Detection, and generation of cellular response

# Transduction of Hormonal Signal

# Signal Transduction

- Transduction: conversion of one form of a signal to another so as cells can produce many kinds of responses in different ways
- Amplification is a MUST
- Signal (polar, large) should bind receptors:
  - Intrinsic
  - Transmembrane
  - Intra- & extracellular domains
- Is that enough? The need for 2nd messenger
  - Few in number
  - Restricted movement

# Second messengers

- Ability to diffuse to other cellular compartments
- Amplification of the signal
  - Enzyme activation
  - Membrane channels
- Some second messengers are common in multiple signaling pathways (≈ 30 hormones uses cAMP!!!)
  - Permits fine tuning but can pose problems
- Types of 2nd messengers:
  - Small molecules: cAMP, cGMP, $Ca^{+2}$
  - Phosphorylation through kinases

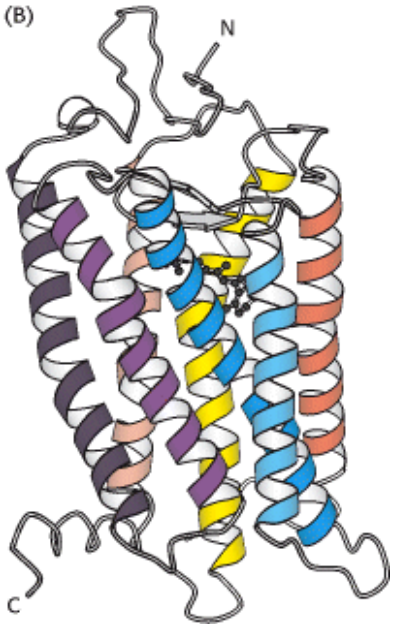
# Signal Termination

- Is it important?
  - Keeps cells responsive to new signals
  - Failure of termination may cause problem e.g GH & cancer

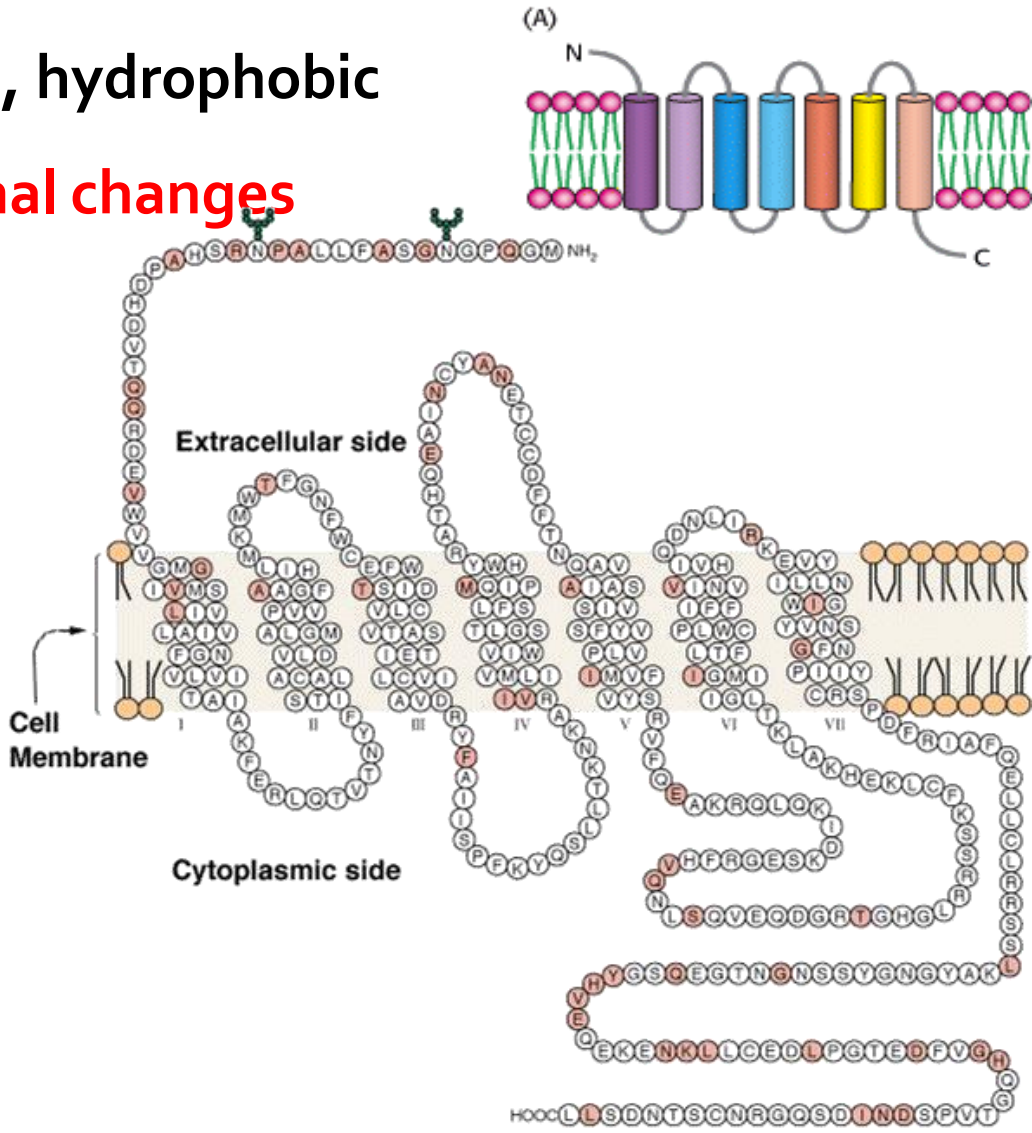
- How it is achieved?
  - Degradation of the second messenger
  - Dephosphorylation by hydrolysis

# Membrane Associated Receptors
# 7-Transmembrane Helix Receptors (7TM)

- 7 α-helices: H-bonding, rigid, hydrophobic
- Signal induces conformational changes
- Is it enough?




Rhodopsin receptor



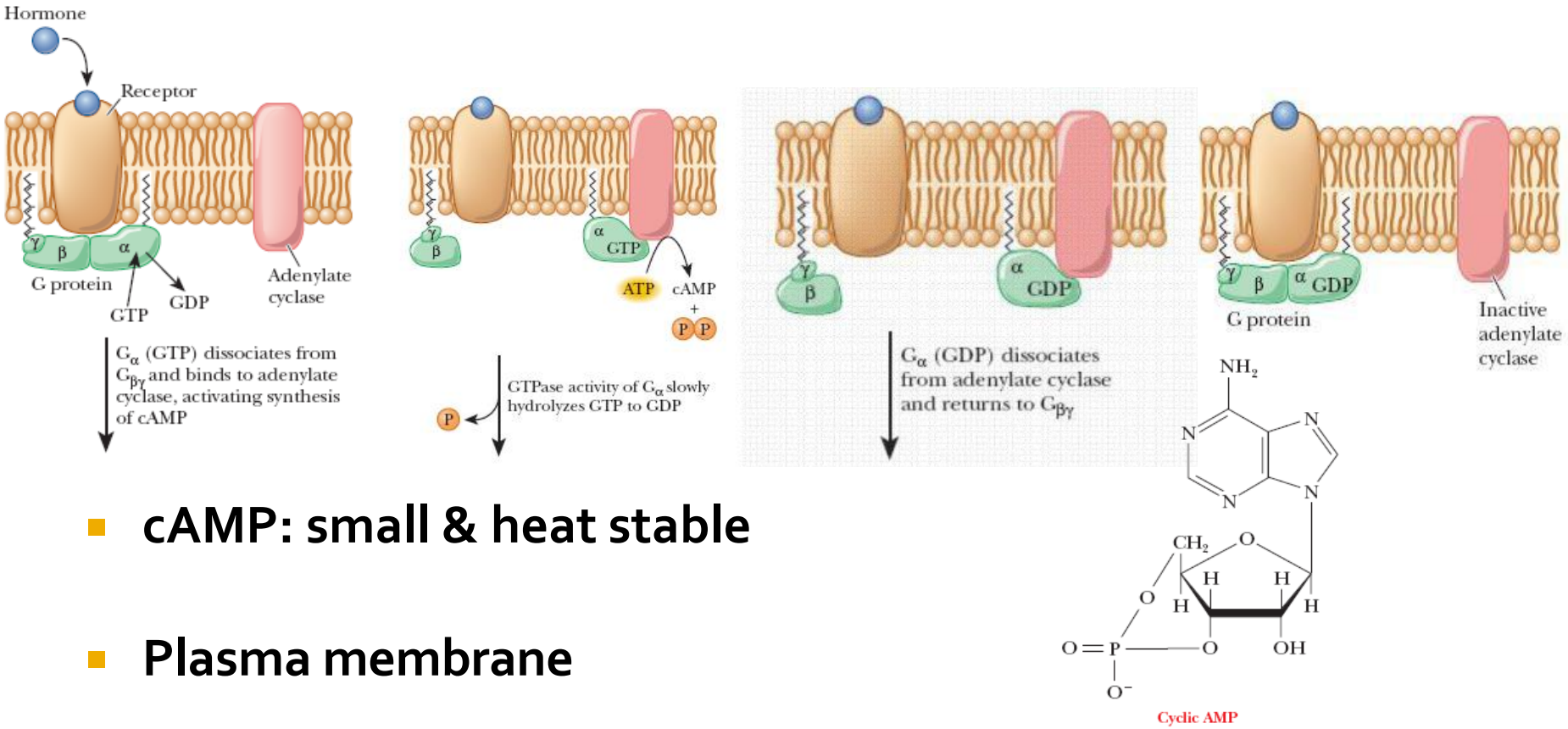

- Many Ser & Thr residues

# Biological Functions Mediated by 7TM

- Examples:
- Smell, Taste, Vision
- Neurotransmission
- Hormone Secretion
- Chemotaxis
- Exocytosis
- Cell Growth, Development
- Viral Infection

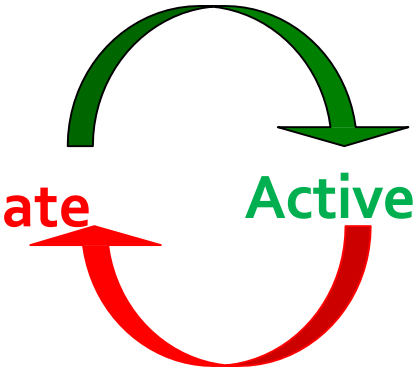
**All these receptors share the same basic structure; however, they differ in their specificity and effects**

# G-proteins & cAMP




- **cAMP: small & heat stable**
- **Plasma membrane**
- **Hormone → Specific receptor (β1- or β2-adrenergic receptor) → G protein → Adenylate cyclase → cAMP → protein kinase A → phosphorylation**

# G Protein cycles between two forms



Inactive State

Active State

Bound to GDP

Bound to GTP

Heterotrimer αβγ

Dissociated α + βγ dimer

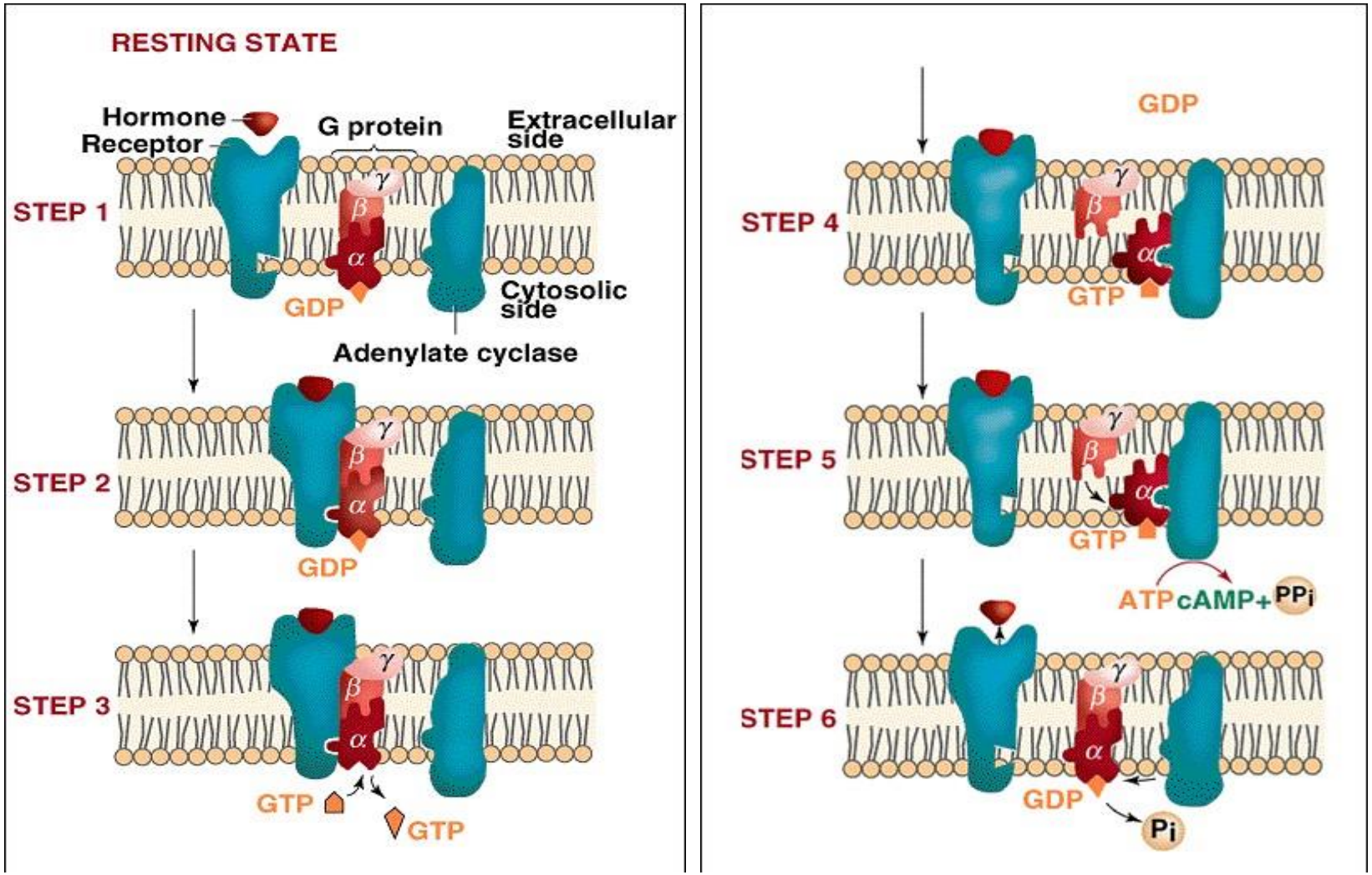
Activated Receptor

↑ Adenylate Cyclase

↑ cAMP

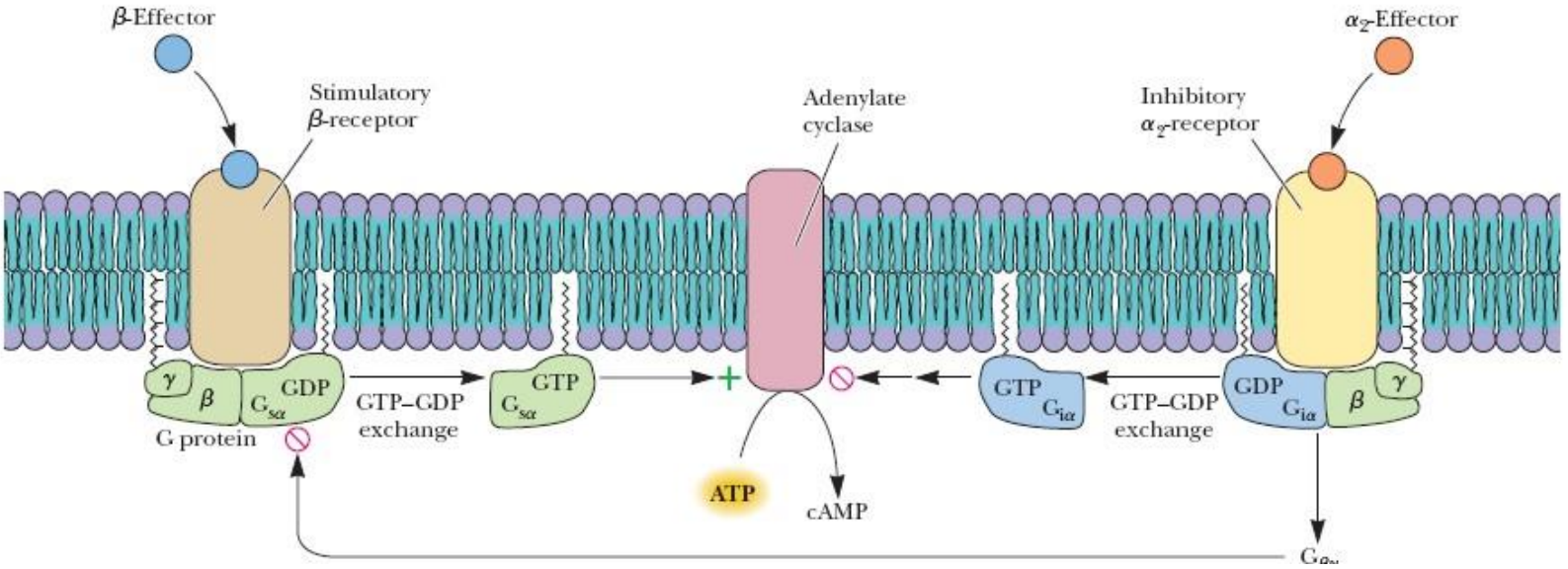
Biochemical Response

**α subunit has GTPase Activity**




**α subunit has GTPase Activity**

# G protein: stimulatory or inhibitory?




- **Cyclic AMP & G Proteins:**
  - Hormone ⟶ receptor (α2-receptor) ⟶ G protein ⟶ inhibits adenylate cyclase

# G Proteins

- G proteins:
  - More than 100 known G protein–coupled receptors and more than 20 known G proteins
  - Can be activated by combinations of hormones
    - Epinephrine & glucagon act via a stimulatory G protein in liver cells
  - Other than cAMP:
    - Stimulating phospholipase C
    - Opening or closing membrane ion channels

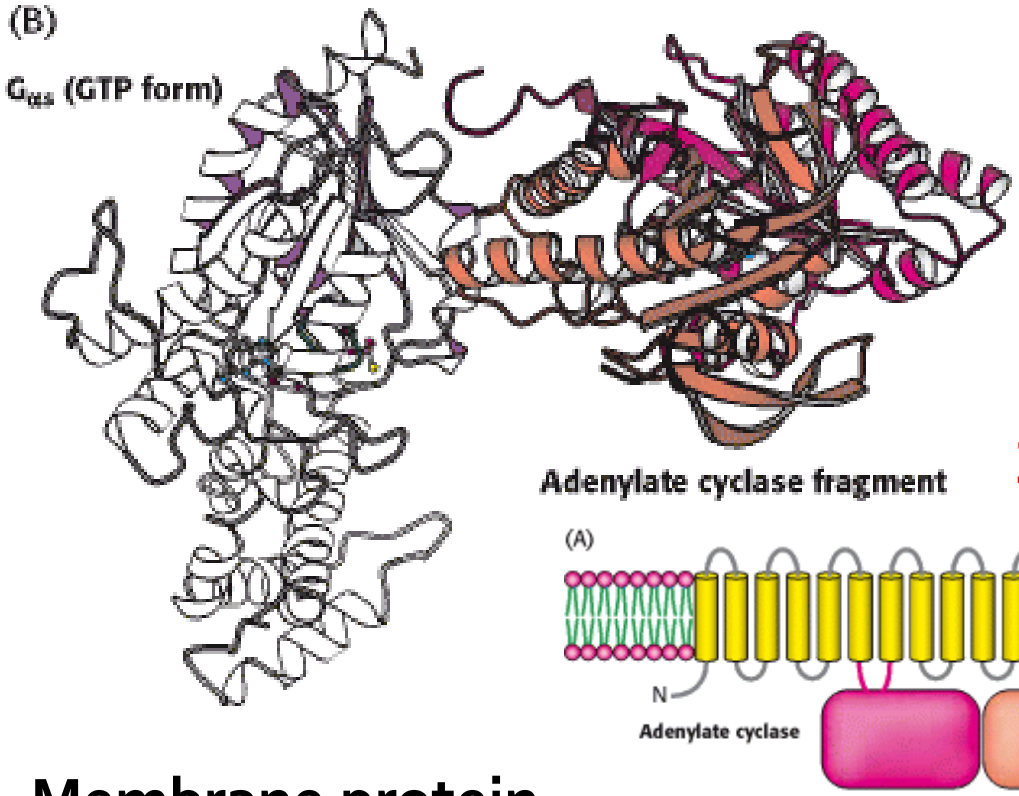
# G Proteins (cont.)

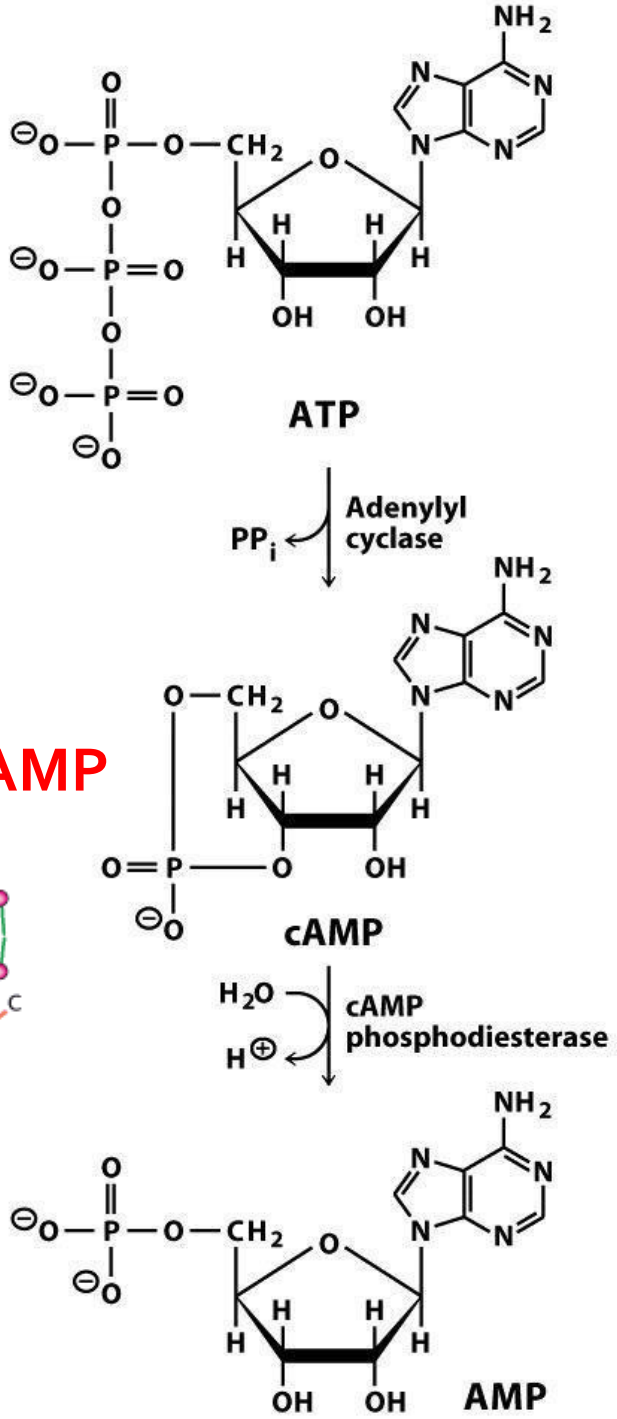
- α and γ Subunits have covalently attached fatty acid
- α and βγ can interact with other proteins
- All 7TM receptors appear to be coupled to G proteins

GPCRs

- Amplification: receptor → 100's of G protein → 100's of adenylate cyclase → 100's X 1000's molecules/sec of cAMP

# Adenylate Cyclase



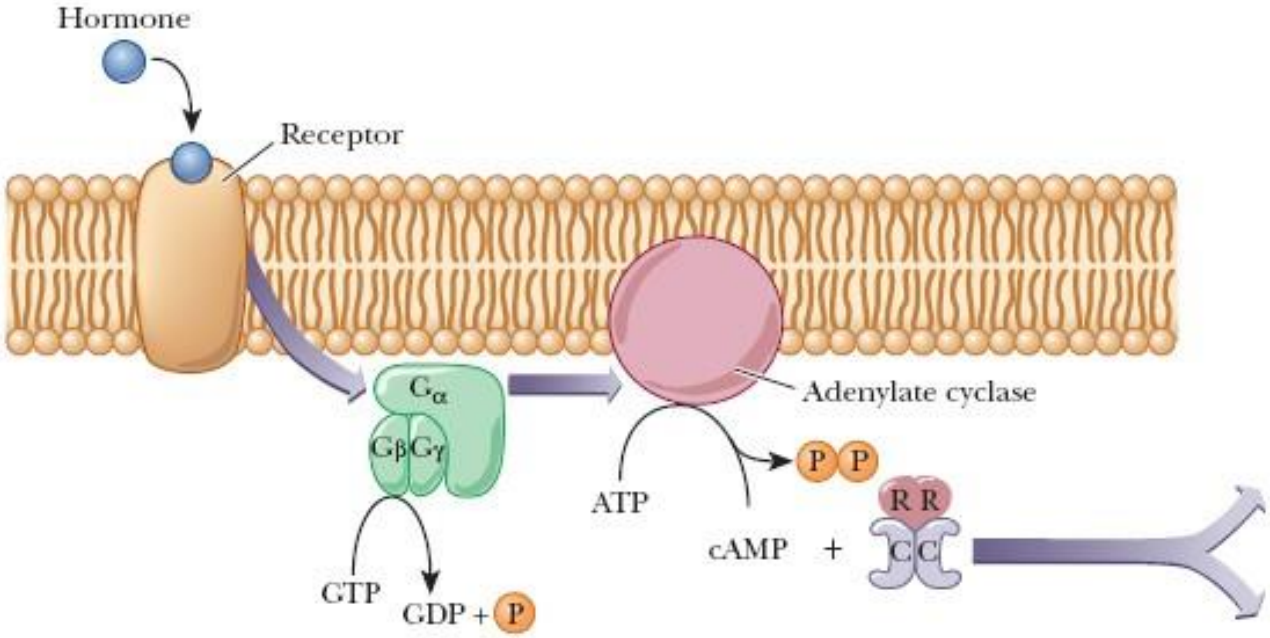

3'5' CAMP

- Membrane protein
- 12 helices
- Two large intracellular domains
- Activated by G protein

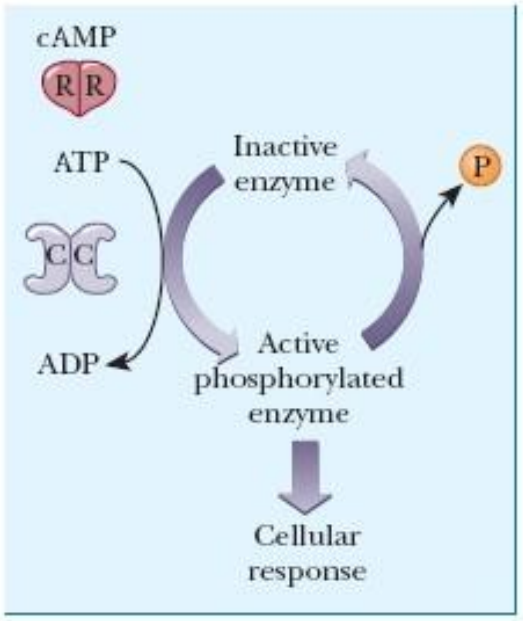
# cAMP can affect a wide range of cellular processes

- ↑ degradation of storage fuels
- **↑ secretion of acid by gastric mucosa**
- Dispersion of melanin pigment granules
- ↓ aggregation of blood plateletes
- Opening of chloride channels

# Then what?




**Usually:**
**Ser or Thr**

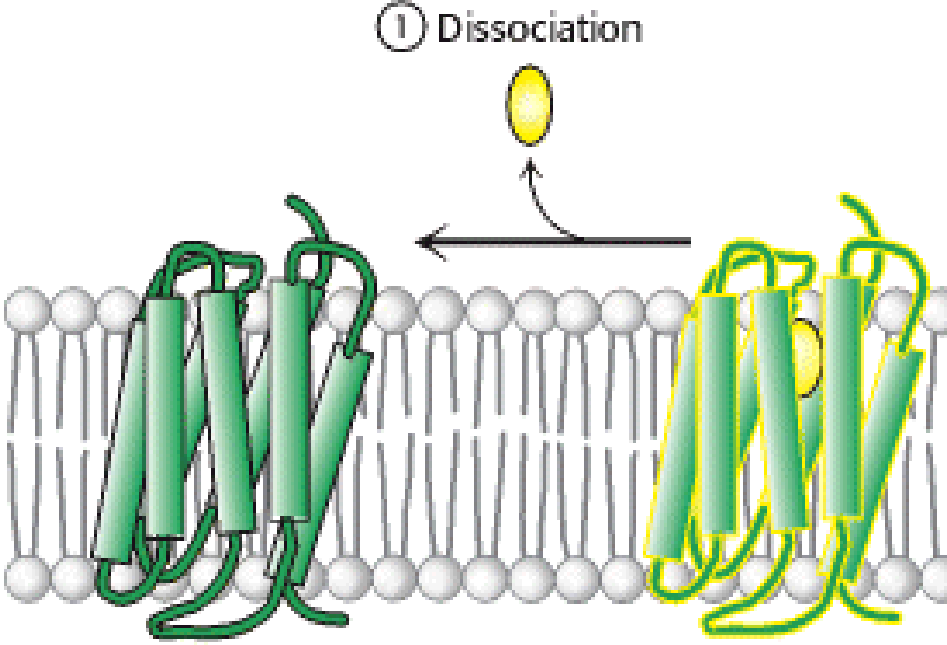


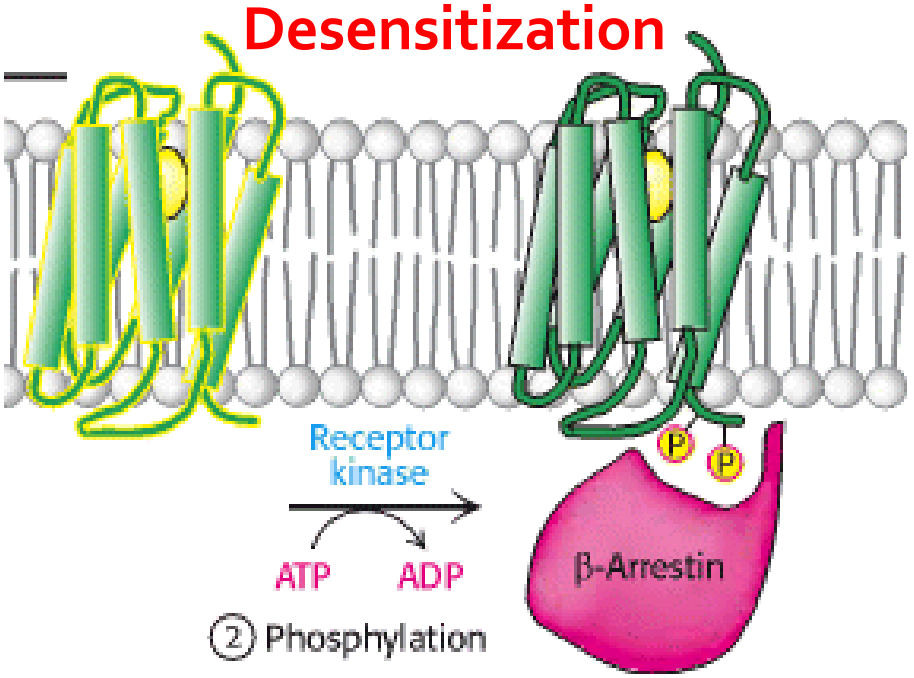

**Glycogen Synthase!!**

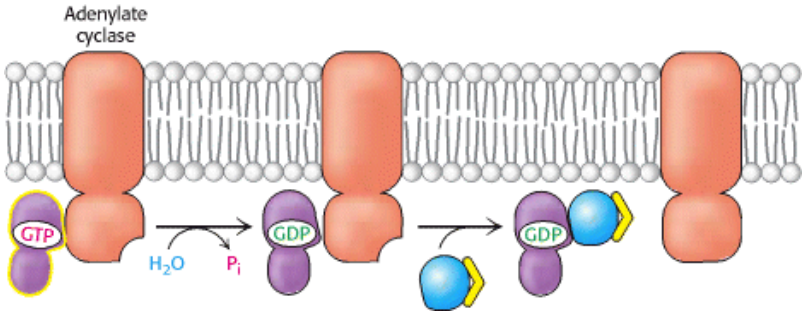
Signal Amplification

# Switching off the signal

- **Dissociation** of the hormone
- **GTPase** activity of Gα subunit
- **Hydrolysis** of cAMP (phosphodiesterase)
- Phosphorylation of the hormone bound-receptor followed by binding to **β-Arrestin**

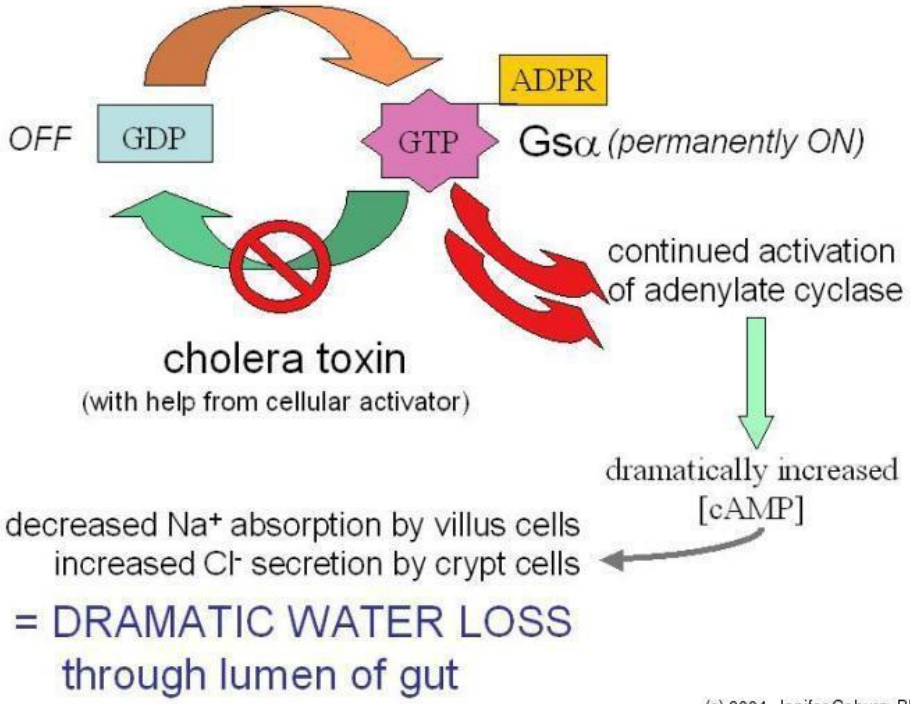


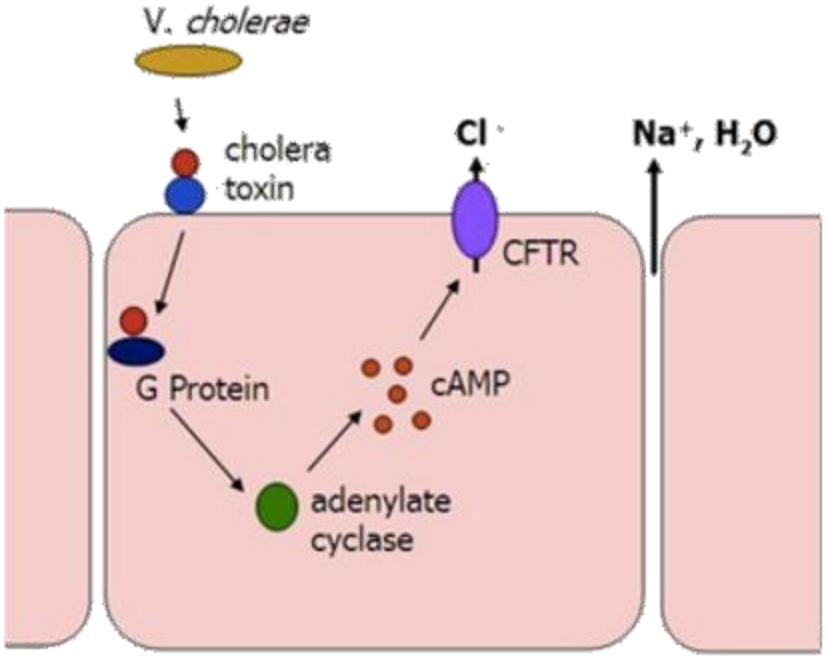



# Cholera

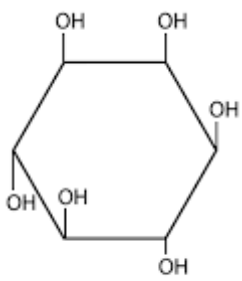
- **Cholera toxin → unregulated activity of adenylate cyclase in epithelial cells → Excessive cAMP in epithelial cells stimulates active transport of $Na^+$ → large flow of $Na^+$ and water from the mucosa → diarrhea**



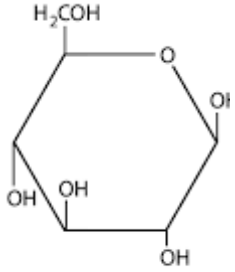
# The Phosphoinositide Cascade

- Used by many hormones (e.g. ADH)
- Binding of a hormone to 7TM receptor



Inositol

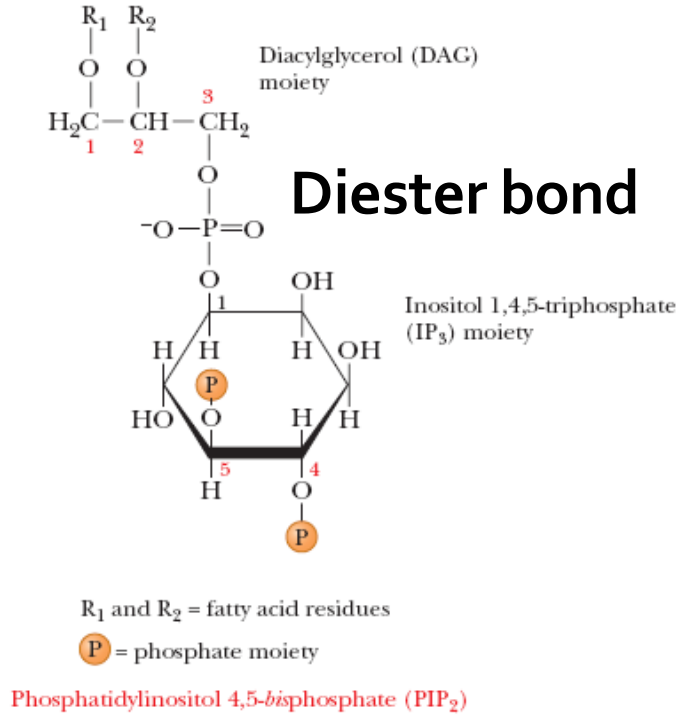
Activation of G Protein

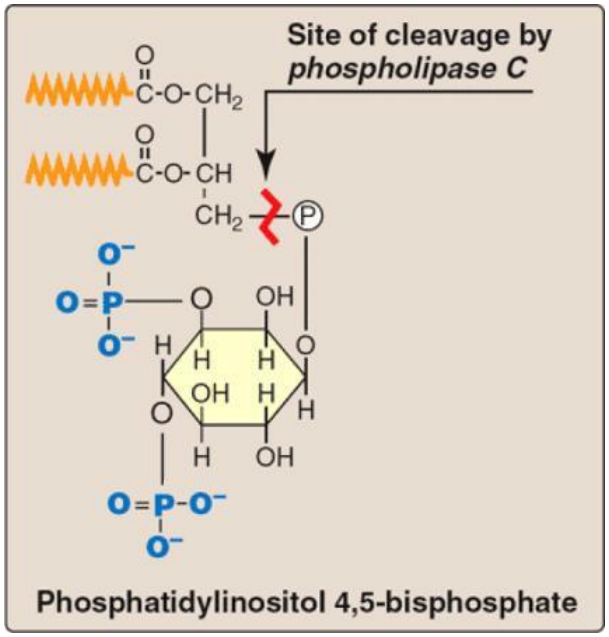


Glucose

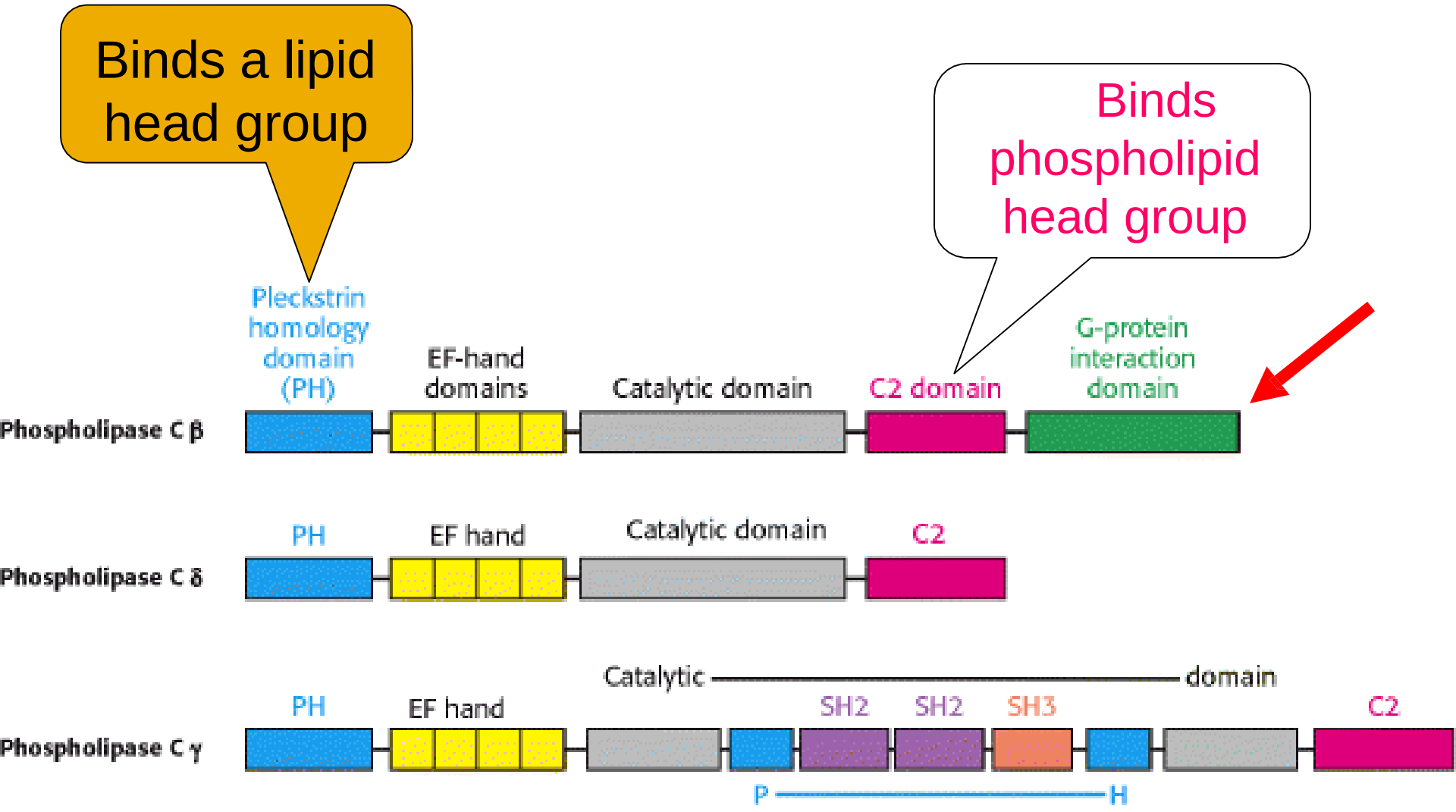
Activation of Phospholipase C (many isoforms) – PIP2

- Two messengers are produced
  - Inositol 1,4,5-trisphosphate, hydrophilic, (Soluble)
    - IP3 is the actual second messenger
  - Diacyclglycerol, amphipathic (membrane)

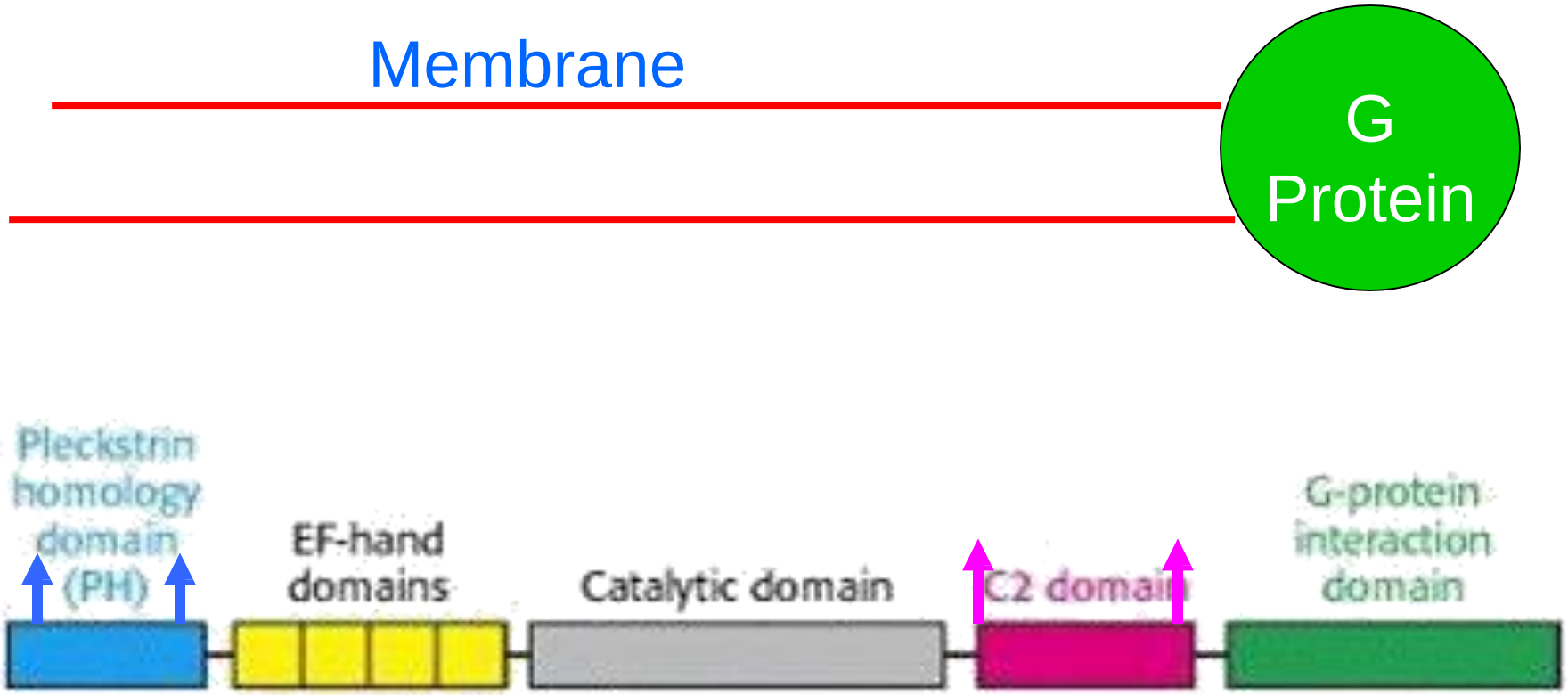


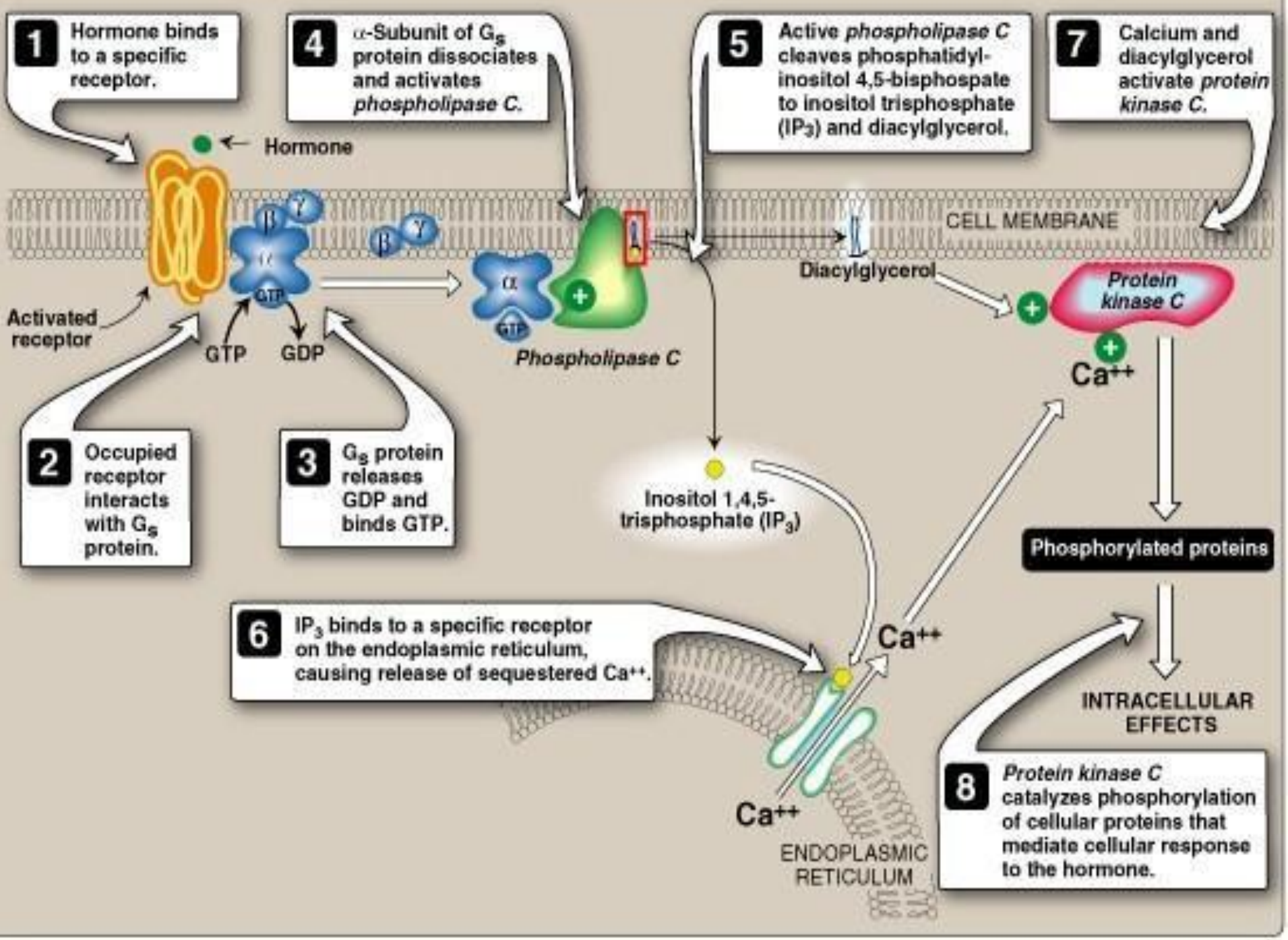



# The domain structures of three isoforms of Phospholipase C



# Binding of a G protein brings the enzyme into a catalytically active form




1 Hormone binds to a specific receptor.
Hormone
Activated receptor
GTP
GDP
2 Occupied receptor interacts with $G_s$ protein.
3 $G_s$ protein releases GDP and binds GTP.
4 α-Subunit of $G_s$ protein dissociates and activates *phospholipase C*.
Phospholipase C
5 Active *phospholipase C* cleaves phosphatidyl-inositol 4,5-bisphospate to inositol trisphosphate ($IP_3$) and diacylglycerol.
CELL MEMBRANE
Diacylglycerol
7 Calcium and diacylglycerol activate *protein kinase C*.
Protein kinase C
$Ca^{++}$
Inositol 1,4,5-trisphosphate ($IP_3$)
Phosphorylated proteins
6 $IP_3$ binds to a specific receptor on the endoplasmic reticulum, causing release of sequestered $Ca^{++}$.
$Ca^{++}$
$Ca^{++}$
ENDOPLASMIC RETICULUM
INTRACELLULAR EFFECTS
8 *Protein kinase C* catalyzes phosphorylation of cellular proteins that mediate cellular response to the hormone.

# Effects of Second Messengers

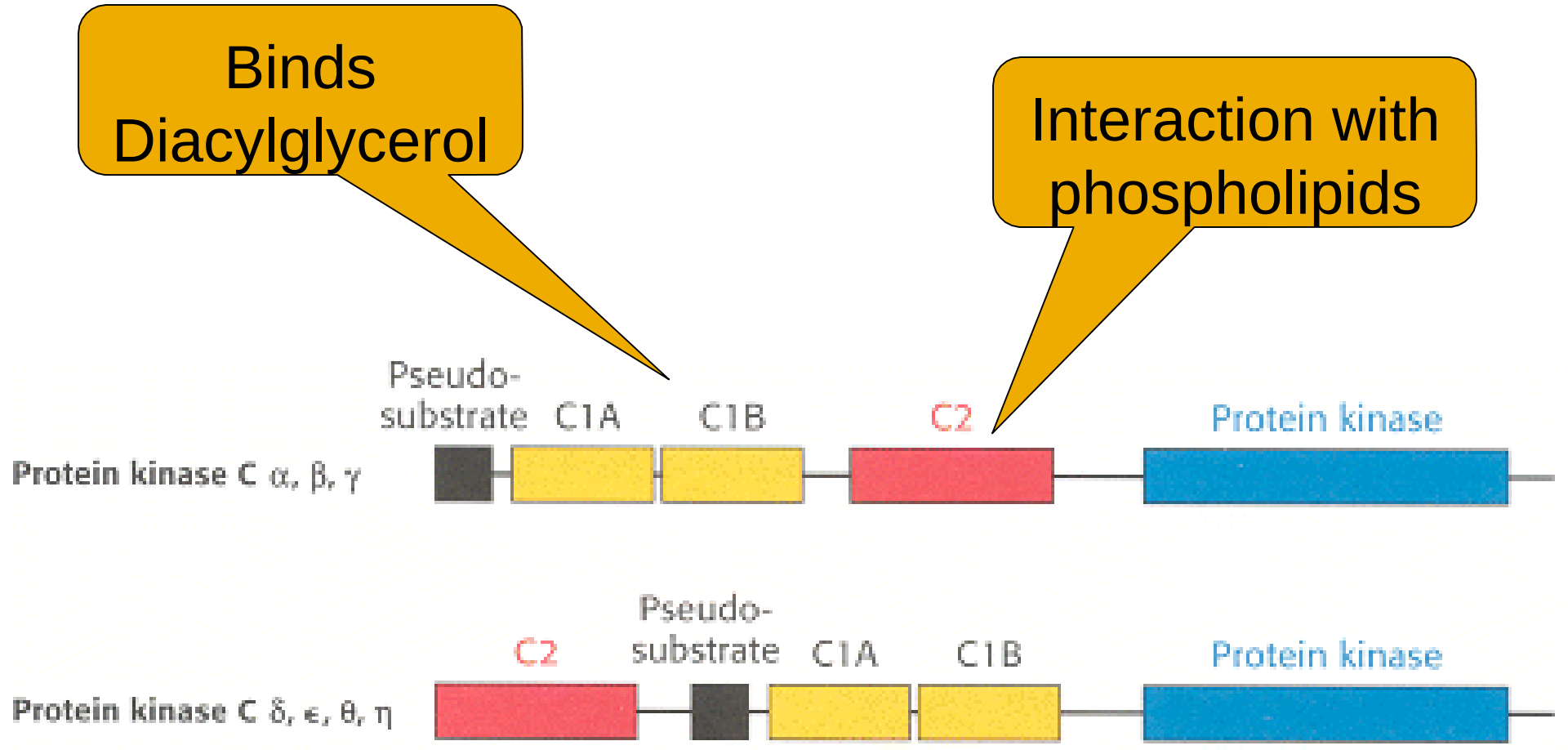
## Inositol trisphosphate ($IP_3$)

- ✓ Opens Calcium Channels
- ✓ Binding to $IP_3$-gated Channel
- ✓ Cooperative binding (sigmoidal)

## Diacylglycerol (DAG)

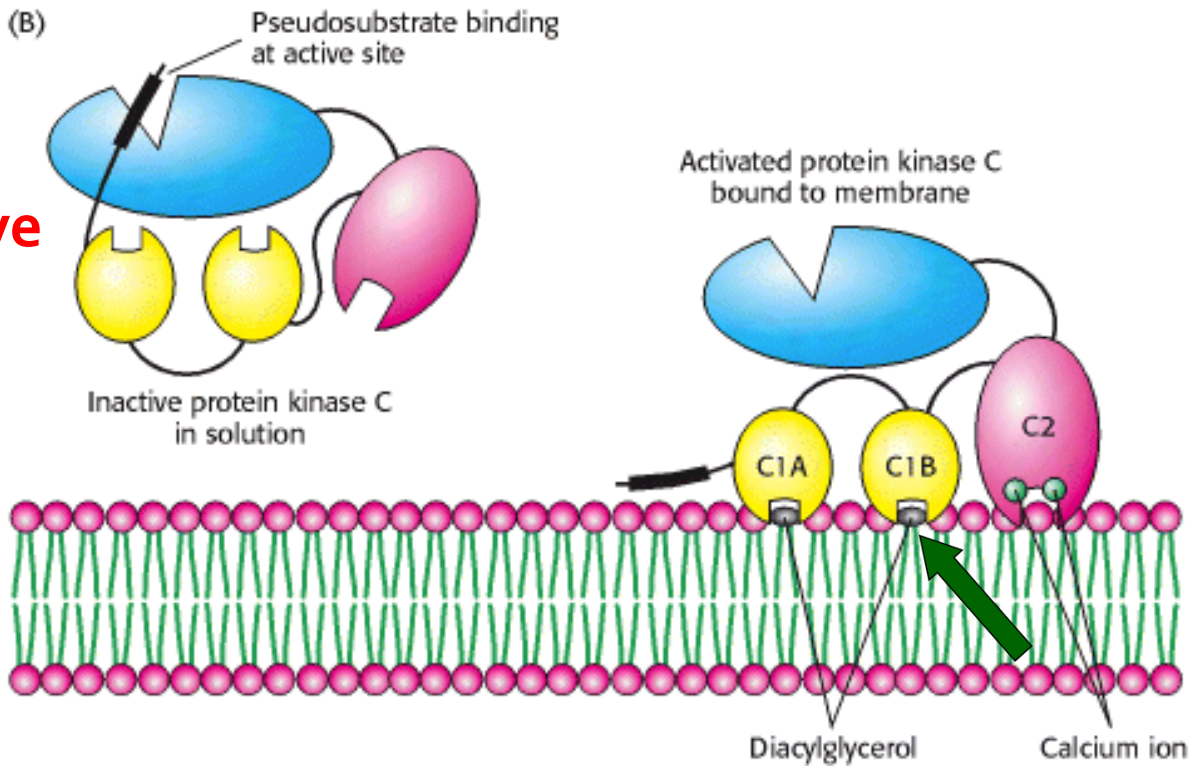
- ✓ Activates Protein Kinase C
- ✓ $Ca^{2+}$ is required
- ✓ Phosphorylation of many target proteins

# The domain structures of protein kinase C isoforms



# Pseudosubstrate Sequence

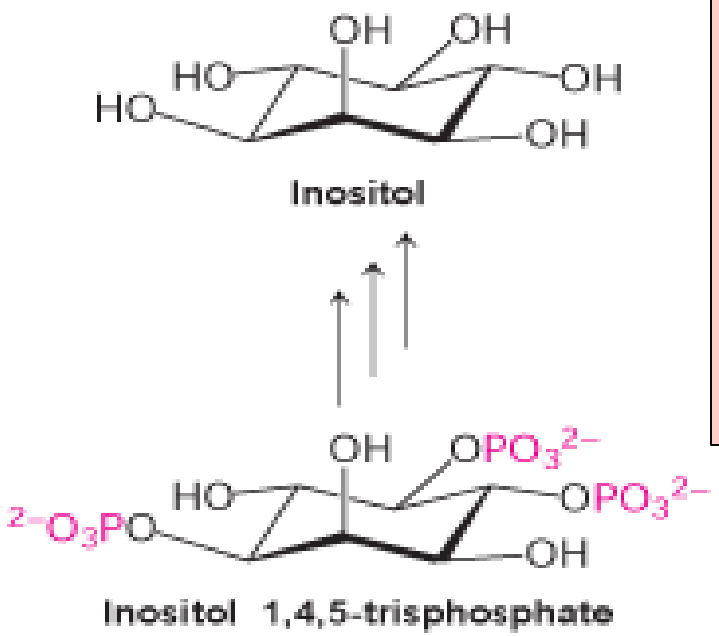
**Competitive Inhibitor**



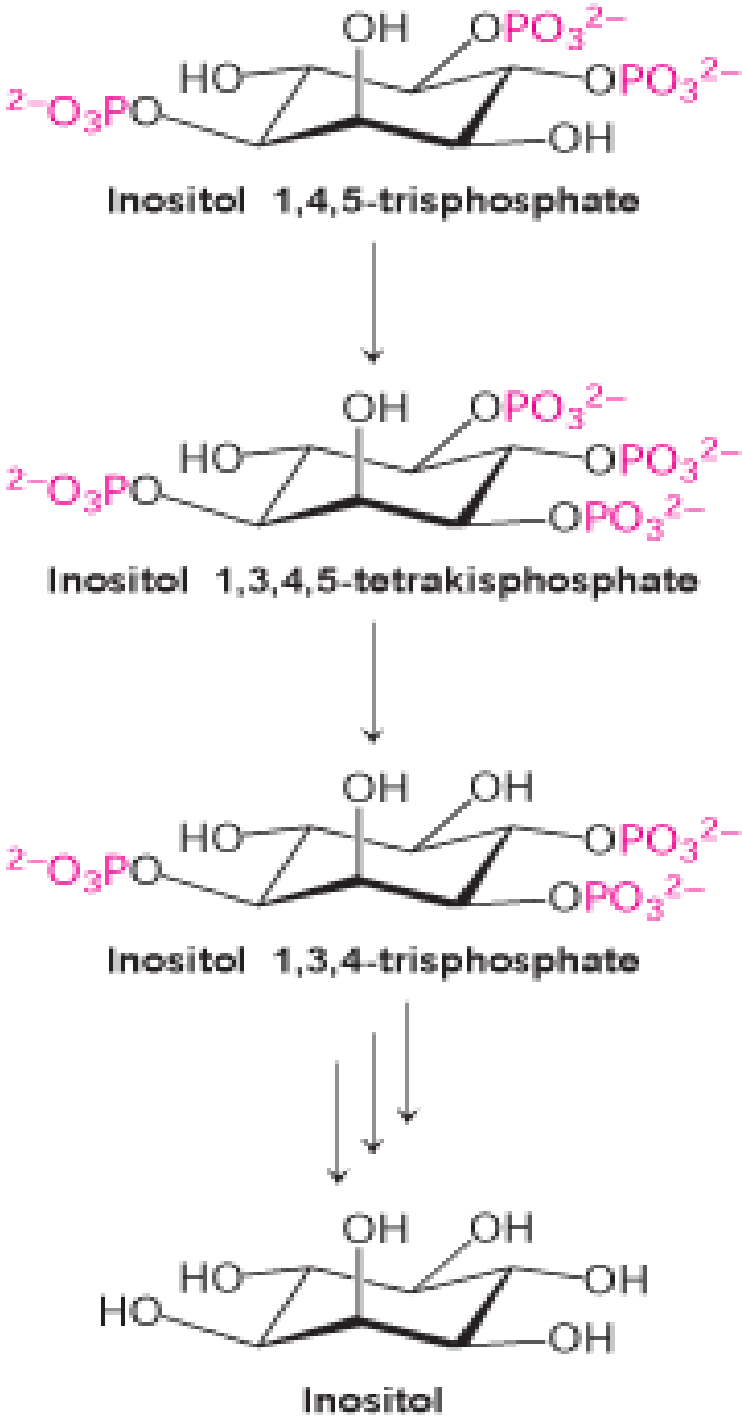

- Resembles the substrate sequence: A-R-K-G-A-L-R-Q-K
- Substrate Sequence: (S,T)
- Binds to the Enzyme's Active Site

# Termination of IP3 Signal

## IP3 is a Short-Lived Messenger



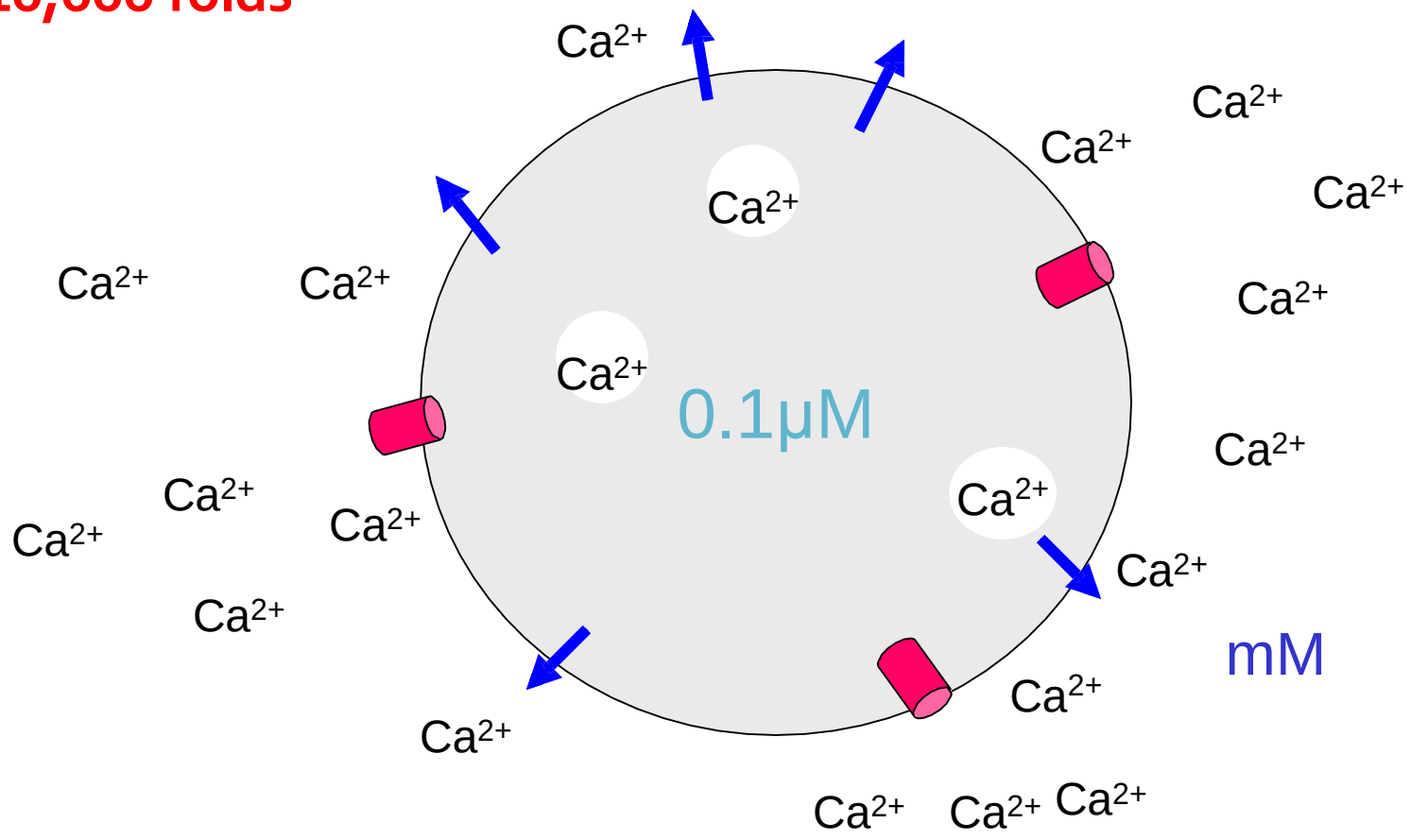

**Lithium ions, used to treat some psychological disorders Inhibits $IP_3$ recycling**



# Why $Ca^{2+}$?

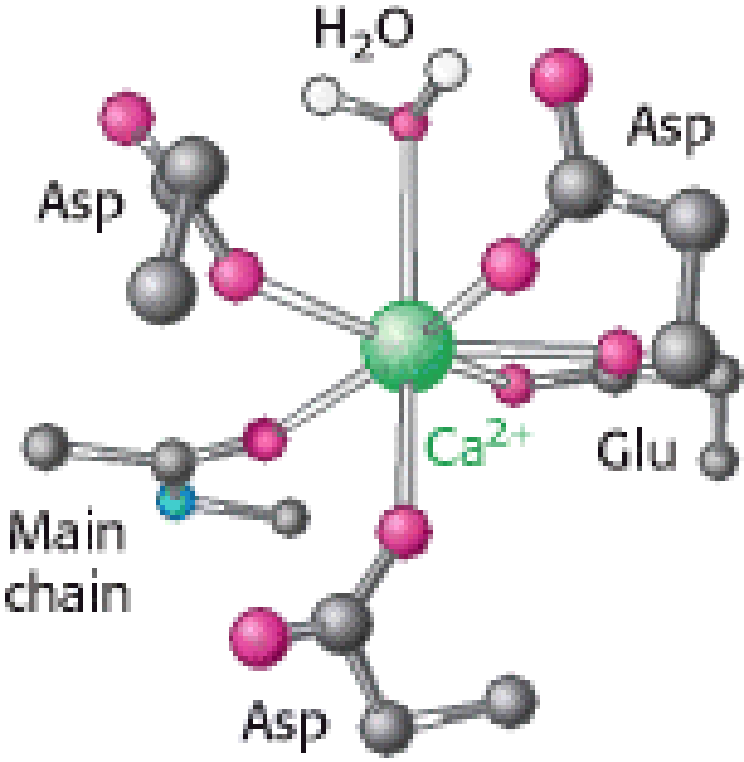
## A large difference in concentration

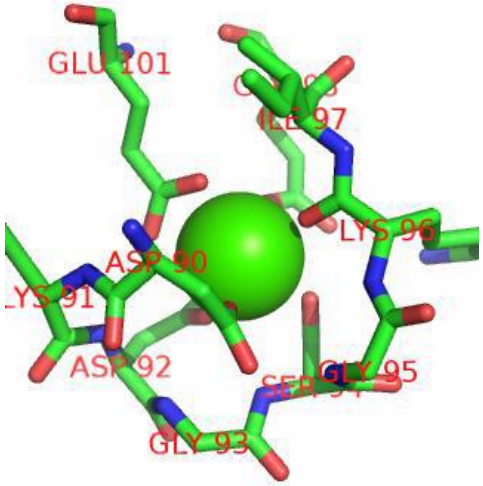
**10,000 folds**

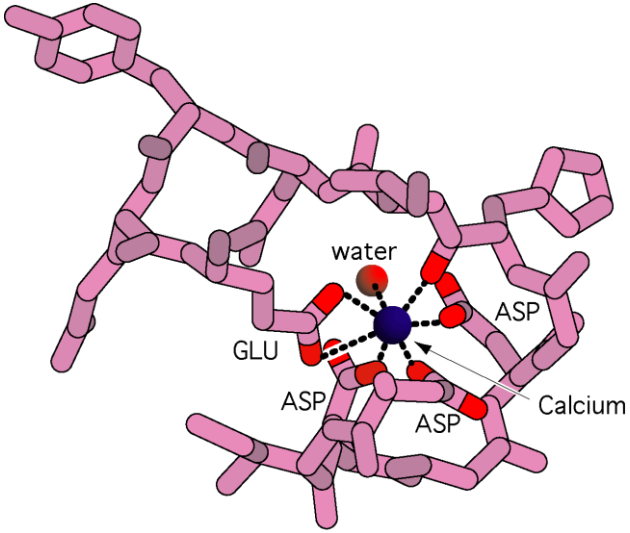


# Why $Ca^{2+}$?

- Ability to bind protein tightly
- 6-8 bonds with oxygen
- Conformational changes



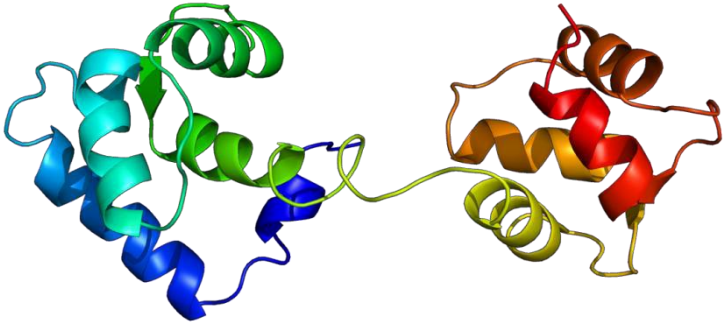



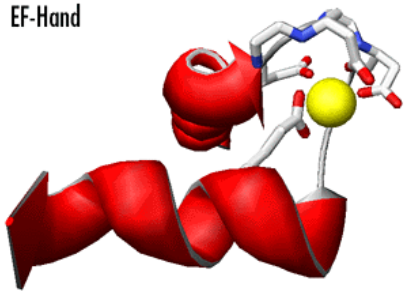
# Calcium Binding Proteins

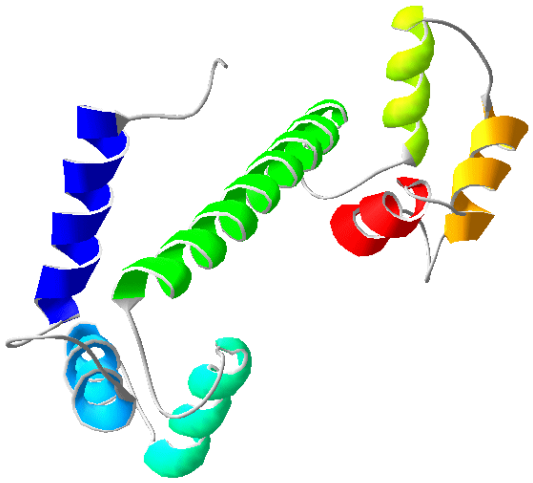
- Mediate the effects of Calcium ($Ca^{+2}$)
- Many proteins

  Calmodulin, Troponin C, Parvalbumin



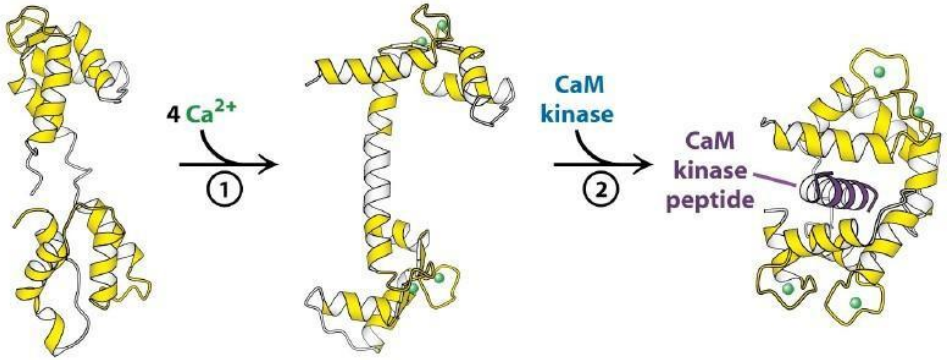
- Similar structures
  - Rich in Asp and Glu
    - Gln, Asn, Ser
  - Several α helical segments
  - Binding site is formed by
    - Helix Loop Helix
      - Super-secondary structure



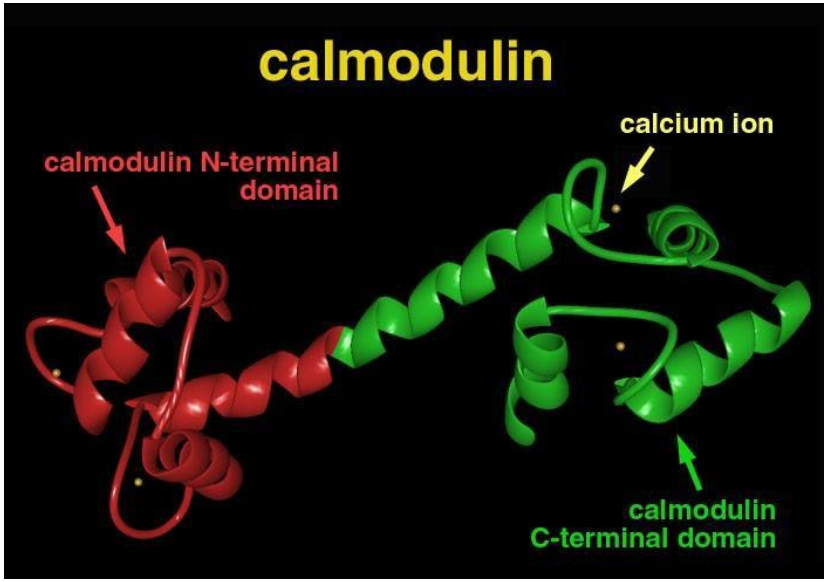
# Calmodulin (≈17 kD)

## Calcium-modulated protein



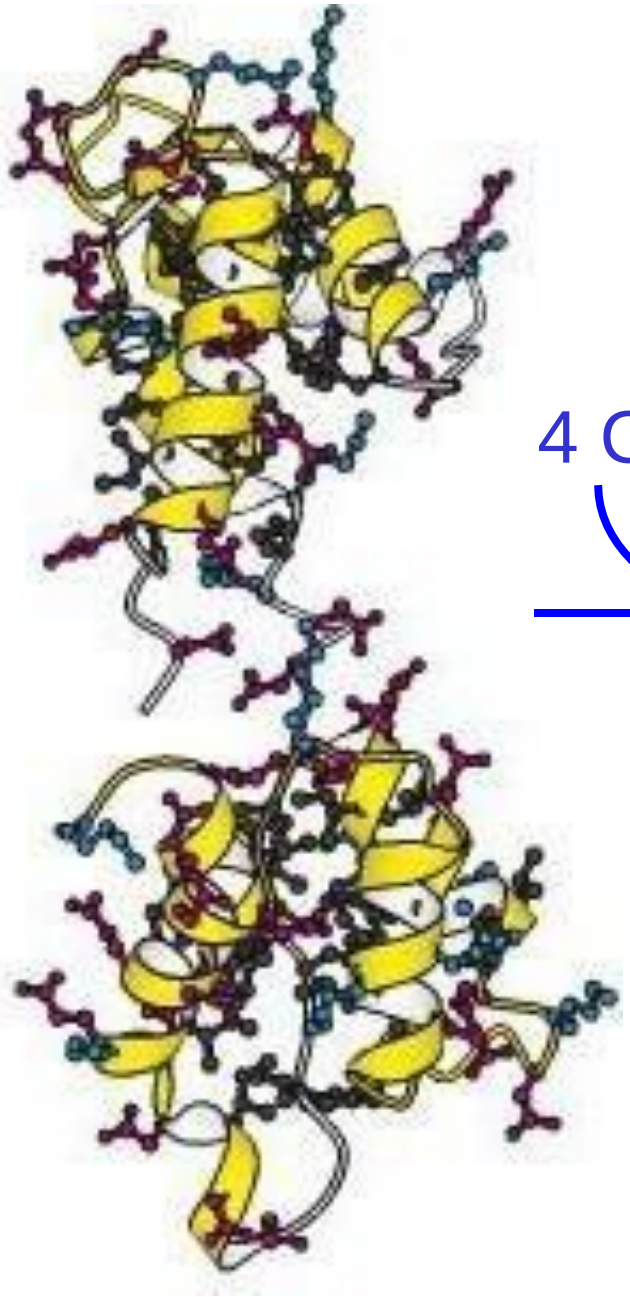

- Found in almost all eukaryotes
- Consists of two globular regions
  - Connected by flexible region
  - Each contains 2 EF hands
  - Four $Ca^{2+}$ binding sites
- Calcium-Calmodulin complex can bind to a large number of target proteins including:

149 amino acids




Calmodulin-dependant Protein Kinase

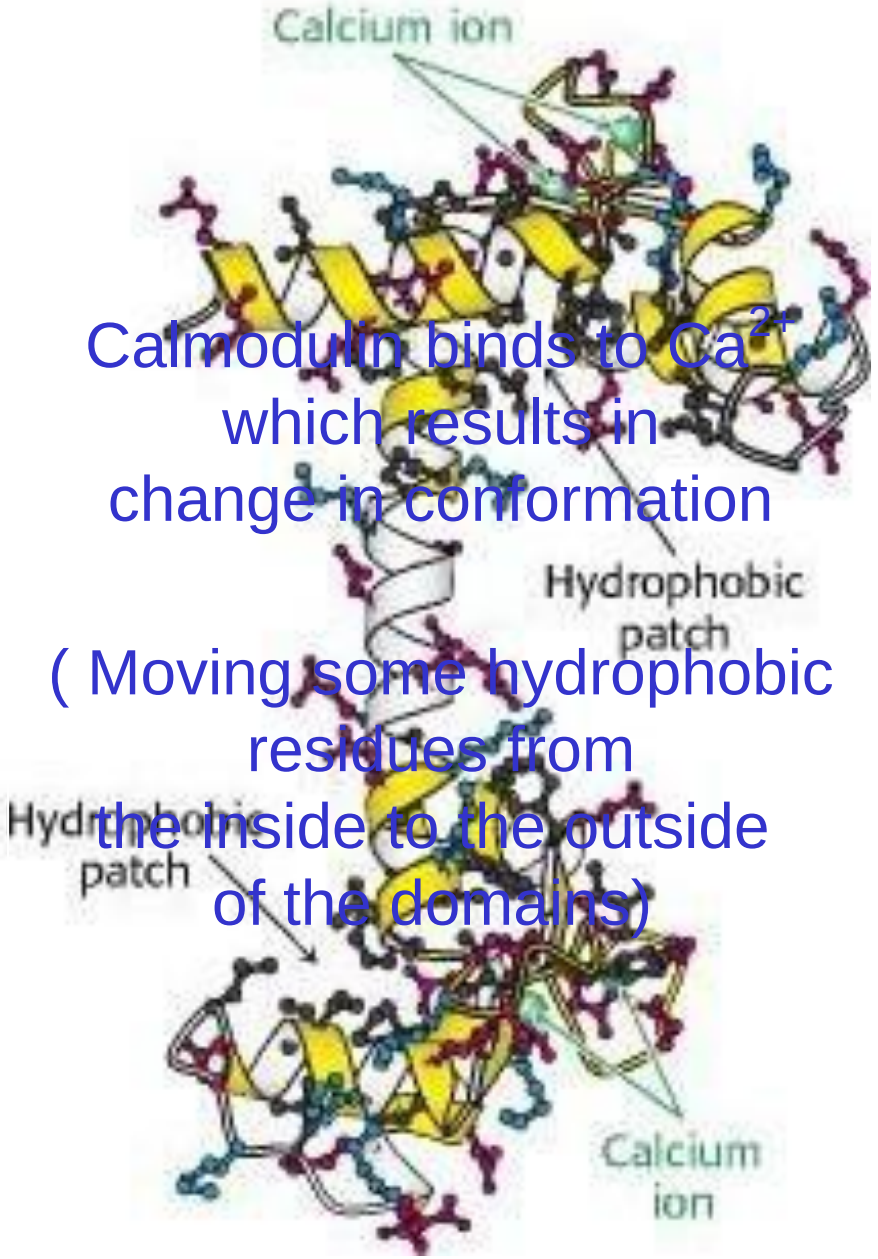
$Ca^{2+}$ ATP'ase Pump



4 $Ca^{2+}$

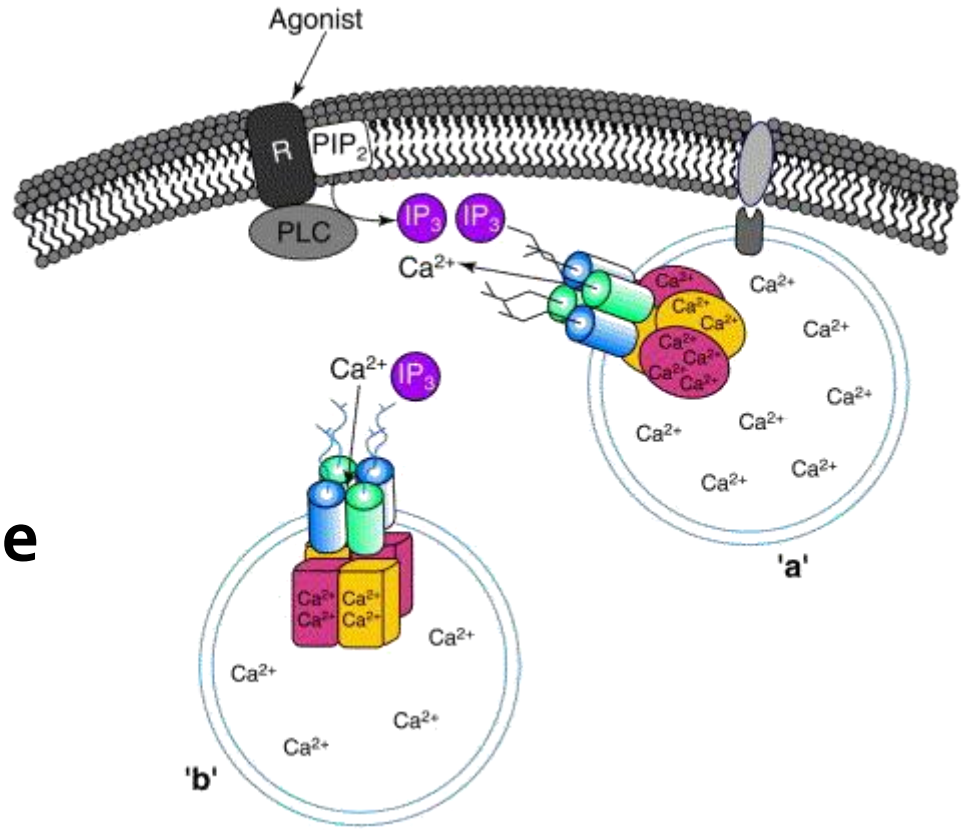
Calmodulin binds to $Ca^{2+}$ which results in change in conformation

( Moving some hydrophobic residues from the inside to the outside of the domains)



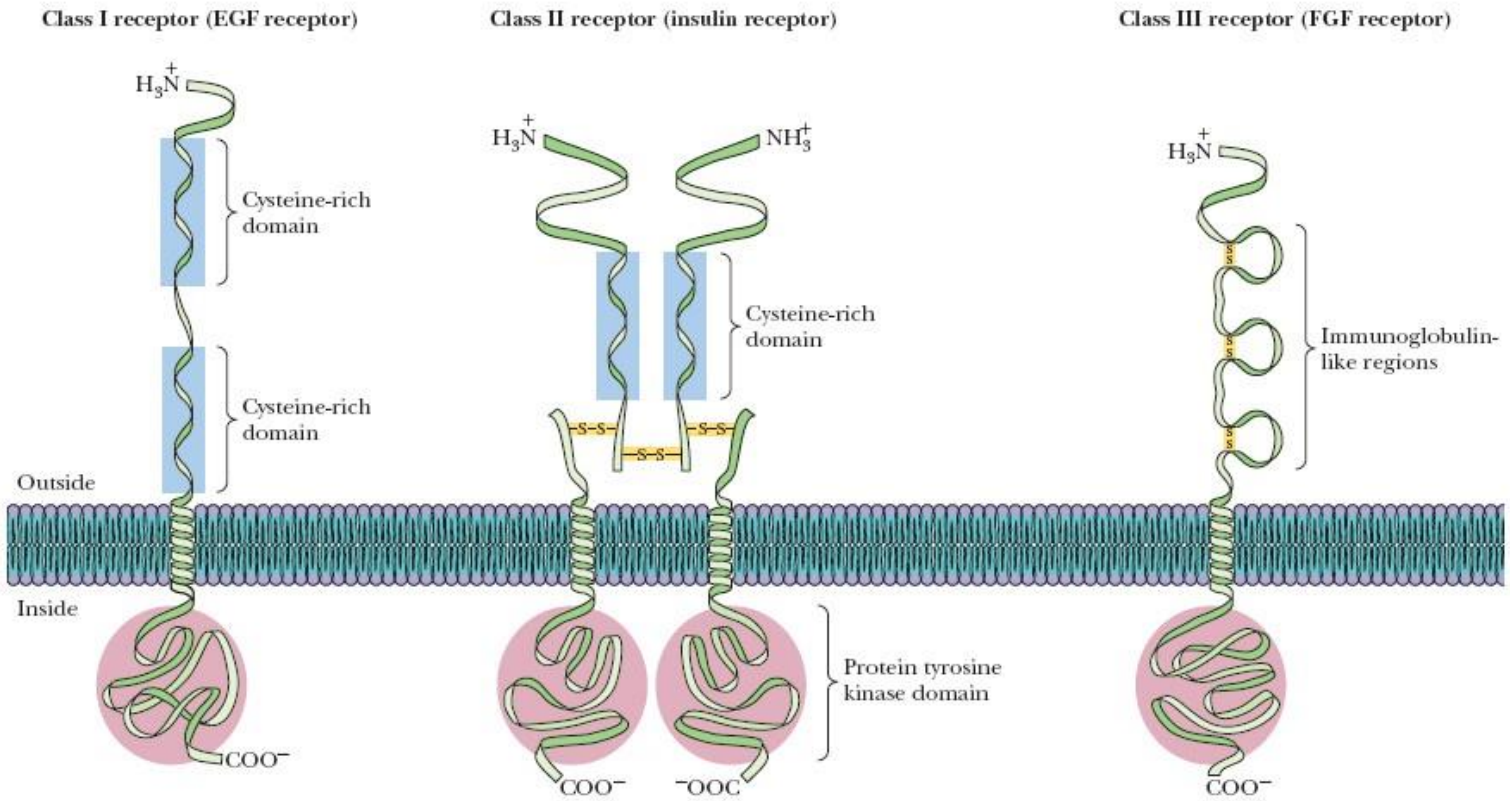
# $Ca^{2+}$ Transporter

- In sarcoplasmic reticulum
  - 80% of the membrane proteins
  - 10 membrane spanning helices
  - $Ca^{2+}$ move against a large concentration gradient
  - 2 $Ca^{2+}$ / ATP (high)
    - Depletion of ATP leads to tetany, Rigor mortis



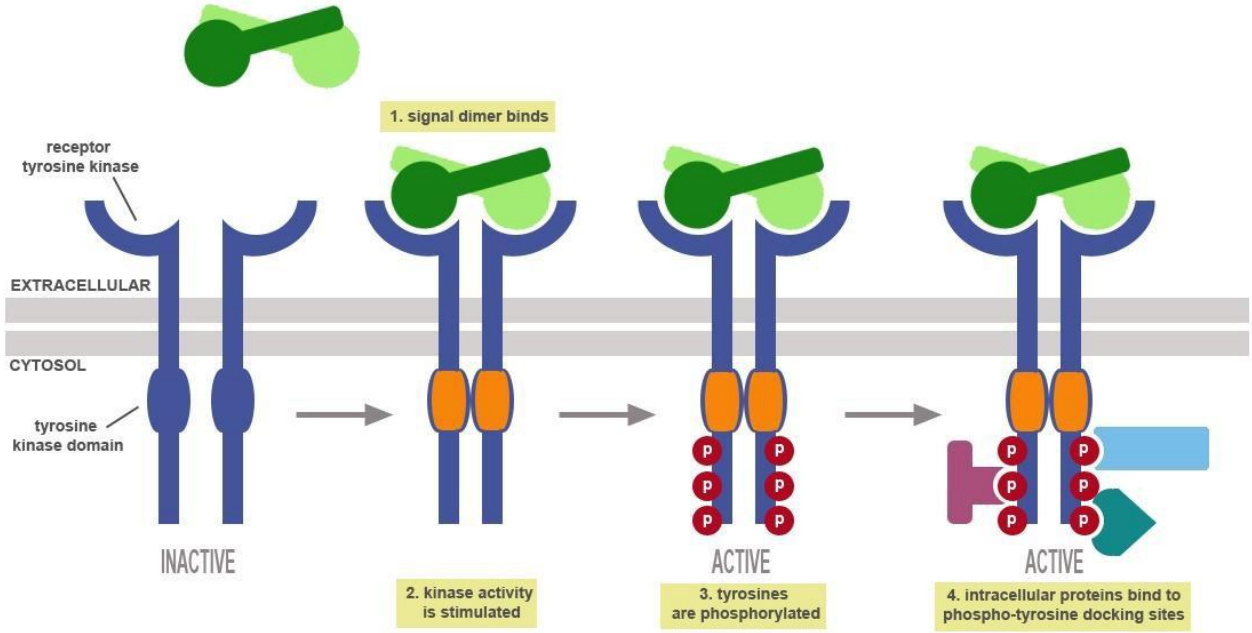
# Receptor Tyrosine Kinases Cascade

- Second Messengers
- Span the membrane, several subclasses (class II, Insulin R), hormone receptor & tyrosine kinase portion



# Second Messengers
# Receptor Tyrosine Kinases

- When activated (**dimer**) → tyrosines on target proteins:
  - Alterations in membrane transport of ions & amino acids & the transcription of certain genes
  - **Dimerization is necessary but not sufficient for activation (kinase activity)**
  - **Phospholipase C** is one of the targets
  - Insulin-sensitive protein kinase: activates **protein phosphatase 1**



# Signal Transduction through Tyrosine Kinase

**Growth hormones:**

- ✓ **Epidermal Growth Factor**
- ✓ **Platelet-derived growth Factor**
- ✓ **GH**
- ✓ **Insulin**

Hormone Binding

↓

Dimerization of the receptor

↓

Auto phosphorylation of the receptor

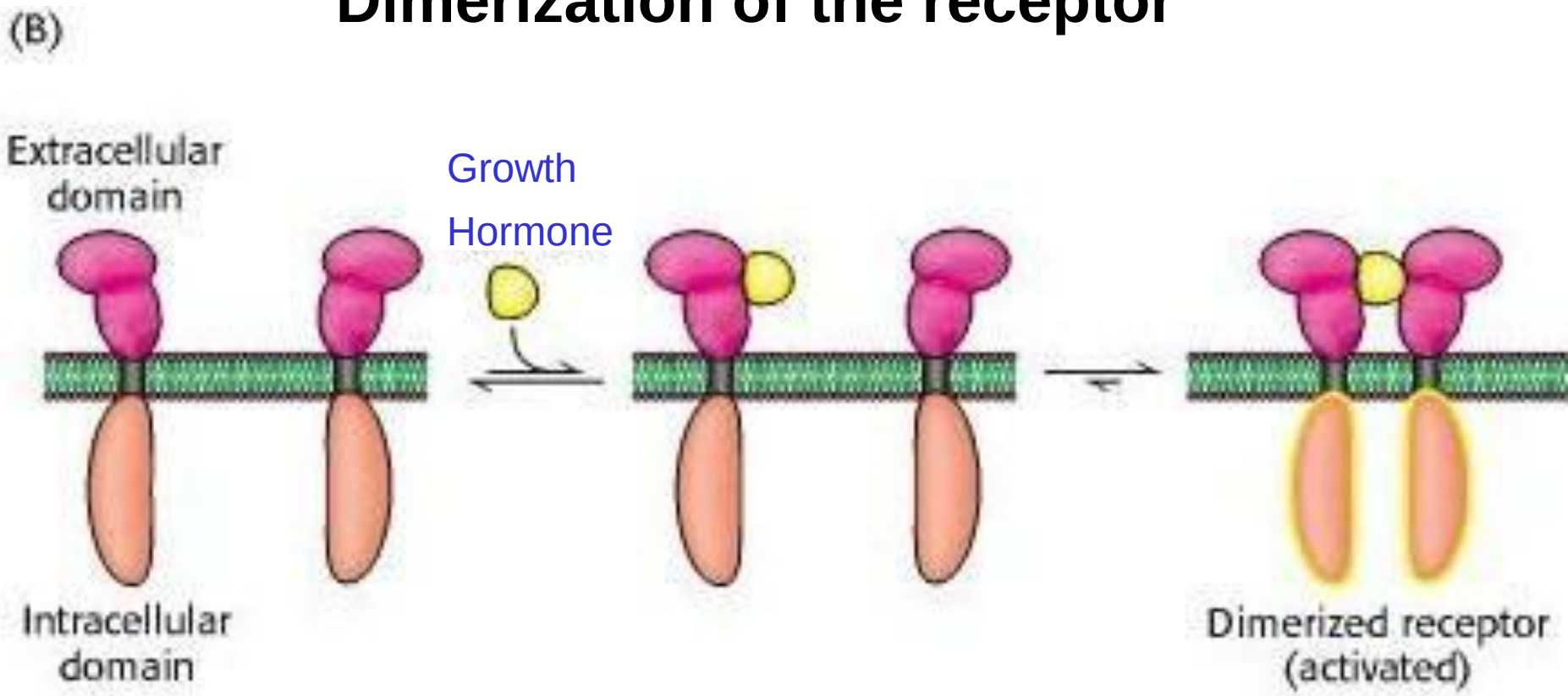
↓

Phosphorylation of the target proteins

# Growth Hormone dimerization

**Binding of one molecule of growth hormone**

**Dimerization of the receptor**



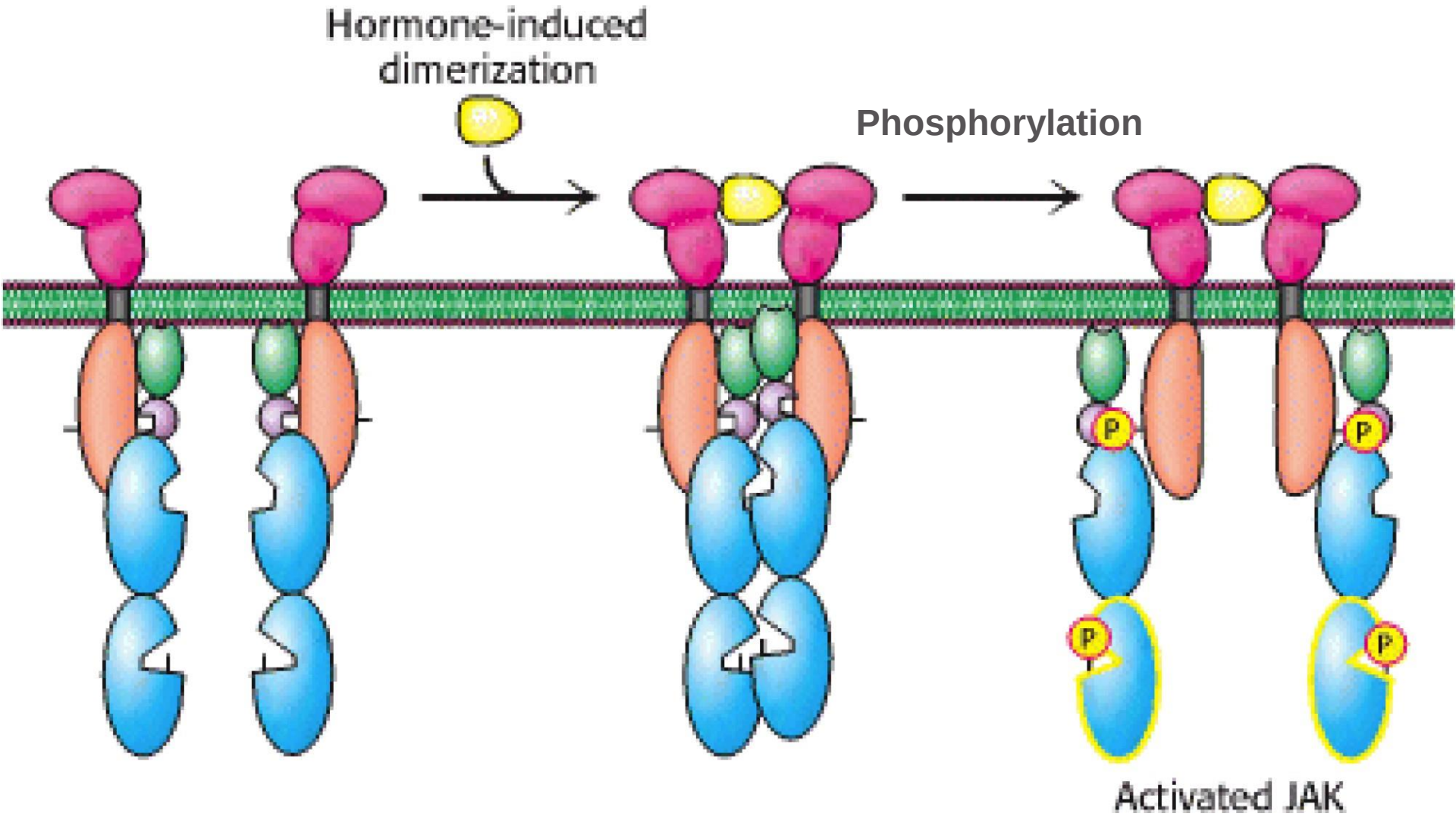
Janus

Each Intracellular Domain is associated with a protein kinase called Janus Kinase


Interaction with membrane

Binds peptides that contain Phosphotyrosine

# Receptor dimerization brings two JAKs together
# Each Phosphorylates key residues on the other



# Activated JAK 2 can Phosphorylate other substrates

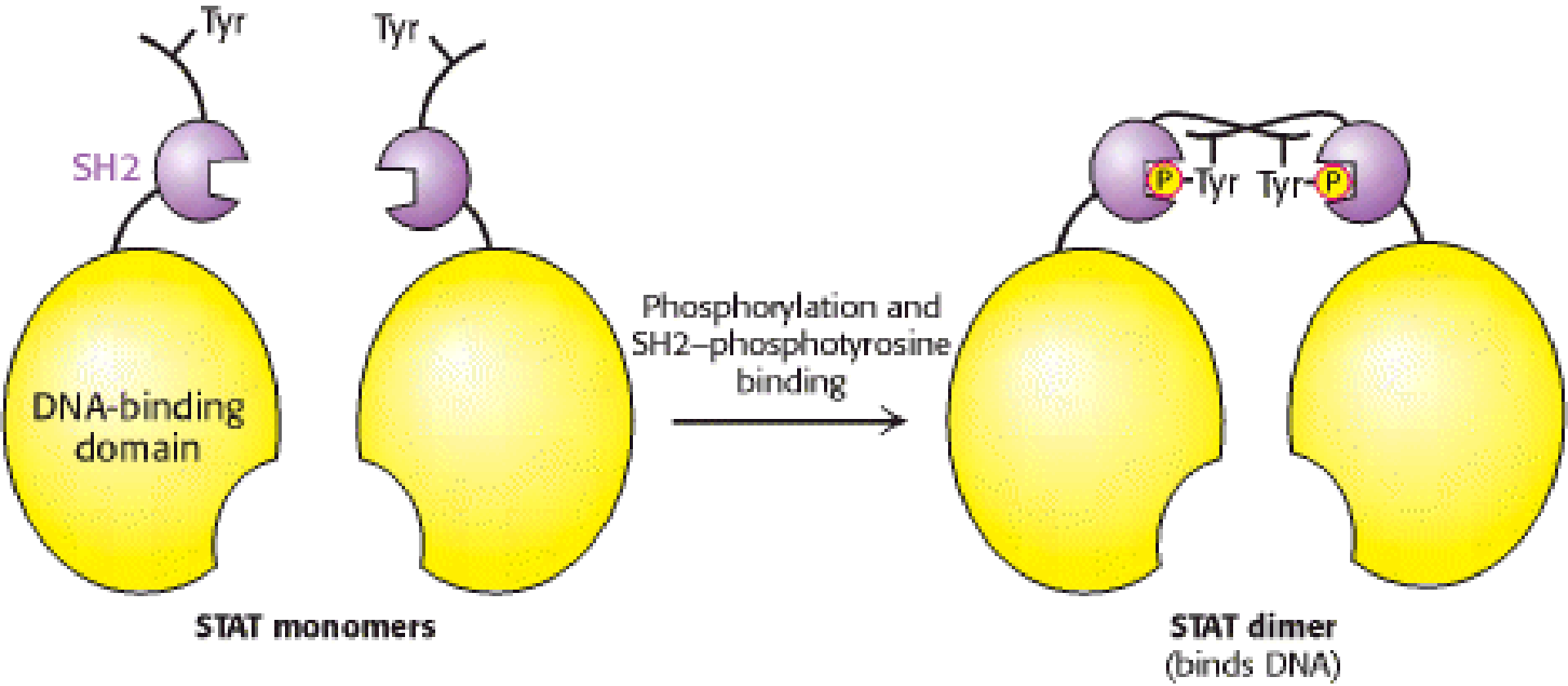
- STAT
  - Signal Transducers & Activators of Transcription
- Regulator of transcription
- STAT Phosphorylation

  → **Dimerization**

  → Binding to specific DNA sites
- If JAK2 remains active it will produce **Cancer**

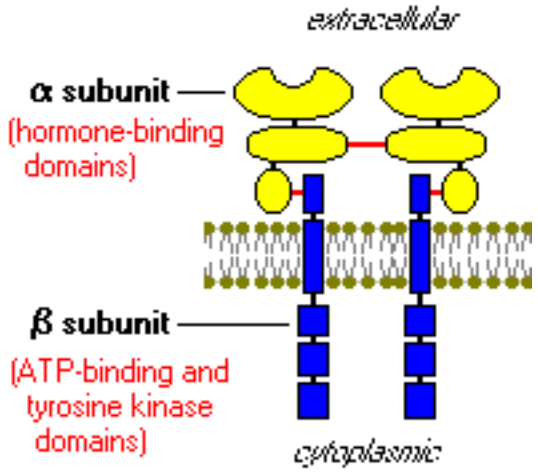
STAT is phosphorylated on a tyrosine residue near the carboxyl terminus

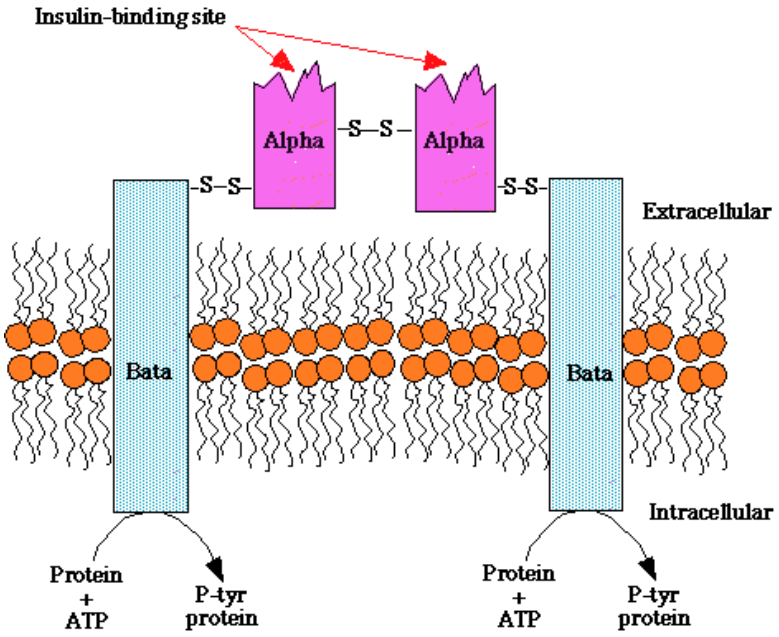
Phosphorylated tyr binds to SH2 domain of another STAT molecule



# Tyrosine Kinase & other Hormones

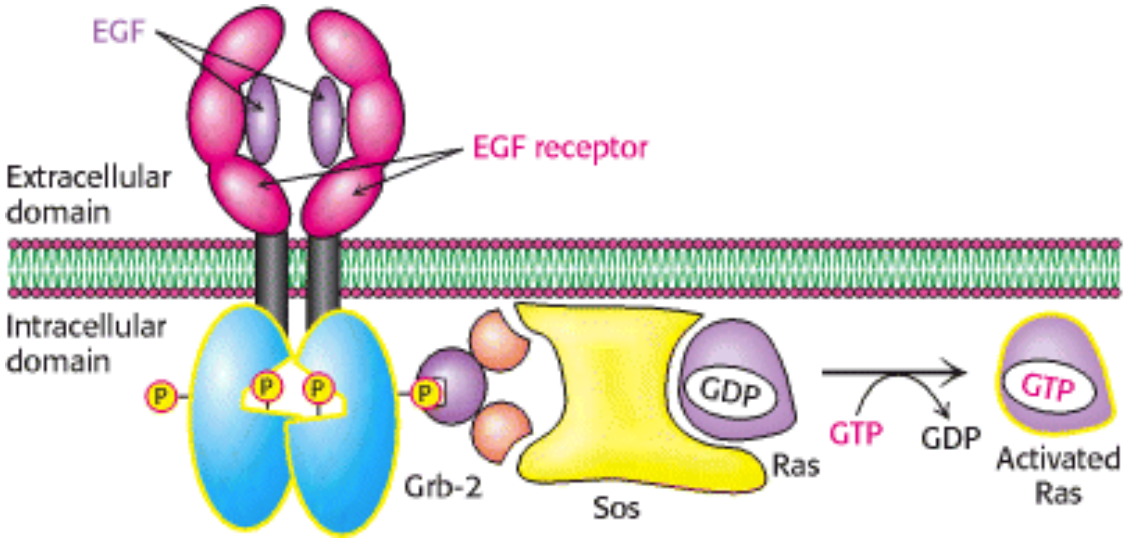
- Insulin Receptor
- Tetramer ($2^{\alpha}$; $2^{\beta}$), dimer ($2^{\alpha\beta}$ pairs)
- Disulfide bridges
- Insulin Binding → Activation of the Kinase



# Ras is a member of small G proteins family

- Monomeric
- 2 forms: GDP ↔ GTP
- Smaller (1 subunit)
- GTPase activity
- Many similarities in structure and mechanism with $G_\alpha$




- Include several groups or subfamilies
- Major role in growth, differentiation, cellular transport, motility etc…

# Impaired $GTP_{ase}$ activity can lead to cancer in human

- Mammalian cells contain 3 different Ras proteins

Mutation →

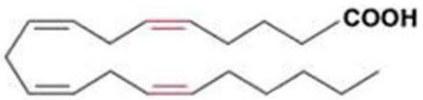
Loss of ability to hydrolyze GTP → Ras

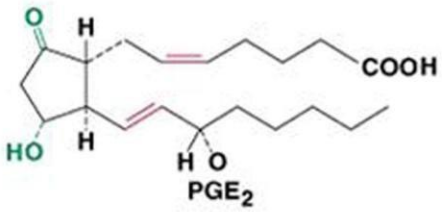
is locked in "ON" position →

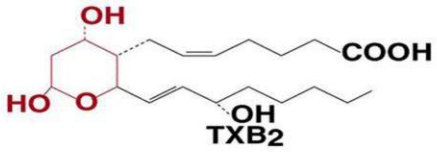
continuous growth stimulation

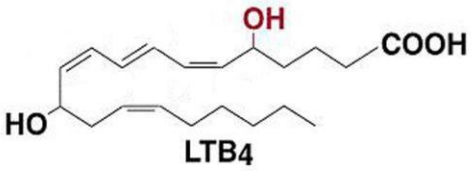
# Eicosanoids

- 20 carbon signaling molecules
- Several Classes:
  - Prostaglandins
  - Thromboxanes
  - Leukotrienes




- Very Potent (very low conc.)
- Short Half Life
- Not Stored

- Produced In Almost all Tissues
- Wide Range of Responses
- Local Hormones (autocrine & paracrine)

# Some Functions of the Prostaglandins and Thromboxanes

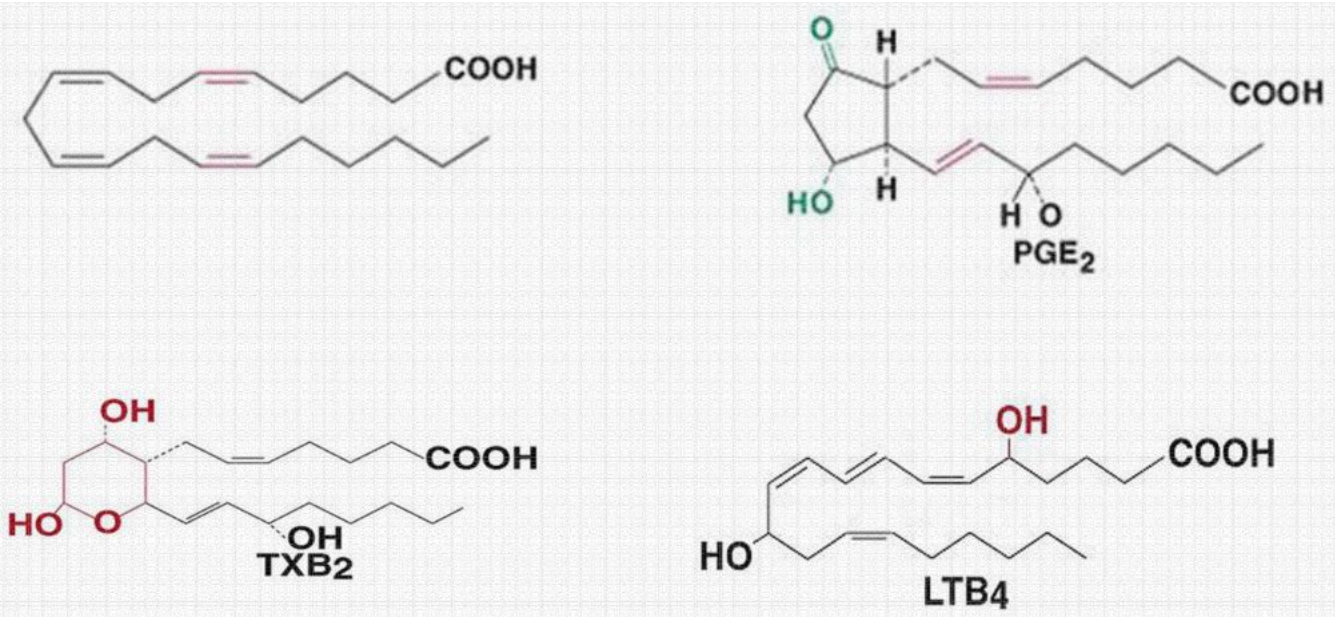
- What 2 stands for?

- $PGI_2$, $PGE_2$, $PGD_2$
  - Increase
    - Vasodilation, cAMP
  - Decrease
    - Platelet Agregation
    - Lymphocyte Migration
    - Leucocyte Aggregation

- $PGF_{2\alpha}$ Increses
  - Vasoconstriction
  - Bronchoconstriction
  - Smooth Muscle Contraction

- Thromboxanes Increases
  - Vasoconstriction
  - Platelet Agregation
  - Lymphocyte Proliferation
  - Bronchoconstriction

# Eicosanoids Structure




- Arachidonic acid (20, 4, no ring)
- Prostaglandins (20, 2, 5-ring)
- Thromboxanes (20, 2, 6-ring, oxygen)
- leukotrienes (20, 3 conjugated, no ring)

# Eicosanoids Synthesis