

# Drug Targets: How do drugs work?

Prof M Kaur

# Druggable Targets (Till 2007, Known 324, new estimated 668)

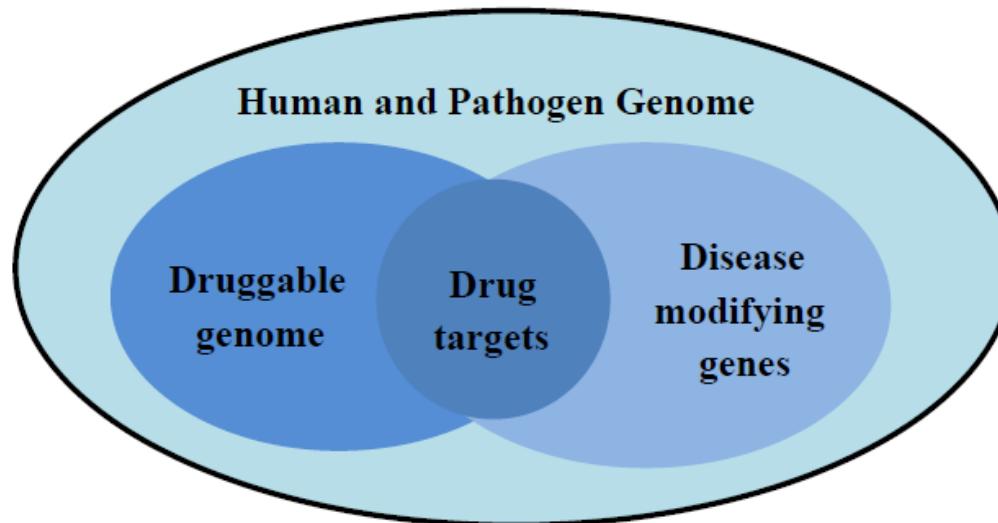
- A ‘druggable’ target is a protein, peptide or nucleic acid with activity that can be modulated by a drug, which can consist of a small molecular weight chemical compound (SMOL) or a biologic (BIOL), such as an antibody or a recombinant protein .

Target classes addressed by SMOLs, BIOLs and nucleic acids and their modes of action		
Drug	Covered target classes	Mode of action
Small molecular weight chemical compound (SMOL)	Enzymes	Inhibitors, activators <sup>a</sup>
	Receptors	Agonists, antagonists, modulators, allosteric activators, sensitizers
	Transcription factors	Inhibitors, activators
	Ion channels	Inhibitors, openers
	Transport proteins	Inhibitors
	Protein–protein interface	Inhibitors of protein–protein interaction <sup>a</sup>
	Nucleic acids	Alkylation, complexation, intercalation
Biologics (BIOL)	(Extracellular) proteins	Antibodies
	Transmembrane receptors, extracellular proteins	Recombinant proteins
	Cell surface receptors	Antibody–drug conjugates (ADCs)
	Substrates and metabolites	Enzymatic cleavage
Nucleic acids	RNA	RNA interference

<sup>a</sup> Novel approaches.

# Druggable Genome

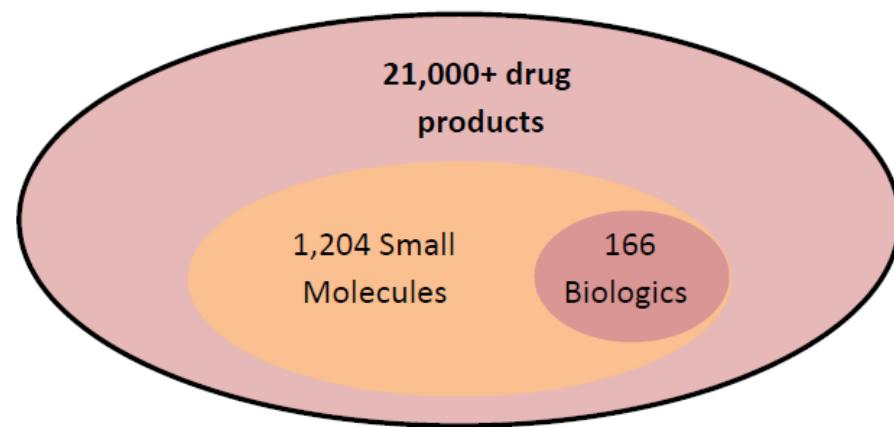
- New estimates potential druggable targets, 2015-
  - 5000 for SMOL
  - 3200 for BIOL



**FIGURE 3.1** Completion of the human and pathogenic genomes provided a wealth of understanding of potential drug targets, but not all of the genes uncovered are useful as drug targets. Useful targets for therapeutic intervention sit within the juxtaposition of the druggable genome and disease-modifying genes. Druggable genes that do not modify diseases are not useful targets just as disease-modifying genes that cannot be successfully modulated to alter disease progression are unlikely to lead to novel therapeutics.

# Why more targets are needed?

- Over 21,000 marketed drugs, but these products contain fewer than 1400 unique molecules that create a positive impact through interaction with just 324 drug targets



**FIGURE 3.2** While the total number of marketed drug products exceeds 21,000 individual products, further analysis shows that the number of therapeutically useful compounds is actually far smaller. Elimination of supplements, imaging agents, vitamins, duplicate salt forms, and other redundancies reveals that there are fewer than 1400 unique drug molecules. Biologic, macromolecular therapeutic entities, represent only 12.2% of the total, but are growing in importance as technologies evolve to support their use. It has been estimated that the collection of marketed drugs exerts their influence through only 324 of the known drug targets.

# How targets are identified?

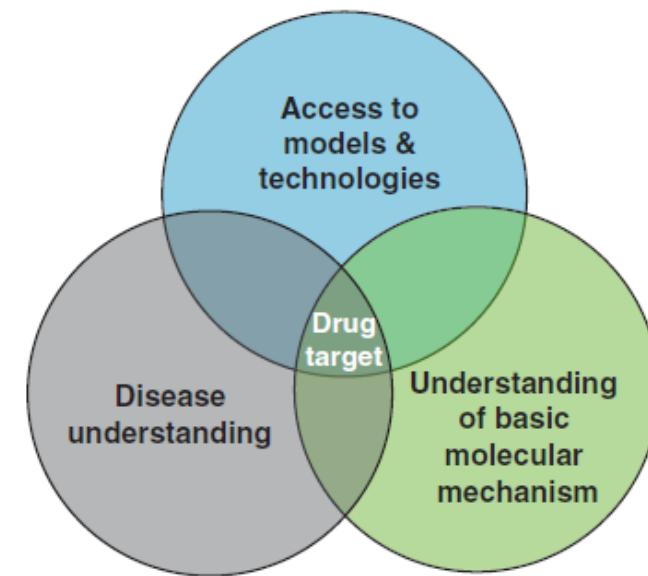
Drugs target four classes of macromolecules:

1. enzymes,
2. G-protein-coupled receptor (GPCR),
3. ion channels, and
4. transporters

## (a) Target identification strategies:

- Gene expression profiling [21]
- Focused proteomics, e.g. activity-based protein profiling [20]
- Pathway analysis – pathway databases, e.g. GeneGo Metacore & Ariadne [23,24]
- Phenotype analysis – phenomic database [25,26]
- Functional screening (siRNAs, shRNAs) [27]
- Genetic association
- Literature

## (b)



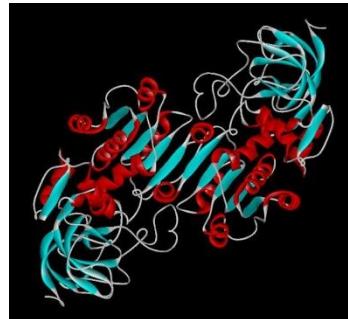
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**FIGURE 2**

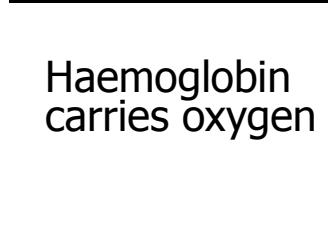
Key factors contributing to a successful target identification strategy. **(a)** Target identification strategies applied at Bayer HealthCare. **(b)** The relationship of disease understanding, suitable models and technologies with basic molecular mechanisms leading to a successful identification of a promising drug target.

# Protein Functions

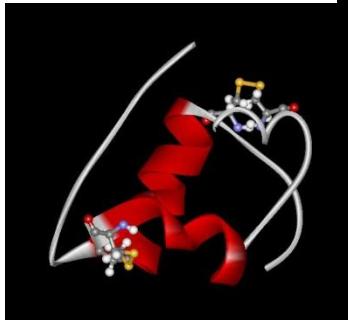
- Three examples of protein functions
  - Catalysis:  
Almost all chemical reactions in a living cell are catalyzed by protein enzymes.
  - Transport:  
Some proteins transport various substances, such as oxygen, ions, and so on.
  - Information transfer:  
For example, hormones.



Alcohol dehydrogenase oxidizes alcohols to aldehydes or ketones

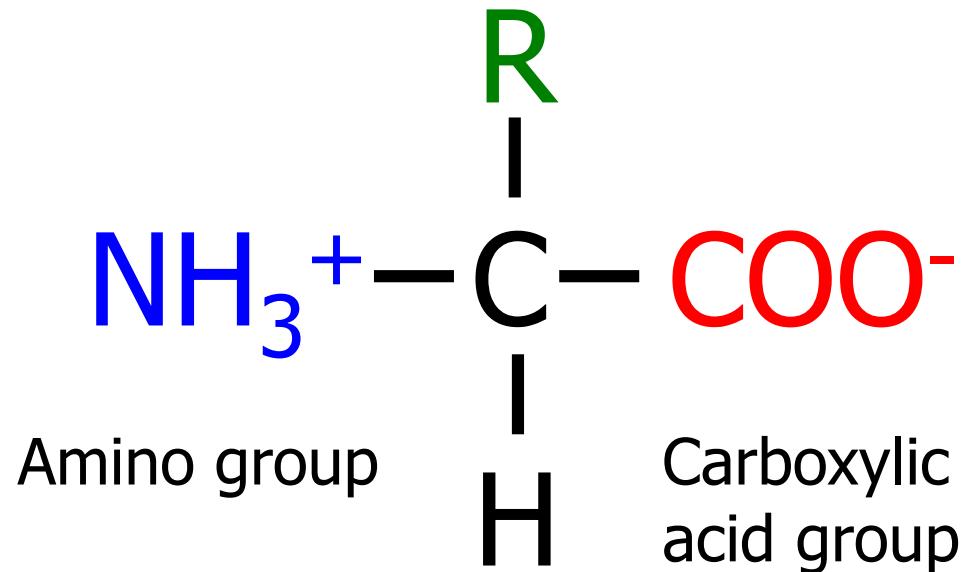


Haemoglobin carries oxygen



Insulin controls the amount of sugar in the blood

# Amino acid: Basic unit of protein



Different side chains, R, determine the properties of 20 amino acids.

An amino acid

# Proteins

- 20  $\alpha$ - amino acids (aa's) -> amide bonds
- Few dozen to thousands
- Tintins- largest polypeptides, 27,000- 33,000 aa's.
- Linear- primary structure
- Amino Acid Basic Structure  
 $H_2N-CH-COOH$

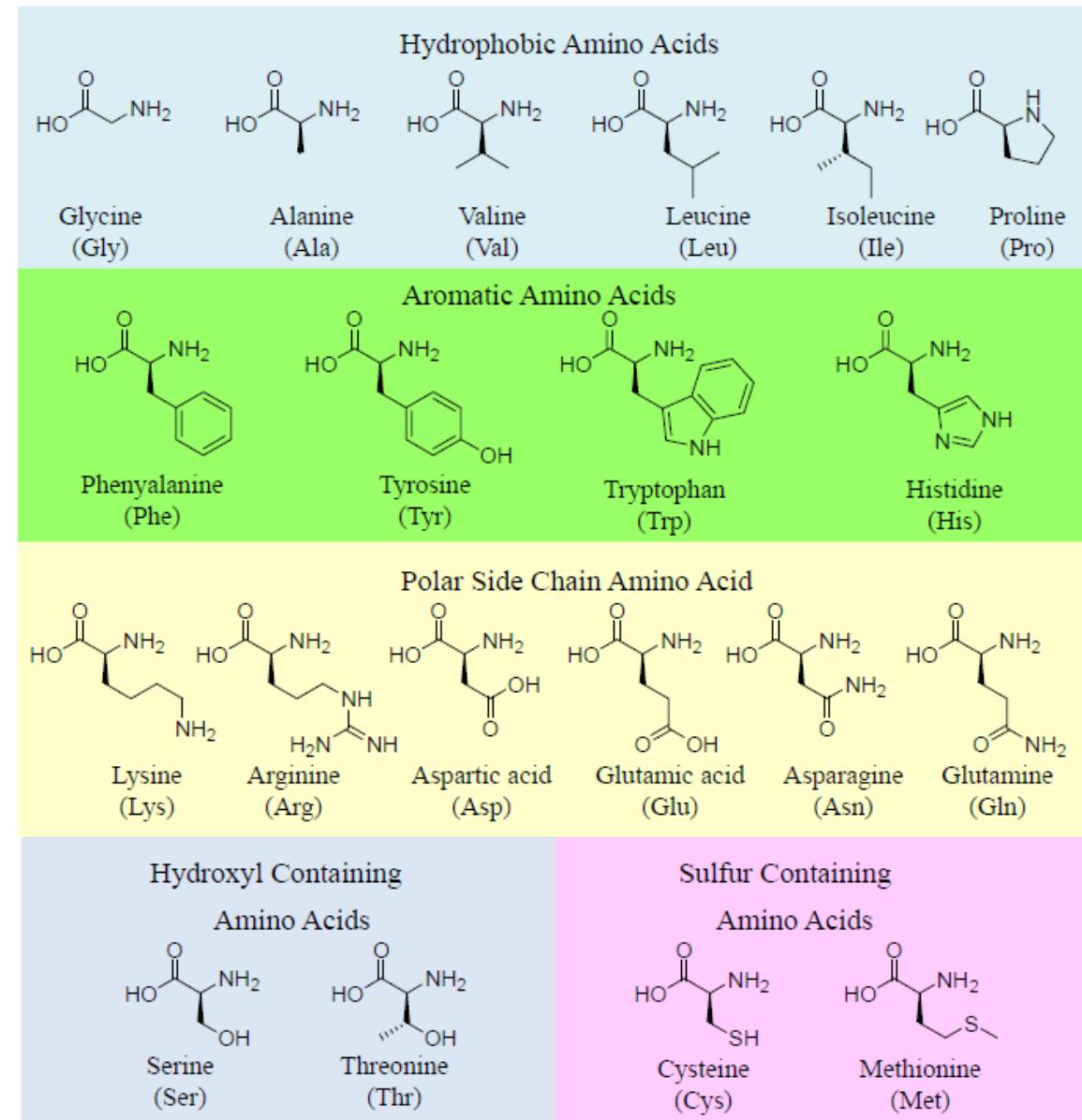
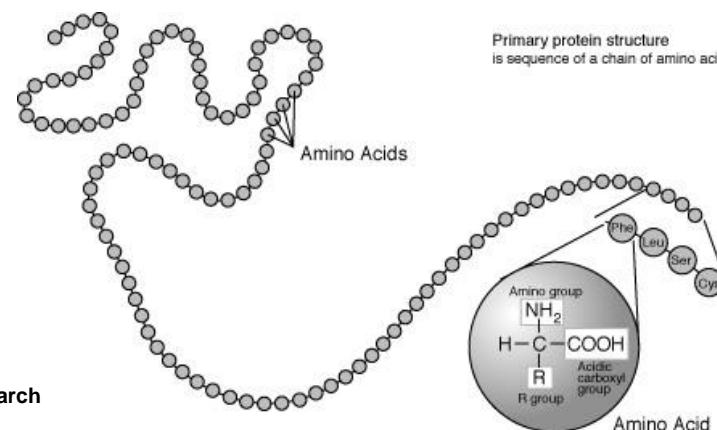


FIGURE 3.3 The 20 fundamental  $\alpha$ -amino acids that are the building blocks for proteins.

# Hierarchical nature of protein structure

**Primary structure** (Amino acid sequence)



**Secondary structure** ( $\alpha$ -helix,  $\beta$ -sheet)

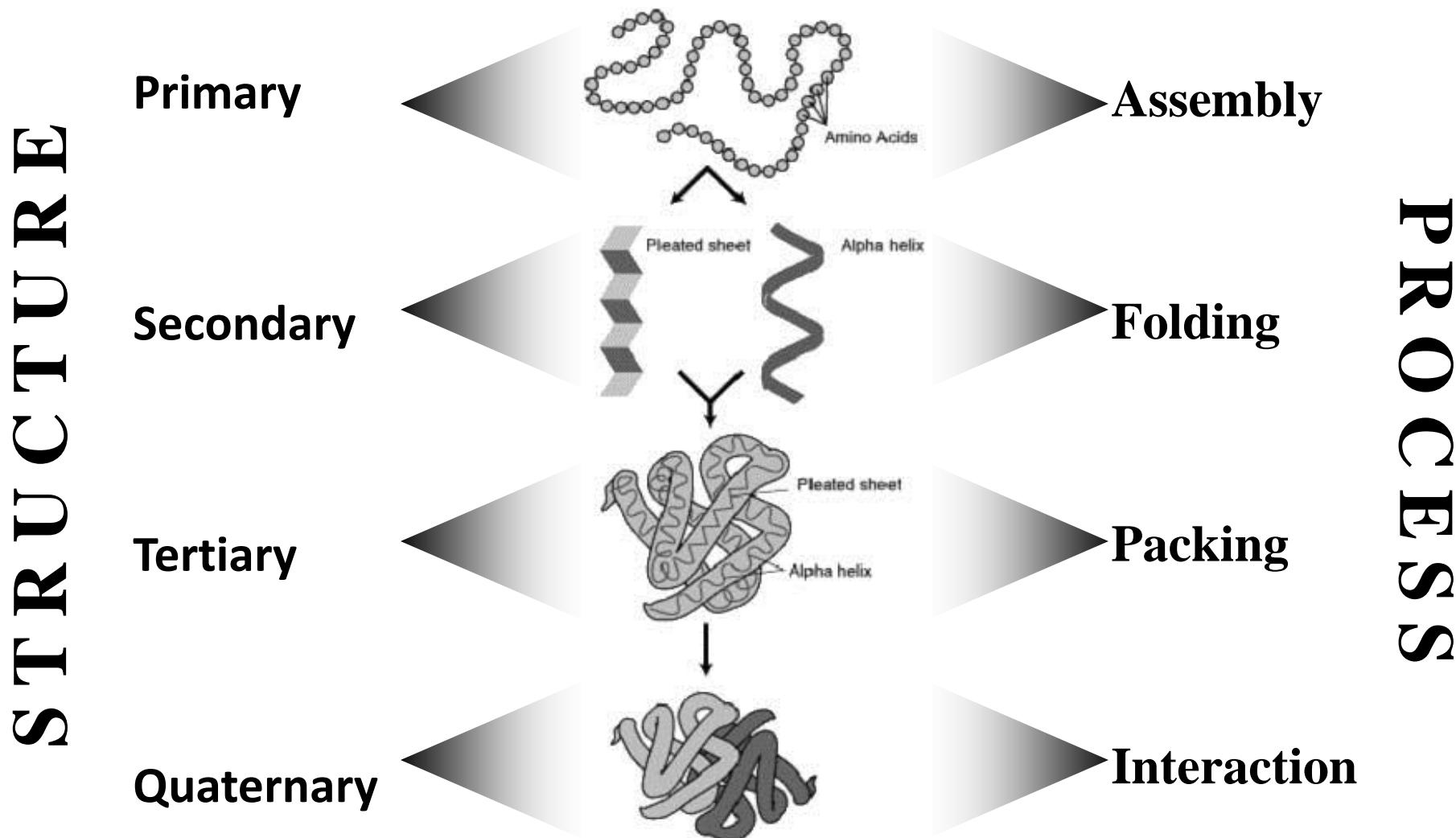


**Tertiary structure** (Three-dimensional structure formed by assembly of secondary structures)



**Quaternary structure** (Structure formed by more than one polypeptide chains)

# Protein Structure



# Physicochemical interactions

- Covalent Bond between two cysteine residues

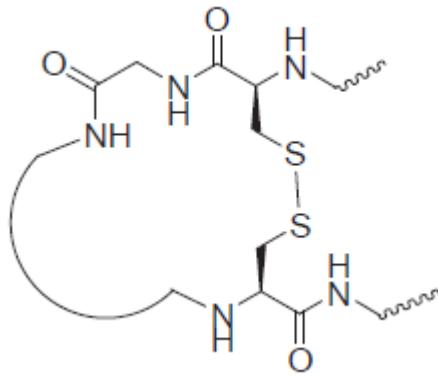


FIGURE 3.4 Disulfide bridge formed between two cysteine residues.

- Electrostatic Interactions
  - Salt bridges

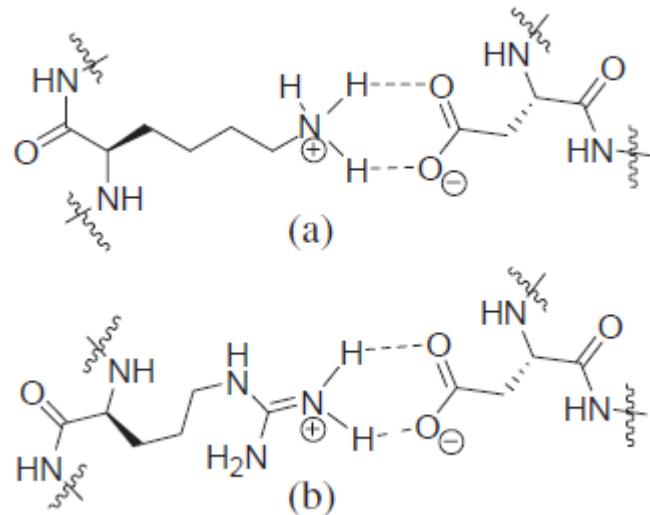


FIGURE 3.5 (a) Salt bridge formed between a lysine residue and an aspartic acid residue. (b) Salt bridge formed between a protonated arginine residue and an aspartic acid residue.

Clustering of non-polar side chains in a protein provide stabilization energy-  
major drivers of protein folding

- Non-Covalent Interactions (hydrophobic)
  - Stabilize 3-dimensional structure
  - Not as strong as other two but outnumber

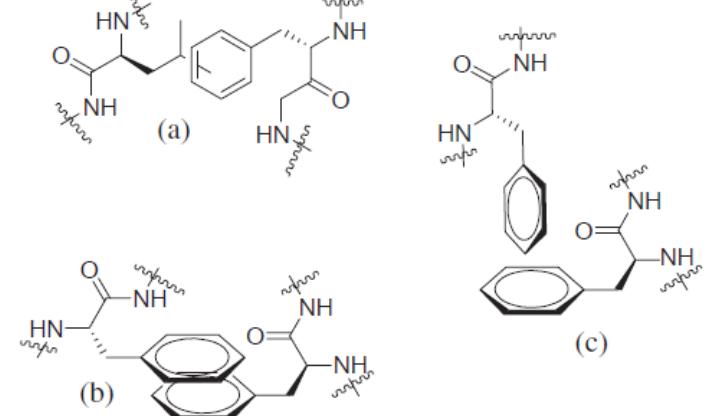


FIGURE 3.6 (a) Hydrophobic interaction between leucine and phenylalanine residues. (b) Face to face π-stacking interaction between two phenylalanines. (c) *t*-Type π-stacking interaction between two phenylalanines.

# How targets are identified?

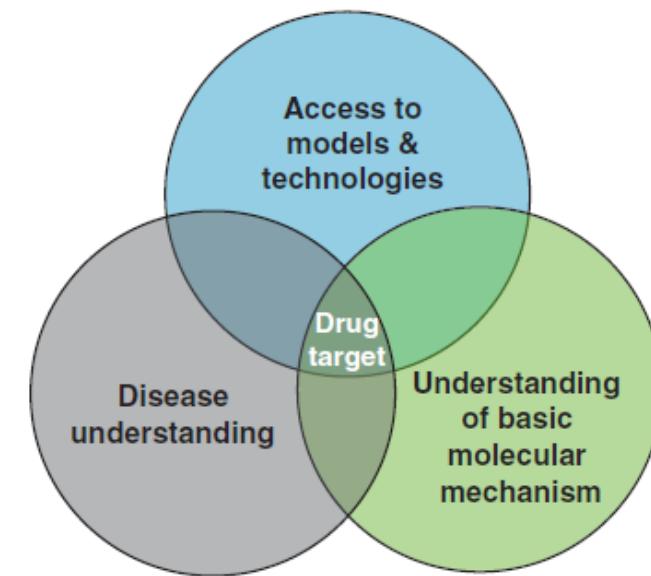
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**FIGURE 2**

Key factors contributing to a successful target identification strategy. **(a)** Target identification strategies applied at Bayer HealthCare. **(b)** The relationship of disease understanding, suitable models and technologies with basic molecular mechanisms leading to a successful identification of a promising drug target.

# Enzymes

- Protein catalysts facilitate transformations required to sustain life.
- First concept proposal- Wilhelm Kuhne 1877
- James B. Sumner 1927- crystallized Jack bean urease enzyme (first recognition as a protein)
- 6 classes

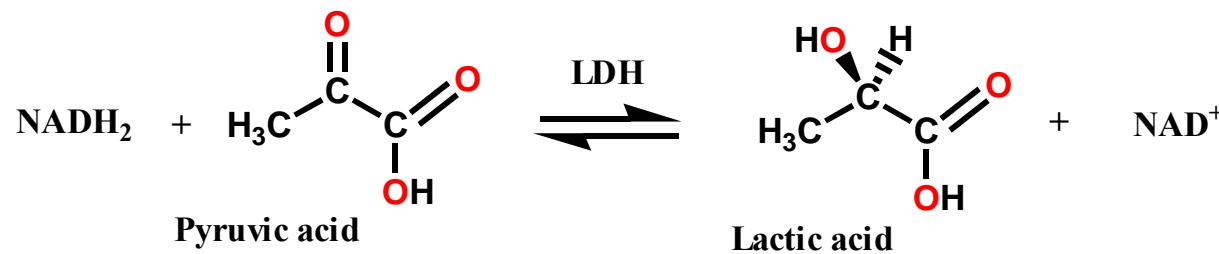
Classification	Function	Examples
Oxidoreductases	Catalyzes redox chemistry, transferring electrons from one molecule to another, often with use of a cofactor	HMG-CoA Reductase, Cyclooxygenase, Monoamine Oxidase, Alcohol Dehydrogenase
Transferase	Catalyzes the transfer of a functional group from one compound to another.	Tyrosine kinase, Reverse Transcriptase, DNA Methyltransferase, Glycosyltransferases
Hydrolase	Catalyzes the hydrolysis of a chemical bond.	HIV Protease, Tyrosine Phosphatase, Carboxypeptidases, Influenza Neuraminidase
Lyase	Catalyzes the cleavage of a chemical bond in a manner other than hydrolysis or oxidation, often forming a double bond or ring.	Adenylate Cyclase, Pyruvate Decarboxylase, Maleate Hydratase, Isocitrate Lyase
Isomerase	Catalyzes structural rearrangement to form isomers of the substrate.	Topoisomerase, Retinol Isomerase, Mannose Isomerase, Isocitrate Epimerase
Ligase	Catalyzes the joining of large molecules with a chemical bond.	DNA Ligase, RNA Ligase, E3 Ubiquitin Ligase, Tyrosine-tRNA Ligase

FIGURE 3.10 Representative examples of six classes of enzymes.

# 1. Structure and function of enzymes

- Globular proteins acting as the body's catalysts
- Speed up time for reaction to reach equilibrium
- Lower the activation energy of a reaction

**Example:**



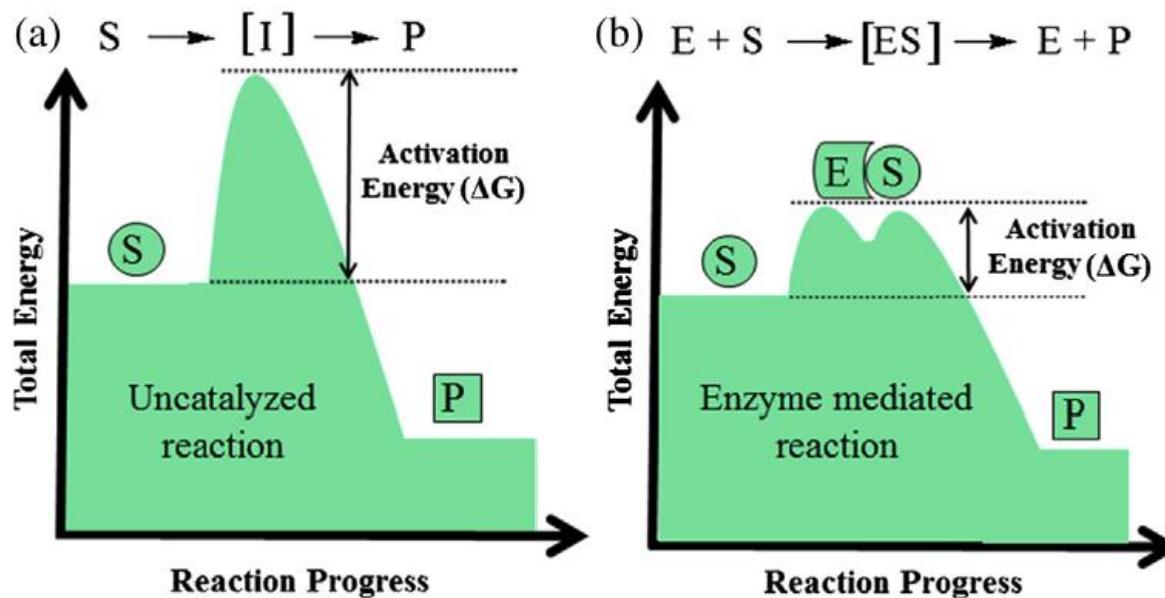
**LDH = Lactate dehydrogenase (enzyme)**

**NADH<sub>2</sub> = Nicotinamide adenosine dinucleotide (reducing agent & cofactor)**

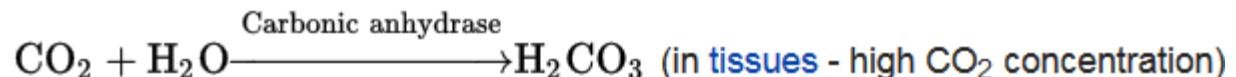
**Pyruvic acid = Substrate**

# 1. Structure and function of enzymes

## Lowering the activation energy of reaction



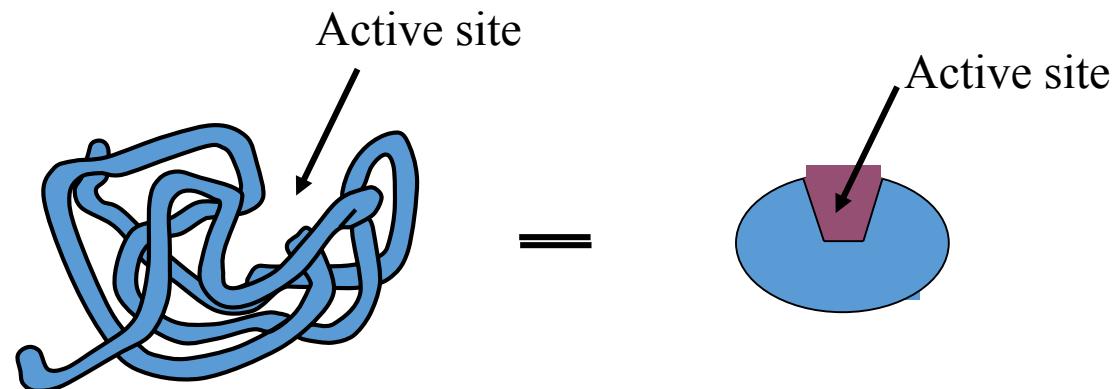
Human Carbonic anhydrase catalyzes the conversion of  $\text{CO}_2$   
10<sup>8</sup> times faster than uncatalyzed reaction (Blass, 2015)



**FIGURE 3.13** (a) In the absence of a catalyst, a given reaction will proceed from starting material (S) to product (P) by way of a transition state intermediate (I) that is higher in energy than the starting material. The energy required to reach the transition state is referred to as the activation energy ( $\Delta G$ ). In general, lowering the activation energy of a reaction leads to higher reaction rate. (b) An enzyme acts as a catalyst for a reaction, lowering the activation energy of a reaction by forming lower energy intermediates (an enzyme/substrate complex, ES). Binding interactions between the substrate and the enzyme decrease the energy requirements, in some cases forming multiple lower energy intermediates that lower the overall activation energy of a reaction.

## 2. The active site

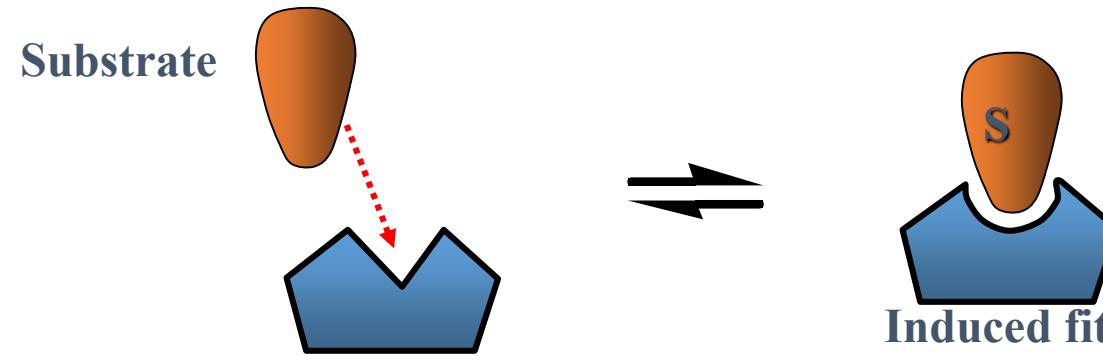
- Hydrophobic hollow or cleft on the enzyme surface
- Accepts reactants (substrates and cofactors)
- Contains amino acids which
  - bind reactants (substrates and cofactors)
  - catalyse the reaction



Dictates enzyme specificity  
Steric limitation on what will fit?

# 3. Enzymatic mechanisms

- ‘Lock and Key’ Model- Emil Fischer 1894
- Enzyme-substrate complex – Brown and Henri 1902
- Daniel Koshland 1958- ‘Induced Fit’ model

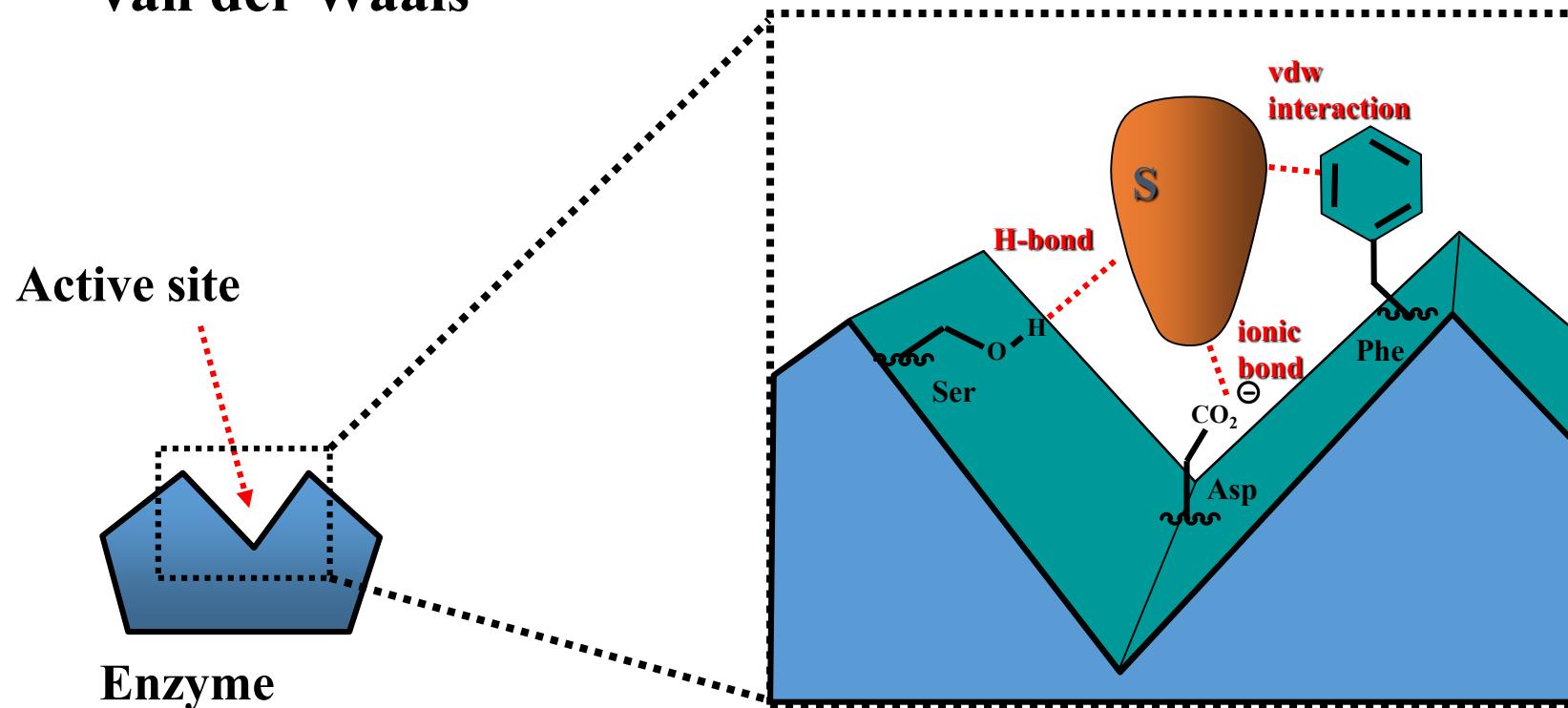


- Active site is **nearly the correct shape** for the substrate
- Binding alters the shape of the enzyme (induced fit)
- Binding will strain bonds in the substrate
- **Binding involves intermolecular bonds between functional groups** in the substrate and functional groups in the active site

# 4. Substrate binding

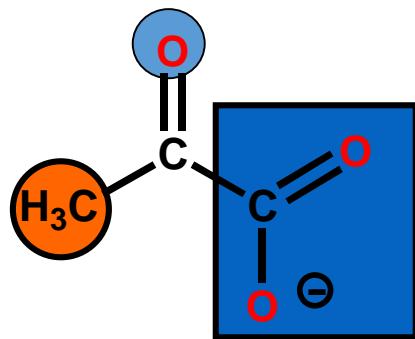
## Bonding forces

- Ionic
- H-bonding
- van der Waals



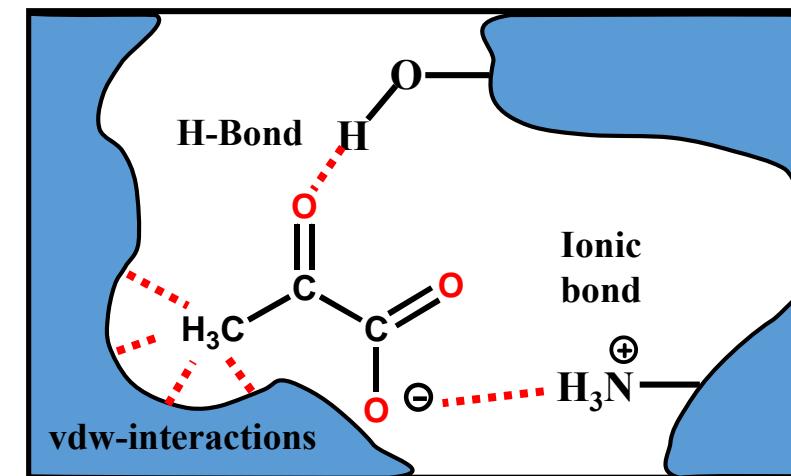
# 4. Substrate binding- Example

## Binding of pyruvic acid in LDH



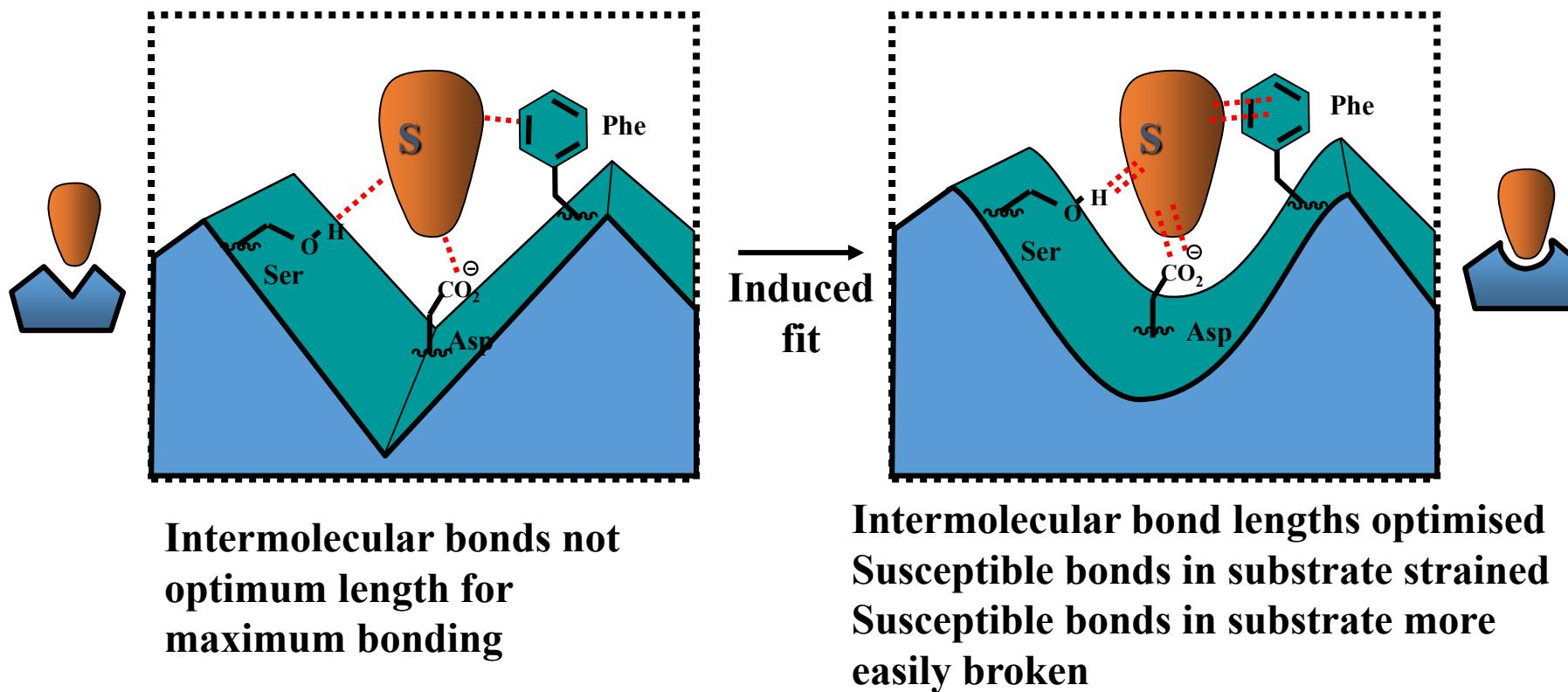
Possible interactions

- H-Bond
- van der Waals
- Ionic

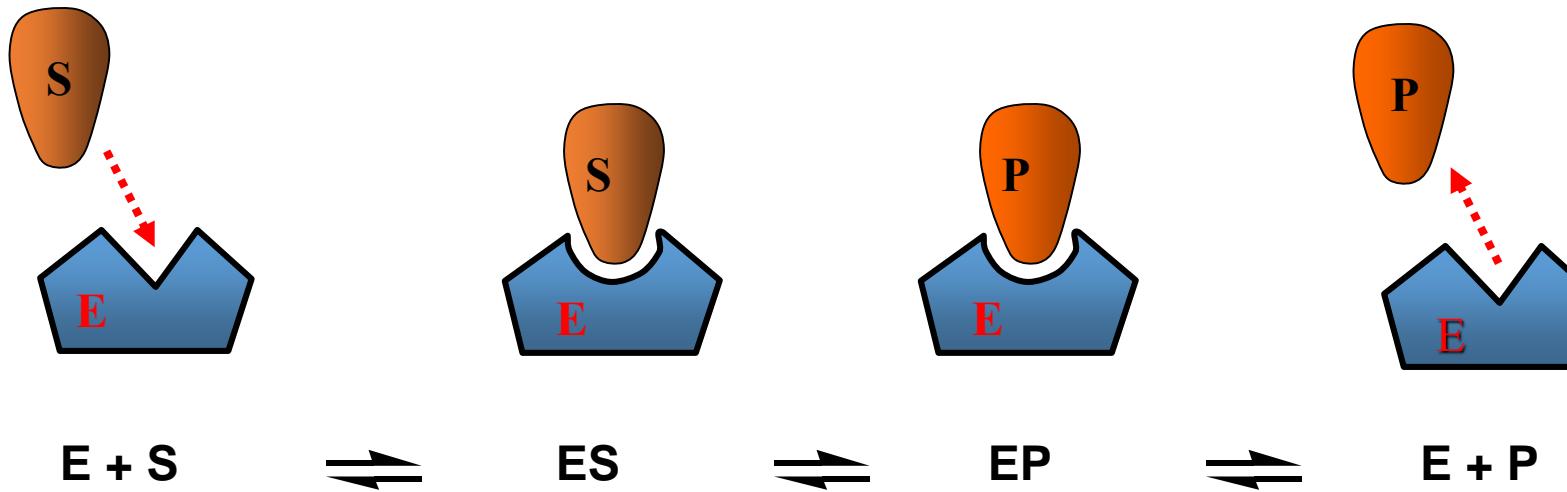


# 4. Substrate binding

Induced fit - Active site alters shape to maximise intermolecular bonding



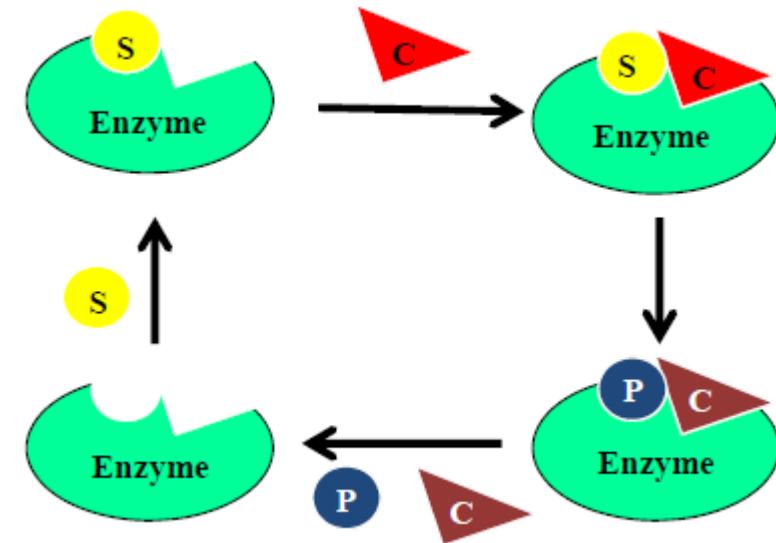
# 5. Overall process of enzyme catalysis



- Binding interactions must be;
  - strong enough to hold the substrate sufficiently long for the reaction to occur
  - weak enough to allow the product to depart
- Implies a fine balance
- Drug design - **designing molecules with stronger binding interactions results in enzyme inhibitors which block the active site**

# Enzyme Co-factors

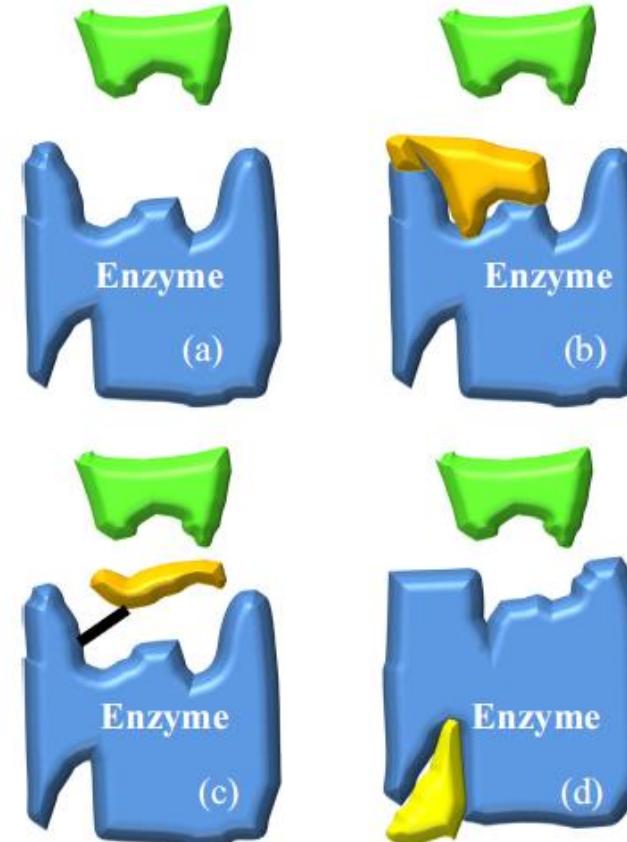
- Can be a variety of atoms and molecules
- E.g collagen to gelatin degradation- by metalloproteinases in the presence of Zn atom.
- Iron, magnesium, manganese, molybdenum, selenium and copper- other examples
- Organic compounds- as coenzyme- Cytochrome P450 17A1 ( $17\text{-}\alpha$ -hydroxylase/ $C_{17,20}$ -lyase)- leads to production of progestins, glucocorticoids, androgens, estrogens etc.
- Other examples- ATP, Coenzyme Q, heme B



**FIGURE 3.15** An enzyme binds with a substrate (S), which then binds to a coenzyme that supports the enzymatic process. After the reaction is complete, the product is released, and both the enzyme and coenzyme are recycled. In some cases, the coenzyme must be regenerated before the next reaction cycle.

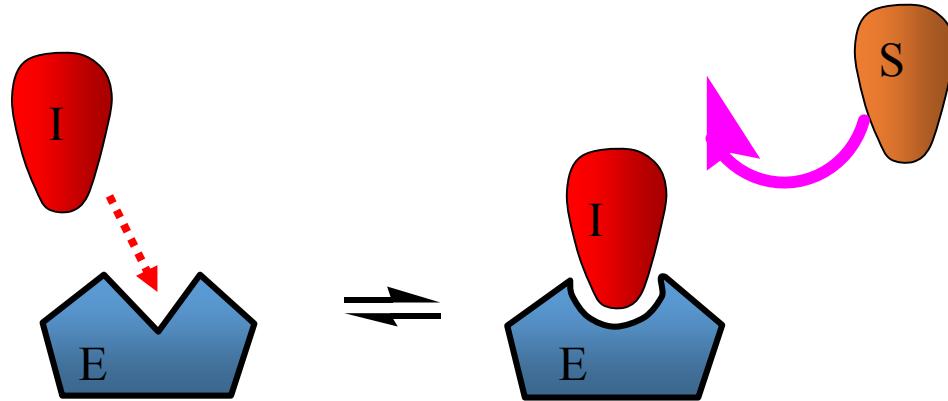
# Inhibition of Enzymes

- **Competitive Inhibitors** → Block active site of enzyme (reversible as no covalent bonding), e.g- Tamiflu
- **Irreversible Inhibitors** → covalently attach to active site, block substrate entry and inactivate the enzyme.  
e.g- Penicillin G
- **Allosteric Inhibitors** → Exert influence at sites other than the active site of the enzyme. e.g- MEK inhibitor CI-1040.
  - Active site remains unoccupied, but inaccessible to natural substrate



**FIGURE 3.17** (a) In the normal enzyme process, substrates (green) interacts with active sites. (b) Competitive inhibitors (orange) reversibly blocks the active site. (c) Irreversible inhibitors (orange) covalently bind to the active site. (d) An allosteric inhibitor (yellow) binds to an allosteric binding site, altering the active site, preventing substrate binding.

# Competitive (reversible) inhibitors



- Inhibitor binds reversibly to the active site
- Intermolecular bonds are involved in binding
- No reaction takes place on the inhibitor
- Inhibition depends on the strength of inhibitor binding and inhibitor concentration
- Substrate is blocked from the active site
- **Increasing substrate concentration reverses inhibition**
- Inhibitor likely to be similar in structure to the substrate

Selectivity? E.g- Kinases (phosphorylates via ATP-mediated process), **ATP binding is same across 500 known kinases, problem for inhibitors targeting ATP-binding domain of active site.**

# How targeting ATP-binding site worked for Gleevec?

- However, as scientists started learning more about kinase structure, they began to realize that **there is considerable variation among the different kinases with respect to the structure of their ATP-binding pockets**. This discovery meant that specificity might be possible after all. Oncologist Brian Druker, the researcher at Oregon Health and Science University who would eventually conduct the pivotal clinical trials leading to FDA approval of Gleevec, was one of the first scientists to recognize this possibility. Prior to this period, Druker had already been heavily involved with CML genetics research. As he later recounted, "I had one goal at the time: to find a company that had an inhibitor for *bcr-abl* and to bring it into the clinic" (Cameron, 2007).
- This company ended up being Ciba-Geigy (which later became Novartis), one of the few pharmaceutical firms in which scientists were conducting tyrosine kinase inhibitor research. In fact, **company scientists had already synthesized some kinase-blocking inhibitor compounds, using computer models to predict which molecular structures might fit the ATP-binding site of the fusion protein**. Druker collaborated with Ciba-Geigy, screening their collection of synthesized compounds in human bone marrow cells for signs of anticancer activity. One compound in particular looked promising. In cell culture, this chemical caused a 92%-98% decrease in the number of *bcr-abl* colonies formed, suggesting that it was effective, while simultaneously causing no decrease in normal colony formation, suggesting that the chemical was safe and did not harm healthy cells (Druker *et al.*, 1996).

# Gleevec Can Act as an Allosteric Activator of ABL Kinase

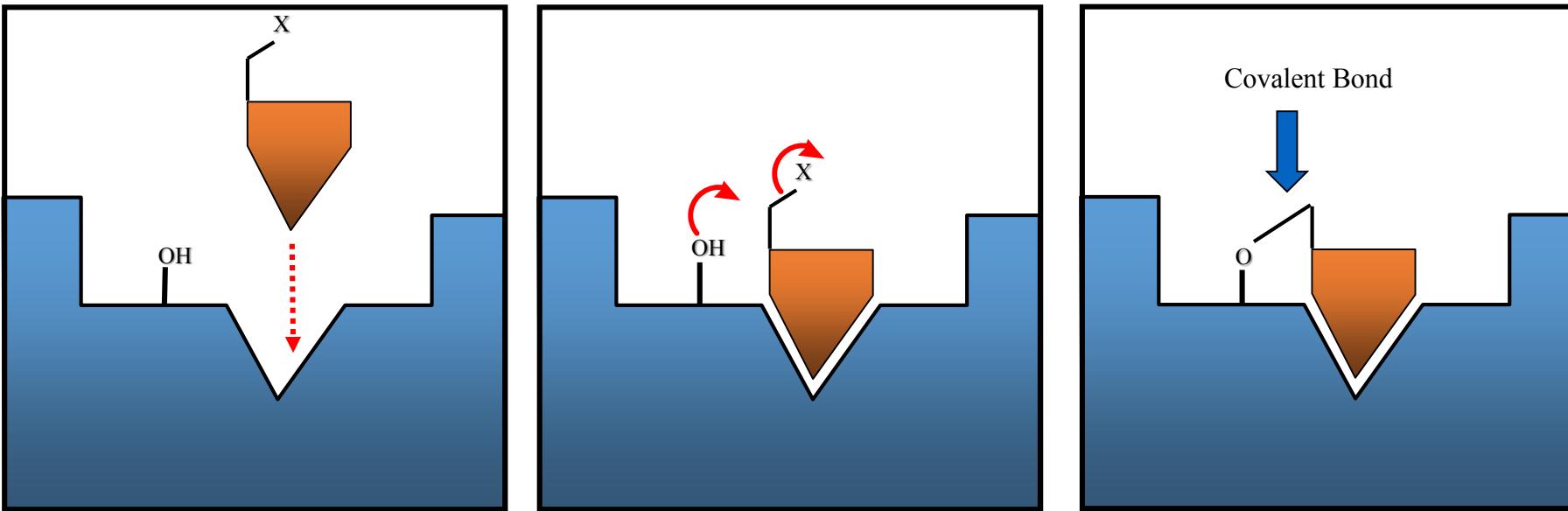
Tao Xie • Tamjeed Saleh • Charalampos G. Kalodimos

DOI: <https://doi.org/10.1016/j.bpj.2017.11.1274>

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Gleevec remains the first-line therapy for chronic myelogenous leukemia (CML) by targeting deregulated kinase activity of Bcr-Abl as a specific ATP-competitive inhibitor and its success has revolutionized targeted cancer therapy. Using nuclear magnetic resonance (NMR) spectroscopy and other biophysical methods we show other than the ATP binding site, Gleevec binds to the myristate pocket with a sub-micromolar affinity and unexpectedly acts as an allosteric activator of Abl by preventing the  $\alpha$ -helix from forming a conformation which is required for assembly of Abl to its inhibited state. It may lead to diminished concentration of active drug for binding to the active site and development of resistance. These results suggest administration of high-dose of Gleevec may result in poor inhibition of Abl kinase activity of some Gleevec-resistant mutants by competing ATP-site for Gleevec binding and enhancing kinase activity. We also describe a NMR-based method to distinguish between myristate-site allosteric inhibitors and activators of Abl.

# Non competitive (irreversible) inhibitors

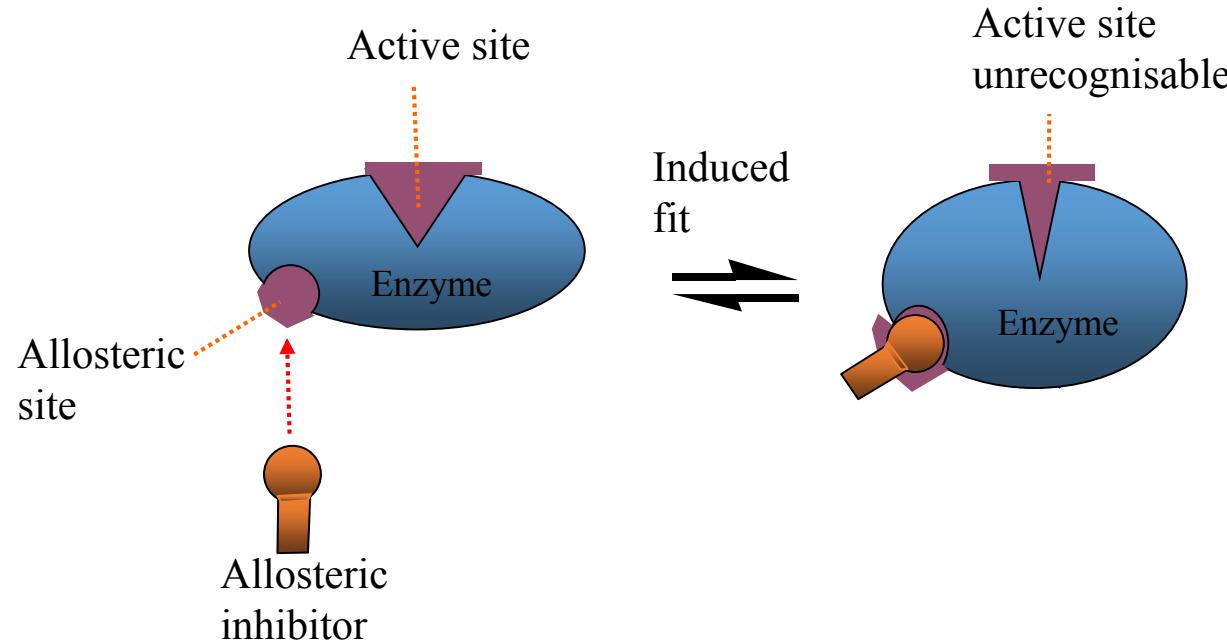


Irreversible inhibition

- Inhibitor binds **irreversibly** to the active site
- Covalent bond formed between the drug and the enzyme**
- Substrate is blocked from the active site
- Increasing substrate concentration does not reverse inhibition
- Inhibitor likely to be similar in structure to the substrate

Inhibition by  $\beta$ -lactams  
(Penicillin G) by reacting with a serine residue in the active site of the penicillin-binding proteins (PBPs), inactivating enzyme., thus decreased cell wall strength killing organisms

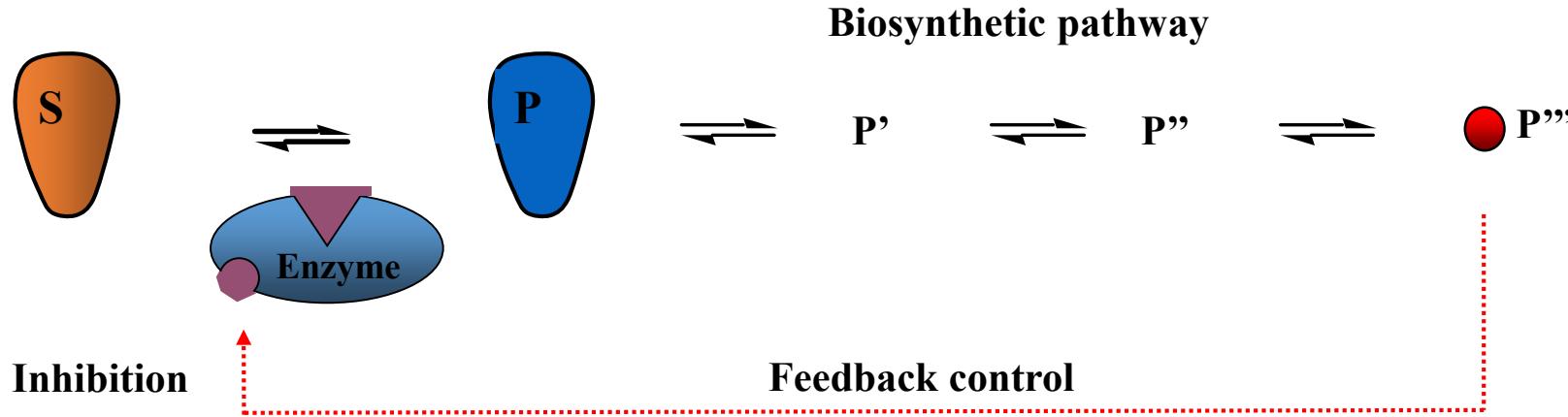
# Non competitive (reversible) allosteric inhibitors



- Inhibitor binds reversibly to the allosteric site
- Intermolecular bonds are formed
- Induced fit alters the shape of the enzyme
- Active site is distorted and is not recognised by the substrate
- Increasing substrate concentration does not reverse inhibition
- Inhibitor is not similar in structure to the substrate

MEK1, a member of the kinase class of enzymes that plays an important role in the progression of cancer, for example, can be allosterically inhibited by compounds such as **CI-1040**. Although this compound is a potent inhibitor of MEK1 and MEK2, it does not bind to the ATP-binding domain, which is the active site of MEK1. Rather, it binds to an adjacent binding site and produce inhibition of MEK1 via conformational changes induced by its presence in the allosteric site (Blass, 2015)

# Non competitive (reversible) allosteric inhibitors



- Enzymes with allosteric sites often at start of biosynthetic pathways
- Enzyme is controlled by the final product of the pathway
- Final product binds to the allosteric site and switches off enzyme
- Inhibitor may have a similar structure to the final product

## Pharma favours competitive and allosteric inhibitors

- If an irreversible kinase inhibitor targeting the ATP-binding site of MEK1 is developed. The irreversible inhibitor would bind to MEK1, suppressing its activity, but given the high degree of homology of the ATP-binding site within the kinase family, it is likely that many other kinases would also be irreversibly inhibited.
- Irreversible inhibition of enzymes that are involved in drug metabolism can also lead to significant negative consequences by altering the rate at which drugs are cleared from the body.

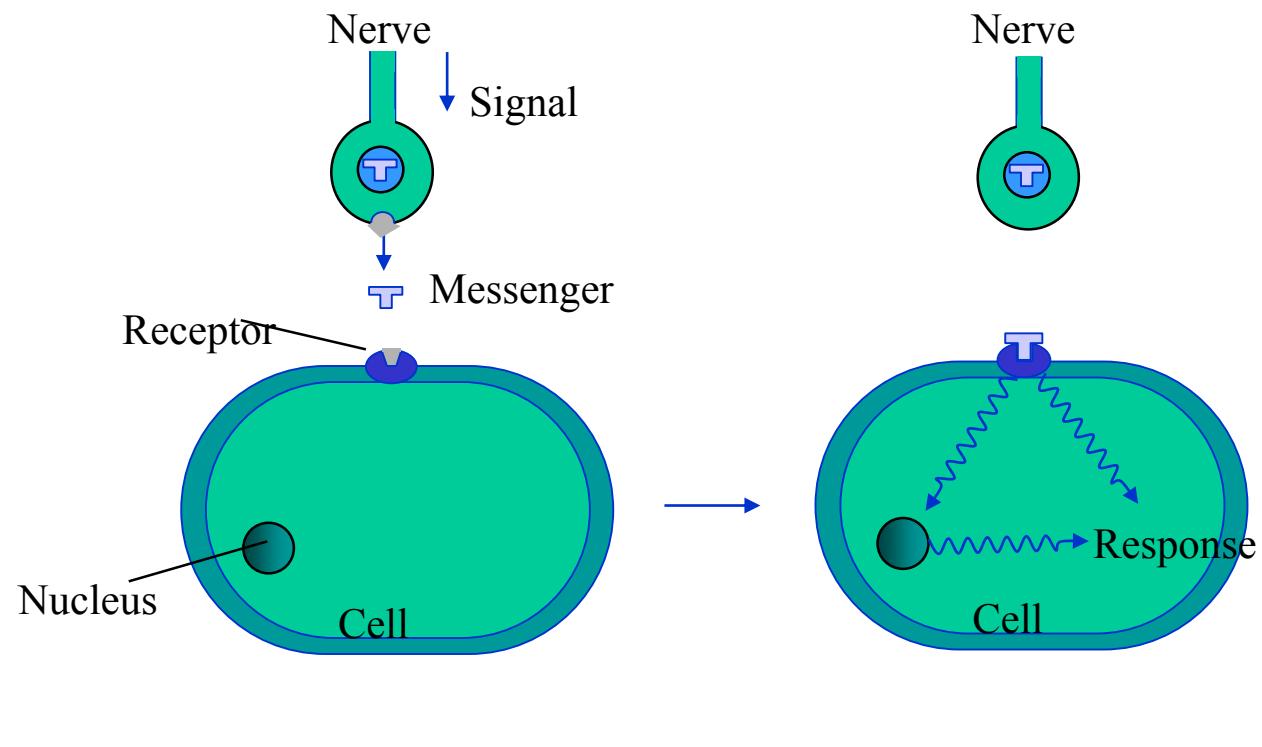
# G-Protein-Coupled Receptors (GPCRs)

# Receptors

- Cell-to-cell communication, how?
- Flow of information across plasma membrane
- Faster transmit- e.g touching hot surface
- **Receptors** are most suited for the task.
  - Globular proteins acting as a cell's 'letter boxes'
  - Located mostly in the cell membrane
  - Receive **messages from chemical messengers** coming from other cells
  - Transmit a message into the cell leading to a cellular effect
  - Different receptors specific for different chemical messengers
  - Each cell has a range of receptors in the cell membrane making it responsive to different chemical messengers



# Receptor functions

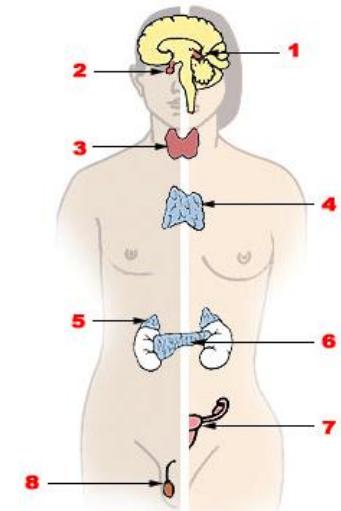
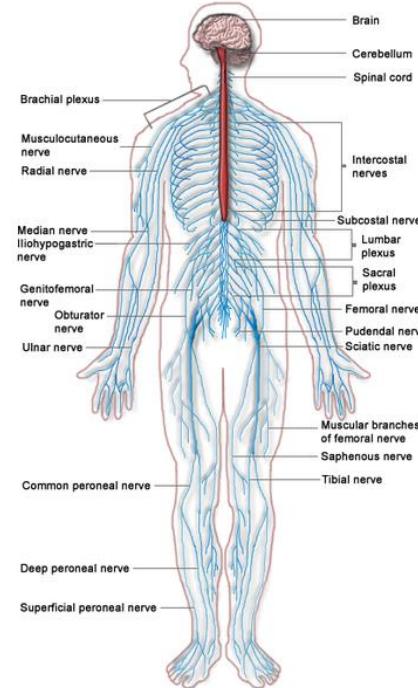


# Chemical Messengers

**Neurotransmitters:** Chemicals released from nerve endings which travel across a nerve synapse to bind with receptors on target cells, such as muscle cells or another nerve. Usually short lived and responsible for messages between individual cells

**Hormones:** Chemicals released from cells or glands and which travel some distance to bind with receptors on target cells throughout the body

- **Chemical messengers ‘switch on’ receptors without undergoing a reaction**

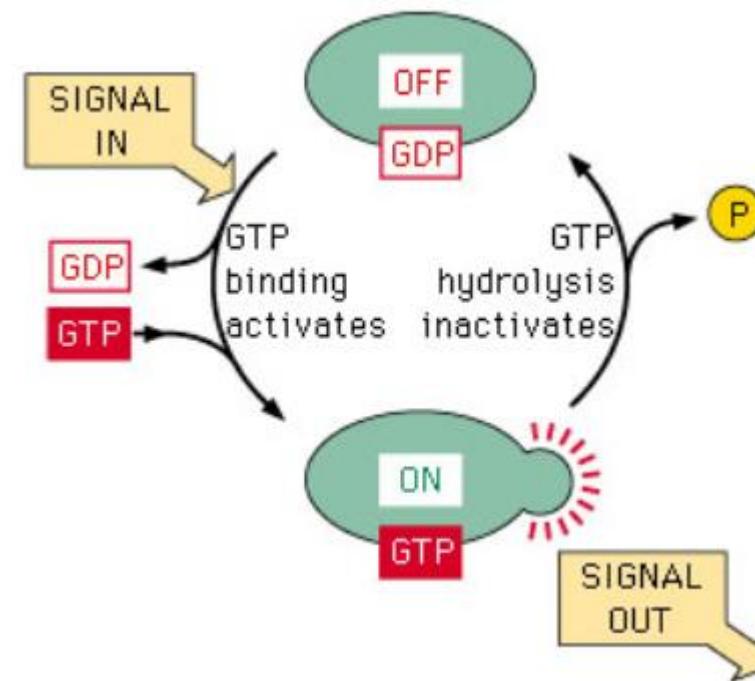


The major endocrine glands: (Male left, female right) 1 Pineal gland 2 Pituitary gland 3 Thyroid gland 4 Thymus 5 Adrenal gland 6 Pancreas 7 Ovary 8 Testes

**G proteins**, also known as **guanine nucleotide-binding proteins**, are a family of proteins that act as molecular switches inside cells, and are involved in transmitting signals from a variety of stimuli outside a cell to its interior. Their activity is regulated by factors that control their ability to bind to and hydrolyze guanosine triphosphate (GTP) to guanosine diphosphate (GDP).

When they are bound to GTP, they are '**on**', and, when they are bound to GDP, they are '**off**'.

G proteins belong to the larger group of enzymes called GTPases.

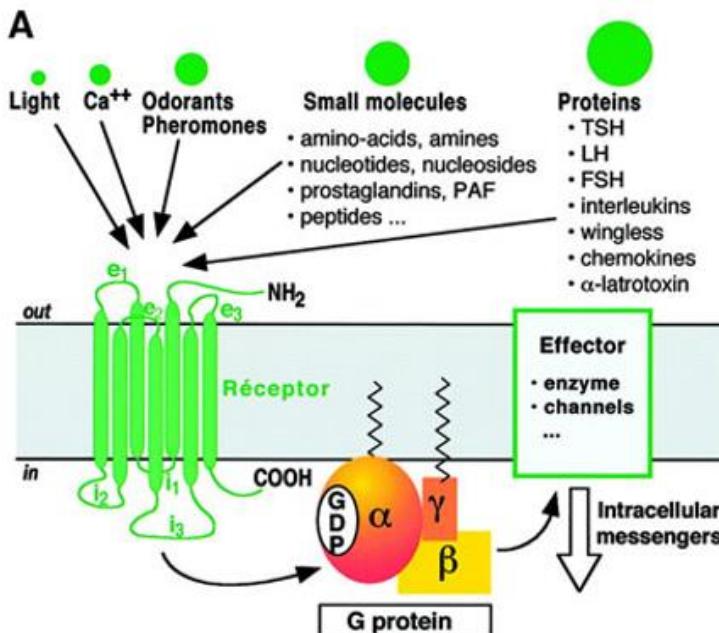


# GPCRs

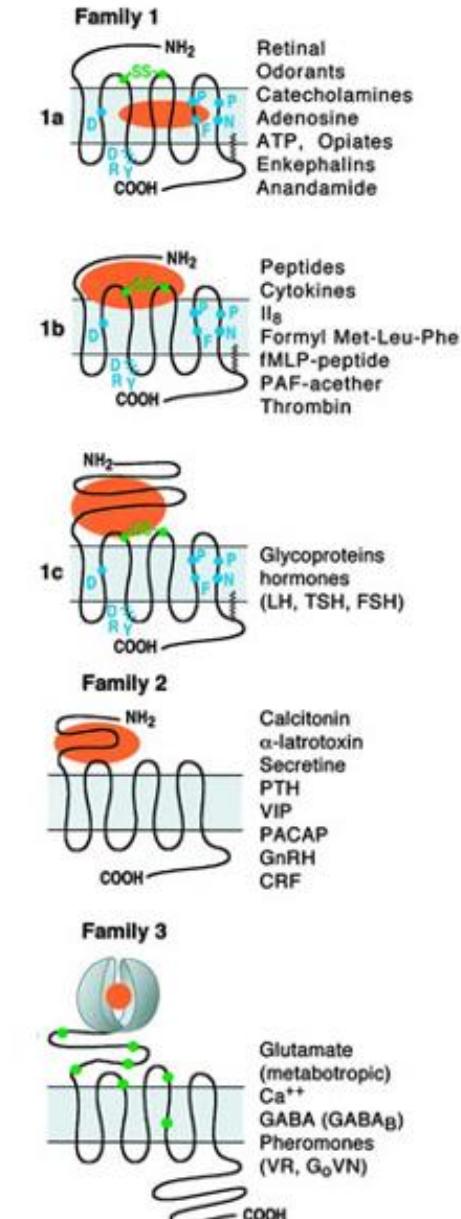
- Membrane-bound proteins (3 broad families)
- Signal transduction

## Basic structure:

- 7 Transmembrane Domains (TMDs),  
3 intracellular loops, 3 extracellular loops, N- and C-terminals
- Bind to G-protein      Bind to drug



A) GPCRs have a central common core made of seven transmembrane helices (TM-I to -VII) connected by three intracellular (i1, i2, i3) and three extracellular (e1, e2, e3) loops.



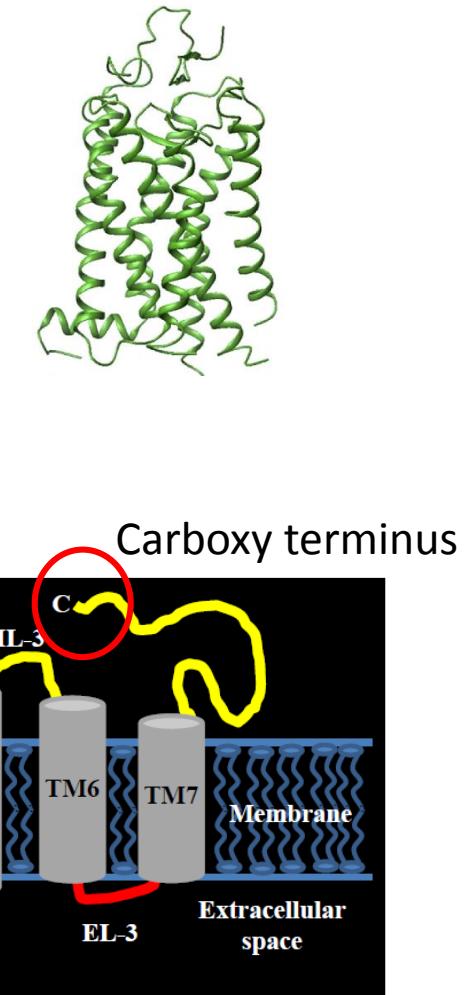
# GPCRs

- 1970, Robert Lefkowitz et al, Adenocorticotrophic hormone (ACTH, corticotropin) binding to its receptor by radiolabelling.
- 2000, first crystallographic structure of mammalian GPCR, bovine rhodopsine
- 2007, human GPCR structure- Beta2-adrenergic receptor.

- **Similarities among GPCRs**

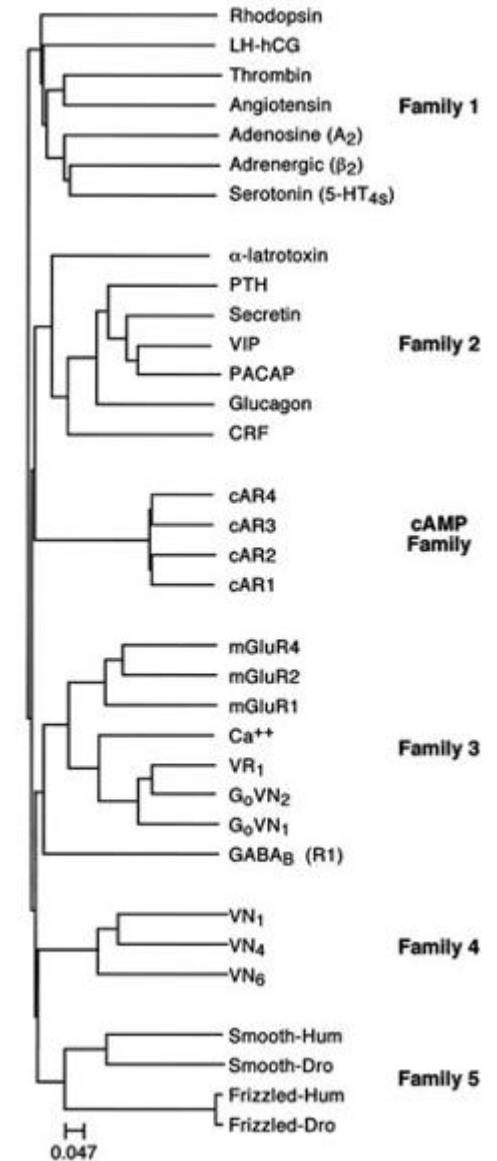
- Membrane-bound
- Interact with G-proteins through a ligand
- Basic structure
- High degree of homology between TM regions  
(therefore, also called seven-transmembrane (7TM) receptors.

- C- and N-Terminus along with TM5/6 loop have higher degree of variability- allow GPCRs to distinguish wide variety of ligands



# GPCRs

- Over 800 unique GPCRs in 5 families (3 primary+2 more added) based on human genome analysis
- **Family 1- Rhodopsin** (over 700 members), divided into 19 subfamilies (A1 to A19).
  - Serotonin, dopamine, angiotensin II, prostaglandin receptors.
  - Function in CNS, Cardiovascular regulation and pain perception.
- **Family 2- Secretin Family** (15 members)
  - Parathyroid hormone and glucagon receptors.
- **Family 3- Frizzled/Taster GPCRs** (24 members)
  - Cell differentiation and proliferation through Wnt signalling pathway.
- **Family 4- Glutamate receptors** (15 members)
  - Modulation of excitability of synaptic cells, nerve transmission.
- **Family 5- Adhesion class of GPCRs** (24 members), posses an extracellular domain far larger than other classes (structurally largest GPCRs)
  - Cell adhesion, cellular response
  - Found in immune cells, CNS and reproductive tissue



*The EMBO Journal* (1999) **18**, 1723–1729

# Diversity (of physiological responses to GPCR stimulation)

*Just for information*

<u>TARGET TISSUE</u>	<u>HORMONE</u>	<u>MAJOR RESPONSE</u>
Thyroid gland	thyroid-stimulating hormone (TSH)	thyroid hormone synthesis and secretion
Adrenal cortex	adrenocorticotropic hormone (ACTH)	cortisol secretion
Ovary	luteinizing hormone (LH)	progesterone secretion
Muscle	adrenaline	glycogen breakdown
Bone	parathormone	bone resorption
Heart	adrenaline	increase in heart rate and force of contraction
Liver	glucagon	glycogen breakdown
Kidney	vasopressin	water resorption
Fat	adrenaline, ACTH, glucagon, TSH	triglyceride breakdown

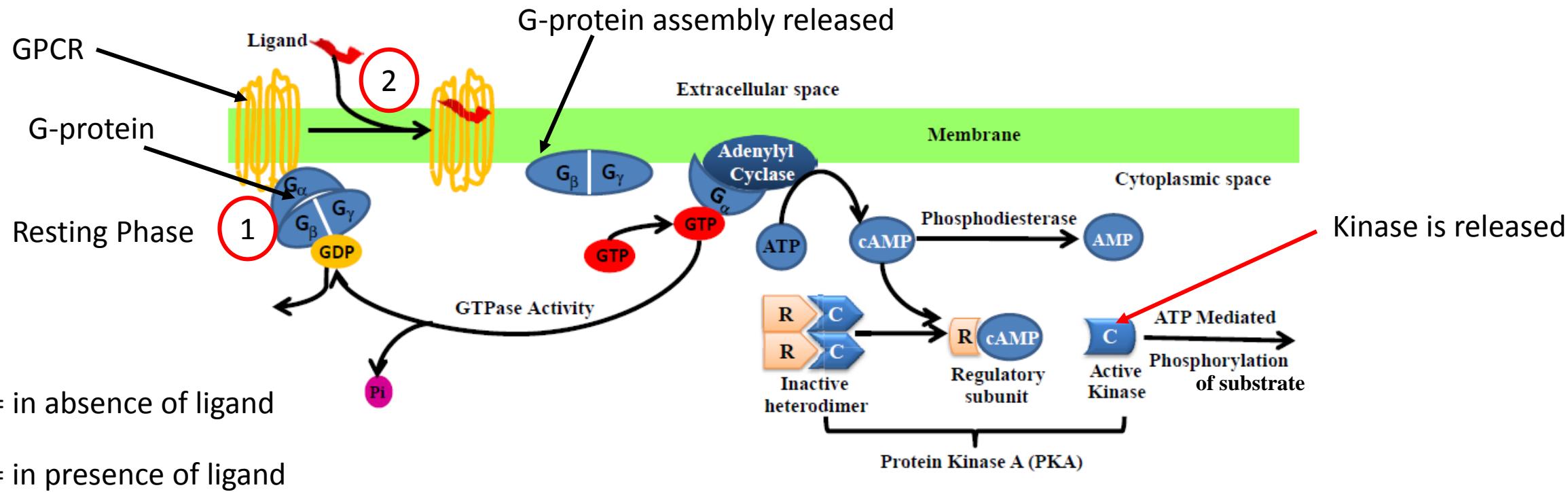
<u>TARGET TISSUE</u>	<u>SIGNALING MOLECULE</u>	<u>MAJOR RESPONSE</u>
Liver	vasopressin	glycogen breakdown
Pancreas	acetylcholine	amylase secretion
Smooth muscle	acetylcholine	contraction
Blood platelets	thrombin	aggregation

# G-Protein-Dependent Signaling Pathways

- Machinery → Signal → Carrier  
(GPCR)- generates a signal, need a transmitter to send the signal
- **Secondary messengers-** molecules that transmit signals from GPCRs to cellular machinery
  - Two major types-
  - cAMP system (cyclic adenosine monophosphate)
  - Phosphatidylinositol system  
phosphatidylinositol 4,5-bisphosphate → inositol-1,4,5-trisphosphate (IP<sub>3</sub>) and diacylglycerol (DAG)

# cAMP Signaling

G-protein- (guanyl nucleotide-binding protein)  
GTP- (Guanine triphosphate)



**FIGURE 3.23** cAMP signaling begins with binding of a ligand to the GPCR. Conformational changes in the GPCR causes the G-protein complex to disassociate from the GPCR, the G<sub>α</sub> protein and GDP are released, and GTP binds to the G<sub>α</sub> protein. The GTP/G<sub>α</sub> protein complex binds to adenylate cyclase, activating the enzyme, which produces cAMP. Binding of cAMP to the regulatory protein ("R") suppressing protein kinase A (RC) releases active protein kinase A (C), allowing it to phosphorylate molecular targets. The system is regulated by the GTPase activity of the G<sub>α</sub> protein and cAMP phosphodiesterase.

Ligand binding → extracellular side of GPCR → kinase activity

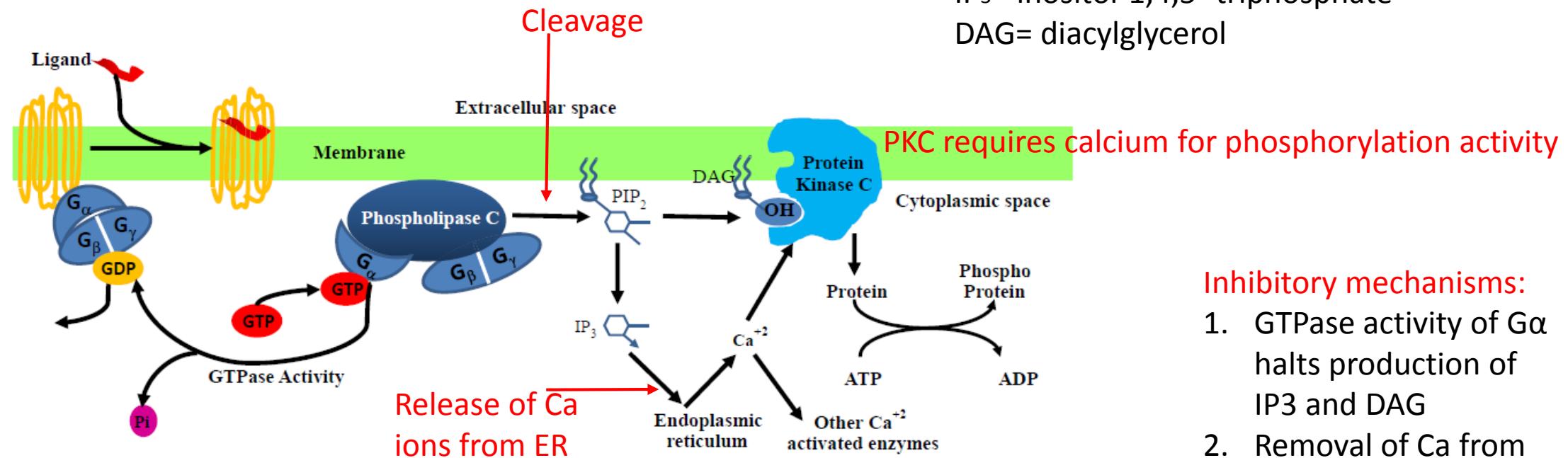
Chapter 3: Blass, 2015

# Regulation of cAMP Signaling

- Signal needs to be turned off to stop the activity
- Dissociation of ligand and GPCR does not stop cAMP activity
- cAMP phosphodiesterase (converts cAMP to AMP) removes cAMP and stops kinase activity.
- $G\alpha$  protein is also a GTPase (converts GTP to GDP). After this reaction,  $G\alpha$  cannot bind to adenylyl cyclase, deactivates cAMP production.

# IP<sub>3</sub> Signaling (Phosphatidylinositol Signaling)

PIP<sub>2</sub>=Phosphatidyl-4,5-inositol biphosphate  
IP<sub>3</sub>= inositol-1,4,5- triphosphate  
DAG= diacylglycerol



**FIGURE 3.24** IP<sub>3</sub> signaling is initiated by ligand binding to the GPCR. Conformational changes in the GPCR cause the G-protein complex to disassociate from the GPCR, the G<sub>α</sub> protein and GDP are released, and GTP binds to the G<sub>α</sub> protein. The GTP/G<sub>α</sub> protein complex binds to phospholipase C, which hydrolyzes PIP<sub>2</sub>, releasing DAG and IP<sub>3</sub>. Cytoplasmic IP<sub>3</sub> causes the release of cellular calcium stores, while membrane-bound DAG activates protein kinase C which phosphorylates molecular targets via ATP. Protein kinase C activity is augmented by the presence of calcium. The system is regulated by enzymatic degradation of IP<sub>3</sub> and DAG, GTPase activity of the G<sub>α</sub> protein, and removal of cytosolic calcium.

## Inhibitory mechanisms:

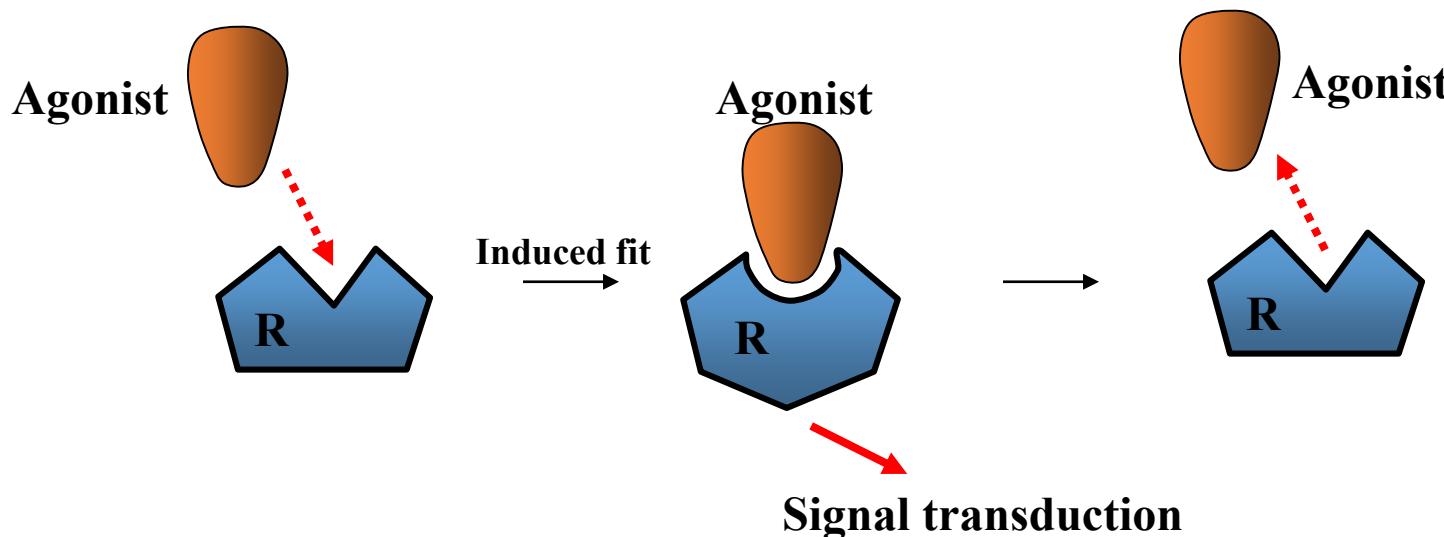
1. GTPase activity of G<sub>α</sub> halts production of IP<sub>3</sub> and DAG
2. Removal of Ca from cytosol by calcium ATPase pumps
3. DAG converted to glycerol or phosphorylated

# Modulating GPCR activity- Drug-receptor interactions

- Complex process- multiple GPCRs have overlapping effects, differential activities for ligands.
- Drugs targeting GPCRs- 3 categories
  - Agonists
  - Antagonists
  - Inverse agonists

# Agonists → mimic natural ligand, produce same cellular response

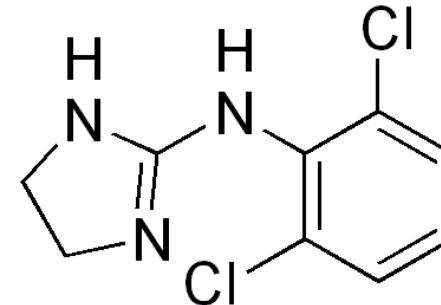
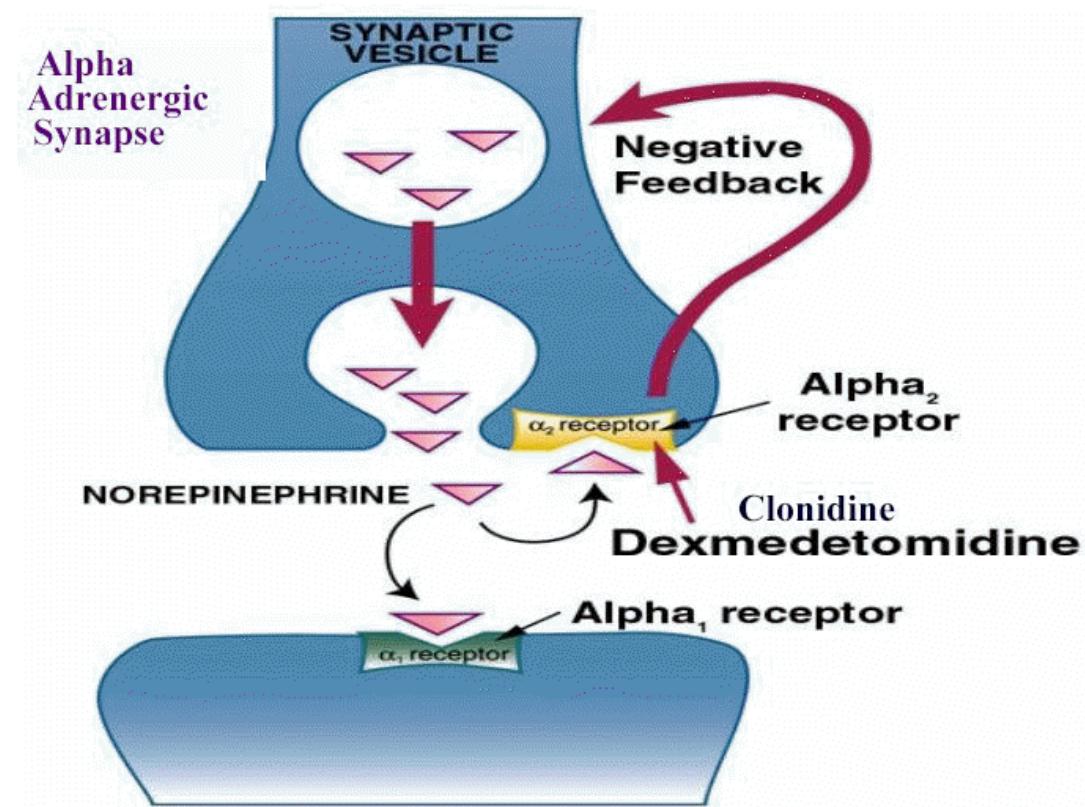
- Agonist binds reversibly to the binding site
- Similar intermolecular bonds formed as to natural messenger
- Induced fit alters the shape of the receptor in the same way as the normal messenger
- Receptor is activated
- Agonists are often similar in structure to the natural messenger



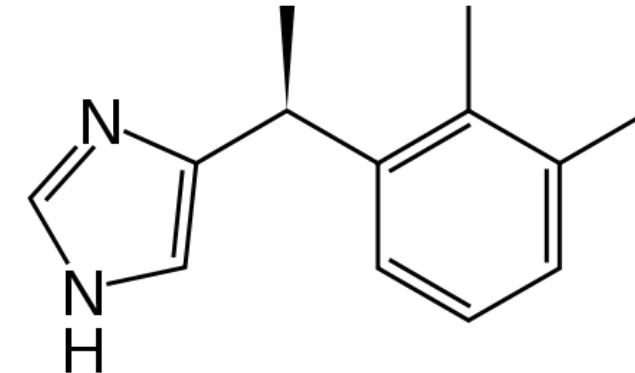
- Full agonists-  
100% efficiency  
relative to natural  
ligand  
- Partial agonists-  
efficiency below  
endogenous ligand

# Example- Agonist

attention deficit hyperactivity disorder, anxiety disorders



Clonidine

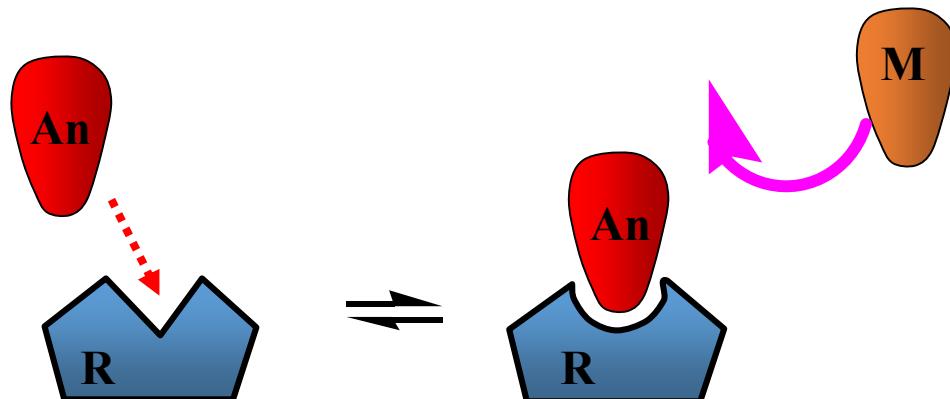


Dexmedetomidine

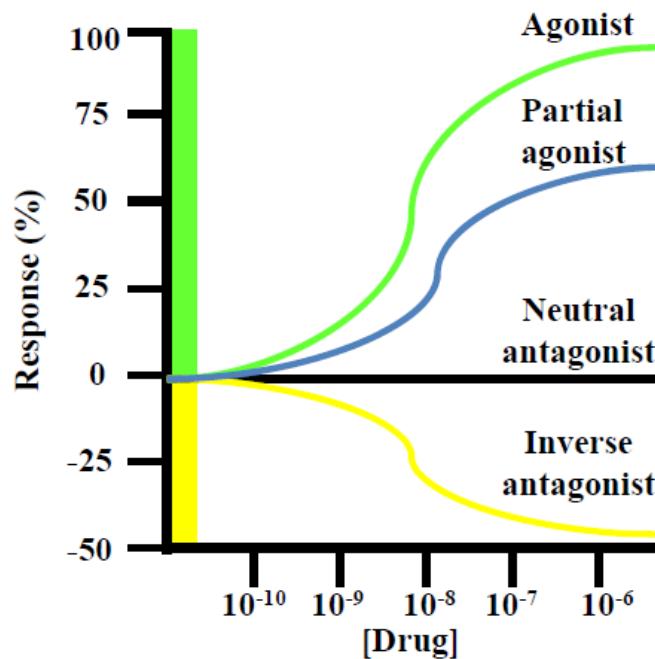
is an anxiety reducing, sedative, and pain medication

# Antagonists- bind to GPCR, but do not elicit a cellular response

- Antagonist binds reversibly to the binding site
- Intermolecular bonds involved in binding
- Different induced fit means receptor is not activated
- No reaction takes place on antagonist
- Level of antagonism depends on strength of antagonist binding and concentration
- Messenger is blocked from the binding site



**Inverse Agonists-** can block activity of endogenous ligand (like antagonists), but produce cellular response opposite to the natural ligand



**FIGURE 3.26** Full agonists (green) induce GPCR signaling equal to that of the endogenous ligand, while partial agonists (blue) activate GPCR signaling to a lesser extent. Neutral antagonists (black) do not induce GPCR activity, but will block agonist activity. Inverse agonists suppress basal activity of a constitutively active GPCR.

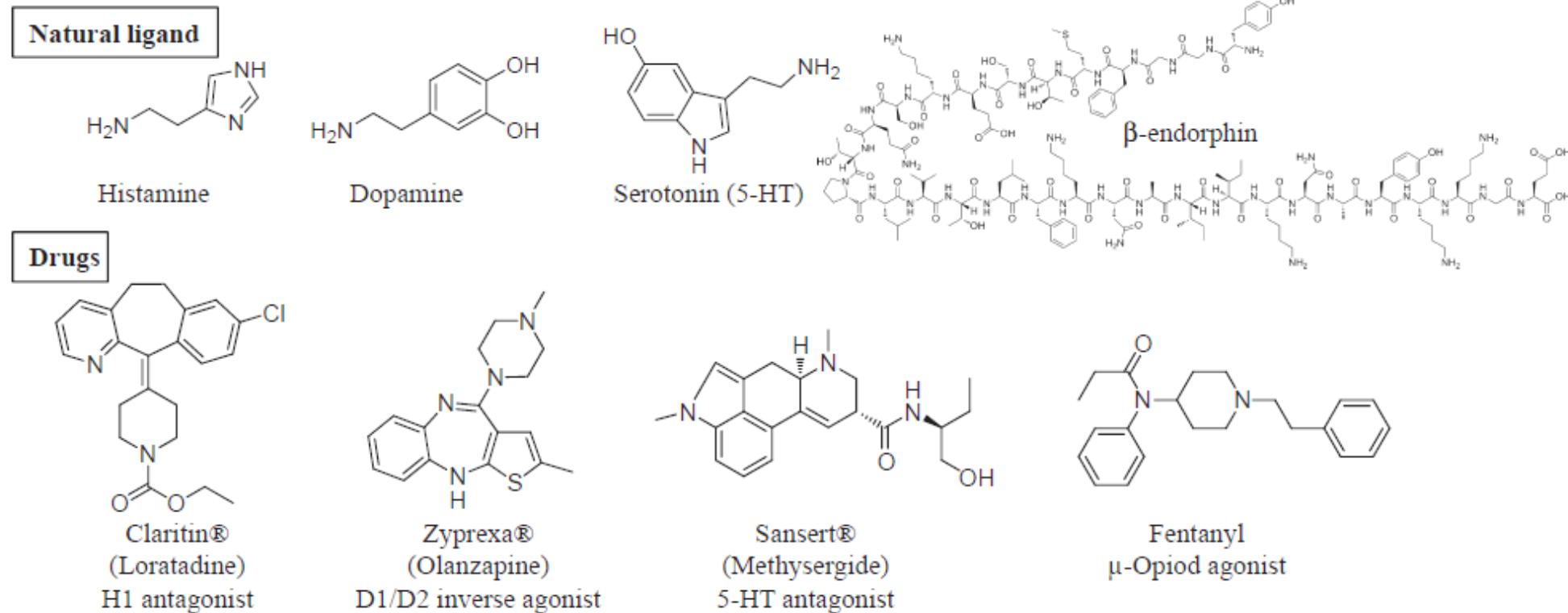
**Example-** Inverse agonists are effective against certain types of receptors (e.g. certain histamine receptors and GABA receptors) which have constitutive activity

**Difference from Antagonist:** Antagonist binds to the receptor, but does not reduce basal activity  
Agonist → positive efficacy  
Antagonist → zero efficacy  
Inverse agonist → negative efficacy

# GPCR activity modulators

- Examples- serotonin, histamine, dopamine.
- Size and Complexity of molecules is no driving force for mediating GPCR function.
- What is important?
  - *GPCRs must obtain a **specific conformation** in order to propagate a signal, compounds that modulate GPCR activity must be **able to adopt the specific conformation** required by the binding site that they are targeting.*

# Size and complexity of ligands- does not matter



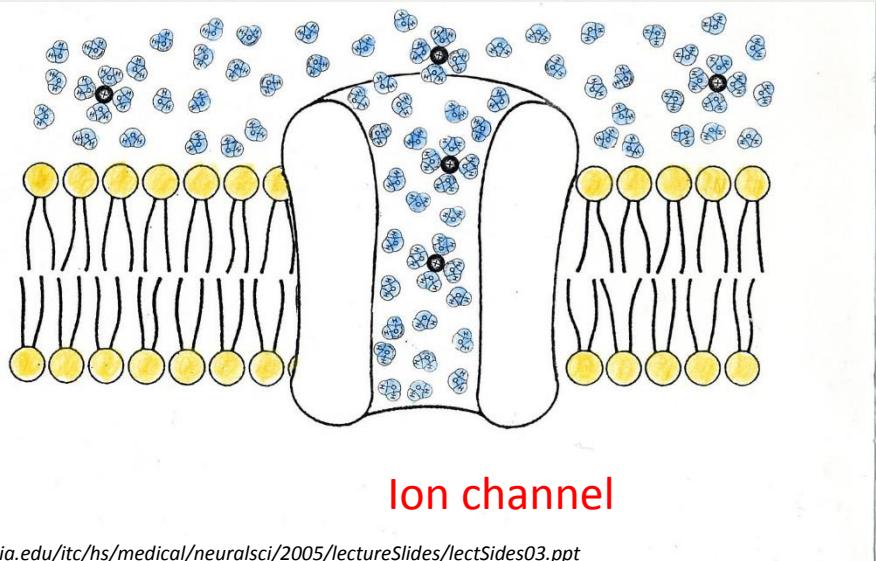
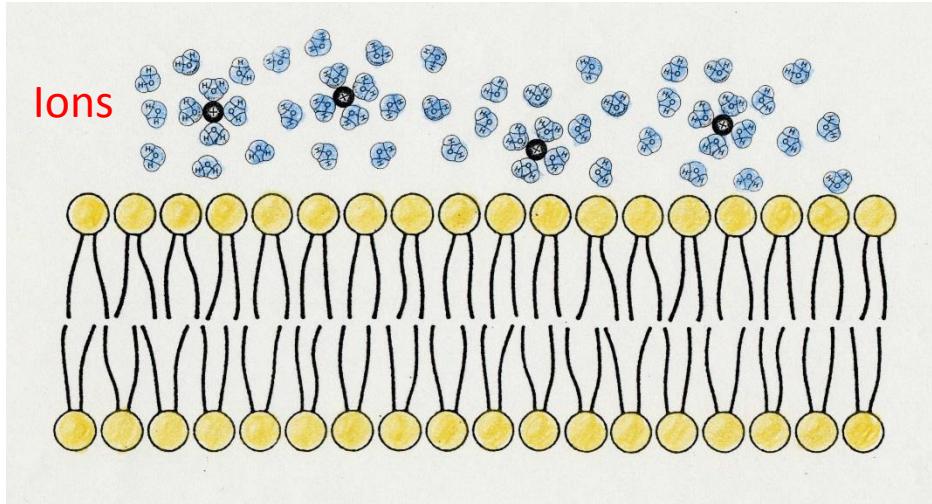
**FIGURE 3.27** Functional activity at a given GPCR is not necessarily mediated by size. Claritin® (loratadine), Zyprexa® (olanzapine), and Sansert® (methysergide) suppress GPCR signaling and are significantly larger than the endogenous ligand that activates the corresponding GPCR, but the same cannot be said of fentanyl and  $\beta$ -endorphin. In this case, the endogenous agonist is substantially larger than the synthetic agonist fentanyl, indicating that the proper agonist binding configuration can be achieved by molecules of diverse size and composition.

# Ion Channels

# Ions Cannot Diffuse Across the Hydrophobic Barrier of the Lipid Bilayer

## Specialized Functions

- Mediate the generation, conduction and transmission of electrical signals in the nervous system
- Control the release of neurotransmitters and hormones
- Initiate muscle contraction
- Transfer small molecules between cells (gap junctions)
- Mediate fluid transport in secretory cells
- Control motility of growing and migrating cells
- Provide selective permeability properties important for various intracellular organelles



Ion Channels are Selectively Permeable

Channels are made of sub-units

Cation Permeable

$\text{Na}^+$

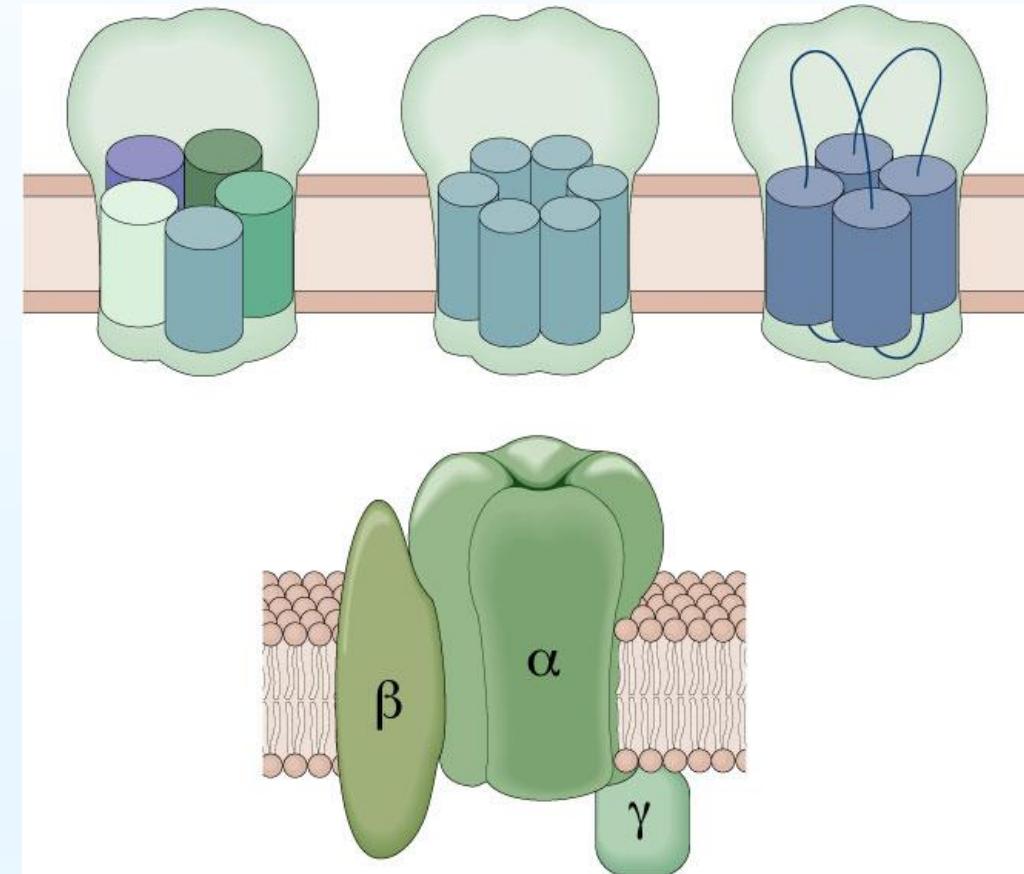
$\text{K}^+$

$\text{Ca}^{++}$

$\text{Na}^+, \text{Ca}^{++}, \text{K}^+$

Anion Permeable

$\text{Cl}^-$



# Ion Channels

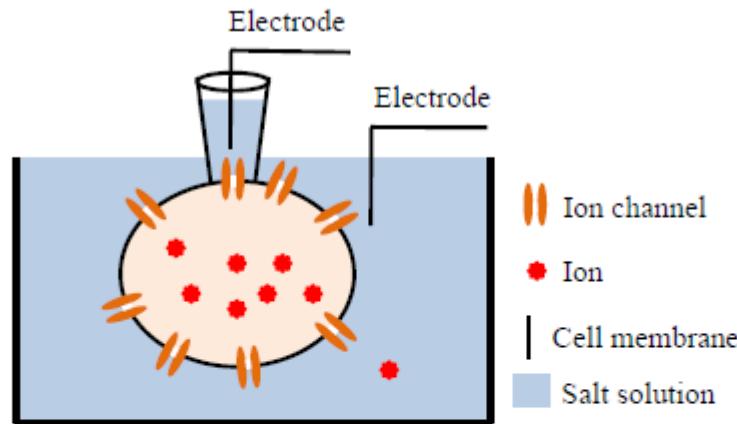
- transmembrane protein assemblies that regulate the flow of ions across biological barriers
- Nerve impulse transmission, muscle contraction, and cardiovascular function, especially heart rate and rhythm, -depend on the exquisitely balanced flow of ions created by a network of ion channels opening and clos

TABLE 3.1 Disease States Associated with Ion Channels

Disease	Channel	Gene
Arrhythmia	Nav1.5	SCN5A
Arrhythmia	Kv1.5	KCNA5
Cystic fibrosis	CFTR	CFTR
Diabetes mellitus	Kir6.2	KCNJ11
Epilepsy	KCNQ2	KCNQ2
Epilepsy	Nav1.2	SCN2A
Episodic Ataxia	Kv1.1	KCNA1
Erythromelalgia	Nav1.7	SCN9A
Migraine	Cav2.1	CACNA1A
Fibromyalgia	Nav1.7	SCN9A
Long QT syndrome	hERG	KCNH2
Malignant hyperthermia	Cav1.1	CACNA1S
Neuropathic pain	TrpV1	TRPV1
Osteoporosis	ClC-7	CLCN7
Timothy syndrome	Cav1.2	CACNA2

# Ion Channels

- Membrane potential- electrical gradients across cellular barriers result from **differences in ion concentrations** on the inside and outside of cells created by the selective movement of ions across the cellular barrier
- Patch clamp technique-



**FIGURE 3.29** A basic patch clamp system consists of a micropipette with an opening on the order of  $1\text{ }\mu\text{m}$  pressed against the surface of a cell. The inside of the micropipette covers a limited number of ion channels, and a seal with high electrical resistance ("gigaohm seal") is created by suction on the surface of the cell. An electrode, salt solution inside the micropipette, and the appropriate electrical amplification and monitoring systems can then be employed to either maintain a constant voltage while monitoring current or maintain a constant current while monitoring changes in membrane potential in the presence of test compounds.

# Ion Channels

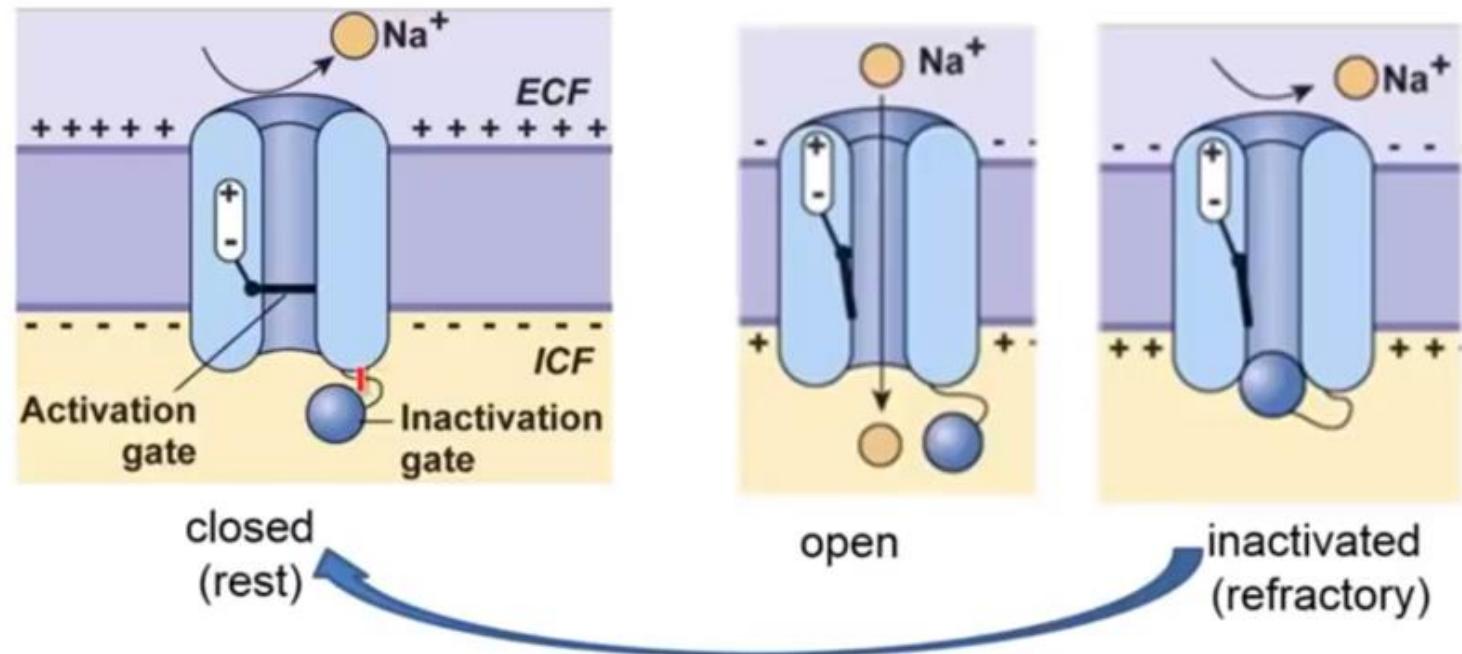
(4287 structures in PDB, 19<sup>th</sup> Sept 2018)

- First structure determined in 1998 by Roderick Mackinnon- potassium channel designated KcsA from the soil bacteria *Streptomyces lividans*.
- As of 2013, the Protein Data Bank contains over **3100 crystal** structures categorized as ion channels.
- Over **300 ion channels** have been identified to date.
- Much like the GPCRs, ion channels are integral membrane proteins comprised of a series of transmembrane domains that are linked by extracellular and intracellular loops.
- To date, ion channels have been identified that support the flow of sodium, calcium, potassium, chloride, and hydrogen ions.

The screenshot shows the RCSB PDB homepage with a search bar at the top. Below the search bar, there are navigation links for PDB-101, Worldwide Protein Data Bank, EMDDataBank, NUCLEIC ACID DATABASE, and Worldwide Protein Data Bank Foundation. A summary bar indicates 4,287 Structures, 3 Unreleased Structures, 1962 Citations, 1374 Ligands, and 15 News & PDB-101 Articles. The main content area displays search parameters for 'ion channels' and refinement options for organism (Homo sapiens, Mus musculus, Rattus norvegicus, Escherichia coli, Gloeobacter violaceus, Gallus gallus, Pseudomonas aeruginosa, Other) and UniProt molecule name (Uncharacterized protein, Proton-gated ion channel). A detailed search result for entry 4FC4 is shown, featuring a 3D ribbon model of the FNT family ion channel, author information (Lu, W., Schwarzer, N.J., Du, J., Gerbig-Smentek, E., Andrade), publication details ((2012) Proc Natl Acad Sci U S A 109 18395-18400), and technical details (Released: 11/21/2012, Method: X-ray Diffraction, Resolution: 2.4 Å). The macromolecule is identified as Nitrite transporter N with unique ligands B.

# Gating Mechanisms

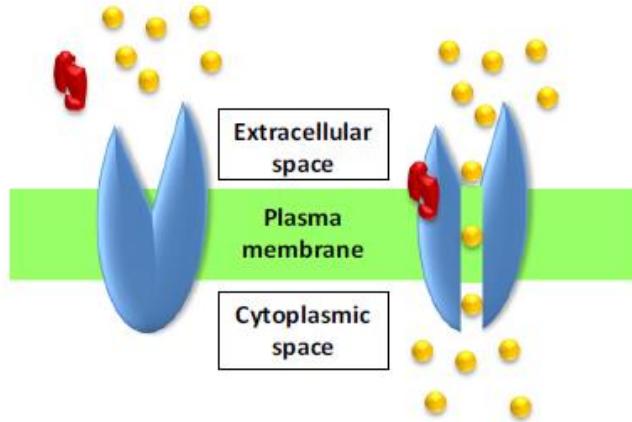
- Open-close mechanism
- The gating of a channel can be dependent on the presence of a ligand, environmental pH, temperature, or membrane voltage differences.
- Ligand-gated and voltage-gated channels are the most extensively studied types of channels.



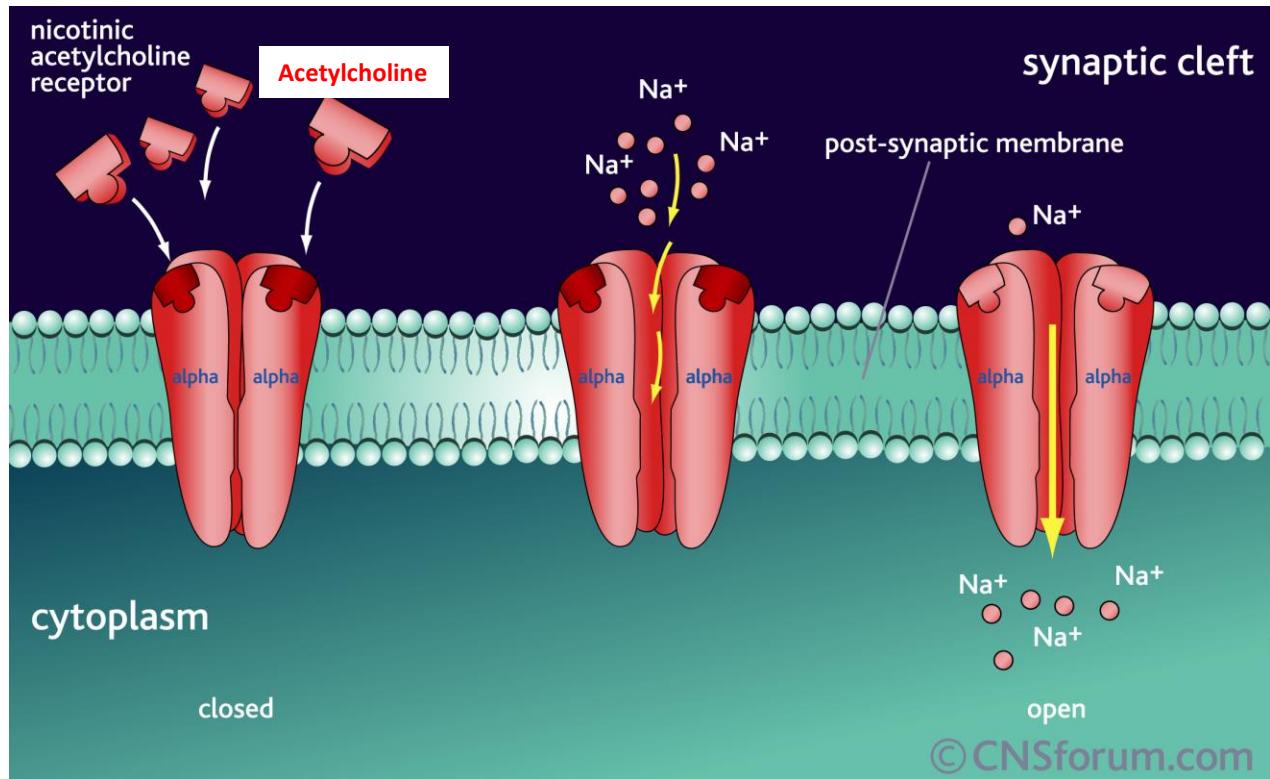
[physiology.lf2.cuni.cz/teaching/Ion%20Channels1.ppt](http://physiology.lf2.cuni.cz/teaching/Ion%20Channels1.ppt)

# Ligand-Gated Channels

- Activated in presence of a ligand



**FIGURE 3.31** Ligand-gated channels are closed in the absence of a ligand (red). Binding of the ligand to channel leads to conformational changes that cause the channel to open, allowing migration of suitable ions through the channel. Removal of the ligand causes the channel to close, stopping ion flow. Ligand-gated channels can be activated by synthetic ligands or blocked with antagonists (compounds that bind to the ligand-binding site, but do not lead to channel opening). Direct blockade of the channel is also possible.



Nicotinic acetylcholine receptor (nAChR), a key player in neurotransmission, is activated in the presence of acetylcholine

# Examples- drugs targeting ligand channels

## Activation of channels

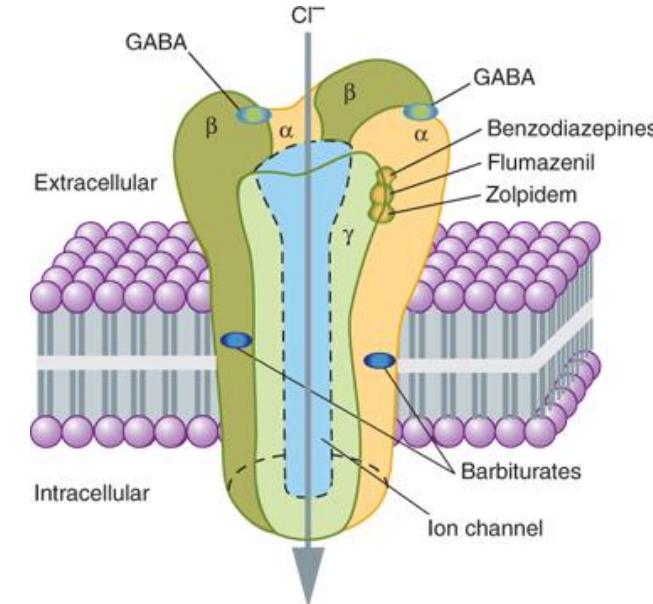
- Nicotine, in tobacco, is an agonist of nAChR, gives activation of reward system of brain
- smoking-cessation medication Chantix® (Varenicline) is a **partial agonist** of nAChR, and provides a **lower level of channel activity** upon binding than nicotine.

Chantix competes with Nicotine for binding to nAChR

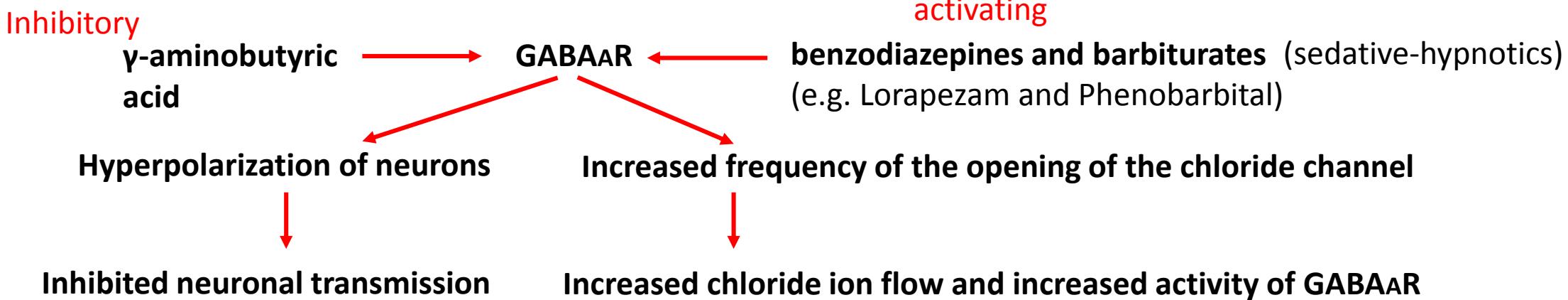
- It has been successfully employed to decrease the cravings and the pleasurable effects of nicotine.

# Allosteric activation

The  $\gamma$ -aminobutyric acid type A receptor (GABA<sub>A</sub>R), for example, is a ligand-gated chloride channel - critical role in the central nervous system.



Source: Bertram G. Katzung, Anthony J. Trevor: Basic & Clinical Pharmacology, 13th Ed.  
www.accesspharmacy.com  
Copyright © McGraw-Hill Education. All rights reserved.



# Examples- drugs targeting ligand channels

## Blocking of channels

### Functional antagonists-

1. compete for the natural ligands binding site, but do not cause the conformational changes and prevent opening of the channel.
2. bind to an allosteric site and either stabilize the closed form of the channel or cause conformational changes that prevent binding of the natural ligand

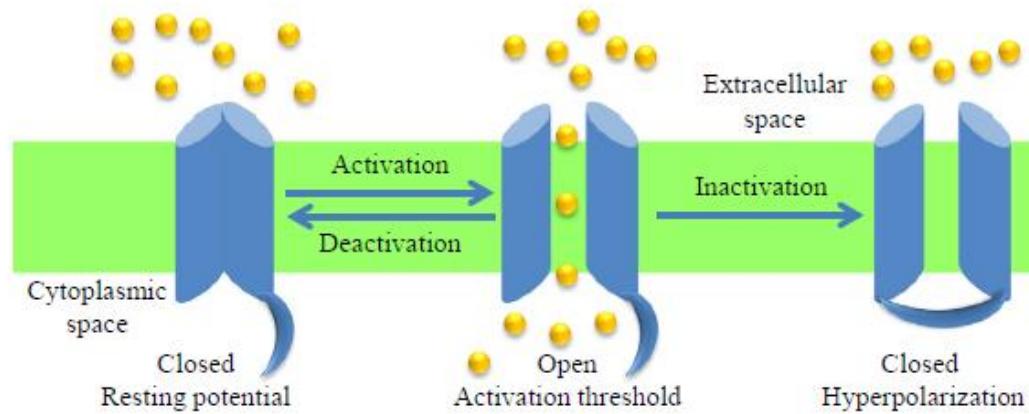
Example-  $\alpha$ -neurotoxins, for example, are a family of peptides from snake venom tightly bind to nAChR in skeletal muscle, prevent acetylcholine-mediated neurotransmission (through the opening of nAChR), causing paralysis in snake bite victims

**Hyperpolarization** is when the membrane potential becomes more negative at a particular spot on the neuron's membrane, while **depolarization** is when the membrane potential becomes less negative (more positive).

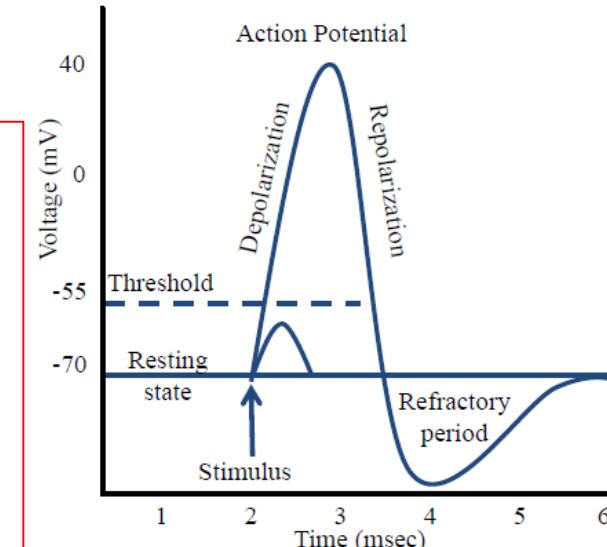
# Voltage-Gated Channels

- have no natural ligand.
- open and close as a result of **changes in membrane potential** produced as electrical currents move through biological systems.
- propagation of nerve impulse through axons, muscle contraction, and cardiac function.

1. Stimulus above the gating threshold → channel opening Rapid
2. Depolarization caused by ion flow through the channel → hyperpolarization and closing of the inactivation gate.
3. The inactivation gate remains closed until the membrane potential is reset by the action of opposing forces.
4. Stimulation of the channel will not evoke a response until this “refractory period” has ended and the resting potential (-50 to -70 millivolts) is restored.



**FIGURE 3.35** In the resting state, voltage-gated channels are closed. When the membrane potential reaches the proper level, conformational changes cause the channel to open, allowing the flow of ions across the membrane. This quickly leads to a hyperpolarized state, which induces another set of conformational changes that inactivate the channel. The channel cannot reopen until the resting potential is restored and its conformation shifts back to the closed resting potential state.

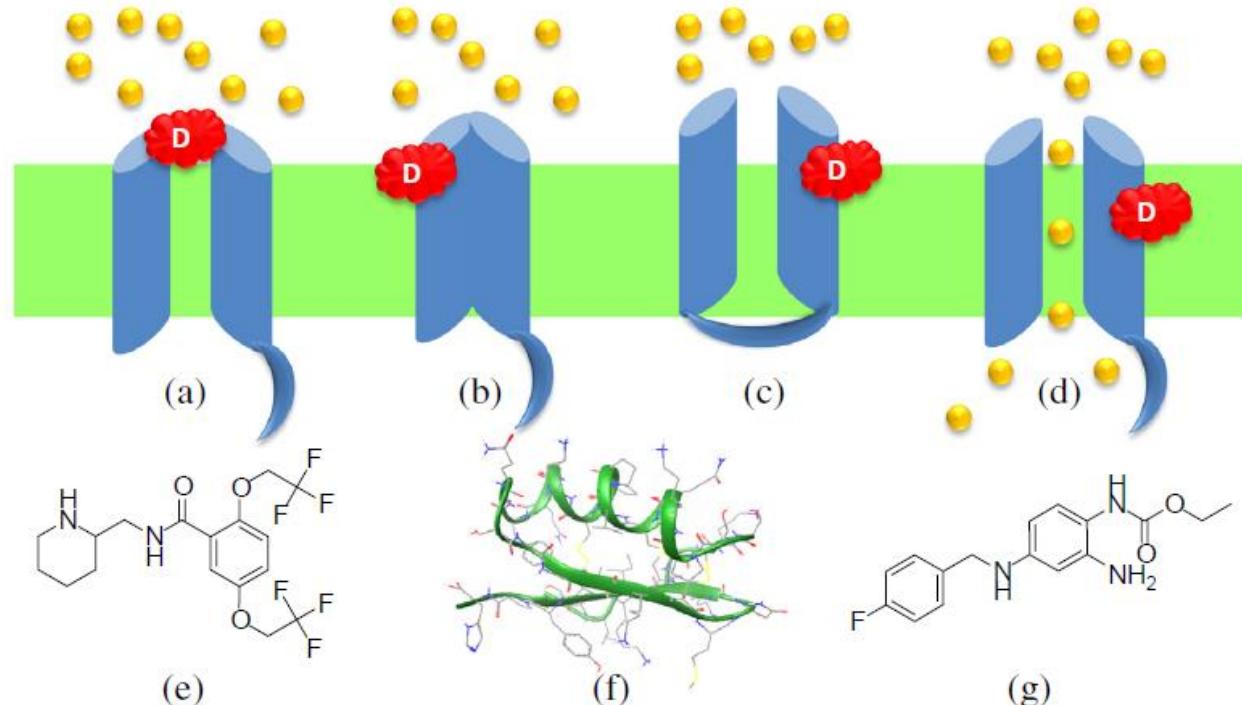


*For more info:* <https://www.khanacademy.org/science/biology/human-biology/neuron-nervous-system/a/depolarization-hyperpolarization-and-action-potentials>

# Modulation of activity of Voltage-Gated Channels

- Not possible with agonists and antagonists
- Options- **Blocking the open channel directly**
- E.g. **Flecainide** blocks Nav1.5, a voltage-gated sodium channel that plays a major role in cardiac function, and is useful for the treatment of arrhythmia and the prevention of tachycardia.
- The scorpion venom **Margatoxin** blocks the Kv1.3 channel, a voltage-gated potassium channel found in a variety of cell types, including neuronal cells. Kv1.3 channel blockade also can induce immunosuppression by decreasing T-cell proliferation.

Either close or open state maintained



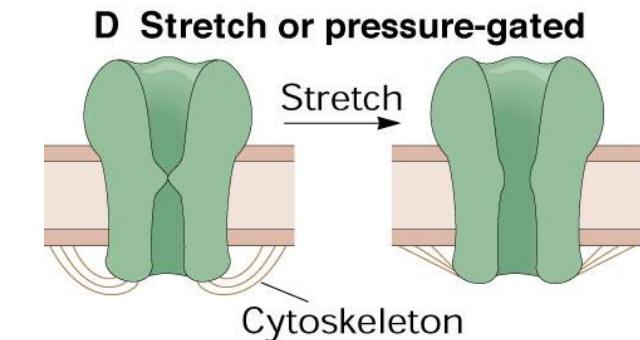
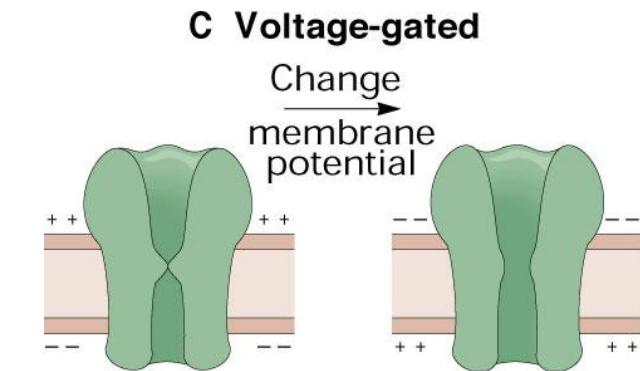
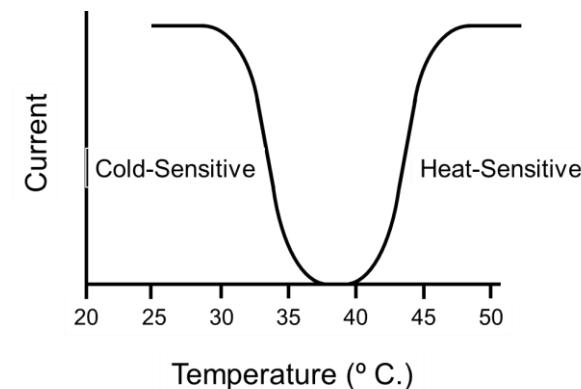
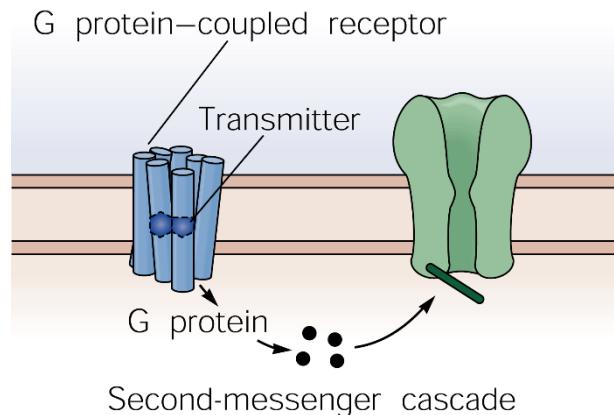
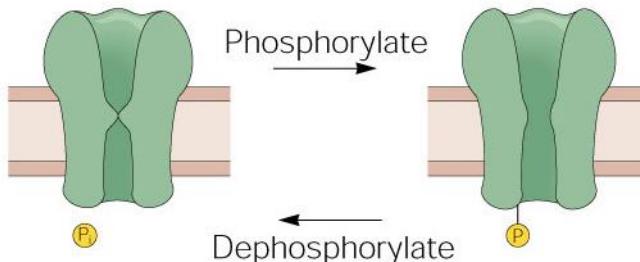
**FIGURE 3.37** (a) Direct blockade of the open configuration of the channel by a drug (red) prevents ion flow through the pore. (b) Stabilization of the closed form of the channel by a compound (red) effectively increases the activation threshold, decreasing channel activity. (c) The hyperpolarized state of a voltage-gated channel can be stabilized by a drug (red), maintaining the position of the inactivation gate, slowing conformational changes required to reach the closed resting state. (d) Interaction of a drug (red) with the open channel can stabilize the open configuration, leading to increased ion flow across a cellular barrier. (e) Flecainide, a Nav1.5 blocker and antiarrhythmic agent. (f) Margatoxin, a 39-amino acid peptide found in the venom of *Centruroides margaritatus* (the Central American Bark Scorpion) and Kv1.3 channel blocker. (g) Retigabine, a Kv7.2 and Kv7.3 channel opener and antiseizure agent.

# Other Modulating options

- Interaction of compounds with the protein **at sites other than the pore region**. This could be considered a form of **allosteric modulation**, as the “active site” of an ion channel is the pore through which ions move.
- Retigabine, an anticonvulsant useful for the treatment of epilepsy and seizures, **stabilizes the open forms** of voltage-gated potassium channels Kv7.2 and Kv7.3, leading to increased potassium flow and seizure suppression.

# Other gating mechanisms

- Temperature-gated channels
- Mechanosensitive ion channels
- pH gating has also been observed
- Phosphorylation
- GPCR mediated



# Ion channel openers / closers

## OPENERS

- Diazoxide

-vasodilator used for hypertension,  
smooth muscle relaxing activity

## CLOSERS

- \* Amiodarone

- Used to treat cardiac arrhythmias ,  
prolonging the repolarization

# Transporters

# Membrane Transport Proteins (MTPs)

- cellular survival requires that numerous compounds travel across cellular membranes
- majority of transmembrane traffic of small molecules across biological barriers is accomplished by membrane transport proteins, also known as **transporters**.
- nerve impulse transmission (serotonin, norepinephrine, and dopamine transporters), metabolism (glucose transporter), and muscle contraction (e.g., glucose transporter).
- **modulation of transporter activity is a major clinical tool in the treatment of psychiatric disorders**

# Transporters

Facilitated diffusion:  
protein-mediated movement down a gradient

- Protein Data Bank contains over 800 crystal structures categorized as transporters
- **12 transmembrane** regions and do not require multi-subunit assemblies for activity
- Members of the major facilitator superfamily (MFS) of transporters share this structural feature –
  - the norepinephrine transporter (NET),
  - glucose transporters,
  - ATP-binding cassette transporters (ABC-transporters).
- The P-glycoprotein efflux pumps (Pgps) are a particularly important subclass of the ABC-transporters family, as they are the most common “molecular pumps” that protect cells from **toxic materials and xenobiotics**. Unlike the majority of transporters, the Pgps are capable of interacting with a broad array of compounds, and are a major issue in drug discovery programs.

NADH: ubiquinone oxidoreductase (also known as respiratory complex I), one of the largest known membrane-protein complexes, contains multiple transporter proteins that contain as many as **14 transmembrane domains**

Pgp → DRUG EXPORTER → reduced efficacy

# How transporters differ from ion-channels?

Ion channels form a **tunnel** for passage of material, membrane transporters employ a **binding site** that is only available on **one side of a cellular membrane** at a time.

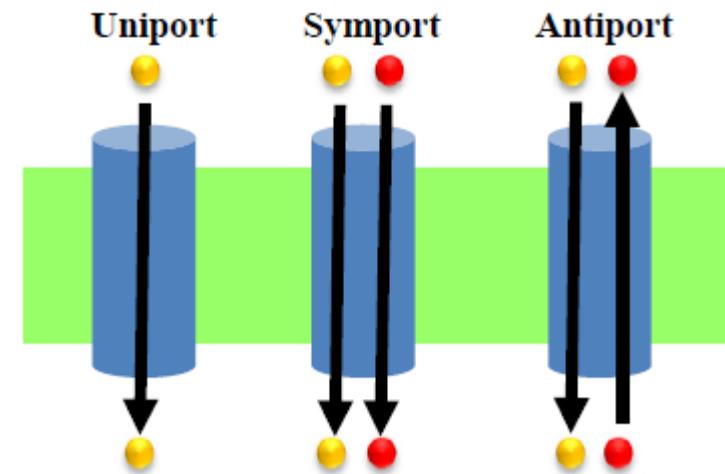


Conformational changes induced by the binding of a solute molecule



lead to the transfer of the solute molecule from one side of the membrane to another.

There are three basic types of membrane transporters, uniporter, symporters, and antiporters



**FIGURE 3.41** Uniporters move a single molecule in one direction down a concentration gradient, while symporters and antiporters move multiple molecules. Symporters move molecules in the same direction, while antiporters move molecules in opposite directions.

# Further classification of MTP's: passive or active transport systems

Facilitated diffusion:

## 1. Passive transport

driving force ->  
concentration/electrochemical  
gradient (high → low conc.)

No energy requirement

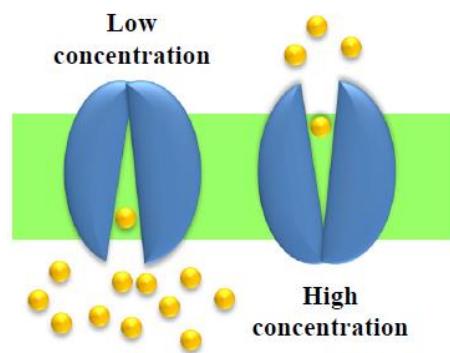
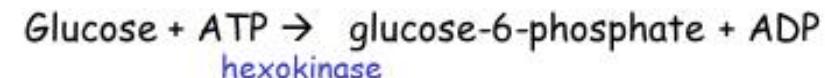
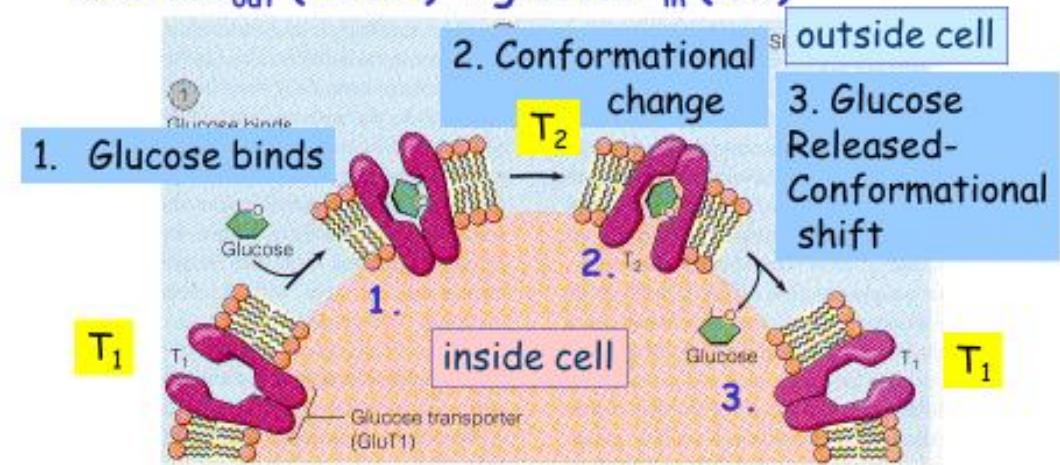


FIGURE 3.42 Facilitated diffusion moves a solute down a concentration gradient without expending cellular energy. Binding of the solute molecule induces conformational changes that move the solute across the membrane at a rate substantially greater than possible by simple diffusion.

Example: Glucose transporter GluT1 : carrier-mediated facilitated diffusion

$\text{Glucose}_{\text{out}} (\text{HIGH}) \rightarrow \text{glucose}_{\text{in}} (\text{low})$



*GLUT-1, 2, 3, 4, and 5, increase the rate of passage of glucose across the cellular membrane by a factor of 50,000 relative to simple diffusion.*

# 2. Active transport

against a gradient  
(low → high conc.)  
unfavorable  
requires energy input

Antiport

## Primary active transport

sodium potassium ATPase ( $\text{Na}^+/\text{K}^+$  ATPase) pump,  
an antiporter system (neurons and muscles)

two potassium ions ( $\text{K}^+$ ) into a cell while moving three sodium ions ( $\text{Na}^+$ ) out of the cell, membrane potential of  $-50$  to  $-70$  mV.

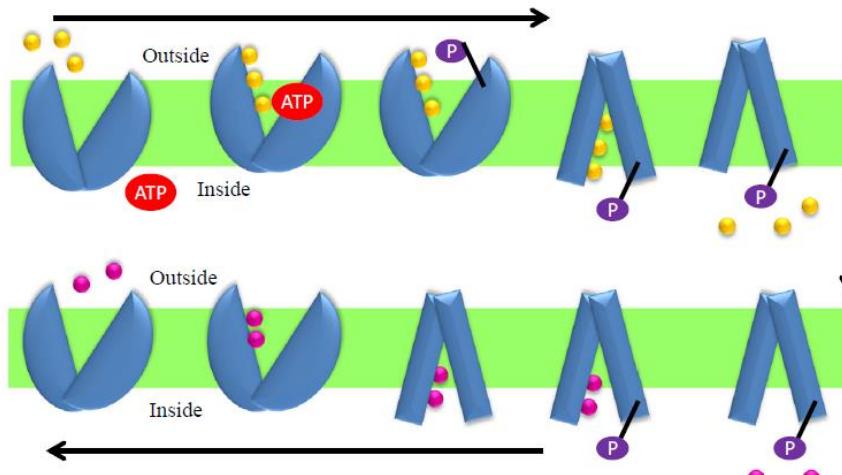


FIGURE 3.43  $\text{Na}^+/\text{K}^+$  ATPase pumps bind three  $\text{Na}^+$  (yellow) and one ATP (red) to the transporter. Phosphorylation (dark purple) leads to conformational changes that transfer the  $\text{Na}^+$  to the interior of the cell. The  $\text{Na}^+$  is released and two  $\text{K}^+$  (light purple) associate with the protein. This induces dephosphorylation, which causes the transporter to revert to its original structure and transfer the  $\text{K}^+$  to the outside of the cell at the same time.

## Secondary active transport

employ energy stored in the form of electrochemical gradients

Syport

sodium–glucose cotransporters utilize the sodium gradient created by  $\text{Na}^+/\text{K}^+$  ATPase pumps to drive the transport of glucose into cells.

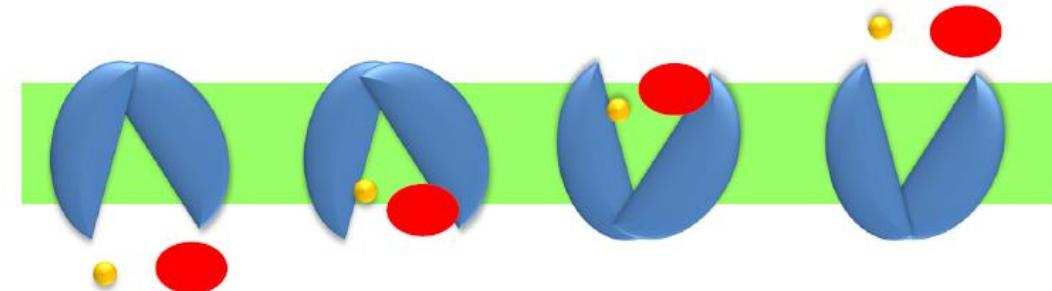


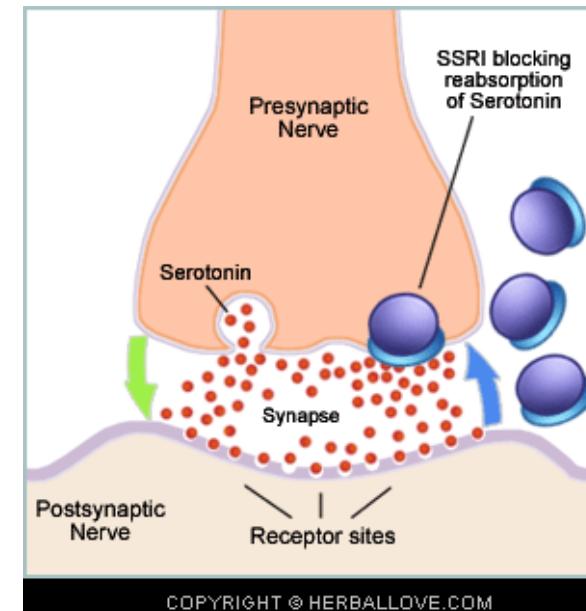
FIGURE 3.44 Glucose (red) and  $\text{Na}^+$  (yellow) bind to the sodium–glucose cotransporters (SGLT) which moves both across the cell membrane.  $\text{Na}^+/\text{K}^+$  ATPase pumps (not shown) create a  $\text{Na}^+$  gradient that drives this process.

# Transporter inhibition- a tool for drug discovery

- occupying the **substrate binding site** with a substrate mimic that is not transported through the membrane, thus “clogging the pipe.”
- **substrate mimic** that is preferentially transported as compared to the normal substrate
- binding to an **allosteric site** on the transporter

Example- Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Prozac®), citalopram (Celexa®), and sertraline (Zoloft®) **inhibit serotonin transporter (SERTs)** mediated transport of serotonin by **presynaptic cells of neural junctions**.

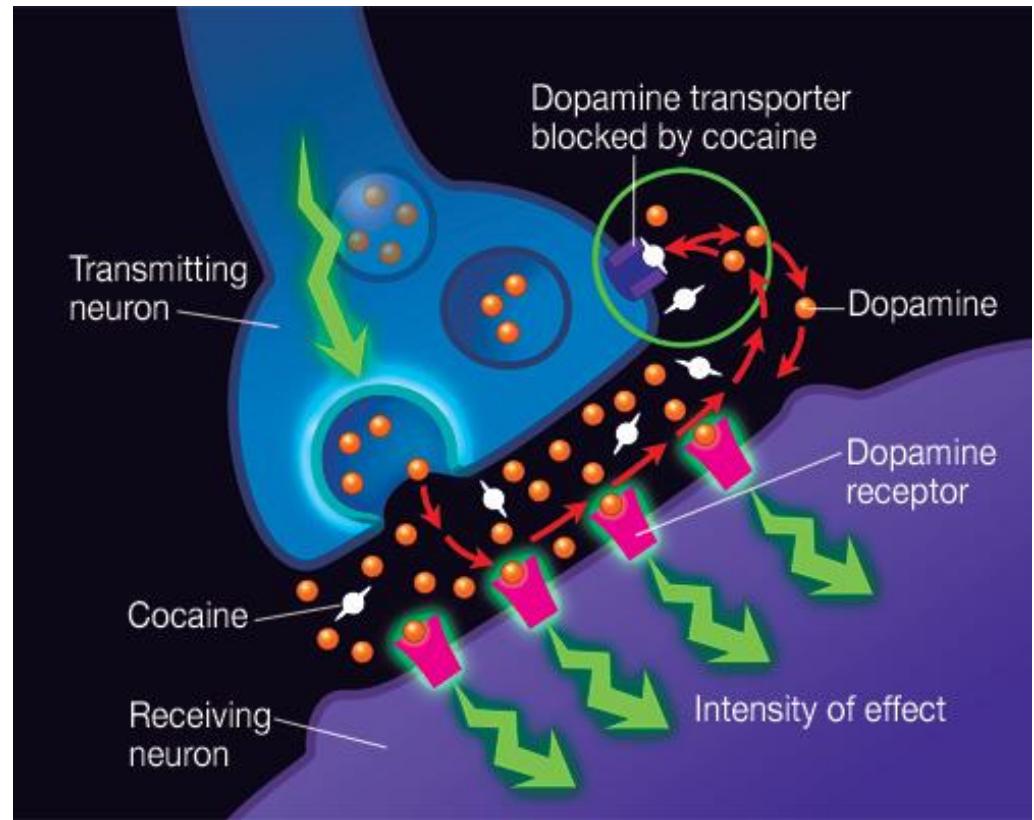
This, in turn, **increases the serotonin concentration** in the synaptic cleft available to act upon the serotonin receptors of postsynaptic cells, and eventually, down regulation of serotonin receptor expression occurs)



**psychiatric disorders such as attention deficit hyperactivity disorder, obsessive compulsive disorder, schizophrenia, and depression**

# Negative impact of Transporter inhibition-an example

- Cocaine (addictive),
- Blocks dopamine transporters (DAT) in presynaptic cells
- Blocking dopamine reuptake
- results in a rapid increase in extracellular dopamine levels that produce the euphoric feeling associated with cocaine exposure.
- Chronic cocaine exposure leads to an upregulation of DAT, and this increase in receptor density may contribute to the increasing level of cocaine required to deliver the same effects as chronic use continues.
- At the molecular level, the binding site for cocaine overlaps with that of the natural substrate, dopamine, blocking its access to the transporter.



<http://www.pathwaytorecovery.com/cocaineinfo.php>

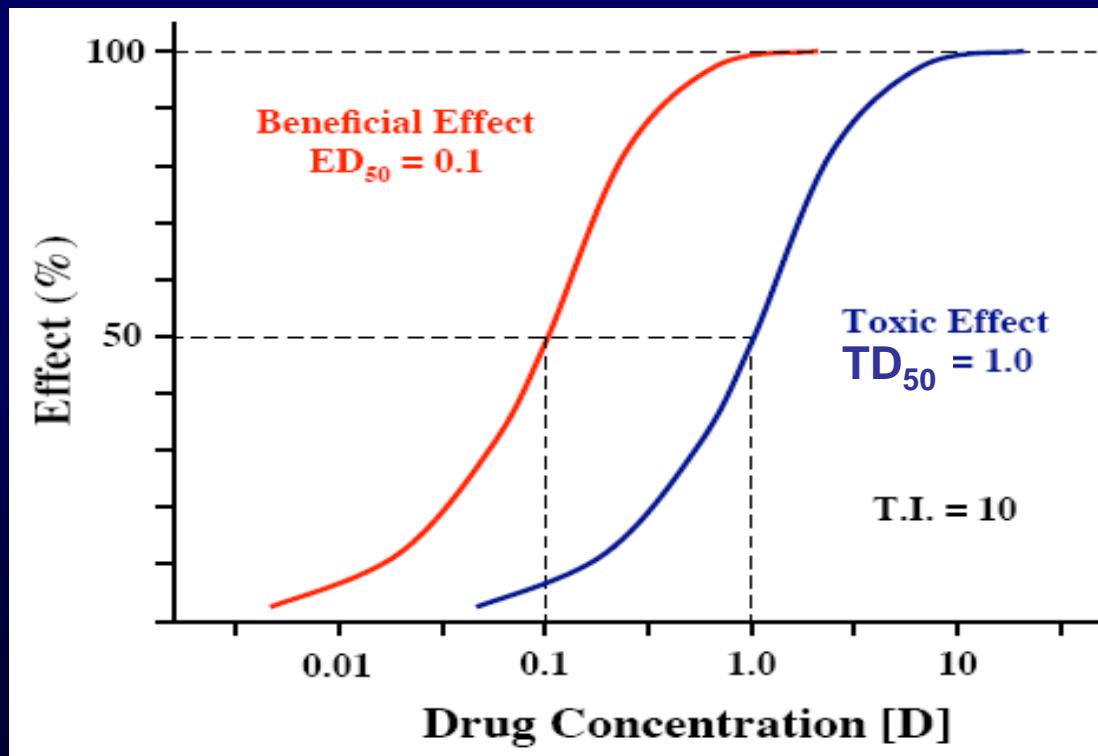
*In the normal communication process, dopamine is released by a neuron into the synapse, where it can bind to dopamine receptors on neighbouring neurons. Normally, dopamine is then recycled back into the transmitting neuron by a specialized protein called the dopamine transporter. If cocaine is present, it attaches to the dopamine transporter and blocks the normal recycling process, resulting in a build up of dopamine in the synapse, which contributes to the pleasurable effects of cocaine.*

# Visualization of SSRI action



# Therapeutic Index (T.I.)

- A measure of drug safety
- The ratio of the dose that produces toxicity to the dose that produces a clinically desired or effective response in a population of individuals
- Therapeutic Index =  $TD_{50}/ED_{50}$  or  $LD_{50}/ED_{50}$   
where  $TD_{50}$  is the dose that produces a toxic effect in 50% of the population,  $LD_{50}$  is the dose that is lethal in 50% of the population and  $ED_{50}$  is the dose that produces therapeutic response in 50% of the population
- ❖ In general, a larger T.I. indicates a clinically safer drug



# Therapeutic Index, contd.

Why don't we use a drug with a T.I. <1?

$ED_{50} > TD_{50}$  = Very Bad!

# Therapeutic Index (T.I.), contd.

- High therapeutic index
  - NSAIDs
    - Aspirin
    - Tylenol
    - Ibuprofen
  - Most antibiotics
  - Beta-blockers
- Low therapeutic index
  - Lithium
  - Neuroleptics
    - Phenytoin
    - Phenobarbital
  - Digoxin
  - Immunosuppressives