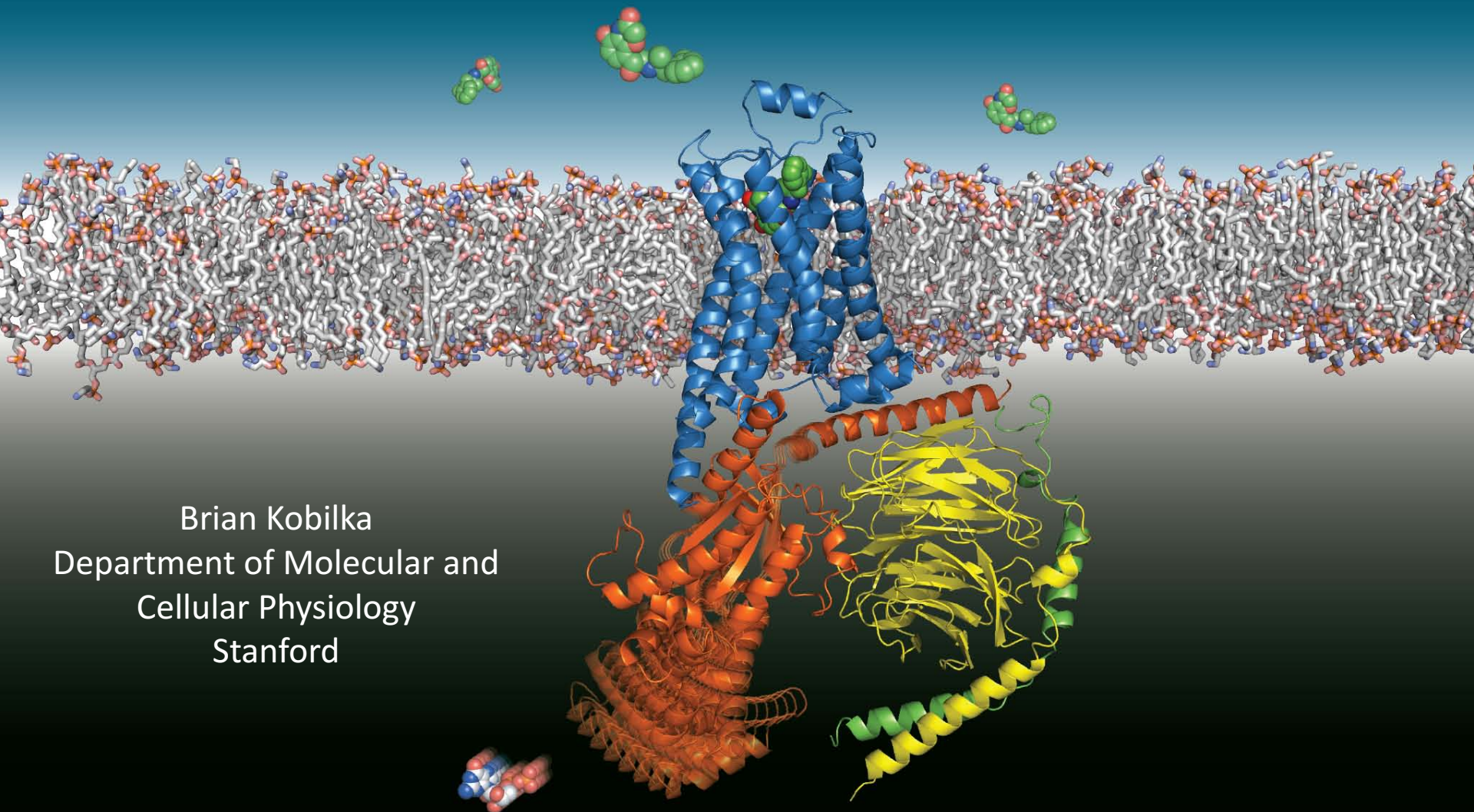
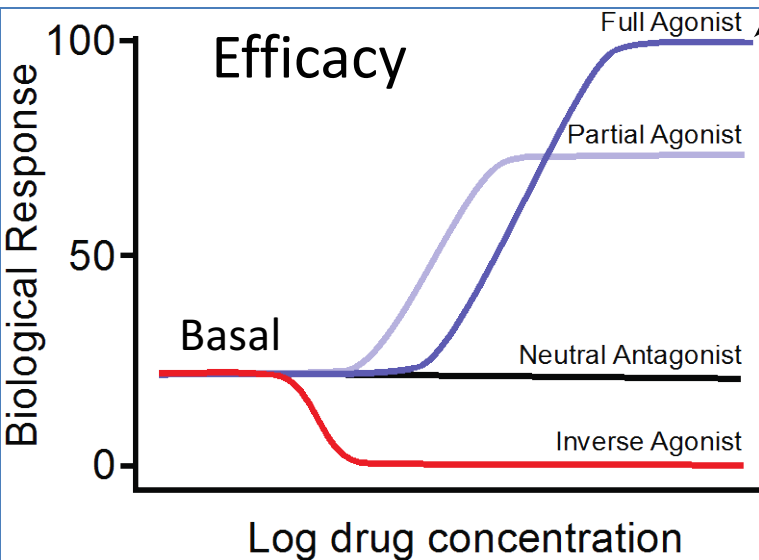
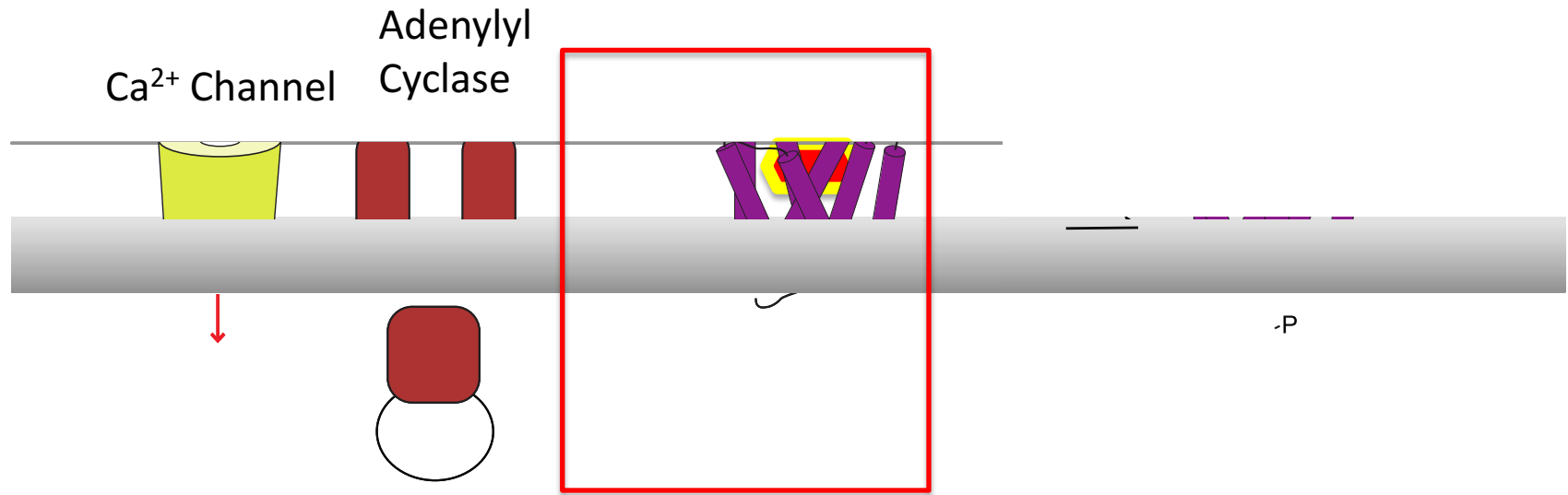


The structural basis of G protein coupled receptor signaling



Brian Kobilka
Department of Molecular and
Cellular Physiology
Stanford

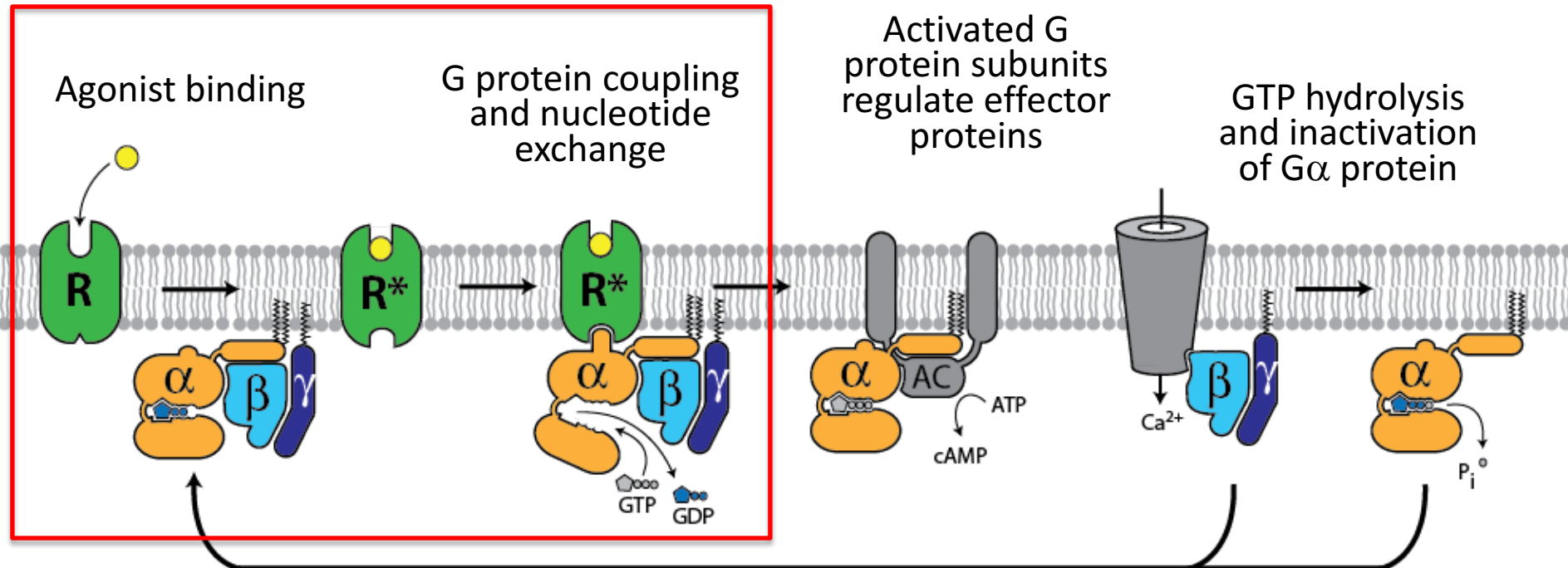
The β_2 AR modulates the activity of multiple signaling pathways



Recycling back to membrane

Targeted for degradation in lysosomes

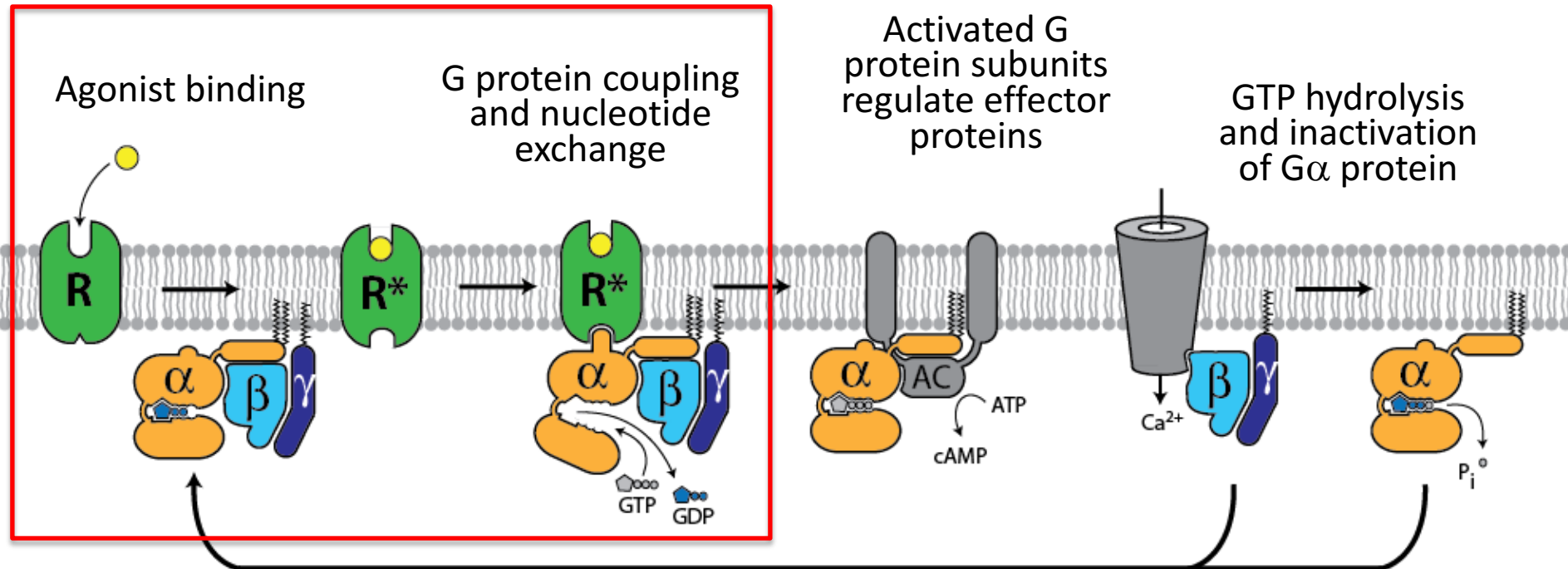
MAP kinase pathway
Gene expression



GPCR-G Protein Cycle

Outline

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



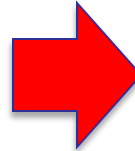
GPCR-G Protein Cycle

Approaches to characterizing β_2 AR structure:

- Sequence analysis
 - secondary structure
(transmembrane domains)

Cloned DNA Sequence (1986)

GAATTCATGCCGCGTTTCTGTGTTGGACAGGGGTGACTTTGTGCC
GGATGGCTTCTGTGTGAGAGCGCGCGAGTGTGCATGTCGGTGA
GCTGGGAGGGTGTGTCTCAGTGTCTATGGCTGTGGTTCCGGTATAAG
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Amino Acid Sequence

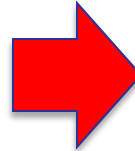
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Approaches to characterizing β_2 AR structure:

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 - secondary structure
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Cloned DNA Sequence (1986)

GAATTCATGCCGCGTTTCTGTGTTGGACAGGGGTGACTTTGTGCC
GGATGGCTTCTGTGTGAGAGCGCGCGCAGTGTGCATGTCGGTGA
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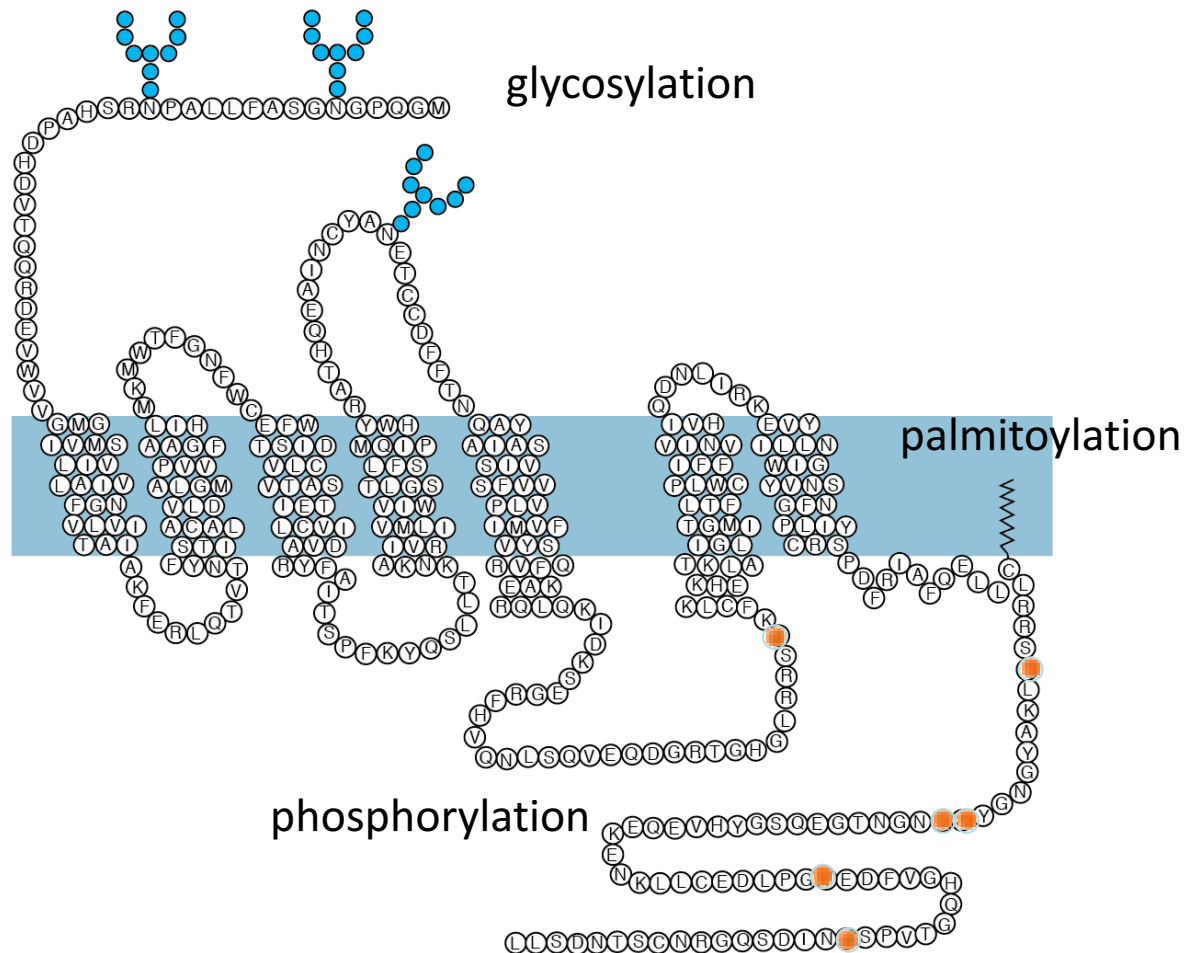


Amino Acid Sequence

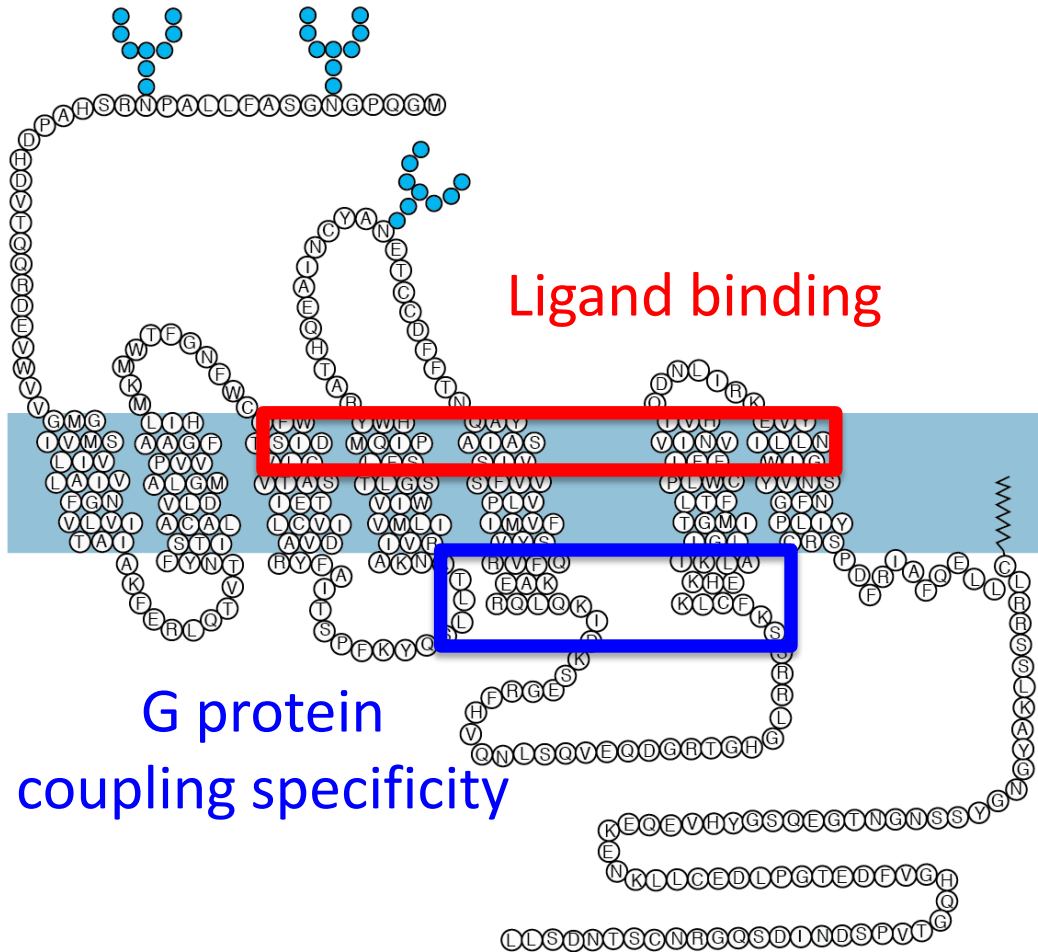
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SGYHVEQEKENKLLCEDLPGTEDFVGH
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Approaches to characterizing β_2 AR structure:

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



Approaches to characterizing β_2 AR structure:



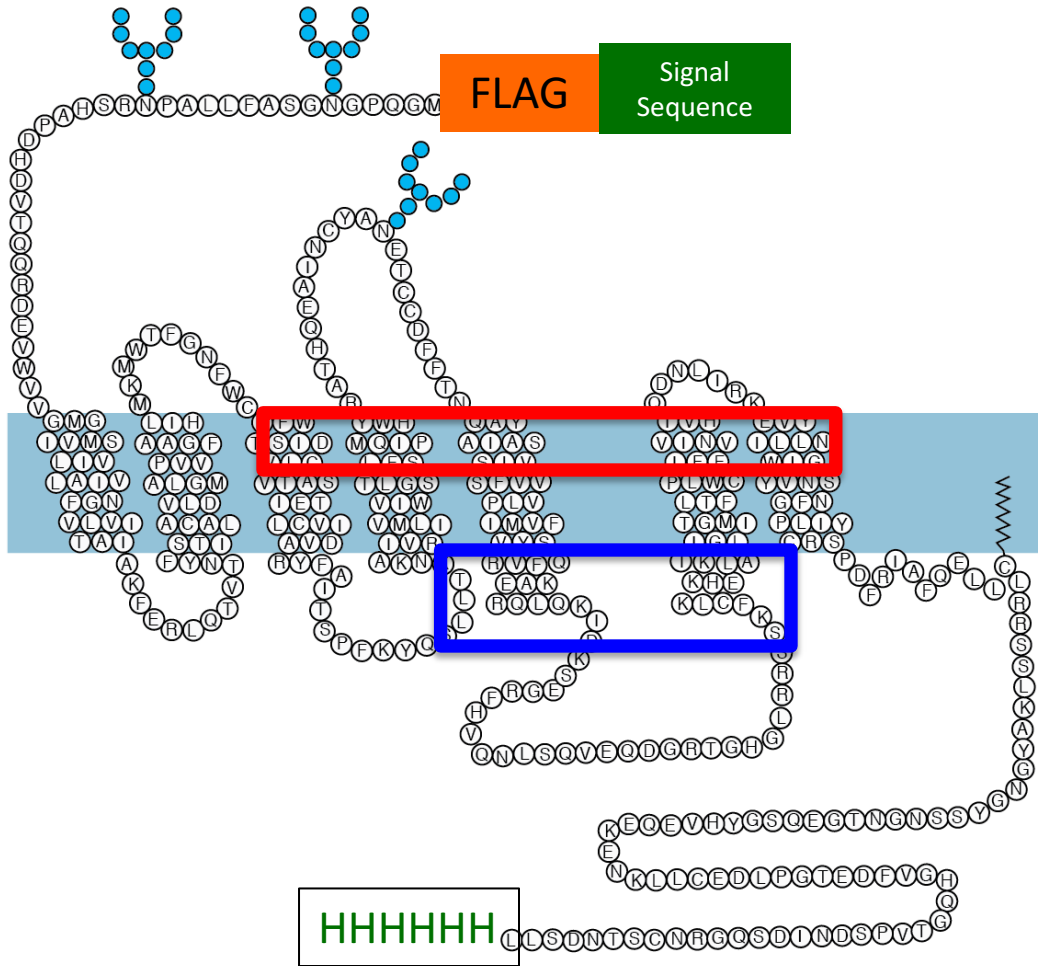
- Sequence analysis

- 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:



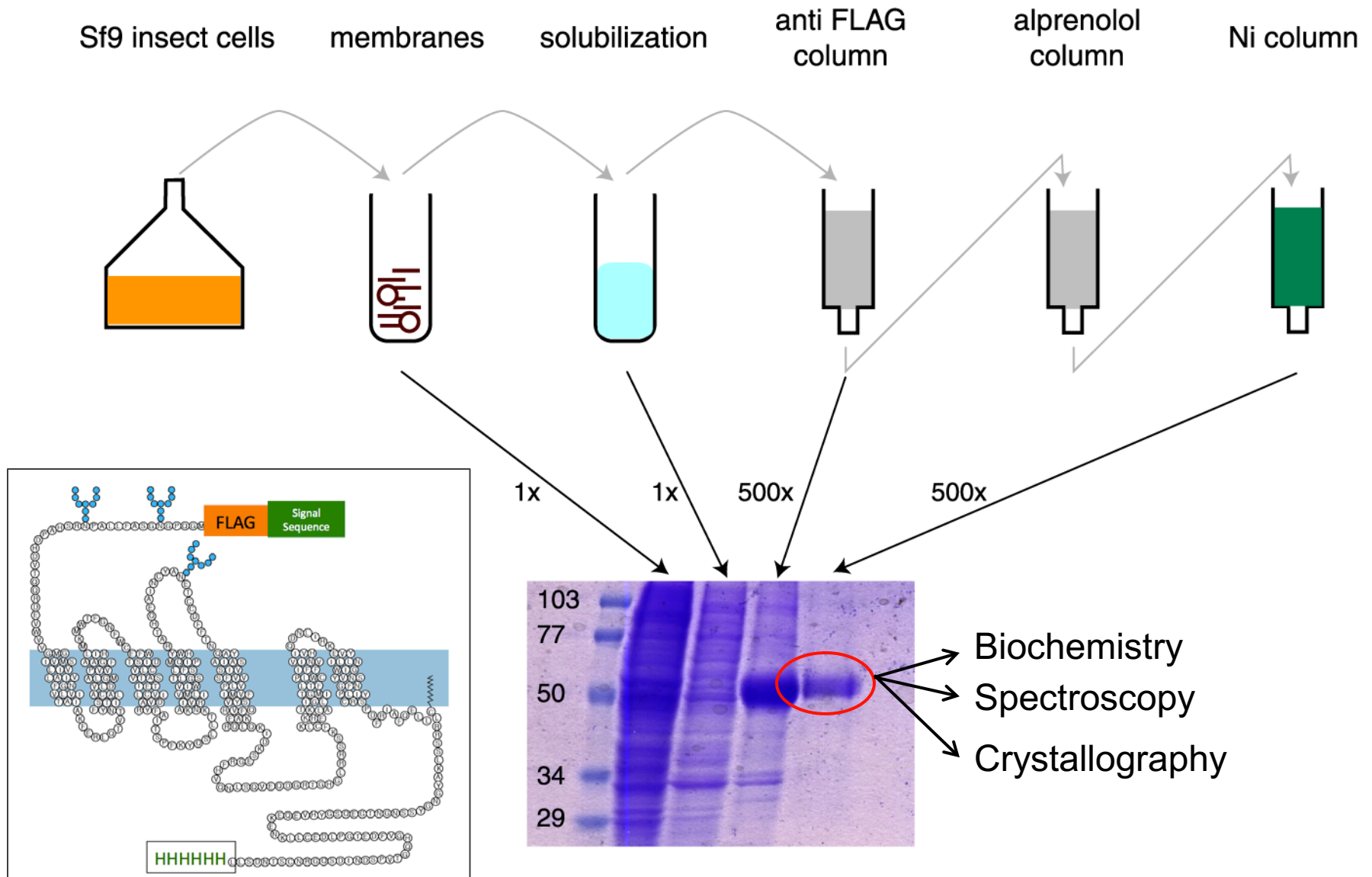
- Sequence analysis

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

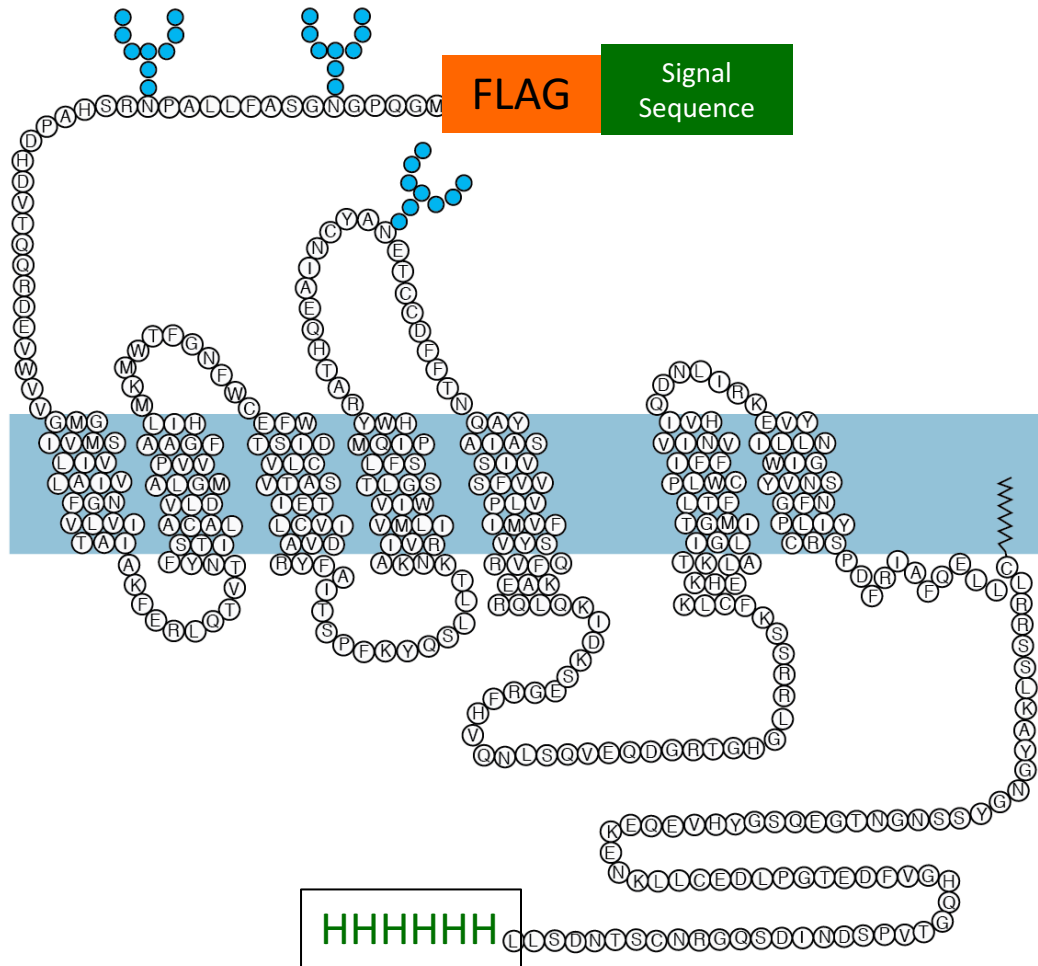
•Chimeric Receptors and Site-directed mutagenesis

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

Expression and purification



Approaches to characterizing β_2 AR structure:



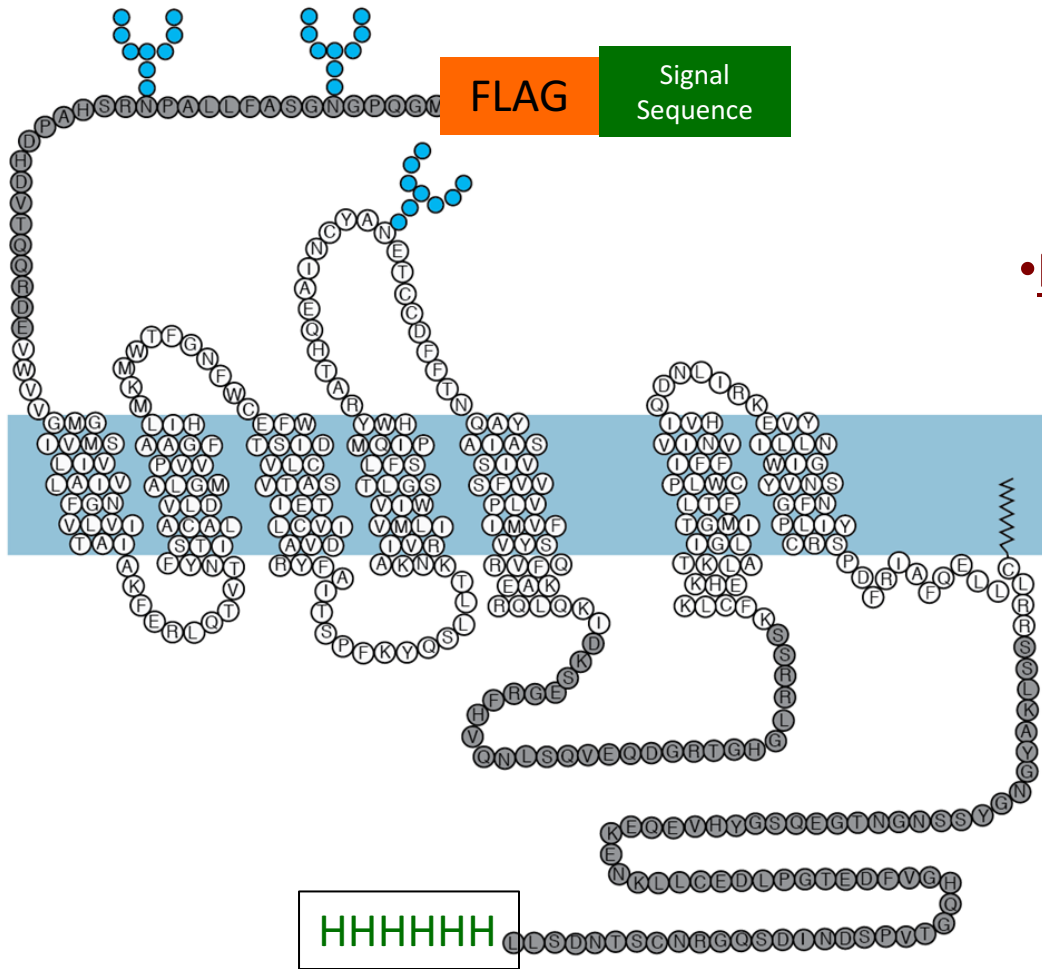
• Sequence analysis

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

• Chimeric Receptors and site-directed mutagenesis

- ligand binding and G protein coupling
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Approaches to characterizing β_2 AR structure:



- Sequence analysis

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

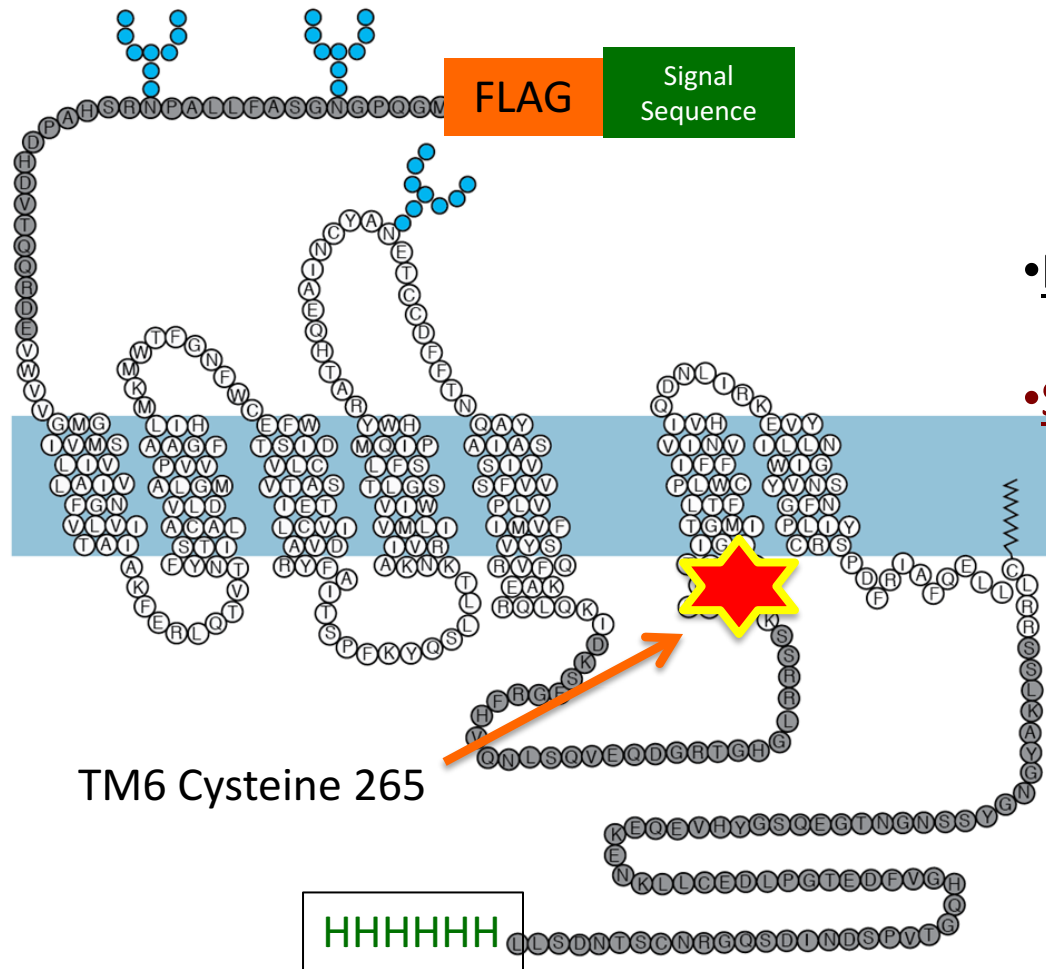
- Chimeric Receptors and site-directed mutagenesis

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

- Biochemistry

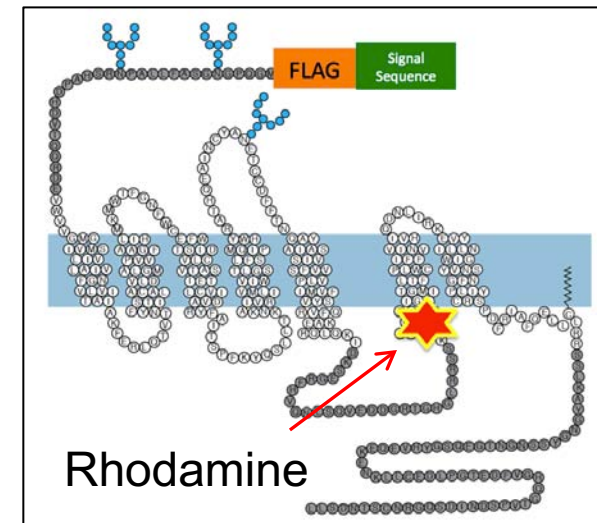
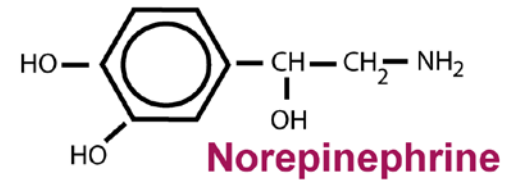
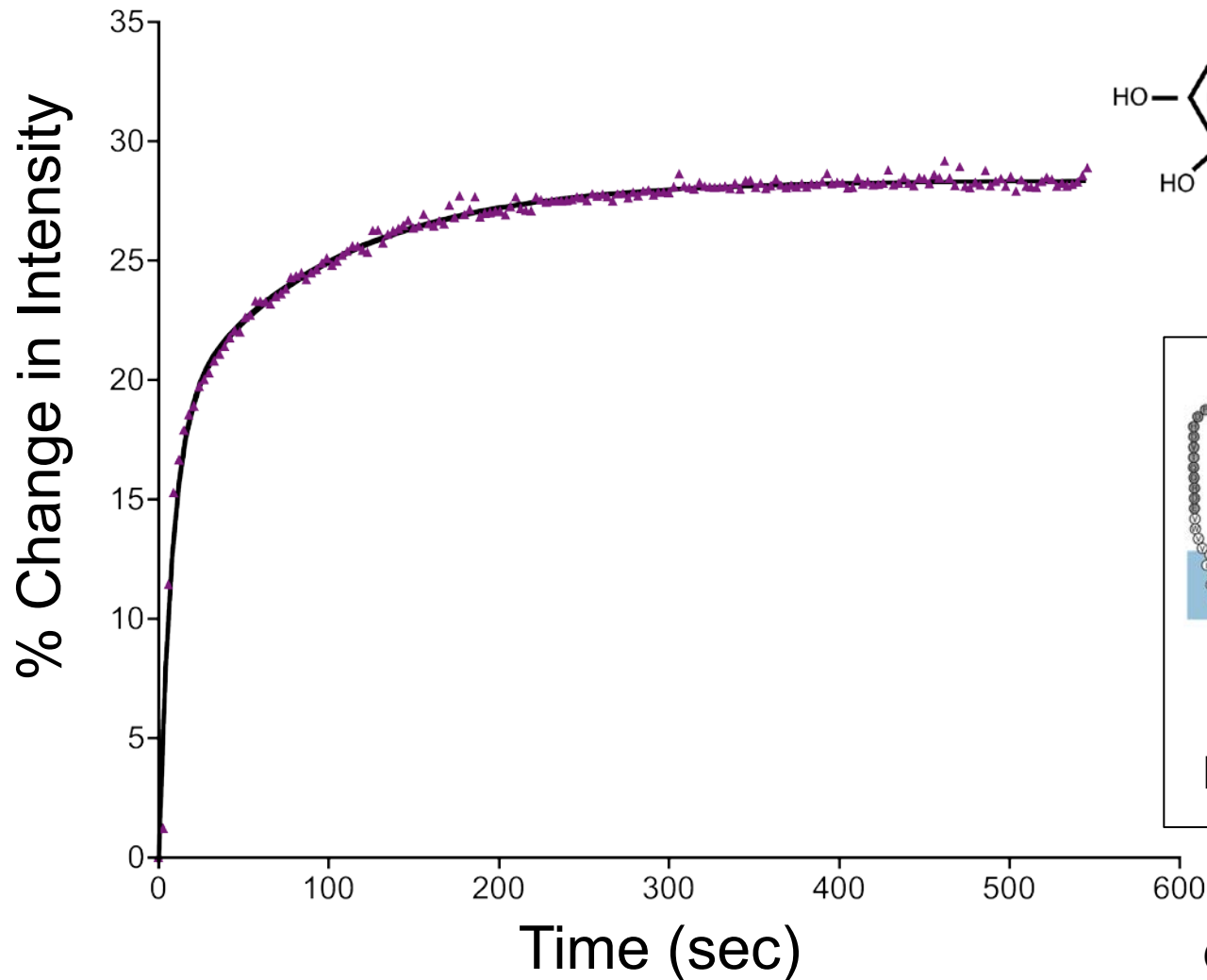
- unstructured, flexible sequence

Approaches to characterizing β_2 AR structure:

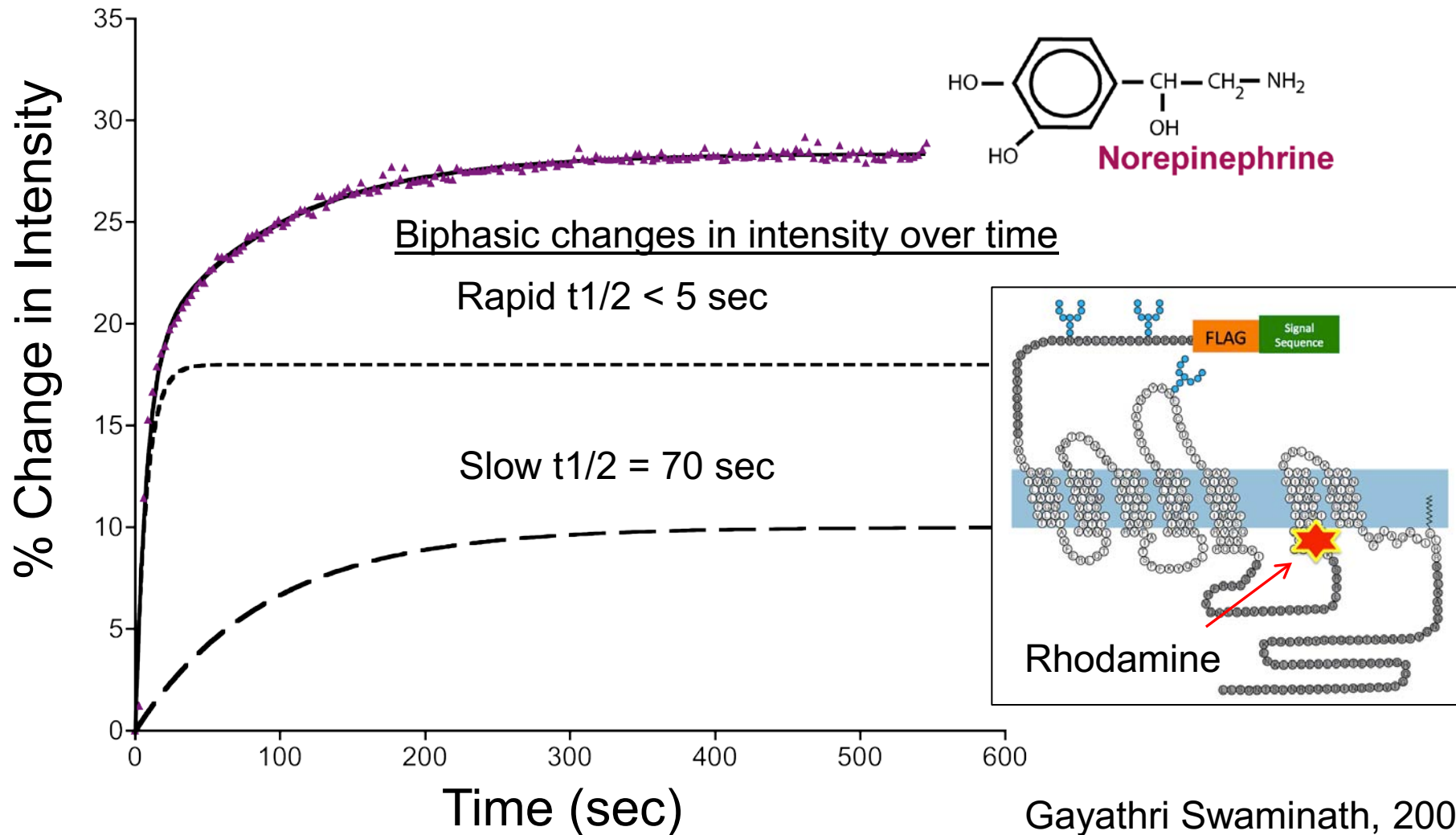


- Sequence analysis
 - secondary structure (transmembrane domains)
 - post-translational modifications
- Chimeric Receptors and site-directed mutagenesis
 - ligand binding and G protein coupling
 - enhance expression and purification
- Biochemistry
 - unstructured, flexible sequence
- Spectroscopy (Fluorescence,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

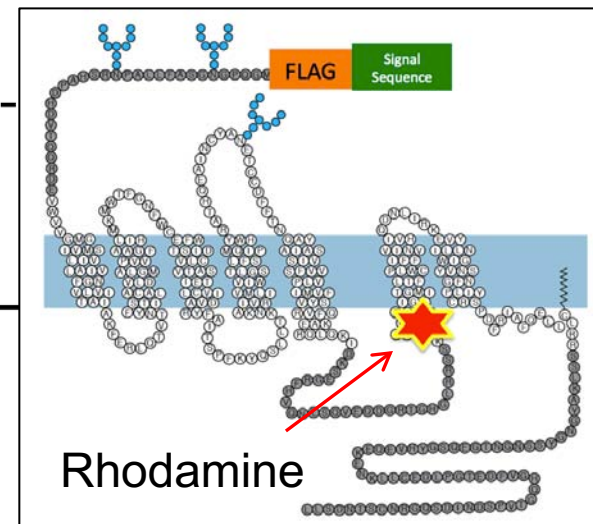
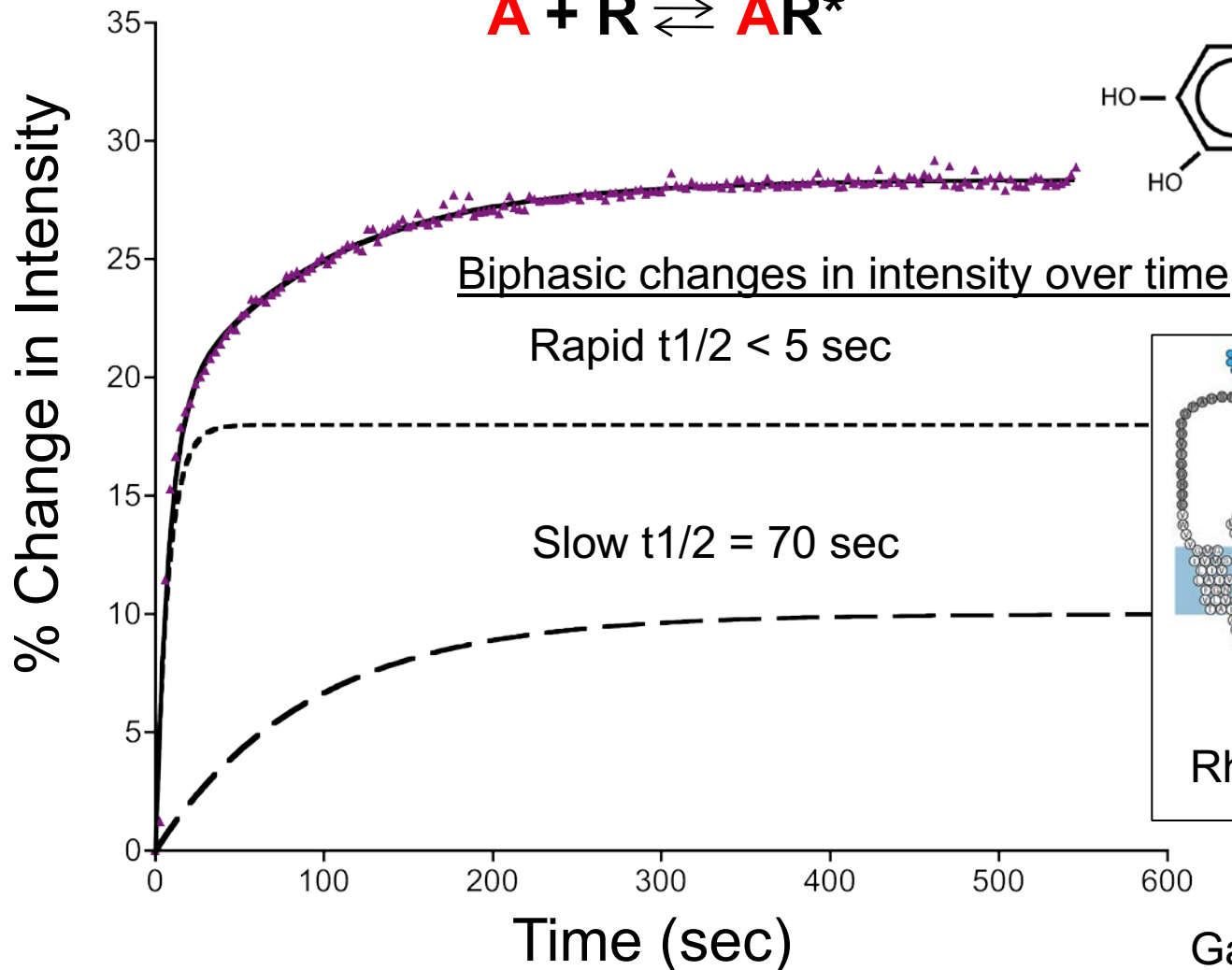
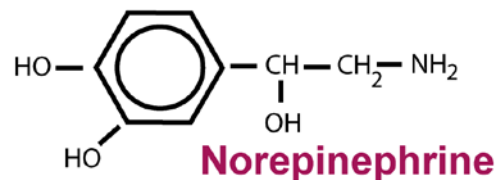
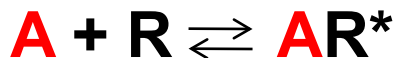
Conformational changes in TM6 in response to norepinephrine:



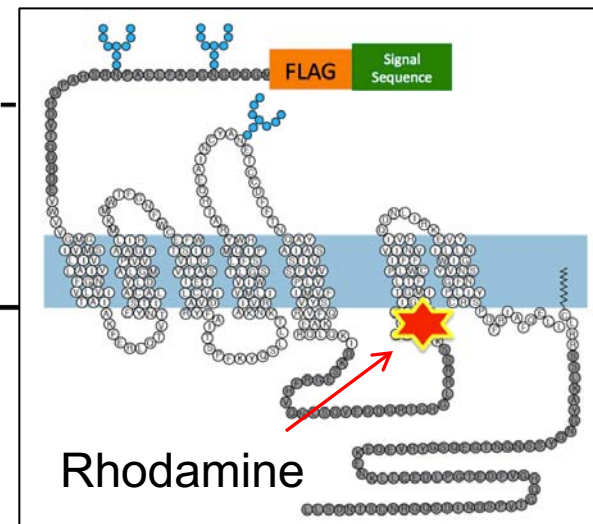
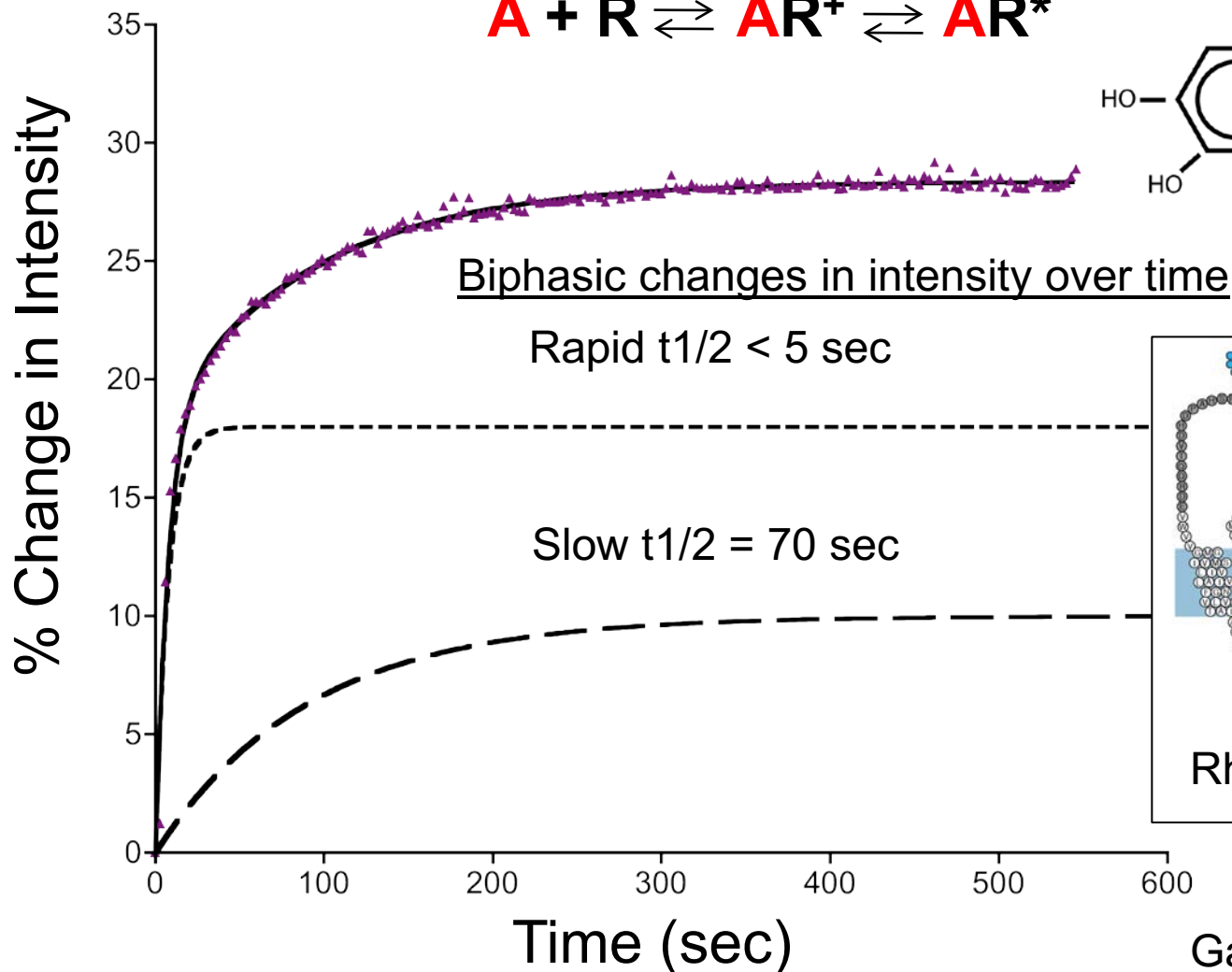
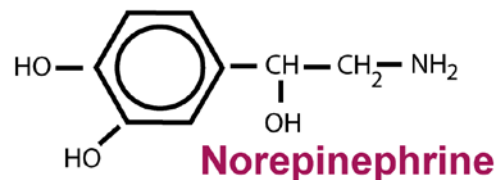
Conformational changes in TM6 in response to norepinephrine:

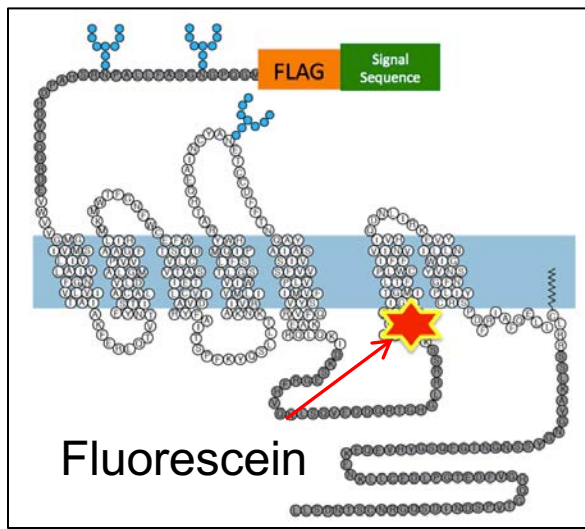


Conformational changes in TM6 in
response to norepinephrine:
Not consistent with single active state



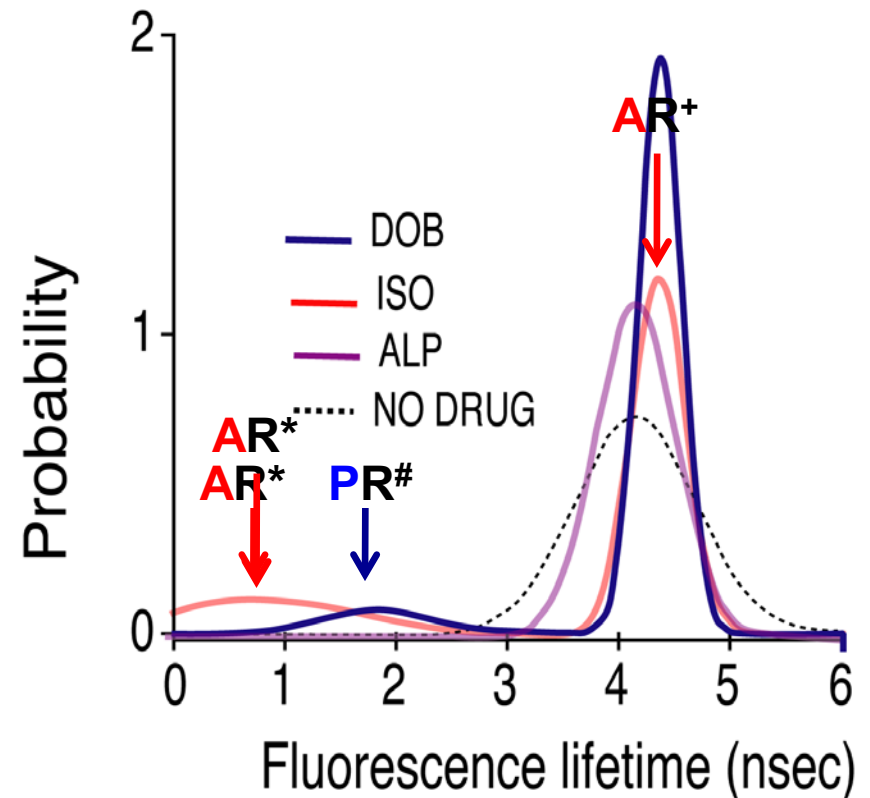
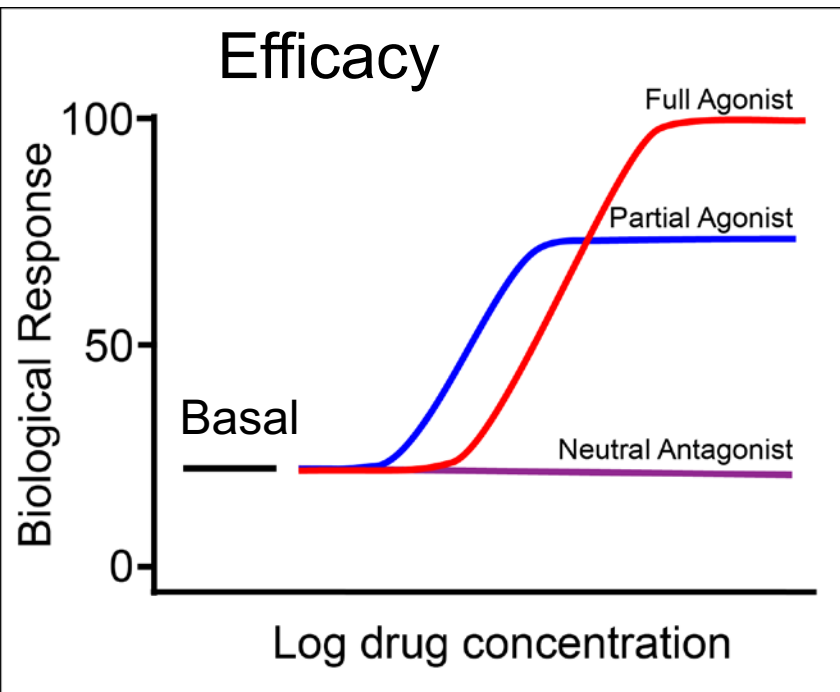
Conformational changes in TM6 in response to norepinephrine: Sequential conformational changes





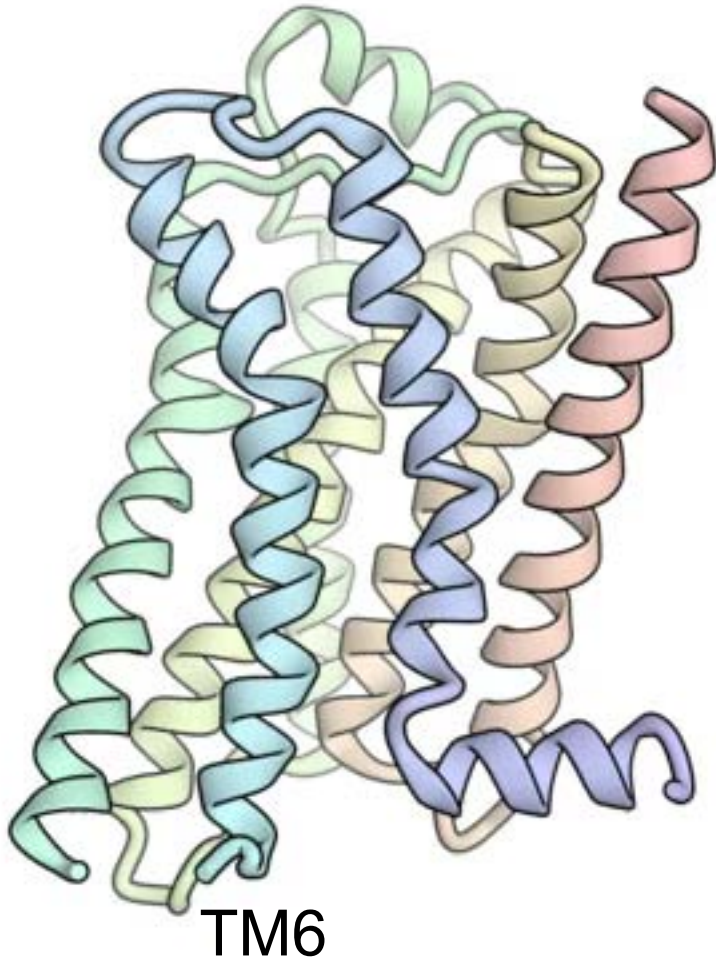
Fluorescence lifetime experiments

- Multiple agonist states
- Ligand-specific conformational states



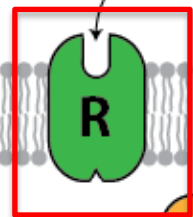
Insights from spectroscopy studies

-fluorescence, NMR, EPR-

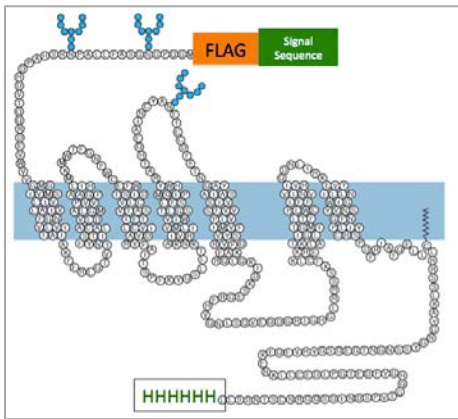


- The β_2 AR is flexible and dynamic
- TM6 undergoes the largest changes in response to agonists
- Agonist binding and activation occur through a series of conformational intermediates
- Agonists and partial agonists stabilize distinct conformational states
- Agonists alone do not stabilize a single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

- ## •GPCR crystallography



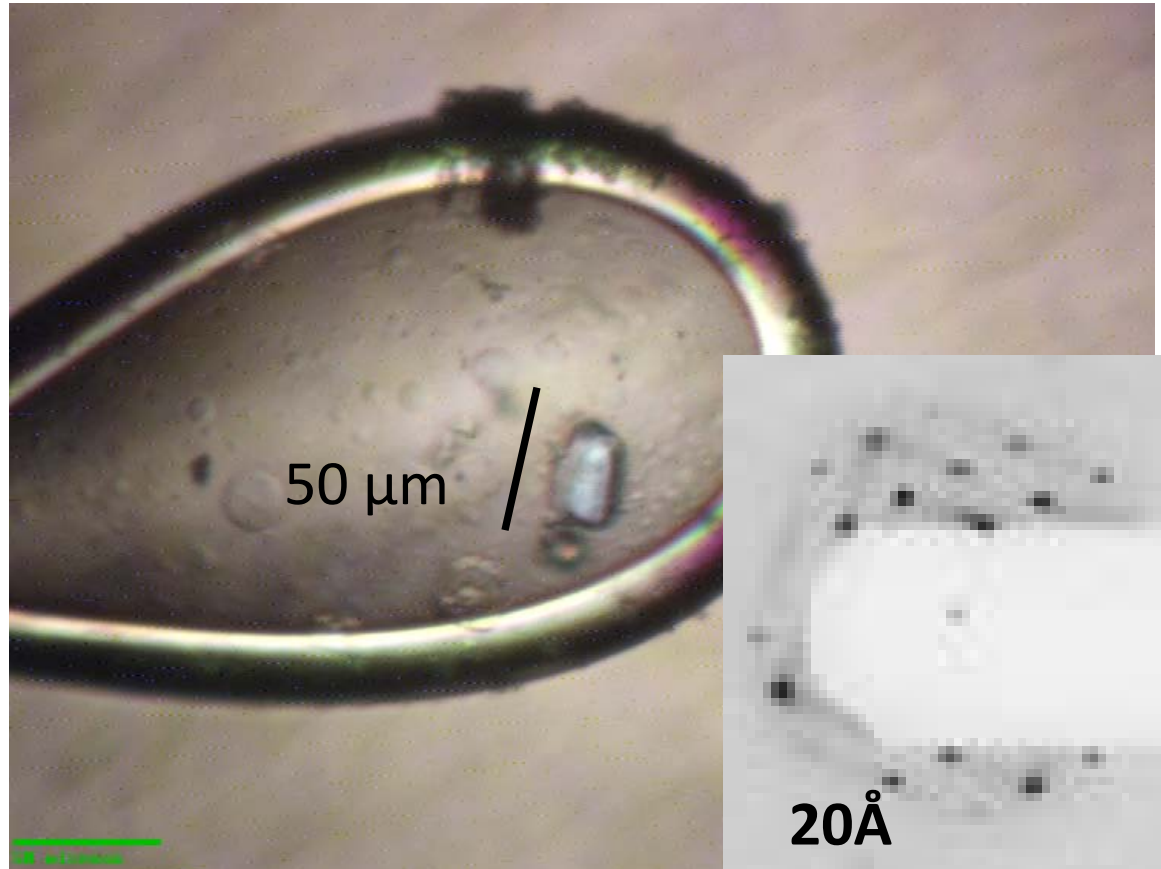
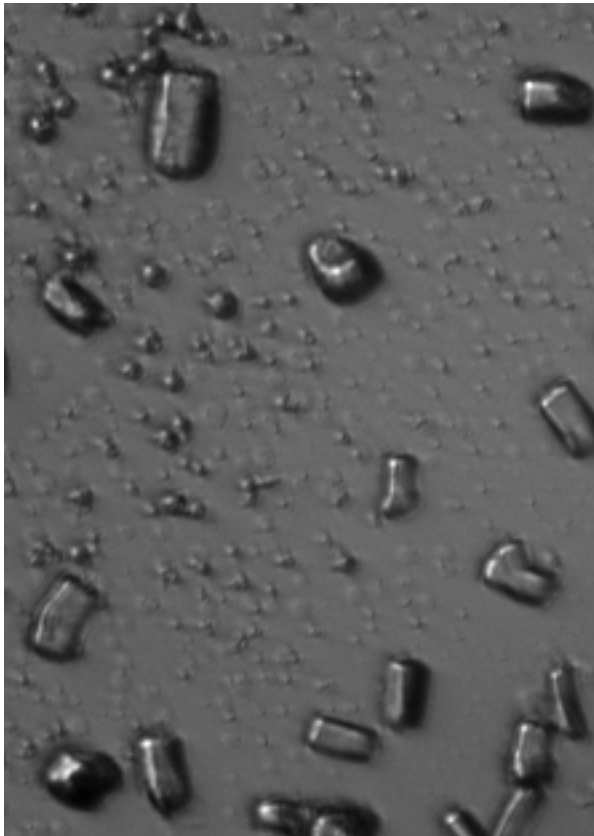
GPCR-G Protein Cycle

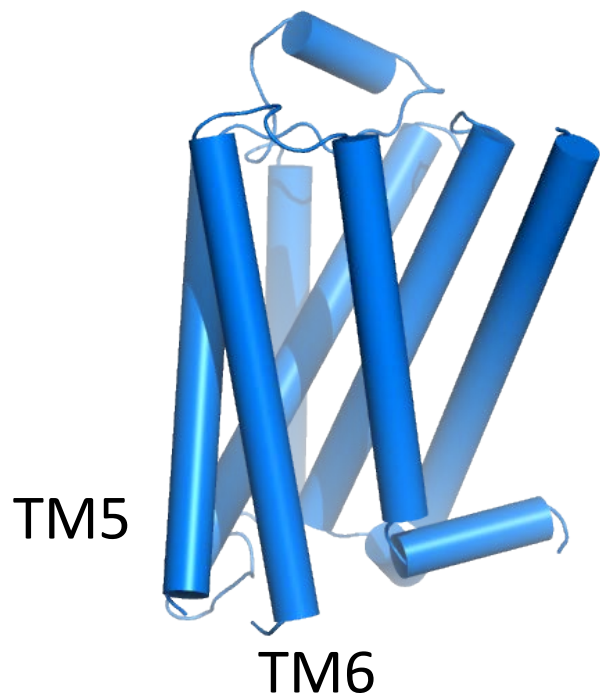


Crystals of wild-type β_2 AR

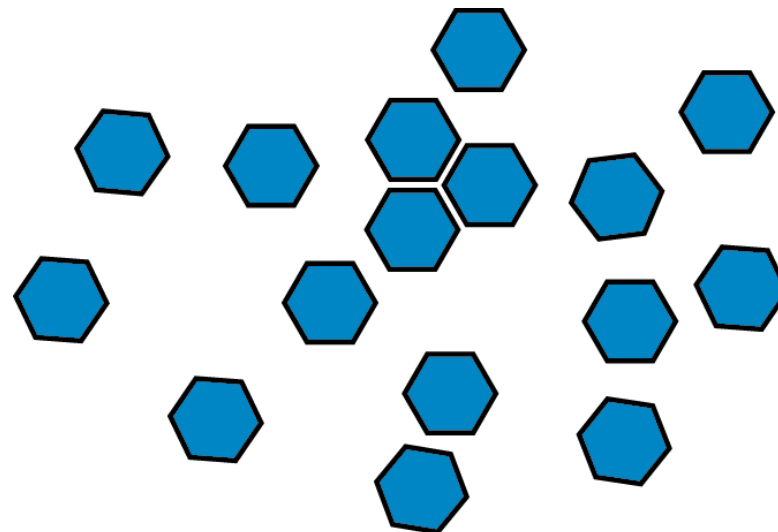
Nov 2004

ESRF microfocus beamline ID13, July 2005

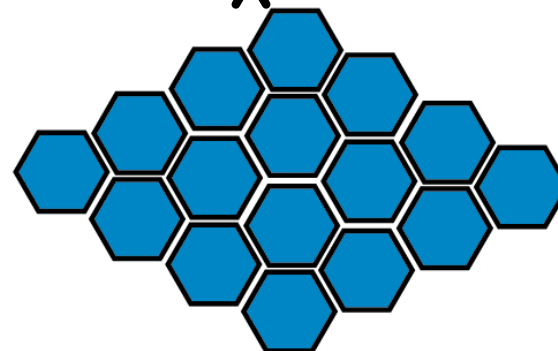




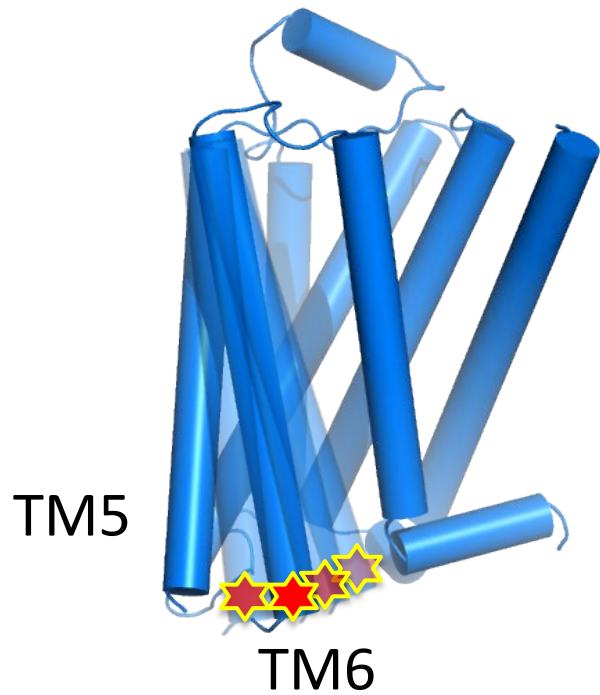
Conformationally
uniform GPCR



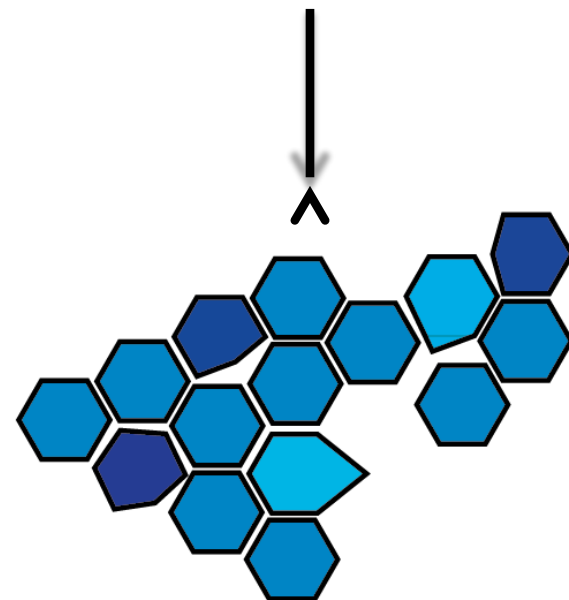
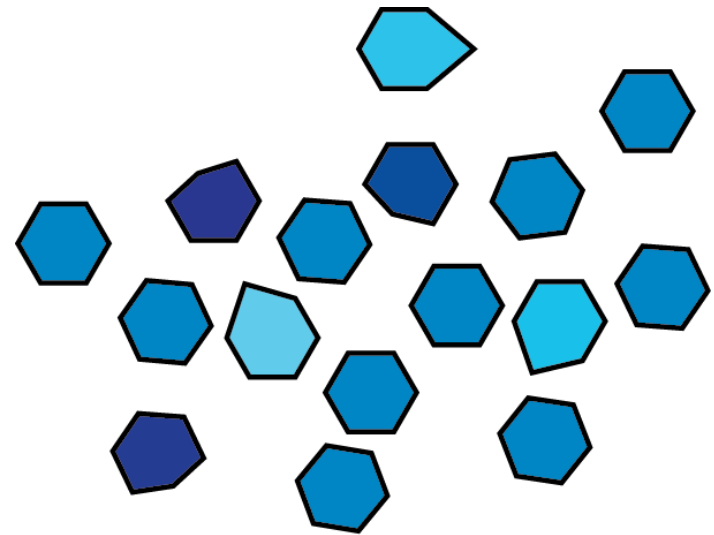
Purified protein



High-quality crystal



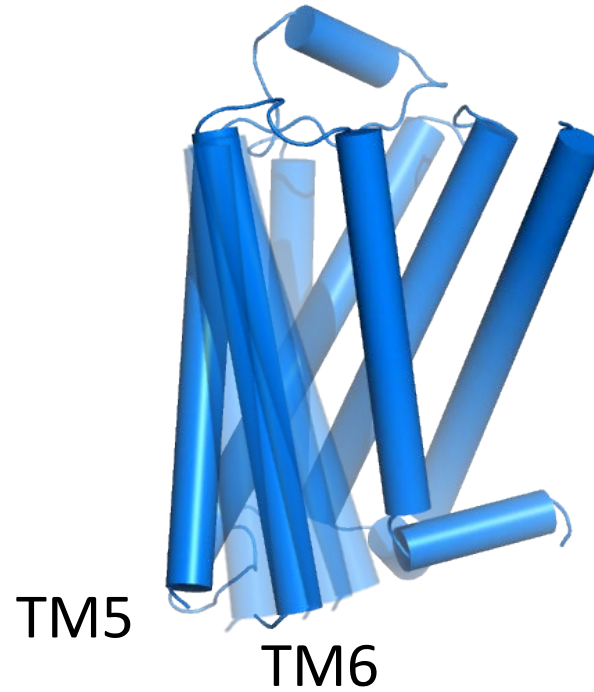
Conformationally
heterogeneous GPCR



Poor quality crystal

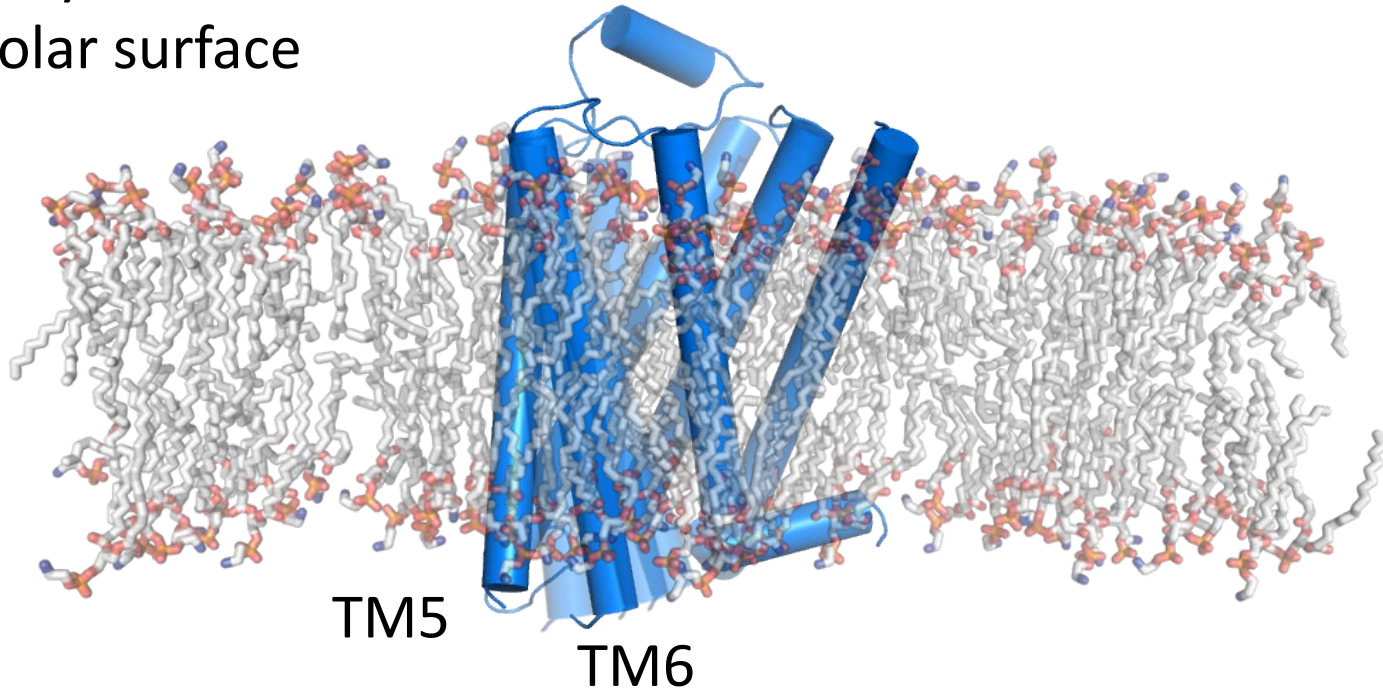
Challenges for crystallography

- Protein dynamics



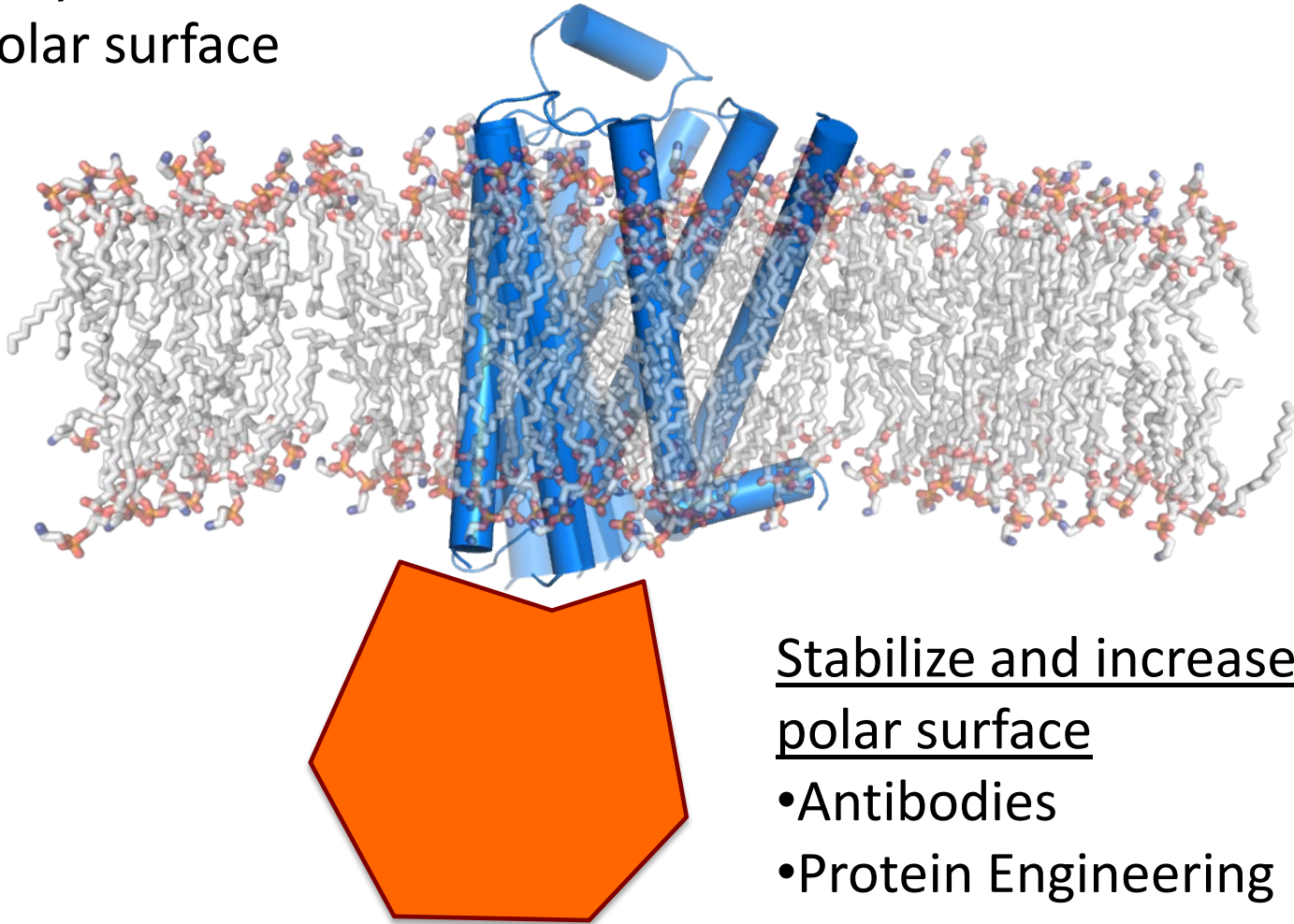
Challenges for crystallography

- Protein dynamics
- Little polar surface



Challenges for crystallography

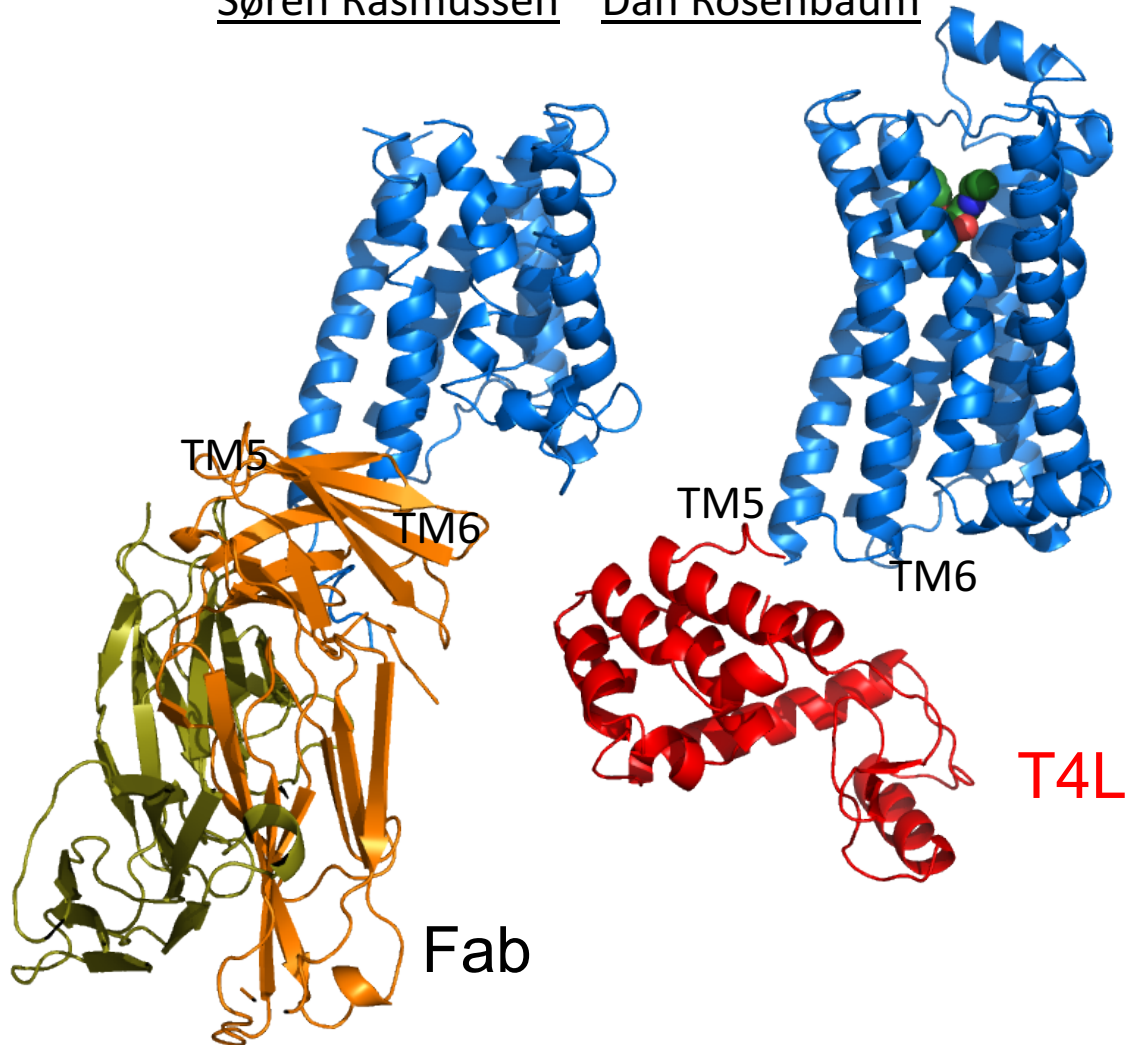
- Protein dynamics
- Little polar surface



- Antibodies and protein engineering

2007

Søren Rasmussen Dan Rosenbaum

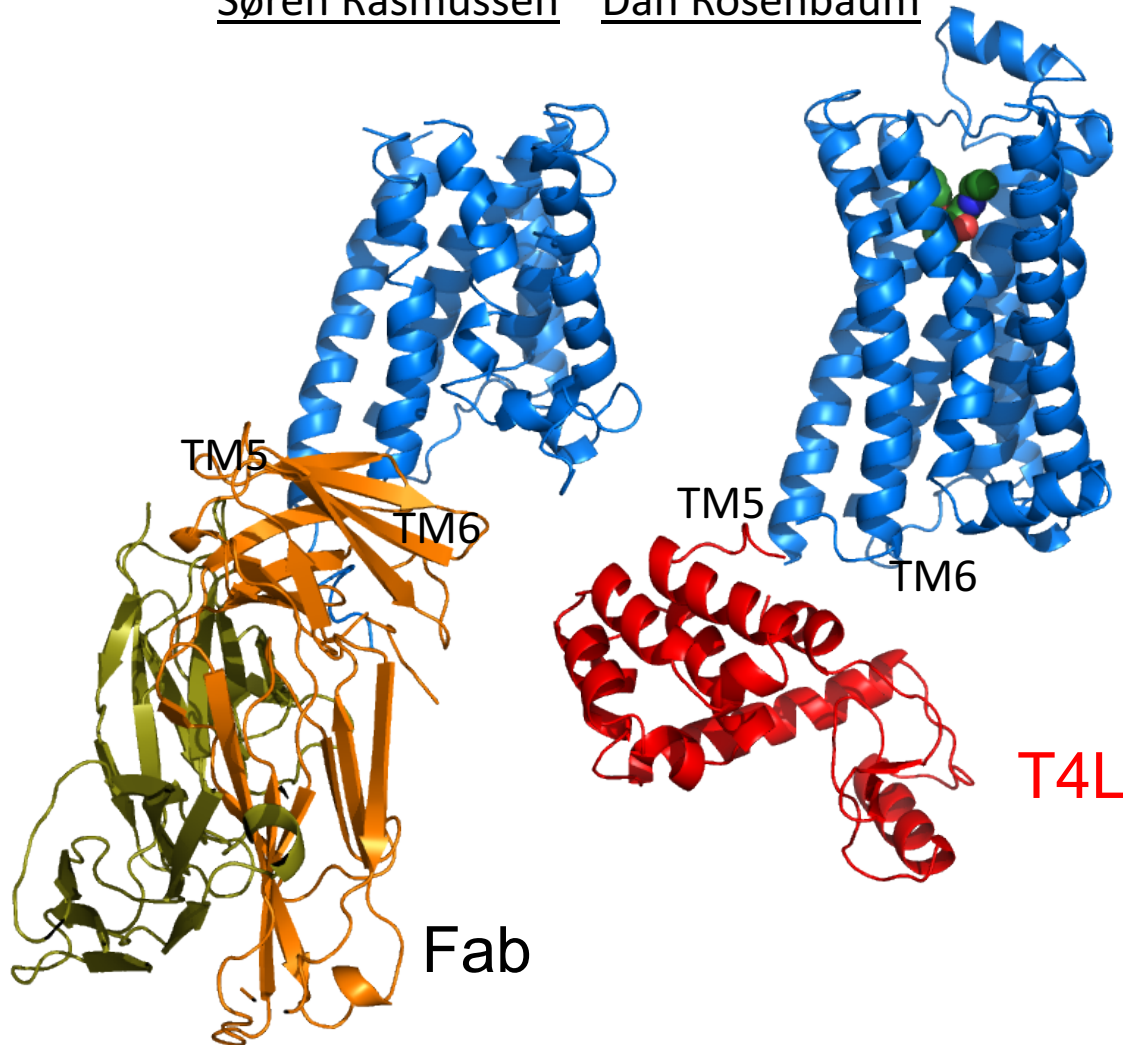


Approaches for GPCR crystallogenesis:

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

2007

Søren Rasmussen Dan Rosenbaum

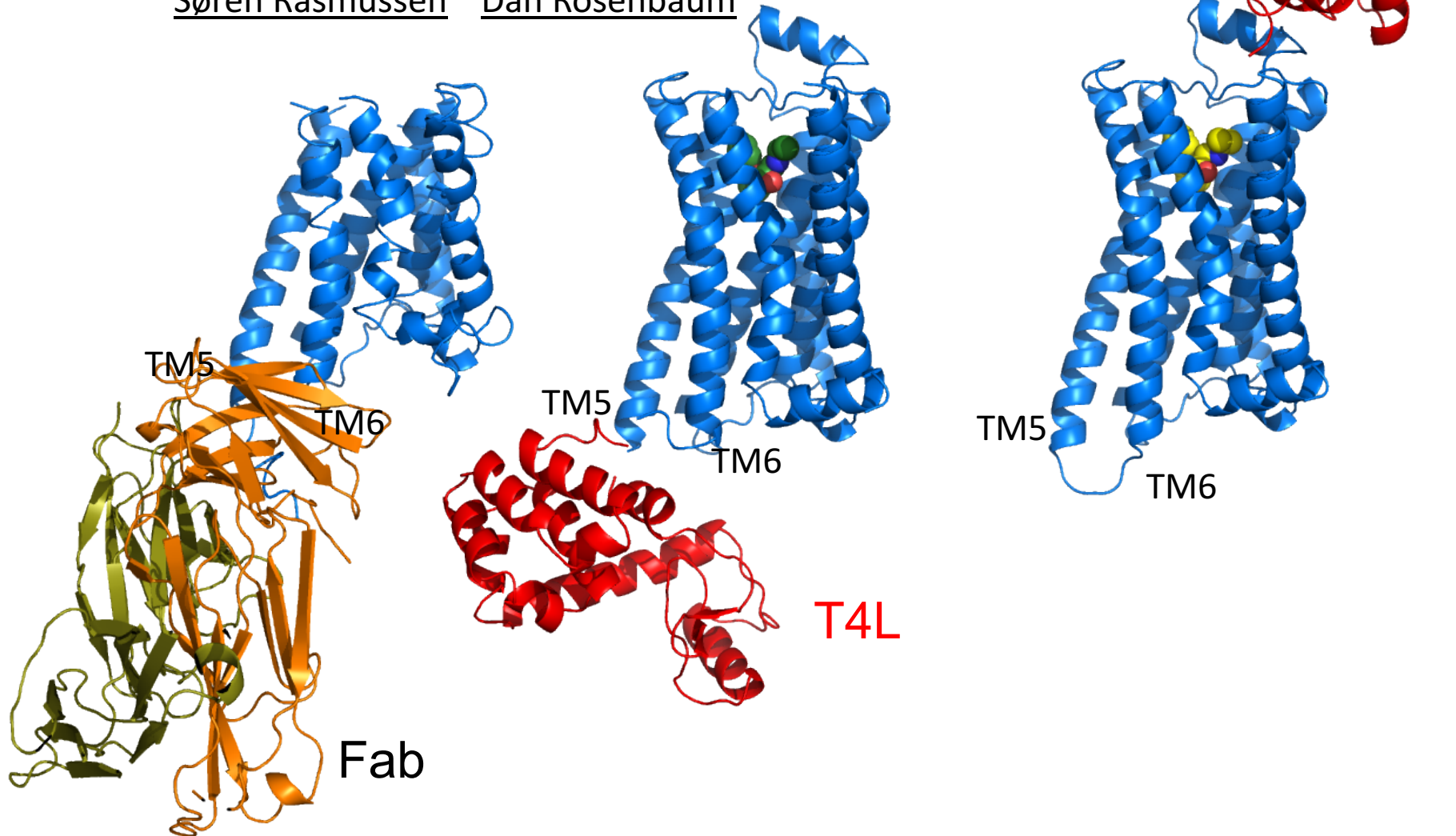


Approaches for GPCR crystallogenesis:

- 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

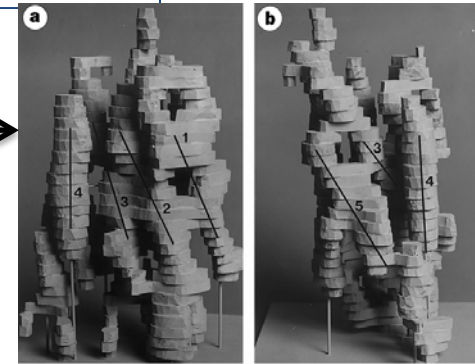
Other Approaches

Thermostabilization through alanine scanning mutations

– β_1 AR, Adenosine A2A –
Tate and Schertler

Rhodopsin (native)

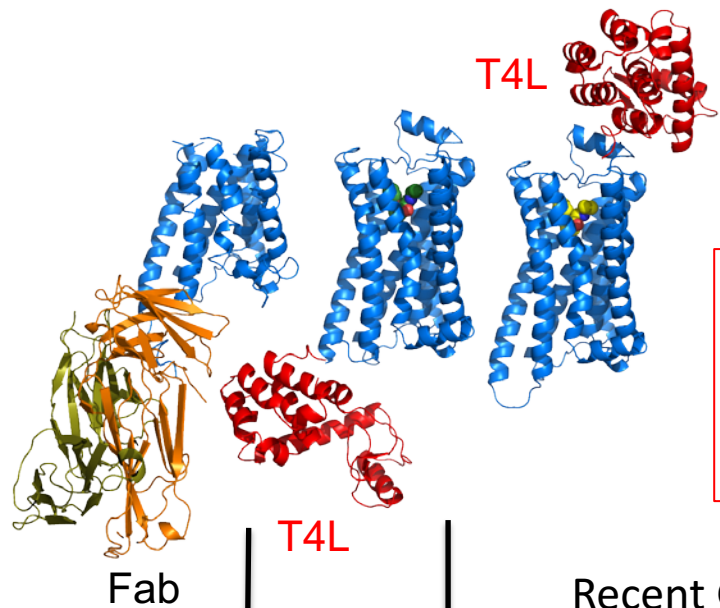
- Schertler (2D crystals) - 1997
- Palczewski and Okada (3D) - 2000
- Ernst and Hofmann (Opsin) - 2008



Recent GPCR-T4L structures from Kobilka Lab and collaborators

Stevens Lab and collaborators

Adenosine A2A
D3 Dopamine
CXCR4
Histamine
S1P1
 κ -opioid



M2 Muscarinic

M3 Muscarinic

δ Opioid

μ Opioid

PAR1

(Haga and Kobayashi)

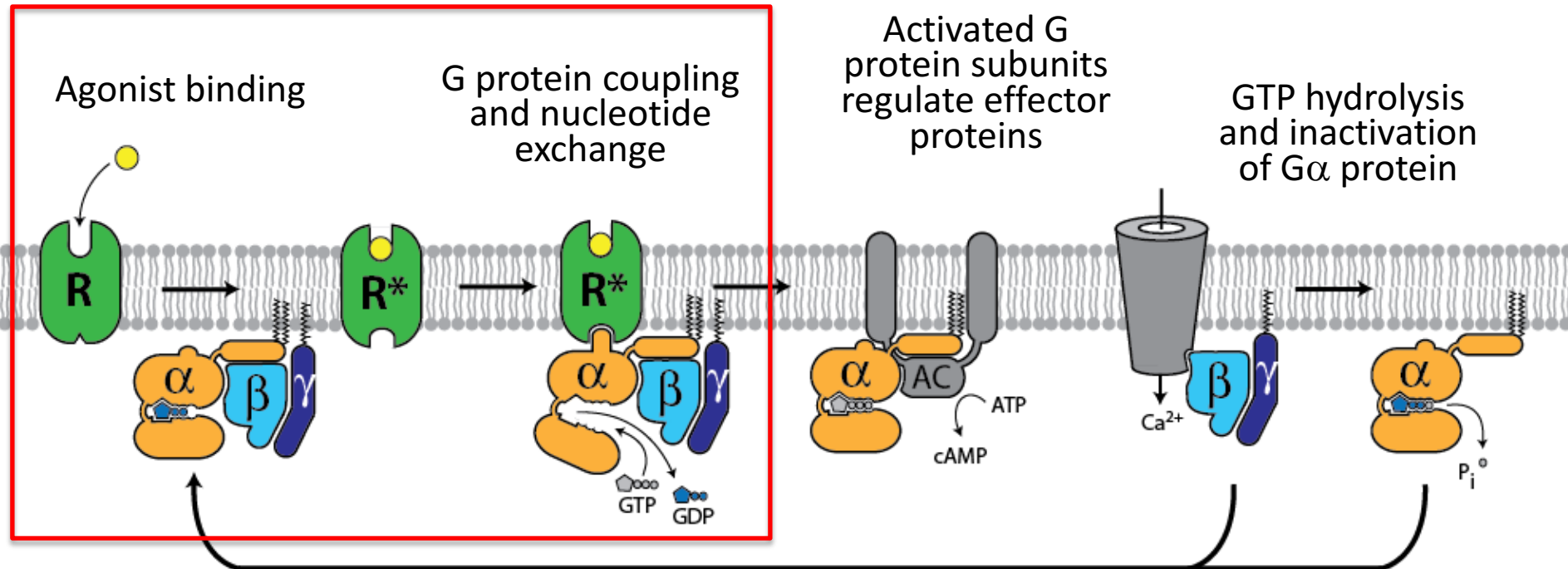
(Jurgen Wess)

(Sebastien Granier)

(Shaun Coughlin)

Outline

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

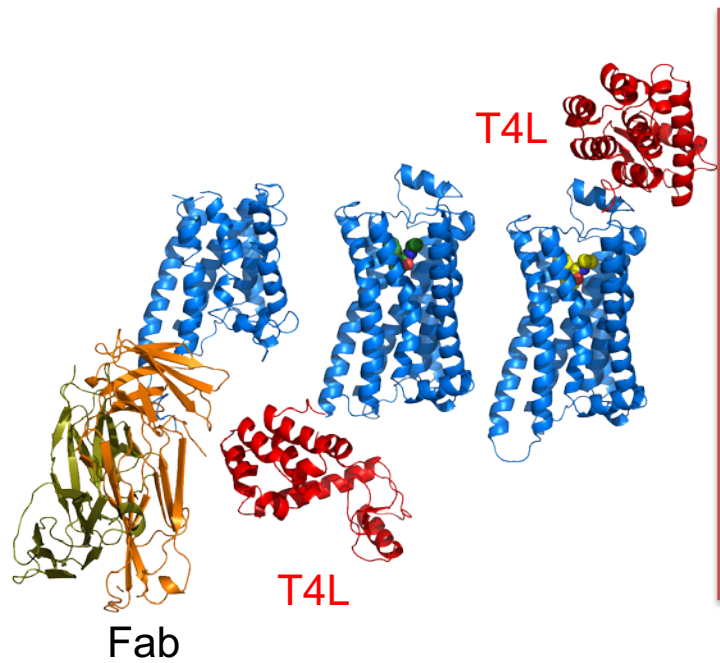


GPCR-G Protein Cycle



β_2 AR INACTIVE

Inverse Agonist



Agonist
(covalent)



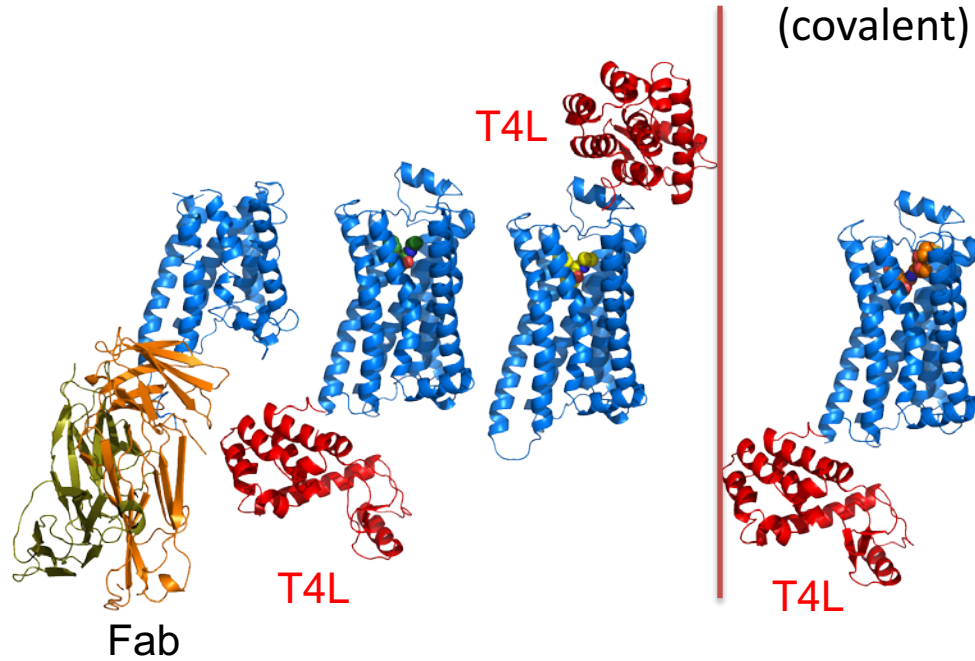
Dan Rosenbaum
Ralph Holl
Peter Gmeiner

β_2 AR ACTIVE ?

β_2 AR INACTIVE

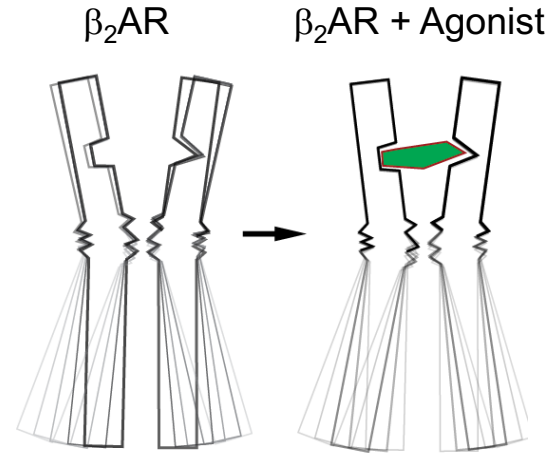
Inverse Agonist

Agonist
(covalent)



β_2 AR ACTIVE ?

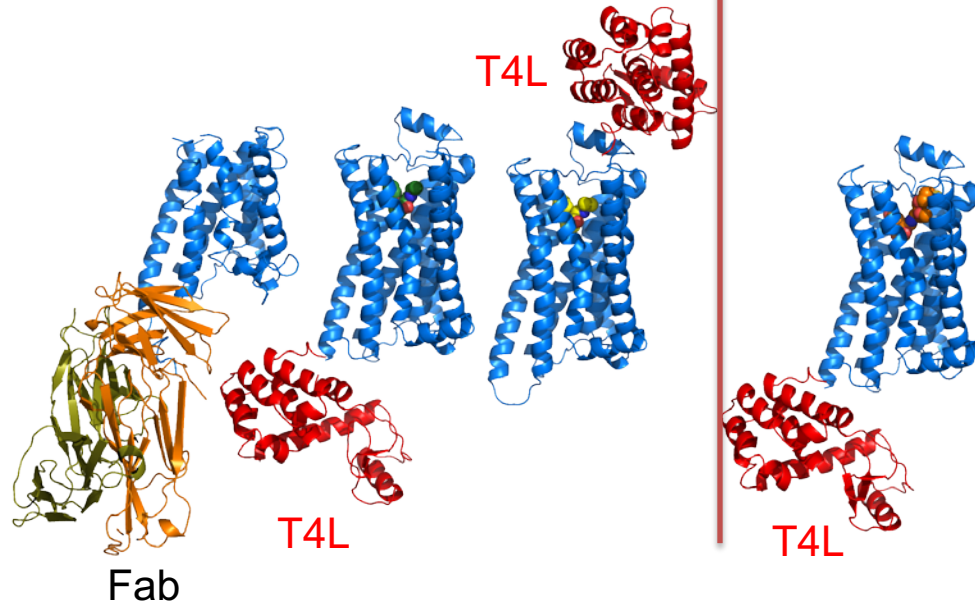
Agonist alone does not fully stabilize
active state



β_2 AR INACTIVE

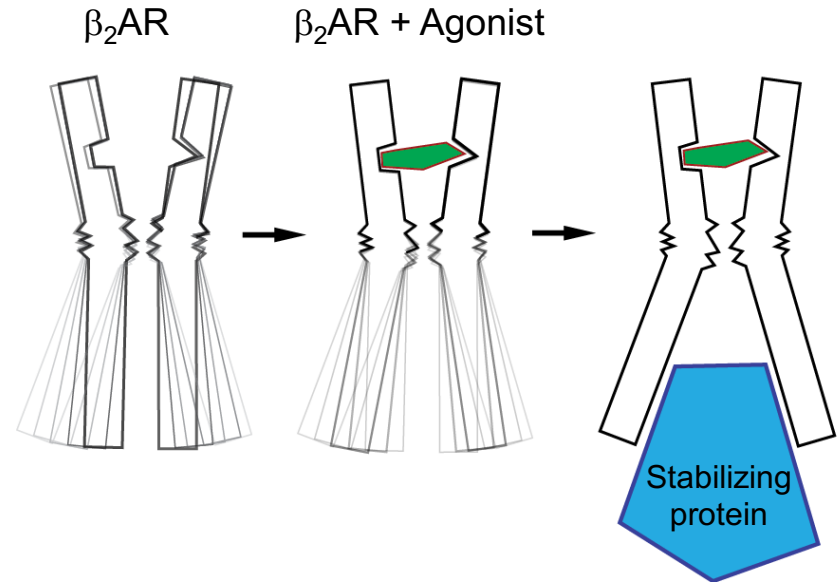
Inverse Agonist

Agonist
(covalent)



β_2 AR ACTIVE ?

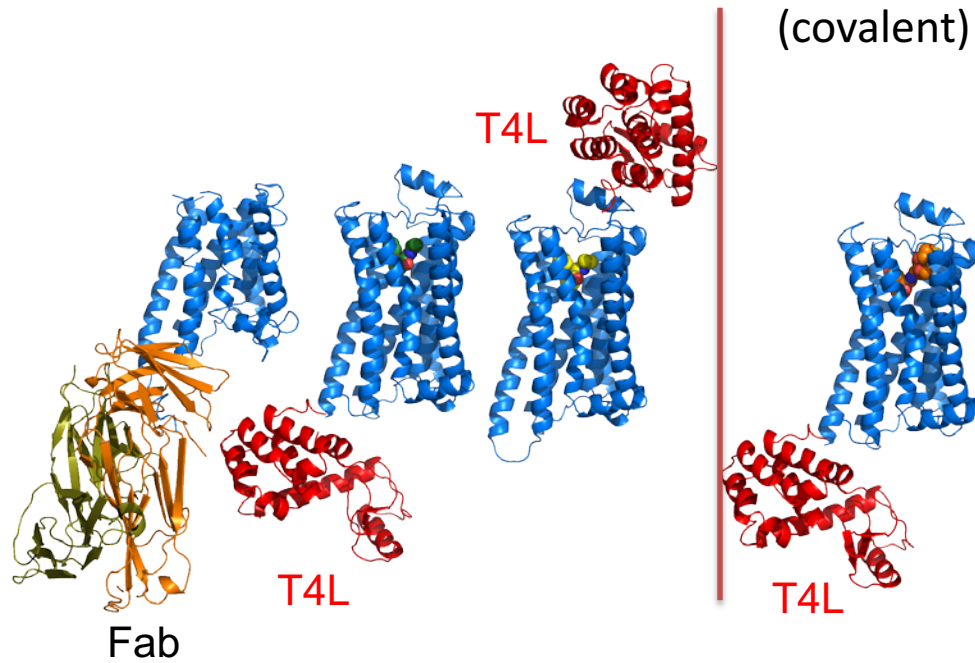
Agonist alone does not fully stabilize active state



β_2 AR INACTIVE

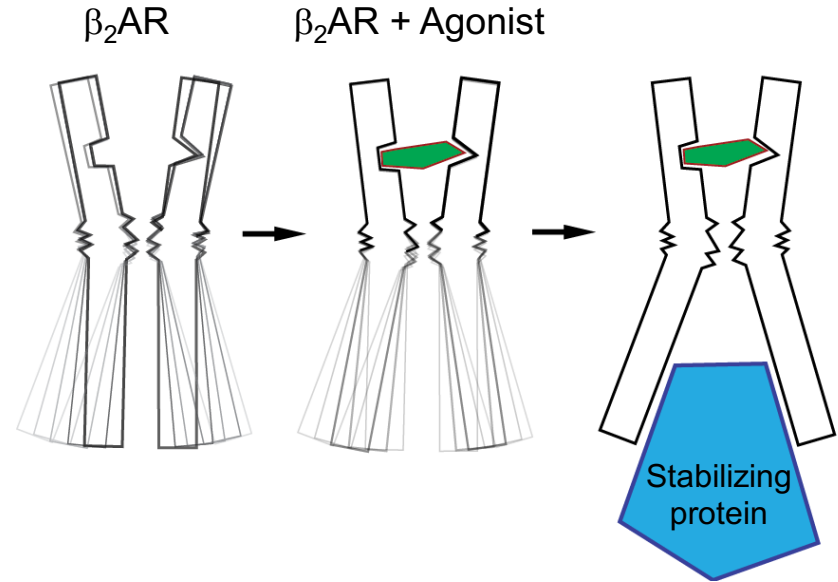
Inverse Agonist

Agonist
(covalent)



β_2 AR ACTIVE ?

Agonist alone does not fully stabilize active state



Nanobody
variable domain of a
single chain
camelid antibody

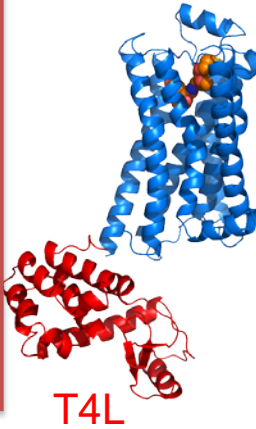
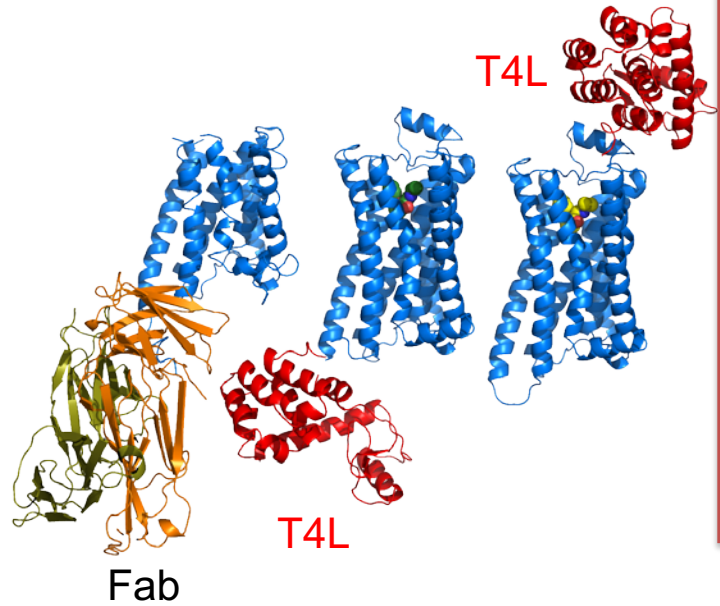
Jan Steyaert
Els Pardon



β_2 AR INACTIVE

Inverse Agonist

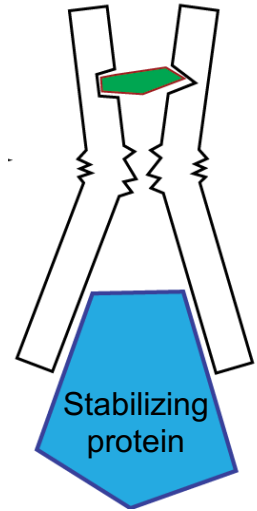
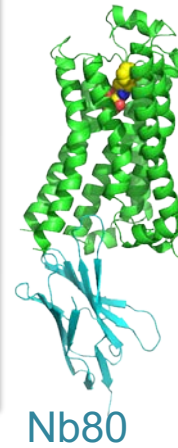
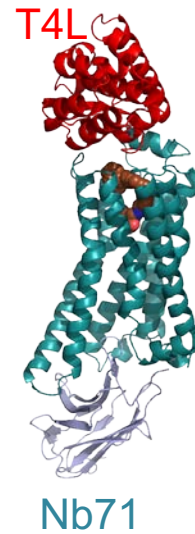
Agonist
(covalent)



β_2 AR ACTIVE

Partial
Agonist

Agonist



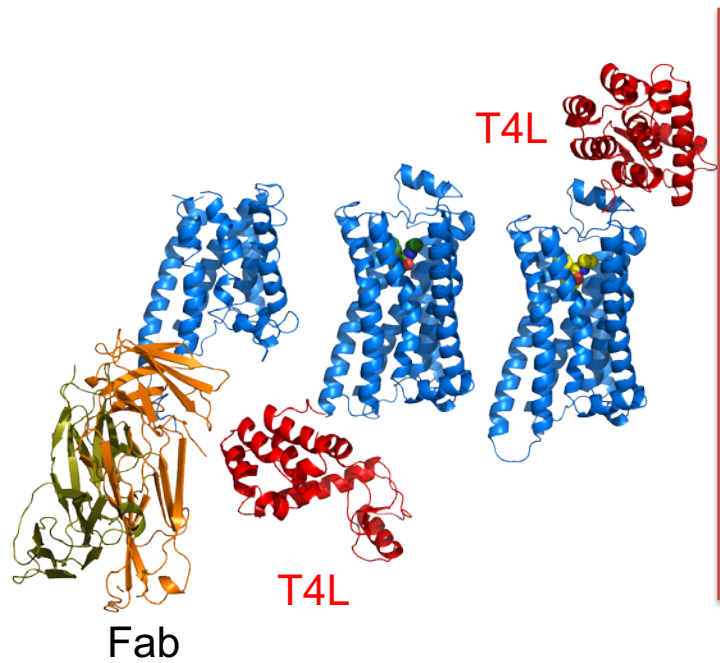
Nanobody
variable domain of a
single chain
camelid antibody

Jan Steyaert
Els Pardon

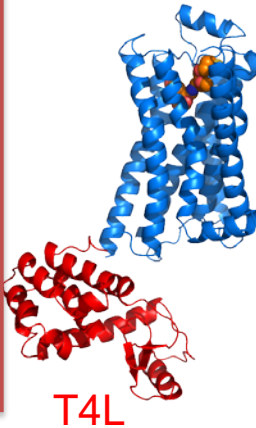


β_2 AR INACTIVE

Inverse Agonist

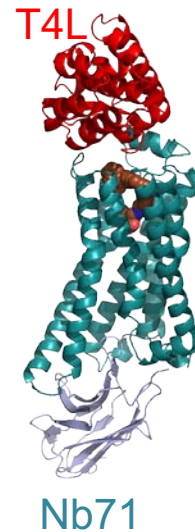


Agonist
(covalent)

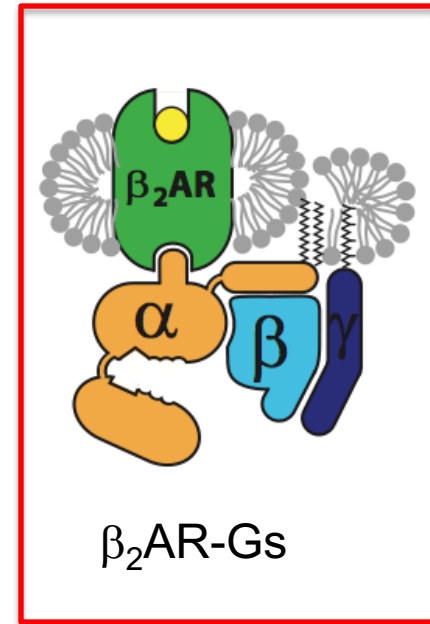
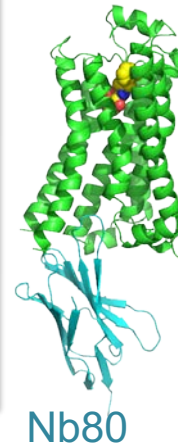


β_2 AR ACTIVE

Partial
Agonist



Agonist



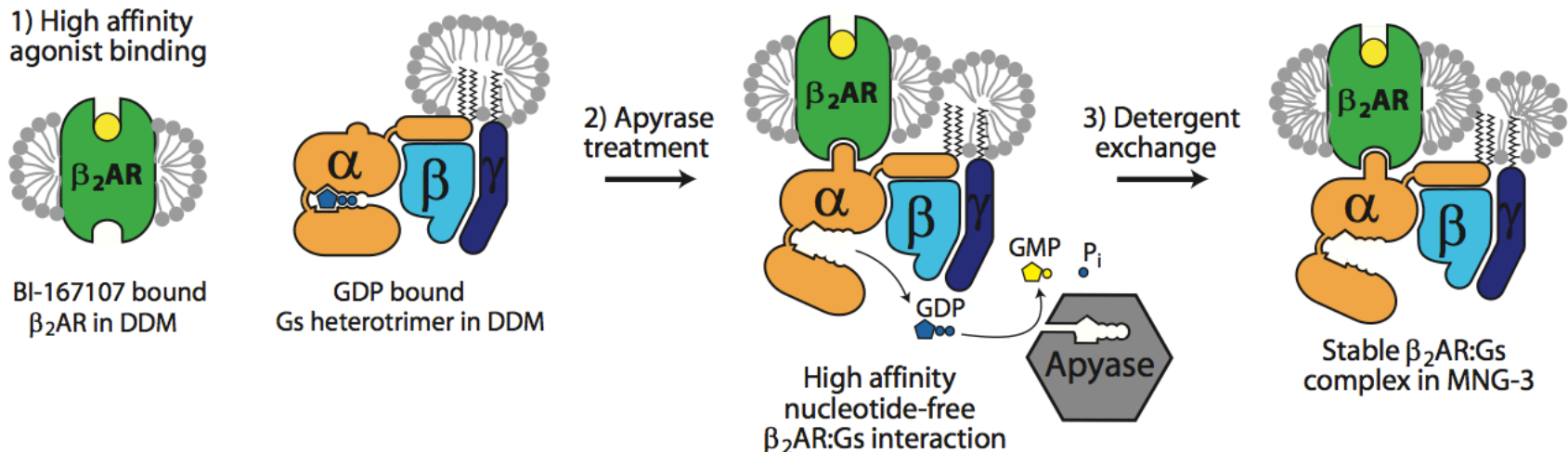
Nanobody
variable domain of a
single chain
camelid antibody

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)



Microcrystallography

GM/CA-CAT at Argonne National Labs



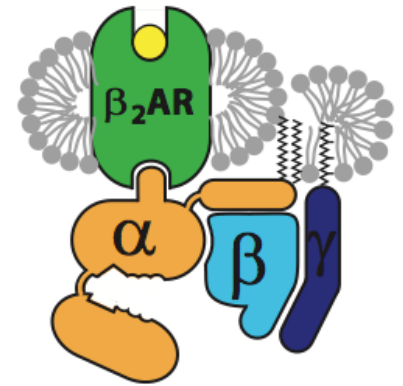
β_2 AR-Gs complex



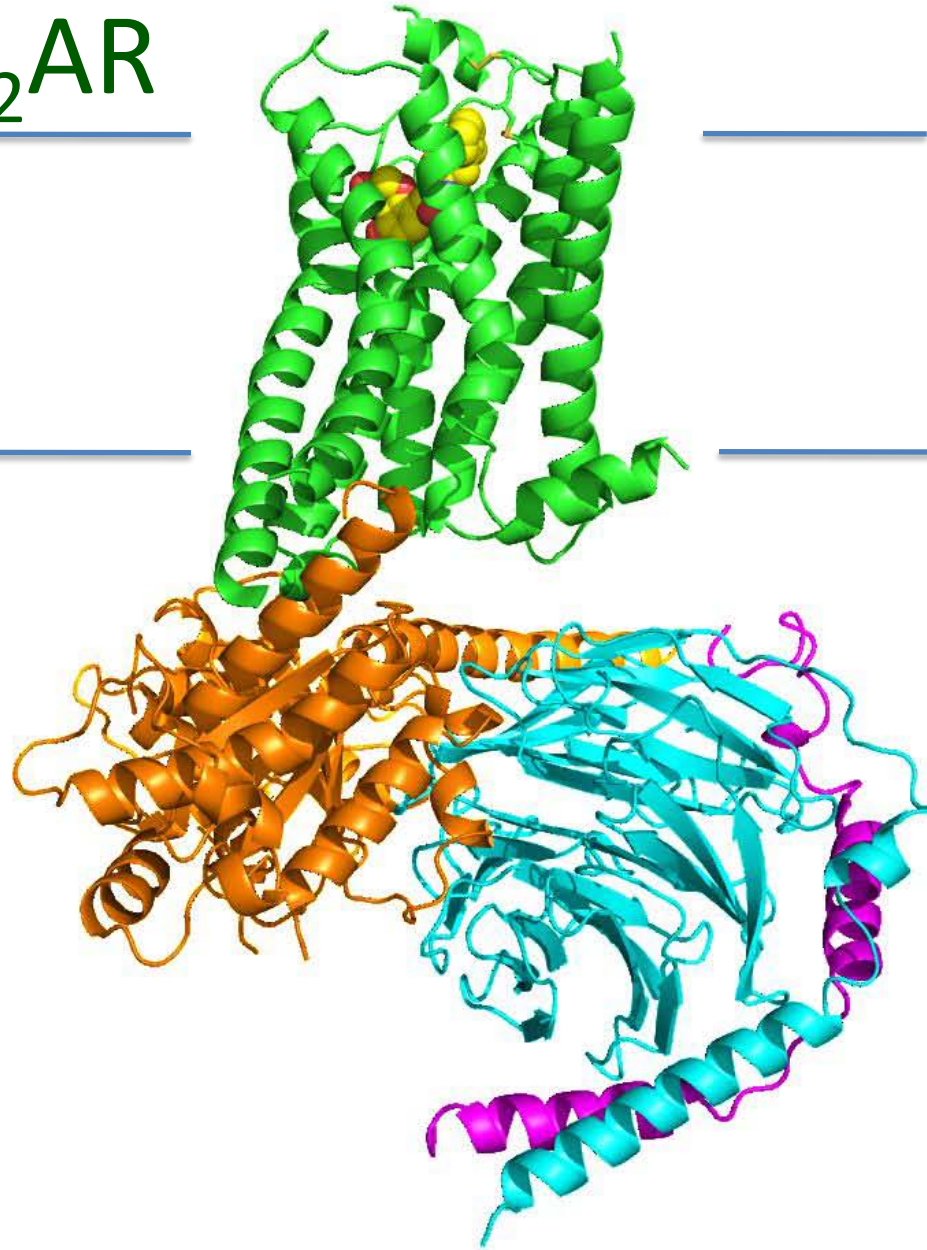
Andy Kruse, Brian DeVree, Søren Rasmussen
me Roger Sunahara

Returning from Argonne with final data set April 2011

β_2AR



Gs $\alpha\beta\gamma$

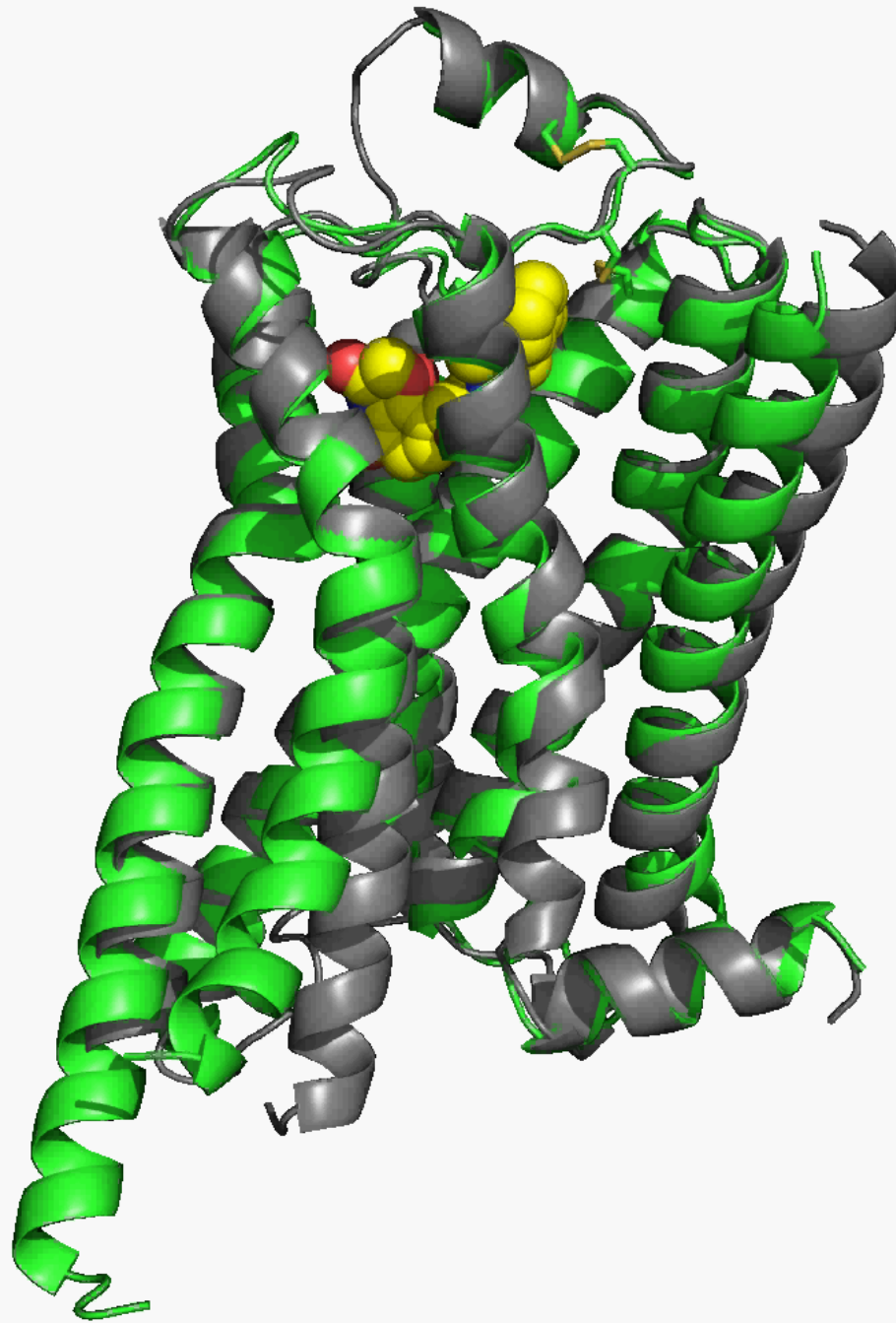


Inactive

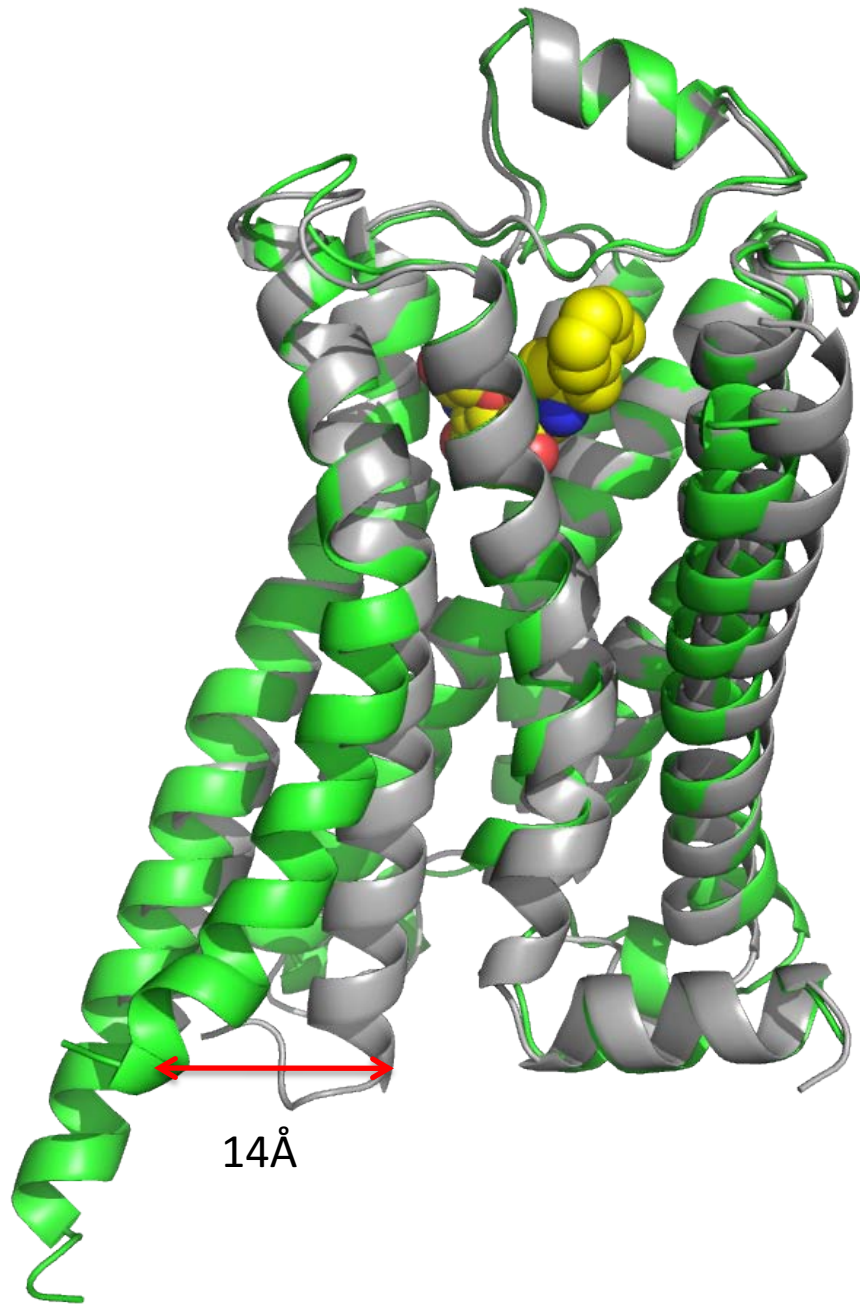
Active

β_2 AR-Cz

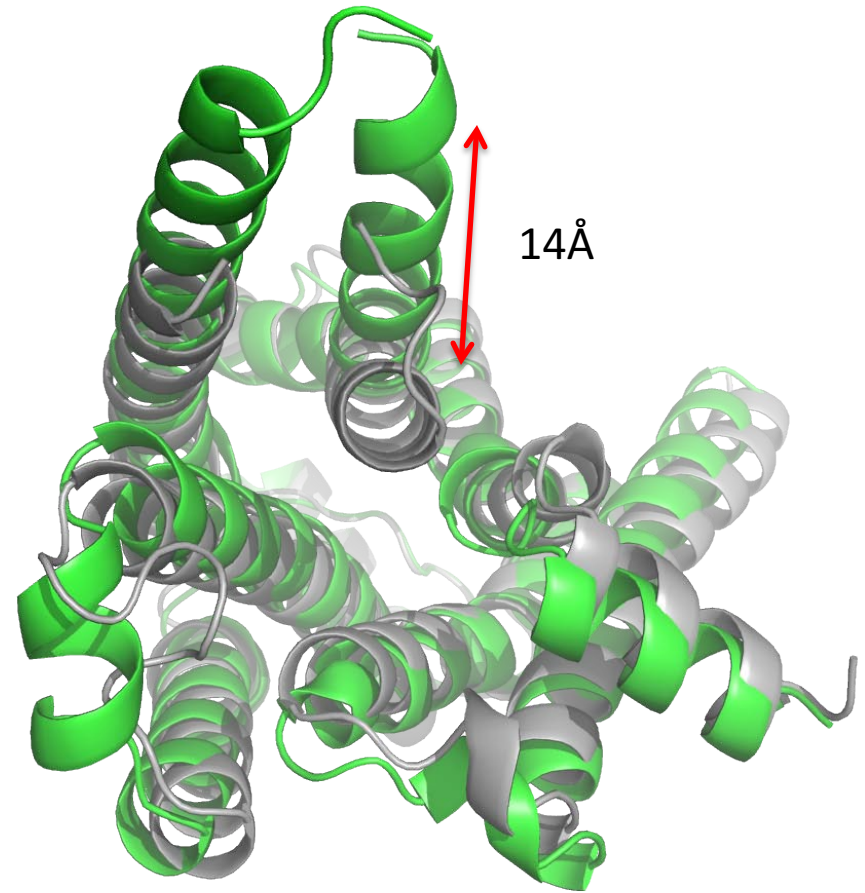
β_2 AR-Gs



Active state of β_2 AR



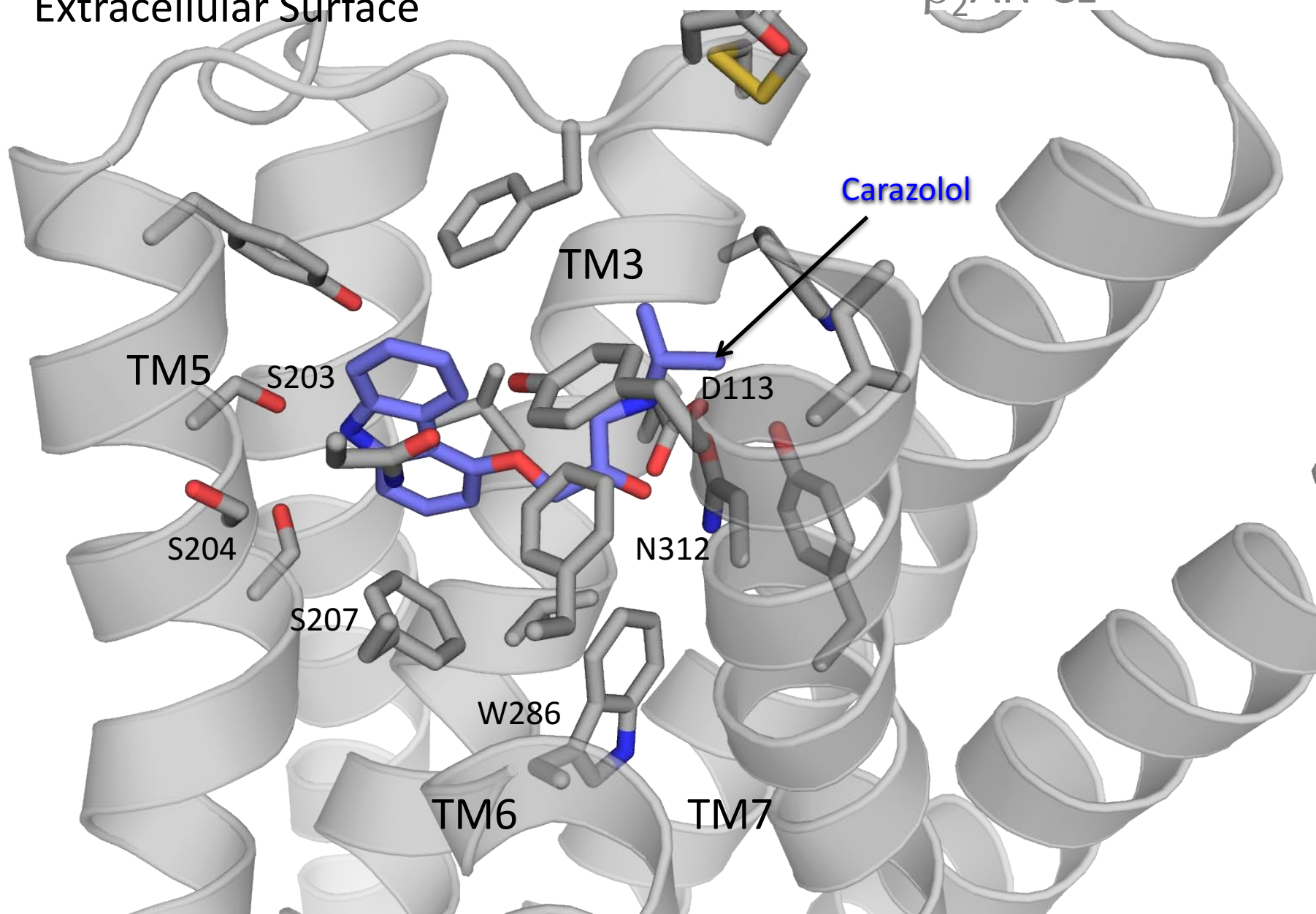
Cytoplasmic View



β_2 AR - Inactive
 β_2 AR-Gs

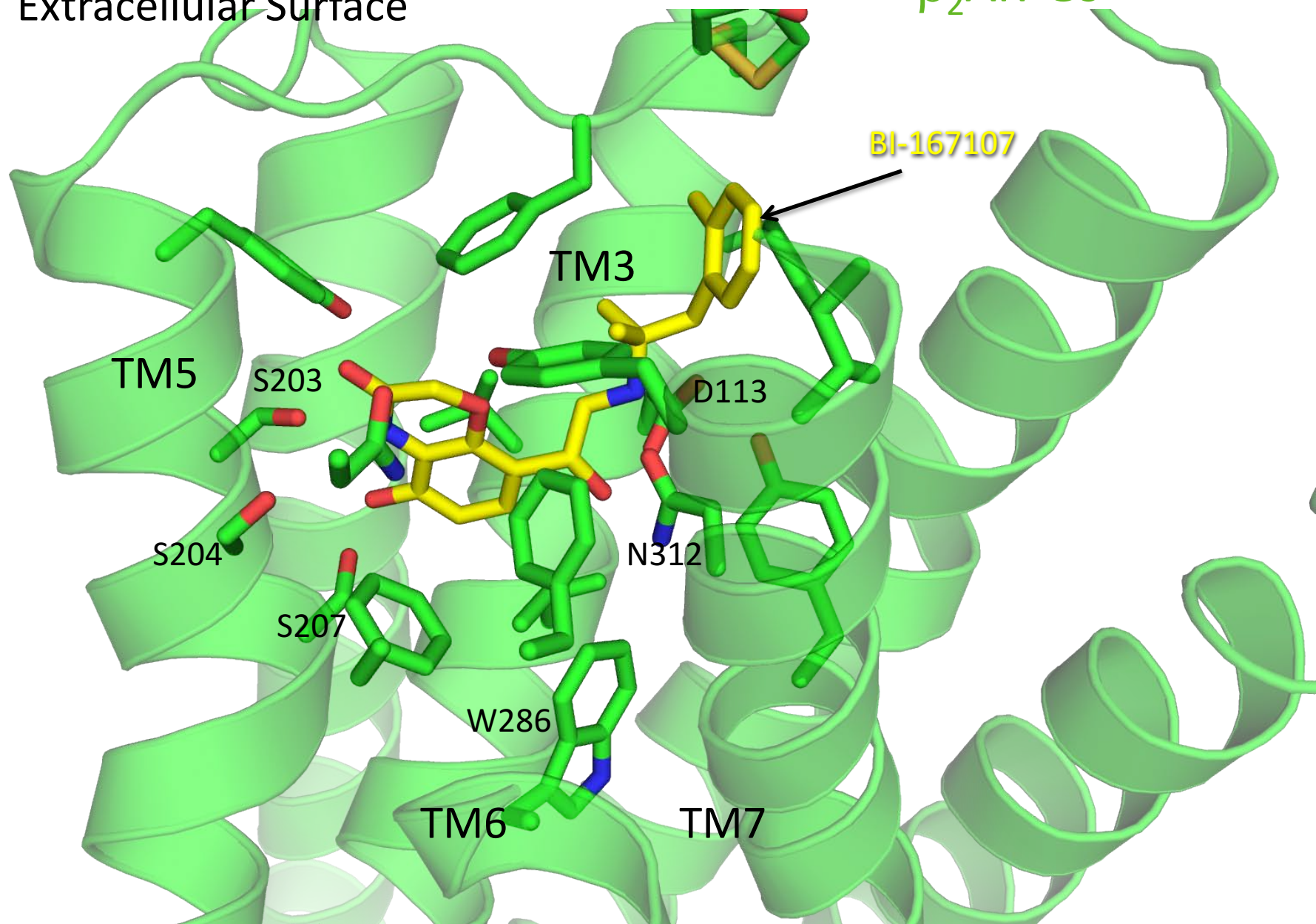
Extracellular Surface

β_2 AR-Cz

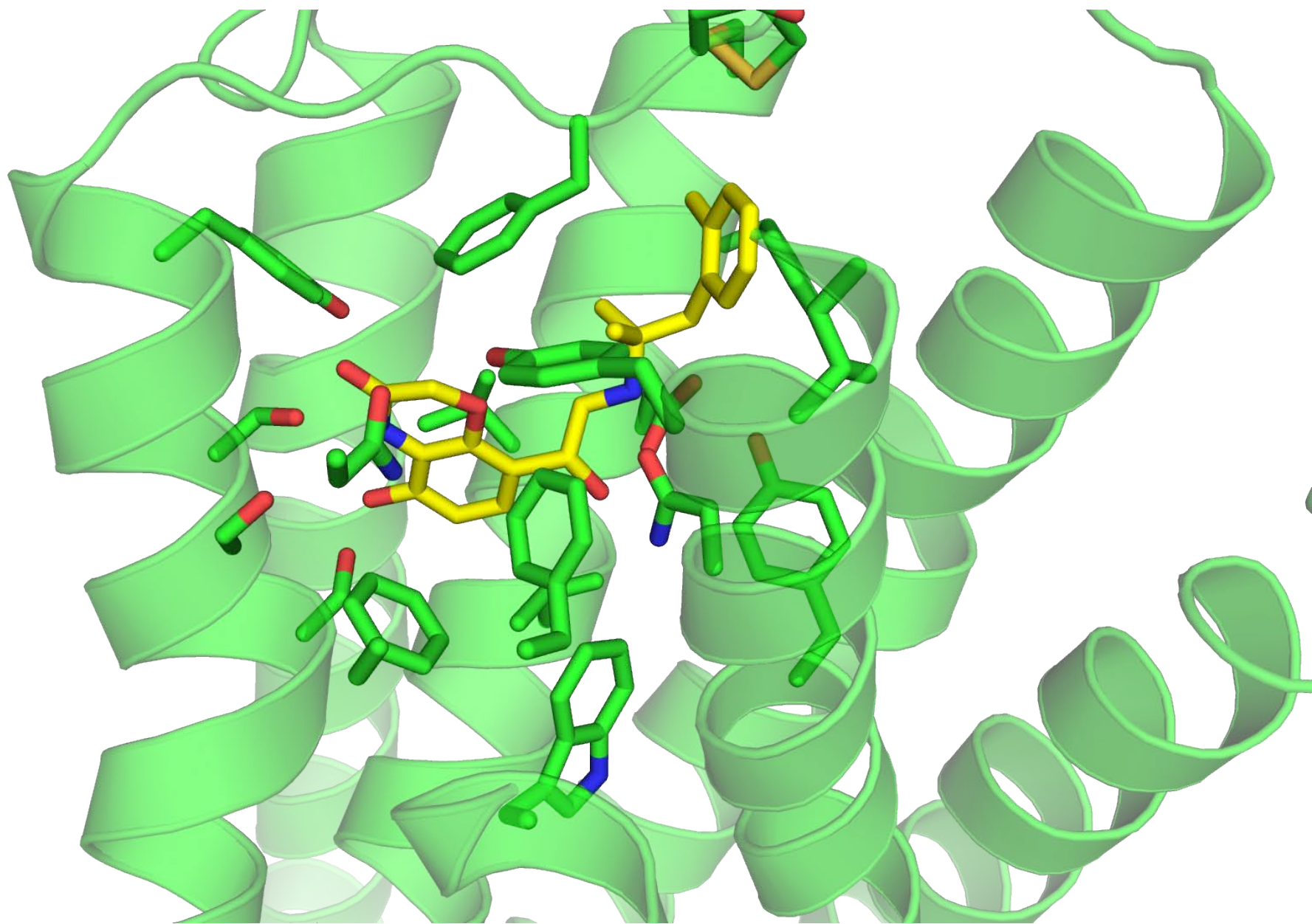


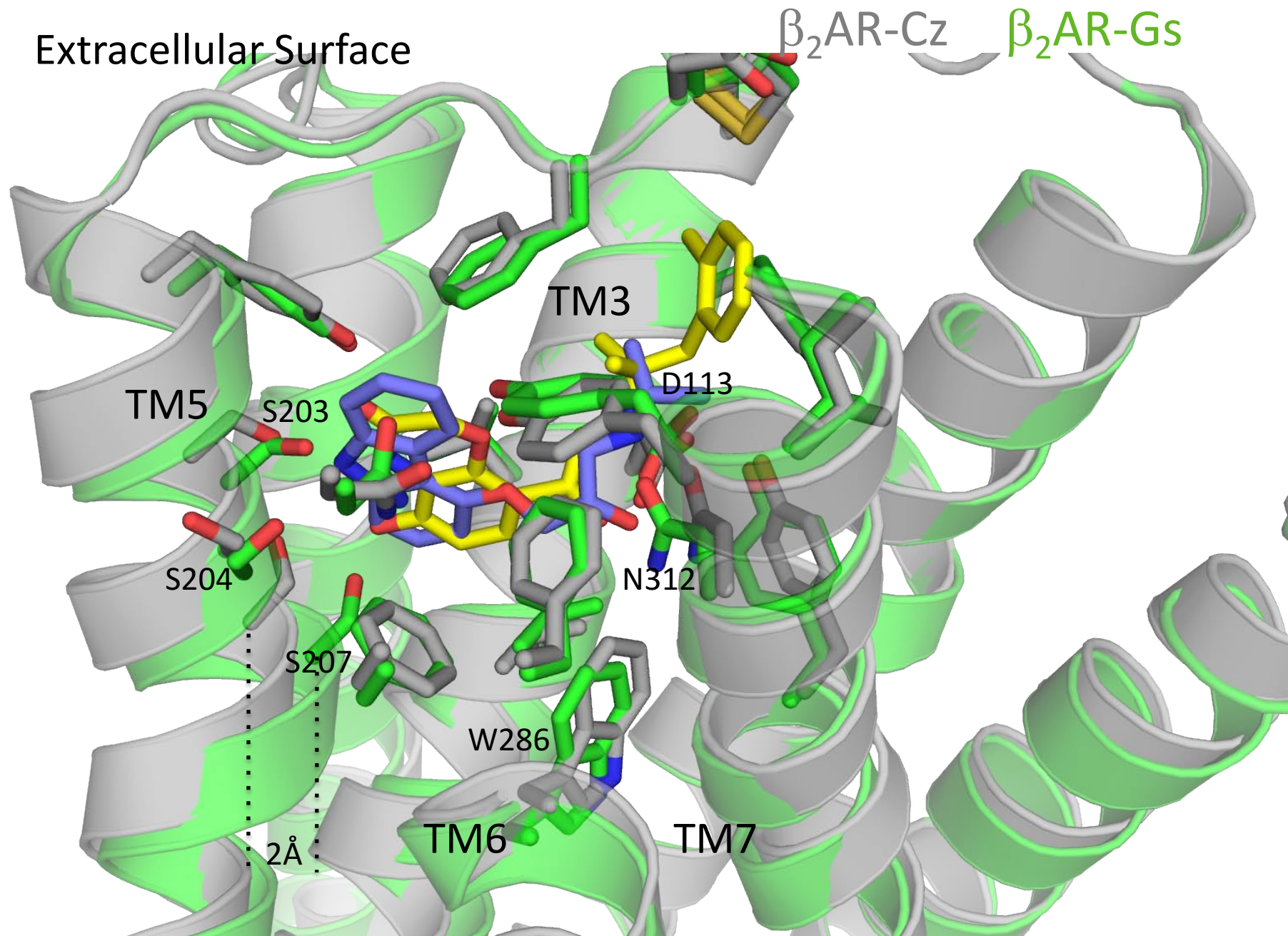
β_2 AR-Gs

Extracellular Surface



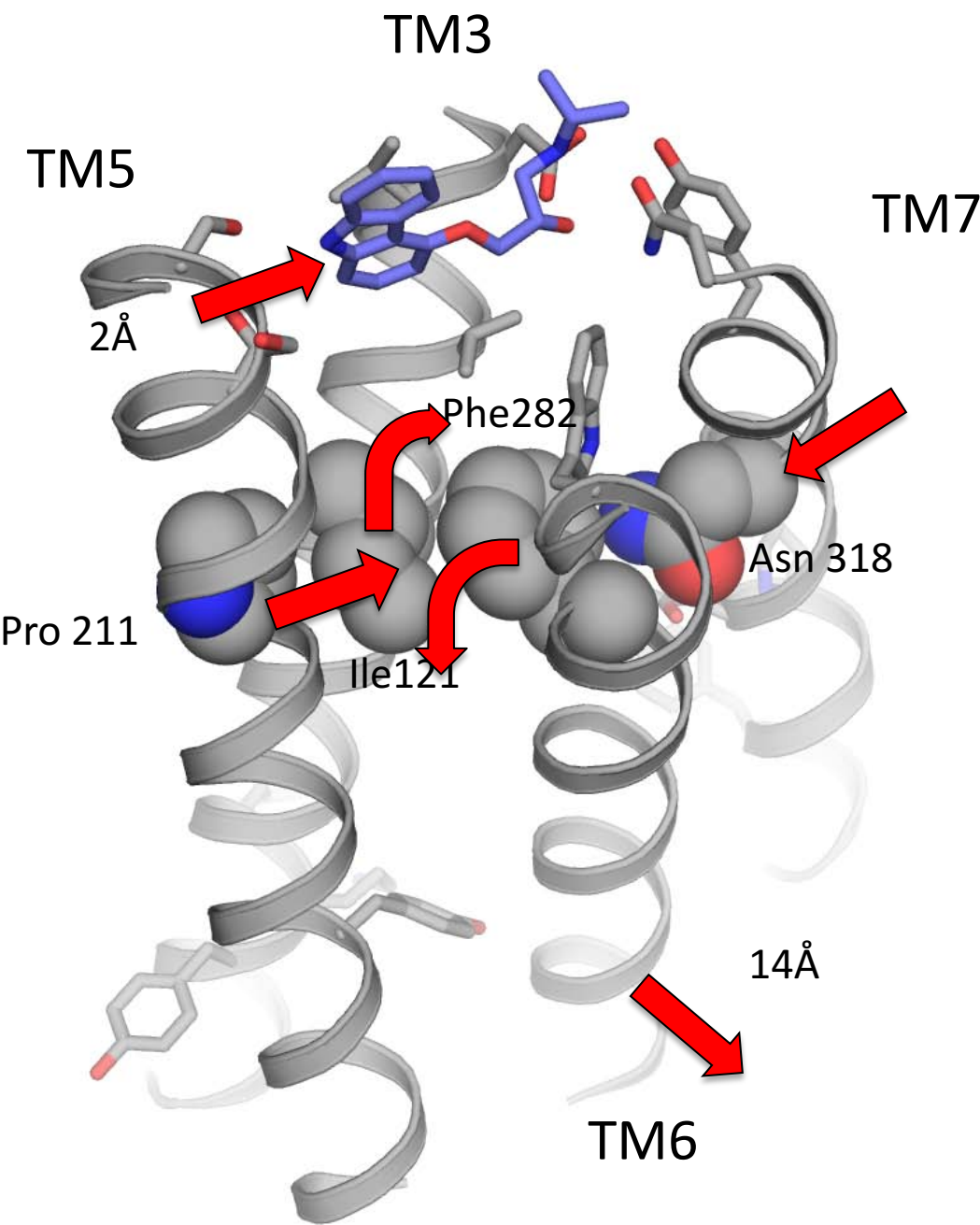
Active





Extracellular

β_2 AR-Inactive

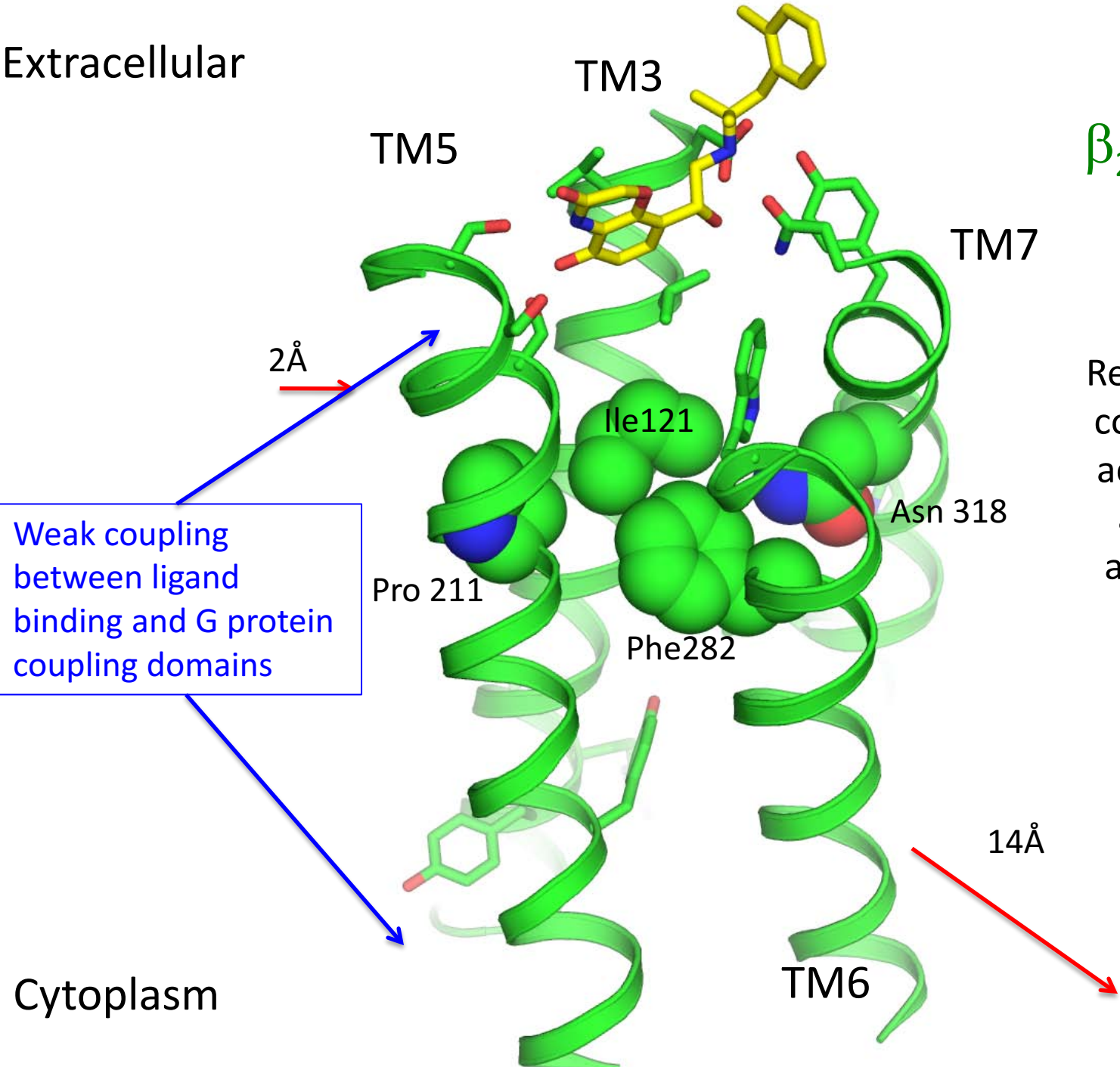


Packing of conserved amino acids maintains inactive state.

Cytoplasm

Extracellular

β_2 AR-Active



Rearrangement of conserved amino acids required to accommodate agonist binding.

$\beta_2\text{AR}$

Gs $\alpha\beta\gamma$

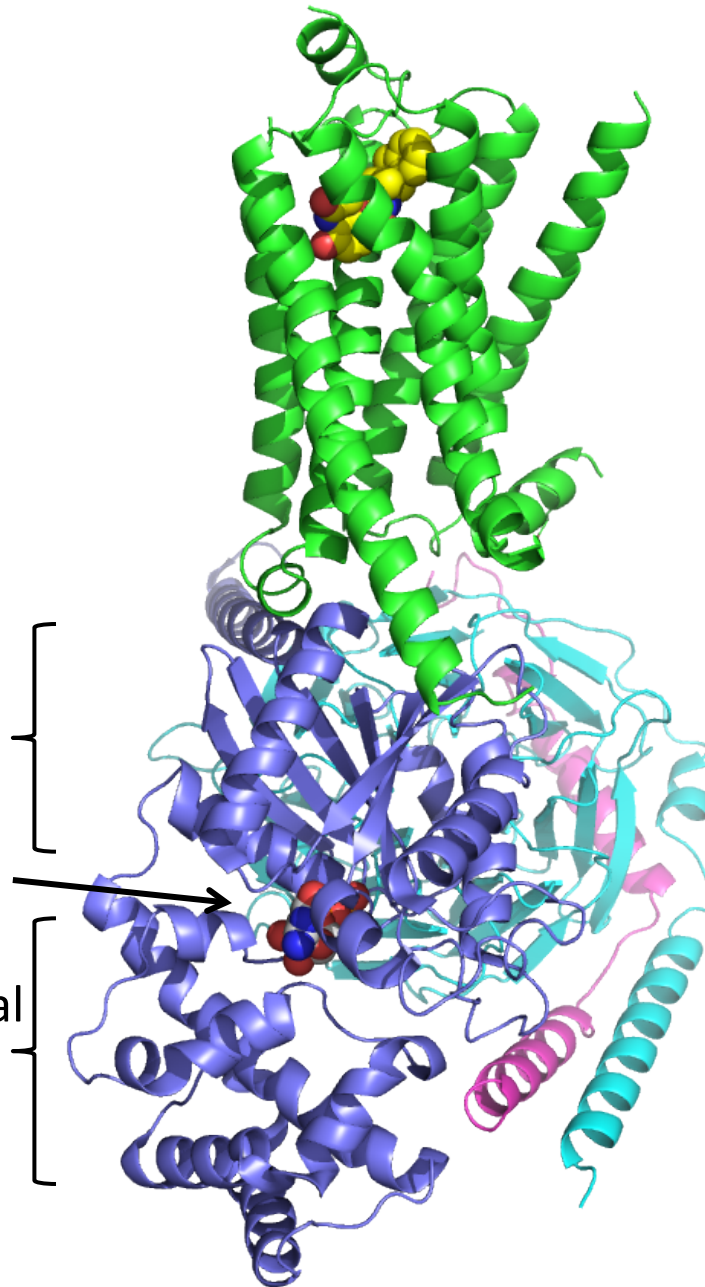
Inactive

G α
Ras-like
domain

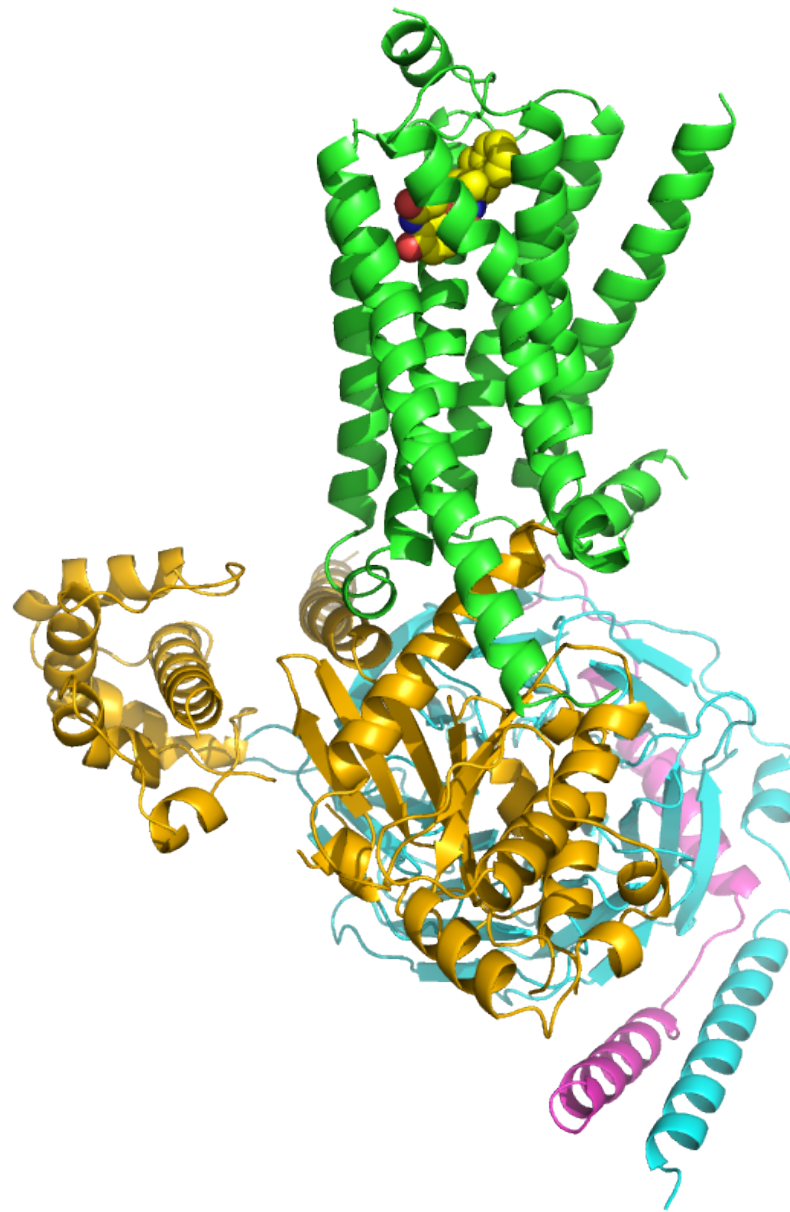
GDP

α -helical
domain

Interactions between
the $\beta_2\text{AR}$ and Gs
promote GDP release....



β_2 AR



G $\alpha\beta\gamma$

Active

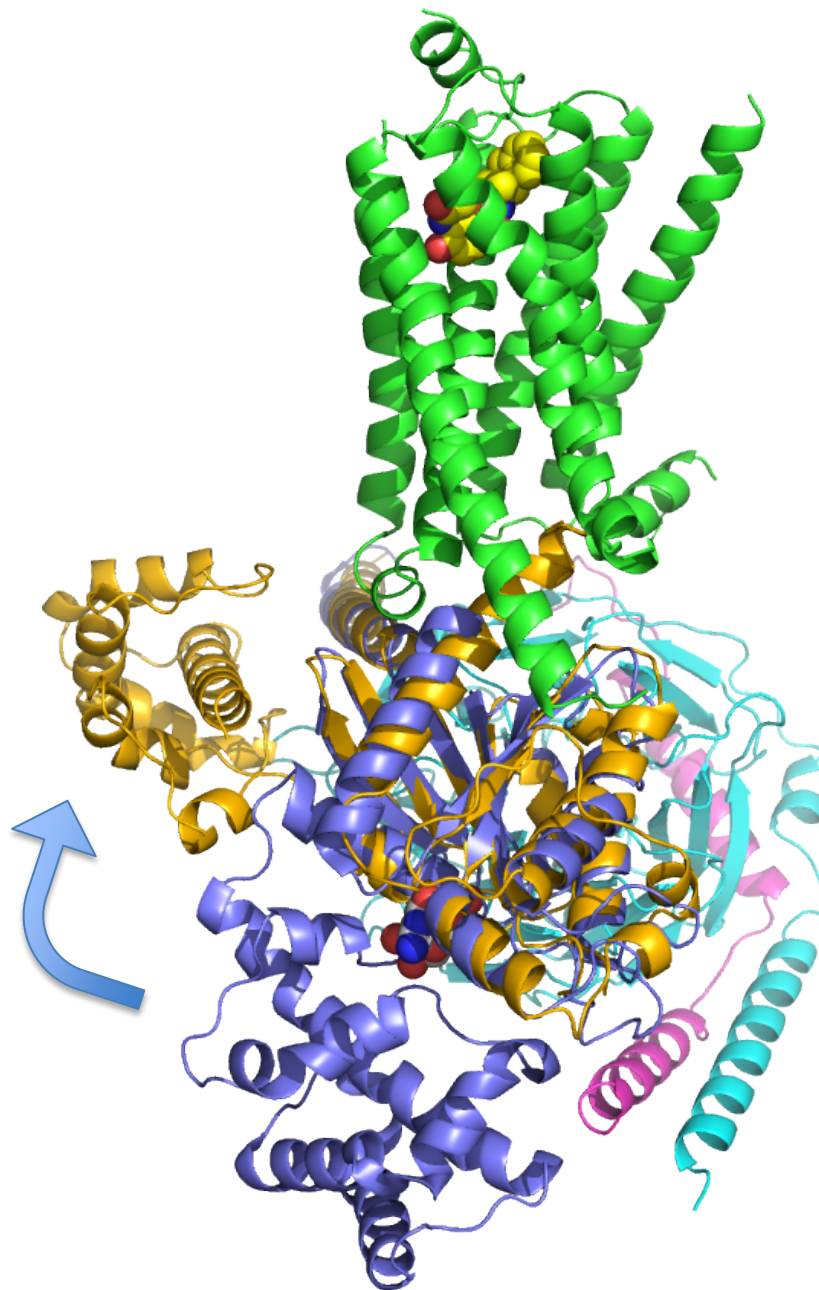
β_2AR

α -helical
domain

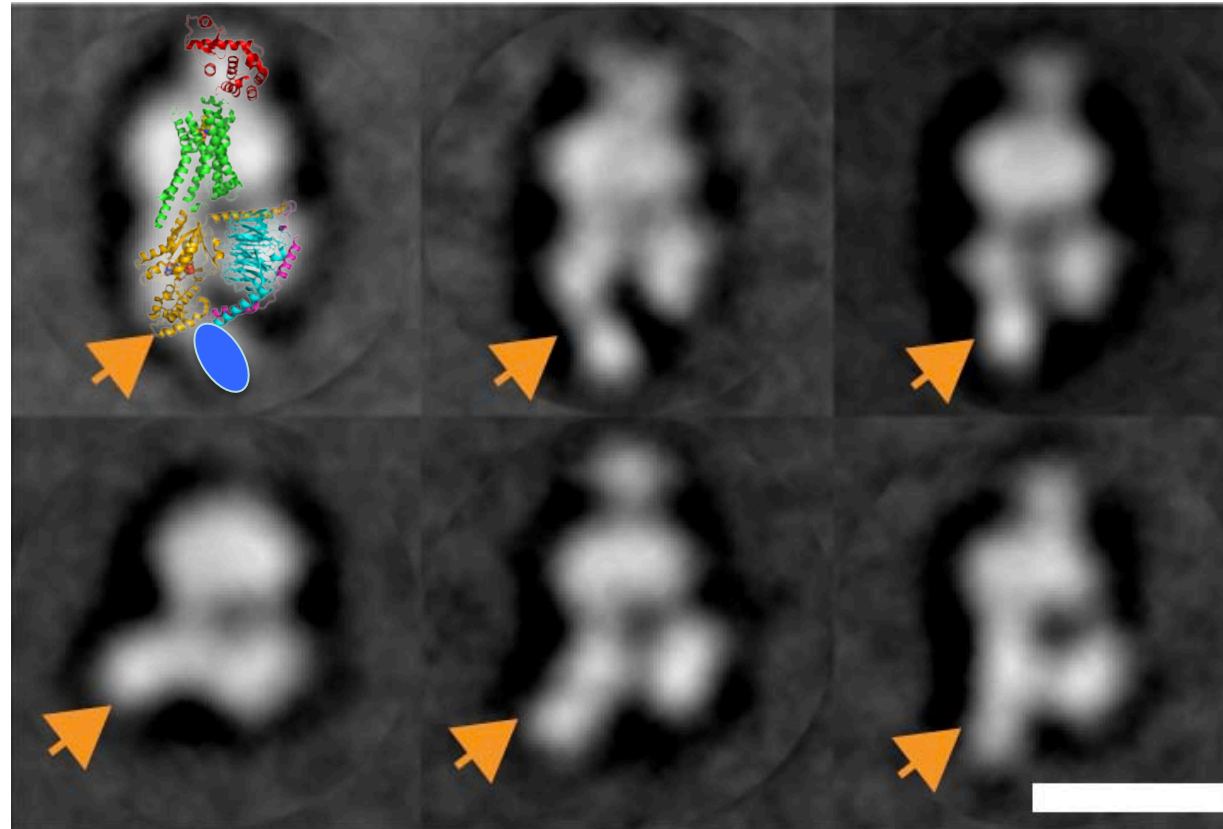
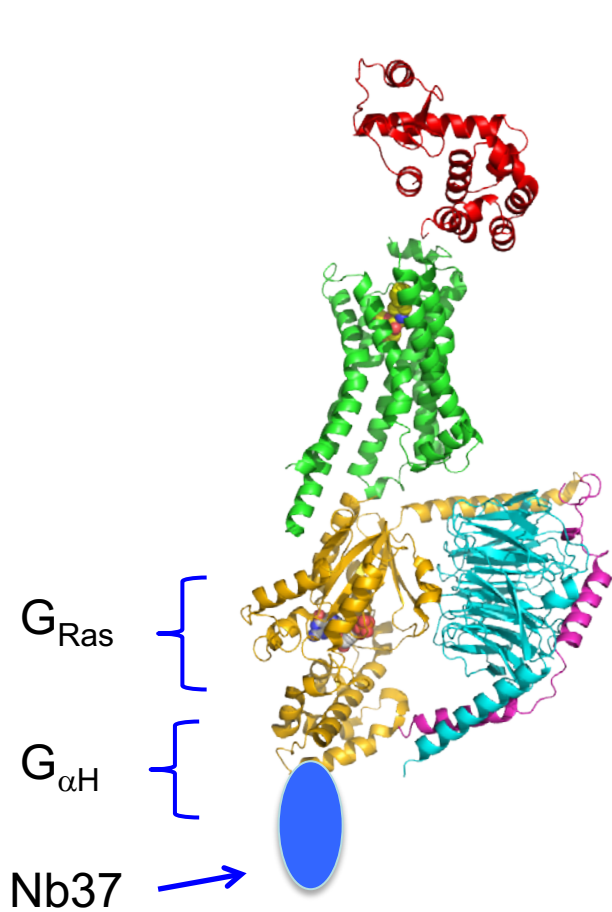
Gs $\alpha\beta\gamma$

Active

Inactive

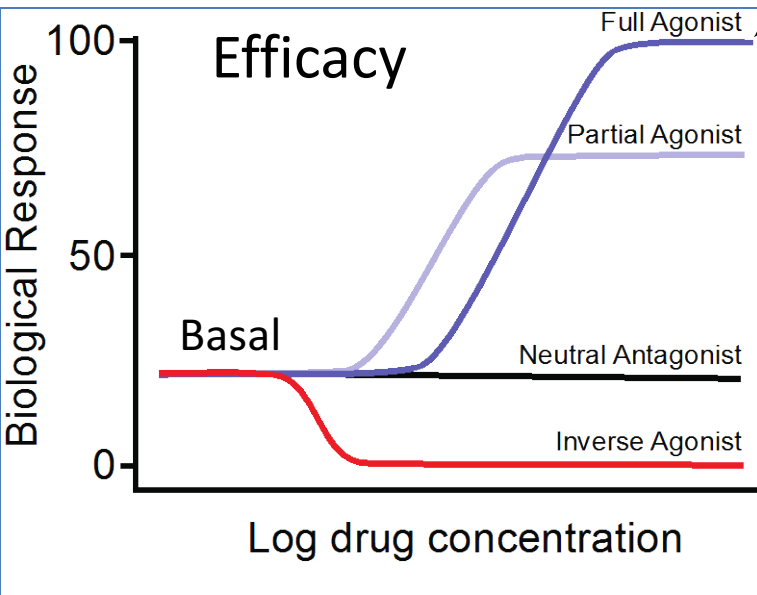
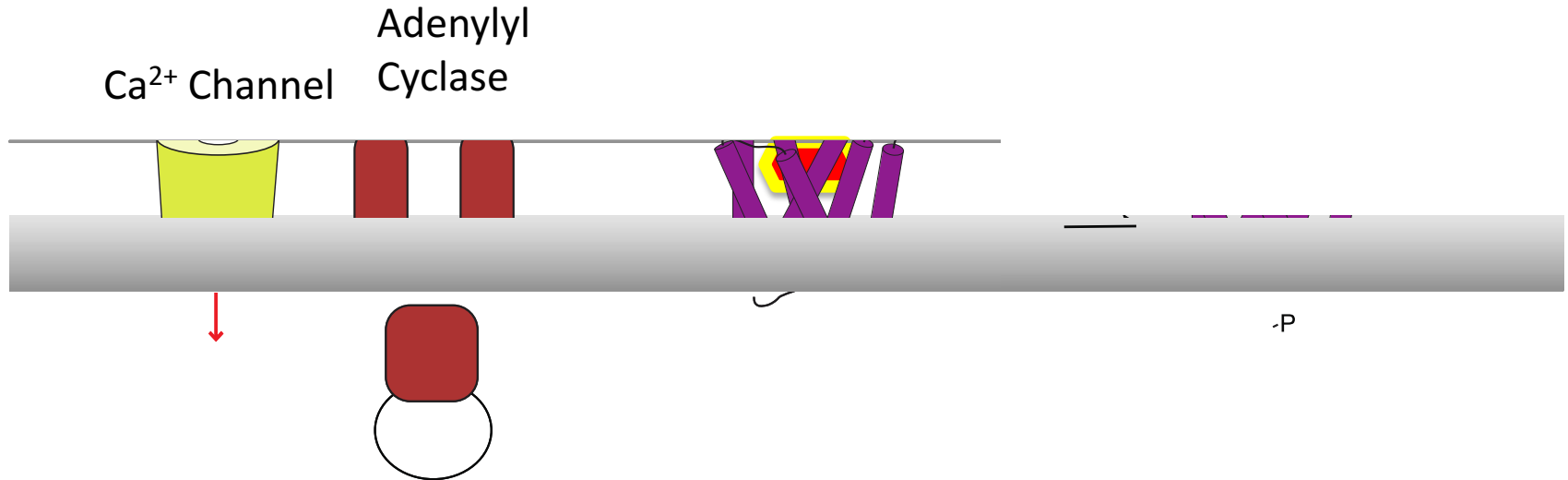


Mobility of alpha helical domain confirmed by EM



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
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- Kobilka lab students, postdoctoral fellows and collaborators (1989-2012)
- Bill Weis and Roger Sunahara

β_2 AR-Gs Team

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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