The Phosphoinositide Cascade

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

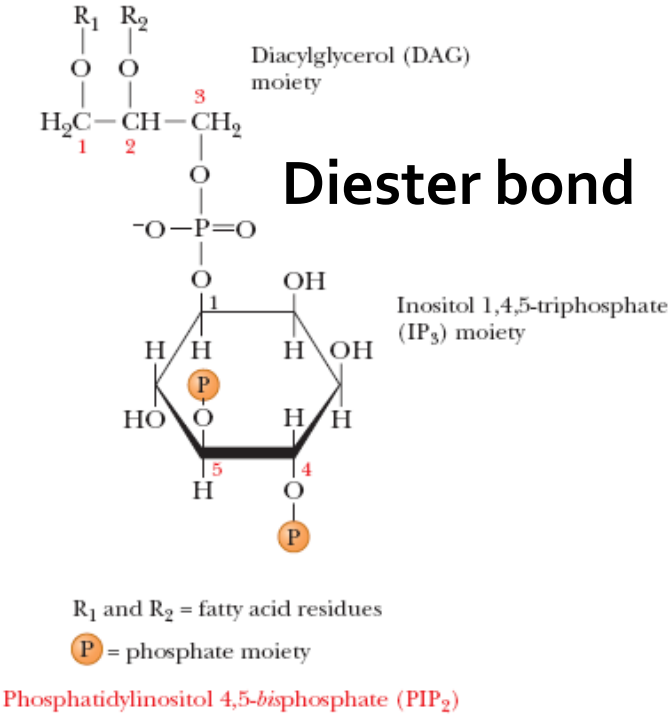
↓

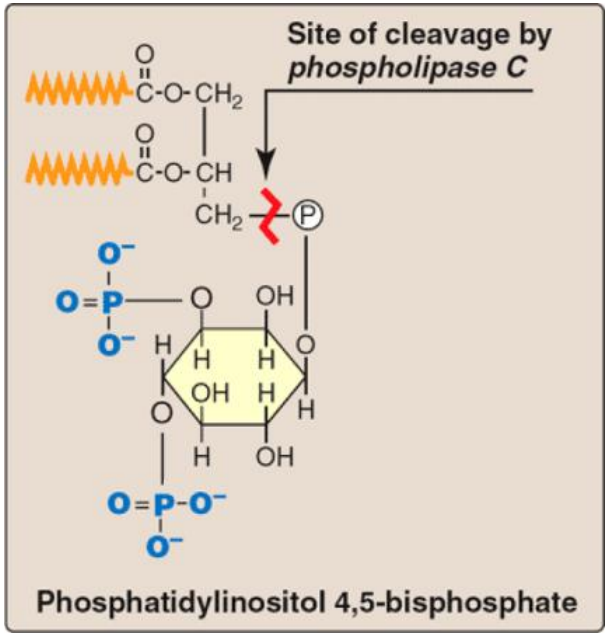
Activation of G Protein

↓

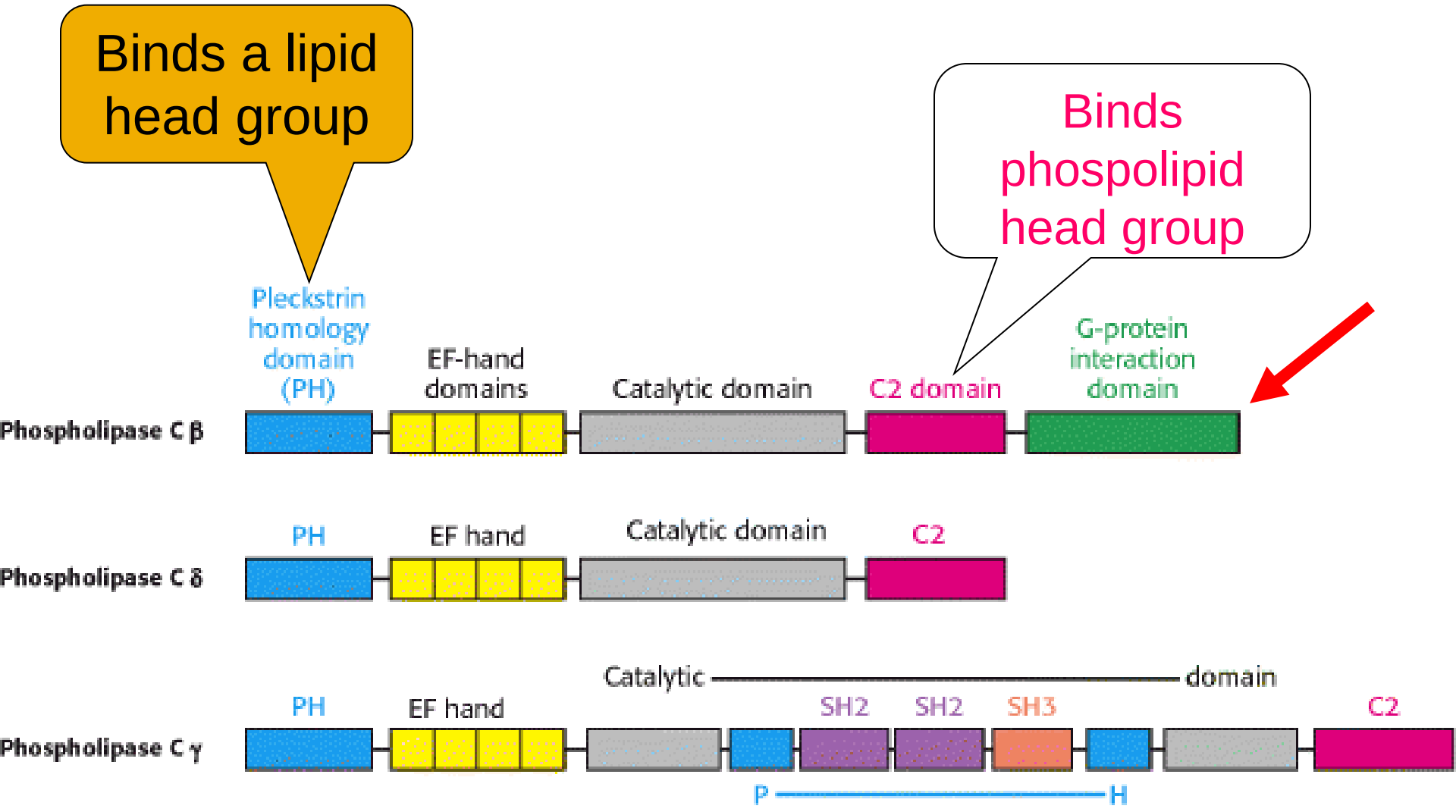
Activation of Phospholipase C (many isoforms) – PIP2

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





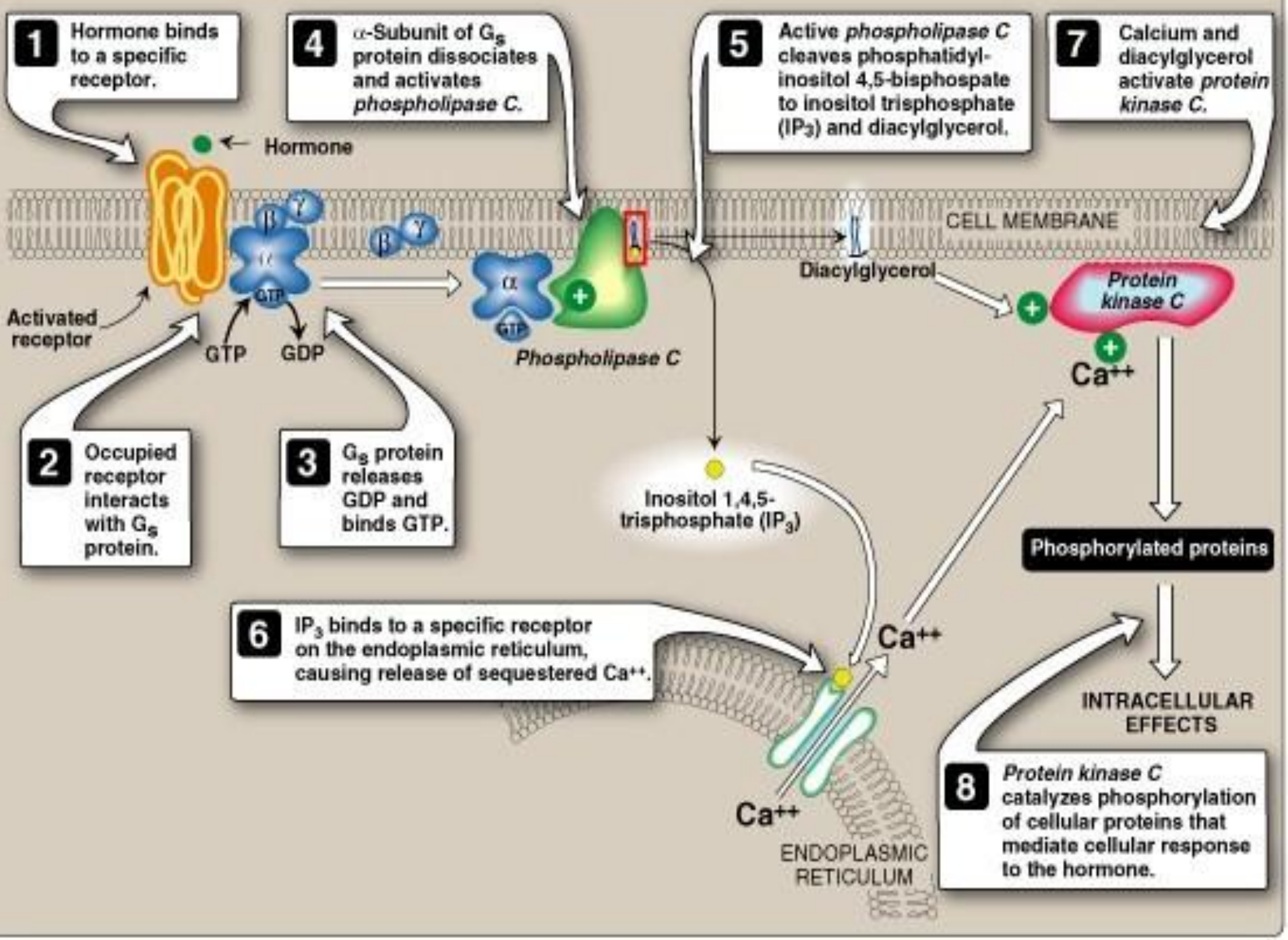
The domain structures of three isoforms of Phospholipase C



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

Membrane

G Protein


1 Hormone binds to a specific receptor.
Hormone
Activated receptor
2 Occupied receptor interacts with G_s protein.
GTP
GDP
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

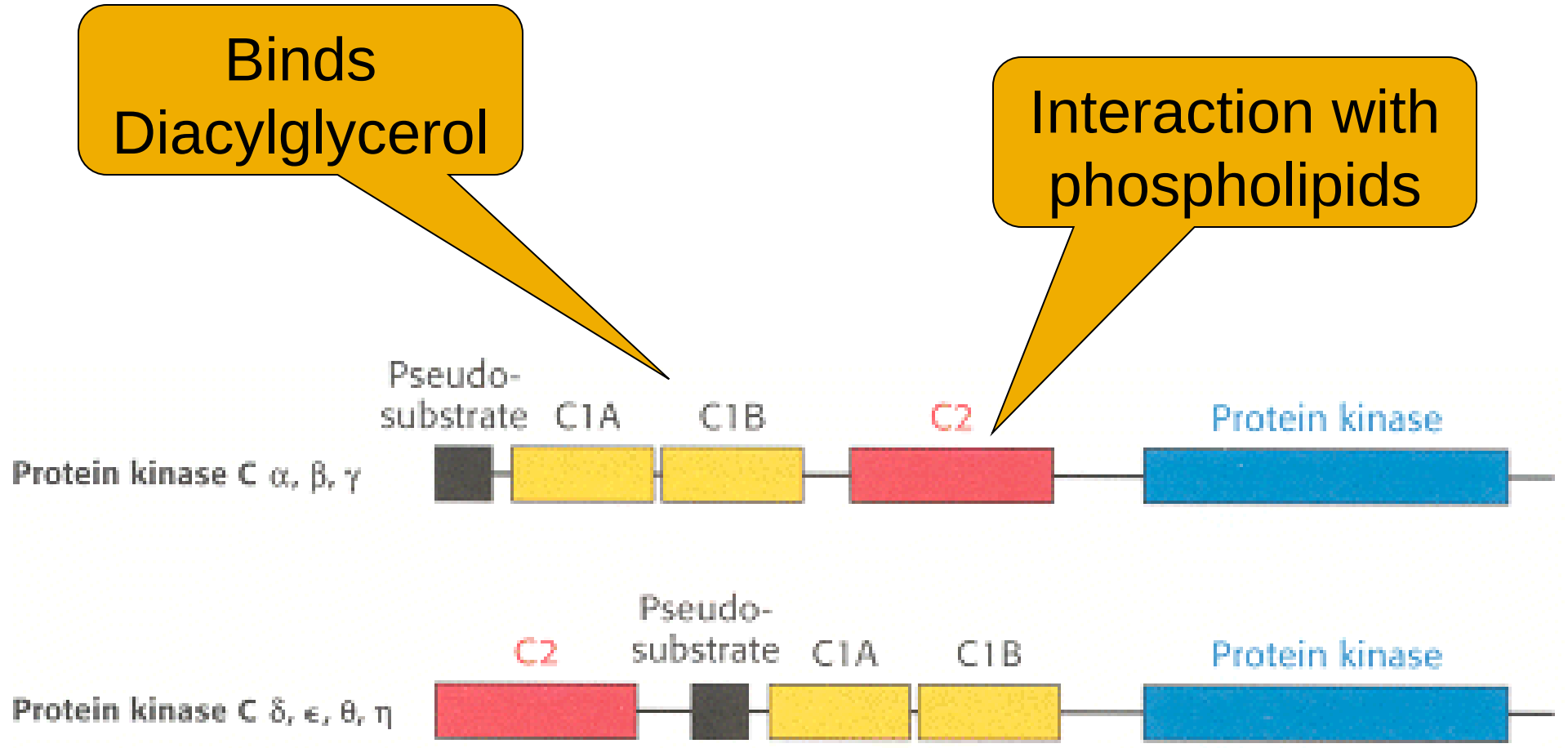
Inositol trisphosphate (IP_3)

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

Diacylglycerol (DAG)

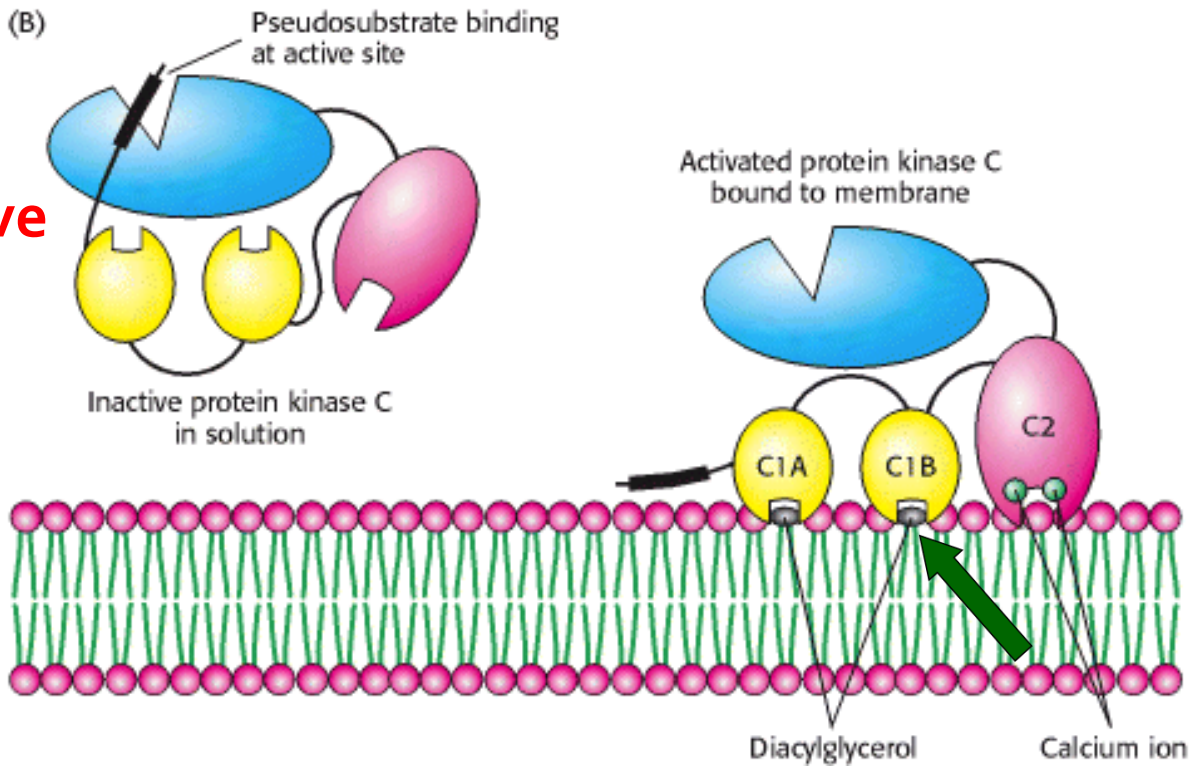
- ✓ Activates Protein Kinase C
- ✓ Ca^{2+} is required
- ✓ Phosphorylation of many target proteins

The domain structures of protein kinase C isoforms



Pseudosubstrate Sequence

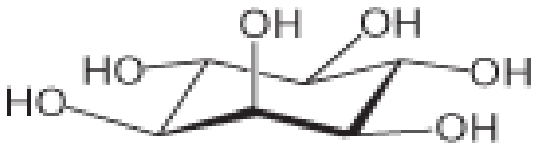
Competitive Inhibitor



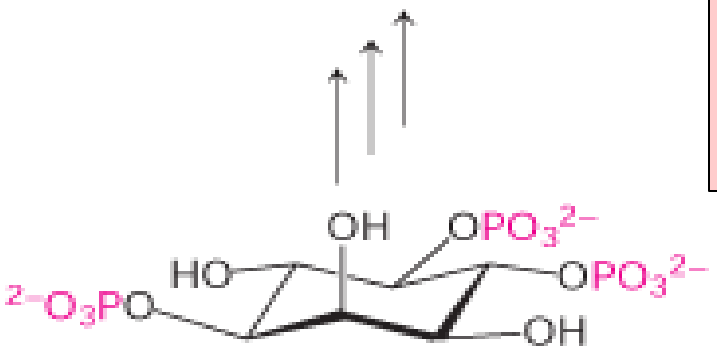
- **Resembles the substrate sequence: A-R-K-G-A-L-R-Q-K**
- **Substrate Sequence: X-R-X-X-(S,T)-Hyd-R-X**
- **Binds to the Enzyme's Active Site**

Termination of IP3 Signal

IP3 is a Short-Lived Messenger



Inositol

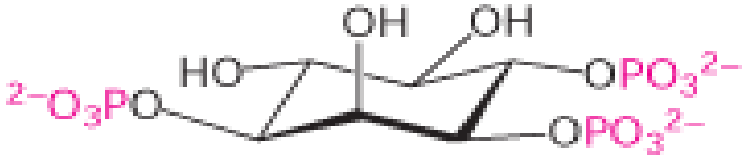


Inositol 1,4,5-trisphosphate

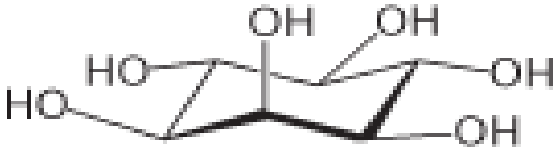
Lithium Ions, Used to treat some psychological disorders Inhibits IP_3 recycling

Inositol 1,4,5-trisphosphate

Inositol 1,3,4,5-tetrakisphosphate



Inositol 1,3,4-trisphosphate

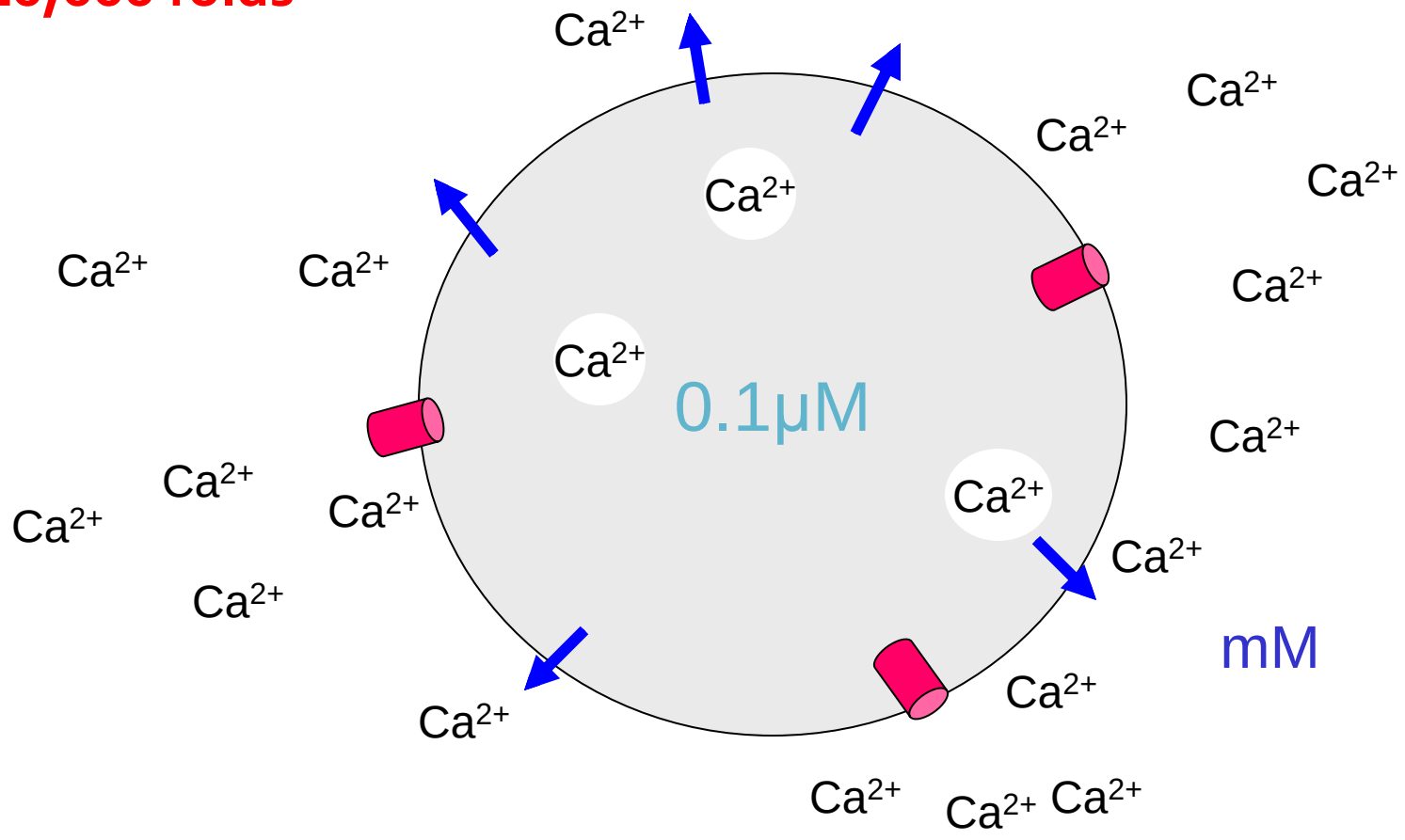


Inositol

Why Ca^{2+}?

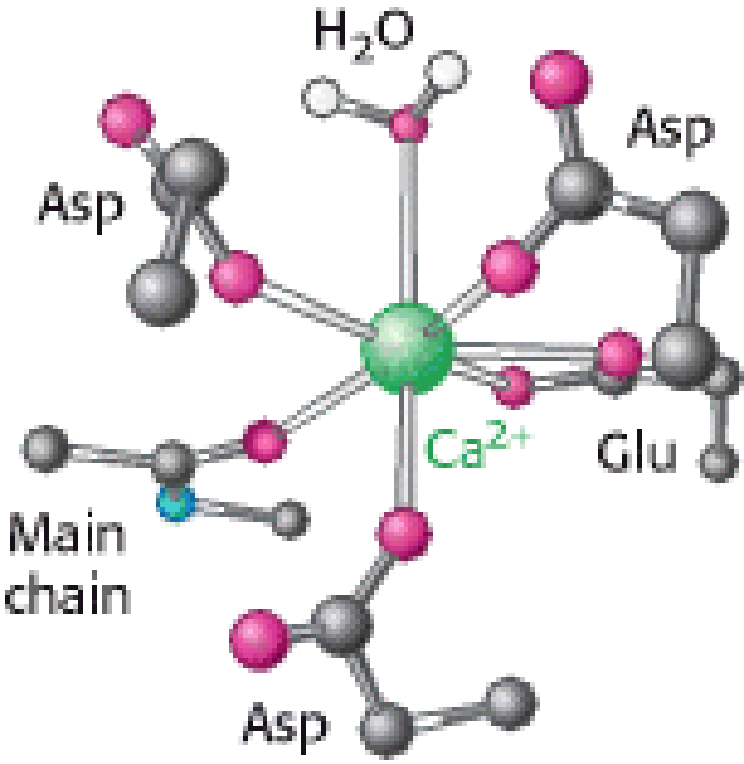
A large difference in concentration

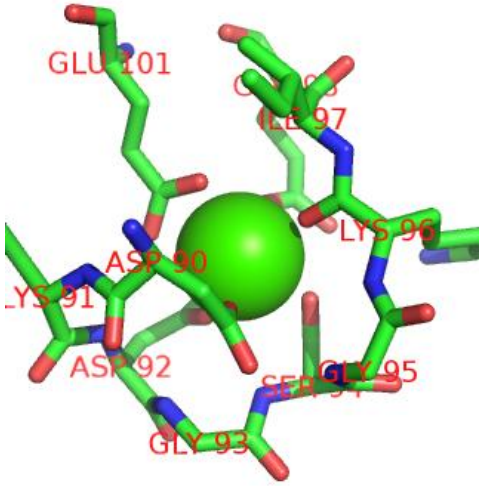
10,000 folds

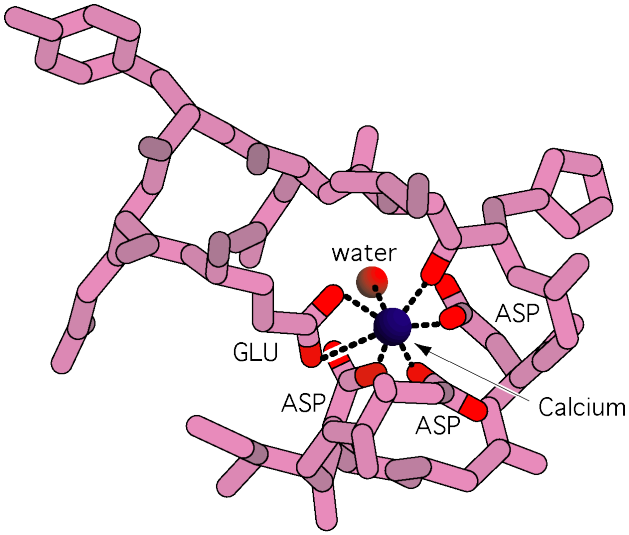


Why Ca^{2+}?

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



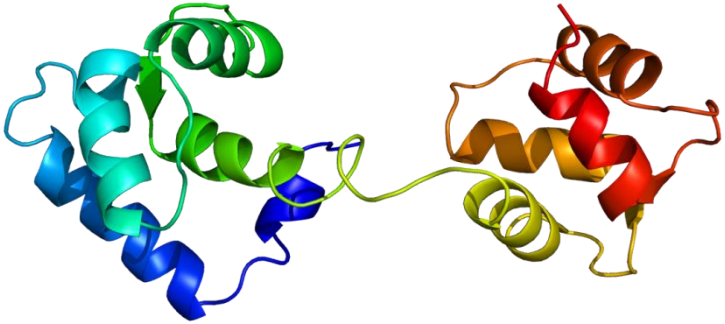




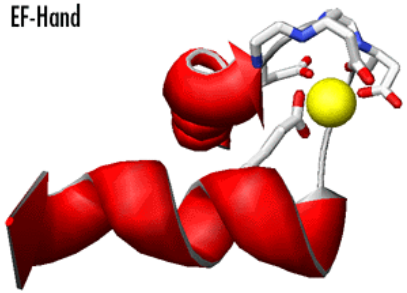
Calcium Binding Proteins

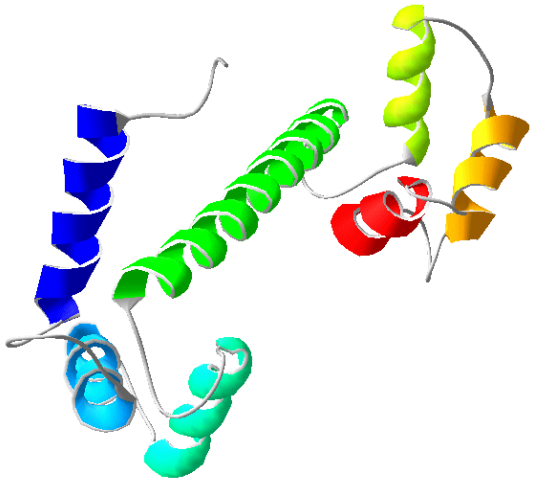
- Mediate the effects of Calcium (Ca^{+2})
- Many proteins

  Calmodulin, Troponin C, Parvalbumin

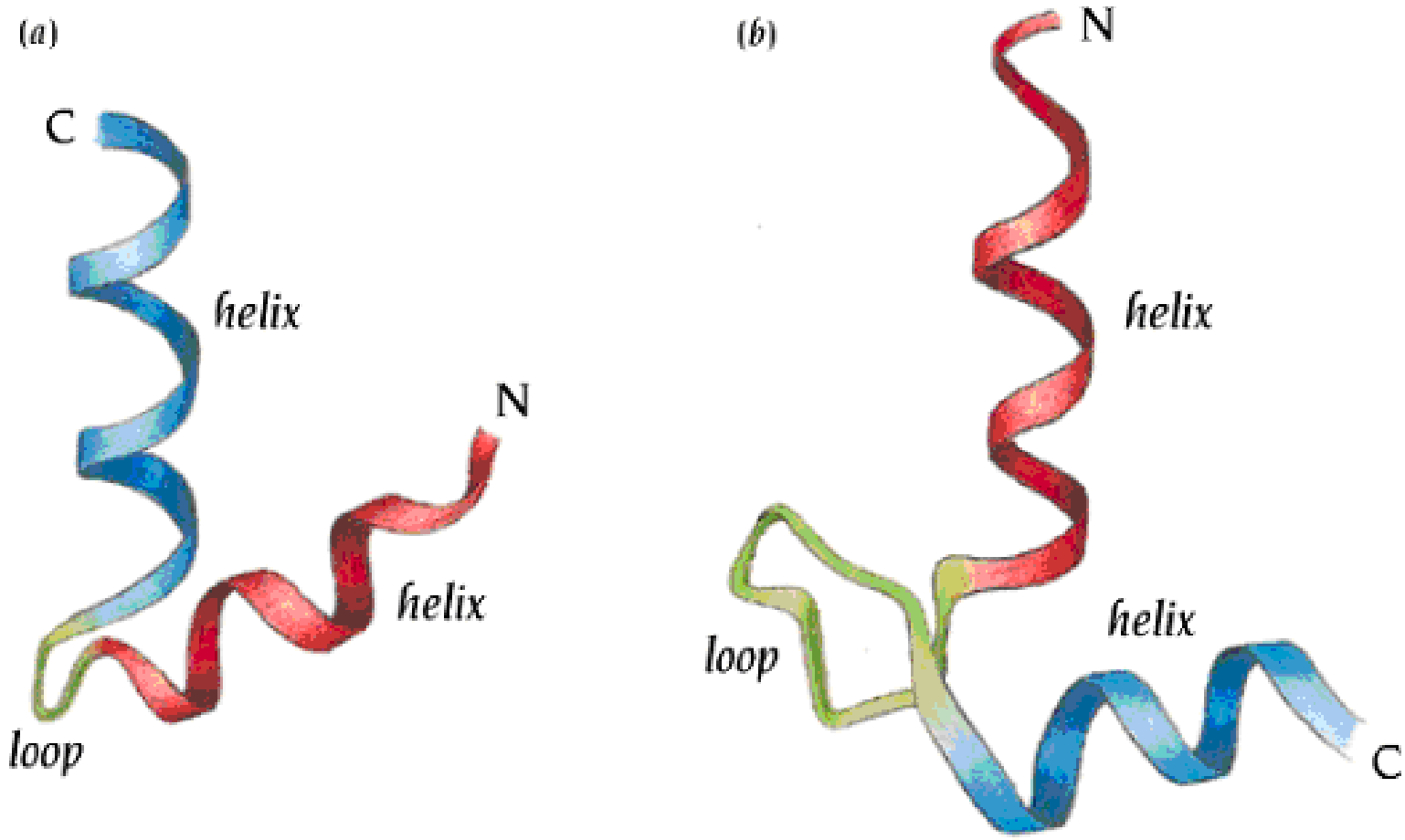


- Similar structures
  - Rich in Asp and Glu
    - Gln, Asn, Ser
  - Several α helical segments
  - Binding site is formed by
    - Helix Loop Helix
      - Super-secondary structure



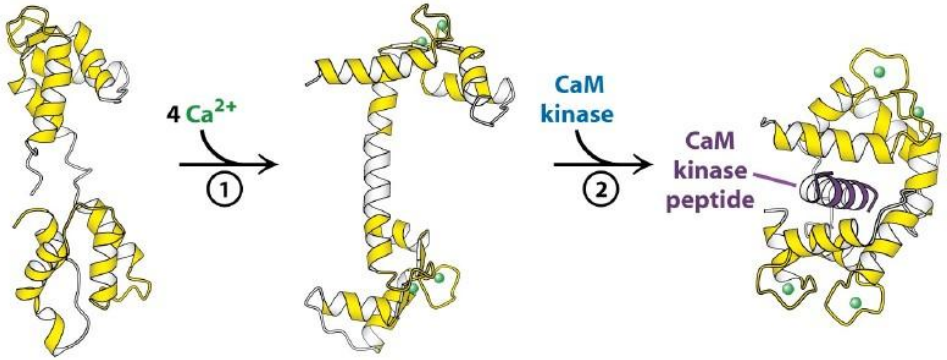


Calcium Binding Proteins



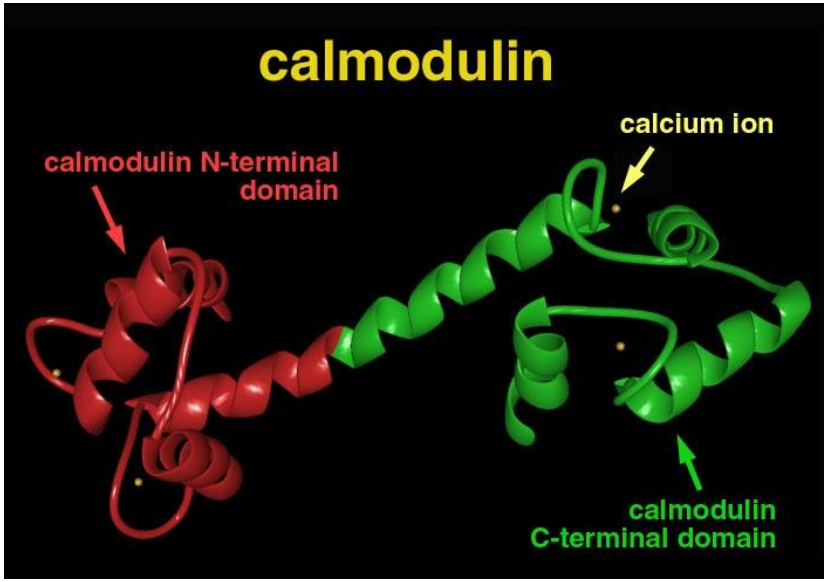
Calmodulin (≈17 kD)

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:

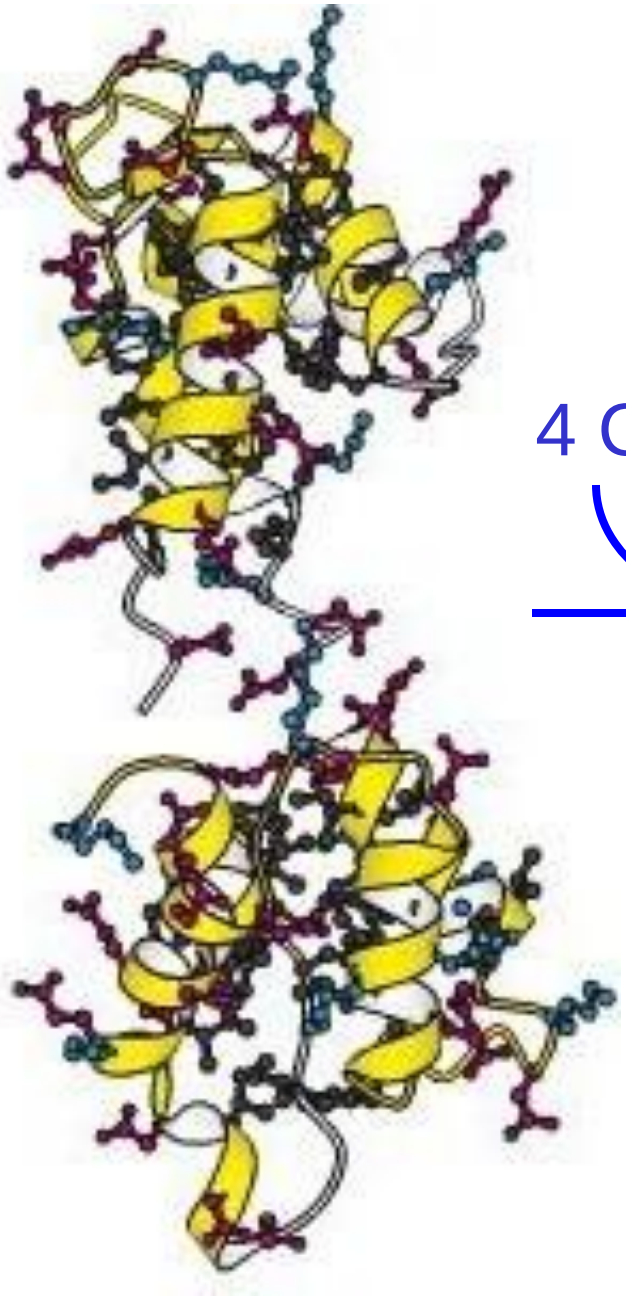
149 amino acids



Calmodulin-dependant Protein Kinase

Sort of memory

Ca^{2+} ATP'ase Pump



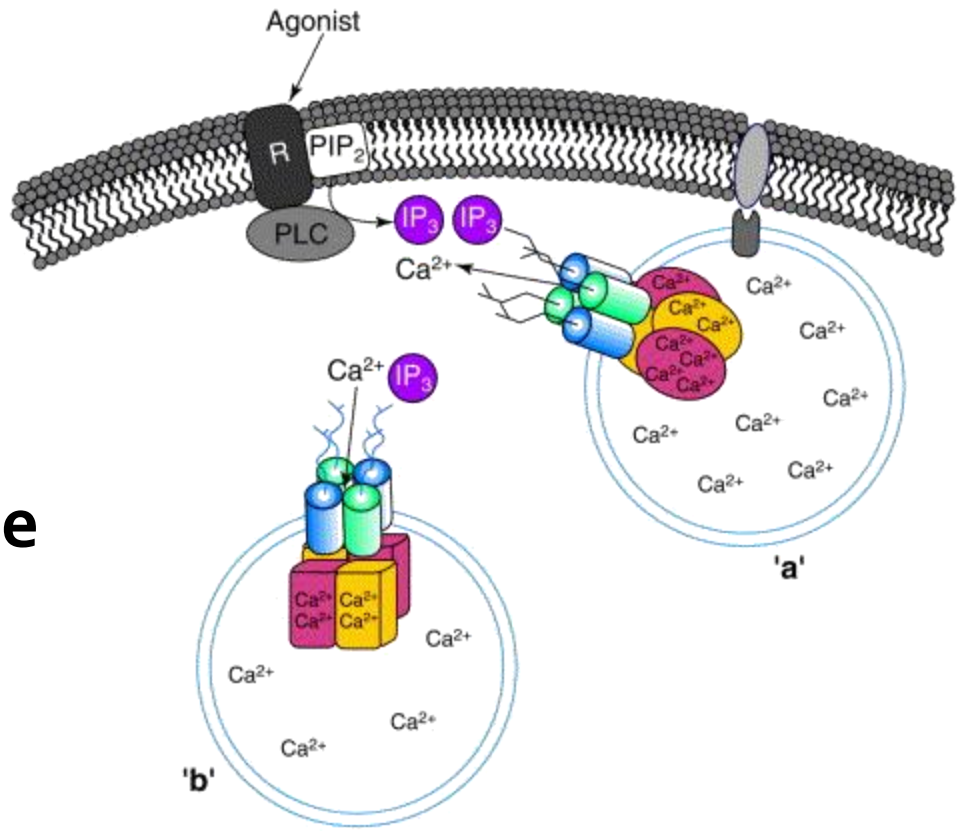
4 Ca^{2+}

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

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

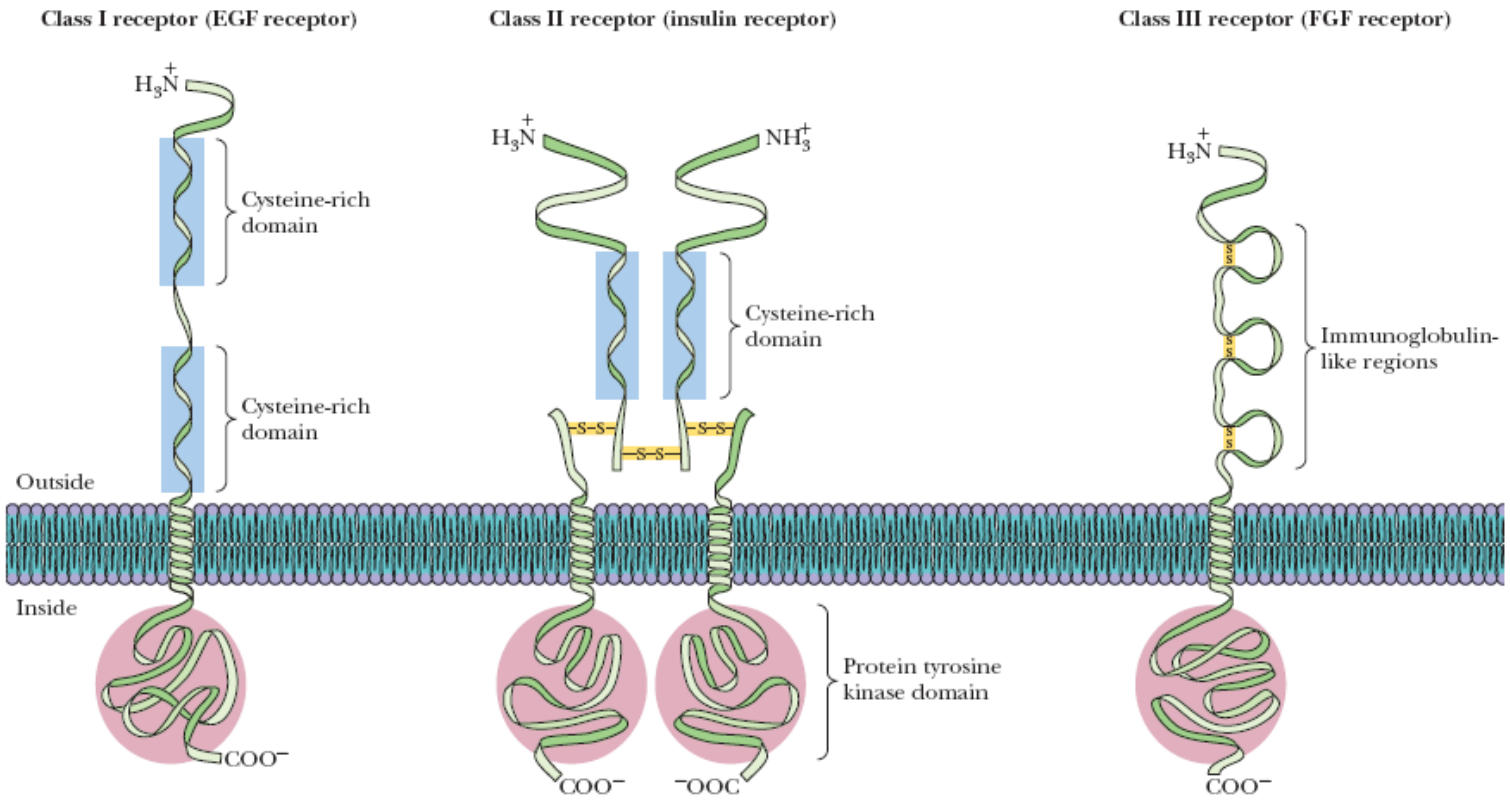
Ca^{2+} Transporter

- 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

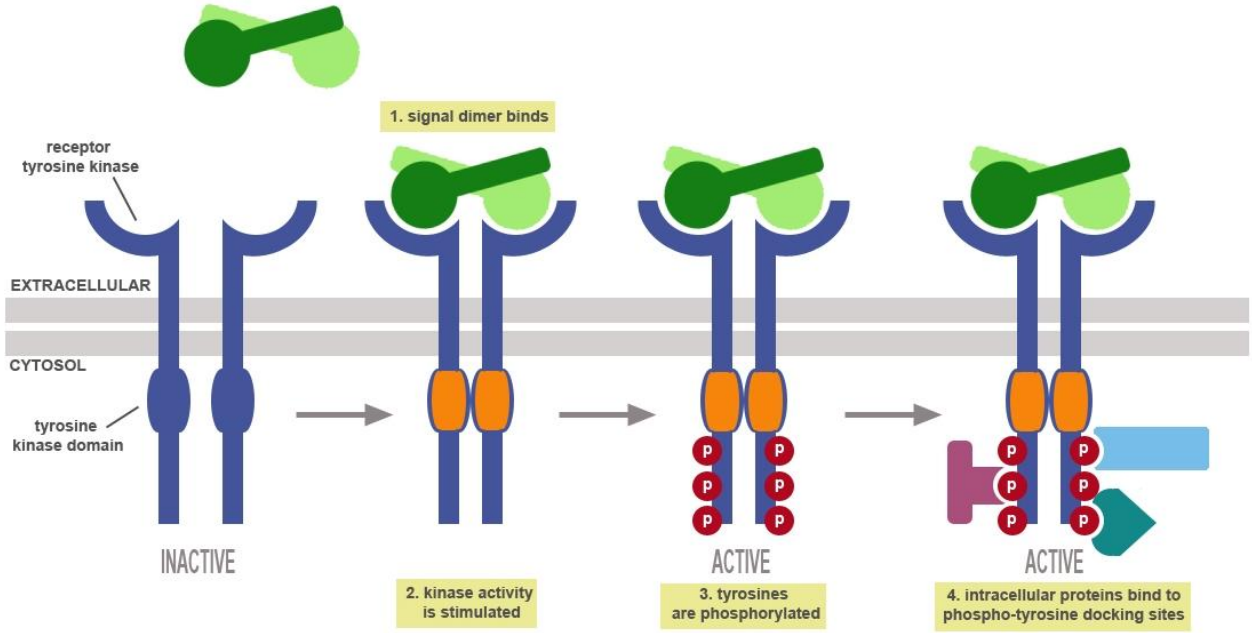
- Second Messengers
- Span the membrane, several subclasses (class II, Insulin R), hormone receptor & tyrosine kinase portion



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

  - **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

↓

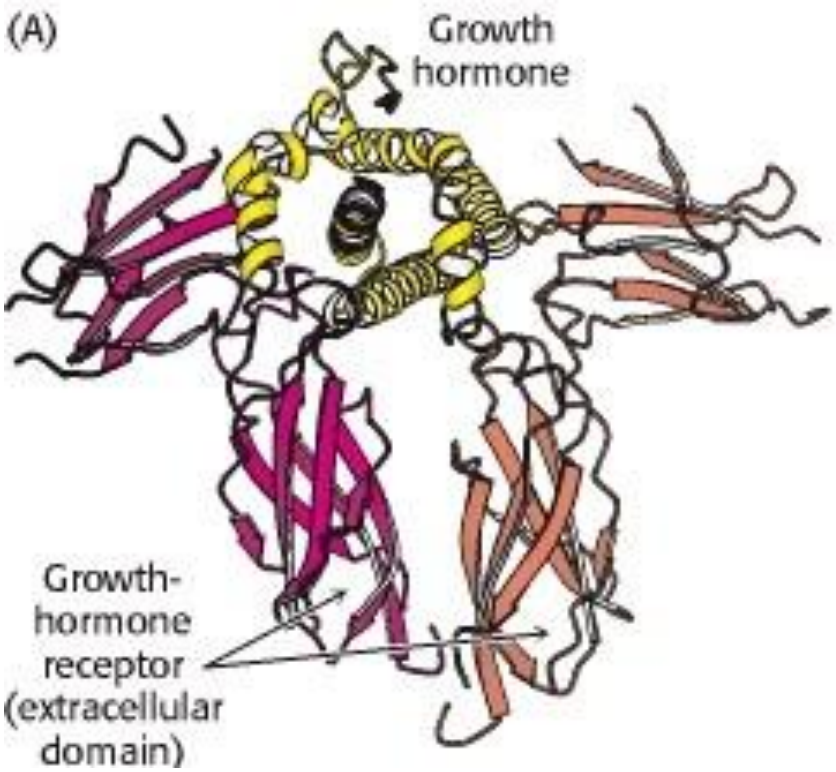
Auto phosphorylation of the receptor

↓

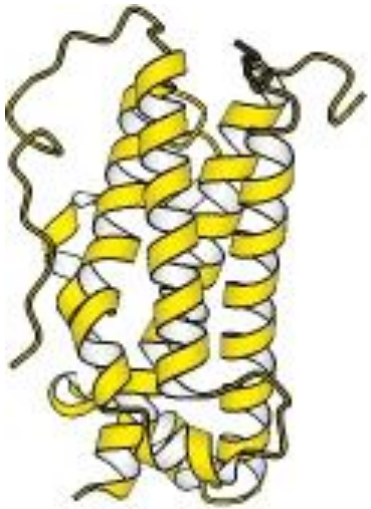
Phosphorylation of the target proteins

Growth Hormone & GH receptor

- GH:
  - Monomeric Protein
  - 217 Amino Acids
  - Compact Four-helix Bundle



- GH receptor (cooperative binding)
  - 638 A.Acid
  - Extracellular Domain (≈250 A.A) & Intracellular Domain (≈350 A.A)
  - Single Membrane-Spanning Helix
  - Monomeric (free) vs. Dimeric (bound)

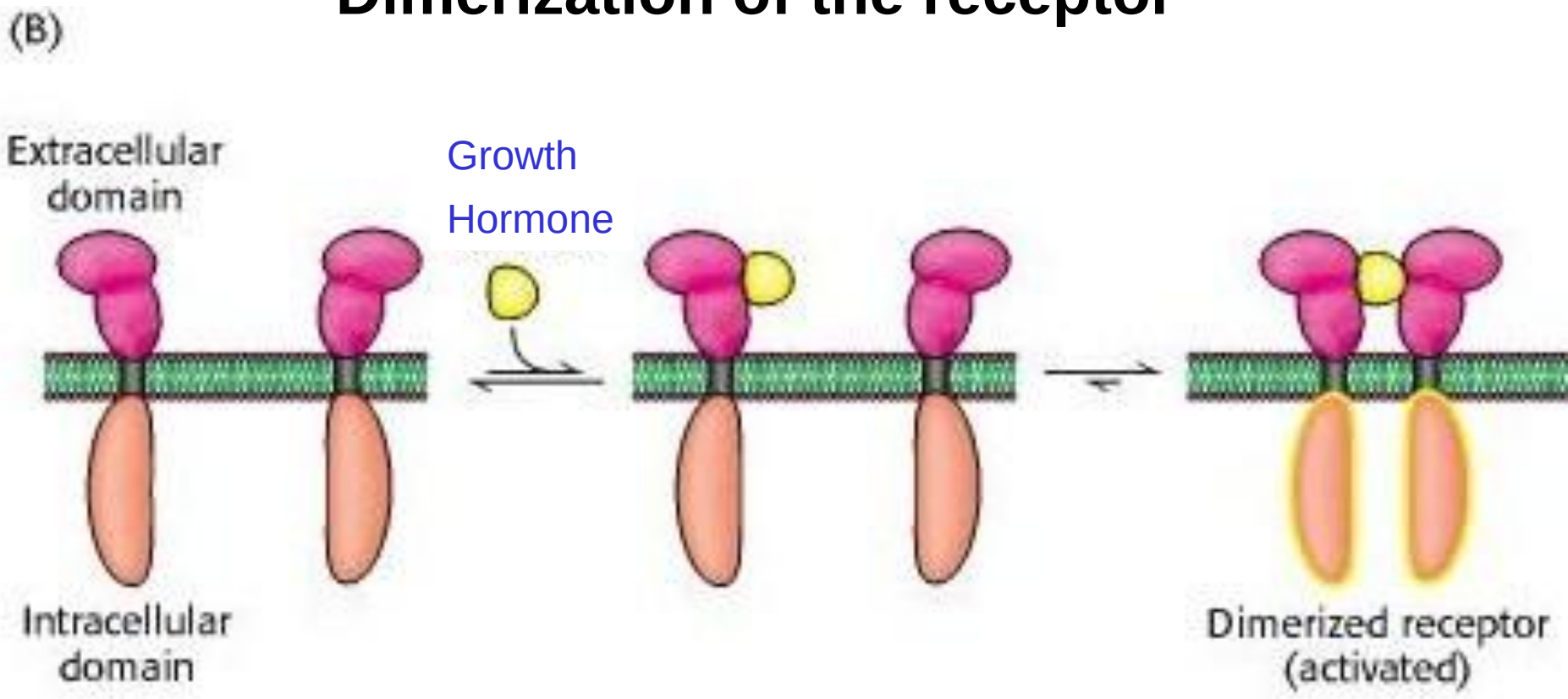

Human growth hormone

Growth Hormone dimerization

Binding of one molecule of growth hormone

↓

Dimerization of the receptor



Each Intracellular Dom ith

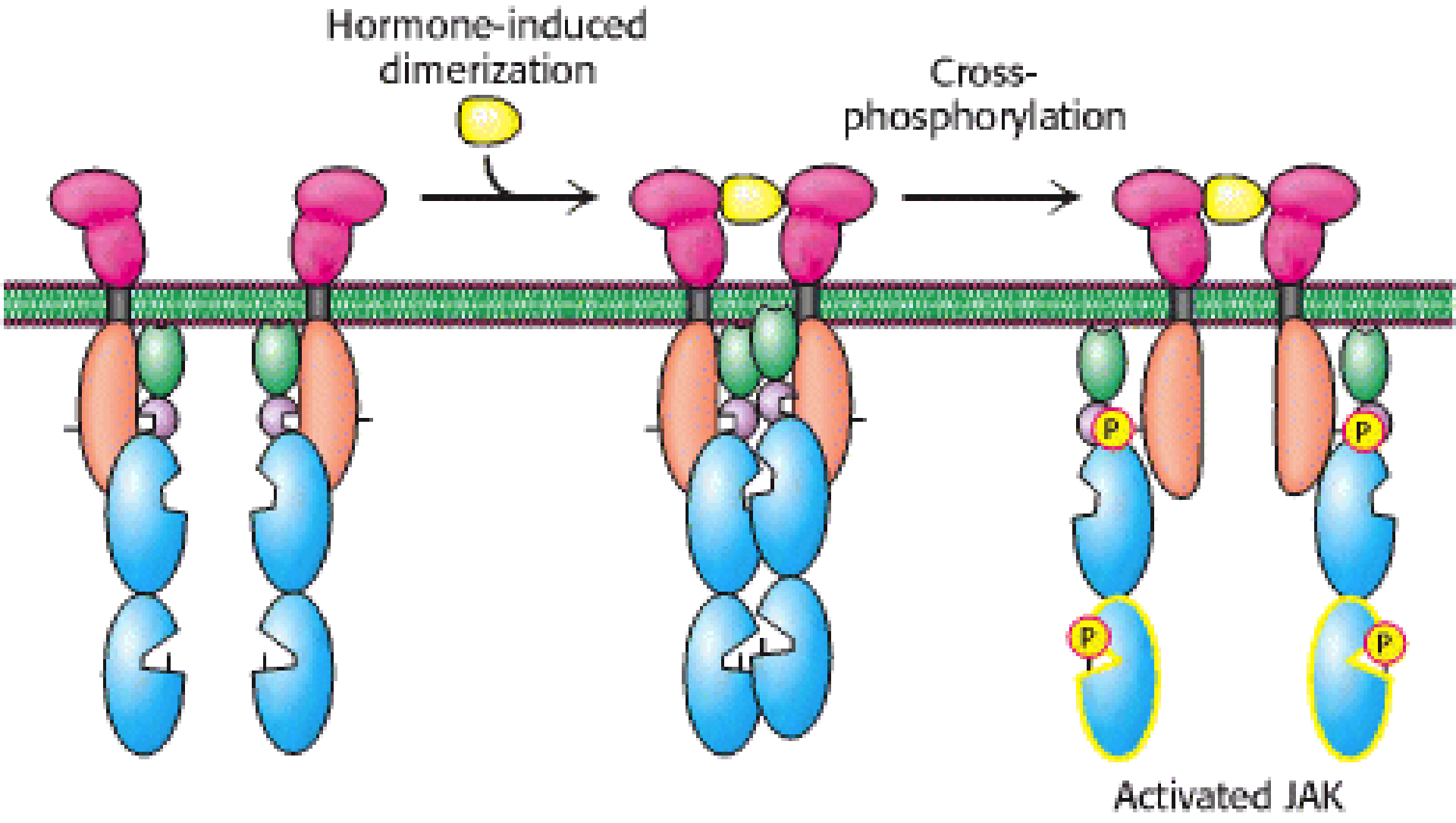
Janus a protein kin

Janus K

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

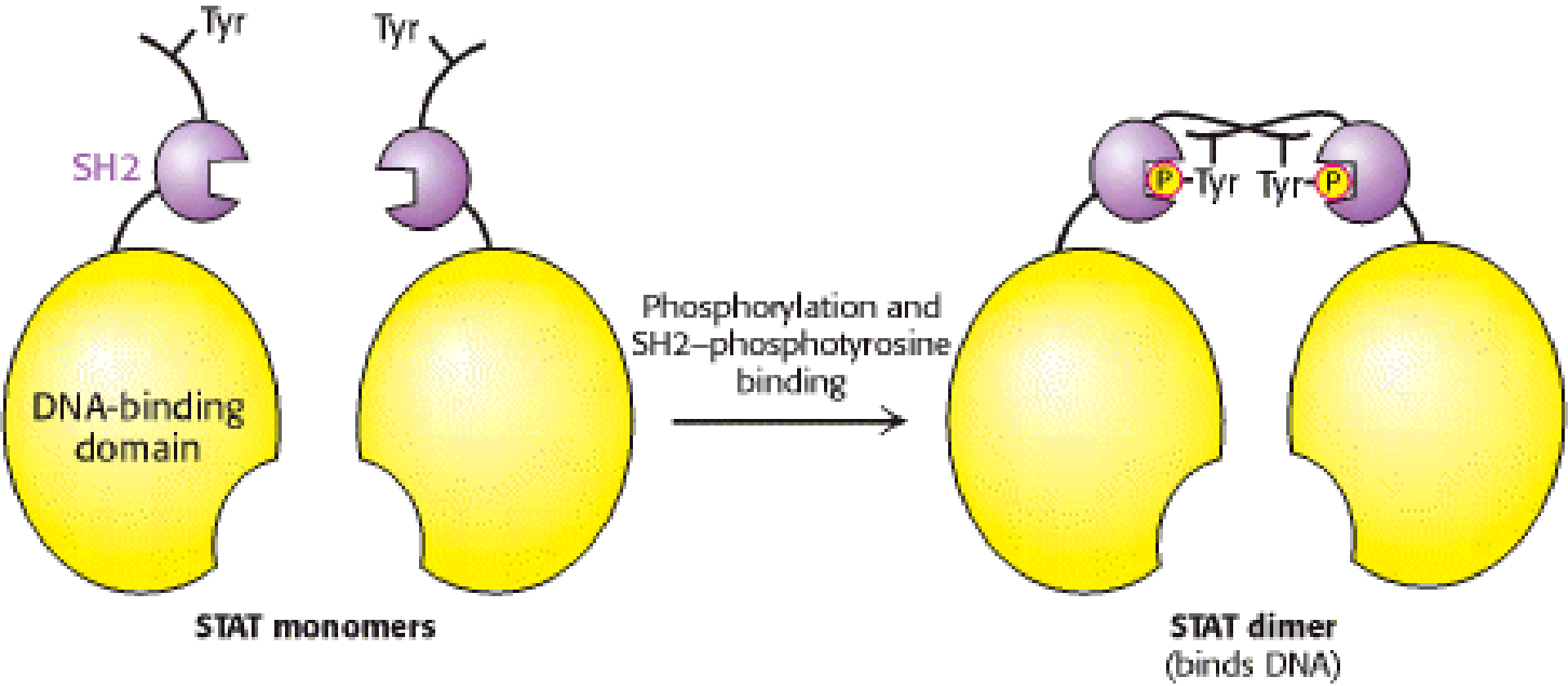
- STAT
  - Signal Transducer & Activators of Transcription
- Regulator of transcription
- STAT Phosphorylation

  → **Dimerization**

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

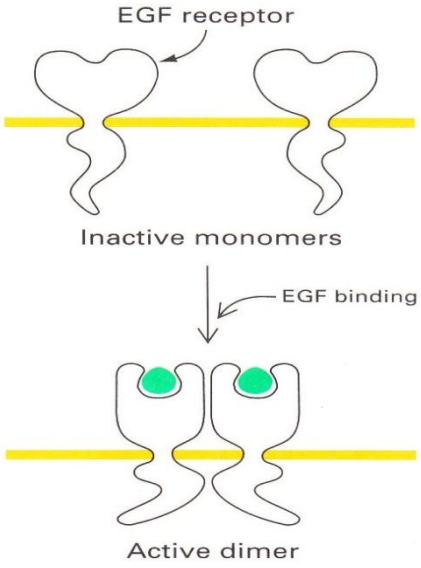
STAT is phosphorylated on a tyrosine residue near the carboxyl terminus

Phosphorylated tyr binds to SH2 domain of another STAT 5 molecule



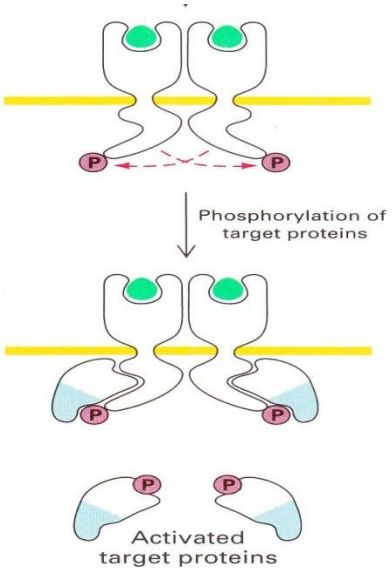
Tyrosine Kinase & other Hormones
EGF

- Epidermal Growth Factor Receptor
  - Monomeric (inactive)
  - EGF binding ➔Dimerization ➔ Cross Phosphorylation ➔ Activation



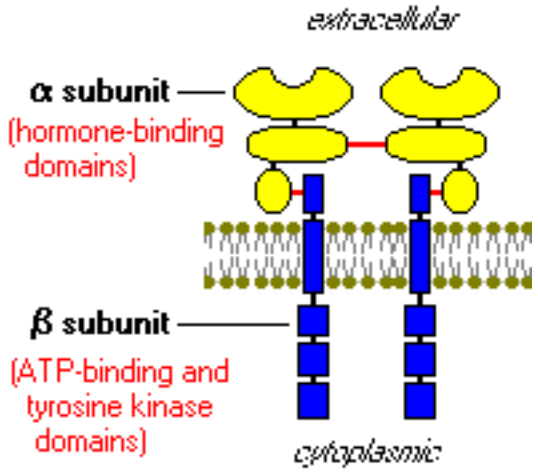
Autophosphorylation

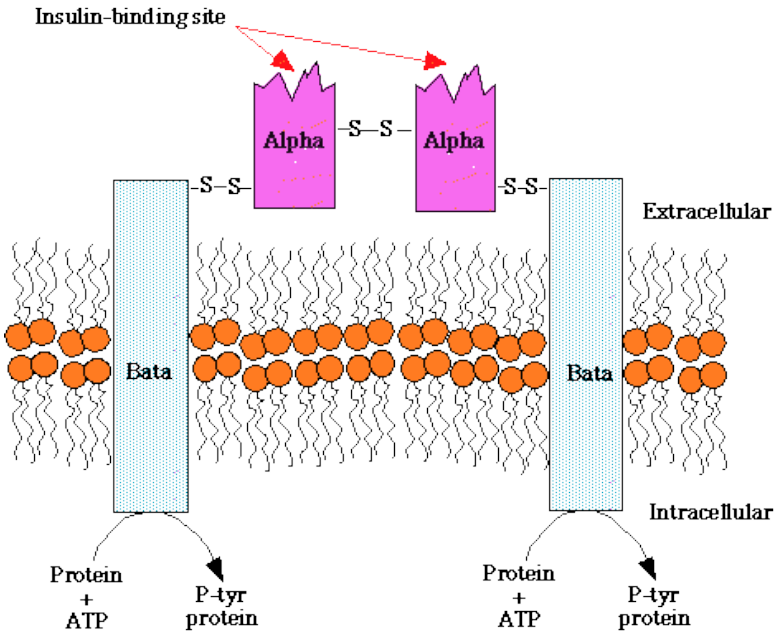
Dimerization is necessary but not sufficient for activation (kinase activity)



Tyrosine Kinase & other Hormones
Insulin

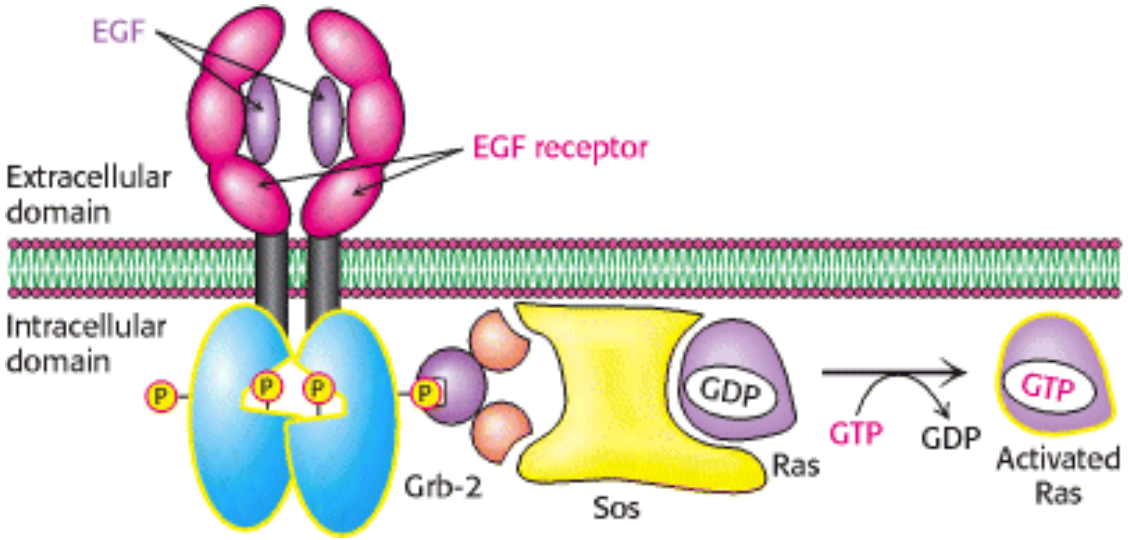
- Insulin Receptor
- Tetramer (2^{α}; 2^{β}), dimer ($2^{\alpha\beta}$ pairs)
- Disulfide bridges
- Insulin Binding → Activation of the Kinase





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_{α}



- 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 Ras proteins

Mutation ➔

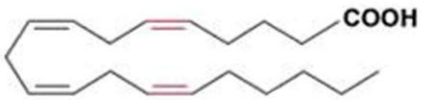
Loss of ability to hydrolyze GTP ➔

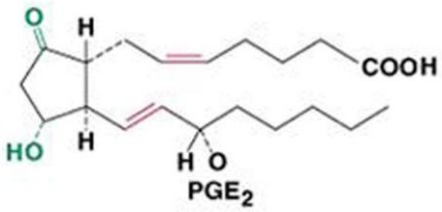
Ras is locked in "ON" position ➔

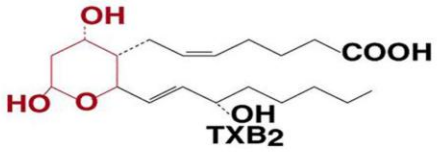
continuous stimulation of growth

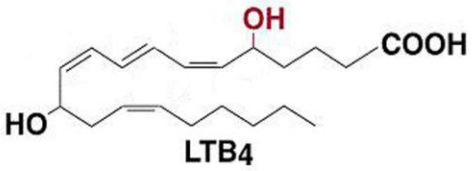
Eicosanoids

- 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

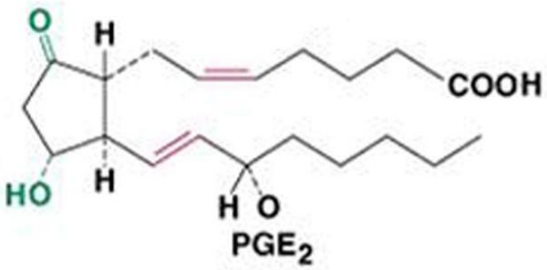
- What 2 stands for?

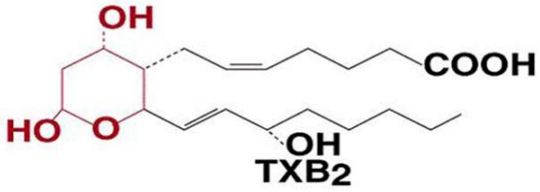
- PGI_2, PGE_2, PGD_2
  - Increase
    - Vasodilation, cAMP
  - Decrease
    - Platelet Agregation
    - Lymphocyte Migration
    - Leucocyte Aggregation

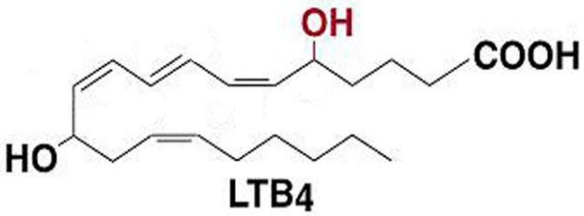
- $PGF_{2\alpha}$ Increses
  - Vasoconstriction
  - Bronchoconstriction
  - Smooth Muscle Contraction

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

Eicosanoids Structure

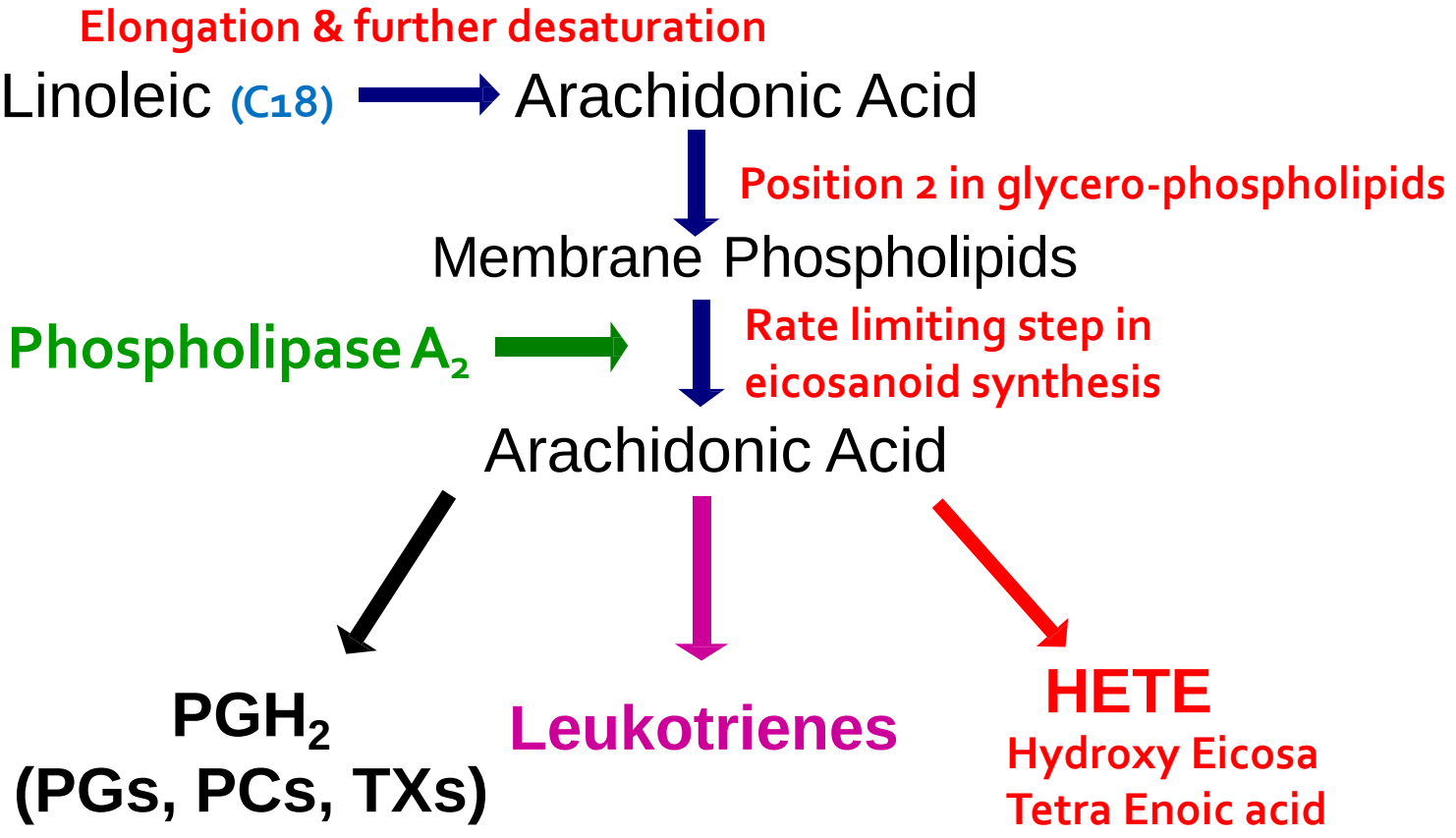






- Arachidonic acid (20, 4, no ring)
- Prostaglandins (20, 2, 5-ring)
- Thromboxanes (20, 2, 6-ring, oxygen)
- leukotrienes (20, 3 conjugated, no ring)

Eicosanoids Synthesis



Eicosanoids Can be Synthesized from other Polyunsaturated Fatty Acids

- **Fatty acids of 20 carbons with:**
  - **3 double bonds like Eicosatrienoic acid (omega-6)**
    - 1 double bonds, **PGE1 (3 → 1)**
  - **4 double bonds as Eicosatetraenoic acid (arachidonic acid)**
    - 2 double bonds, **PGE2, PGF2, TXB2 (4 → 2)**
  - **5 double bonds (Eicosapentaneoic acid : (omega-3)**
    - 3 double bonds, **PGE3, TXB3 (5 → 3)**
- **Which is more healthy? Less MI**
  - **Omega-3: TxB3 → inhibits platelet aggregation**
  - **Omega-6: TxB2 → stimulates platelet aggregation**