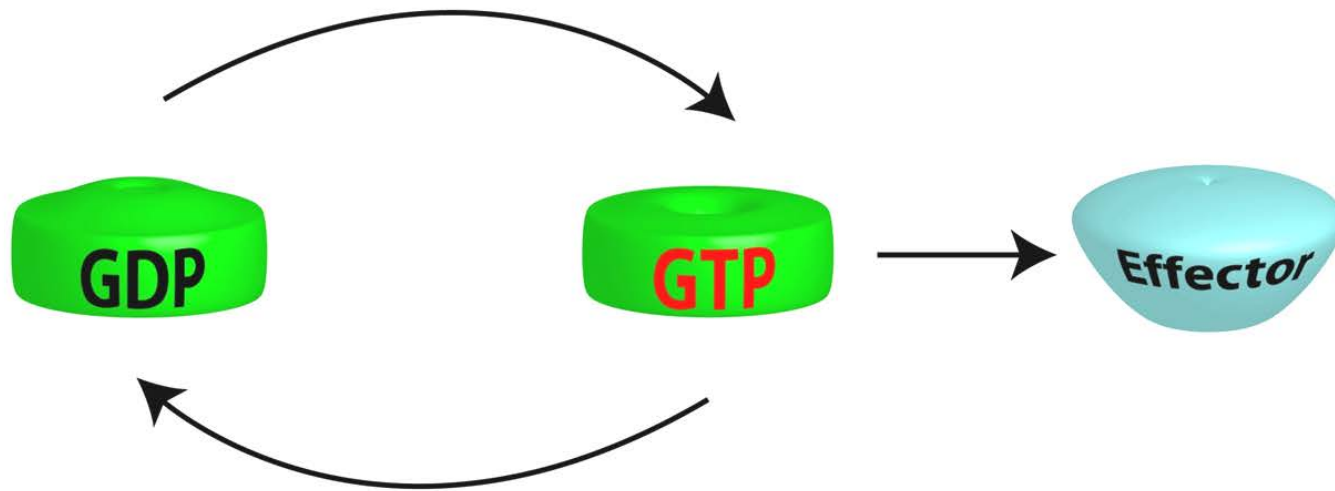


# **Heterotrimeric G proteins and the role of lipids in signaling**

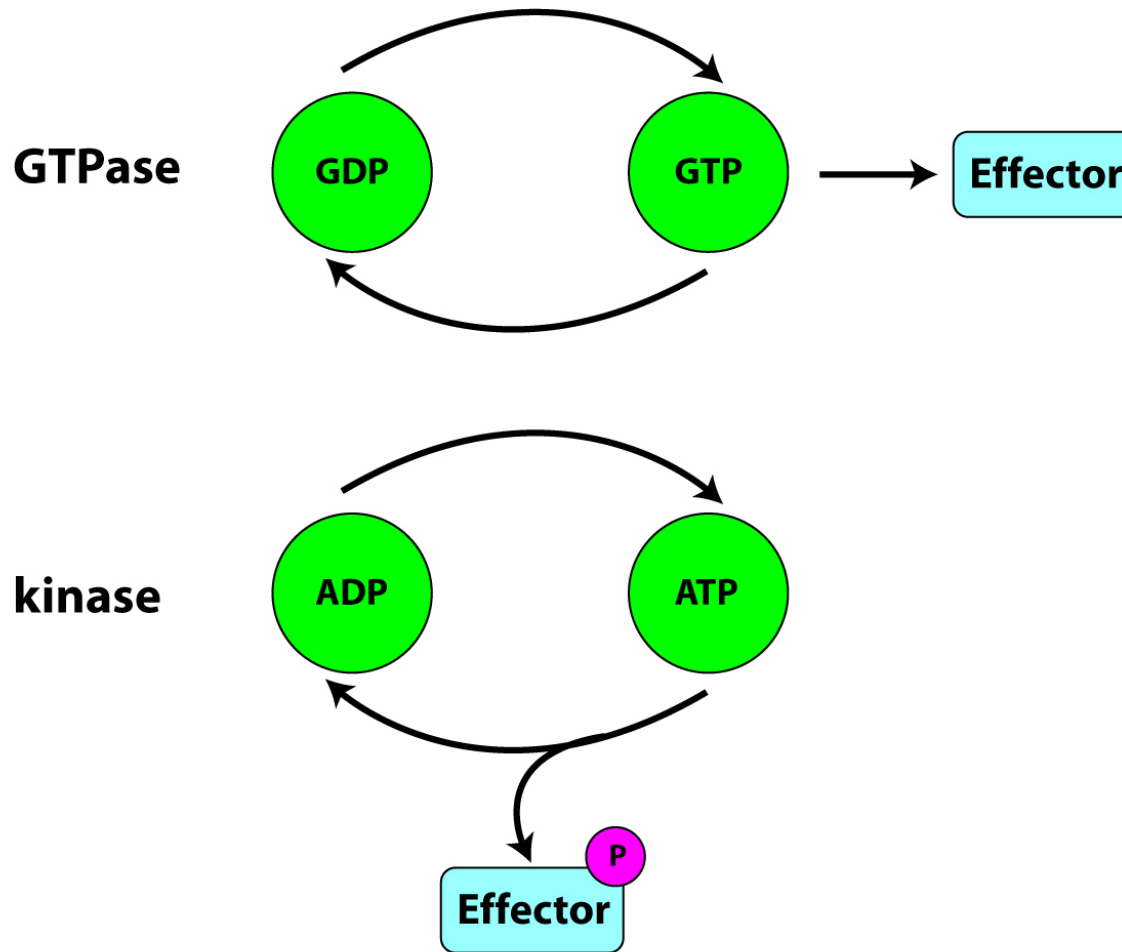
**John Sondek, Ph.D.**

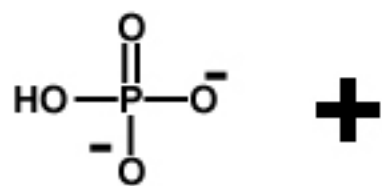
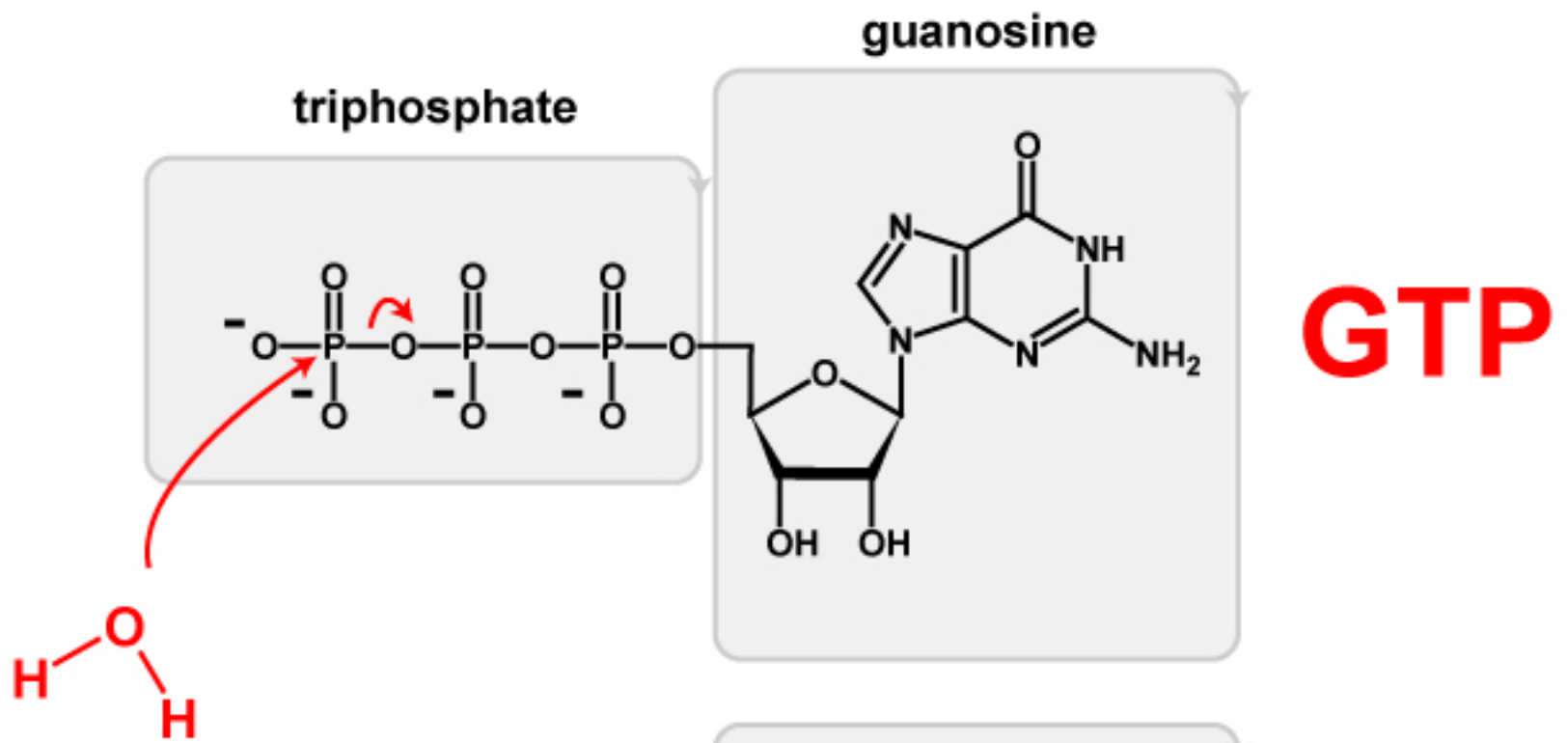
**Depts. of Pharmacology and  
Biochemistry & Biophyscis**

# The GTPase cycle – molecular switch

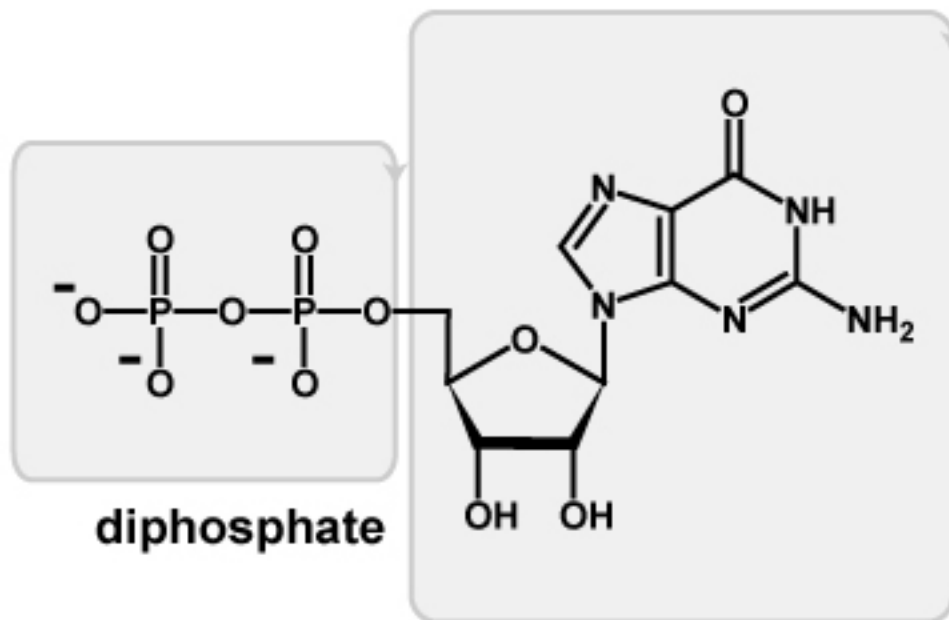


# A GTPase is NOT a kinase

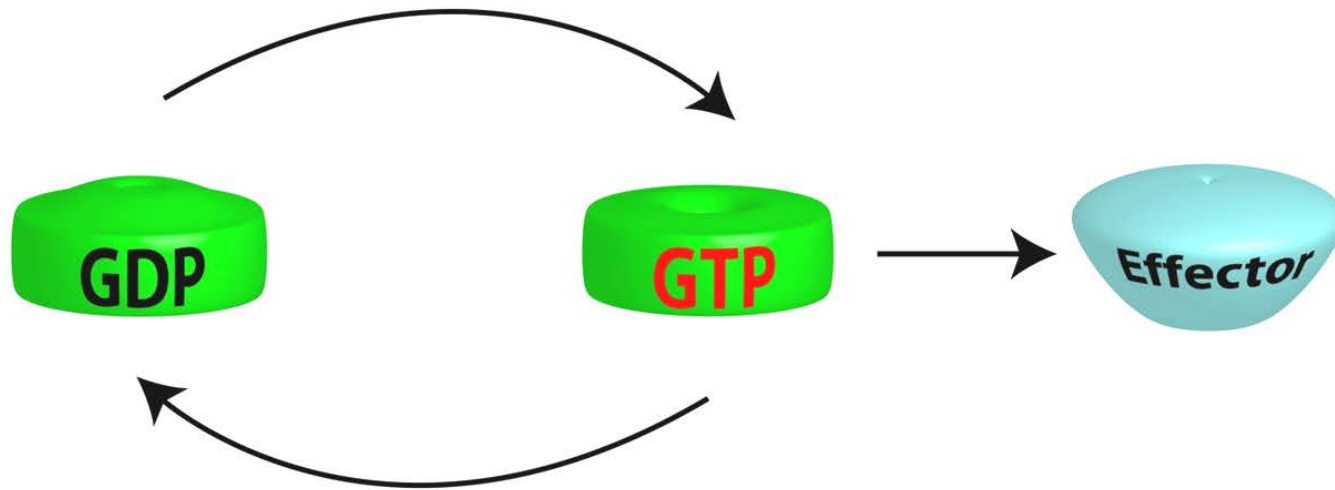




**inorganic  
phosphate**

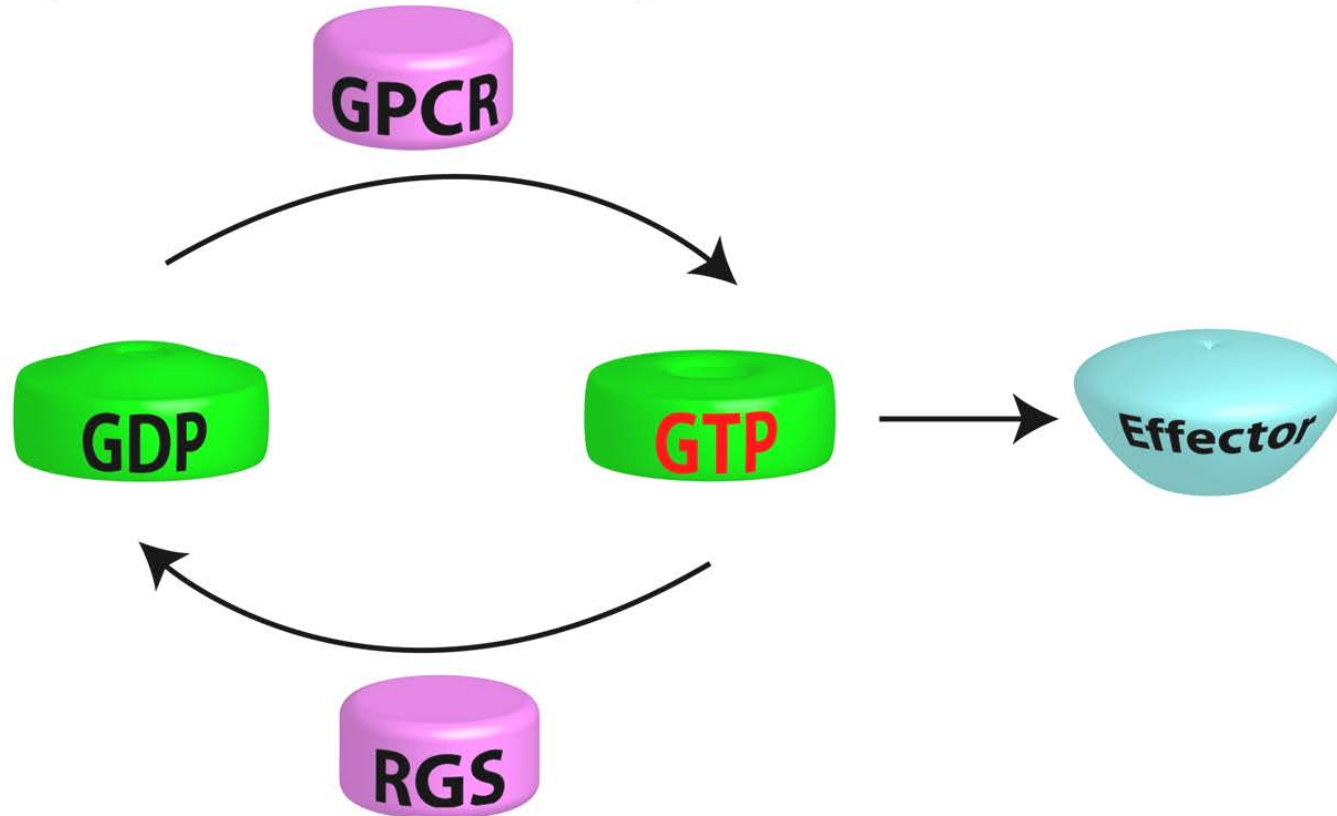


# Two major regulators of GTPase cycle



# Specific GEFs and GAPs for heterotrimeric G proteins

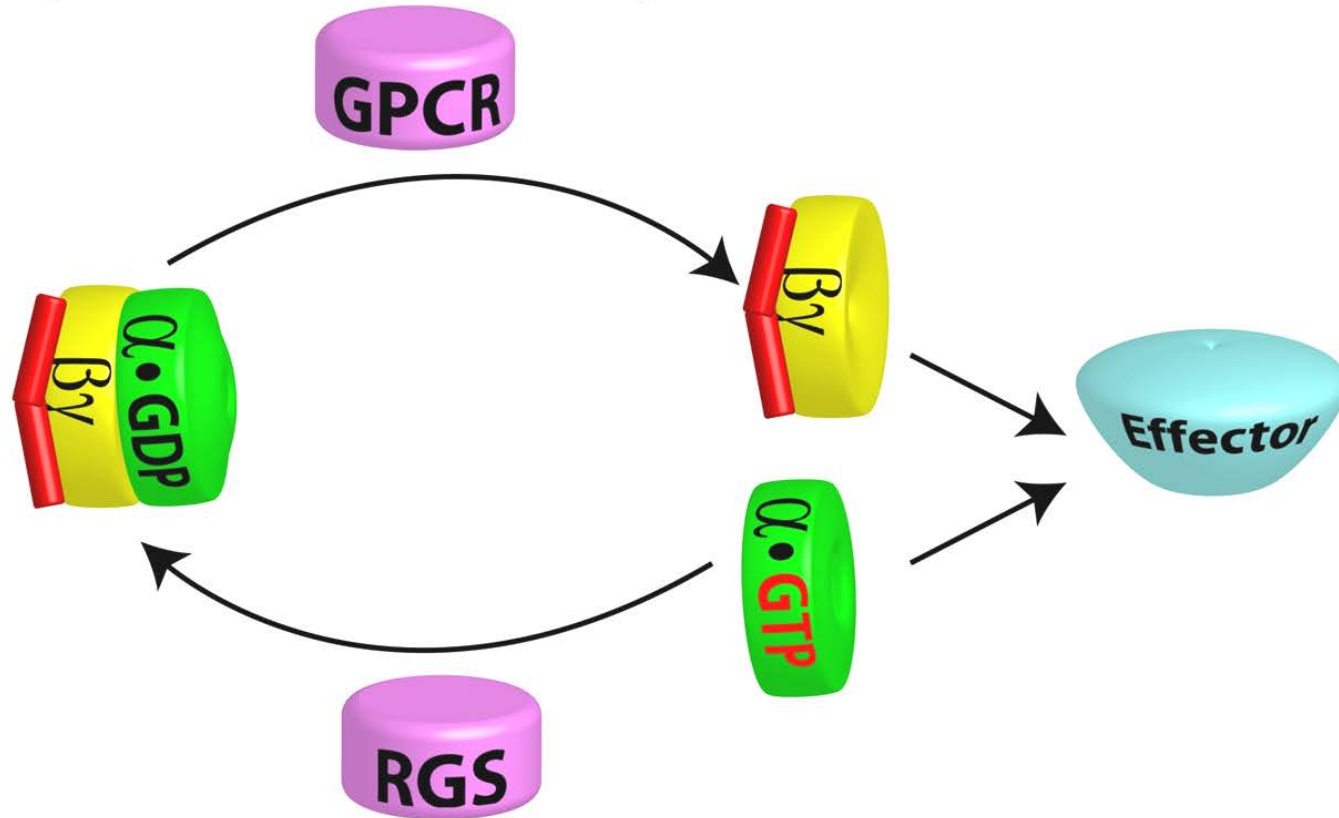
*G* protein coupled receptor



Regulator of *G* protein signaling

# Active heterotrimer dissociates into a $G\alpha$ subunit and a $G\beta\gamma$ dimer

**G** protein coupled *receptor*



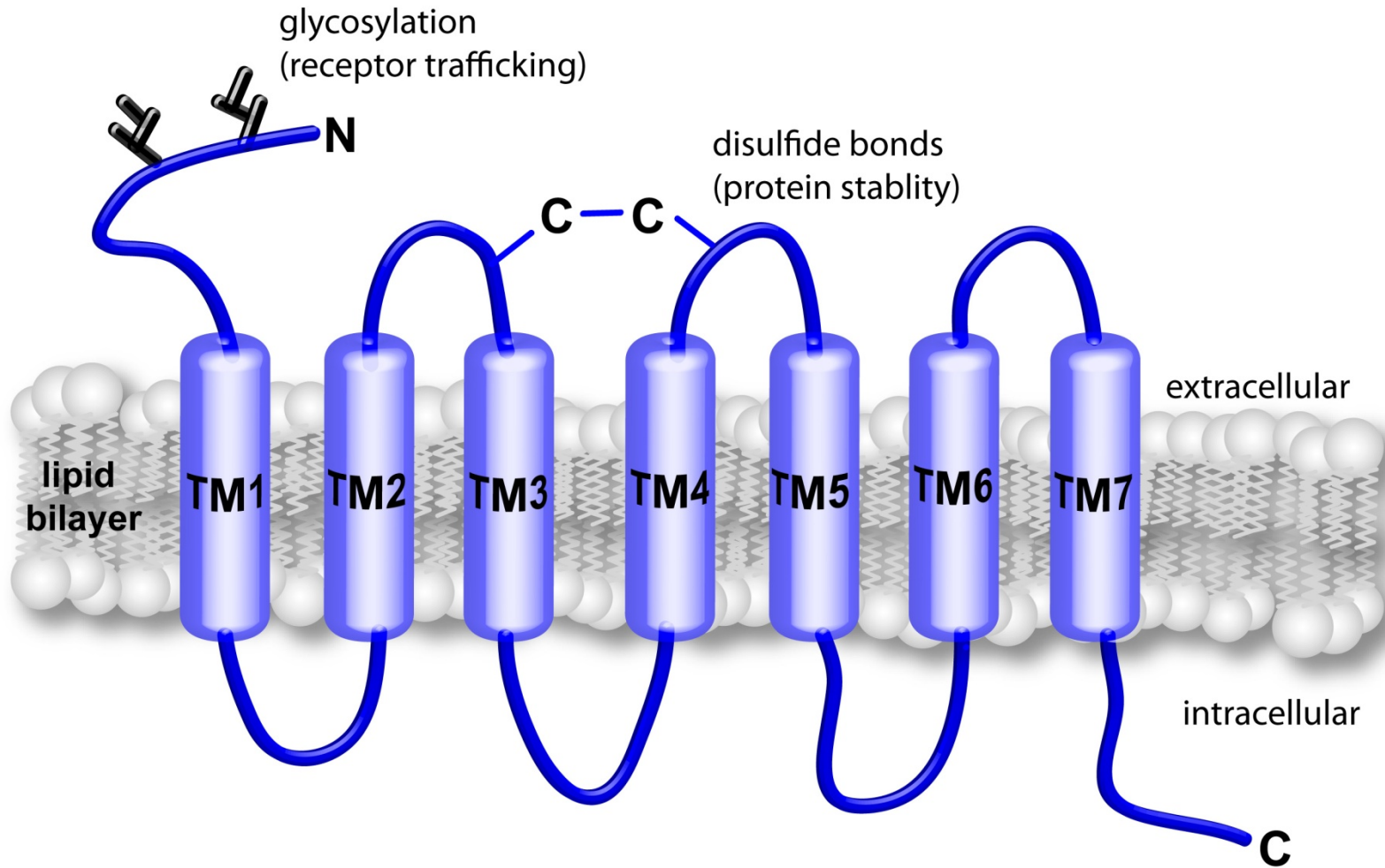
Regulator of **G** protein signaling

## **G protein-coupled receptors as GEFs for heterotrimeric G proteins**

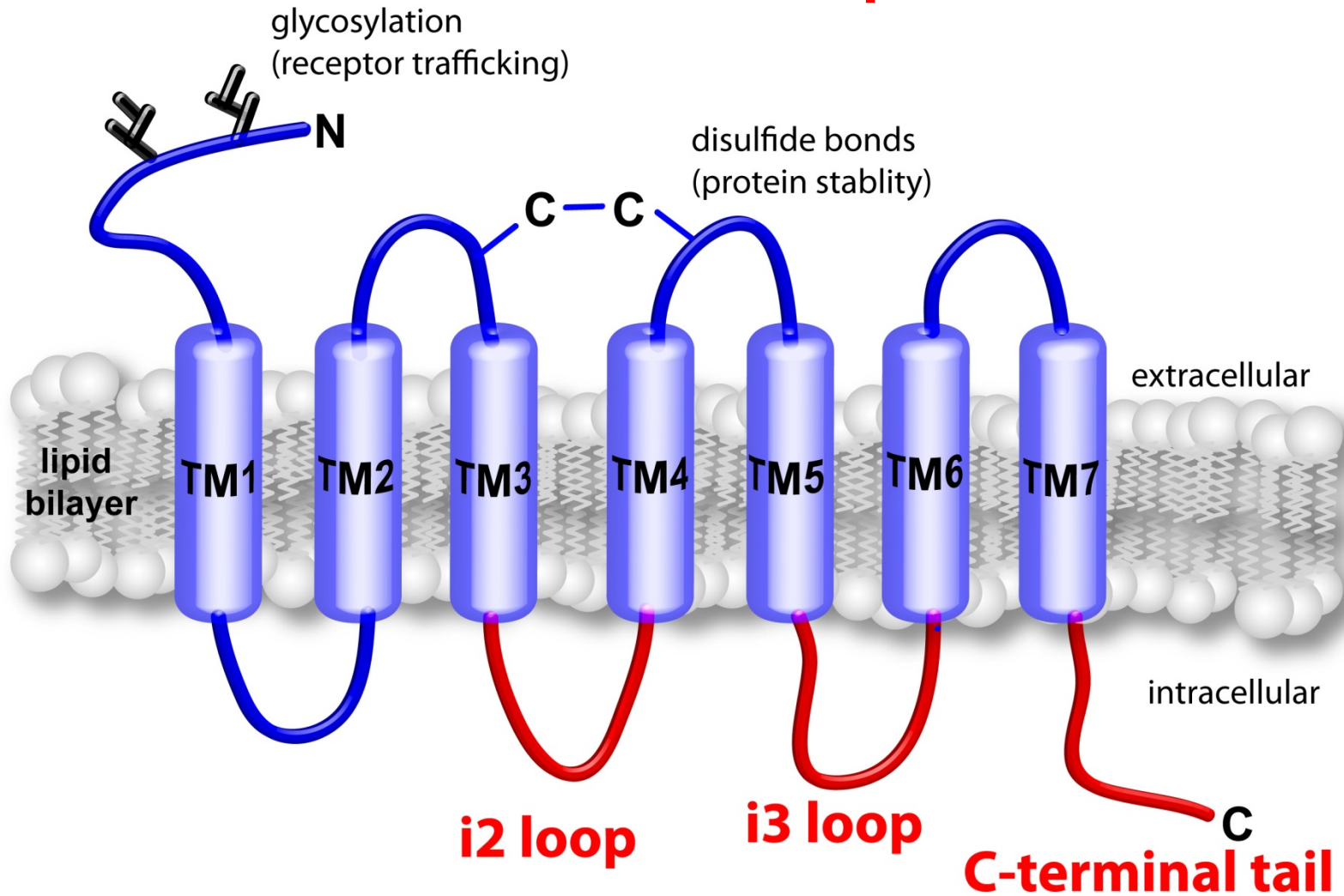
- **GPCRs are the largest family of cell-surface receptors for extracellular signals (superfamily of >1000 members)**
- **GPCRs respond to a wide range of inputs: hormones, neurotransmitters, odorants, tastants, photons of light, etc.**
- **~60% of all clinical therapeutics act by affecting some aspect of GPCR signaling (e.g., agonist, antagonist, inhibition of natural ligand metabolism)**



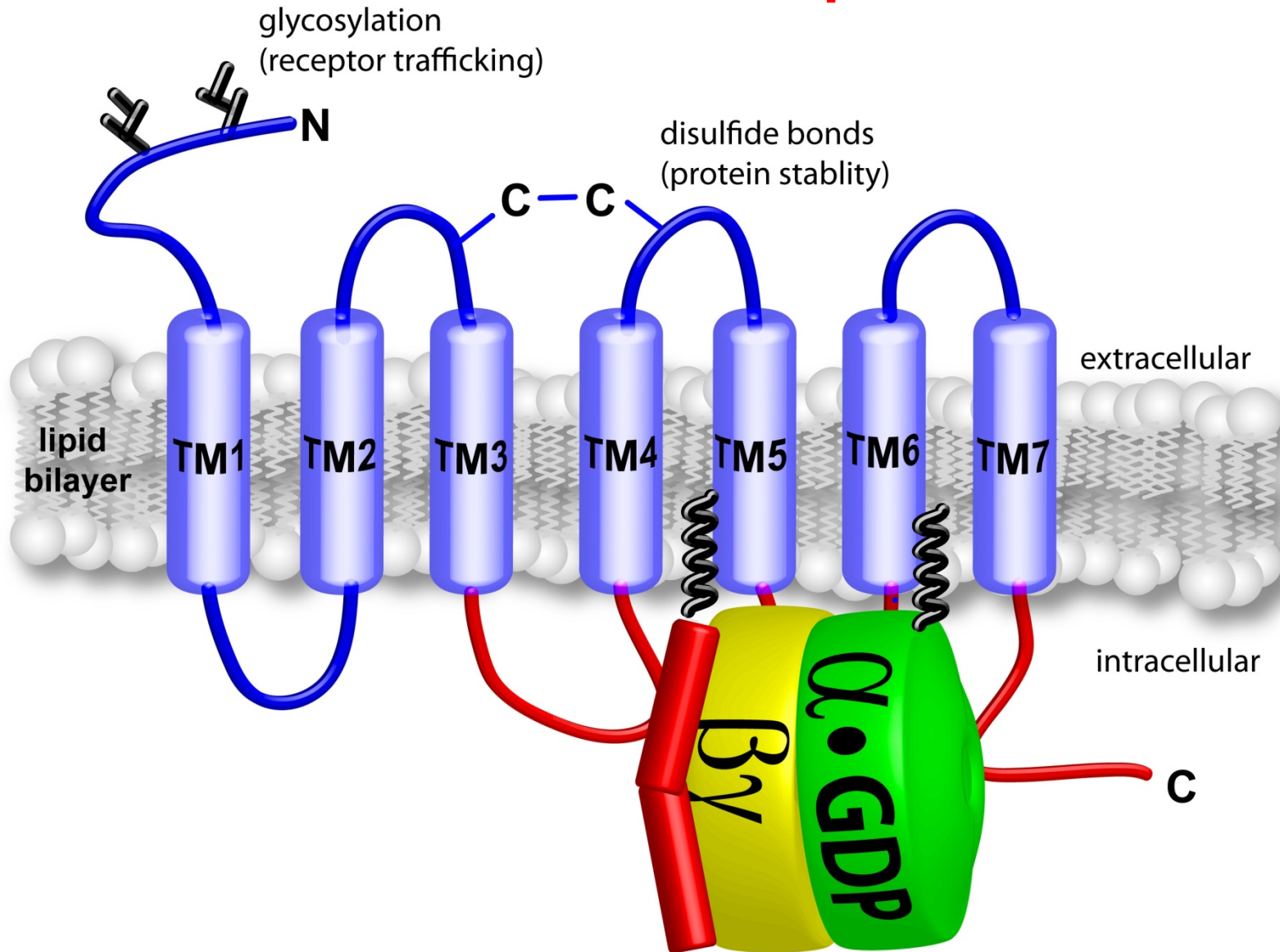
# GPCRs are seven transmembrane (7TM) receptors



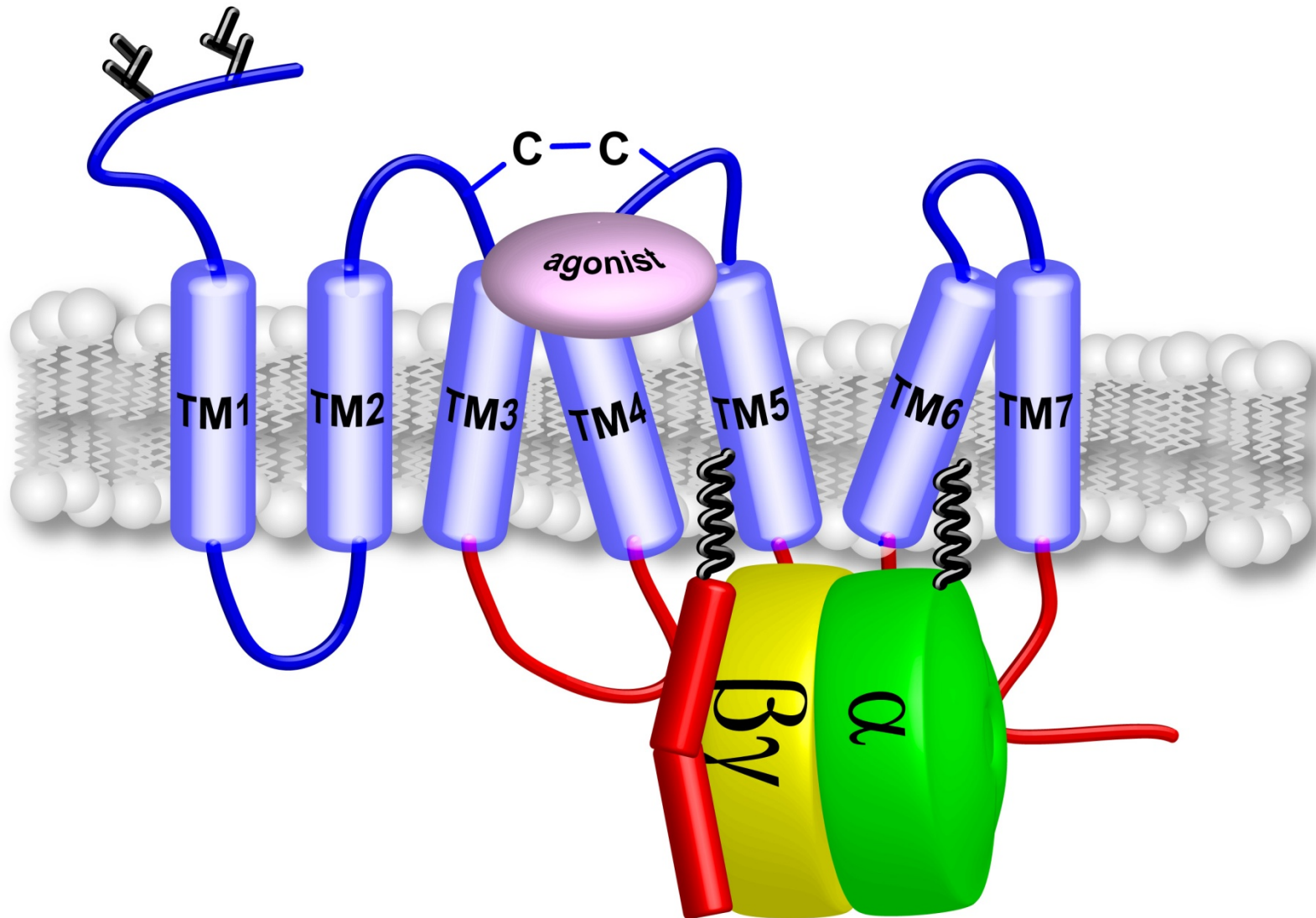
# G proteins sense conformational changes of intracellular loops



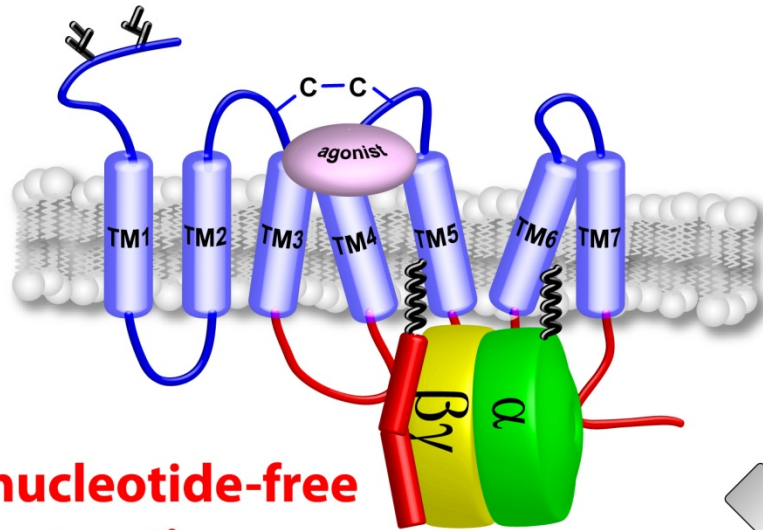
# G proteins sense conformational changes of intracellular loops



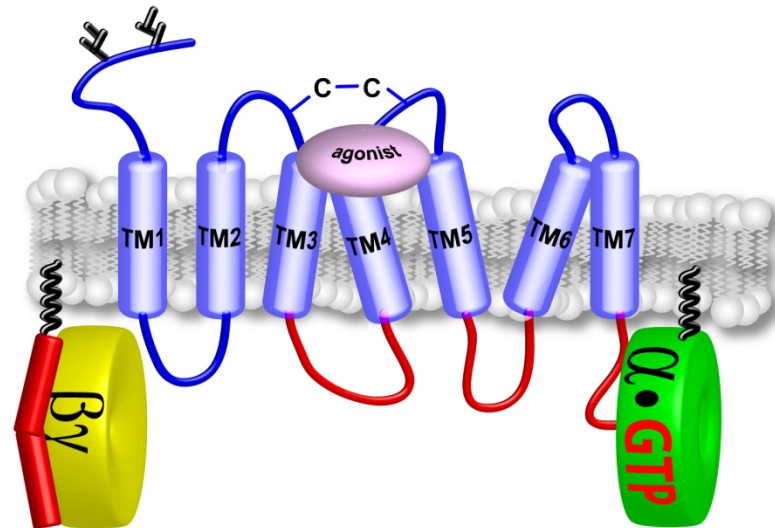
# Active receptor stabilized nucleotide-depleted heterotrimer



# GTP-loading catalyzes heterotrimer dissociation



**nucleotide-free  
heterotrimer**



**active trimer  
( $G\alpha + G\beta\gamma$ )**

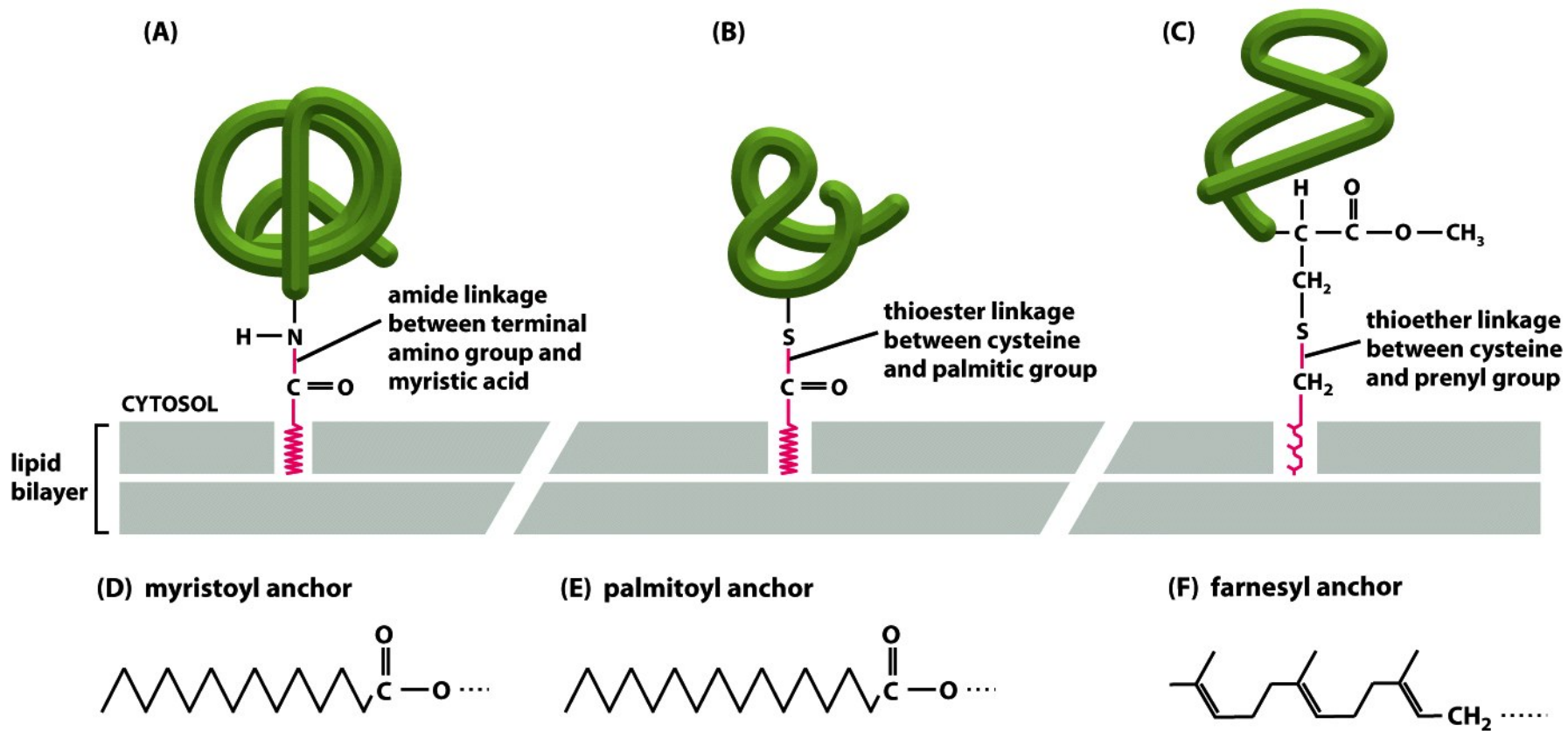
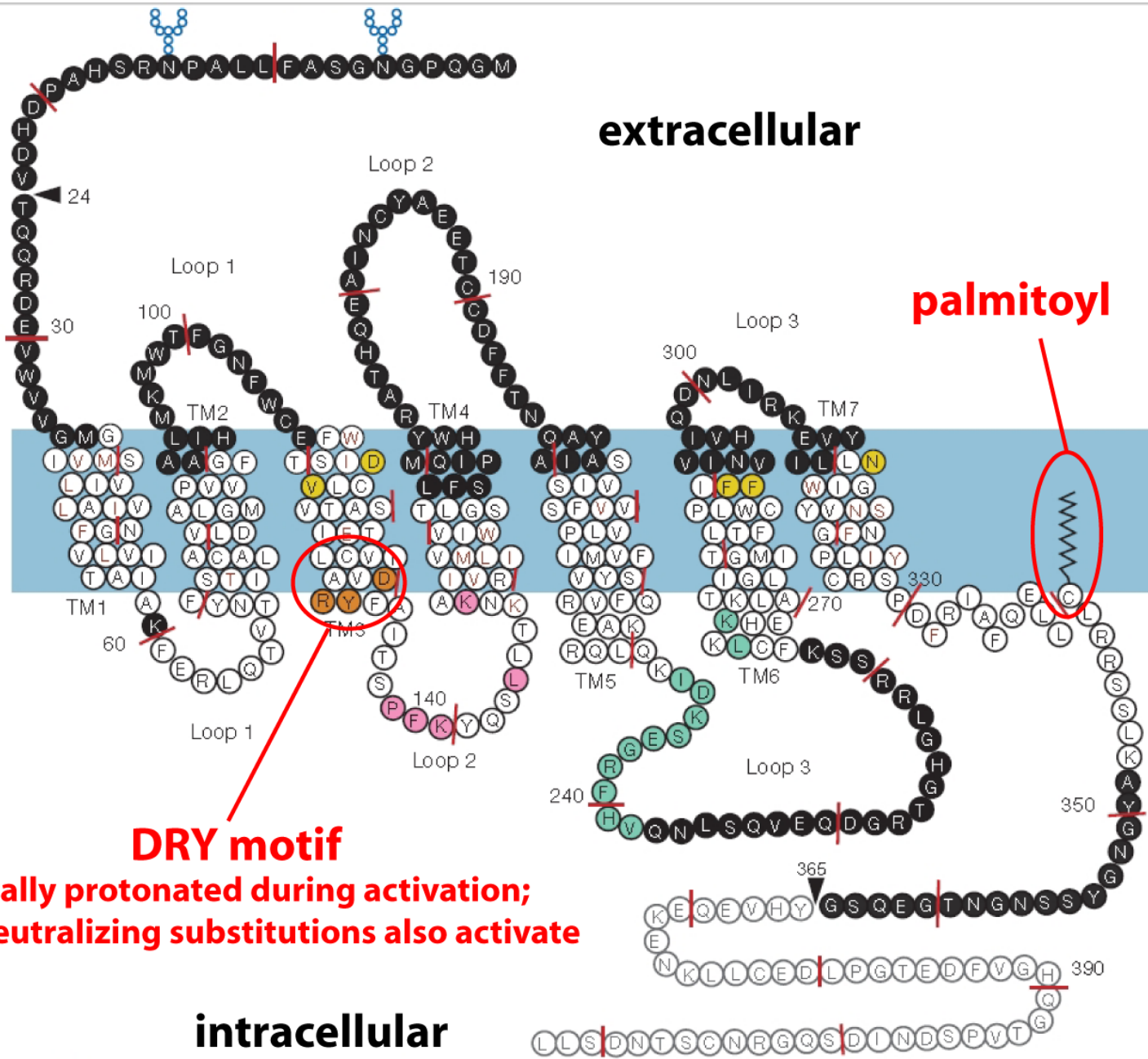
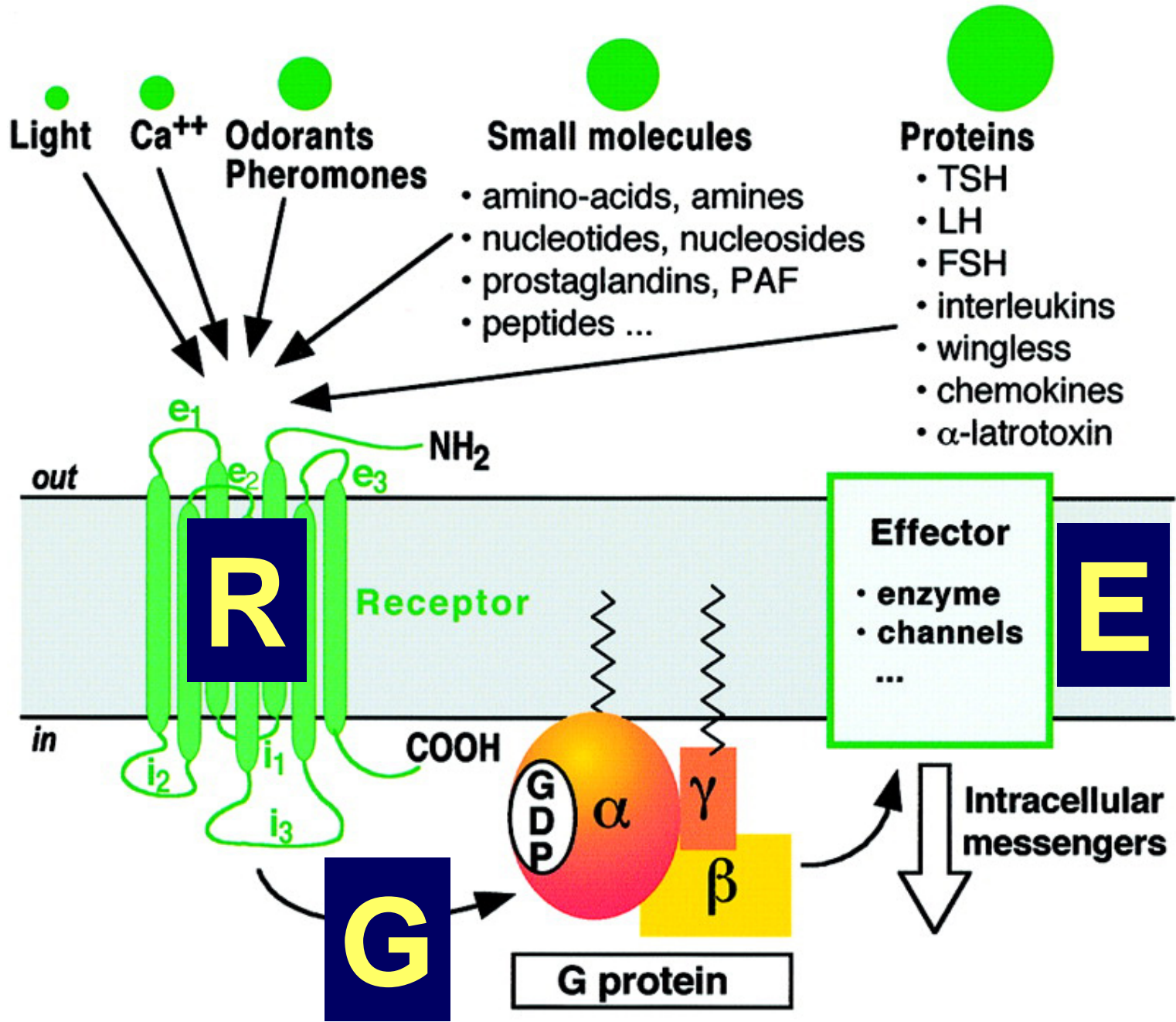


Figure 10-20 *Molecular Biology of the Cell* (© Garland Science 2008)

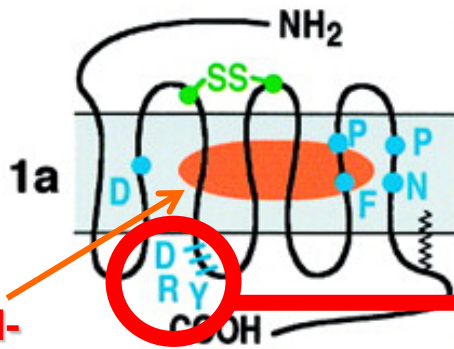






# Family 1

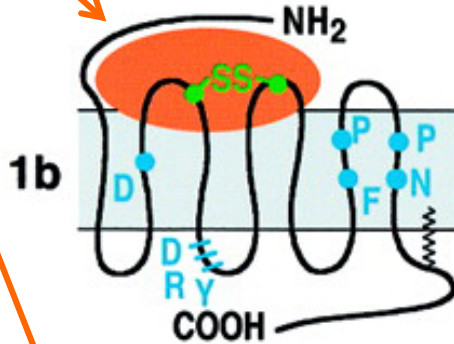
## Rhodopsin-like:



- Retinal
- Odorants
- Catecholamines - dopamine, adrenaline, serotonin (5HT)
- Adenosine
- ATP, Opiates
- Enkephalins
- Anandamide

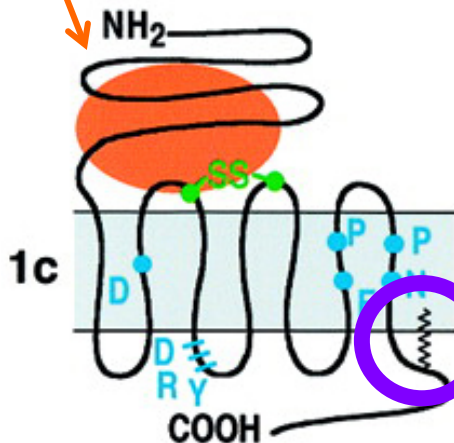
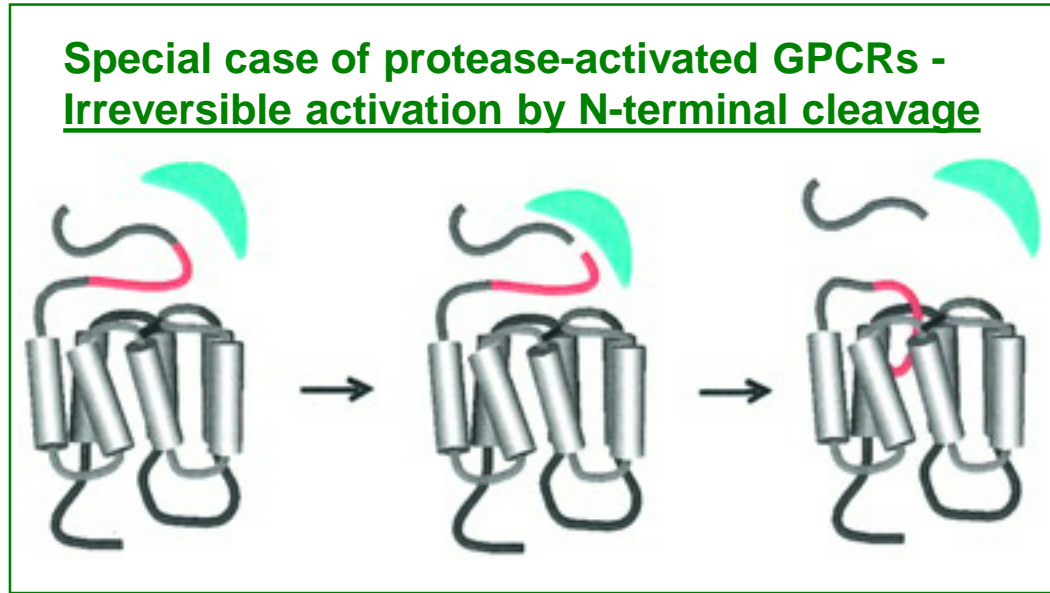
**“DRY”-motif critical for G-protein activation**

**Ligand-binding pocket**



- Peptides
- Cytokines
- IL8, fMLP
- PAF
- Thrombin:**

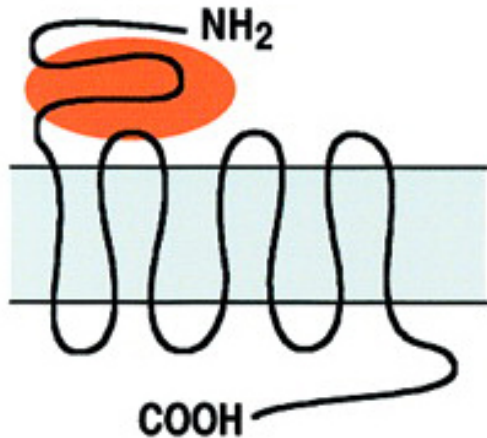
**Special case of protease-activated GPCRs - Irreversible activation by N-terminal cleavage**



- Glycoproteins
- hormones (LH, TSH, FSH)

**Palmitoylated cysteine**

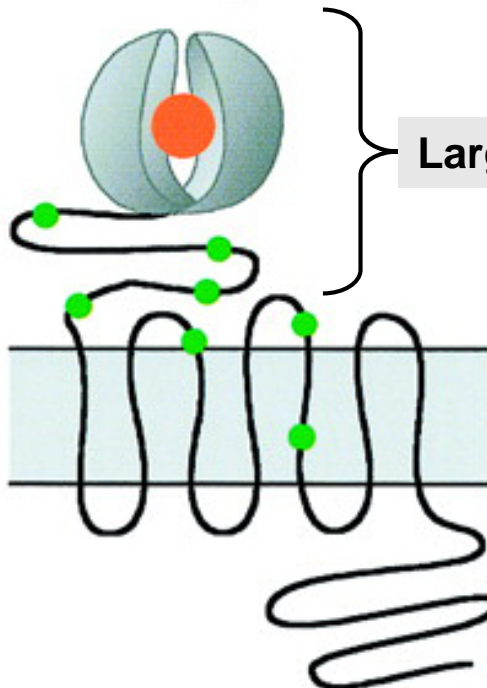
## Family 2



### High-mol.wt. hormones:

Calcitonin  
Glucagon  
Secretine  
PTH  
VIP  
PACAP  
GnRH  
CRF

## Family 3

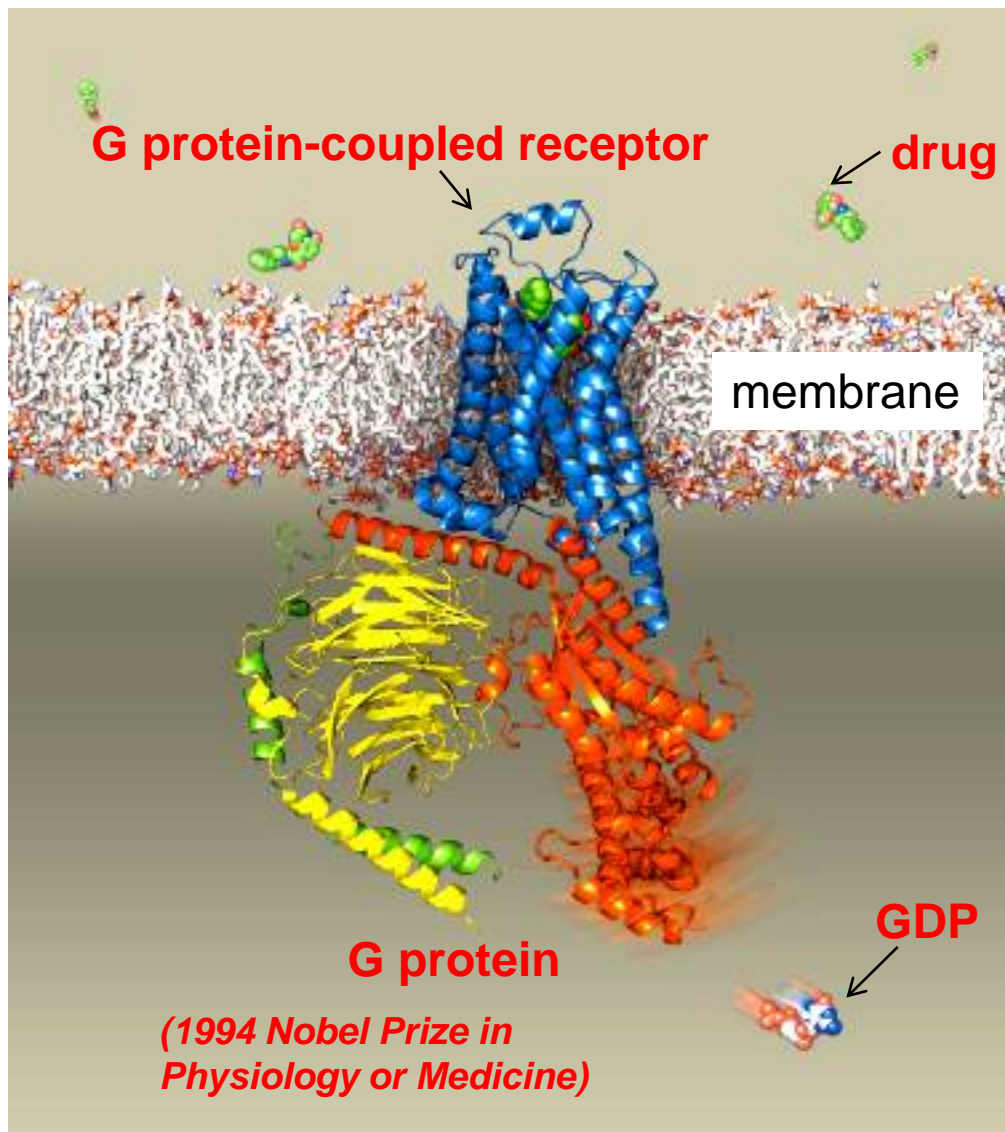


### Metabotropic glutamate/GABA/pheromone:

Large "Venus flytrap" disulfide-linked ectodomain

Glutamate  
(metabotropic)  
Ca<sup>++</sup>  
GABA (GABA<sub>B</sub>)  
Pheromones  
(VR, G<sub>o</sub>VN)

# 2012 Nobel Prize in Chemistry



Brian Kobilka



Brian Kobilka

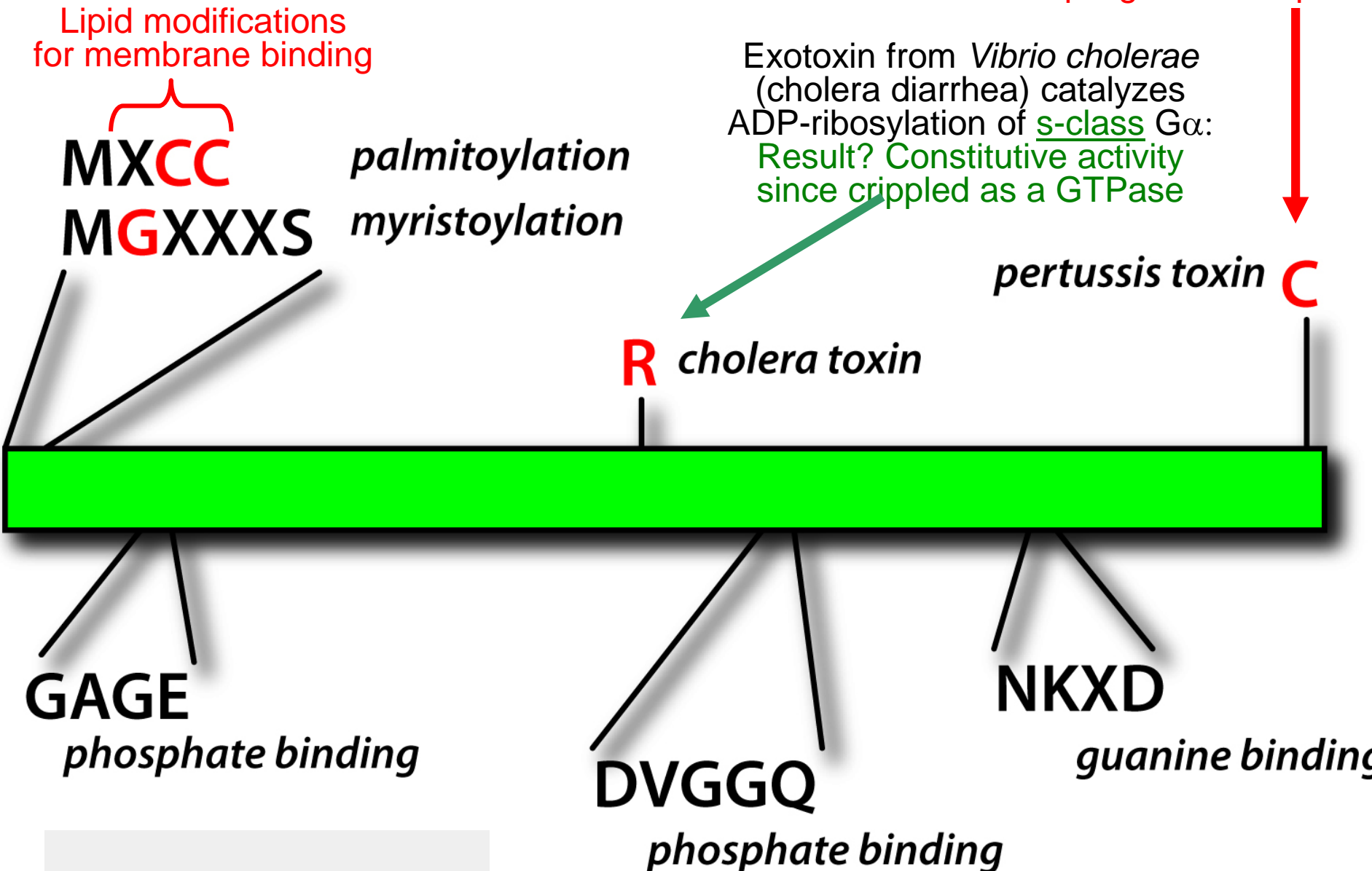


Robert Lefkowitz

# The G $\alpha$ subunit:

Exotoxin from *Bordetella pertussis* (whooping cough) catalyzes ADP-ribosylation of i-class G $\alpha$ :  
Result? De-coupling from receptor

Exotoxin from *Vibrio cholerae* (cholera diarrhea) catalyzes ADP-ribosylation of s-class G $\alpha$ :  
Result? Constitutive activity since crippled as a GTPase





Oct. 16



Oct. 17



Oct. 18



Oct. 19



Oct. 20



Oct. 21



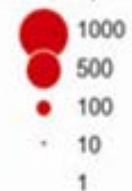
Oct. 22



Main towns

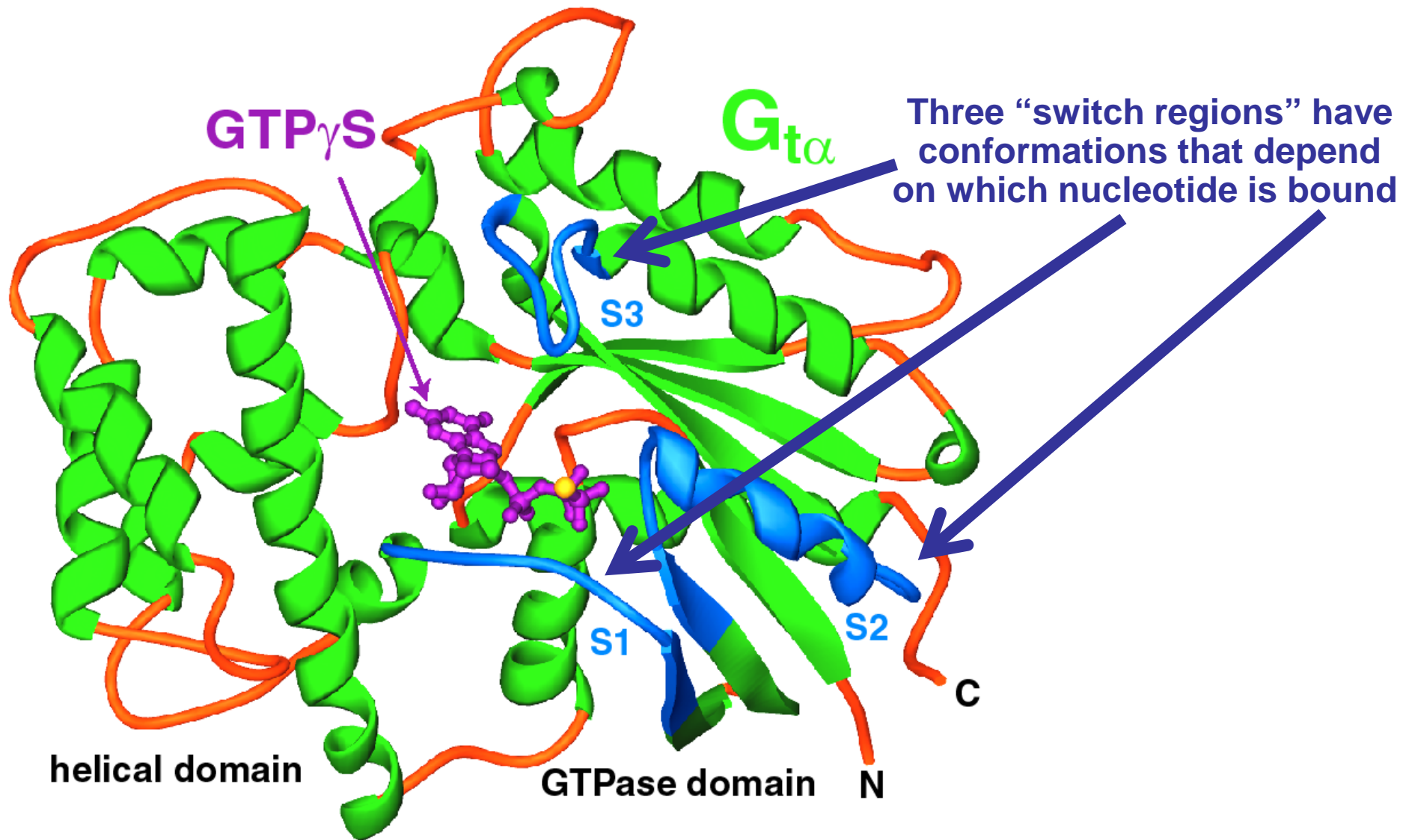
- Port-au-Prince
- Saint-Marc

Number of patients

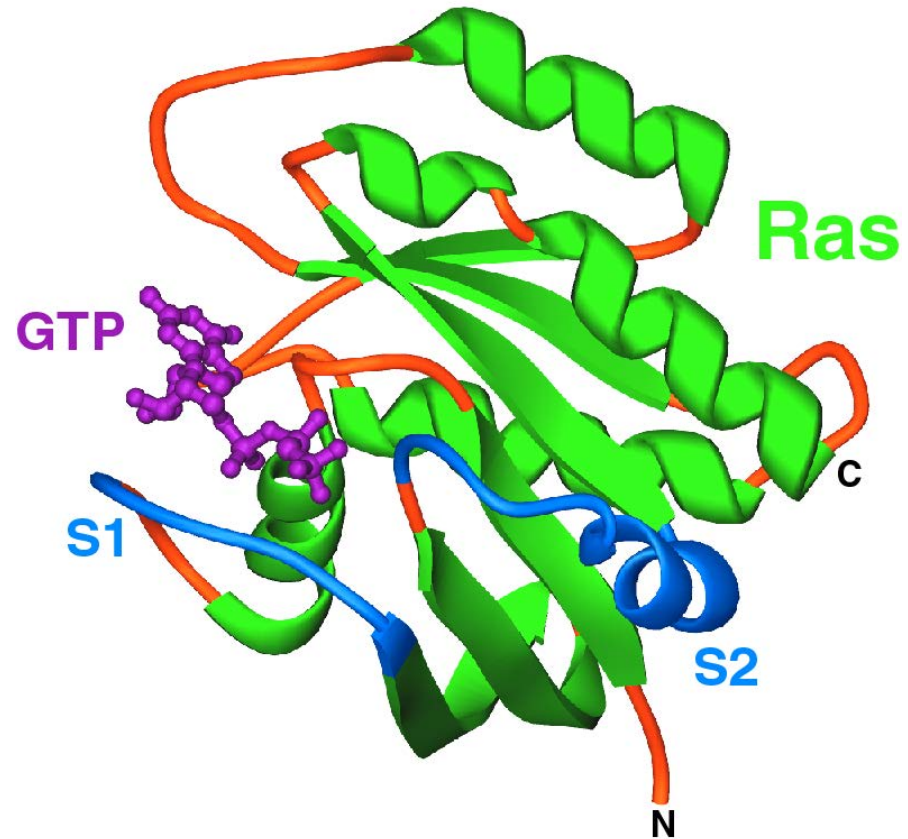
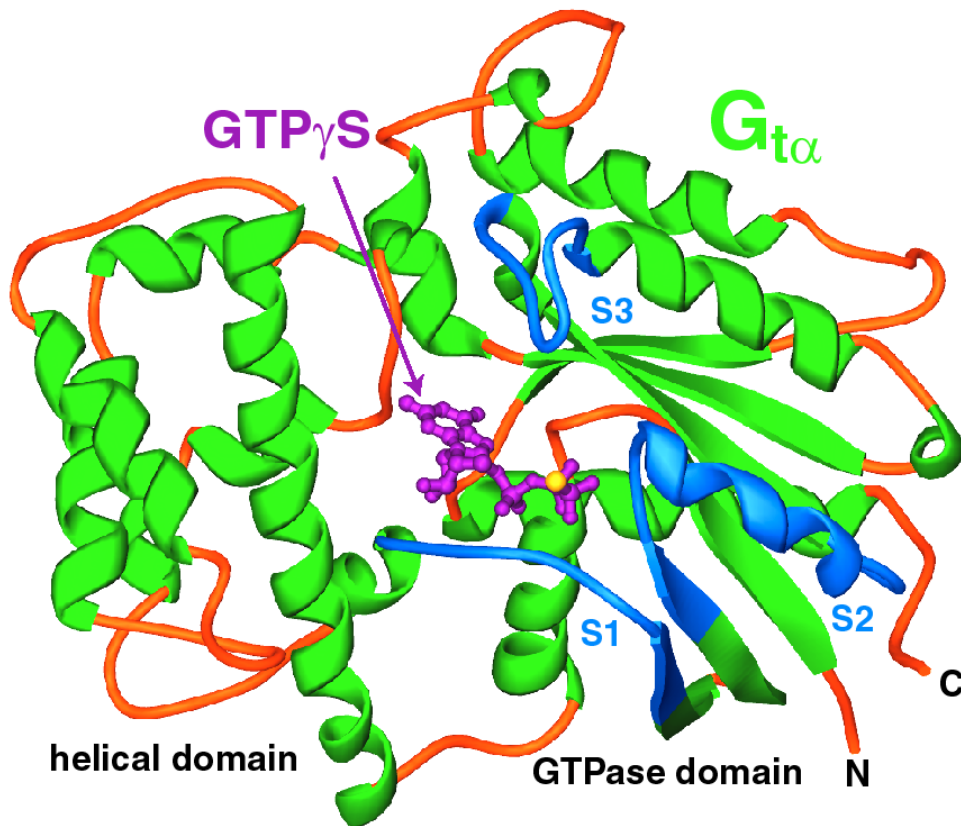


○ Spatio-temporal cluster

G $\alpha$  contains a Ras-like domain and an all-helical domain

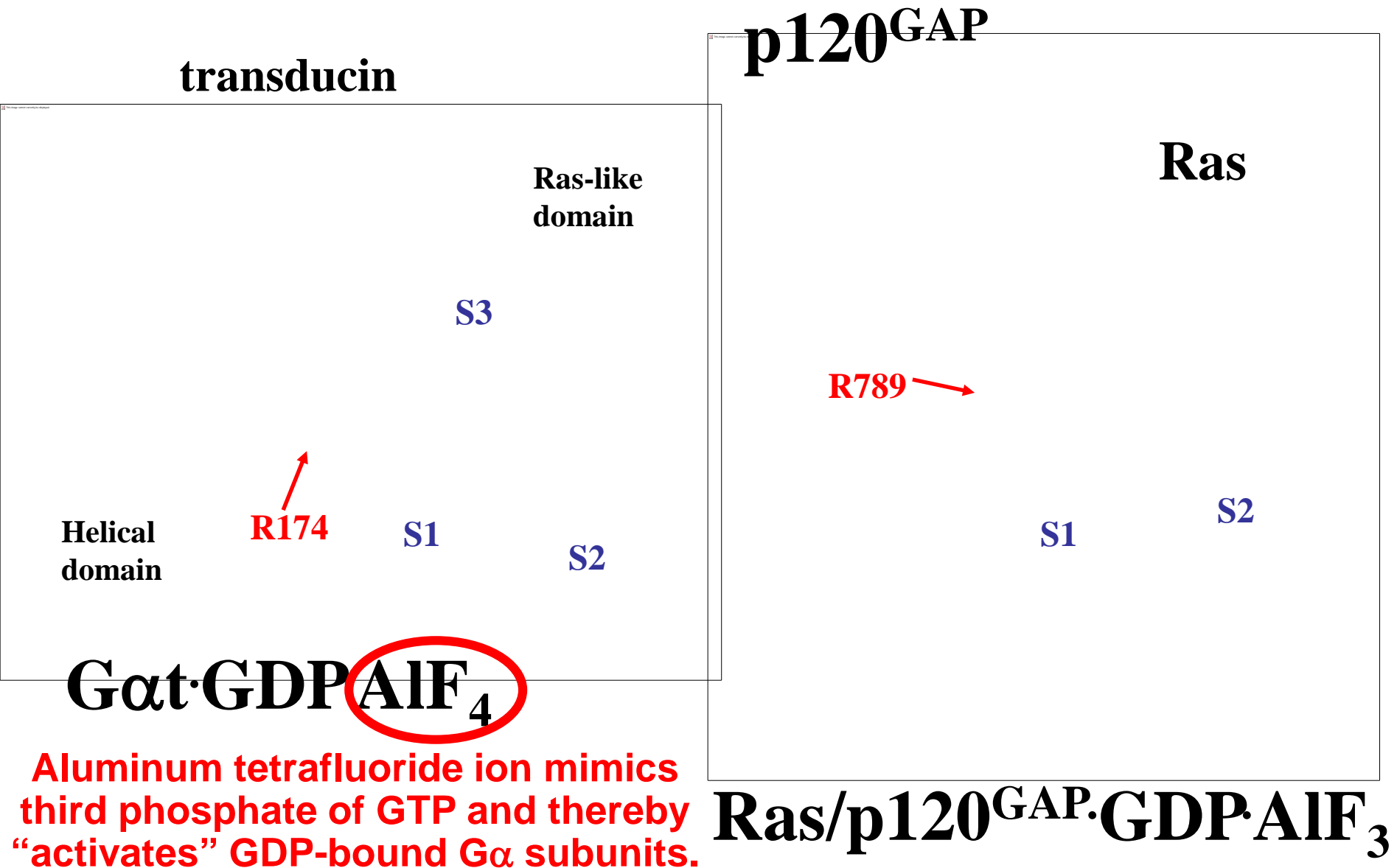


# G $\alpha$ contains a Ras-like domain and an all-helical domain





# “Arginine finger” is critical for GTP hydrolysis.



transducin

Ras-like domain

S3

Helical domain

R174

S1

S2

$G\alpha \cdot GDP \cdot AIF_4$

p120<sup>GAP</sup>

Ras

R789

S1

S2

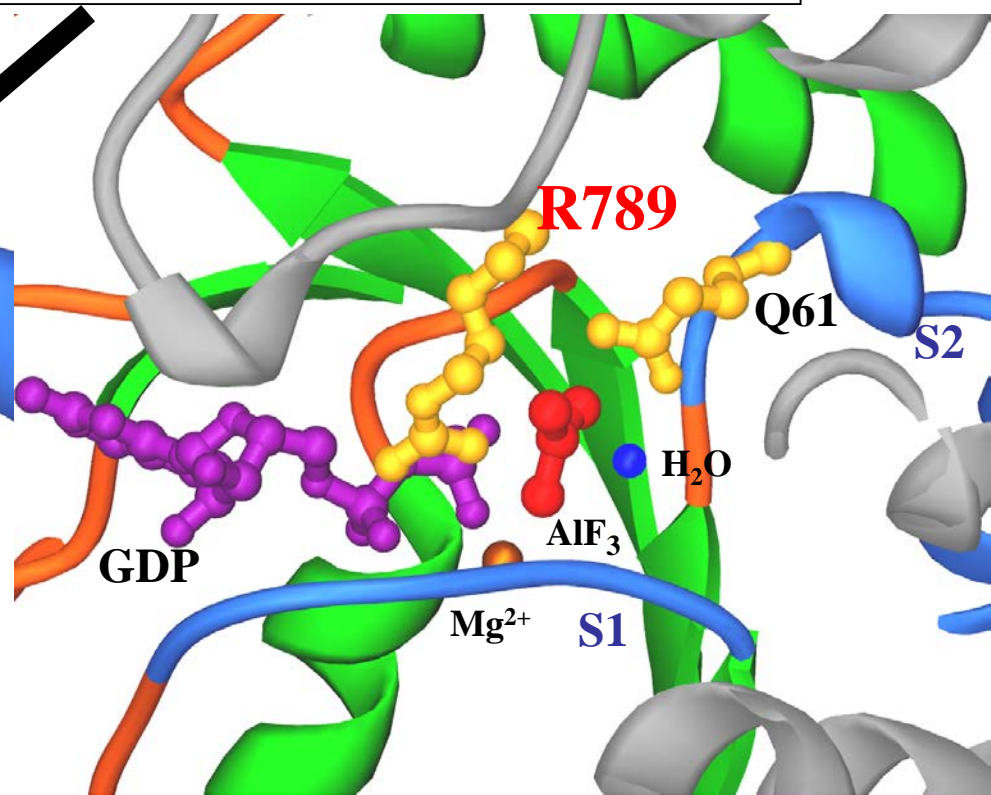
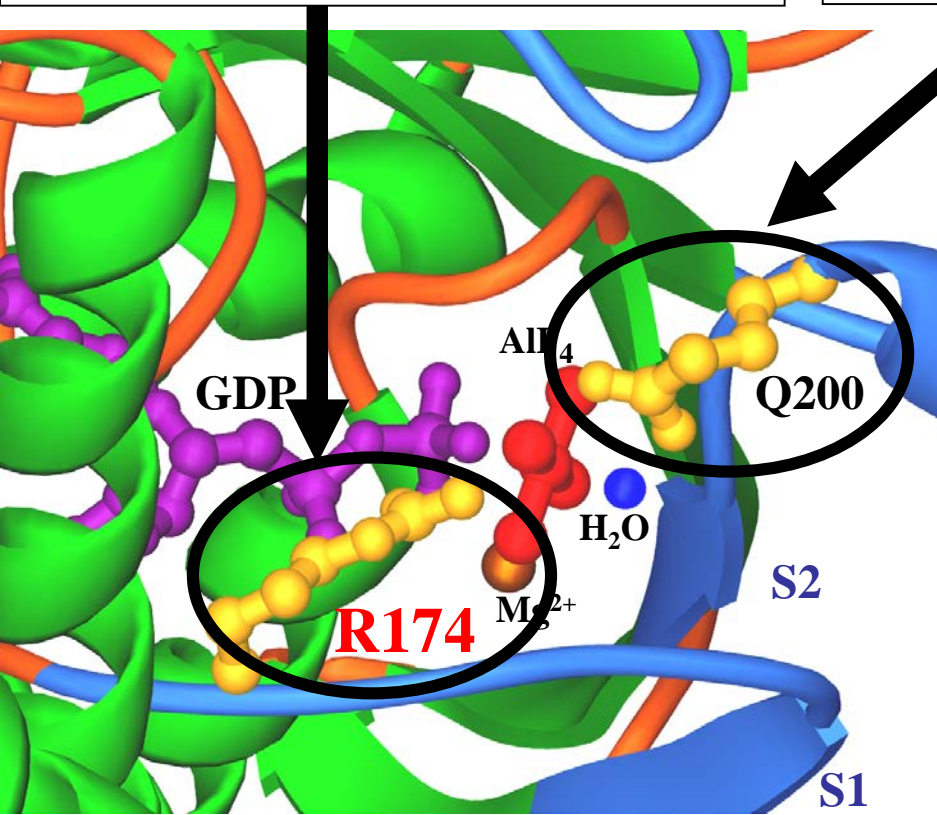
$Ras/p120^{GAP} \cdot GDP \cdot AIF_3$

Aluminum tetrafluoride ion mimics third phosphate of GTP and thereby “activates” GDP-bound  $G\alpha$  subunits.

# “Arginine finger” is critical for GTP hydrolysis

Site of cholera activation;  
R>C mutation activates.

Q>L mutation also activates  
by crippling GTPase function.



**Gαt·GDP·AIF<sub>4</sub>**

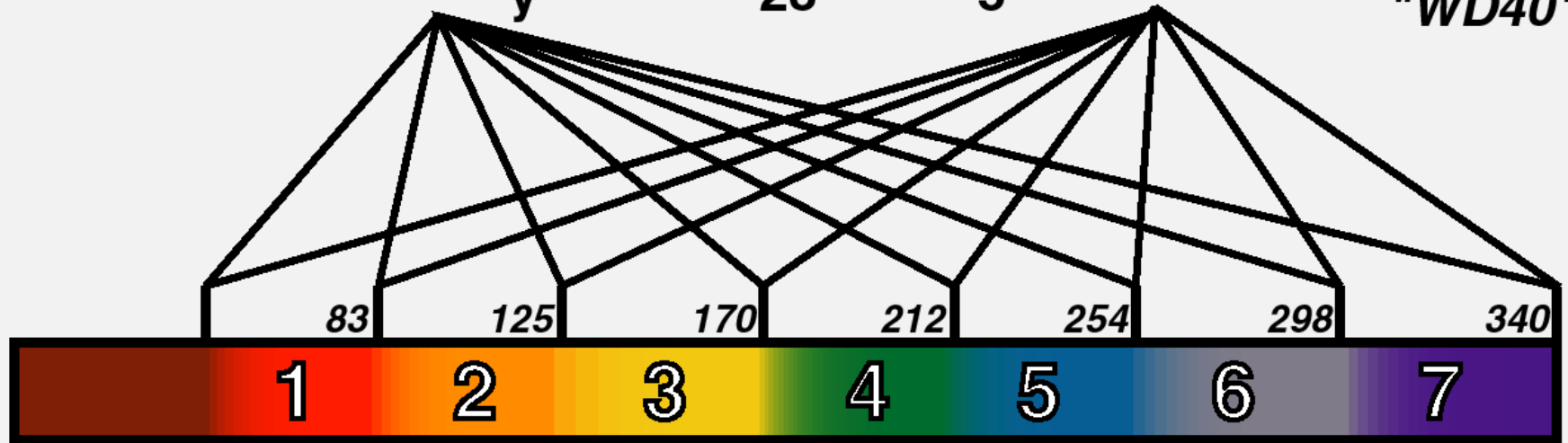
**Ras/p120<sup>GAP</sup>·GDP·AIF<sub>3</sub>**

For Ras-like GTPases, arginine finger is supplied in *trans* by GAPs.  
For heterotrimeric G proteins, the catalytic arginine is part of the alpha subunit.

# Primary sequence characteristics of Gβγ dimers

$X_y$ -GH- $X_{23}$ -D- $X_5$ -WD

WD repeats  
"WD40"



coiled coil

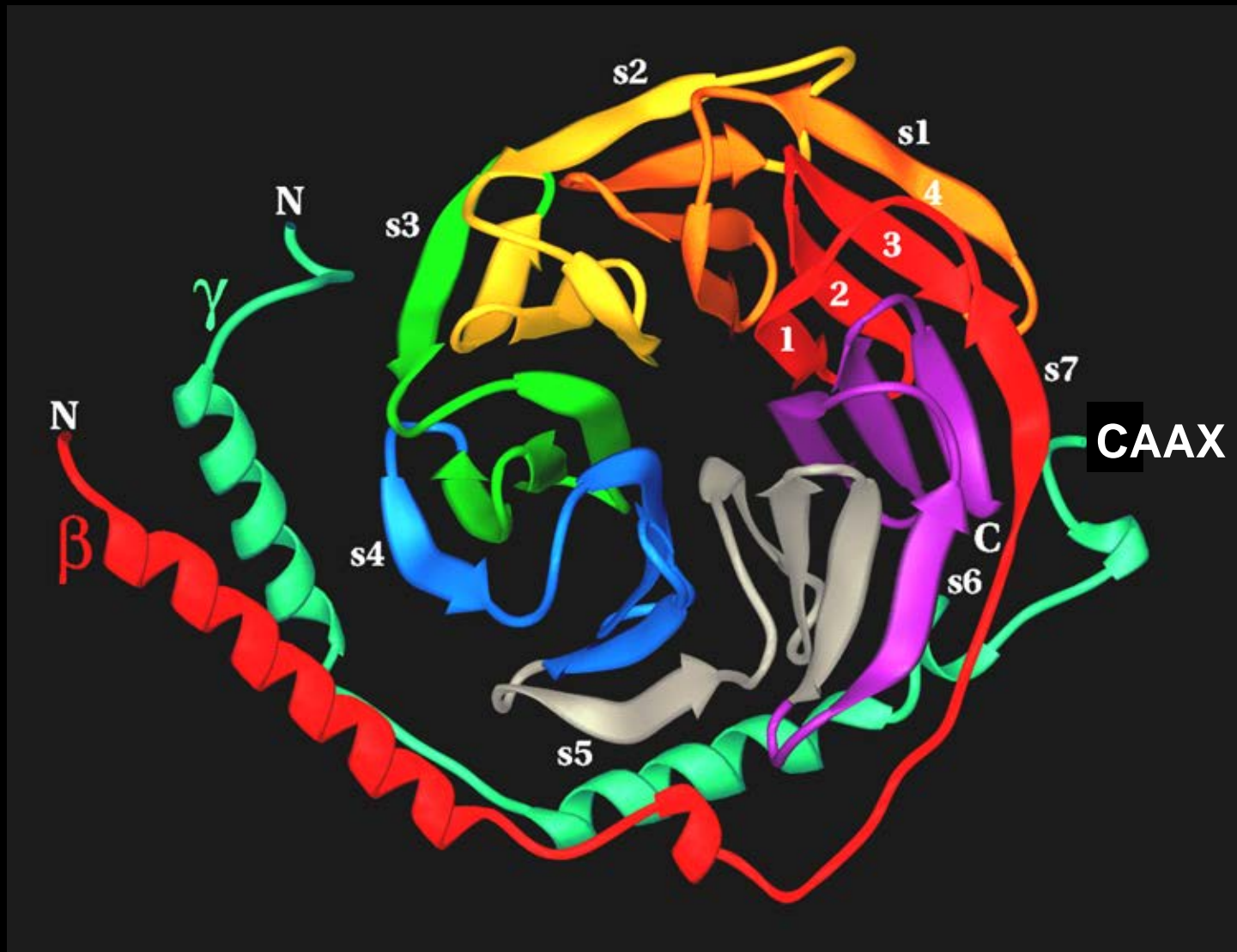
isoprenylation

CAAX<sup>74</sup>

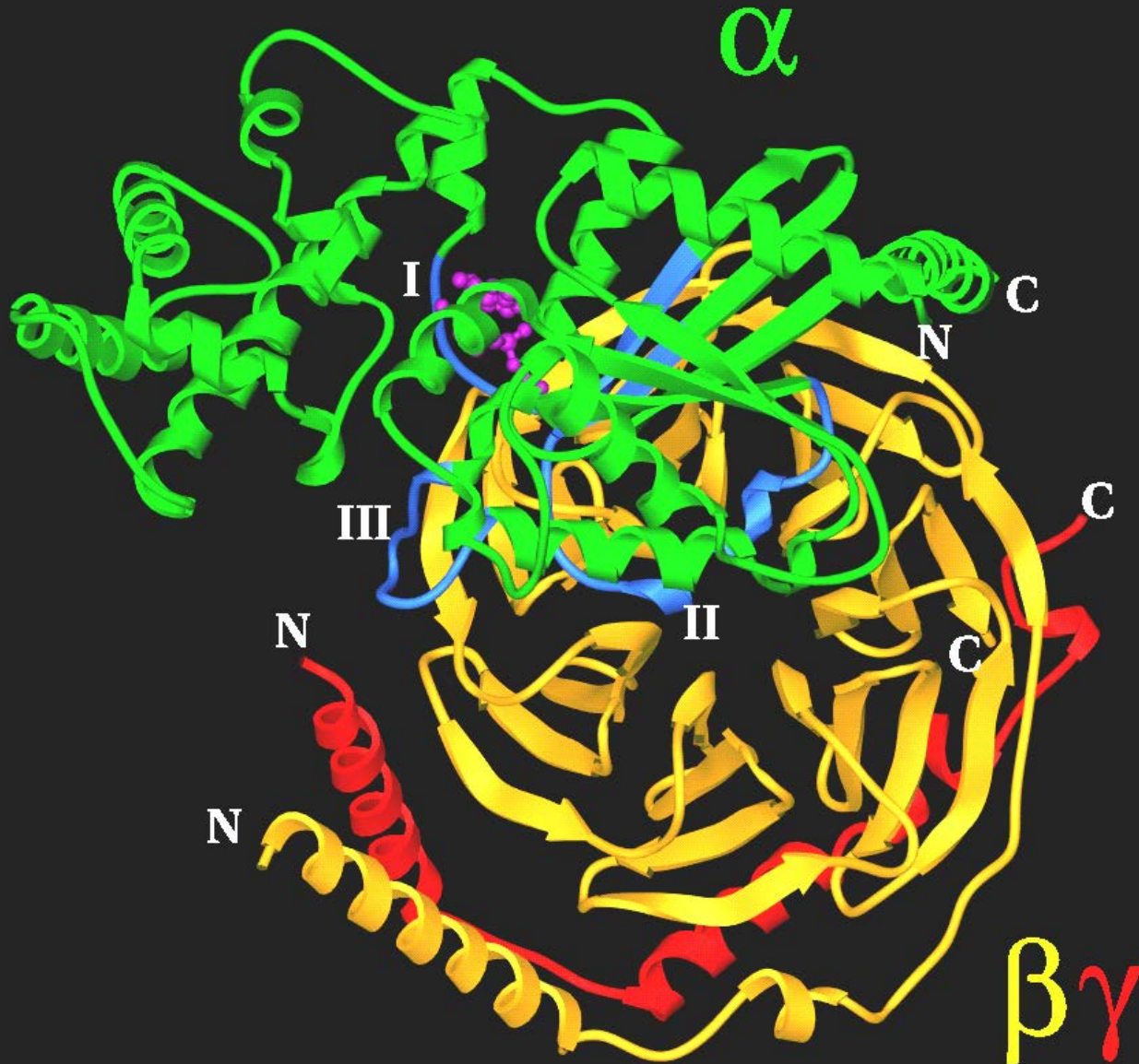
Gβγ dimers



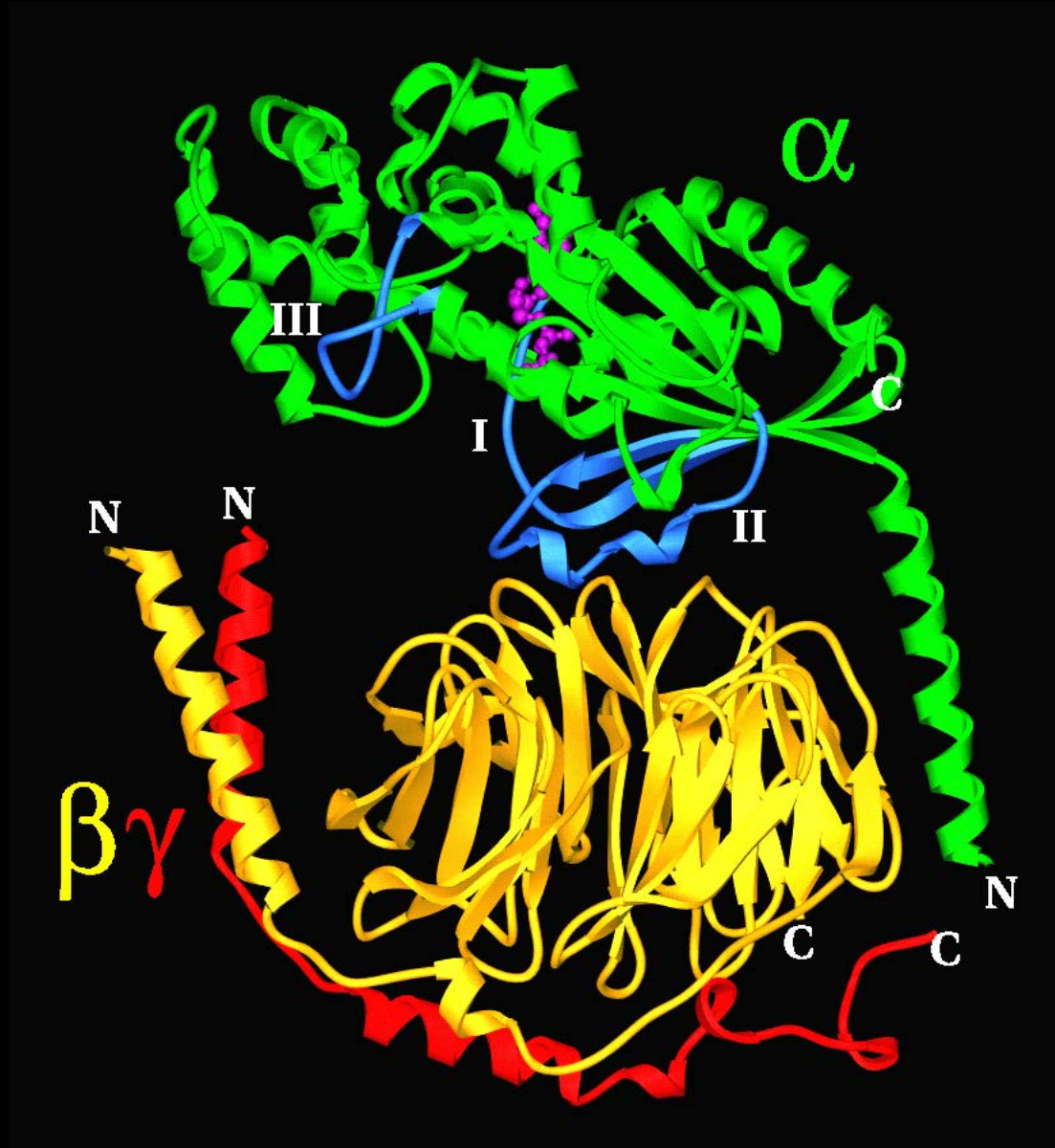
G $\beta$  forms a propeller; G $\gamma$  is an extended helical peptide.



The switches of  $G\alpha$  are primary interaction sites with  $G\beta\gamma$   
(no interactions between  $G\alpha$  and  $G\gamma$ !)



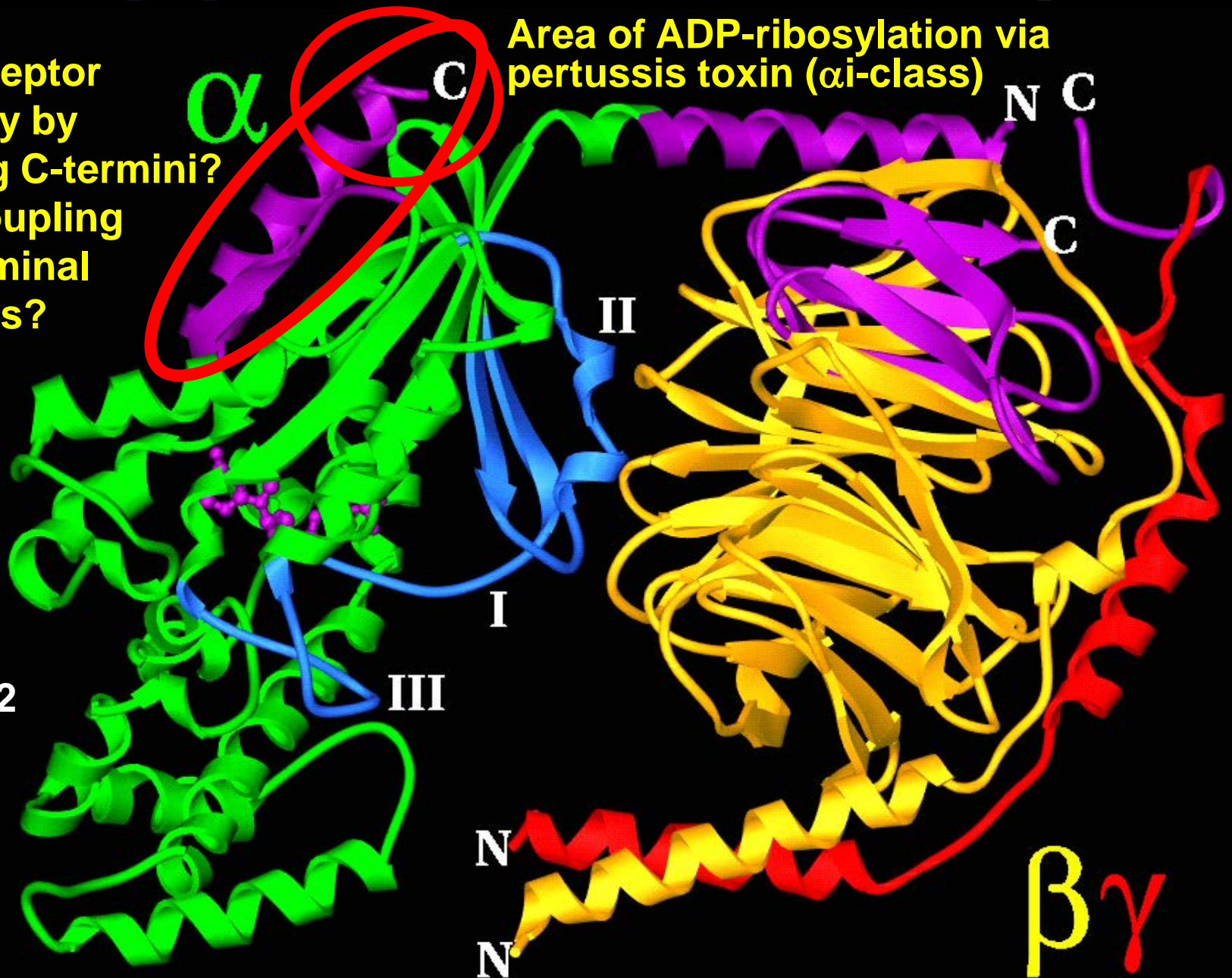
The switches of  $G\alpha$  are primary interaction sites with  $G\beta\gamma$   
(the N-terminal helix of  $G\alpha$  is also used)



Portions (purple) of all three subunits contact receptor

Swap receptor  
specificity by  
switching C-termini?  
Inhibit coupling  
via C-terminal  
minigenes?

Area of ADP-ribosylation via  
pertussis toxin ( $\alpha$ i-class)

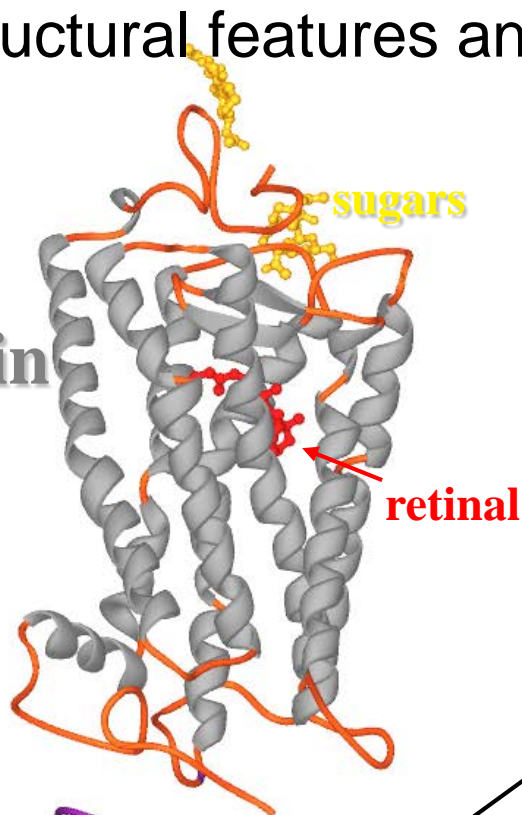


Gilchrist  
*et al.*  
(2001)  
*JBC*  
276:25672

# Interface defined by structural features and biochemical evidence

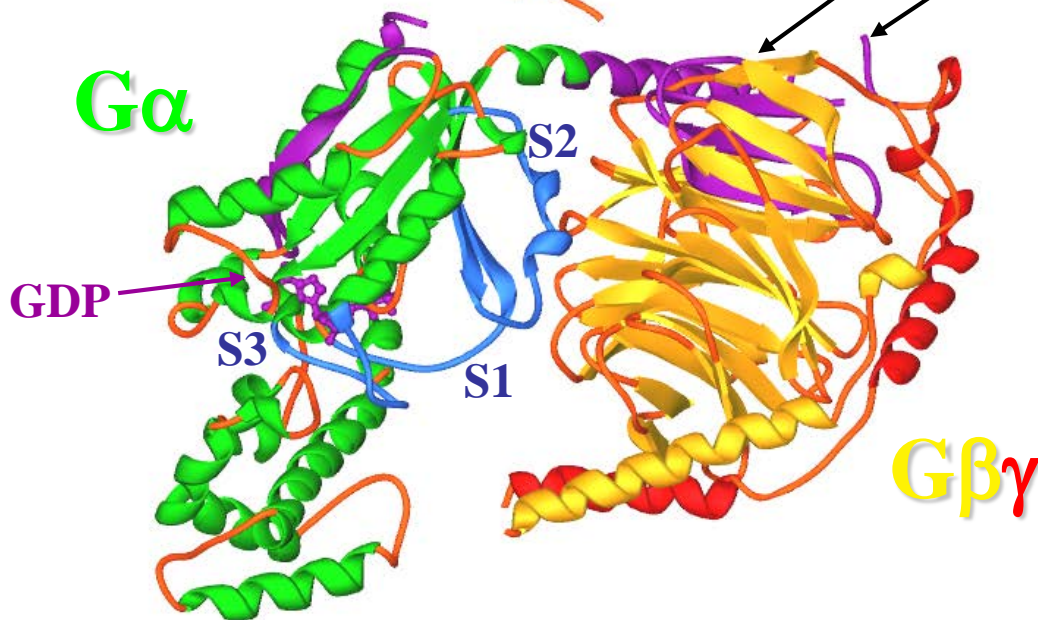
membrane

rhodopsin



N-Myristoyl, S-palmitoyl

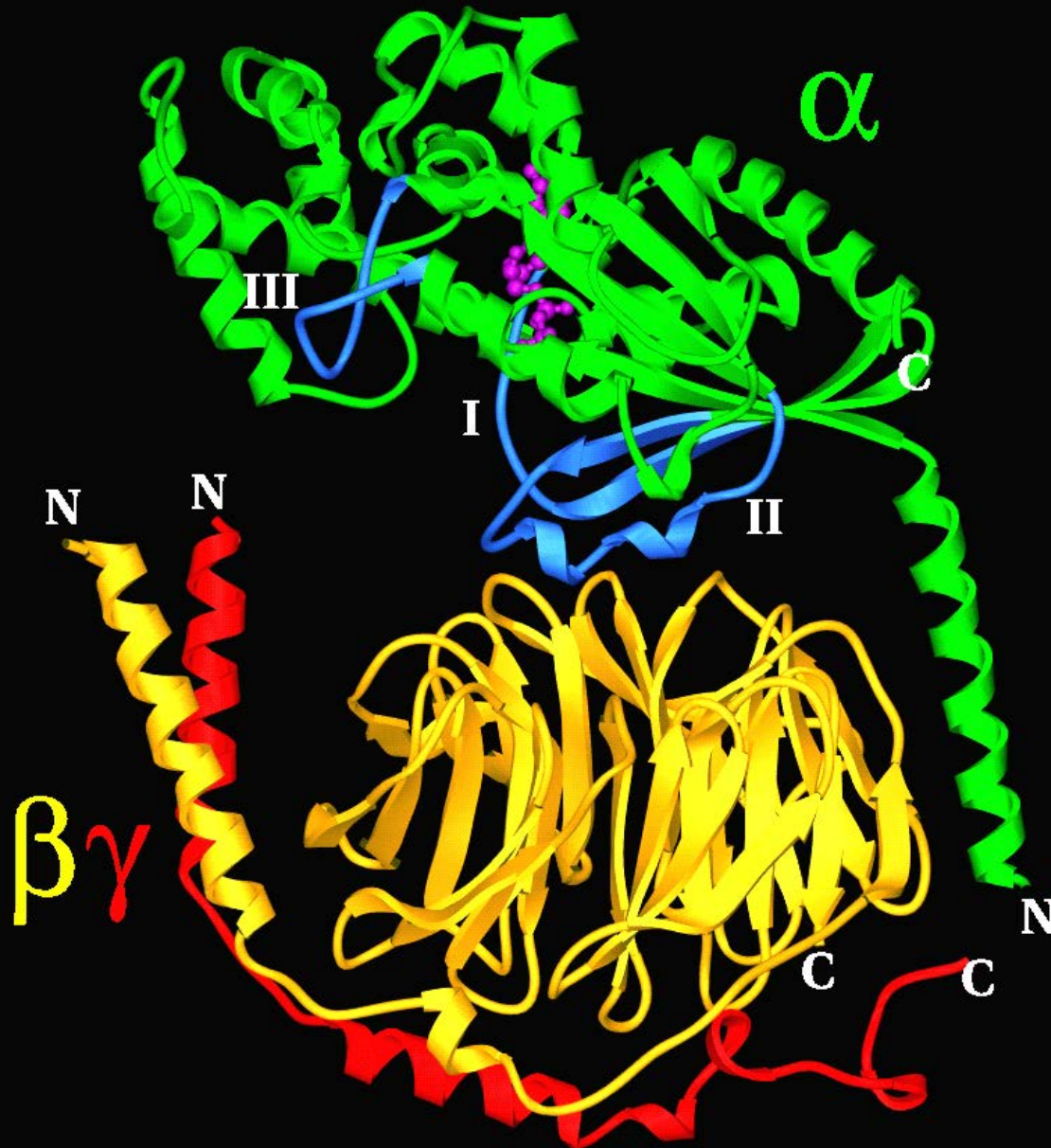
S-Prenyl (GG or F)



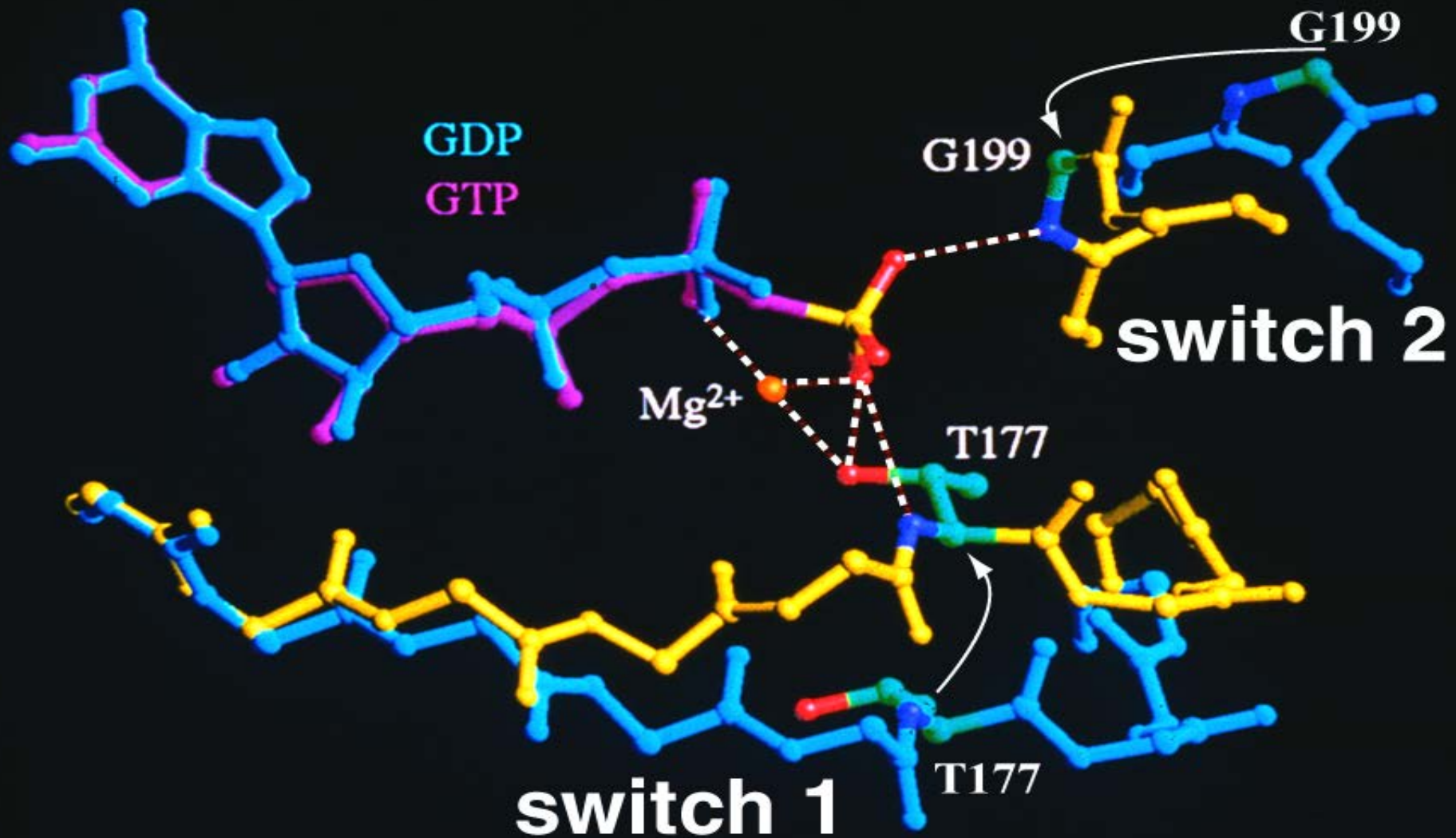
Regions of protein in purple are implicated in receptor interactions



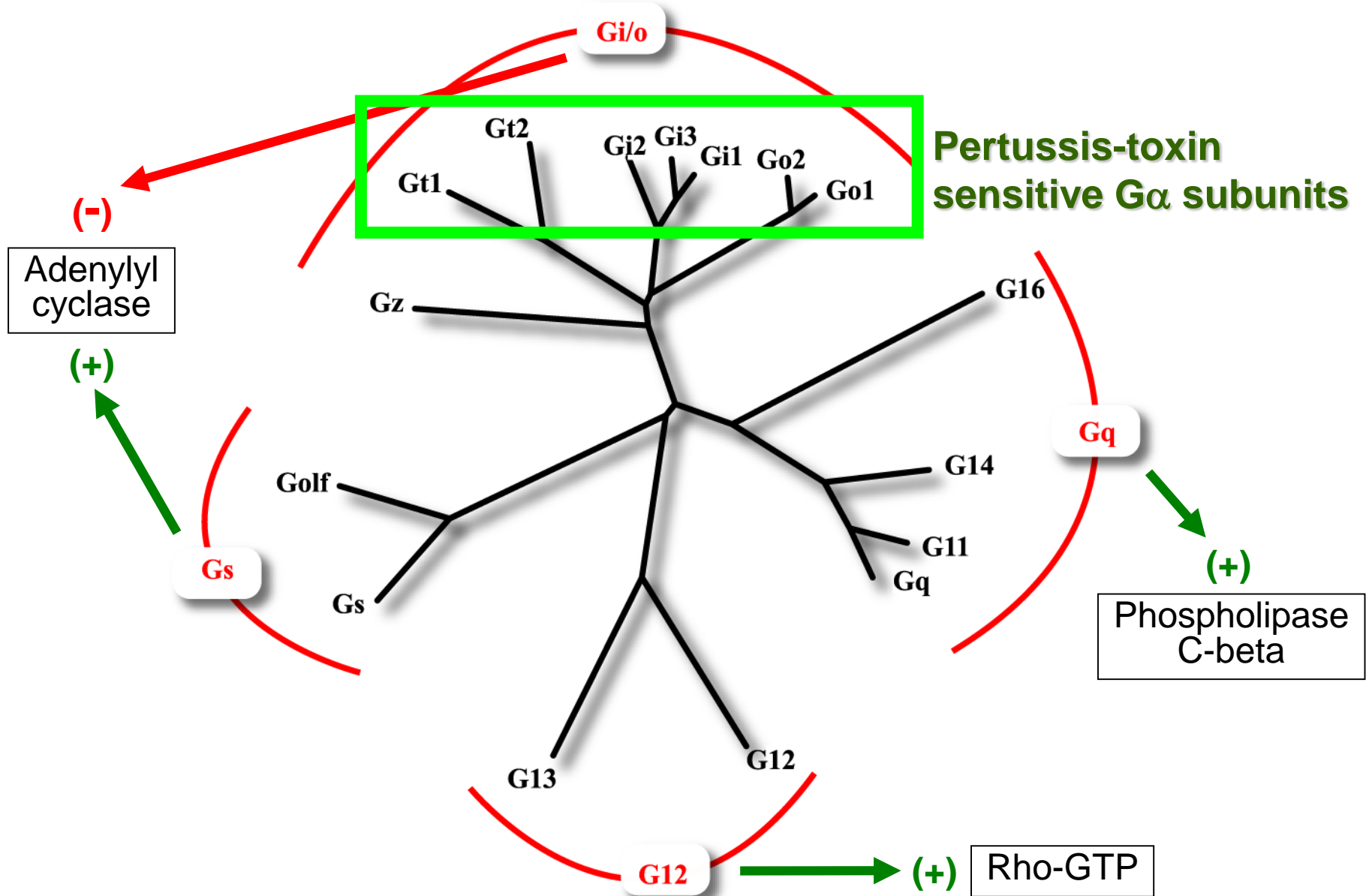
G $\alpha$  switches (I, II, III) are sensitive to bound nucleotide



# Gα switch regions (I, II) directly interact with GTP



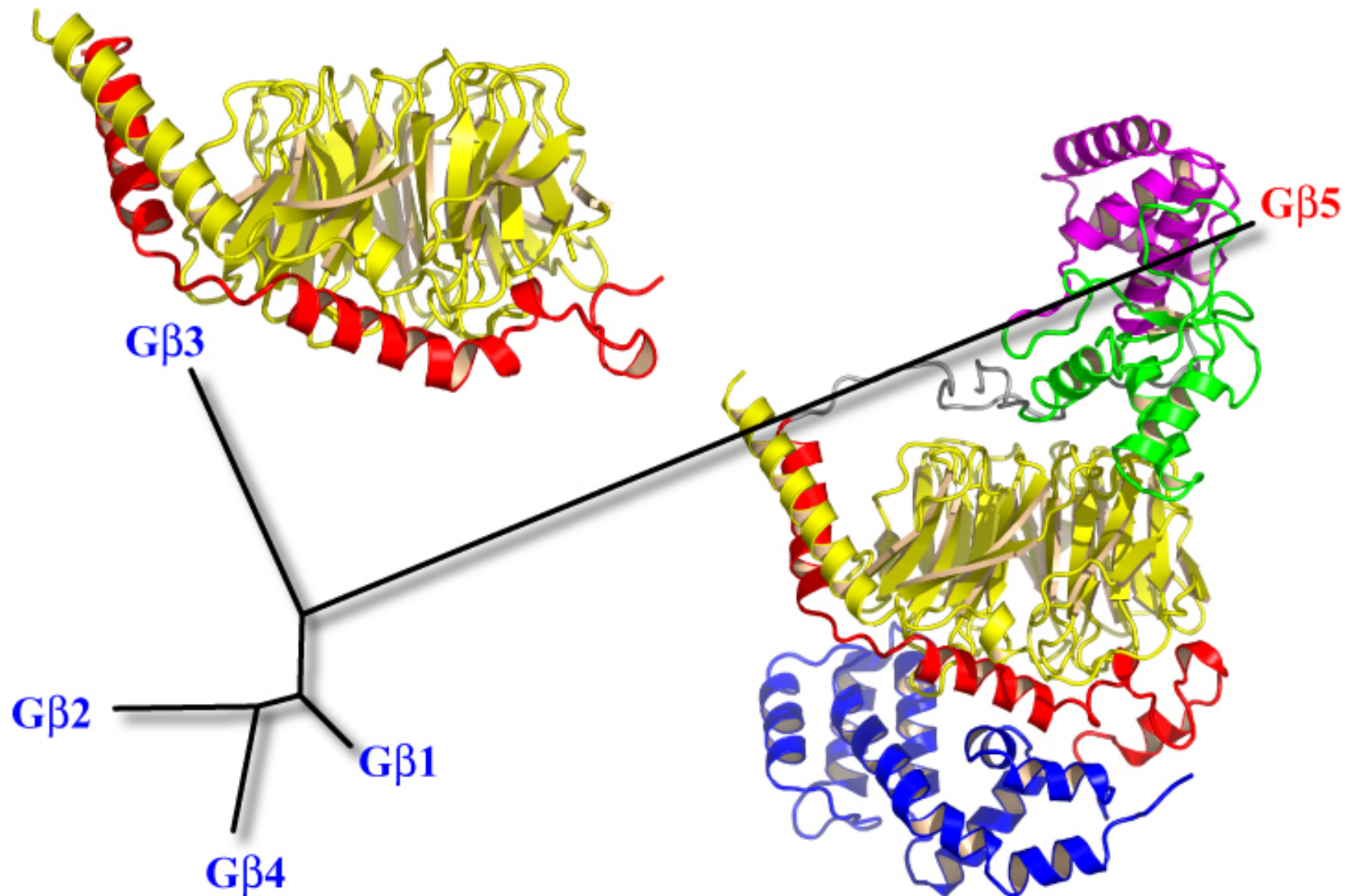
# The four families of G $\alpha$ subunits



# Examples of G-protein effector systems

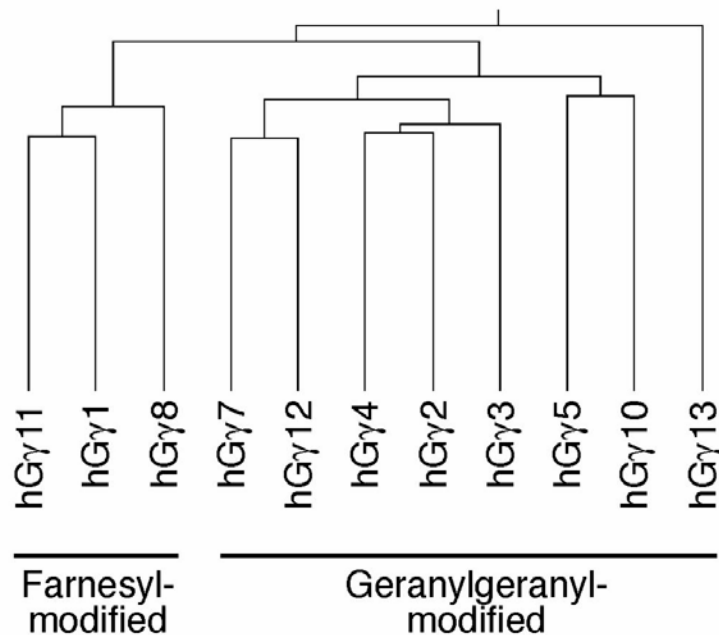
G-protein subunit	Effector	"Second messenger"
G $\alpha$ s, G $\alpha$ olf	↑adenylyl cyclase	↑[cAMP]
G $\alpha$ i1/i2/i3	↓adenylyl cyclase	↓[cAMP]
G $\alpha$ q/11	↑phospholipase-C $\beta$	↑[IP <sub>3</sub> ] & ↑[DAG]
G $\alpha$ 12/13	↑RGS-box RhoGEFs	↑[RhoA-GTP]
	↑phospholipase-C $\epsilon$	↑[IP <sub>3</sub> ] & ↑[DAG]
G $\alpha$ $\tau$ (transducin)	↑cyclic GMP phosphodiesterase	↓[cGMP]
G $\beta\gamma$ dimer	↑or ↓ adenylyl cyclase	↑or ↓ [cAMP]
	↑PLC- $\beta$ & PLC- $\epsilon$	↑[IP <sub>3</sub> ] & ↑[DAG]
	↑or ↓ ion channel flux	K <sup>+</sup> and Ca <sup>2+</sup>

# Four G $\beta$ subunits and one oddball

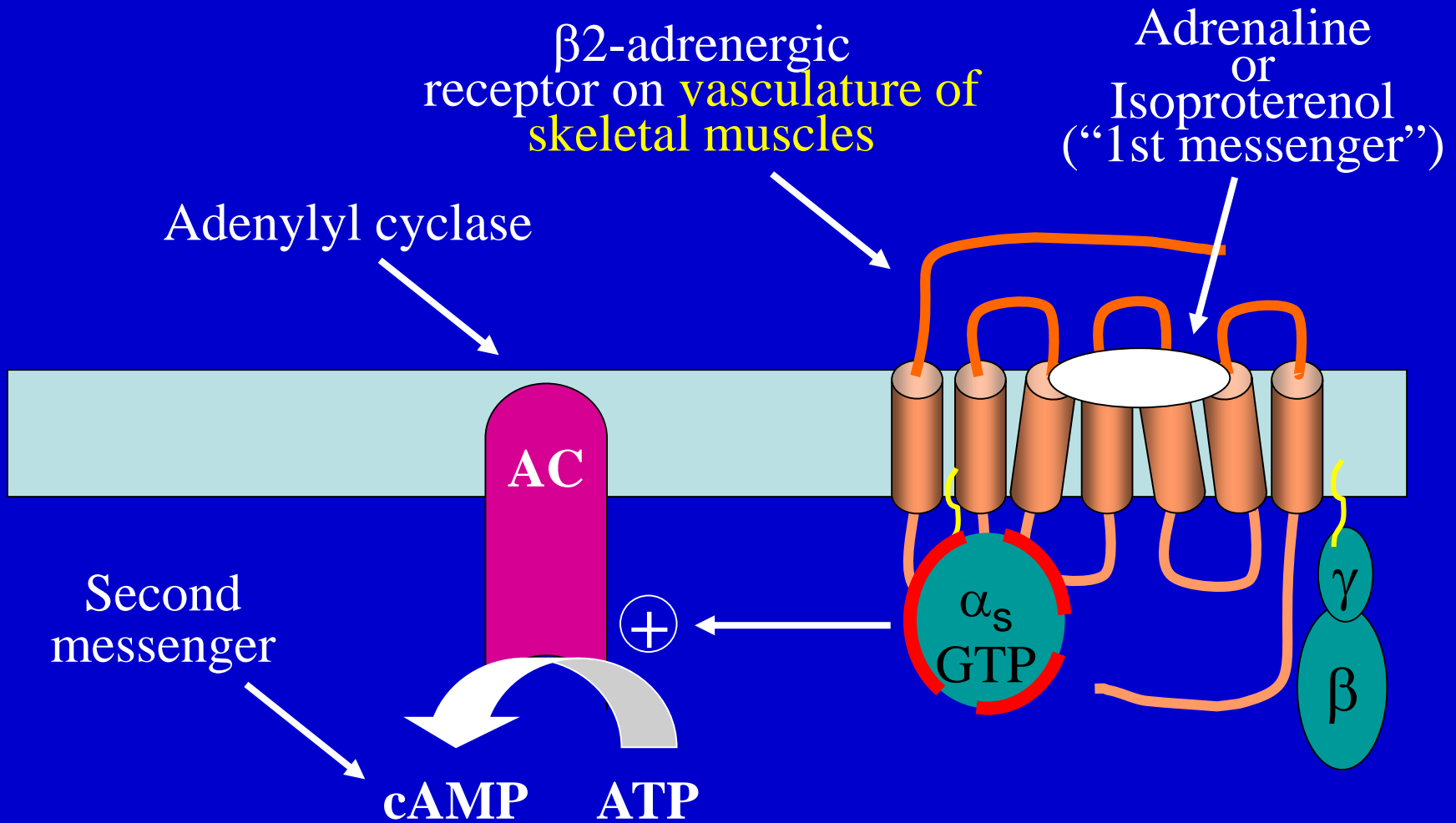


# Plenty o' G $\gamma$ subunits

hG $\gamma$ 11	K - EK LKME <b>VEQL</b> RK <b>EV</b> KLQ <b>RQ</b> QVSKCSEEEKN <b>YI</b> EERSGED <b>DPL</b> VK <b>GIP</b> EDKNPFKE - <b>KGS</b> CVIS* 73
hG $\gamma$ 1	K - DK LKME <b>VDQL</b> KK <b>EV</b> TLERML <b>VSK</b> CCEEVRD <b>YV</b> EERSGED <b>DPL</b> VK <b>GIP</b> EDKNPFKEL <b>KGG</b> CVIS* 74
hG $\gamma$ 8	K - DL LKME <b>VEQL</b> KK <b>EV</b> KNTRI <b>PIS</b> KAGKEIKEY <b>V</b> EAQAGND <b>PFL</b> K <b>GIP</b> EDKNPFKE - <b>KGG</b> CLIS* 69
hG $\gamma$ 7	NIAQARR <b>T</b> <b>VQQL</b> RRLEASIERI <b>KV</b> SKASAD <b>LMS</b> YCEEHARS <b>DPL</b> LI <b>GIP</b> TSENPFKDKK - <b>TC</b> IIL* 65
hG $\gamma$ 12	NIAQARKL <b>VEQL</b> RIEAGIERI <b>KV</b> SKAASD <b>LMS</b> YCEQHARN <b>DPL</b> LV <b>GVP</b> ASENPFKDKK - <b>PC</b> IIL* 72
hG $\gamma$ 4	SIAQARKL <b>VEQL</b> KMEANIDRI <b>KV</b> SKAAAD <b>LMA</b> YCEAHAKED <b>DPL</b> LTPVPASENPFREKK <b>FFC</b> AIL* 75
hG $\gamma$ 2	SISQARKA <b>VEQL</b> KMEACMDRV <b>KV</b> SQAAAD <b>LLA</b> YCEAHVRED <b>DPL</b> IIPVPASENPFREKK <b>FFC</b> TIL* 71
hG $\gamma$ 3	SIGQARKM <b>VEQL</b> KIEASLCRI <b>KV</b> SKAAAD <b>LMT</b> YCDAHACED <b>DPL</b> ITPVPASENPFREKK <b>FFC</b> ALL* 75
hG $\gamma$ 5	SVAAMKK <b>V</b> <b>VQQL</b> RRLEAGLNR <b>V</b> KVSQAAAD <b>LKQ</b> FCLQNAQH <b>DPL</b> LT <b>GV</b> SSSTNPFRPQ <b>KV</b> - <b>CS</b> FL* 68
hG $\gamma$ 10	SASALQRL <b>VEQL</b> KLEAGVERI <b>KV</b> SQAAAEL <b>QQY</b> CMQNACK <b>DAL</b> LV <b>GV</b> PAGSNPFREPRS - <b>CA</b> LL* 68
hG $\gamma$ 13	DVPQMKKE <b>VE</b> SLKYQLAFQ <b>RE</b> MA <b>S</b> KTIPE <b>L</b> LKWIE <b>DG</b> IPK <b>D</b> PFLNPDL <b>MKN</b> <b>NP</b> WVE - <b>KG</b> K <b>C</b> TIL* 67

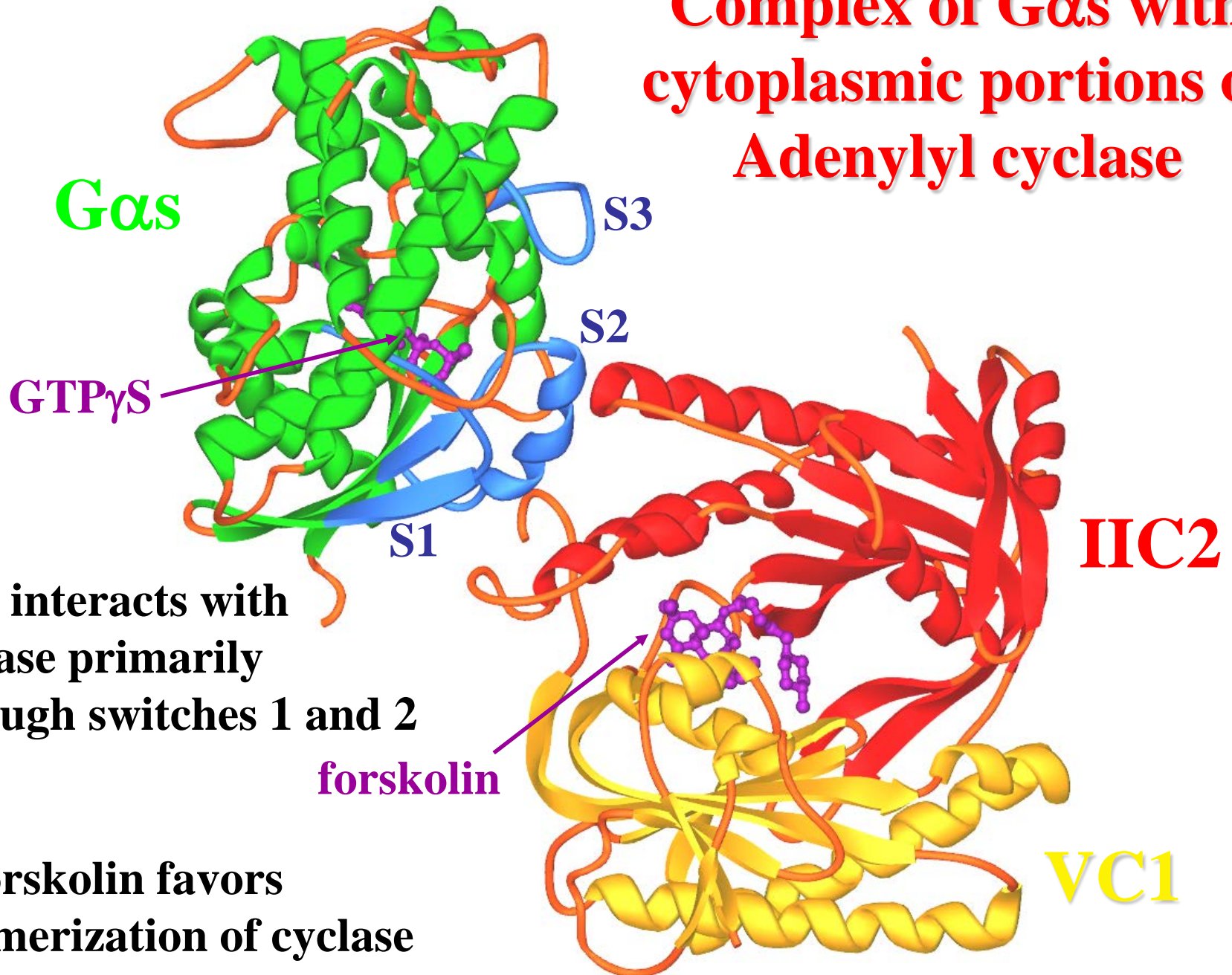


**Gs = “stimulatory” G-protein linked to adenylyl cyclase activation (2nd-messenger generation)**



Net result: Five-fold increase in  $[cAMP]_i$  in seconds

# Complex of G $\alpha$ s with cytoplasmic portions of Adenylyl cyclase



**G $\alpha$ s interacts with cyclase primarily through switches 1 and 2**

**forskolin**

**Forskolin favors dimerization of cyclase domains**

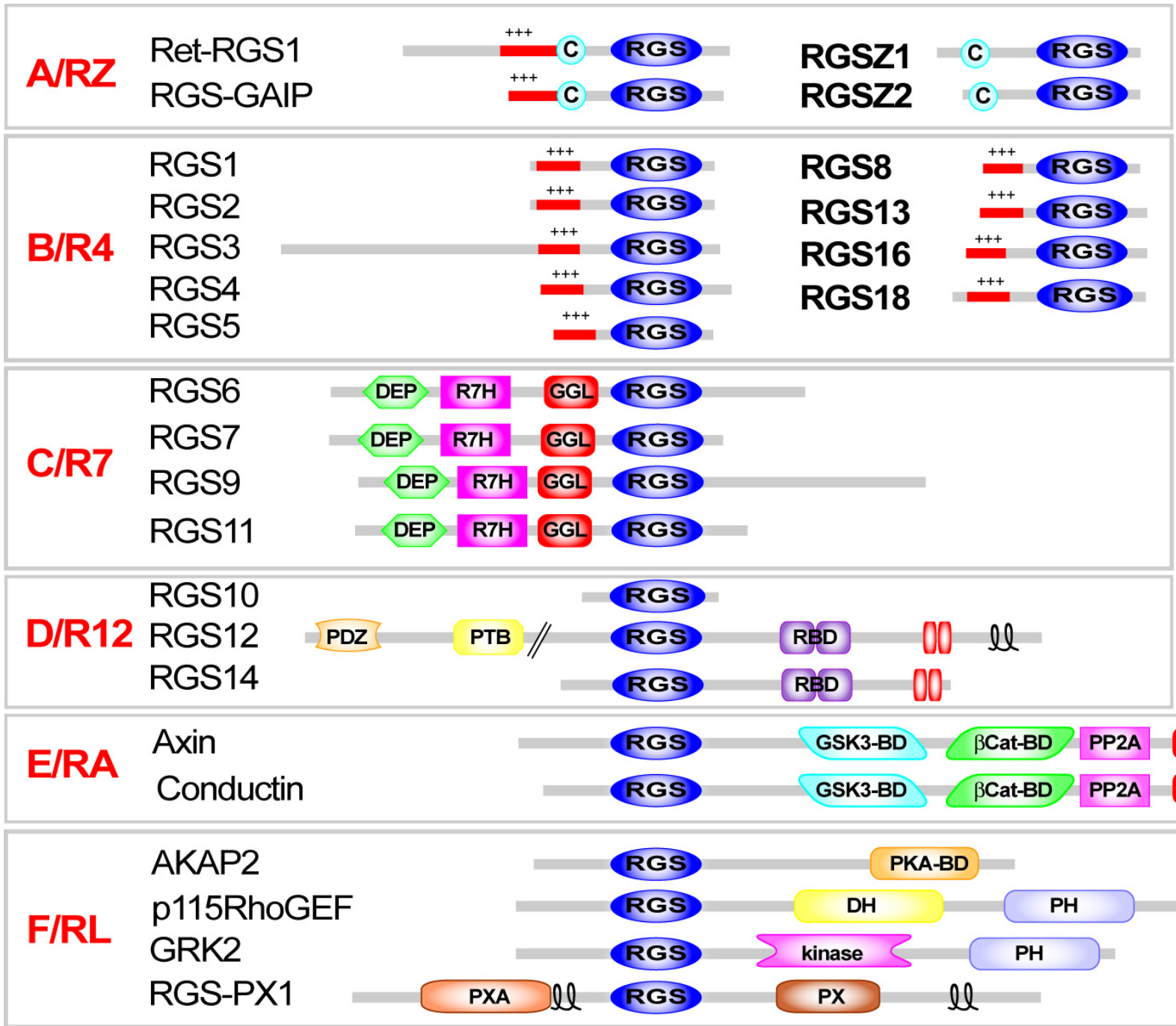


# Turning off the signal

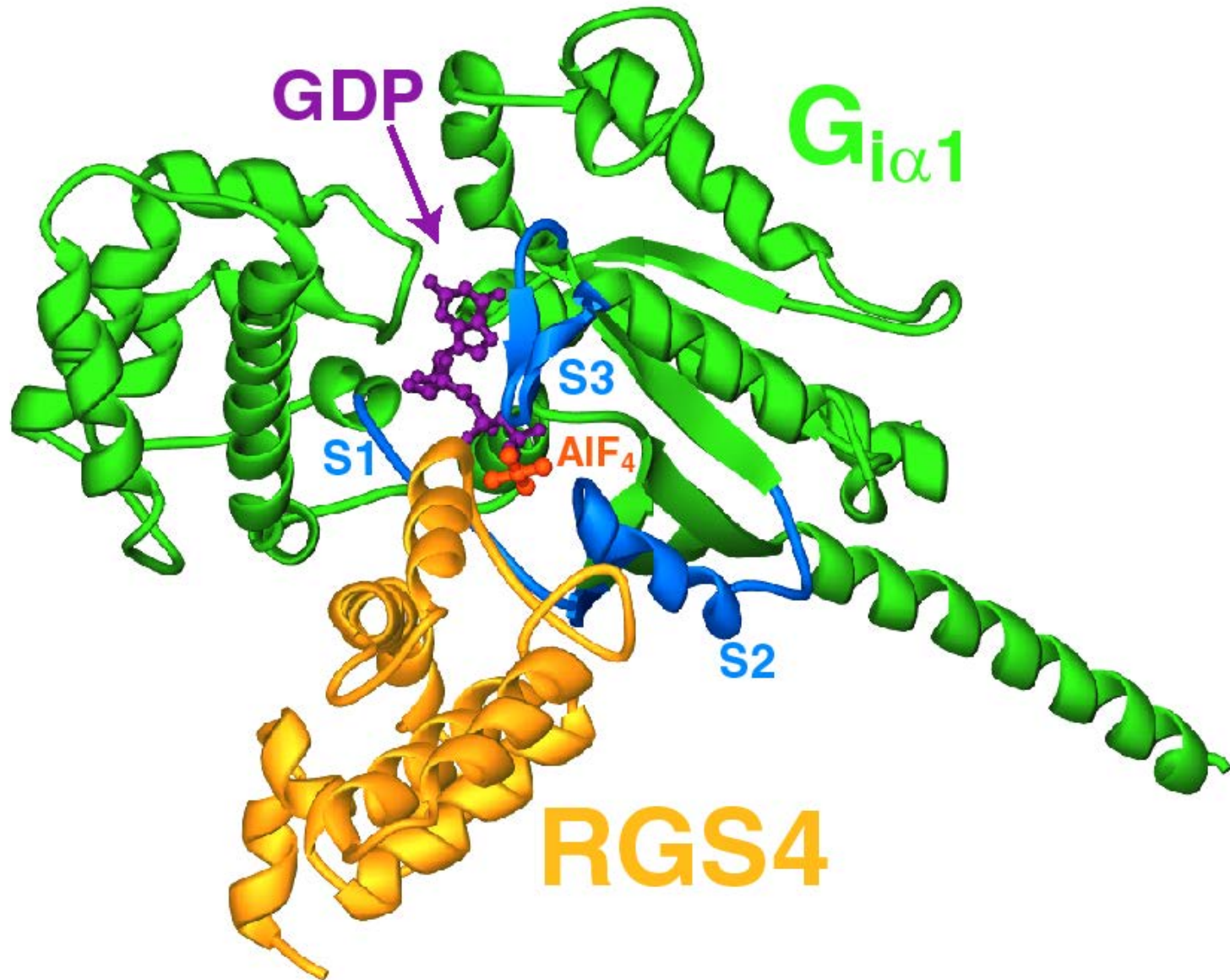
## Multiple levels & multiple time-frames

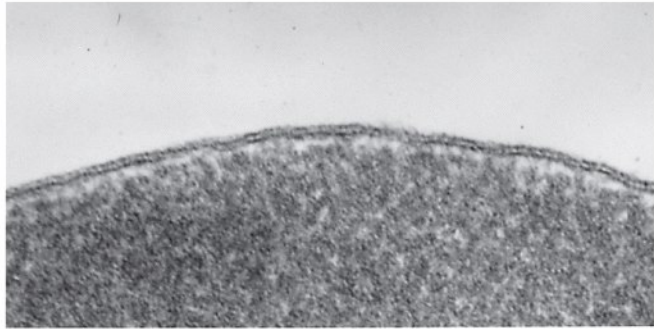


- Reuptake/destruction of agonist (~millisec)
- Hydrolysis of GTP bound to  $G\alpha$  subunit (~sec)
- Reuptake/destruction of second messenger (~sec)
- Uncouple receptor from signal machinery (~sec/min)
- Remove receptor from cell-surface (~min/hr)

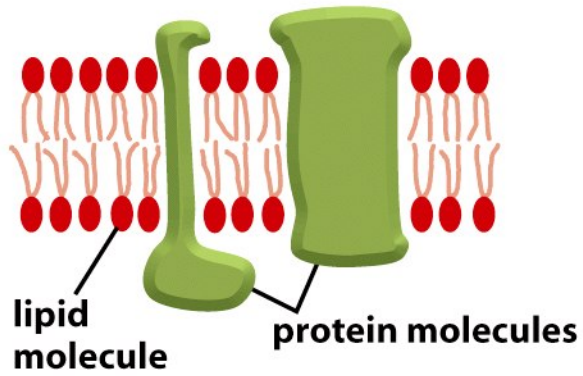


# RGS proteins stabilize the transition state for GTP hydrolysis

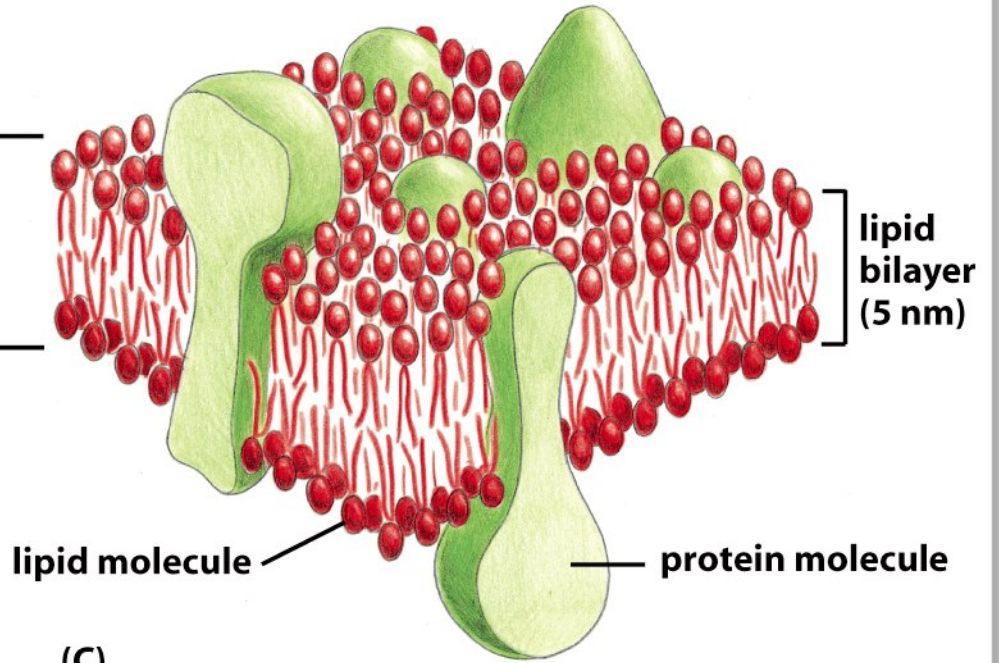




(A)



(B)



(C)



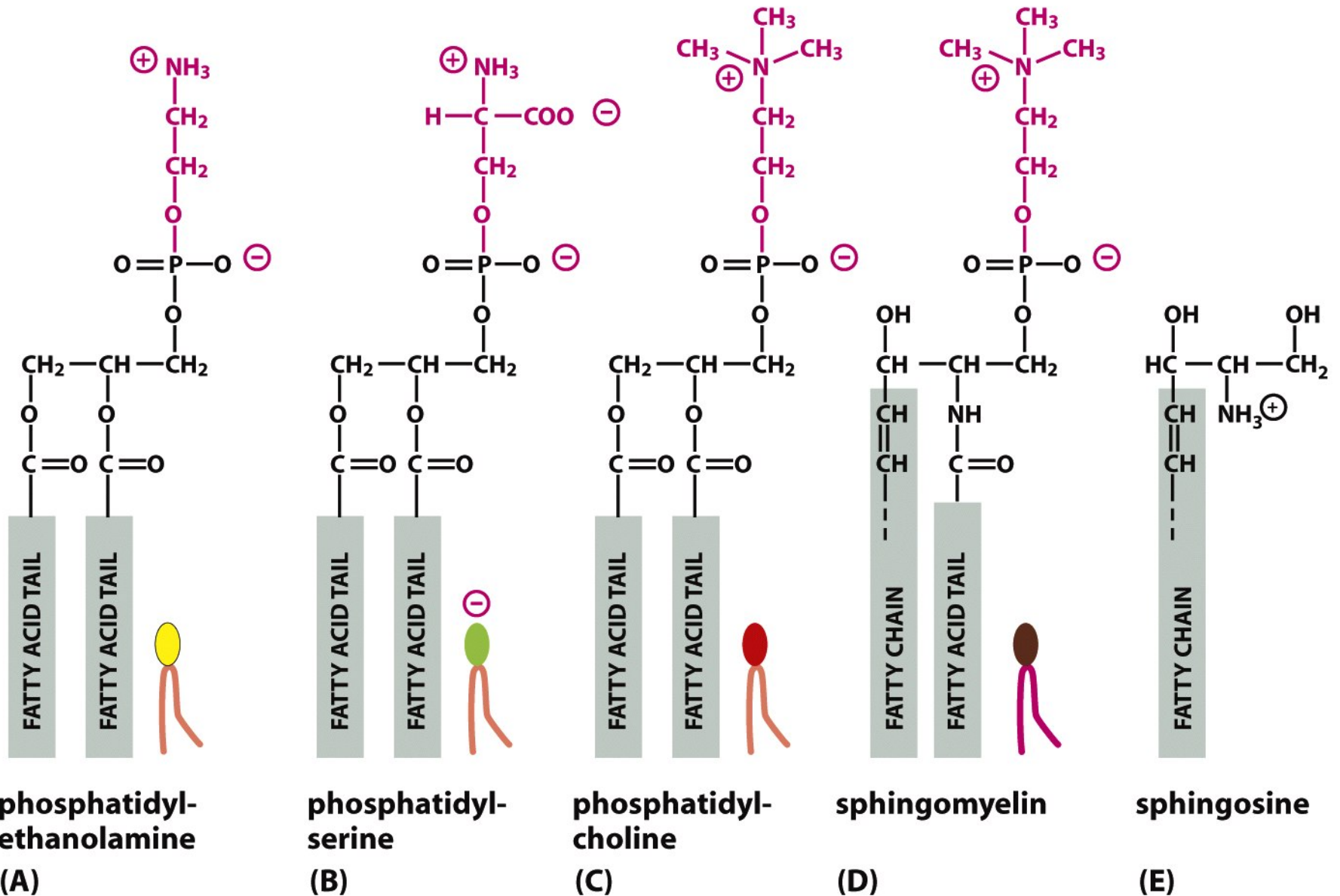
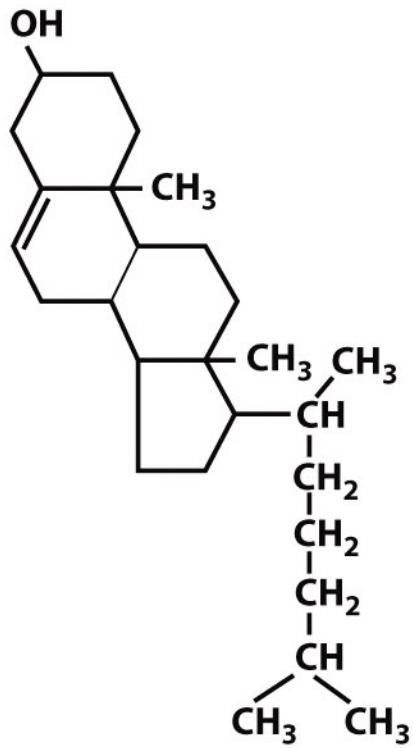
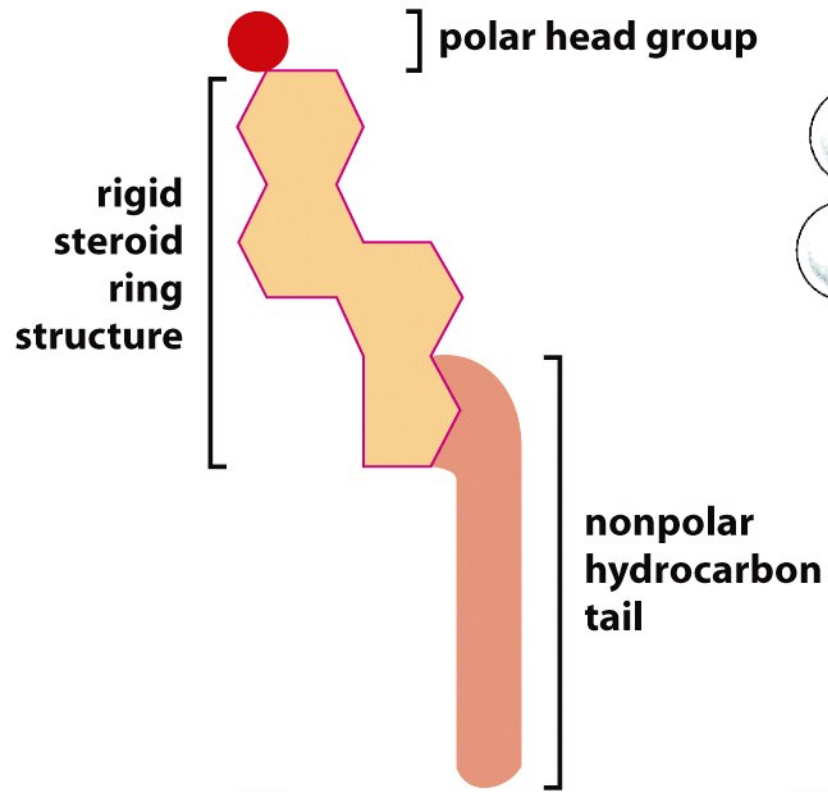


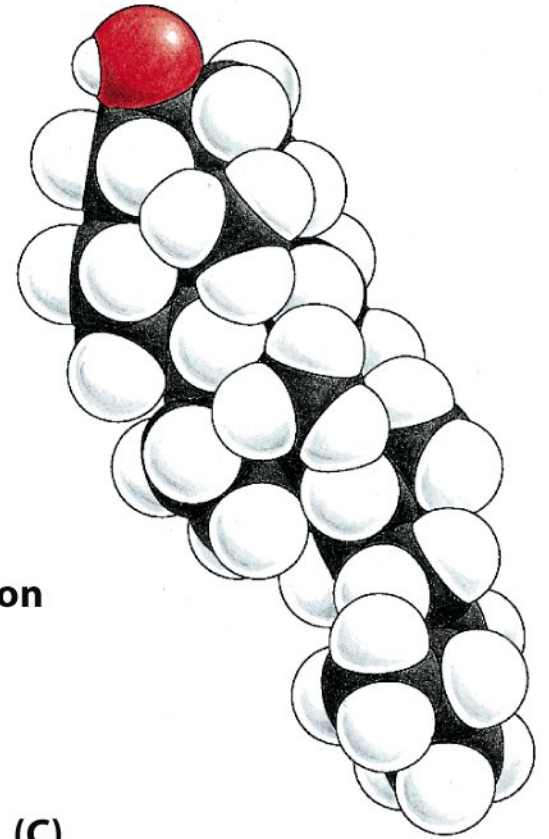
Figure 10-3 *Molecular Biology of the Cell* (© Garland Science 2008)



(A)



(B)



(C)

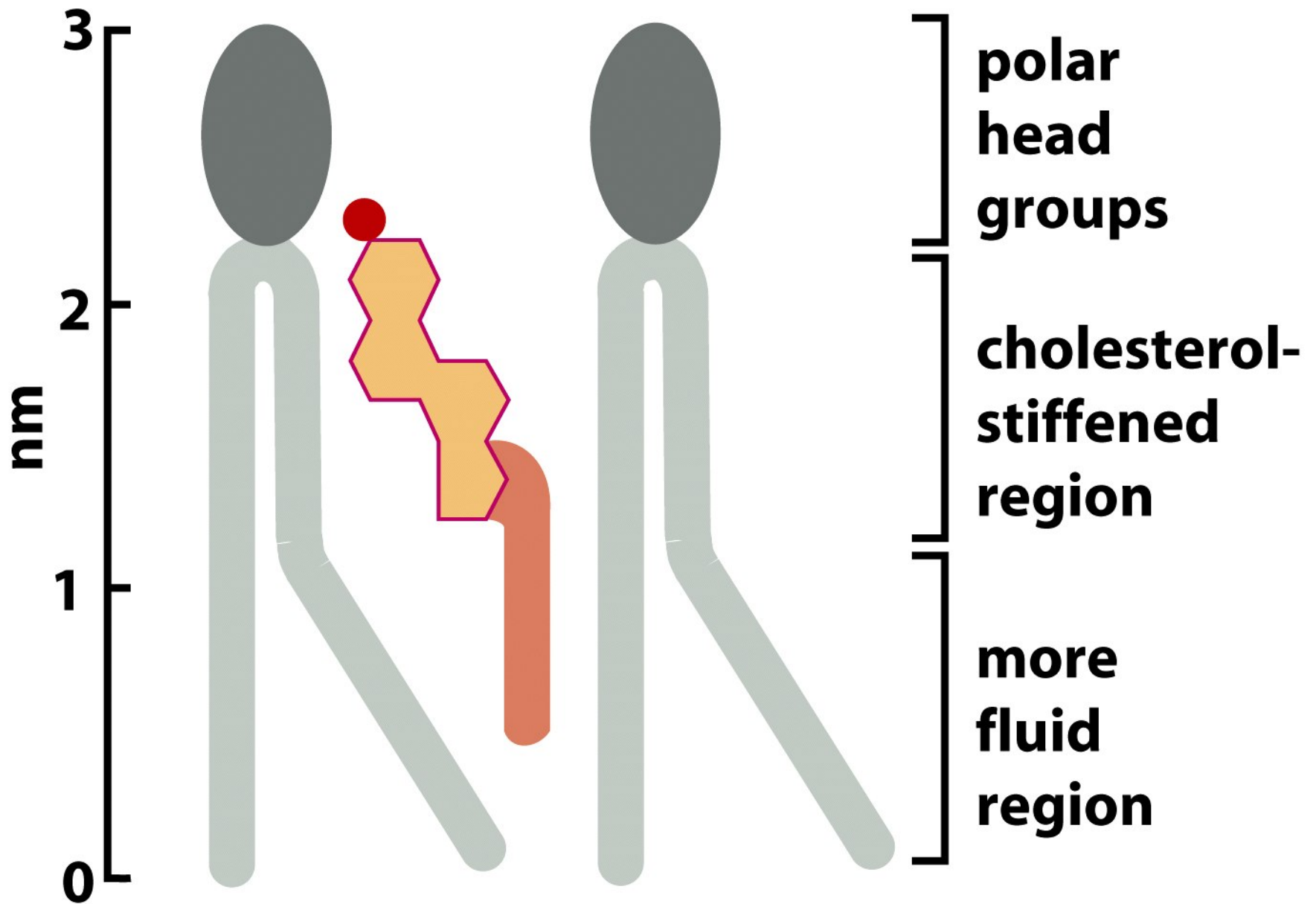


Figure 10-5 *Molecular Biology of the Cell* (© Garland Science 2008)



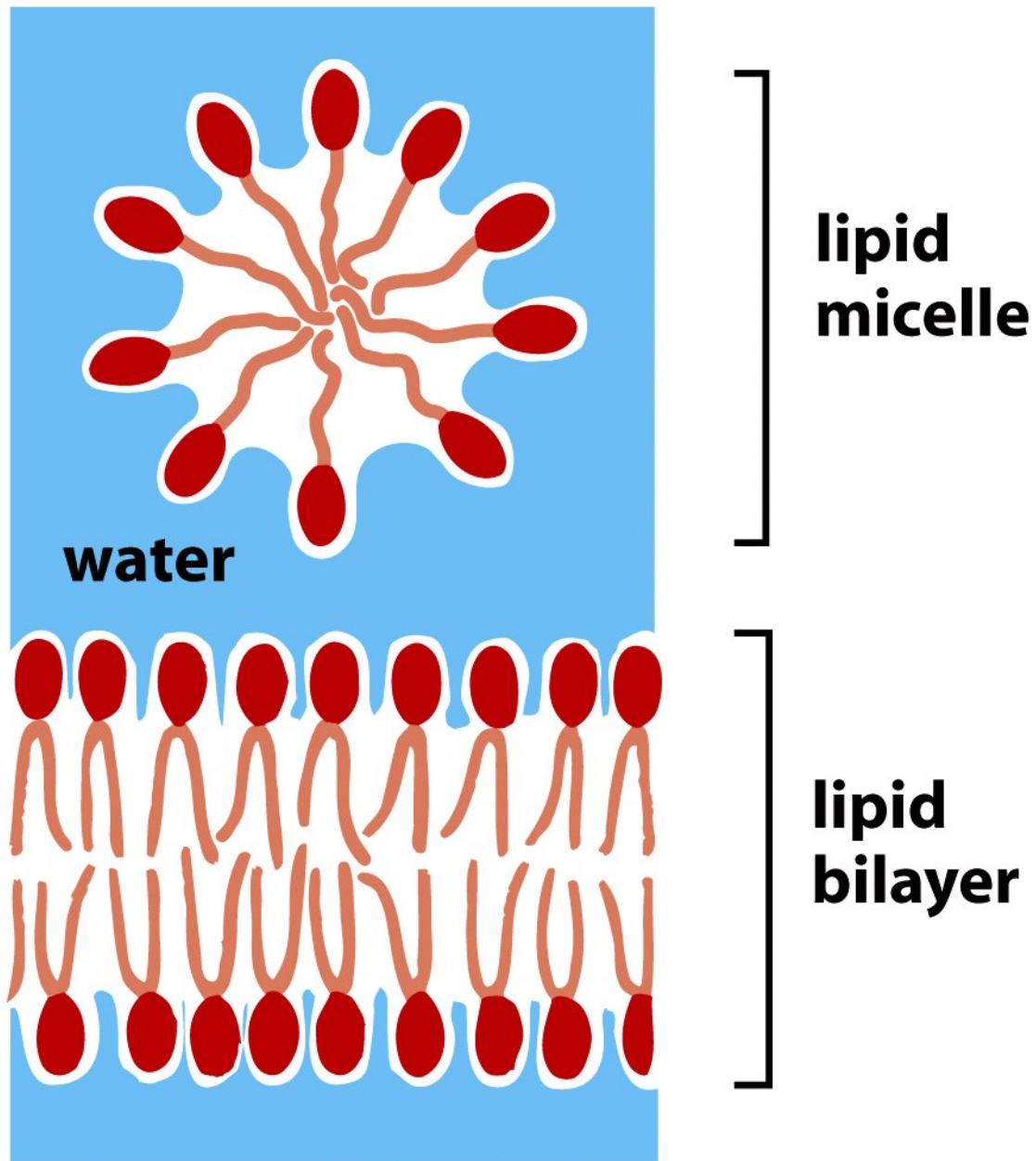
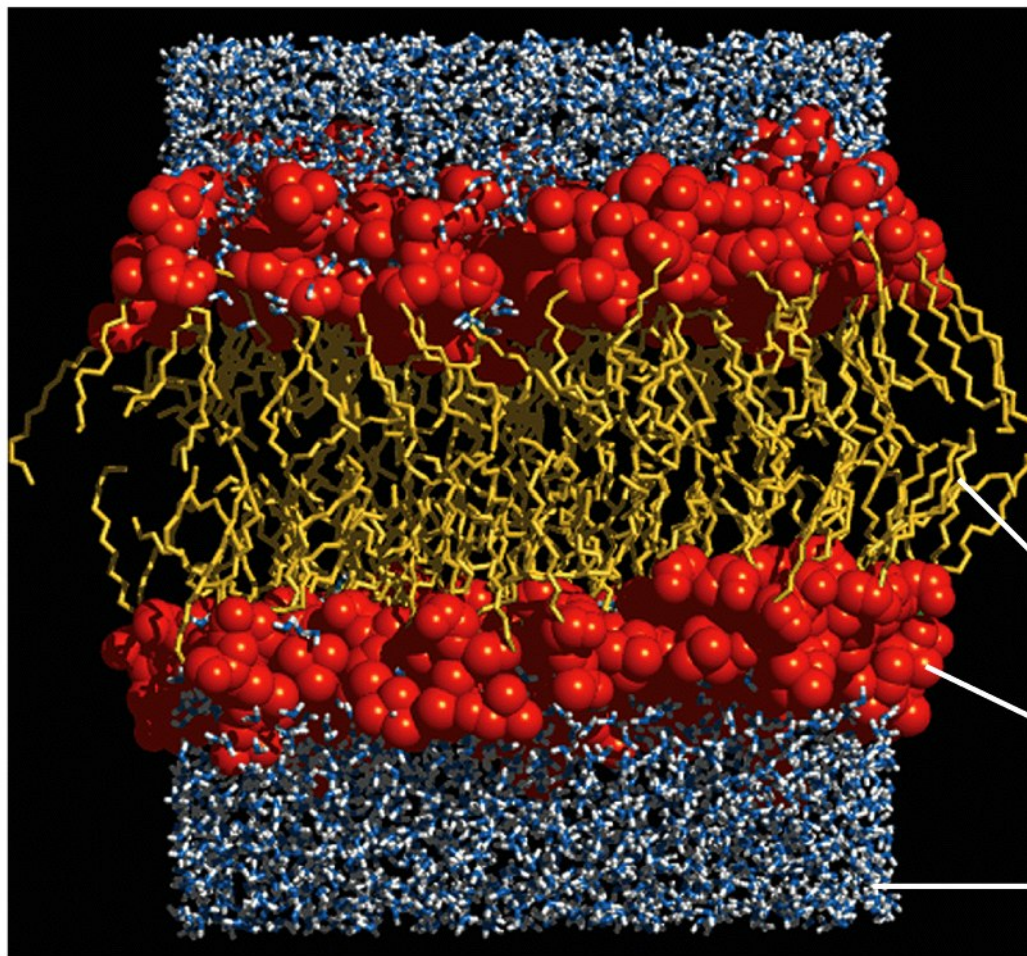
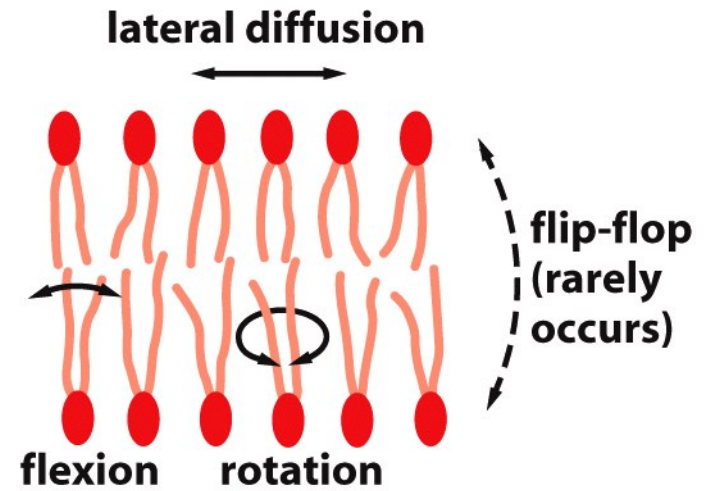


Figure 10-7b *Molecular Biology of the Cell* (© Garland Science 2008)



(A)

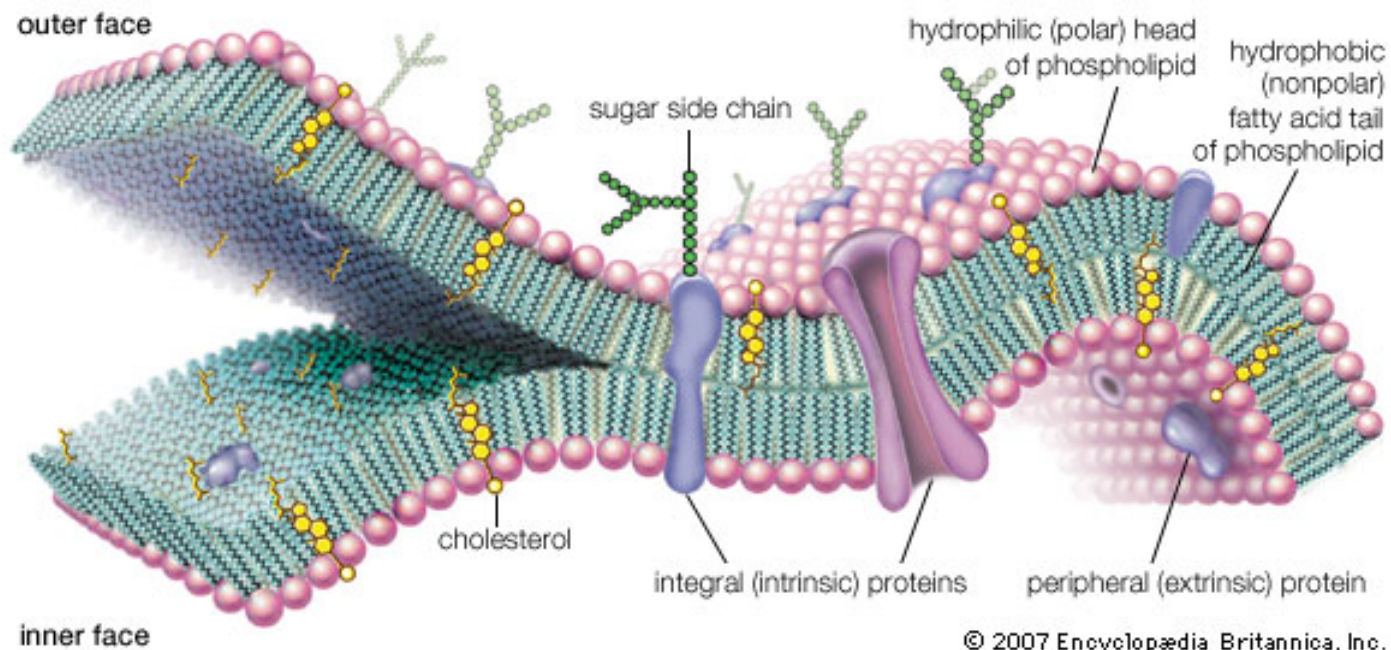


(B)

fatty acid tails

lipid head groups

water molecules



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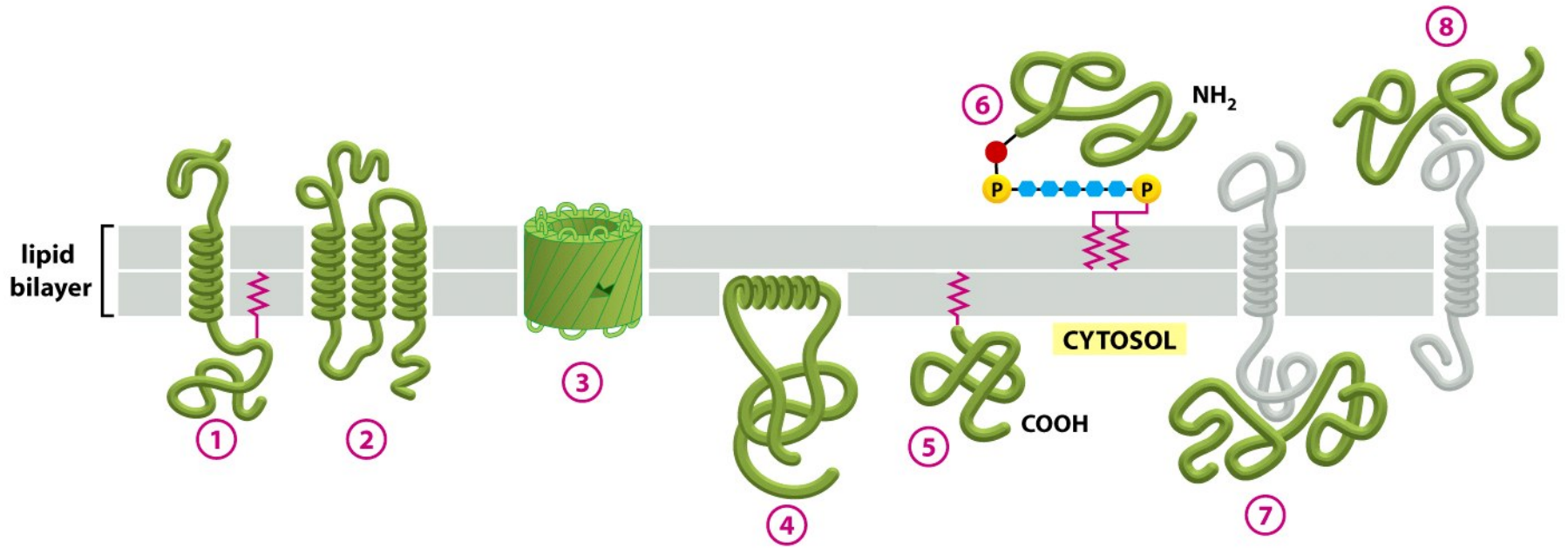
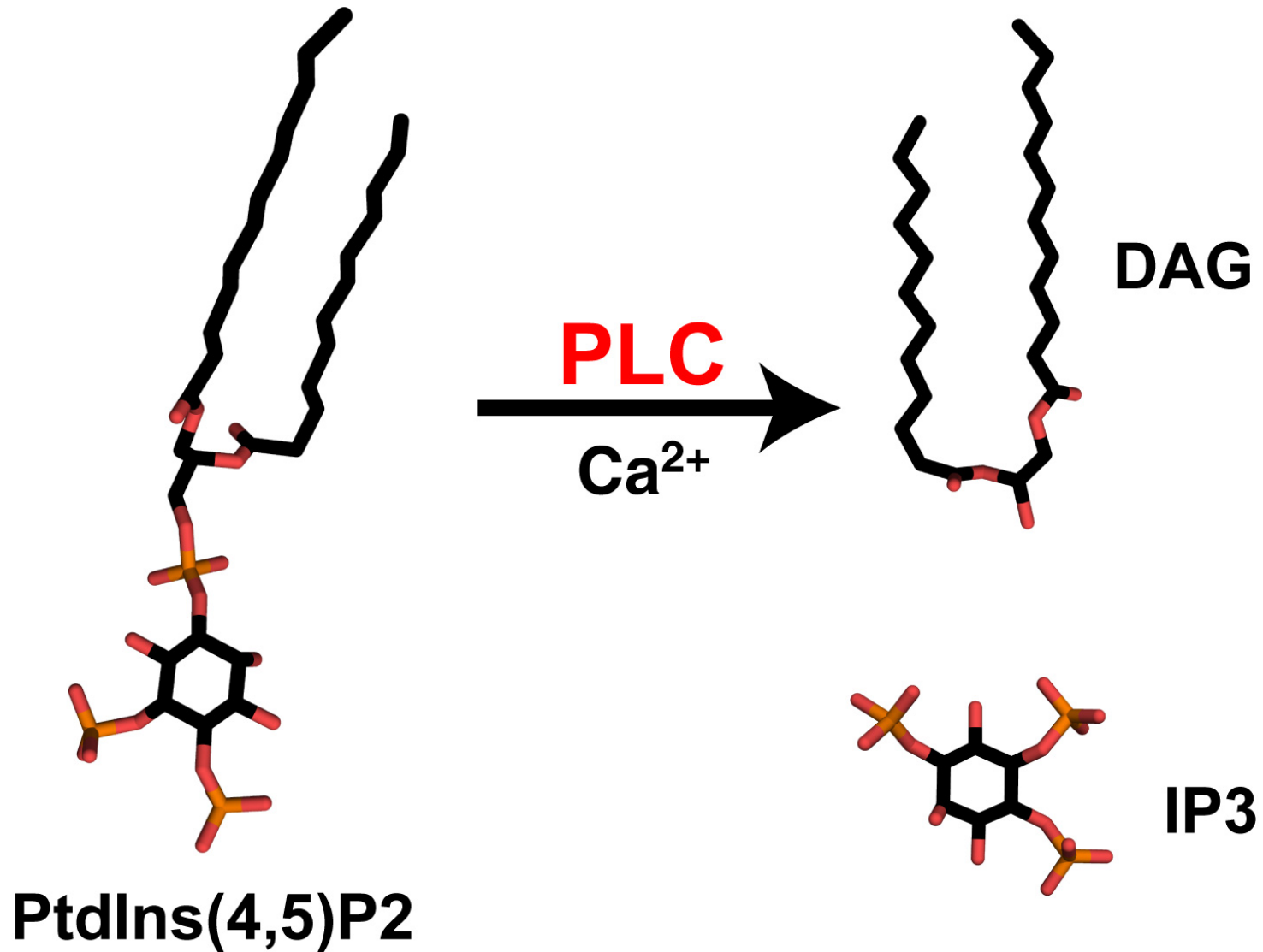


Figure 10-19 *Molecular Biology of the Cell* (© Garland Science 2008)

# PLCs are phospholipases



# PLC- $\beta$ isozymes are classic effectors of heterotrimeric G proteins

