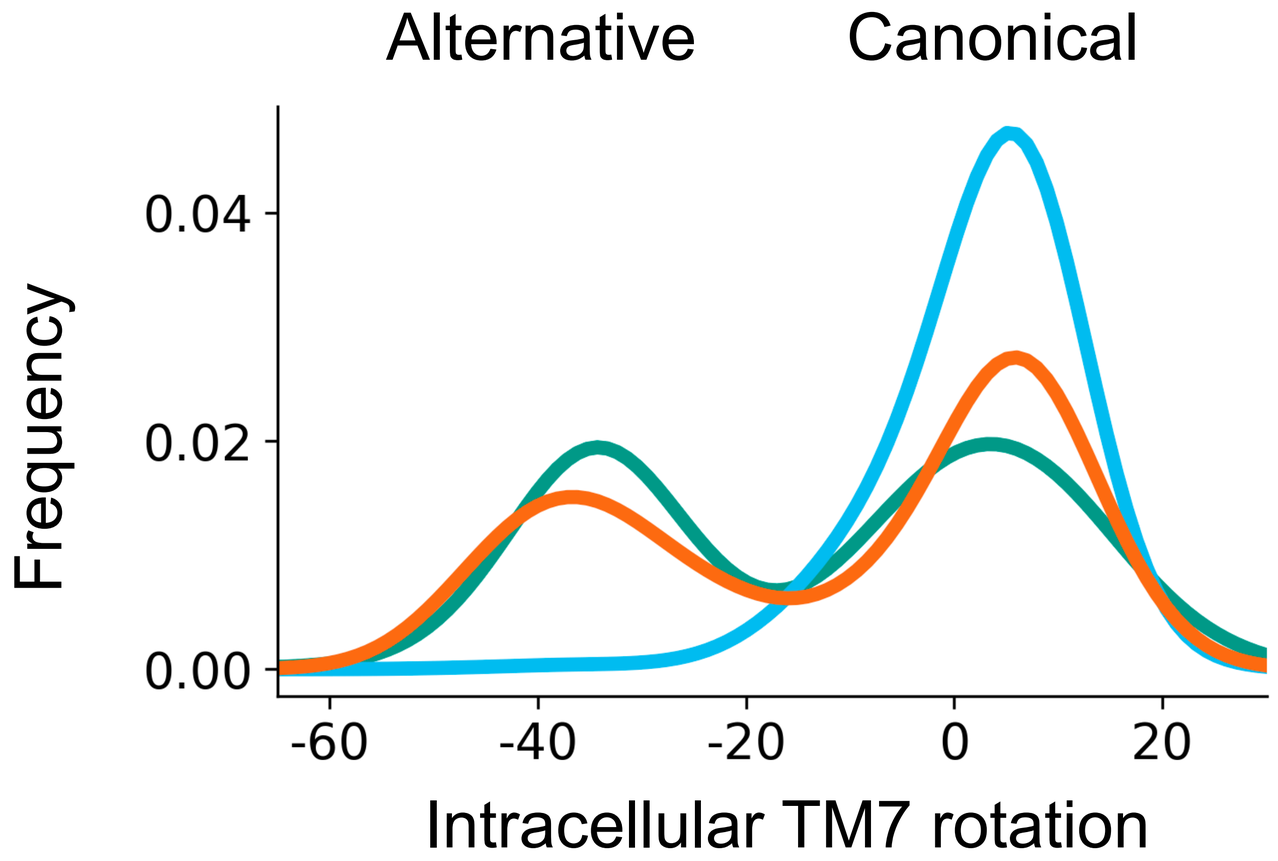


Arrestin biased

- 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

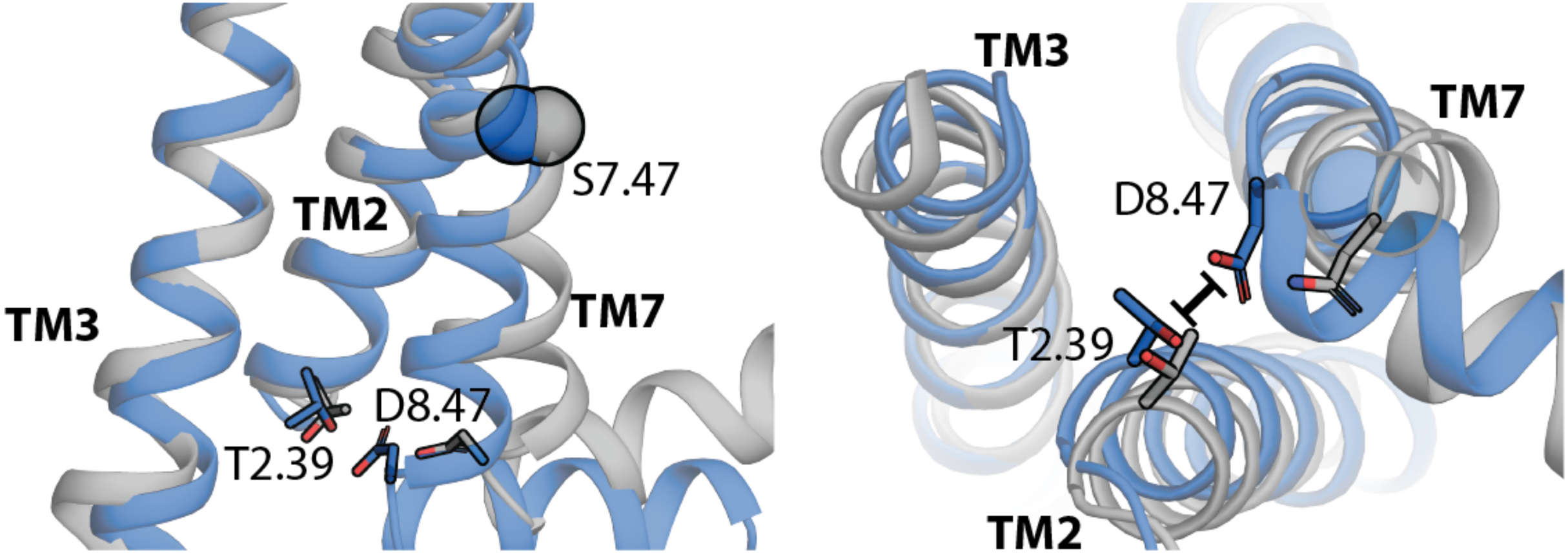
Brust et al, *Science Signaling,* 2016. Nakoa et al, *J Pharmacy Sci*, 2016

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

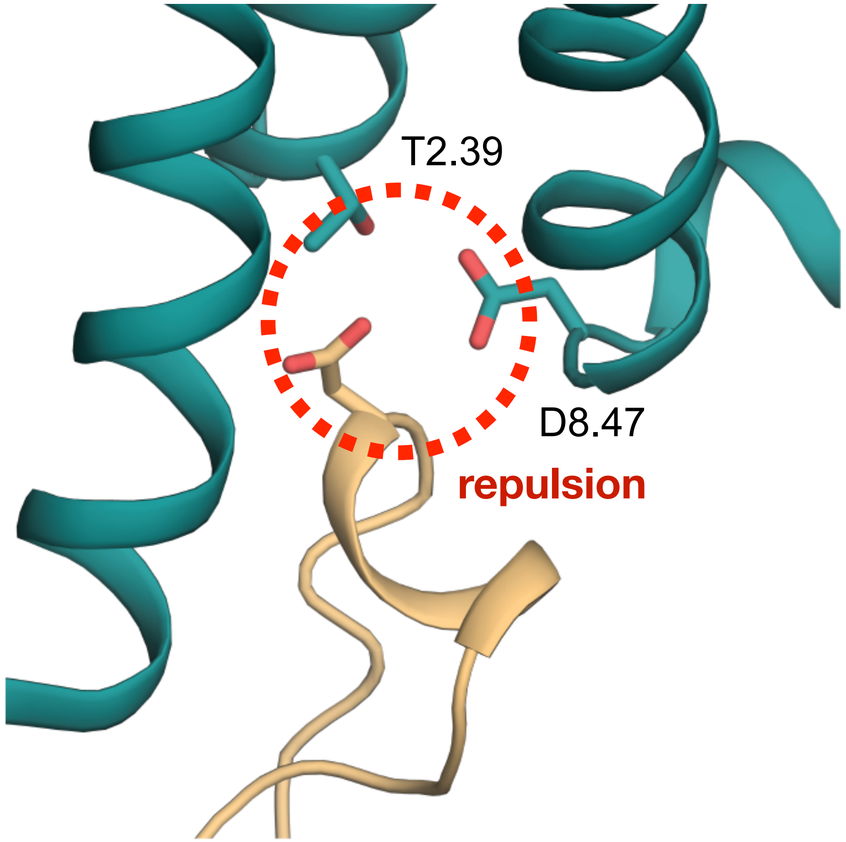


Nalfurafine: G protein biased
U50,488: Balanced
WMS-X600: Arrestin biased

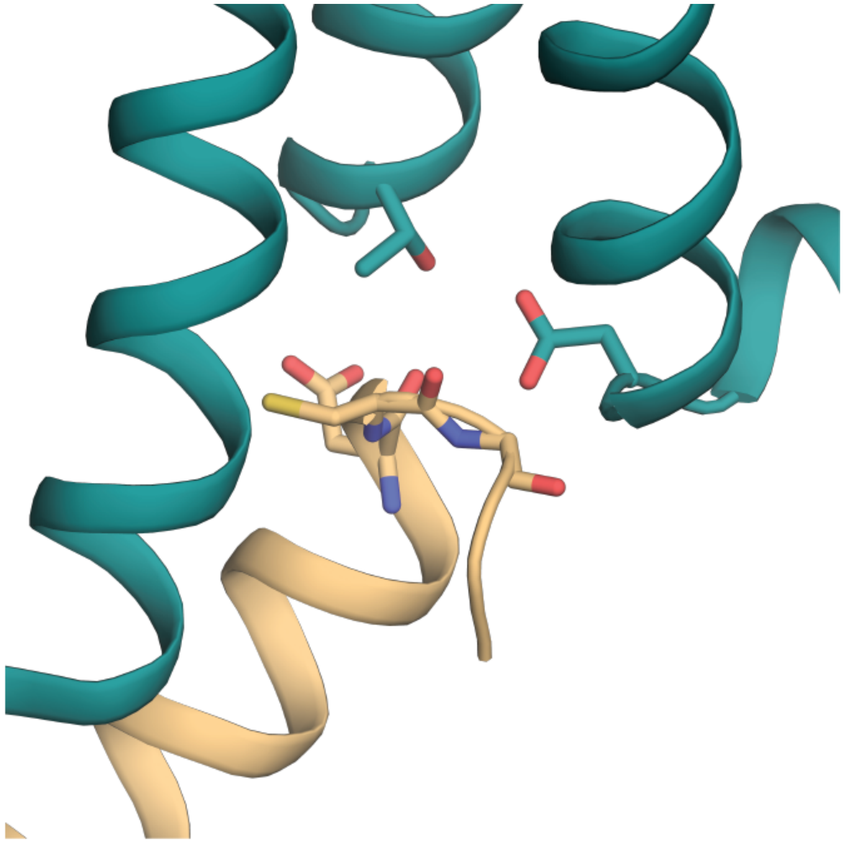
At kOR, we observe a third receptor conformation: the “occluded state”



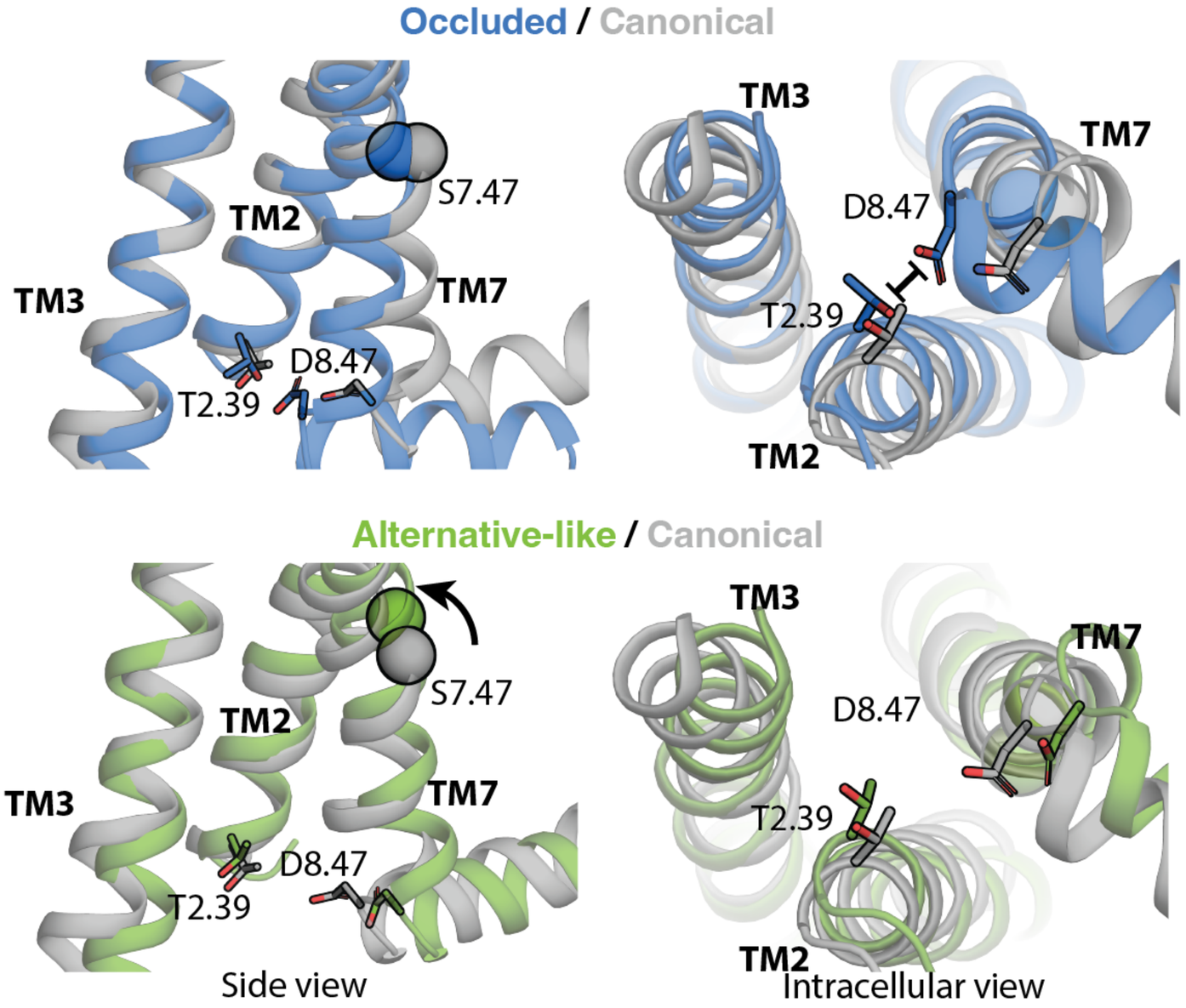
The occluded state presents an electrostatic barrier to arrestin coupling

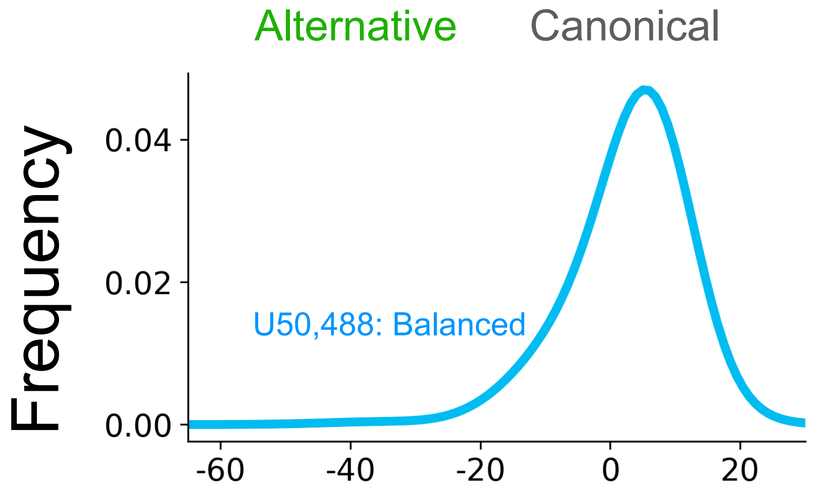


kOR occluded state
NTSR1–arrestin-2

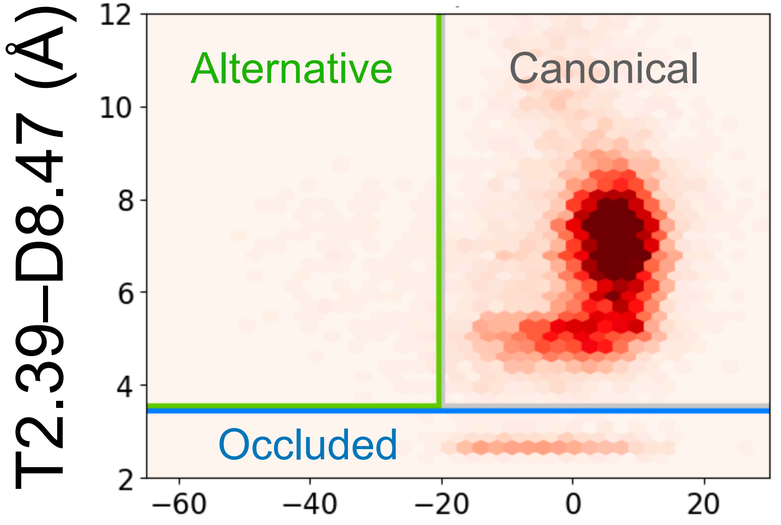


kOR occluded state
*μ*OR–Gi

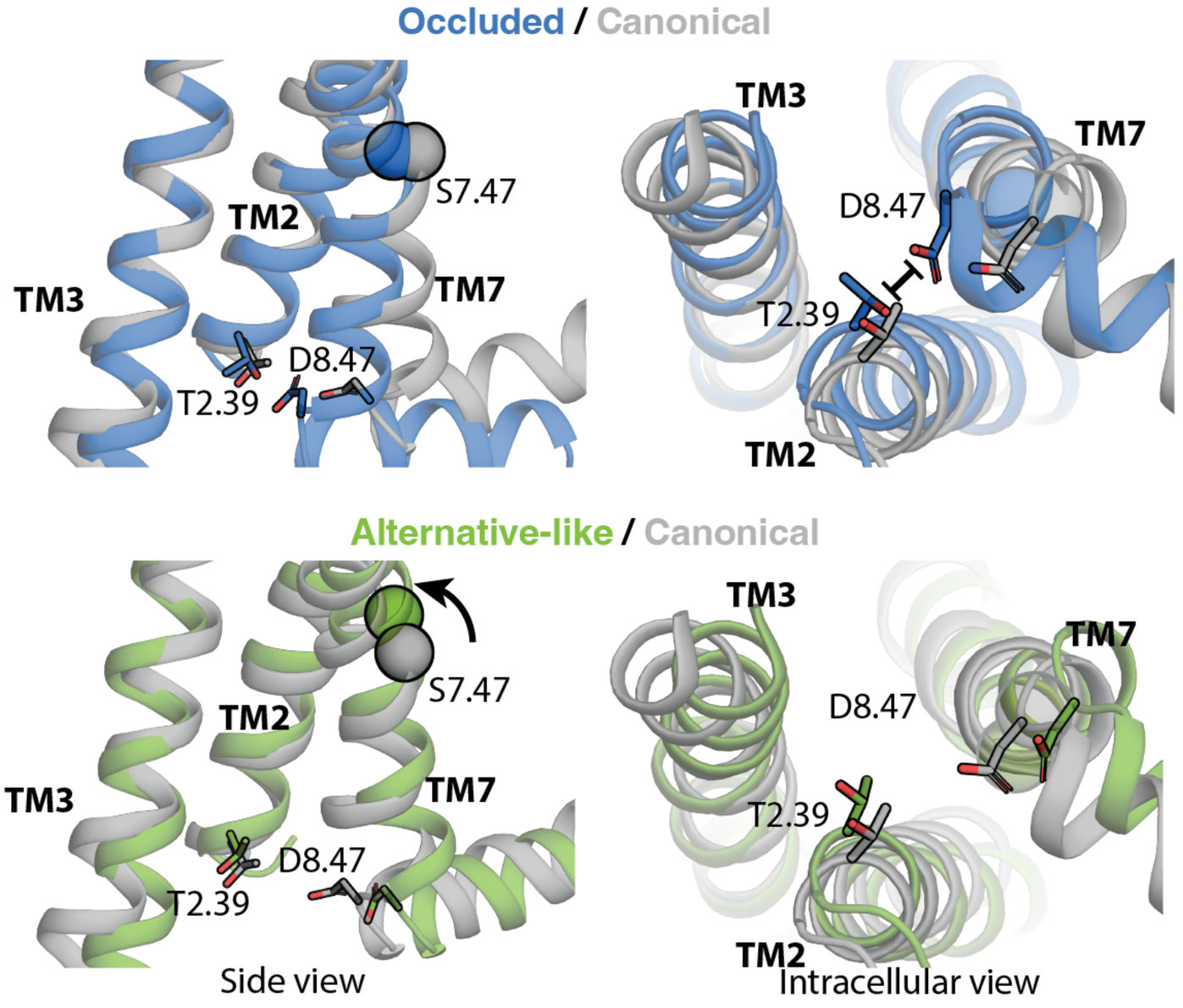


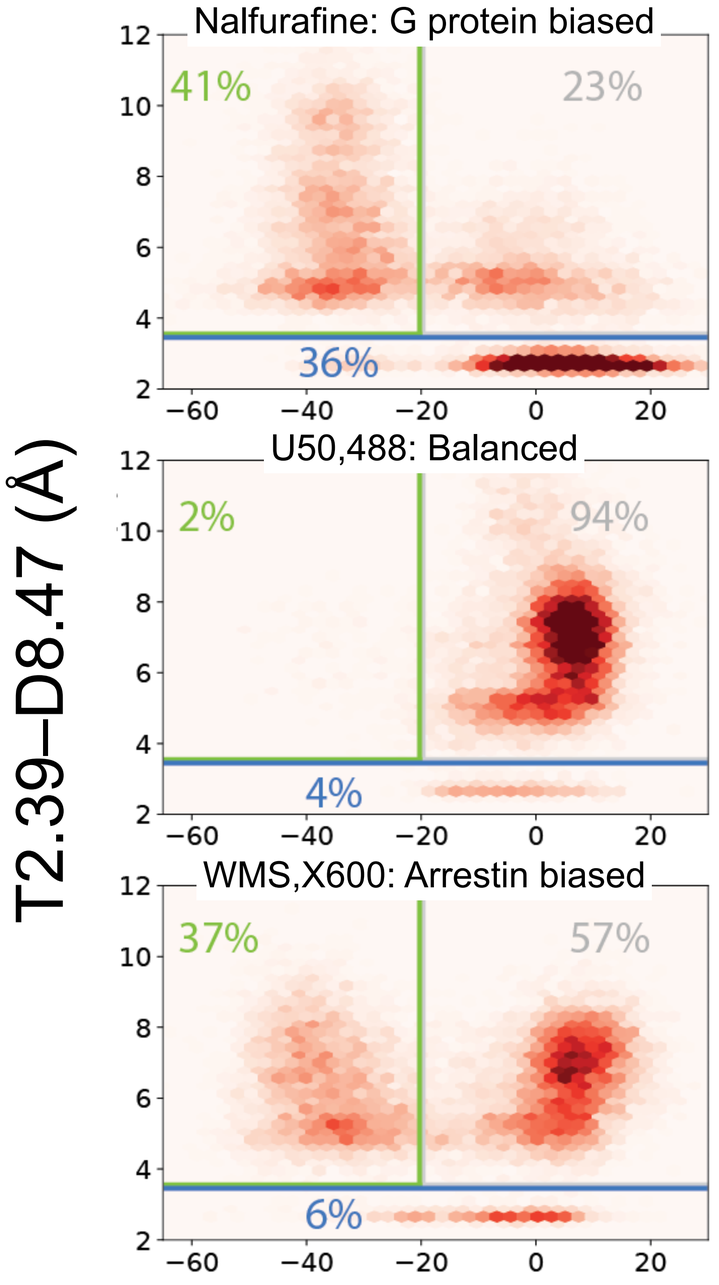


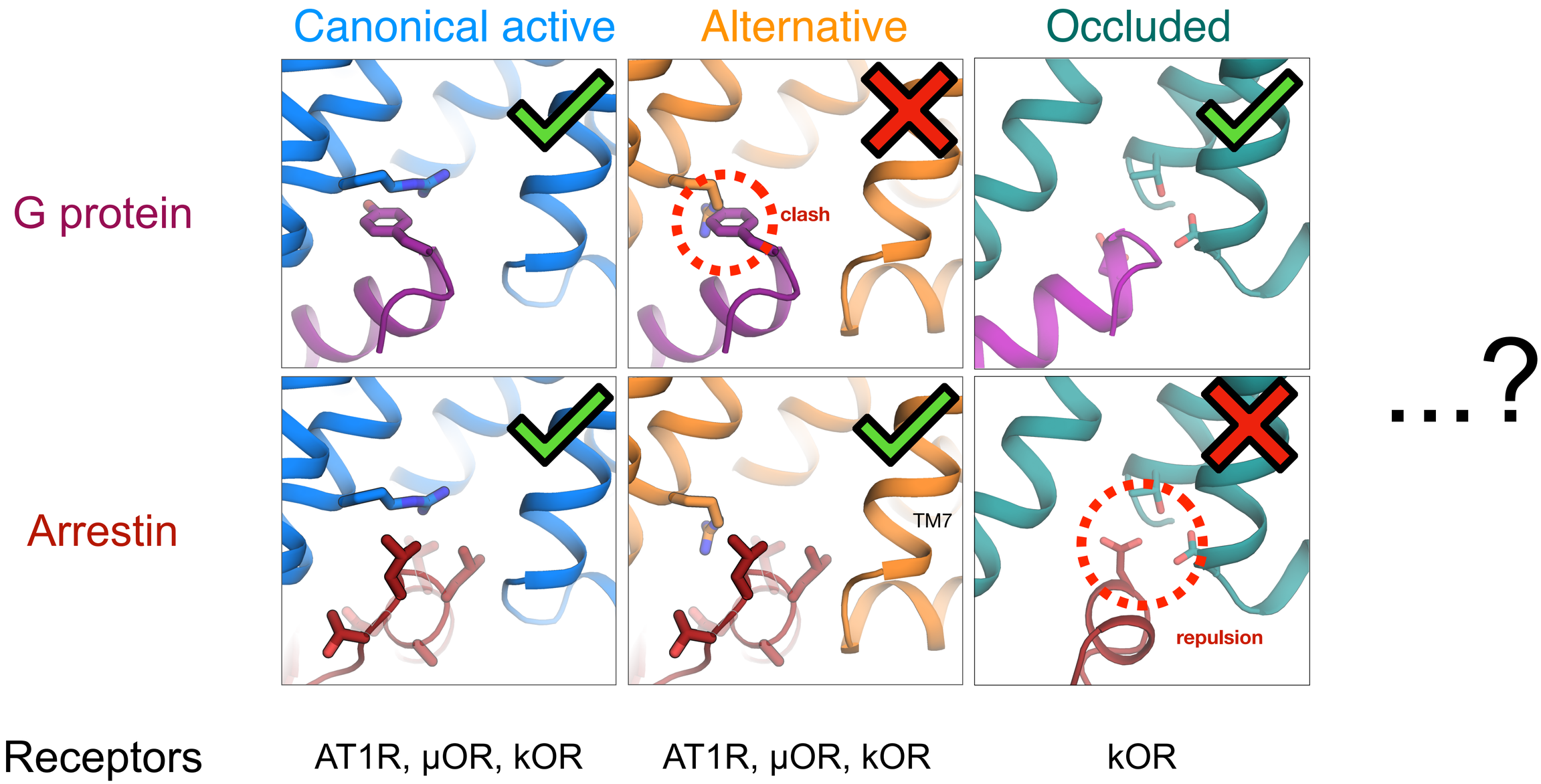
Intracellular TM7 rotation



Intracellular TM7 rotation


Occluded / Canonical
TM3
TM2
S7.47
TM7
D8.47
T2.39
TM3
TM7
D8.47
T2.39
TM2
Alternative-like / Canonical
TM3
TM2
S7.47
TM7
D8.47
T2.39
TM3
TM7
D8.47
T2.39
TM2
Side view
Intracellular view


Nalfurafine: G protein biased
41%
23%
36%
U50,488: Balanced
2%
94%
4%
WMS,X600: Arrestin biased
37%
57%
6%
T2.39–D8.47 (Å)
Intracellular TM7 rotation

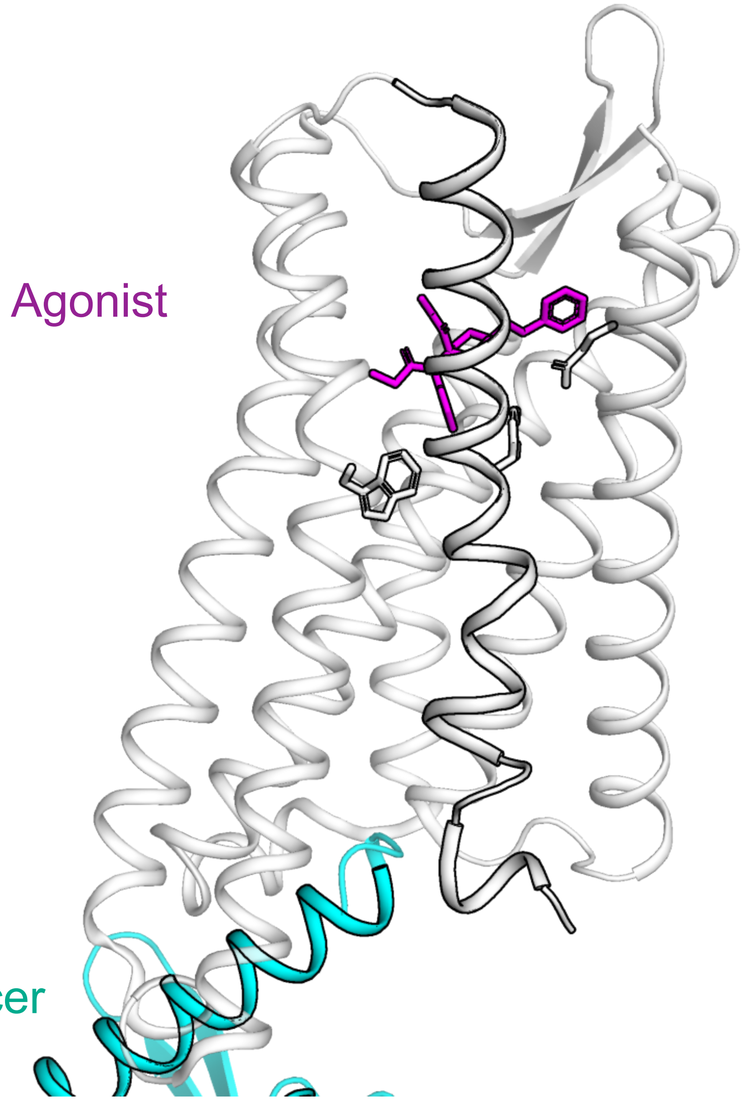

Canonical active
Alternative
Occluded
G protein
Arrestin
clash
TM7
repulsion
...?
Receptors
AT1R, μOR, kOR
AT1R, μOR, kOR
kOR

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…

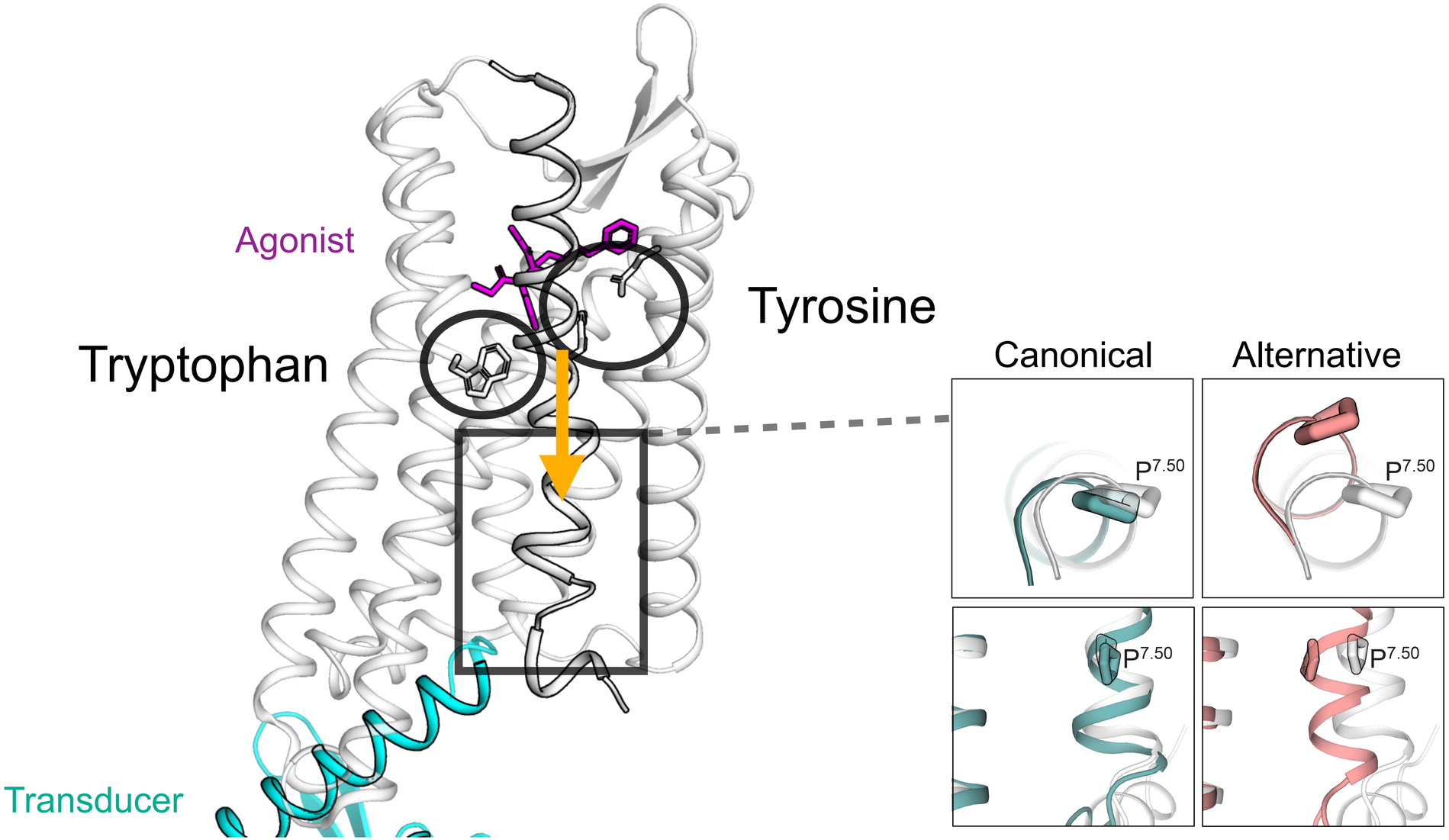


- I'm only going to share a subset of the results here
  - See our papers for more details!
  - We validate much of our proposed mechanism with mutagenesis experiments

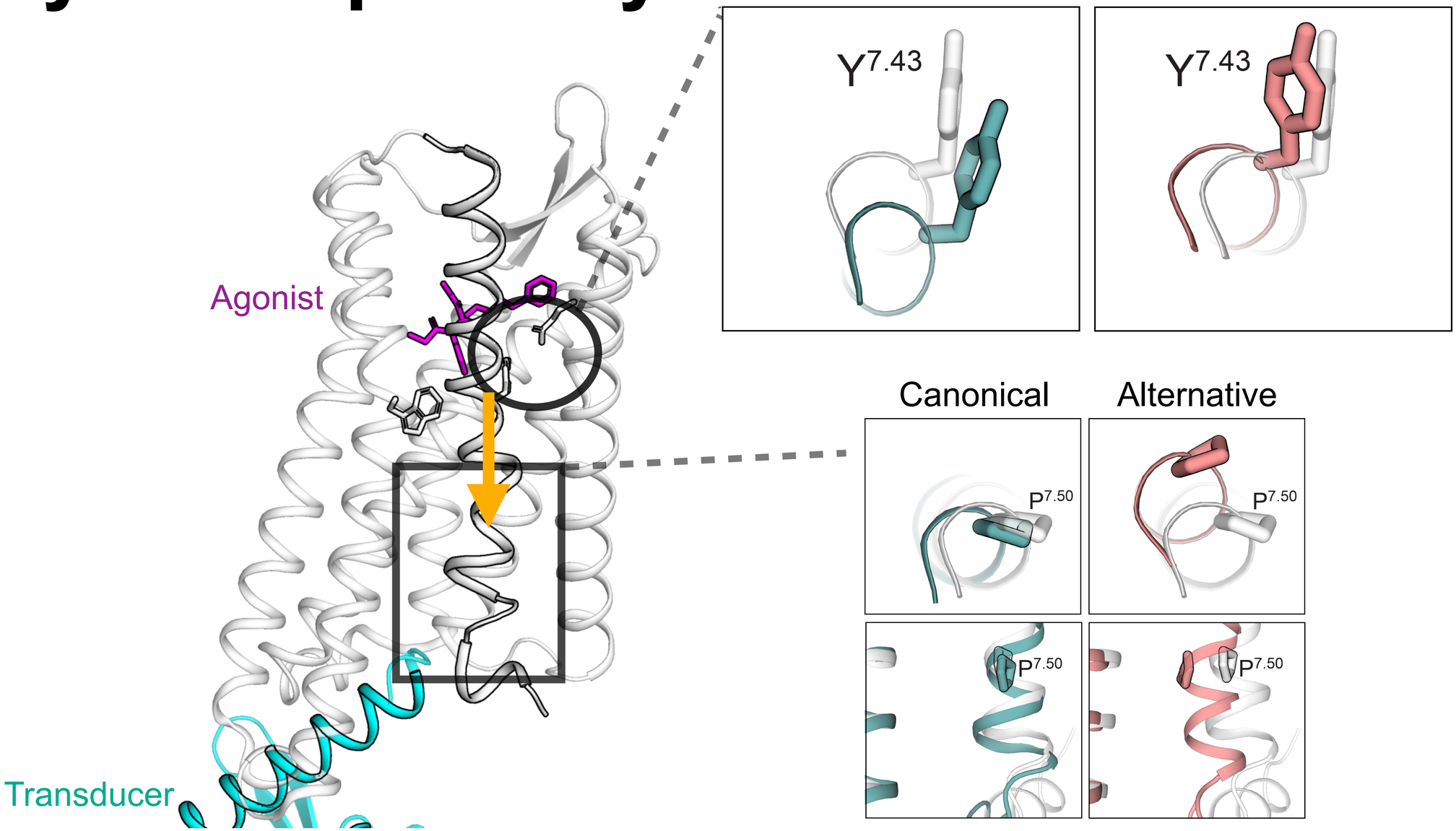
1. Multiple layers of abstraction, things get confusing if you stay too low
   - Transducer site conformations
   - Allosteric pathways
   - Direct protein–ligand interactions

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

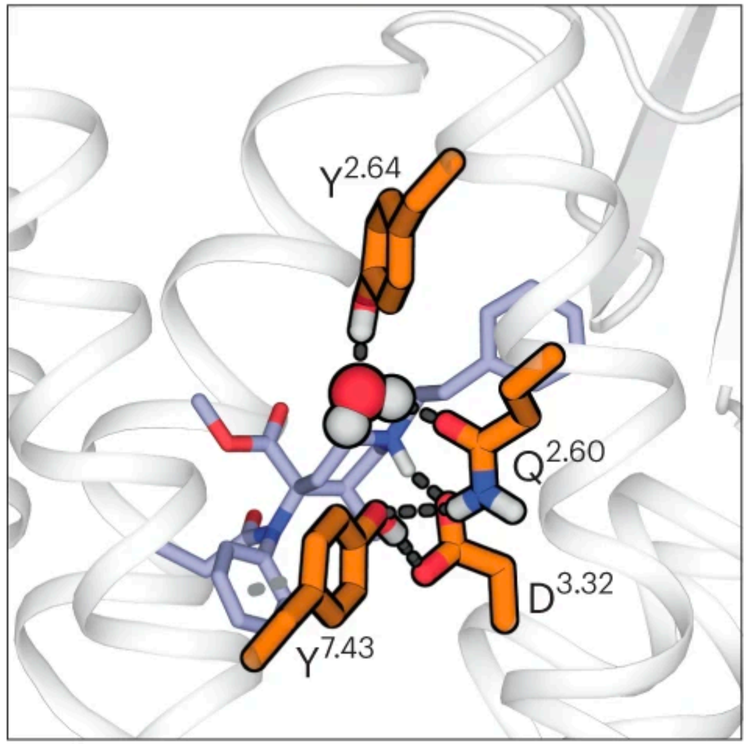


Tyrosine pathway

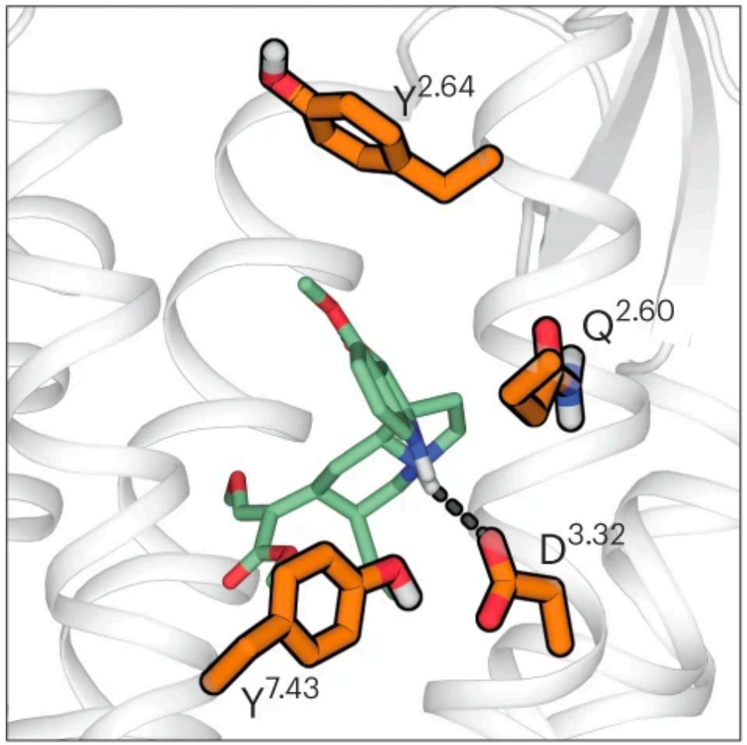


Lofentanil stabilizes a polar network holding Y7.43 inwards
MP disrupts this polar network

Lofentanil: Arrestin biased



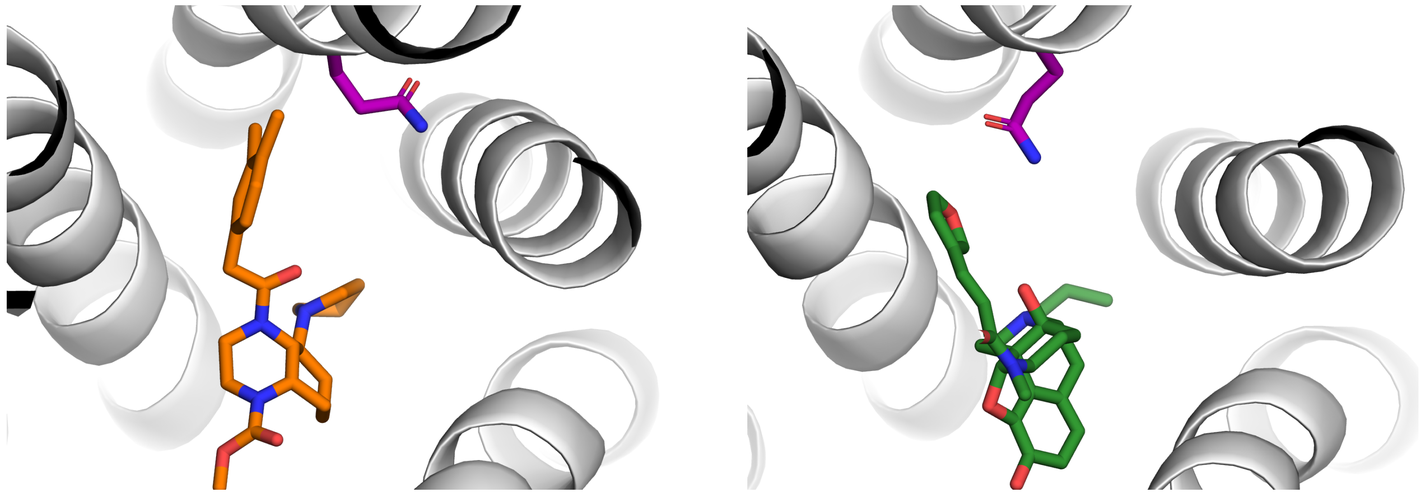
MP: G protein biased



Representative MD frames

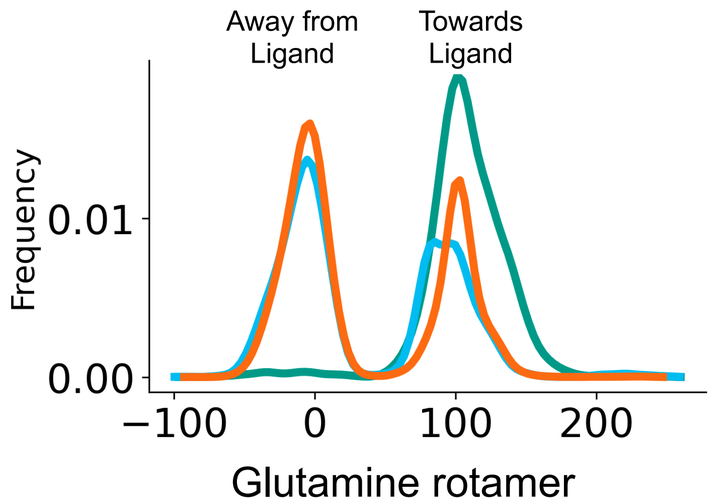
Nalfurafine has a similar effect as MP, but through a different mechanism

MD frames

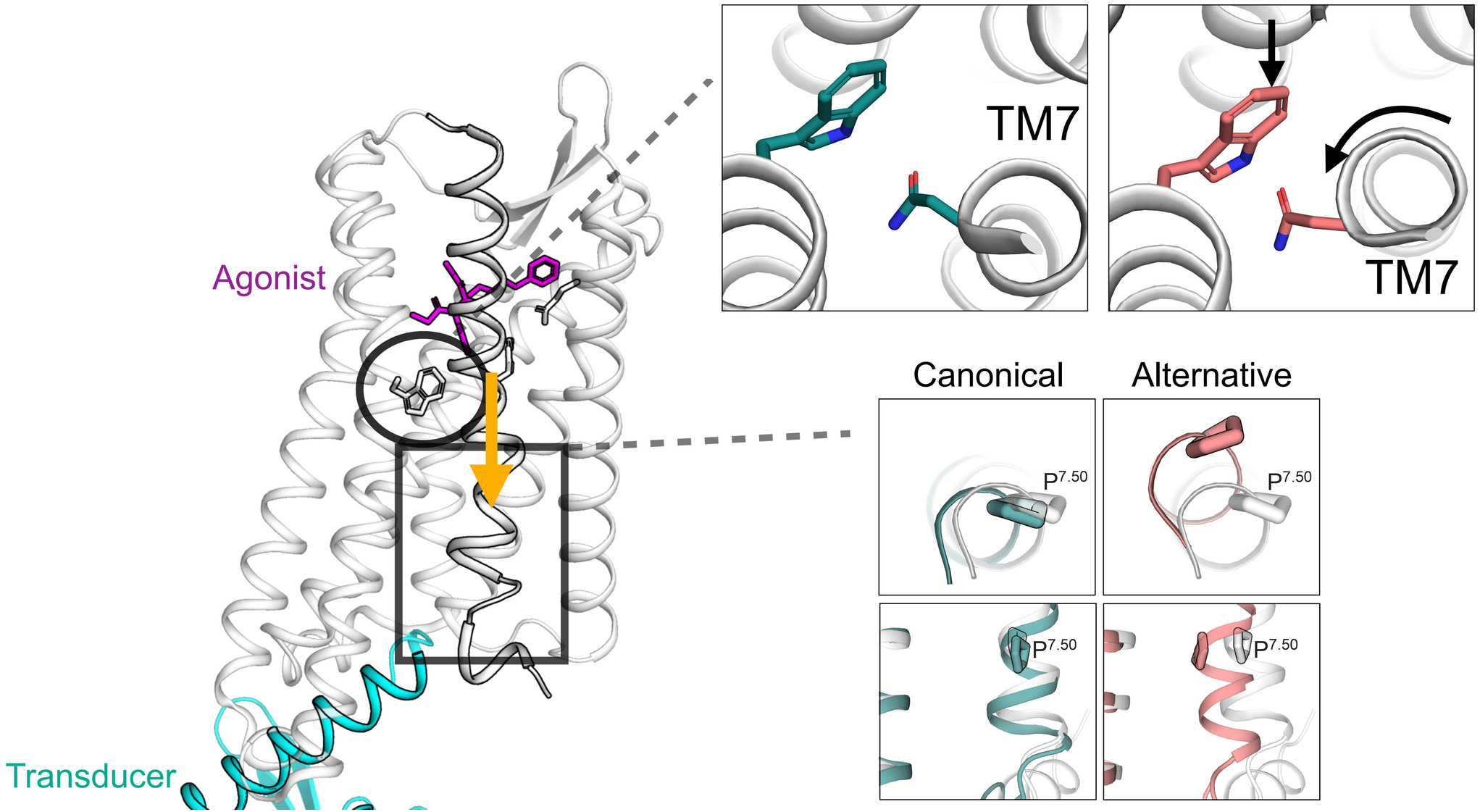


Extracellular view

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



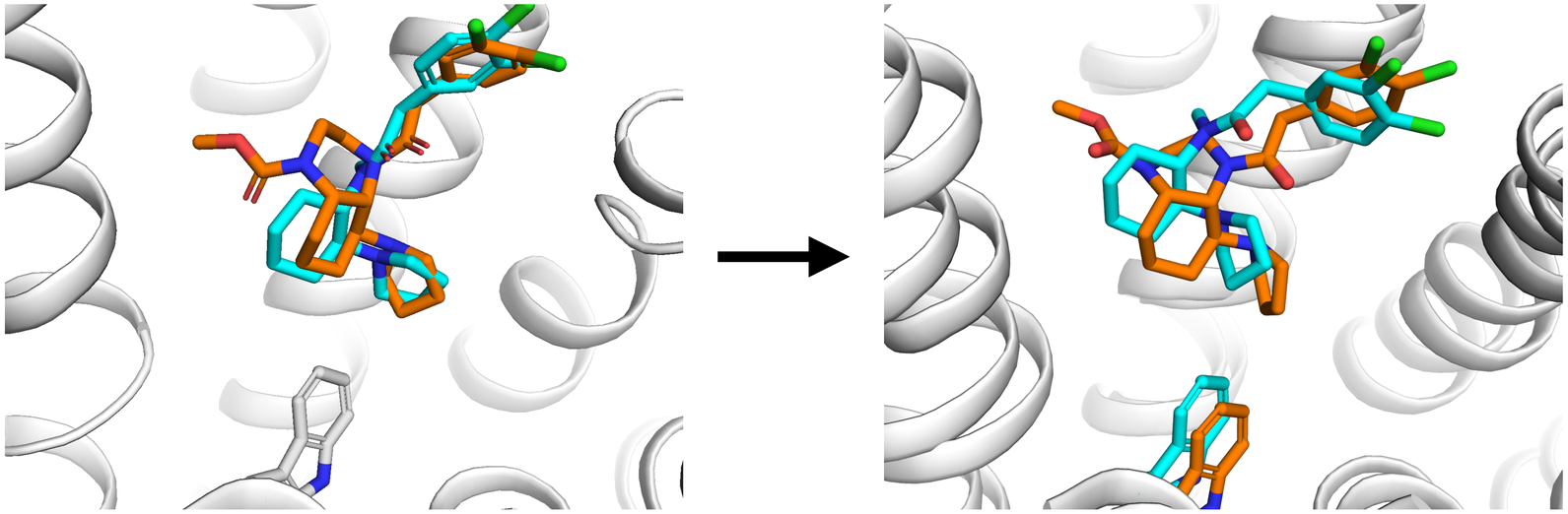
Tryptophan pathway



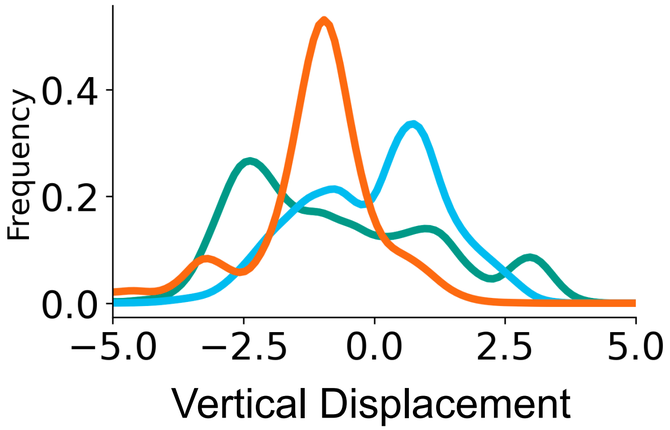
Vertical displacement of tryptophan explains arrestin-bias of WMS-X600

Initial docked poses

MD frame



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



*Nalfurafine not significantly different than others

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

Acknowledgements

- Ron Dror
- Deniz Aydin
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- **Weijiao Huang**
- **Alpay B. Seven**
- **Susruta Majumdar**
- **Brian K. Kobilka**
- **Georgios Skiniotis**
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- Soumen Chakraborty
- Michael J. Robertson
- Asuka Inoue
- Bryan L. Roth
- **Amal El Daibani**
- **Kuglae Kim**
- **Tao Che**
- Petr Popov
- Sarah M. Bernhard
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- Reid H. J. Olsen
- Jeffrey F. DiBerto
- Ivy Carroll
- Vsevolod Katritch
- Bernhard Wünsch