LECTURE 12: RELATING STRUCTURE TO FUNCTION

Motif and Domain: Recap

- A **motif** is a similar 3-D structure conserved among different proteins that serves a similar function.
  - e.g., the presence of a helix-turn-helix motif in DNA binding proteins is an indication of a protein's function.
- **Domains**, on the other hand, are regions of a protein that has a specific function and can (usually) function independently of the rest of the protein.
  - Theoretically, DNA binding domain can be separated and can still bind the DNA

Classification of Protein Structure

- Protein domain folds into 5 broad classes:

1. α **domains**: only α helices

2. β **domains**: only β sheet

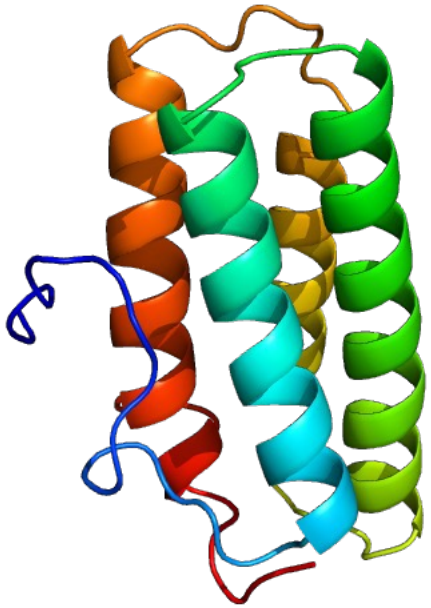
3. α/β **domains**: β strands connecting helical segments

4. α **+** β **domains**: separate β sheet and helical regions

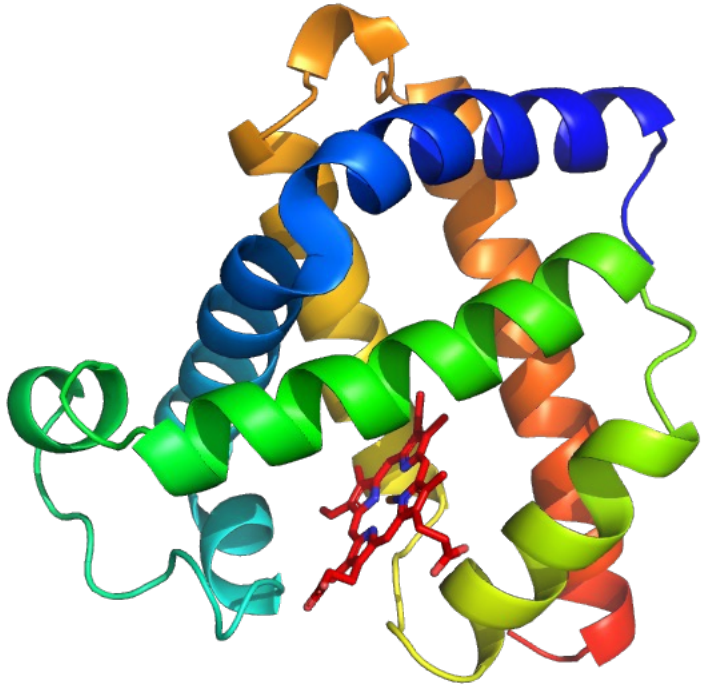
5. **Cross-linked domains**: little 2o structures stabilized by disulfide bonds or metal ions.

α domains

- Two common motifs for α domains are the **four-helix bundle** and the **globin fold**



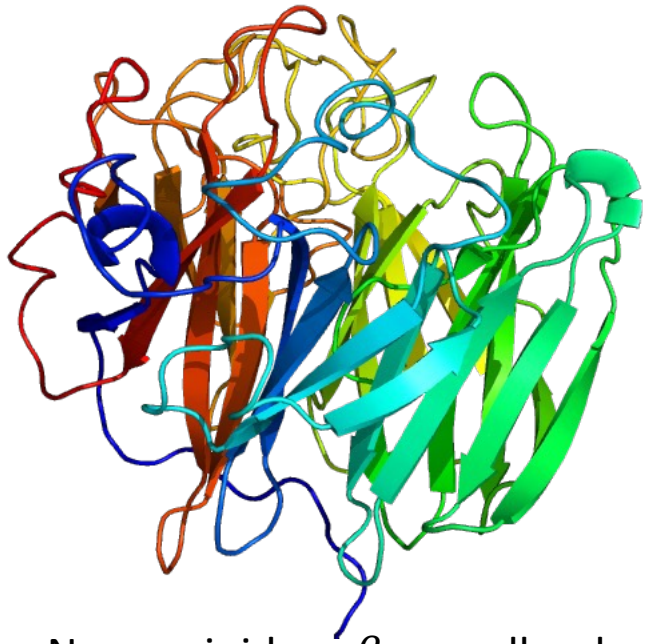
Myohemerythrin
PDB 2mhr

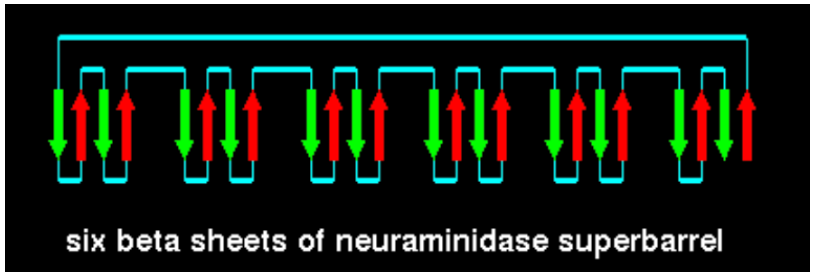


Myoglobin
PDB 1a6k

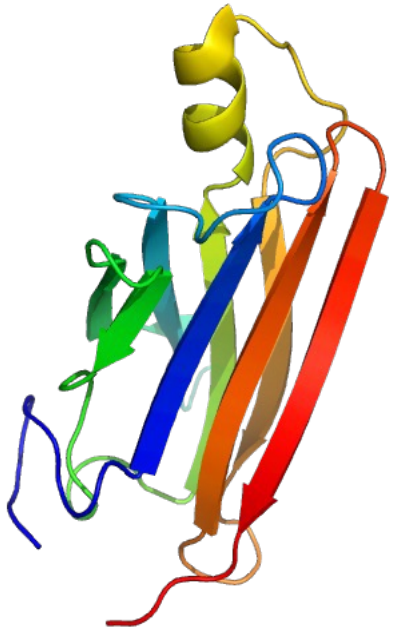
β domains

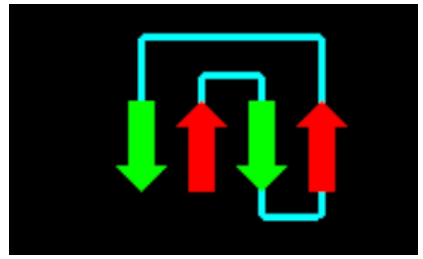
- β domains contain strands connected in two distinct ways:
  - β propeller domain: link adjacent β strands
  - Greek Key: Connection to the fourth strand





Neuraminidase β propeller domain
PDB 1a4q

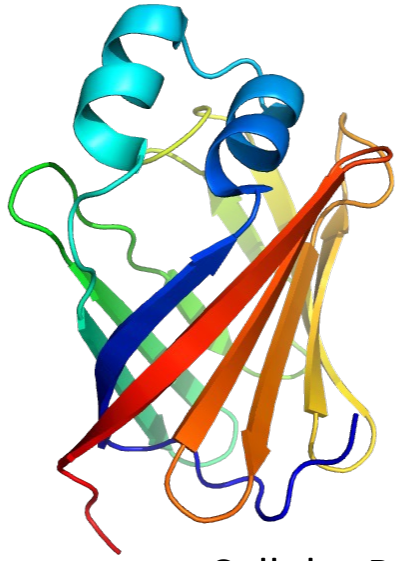




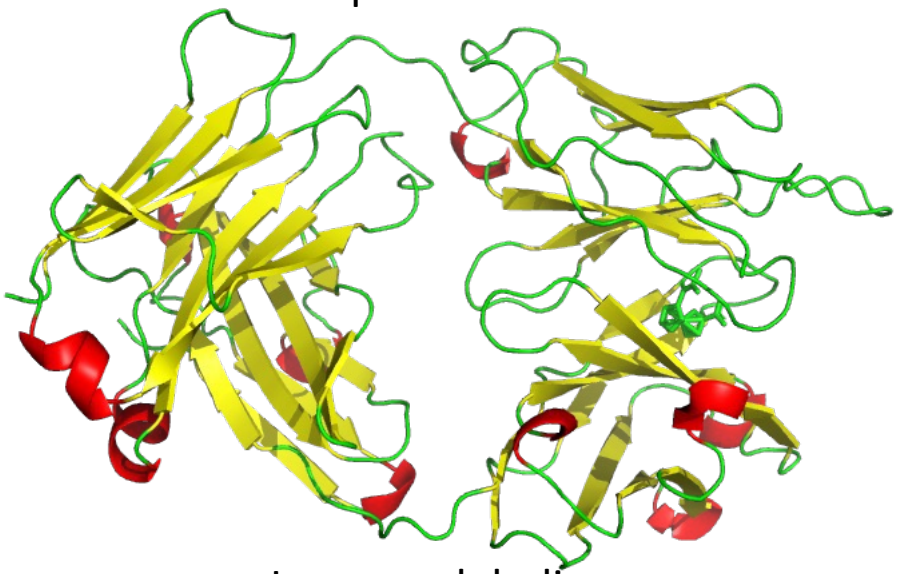
Pre-albumin
PDB 1tta

β domains

- Antiparallel sheets in β domains are <u>amphipathic</u>
  - One face exposed to aqueous surroundings
  - The other face is packed against another β sheets inward facing side, forming a hydrophobic core
- Two packing ways:
  - β **barrels**: β sheet forms a closed cylindrical structure
  - β **sandwiches**: two separate β sheets pack together face to face (like two slices of bread)



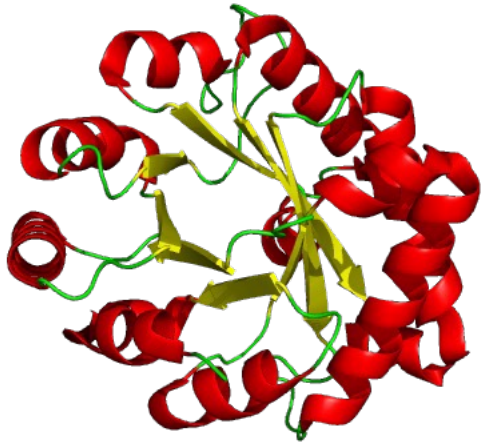
human apo Cellular Retinol Binding Protein II (CRBP-II)
PDB 2rcq



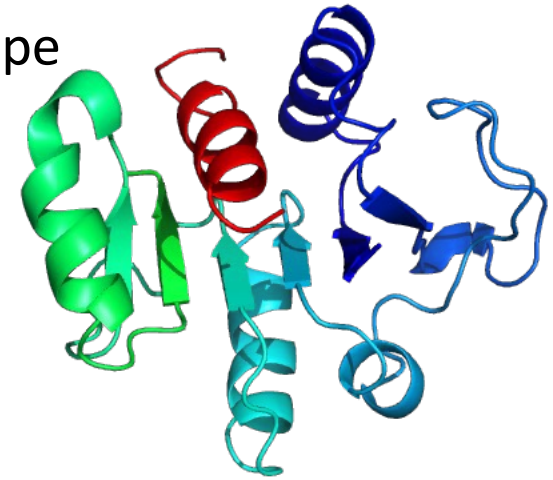
Immunoglobulin
PDB 1a3l

α/β domains

- β-α-β-α units
- Two major families:
  - α/β **Barrels**: parallel β sheet (consecutive) surrounded by α helices
    - The helices are amphipathic: their nonpolar side pack against the hydrophobic side of β sheet
    - The center of α/β Barrel is usually filled with hydrophobic side chains
    - **TIM barrel:** relatively nonpolar β sheet followed by amphipathic α helix, repeat 8 times
  - α/β **twists**: open β sheet that is twisted into a saddle shape



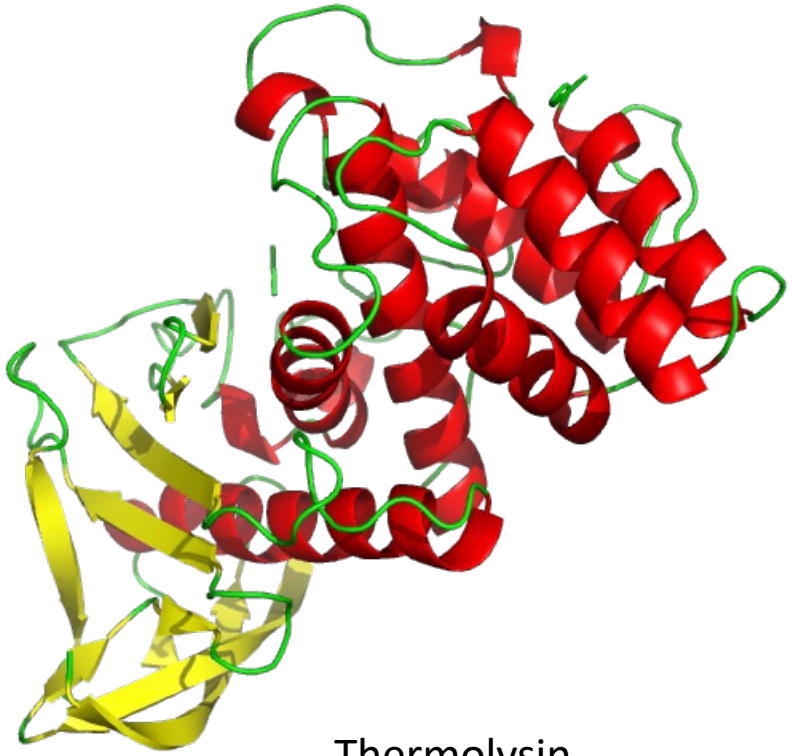
Triose phosphate isomerase
PDB 1tim



Aspartate beta-semialdehyde dehydrogenase (partial)
PDB 1brm

α + β domains

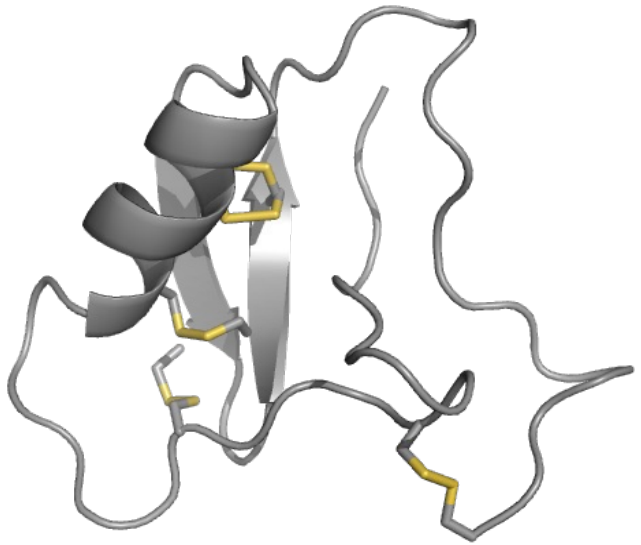
- Segregated α helices and β sheets



Thermolysin
PDB 3tmn

Cross-linked domains

- Found in small single-domain intra- and extracellular proteins



Scorpion toxin: a small irregular extracellular protein stabilized by 4 S-S bonds
PDB 1b7d

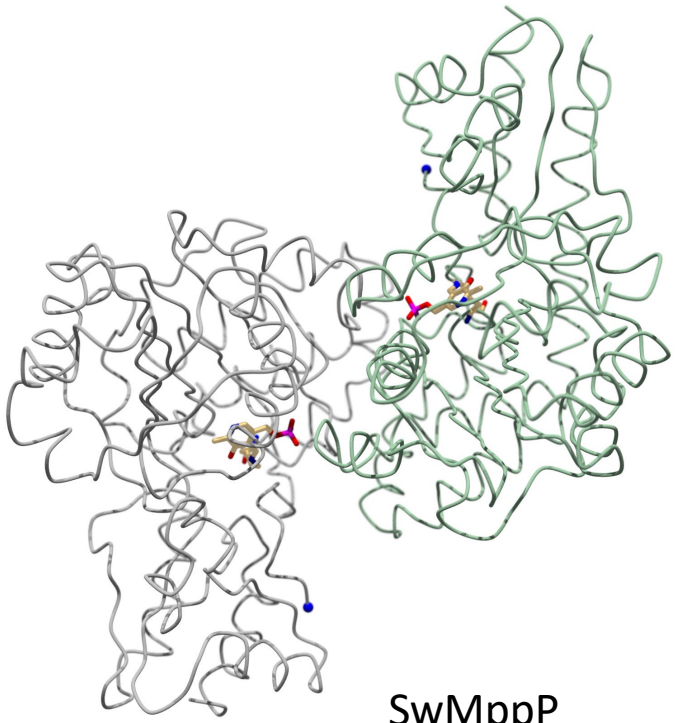
Proteins are Flexible Molecules

- The pictures of protein structures from X-ray crystallography seem rigid and static, however, in reality, proteins are highly flexible.
  - The forces that maintain 2^o, 3^o and 4^o structures are weak

- Ligand binding may induce
  - disordered polypeptide segments to become ordered (common)
  - disordering of previously ordered strand (less common)
  - Large movements of side chains, loops, or domains
  - association and dissociation of subunits

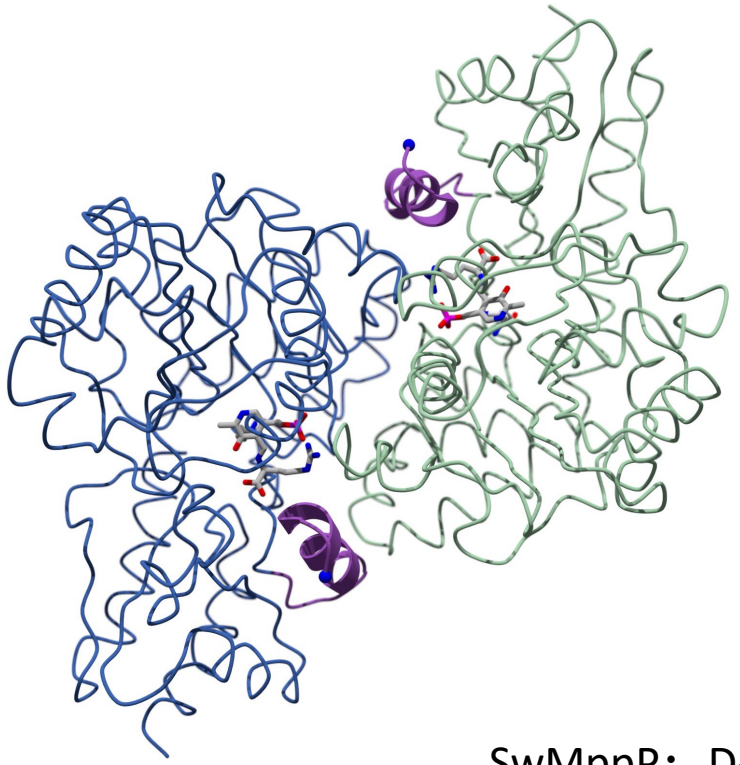
Case 1:

- The disordered N terminus orders (by forming an α helix) after the substrate analog bound.



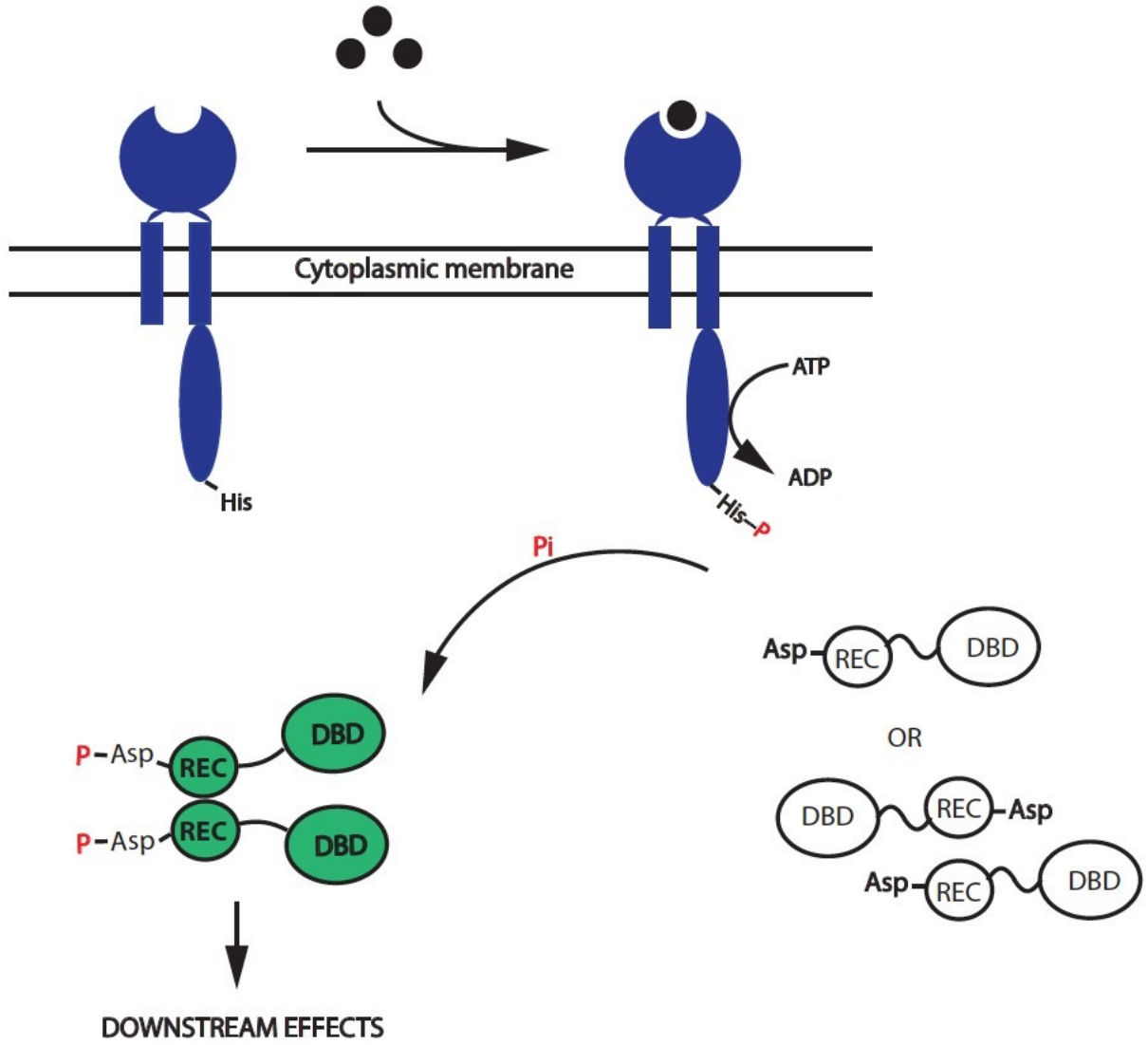
SwMppP

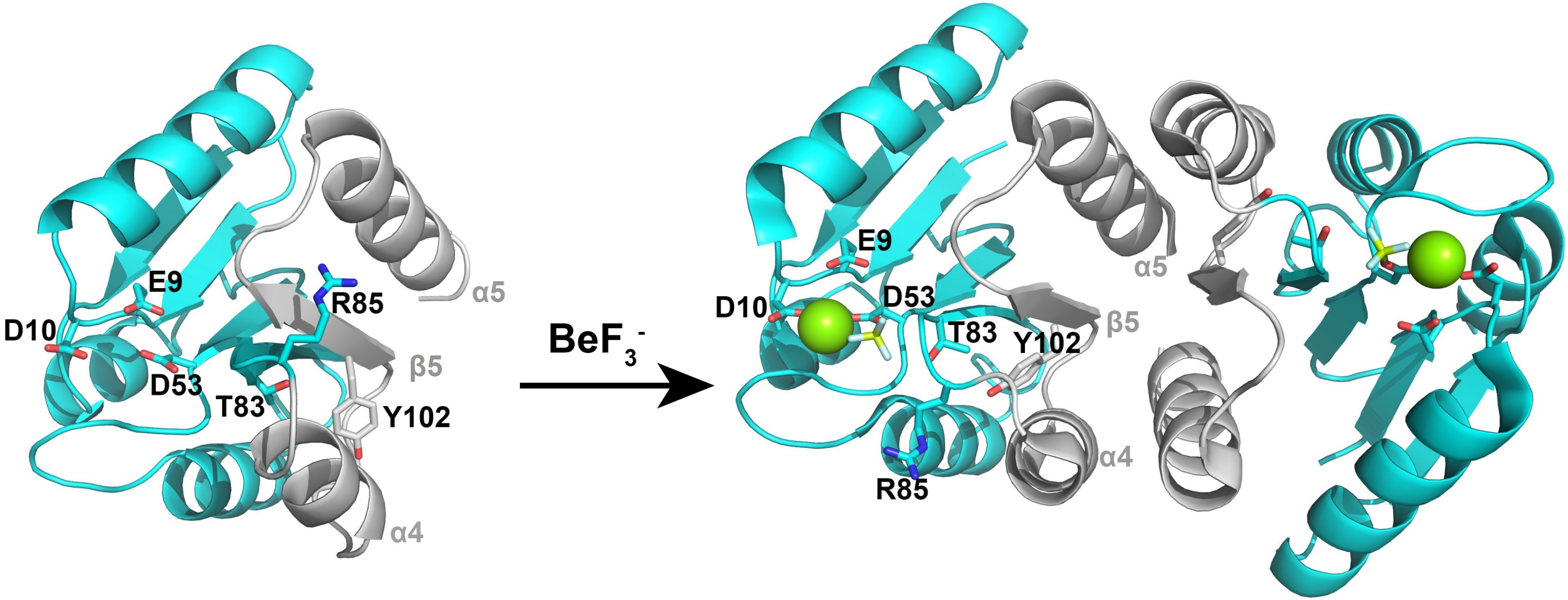
With
D-Arg
bound



SwMppP· D-Arg

Case 2: Two-component Signal Transduction System



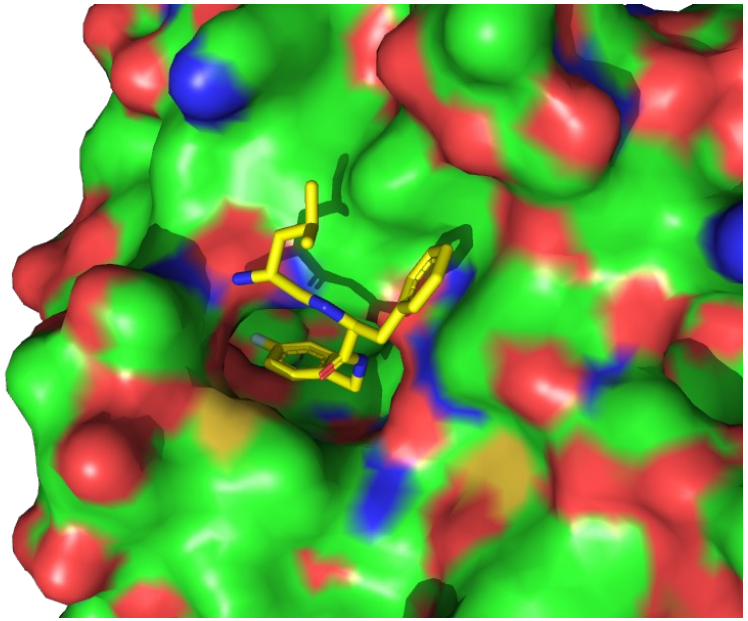


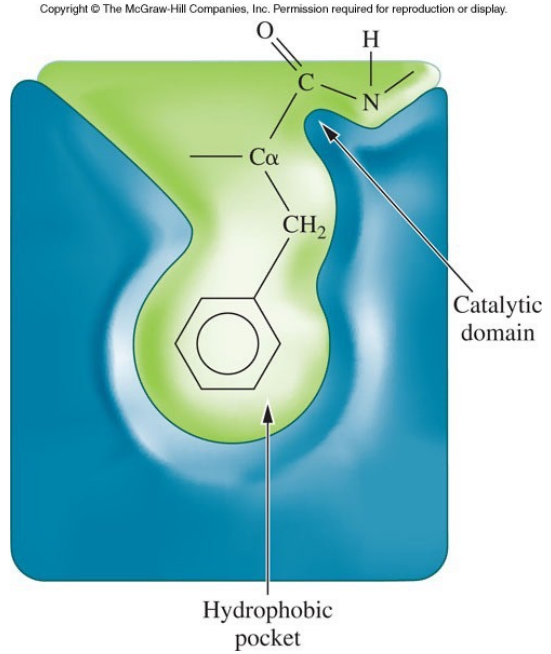
Inactive PhoB REC domain (monomer)
PDB 1B00

Activated PhoB REC domain (dimer)
PDB 1ZES

Structural Basis of Protein Function: Overview

- Protein functions such as molecular recognition and catalysis depends on **complementarity** of shape and charge distribution.
  - Chymotrypsin cleaves the peptide bond at the carboxylic end of Tyr, Trp, and Phe





Since trypsin cleaves after lysine and arginine, how might trypsin's binding pocket differ from chymotrypsin?

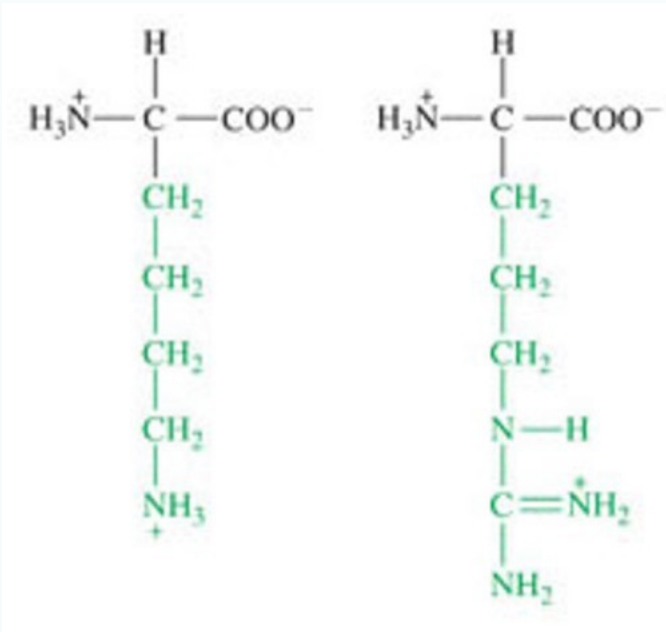
A. Trypsin would contain a positive charge near the “bottom” of the pocket.

B. Trypsin would contain a positive charge near the “top” of the pocket, near the catalytic site.

C. Trypsin would contain a negative charge near the “bottom” of the pocket .

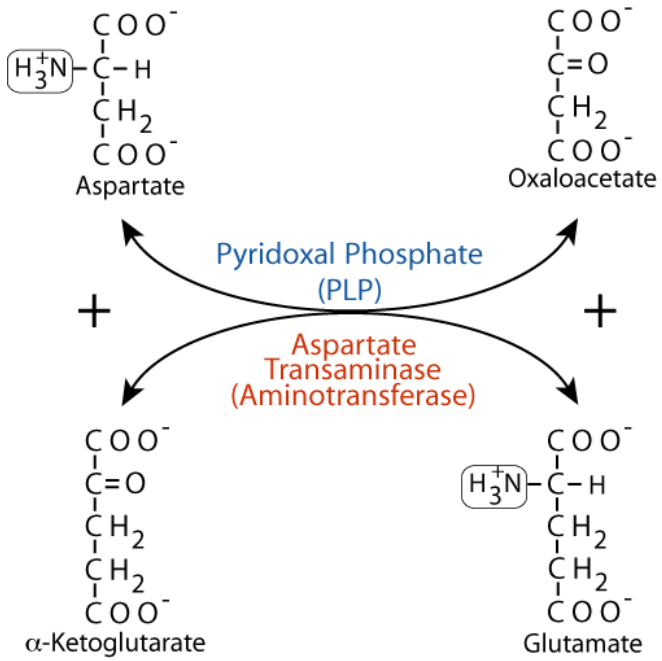
D. Trypsin would contain a negative charge near the “top” of the pocket, near the catalytic site.

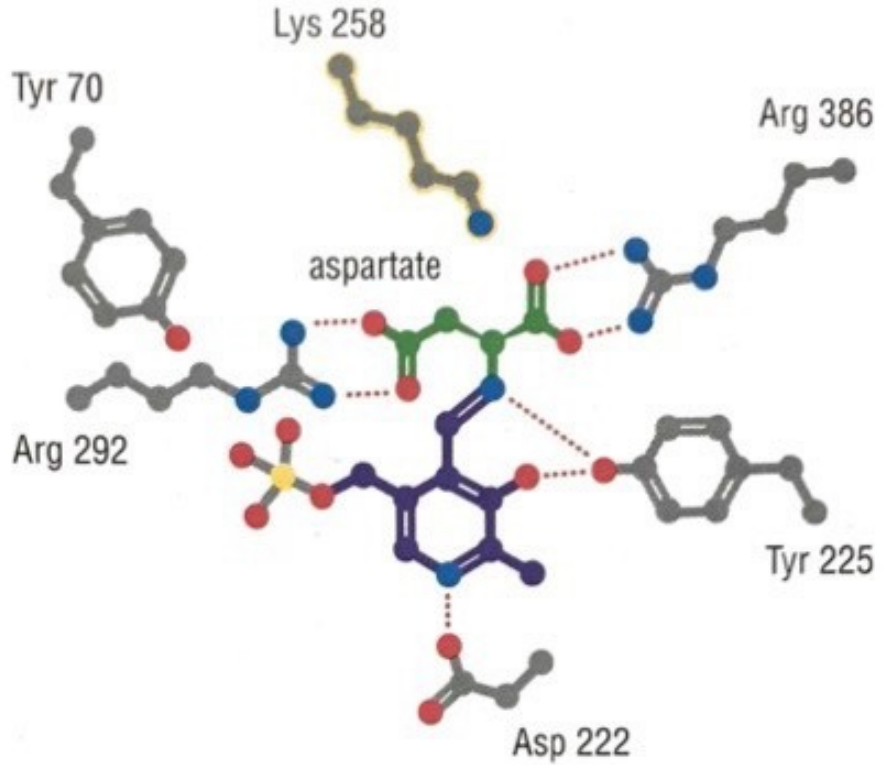
E. . Trypsin would have an identical binding pocket, but different catalytic site.



Amino Acid Changes Can Affect the Specificity

- Mutation of Arg292 to aspartic acid produces an enzyme that prefers arginine to aspartate as a substrate.

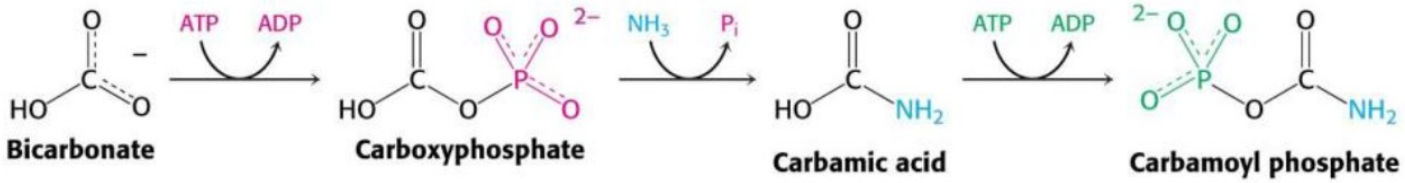


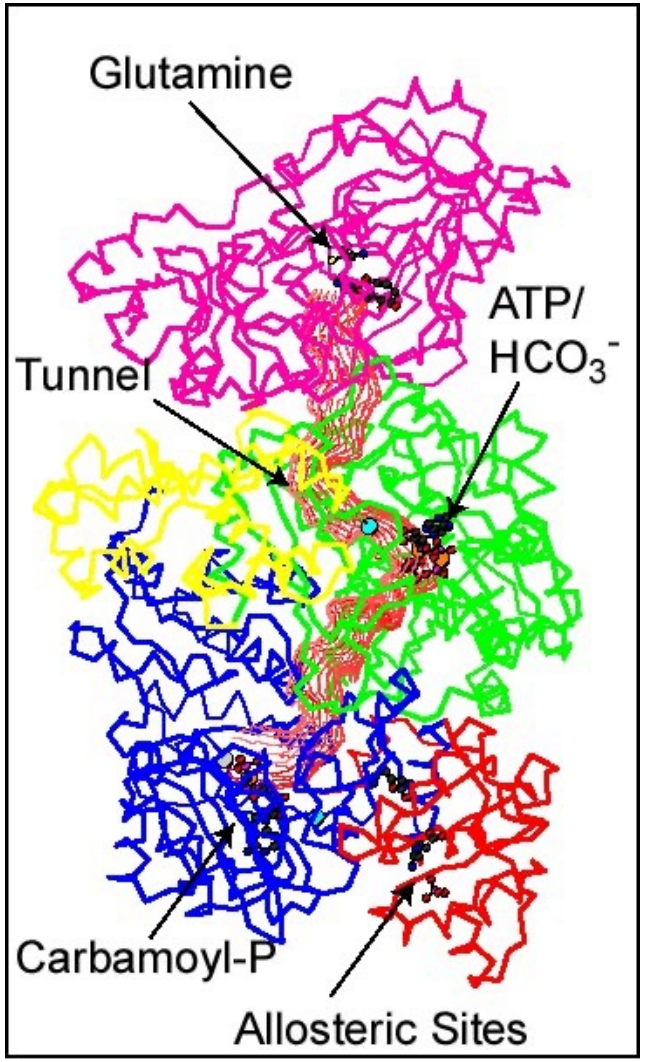


Steven C. Almo, Douglas L. Smith, Avis T. Danishefsky, Dagmar Ringe. *Protein Engineering, Design and Selection*, Volume 7, Issue 3, March 1994, Pages 405–412

Some Enzymes Can Catalyze More Than One Reaction

- Some enzymes may have one or more active sites, some enzymes may be comprised of more than one polypeptide chain, each has one active site.
  - e.g., a trifunctional enzyme, carbamoyl phosphate synthetase, has a 96 Å long tunnel that allows substrate to move through as it is processed.





Glutamine is hydrolyzed to ammonia

Ammonia migrates to the 2nd active site, where it reacts with carboxyphosphate to produce a carbamate intermediate.

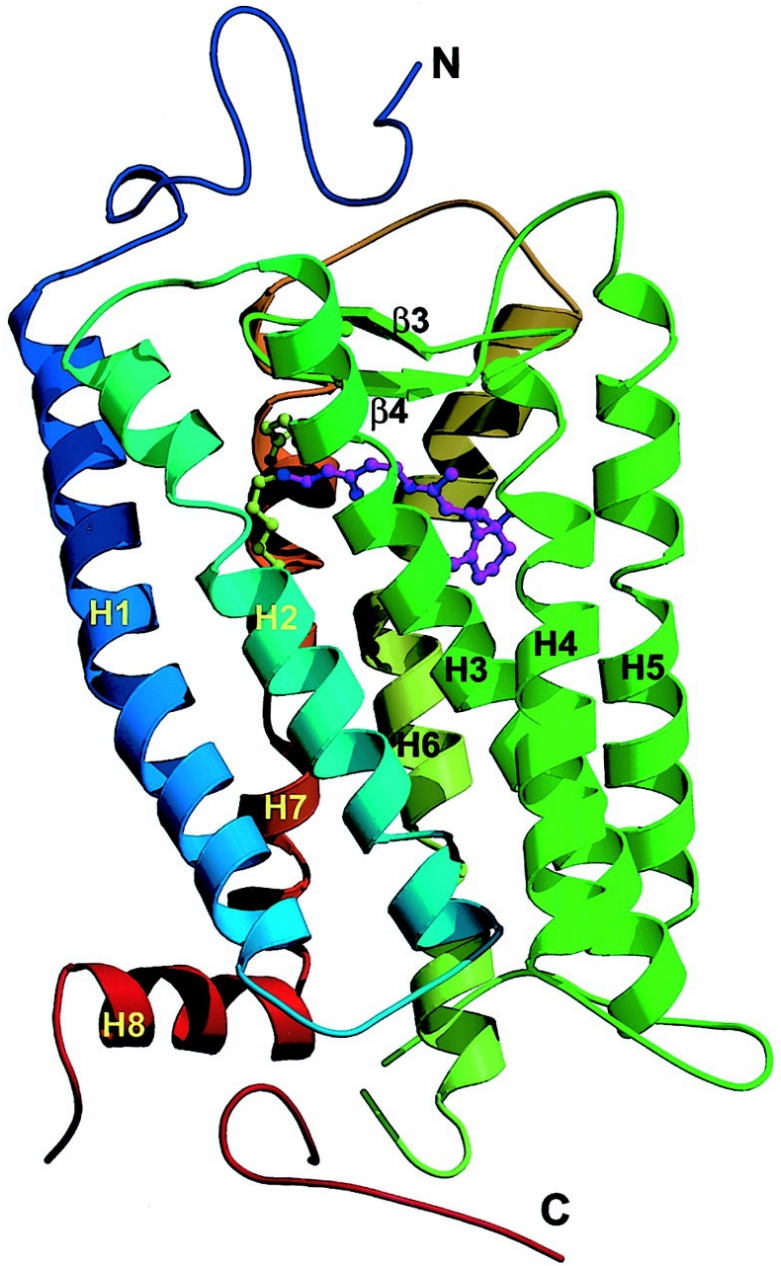
Carbamate intermediate diffuse into the 3rd active site, where it is phosphorylated by another ATP to produce carbamoyl phosphate

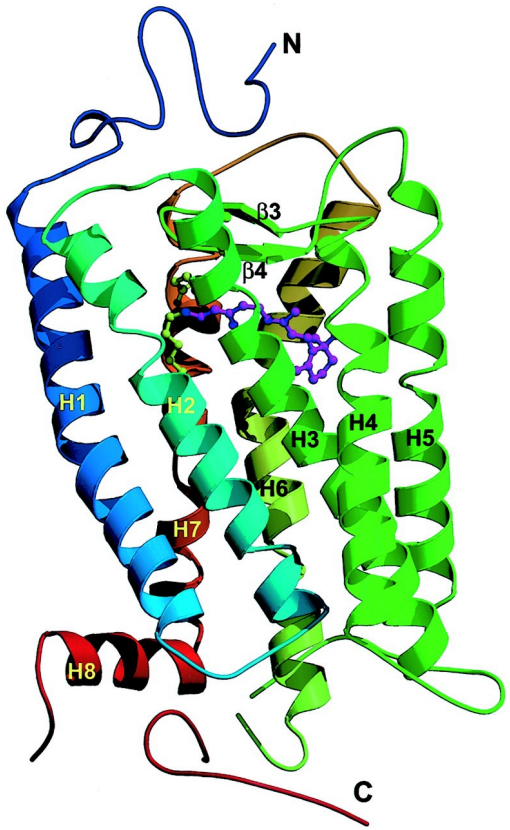
Structural Basis for Receptor Signaling:

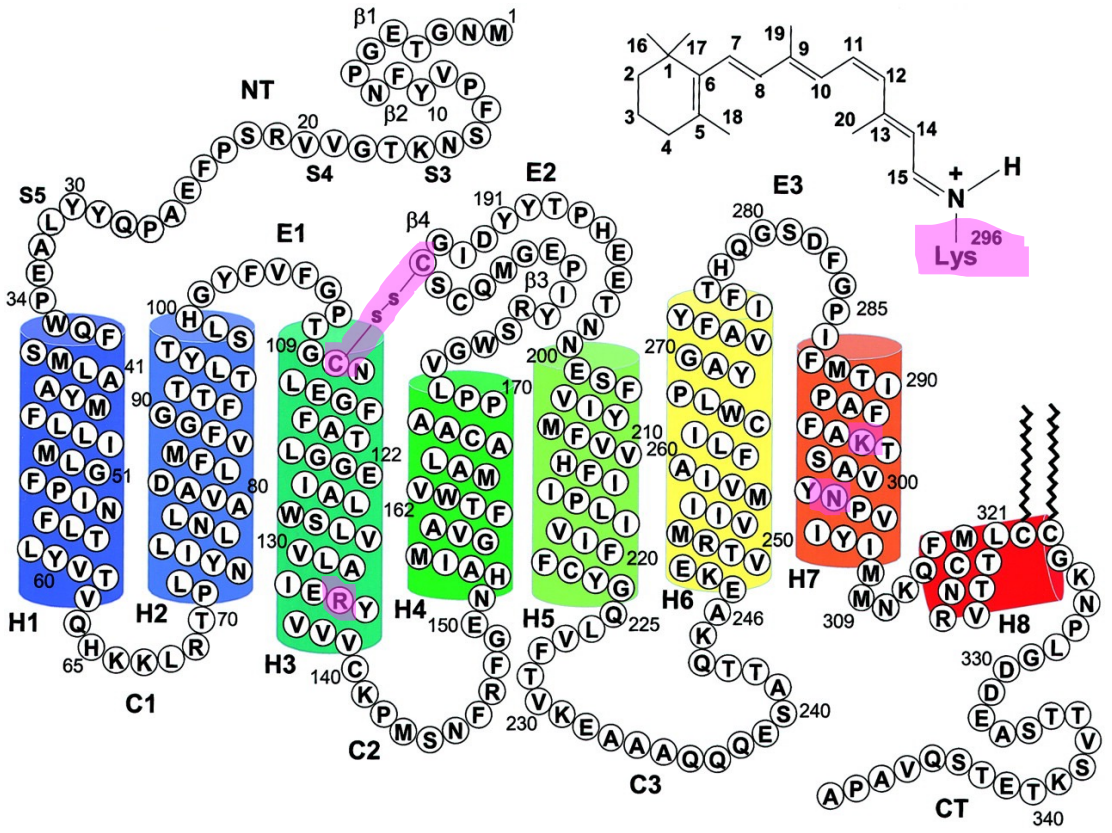
Case Study: Rhodopsin as a model GPCR

Rhodopsin (Rho)

- Rhodopsin is a prototypical G-protein-coupled receptor (GPCR) in vertebrate vision, activates the G-protein transducin (GT) by catalyzing GDP-GTP exchange.
- Rho is a photoreceptor composed of two parts: the opsin protein, and the 11-cis retinal chromophore which derives from vitamin A.







Structure of Rhodopsin (opsin)

- A distorted barrel consisting of seven transmembrane α-helices,
- extracellular N-terminus,
- and cytoplasmic C-terminus.

Menon, Han, & Sakmar (2001) Physiol. Rev. 81, 1659-1688.

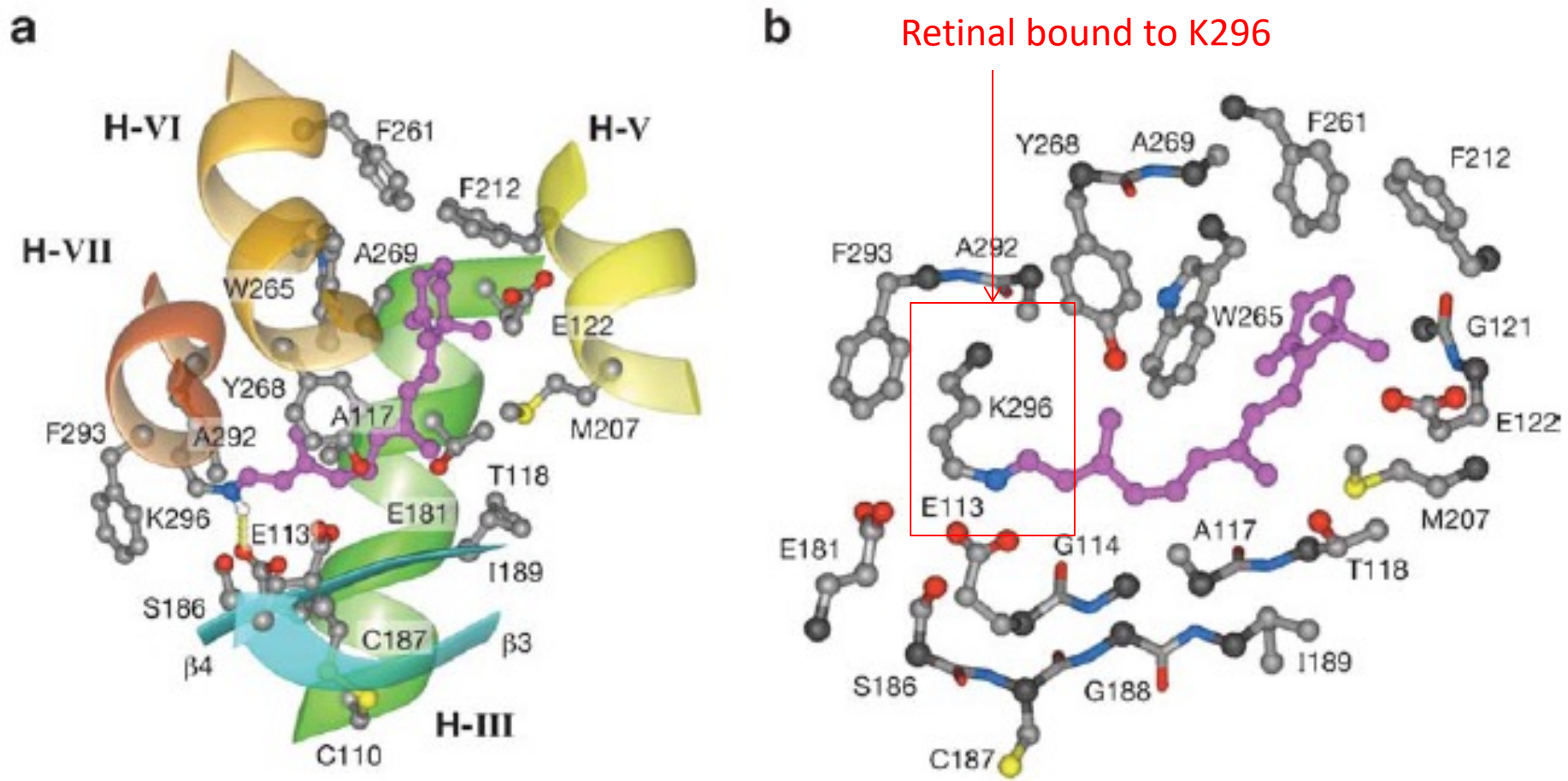
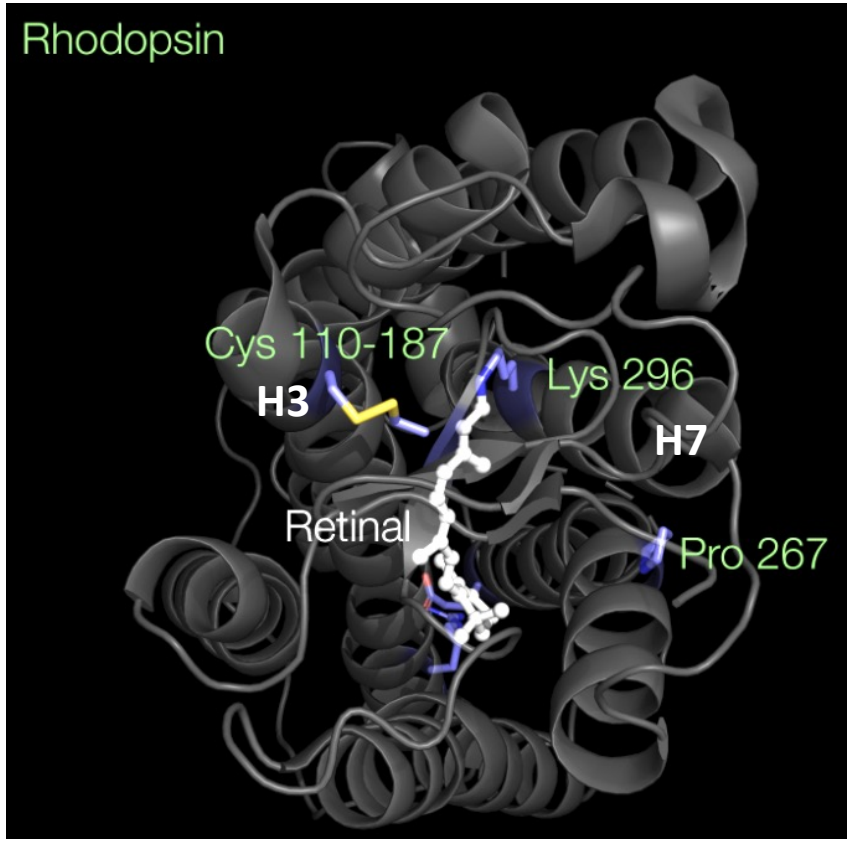
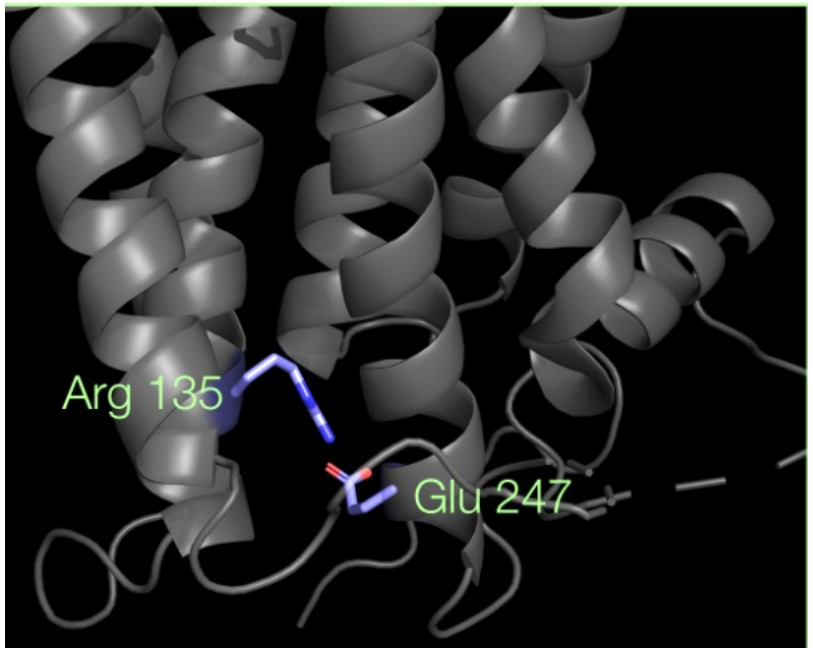


Figure 5

The amino acid residues in the vicinity of the chromophore. (*a*) Schematic showing the side chains surrounding the 11-*cis*-retinylidene group (*pink*); side view through helices III, V, and VI. (*b*) Schematic presenting the residues within 5 Å distance from the 11-*cis*-retinylidene group (*pink*). Note that the chromophore is coupled via the protonated Schiff base with Lys^{296}.

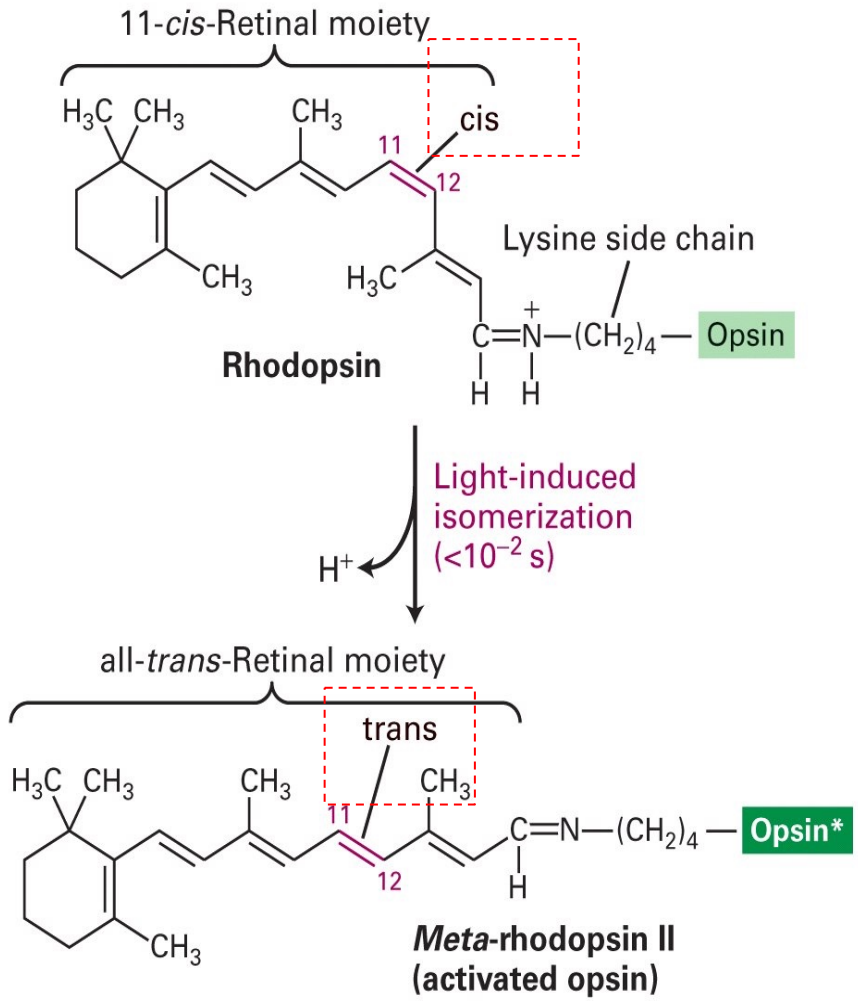




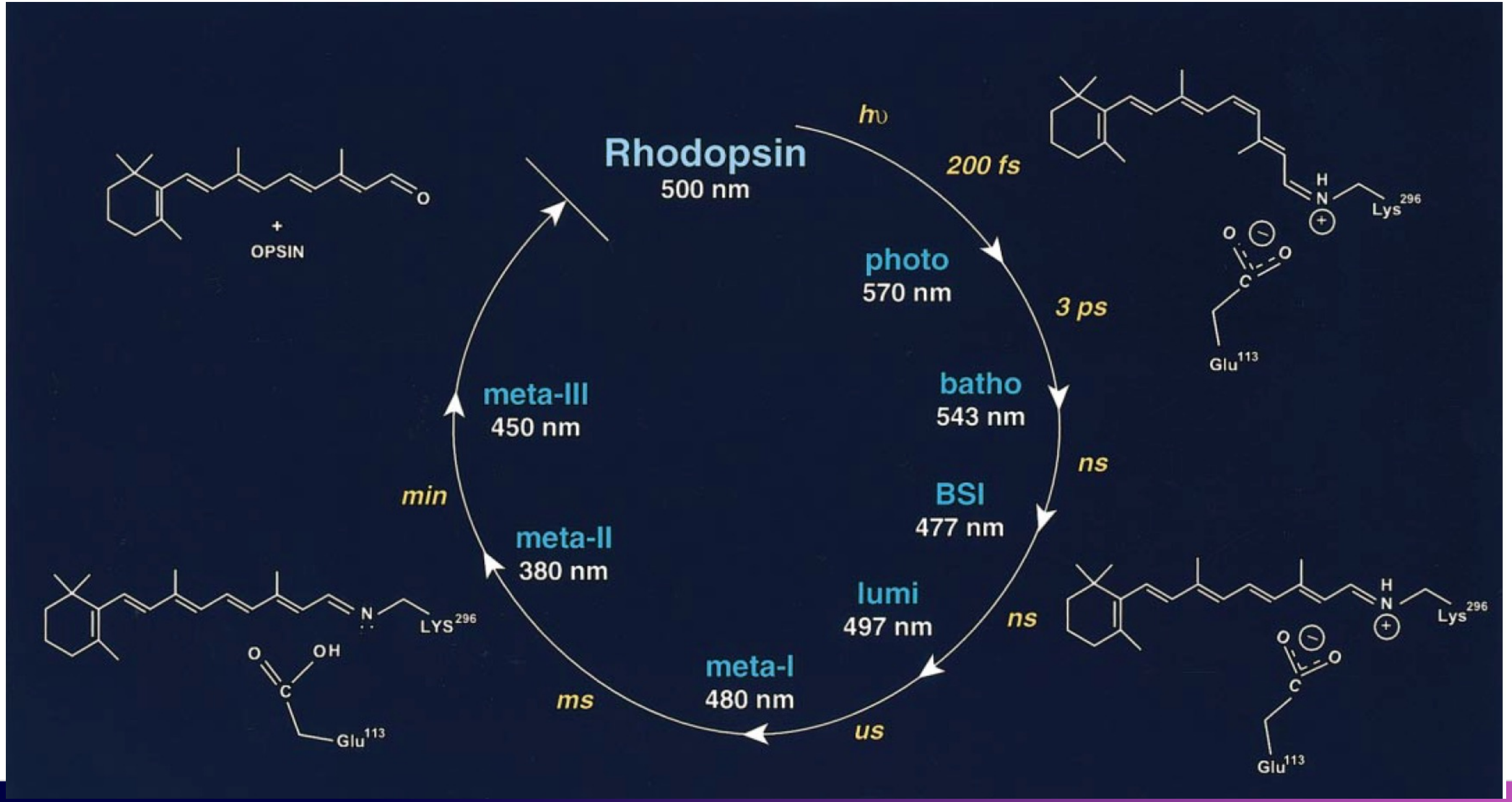
- Disulfide bond between Cys110 and Cys 187 stabilizes the beta sheets and the binding of retinal.
  - These beta sheets serve as a lid, blocking retinal from dissociating when rhodopsin is inactive.
- Another important feature is a salt bridge between Arg135 and a Glu247 (located in helices 3 and 6, respectively), which prevents G-proteins from binding to inactive rhodopsin.

Rhodopsin Activation

- Light absorbed
- **Isomerization to all-trans retinal**.
- The isomerization of retinal triggers **conformational changes in opsin**
- and through a series of intermediates, it turns into **meta-rhodopsin** - the active form of rhodopsin.

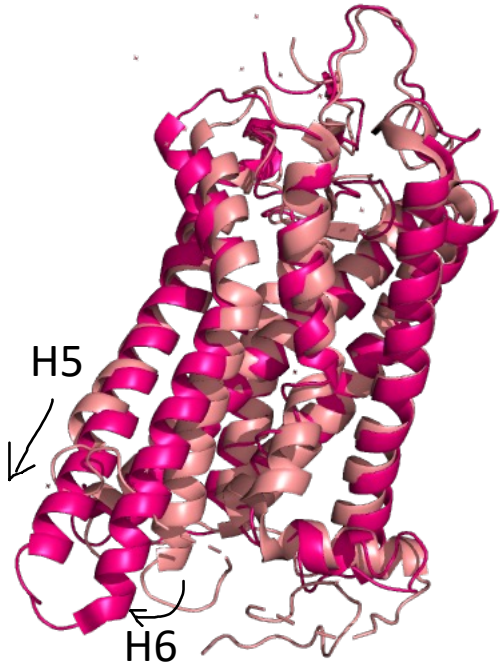


The photolyzed pigment then proceeds through a number of well-characterized spectral intermediates.

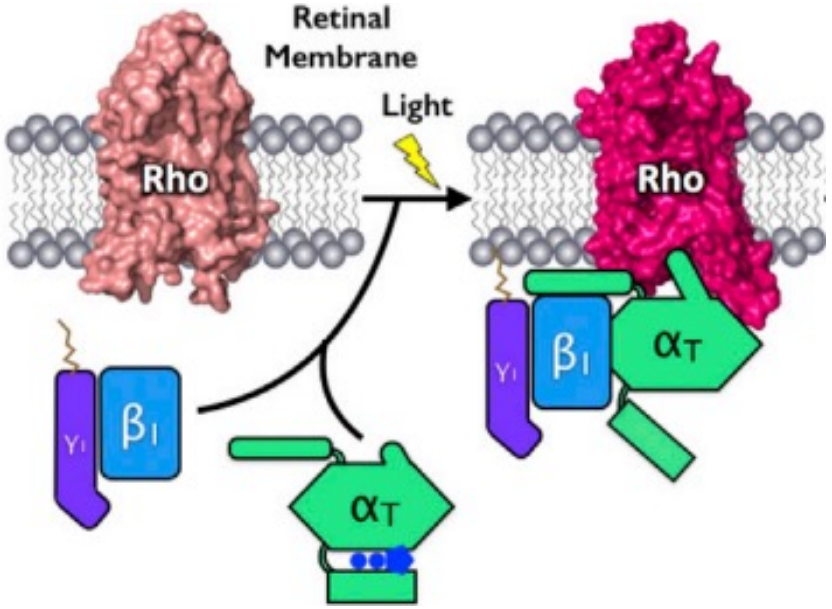


Rhodopsin Activation

- Two major conformational changes:
  - **helix 6** tilts away from the trans-membrane core towards the cytoplasmic side, due to a kink on Pro267, widening the G-protein binding site.
  - **helix 5** extends into the cytoplasmic matrix, increasing the G-protein binding interface.



Rhodopsin: 1F88
Meta-Rhodopsin: 6OY9

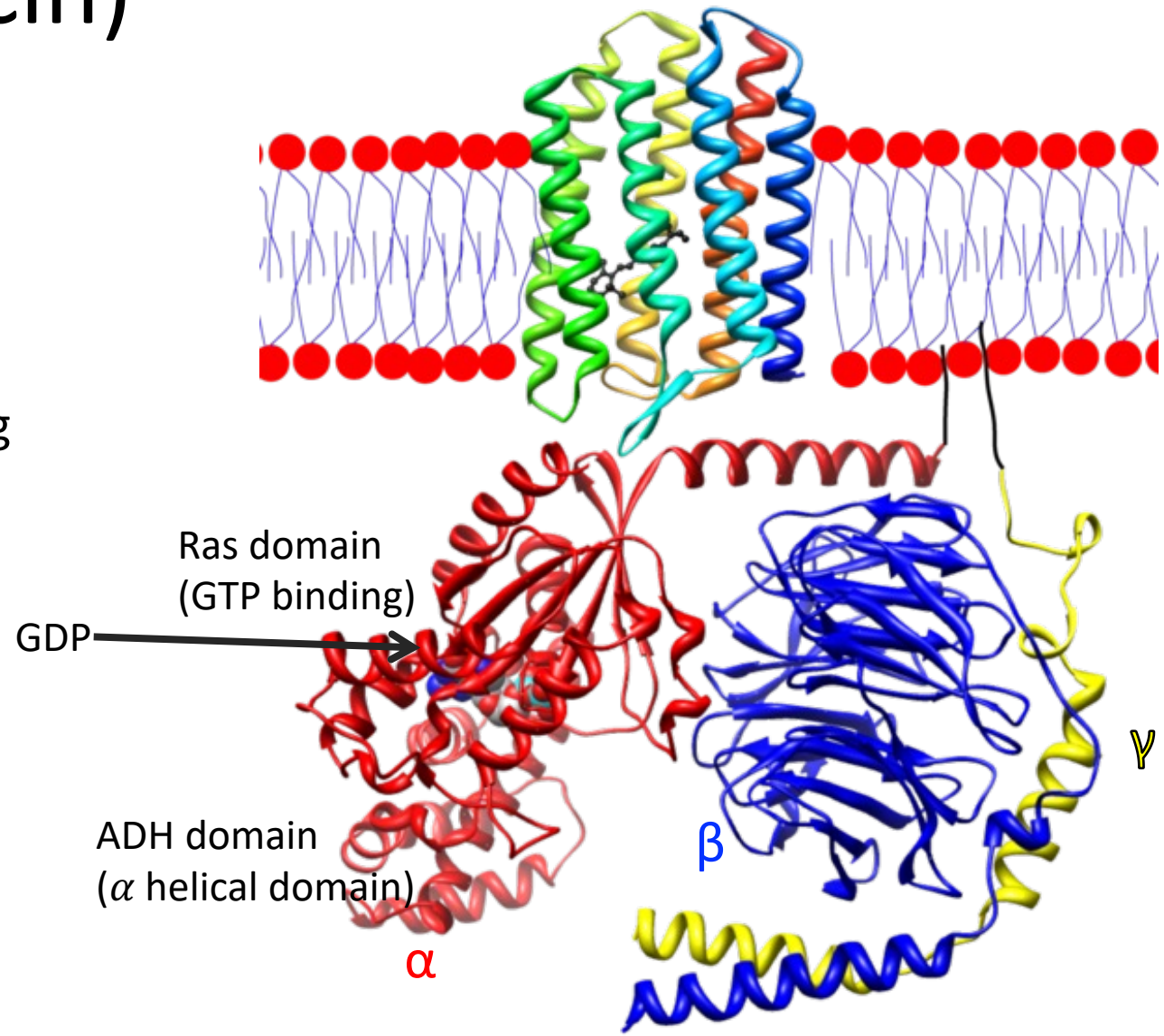


Gao et al., 2019, Molecular Cell 75, 781–790. https://doi.org/10.1016/j.molcel.2019.06.007

G-Protein (Transducin)

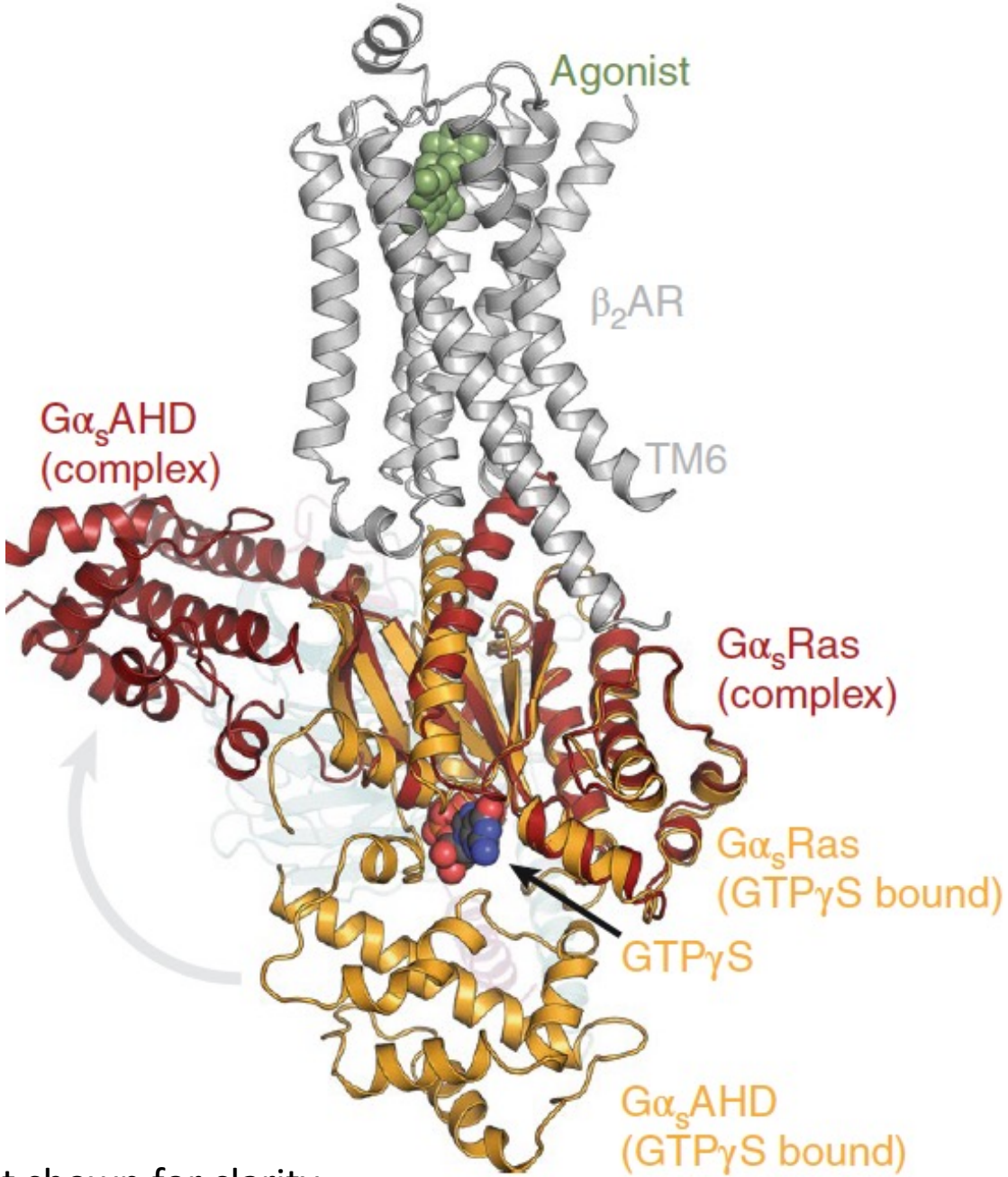
- Upon **photoactivation**, a G-protein called transducin will bind to meta-rhodopsin.
- It is a heterotrimer:
  1. $G_t\alpha$ = red; nucleotide binding subunit
  2. $G_t\beta$ = blue
  3. $G_t\gamma$ = yellow

- GDP-bound: "off" state
  - heterotrimer bind to GPCRs in their GDP-bound state
- GTP-bound: "on" state



- Nucleotide-free Gα: red
- GTP bound Gα: orange

- Dissociation of GDP, which induces a large conformational change: AHD domain changes position relative to the Ras domain

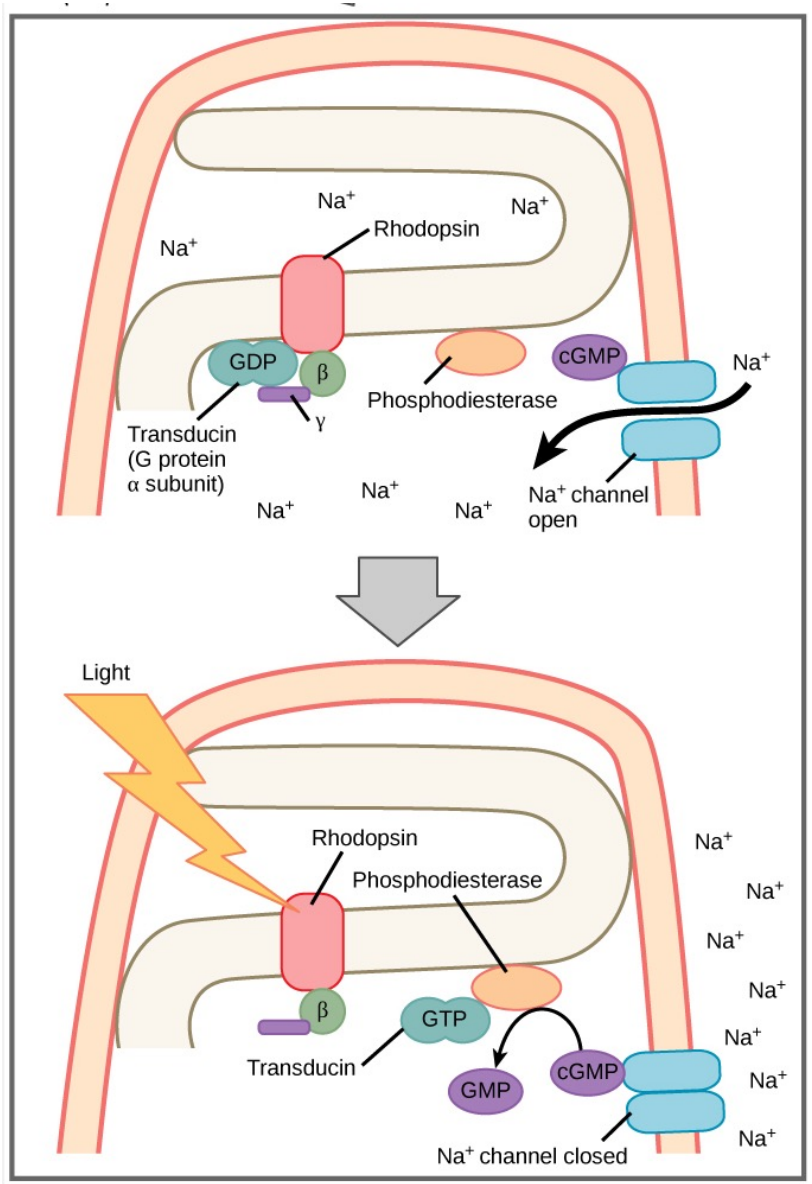


βγ subunits are not shown for clarity.

Hilger D, Masureel M, Kobilka BK. Structure and dynamics of GPCR signaling complexes. Nat Struct Mol Biol. 2018 Jan;25(1):4-12. doi: 10.1038/s41594-017-0011-7.

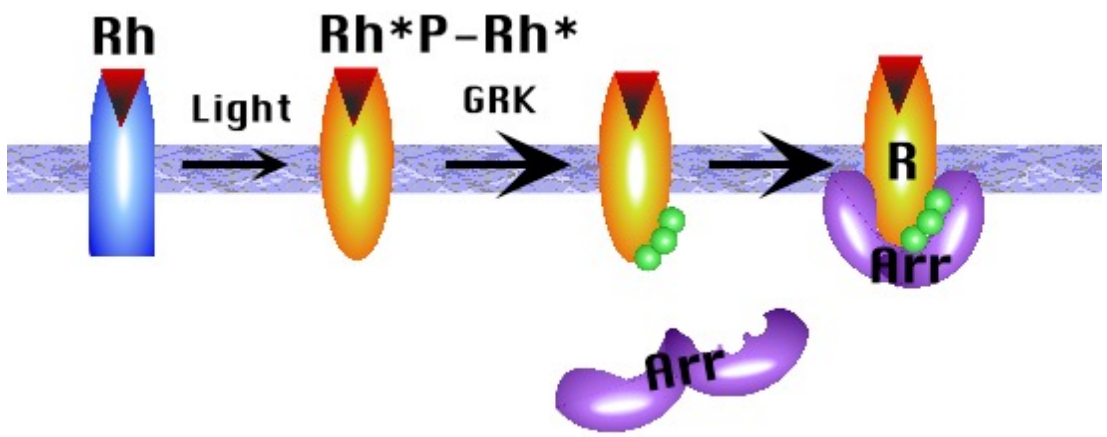
Rhodopsin as a Transducin Activator

1. Absorption of a photon by retinal changes conformation to "metarhodopsin II" (isomerization from 11-cis to 11-trans)
2. Transducin (Gα) is activated by metarhodopsin II
3. When metarhodopsin activates transducin, triggering GDP dissociation and GTP association
4. When GTP bound, the α subunit dissociates from the βγ subunits ($G_T\beta\gamma$)
5. Activated transducin α-subunit activates cGMP phosphodiesterase. Gα subunits has GTPase activity, which can hydrolyze GTP to GDP, and then reassociates with $G_T\beta\gamma$, completing the G-protein activation circle.
6. cGMP phosphodiesterase breaks down cGMP, an intracellular second messenger which opens cGMP-gated cation channels
7. Decrease in cGMP concentration leads to decreased opening of cation channels and hyperpolarization of the membrane potential
8. This signaling cascade ultimately leads to a rapid visual response in rod cells.



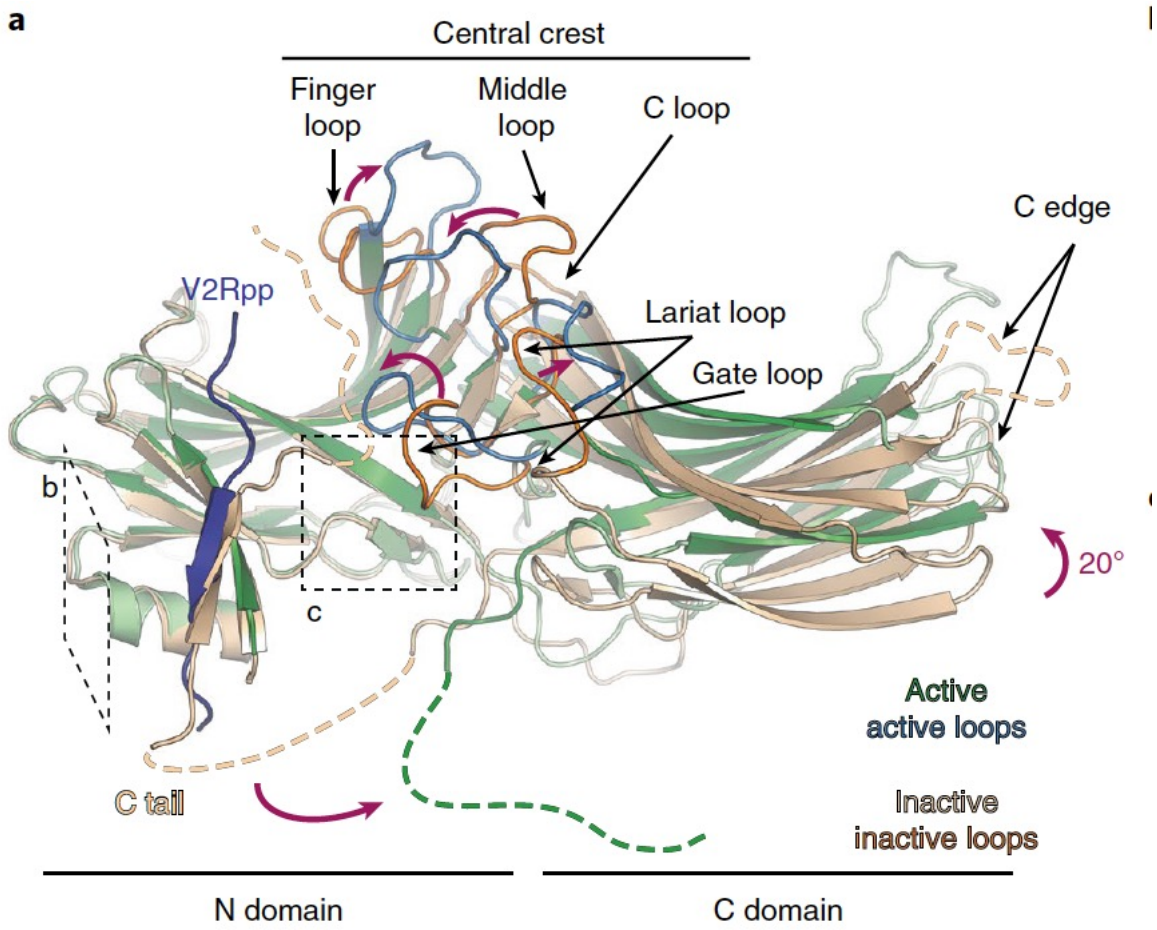
Arrestin

- To turn off GPCR: G protein-coupled receptor kinases (GRKs) and arrestins come into play.
- Visual arrestin modulates the intracellular response of retinal rod cells to light by specifically binding to the phosphorylated light-activated form of the photoreceptor rhodopsin(P-Rh*)



- Arrestin binding to the receptor blocks further G protein-mediated signaling, targets receptors for internalization, and redirects signaling to alternative G protein-independent pathways.

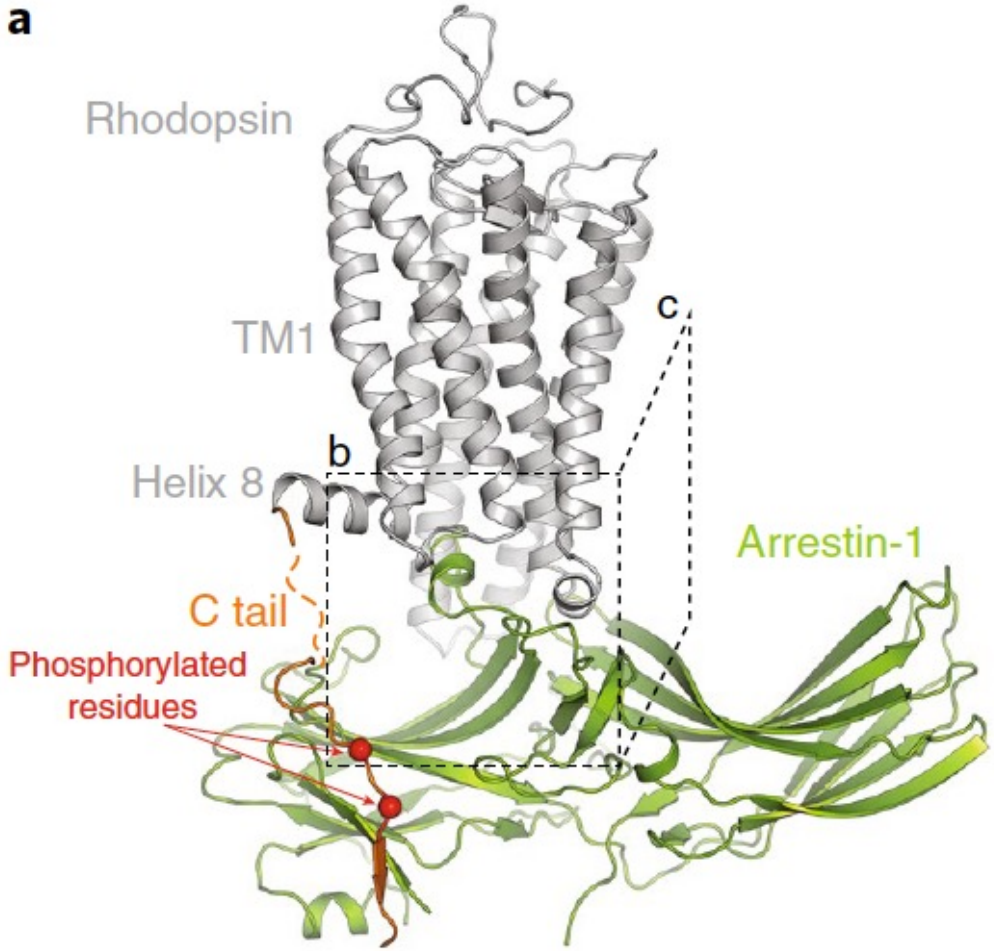
Arrestin



Activation induces major conformational changes:

1. rearrangements of the loops at the N–C-domain interface,
2. displacement of the arrestin C tail,
3. and an ~20° interdomain rotation.

Rhodopsin・Arrestin-1 complex



Arrestin binding to the receptor involves the movement of the two domains relative to each other and the release of arrestin C-tail.

Hilger D, Masureel M, Kobilka BK. Structure and dynamics of GPCR signaling complexes. Nat Struct Mol Biol. 2018 Jan;25(1):4-12. doi: 10.1038/s41594-017-0011-7.