ZOOCC-408

SL

B.Sc. 2nd year (IVth Sem)

Classification of Receptors

By

Dr. Amresh Kumar

Dept. of Zoology, PWC, Patna-01

amresh27@gmail.com

CONTENTS

Contents

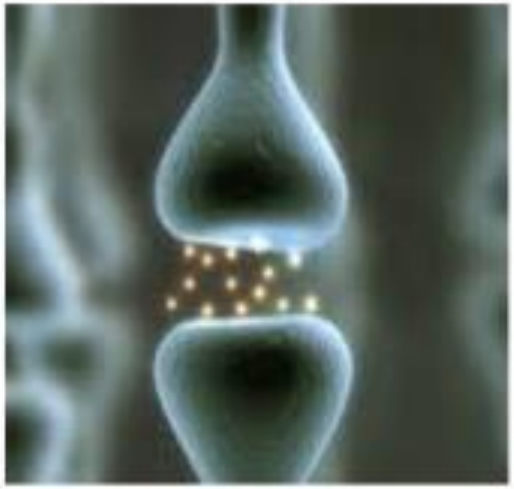
- Definition
- Classification and description of each class.
- Description of individual receptor.
- Forces affecting the drug receptor binding.
- Binding of drug receptor affect drug action.
- Agonist and antagonist.
- Disease due to malfunctioning of receptors.
- New drug design based on structure of receptors
- Receptor as target for drug discovery.
- Drug action not mediated by receptor.

WHAT IS A RECEPTOR?

- Specialized areas of cell to which drugs get bound.

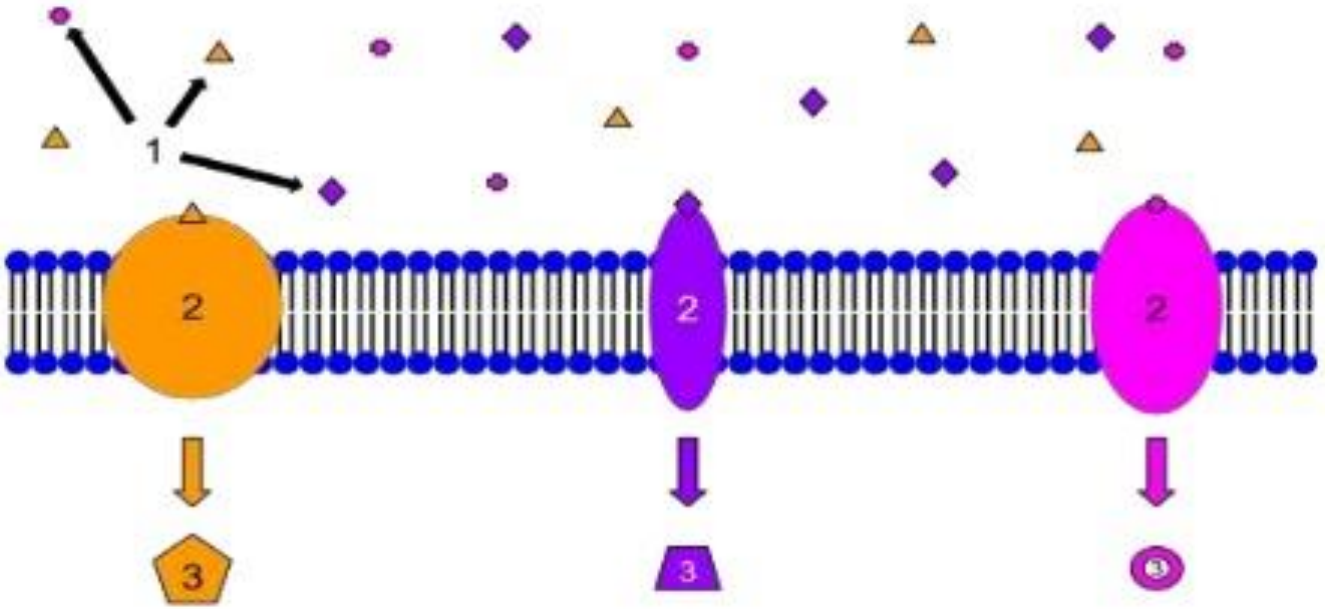
- They are regulatory protein macro molecules .

- Drug should have –selectivity to a receptor ; receptor should have ligand specificity to elicit action.



Receptor

A receptor is a protein molecule usually found embedded within the plasma membrane surface of a cell that receives chemical signals from outside the cell and when such chemical signals bind to a receptor, they cause some form of cellular/tissue response.



DRUG RECEPTOR INTERACTIONS

- Effect of drug attributed to two factors

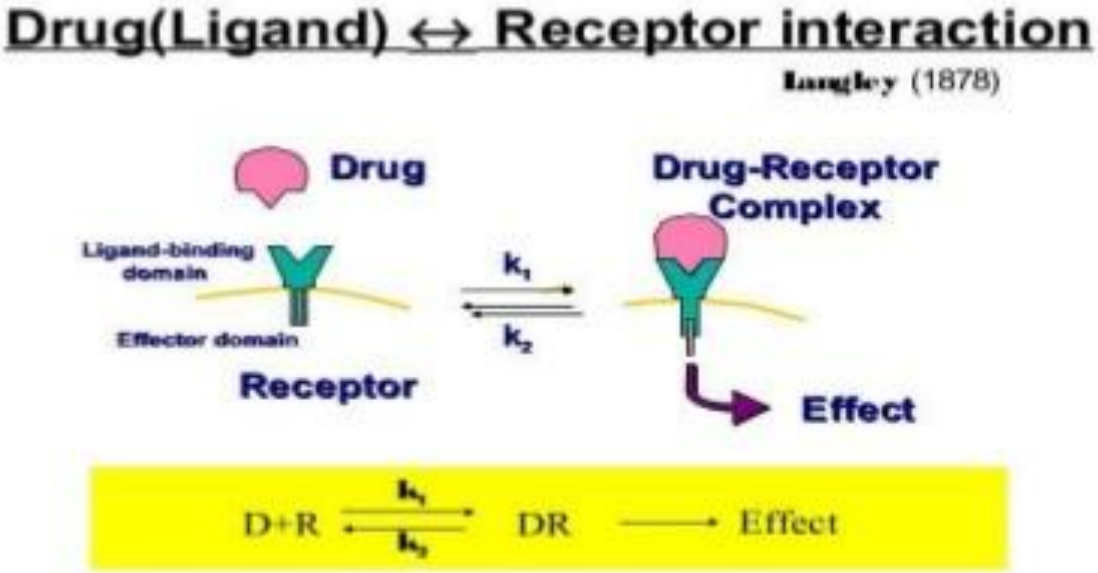
1. Affinity : tendency of the drug to bind to receptor and form D-R complex .

2. Efficacy or intrinsic activity : ability of the drug to trigger pharmacological responses after forming D-R complex .

Binding affects drug action

Drugs produce effects by interacting with special macromolecular components(receptor) forming drug-receptor complex & modify the function of the receptor.

Drug+Receptor -> Drug-receptor complex -> Modified biological function



CONTD…

- Based on affinity and intrinsic activity :

- Full agonist : high affinity

  high intrinsic activity(=1)

Eg. Methacholine on acetylcholine receptors

- Antagonist : only affinity

  no intrinsic activity (=0)

Eg. Atropine on muscarinic receptors

Agonist and antagonist

Agonist :

Agonists are chemical that binds to a receptor of a cell and triggers a response by that cell.

Antagonist :

Antagonists are drugs that decrease the actions of another drug or endogenous ligand.

Malfunctioning of receptors

1. Autoimmune :

- Nicotinic cholinergic receptors → myasthenia gravis
- Insulin receptors → insulin-resistant diabetes mellitus
- TSH receptor → Grave's disease (activation)
- TSH receptor → Atropic thyroiditis (blocking)

Malfunctioning of receptors

2. Loss-of-function mutations :

Hypothyroidism, hypogonadism, short stature, diabetes insipidus.

TABLE 1 Diseases caused by GPCR loss-of-function mutations

Receptor	Disease	Inheritance
Cone opsins	Color blindness	X-linked; autosomal recessive
Rhodopsin	Retinitis pigmentosa	Autosomal dominant; recessive
V2 vasopressin	Nephrogenic diabetes insipidus	X-linked
ACTH	Familial ACTH resistance	Autosomal recessive
LH	Male pseudohermaphroditism	Autosomal recessive
Ca^{2+} sensing	Familial hypocalciuric hypercalcemia	Autosomal dominant
Ca^{2+} sensing	Neonatal hyperparathyroidism	Autosomal recessive
Endothelin-B	Hirschsprung disease	Complex
FSH	Hypergonadotropic ovarian failure	Autosomal recessive
TSH	Congenital hypothyroidism	Autosomal recessive
TRH	Central hypothyroidism	Autosomal recessive
GHRH	Growth hormone deficiency	Autosomal recessive
GnRH	Central hypogonadism	Autosomal recessive
Melanocortin 4	Extreme obesity	Codominant
PTH/PTHrP	Blomstrand chondrodysplasia	Autosomal recessive

Malfunctioning of receptors

3. Gain-of-function mutations :

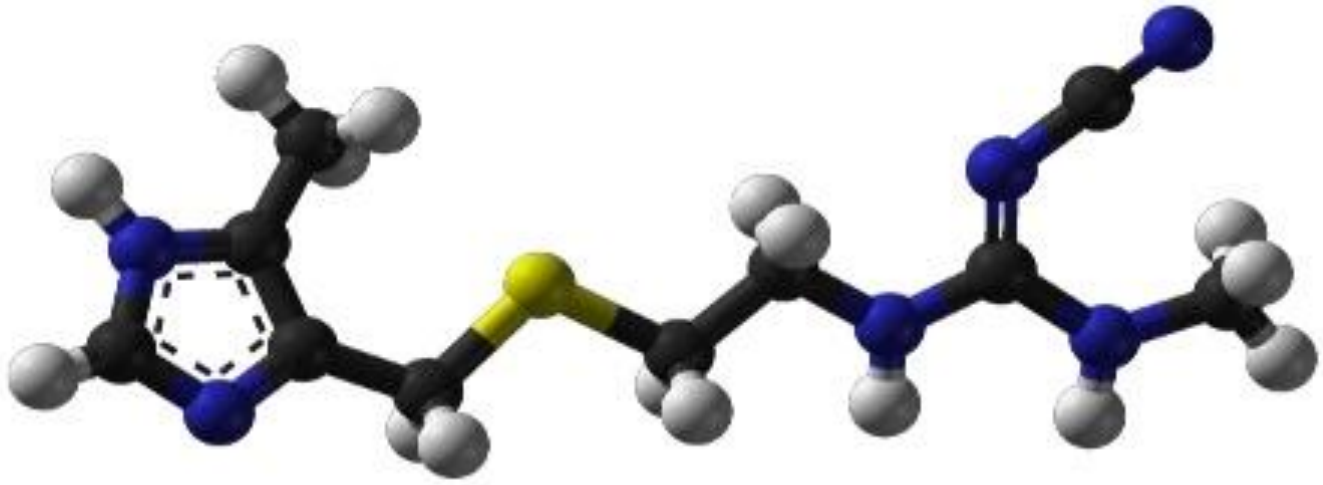
- Activation in the absence of a ligand.
- Increased sensitivity to the receptor's usual agonist.

TABLE 2 Diseases caused by GPCR gain-of-function mutation

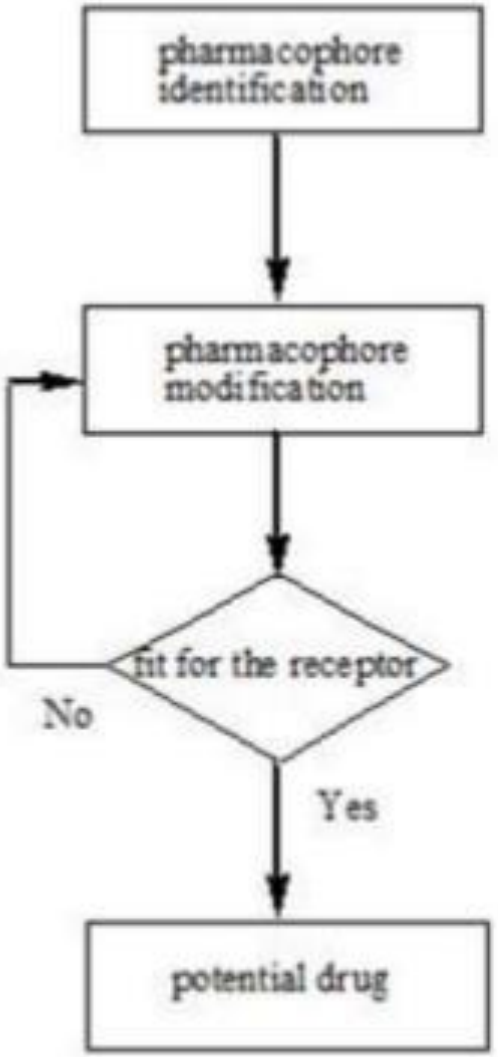
Receptor	Disease	Inheritance
Rhodopsin	Congenital night blindness	Autosomal dominant
LH	Familial male precocious puberty	Autosomal dominant
LH	Sporadic Leydig cells tumors	Somatic
TSH	Familial nonautoimmune hyperthyroidism	Autosomal dominant
TSH	Sporadic hyperfunctional thyroid adenomas	Somatic
Ca^{2+} sensing	Familial hypocalcemia	Autosomal dominant
PTH/PTHrP	Jansen metaphyseal chondrodysplasia	Autosomal dominant

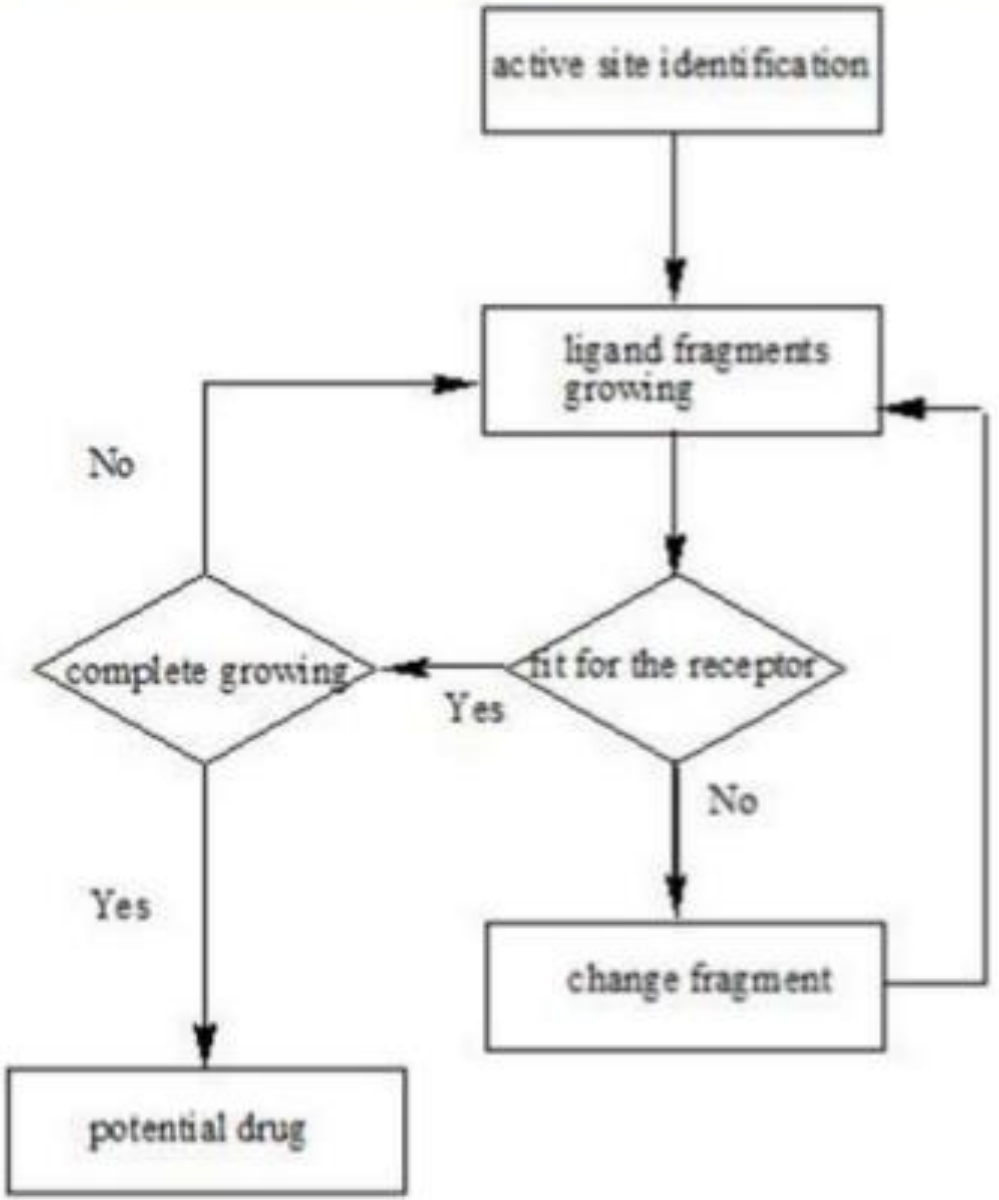
New drug design

- Examination of the 3D structure of the biological target.
- Need to find out the specific chemical groups that could be part of an attractive interaction between the target protein and the drug.
- Lastly need to design a drug candidate that will have multiple sites of complementary interactions with the biological target.



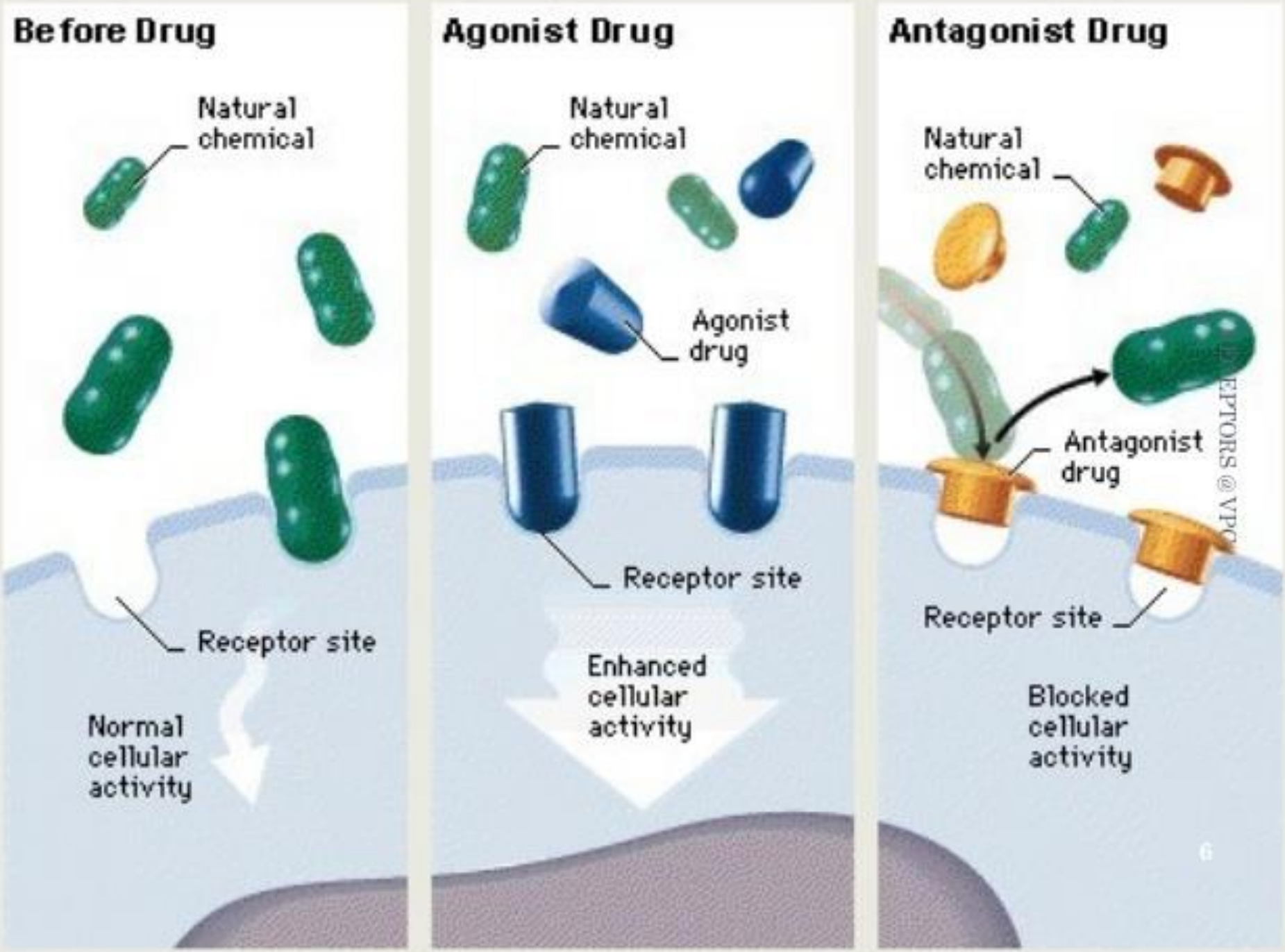
Receptor as target for drug discovery





Drug action not mediated by receptor

- **Physical and chemical means** - Antacids, chelating agents and cholestyramine etc.
- **Alkylating agents:** binding with nucleic acid and render cytotoxic activity – Mechlorethamine, cyclophosphamide etc.
- **Antimetabolites:** purine and pyrimidine analogues – 6 MP and 5 FU –antineoplastic and immunosuppressant activity.


Before Drug
Natural chemical
Receptor site
Normal cellular activity
Agonist Drug
Natural chemical
Agonist drug
Receptor site
Enhanced cellular activity
Antagonist Drug
Natural chemical
Antagonist drug
Receptor site
Blocked cellular activity

Classification

There are 2 types of receptors. Those are : **Internal** & **Cell surface receptor**.

i. **Internal /Intracellular/Cytoplasmic receptors** :

- ✓ found in the cytoplasm of the cell
- ✓ respond to hydrophobic ligand molecules

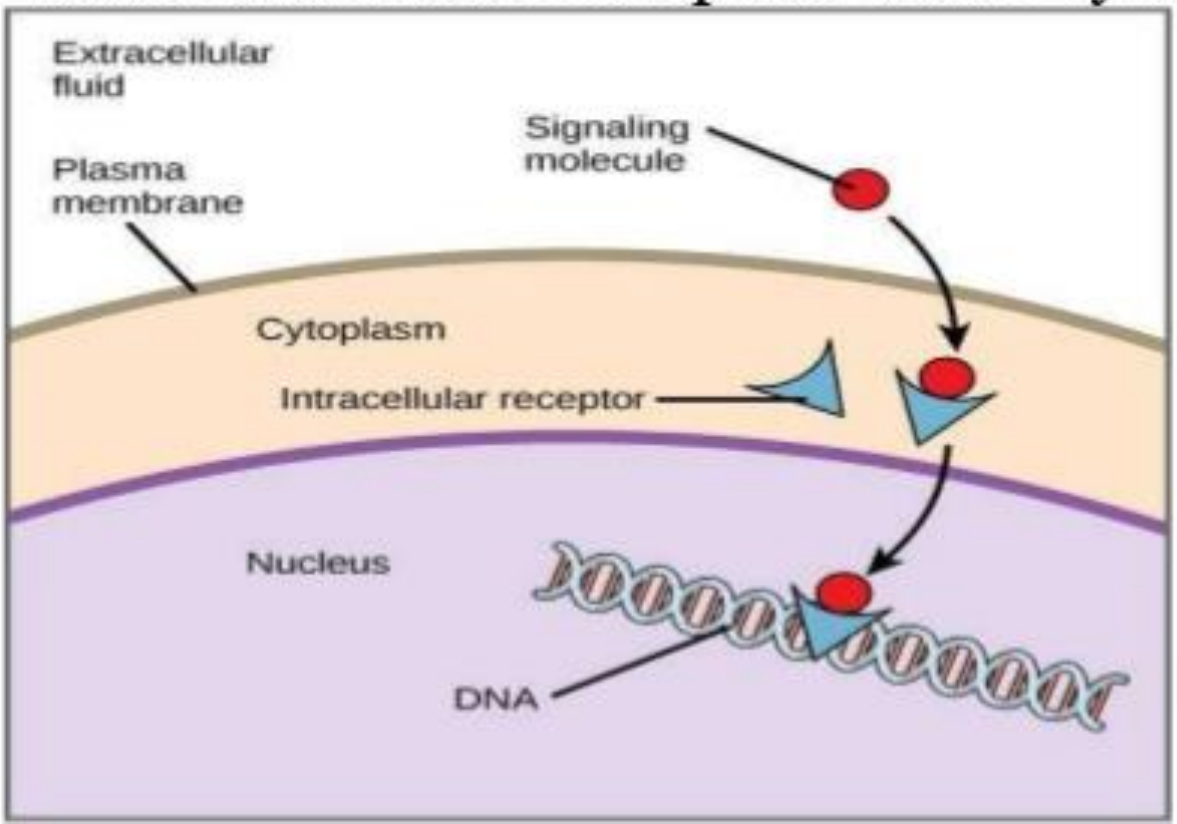
RECEPTOR CLASSIFICATION

Cell surface	Intracellular
1. Inotropic.	1. Nuclear receptors .
2. Metabotropic.	
3. Ligand regulated trans membrane.	

Internal receptor :

Intracellular Receptors :

- ✓Hydrophobic signaling molecules typically diffuse across the plasma membrane
- ✓interact with intracellular receptors in the cytoplasm.



Cell surface receptor

ii. **Cell-surface /transmembrane receptors/cell-specific proteins**

✓ performs signal transduction, converting an extracellular signal into an intracellular signal.

3 main components:

i. an external ligand-binding domain (extracellular domain),
ii. a hydrophobic membrane-spanning region,
iii. and an intracellular domain inside the cell.

Cell surface receptor

There are three general categories of cell-surface receptors:

1. Ion channel-linked receptors,
2. G-protein-linked receptors,
3. Enzyme-linked receptors.

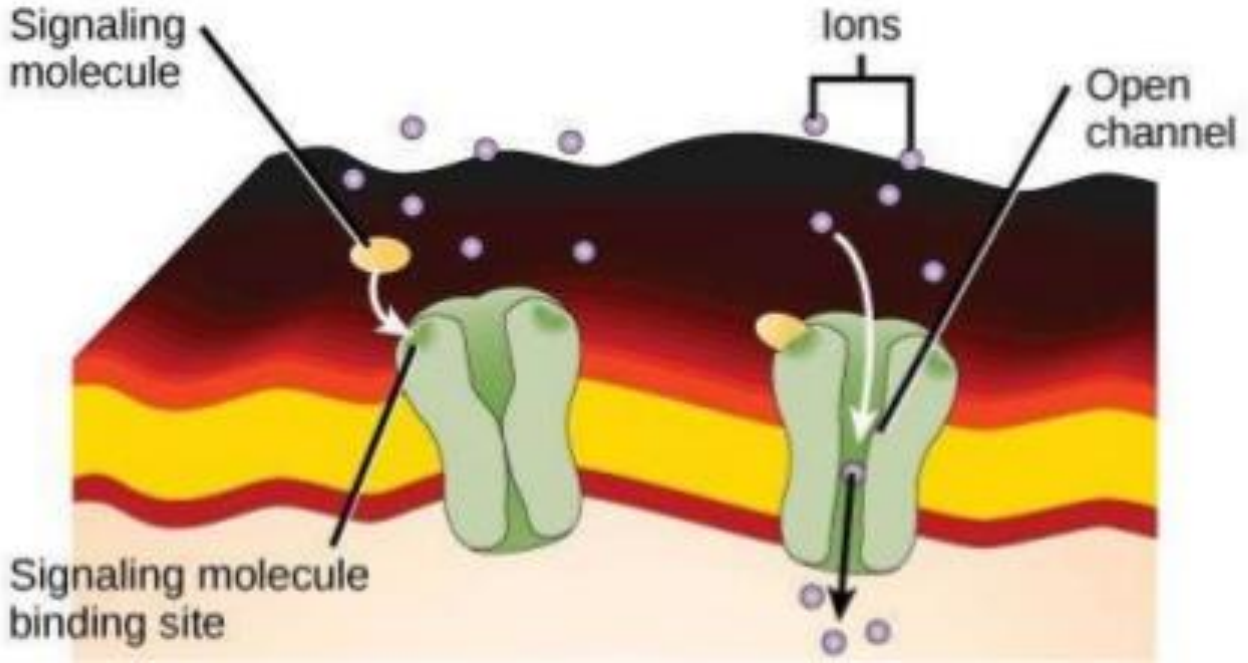
Ligand Gated Ion Channels

- Also called ionotropic receptors.
- Involved mainly in fast synaptic transmission.

Eg: nAchR, $GABA_A$, and glutamate receptors of the NMDA, AMPA and kainate types.

Ion Channel-Linked Receptors

- ✓ Receptors bind with ligand. (Ex:**Nicotinic Receptor**)
- ✓ Open a channel through the membrane that allows specific ions to pass through.
- ✓ Conformational change in the protein's structure that allows ions such as Na,Ca, Mg, and H_2 to pass through.

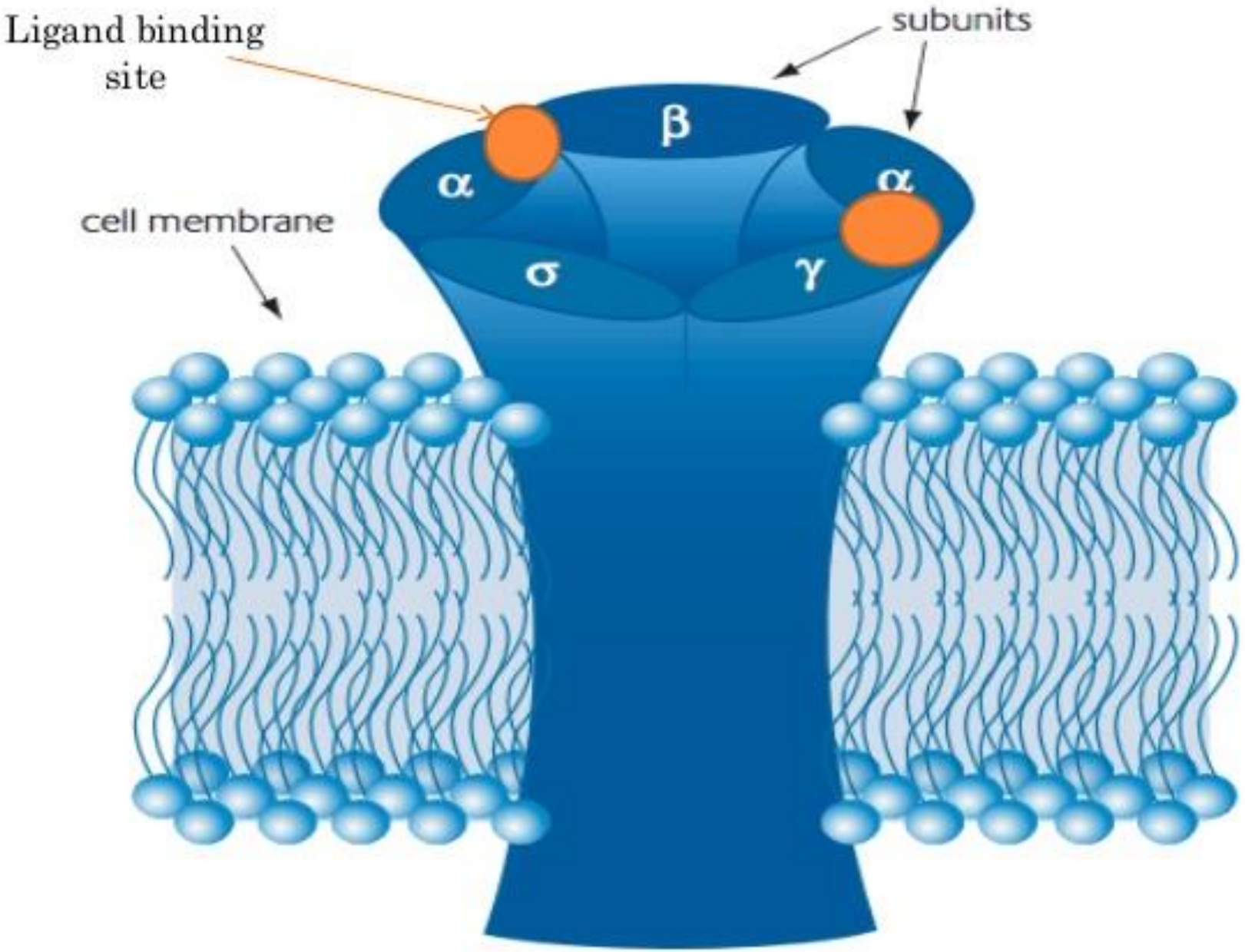


FEATURES – ION CHANNELS

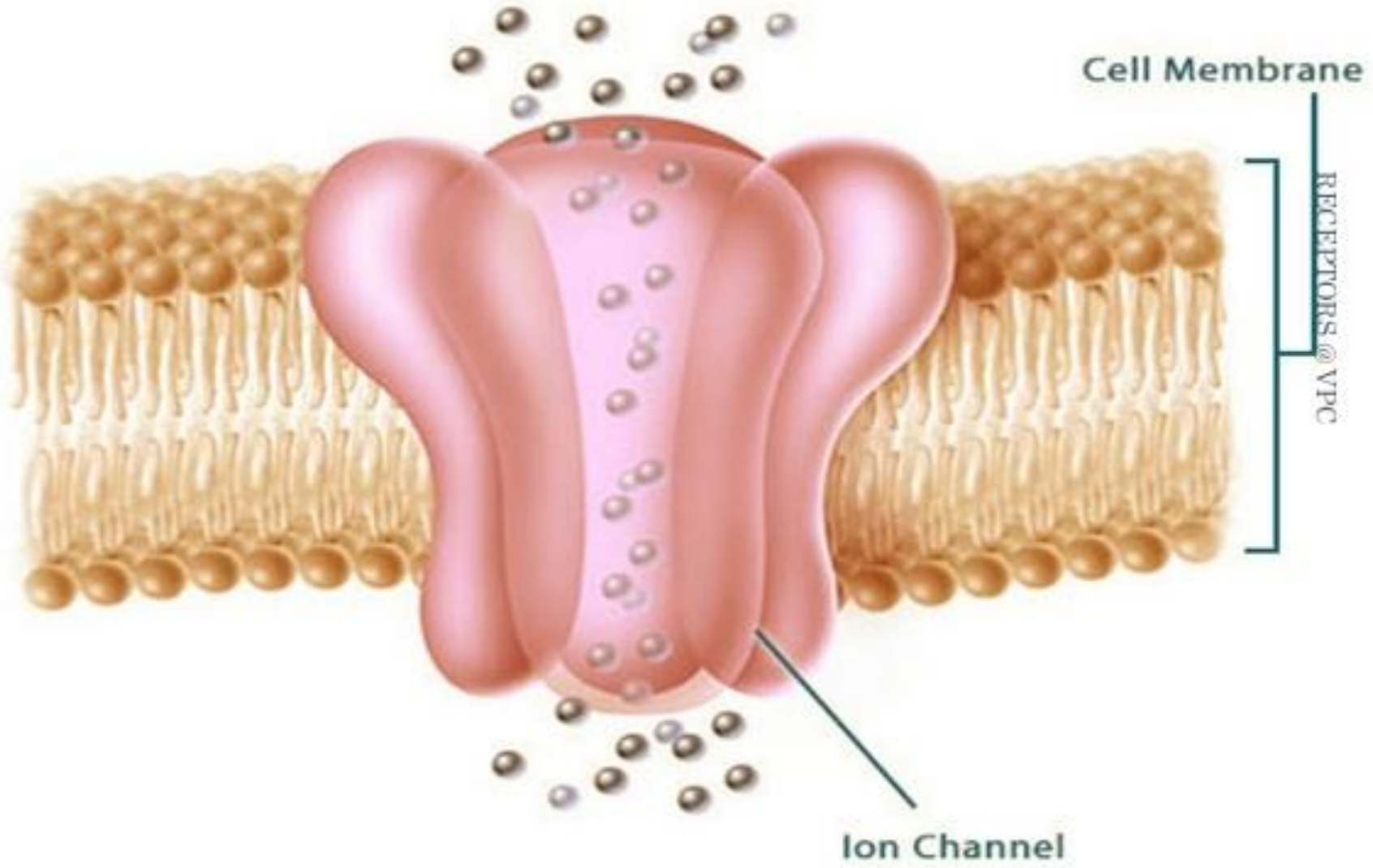
- Protein molecules form water filled pores that span the membrane.
- Switch between open and closed states.
- Rate and Direction of movement depends on electrochemical gradient of the ions

MOLECULAR STRUCTURE

- ligand binding site in extracellular domain.
- 4 subunits α, β, γ and δ.
- α_2, β, γ - pentameric str - 2 ligand binding sites
- Each subunit spans the membrane 4 times; all subunits form a central pore.



Representative Ion Channel


Cell Membrane
RECEPTORS @ VPC
Ion Channel

Mechanism of receptor action

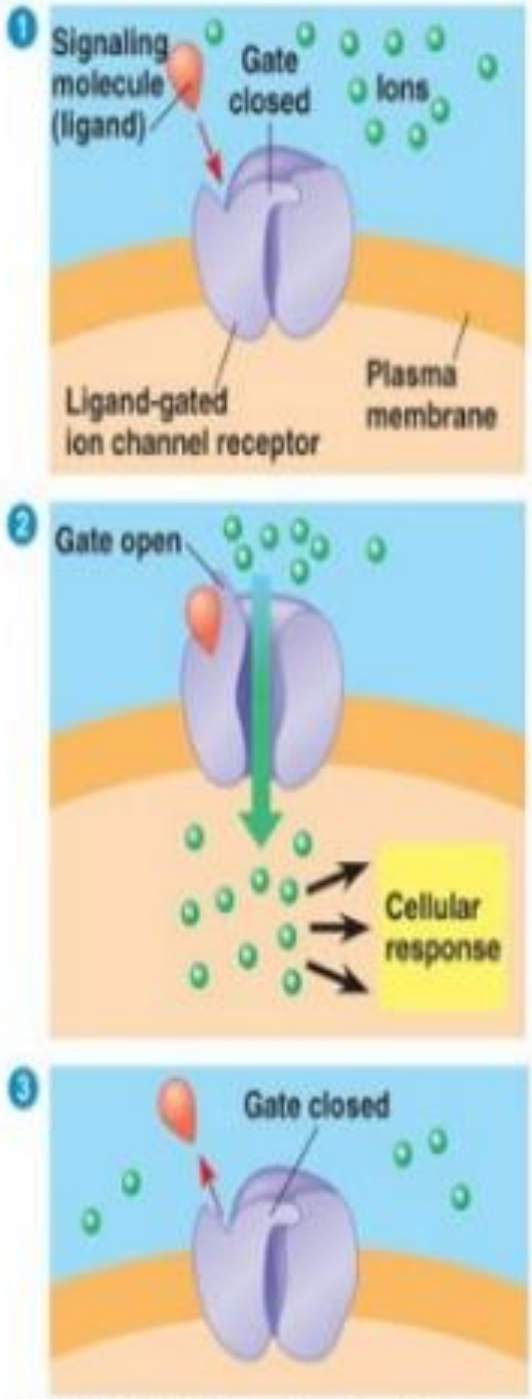
Signal molecule binds as a ligand at a specific site on the receptor

↓

Confomational changes open the channel allowing ions to flow into the cell

↓

The change in ion concentration within the cell triggers cellular responses



CONTD…

- Due to the concentration changes of different ions the following effects are seen.

- Increase in Na^+ and Ca^+ levels- excitatory
- Decrease in Na^+ and Ca^+ levels- inhibitory

- Increase in K^+ levels – inhibitory
- Decrease in K^+ levels – excitatory

- Increase in Cl^- levels – inhibitory
- Decrease in Cl^- levels- excitatory

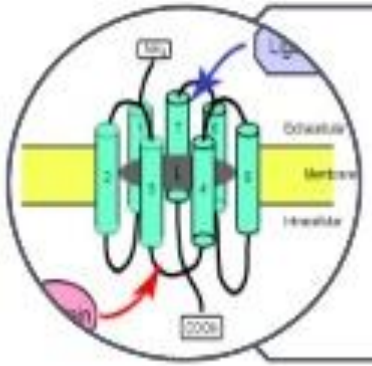
ION CHANNELS - IMPORTANCE

- Generation , propagation of nerve impulse.
- Synaptic transmission of neurons.
- Muscle contraction.
- Salt balance.
- Hormone release.
- Muscle relaxants , anti-arrhythmatics ,anesthetics – act by blocking ion channels.

G-Protein Linked Receptors

- ✓ Binds with a ligand and activate a membrane protein called a G-protein.
- ✓ The activated G-protein then interacts with either an ion channel or an enzyme in the membrane.
- ✓ Each receptor has its own specific extracellular domain and G-protein-binding site.

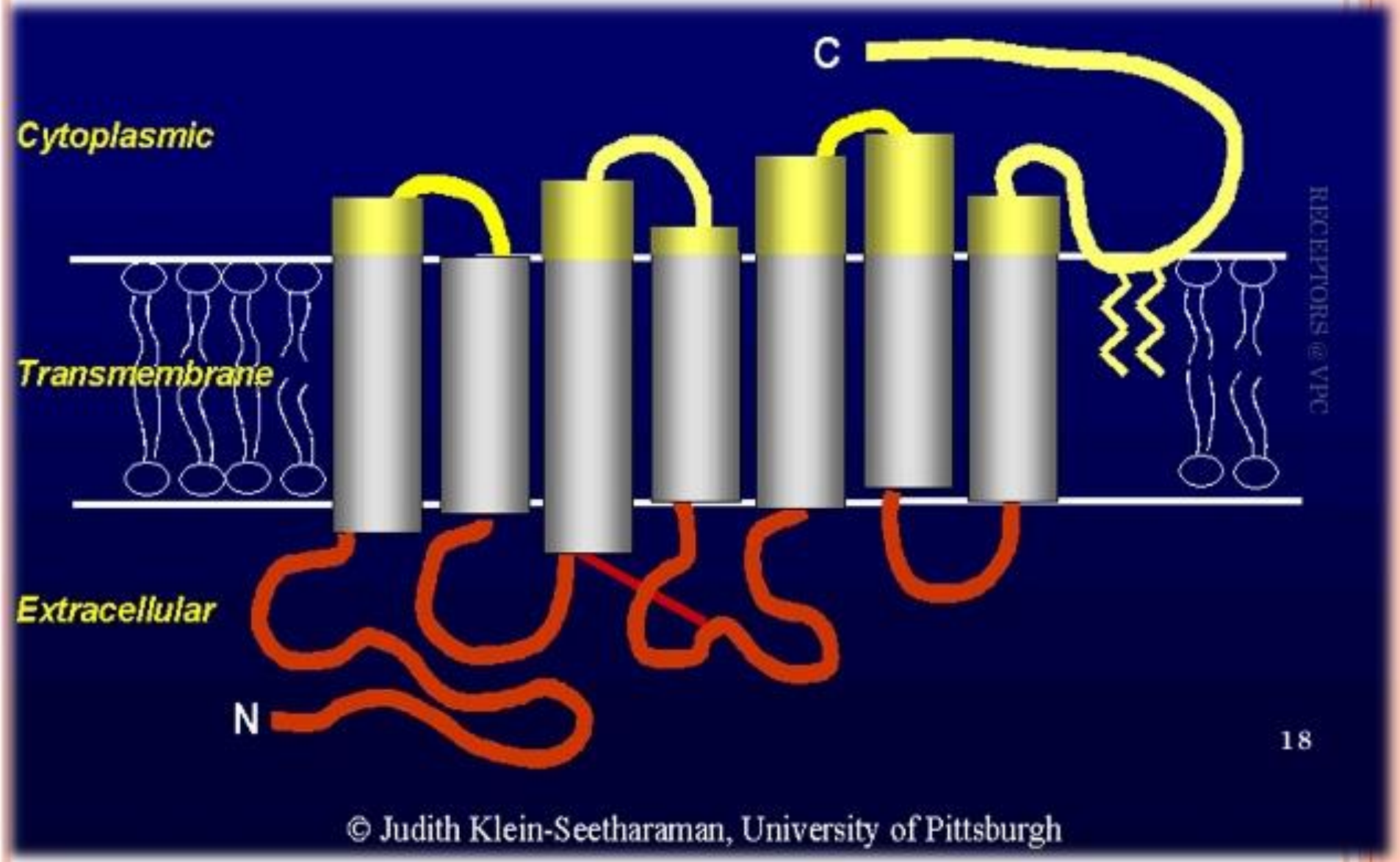
Example : **Beta-adrenergic receptor**



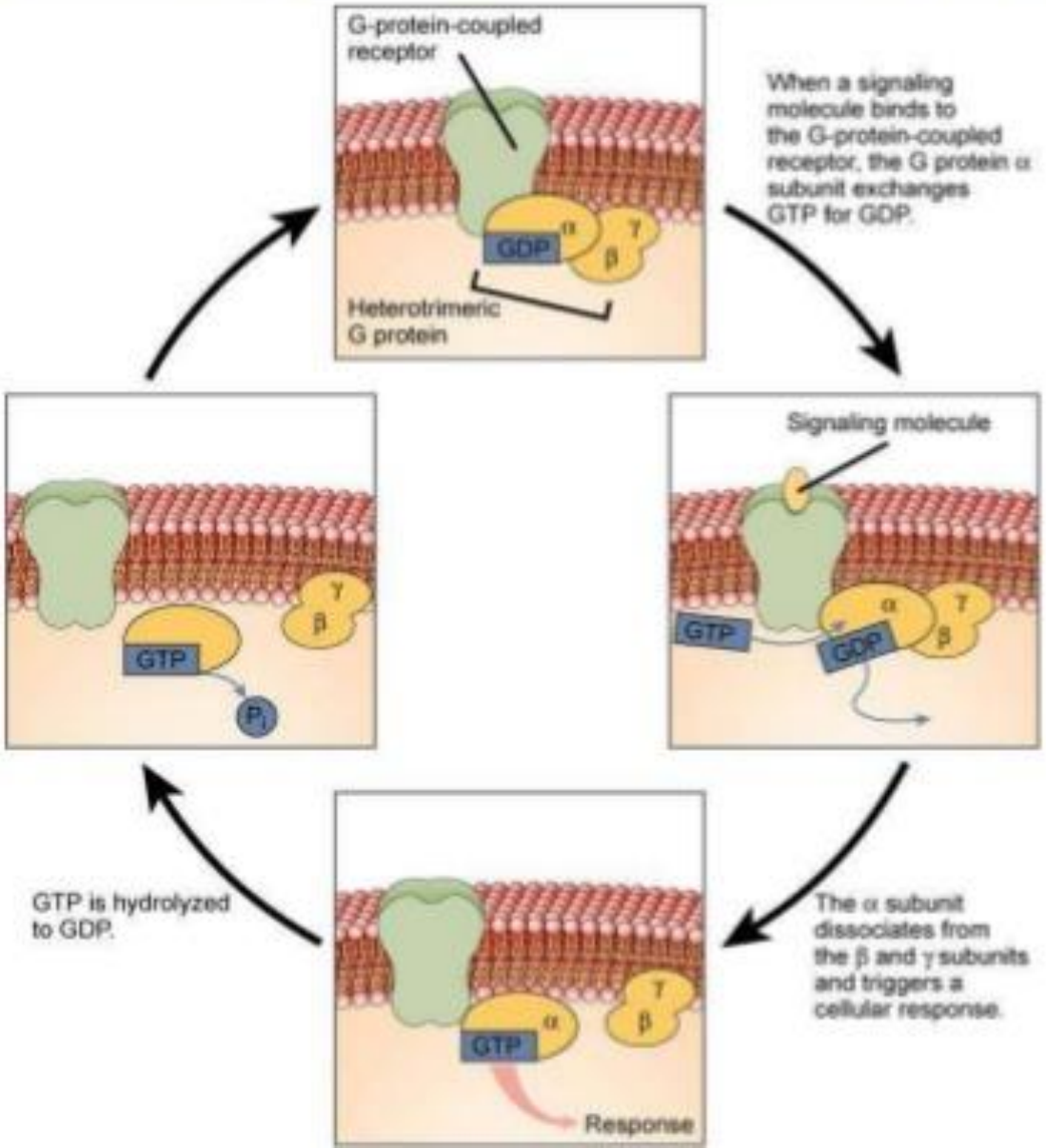
G-Protein Coupled Receptors

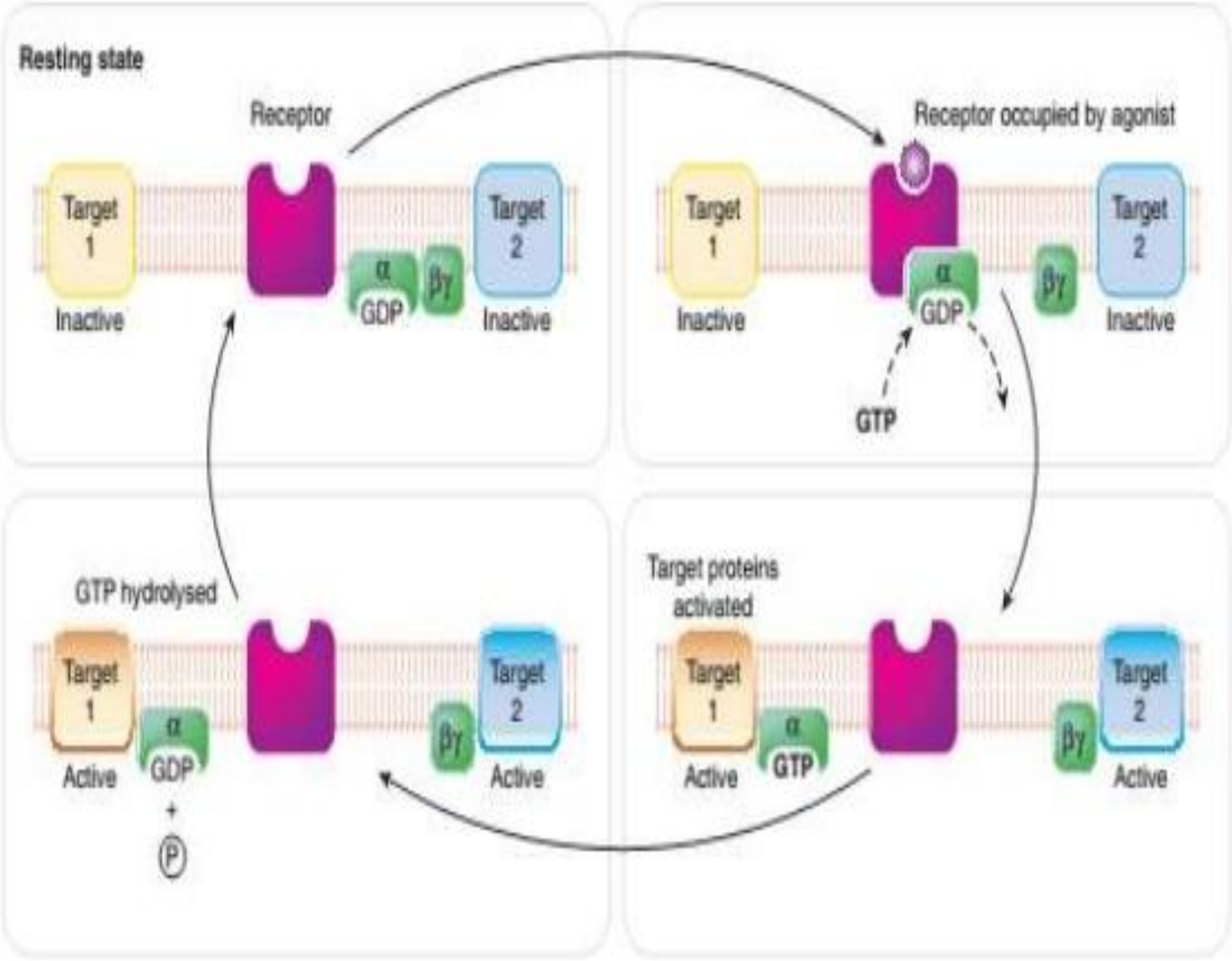
- metabotropic or 7-transmembrane-spanning (heptahelical) receptors.
- coupled to intracellular effector systems via a G-protein.
- mAChRs, adrenoceptors, dopamine, 5-HT, opiate, peptide, purinoceptors, orphans .

MOLECULAR STRUCTURE



G-Protein Linked Receptors




Resting state
Receptor
Target 1
Inactive
α
GDP
βγ
Target 2
Inactive
Receptor occupied by agonist
Target 1
Inactive
α
GDP
βγ
Target 2
Inactive
GTP
Target proteins activated
Target 1
Active
α
GTP
βγ
Target 2
Active
GTP hydrolysed
Target 1
Active
α
GDP
+
P
βγ
Target 2
Active

FAMILIES OF GPCR

3 families:

- A – rhodopsin family

  eg. Amine NT, purines , cannabinoids

- B - secretin/glucagon receptor family Eg. Peptide hormones.

- C - metabotropic glutamate receptor/calcium sensor family.

  Eg. $GABA_B$, Glutamate.

G-PROTEIN -ROLE

- Membrane resident proteins – recognize activated GPCRs- pass message to effector system.
- Occurs in interaction with guanine nucleotides ; freely moving in cytoplasm.
- α, β and γ subunits – trimer in resting state.
- 3 subunits attached to GPCR through fatty acid chain – reaction called prenylation.

G-PROTEIN SUBTYPES

G-PROTEIN	RECEPTOR FOR	SIGNALLING PATHWAY
G_s	Beta adrenergic amines, glucagon histamine, serotonin	↑ Adenylyl cyclase ↑ CAMP •Excitatory effects
G_{i1}, G_{i2}, G_{i3}	$Alpha_2$ adrenergic amines, mAchR, opioid, serotonin	↓ adenylyl cyclase ↓ CAMP ↑ Cardiac K^+ channel open-rate ↓ heart
G_{olf}	Olfactory epithelium	↑ Adenylyl cyclase ↑ CAMP

G-PROTEIN	RECEPTOR FOR	SIGNALLING PATHWAY
G_O	NT ,Opioid cannabinoid	Not clear
G_q	mAchR, serotonin $5HT_{1C}$	↑ PLC IP_3 , DAG Cytoplasmic Ca
G_{t1} , G_{t2}	Rhodopsin and colour opsins in retinal rod and cone cells	↑ cGMP phosphodiesterase-cGMP ↓

SECONDARY MESSENGER SYSTEMS INVOLVED IN SIGNAL TRANSDUCTION

- The adenyly cyclase / cAMP system
- The Phospholipase C / inositol phosphate system
- The Ion channels
- The Rho A /Rho kinase system

ADENYLYL CYCLASE/ CAMP SYSTEM

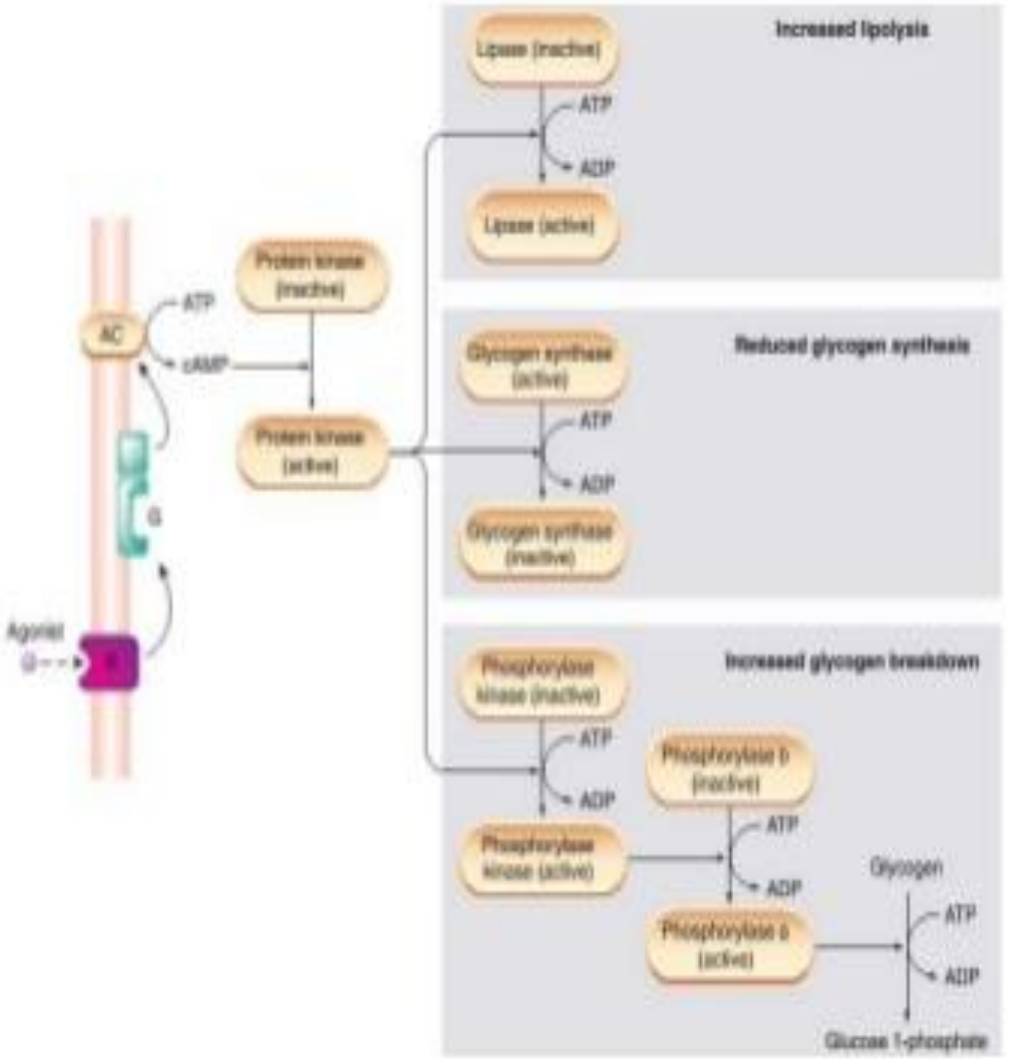
- c AMP –nucleotide synthesized from ATP - by adenylyl cyclase, metabolized by PDE.
- Regulate enzymes of metabolism, growth, contractile proteins of muscle
- NT - acts on GPCR –G_s/G_i activated - produce effects – by inc or dec. activity of adenylyl cylase-and cAMP.
- c AMP- activate - Protein kinases-activate/inactivate enzymes by phosphorylation – cellular events.

Targets for G-Proteins

➢ Adenylate cyclase

- catalyses formation of the intracellular messenger cAMP

- cAMP activates various protein kinases that control cell function in many different ways by causing phosphorylation of various enzymes, carriers & other proteins

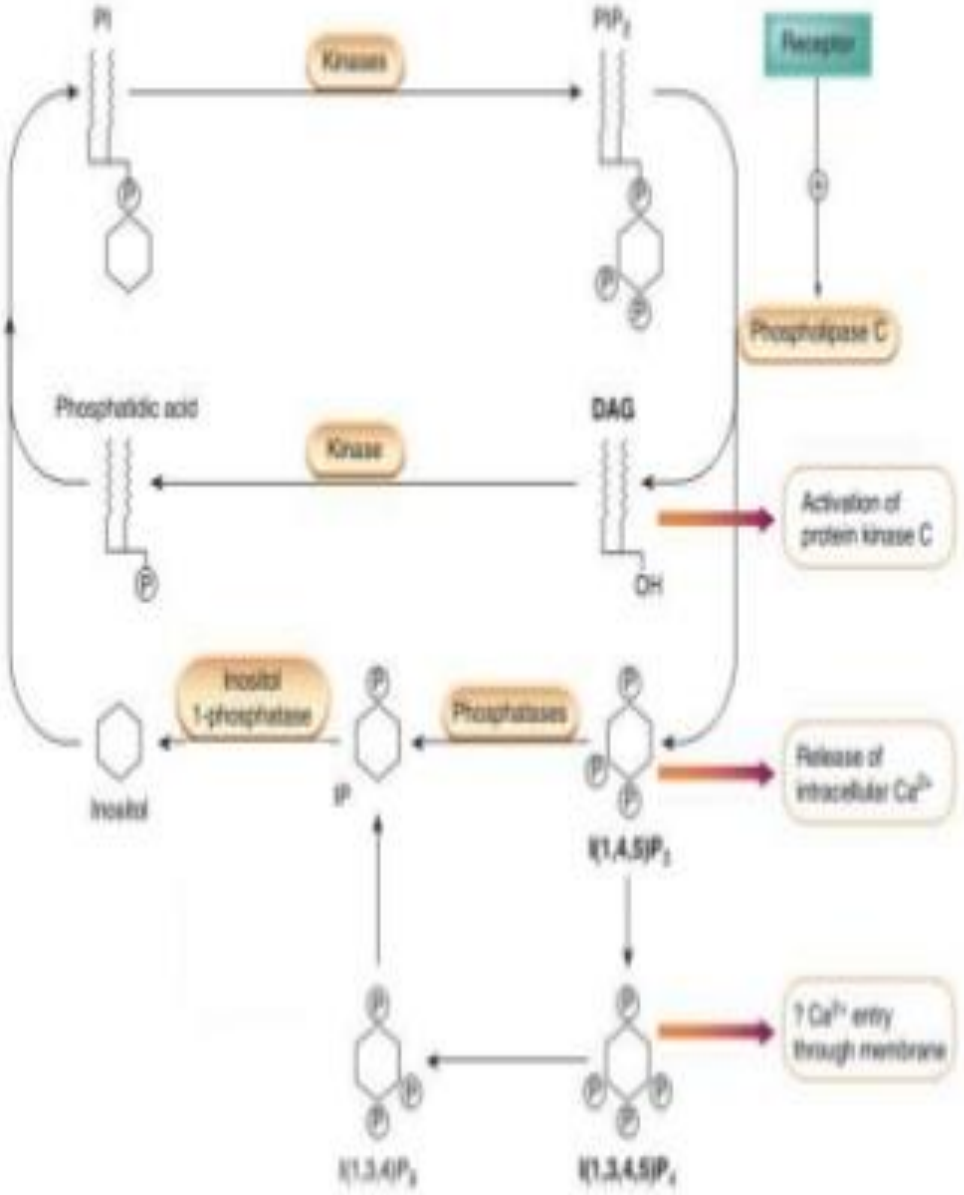


PHOSPHOLIPASE C-INOSITOL SYSTEM

- Phospholipase C : Cleaves membrane phospholipids - phosphoinositides.
- PLC beta – cleaves phosphatidylinositol(4,5)bis Phosphate PIP_2 - into DAG and IP_3
- DAG and IP_3 - Secondary messenegers – elicit cellular responses.

➢Phospholipase C/IP3/DAG

- catalyzes the formation of IP3 and DAG from membrane phospholipid
- IP3 increases free cytosolic **Ca2+** (releasing Ca2+ from intracellular compartments) which initiates many events
- DAG activates **protein kinase C**, which controls many cellular functions by phosphorylating proteins



- **Ion Channels** appears to be general mechanism for controlling K^+ and Ca^{++} channels by direct interaction between the βγ-subunit of G_0 and the channel

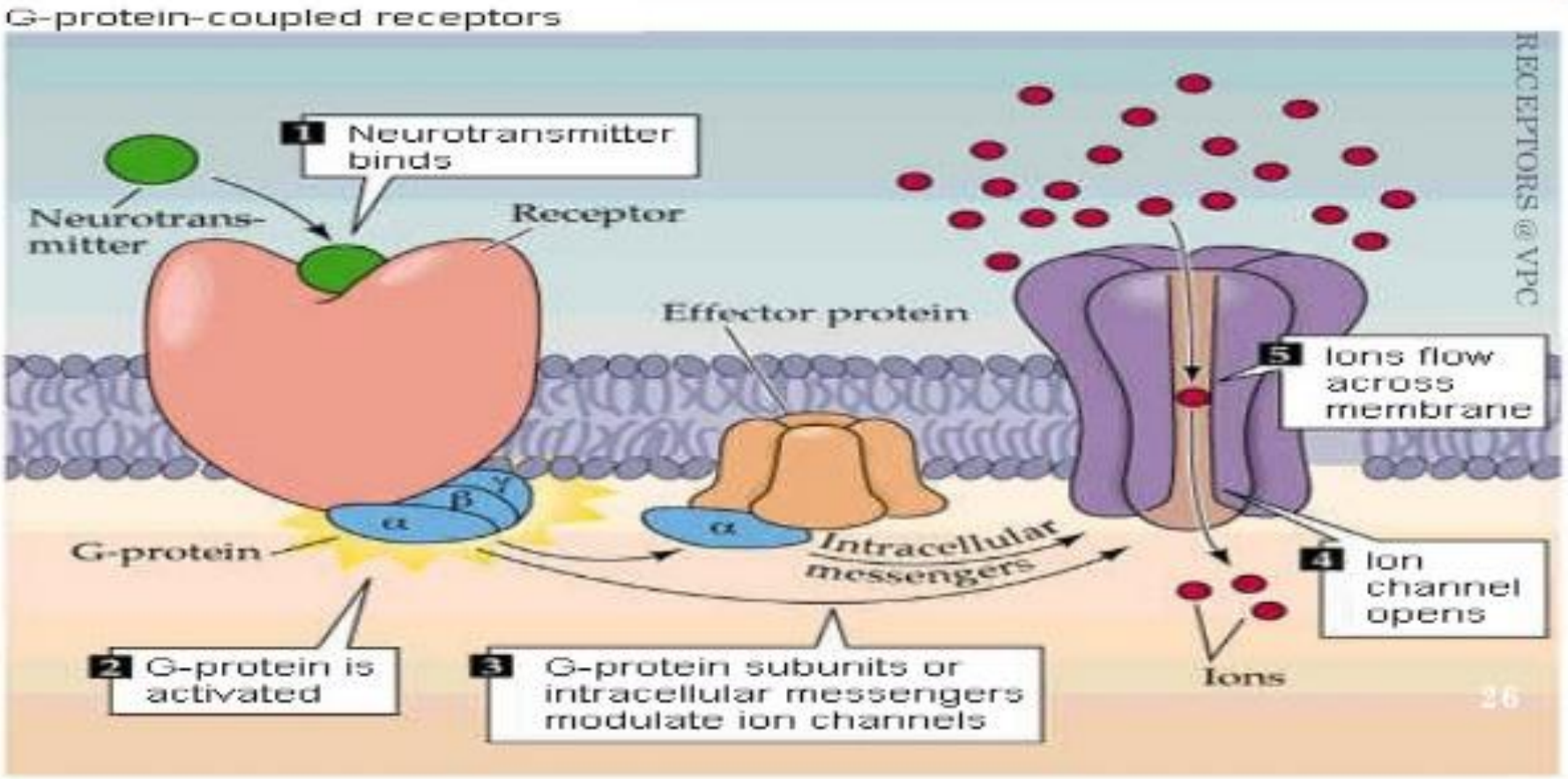
- **Phospholipase A_2** (formation of arachidonic acid and eicosanoids)

- **The Rho/Rho kinase system** $G_{12/13}$ type G-protein. α subunit interacts with ***guanosine nucleotide exchange factor***, which facilitates GDP–GTP exchange at another GTPase, **Rho**. On exchange **Rho** activated & activates **Rho kinase** - phosphorylates substrate proteins

- **The MAP kinase system** activated by cytokines and growth factors acting on kinase-linked receptors and by GPCR ligands. Controls processes involved in cell division, apoptosis and tissue regeneration

ION CHANNELS

- **GPCR- directly control ion channel-without secondary messenger.**
  Eg. mAchR in heart – activate K^+ channel.

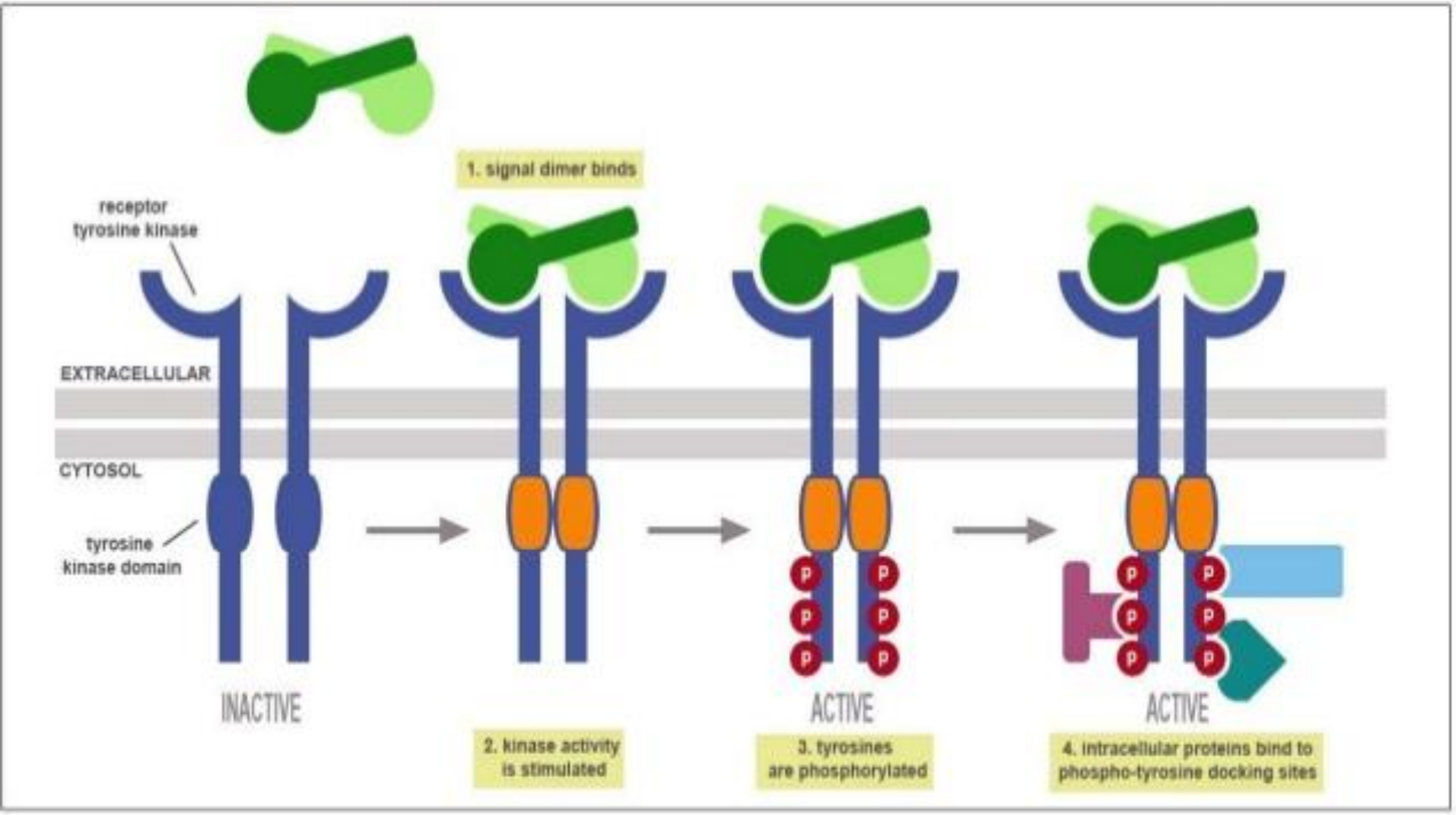


Enzyme-Linked Receptors

- ✓Cell surface receptors with intracellular domains that are associated with an enzyme.
- ✓ Normally have large extracellular and intracellular domains.
- ✓When a ligand binds to the extracellular domain, a signal is transferred through the membrane and activates the enzyme, which eventually leads to a response.

Example : **Tyrosine Kinase receptor**

Enzyme-Linked Receptors



Forces affecting the binding

3 major types of chemical forces/bonds. Those are : Covalent, Electrostatic & Hydrophobic interaction.

1. Covalent bond :

- very strong
- "irreversible" under biological conditions.
- extremely stable.

Example : It is formed between the activated form of Phenoxybenzamine and the α-adrenergic-receptor.

Forces affecting the binding

2. Electrostatic bond :

- Very common & weaker than covalent.
- Interaction strength is variable

Example : van-der Waals forces.

3. Hydrophobic interactions :

- Generally weak, but important.
- Significant in driving interactions.
- Lipophilic drugs and the lipid component of biological membranes.

KINASE LINKED RECEPTORS

- Involved in growth, proliferation, differentiation or survival-called growth factors.
- Mediate actions of protein mediators- GF, cytokines , harmones - insulin and leptin.
- Slow – require the expression of new genes.
- Single membrane spanning helix - extracellular ligand binding domain - intracellular domain.

Structure of Kinases linked receptors

Extracellular domain

Binds to the ligand (growth factor)

Trans membrane domain

Intracellular domain

Endogenous kinases bind and get phosphorlated

TYPES

- receptor tyrosine kinases

  Eg. EGF , NGF , insulin receptor

- serine/ threonine kinases

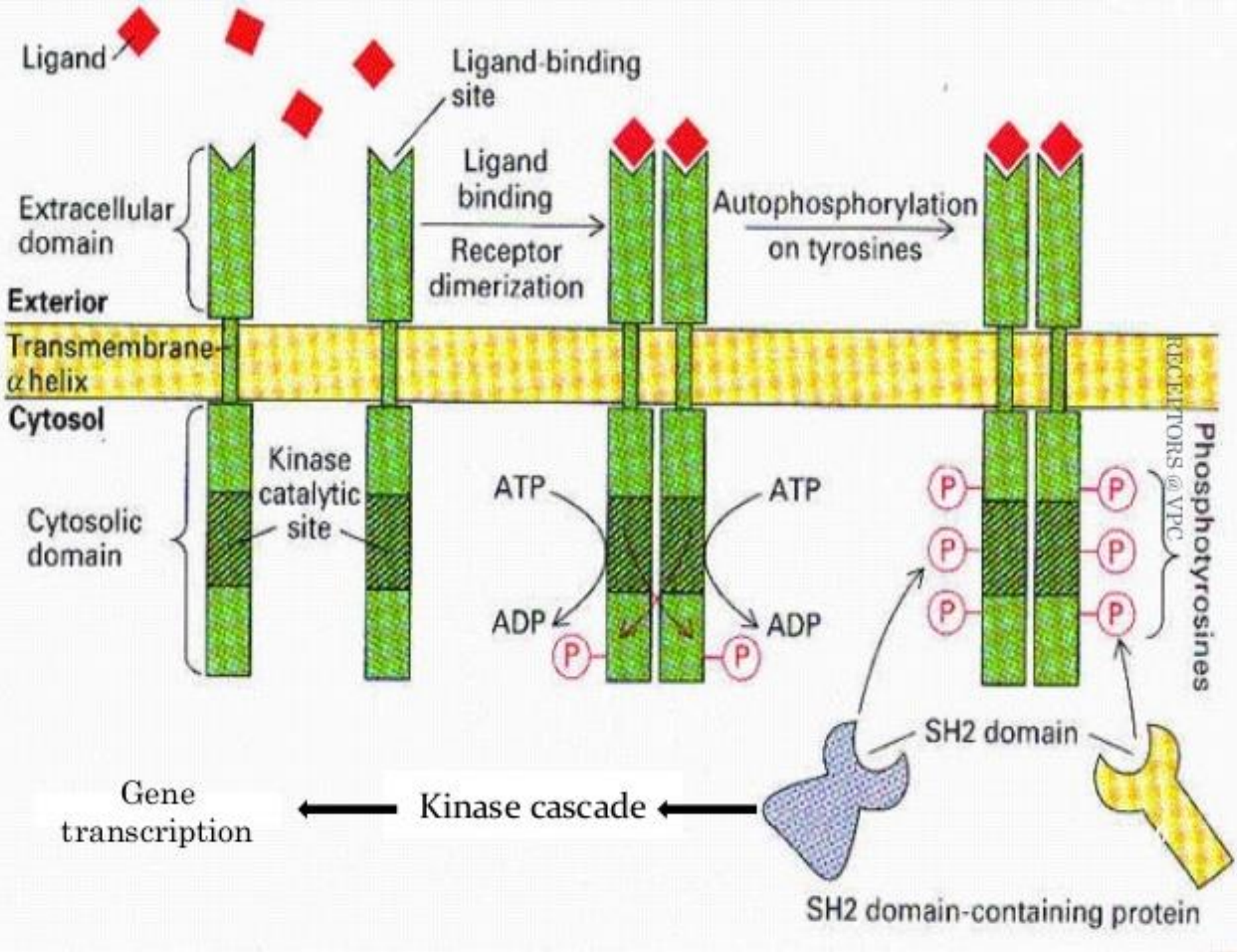
  Eg. TGF

- cytokine receptors

  Eg. Cytokines , CSF

- guanylyl cyclase receptors

  Eg. ANP


Ligand
Ligand-binding site
Extracellular domain
Ligand binding
Receptor dimerization
Autophosphorylation on tyrosines
Exterior
Transmembrane α helix
Cytosol
Cytosolic domain
Kinase catalytic site
ATP
ATP
ADP
ADP
P
Phosphotyrosines
RECEPTORS @ VPC
SH2 domain
Gene transcription
Kinase cascade
SH2 domain-containing protein

- Important pathways activated :

1. The Ras/Raf/mitogen- activated protein (MAP) kinase pathway

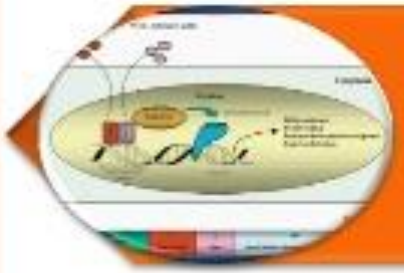
- activated by tyrosine kinases.

- important in cell division, growth, differentiation.

2. The Jak/Stat pathway

- activated by cytokines.

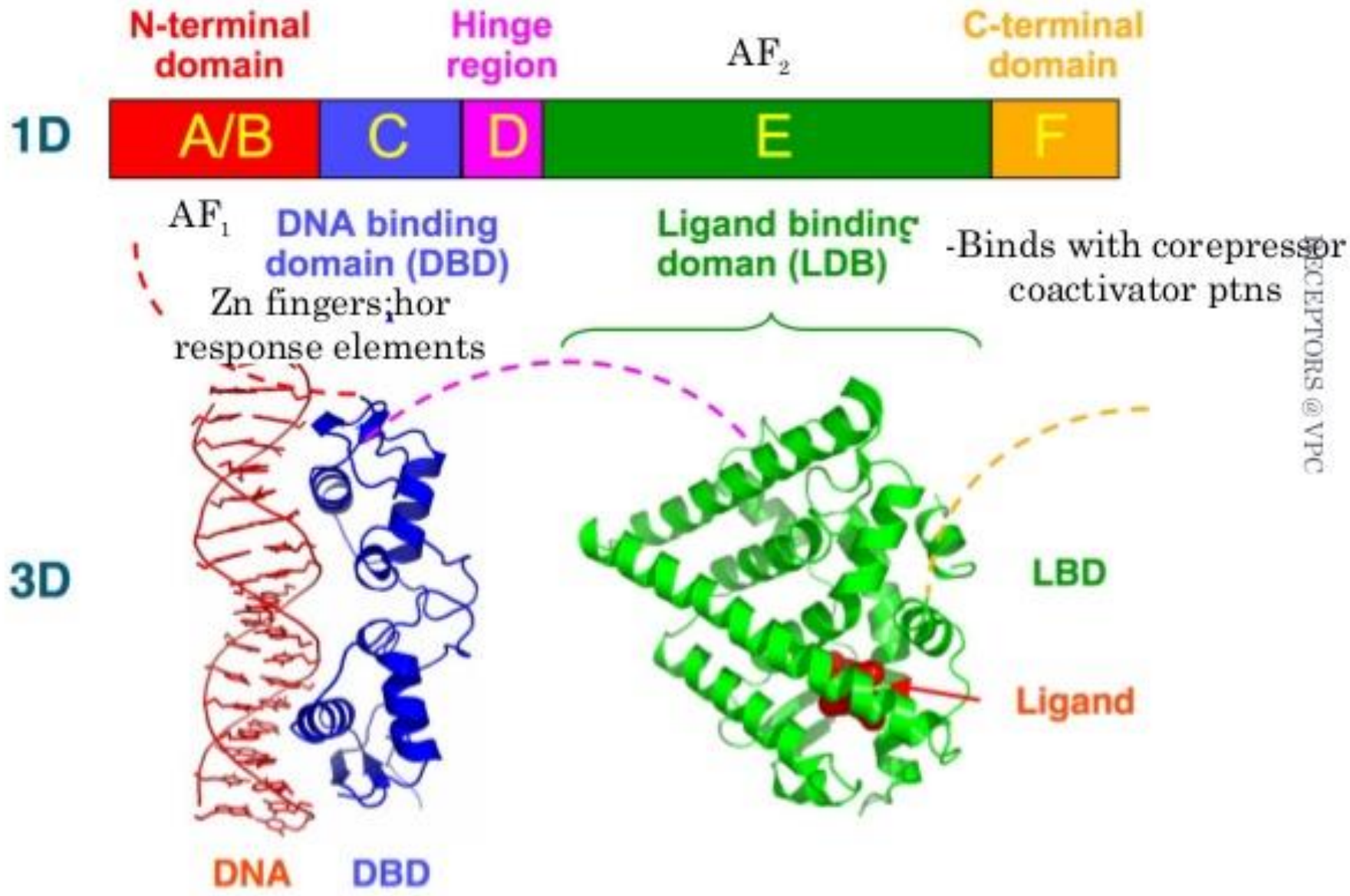
-controls synthesis and release of inflammatory mediators.



NUCLEAR RECEPTORS

- Ligand activated transcription factors.
- Present in soluble form – either in cytoplasm or nucleus – freely diffusable.
- Transduce signals by- modifying gene transcription.
- Eg: steroid hormones, glucocorticoids, vit D and A, orphan receptors
- Play vital role in endocrine signaling and metabolic regulation.

Structural Organization of Nuclear Receptors



CONCLUSION

- Extensive research done on Receptor pharmacology -lead to discovery of new drug targets for treatment of several diseases.
- Still requires discovery of new receptor types and the mechanisms of many orphan receptors that can result in effective treatment of many diseases.
- Requires development of receptor crystallization etc.
- Much to be discovered about the nuclear receptors.

THANK YOU