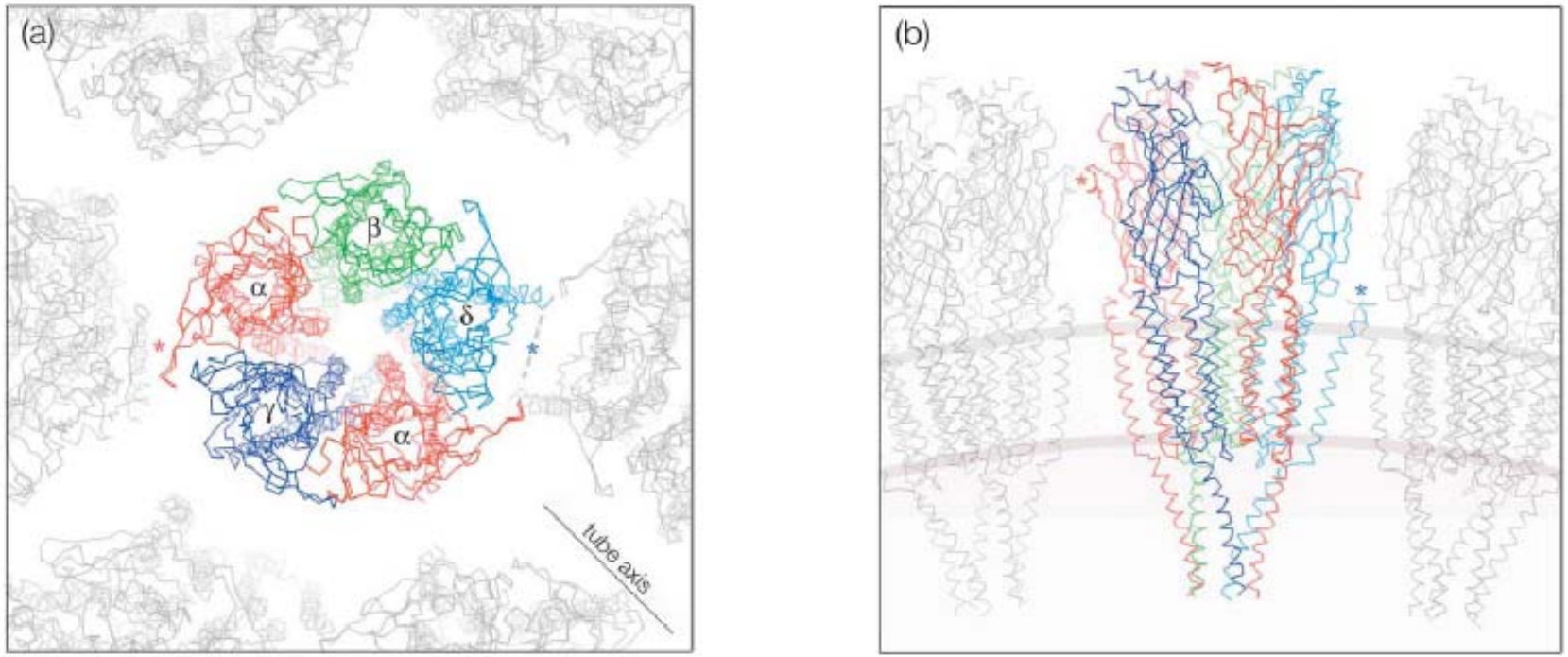
Receptors

- Activation → conformational change that transfers *information* across the cell membrane
- Allow the cell to sense and respond to environmental conditions and stimuli
- Three classes:
  - Ligand-gated ion channels (nicotinic acetylcholine receptor)
  - Enzyme-linked receptors (epidermal growth factor receptor)
  - G-protein coupled receptors (many hormones, peptides, biogenic amines)

Ligand-gated ion channels
(Ionotropic receptors)

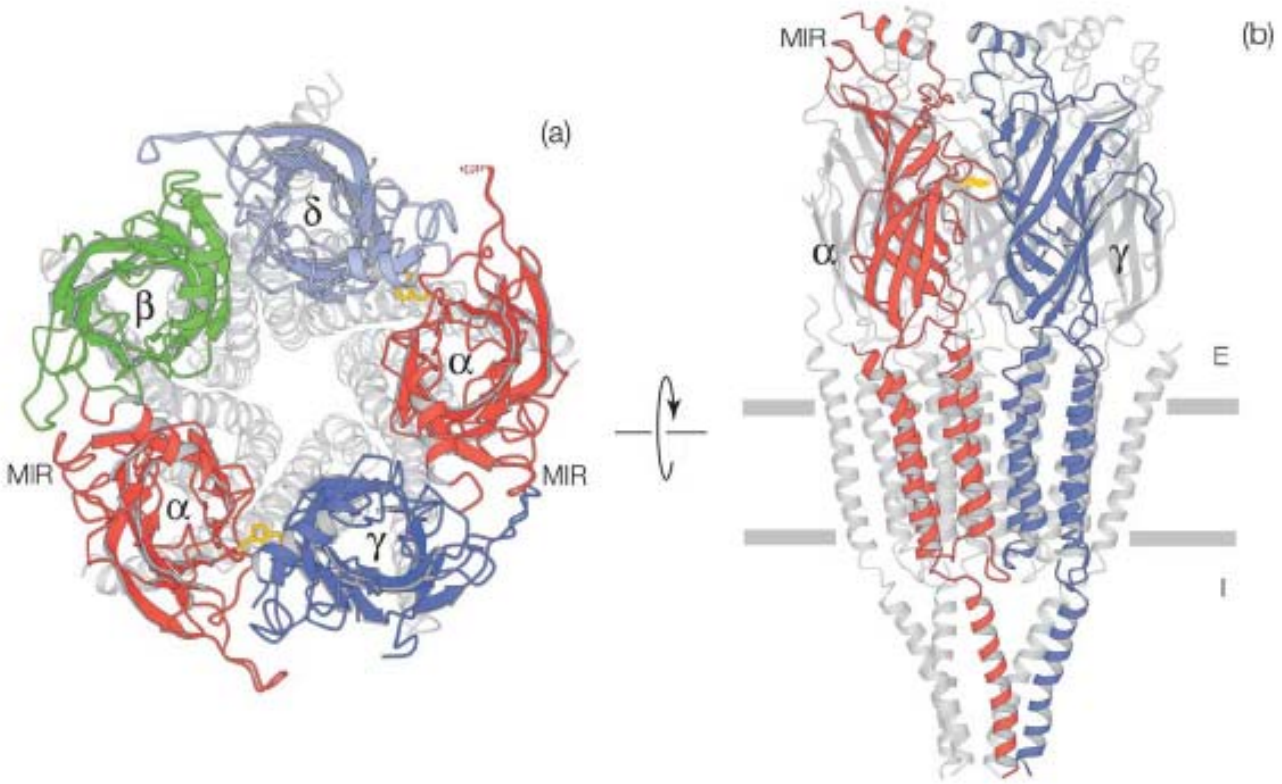
- Neuronal receptors for acetylcholine, glycine, serotonin, GABA
- Very large (~ 300 kDa) glycoproteins
- Heteropentamers ($\alpha\ \gamma\ \alpha\ \beta\ \delta$)
- Ligand binding → opens channel allowing rapid flow of ions down concentration gradient
- Either cation or anion selective

Nicotinic acetylcholine receptor
(Torpedo marmorata)



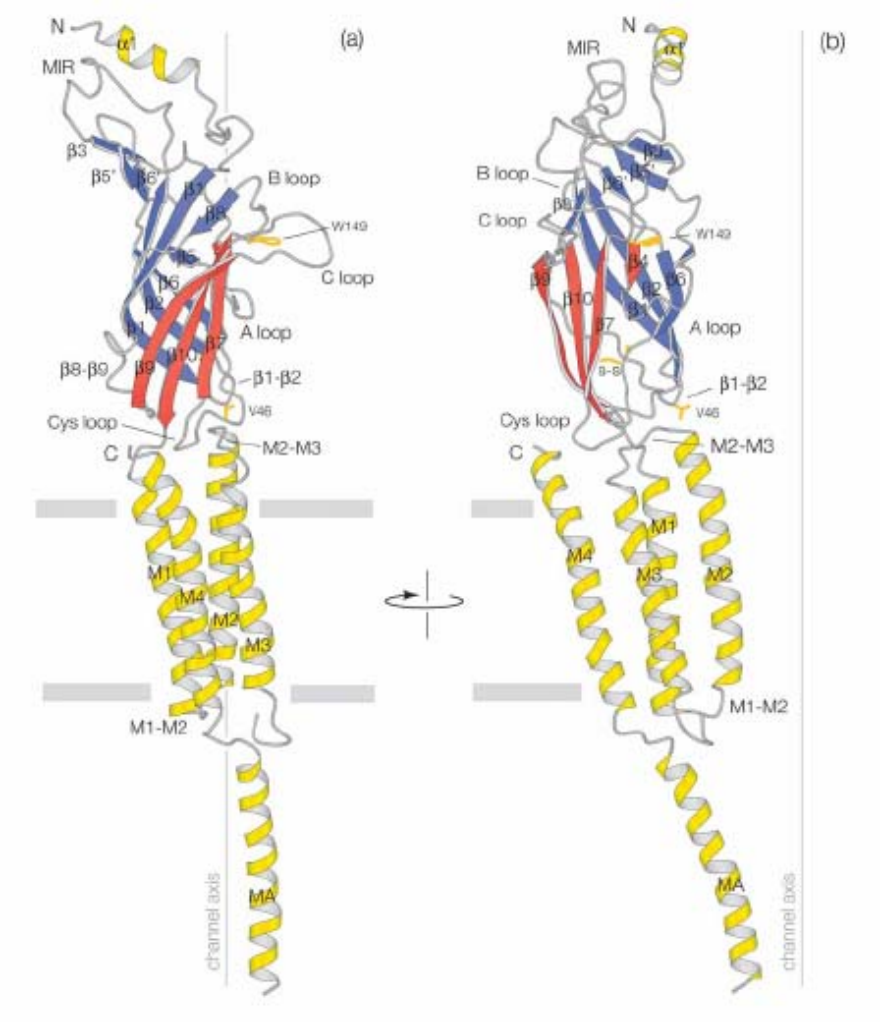
4.0 Å resolution reconstructed from cryoelectron microscopy

N. Unwin, J. Mol. Biol. 346, 967 (2005)



Subunits symmetrically arranged around central pore

Two Ach binding sites at the α-δ and α-γ subunit interfaces



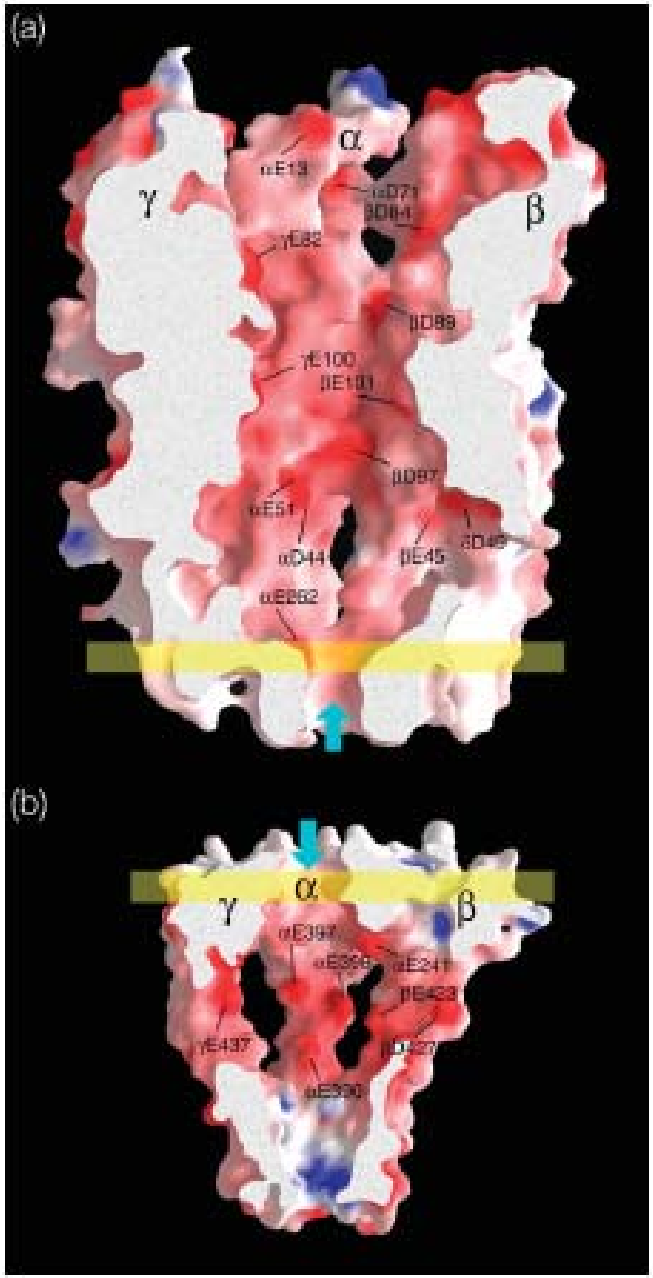
Torpedo AchR α-subunit

Each monomeric subunit

N-terminal extracellular domain: two β-sheets joined through a disulfide bond

TM domain: four α-helices per subunit, arranged symmetrically with the five M2 helices lining the central pore

Cytoplasmic domain: each subunit contributes one α-helix to a "vestibule"

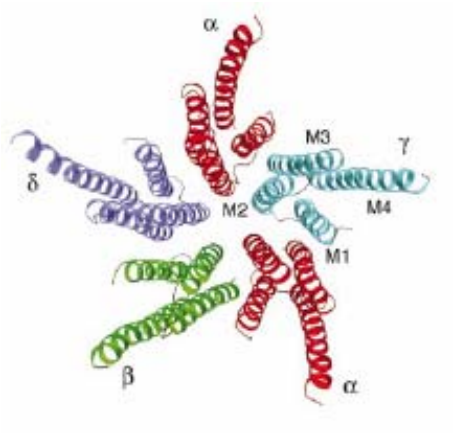


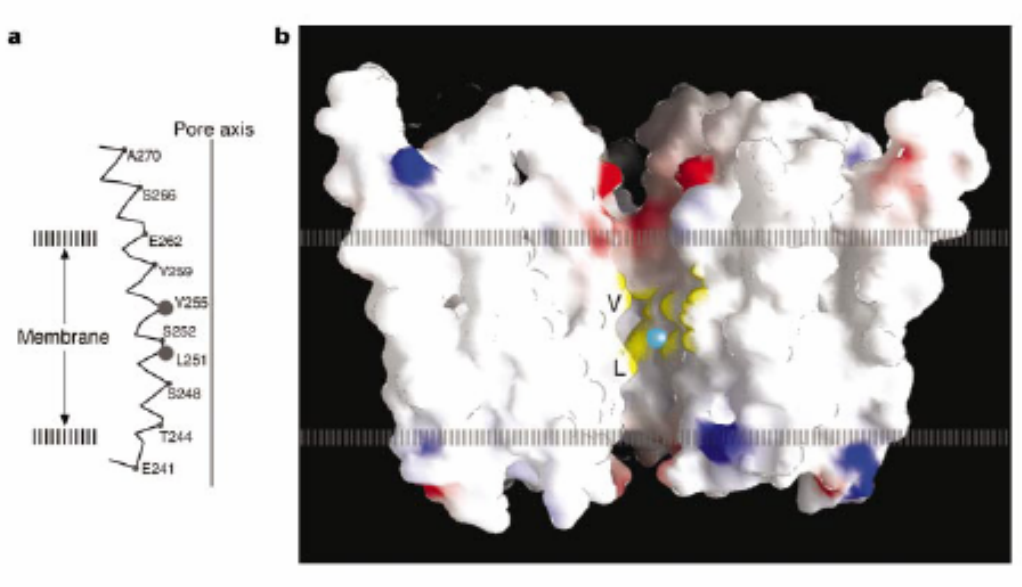
Electrostatic potential of the outer and inner vestibules

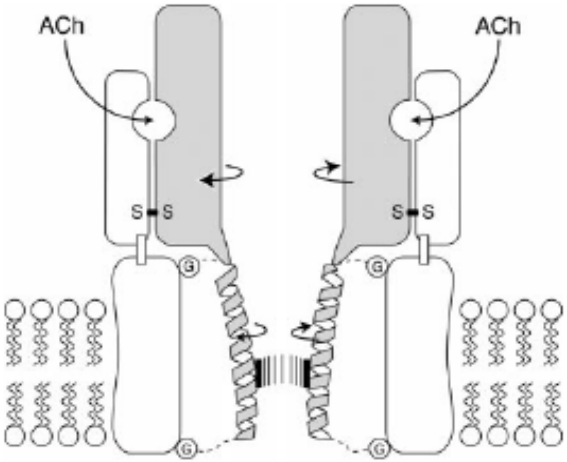
- highly negative
- many Glu and Asp residues

(AchR is cation-selective, allowing passage of both K^+ and Na^+ ions)

Unwin, J. Mol. Biol. 346, 967 (2005)







TM helices M1, M3, M4 shield pore helices from the lipid bilayer

Hyrophobic constriction keeps pore closed

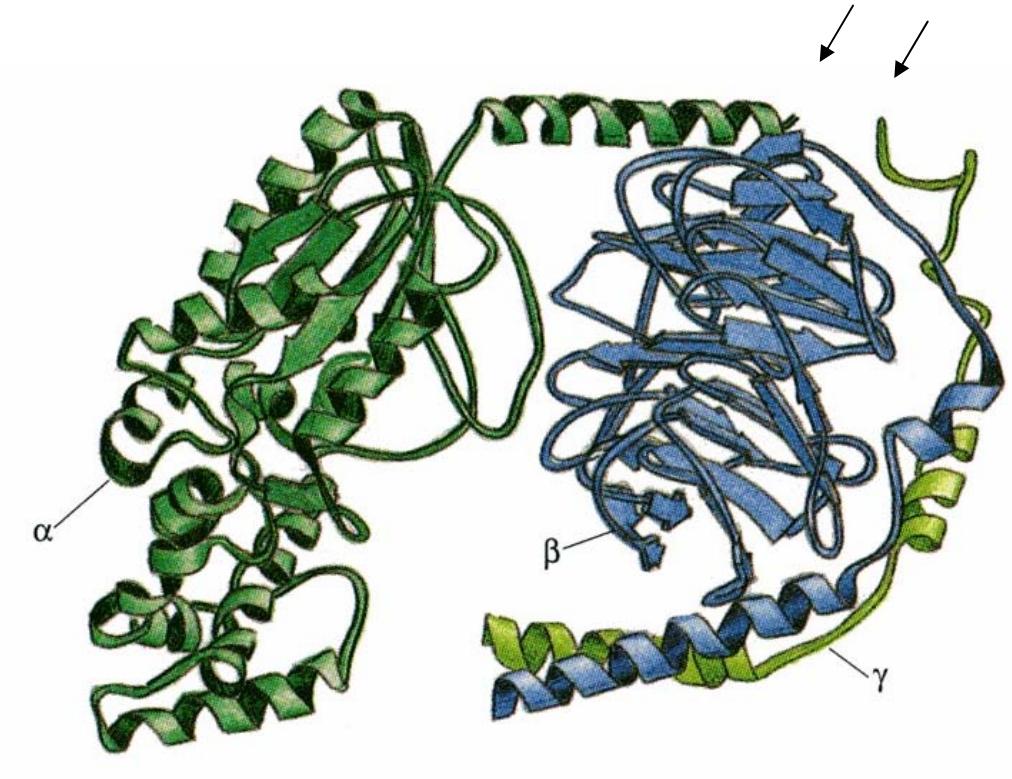
Rotation of α-subunit M2 helices proposed as channel-opening mechanism

G-protein coupled receptors (GPCRs)

- Largest group of cell surface receptors found in nature (several hundred identified, account for ~ 3% of the human genome)
- Present in all eukaryotes (including plants, yeast, on up to mammals)
- Found in essentially all tissues and cell types
- Are targets of ~ 40% of all drugs currently in clinical use

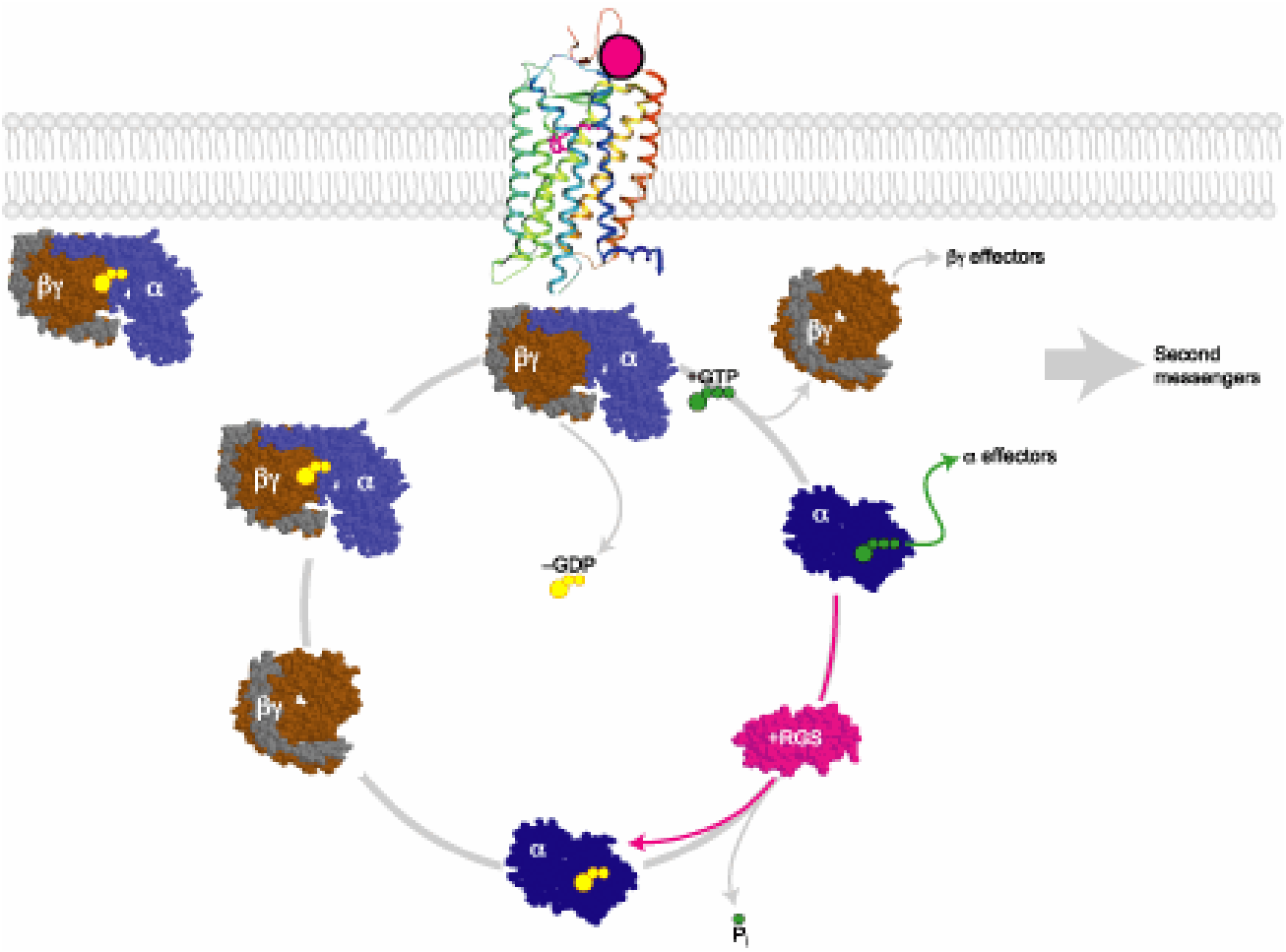
GPCRs signal by activating heterotimeric guanine nucleotide-binding proteins (G-proteins) that consist of α, β, and γ subunits

Transducin



N-termini of Gα and Gγ are lipid-modified

Binding to GPCR → a conformational change in Gα and dissociation of Gα from Gβγ



Overview of the G protein cycle. Gα, blue; Gβ, brown; Gγ, gray; GDP, yellow, with smaller circles representing the two phosphates; GTP, green, with smaller circles representing three phosphates; RGS (regulator of G protein signaling), magenta.

Major subclasses of Gα

- $G\alpha_s$ activate adenylyl cyclase to increase cAMP
  (β-adrenergic receptors)
- $G\alpha_{q/11}$ activate phospholipase C: consequent hydrolysis of PI lipids produce IP_3 and DAG leading to Ca^{2+} release and activation of PKC
  (Adrenergic-α2 receptors)
- $G\alpha_{i/o}$ inhibit adenylyl cyclase to decrease cAMP, $G\beta\gamma$ subunit opens K^+ channels
  (metabotropic epinephrine, serotonin, dopamine receptors, muscarinic acetylcholine receptor)
- Gt cGMP phosphodiesterase, decreased cGMP
  (Rhodopsin)
- $G\alpha_{12/13}$ RAS, Src, PKC, phospholipase D
  (lysophosphatidic acid, thromboxine A2 receptors)

Mammalian trimeric G proteins

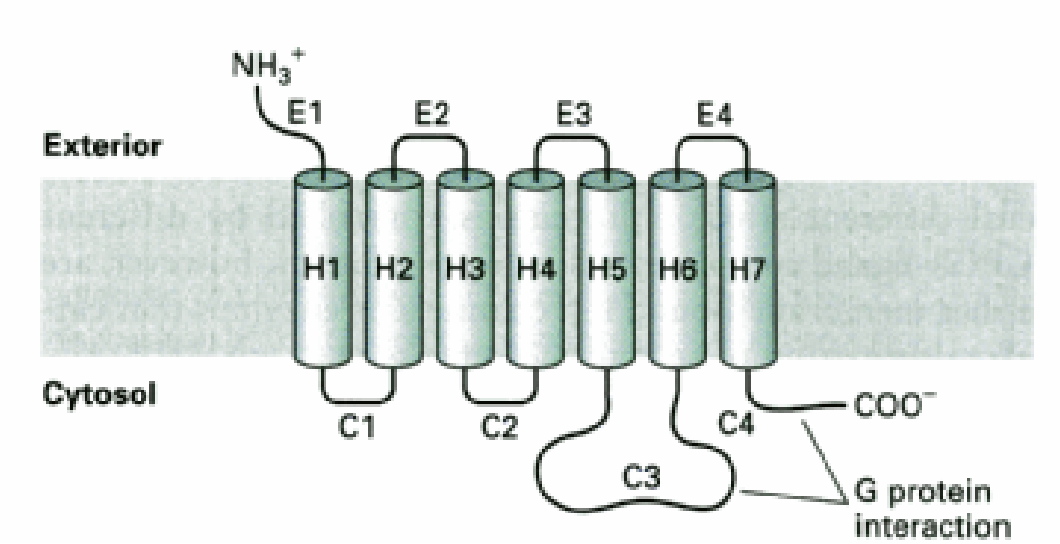
A given Gα subclass may associate with more than one receptor

Only 1 Gαs identified so far, but many Gαq and Gαi proteins have been found

Overall, ~ 20 Gα, 6 Gβ, and 11 Gγ proteins have been identified

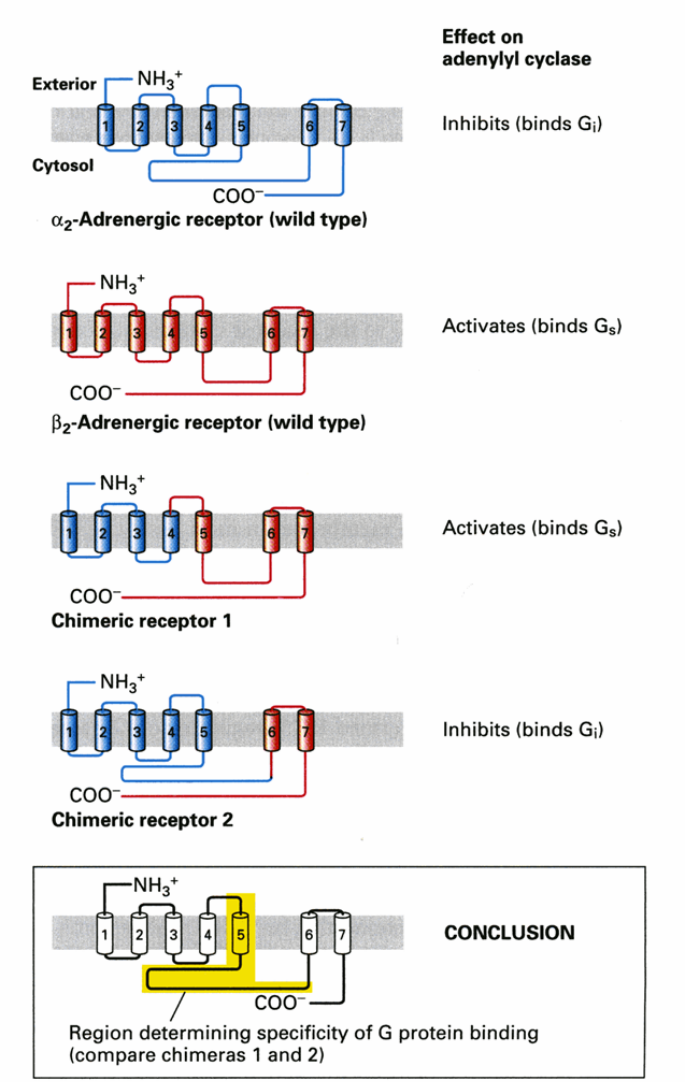
Signaling specificity lies in the recognition of a GPCR cytoplasmic surface by the Gα subunit

GPCR structure



Seven transmembrane α-helices
(GPCRs are sometimes referred to as 7TM receptors)

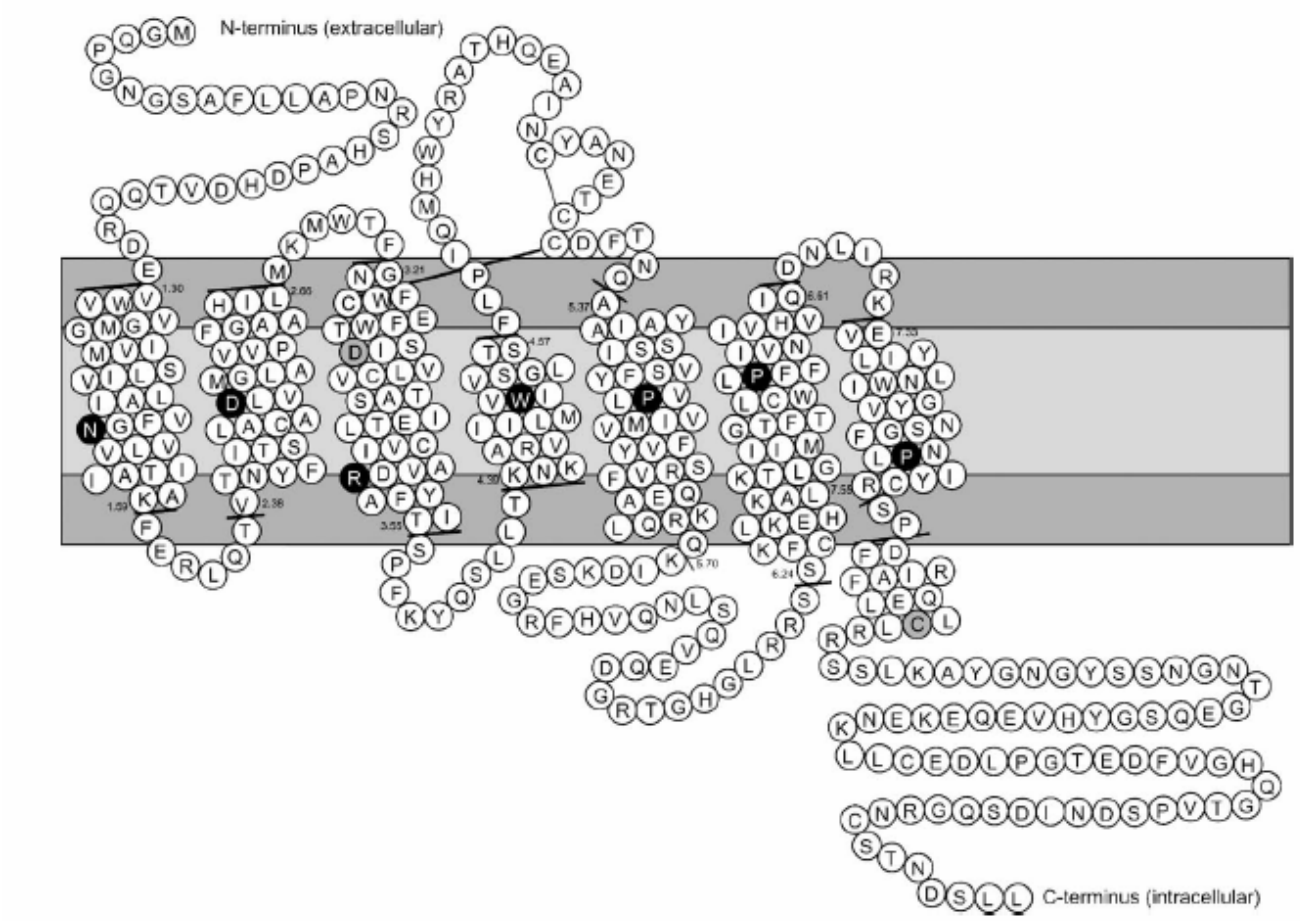
Cytoplasmic loop 3 (between TM helices 5 and 6) and the C-terminal tail involved in signal transduction



Chimeric GPCRs expressed in Xenopus oocytes
→ Critical role of TMH5 and CL3

Kobika et al., Science 240, 1310 (1998)

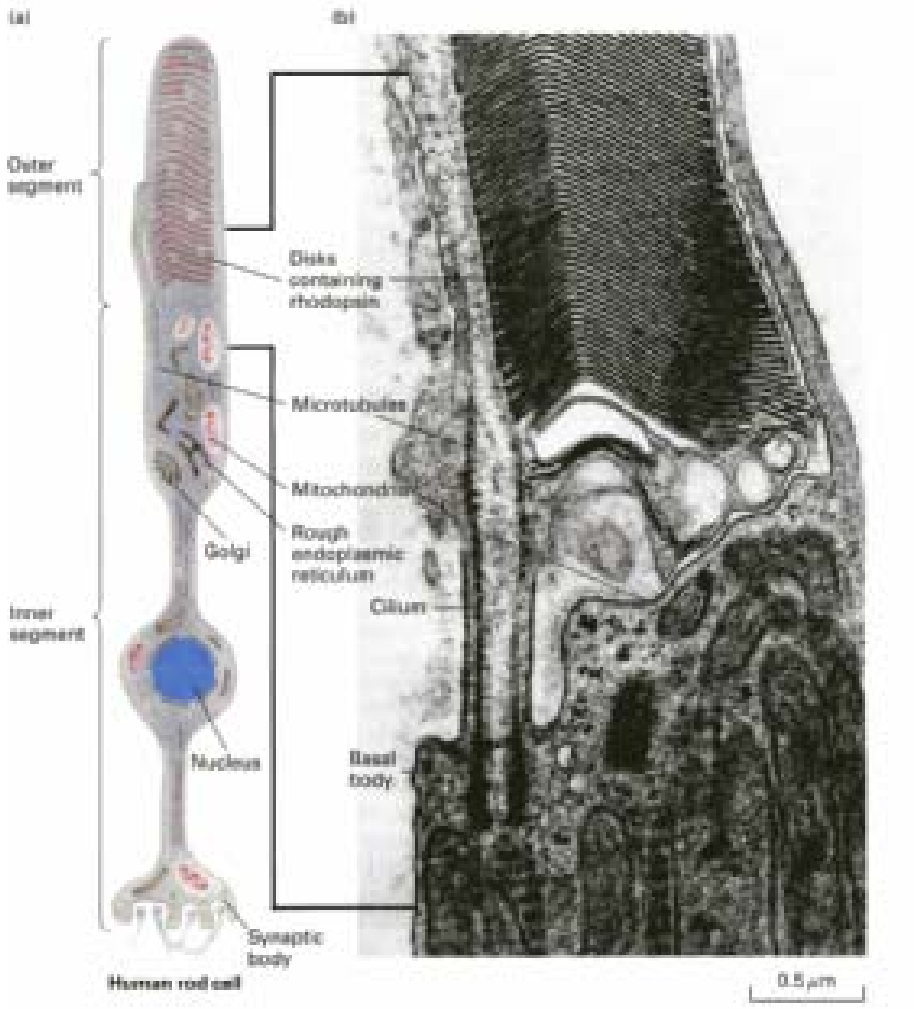
Membrane topology of the human h2 adrenergic receptor



Highly conserved residues found in each TM helix

Two highly conserved disulfide bonds

Palmitoylation site in C-terminal tail (Cys341)



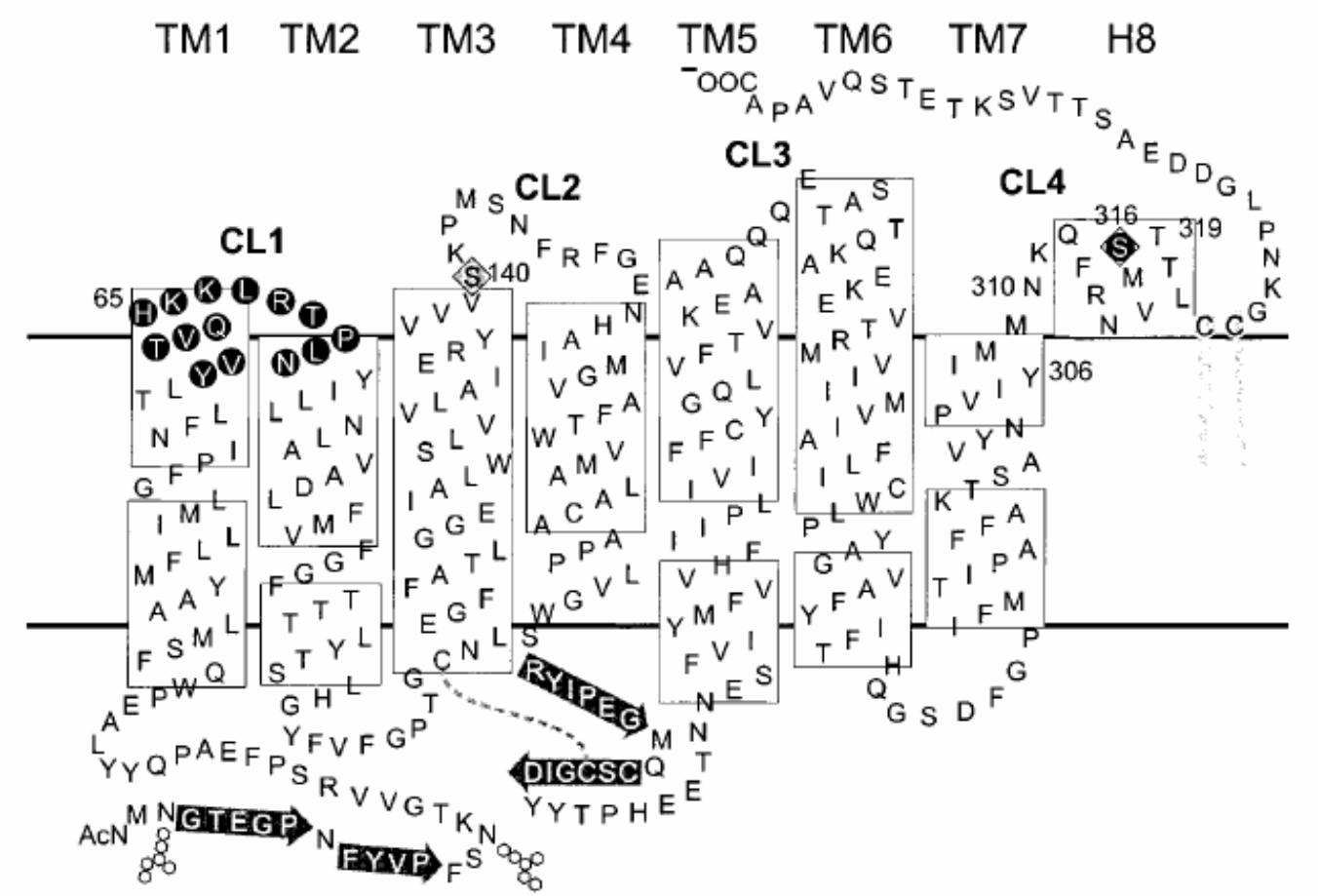
~ 48,000 rhodopsin molecules per μm^2

(70% of total cell protein)

Membrane topology of rhodopsin

cytoplasm

extracellular



Helices 1,2,5,6,7 contains bends
CL3 is α-helical based on EPR and more recent crystal data

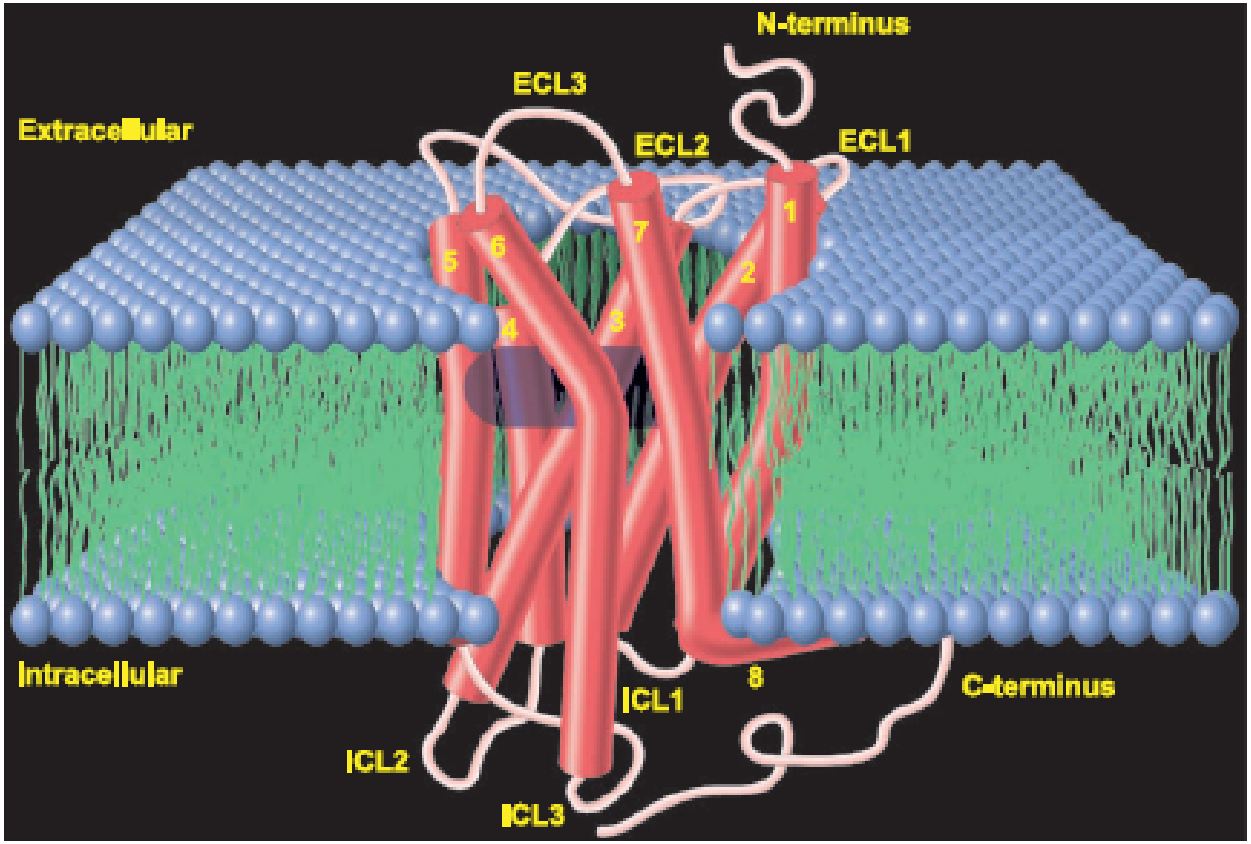
Altenbach et al., Biochemistry 40, 15493 (2001)

Bovine rhodopsin @ 2.2 Å (1U19)

Okada et al., J. Mol. Biol. 342, 571 (2004)

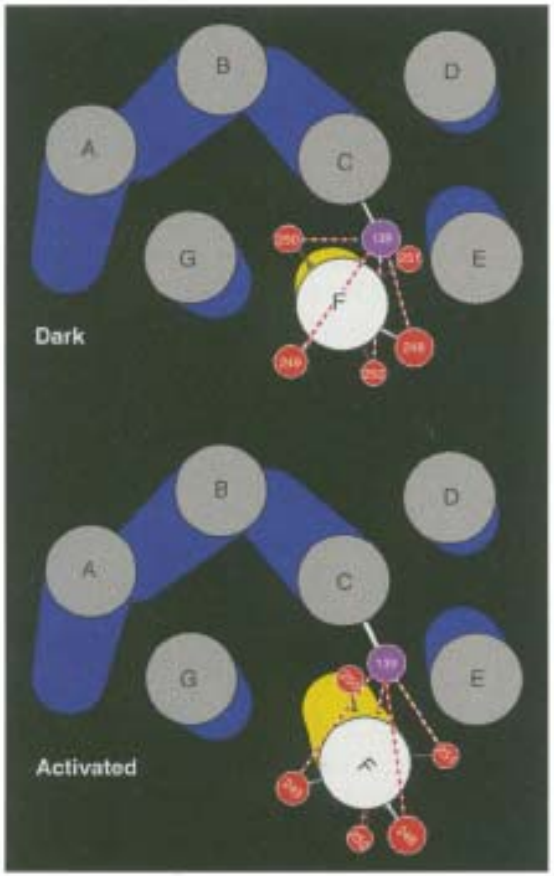
Okada et al., J. Mol. Biol. 342, 571 (2004)

Structural model of bovine rhodopsin



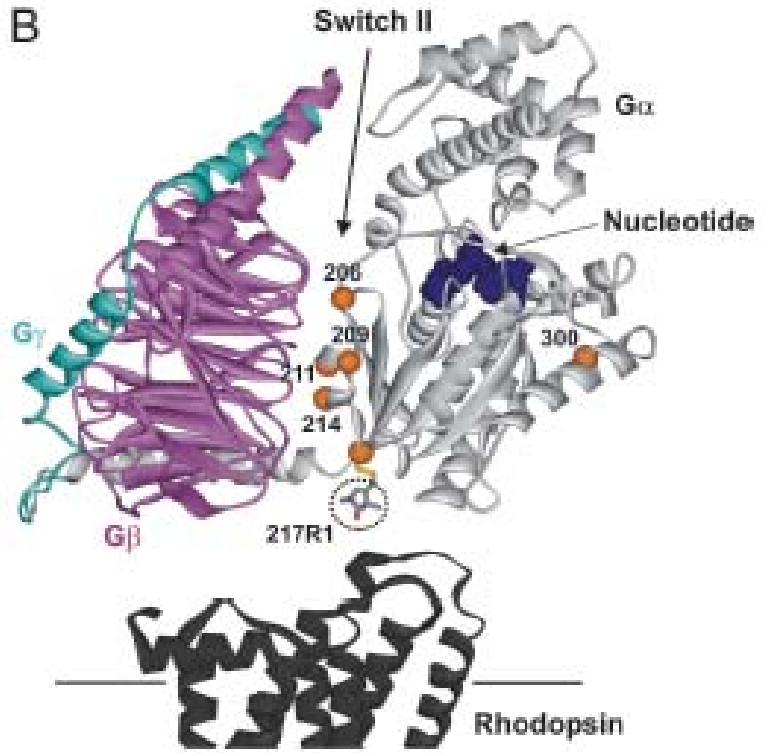
Schematic representation of a GPCR in the cell membrane based on the packing arrangement of TMHs observed in the crystal structure of rhodopsin (Okada et al., J. Mol. Biol. 342, 571 (2004) pdb code 1U19).

Proposed mechanism of rhodopsin activation



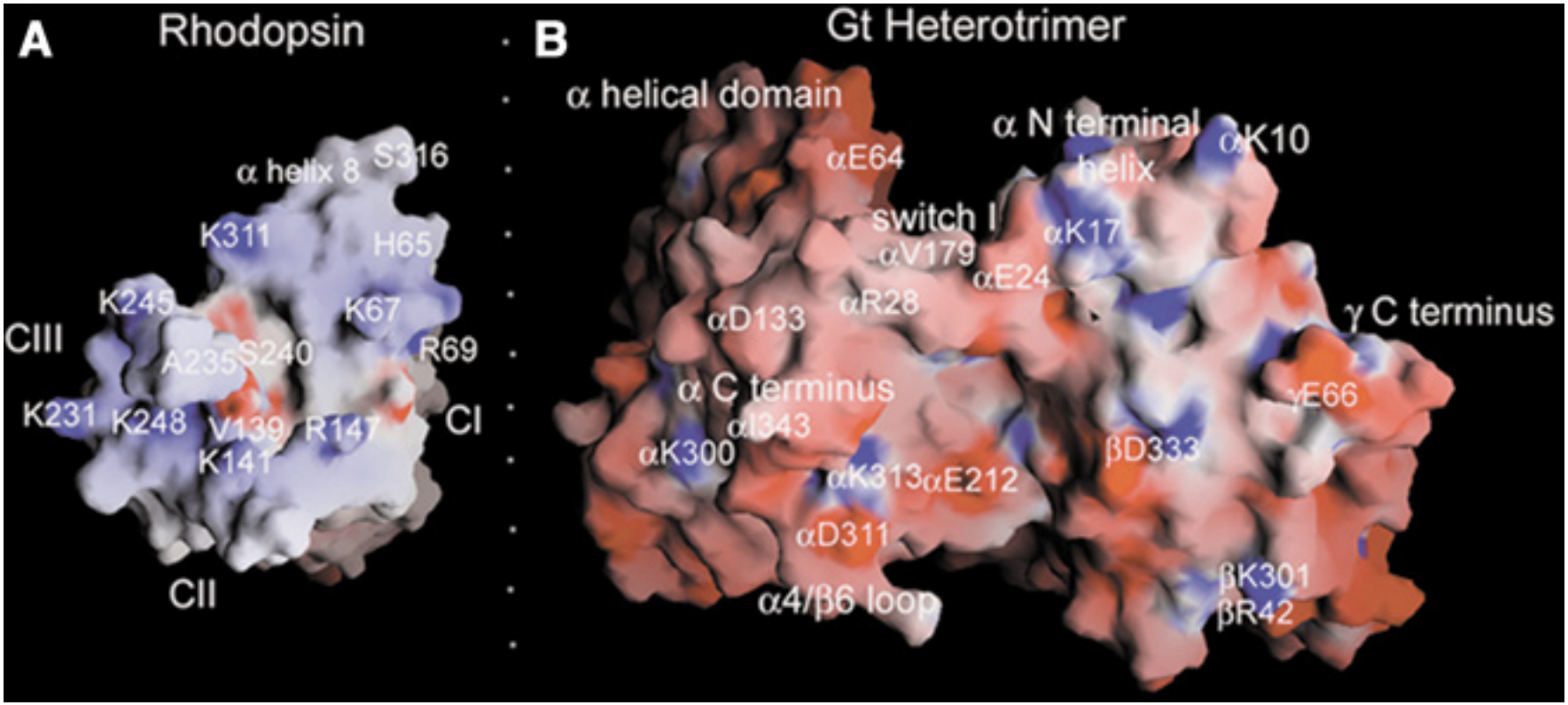
Tilting and rotation of TMH6. A change in the relative orientations of TM helices 3 and 6 produces a conformational change at the cytoplasmic surface

Farrens, Altenbach, Yang, Hubbell, and Khorana Science 274, 768 (1996)



Interaction of switch helix II in the *Ga* subunit of transducin with the cytoplasmic face of activated rhodopsin
→ *G*-protein dissociation and activation

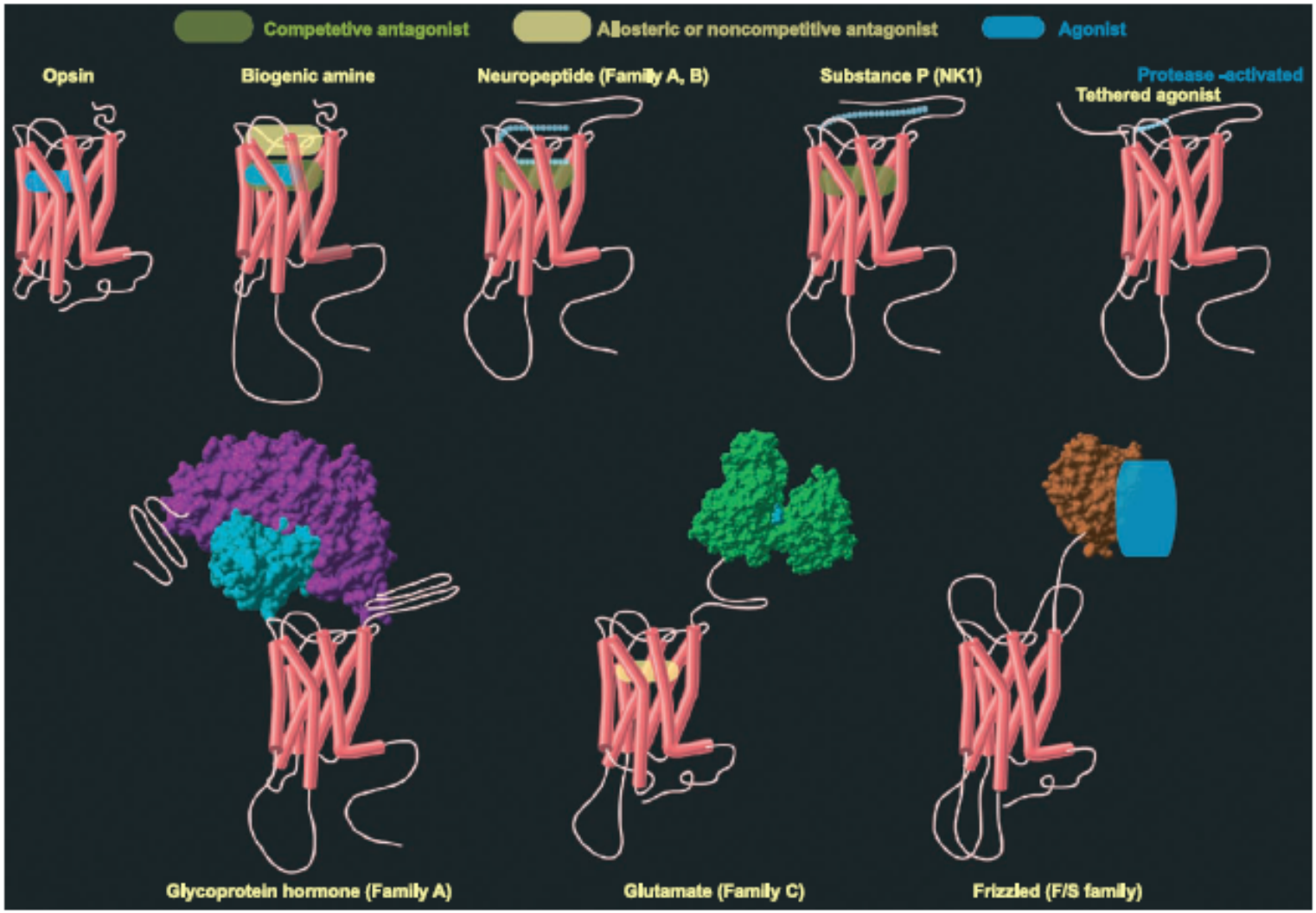
Van Eps et al., PNAS 44, 16194 (2006)



Interaction surfaces of rhodopsin and transducin

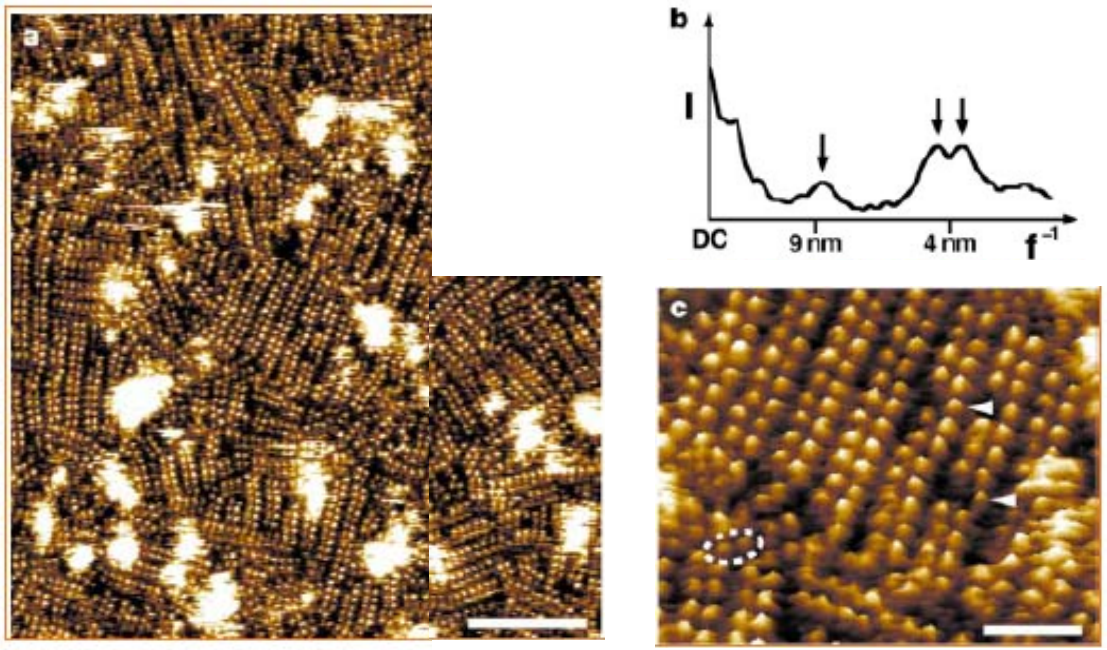
Preininger and Hamm, Science *STKE* (2004)

GPCR families based on sequence homology in TM domain



Agonist/antagonist binding sites identified through mutagenesis, some cross-linking studies

AFM of rhodopsin dimers in native disk membranes

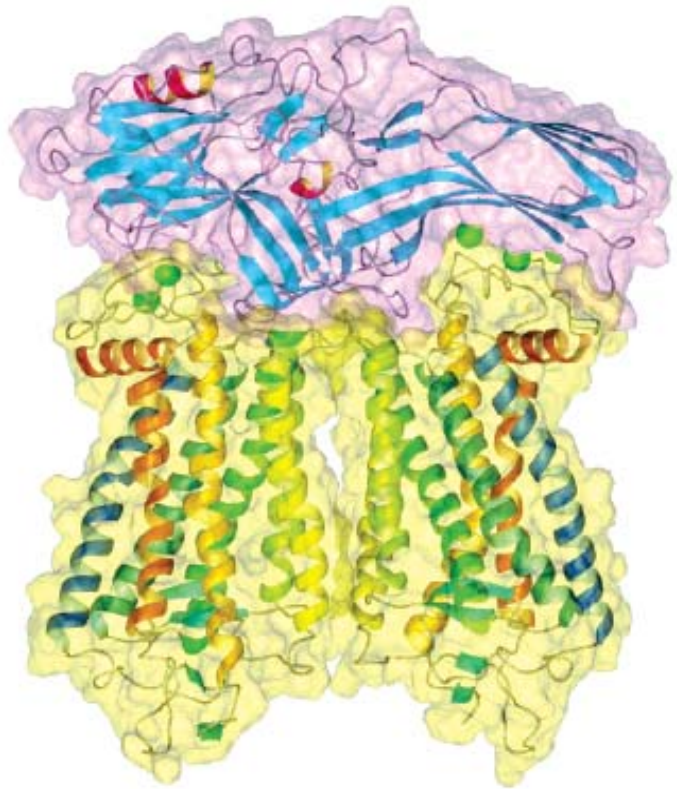


Organization and topography of the cytoplasmic surface of rhodopsin. **a,** Topograph obtained using atomic-force microscopy, showing the paracrystalline arrangement of rhodopsin dimers in the native disc membrane. **b,** Angularly averaged powder-diffraction pattern, showing peaks at (8.4 nm)11, (4.2 nm)11 and (3.8 nm)11. **c,** Magnification of a region of the topograph in **a**, showing rows of rhodopsin dimers, as well as individual dimers (inside dashed ellipse), and occasional rhodopsin monomers (arrowheads). Scale bars: **a**, 50 nm; inset, (5 nm)11; **c**, 15 nm.

Fotiadis et al., Nature 421, 127 (2003)

Evidence for GPCR dimers

- co-IP
- FRET and LRET
- Co-expression of active and inactive isoforms
- Binding cooperativity
- Cross-linking

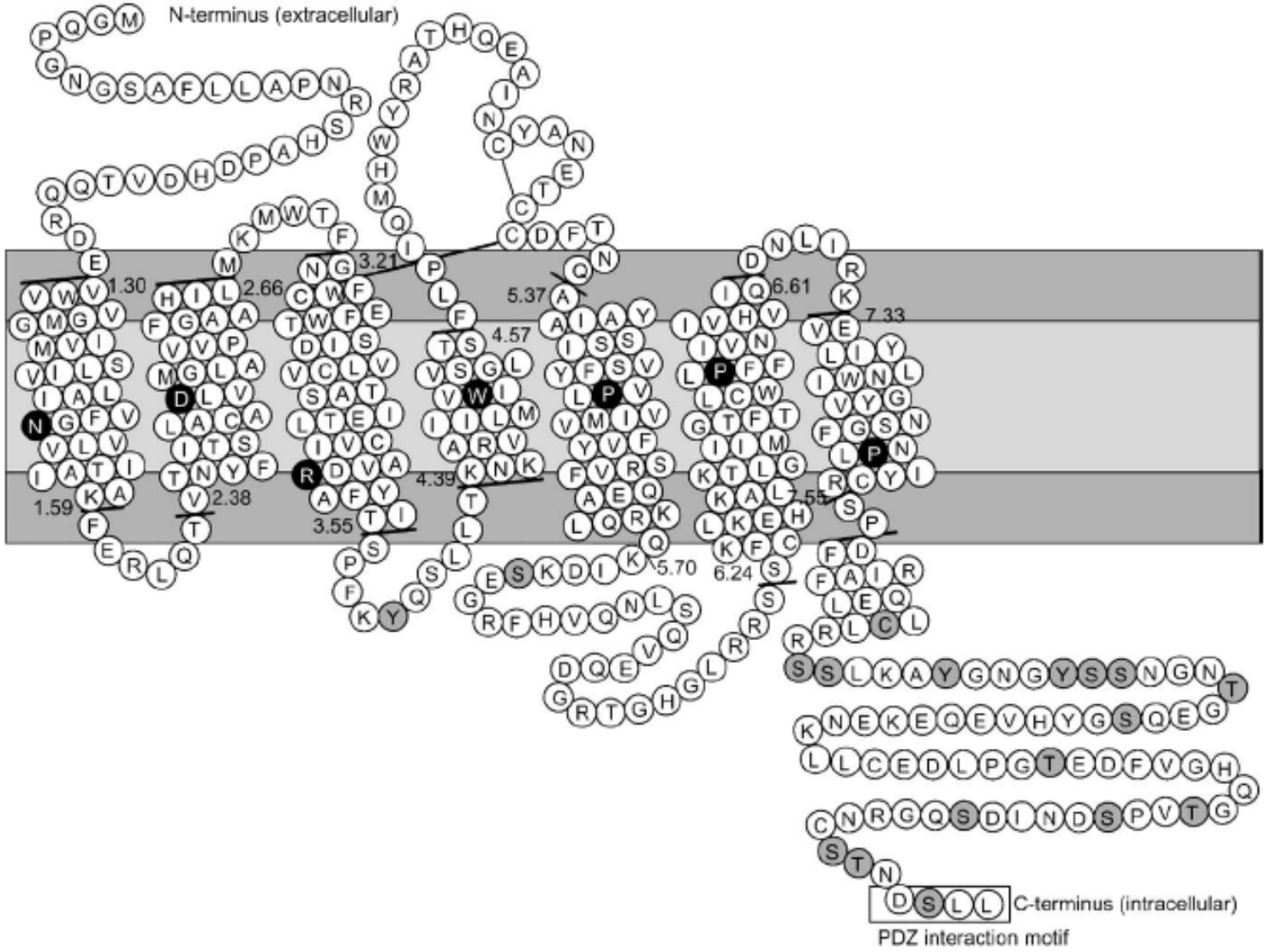


Model of rhodopsin dimer complexed with arrestin

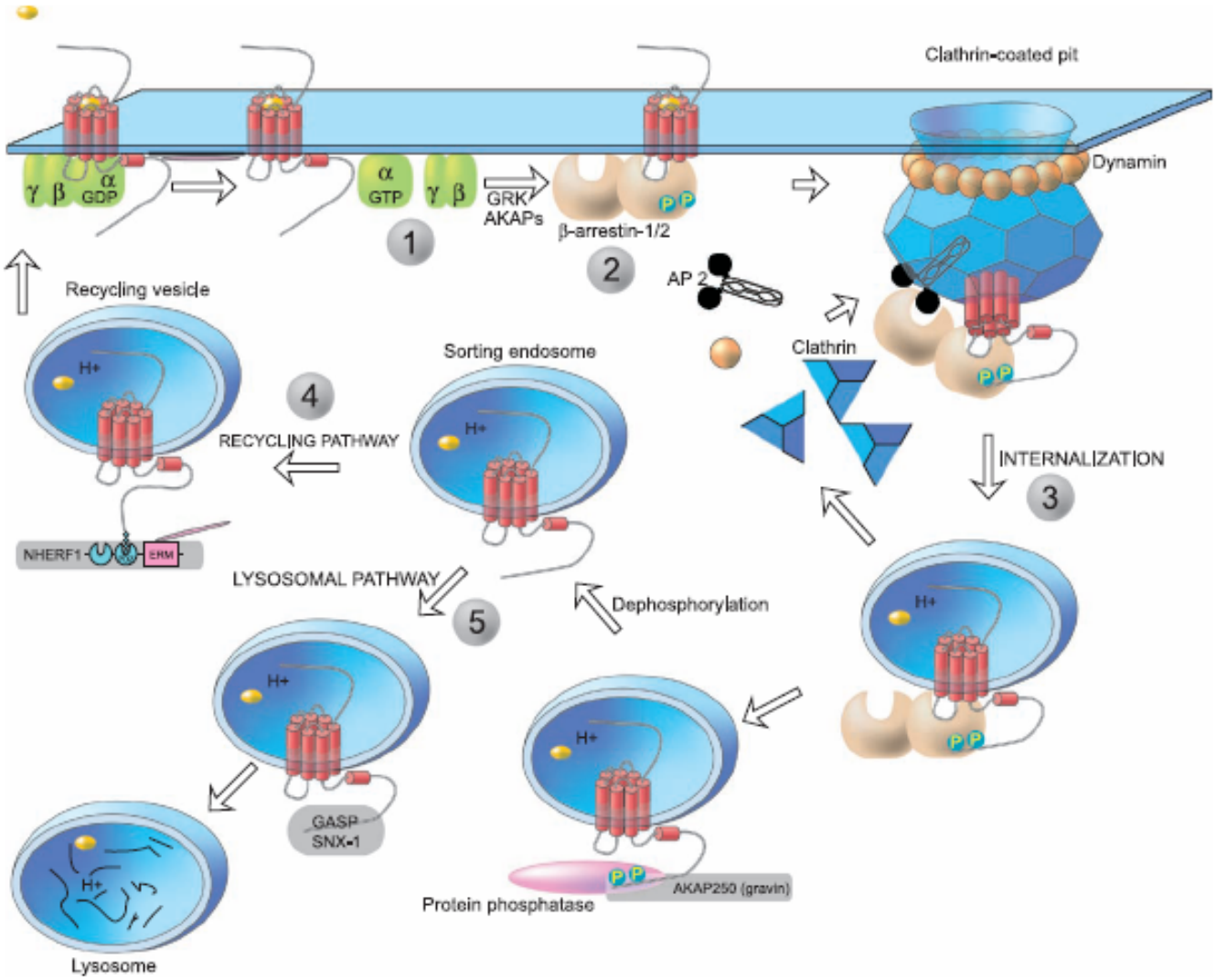
Park et al., Biochemistry 43, 15643 (2004)

Shutting down GPCR signaling

- Deactivation of receptors occurs through phosphorylation and subsequent binding of β-arrestin (this leads clathrin-mediated endocytosis of the arrestin-receptor complex)
- Deactivation of G-proteins occurs through intrinsic GTPase activity of the $G\alpha$ subunit and subsequent reassembly of the inactive hetero-trimeric complex
- Deactivation of second messengers occurs in part through arrestin-mediated recruitment of enzymes that degrade (or metabolize) the second messenger

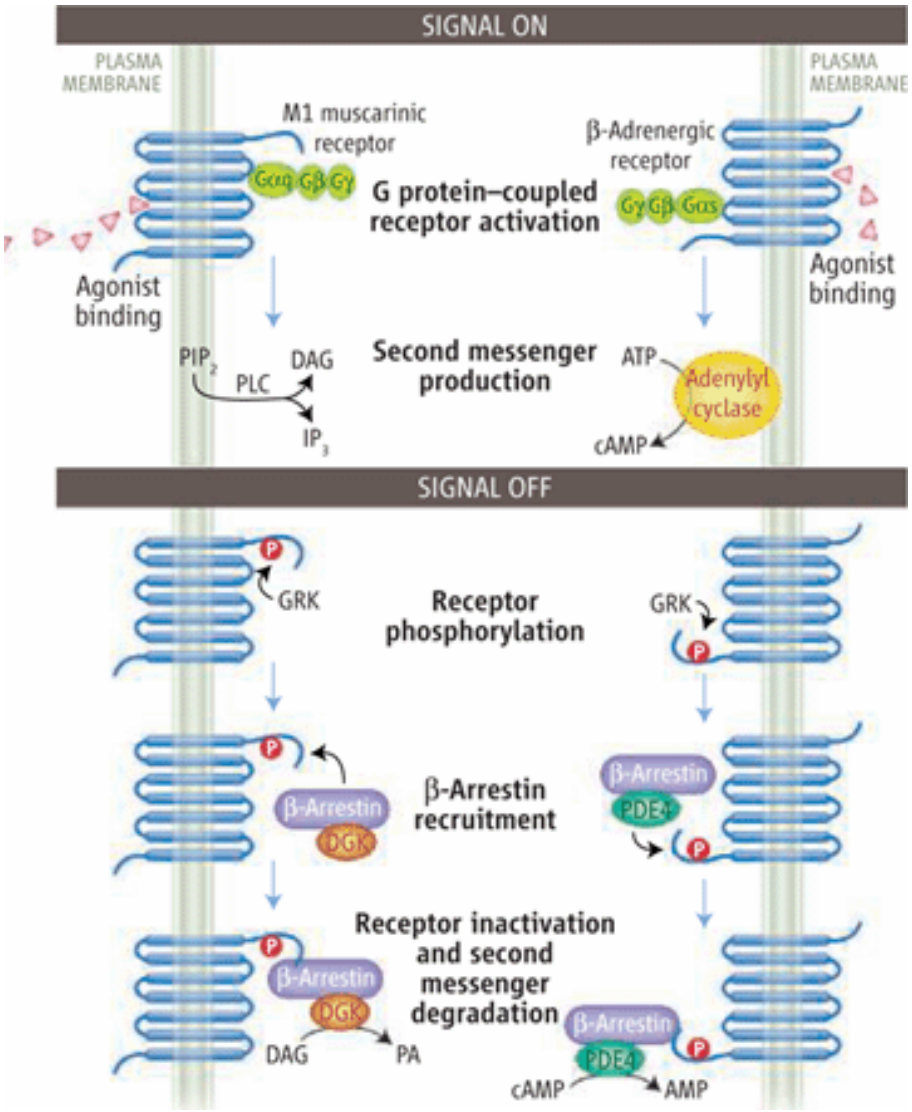


Residues in the human h2 adrenergic receptor that are important for receptor regulation. Possible sites for phosphorylation by protein kinases are indicated in gray circles. A C-terminal PDZ interaction motif is indicated that mediates interactions with NHERF1, NHERF2, and NSF scaffolding proteins. The highly conserved reference residue within each TMH is indicated with black circles and white text.



Typically activation of a GPCR leads to (1) activation and inhibition of specific signaling pathways in the cell, (2) short-term desensitization mediated by phosphorylation of GPCRs by GRKs followed by β-arrestin binding to the GPCR, (3) endocytosis of the receptor, followed by postendocytic sorting of the receptor either (4) back to the plasma membrane or (5) to lysosomes for degradation.

Arrestins also stimulate degradation of second-messenger molecules



Following muscarinic receptor activation:

- activation of the G-protein leads to production of DAG and IP_3

- arrestin was essential for conversion of DAG to PA

- required recruitment of the arrestin-DAG kinase complex to the activated GPCR

Nelson et al., Science 315 663 (2007)

Previously shown that that arrestins recruit PDE4 to activated β-adrenergic receptors, stimulating degradation of cAMP

Perry et al., Science 298 834 (2002)

GPCRs

- Gateway to a wide variety of G-protein coupled second messenger systems
- Contain 7 TM helices
- Extracellular N-terminal domain, cytoplasmic C-terminal tail
- May function as dimers
- Signaling mediated through rigid-body rearrangements of TM helices (esp. TMH6)