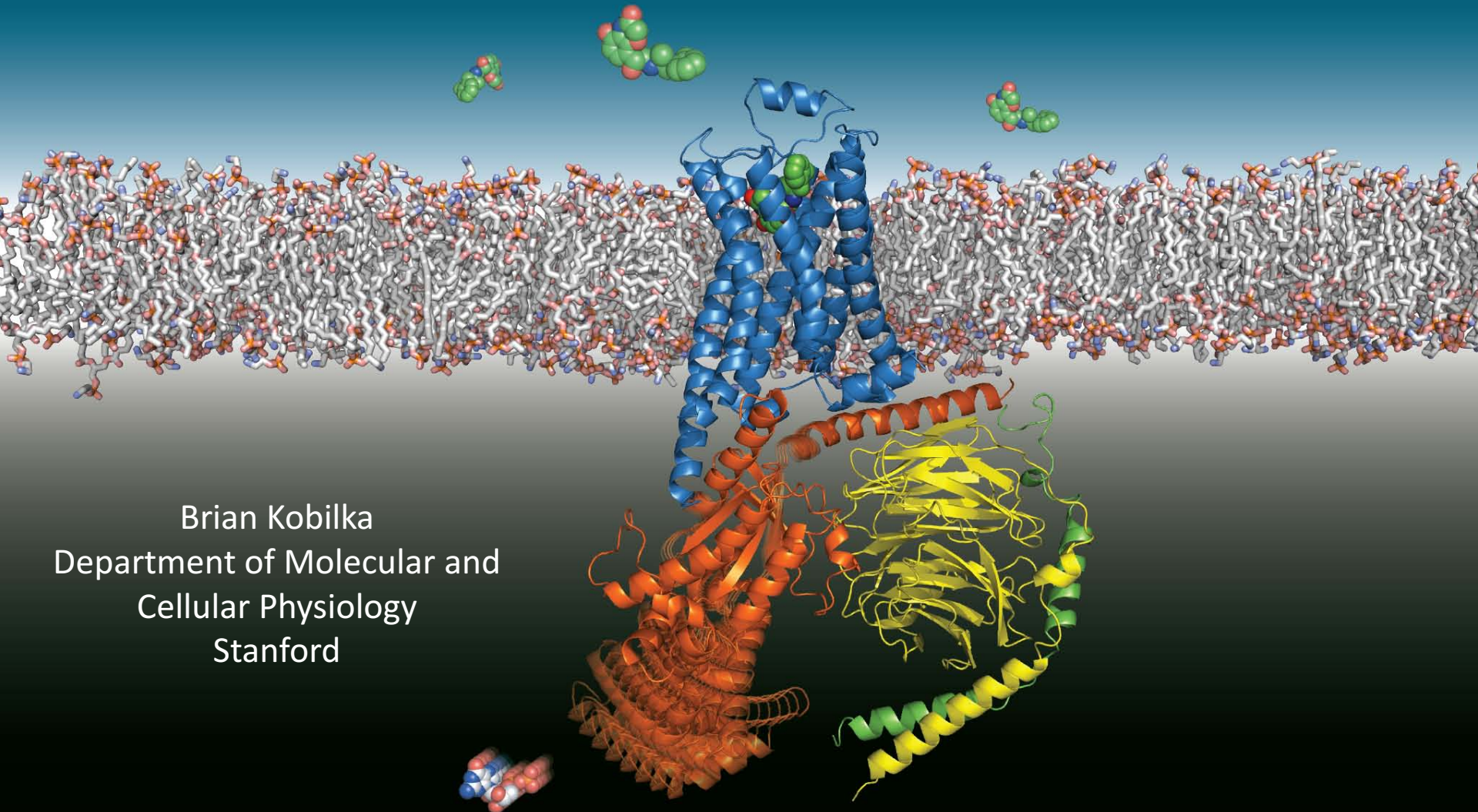
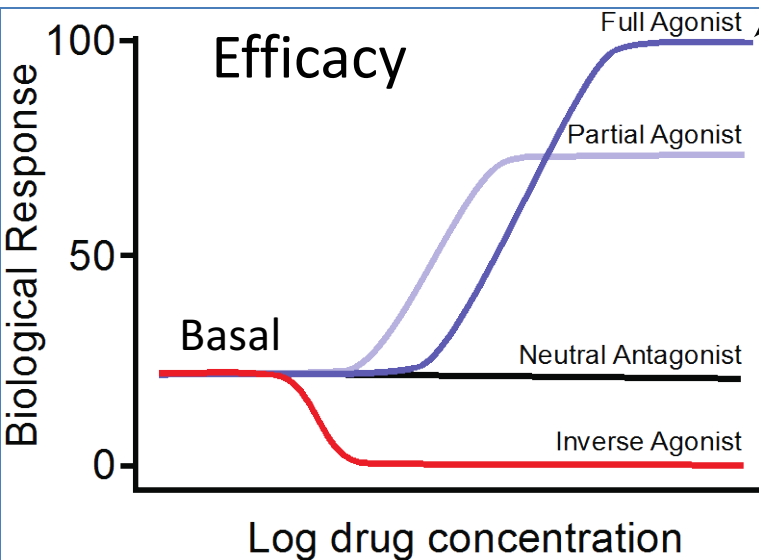
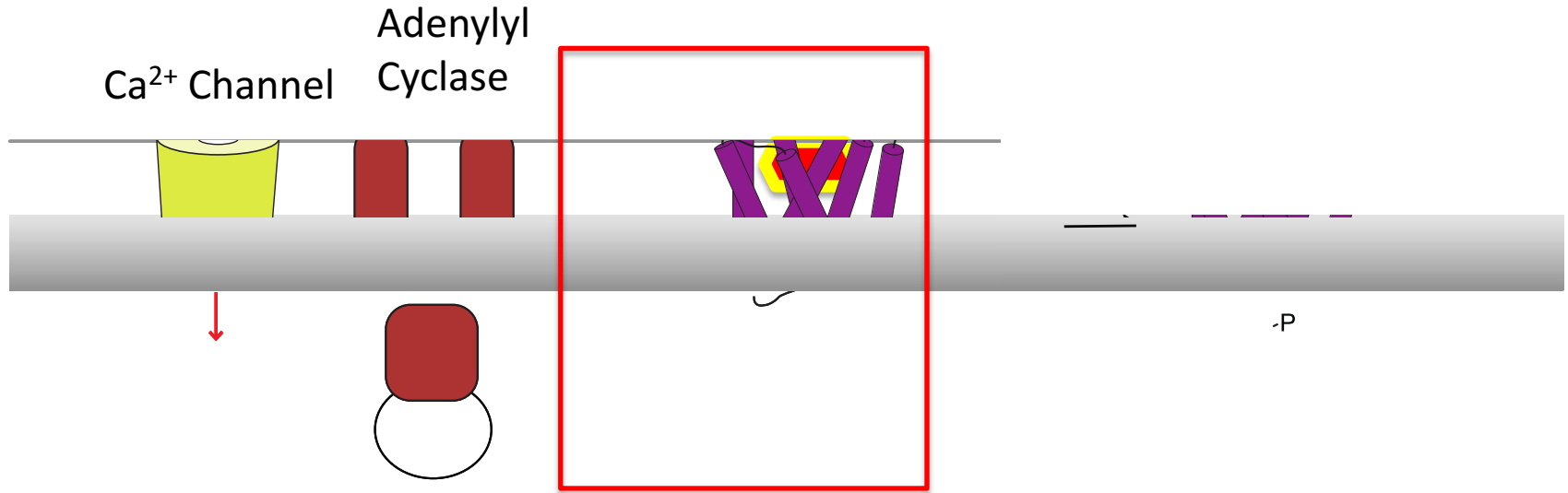


The structural basis of G protein coupled receptor signaling



Brian Kobilka
Department of Molecular and
Cellular Physiology
Stanford

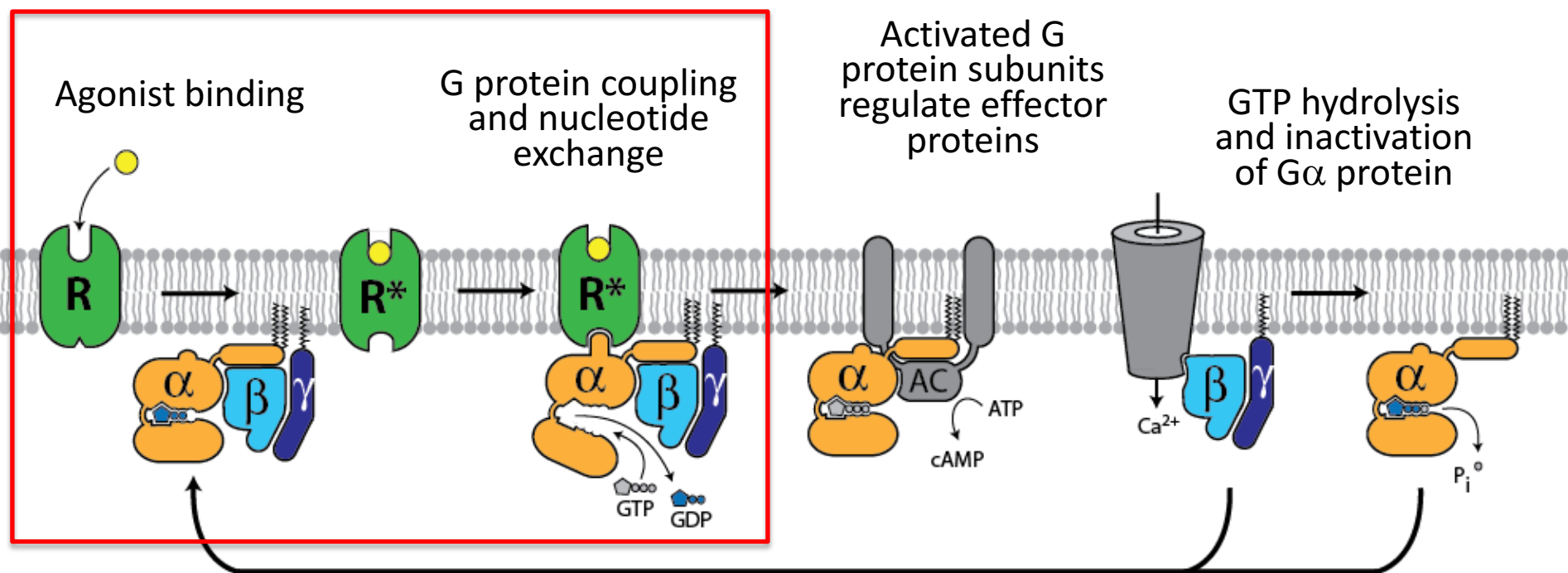
The β_2 AR modulates the activity of multiple signaling pathways



Recycling back to membrane

Targeted for degradation in lysosomes

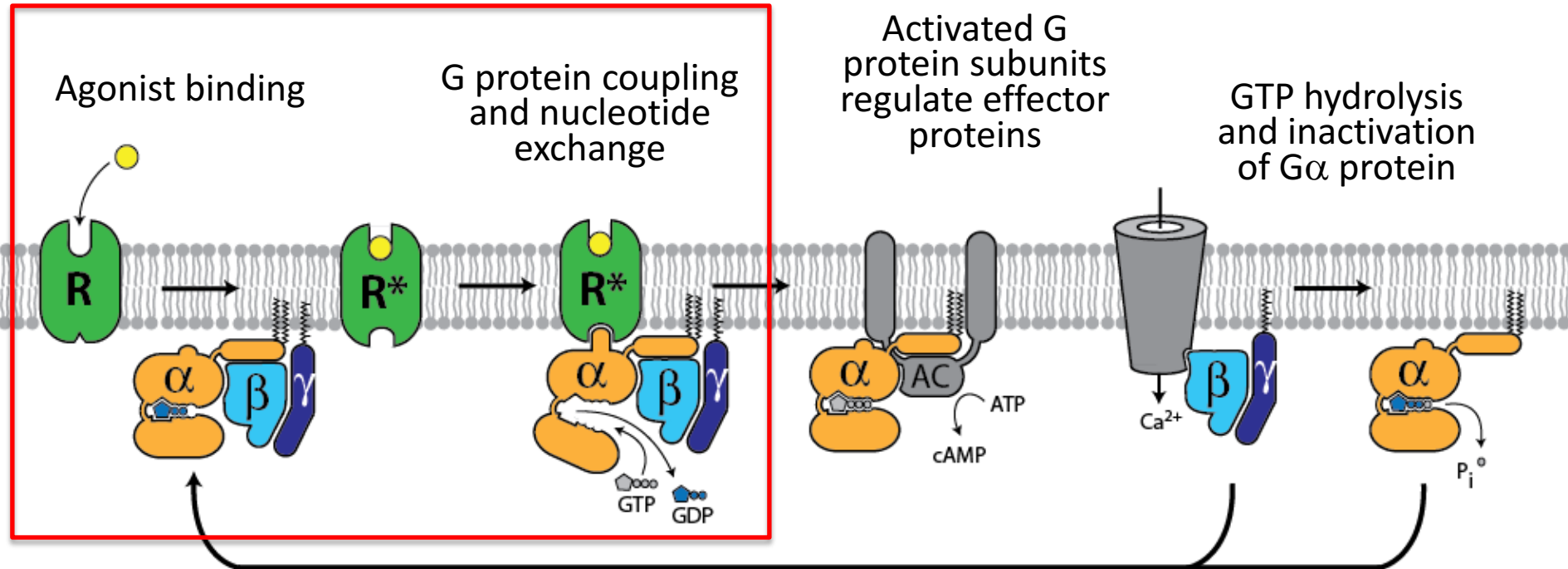
MAP kinase pathway
Gene expression



GPCR-G Protein Cycle

Outline

- Overview of approaches to characterize GPCR structure
- GPCR crystallography
- Mechanistic insights into GPCR-G protein activation



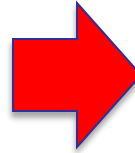
GPCR-G Protein Cycle

Approaches to characterizing β_2 AR structure:

- Sequence analysis
 - secondary structure (transmembrane domains)

Cloned DNA Sequence (1986)

```
GAATTCATGCCGCGTTTCTGTGTTGGACAGGGGTGACTTTGTGCC
GGATGGCTTCTGTGTGAGAGCGCGCGAGTGTGCATGTCGGTGA
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Amino Acid Sequence

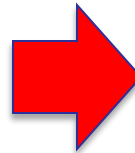
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SGYHVEQEKENKLLCEDLPGTEDFVGH
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```

Approaches to characterizing β_2 AR structure:

- Sequence analysis
 - secondary structure (transmembrane domains)

Cloned DNA Sequence (1986)

GAATTCATGCCGCGTTTCTGTGTTGGACAGGGGTGACTTTGTGCC
GGATGGCTTCTGTGTGAGAGCGCGCGAGTGTGCATGTCGGTGA
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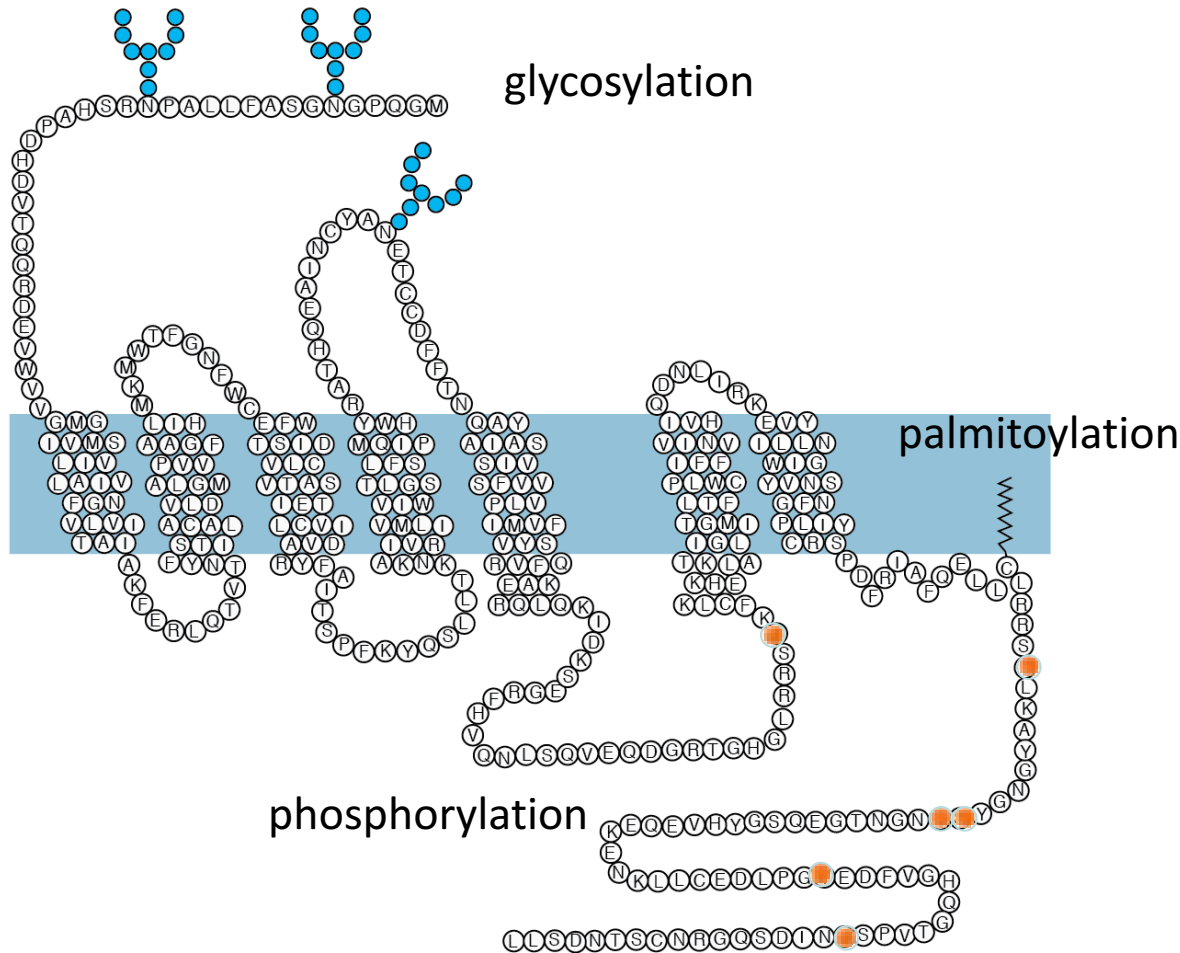


Amino Acid Sequence

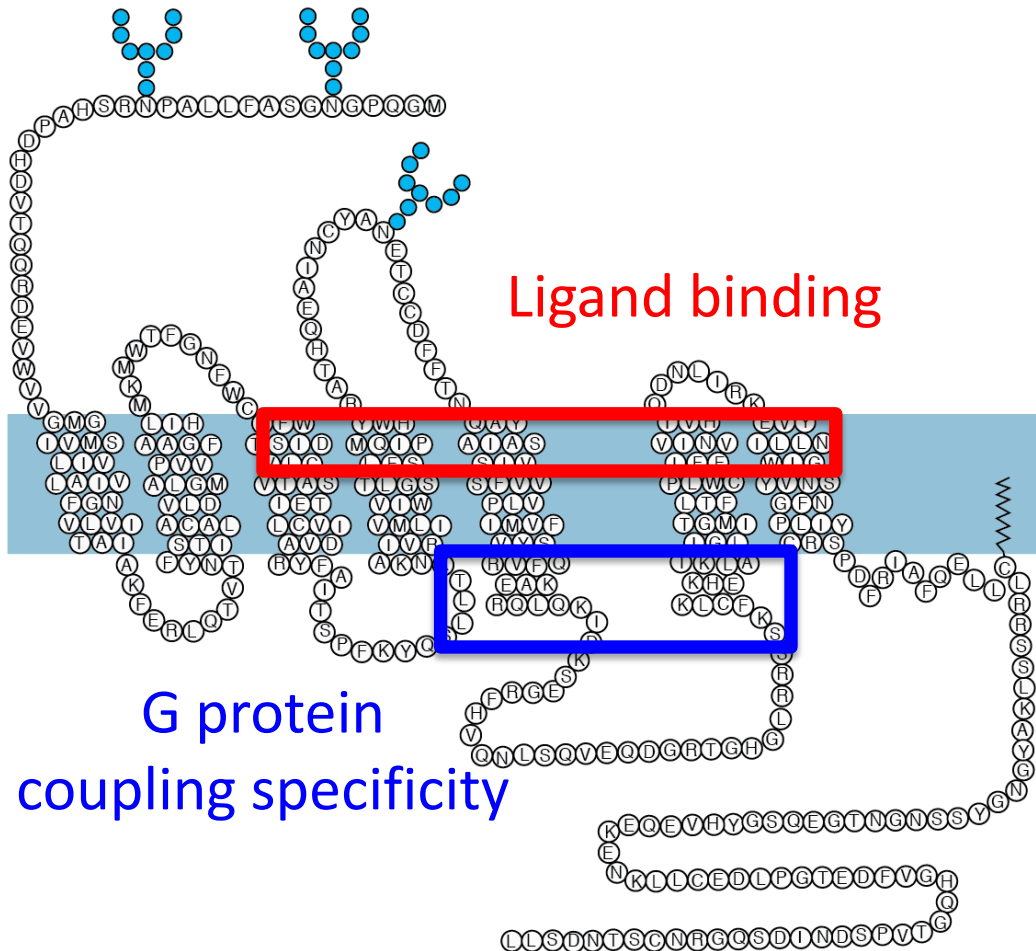
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VYILLNWIGYVNSGFNPLIYCRSPDFRIA
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Approaches to characterizing β_2 AR structure:

- Sequence analysis
 - secondary structure (transmembrane domains)
 - post-translational modifications



Approaches to characterizing β_2 AR structure:



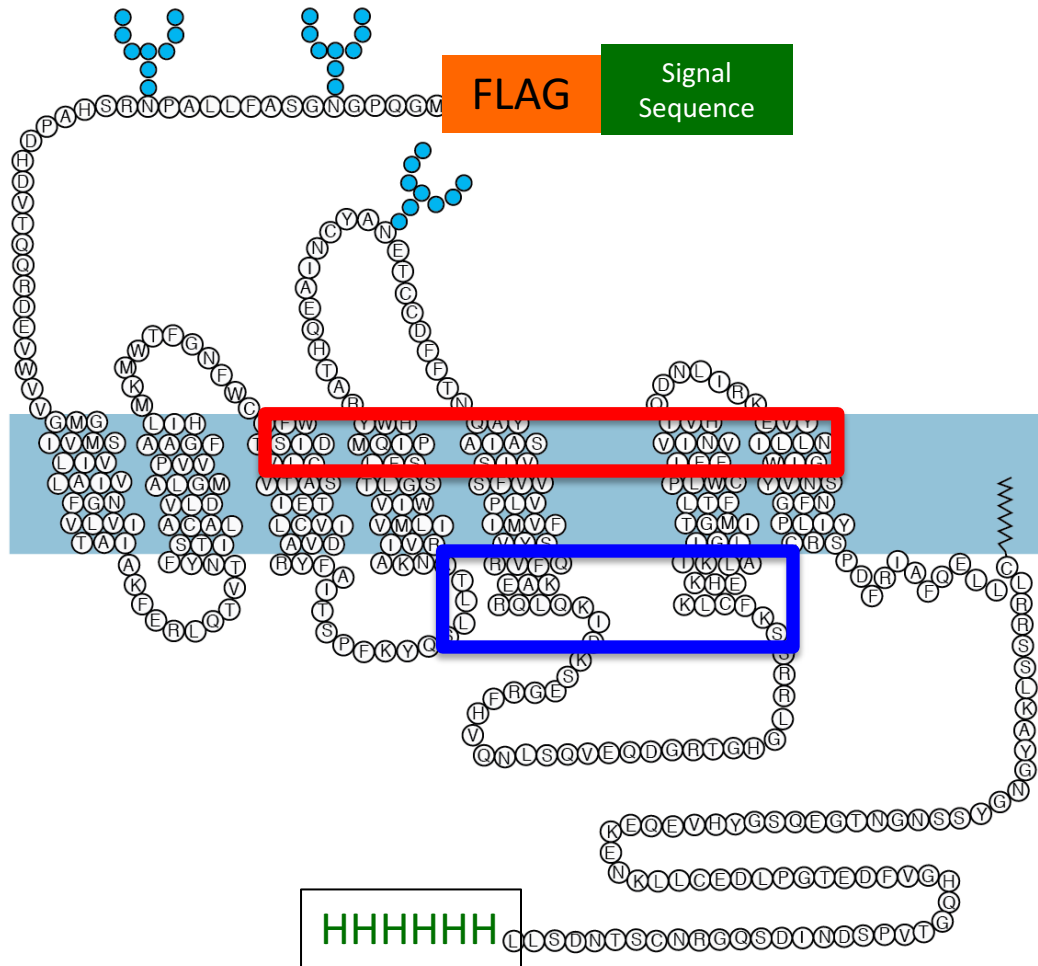
• Sequence analysis

- secondary structure (transmembrane domains)
- post-translational modifications

• Chimeric Receptors and site-directed mutagenesis

- ligand binding and G protein coupling

Approaches to characterizing β_2 AR structure:



• Sequence analysis

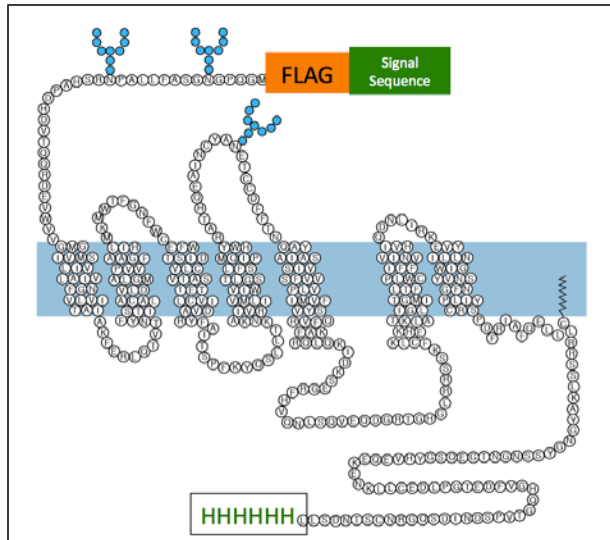
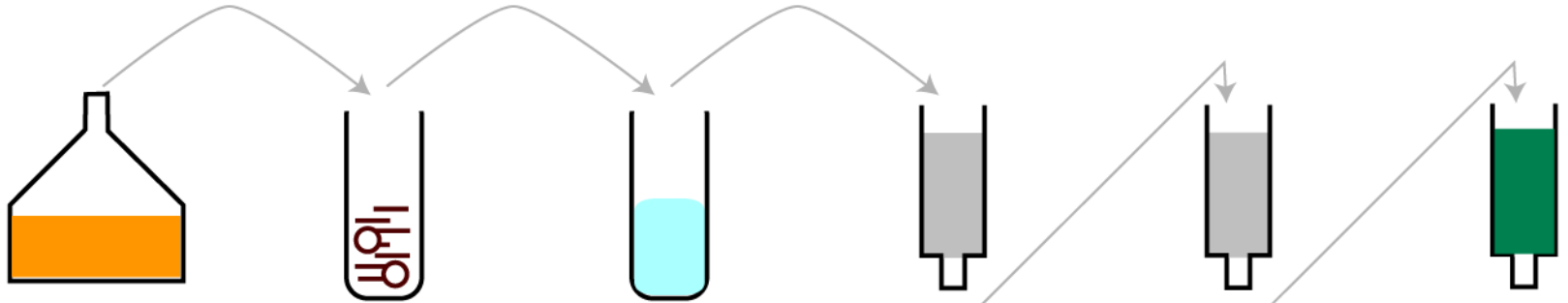
- secondary structure (transmembrane domains)
- post-translational modifications

• Chimeric Receptors and Site-directed mutagenesis

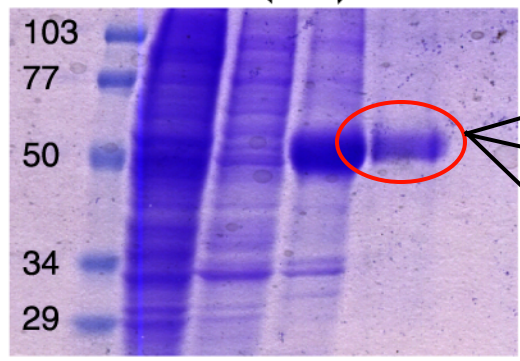
- ligand binding and G protein coupling
- enhance expression and purification

Expression and purification

Sf9 insect cells membranes solubilization anti FLAG column alprenolol column Ni column

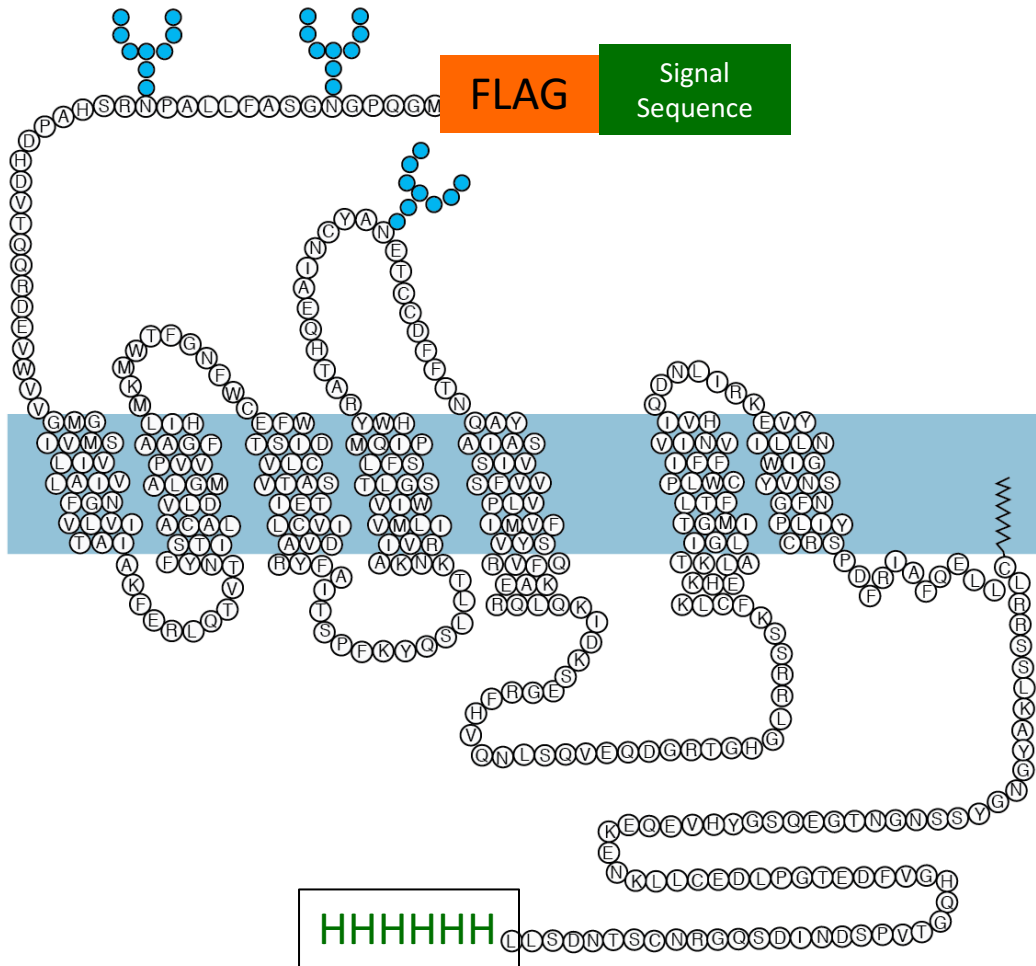


1x 1x 500x 500x



Biochemistry
Spectroscopy
Crystallography

Approaches to characterizing β_2 AR structure:



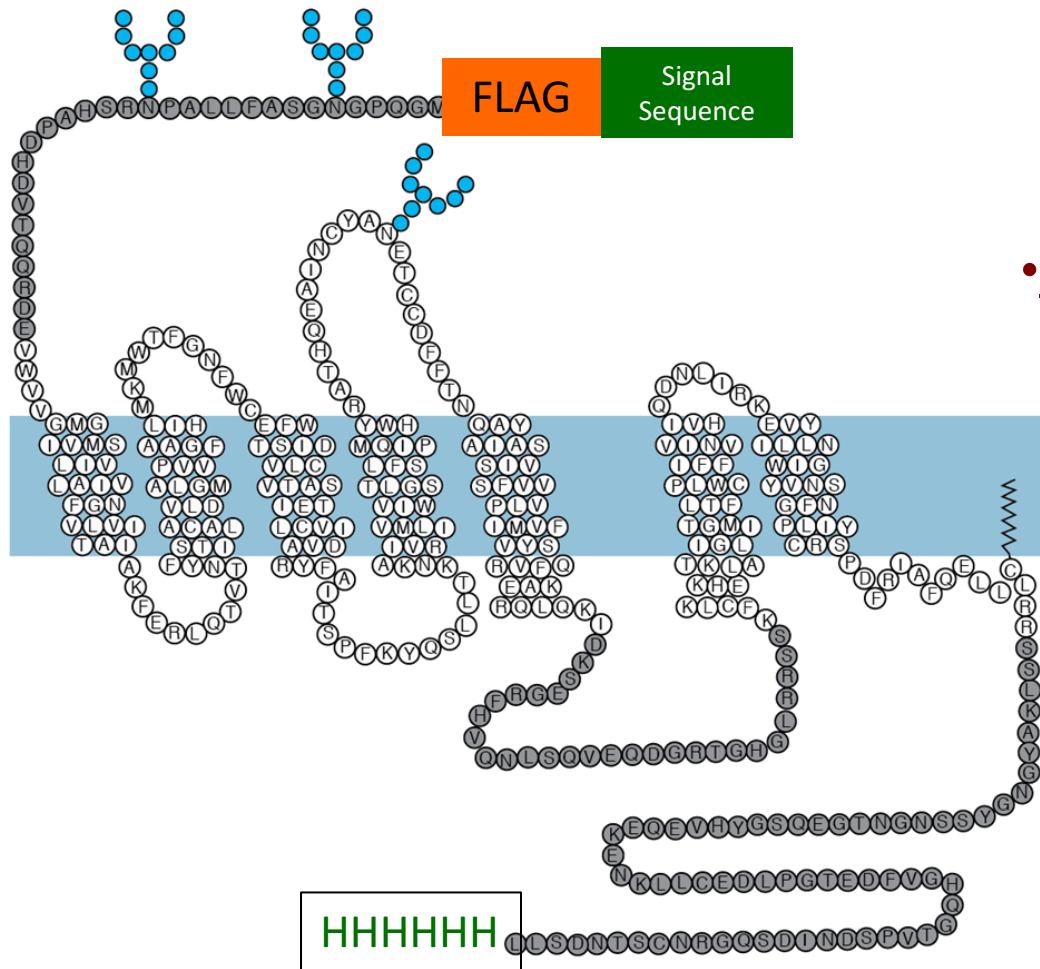
• Sequence analysis

- secondary structure (transmembrane domains)
- post-translational modifications

• Chimeric Receptors and site-directed mutagenesis

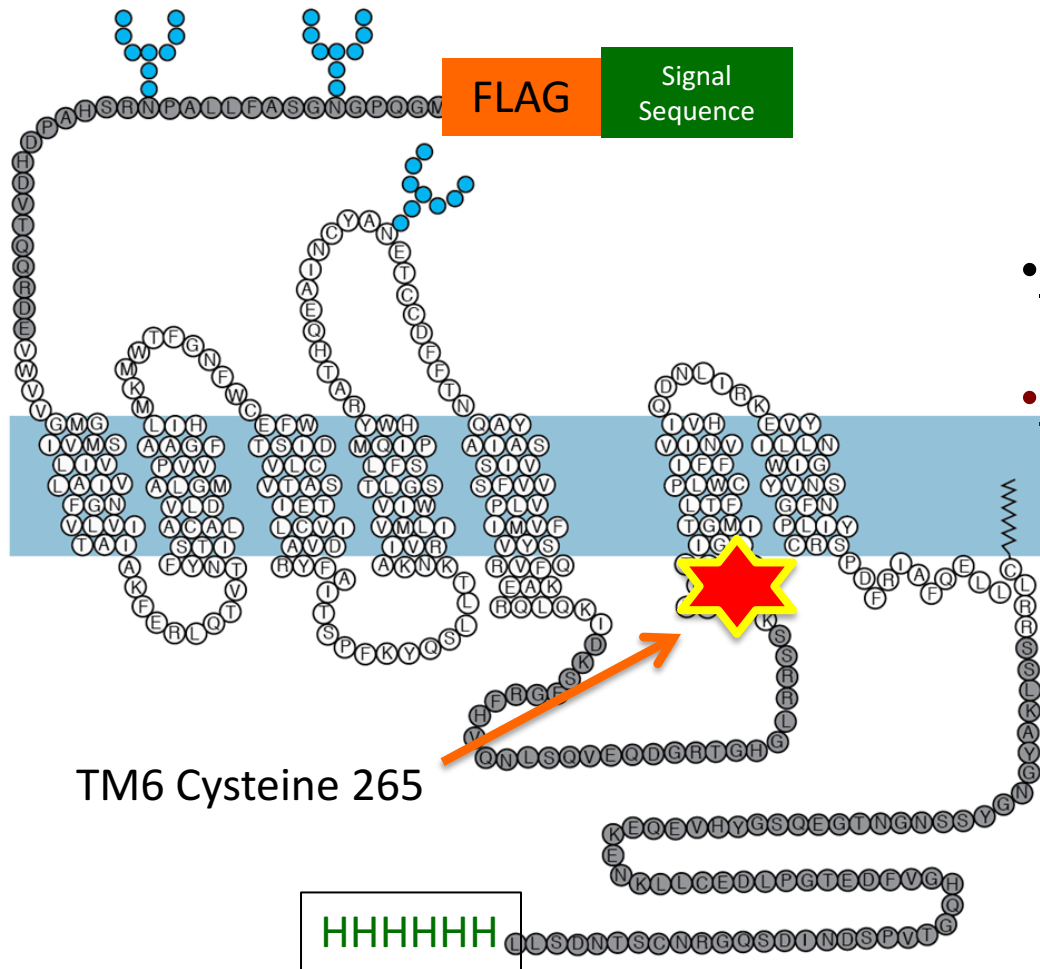
- ligand binding and G protein coupling
- enhance expression and purification

Approaches to characterizing β_2 AR structure:



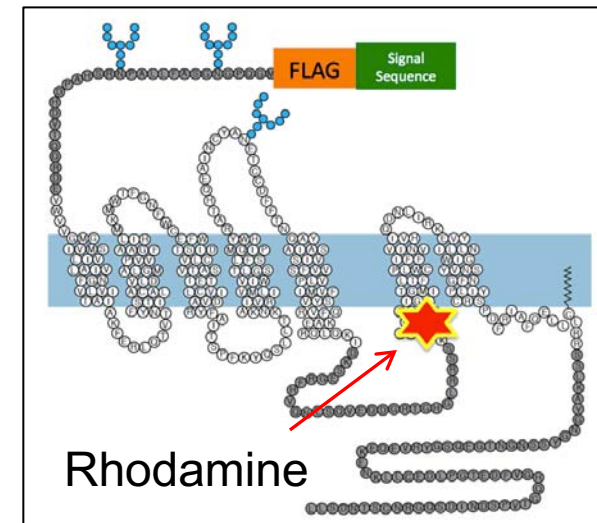
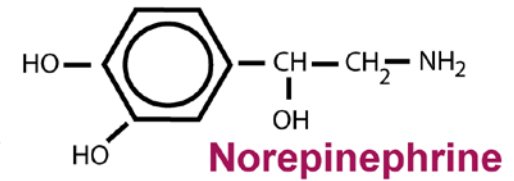
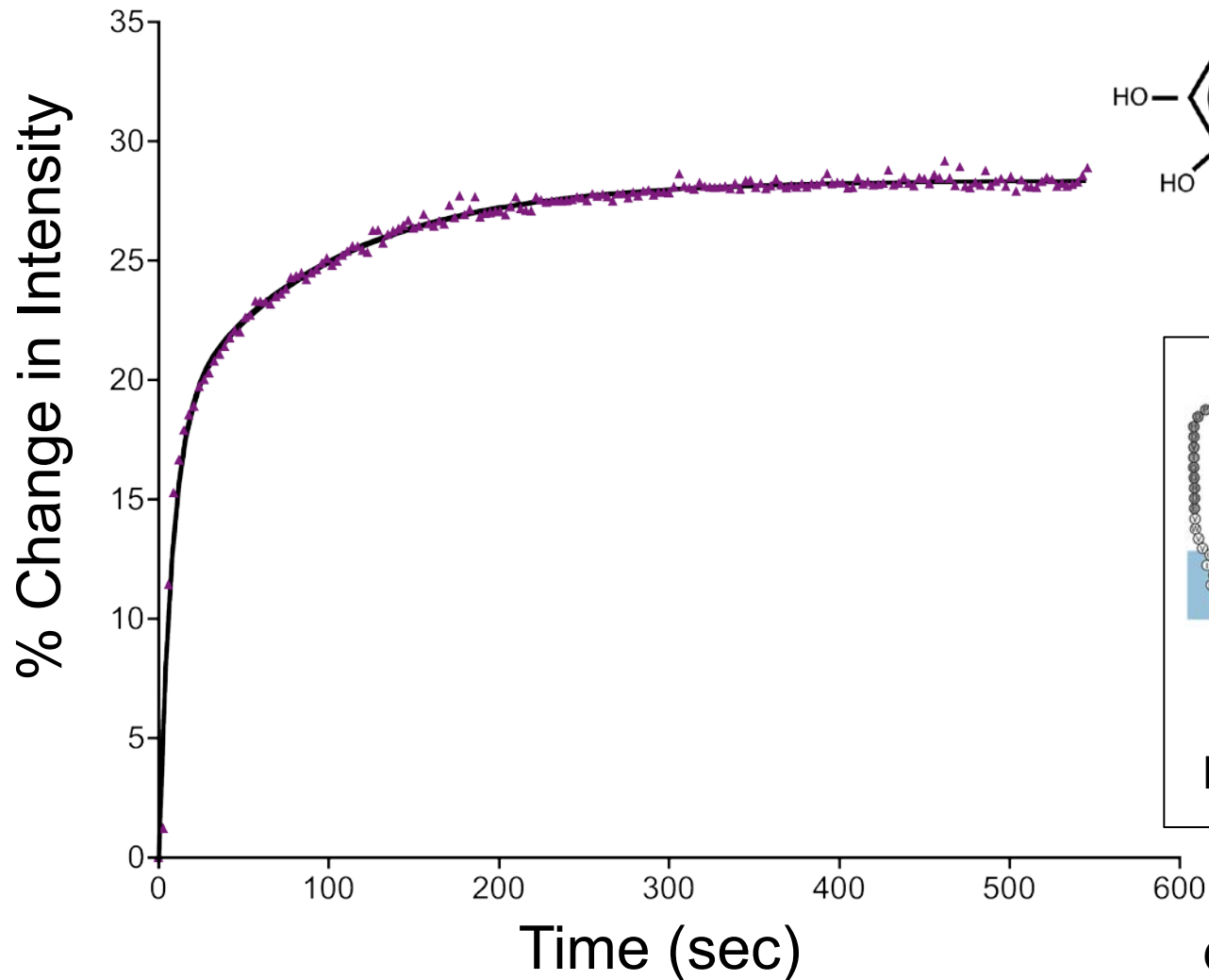
- Sequence analysis
 - secondary structure (transmembrane domains)
 - post-translational modifications
- Chimeric Receptors and site-directed mutagenesis
 - ligand binding and G protein coupling
 - enhance expression and purification
- Biochemistry
 - unstructured, flexible sequence

Approaches to characterizing β_2 AR structure:

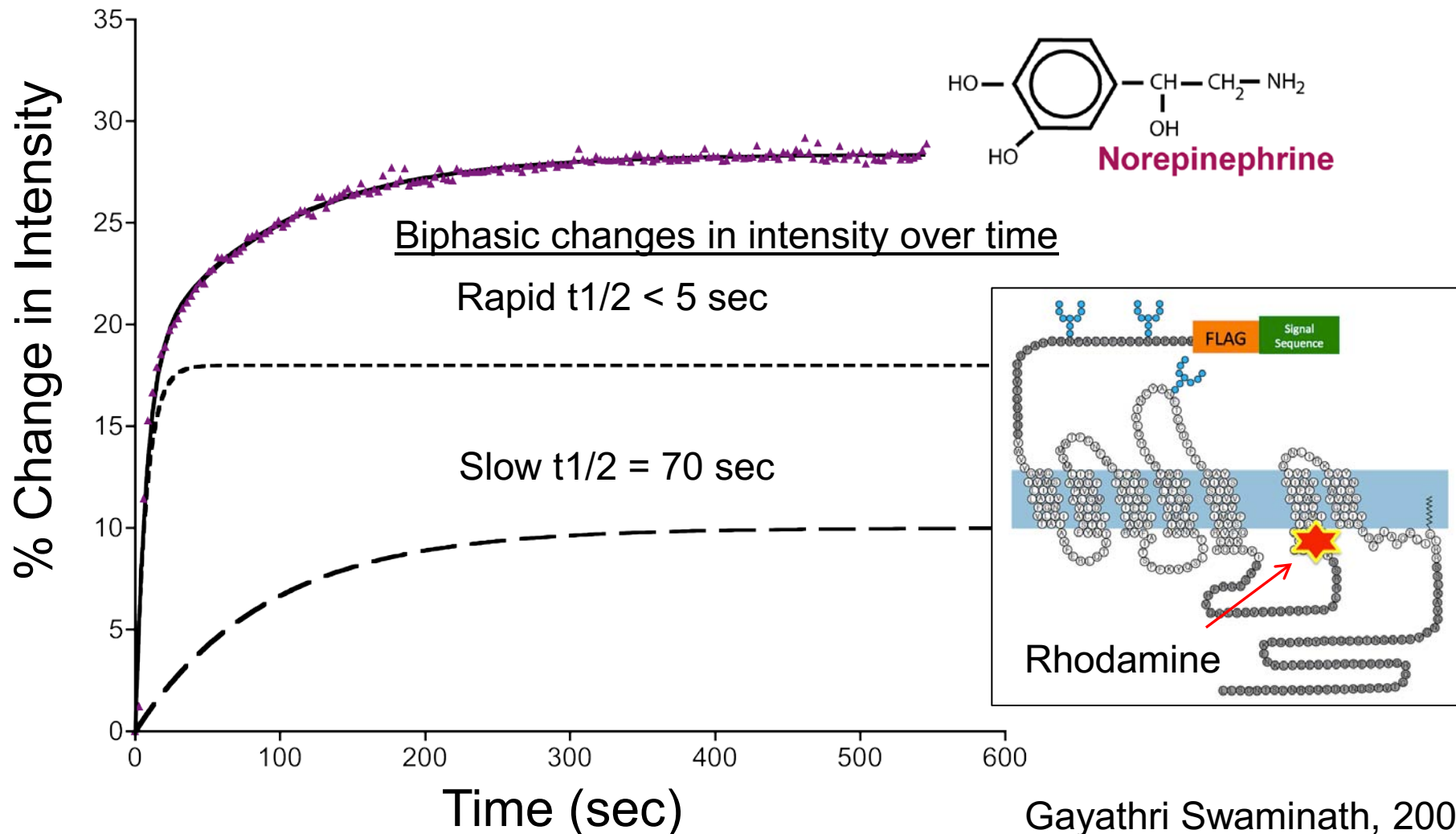


- Sequence analysis
 - secondary structure (transmembrane domains)
 - post-translational modifications
- Chimeric Receptors and site-directed mutagenesis
 - ligand binding and G protein coupling
 - enhance expression and purification
- Biochemistry
 - unstructured, flexible sequence
- Spectroscopy (Fluorescence, EPR, NMR)
 - ligand-specific conformational states
 - dynamic, flexible character
 - useful tool for monitoring receptor activity

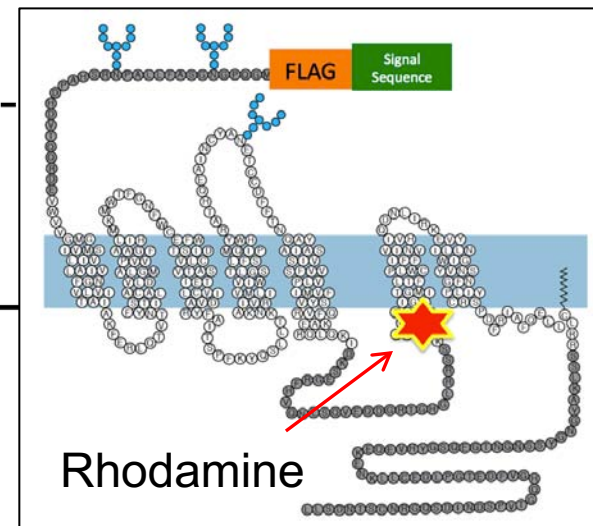
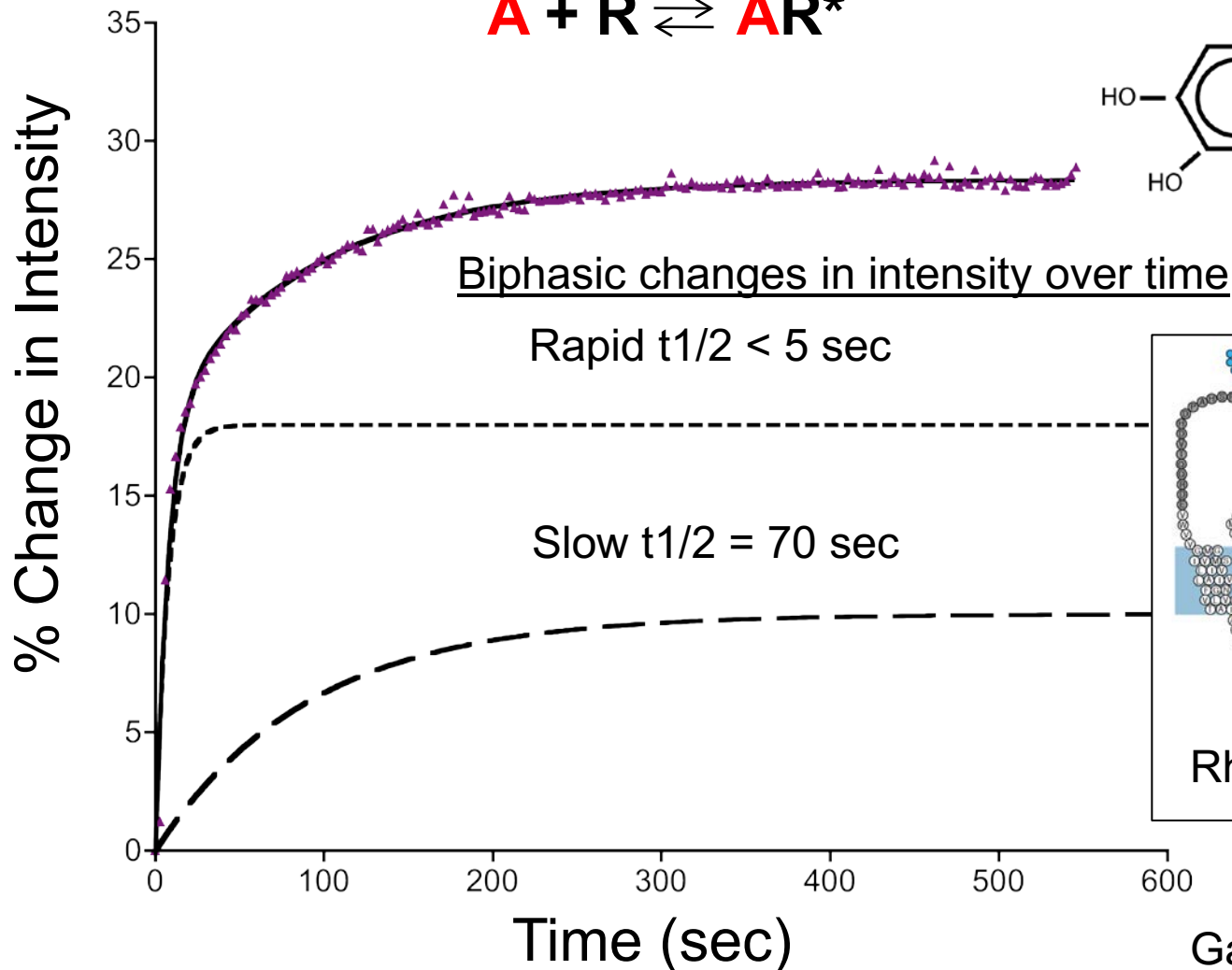
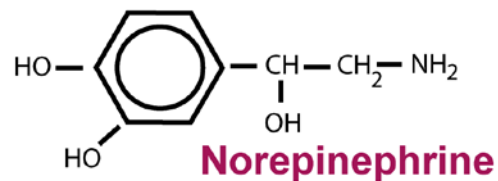
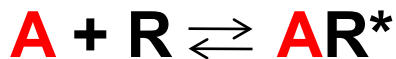
Conformational changes in TM6 in response to norepinephrine:



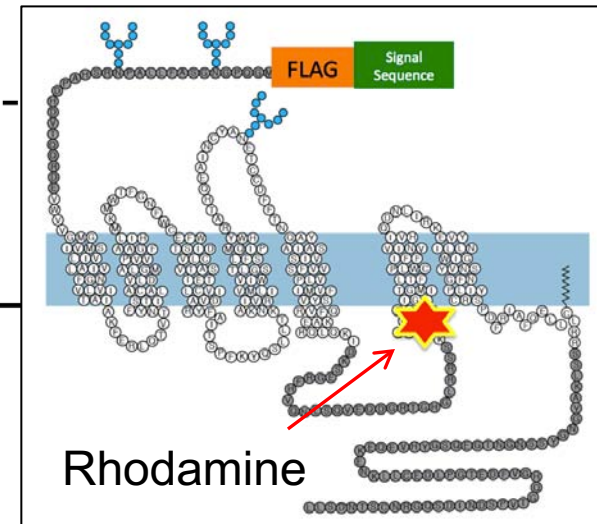
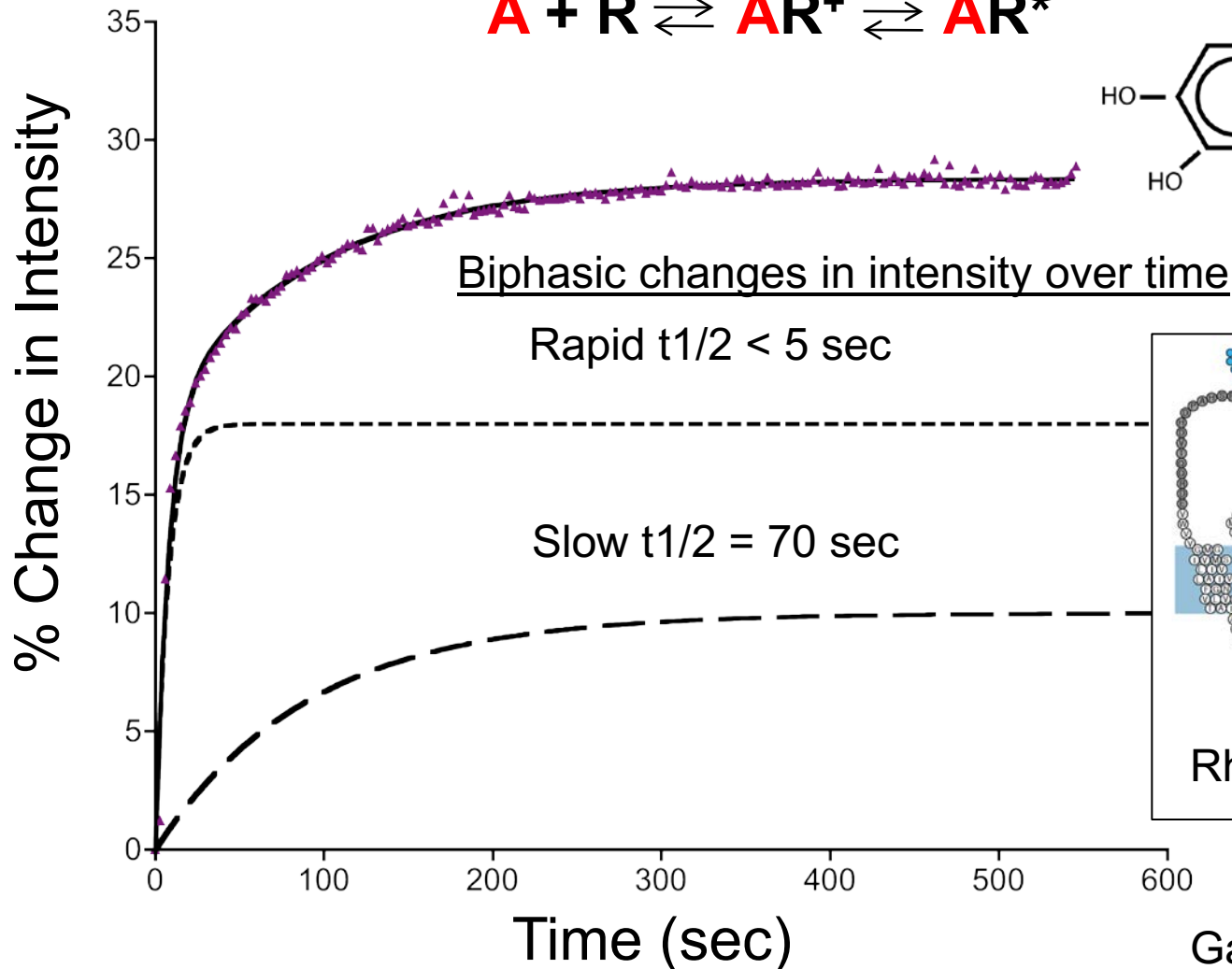
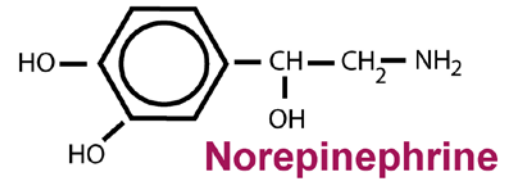
Conformational changes in TM6 in response to norepinephrine:

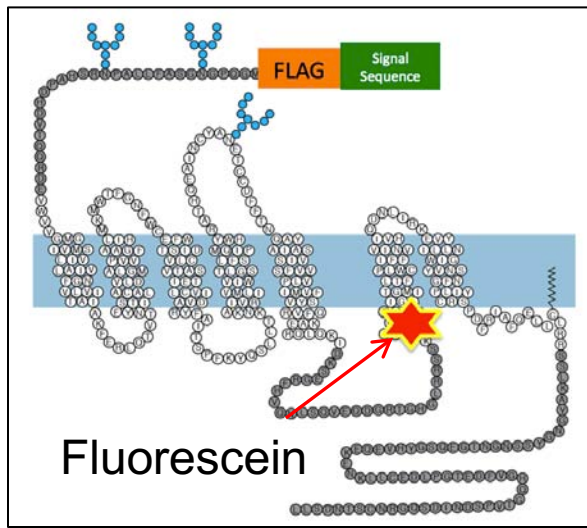


Conformational changes in TM6 in
response to norepinephrine:
Not consistent with single active state



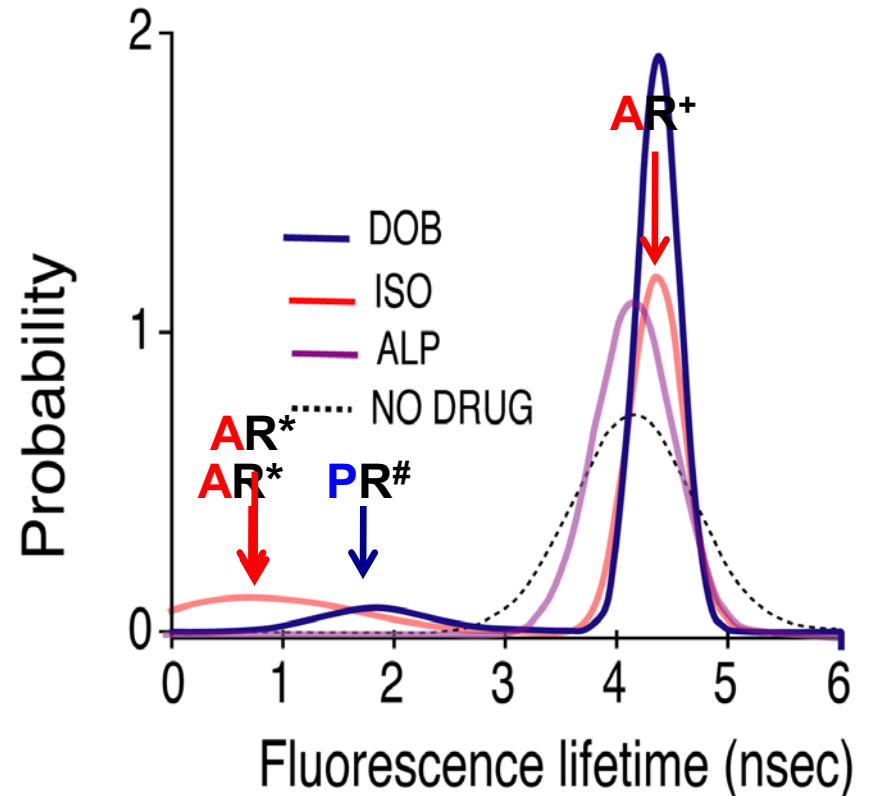
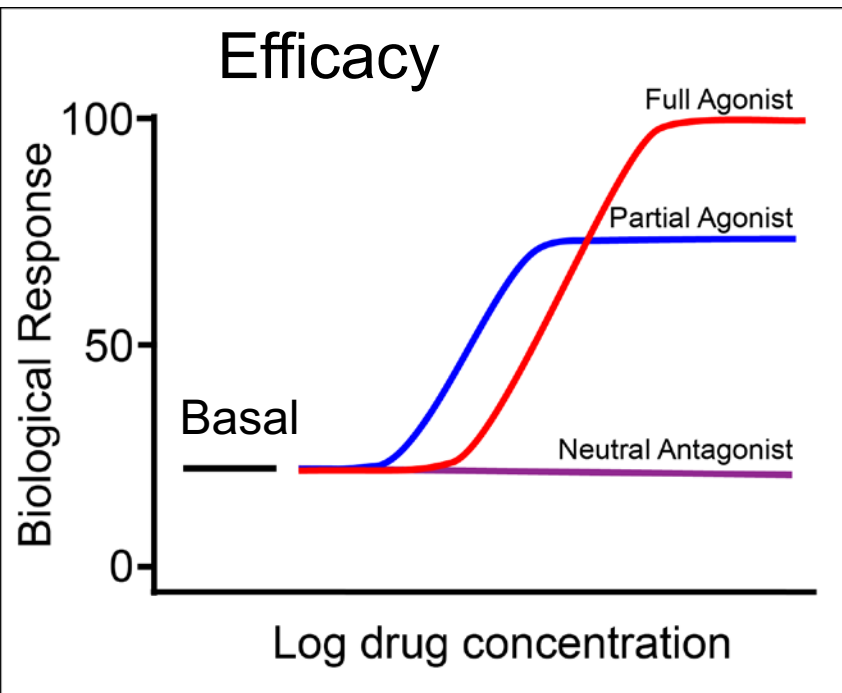
Conformational changes in TM6 in response to norepinephrine: Sequential conformational changes





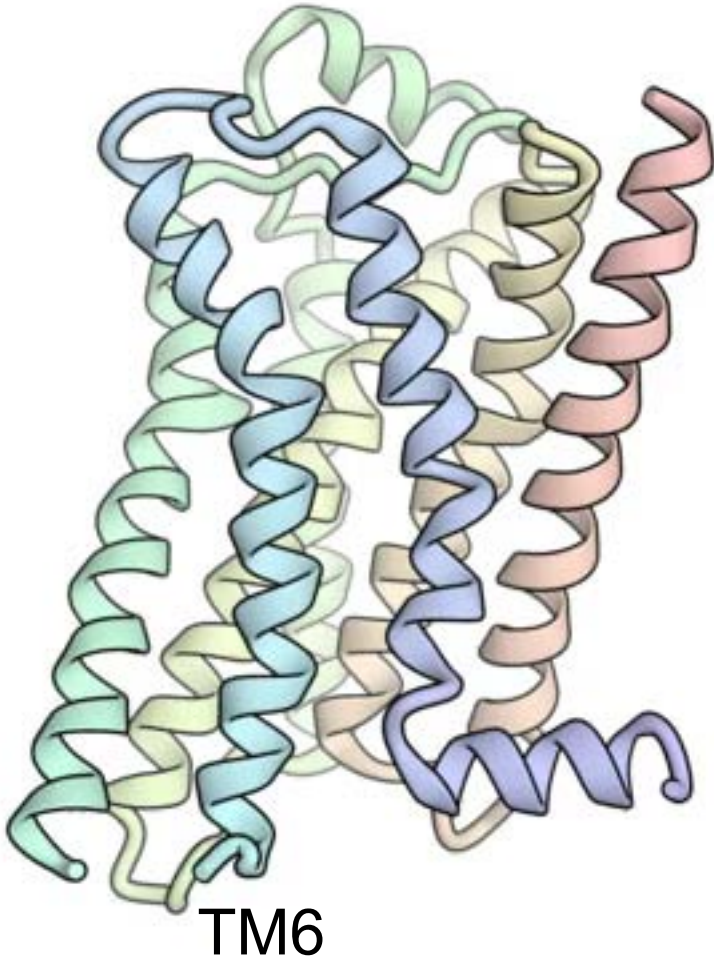
Fluorescence lifetime experiments

- Multiple agonist states
- Ligand-specific conformational states



Insights from spectroscopy studies

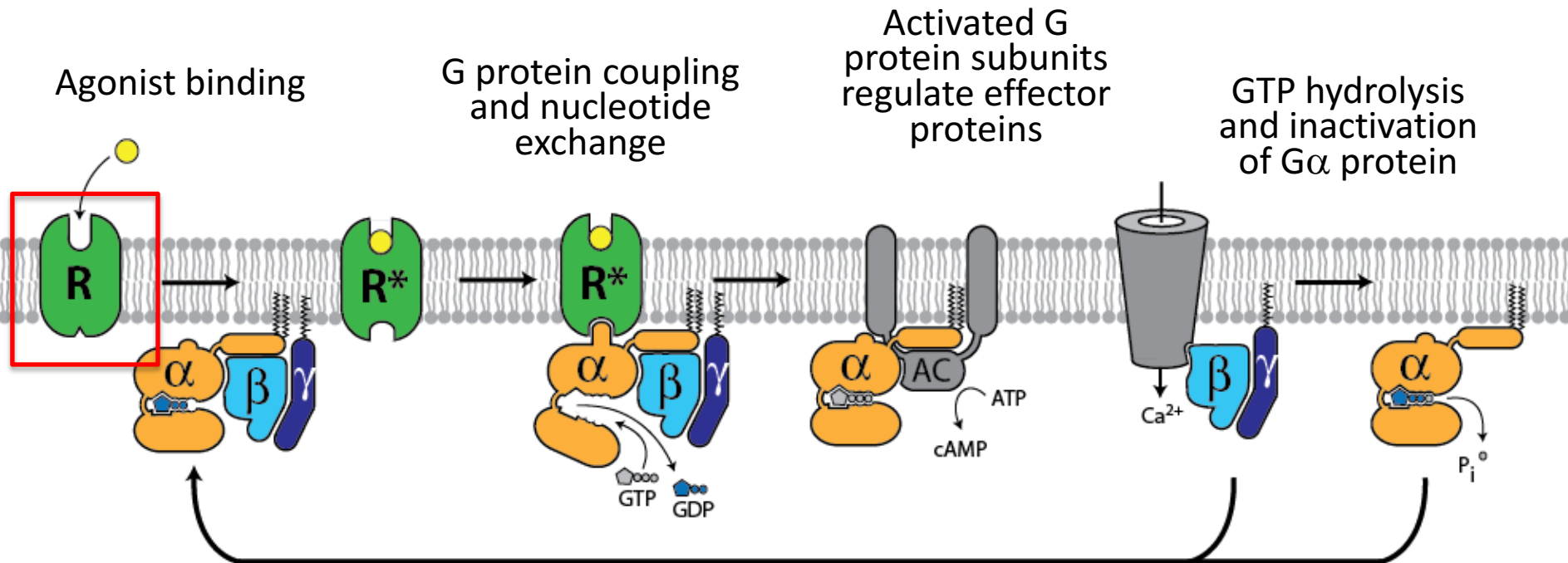
-fluorescence, NMR, EPR-



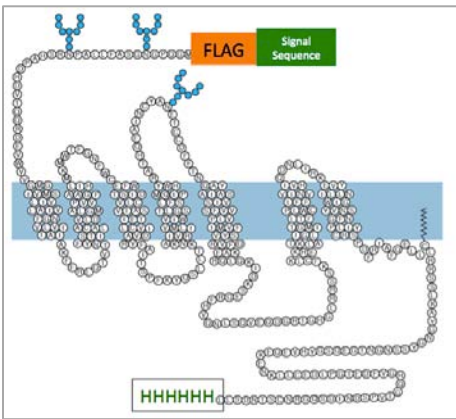
- The β_2 AR is flexible and dynamic
- TM6 undergoes the largest changes in response to agonists
- Agonist binding and activation occur through a series of conformational intermediates
- Agonists and partial agonists stabilize distinct conformational states
- Agonists alone do not stabilize a single active conformation.
- Fluorescence spectroscopy aided in identifying optimal conditions for crystallography

Outline

- Overview of approaches to characterize GPCR structure
- **GPCR crystallography**
- Mechanistic insights into GPCR-G protein activation



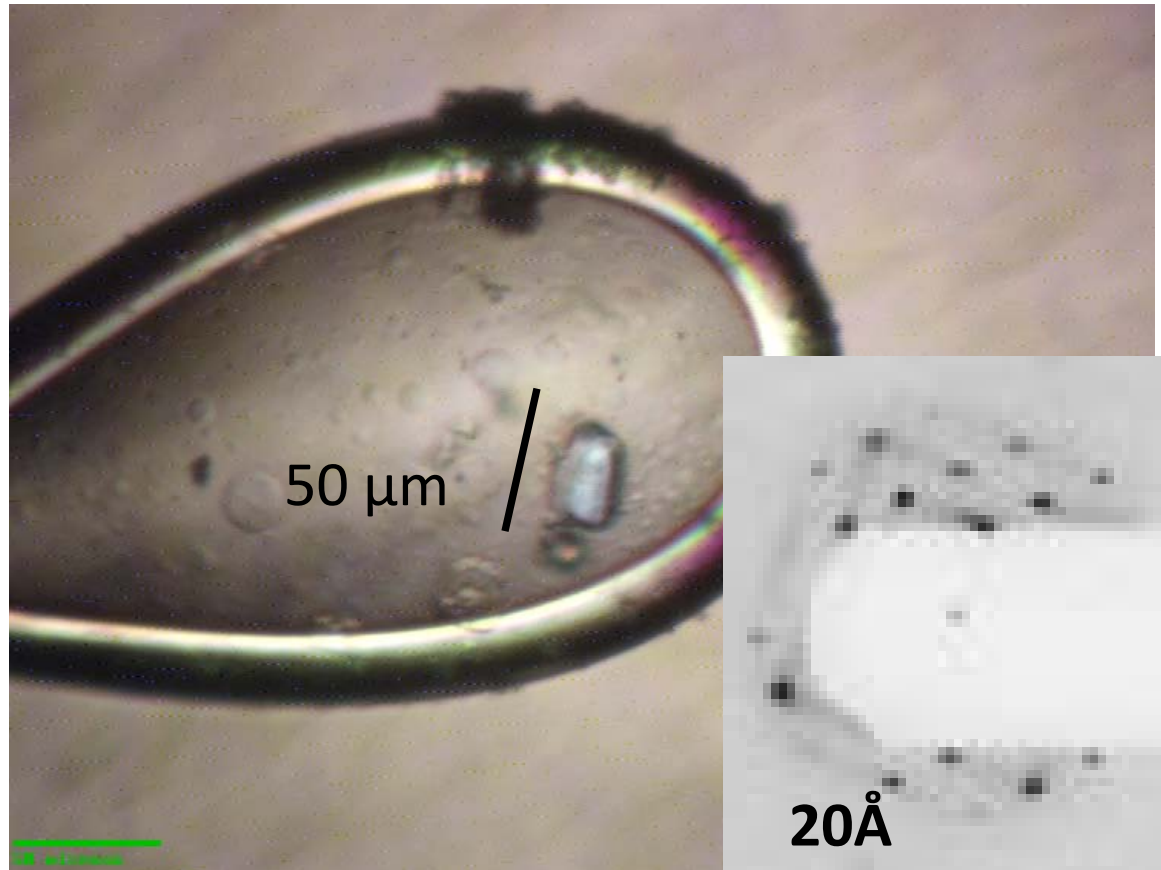
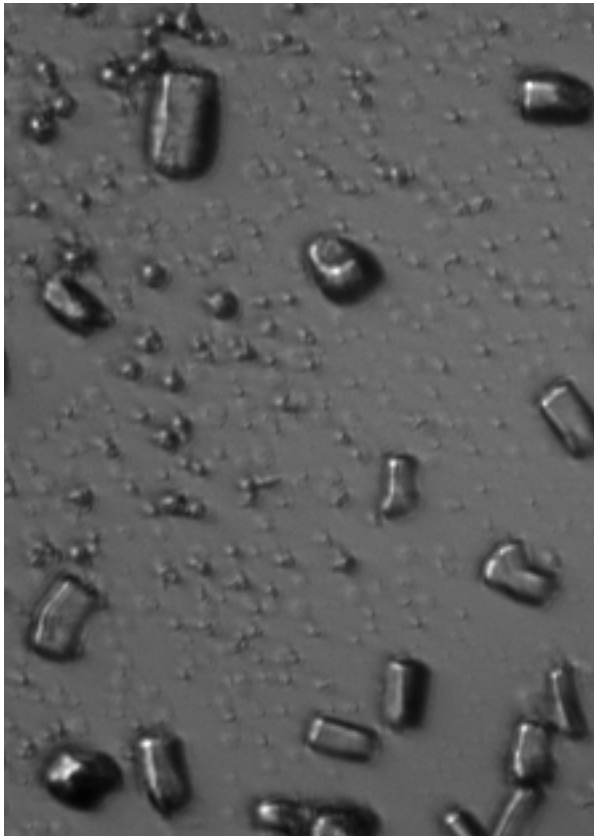
GPCR-G Protein Cycle

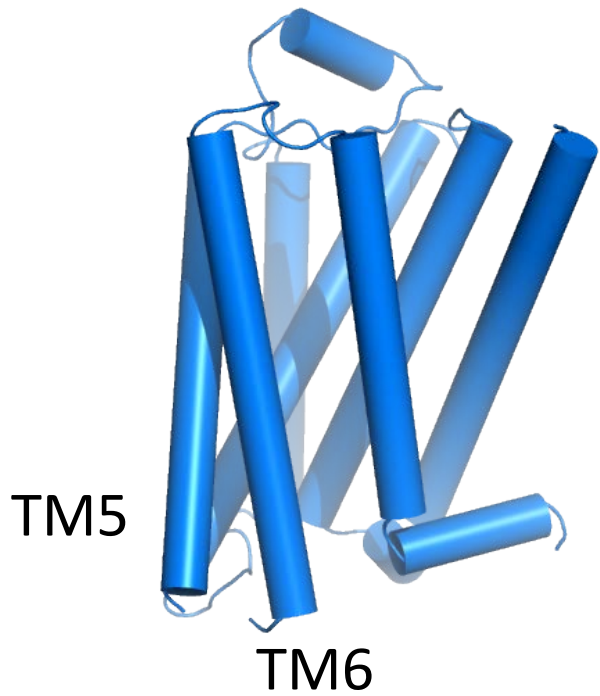


Crystals of wild-type β_2 AR

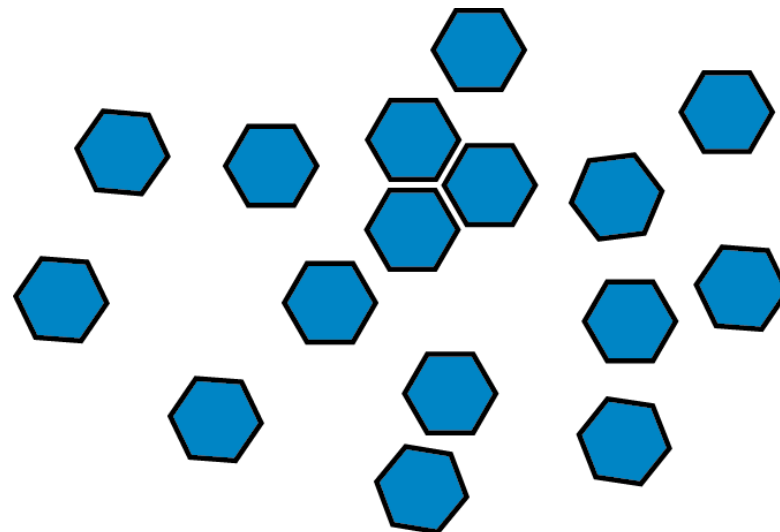
Nov 2004

ESRF microfocus beamline ID13, July 2005

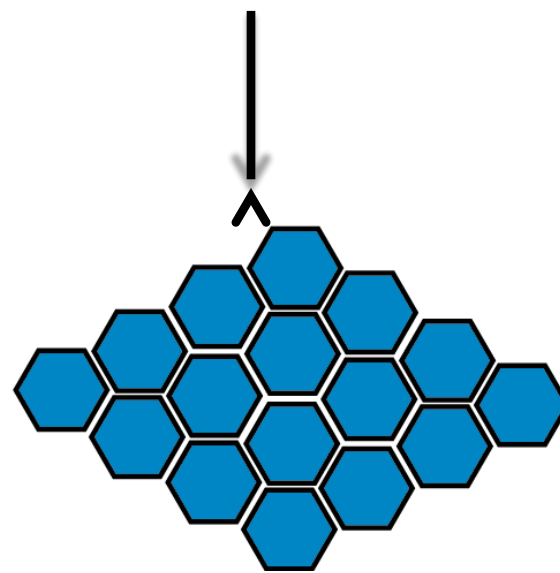




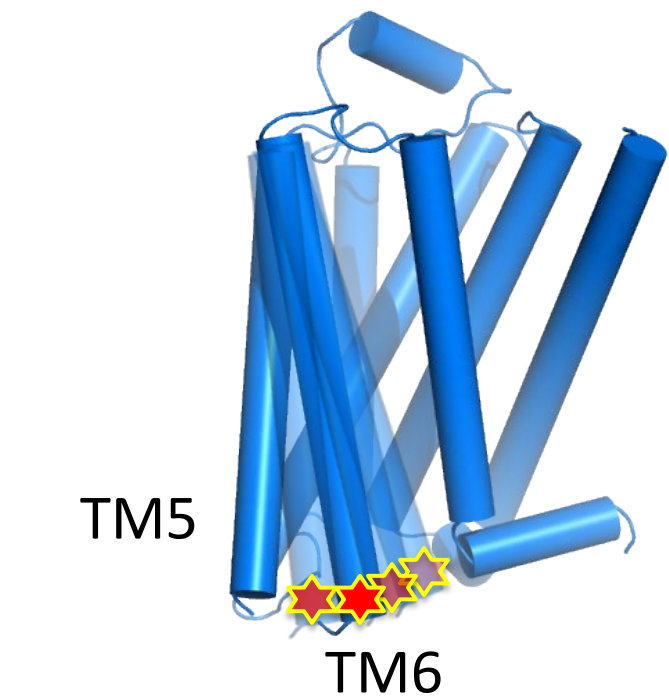
Conformationally
uniform GPCR



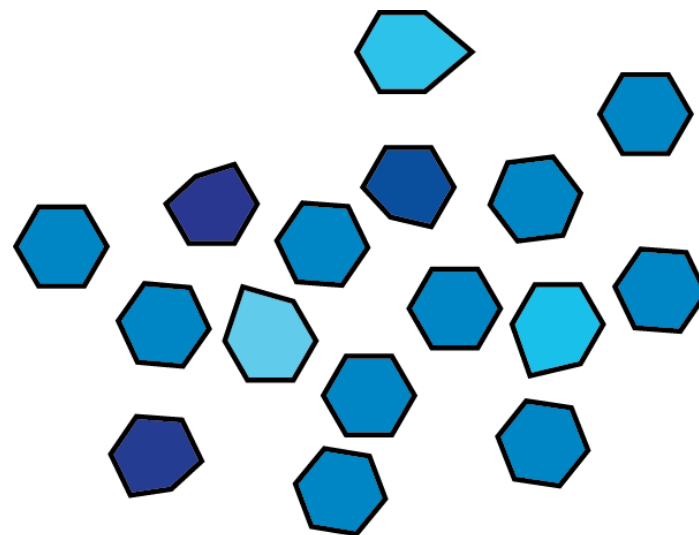
Purified protein



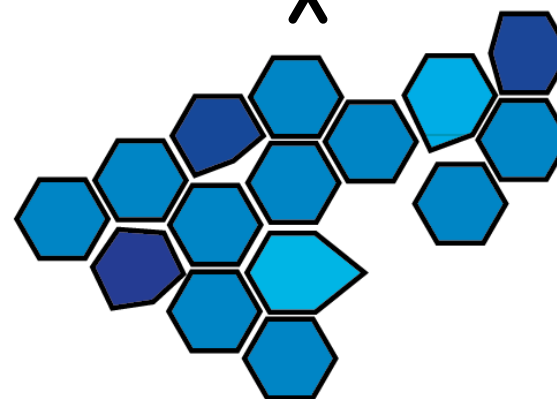
High-quality crystal



Conformationally heterogeneous GPCR



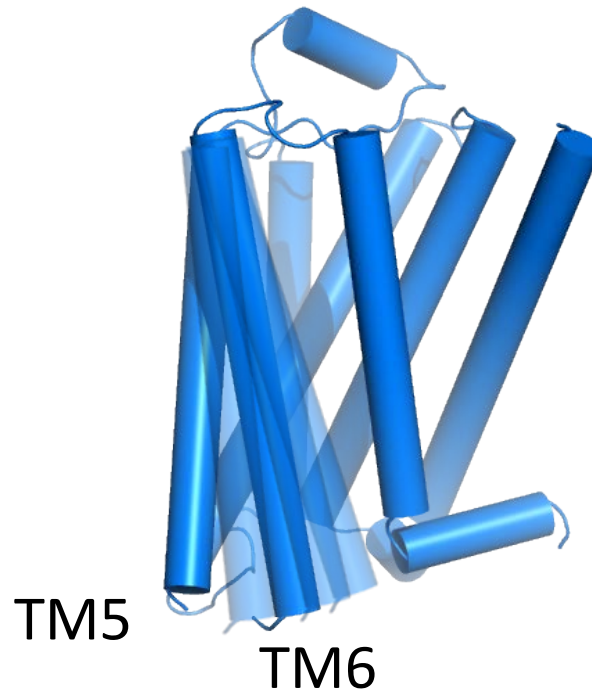
Purified protein



Poor quality crystal

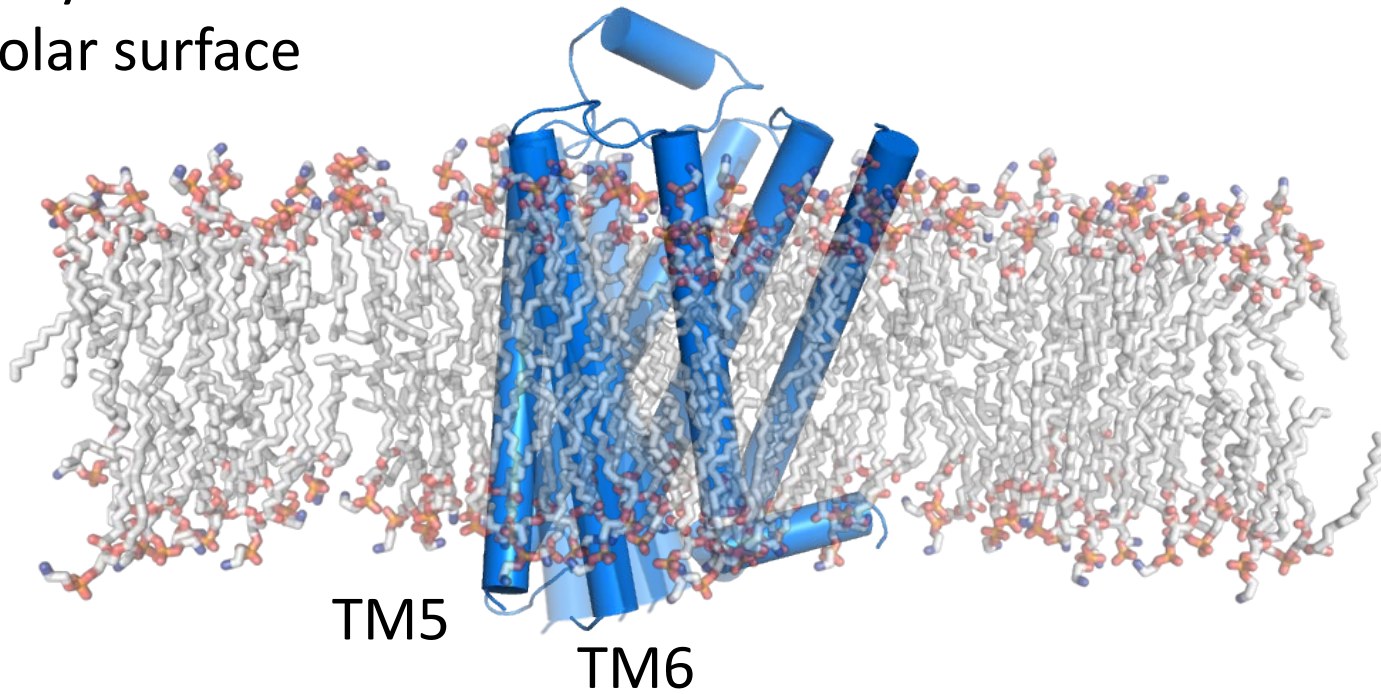
Challenges for crystallography

- Protein dynamics



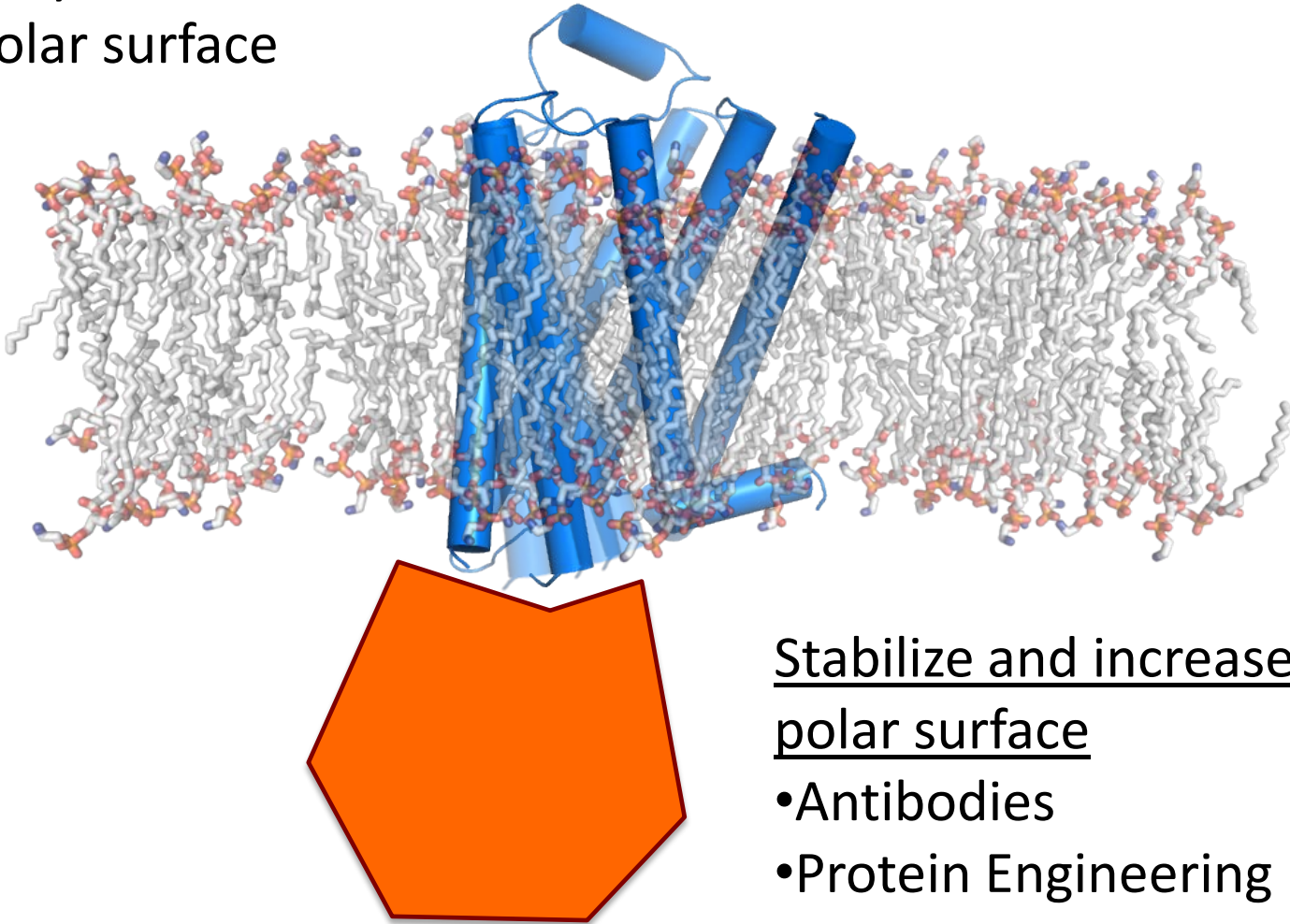
Challenges for crystallography

- Protein dynamics
- Little polar surface



Challenges for crystallography

- Protein dynamics
- Little polar surface

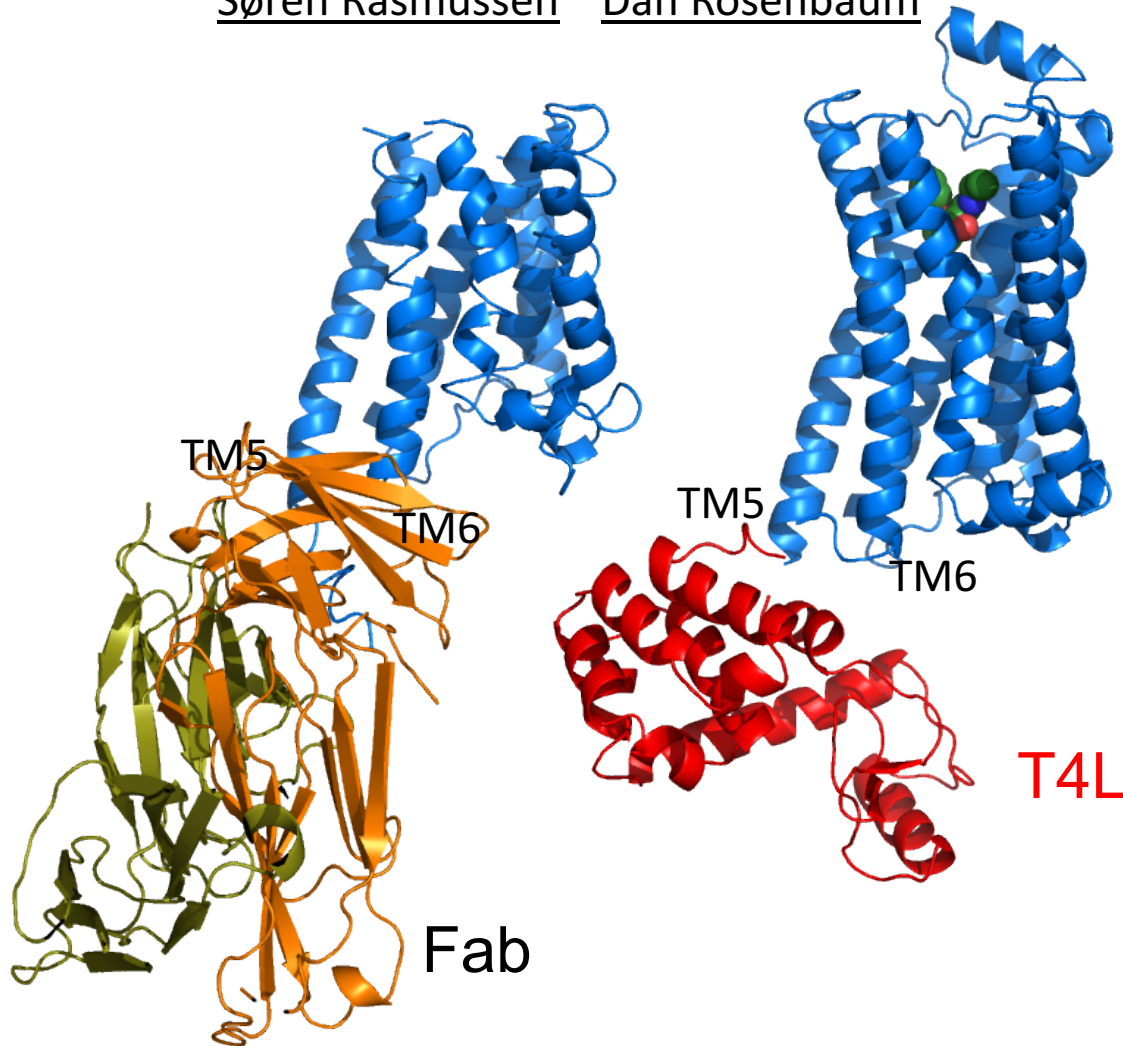


Approaches for GPCR crystallogenesis:

- Antibodies and protein engineering

2007

Søren Rasmussen Dan Rosenbaum

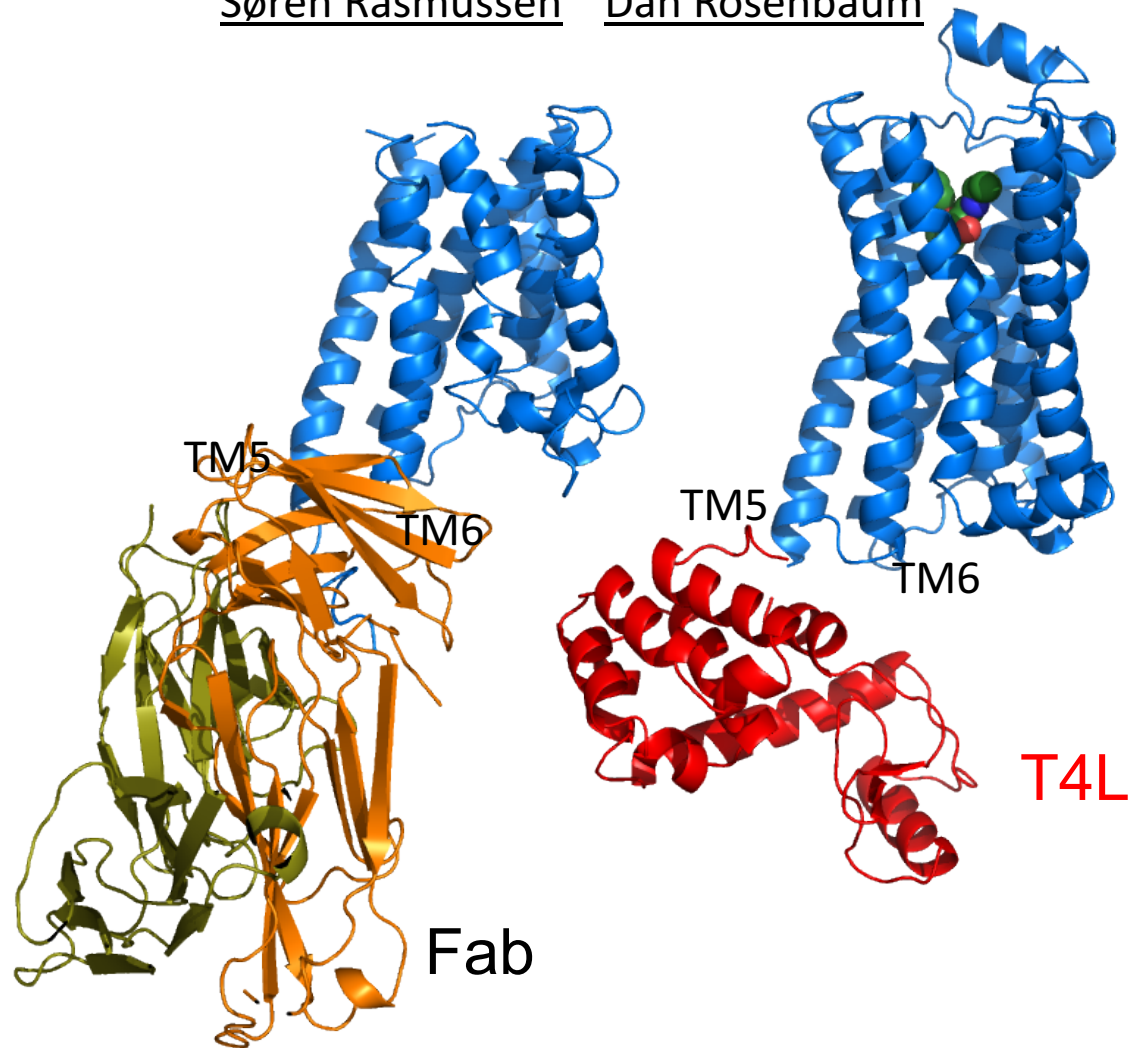


Approaches for GPCR crystallogenesis:

- Antibodies and protein engineering
- Lipid-based media: bicelles and lipidic cubic phase

2007

Søren Rasmussen Dan Rosenbaum

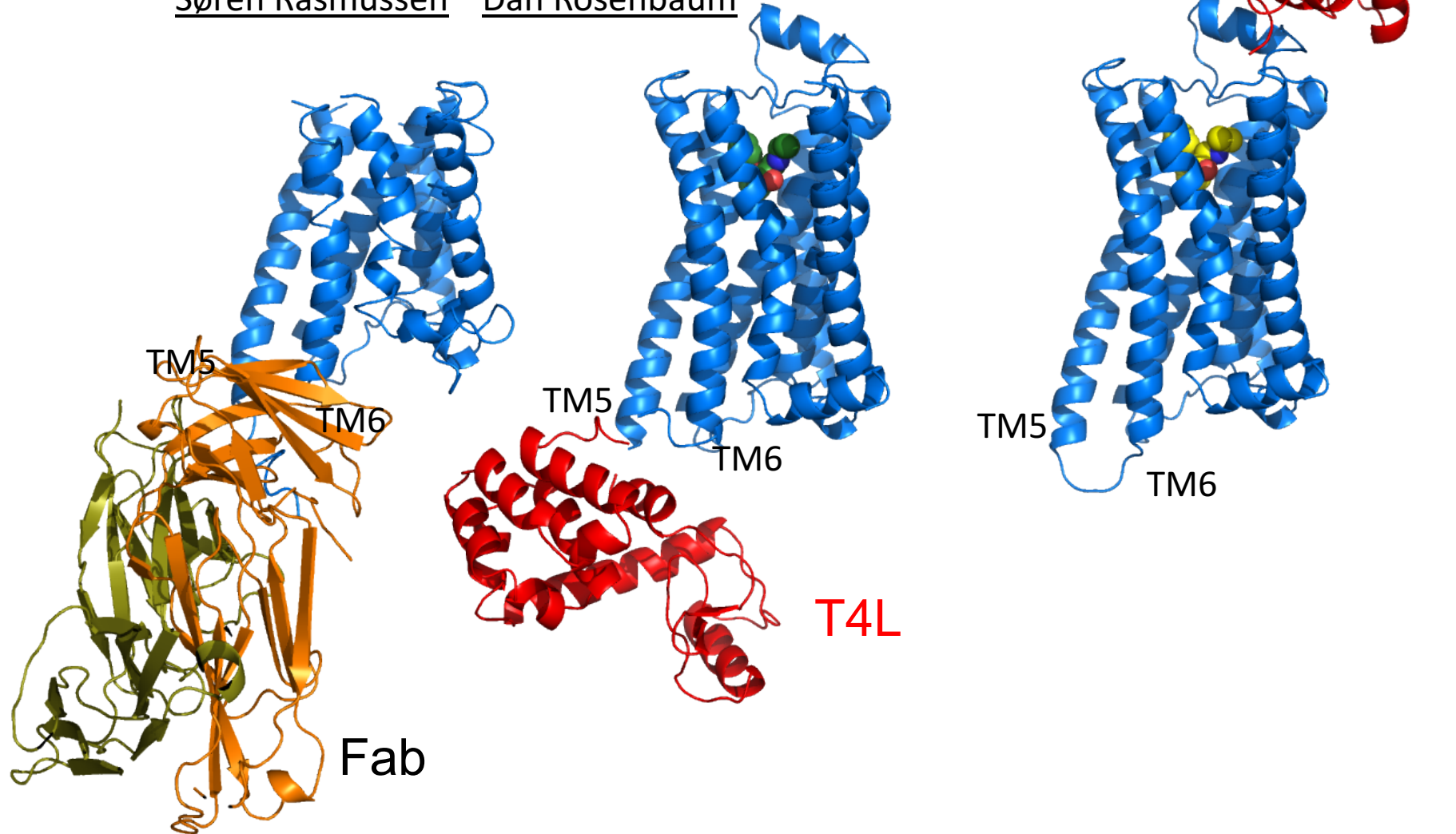


Approaches for GPCR crystallogenesis:

- Antibodies and protein engineering
- Lipid-based media: bicelles and lipidic cubic phase

2007

Søren Rasmussen Dan Rosenbaum



Inactive-state GPCR structures

Antibodies and Protein Engineering

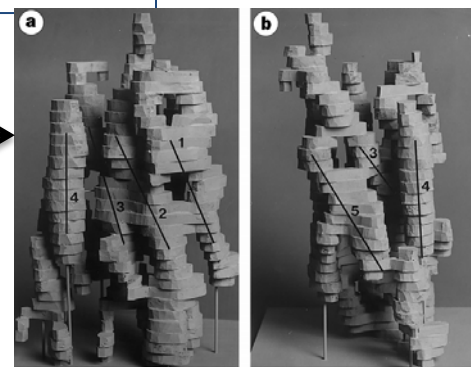
Other Approaches

Thermostabilization through alanine scanning mutations

– β_1 AR, Adenosine A2A –
Tate and Schertler

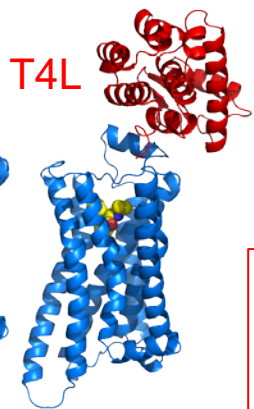
Rhodopsin (native)

- Schertler (2D crystals) - 1997
- Palczewski and Okada (3D) - 2000
- Ernst and Hofmann (Opsin) - 2008

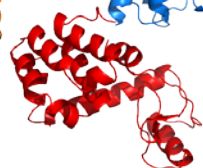


Stevens Lab
and collaborators

Adenosine A2A
D3 Dopamine
CXCR4
Histamine
S1P1
 κ -opioid

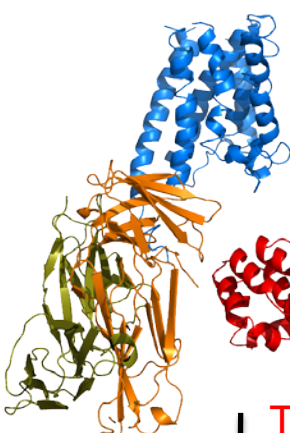


T4L

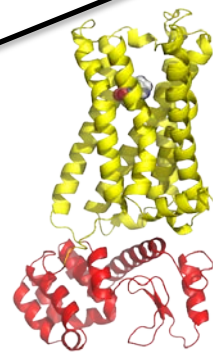


T4L

Recent GPCR-T4L structures
from Kobilka Lab and collaborators

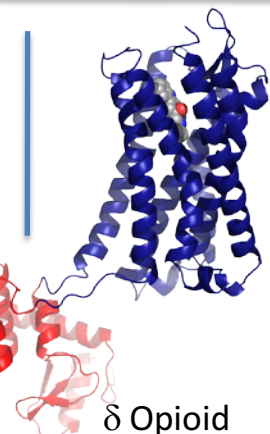
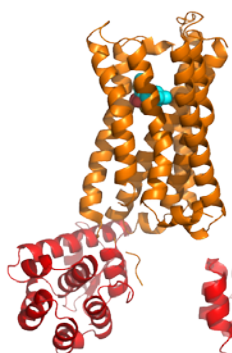


Fab



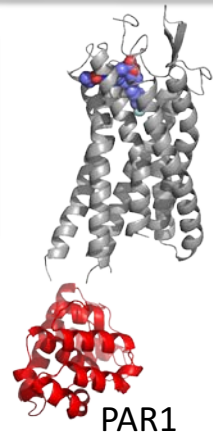
M2 Muscarinic M3 Muscarinic

(Haga and Kobayashi) (Jurgen Wess)



δ Opioid μ Opioid

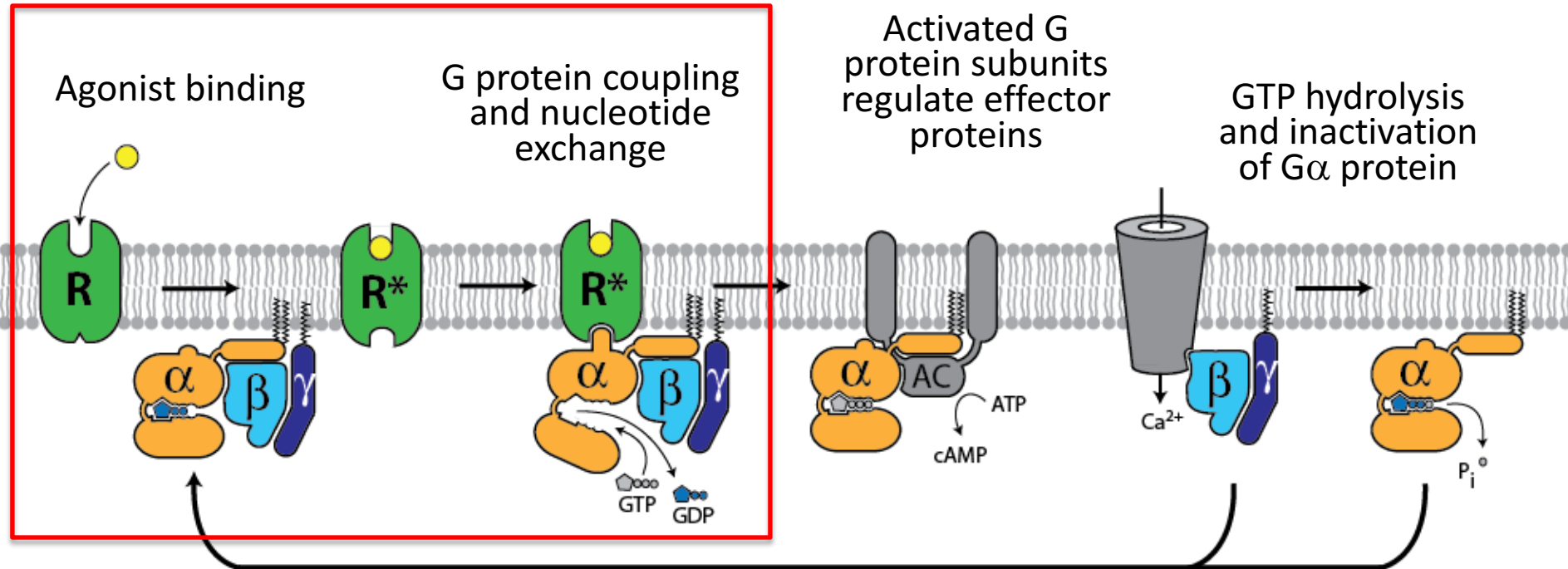
(Sebastien Granier)



PAR1
(Shaun Coughlin)

Outline

- Overview of approaches to characterize GPCR structure
- GPCR crystallography
- Mechanistic insights into GPCR-G protein activation



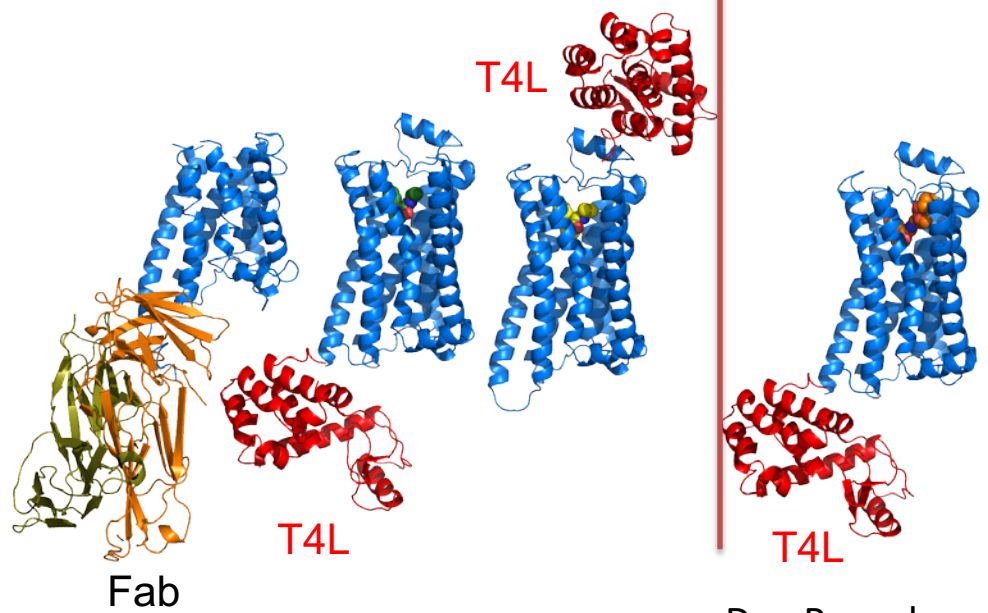
GPCR-G Protein Cycle



β_2 AR INACTIVE

Inverse Agonist

Agonist
(covalent)



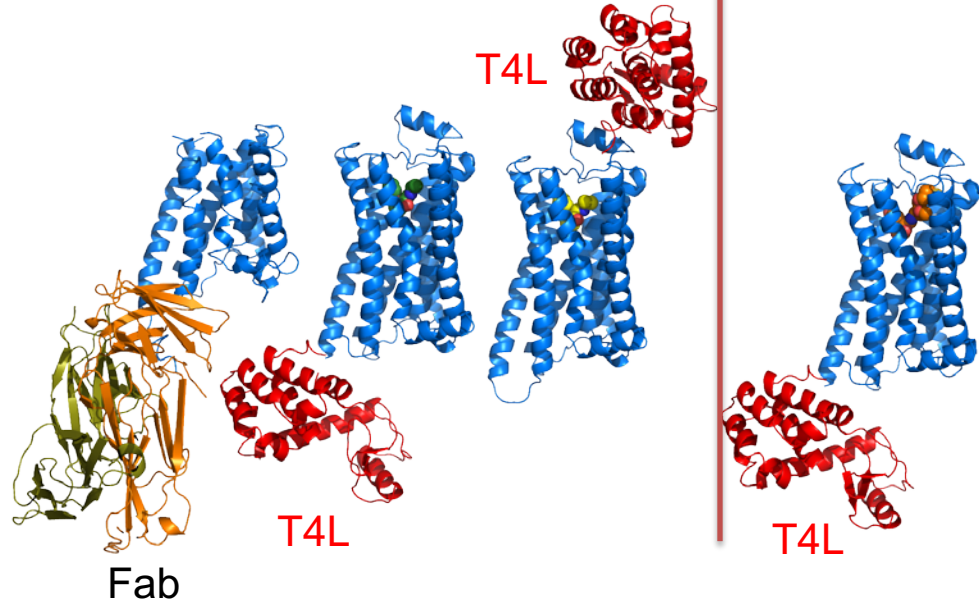
Dan Rosenbaum
Ralph Holl
Peter Gmeiner

β_2 AR ACTIVE ?

β_2 AR INACTIVE

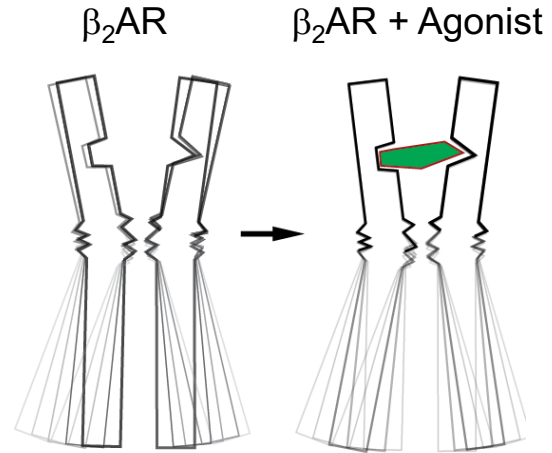
Inverse Agonist

Agonist
(covalent)



β_2 AR ACTIVE ?

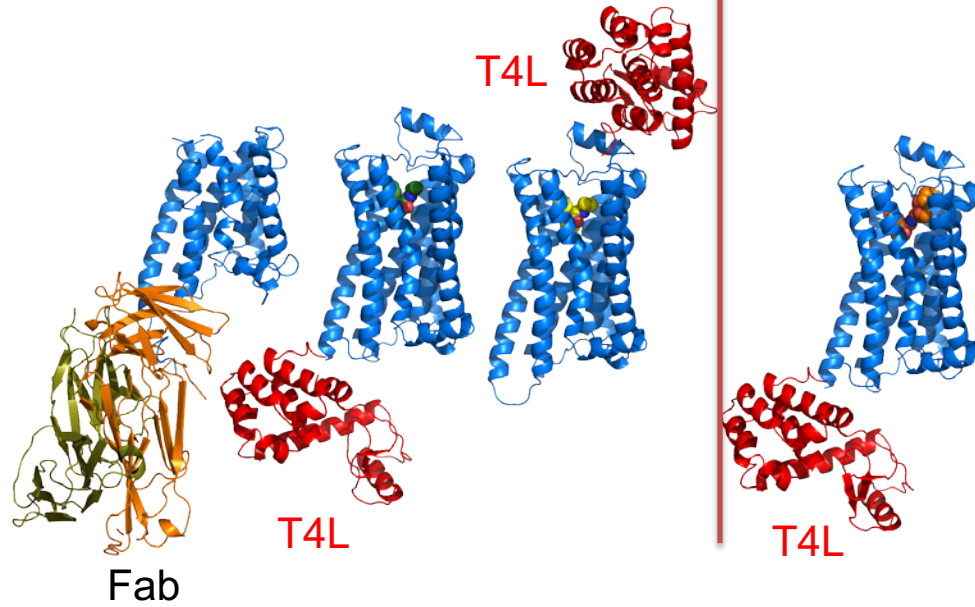
Agonist alone does not fully stabilize active state



β_2 AR INACTIVE

Inverse Agonist

Agonist
(covalent)

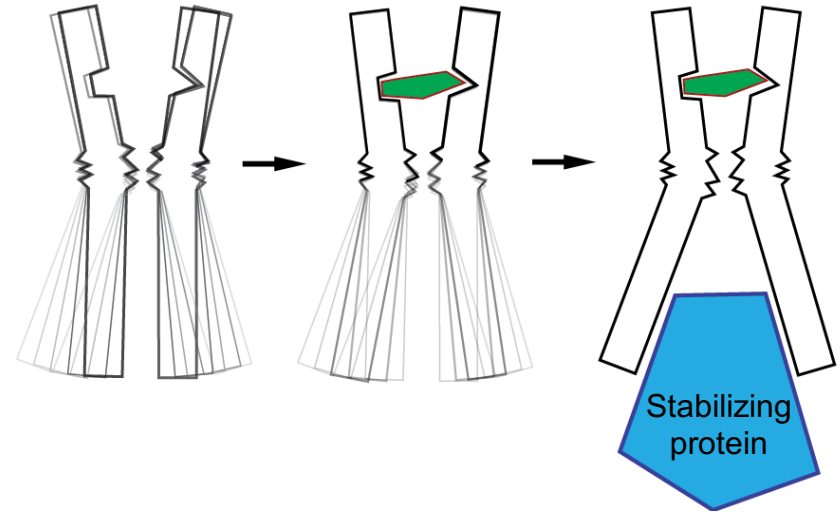


β_2 AR ACTIVE ?

Agonist alone does not fully stabilize active state

β_2 AR

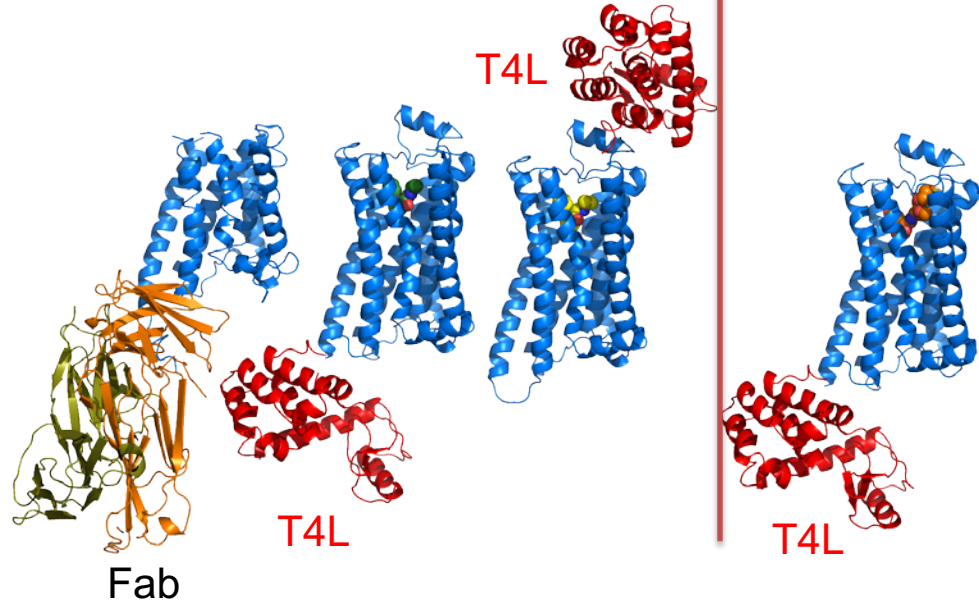
β_2 AR + Agonist



β_2 AR INACTIVE

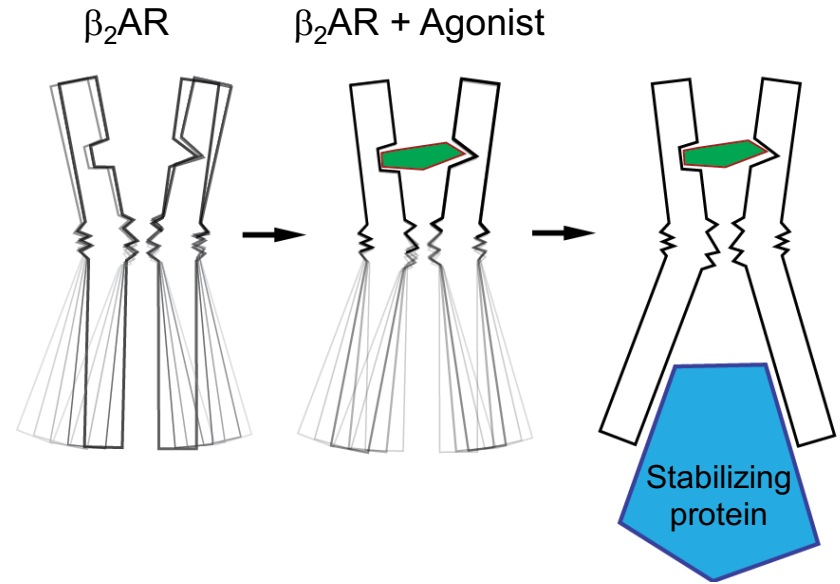
Inverse Agonist

Agonist
(covalent)



β_2 AR ACTIVE ?

Agonist alone does not fully stabilize active state



Nanobody
variable domain of a
single chain
camelid antibody

Jan Steyaert
Els Pardon



β_2 AR INACTIVE

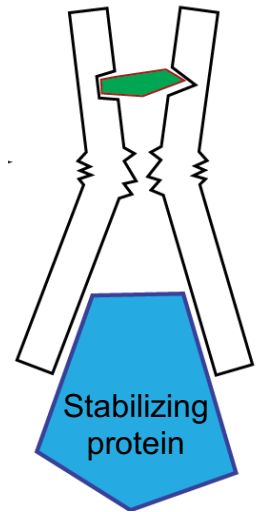
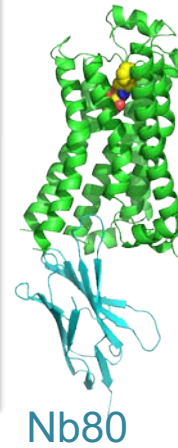
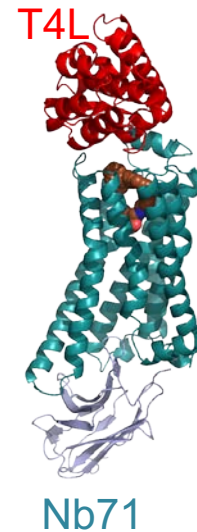
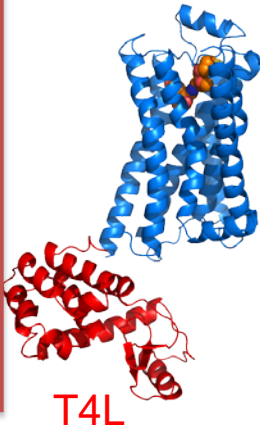
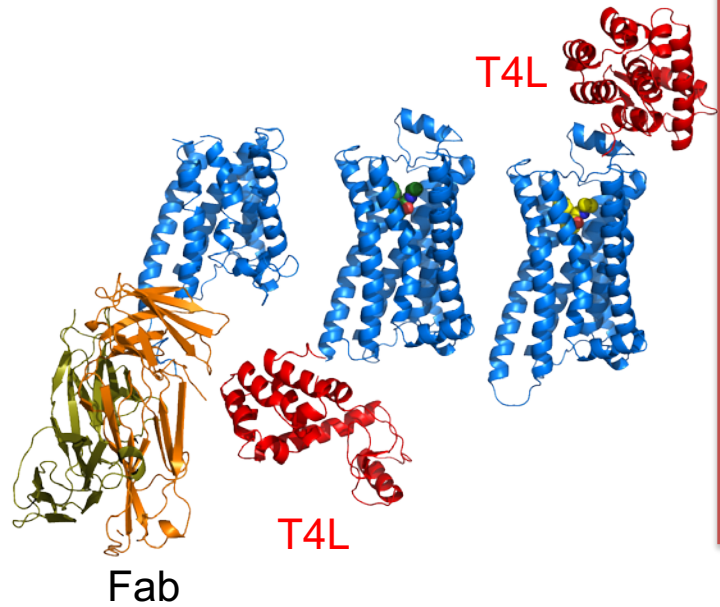
β_2 AR ACTIVE

Inverse Agonist

Agonist
(covalent)

Partial
Agonist

Agonist



Nanobody
variable domain of a
single chain
camelid antibody

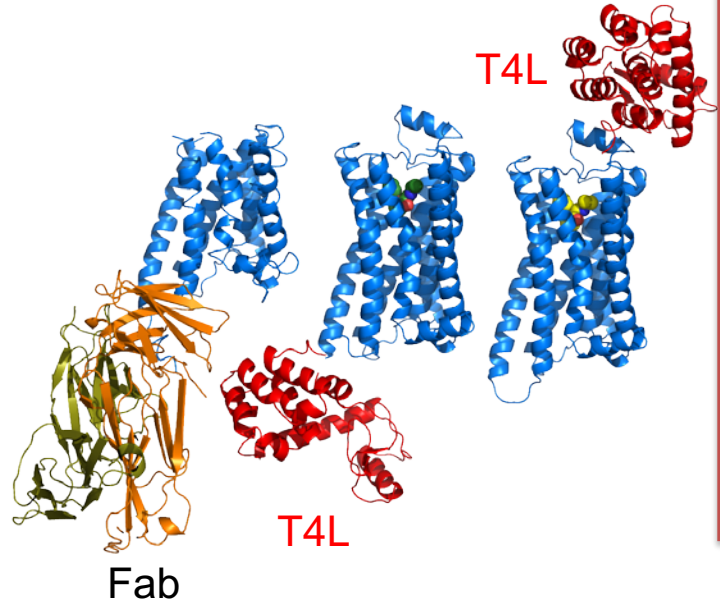
Jan Steyaert
Els Pardon



β_2 AR INACTIVE

Inverse Agonist

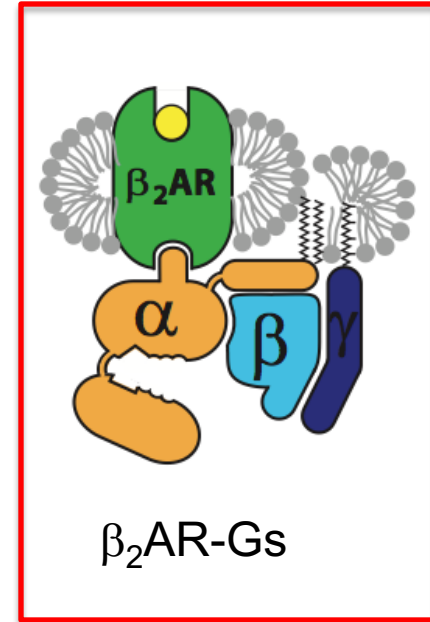
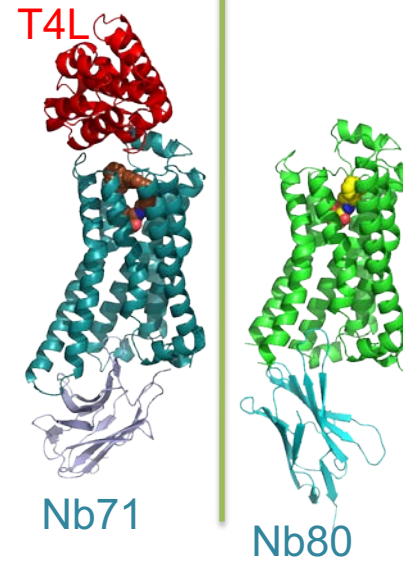
Agonist
(covalent)



β_2 AR ACTIVE

Partial Agonist

Agonist



Nanobody
variable domain of a
single chain
camelid antibody

Jan Steyaert
Els Pardon



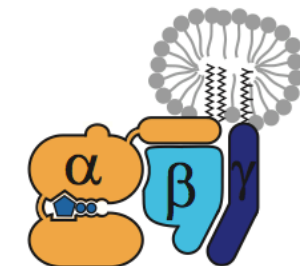
Technical contributions to crystallizing the β_2 AR-Gs complex

- High-affinity agonist BI-167107 (1 of ~ 60 screened)
- Removal of GDP – Apyrase
- Detergent: MNG-3 (long-term storage, aids transition into LCP)
- New mesophase lipid (7.7 MAG) to accommodate G protein (provided by Martin Caffrey)
- Nanobody to stabilize G protein complex (Jan Steyaert)
- Amino Terminal T4 Lysozyme
- Project guided by data from negative stain single particle EM (Georgios Skiniotis)

1) High affinity agonist binding

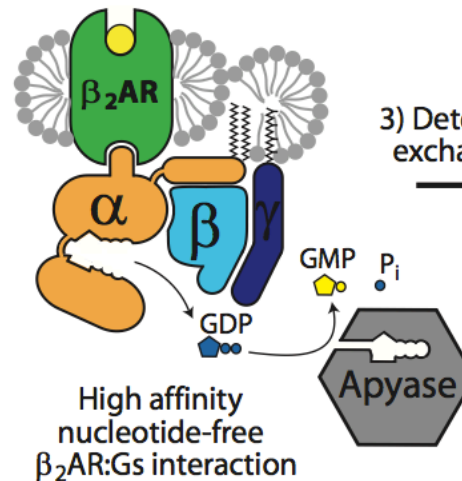


BI-167107 bound β_2 AR in DDM



GDP bound Gs heterotrimer in DDM

2) Apyrase treatment



High affinity nucleotide-free β_2 AR:Gs interaction

3) Detergent exchange



Stable β_2 AR:Gs complex in MNG-3

Microcrystallography

GM/CA-CAT at Argonne National Labs



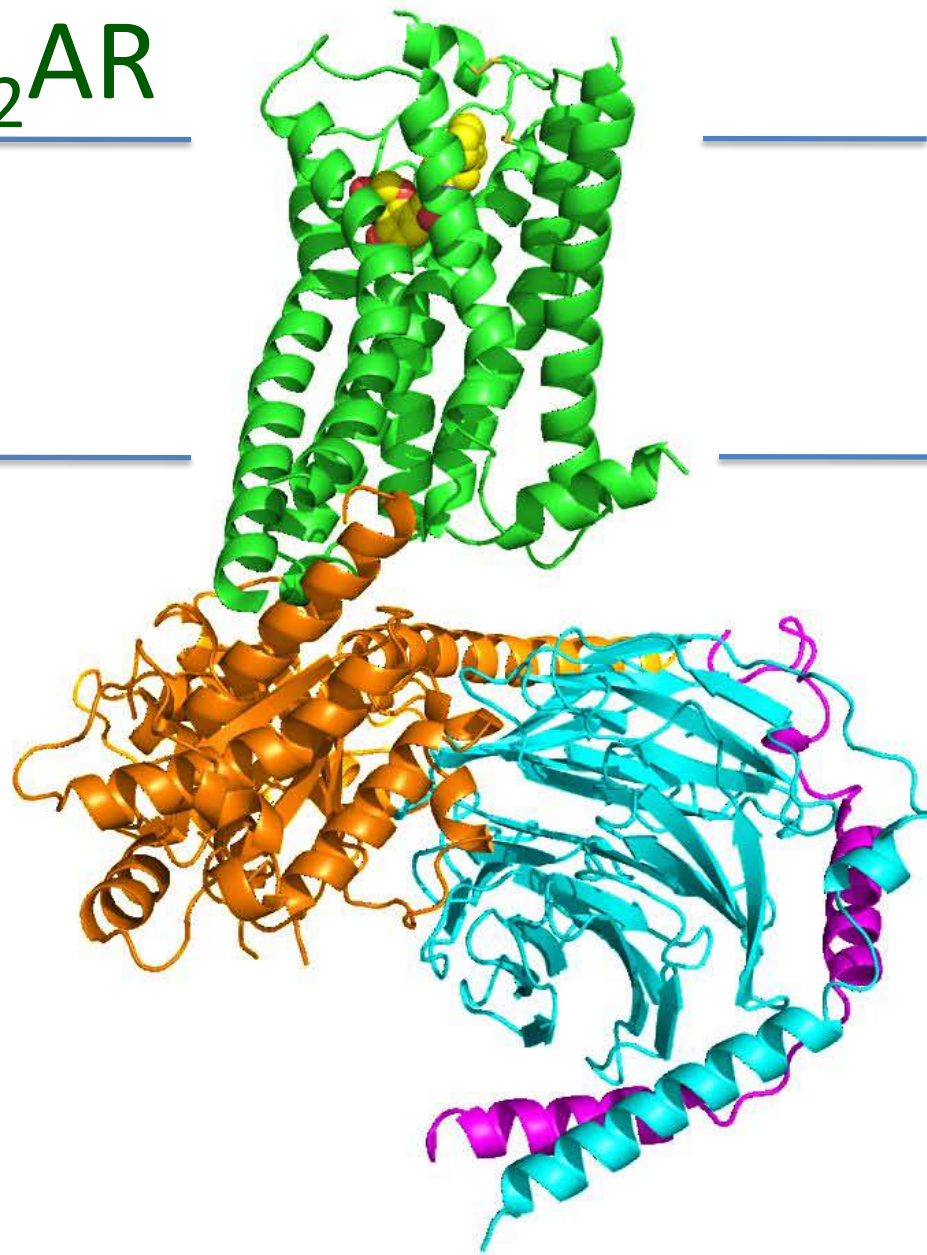
β_2 AR-Gs complex

Andy Kruse, Brian DeVree, Søren Rasmussen
me

Roger Sunahara

Returning from Argonne with final data set April 2011

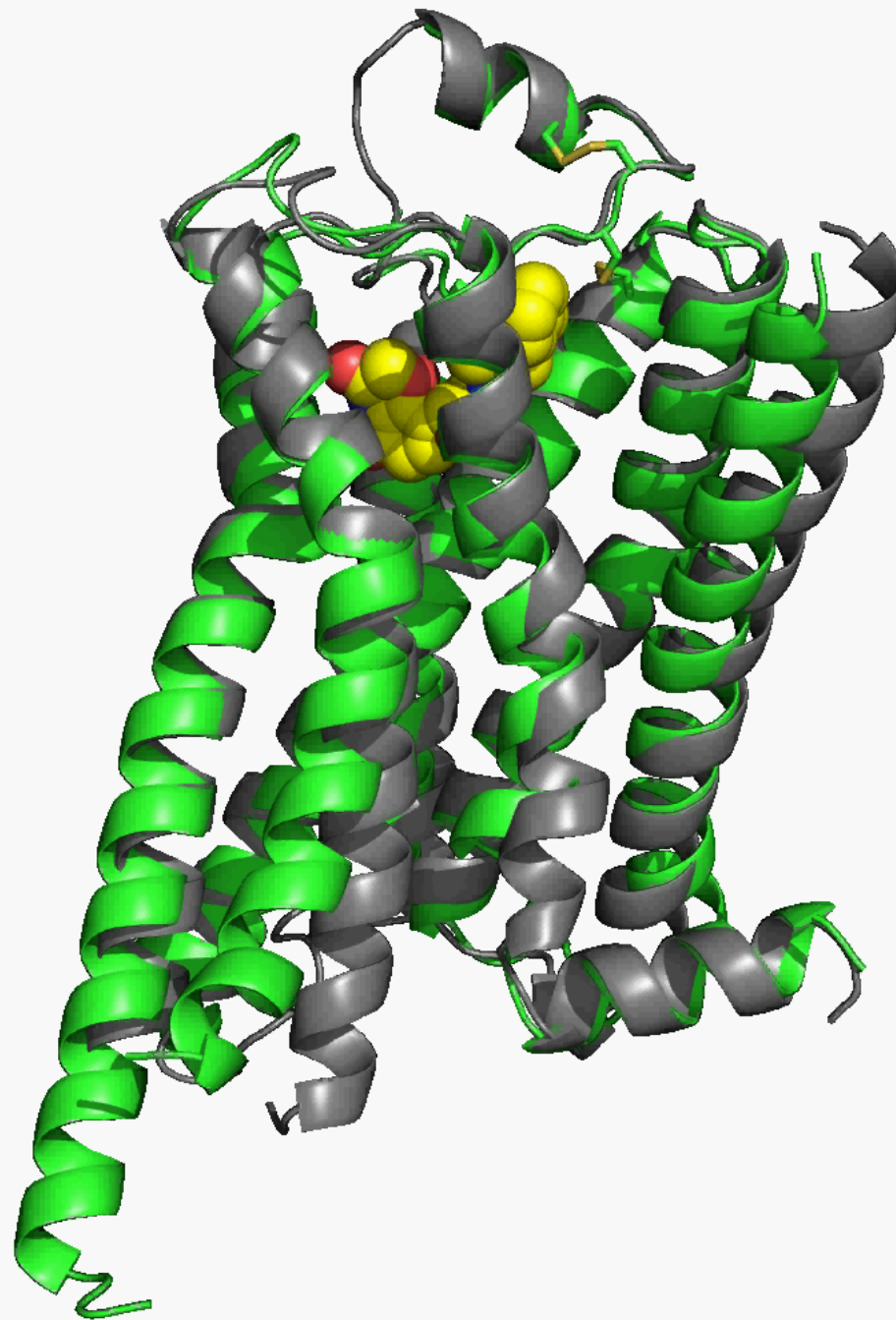
β_2AR



Gs $\alpha\beta\gamma$

Inactive

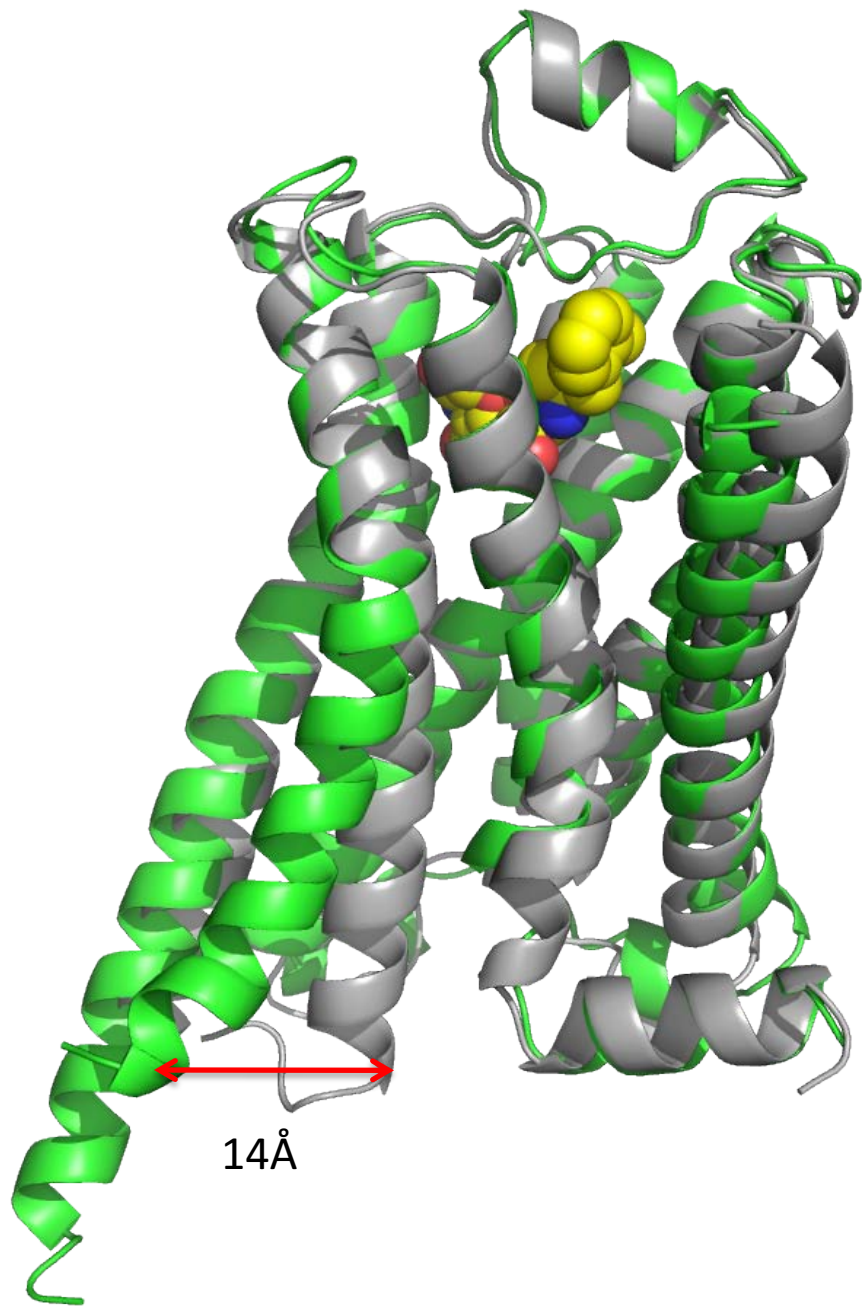
Active



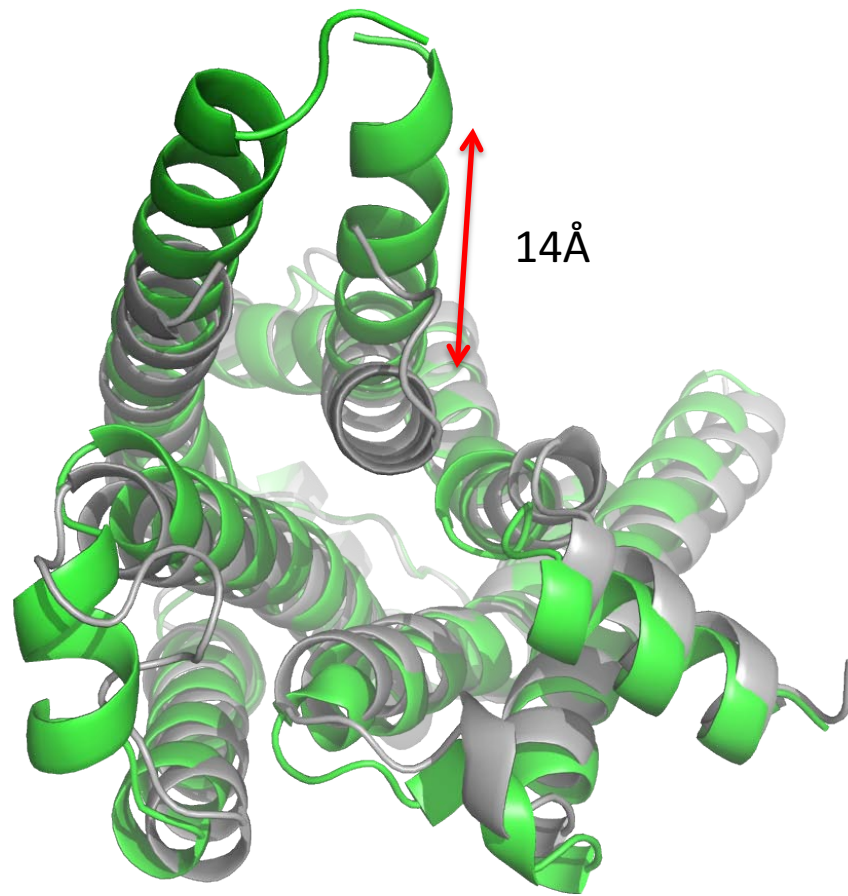
β_2 AR-Cz

β_2 AR-Gs

Active state of β_2 AR



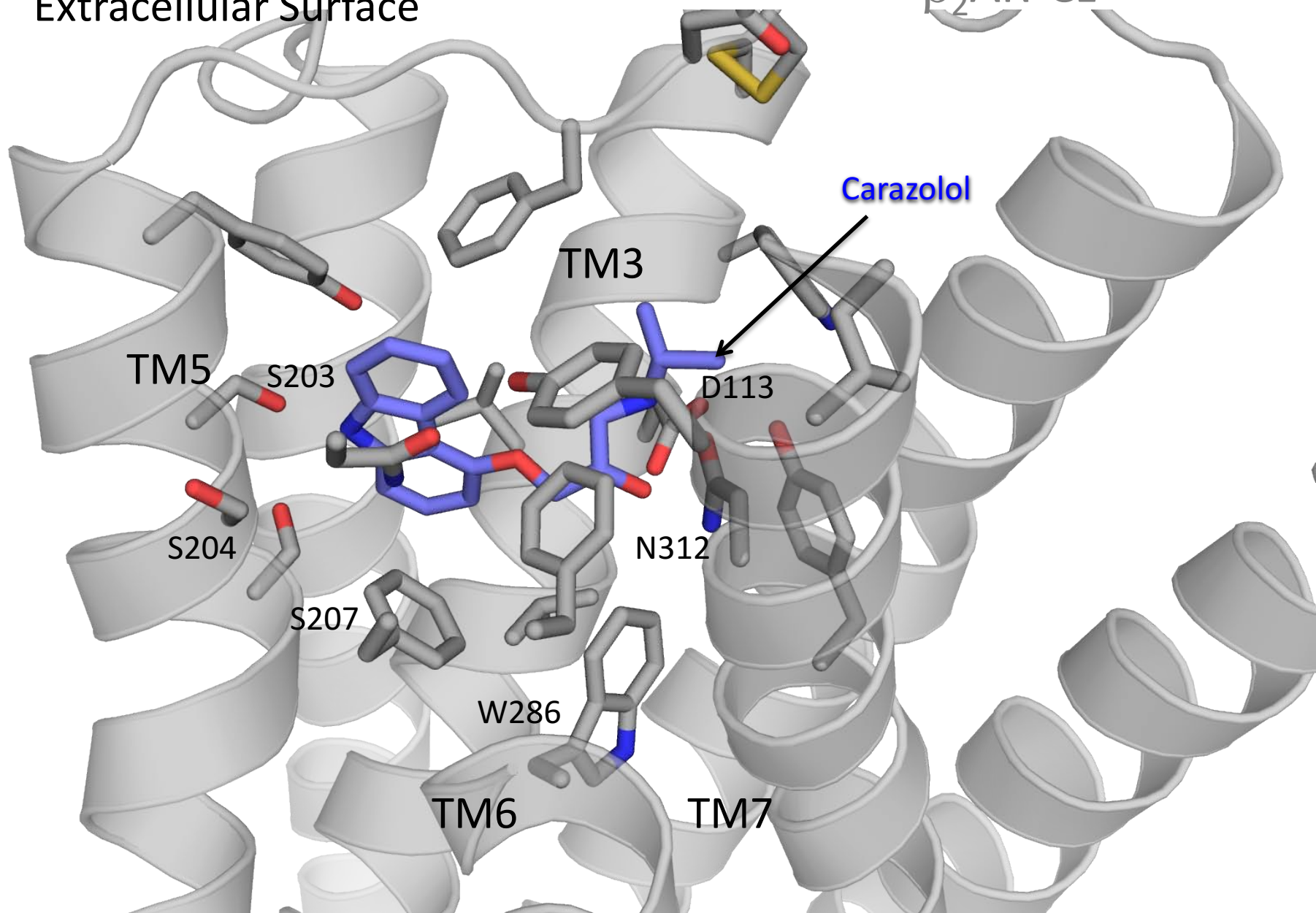
Cytoplasmic View



β_2 AR - Inactive
 β_2 AR-Gs

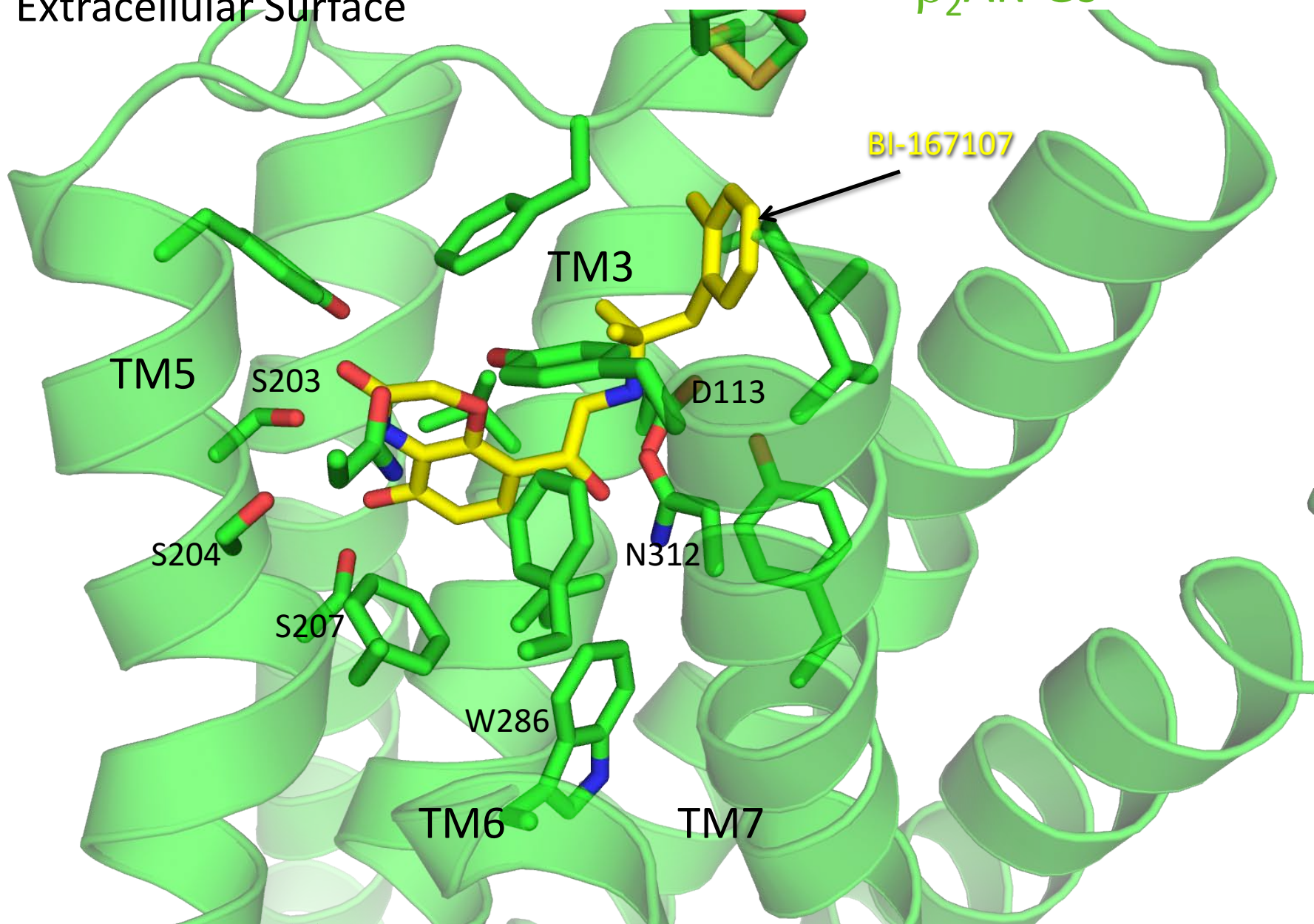
Extracellular Surface

β_2 AR-Cz

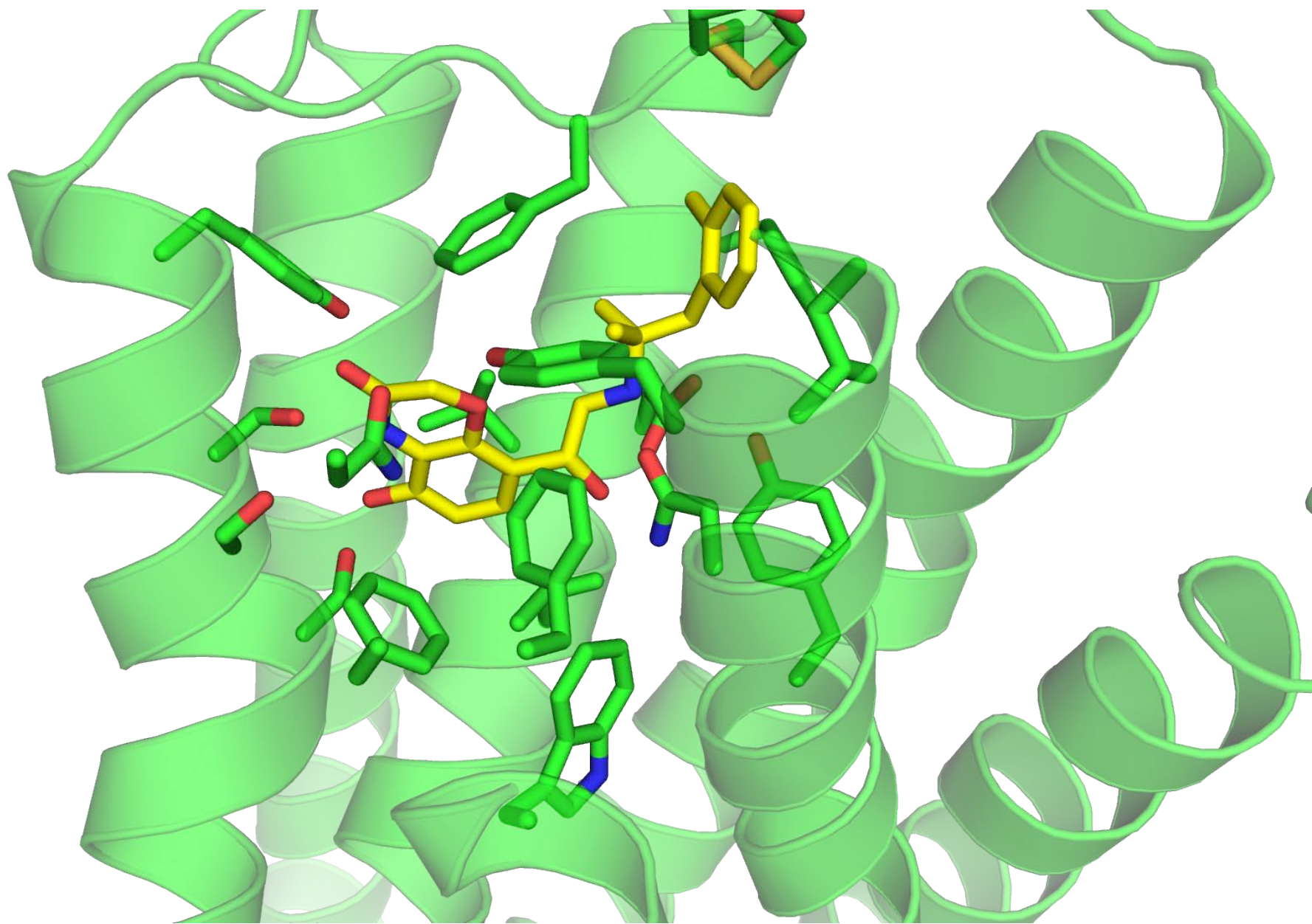


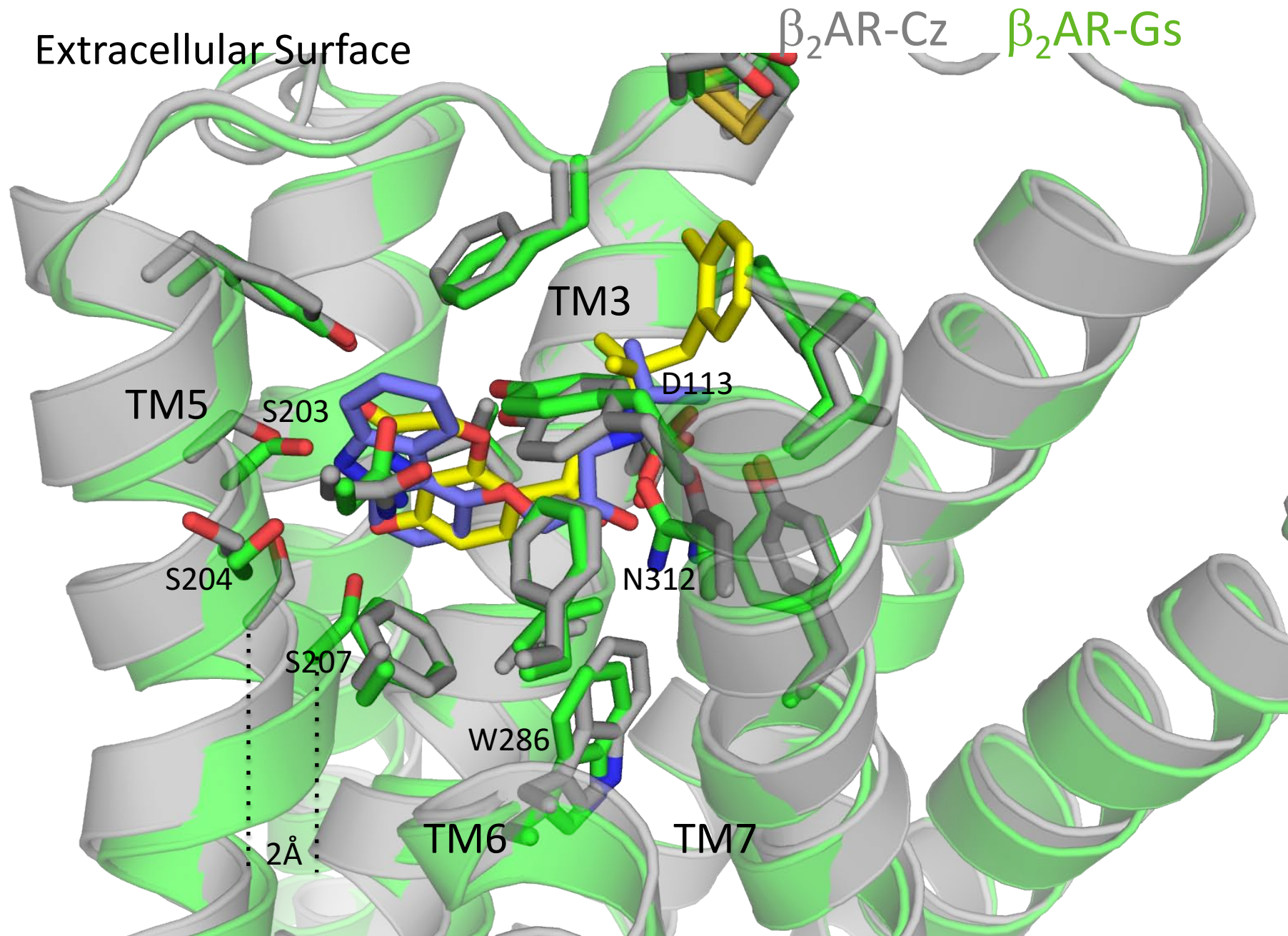
Extracellular Surface

β_2 AR-Gs



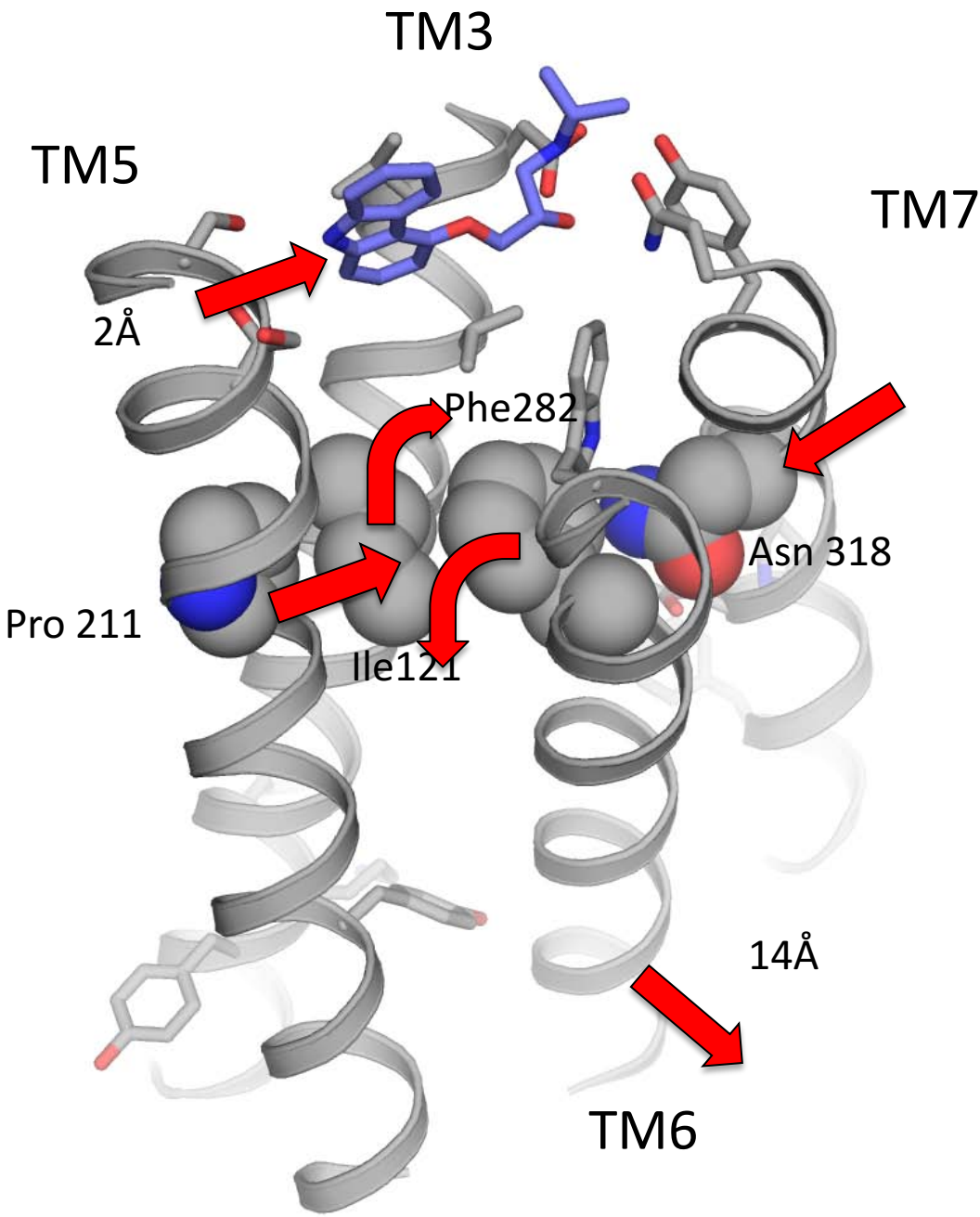
Active





Extracellular

β_2 AR-Inactive



Packing of conserved amino acids maintains inactive state.

Extracellular

β_2 AR-Active

TM5

TM3

TM7

2Å

Weak coupling between ligand binding and G protein coupling domains

Pro 211

Ile121

Asn 318

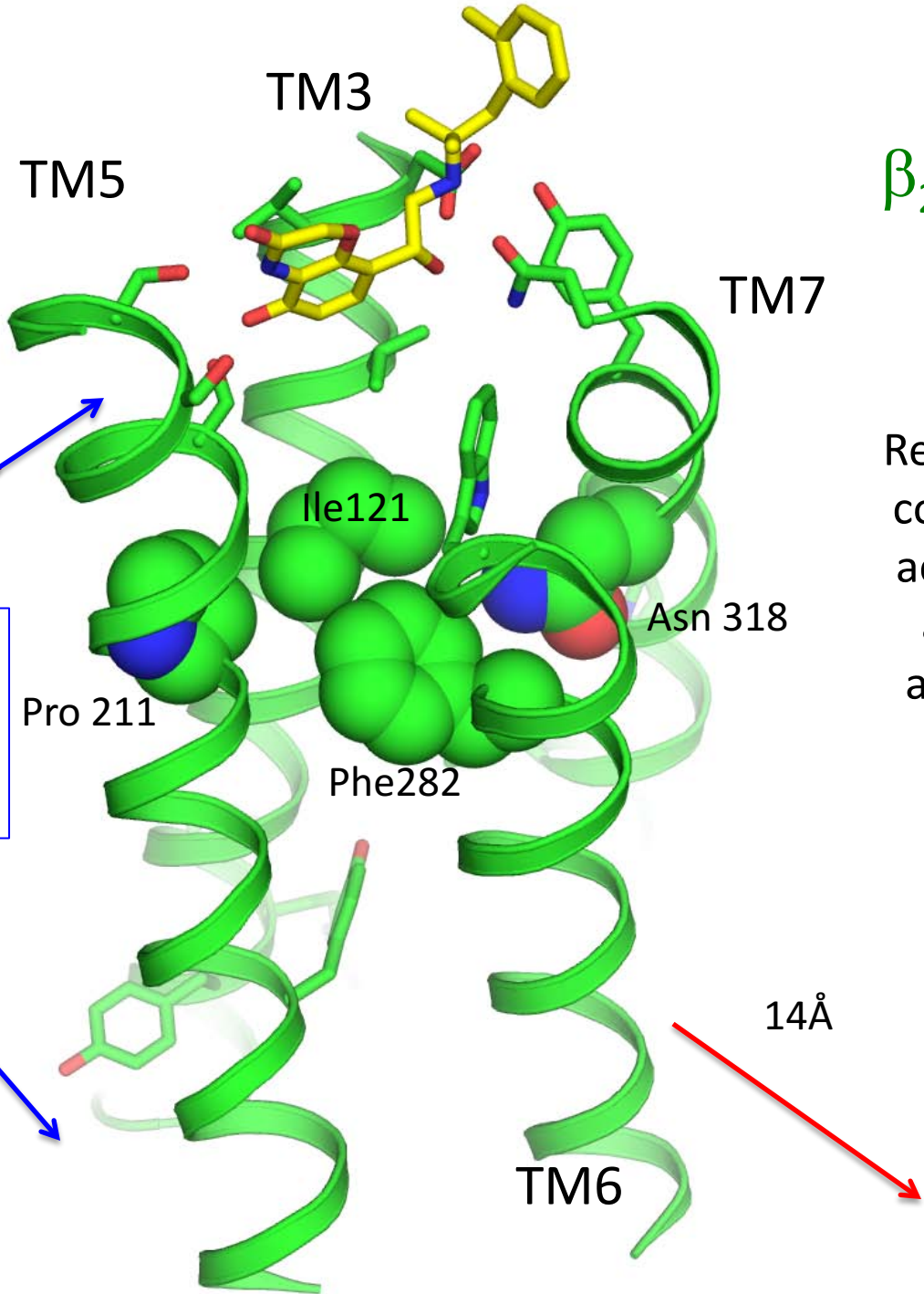
Phe282

Rearrangement of conserved amino acids required to accommodate agonist binding.

14Å

Cytoplasm

TM6



β_2AR

$Gs\alpha\beta\gamma$

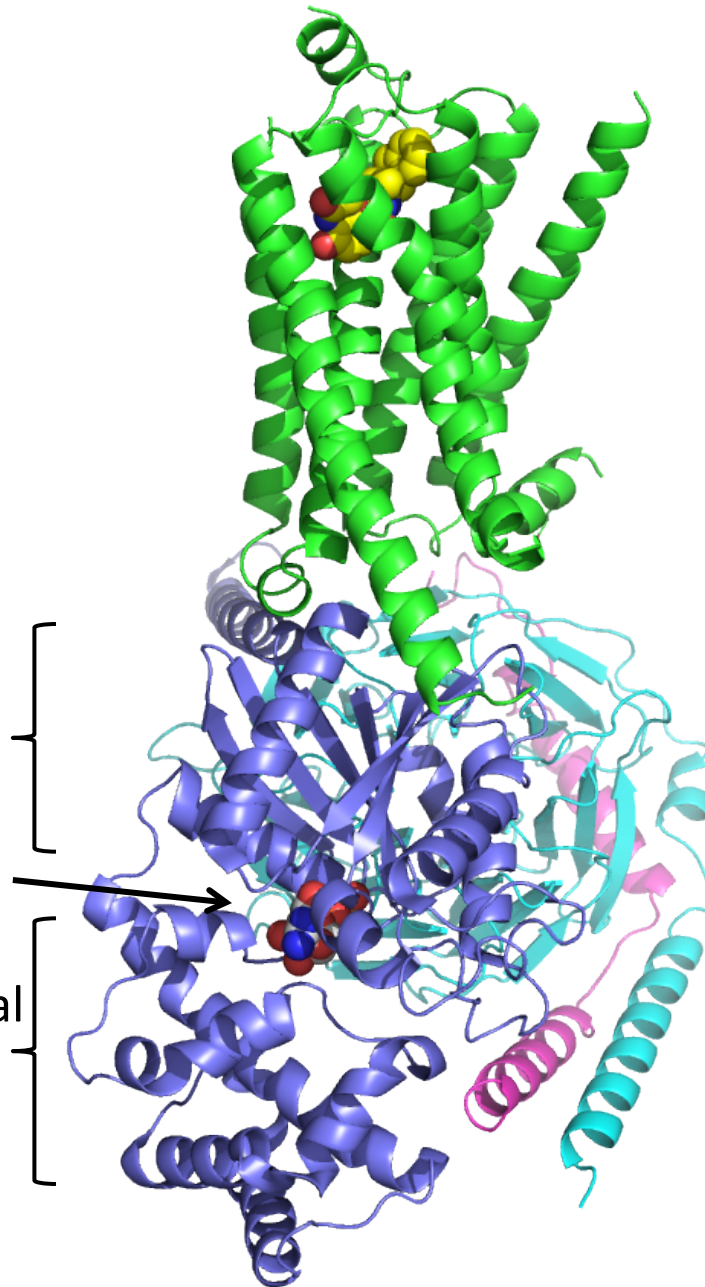
Inactive

$G\alpha$
Ras-like
domain

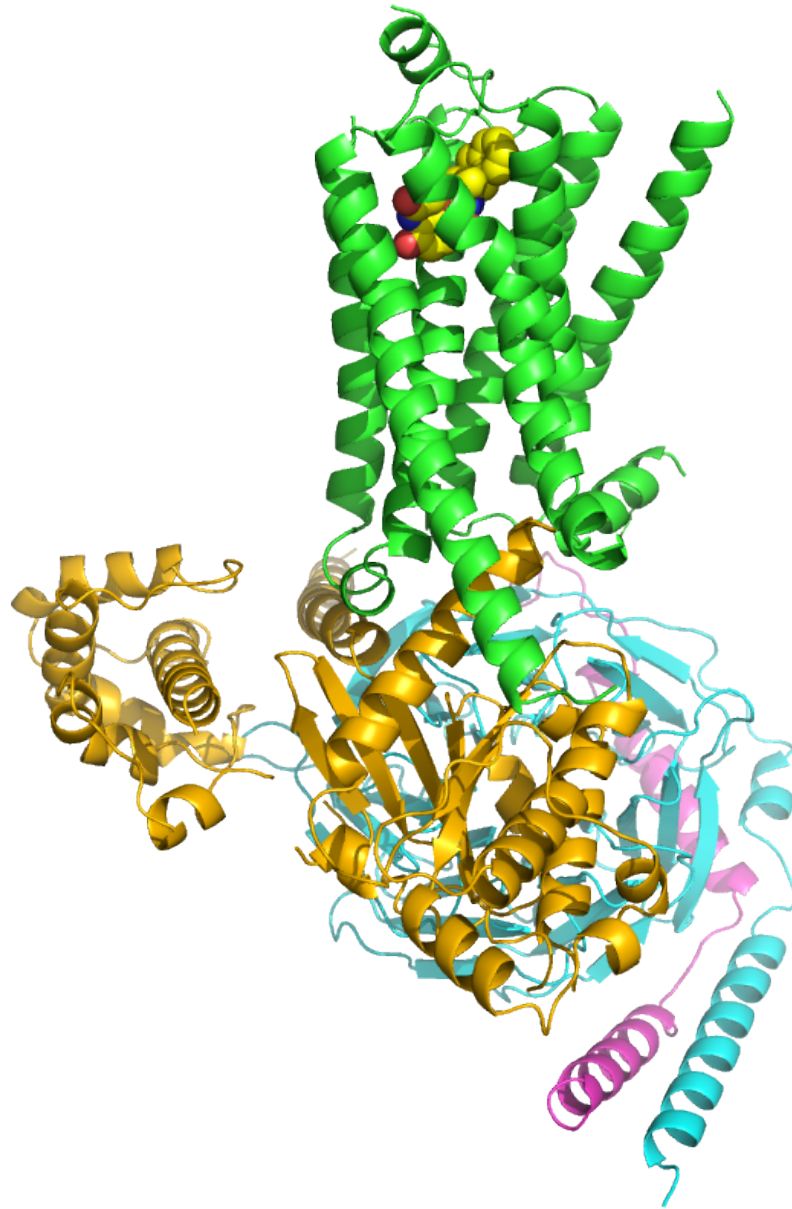
GDP

α -helical
domain

Interactions between
the β_2AR and Gs
promote GDP release....



β_2 AR

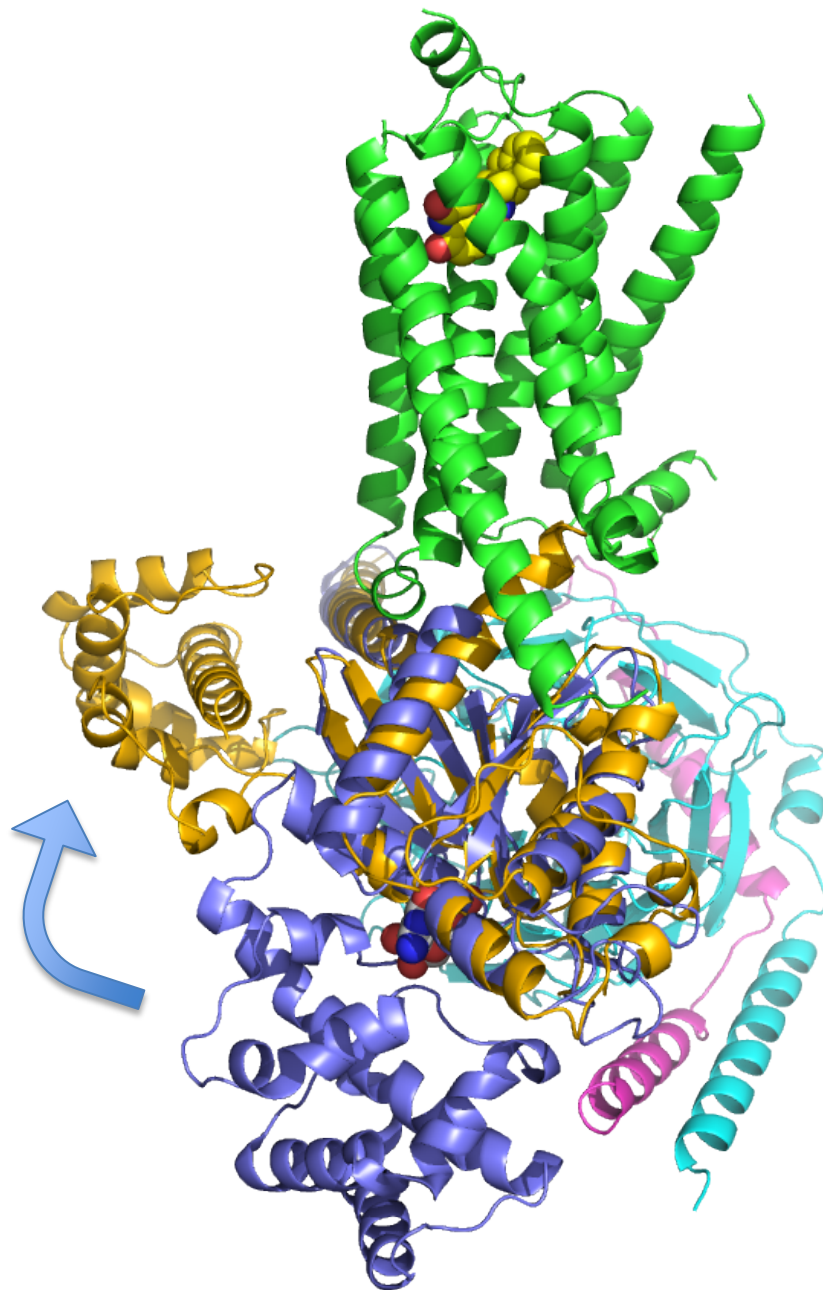


G $\alpha\beta\gamma$

Active

β_2AR

α -helical
domain

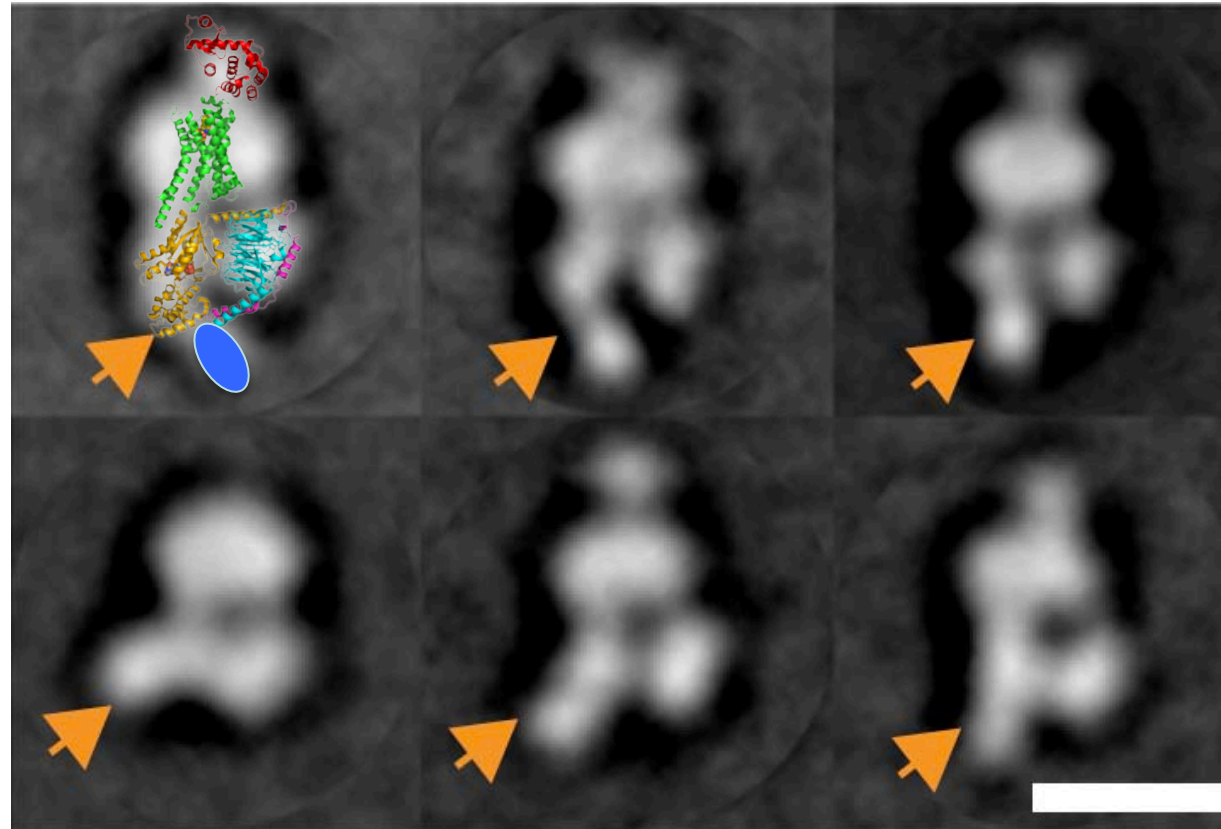
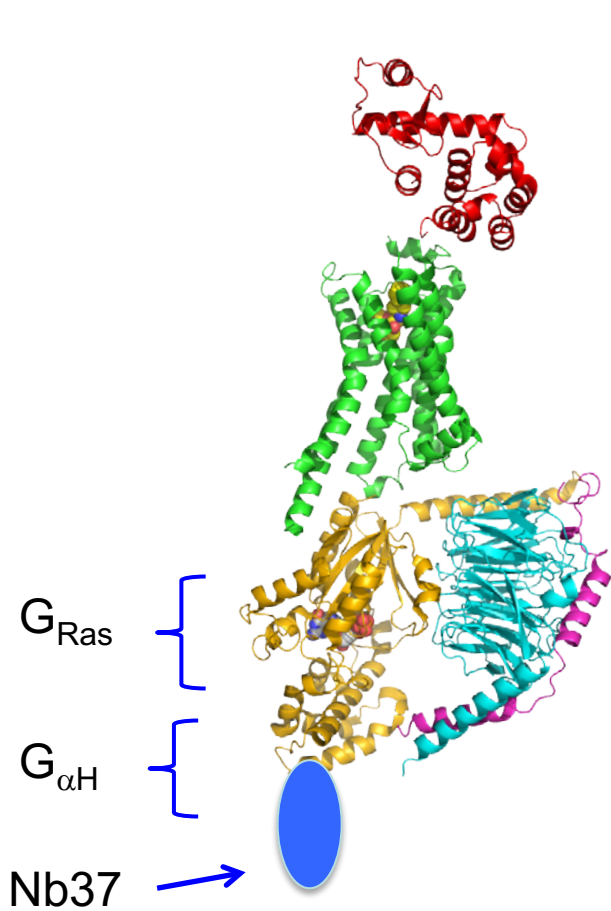


G $\alpha\beta\gamma$

Active

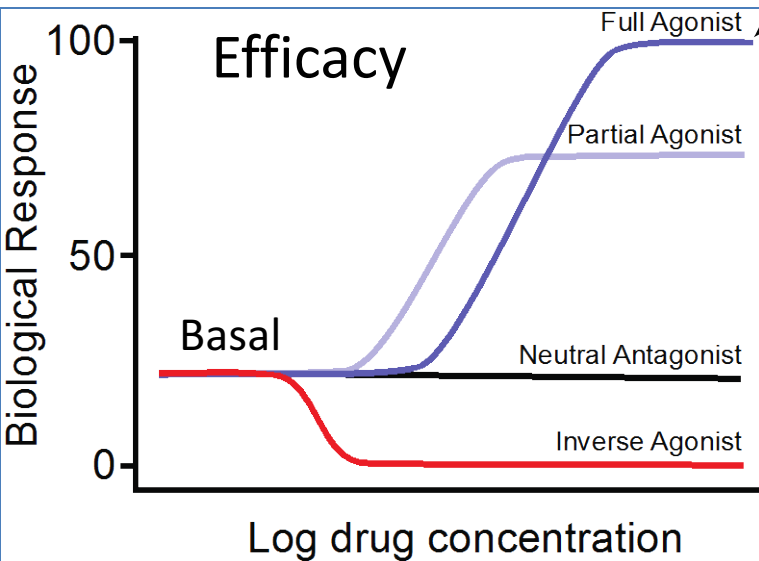
Inactive

Mobility of alpha helical domain confirmed by EM



Gerwin Westfield and Georgios Skiniotis, Univ. Michigan

Future Directions



Recycling back to membrane

Targeted for degradation in lysosomes

MAP kinase pathway

Gene expression

β_2 AR-Gs Team



GPCR Workshop, Maui, Dec. 2011

Many Thanks

- Tong Sun Kobilka
- Bob Lefkowitz and colleagues in the Lefkolab
- Kobilka lab students, postdoctoral fellows and collaborators (1989-2012)
- Bill Weis and Roger Sunahara

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Roger Sunahara

University of Wisconsin

Pil Seok Chae
Sam Gellman

Free University of Brussels

Els Pardon
Jan Steyaert

Trinity College Dublin

Joseph Lyons
Syed Shah
Martin Caffrey

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We will miss Virgil Woods (UCSD) , 1948-2012