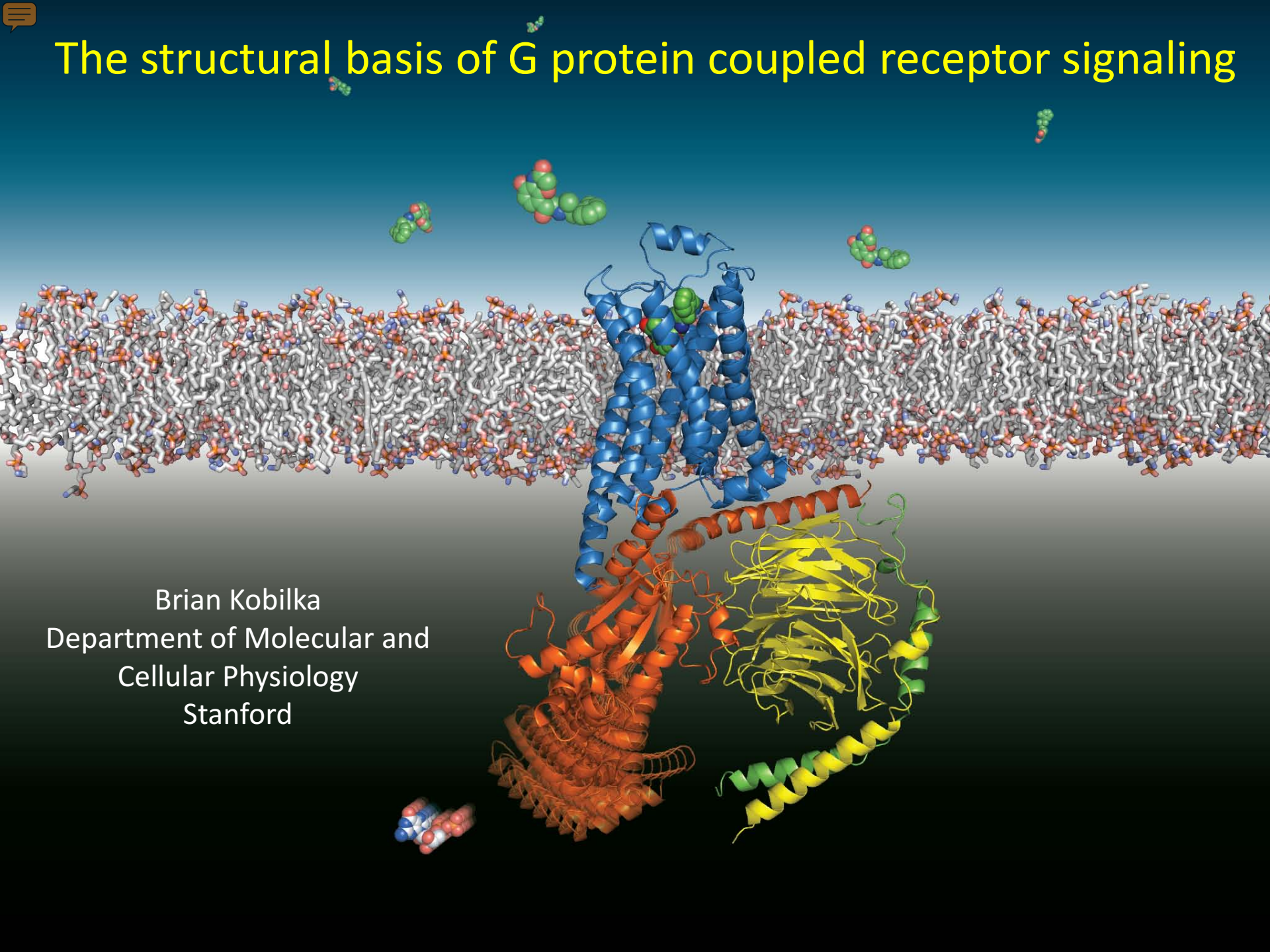
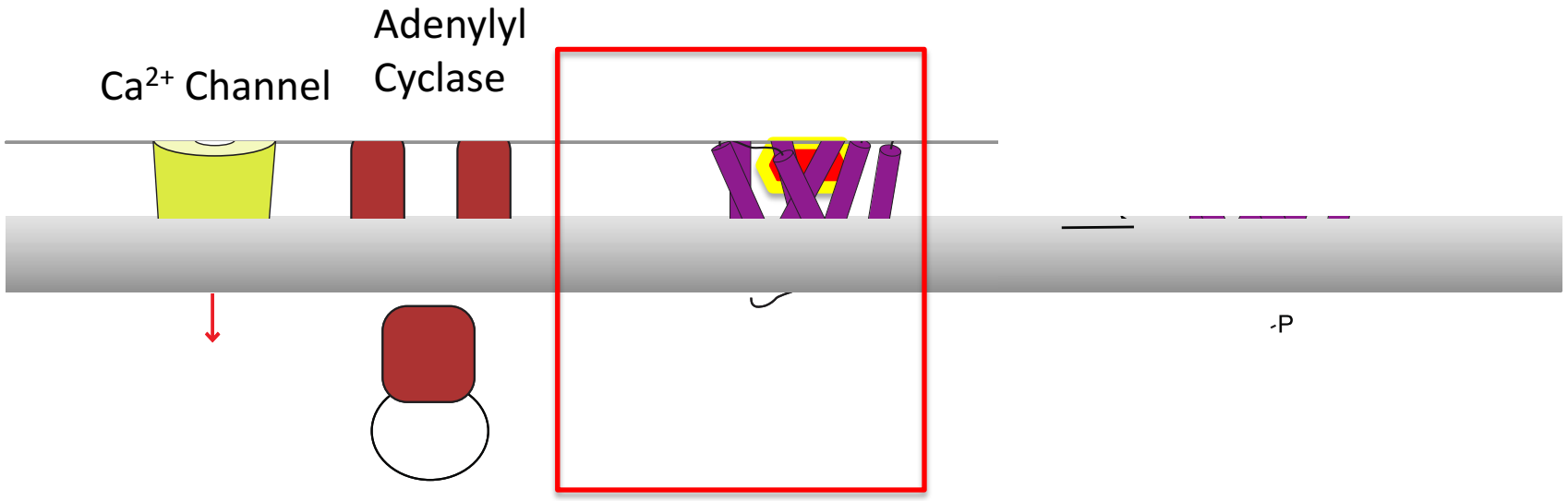
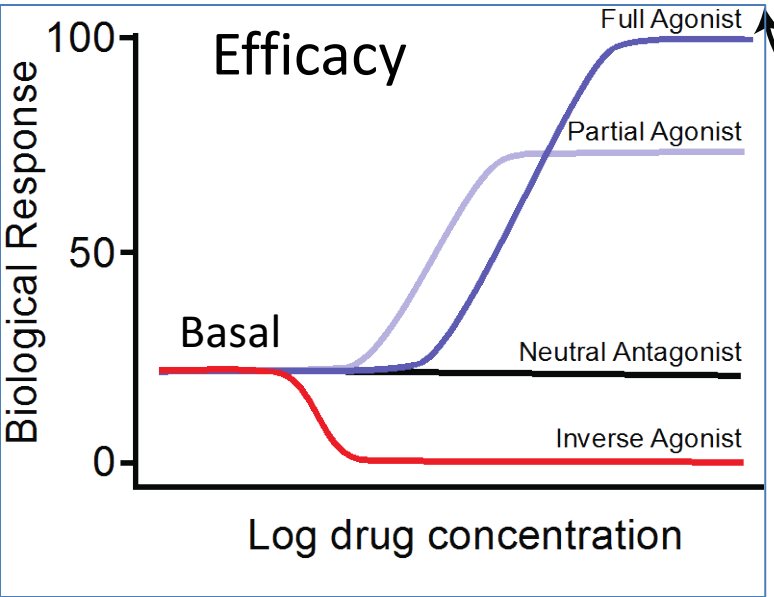
The structural basis of G protein coupled receptor signaling

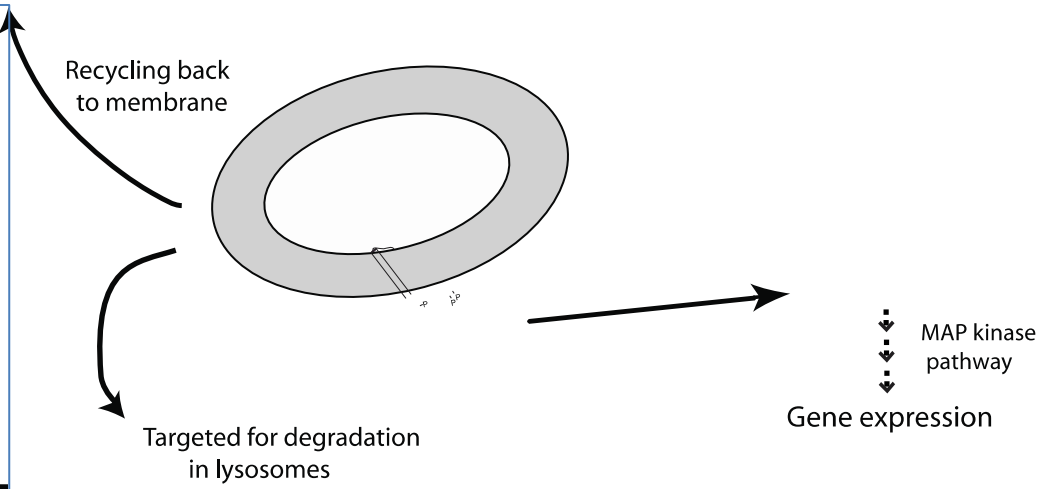


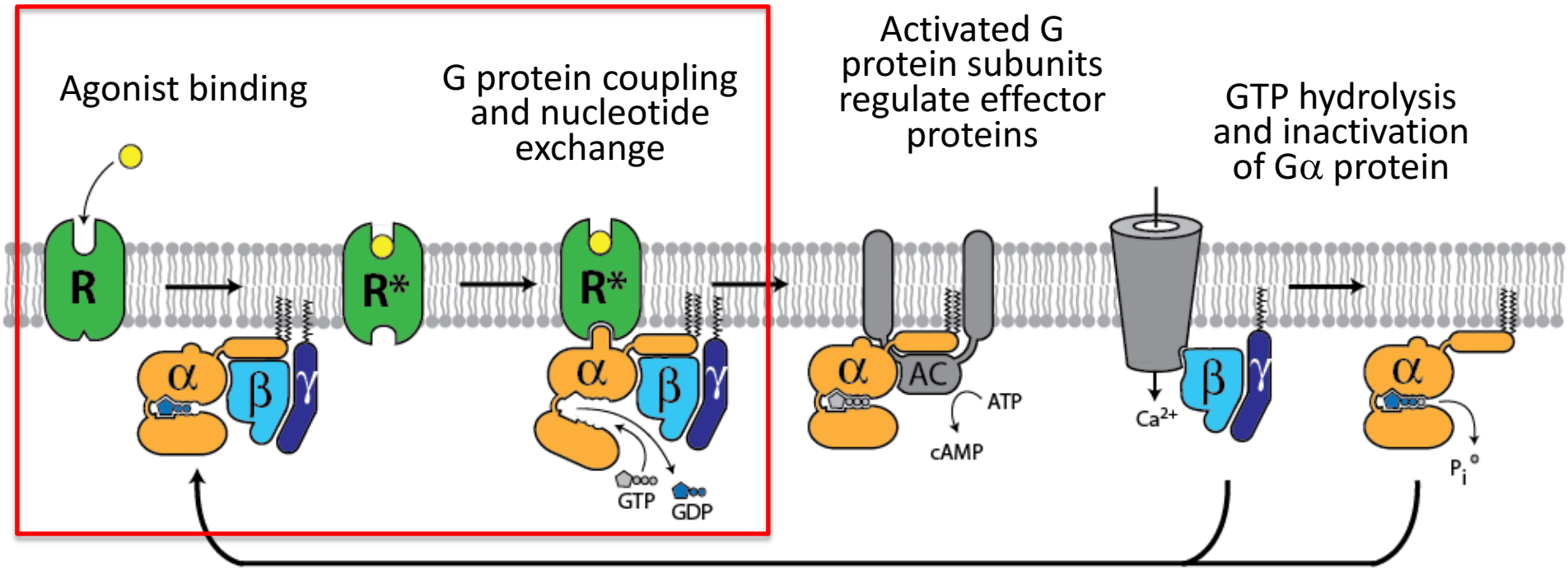
Brian Kobilka
Department of Molecular and Cellular Physiology
Stanford

The β_2AR modulates the activity of multiple signaling pathways





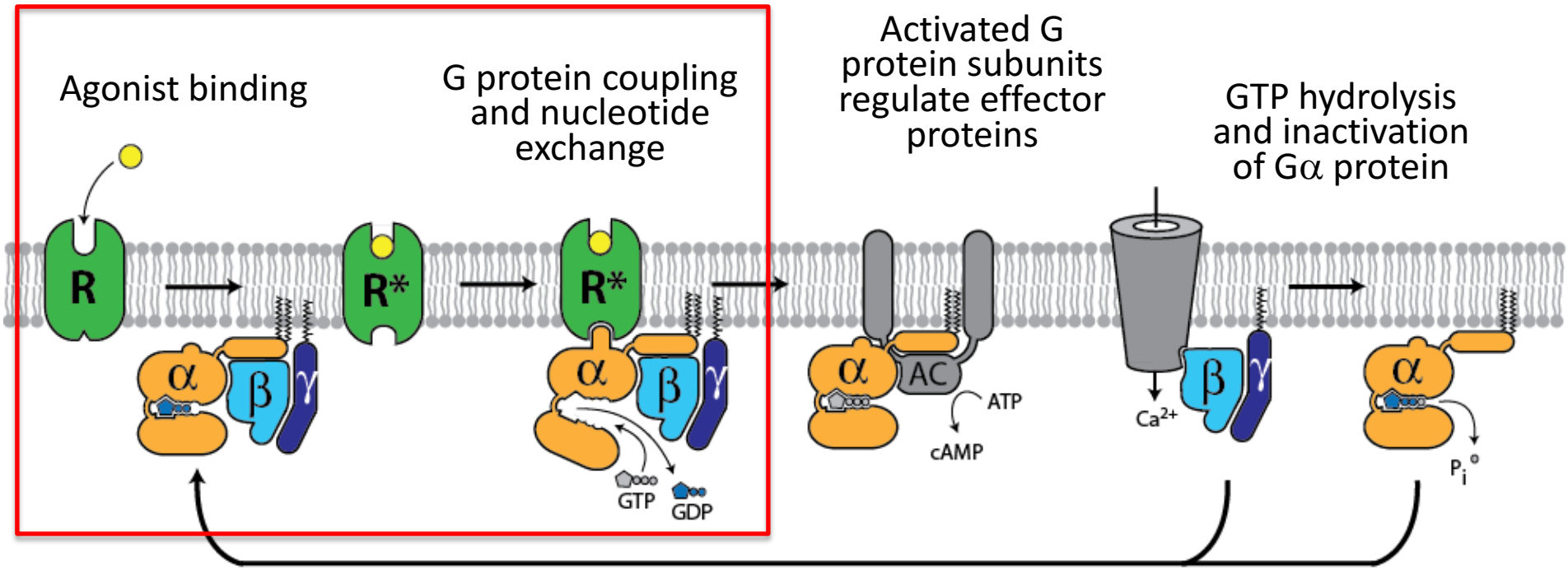




GPCR-G Protein Cycle

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_2AR structure:

- Sequence analysis
  - secondary structure (transmembrane domains)

Cloned DNA Sequence (1986)

GAATTCATGCCGCGTTTCTGTGTTGGACAGGGGTGACTTTGTGCC
GGATGGCTTCTGTGTGAGAGCGCGCGCGAGTGTGCATGTCGGTGA
GCTGGGAGGGTGTGTCTCAGTGTCTATGGCTGTGGTTCGGTATAAG
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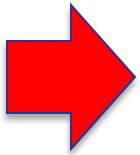

Amino Acid Sequence

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VYILLNWIGYVNSGFNPLIYCRSPDFRIA
FQELLCLRRSSLKAYGNGYSSNGNTGEQ
SGYHVEQEKENKLLCEDLPGTEDFVGH
QGTVPSDNIDSQGRNCSTNDSLL

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GAATTCATGCCGCGTTTCTGTGTTGGACAGGGGTGACTTTGTGCC
GGATGGCTTCTGTGTGAGAGCGCGCGCGAGTGTGCATGTCGGTGA
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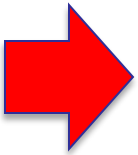

Amino Acid Sequence

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MGLAVVPFGAHILMKMWTFGNFWCE
FWTSIDVLCVTASIETLCVIAVDRYFAITS
PFKYQSLLTKNKARVIILMVWIVSGLTSF
LPIQMHWYRATHQEAINCYANETCCDF
FTNQAYAIASSIVSFYVPLVIMVFVYSRV
FQEAKRQLQKIDKSEGRFHVQNLSQVE
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FQELLCLRRSSLKAYGNGYSSNGNTGEQ
SGYHVEQEKENKLLCEDLPGTEDFVGH
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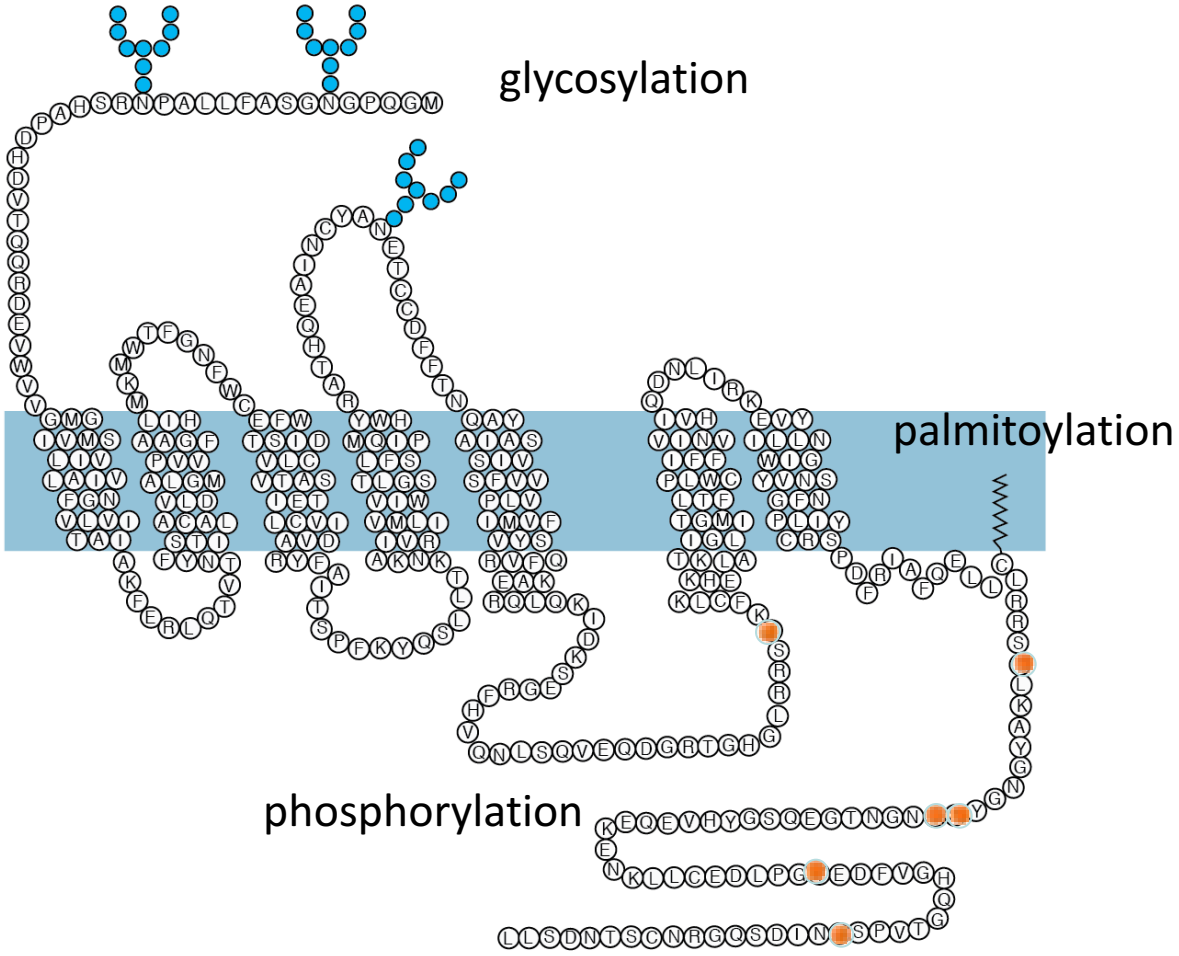
Approaches to characterizing β_2AR structure:

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



Approaches to characterizing β_2AR structure:

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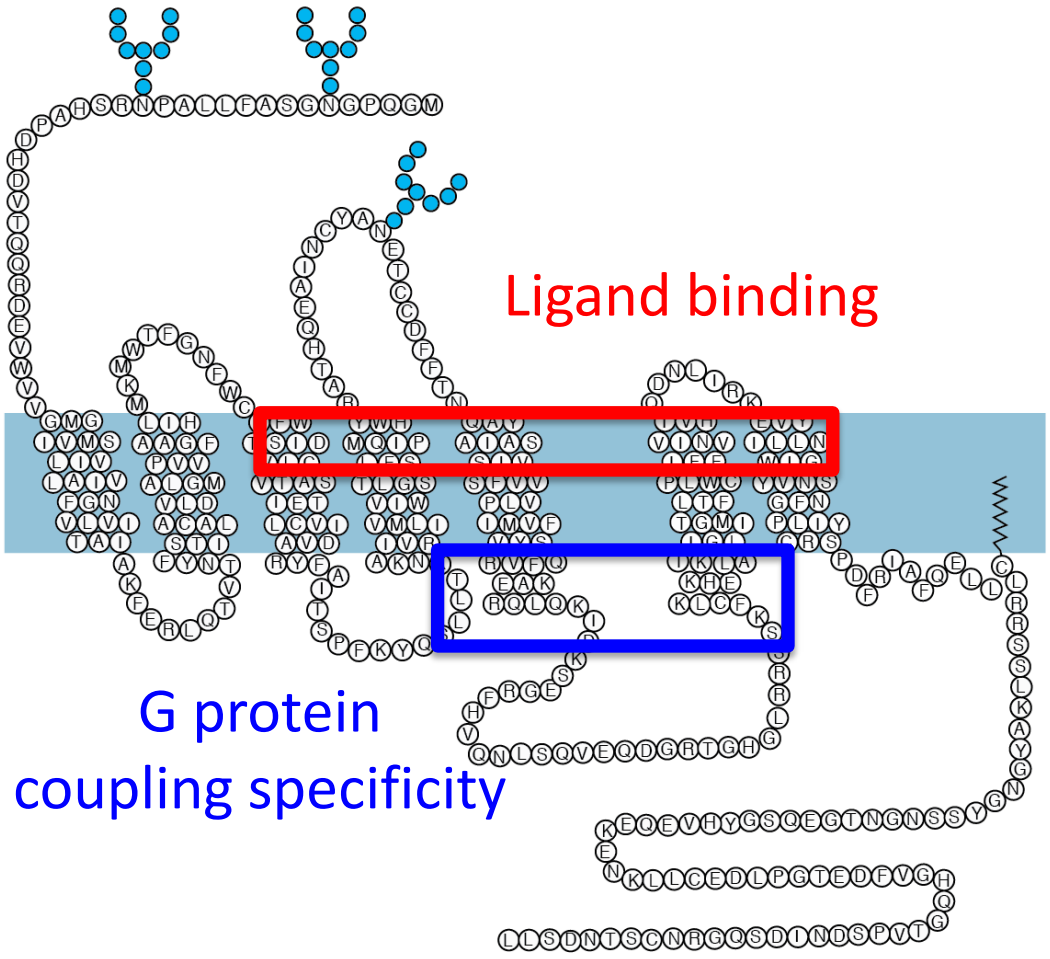
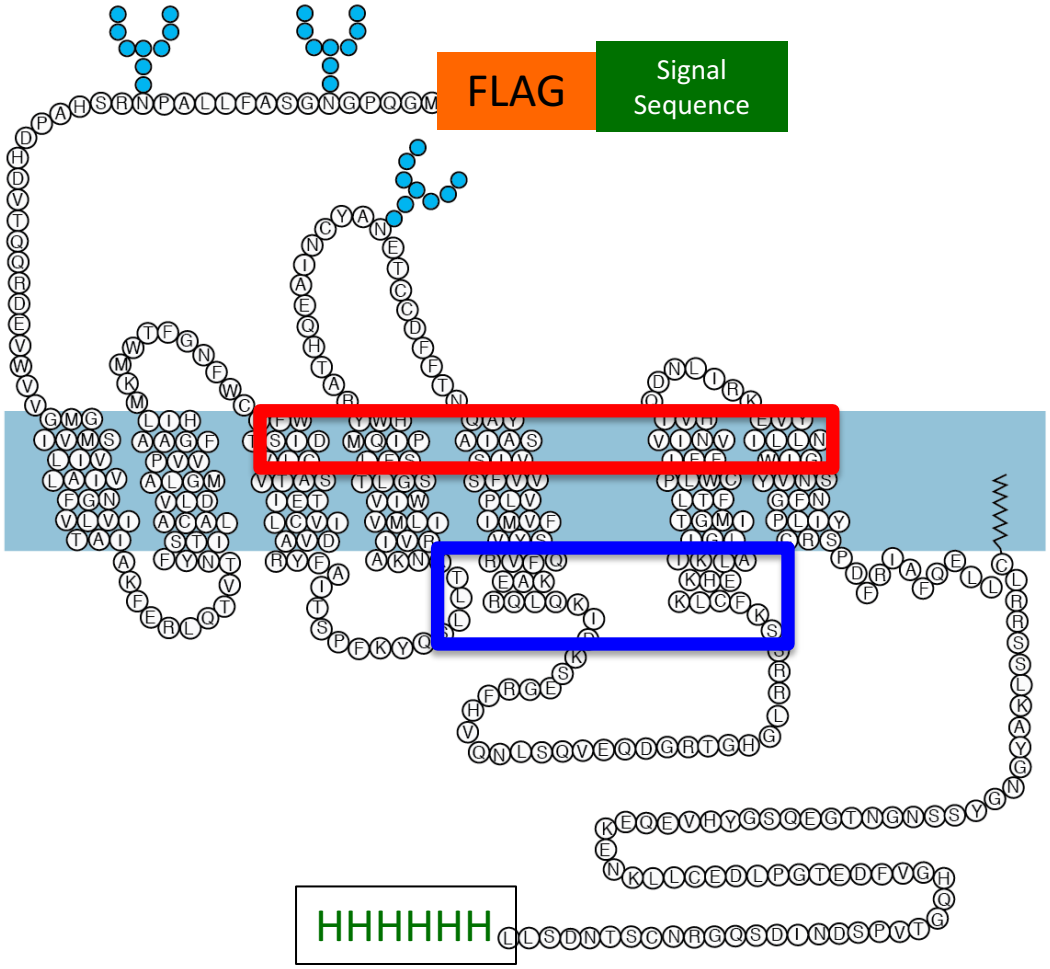
- Chimeric Receptors and site-directed mutagenesis
  - ligand binding and G protein coupling



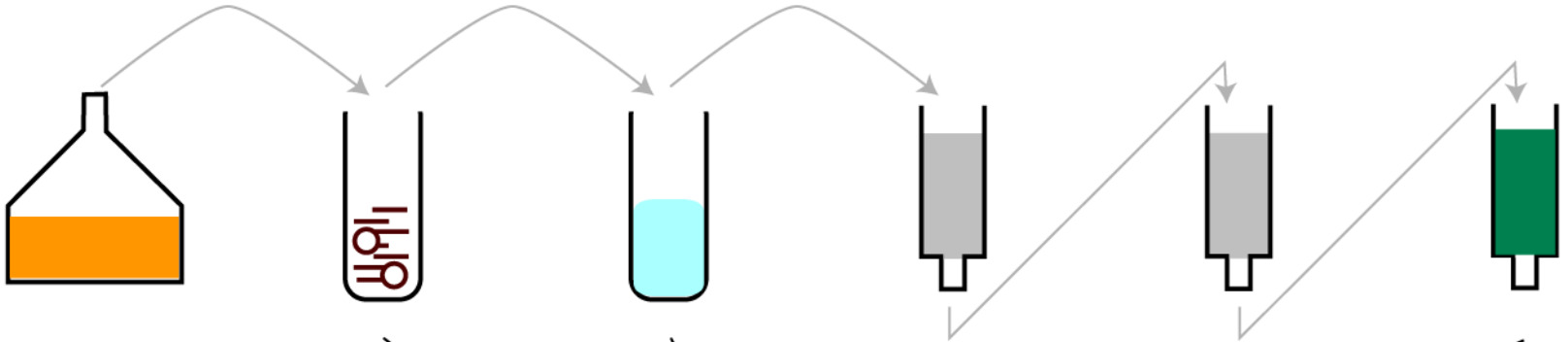
Approaches to characterizing β_2AR structure:



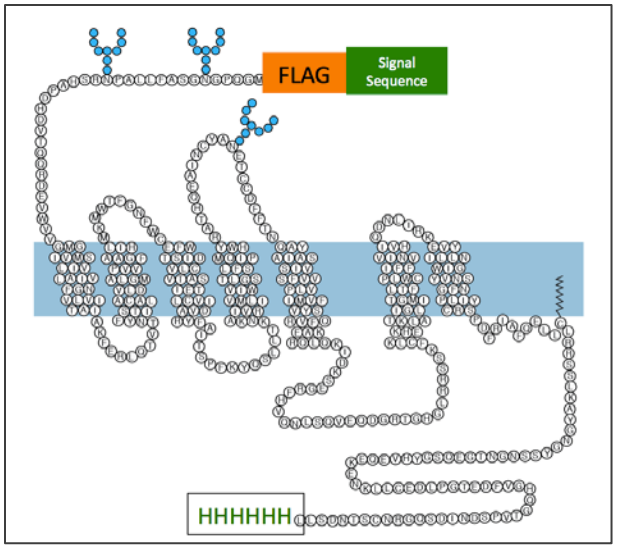
- Sequence analysis
  - secondary structure (transmembrane domains)
  - post-translational modifications
- Chimeric Receptors and Site-directed mutagenesis
  - ligand binding and G protein coupling
  - enhance expression and purification

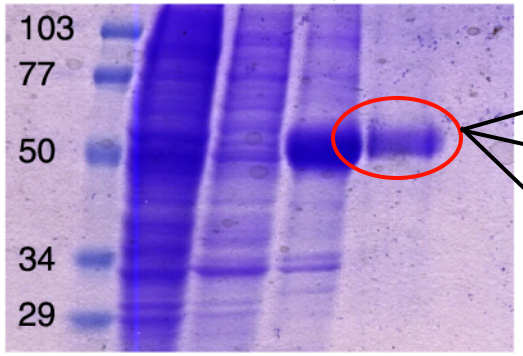
Expression and purification

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



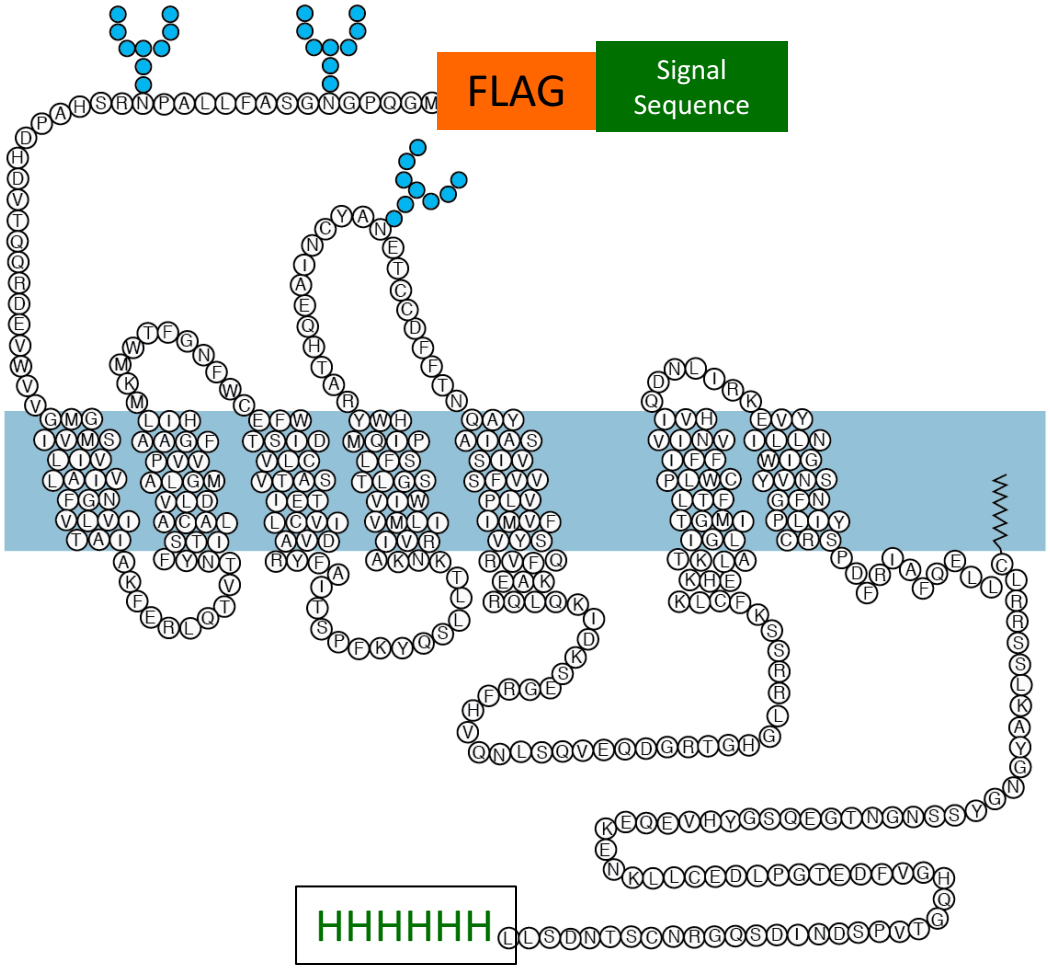
1x | 1x | 500x | 500x





- Biochemistry
- Spectroscopy
- Crystallography

Approaches to characterizing β_2AR structure:



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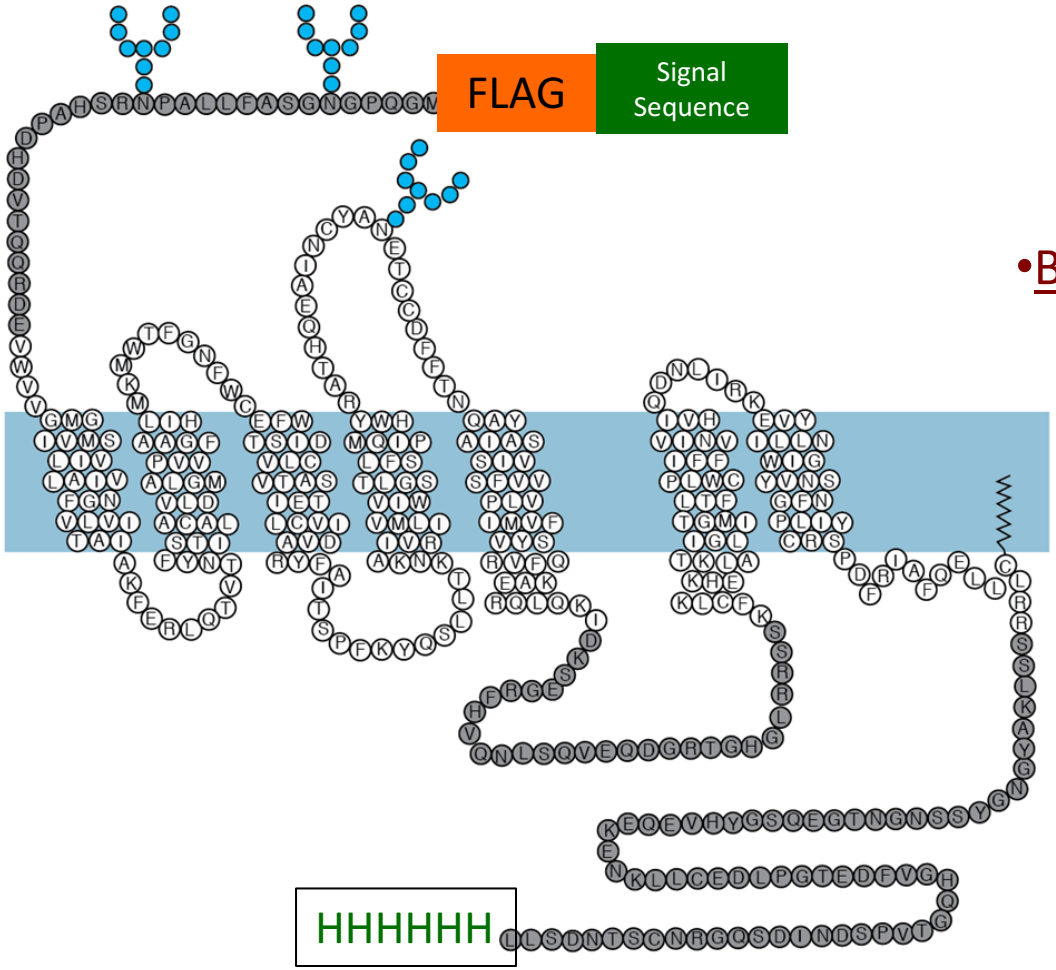

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  - unstructured, flexible sequence

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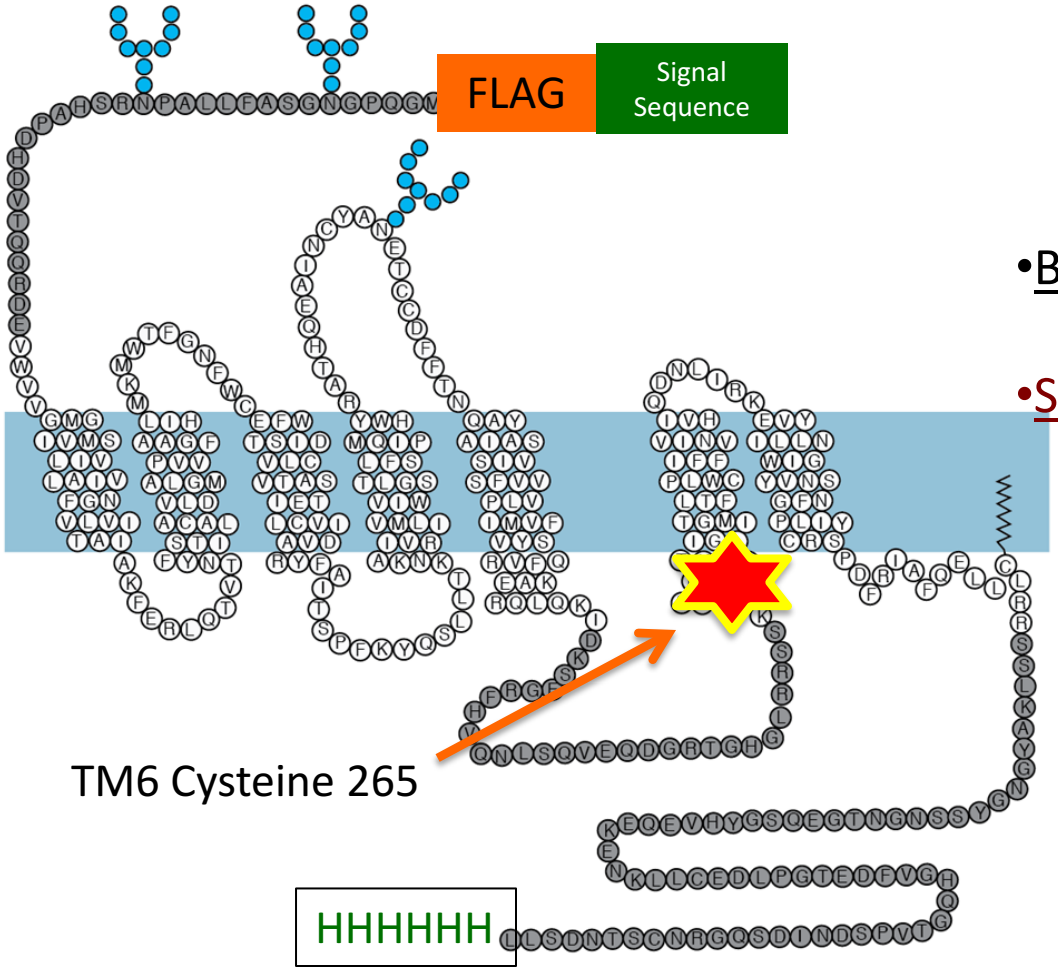
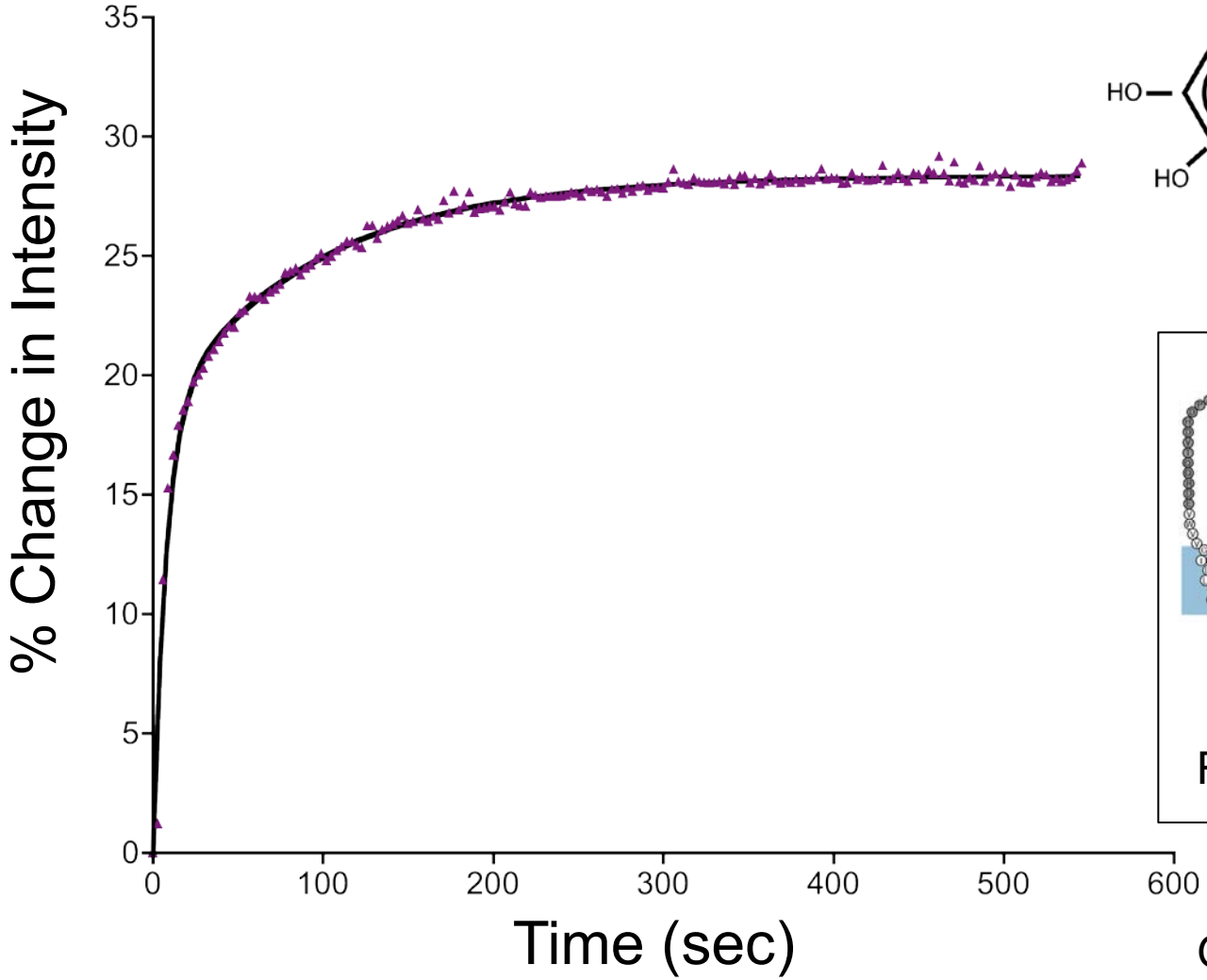
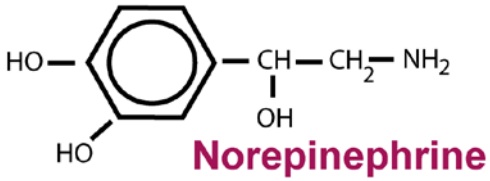
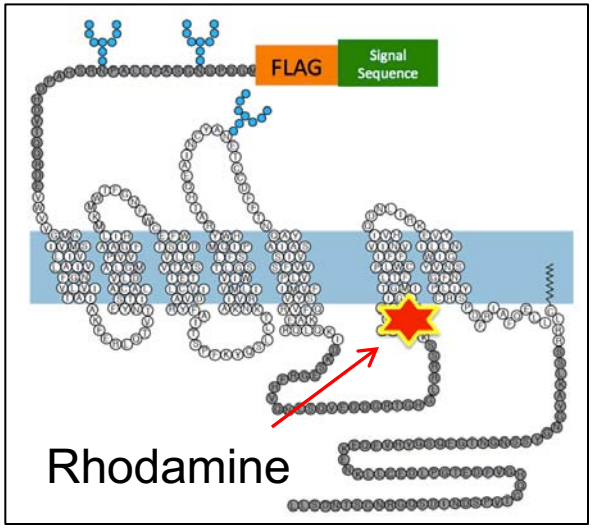


- Sequence analysis
  - secondary structure (transmembrane domains)
  - post-translational modifications
- Chimeric Receptors and site-directed mutagenesis
  - ligand binding and G protein coupling
  - enhance expression and purification
- Biochemistry
  - unstructured, flexible sequence
- Spectroscopy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

Conformational changes in TM6 in response to norepinepherine:

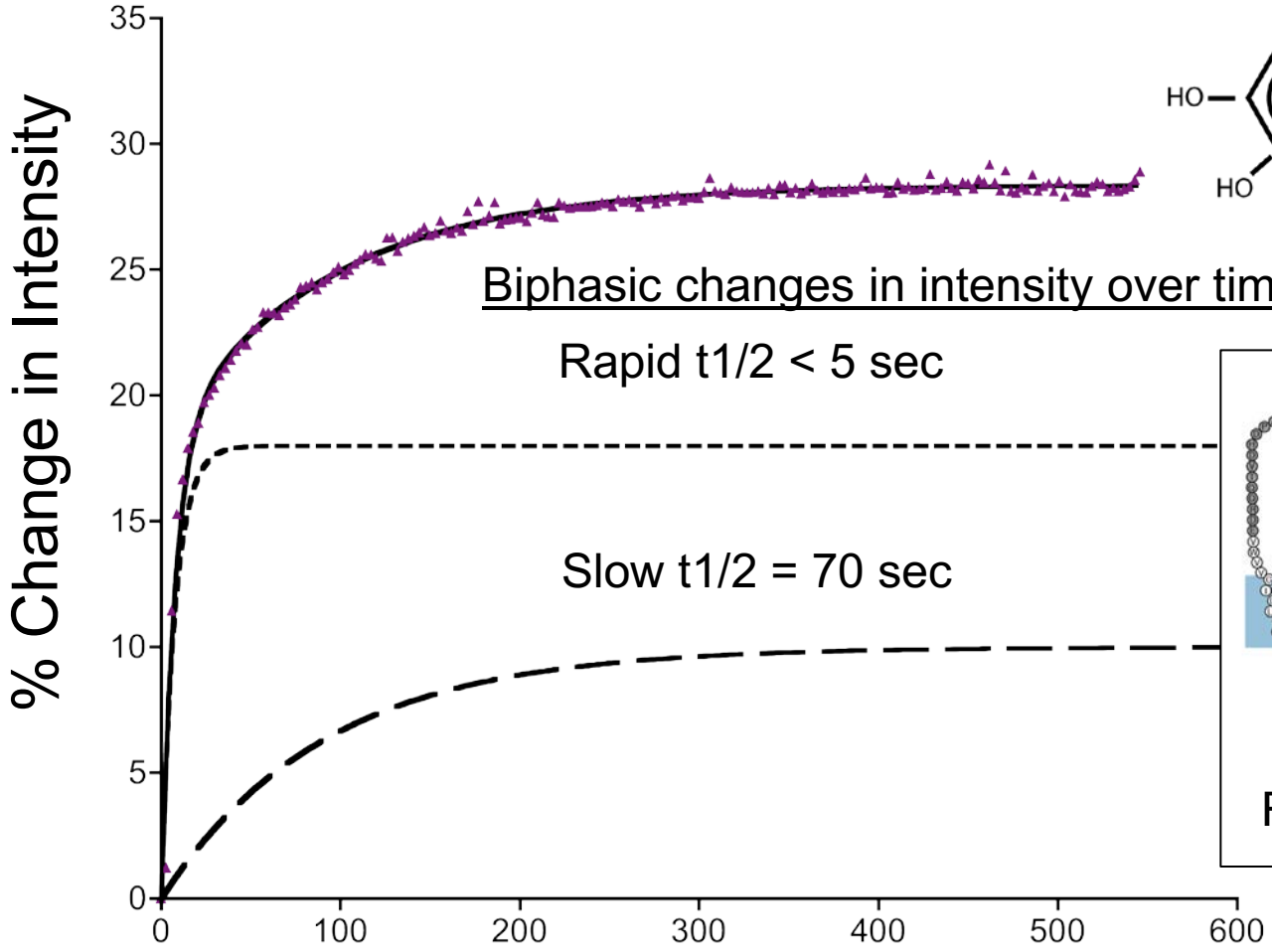


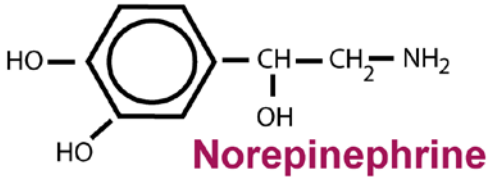




Gayathri Swaminath, 2004

Conformational changes in TM6 in response to norepinepherine:

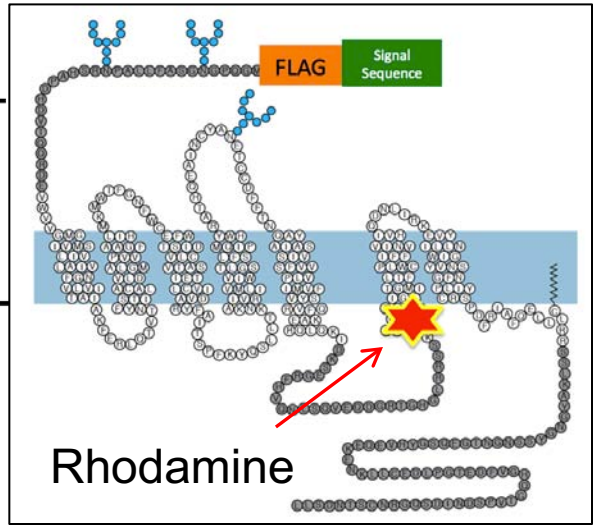




Biphasic changes in intensity over time

Rapid t1/2 < 5 sec

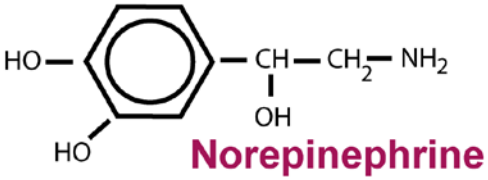
Slow t1/2 = 70 sec



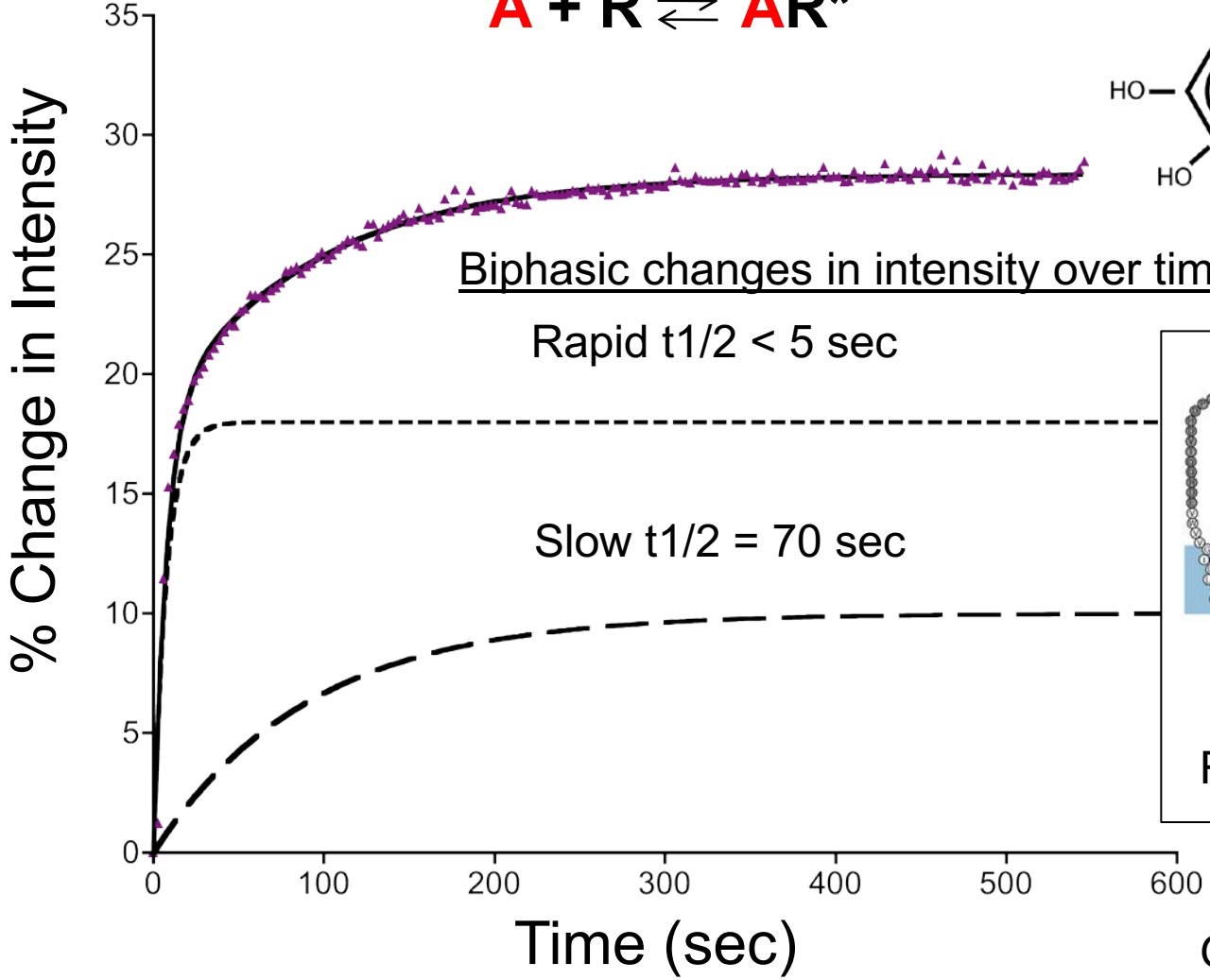
Gayathri Swaminath, 2004

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

$$A + R \rightleftarrows AR^*$$



Norepinephrine



Biphasic changes in intensity over time

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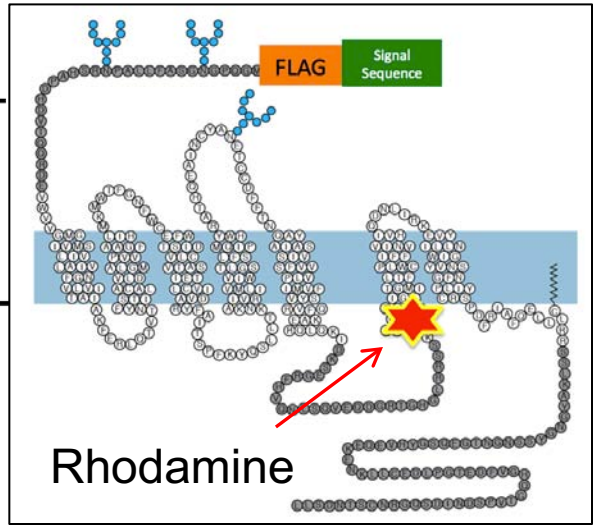
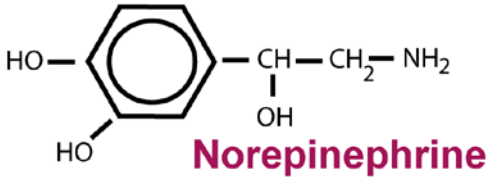


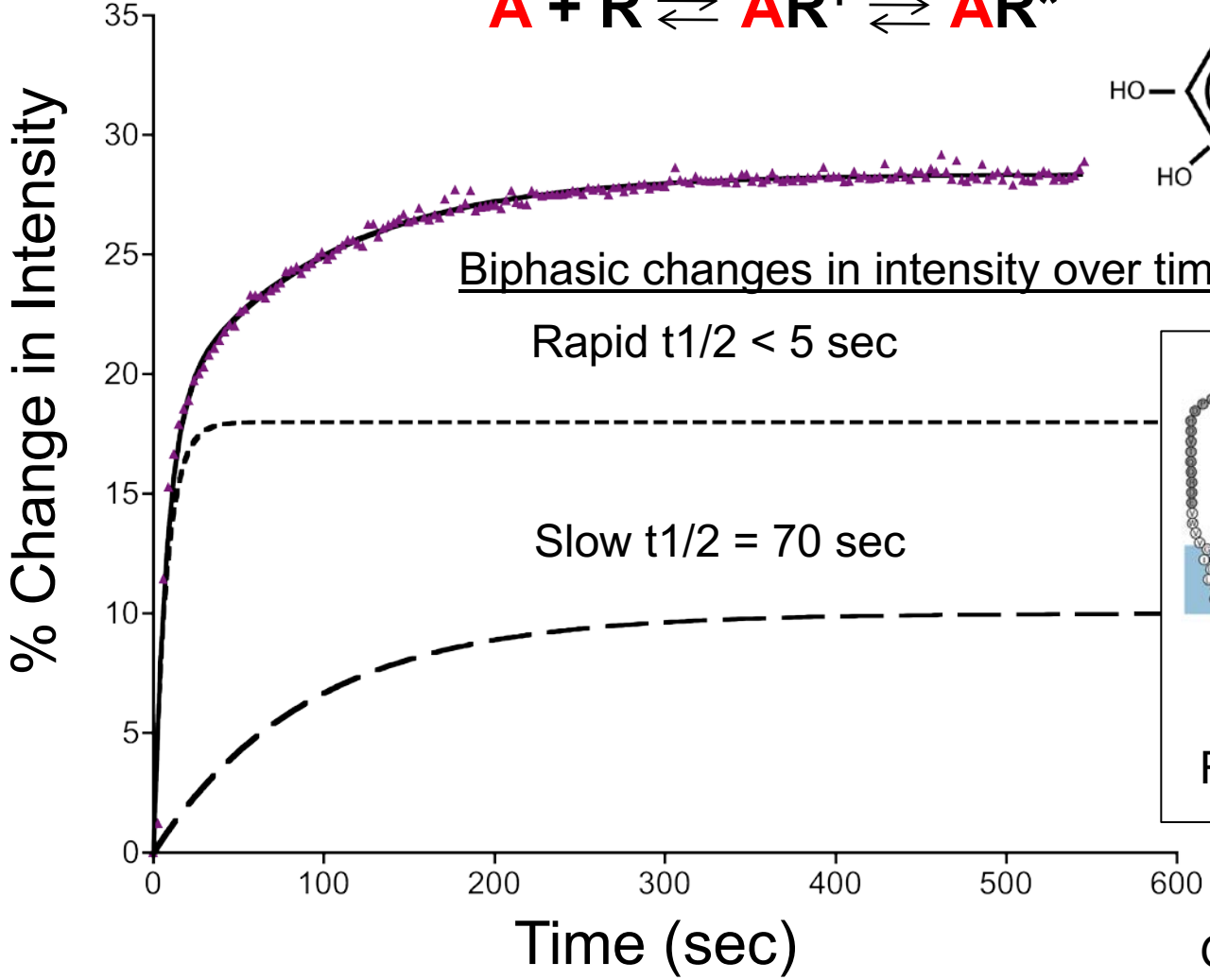
Gayathri Swaminath, 2004

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

$$A + R \rightleftarrows AR^{+} \rightleftarrows AR^{*}$$



Norepinephrine



Biphasic changes in intensity over time

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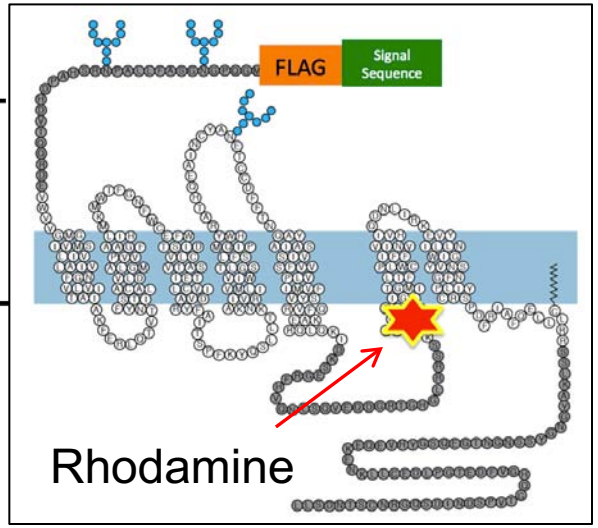
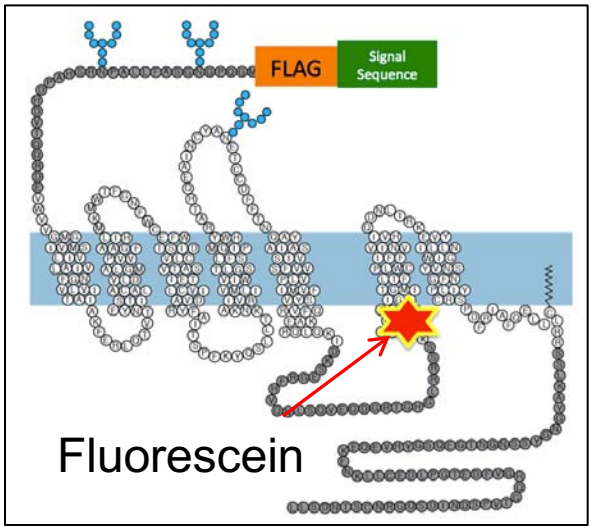


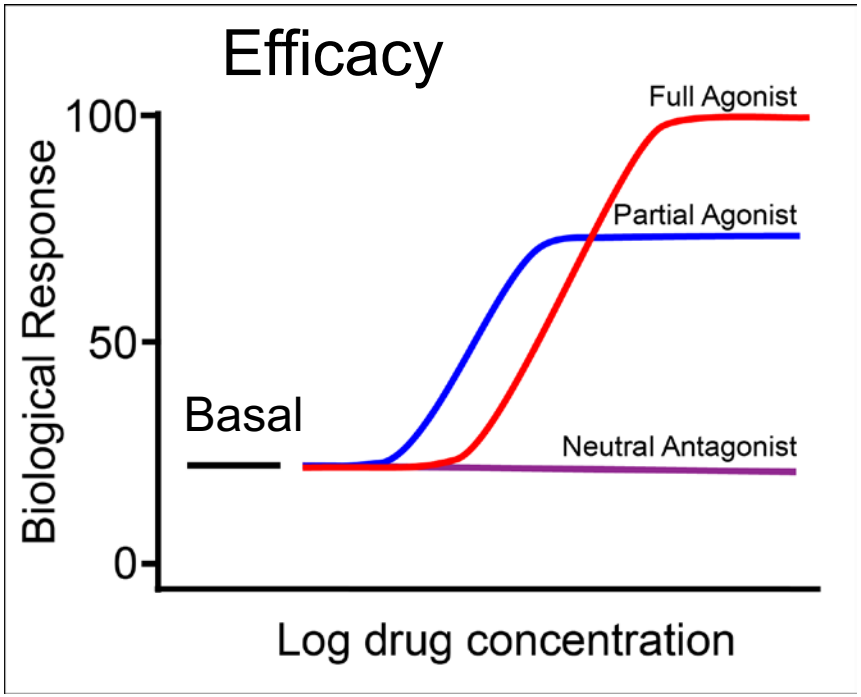
Gayathri Swaminath, 2004

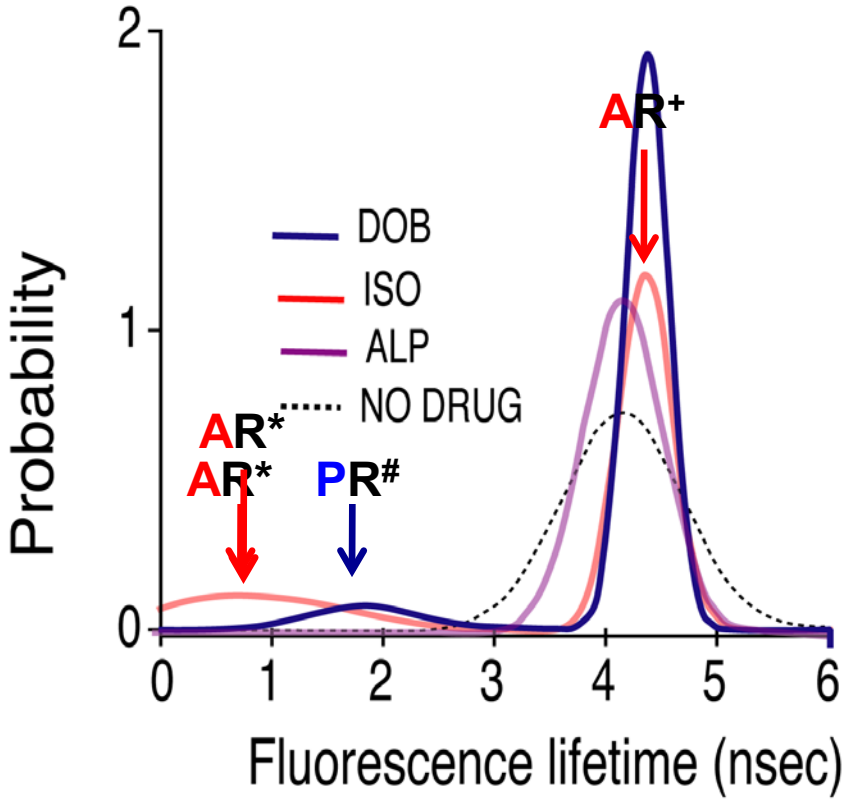


Fluorescence lifetime experiments

- Multiple agonist states
- Ligand-specific conformational states

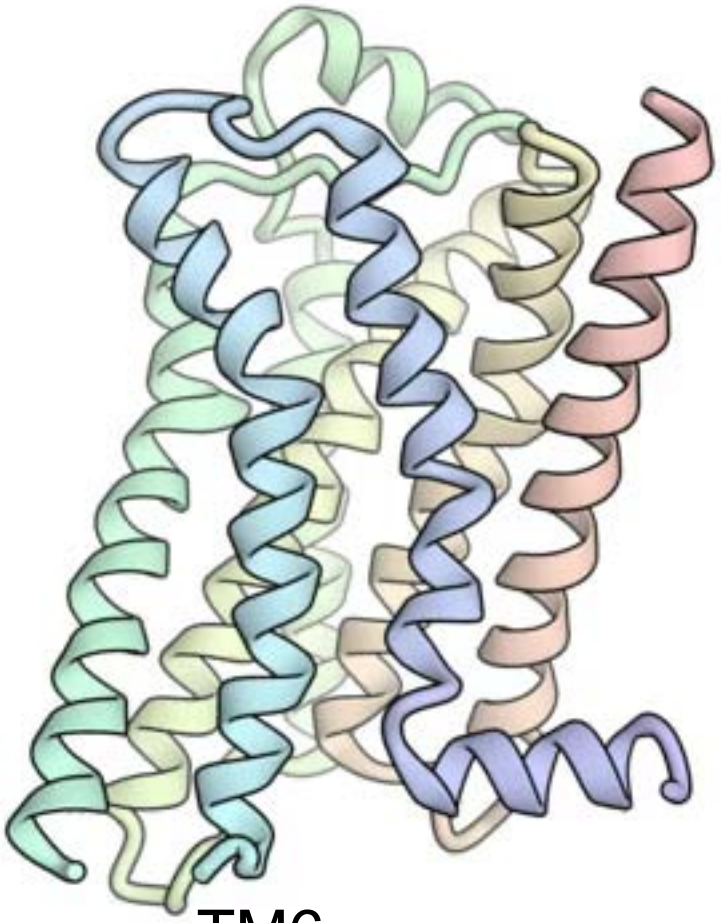
$$A + R \rightleftarrows AR^{+} \rightleftarrows AR^{*}$$
$$P + R \rightleftarrows PR^{+} \rightleftarrows PR^{\#}$$





Pejman Ghanouni, 2001

Insights from spectroscopy studies -fluorescence, NMR, EPR-



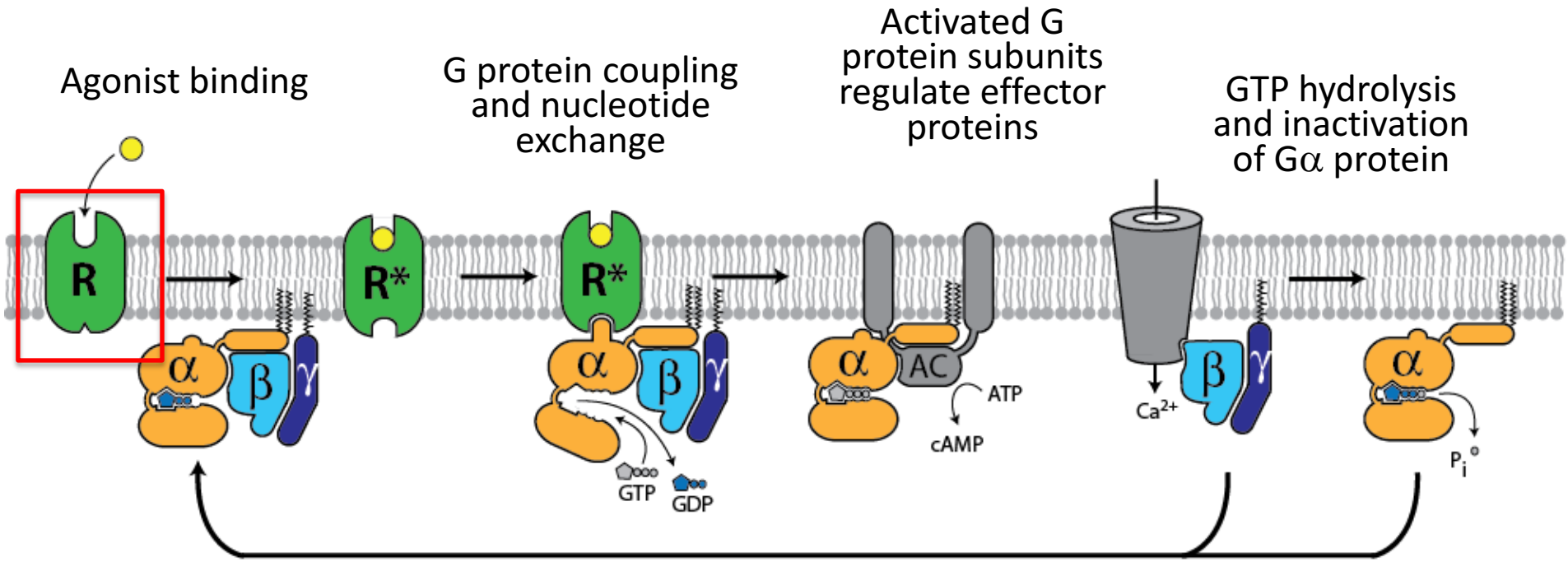
TM6

- The β_2AR 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

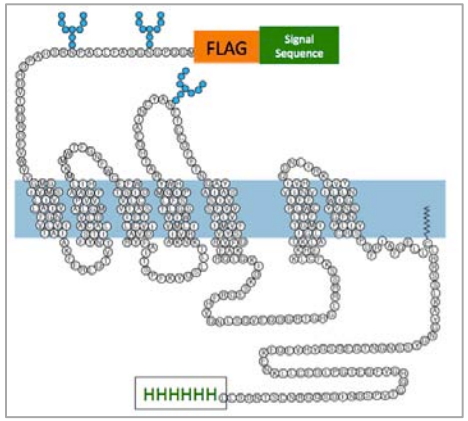
Ansgar Philippsen & Ron Dror (D.E. Shaw Research)

Outline

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



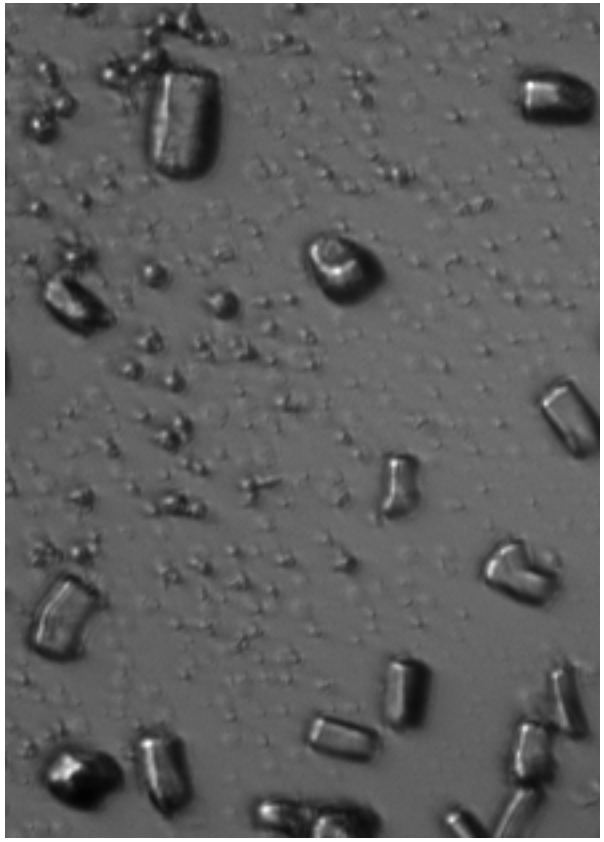
GPCR-G Protein Cycle

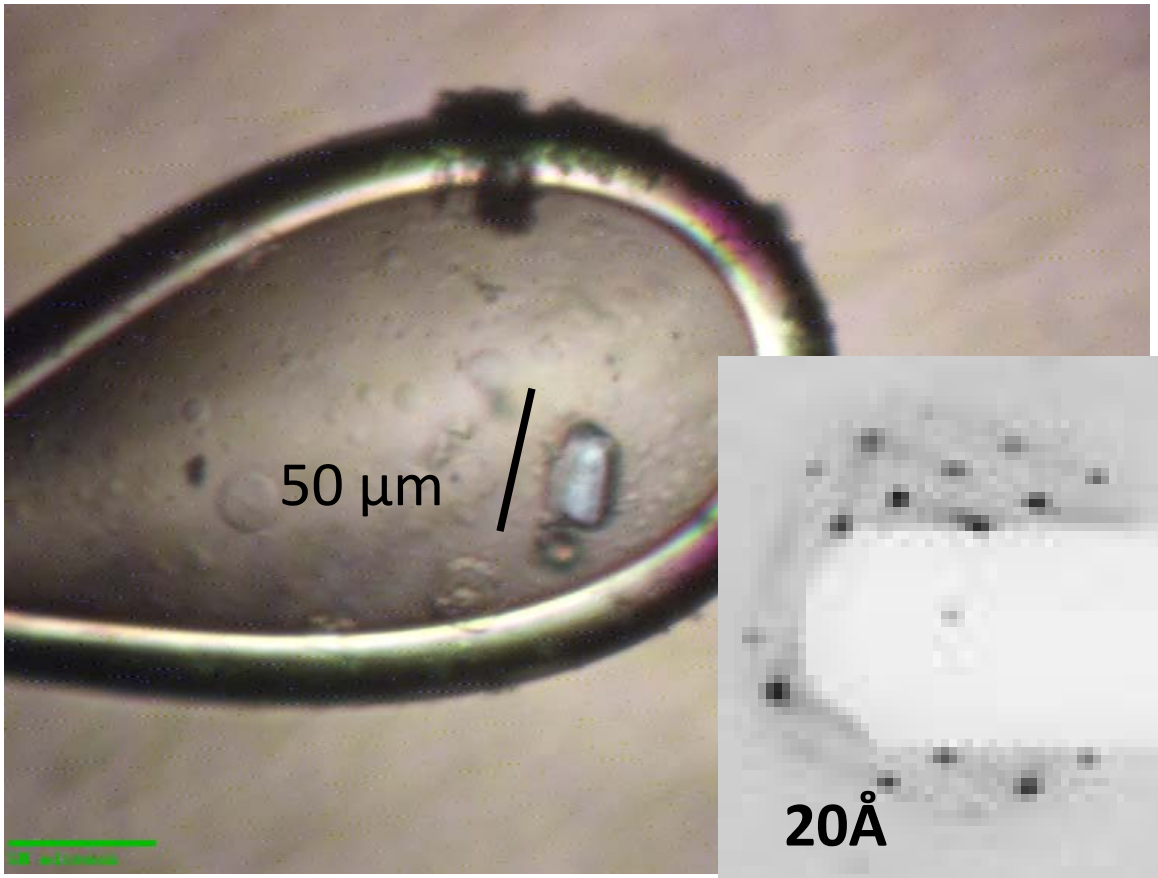


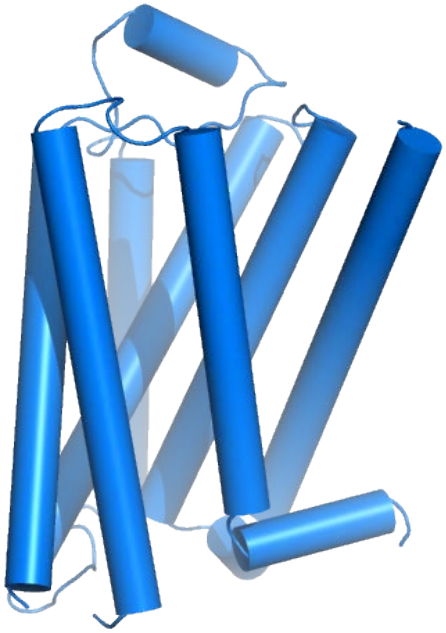
Crystals of wild-type β_2AR

Nov 2004

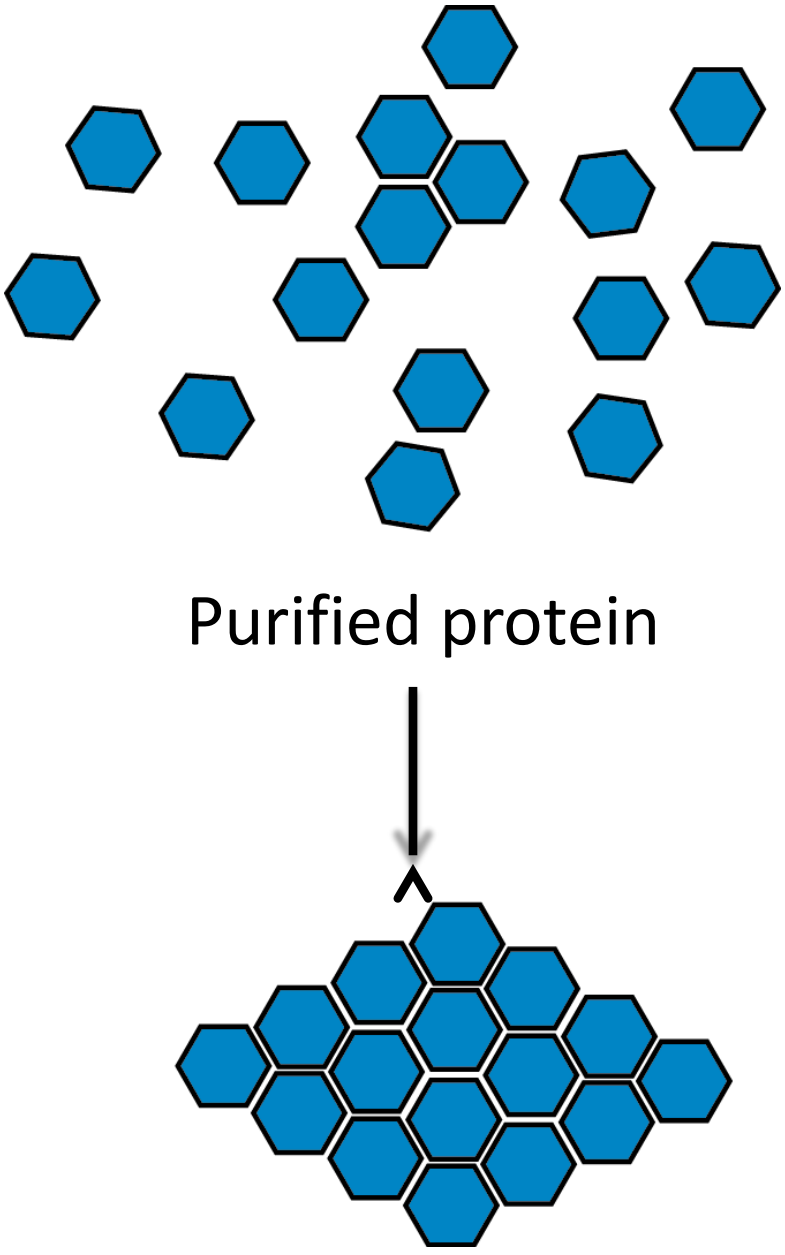
ESRF microfocus beamline ID13, July 2005

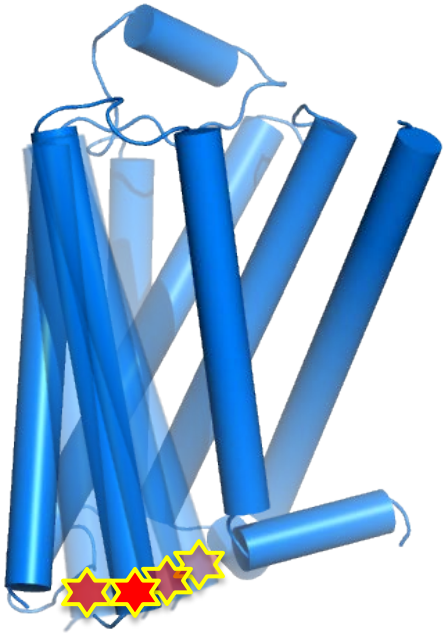




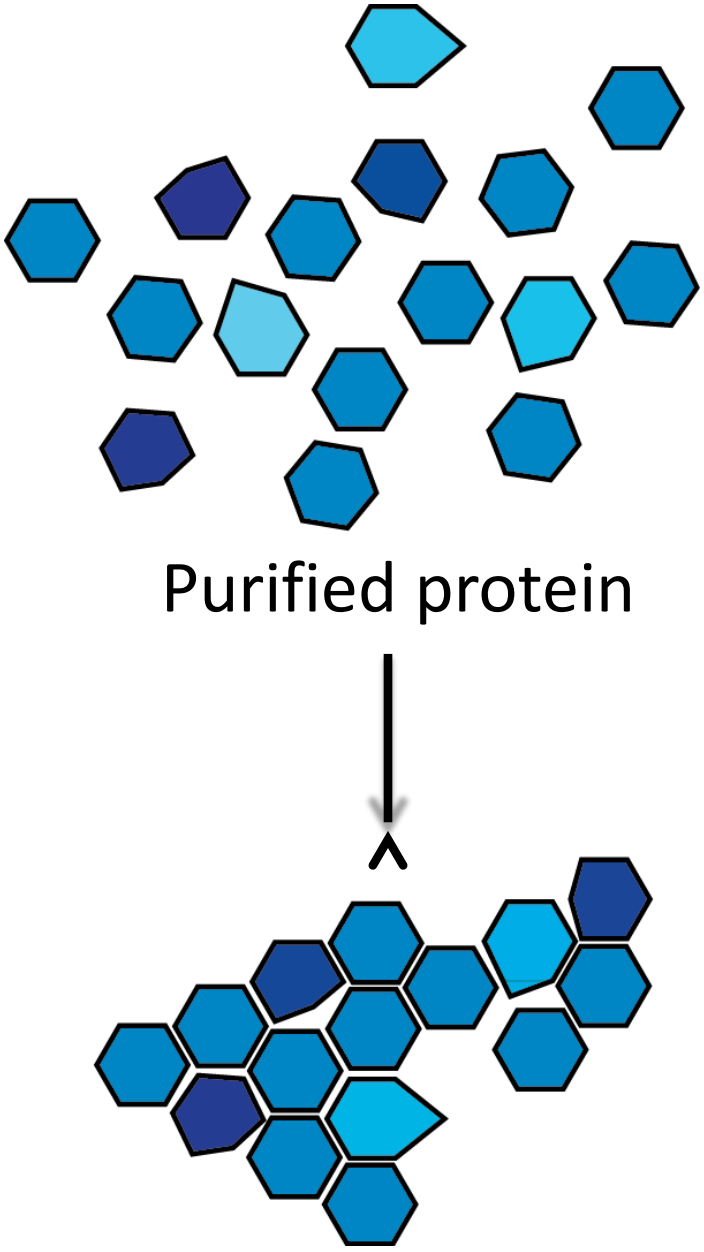


Conformationally uniform GPCR



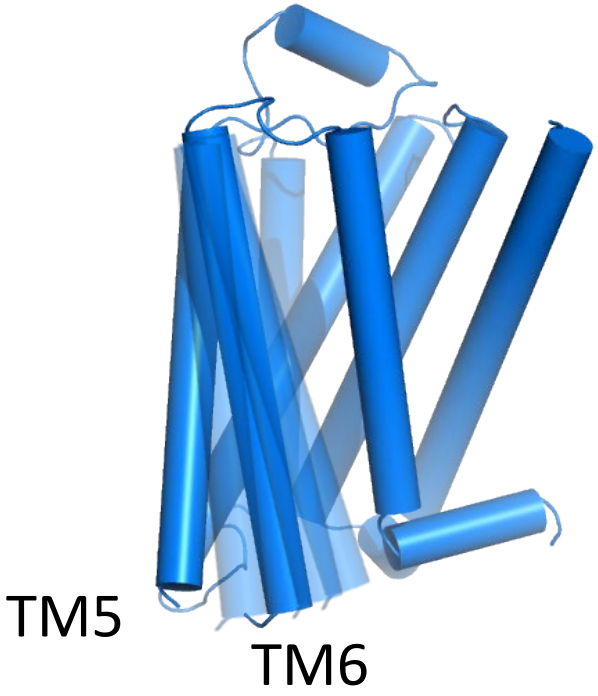


Conformationally heterogeneous GPCR



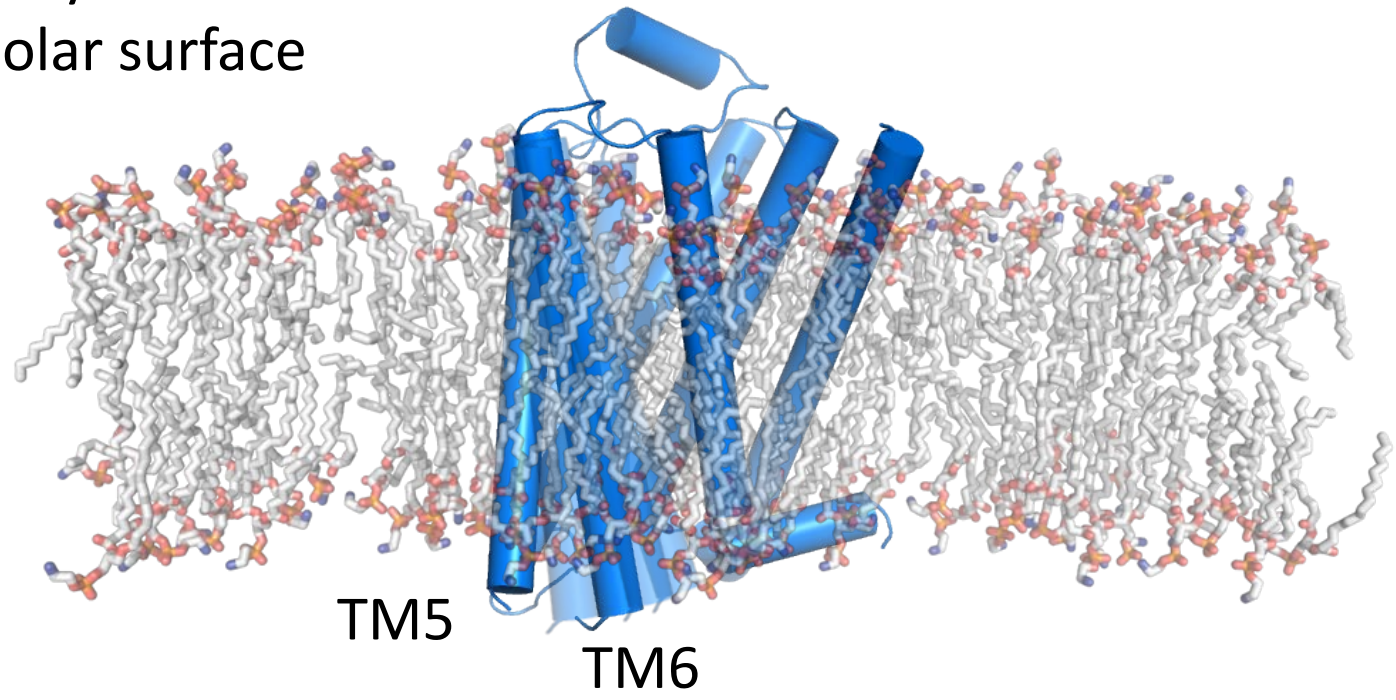
Challenges for crystallography

- Protein dynamics



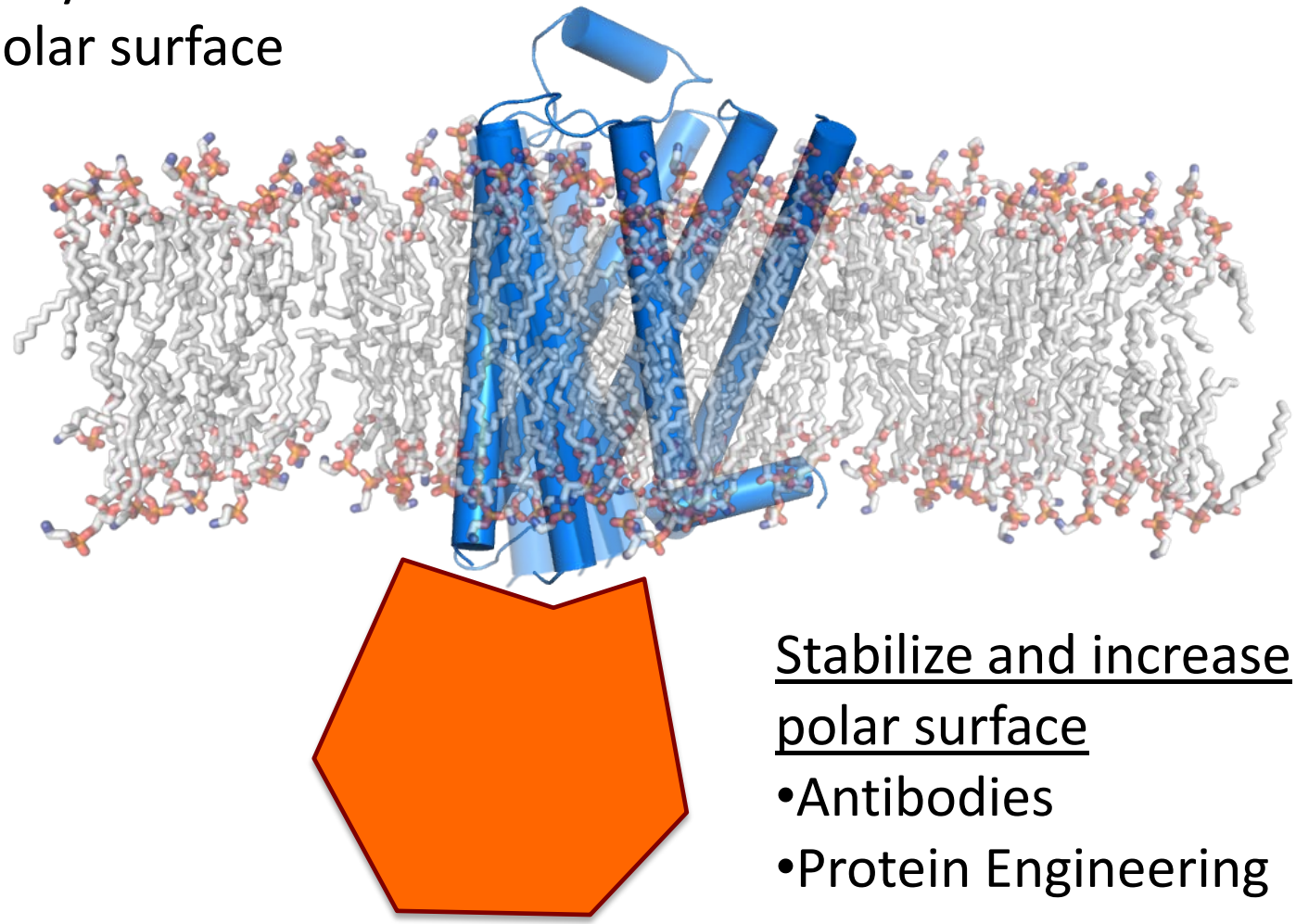
Challenges for crystallography

- Protein dynamics
- Little polar surface



Challenges for crystallography

- Protein dynamics
- Little polar surface

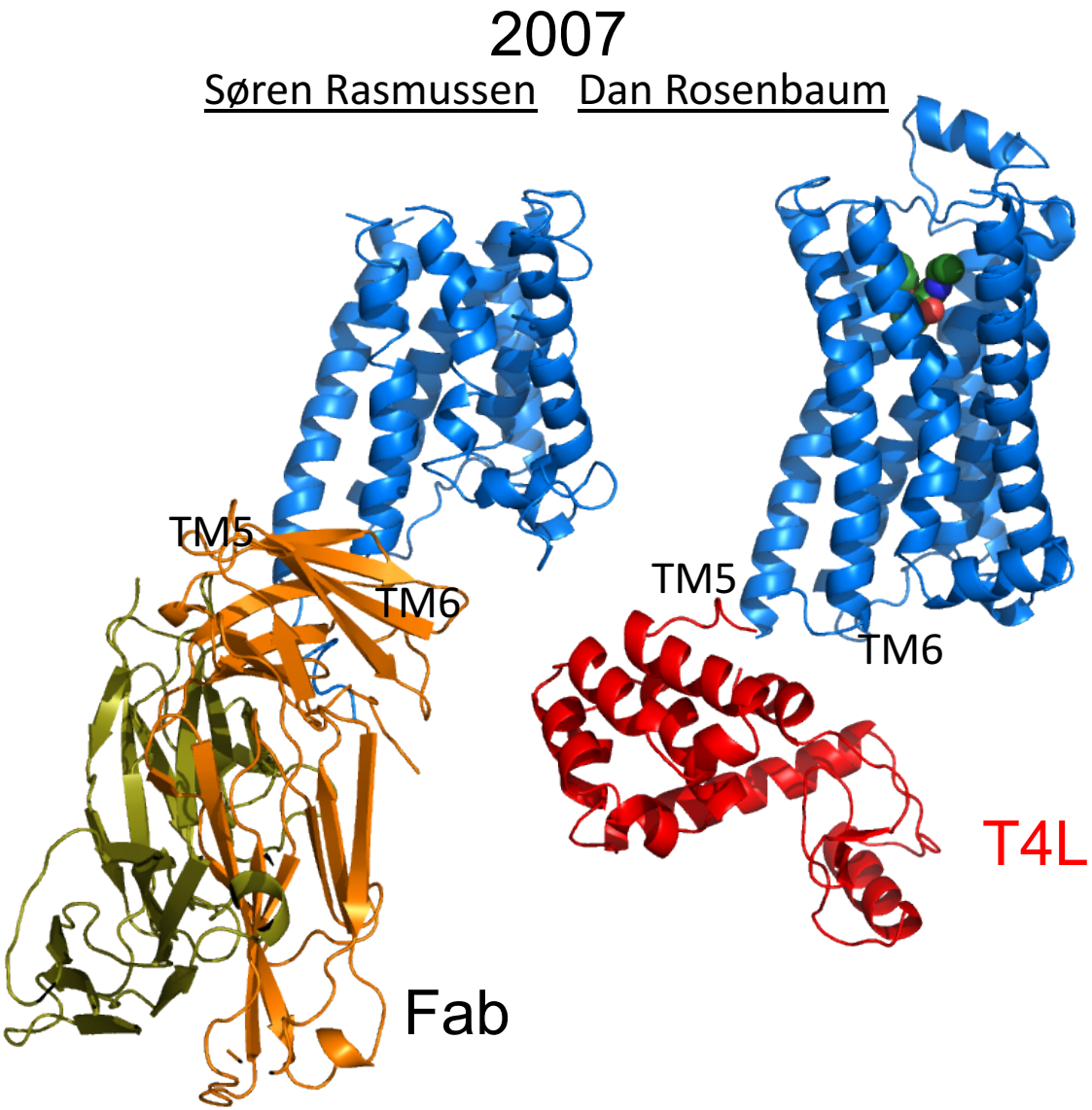


Stabilize and increase polar surface

- Antibodies
- Protein Engineering

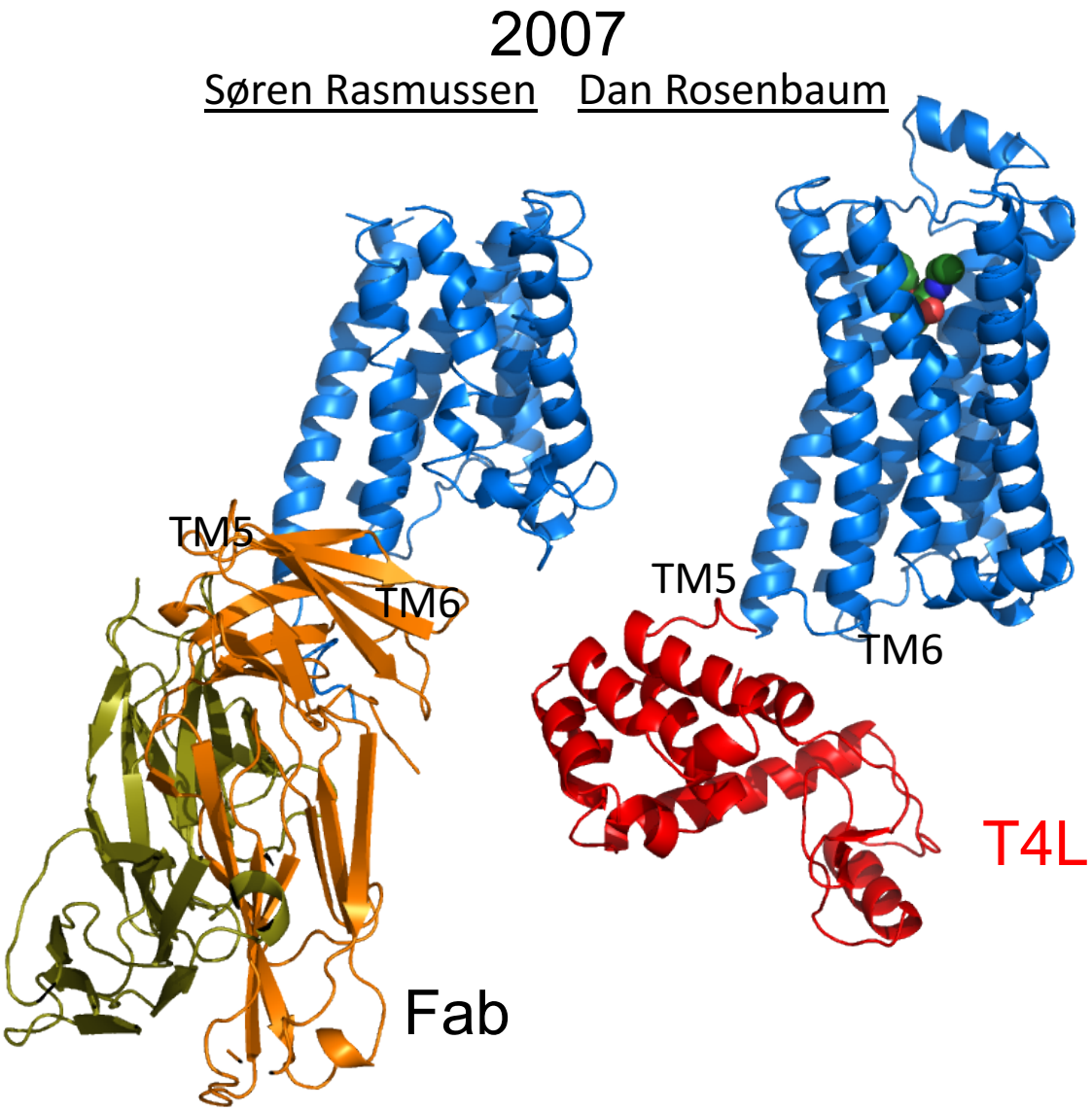
Approaches for GPCR crystallogenesis:

•Antibodies and protein engineering



Approaches for GPCR crystallogenesis:

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

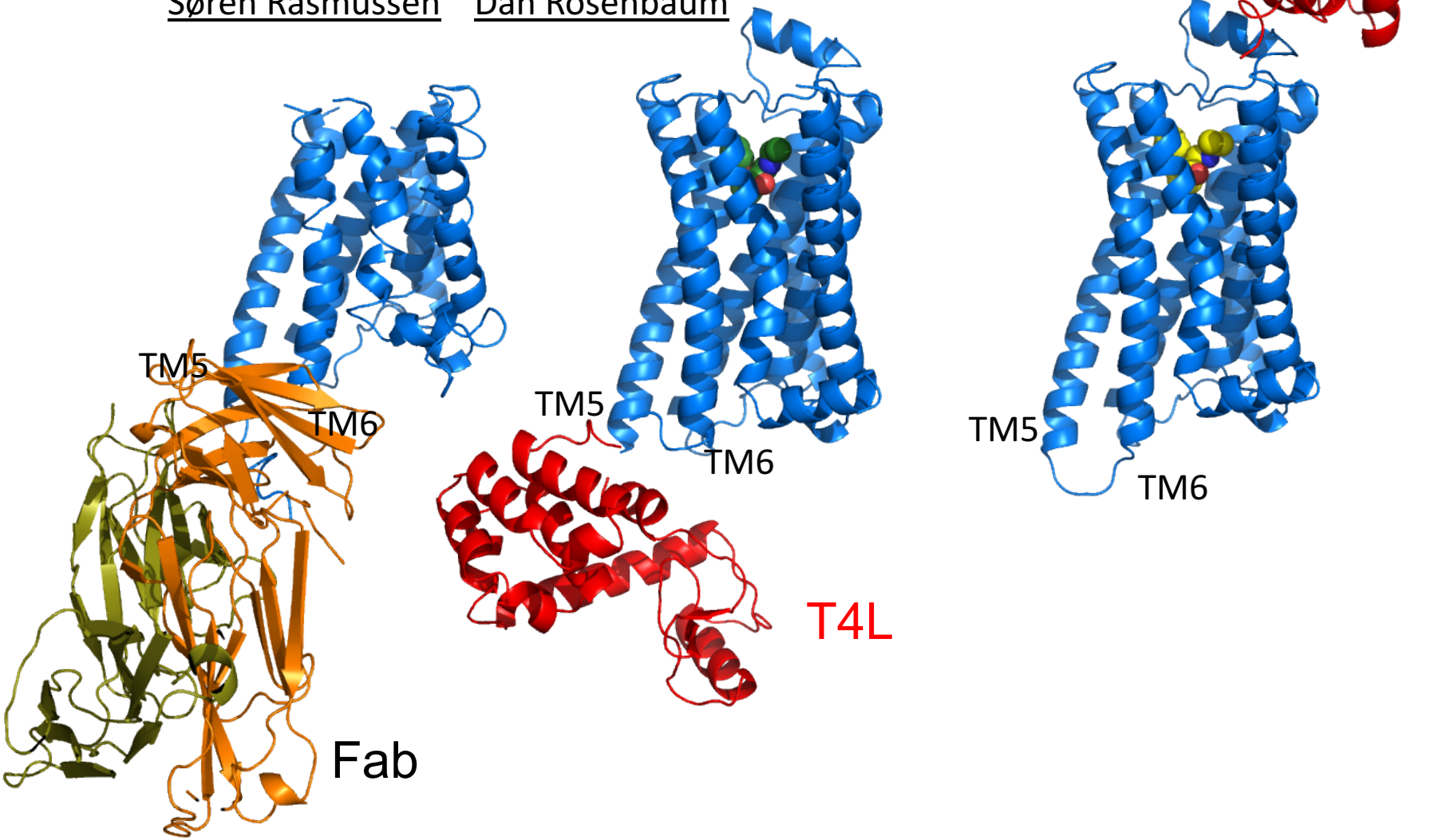


Approaches for GPCR crystallogenesis:

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

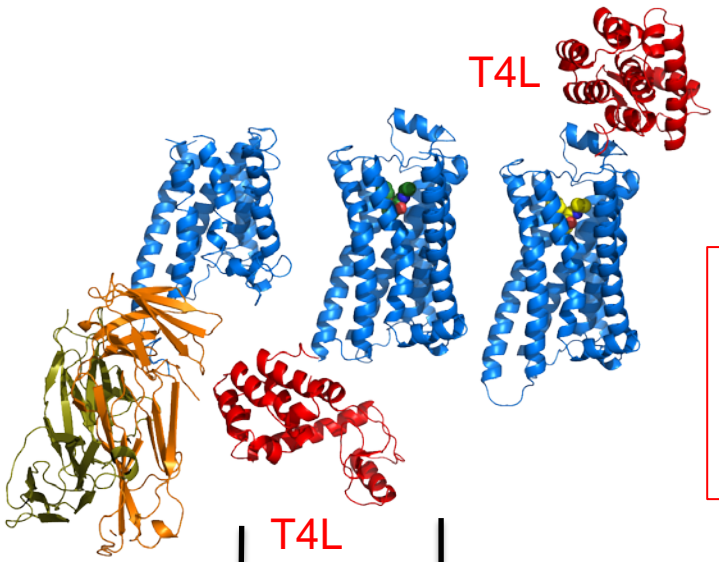
2007

Søren Rasmussen Dan Rosenbaum



Inactive-state GPCR structures

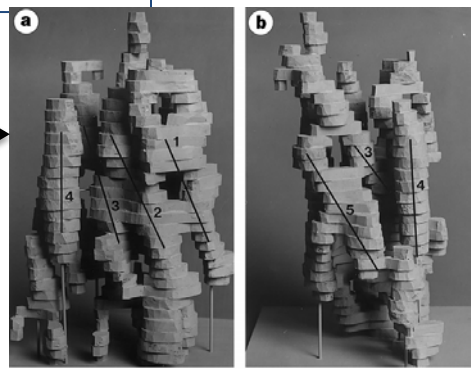
Antibodies and Protein Engineering



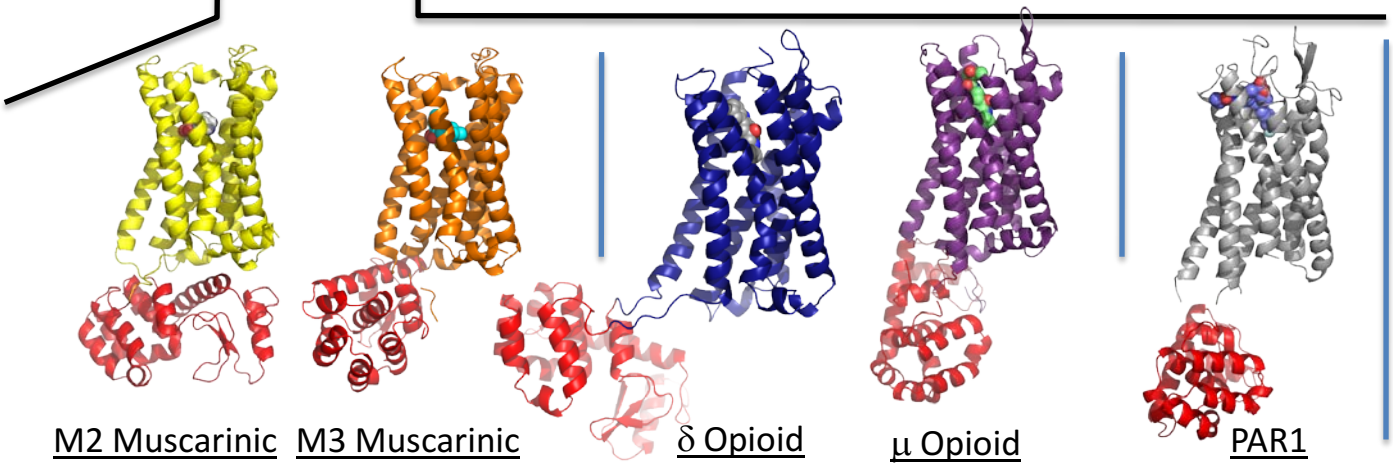
Other Approaches

Thermostabilization through alanine scanning mutations
– β_1AR, 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



M2 Muscarinic (Haga and Kobayashi)

M3 Muscarinic (Jurgen Wess)

δ Opioid

μ Opioid (Sebastien Granier)

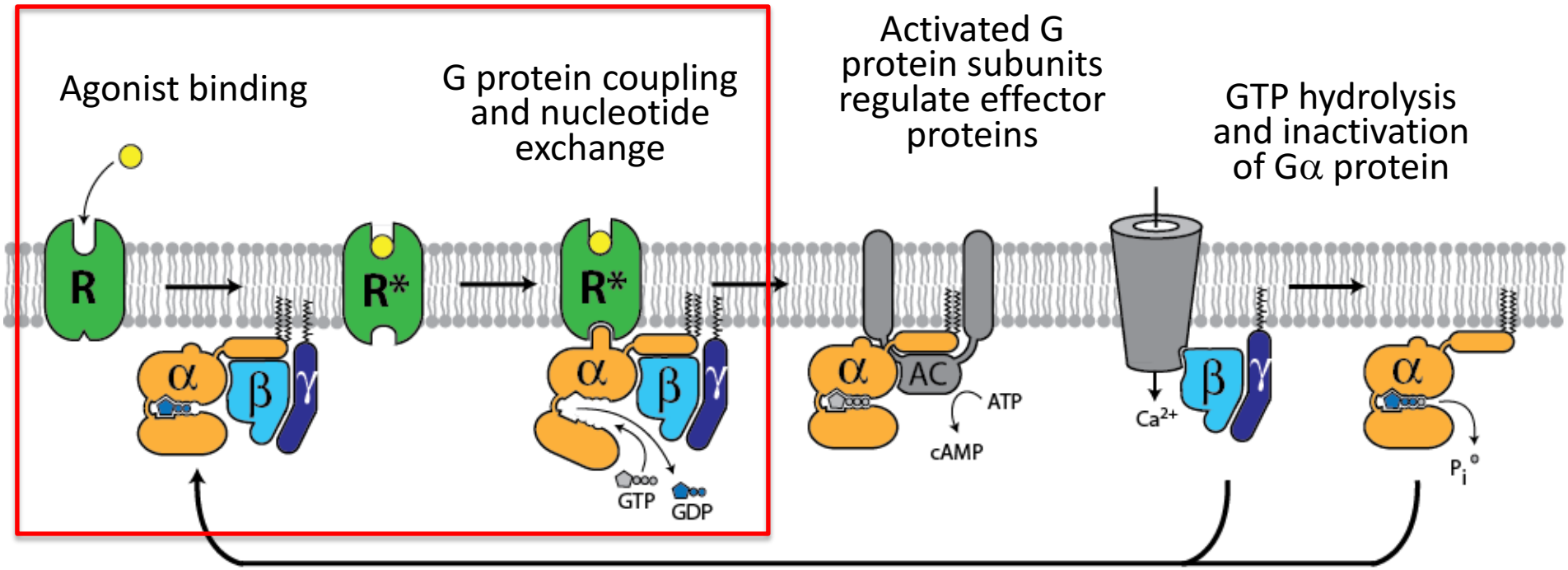
PAR1 (Shaun Coughlin)

Stevens Lab and collaborators

Adenosine A2A
D3 Dopamine
CXCR4
Histamine
S1P1
κ-opioid

Outline

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



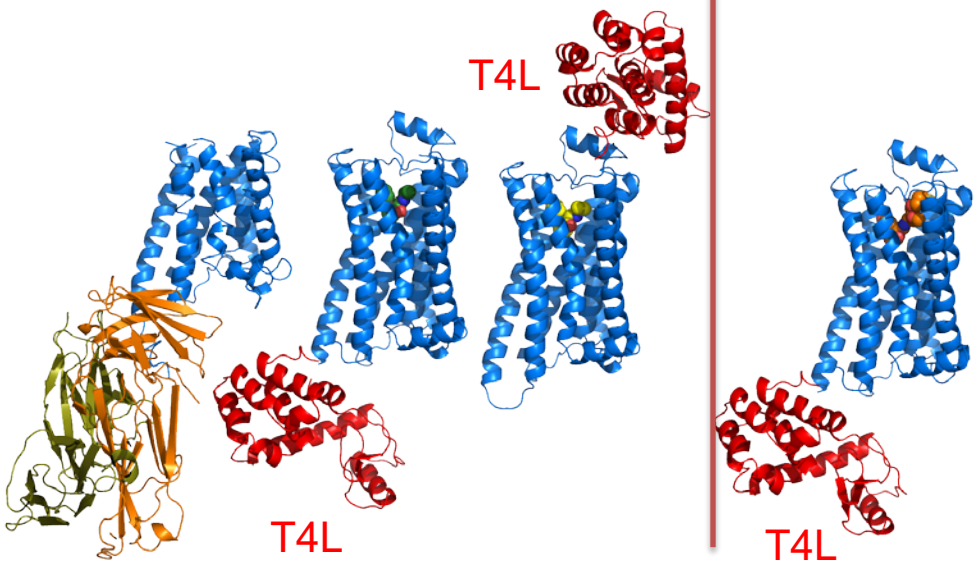
GPCR-G Protein Cycle

β_2AR INACTIVE

β_2AR ACTIVE ?

Inverse Agonist

Agonist
(covalent)



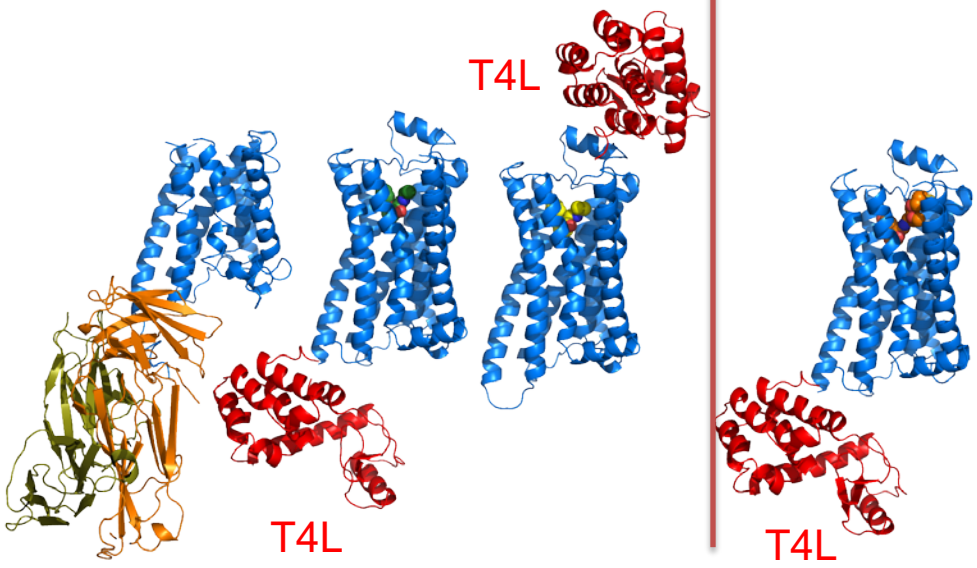
Fab

Dan Rosenbaum
Ralph Holl
Peter Gmeiner

β_2AR INACTIVE

Inverse Agonist

Agonist (covalent)

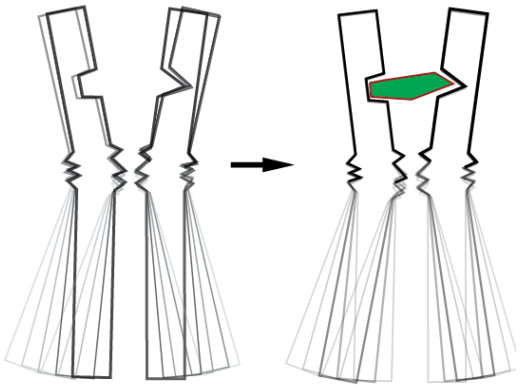


β_2AR ACTIVE ?

Agonist alone does not fully stabilize active state

β_2AR

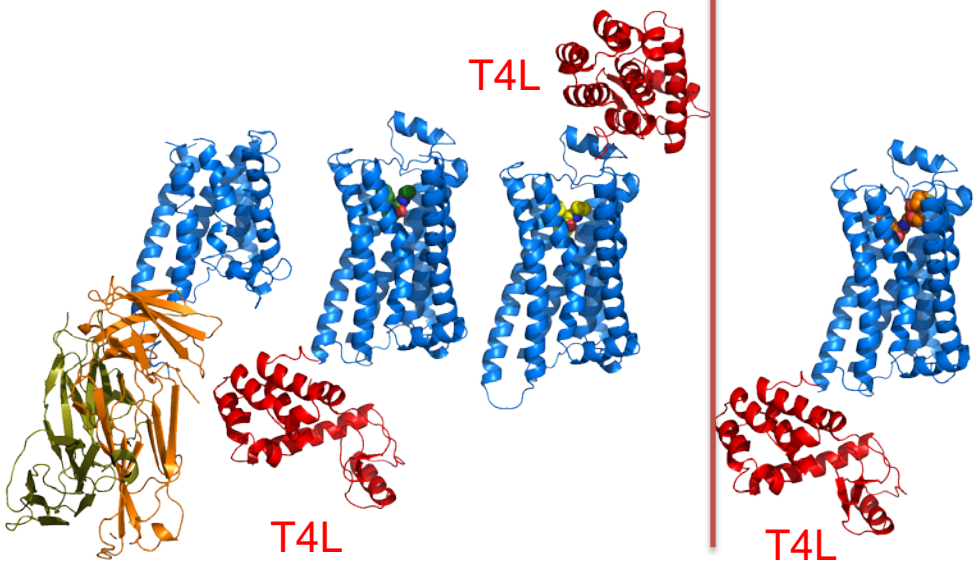
β_2AR + Agonist



β_2AR INACTIVE

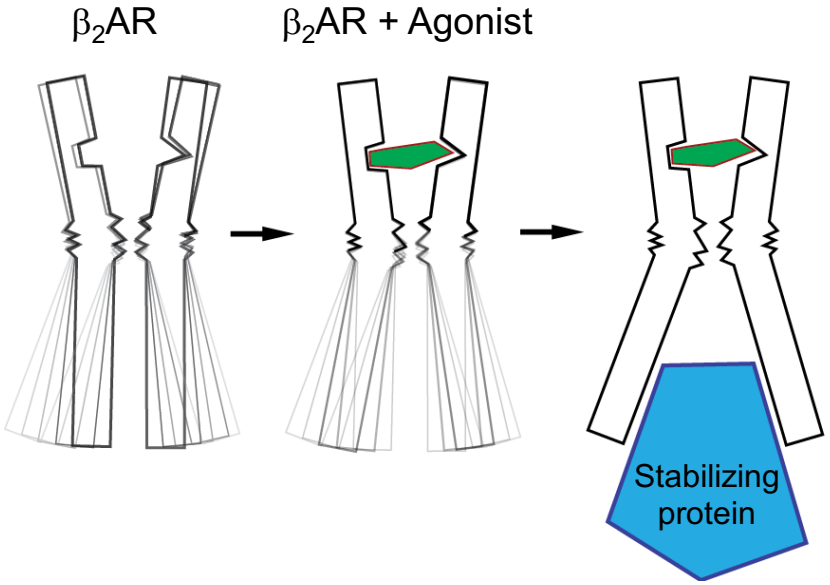
Inverse Agonist

Agonist (covalent)



β_2AR ACTIVE ?

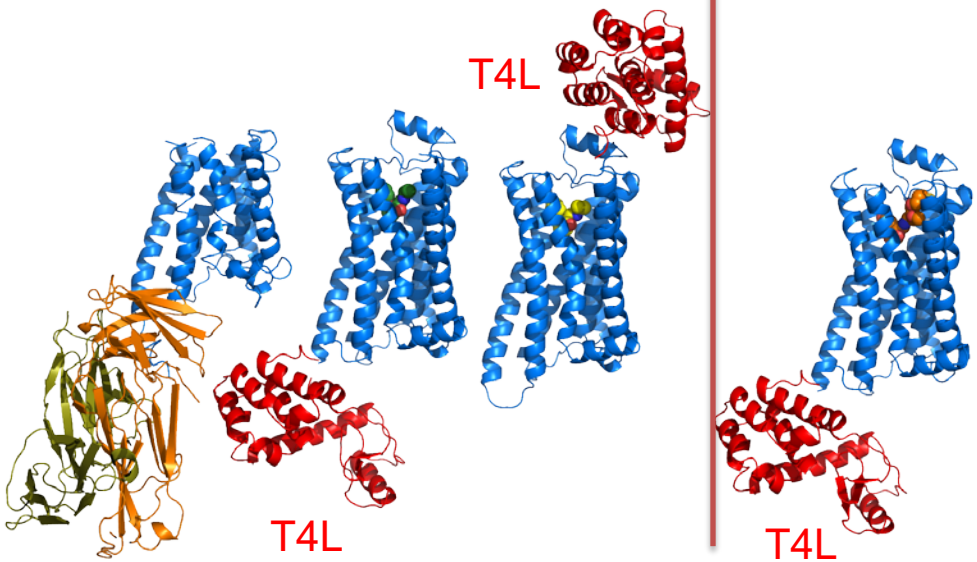
Agonist alone does not fully stabilize active state



β_2AR INACTIVE

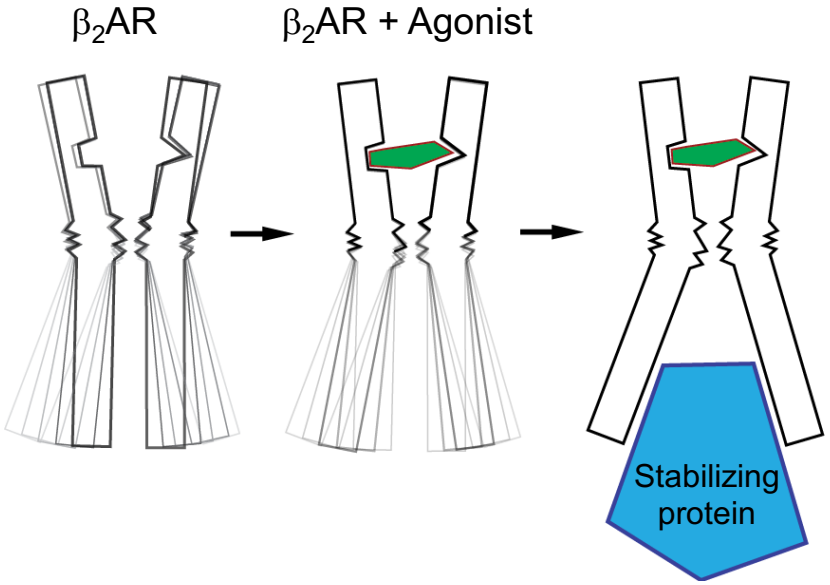
Inverse Agonist

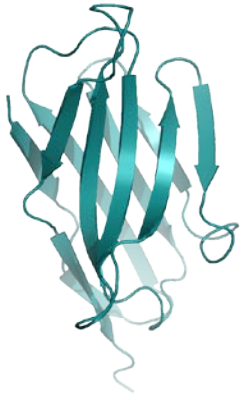
Agonist (covalent)



β_2AR ACTIVE ?

Agonist alone does not fully stabilize active state





Nanobody

variable domain of a single chain camelid antibody

Jan Steyaert
Els Pardon

β_2AR INACTIVE

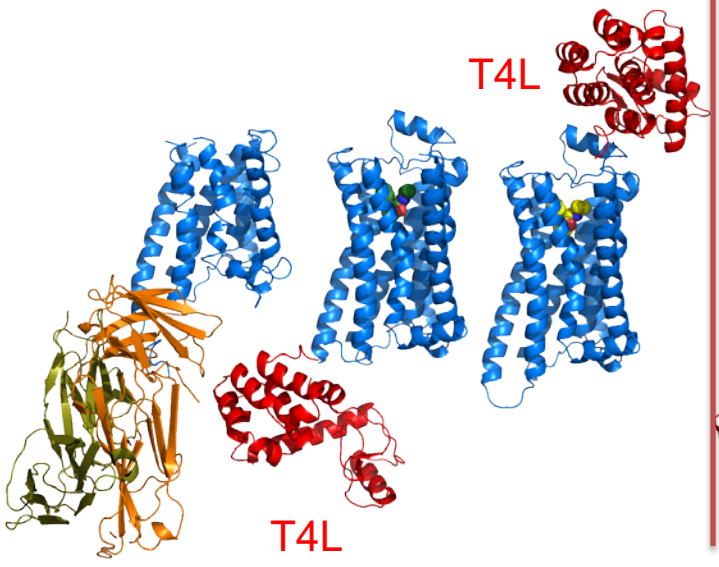
β_2AR ACTIVE

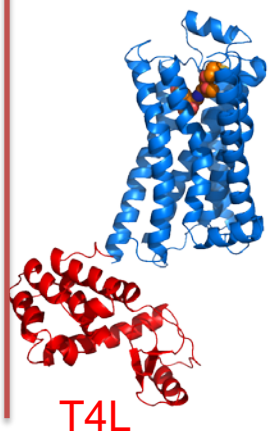
Inverse Agonist

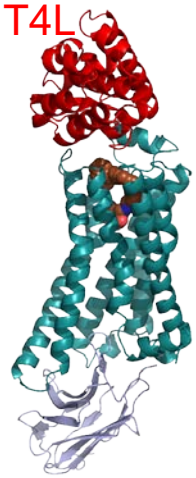
Agonist (covalent)

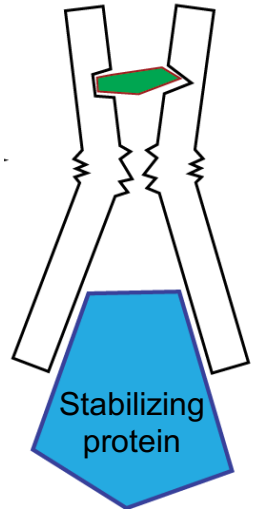
Partial Agonist

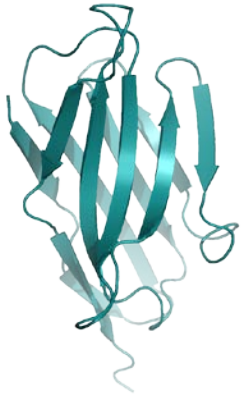
Agonist











Nanobody

variable domain of a single chain camelid antibody

Jan Steyaert
Els Pardon

β_2AR INACTIVE

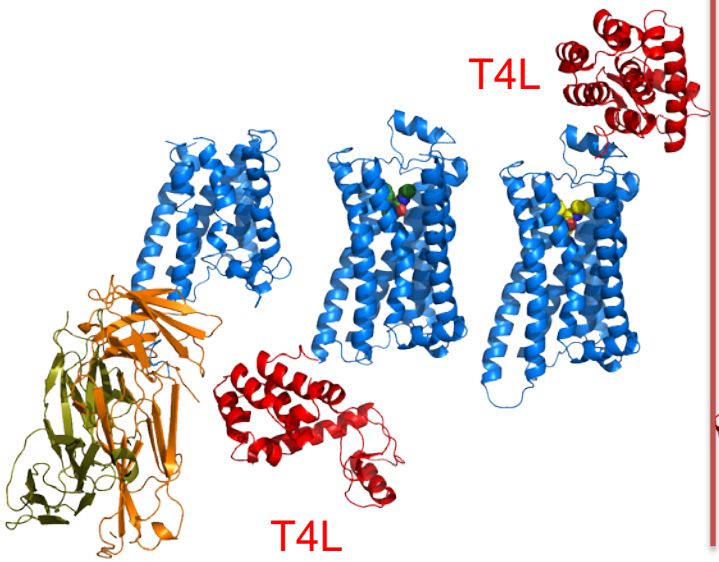
β_2AR ACTIVE

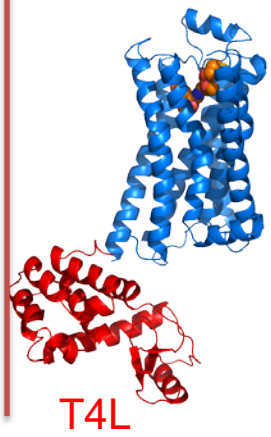
Inverse Agonist

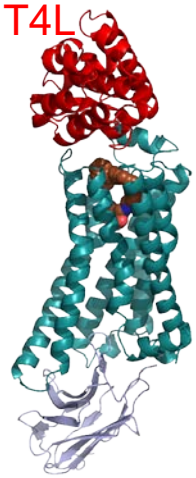
Agonist (covalent)

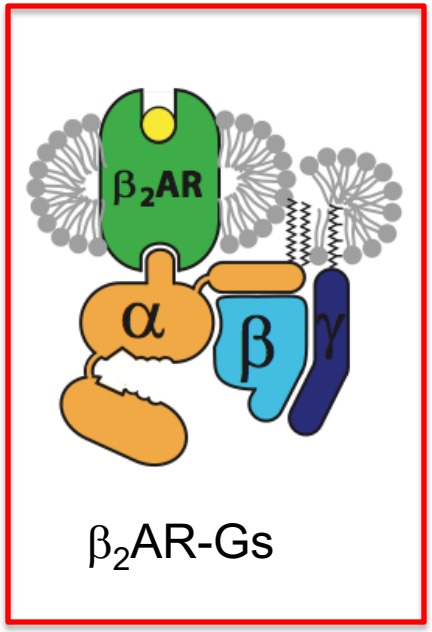
Partial Agonist

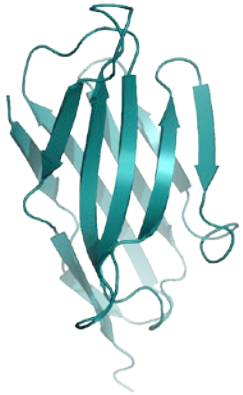
Agonist











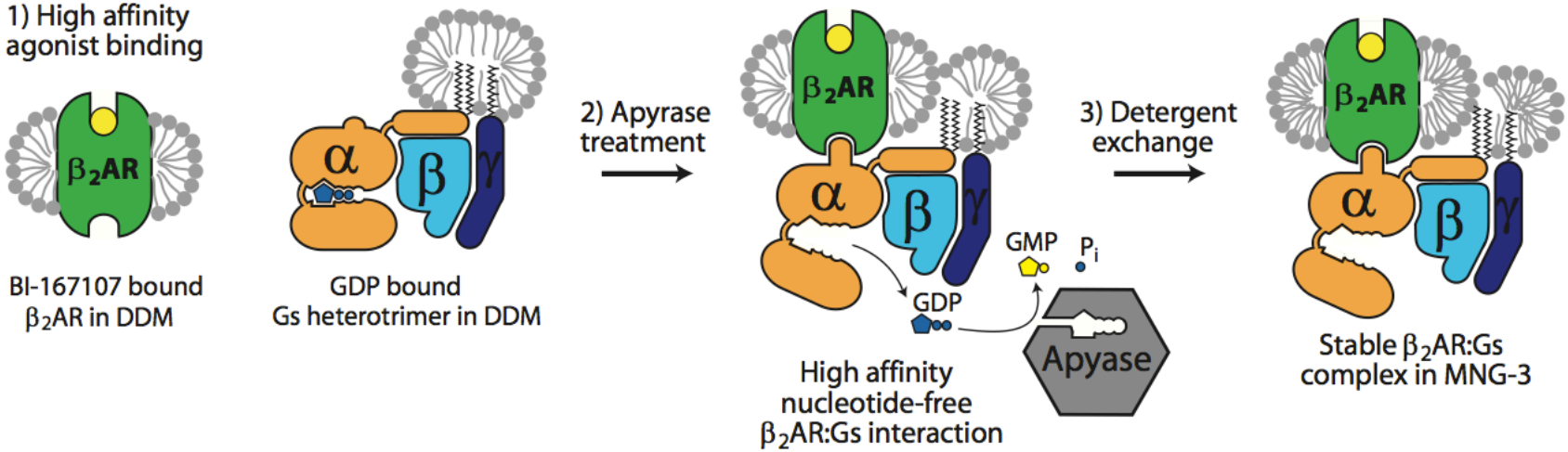
Nanobody

variable domain of a single chain camelid antibody

Jan Steyaert
Els Pardon

Technical contributions to crystallizing the β_2AR-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

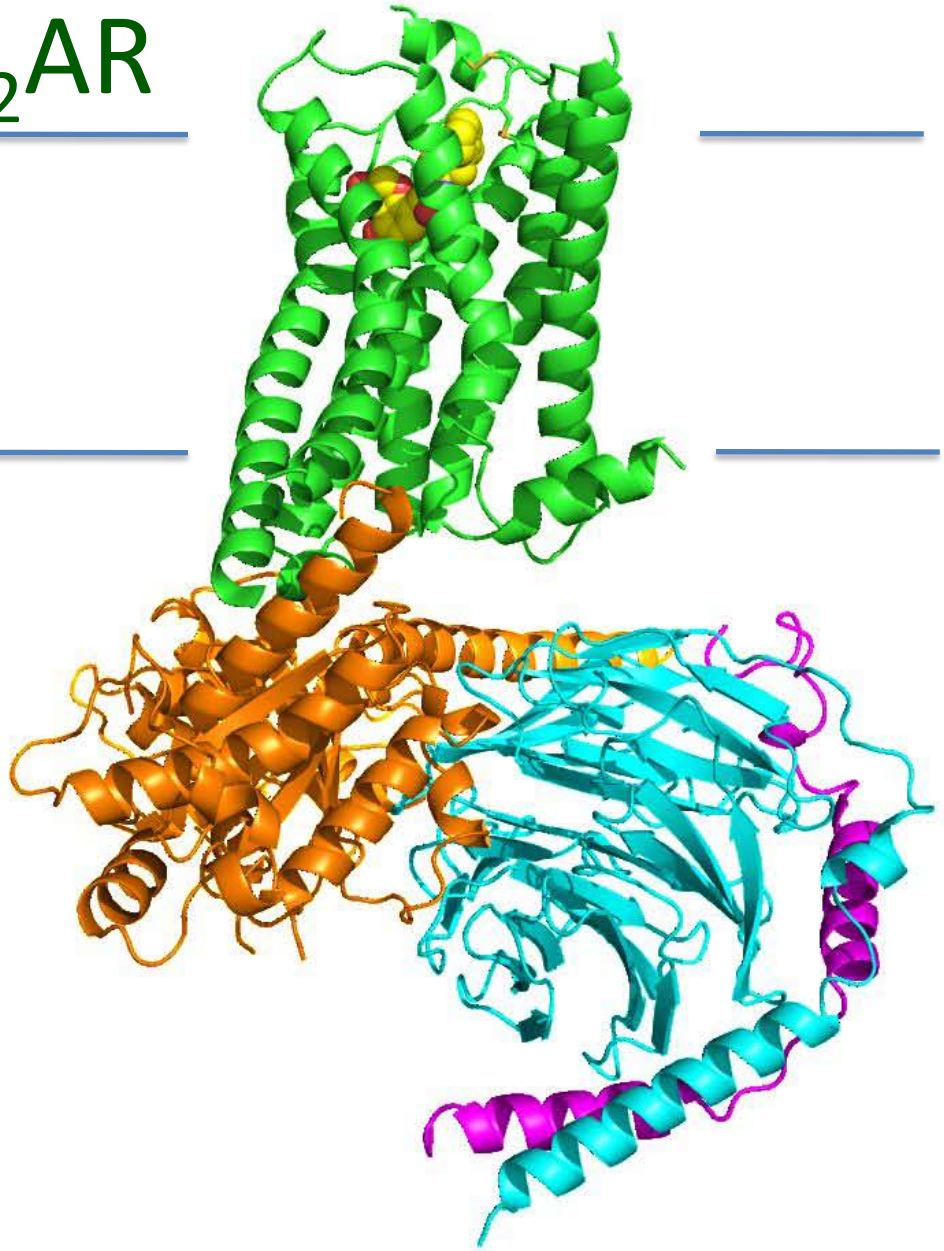
β_2AR-Gs complex

Andy Kruse, Brian DeVree, Søren Rasmussen

me Roger Sunahara

Returning from Argonne with final data set April 2011

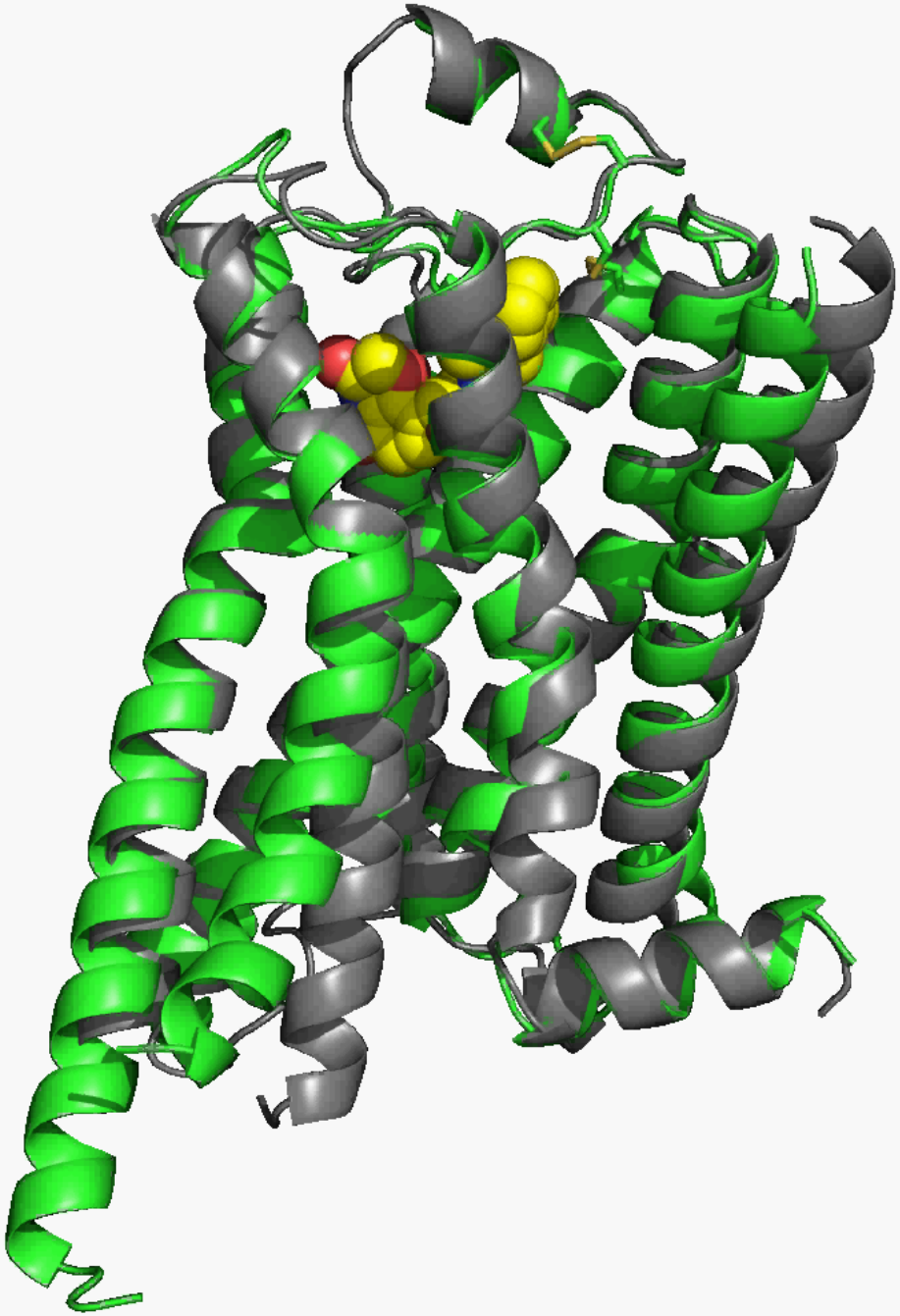
β_2AR



Gsαβγ

Inactive

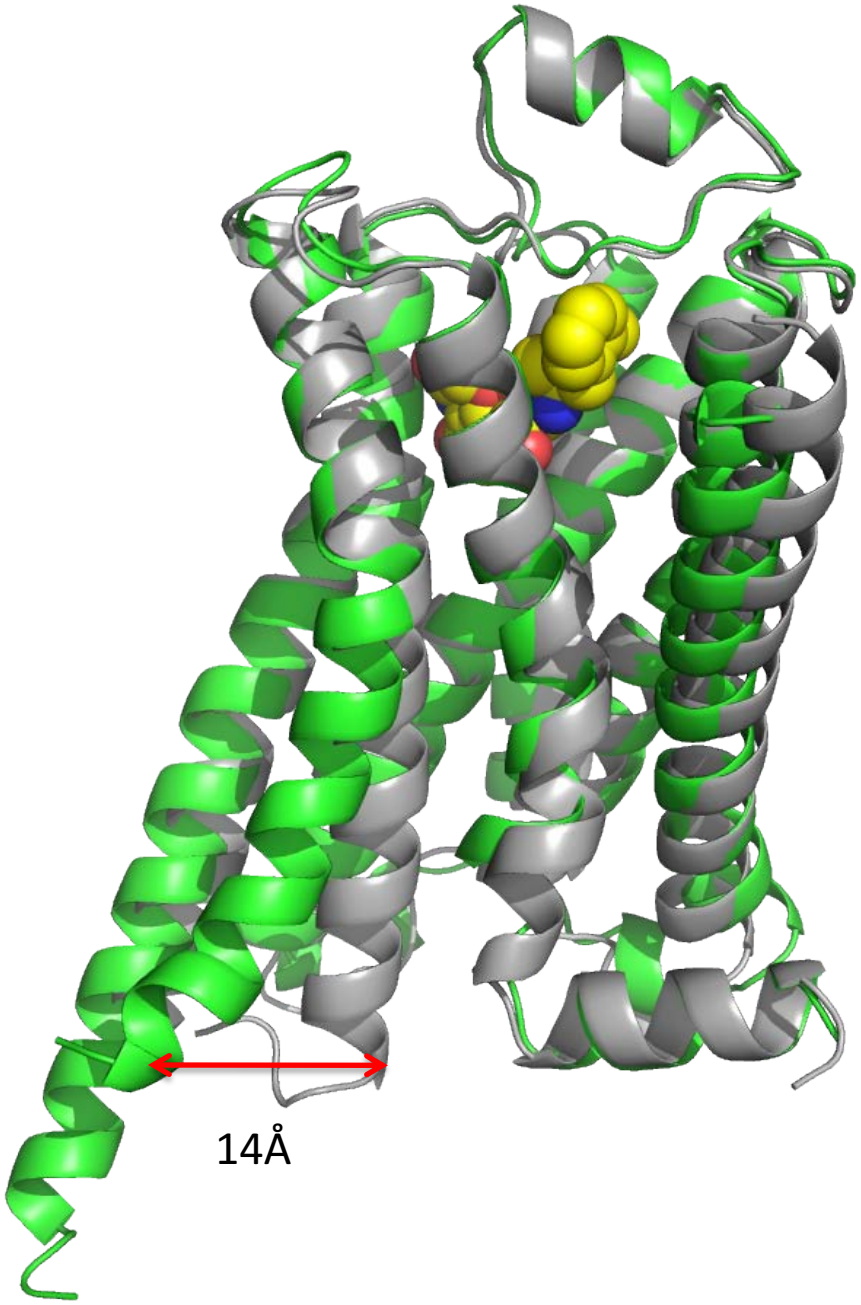
Active



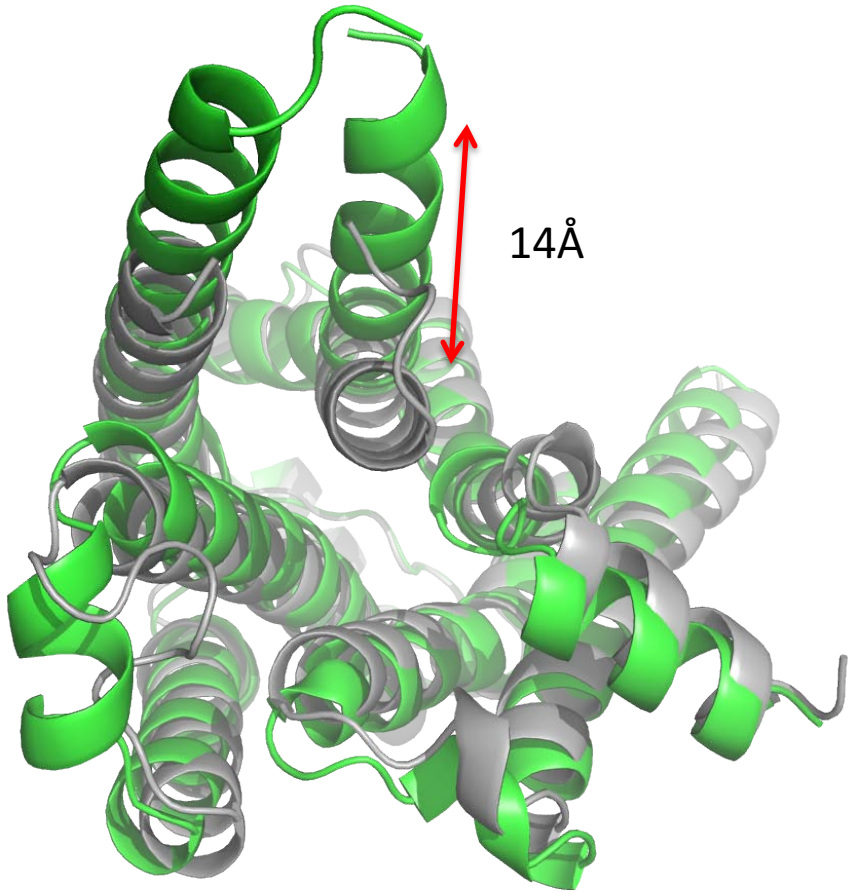
β_2AR-Cz

β_2AR-Gs

Active state of β_2AR

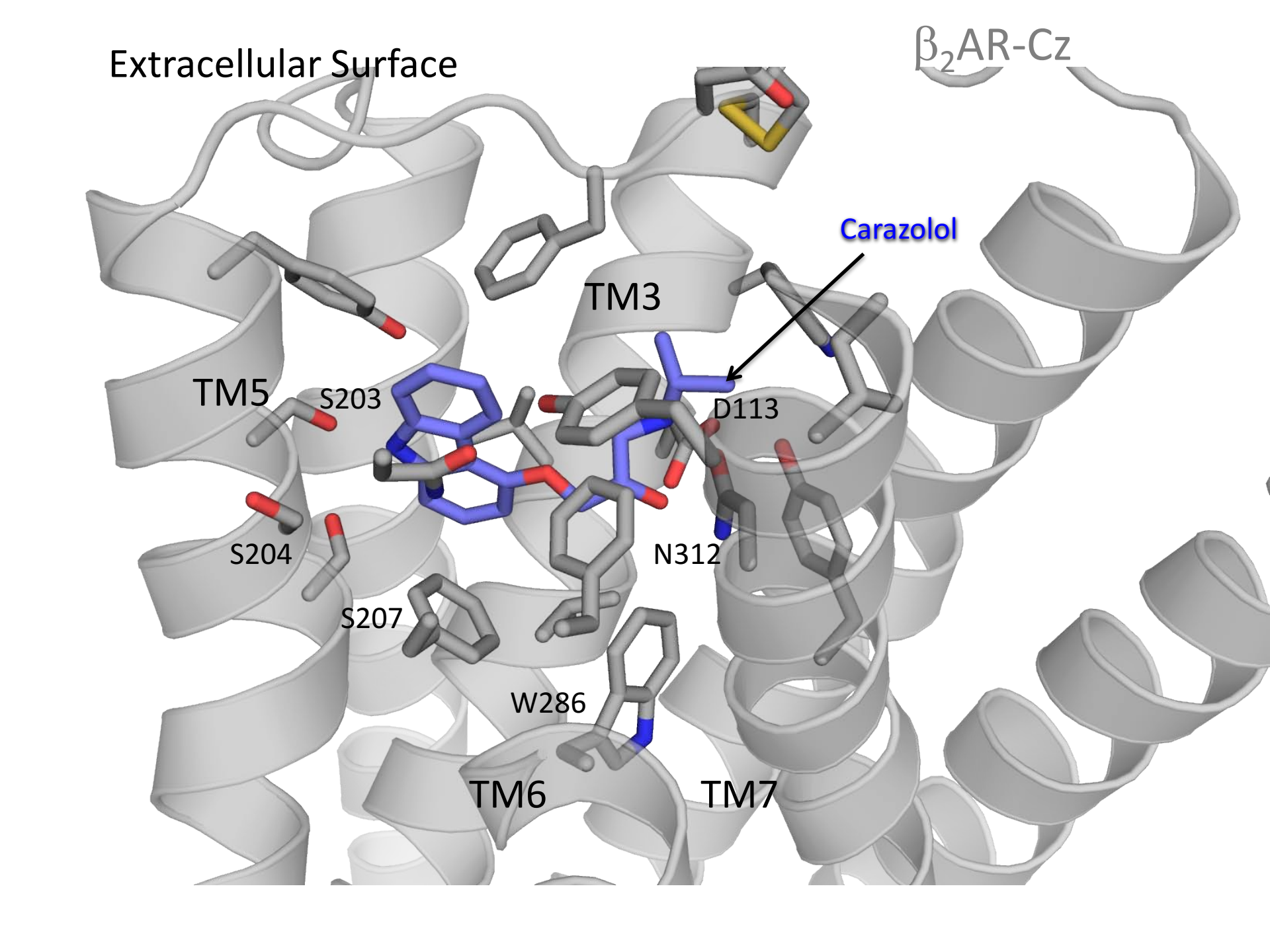


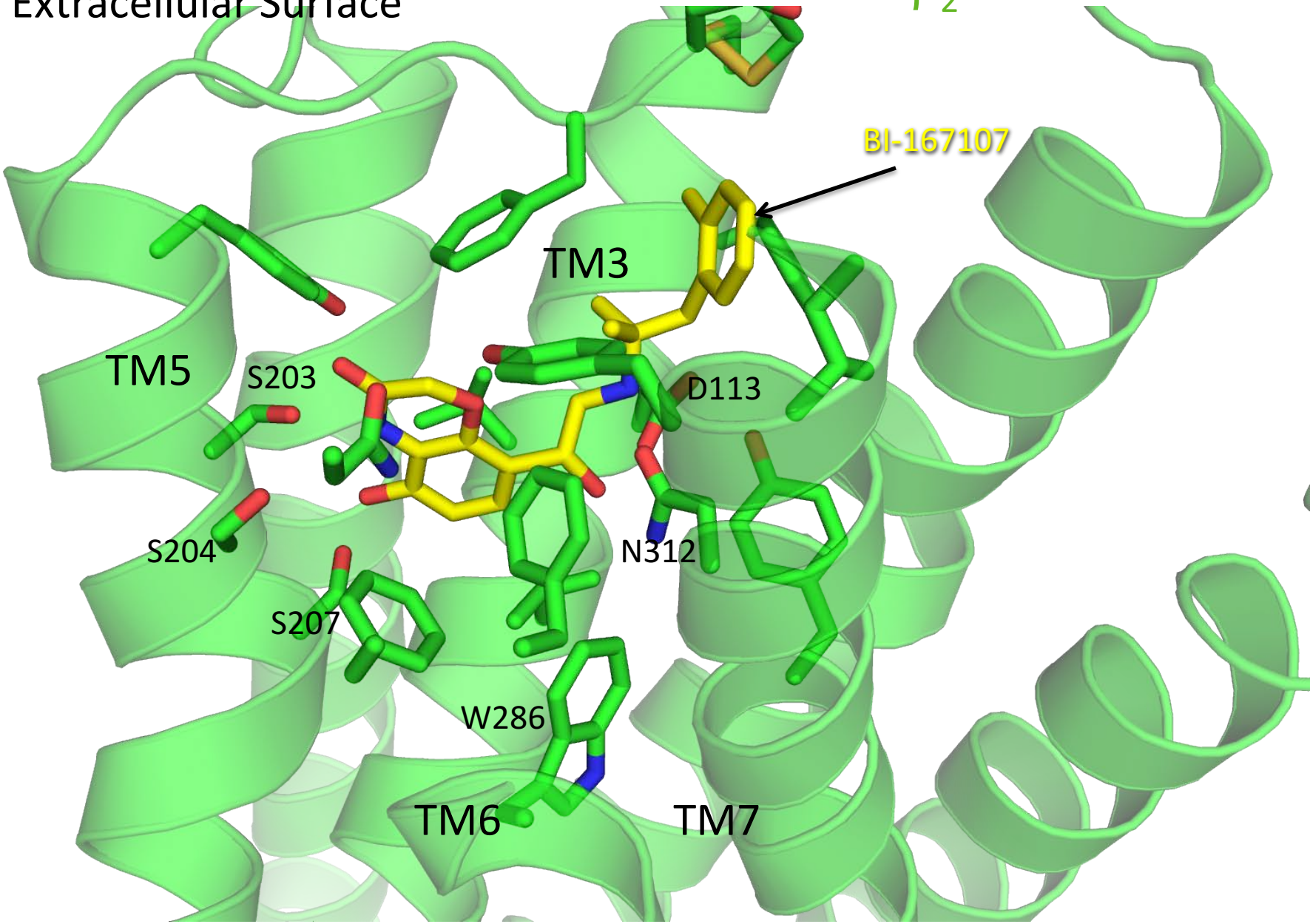
Cytoplasmic View



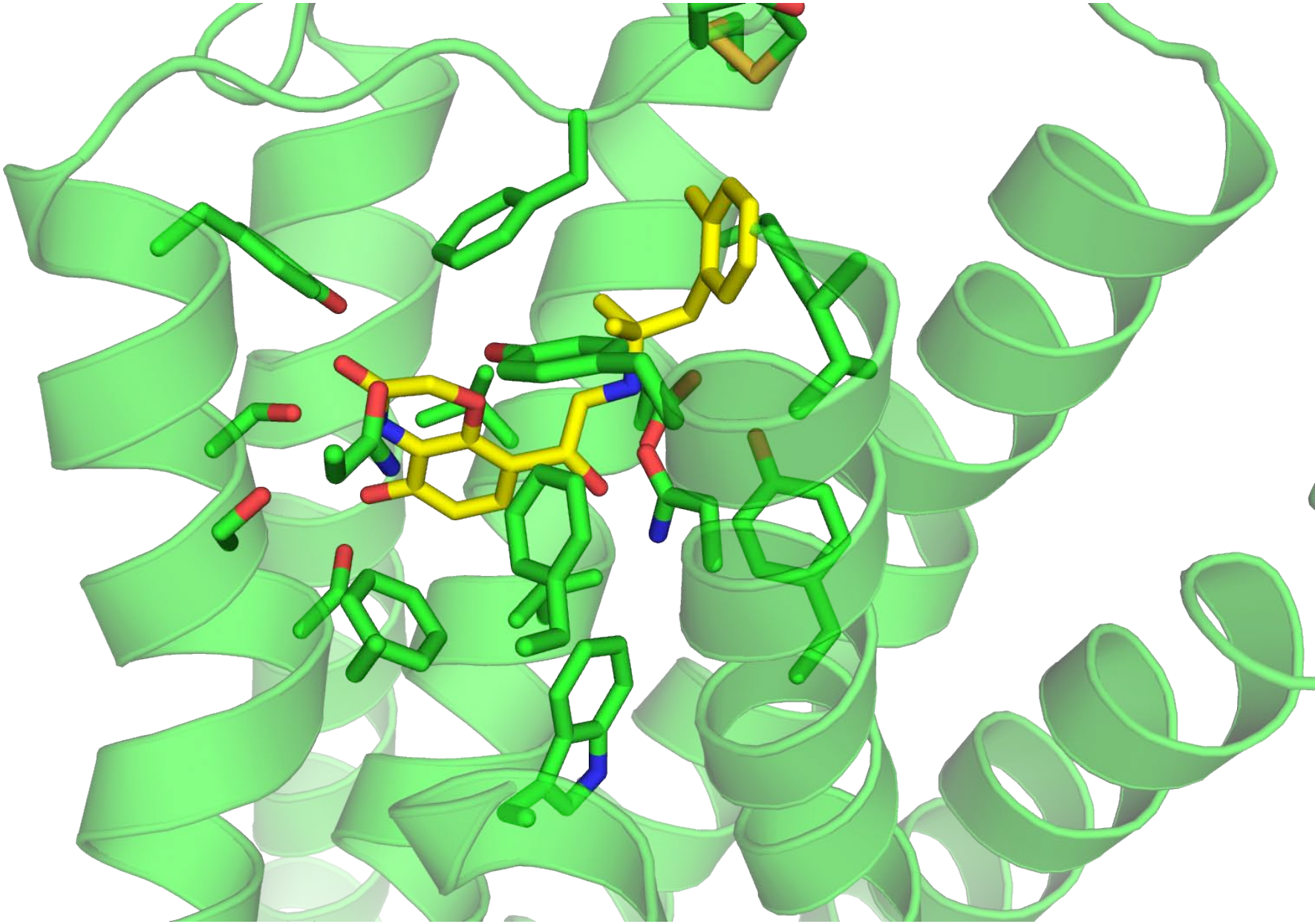
β_2AR - Inactive

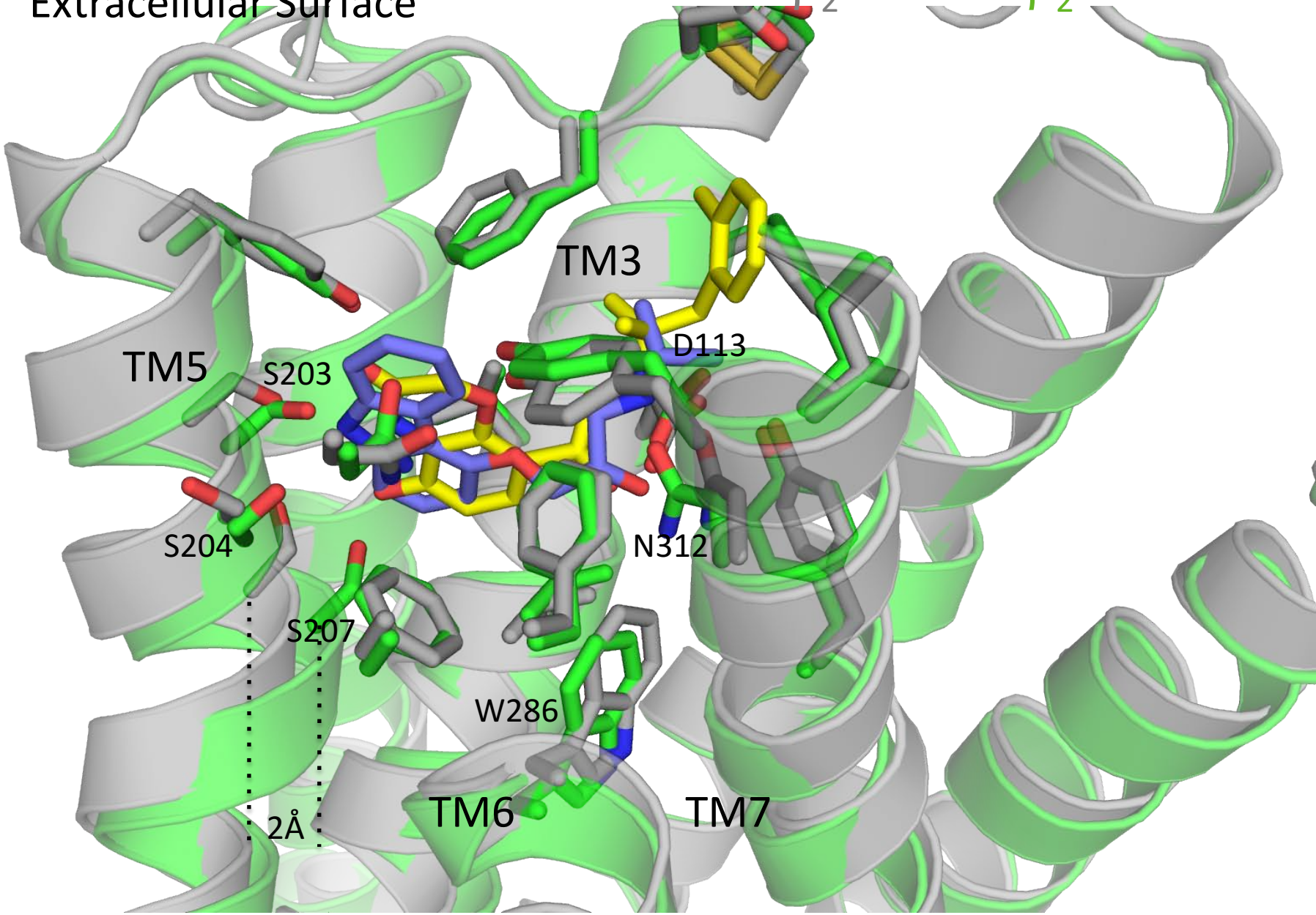
β_2AR-Gs


Extracellular Surface
β2AR-Cz
Carazolol
TM3
TM5
S203
D113
S204
N312
S207
W286
TM6
TM7


Extracellular Surface
β_2AR-Gs
BI-167107
TM3
TM5
S203
D113
S204
N312
S207
W286
TM6
TM7

Active

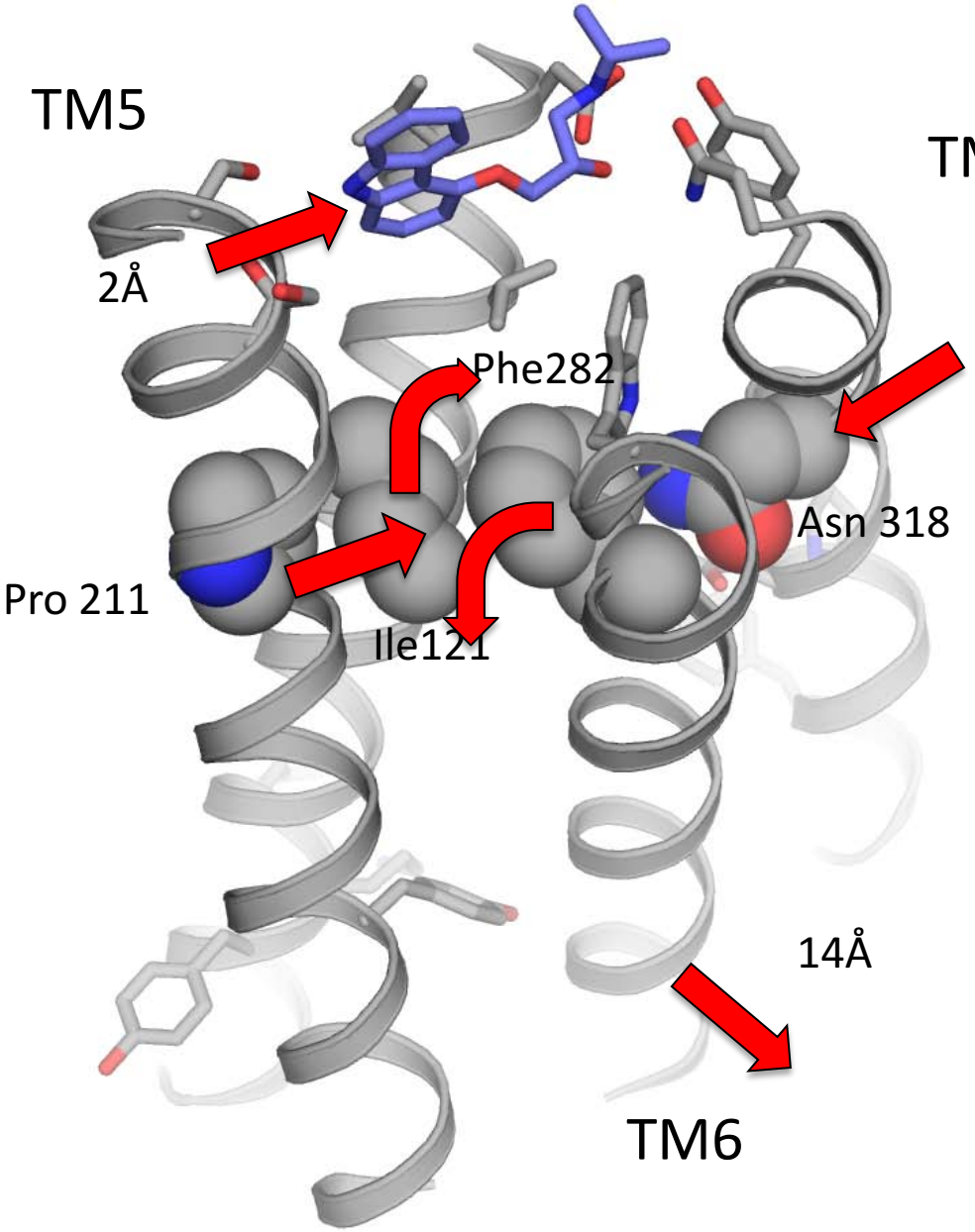



Extracellular Surface
β2AR-Cz
β2AR-Gs
TM3
TM5
S203
D113
S204
N312
S207
W286
TM6
TM7
2Å

Extracellular

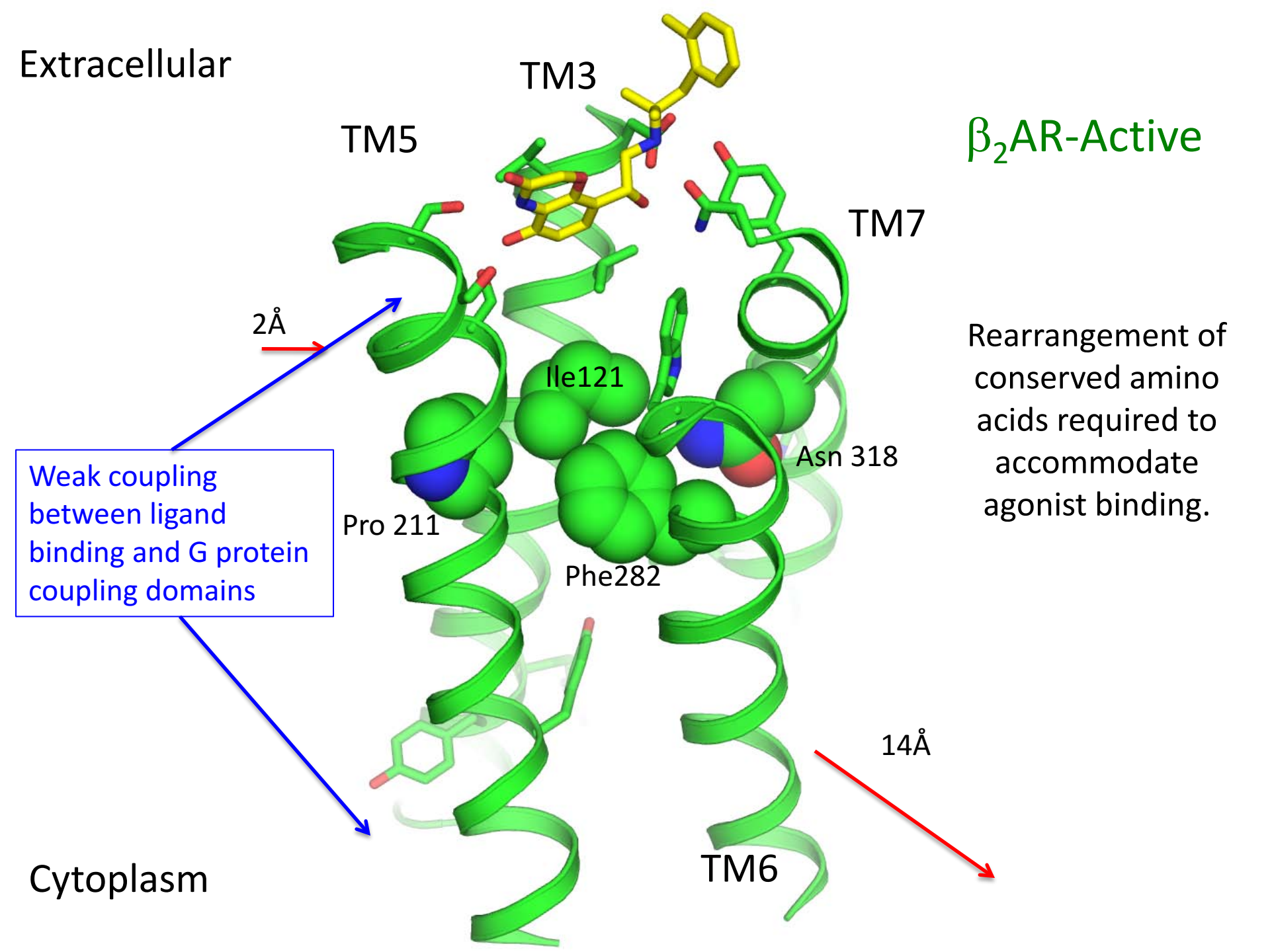
TM3

β_2AR-Inactive



Packing of conserved amino acids maintains inactive state.

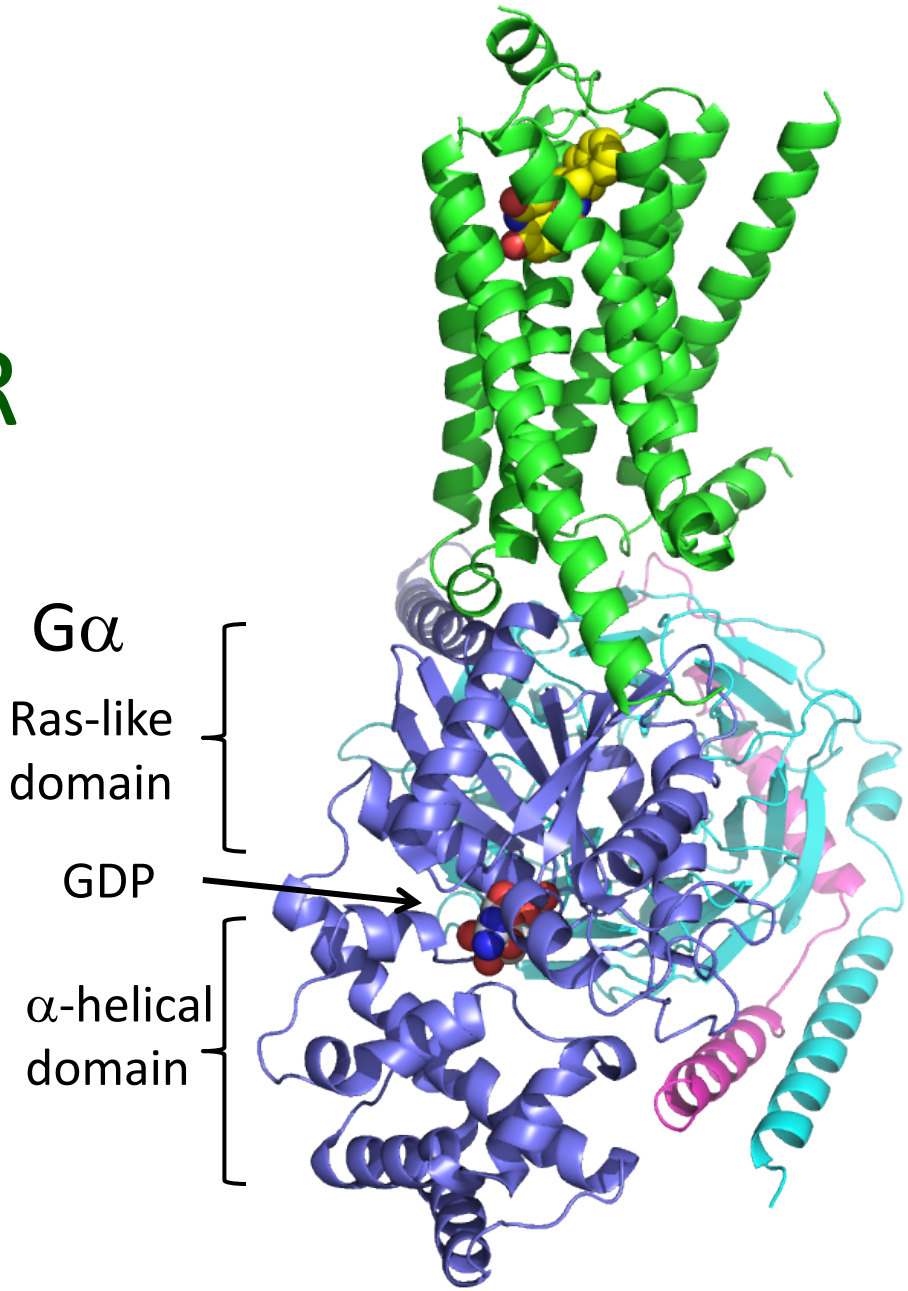
Cytoplasm


Extracellular
TM3
TM5
β_2AR-Active
TM7
2Å
Ile121
Asn 318
Weak coupling between ligand binding and G protein coupling domains
Pro 211
Phe282
Rearrangement of conserved amino acids required to accommodate agonist binding.
14Å
Cytoplasm
TM6

β_2AR

Gs$\alpha\beta\gamma$

Inactive

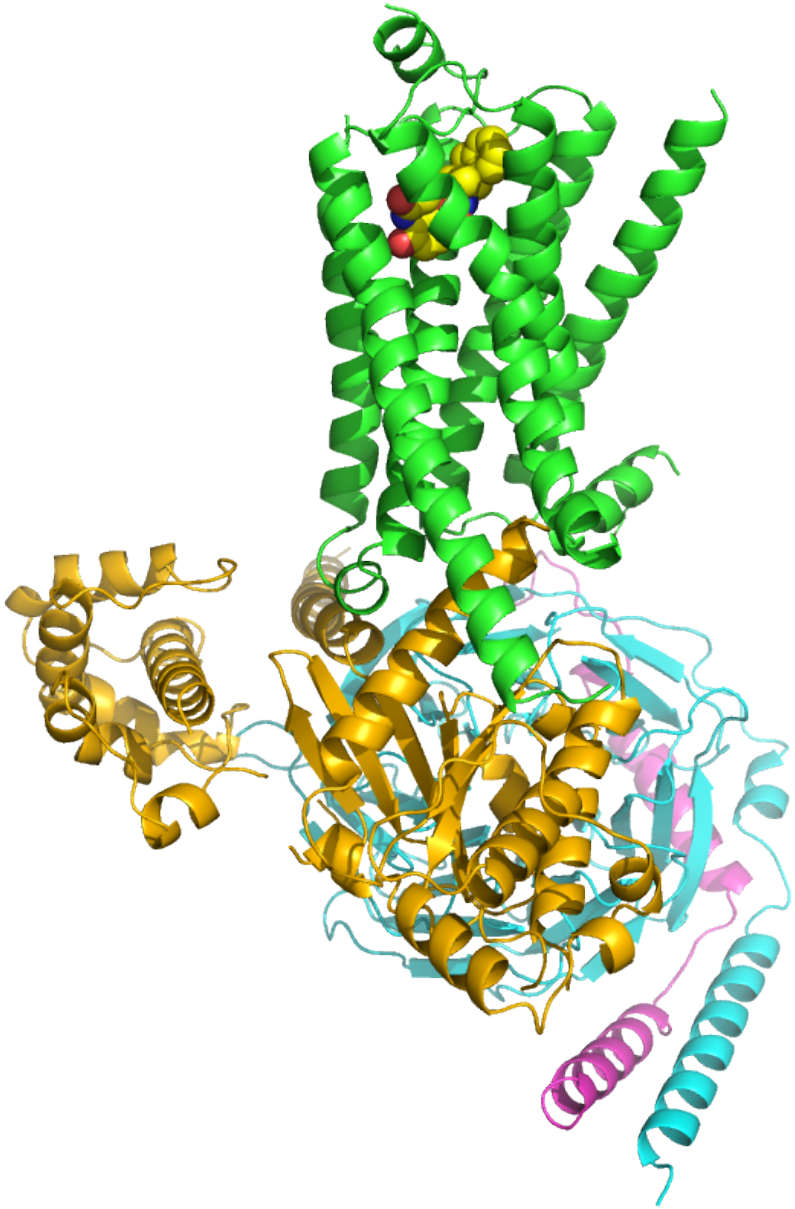


Interactions between the β_2AR and Gs promote GDP release….

β_2AR

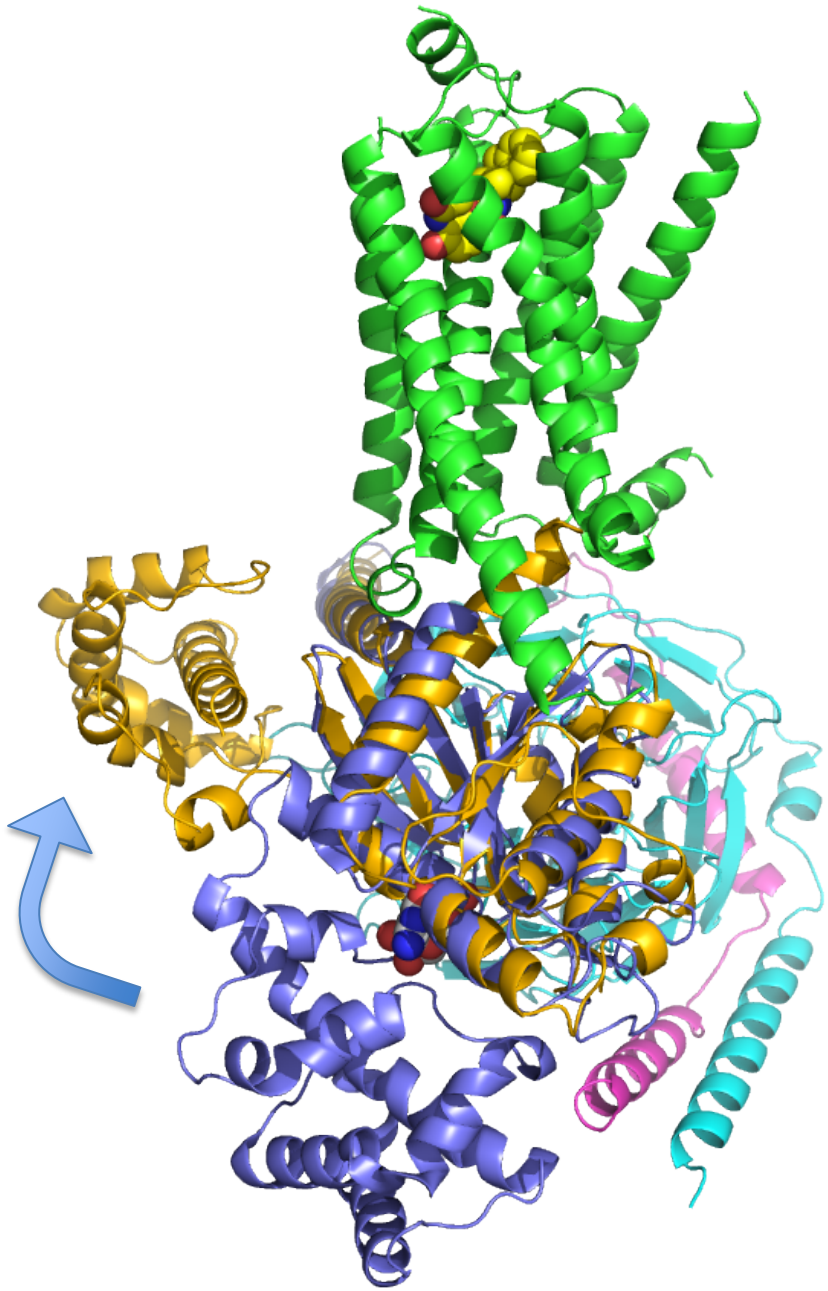
Gs$\alpha\beta\gamma$

Active



β_2AR

α–helical domain

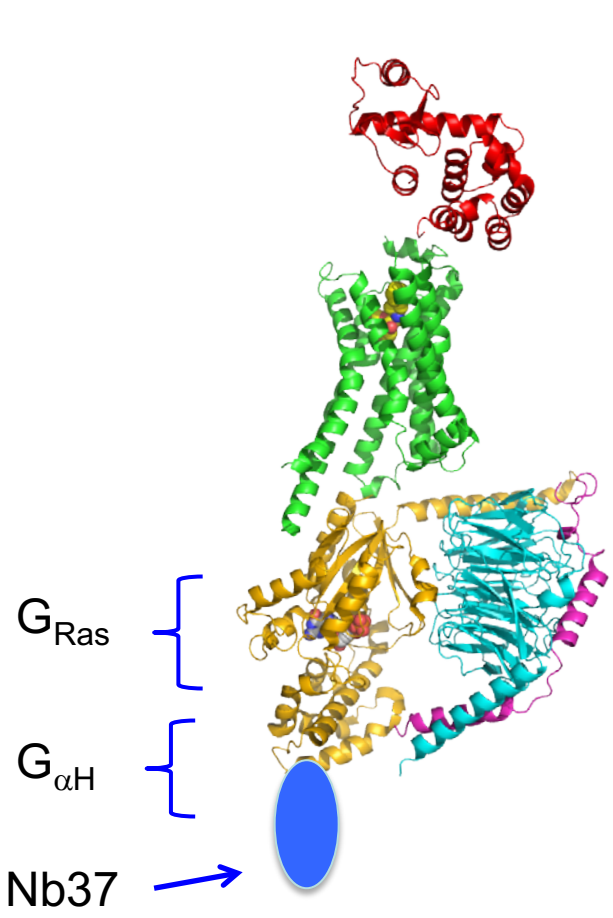


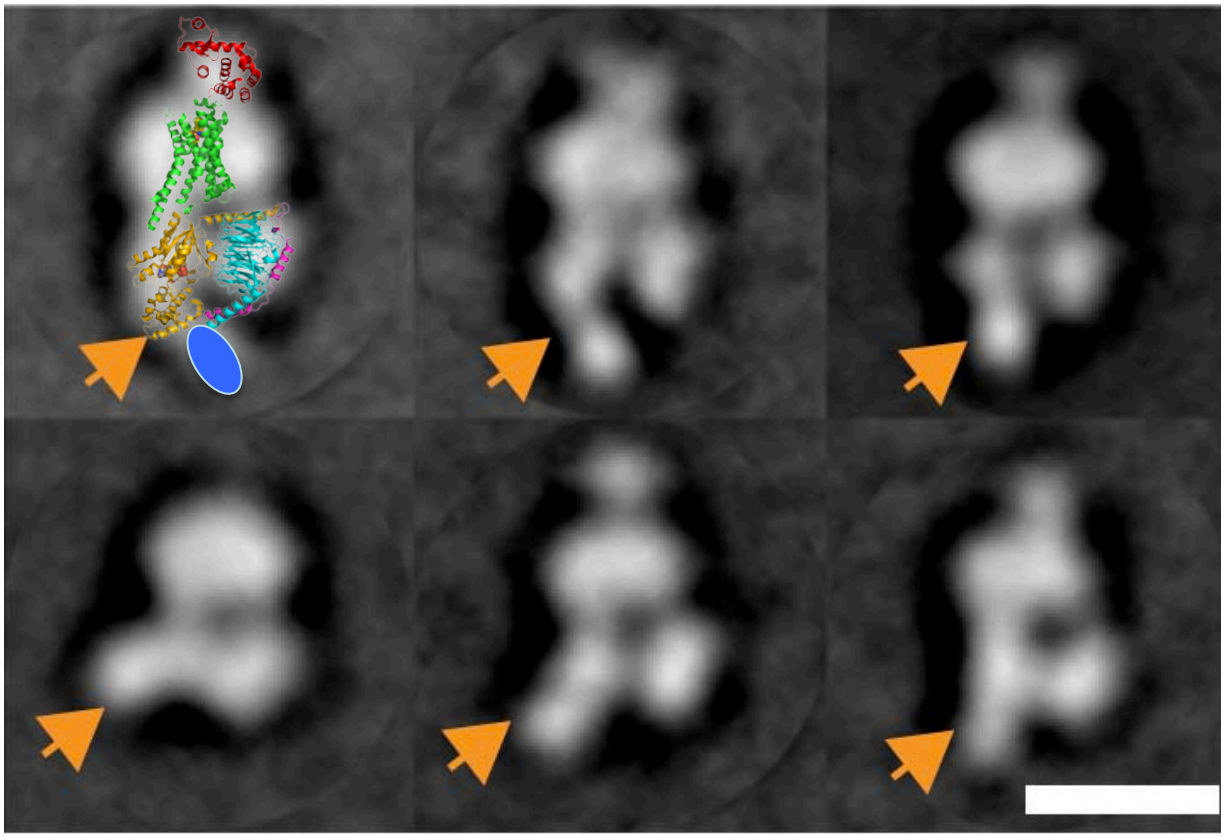
Gsαβγ

Active

Inactive

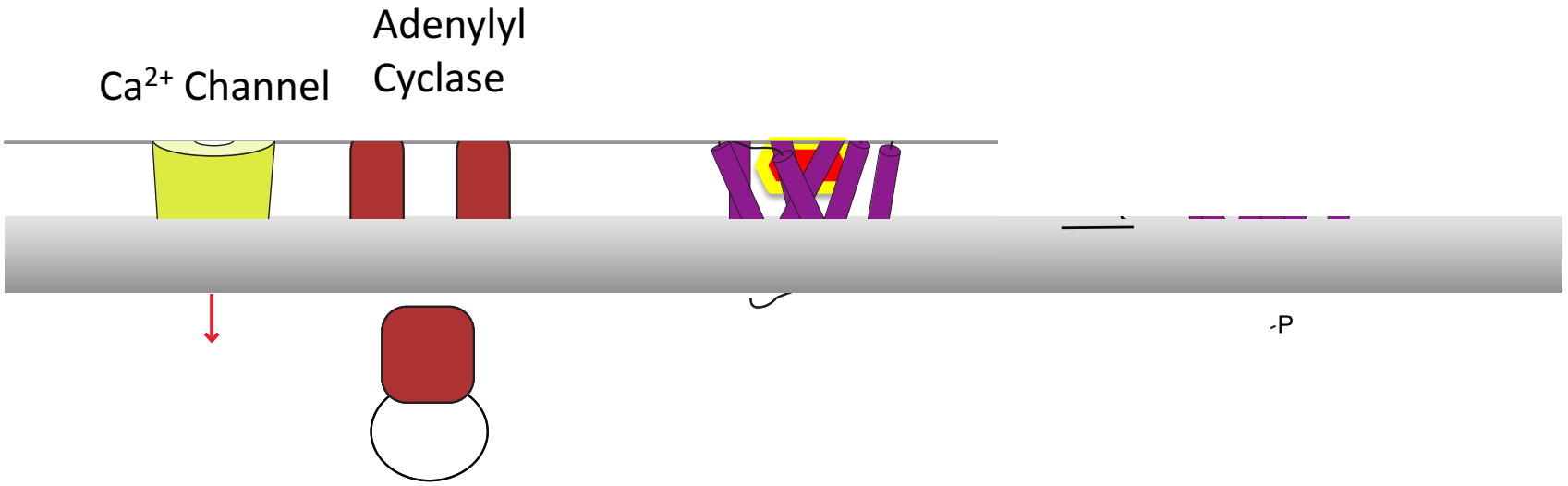
Mobility of alpha helical domain confirmed by EM

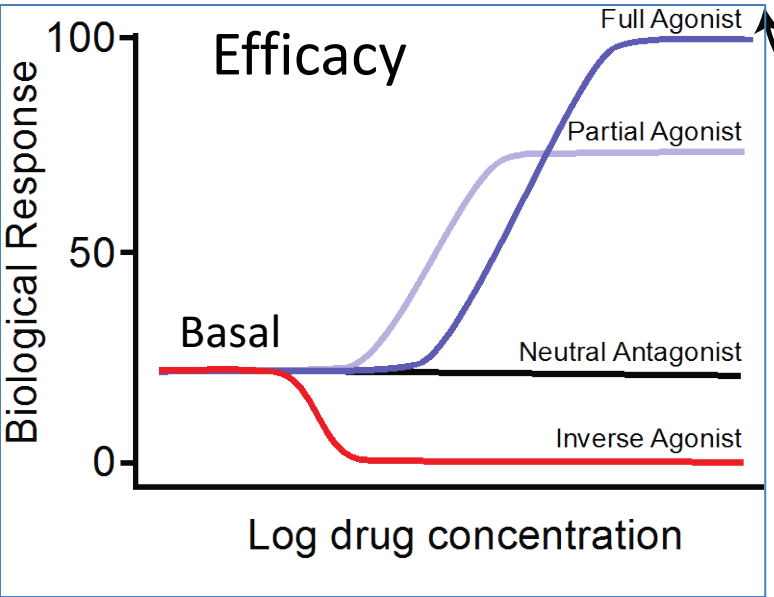


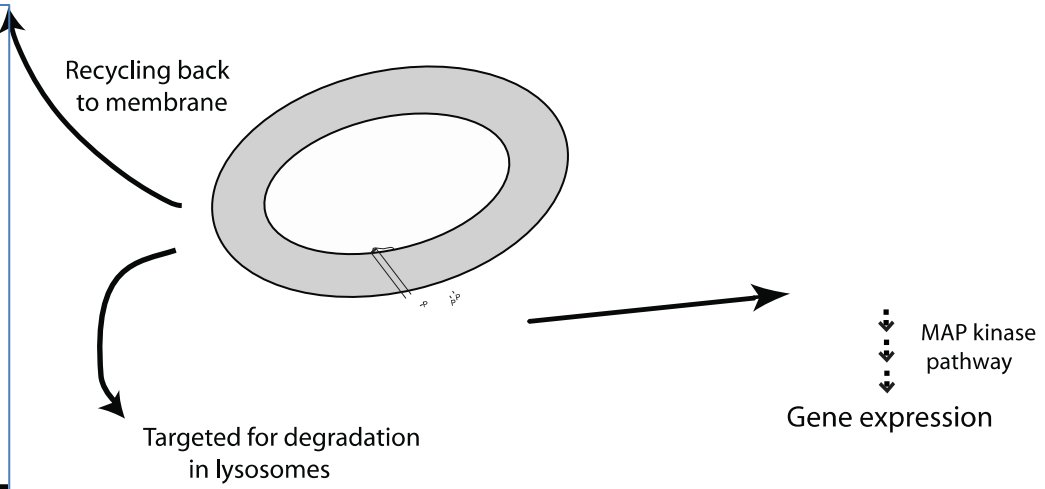


Gerwin Westfield and Georgios Skiniotis, Univ. Michigan

Future Directions







β_2AR-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

β_2AR-Gs Team

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Free University of Brussels
Els Pardon
Jan Steyaert

Trinity College Dublin
Joseph Lyons
Syed Shah
Martin Caffrey

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

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