Topic 4 Proteins as Drug Targets

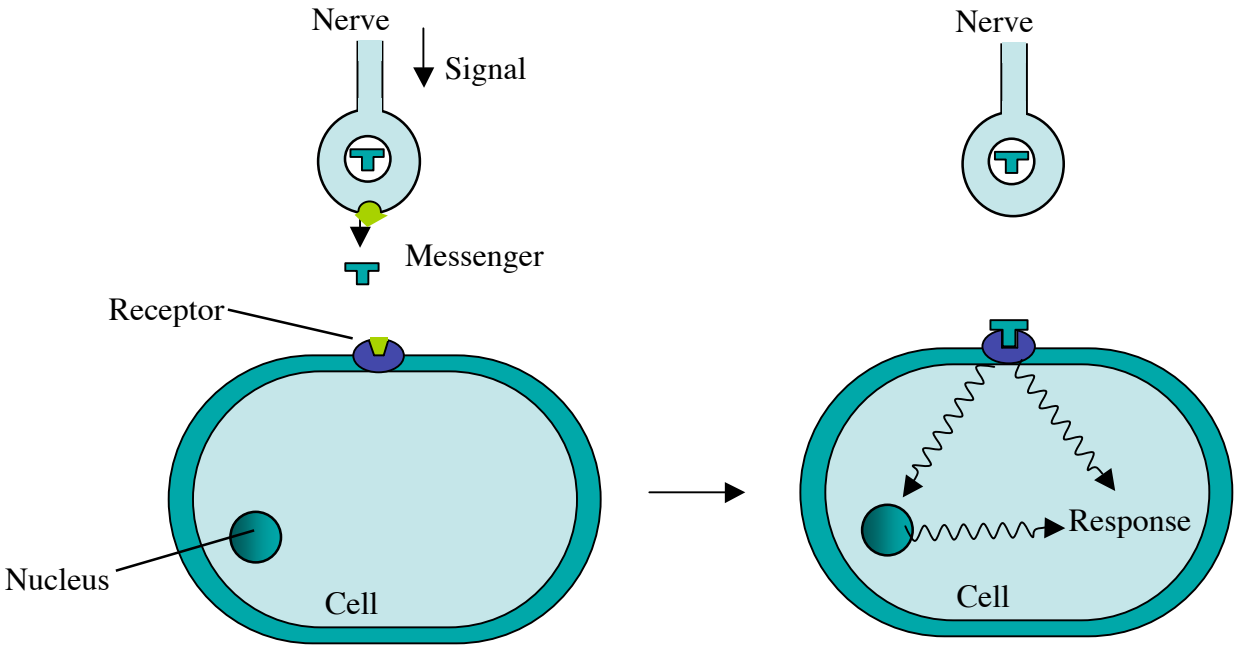
Receptors-Chapters 5 and 6
Patrick and Corey 78-80

Contents

1. Structure and function of receptors

- 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

1. Structure and function of receptors



1. Structure and function of receptors

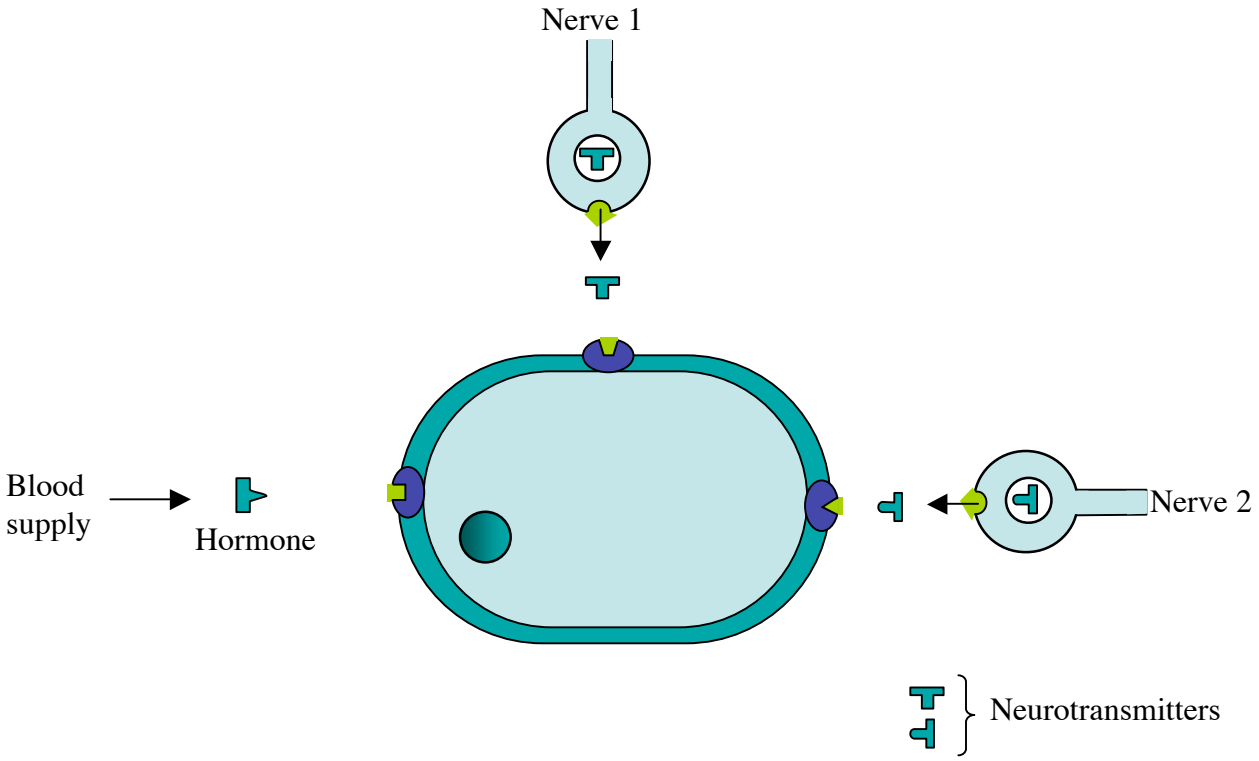
Chemical Messengers

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

1. Structure and function of receptors



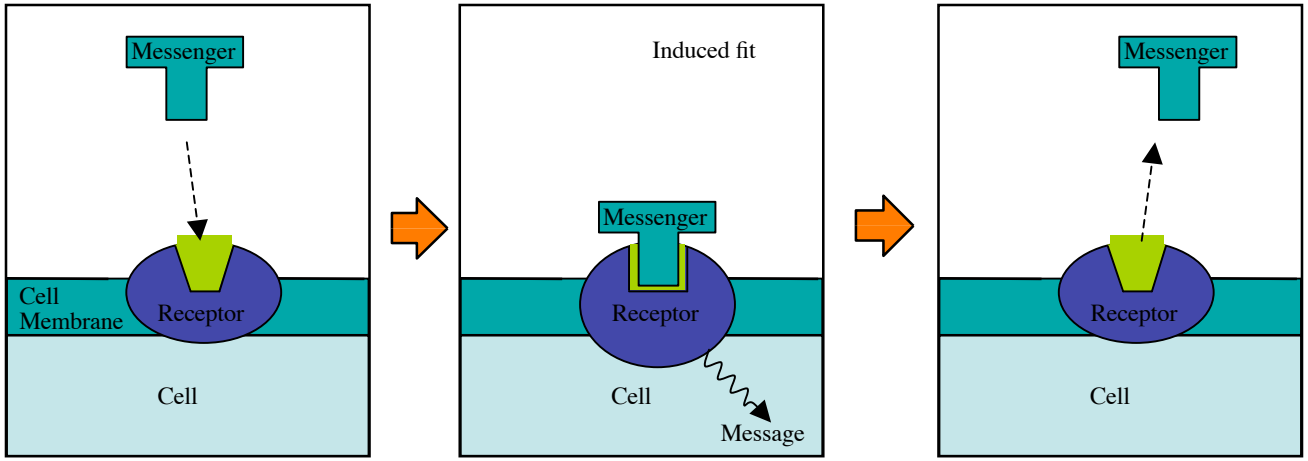
1. Structure and function of receptors

Mechanism

- Receptors contain a binding site (hollow or cleft in the receptor surface) that is recognised by the chemical messenger
- Binding of the messenger involves intermolecular bonds
- Binding results in an induced fit of the receptor protein
- Change in receptor shape results in a ‘domino’ effect
- Domino effect is known as Signal Transduction, leading to a chemical signal being received inside the cell
- Chemical messenger does not enter the cell. It departs the receptor unchanged and is not permanently bound

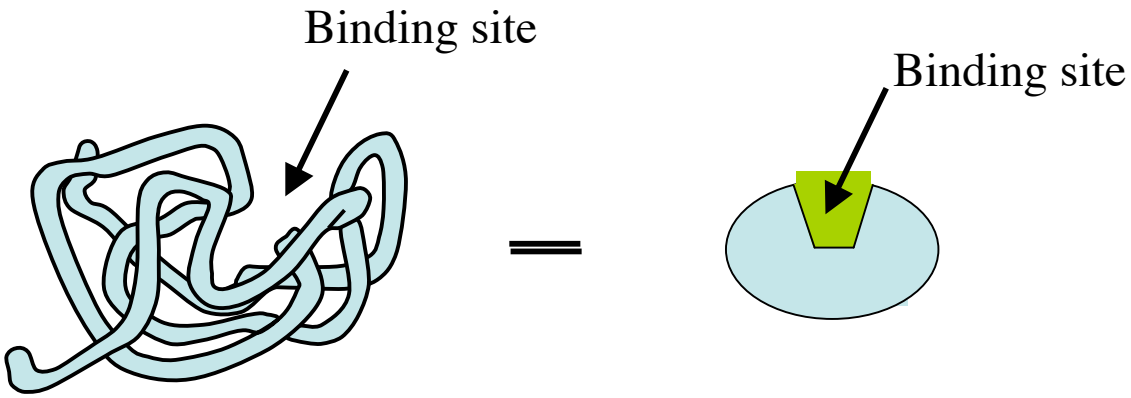
1. Structure and function of receptors

Mechanism



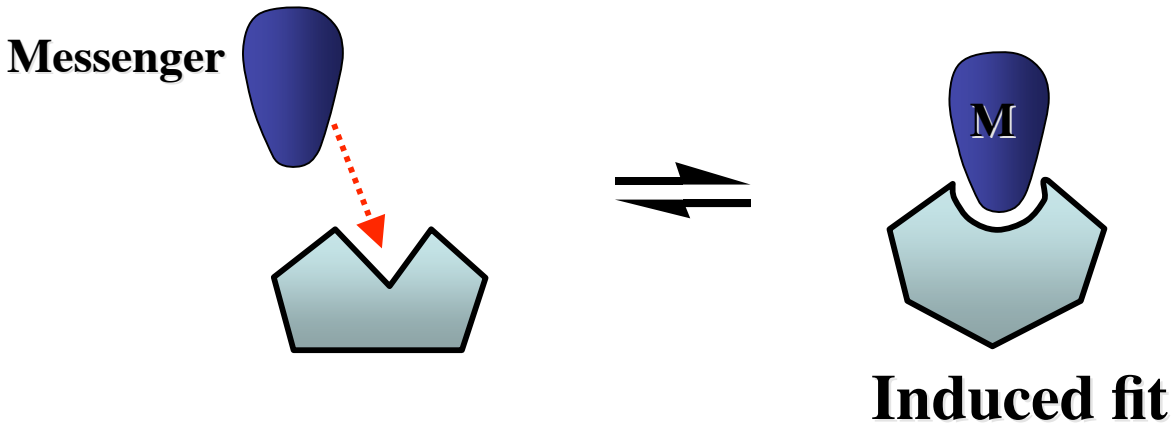
2. The binding site

- A hydrophobic hollow or cleft on the receptor surface - equivalent to the active site of an enzyme
- Accepts and binds a chemical messenger
- Contains amino acids which bind the messenger
- No reaction or catalysis takes place



3. Messenger binding

3.1 Introduction



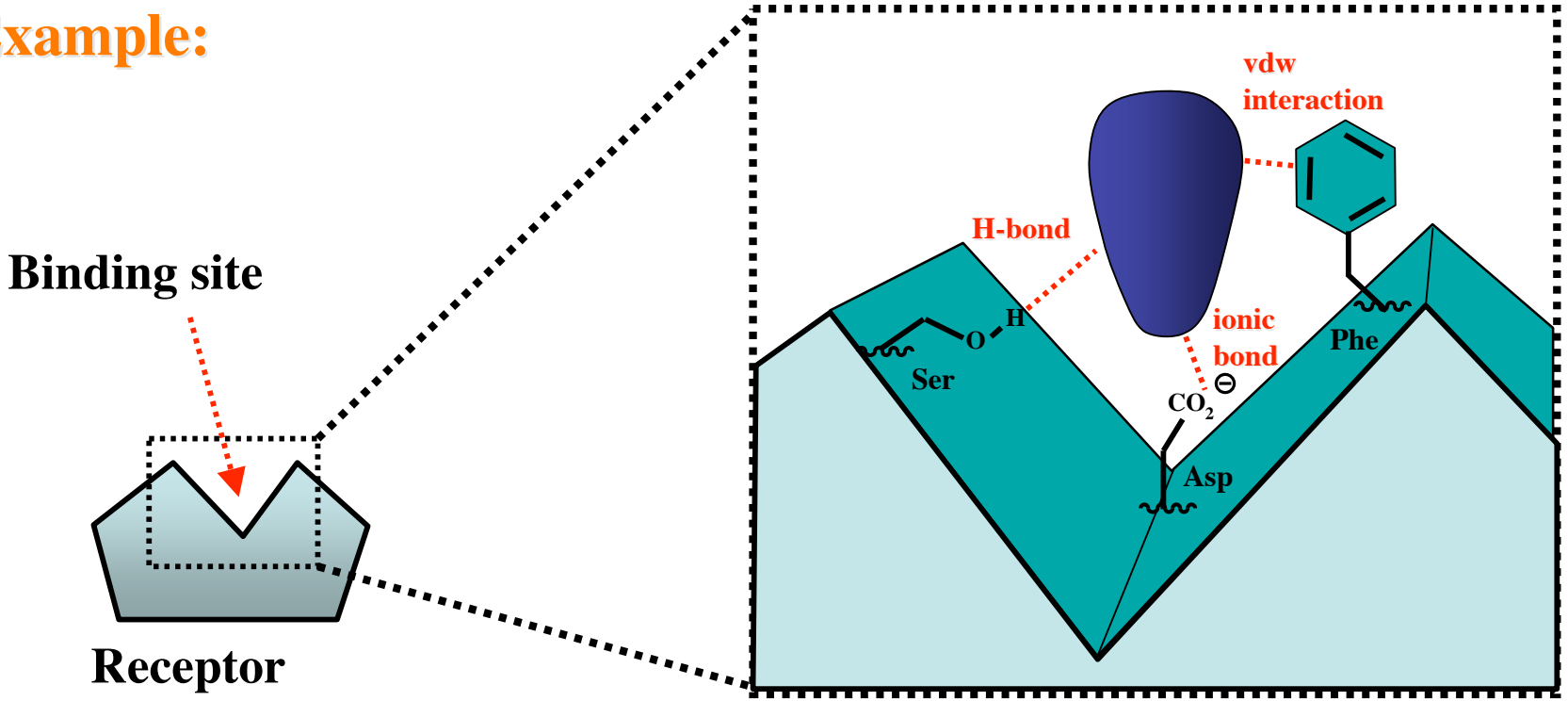
- Binding site is nearly the correct shape for the messenger
- Binding alters the shape of the receptor (induced fit)
- Altered receptor shape leads to further effects - signal transduction

3. Messenger binding

3.2 Bonding forces

- Ionic
- H-bonding
- van der Waals

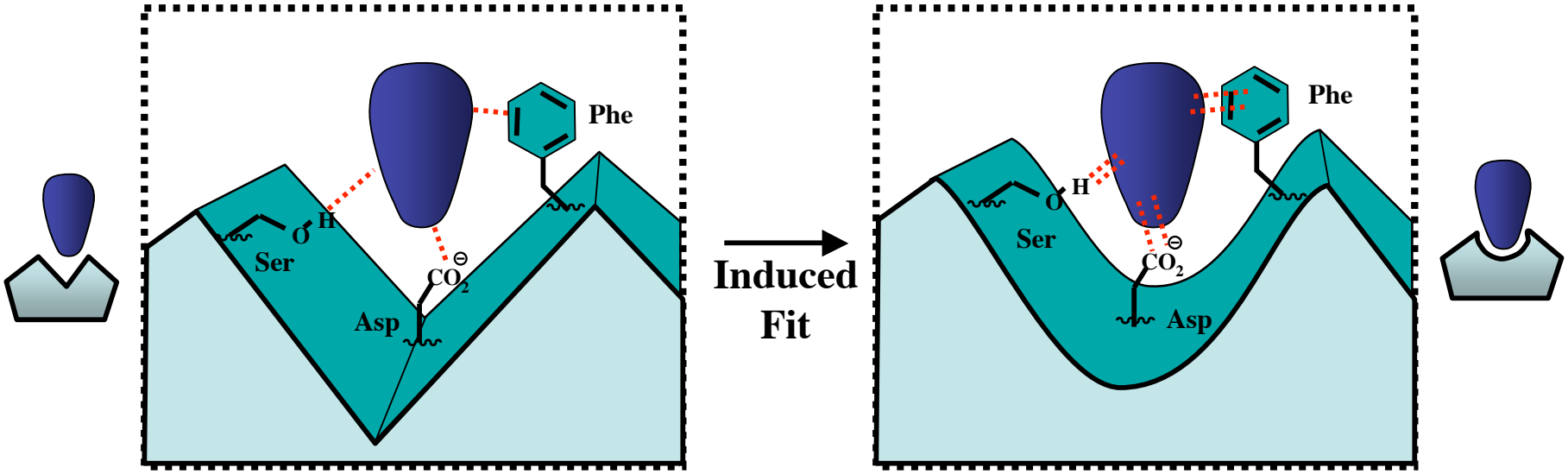
Example:



3. Substrate binding

3.2 Bonding forces

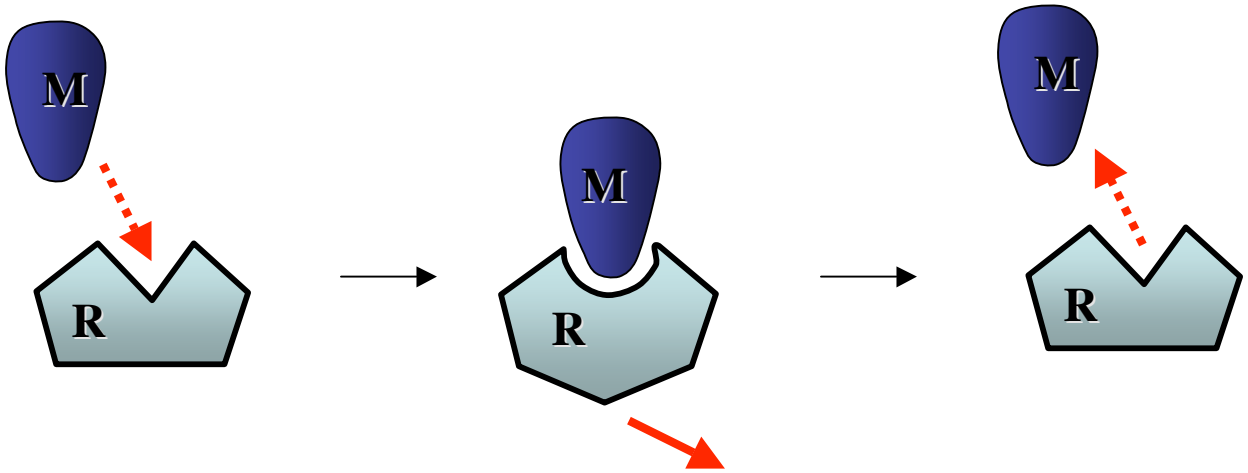
- Induced fit - Binding site alters shape to maximise intermolecular bonding



Intermolecular bonds not optimum length for maximum binding strength

Intermolecular bond lengths optimised

4. Overall process of receptor/messenger interaction



- Binding interactions must be:
  - strong enough to hold the messenger sufficiently long for signal transduction to take place
  - weak enough to allow the messenger to depart
- Implies a fine balance
- Drug design - designing molecules with stronger binding interactions results in drugs that block the binding site - antagonists

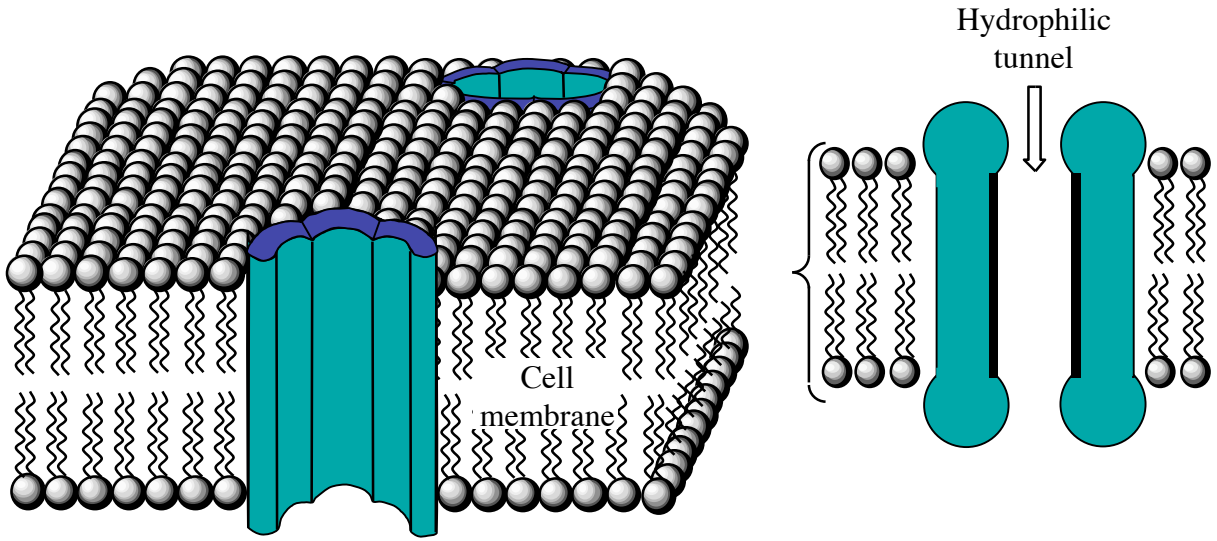
5. Signal transduction

5.1 Control of ion channels

- Receptor protein is part of an ion channel protein complex
- Receptor binds a messenger leading to an induced fit
- Ion channel is opened or closed
- Ion channels are specific for specific ions (Na^+, Ca^{2+}, Cl^-, K^+)
- Ions flow across cell membrane down concentration gradient
- Polarises or depolarises nerve membranes
- Activates or deactivates enzyme catalysed reactions within cell

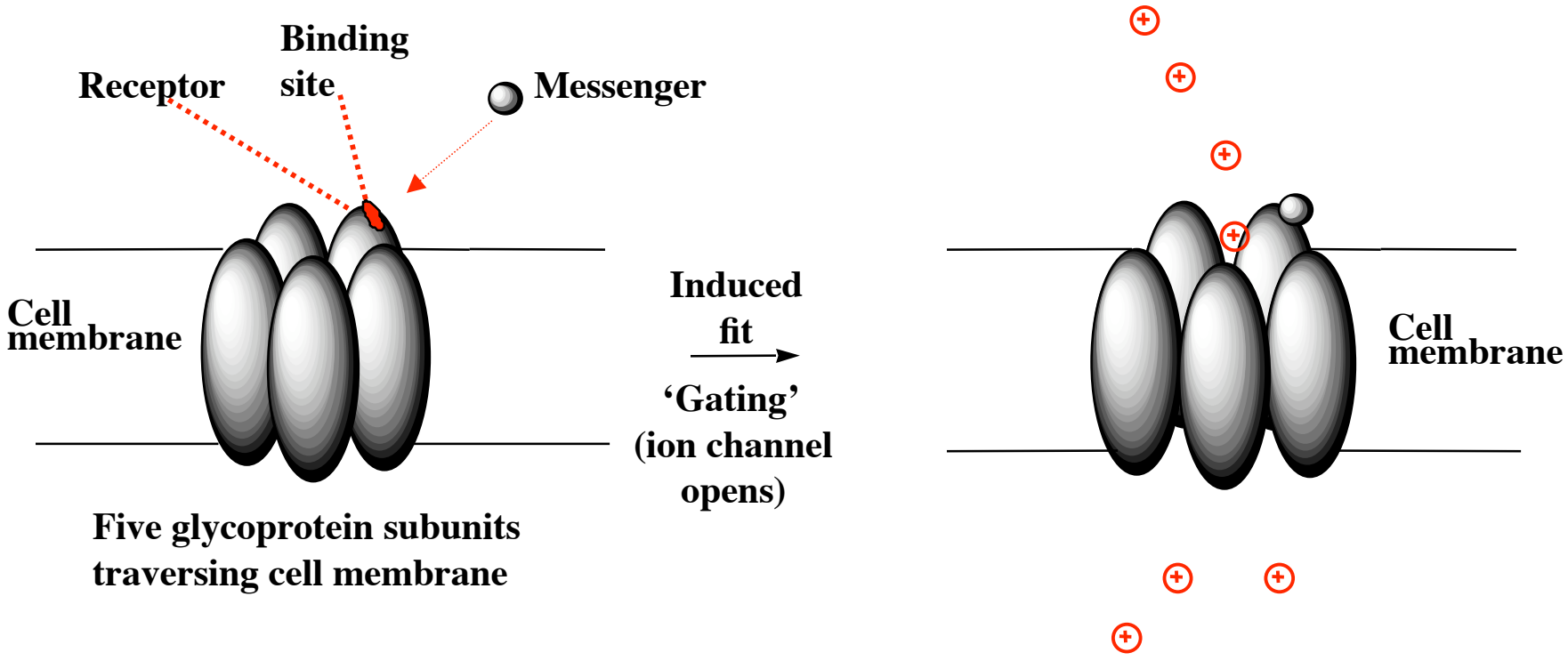
5. Signal transduction

5.1 Control of ion channels



5. Signal transduction

5.1 Control of ion channels

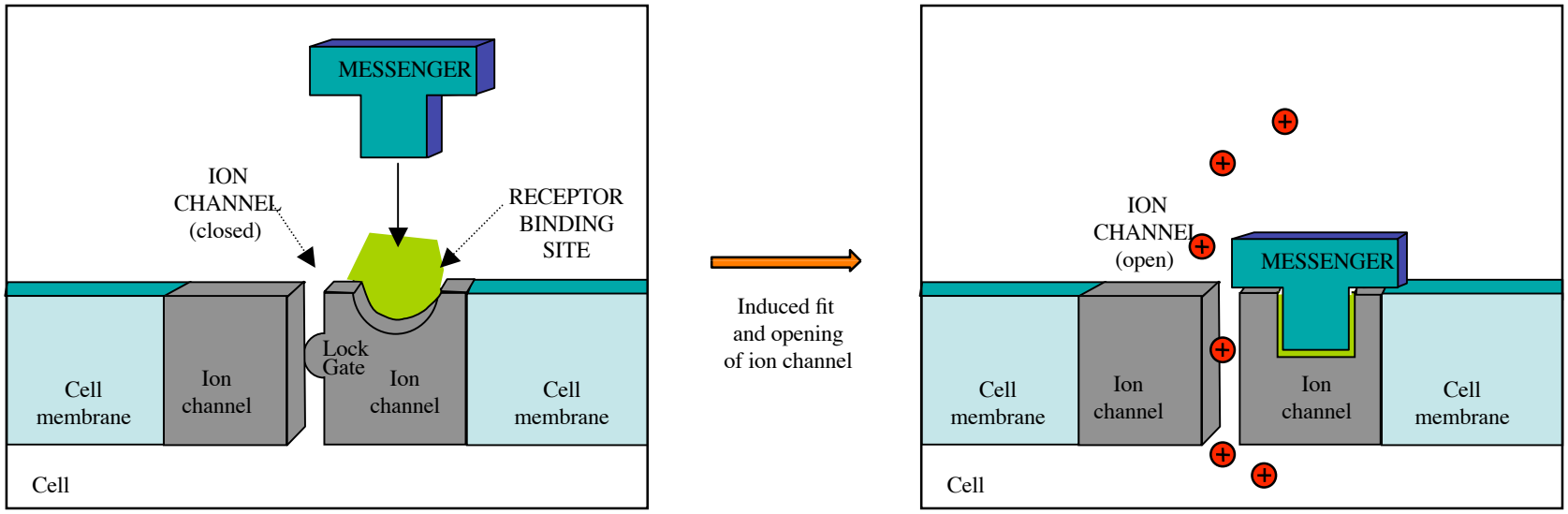


Cationic ion channels for K^+, Na^+, Ca^{2+} (e.g. nicotinic) = excitatory

Anionic ion channels for Cl^- (e.g. $GABA_A$) = inhibitory

5. Signal transduction

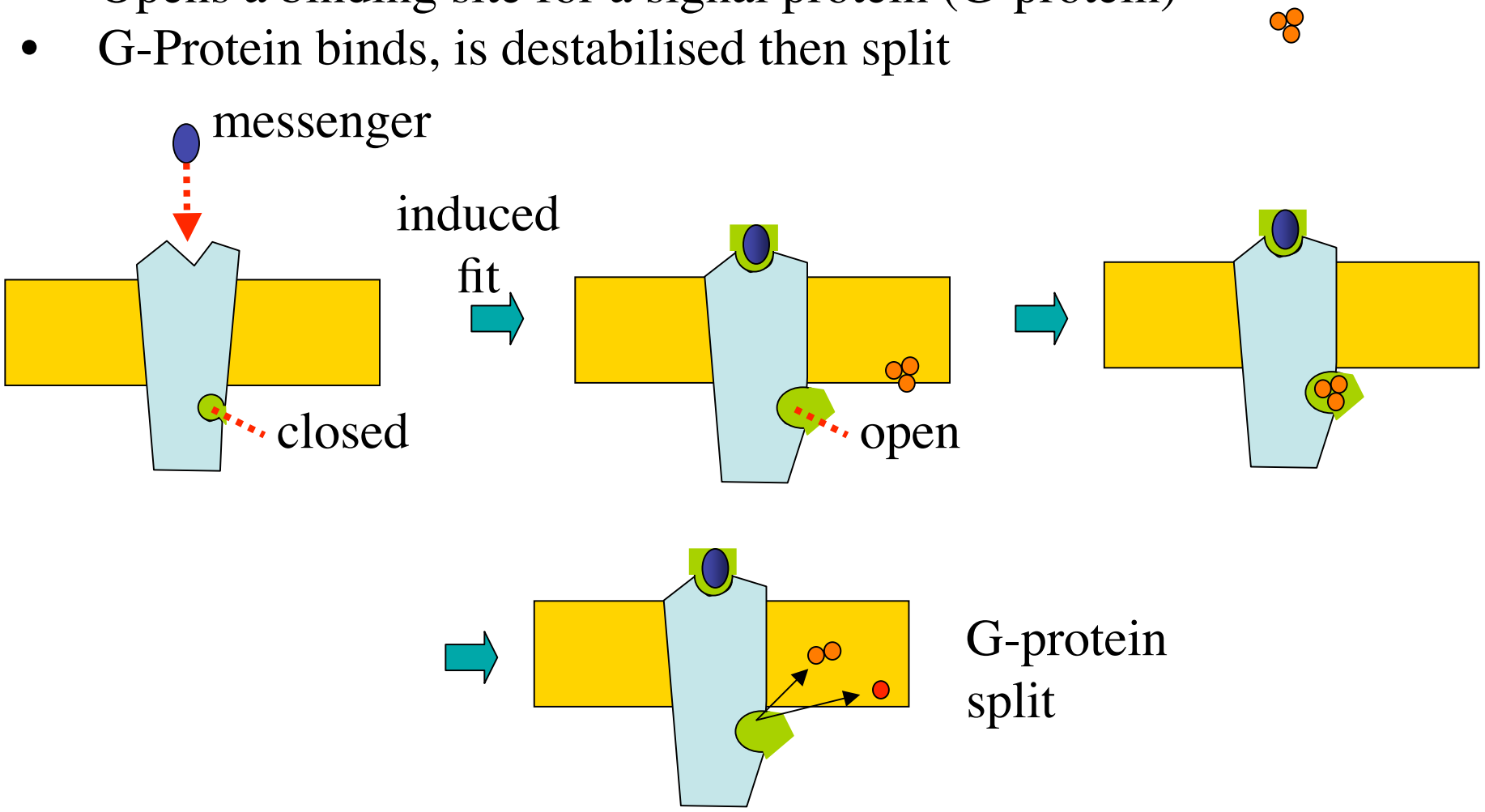
5.1 Control of ion channels:



5. Signal transduction

5.2 Activation of signal proteins

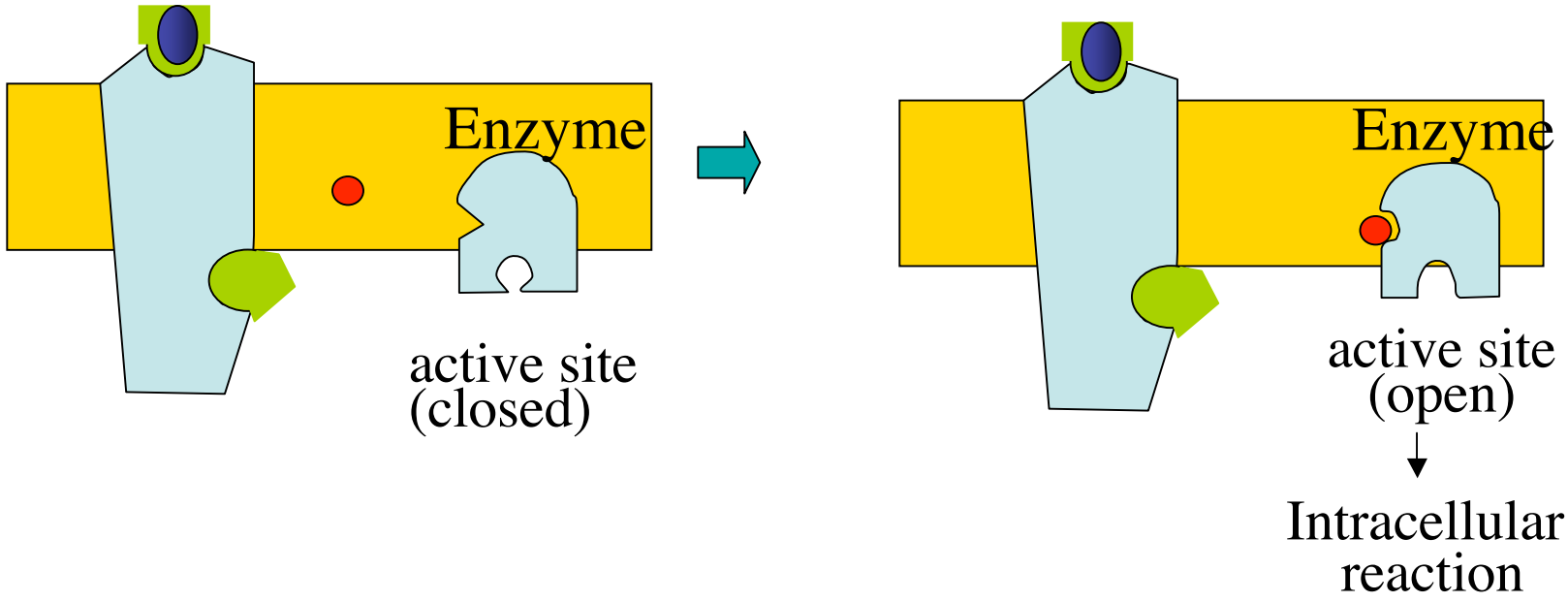
- Receptor binds a messenger leading to an induced fit
- Opens a binding site for a signal protein (G-protein)
- G-Protein binds, is destabilised then split



5. Signal transduction

5.2 Activation of signal proteins

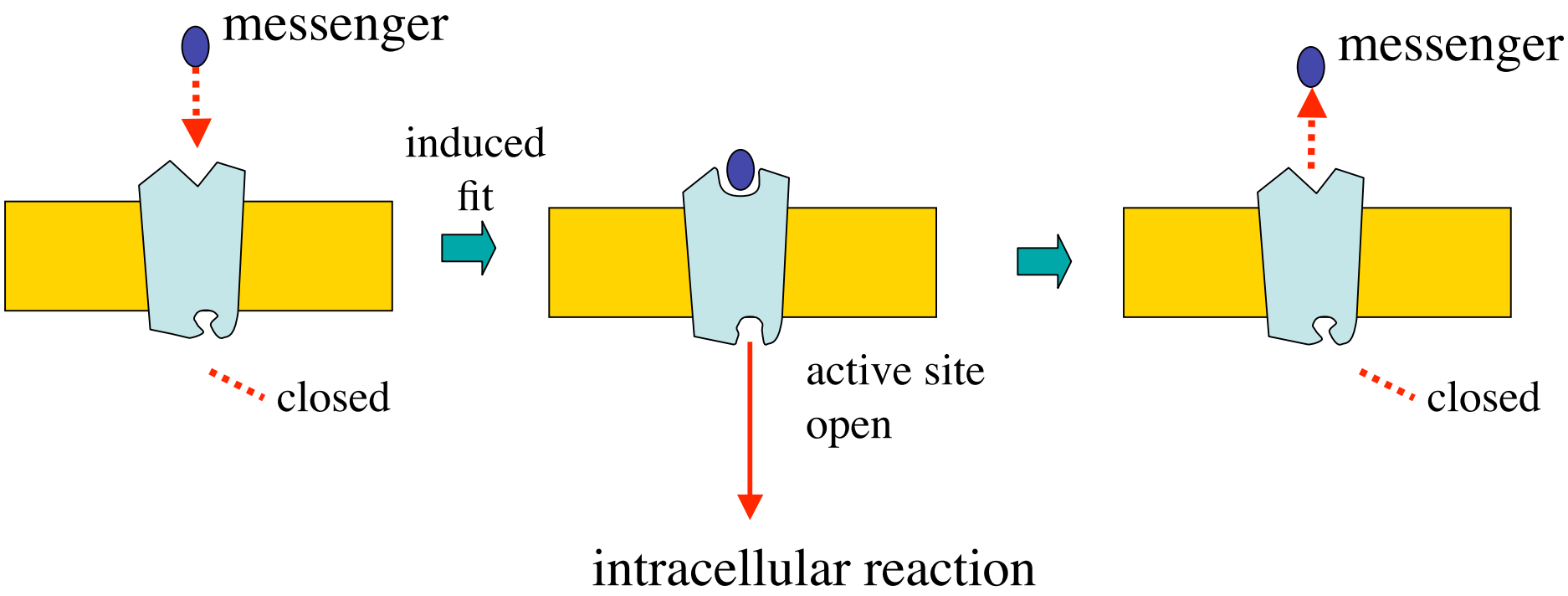
- **G-Protein subunit activates membrane bound enzyme**
  - **Binds to allosteric binding site**
  - **Induced fit results in opening of active site**
- **Intracellular reaction catalysed**



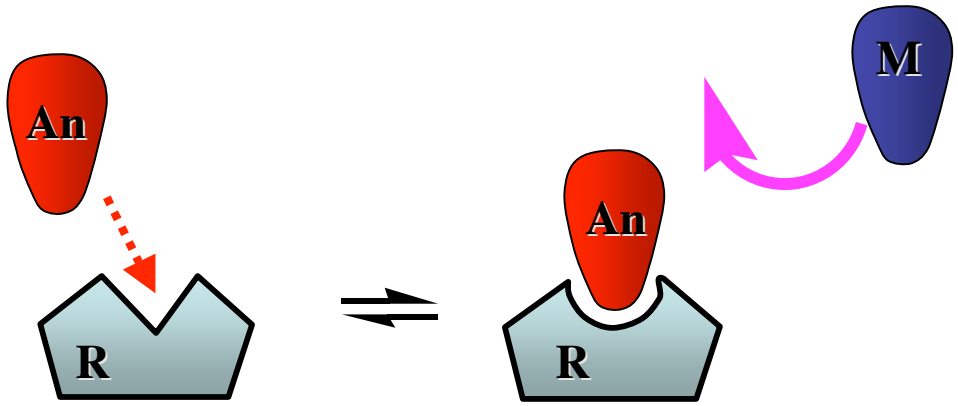
5. Signal transduction

5.3 Activation of enzyme active site

- **Protein serves dual role - receptor plus enzyme**
- **Receptor binds messenger leading to an induced fit**
- **Protein changes shape and opens active site**
- **Reaction catalysed within cell**

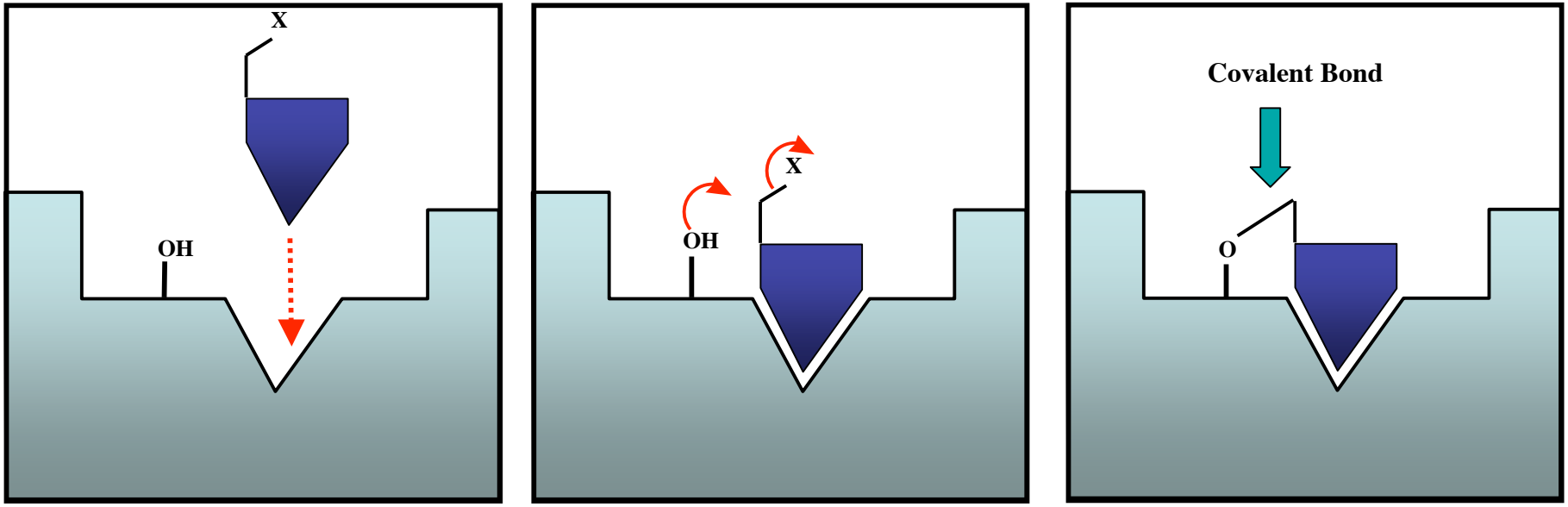


6. Competitive (reversible) antagonists



- 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
- Increasing the messenger concentration reverses antagonism

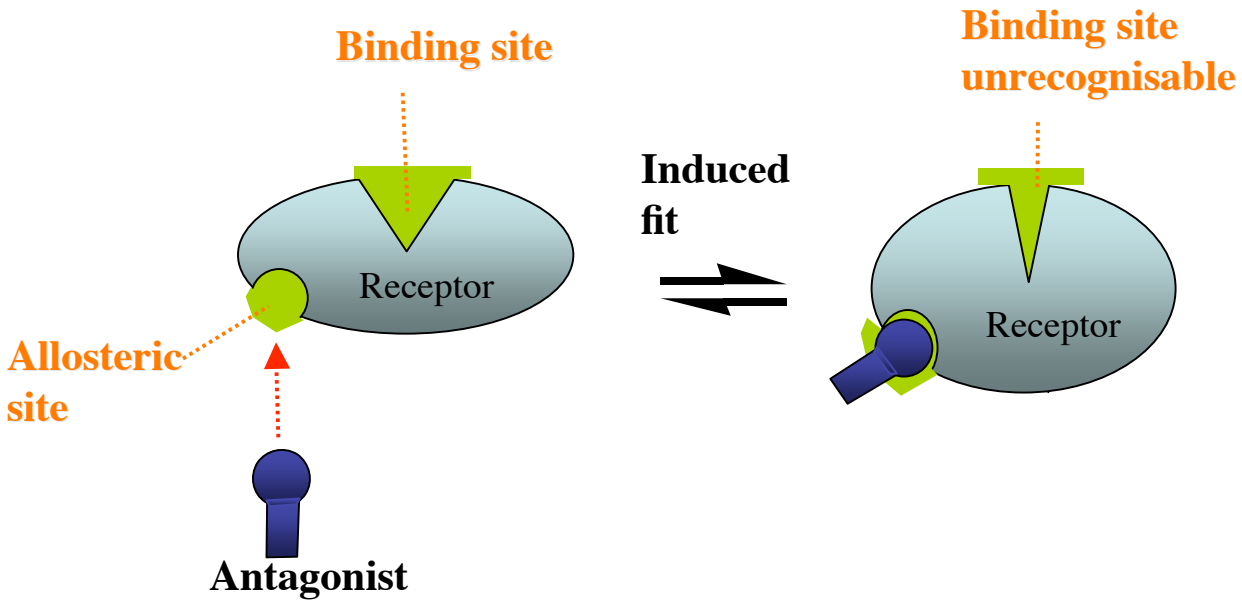
7. Non competitive (irreversible) antagonists



Irreversible antagonism

- Antagonist binds irreversibly to the binding site
- Different induced fit means that the receptor is not activated
- Covalent bond is formed between the drug and the receptor
- Messenger is blocked from the binding site
- Increasing messenger concentration does not reverse antagonism

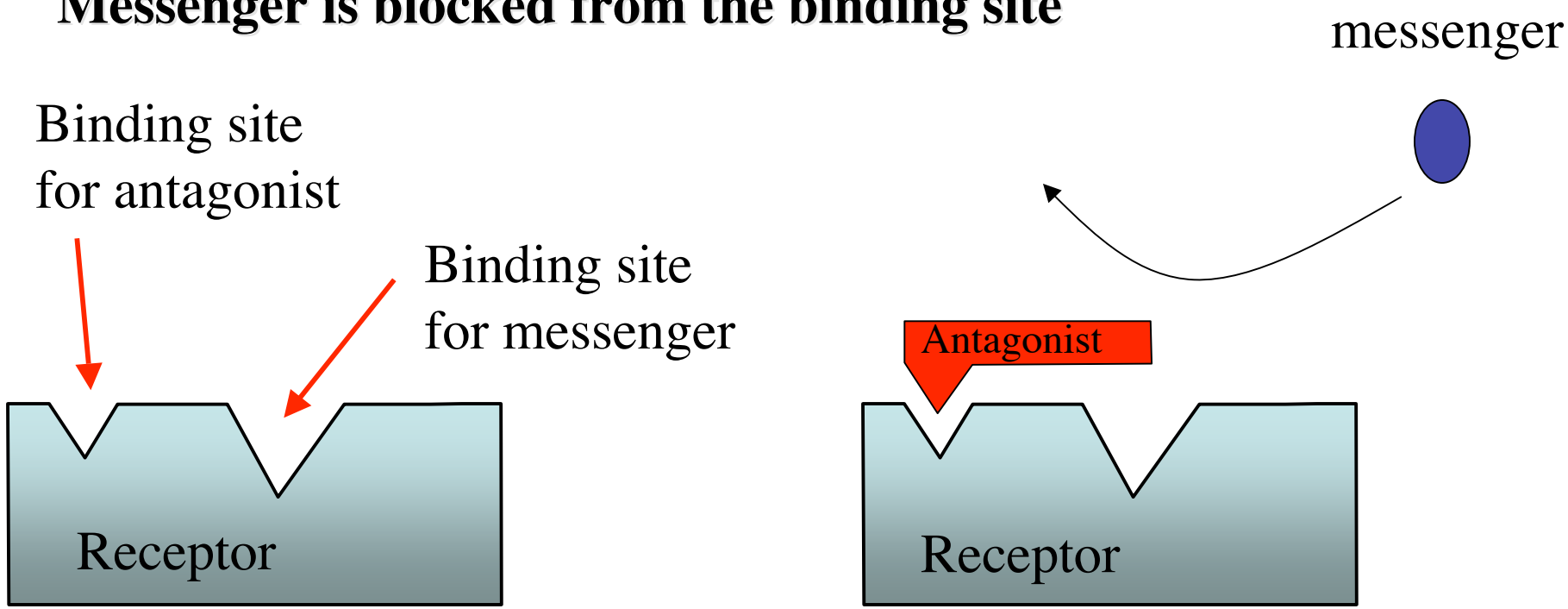
8. Non competitive (reversible) allosteric antagonists



- Antagonist binds reversibly to an allosteric site
- Intermolecular bonds formed between antagonist and binding site
- Induced fit alters the shape of the receptor
- Binding site is distorted and is not recognised by the messenger
- Increasing messenger concentration does not reverse antagonism

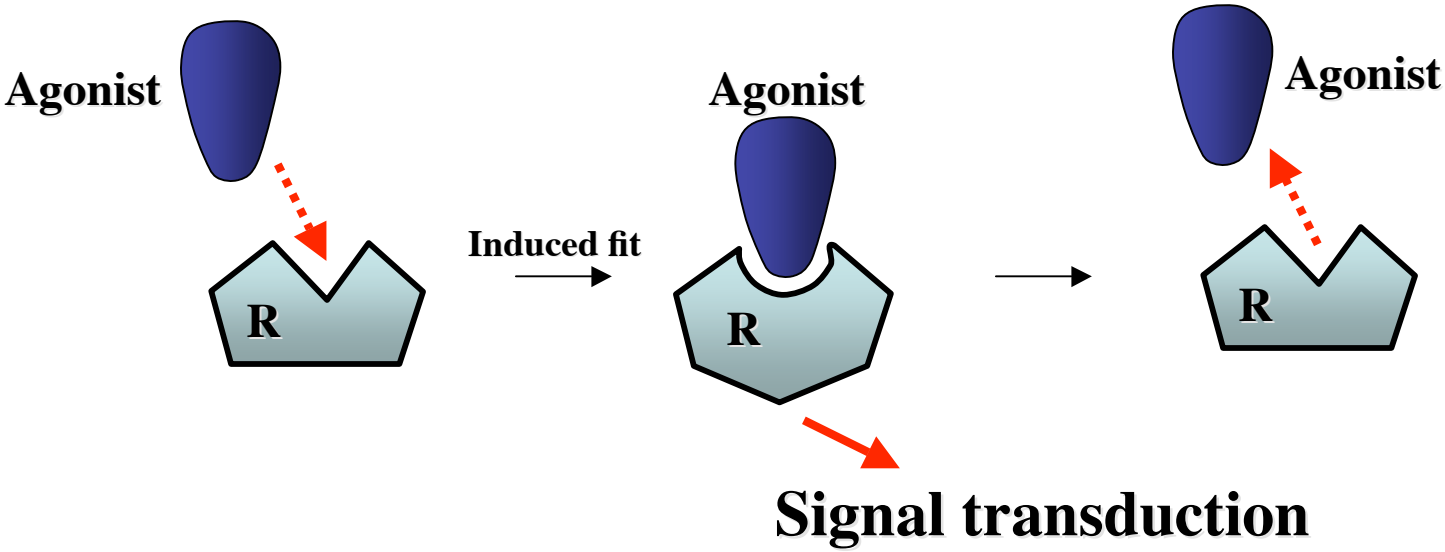
9. Antagonists by umbrella effect

- **Antagonist binds reversibly to a neighbouring binding site**
- **Intermolecular bonds formed between antagonist and binding site**
- **Antagonist overlaps with the messenger binding site**
- **Messenger is blocked from the binding site**



10. Agonists

- 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



Contents

Part 1: Sections 6.1 - 6.2

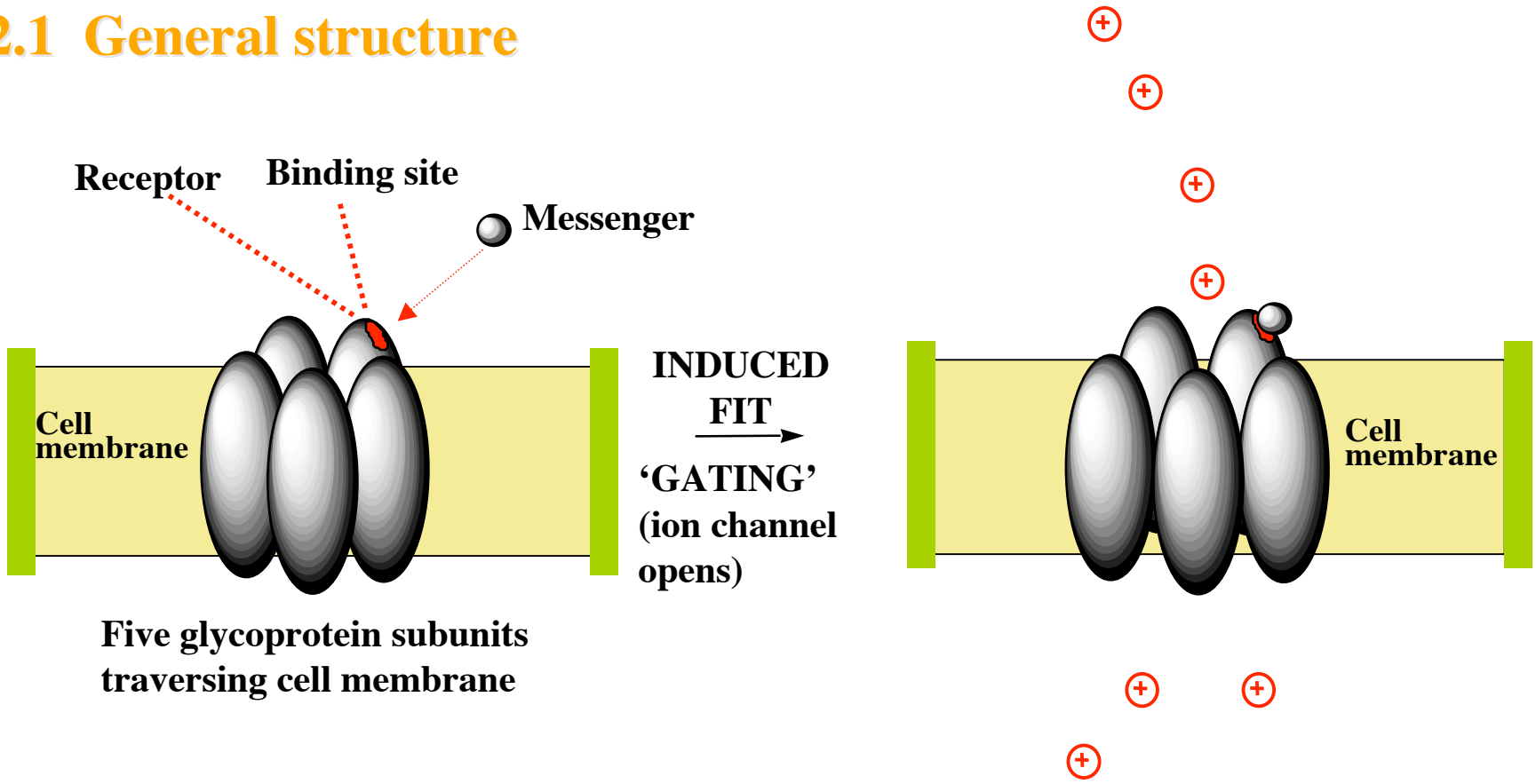
1. Receptor superfamilies
2. Ion channel receptors (Ligand gated ion channels)
   - 2.1. General structure
   - 2.2. Structure of protein subunits (4-TM receptor subunits)
   - 2.3. Detailed structure of ion channel
   - 2.4. Gating

1. Receptor superfamilies

		RESPONSE TIME
• ION CHANNEL RECEPTORS	MEMBRANE BOUND	msecs
• G-PROTEIN COUPLED RECEPTORS		seconds
• KINASE LINKED RECEPTORS		minutes
• INTRACELLULAR RECEPTORS		

2. Ion channel receptors (Ligand gated ion channels

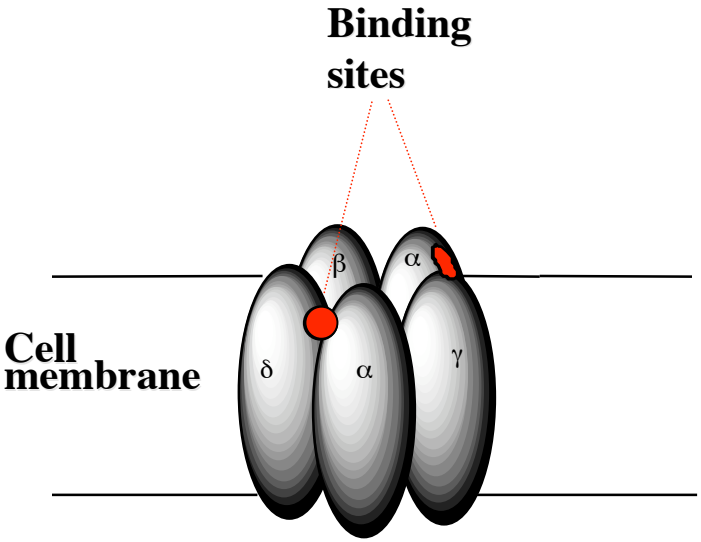
2.1 General structure



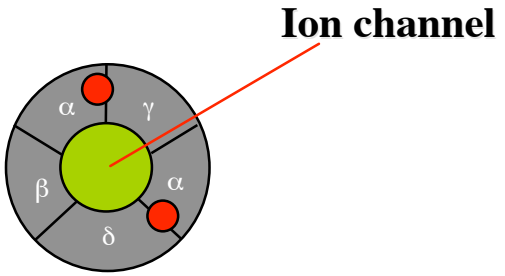
Cationic ion channels for K^+, Na^+, Ca^{2+} (e.g. nicotinic) = excitatory
Anionic ion channels for Cl^- (e.g. $GABA_A$) = inhibitory

2. Ion channel receptors (Ligand gated ion channels

Transverse view (nicotinic receptor)



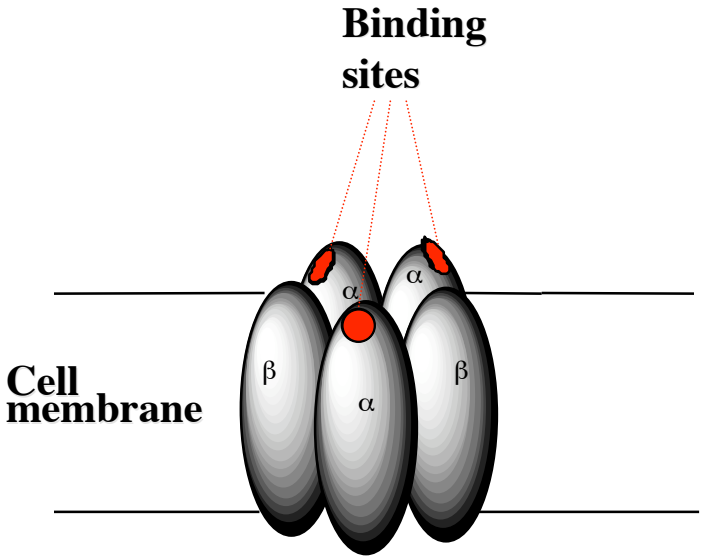
2xα, β, γ, δ subunits



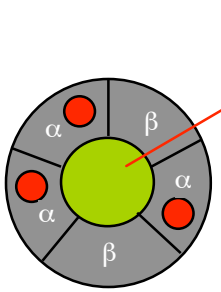
Two ligand binding sites mainly on α-subunits

2. Ion channel receptors (Ligand gated ion channels

Transverse view (glycine receptor)



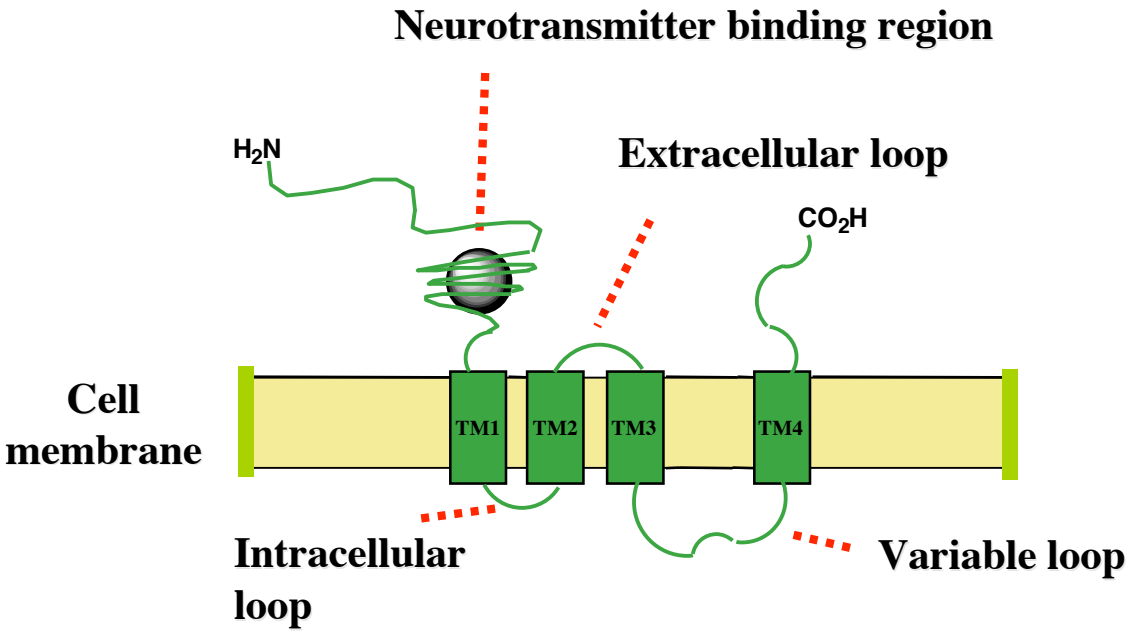
3xα, 2x β subunits



Three ligand binding sites on α-subunits

2. Ion channel receptors (Ligand gated ion channels

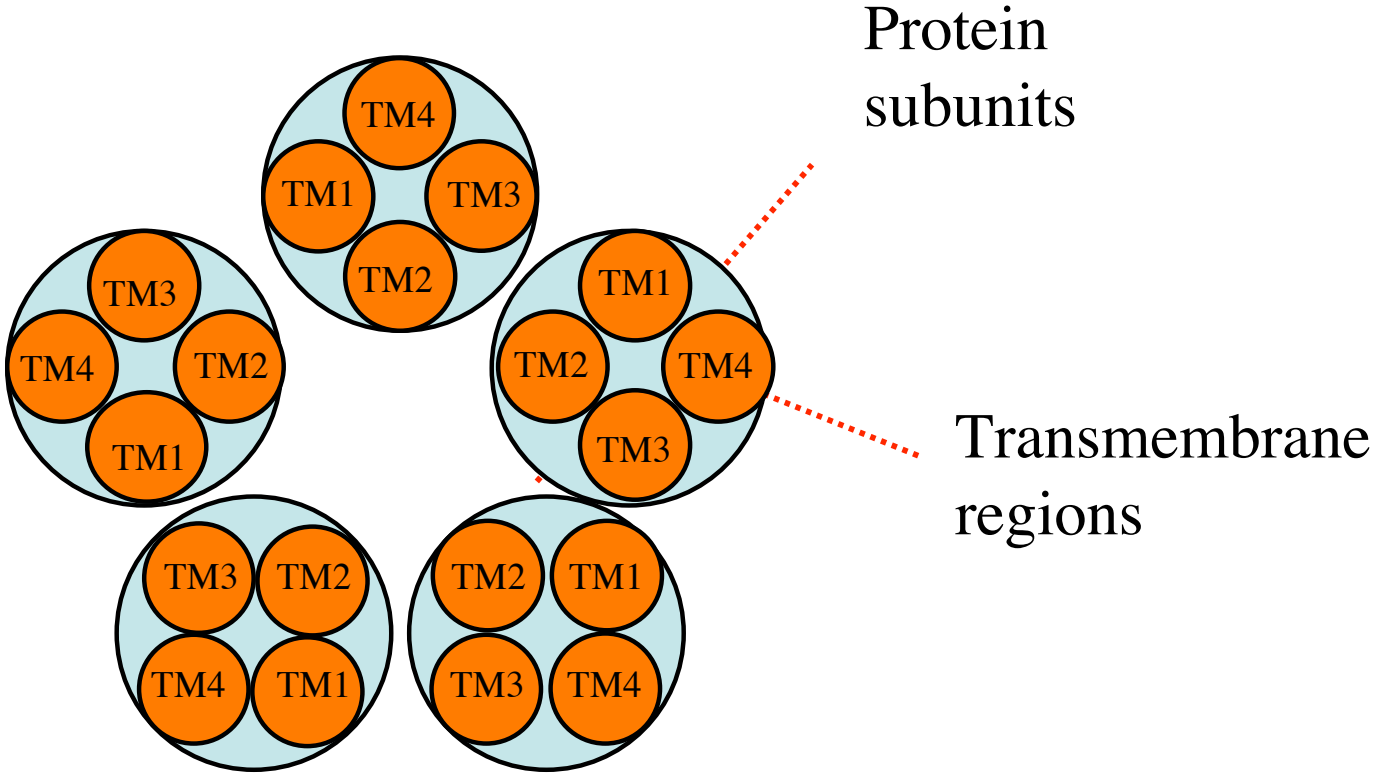
2.2 Structure of protein subunits (4-TM receptor subunits)



4 Transmembrane (TM) regions (hydrophobic)

2. Ion channel receptors (Ligand gated ion channels

2.3 Detailed structure of ion channel

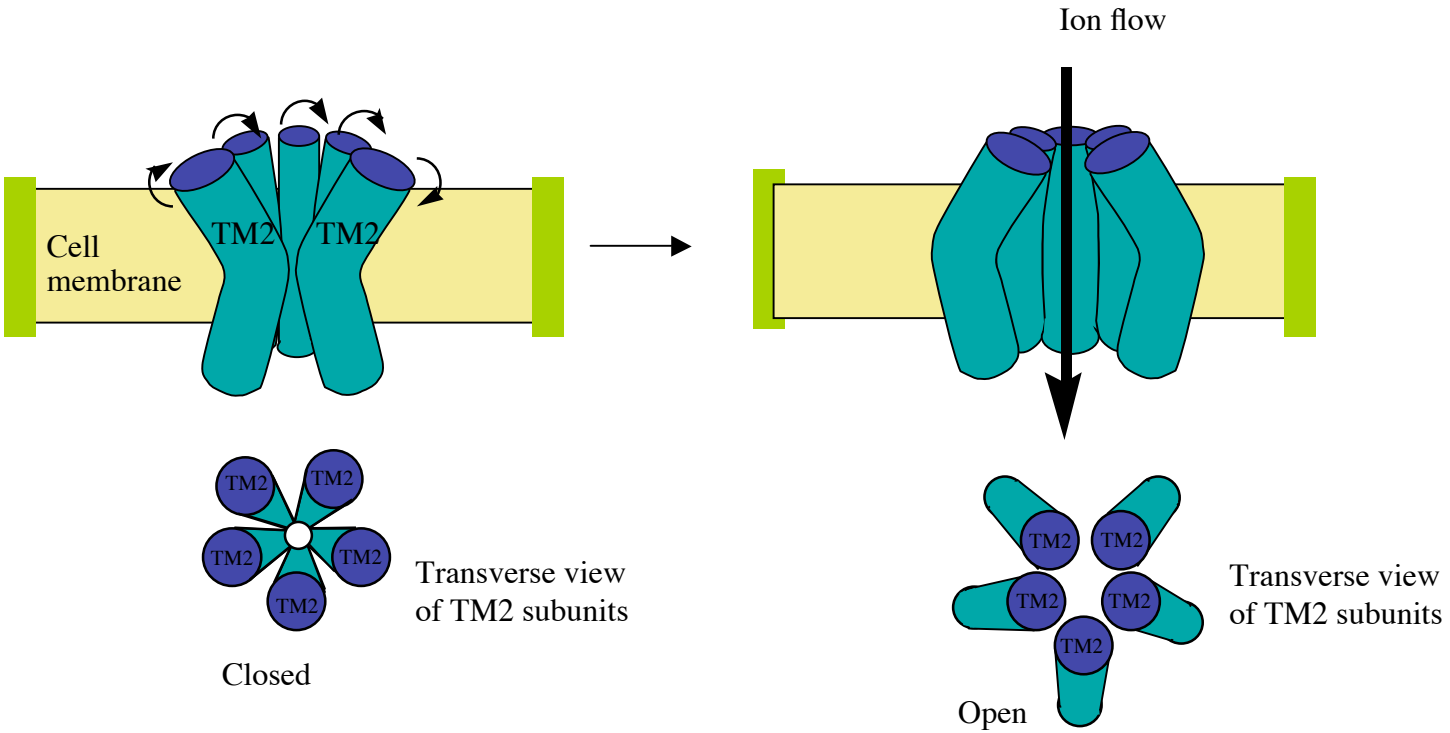


Note: TM2 of each protein subunit 'lines' the central pore

2. Ion channel receptors (Ligand gated ion channels

2.4 Gating

Neurotransmitter binds → **Induced fit at binding site** → **'Domino effect'** → **Rotation of 2TM regions of each protein subunit**



2. Ion channel receptors (Ligand gated ion channels

2.4 Gating

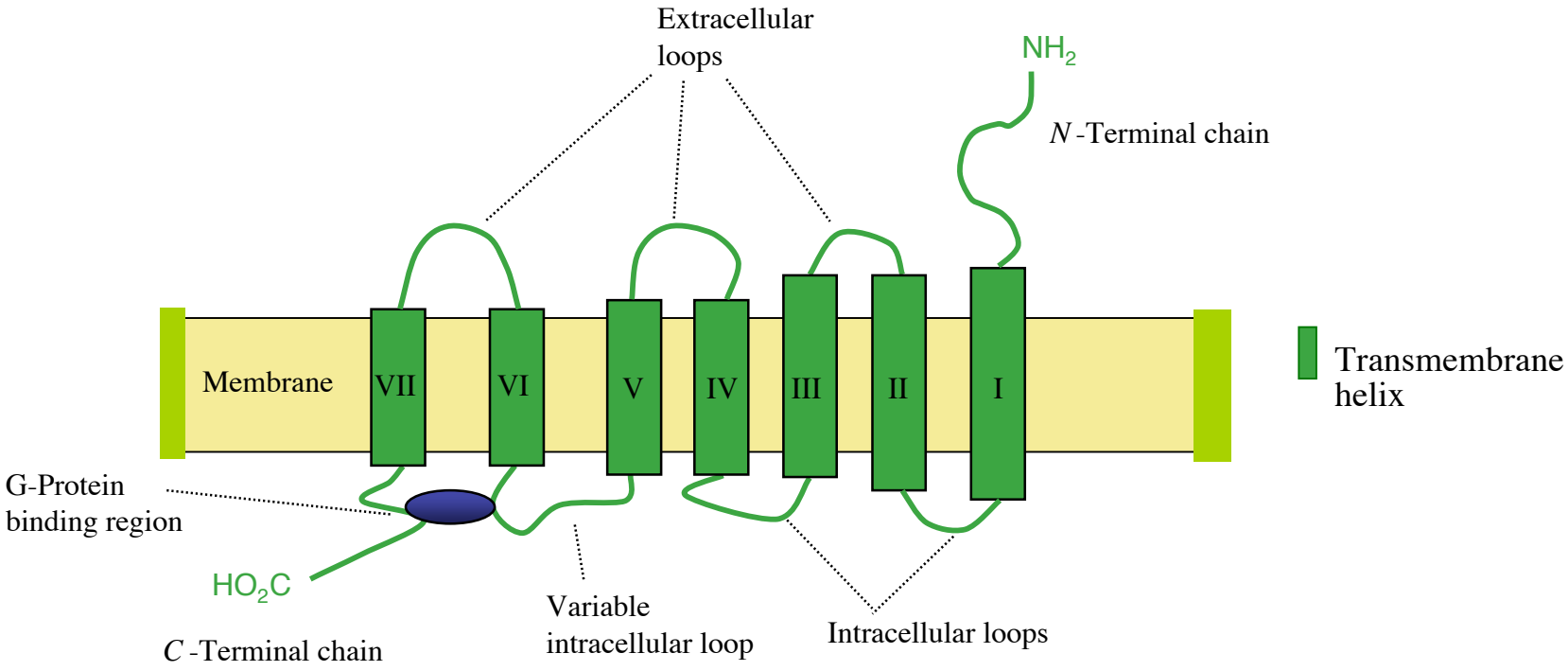
- **Fast response measured in msec**
- **Ideal for transmission between nerves**
- **Binding of messenger leads directly to ion flows across cell membrane**
- **Ion flow = secondary effect (signal transduction)**
- **Ion concentration within cell alters**
- **Leads to variation in cell chemistry**

Contents

Part 2: Sections 6.3 - 6.6

3. G-protein-coupled receptors (7-TM receptors)

3.1 Structure - Single protein with 7 transmembrane regions



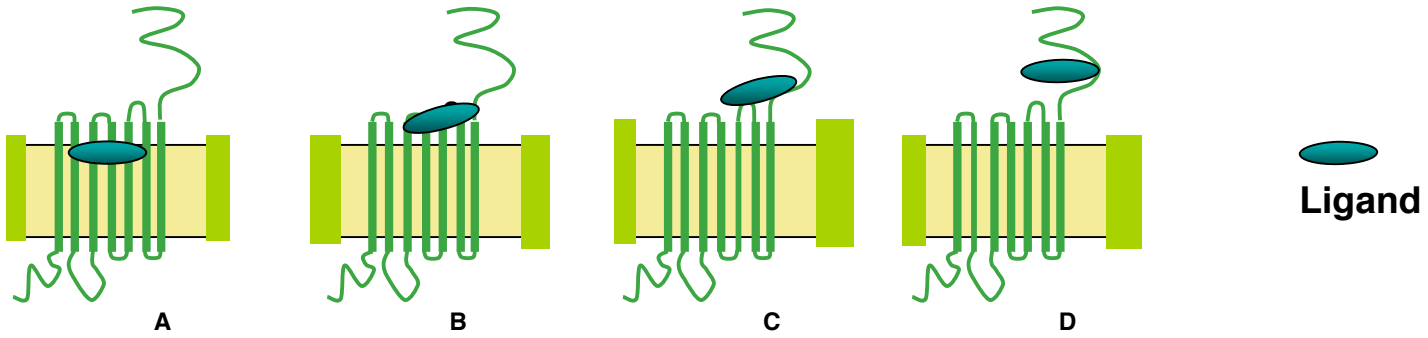
3. G-protein-coupled receptors (7-TM receptors)

3.2 Ligands

- **Monoamines** **e.g. dopamine, histamine, noradrenaline, acetylcholine (muscarinic)**
- **Nucleotides**
- **Lipids**
- **Hormones**
- **Glutamate**
- **Ca^{++}**

3. G-protein-coupled receptors (7-TM receptors)

3.3 Ligand binding site - varies depending on receptor type



A) Monoamines - pocket in TM helices

B) Peptide hormones - top of TM helices + extracellular loops + *N*-terminal chain

C) Hormones - extracellular loops + *N*-terminal chain

D) Glutamate - *N*-terminal chain

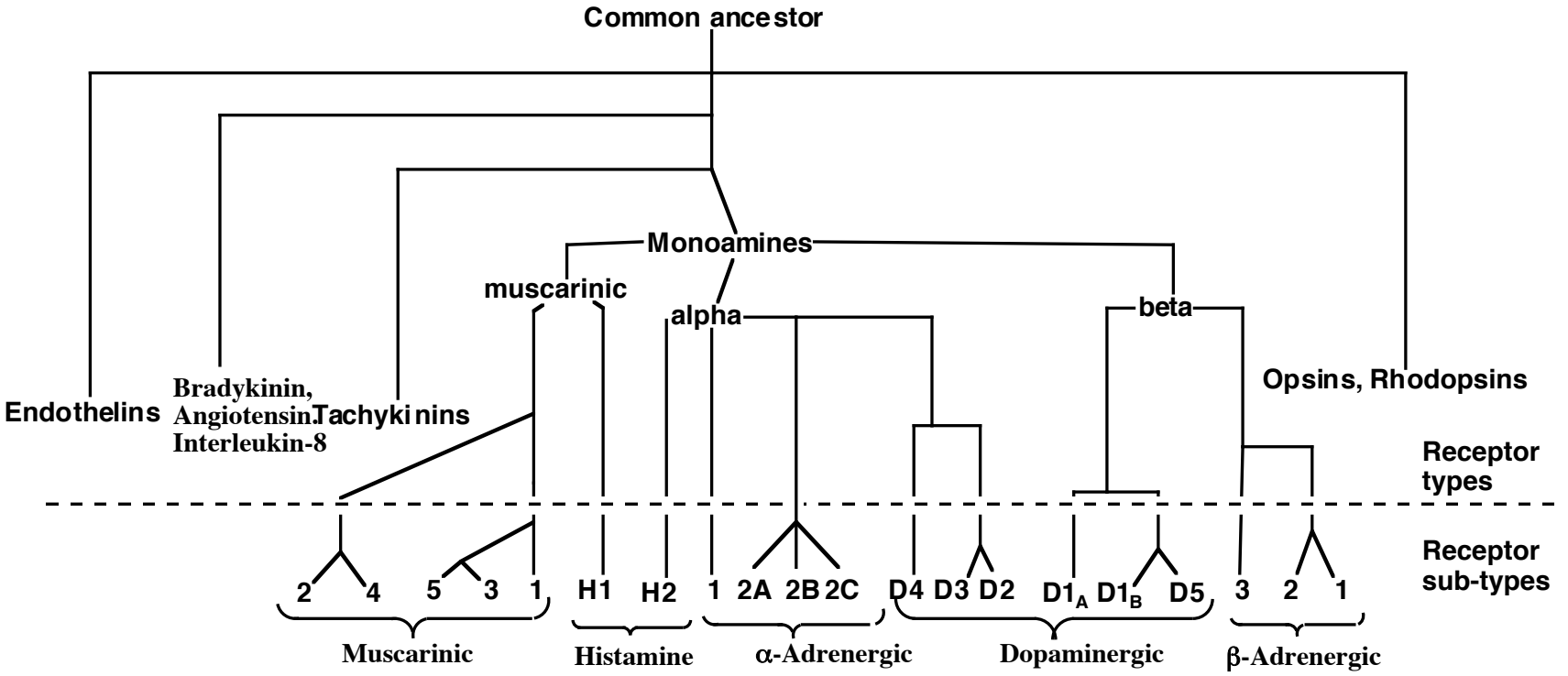
3. G-protein-coupled receptors (7-TM receptors)

3.4 Bacteriorhodopsin & rhodopsin family

- **Rhodopsin = visual receptor**
- **Many common receptors belong to this same family**
- **Implications for drug selectivity depending on similarity (evolution)**
- **Membrane bound receptors difficult to crystallise**
- **X-Ray structure of bacteriorhodopsin solved - bacterial protein similar to rhodopsin**
- **Bacteriorhodopsin structure used as 'template' for other receptors**
- **Construct model receptors based on template and amino acid sequence**
- **Leads to model binding sites for drug design**
- **Crystal structure for rhodopsin now solved - better template**

3. G-protein-coupled receptors (7-TM receptors)

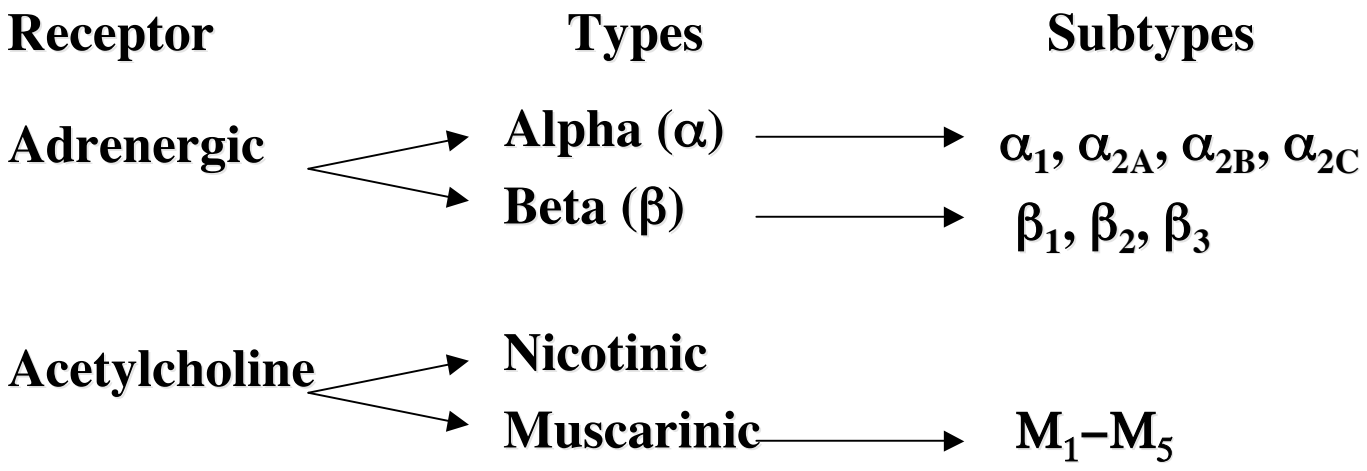
3.4 Bacteriorhodopsin & rhodopsin family



3. G-protein-coupled receptors (7-TM receptors)

3.5 Receptor types and subtypes

Reflects differences in receptors which recognise the same ligand



3. G-protein-coupled receptors (7-TM receptors)

3.5 Receptor types and subtypes

- **Receptor types and subtypes not equally distributed amongst tissues.**
- **Target selectivity leads to tissue selectivity**

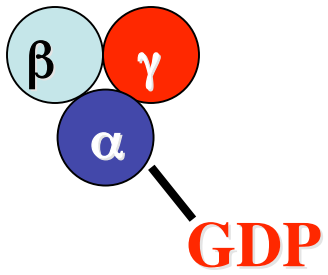
Heart muscle	**- β_1 adrenergic receptors**
Fat cells	**- β_3 adrenergic receptors**
Bronchial muscle	**- α_1& β_2 adrenergic receptors**
GI-tract	**- α_1 α_2 & β_2 adrenergic receptors**

3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

a) Interaction of receptor with G_s-protein

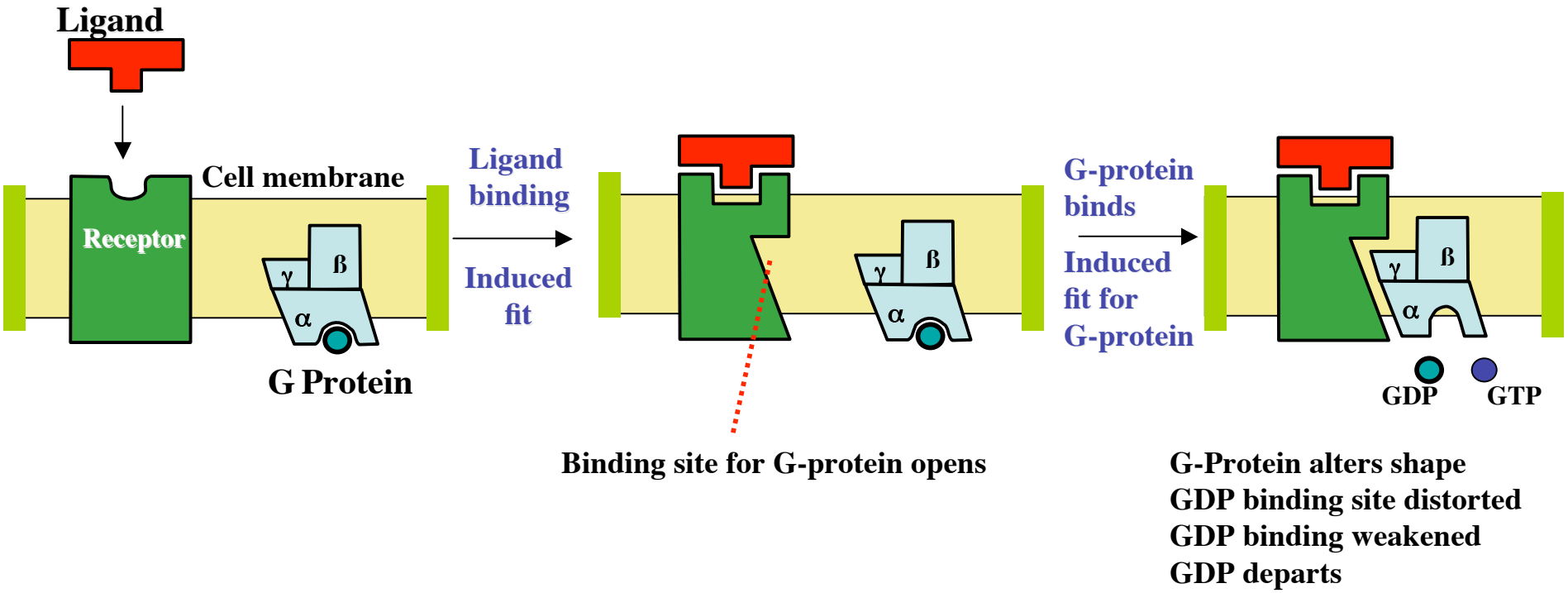
G_S-Protein - membrane bound protein of 3 subunits (α, β, γ)
- α_S subunit has binding site for GDP
-GDP bound non covalently



3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

a) Interaction of receptor with G_s-protein

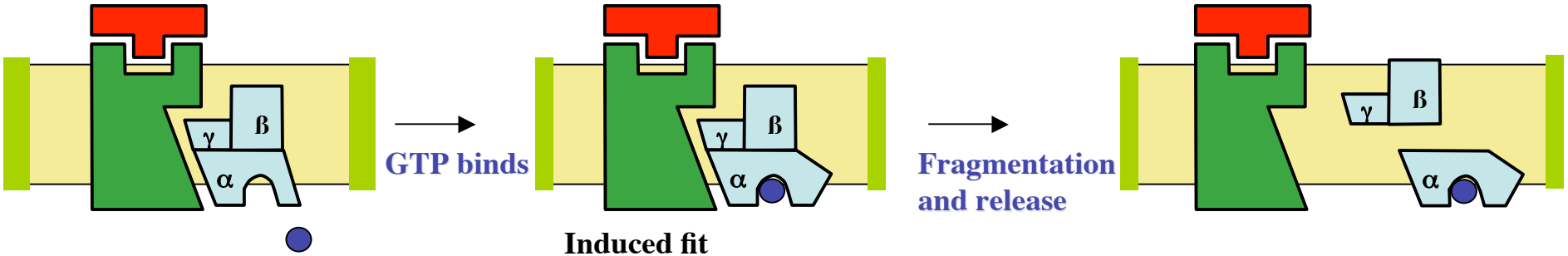


● = GDP

3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

a) Interaction of receptor with G_s-protein

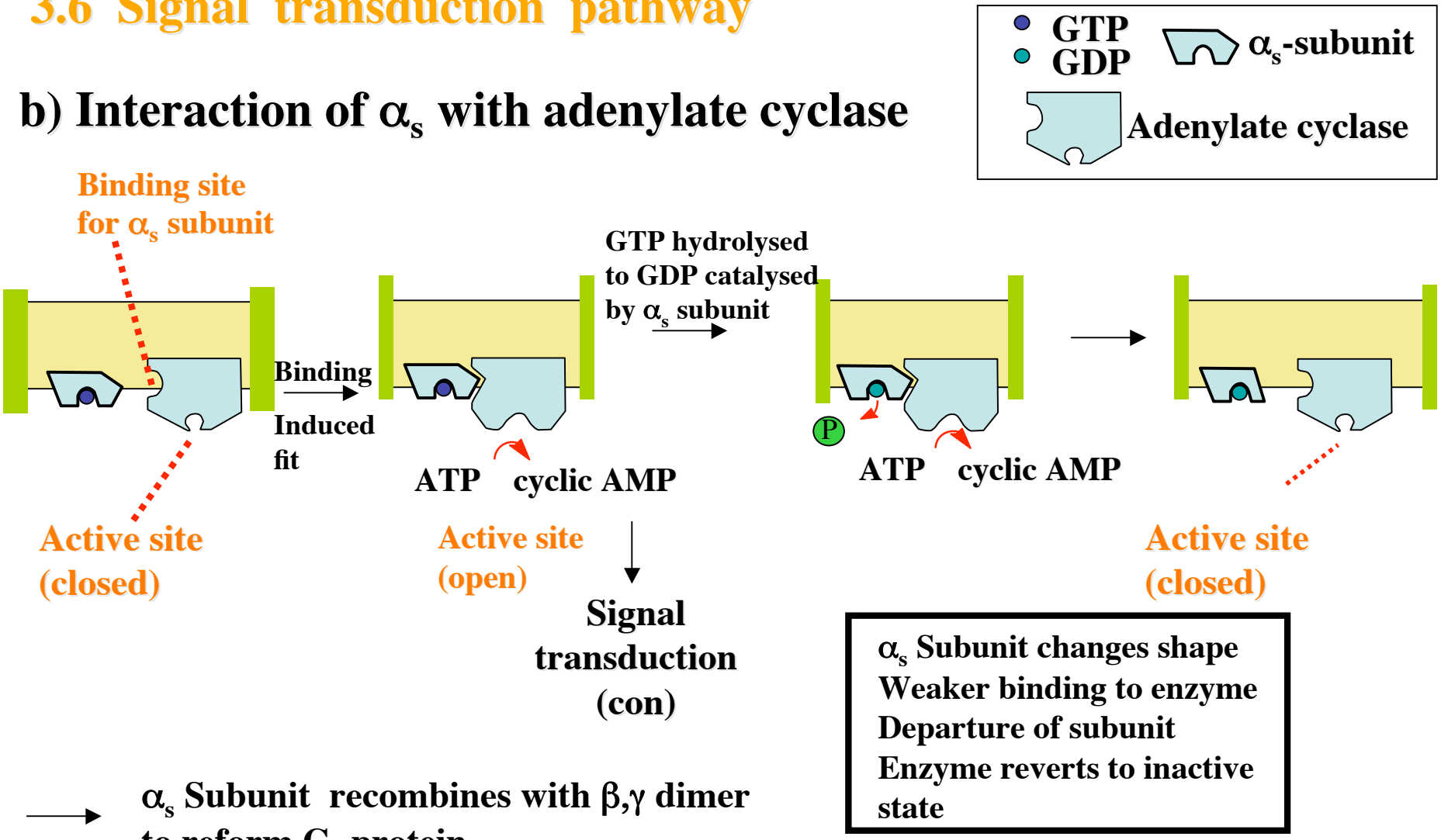


- Process repeated for as long as ligand bound to receptor
- Signal amplification - several G-proteins activated by one ligand
- α_s Subunit carries message to next stage

3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

b) Interaction of α_s with adenylate cyclase

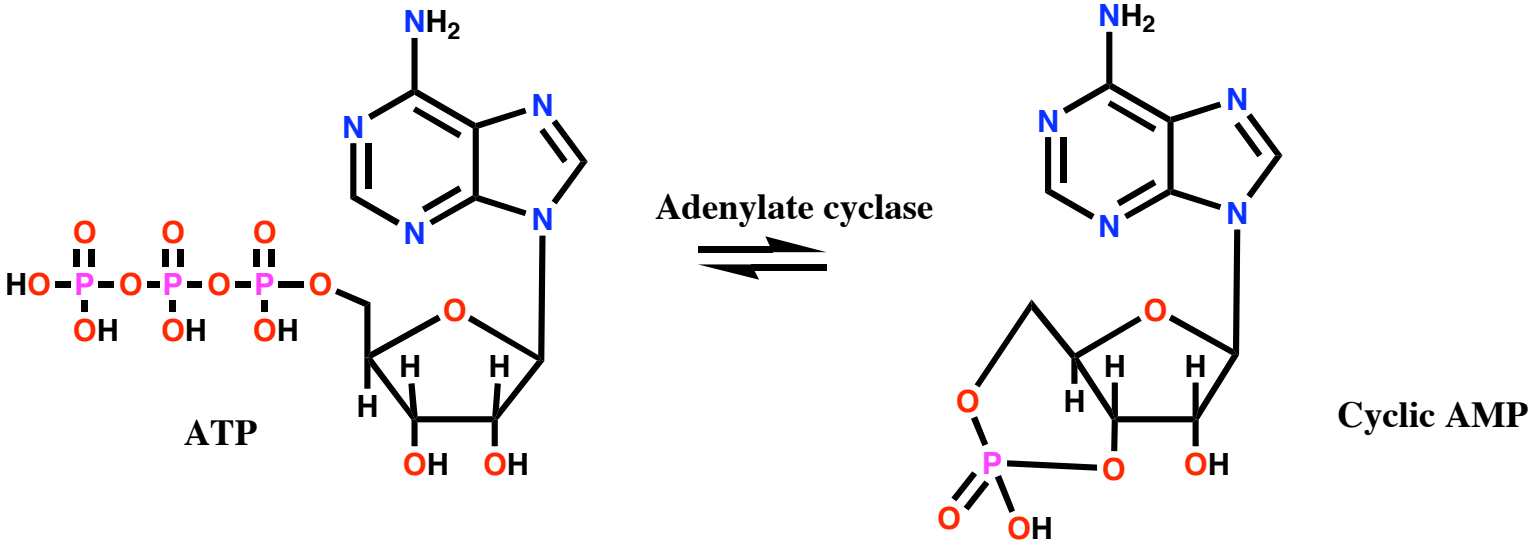


3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

b) Interaction of α_s with adenylate cyclase

- Several-100 ATP molecules converted before α_s-GTP deactivated
- Represents another signal amplification
- Cyclic AMP becomes next messenger (secondary messenger)
- Cyclic AMP enters cell cytoplasm with message

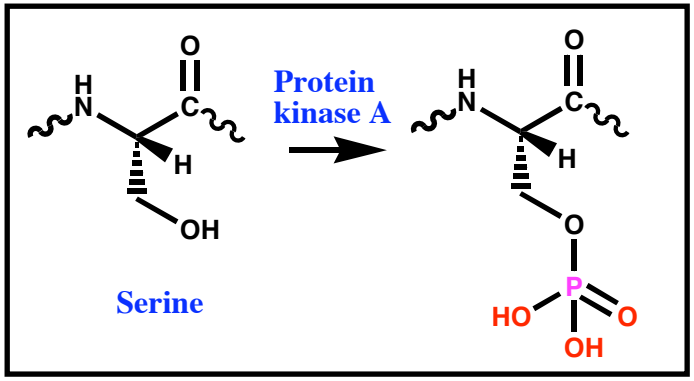


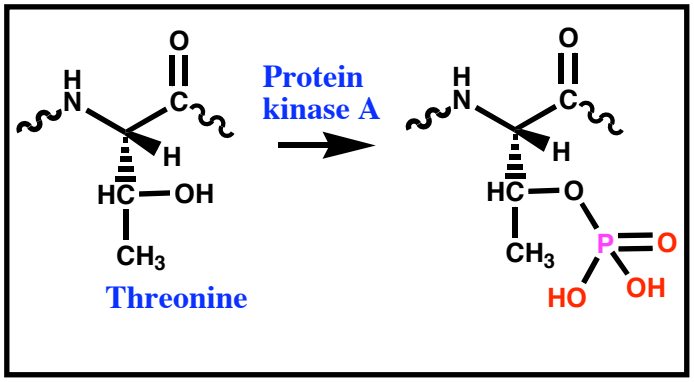
3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

c) Interaction of cyclic AMP with protein kinase A (PKA)

- Protein kinase A = serine-threonine kinase
- Activated by cyclic AMP
- Catalyses phosphorylation of serine and threonine residues on protein substrates
- Phosphate unit provided by ATP

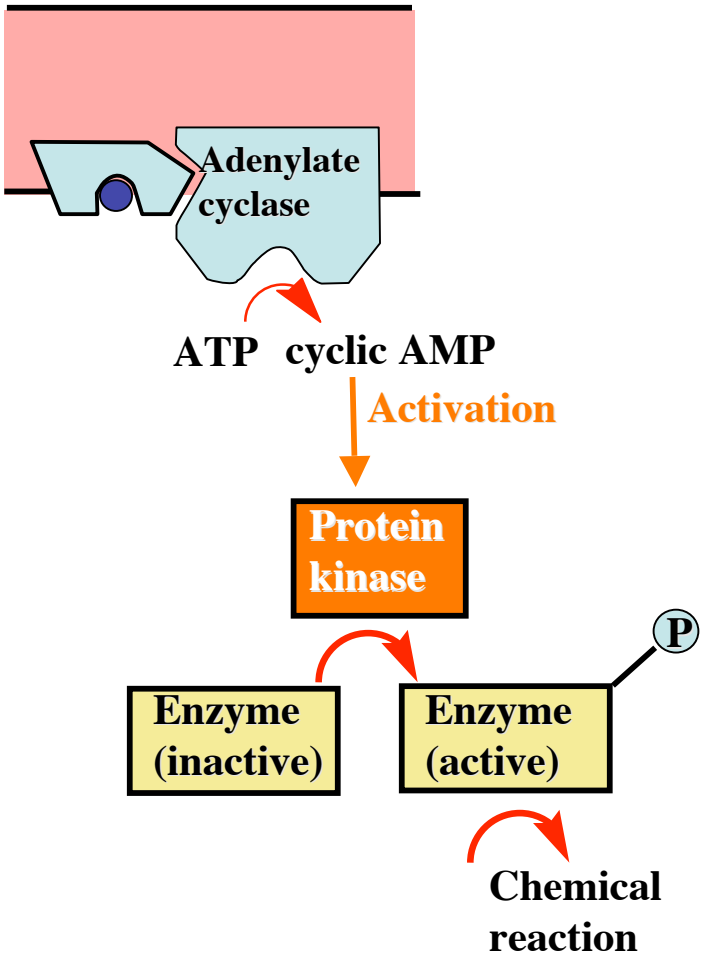




3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

c) Interaction of cyclic AMP with protein kinase A (PKA)

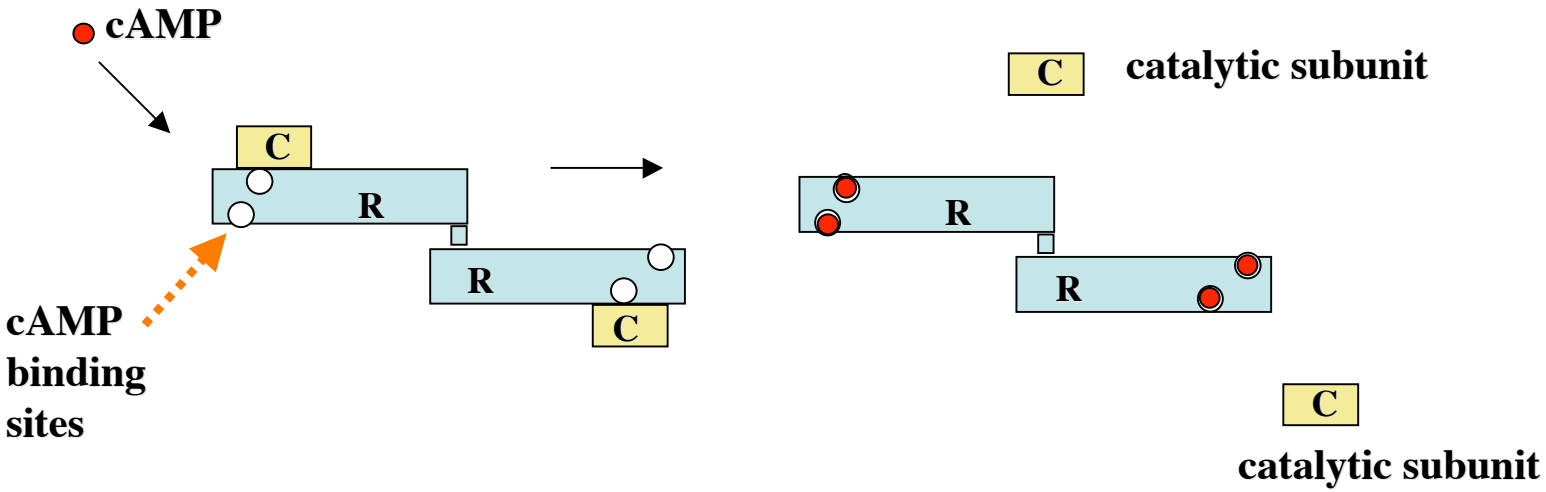


3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

c) Interaction of cyclic AMP with protein kinase A (PKA)

Protein kinase A - 4 protein subunits
- 2 regulatory subunits (R) and 2 catalytic subunits (C)

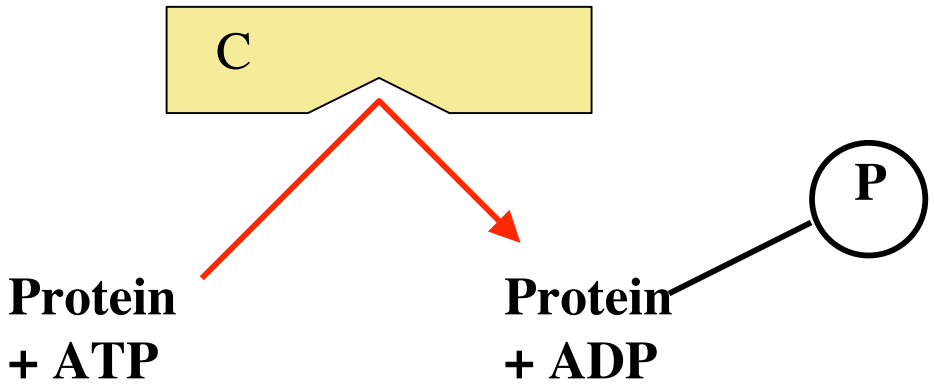


Note Cyclic AMP binds to PKA
Induced fit destabilises complex
Catalytic units released and activated

3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

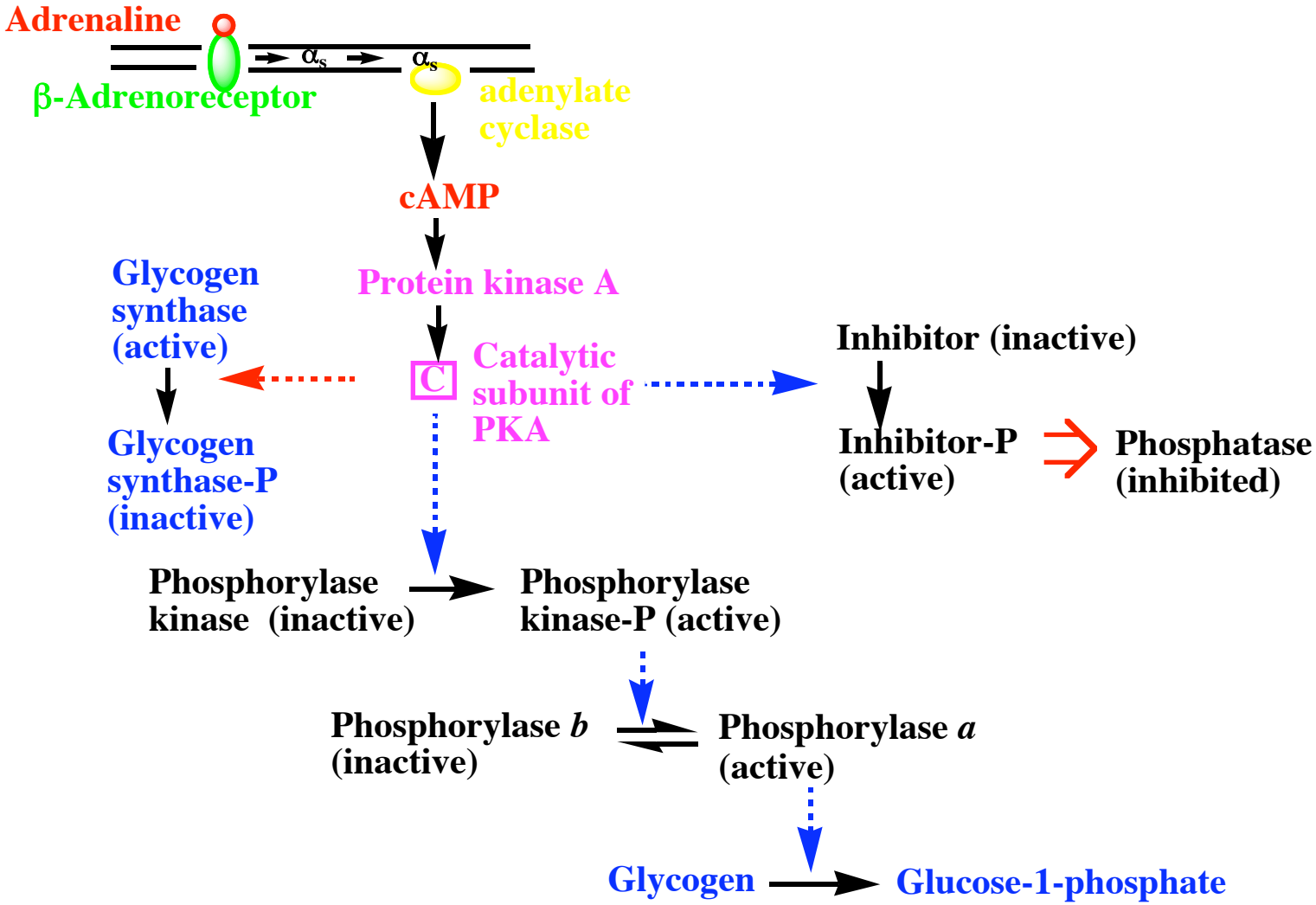
c) Interaction of cyclic AMP with protein kinase A (PKA)



Phosphorylation of other proteins and enzymes
Signal continued by phosphorylated proteins
Further signal amplification

3. G-protein-coupled receptors (7-TM receptors)

3.7 Glycogen metabolism - triggered by adrenaline in liver cells



3. G-protein-coupled receptors (7-TM receptors)

3.7 Glycogen metabolism - triggered by adrenaline in liver cells

Coordinated effect - **activation of glycogen metabolism**
- **inhibition of glycogen synthesis**

Adrenaline has different effects on different cells
- **activates fat metabolism in fat cells**

3. G-protein-coupled receptors (7-TM receptors)

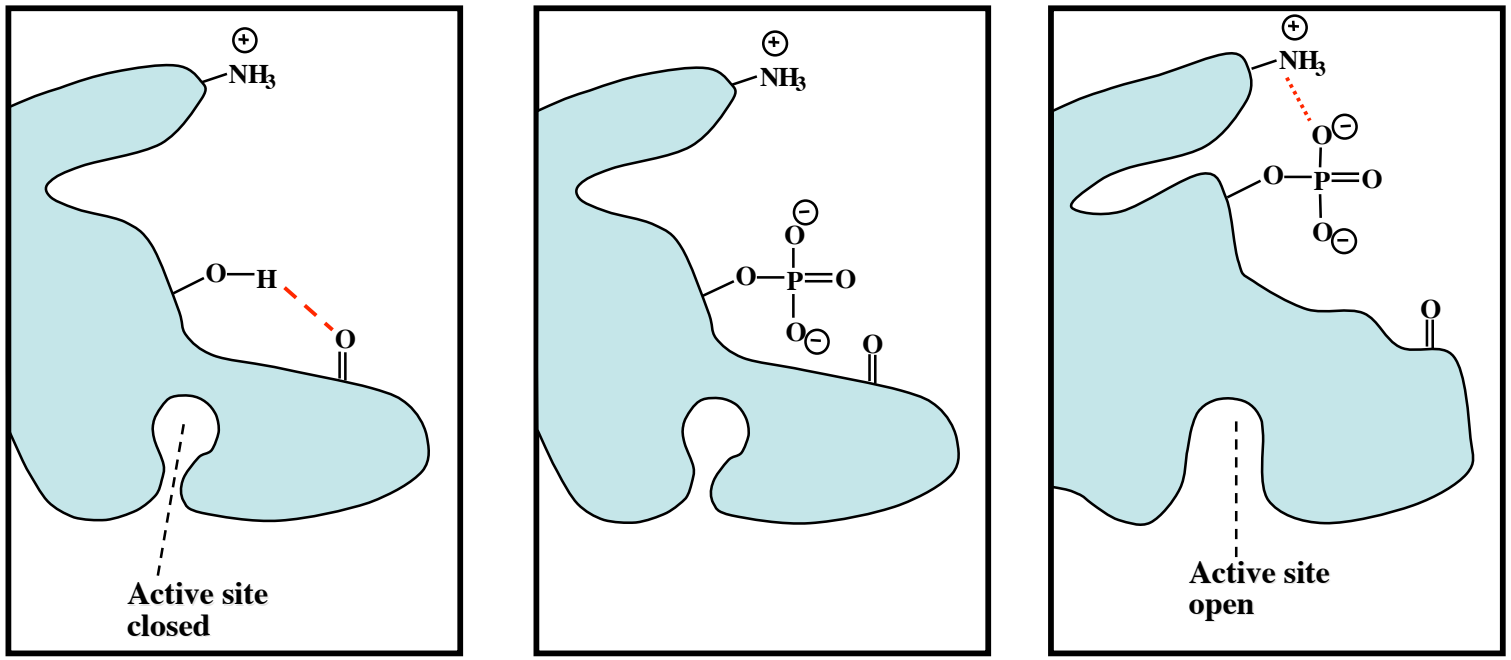
3.8 G_I proteins

- Binds to different receptors from those used by G_s protein
- Mechanism of activation by splitting is identical
- α_I subunit binds adenylate cyclase to inhibit it
- Adenylate cyclase under dual control (brake/accelerator)
- Background activity due to constant levels of α_s and α_i
- Overall effect depends on dominant G-Protein
- Dominant G-protein depends on receptors activated

3. G-protein-coupled receptors (7-TM receptors)

3.9 Phosphorylation

- **Prevalent in activation and deactivation of enzymes**
- **Phosphorylation radically alters intramolecular binding**
- **Results in altered conformations**



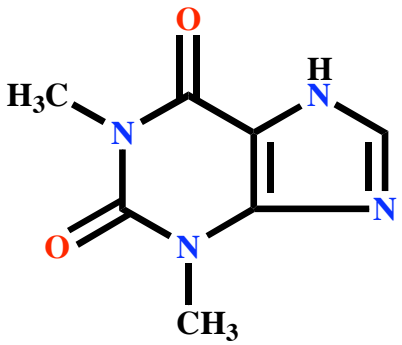
3. G-protein-coupled receptors (7-TM receptors)

3.10 Drugs interacting with cyclic AMP signal transduction

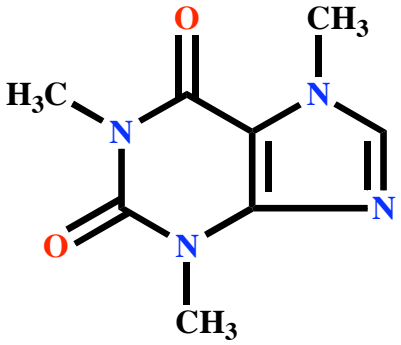
Cholera toxin - constant activation of cAMP - diarrhea

Theophylline and caffeine

- **inhibit phosphodiesterases**
- **phosphodiesterases responsible for metabolising cyclic AMP**
- **cyclic AMP activity prolonged**



Theophylline

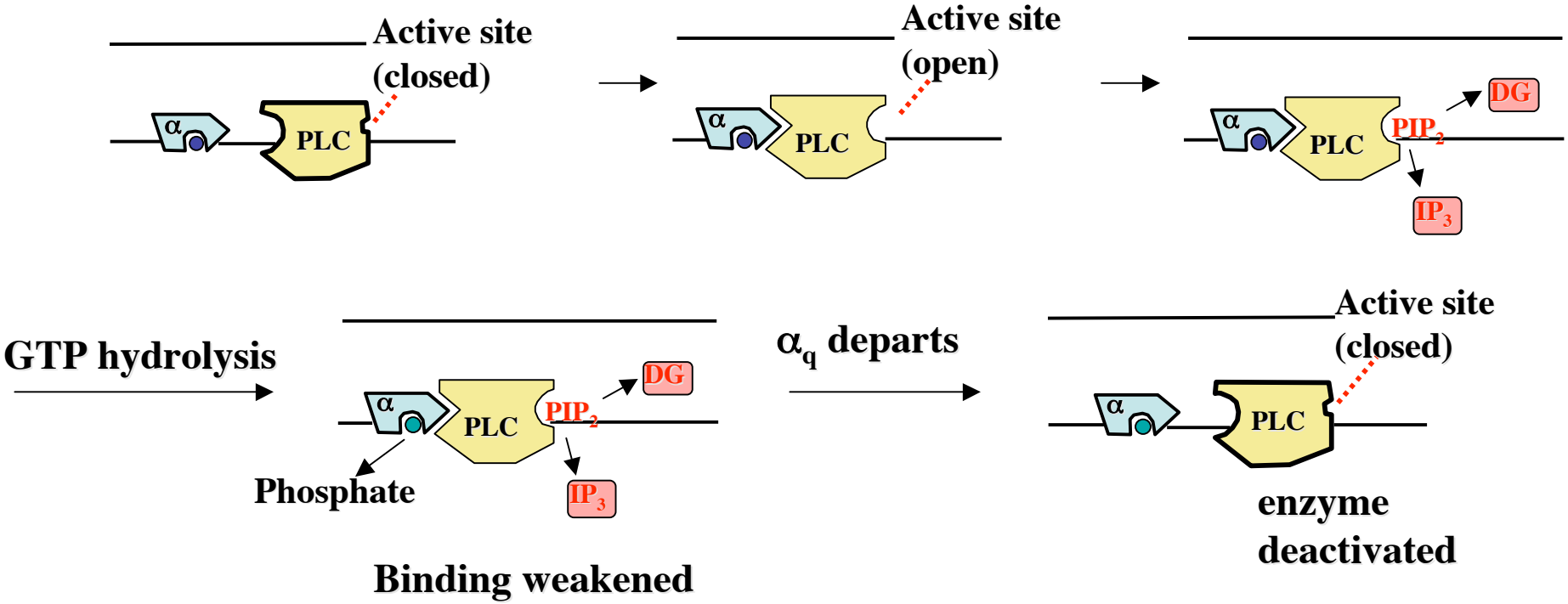


Caffeine

3. G-protein-coupled receptors (7-TM receptors)

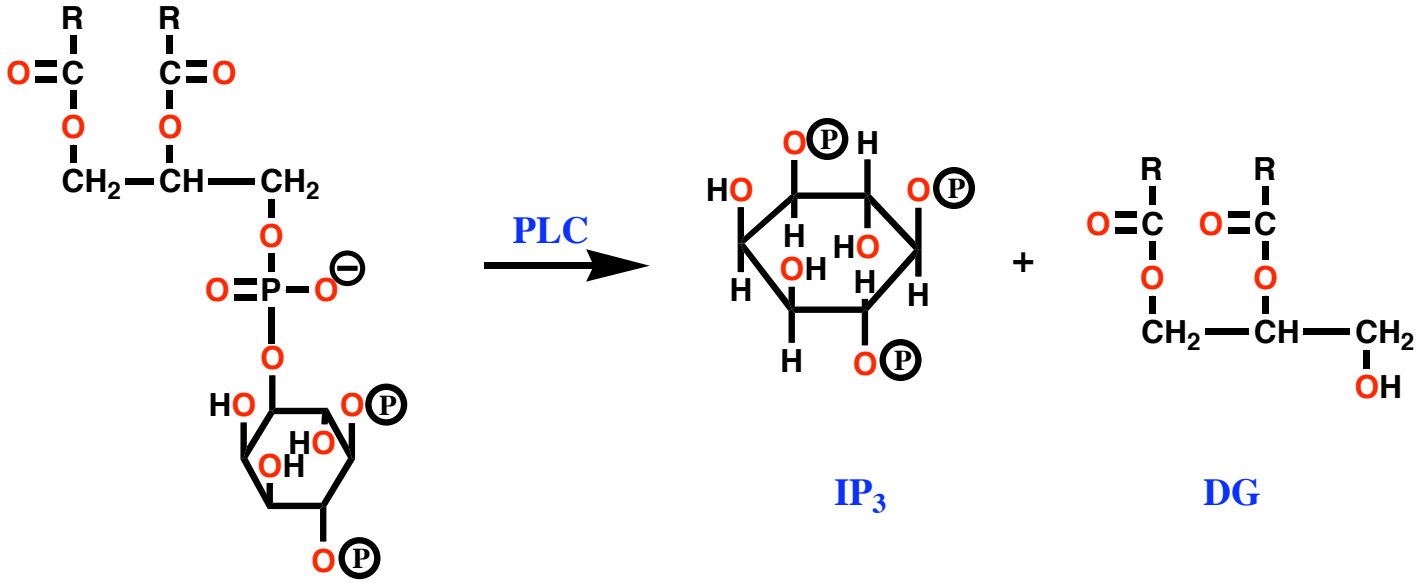
3.11 Signal transduction involving phospholipase C (PLC)

- G_q proteins - interact with different receptors from G_S and G_I
- Split by same mechanism to give α_q subunit
- α_q Subunit activates or deactivates PLC (membrane bound enzyme)
- Reaction catalysed for as long as α_q bound - signal amplification
- Brake and accelerator



3. G-protein-coupled receptors (7-TM receptors)

3.11 Signal transduction involving phospholipase C (PLC)



PIP_2

IP_3

DG

Phosphatidylinositol diphosphate (integral part of cell membrane)

Inositol triphosphate (polar and moves into cell cytoplasm)

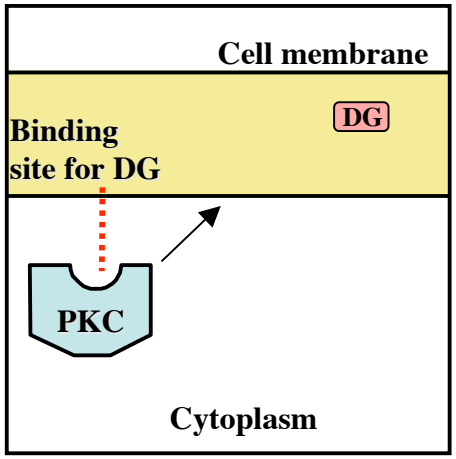
Diacylglycerol (remains in membrane)

R= long chain hydrocarbons Ⓟ = PO_3^{2-}

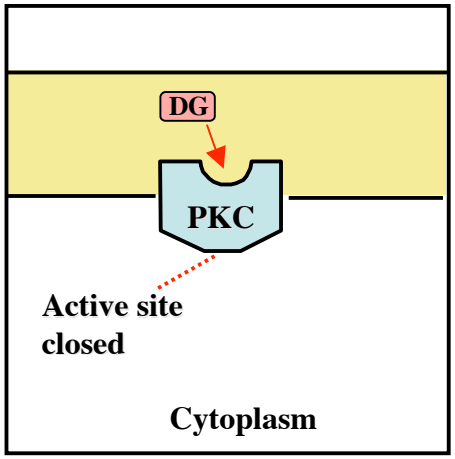
3. G-protein-coupled receptors (7-TM receptors)

3.12 Action of diacylglycerol

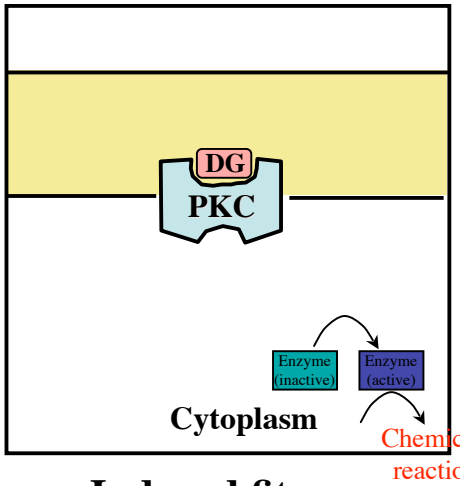
- Activates protein kinase C (PKC)
- PKC moves from cytoplasm to membrane
- Phosphorylates enzymes at Ser & Thr residues
- Activates enzymes to catalyse intracellular reactions
- Linked to inflammation, tumour propagation, smooth muscle activity etc



PKC moves
to membrane



DG binds to
DG binding site

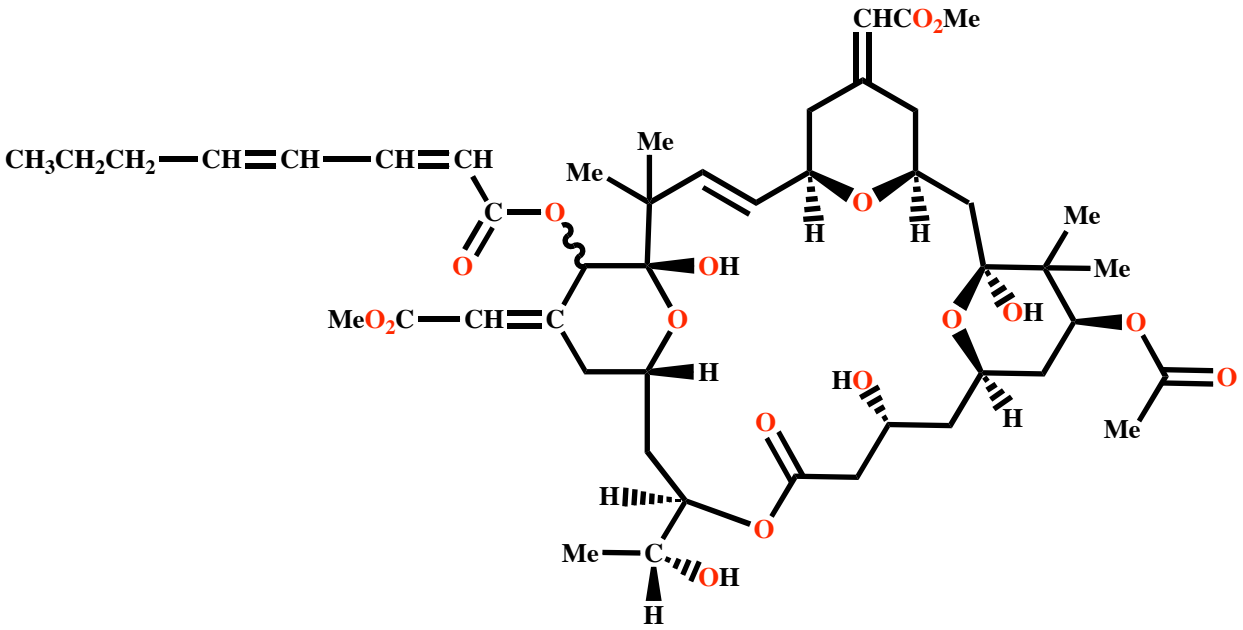


Induced fit
opens active site

3. G-protein-coupled receptors (7-TM receptors)

3.12 Action of diacylglycerol

Drugs inhibiting PKC - potential anti cancer agents



Bryostatin (from sea moss)

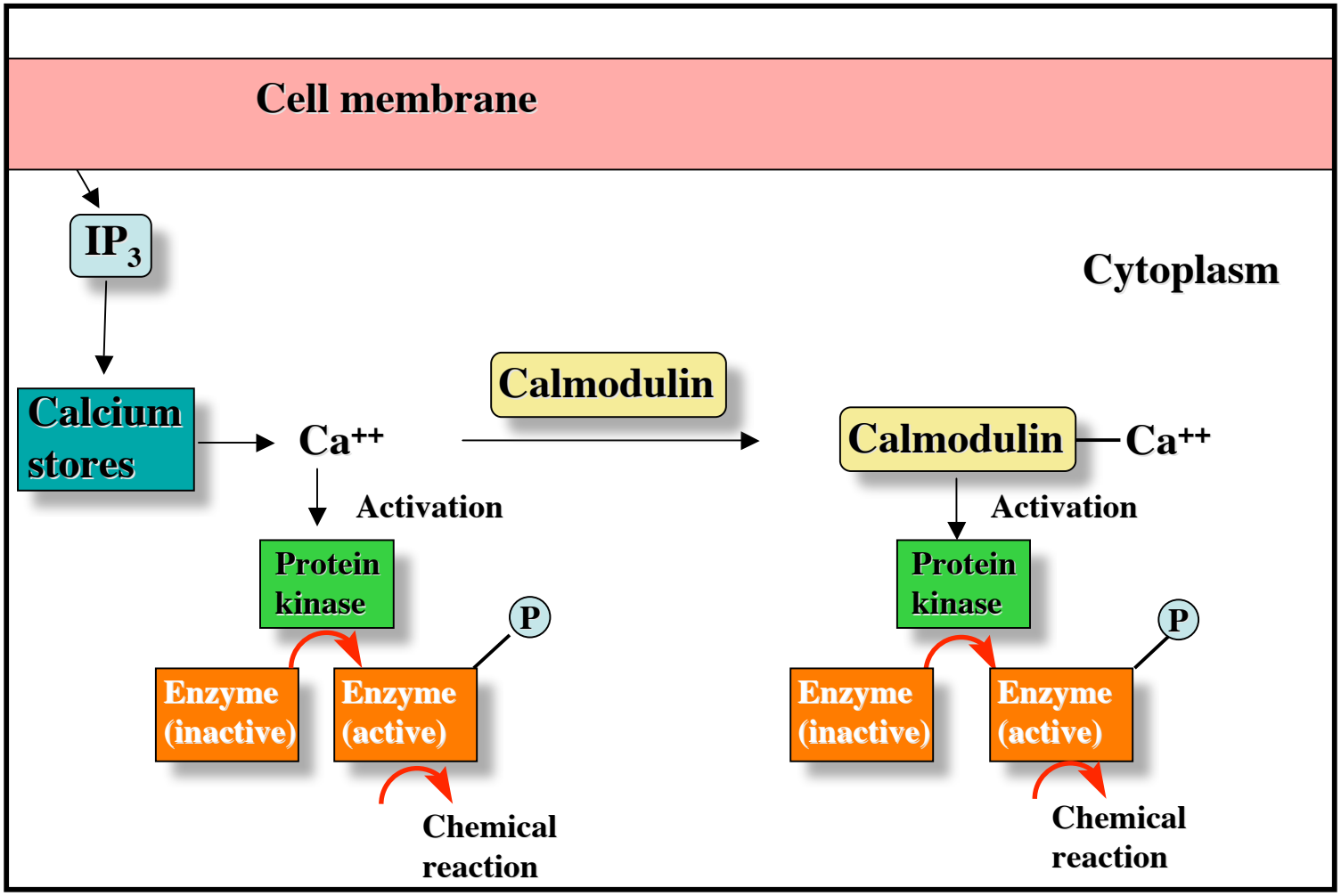
3. G-protein-coupled receptors (7-TM receptors)

3.13 Action of inositol triphosphate

- **IP_3 - hydrophilic and enters cell cytoplasm**
- **Mobilises Ca^{2+} release in cells by opening Ca^{2+} ion channels**
- **Ca^{2+} activates protein kinases**
- **Protein kinases activate intracellular enzymes**
- **Cell chemistry altered leading to biological effect**

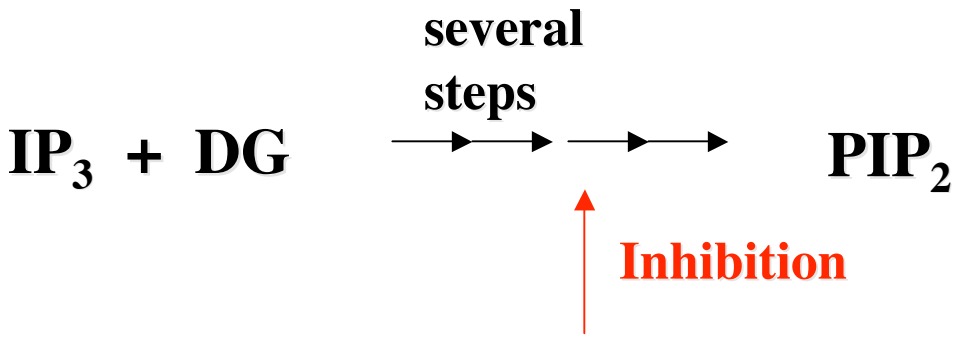
3. G-protein-coupled receptors (7-TM receptors)

3.13 Action of inositol triphosphate



3. G-protein-coupled receptors (7-TM receptors)

3.14 Resynthesis of PIP_2



Lithium salts used vs manic depression

Contents

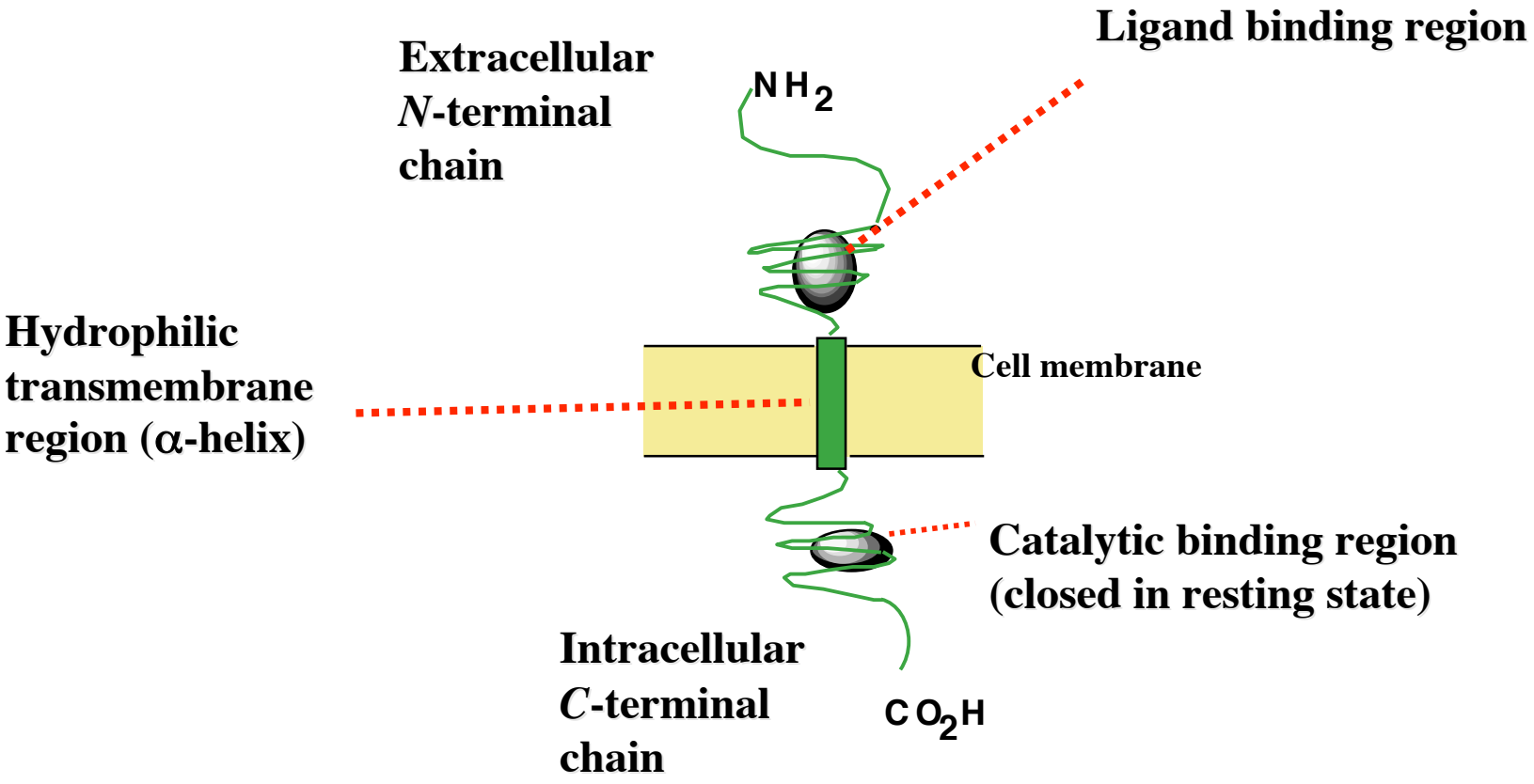
Part 3: Section 6.7

4. Tyrosine kinase linked receptors

- **Bi-functional receptor / enzyme**
- **Activated by hormones**
- **Over-expression can result in cancer**

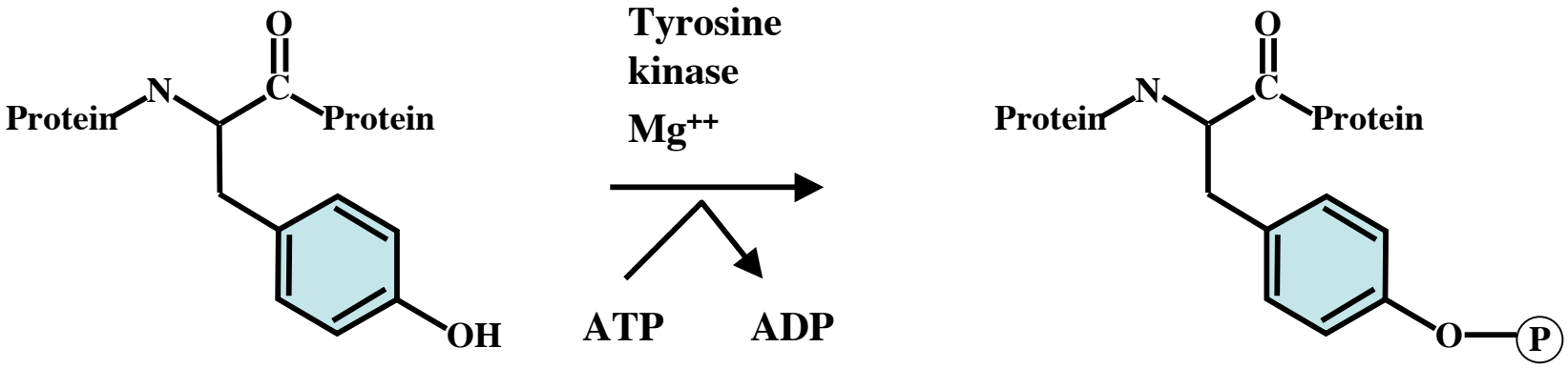
4. Tyrosine kinase linked receptors

4.1 Structure



4. Tyrosine kinase linked receptors

4.2 Reaction catalysed by tyrosine kinase

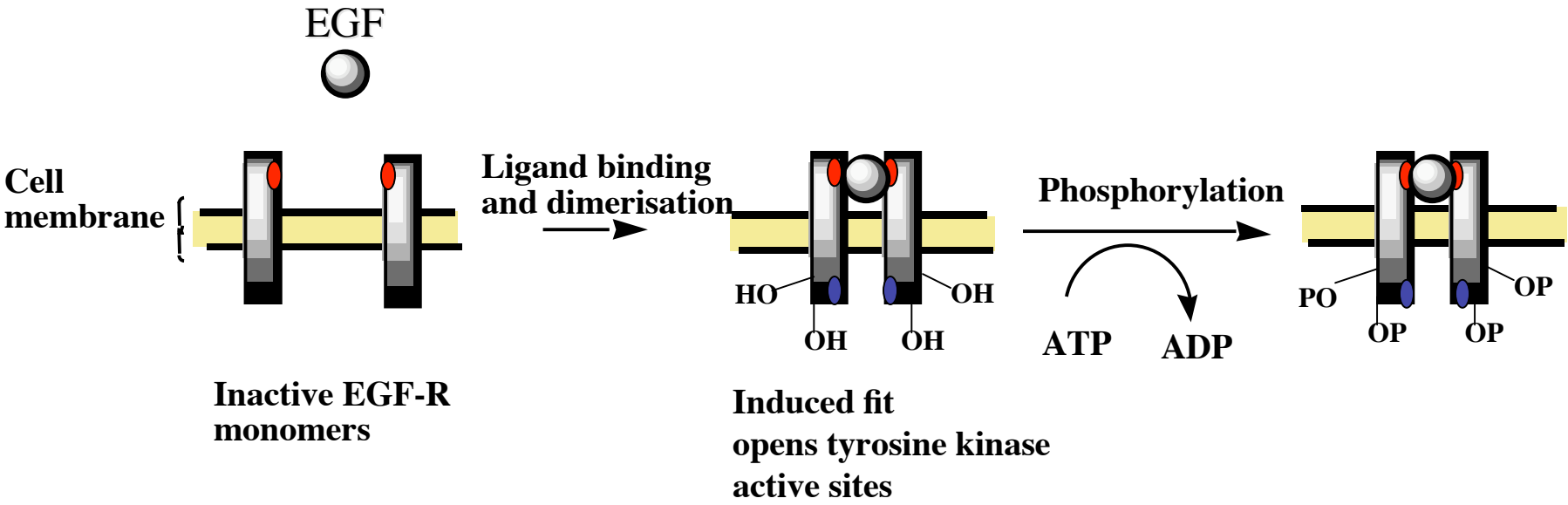


Tyrosine residue

Phosphorylated tyrosine residue

4. Tyrosine kinase linked receptors

4.3 Epidermal growth factor receptor (EGF- R)



- Binding site for EGF
- EGF - protein hormone - bivalent ligand
- Active site of tyrosine kinase

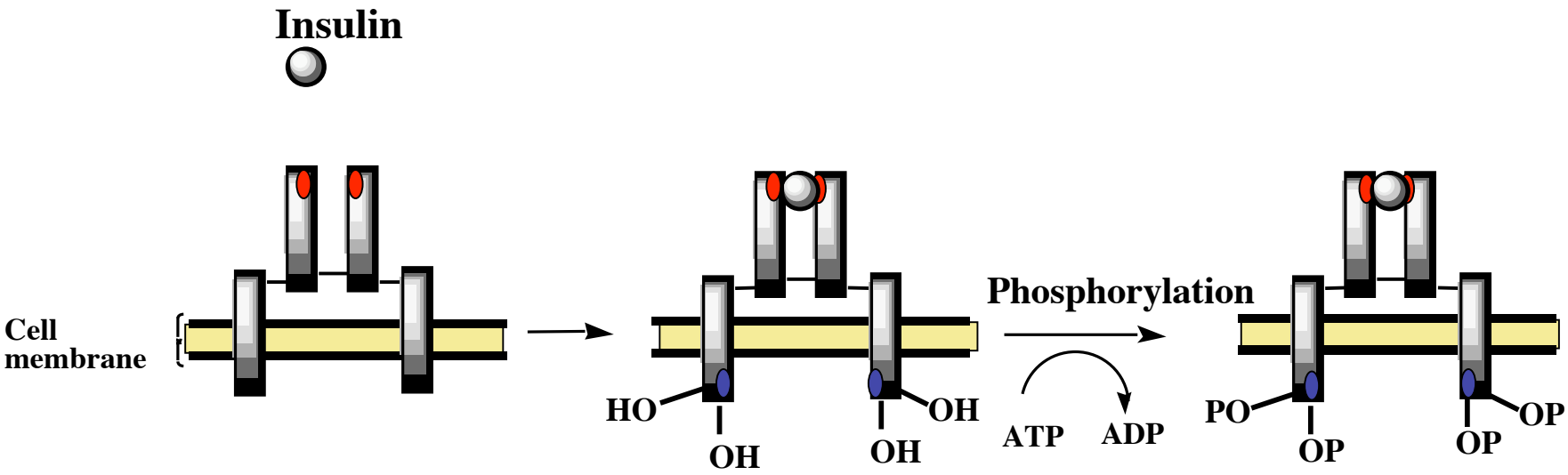
4. Tyrosine kinase linked receptors

4.3 Epidermal growth factor receptor (EGF- R)

- **Active site on one half of dimer catalyses phosphorylation of Tyr residues on other half**
- **Dimerisation of receptor is crucial**
- **Phosphorylated regions act as binding sites for further proteins and enzymes**
- **Results in activation of signalling proteins and enzymes**
- **Message carried into cell**

4. Tyrosine kinase linked receptors

4.4 Insulin receptor (tetrameric complex)



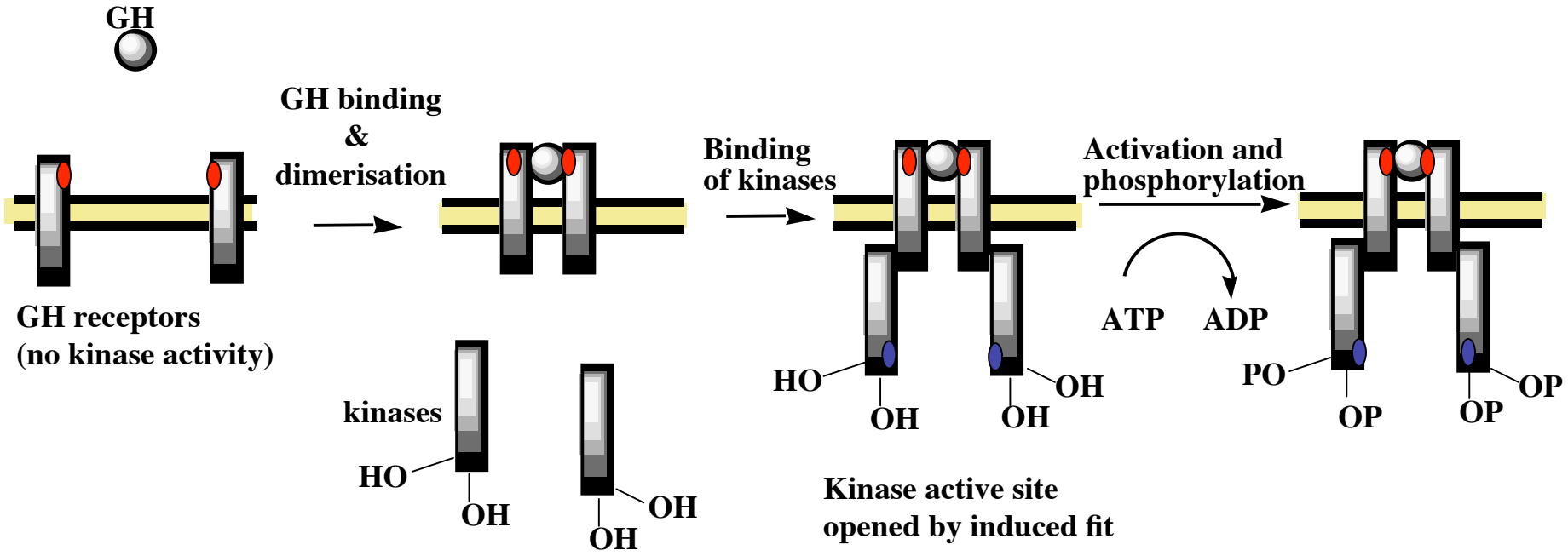
Kinase active site
opened by induced fit

- Insulin binding site
- Kinase active site

4. Tyrosine kinase linked receptors

4.5 Growth hormone receptor

Tetrameric complex constructed in presence of growth hormone

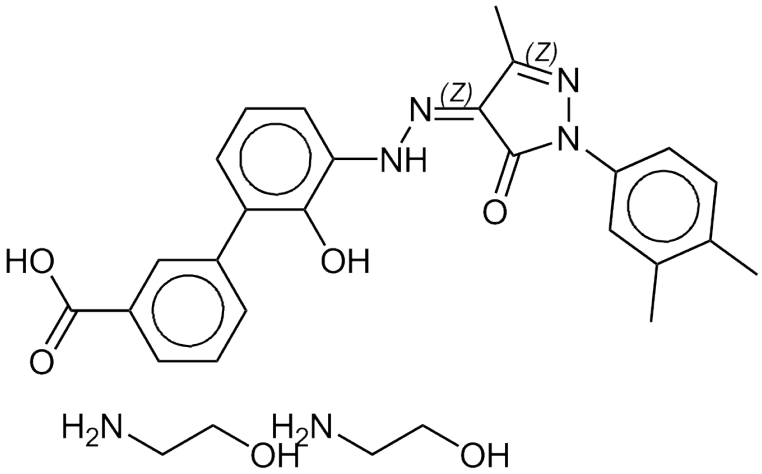


http://en.wikipedia.org/wiki/Cytokine_receptor

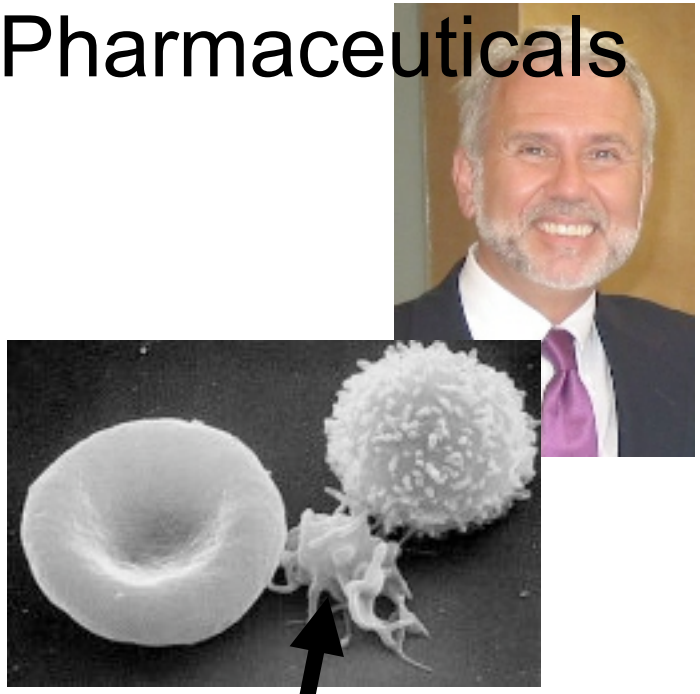
http://www.ebi.ac.uk/interpro/potm/2004_4/Page2.htm

- Growth hormone binding site
- Kinase active site(Janus, JAK kinase)

Tales from the drug development trenches-Tucson-John Kozarich,Ligand Pharmaceuticals



2-aminoethanol hemi((*Z*)-3'-(2-(1-(3,4-dimethylphenyl)-3-methyl-5-oxo-1*H*-pyrazol-4(5*H*)-ylidene)hydrazinyl)-2'-hydroxybiphenyl-3-carboxylate)

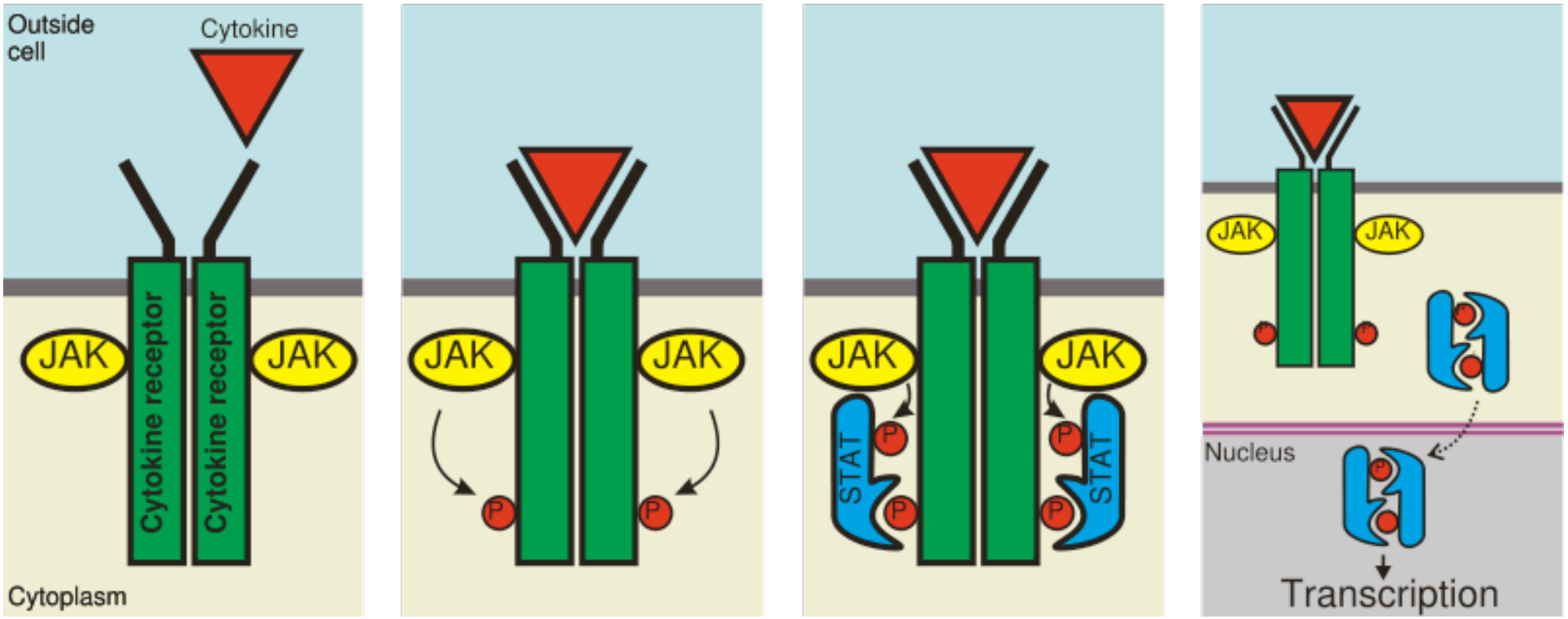


Thrombocyte,i.e. platelet

Eltrombopag,PROMACTA
Binds to DIFFERENT site than thrombopoetin with Zn^{2+} .

http://www.ligand.com/collaborations.php#Leading

Tales from the drug development trenches-Tucson

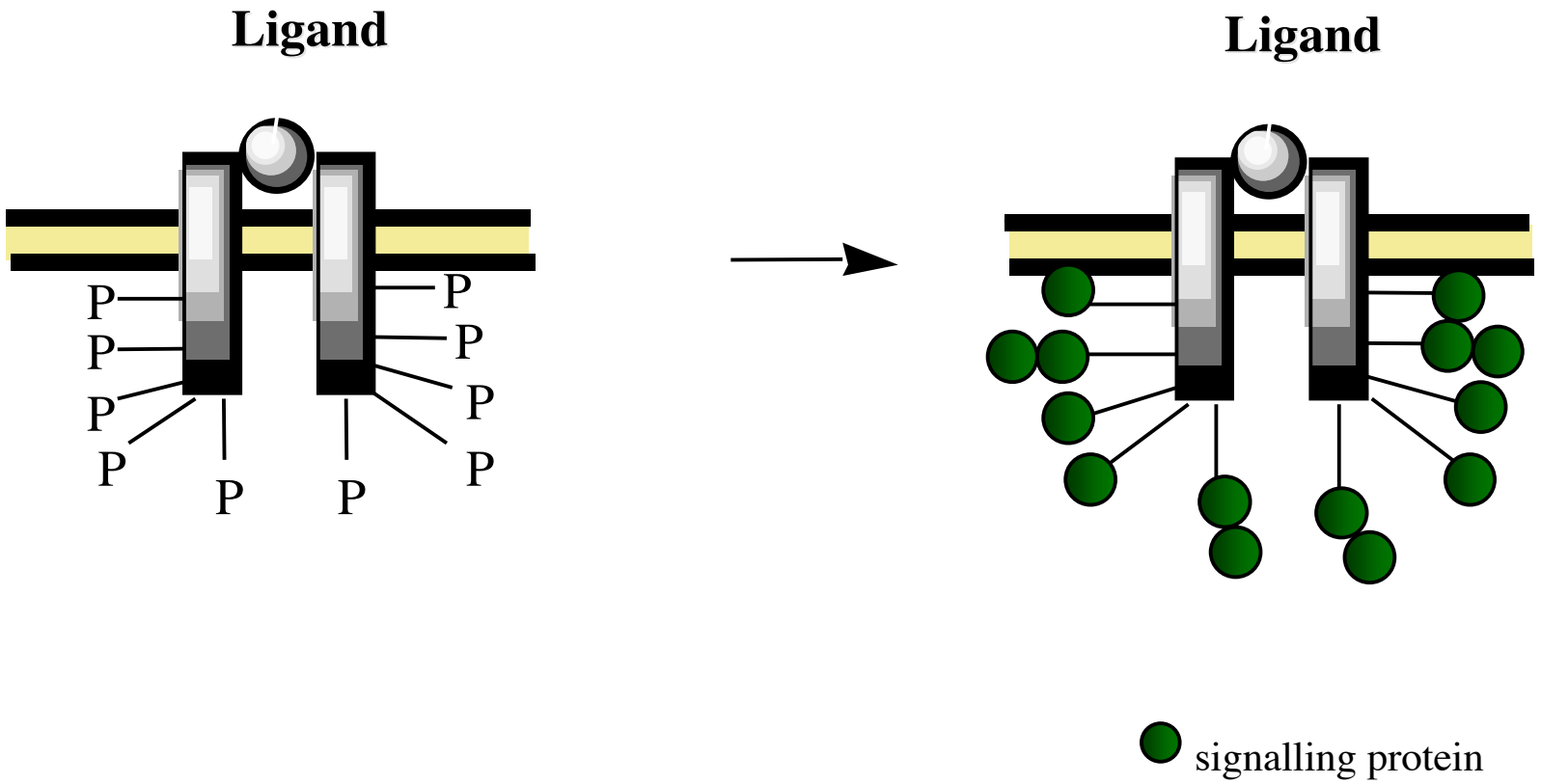


http://en.wikipedia.org/wiki/Cytokine_receptor

TPO and EPO receptors (cytokine type, also growth hormone) connected to Janus kinase (JAK) family of tyrosine kinases

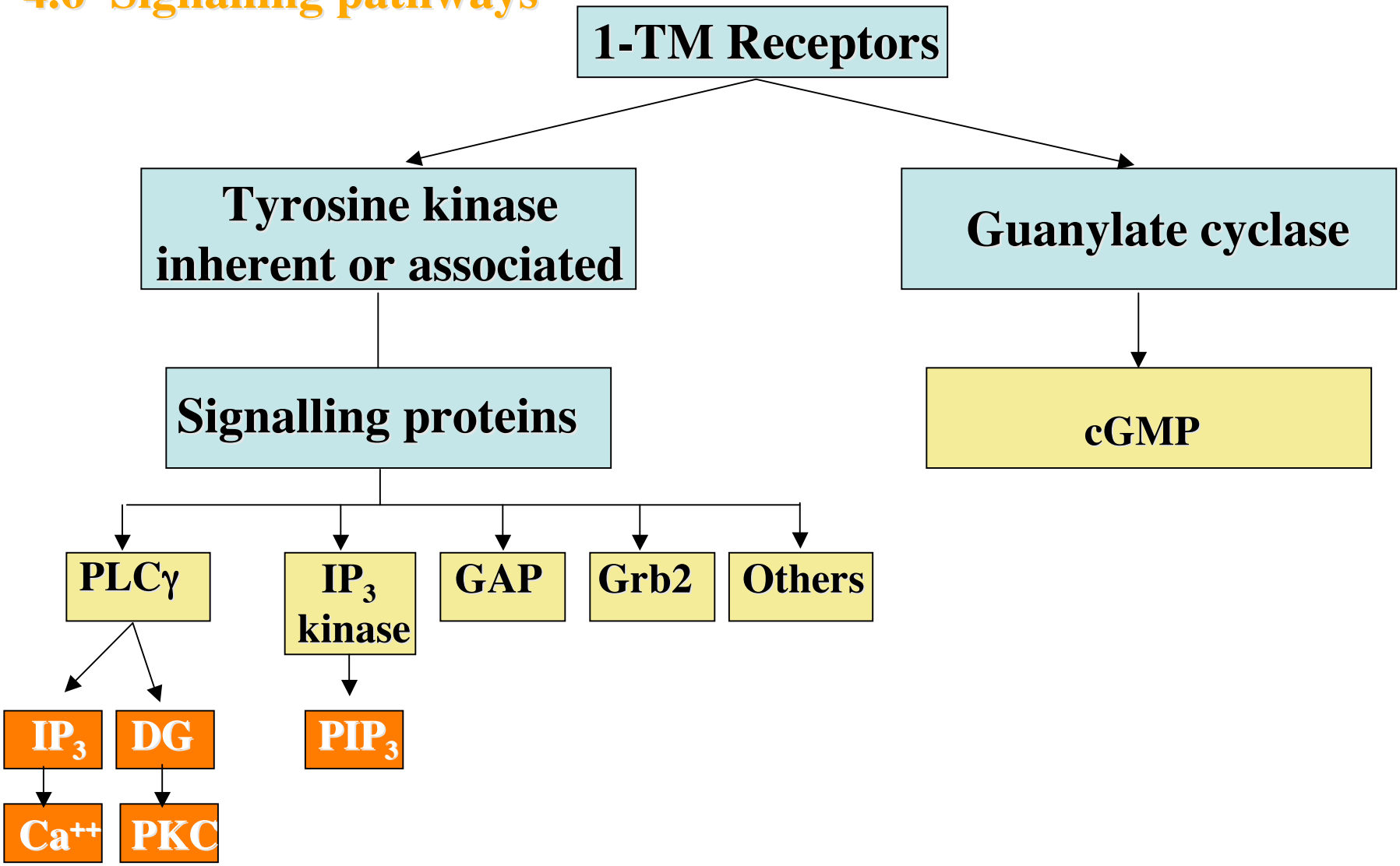
4. Tyrosine kinase linked receptors

4.6 Signalling pathways



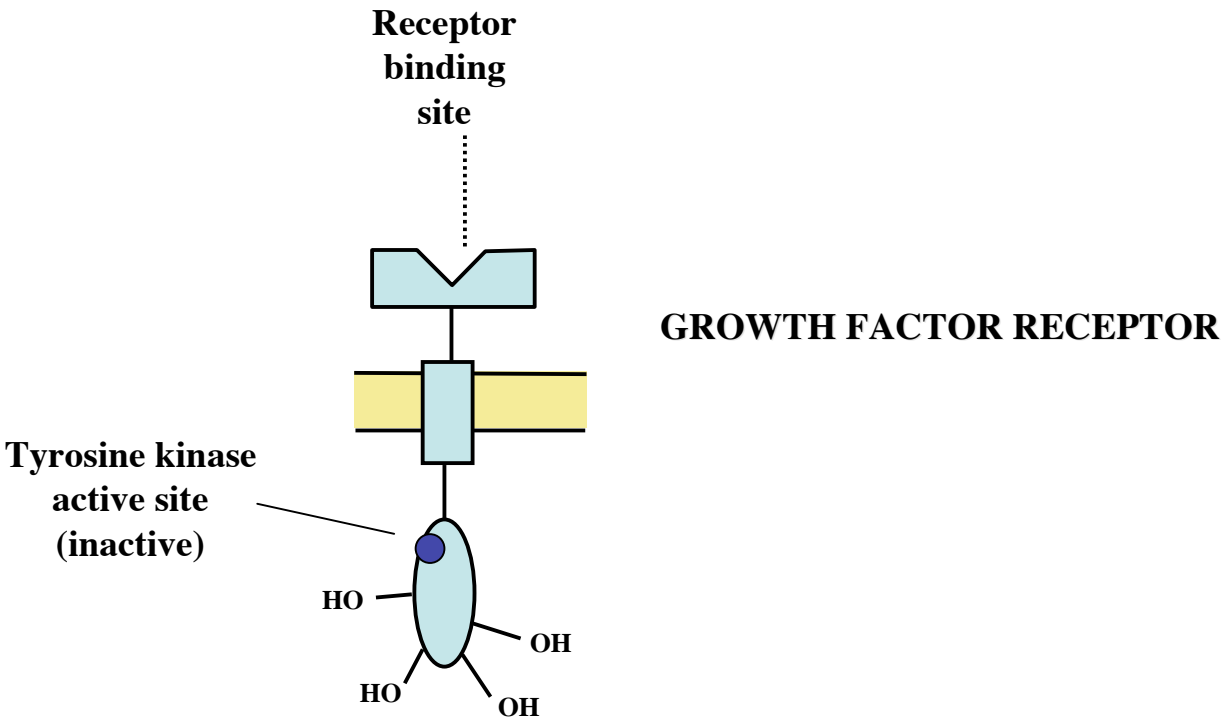
4. Tyrosine kinase linked receptors

4.6 Signalling pathways



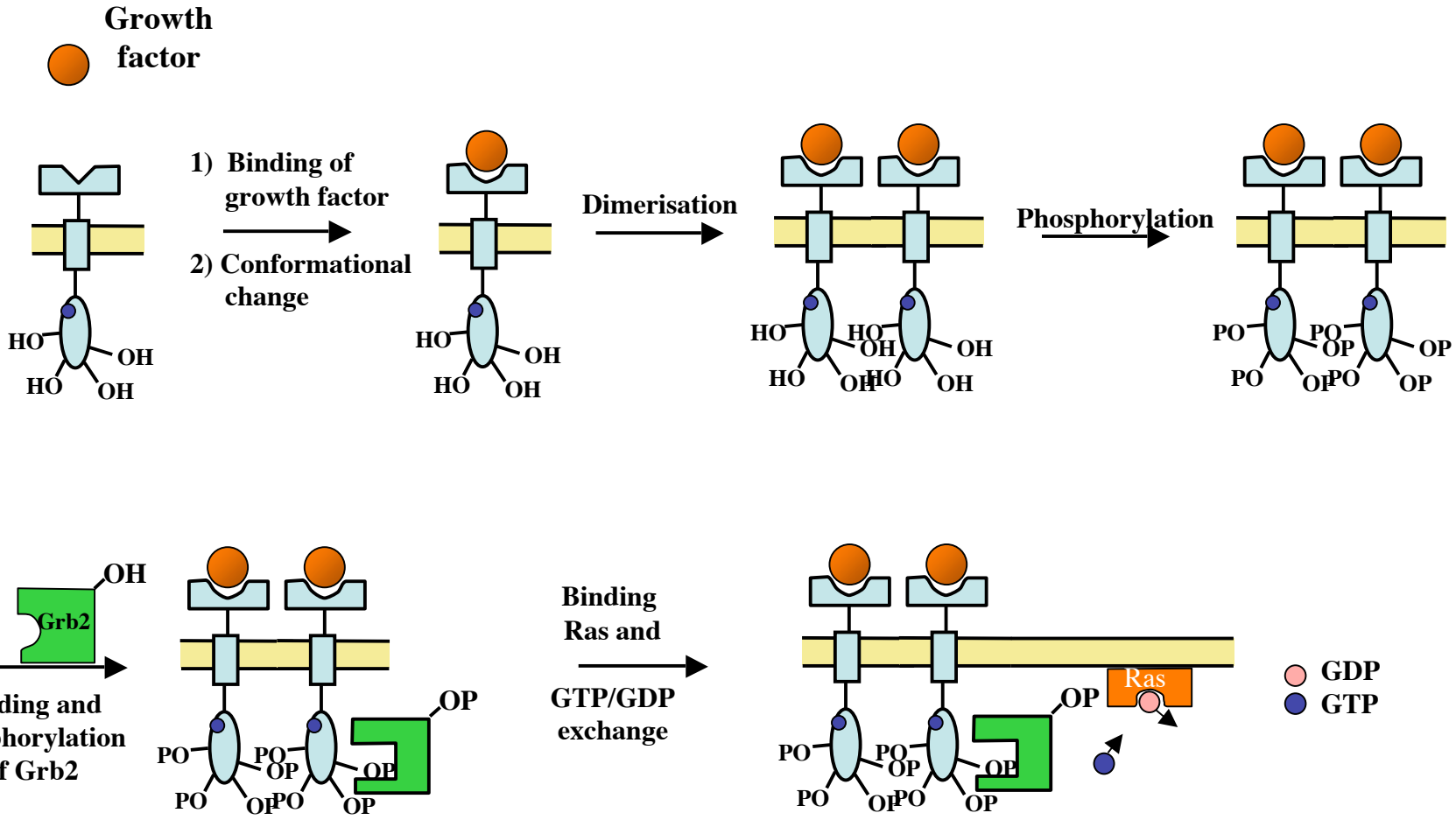
4. Tyrosine kinase linked receptors

4.6 Signalling pathways



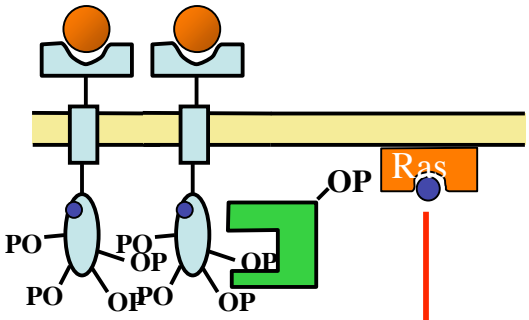
4. Tyrosine kinase linked receptors

4.6 Signalling pathways



4. Tyrosine kinase linked receptors

4.6 Signalling pathways



Raf (inactive) → Raf (active)

Mek (inactive) → Mek (active)

Map kinase (inactive) → Map kinase (active)

Transcription factor (inactive) → Transcription factor (active)

Gene transcription

Contents

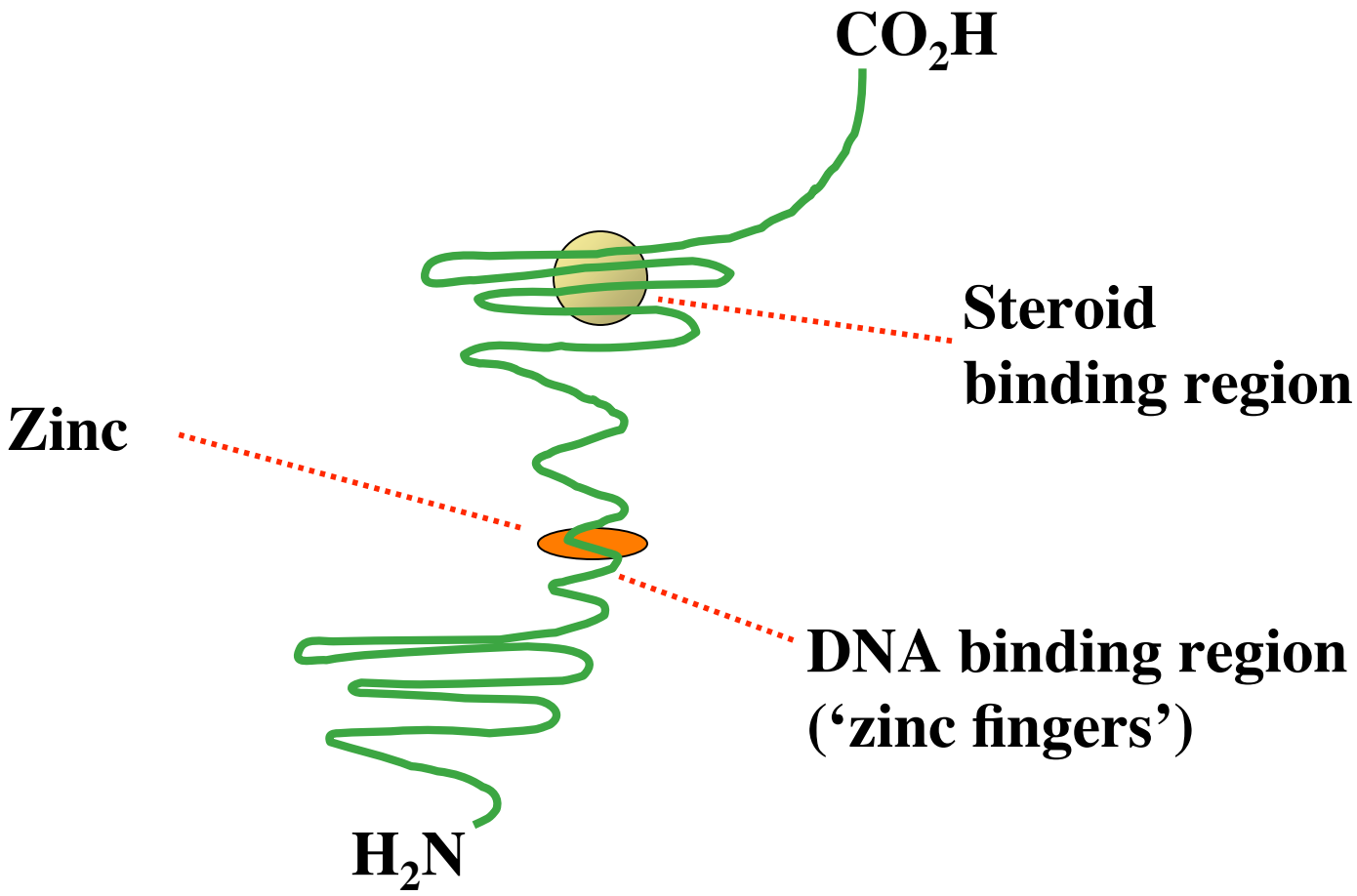
Part 4: Section 6.8

5. Intracellular receptors

- **Chemical messengers must cross cell membrane**
- **Chemical messengers must be hydrophobic**
- **Example - steroids and steroid receptors**

5. Intracellular receptors

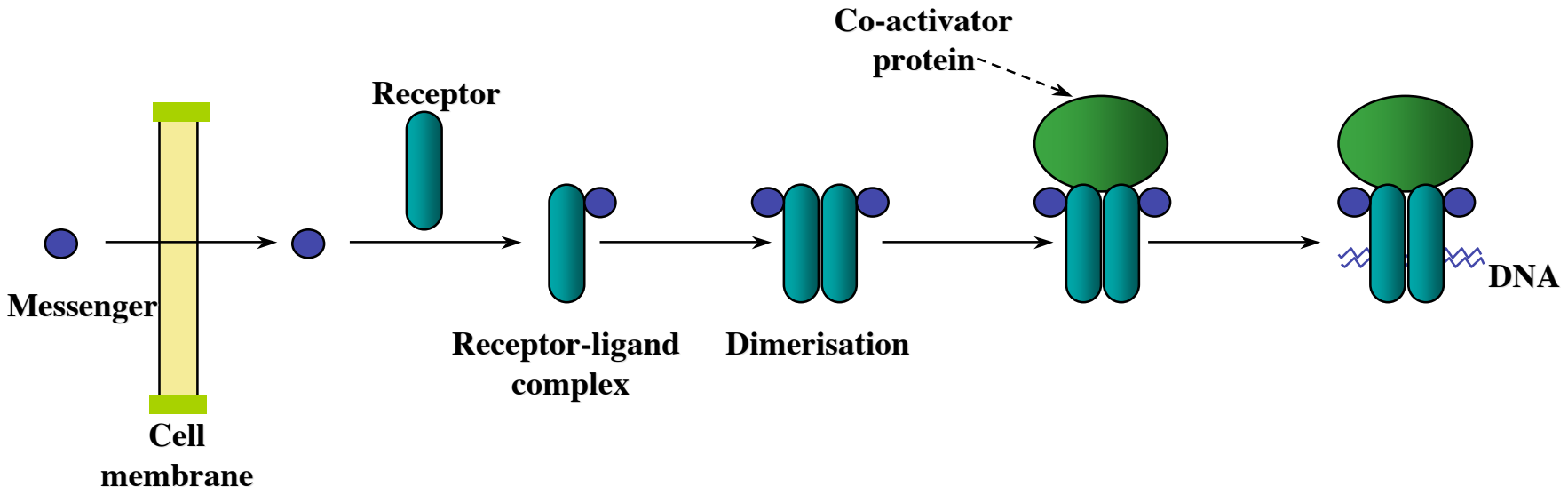
5.1 Structure



Zinc fingers contain Cys residues (SH)
Allow S-Zn interactions

5. Intracellular receptors

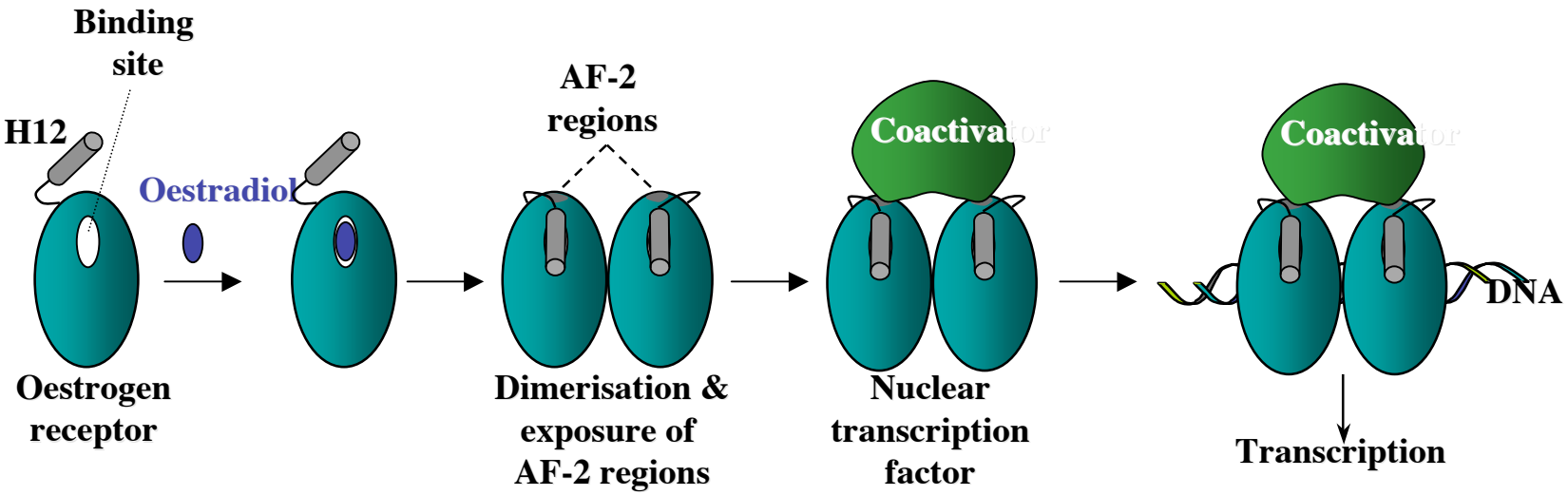
5.2 Mechanism



1. Messenger crosses membrane
2. Binds to receptor
3. Receptor dimerisation
4. Binds co-activator protein
5. Complex binds to DNA
6. Transcription switched on or off
7. Protein synthesis activated or inhibited

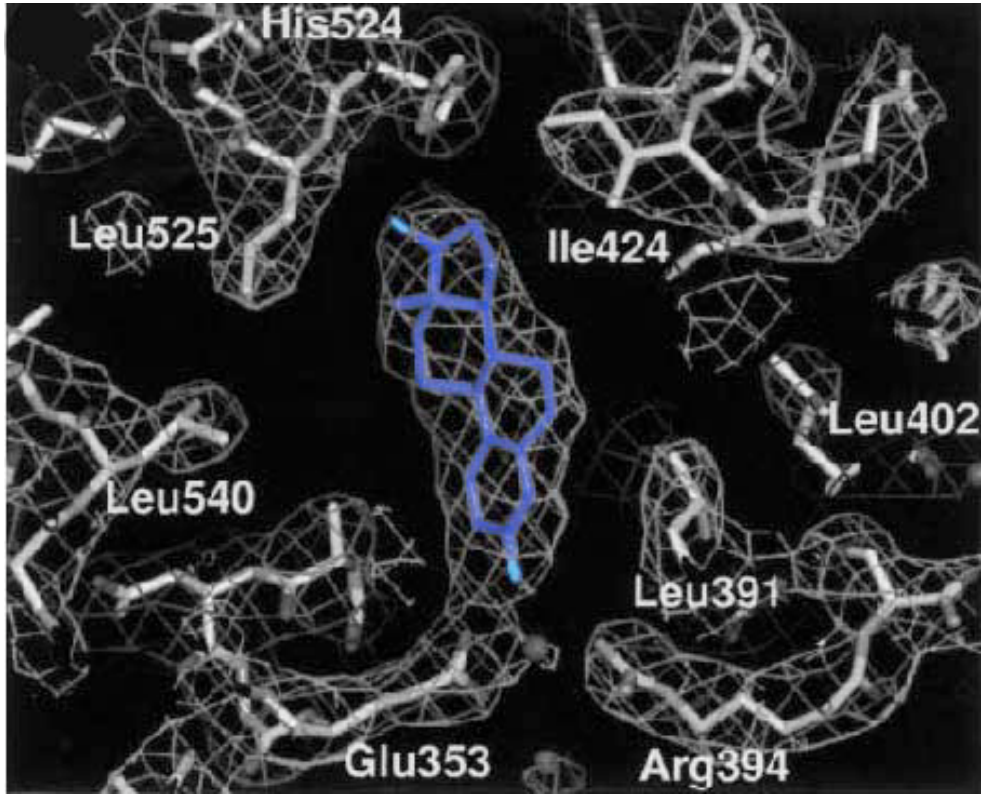
5. Intracellular receptors

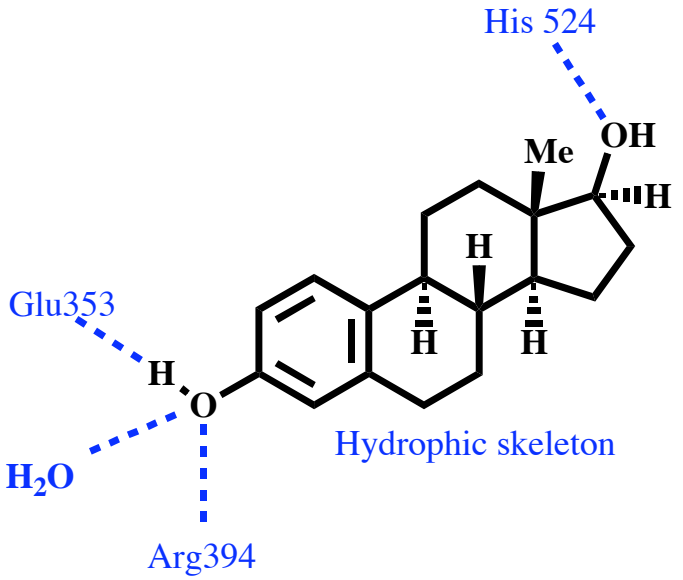
5.3 Oestrogen receptor



5. Intracellular receptors

5.3 Oestrogen receptor



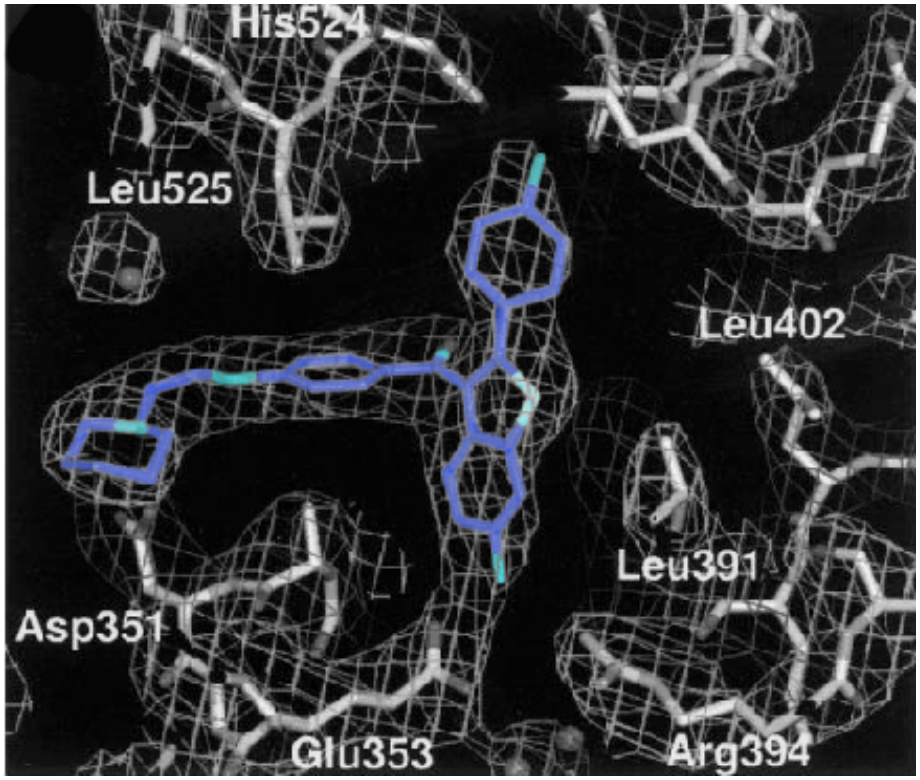


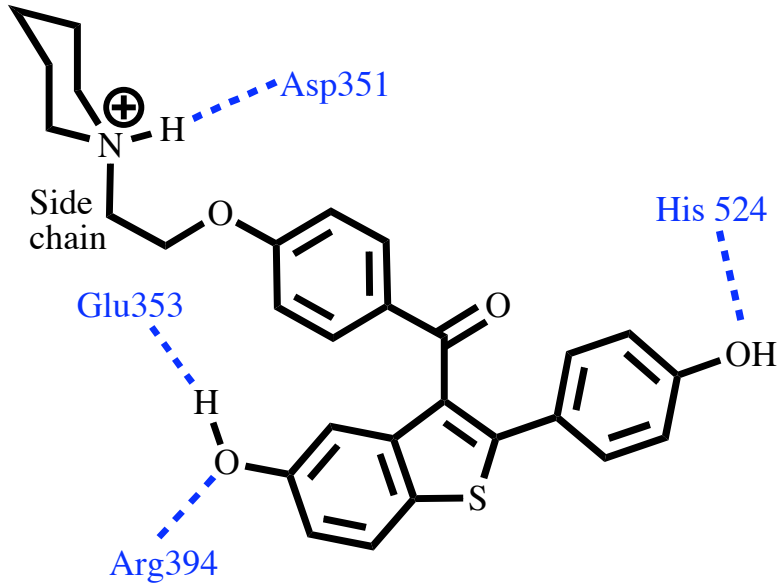
Oestradiol

- **Phenol and alcohol of oestradiol are important binding groups**
- **Binding site is spacious and hydrophobic**
- **Phenol group of oestradiol positioned in narrow slot**
- **Orientates rest of molecule**
- **Acts as agonist**

5. Intracellular receptors

5.3 Oestrogen receptor



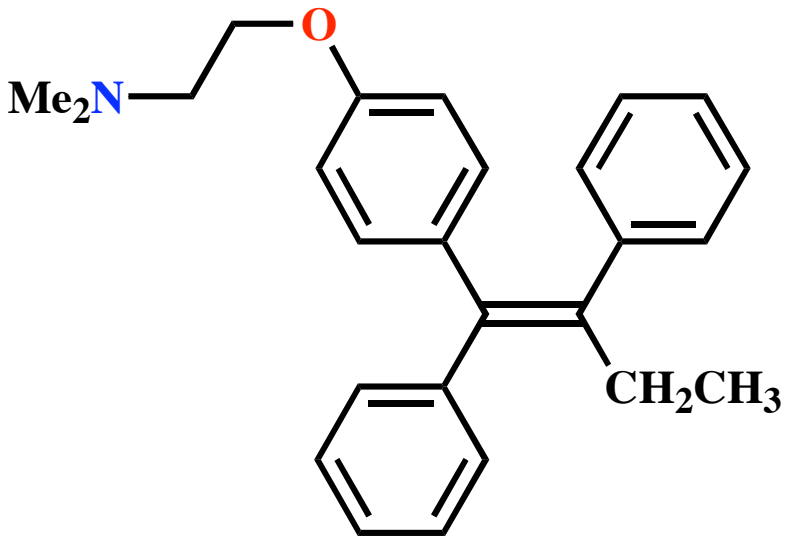


Raloxifene

- **Raloxifene is an antagonist (anticancer agent)**
- **Phenol groups mimic phenol and alcohol of oestradiol**
- **Interaction with Asp351 is important for antagonist activity**
- **Side chain prevents receptor helix H12 folding over as lid**
- **AF-2 binding region not revealed**
- **Co-activator cannot bind**

5. Intracellular receptors

5.3 Oestrogen receptor



Tamoxifen (Nolvadex)

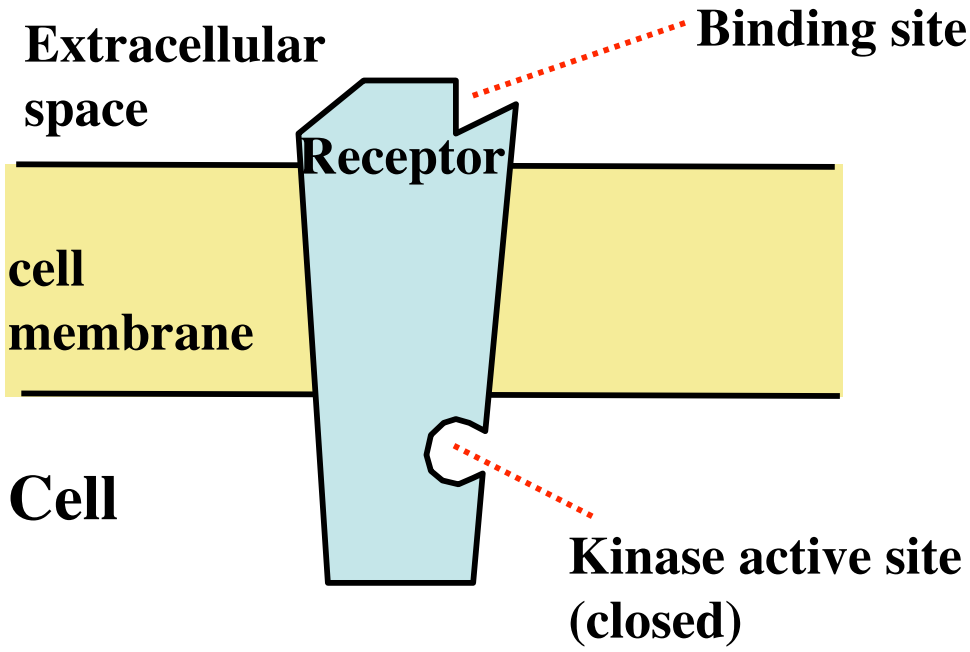
- anticancer agent which targets oestrogen receptor

Contents

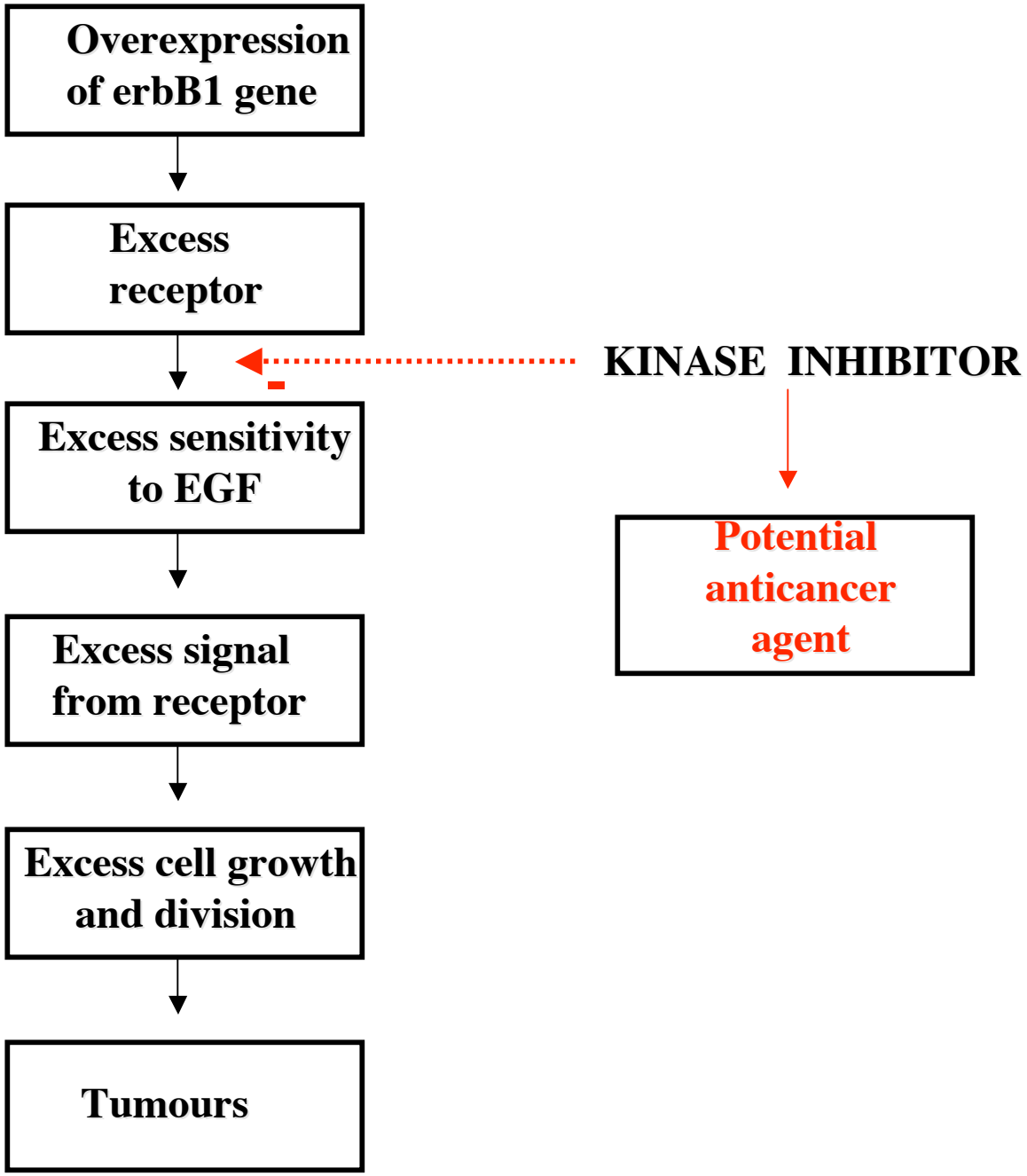
6. Case Study - Inhibitors of EGF Receptor Kinase

6.1 The target

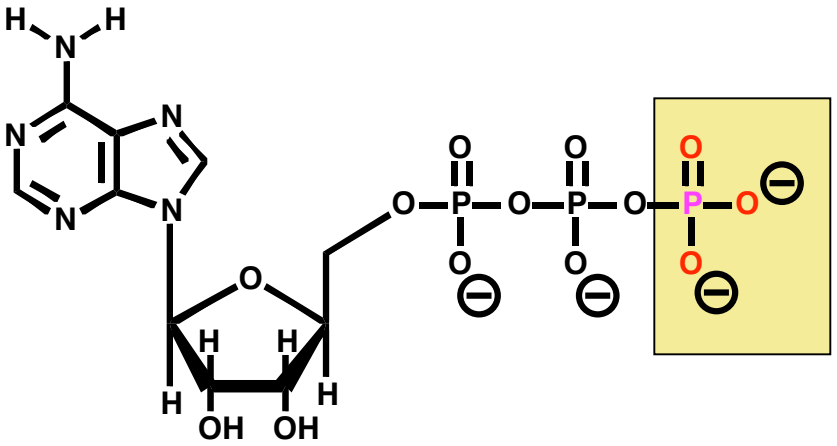
- Epidermal growth factor receptor
- Dual receptor / kinase enzyme role



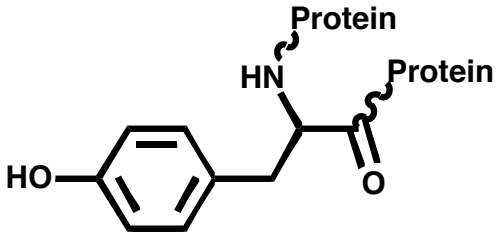
6.1 The target



6.1 The target

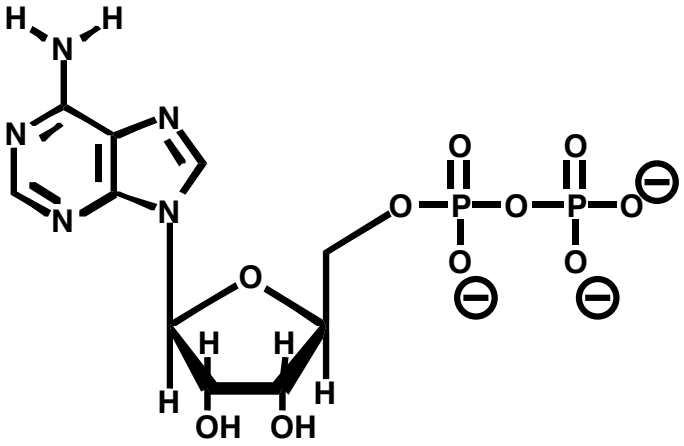


ATP

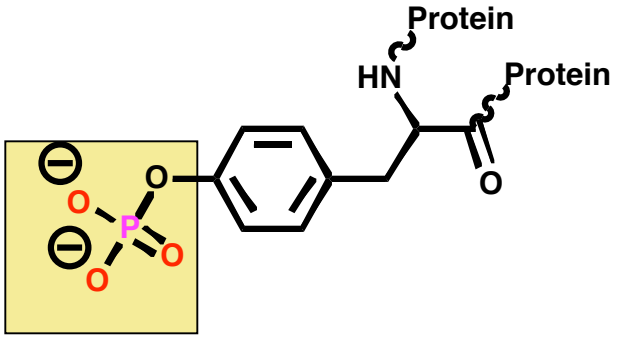


Tyrosine residue

Mg
Tyrosine kinase



ADP



Phosphorylated tyrosine residue

6.1 The target

Inhibitor Design

Possible versus binding site for tyrosine region
Possible versus binding site for ATP

Inhibitors of the ATP binding site

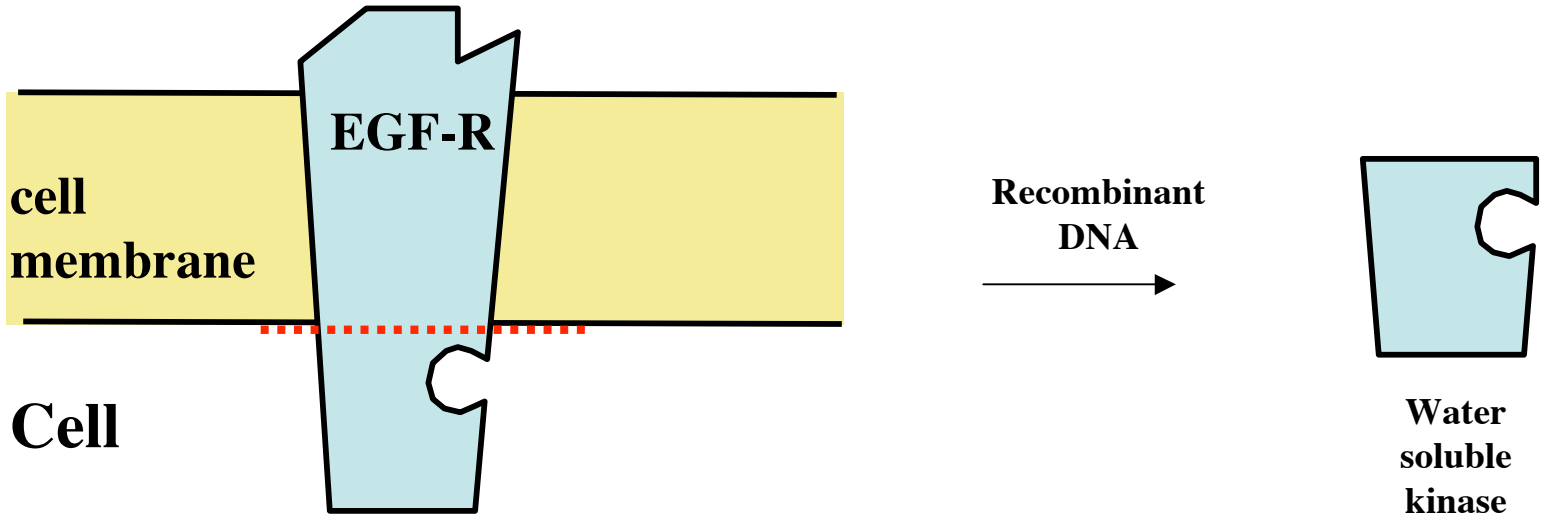
Aims:
To design a potent but selective inhibitor versus EGF receptor kinase and not other protein kinases.

6.2 Testing procedures

In vitro tests

Enzyme assay

using kinase portion of the EGF receptor produced by recombinant DNAtechnology. Allows enzyme studies in solution.

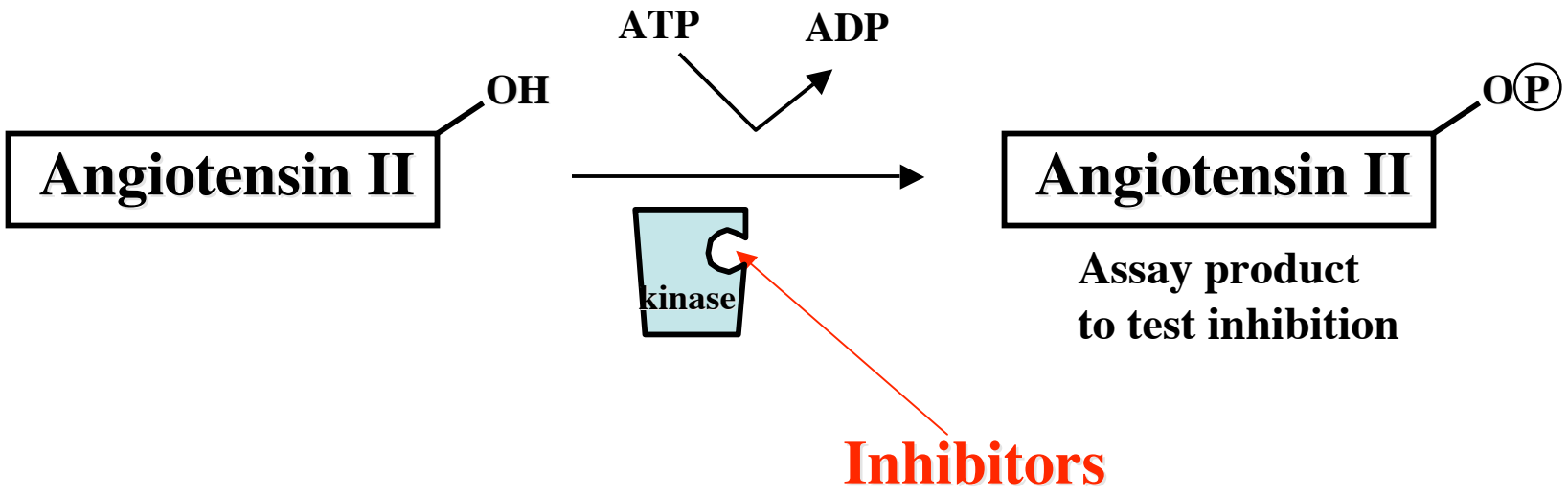


6.2 Testing procedures

In vitro tests

Enzyme assay

Test inhibitors by ability to inhibit standard enzyme catalysed reaction



- **Tests inhibitory activity only and not ability to cross cell membrane**
- **Most potent inhibitor may be inactive *in vivo***

6.2 Testing procedures

In vitro tests

Cell assays

- **Use cancerous human epithelial cells which are sensitive to EGF for growth**
- **Measure inhibition by measuring effect on cell growth - blocking kinase activity blocks cell growth.**
- **Tests inhibitors for their ability to inhibit kinase and to cross cell membrane**
- **Assumes that enzyme inhibition is responsible for inhibition of cell growth**

Checks

- **Assay for tyrosine phosphorylation in cells - should fall with inhibition**
- **Assay for m-RNA produced by signal transduction - should fall with inhibition**
- **Assay fast growing mice cells which divide rapidly in presence of EGF**

6.2 Testing procedures

In vivo tests

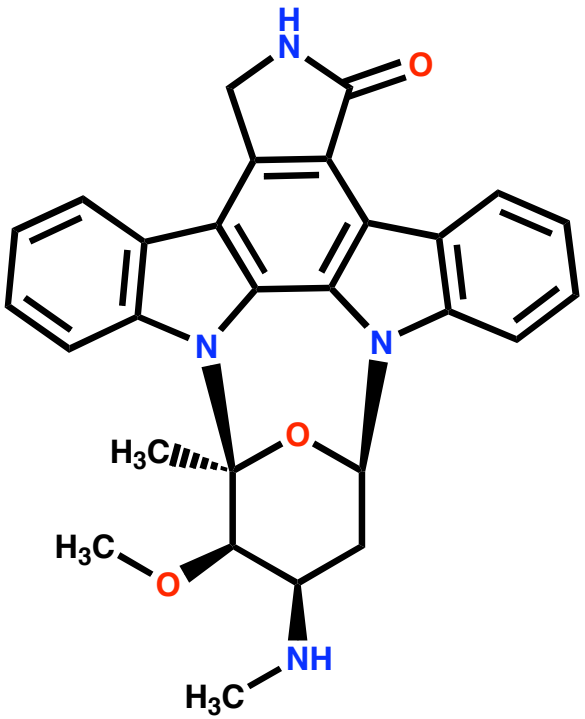
- **Use cancerous human epithelial cells grafted onto mice**
- **Inject inhibitor into mice**
- **Inhibition should inhibit tumour growth**
- **Tests for inhibitory activity + favourable pharmacokinetics**

6.2 Testing procedures

Selectivity tests

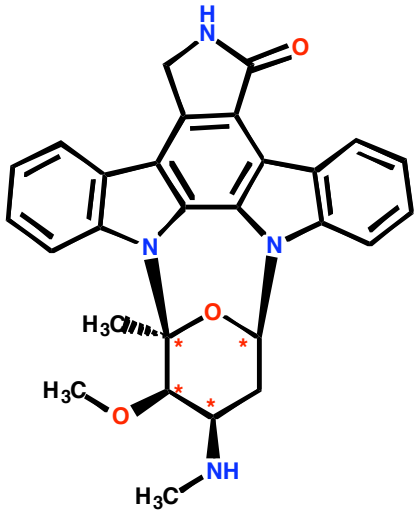
Similar *in vitro* and *in vivo* tests carried out on serine-threonine kinases and other tyrosine kinases

6.3 Lead compound - Staurosporine



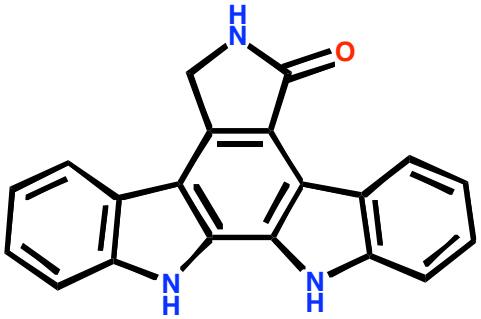
- **Microbial metabolite**
- **Highly potent kinase inhibitor but no selectivity**
- **Competes with ATP for ATP binding site**
- **Complex molecule with several rings and asymmetric centres**
- **Difficult to synthesise**

6.4 Simplification of lead compound

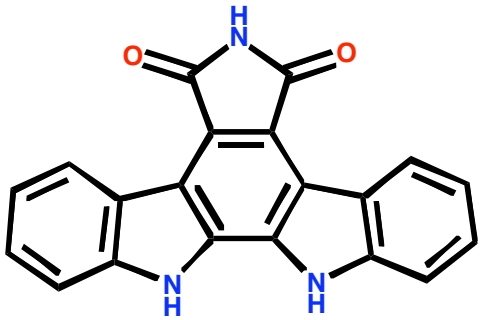


Staurosporine

Simplification
Remove asymmetric ring



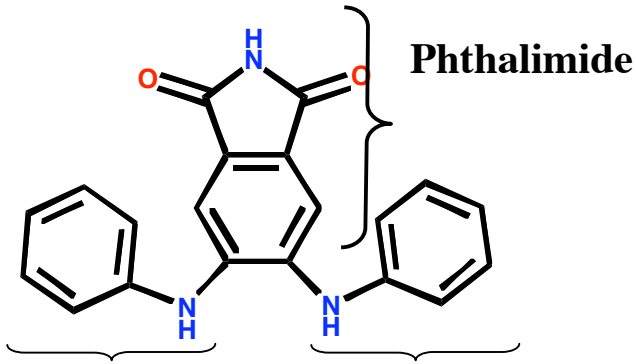
Simplification
Symmetry



Arcyriaflavin A

- **Symmetrical molecule**
- **Active and selective vs PKC but not EGF-R**

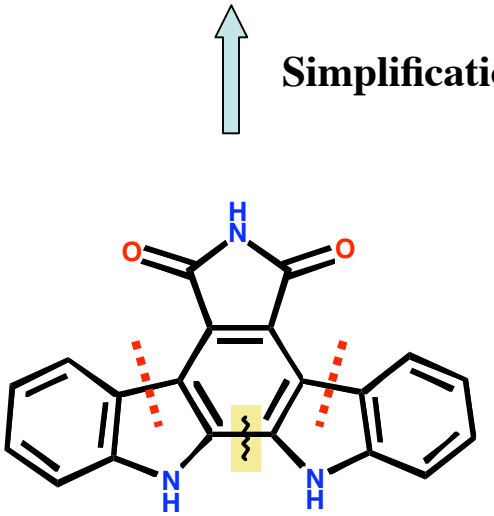
6.4 Simplification of lead compound



Dianilinophthalimide (CGP 52411)

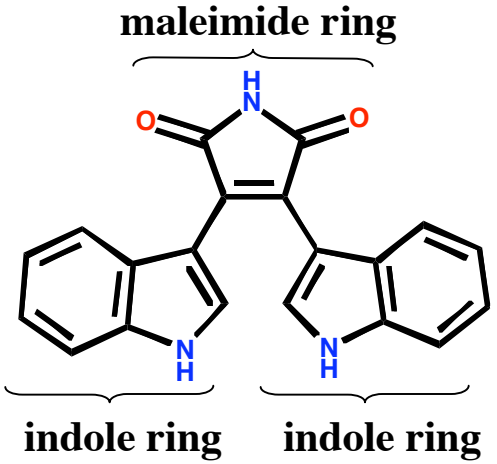
- **Selective inhibitor for EGF receptor and not other kinases**
- **Reversal of selectivity**

Simplification



Simplification

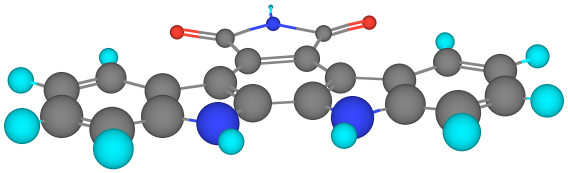
Bisindolylmaleimides
PKC selective



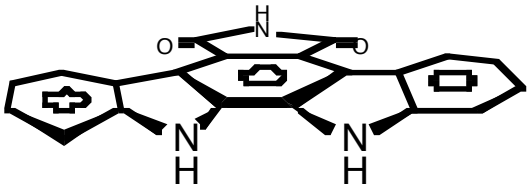
6.5 X-Ray crystallographic studies

Different shapes implicated in different selectivity

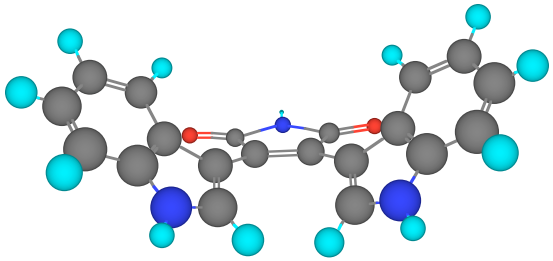
Arcyriaflavin



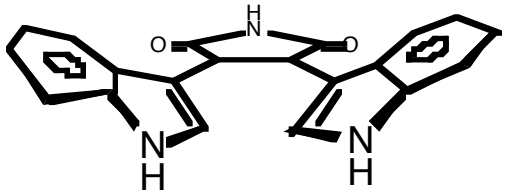
Planar



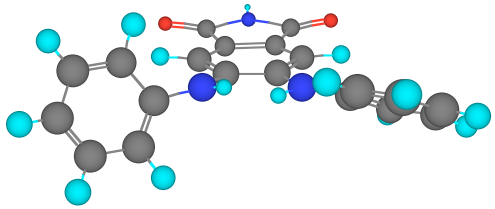
Bisindolyl-maleimides



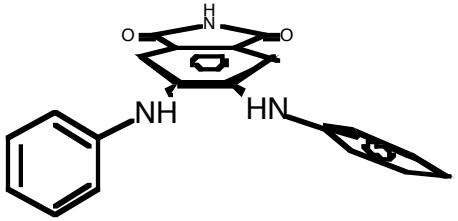
Bowl shaped



Dianilino-phthalimides

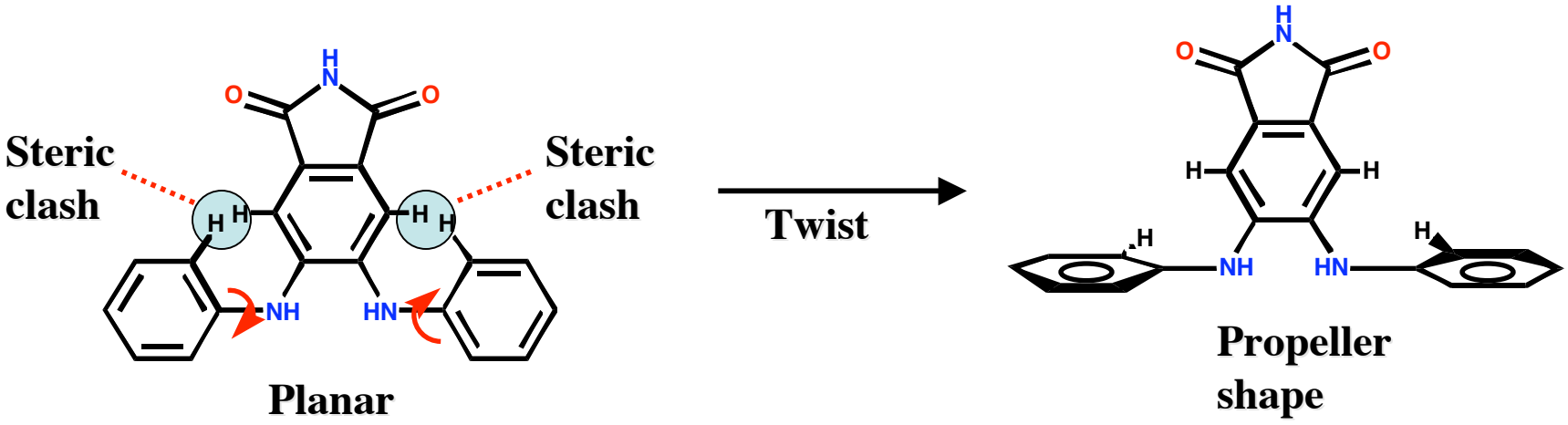


Propellor shaped asymmetric

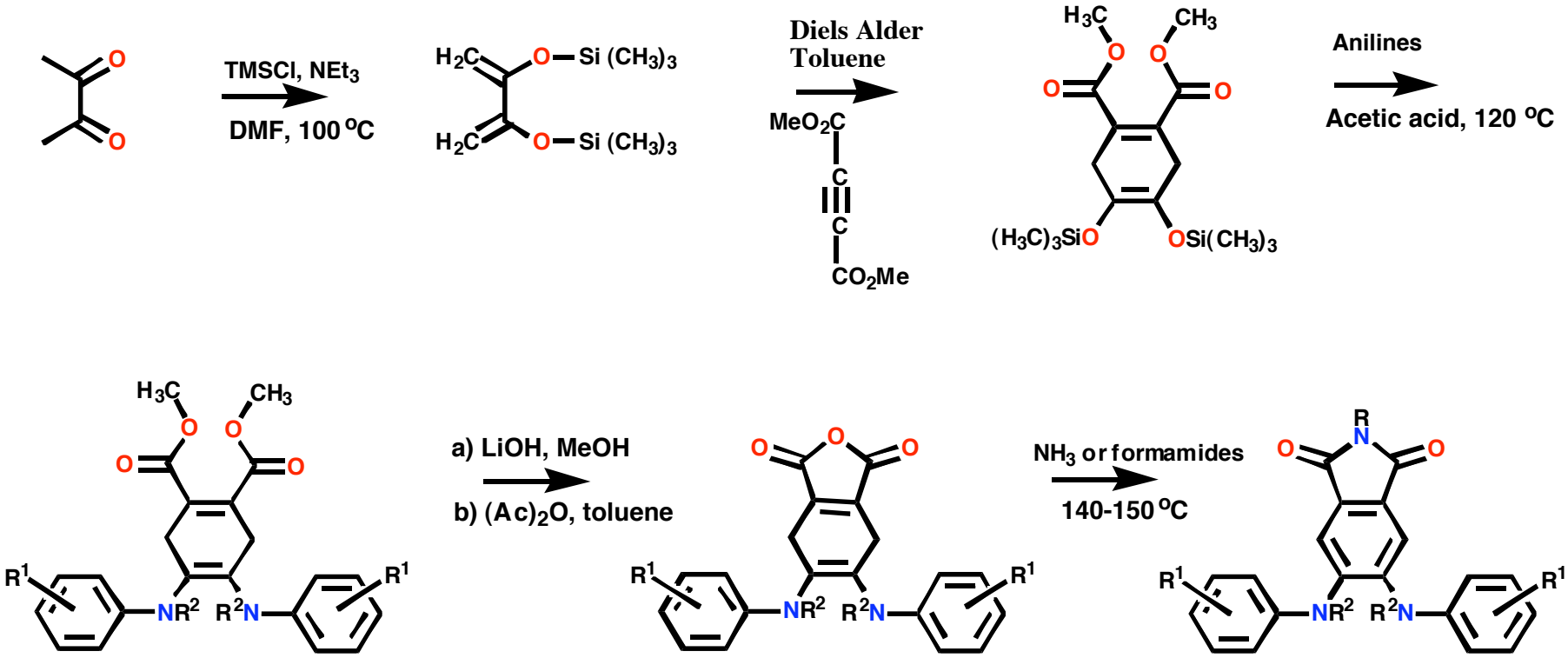


6.5 X-Ray crystallographic studies

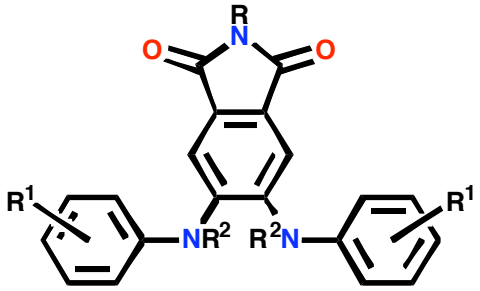
Propeller conformation relieves steric clashes



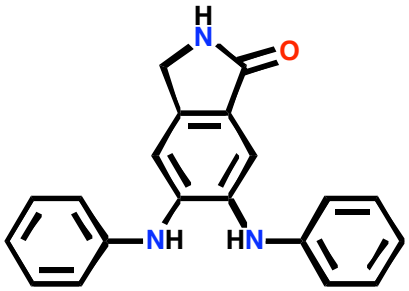
6.6 Synthesis of analogues



6.7 Structure Activity Relationships (SAR)

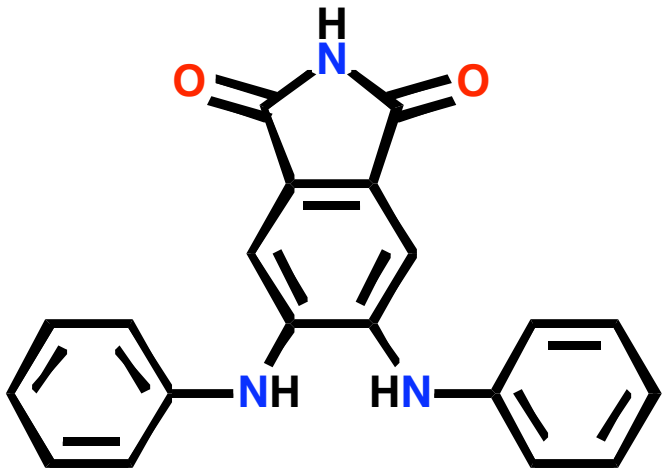


- **R=H Activity lost if N is substituted**
- **Aniline aromatic rings essential (activity lost if cyclohexane)**
- **R^1=H or F (small groups). Activity drops for Me and lost for Et**
- **R^2=H Activity drops if N substituted**
- **Aniline N's essential. Activity lost if replaced with S**
- **Both carbonyl groups important. Activity drops for lactam**



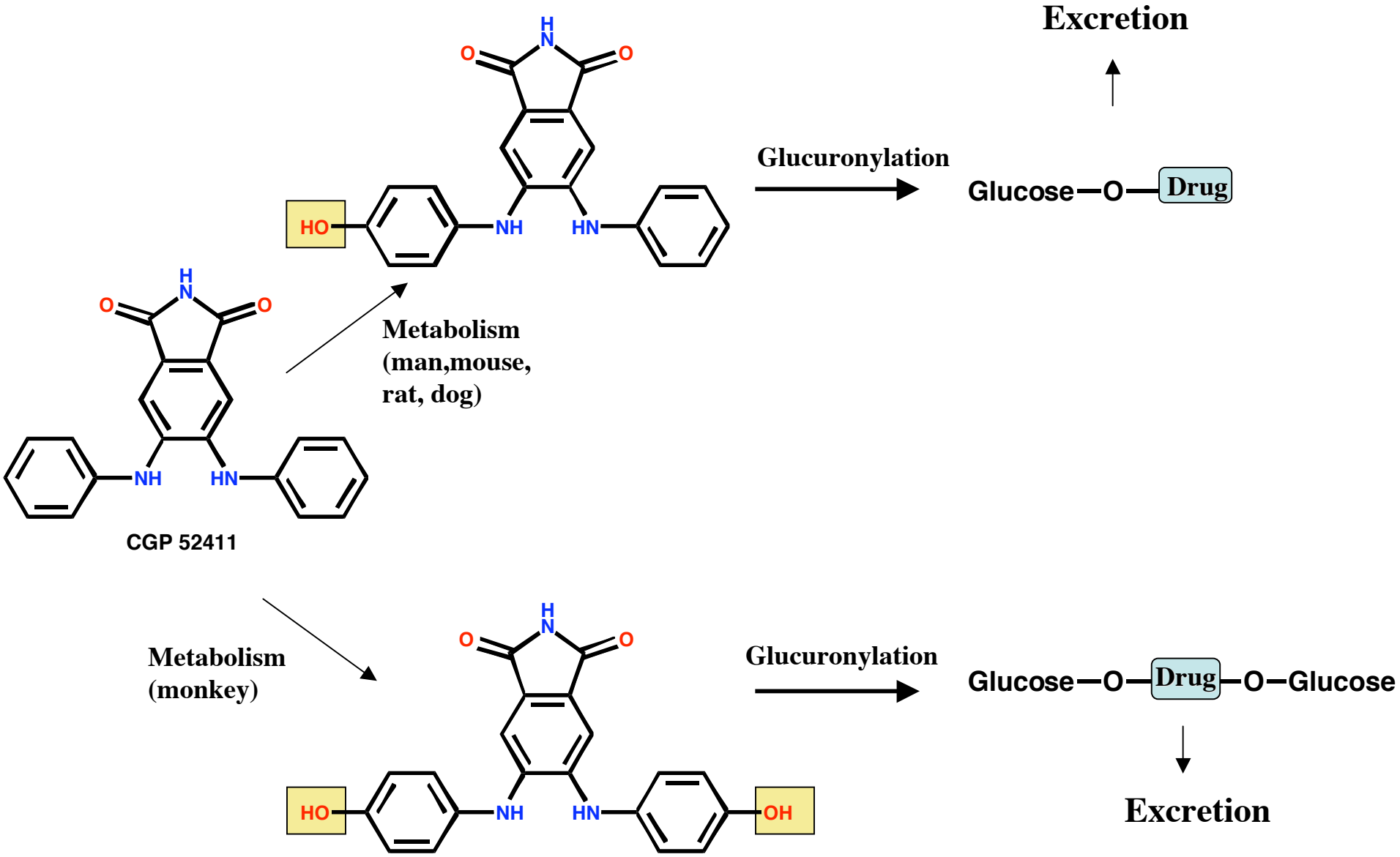
6.7 Structure Activity Relationships (SAR)

Parent Structure: $R=R^1=R^2=H$ chosen for preclinical trials
IC_{50} = 0.7 μM



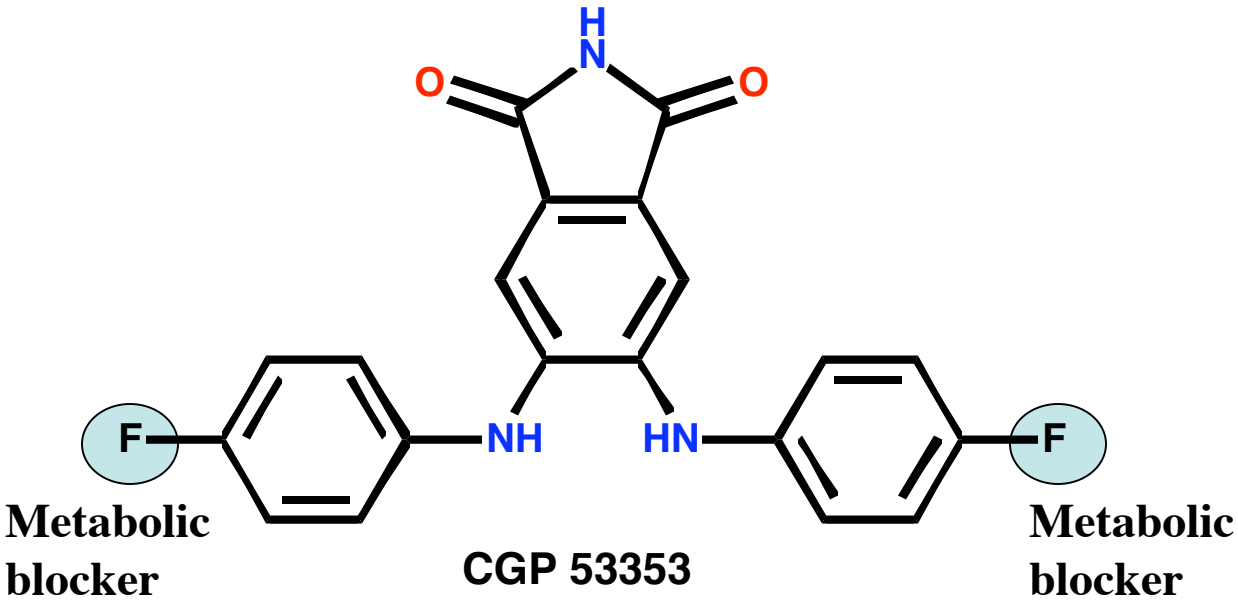
CGP 52411

6.8 Drug metabolism



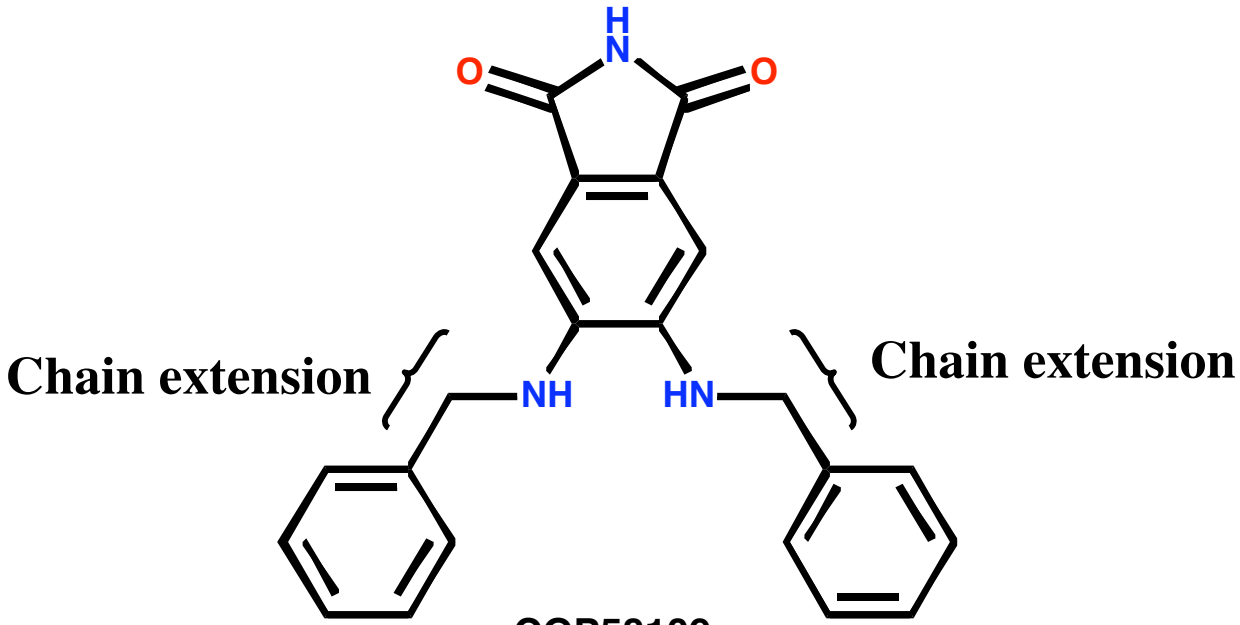
6.8 Drug metabolism

Introduce F at *para* position as metabolic blocker



6.9 Further modifications

a) Chain extension

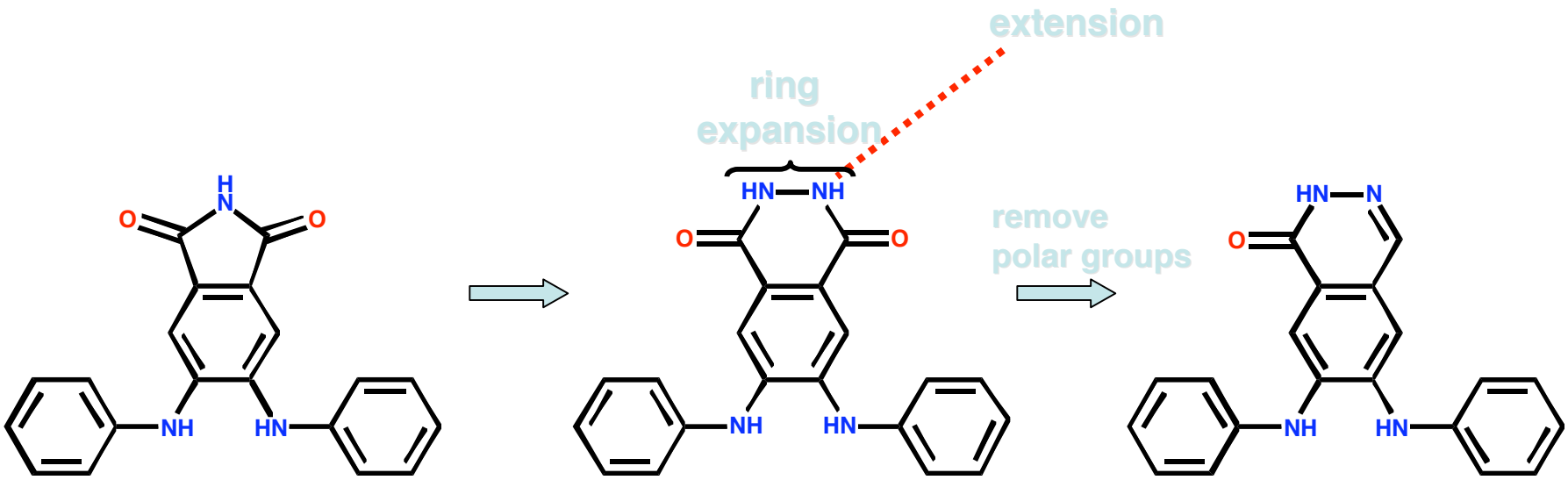


CGP58109

Activity drops

6.9 Further modifications

b) Ring extension / expansion



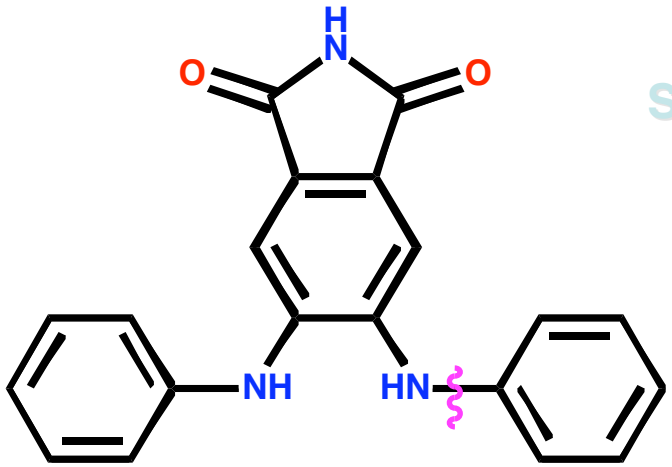
CGP 52411 (IC_{50} 0.7μM)

CGP54690 (IC_{50} 0.12μM)
Inactive in cellular assays due to polarity (unable to cross cell membrane)

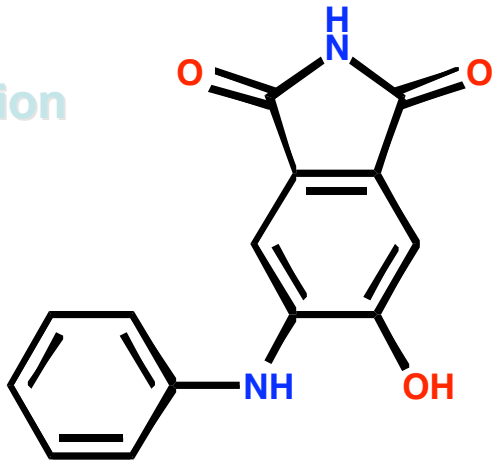
CGP57198 (IC_{50} 0.18μM)
Active *in vitro* and *in vivo*

6.9 Further modifications

c) Simplification



Simplification



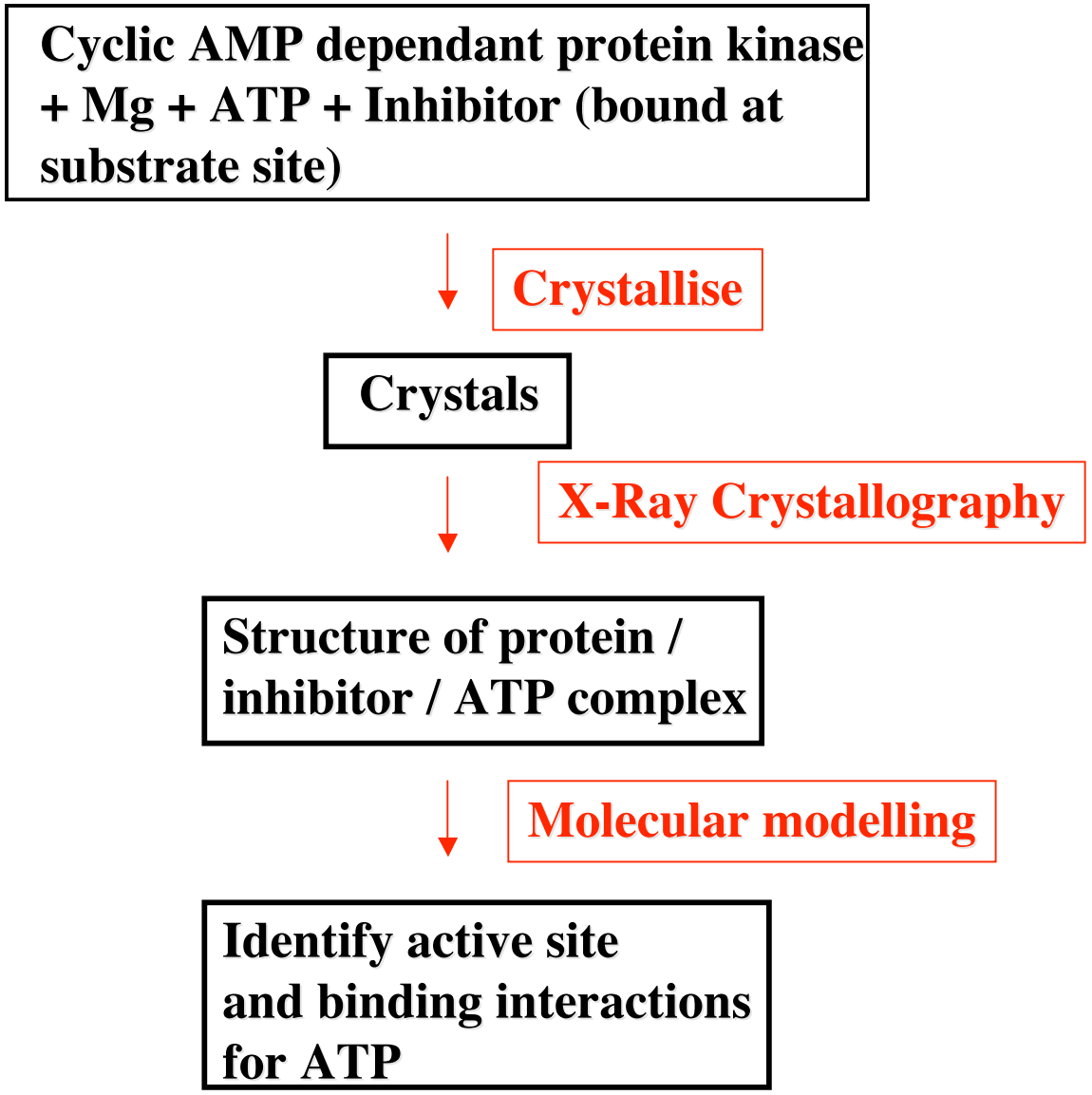
CGP52411

CGP58522
Similar activity in enzyme assay
Inactive in cellular assay

6.10 Modelling studies on ATP binding

- **No crystal structure for EGF- receptor available**
- **Make a model active site based on structure of an analogous protein which has been crystallised**
- **Cyclic AMP dependant protein kinase used as template**

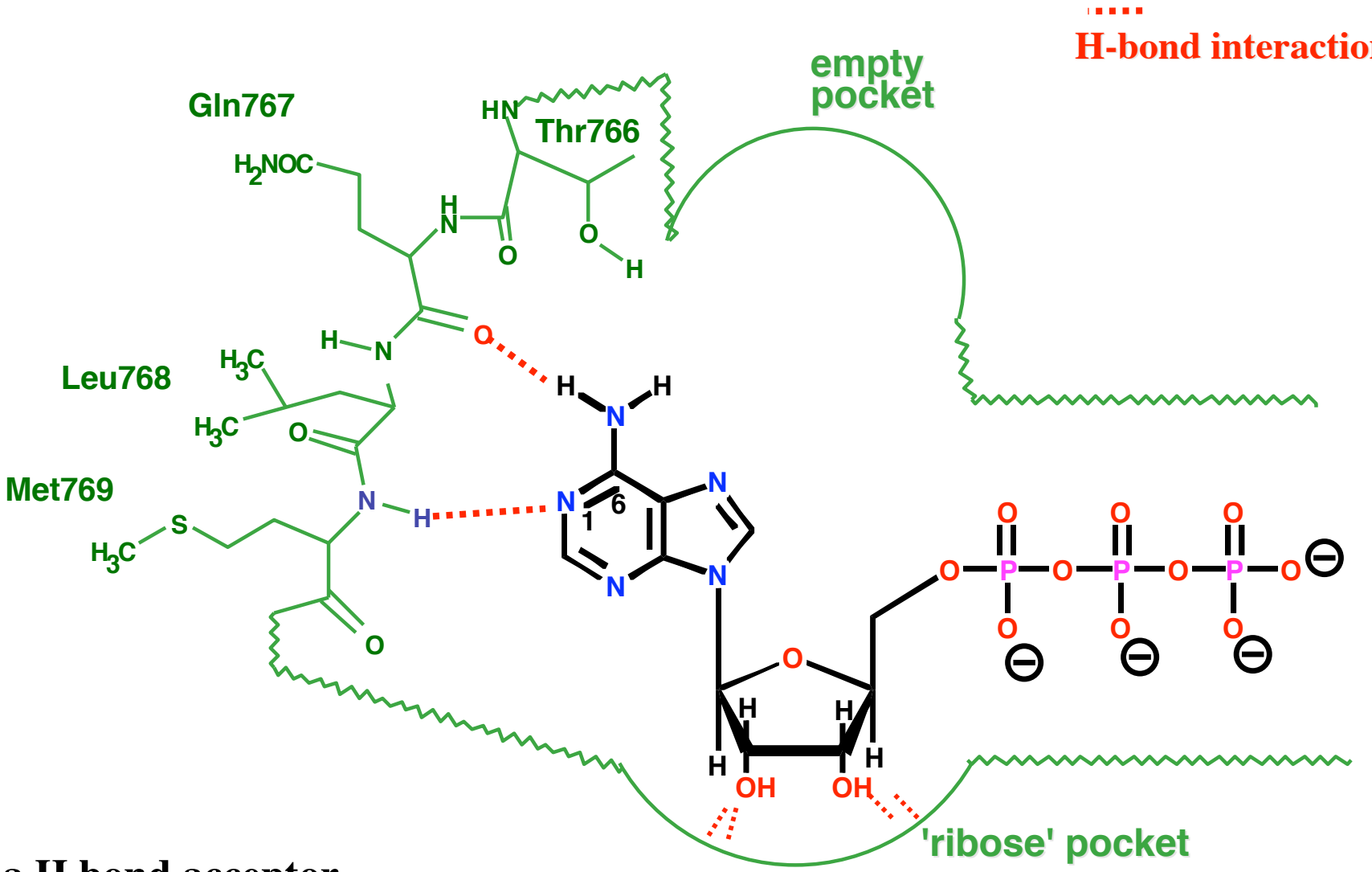
6.10 Modelling studies on ATP binding



6.10 Modelling studies on ATP binding

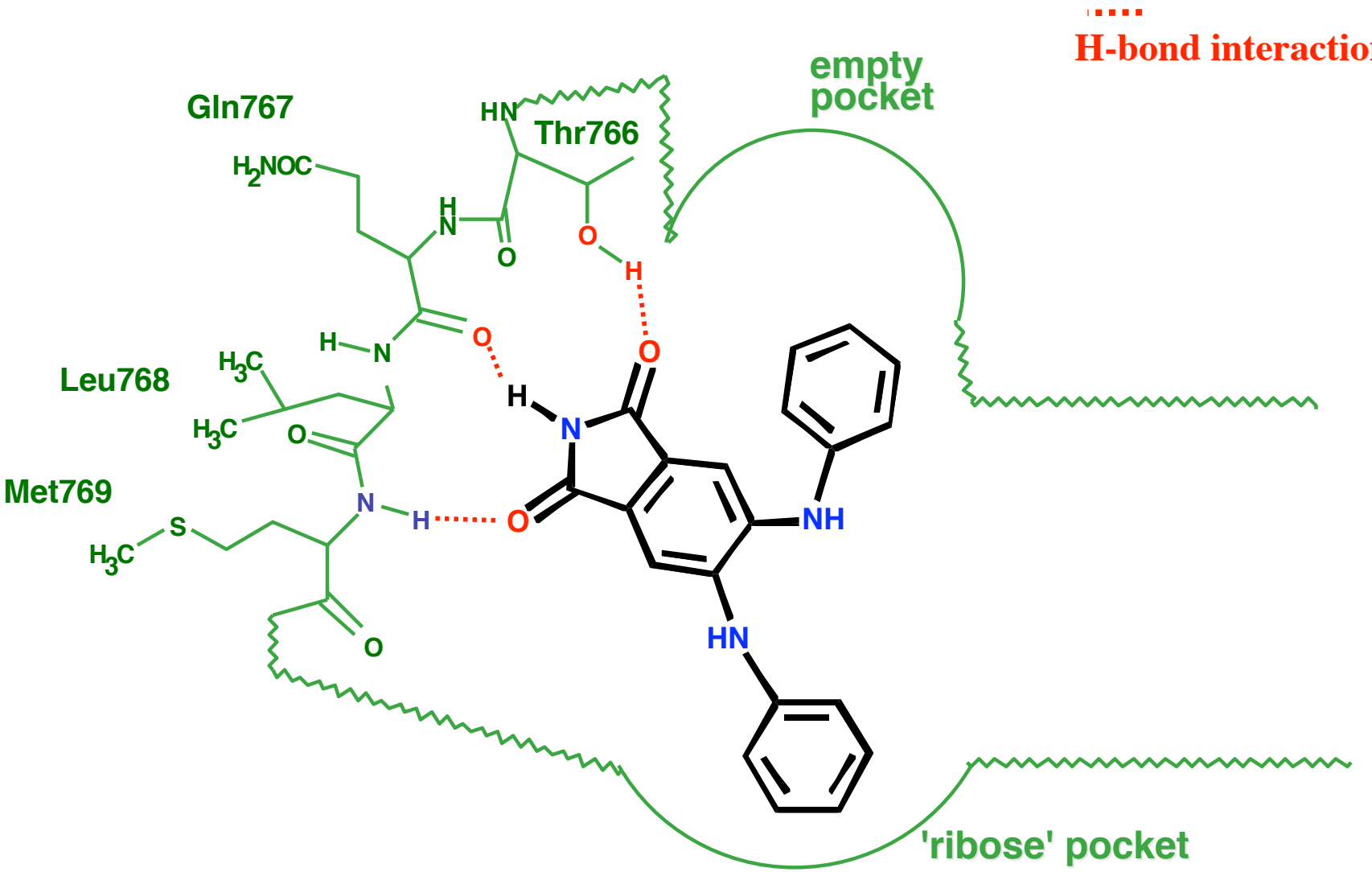
- **ATP bound into a cleft in the enzyme with adenine portion buried deep close to hydrophobic region.**
- **Ribose and phosphate extend outwards towards opening of cleft**
- **Identify binding interactions (measure distances between atoms of ATP and complementary atoms in binding site to see if they are correct distance for binding)**
- **Construct model ATP binding site for EGF-receptor kinase by replacing amino acid's of cyclic AMP dependent protein kinase for those present in EGF receptor kinase**

6.10 Modelling studies on ATP binding



^{1}N is a H bond acceptor
6-NH_2 is a H-bond donor
Ribose forms H-bonds to Glu in ribose pocket

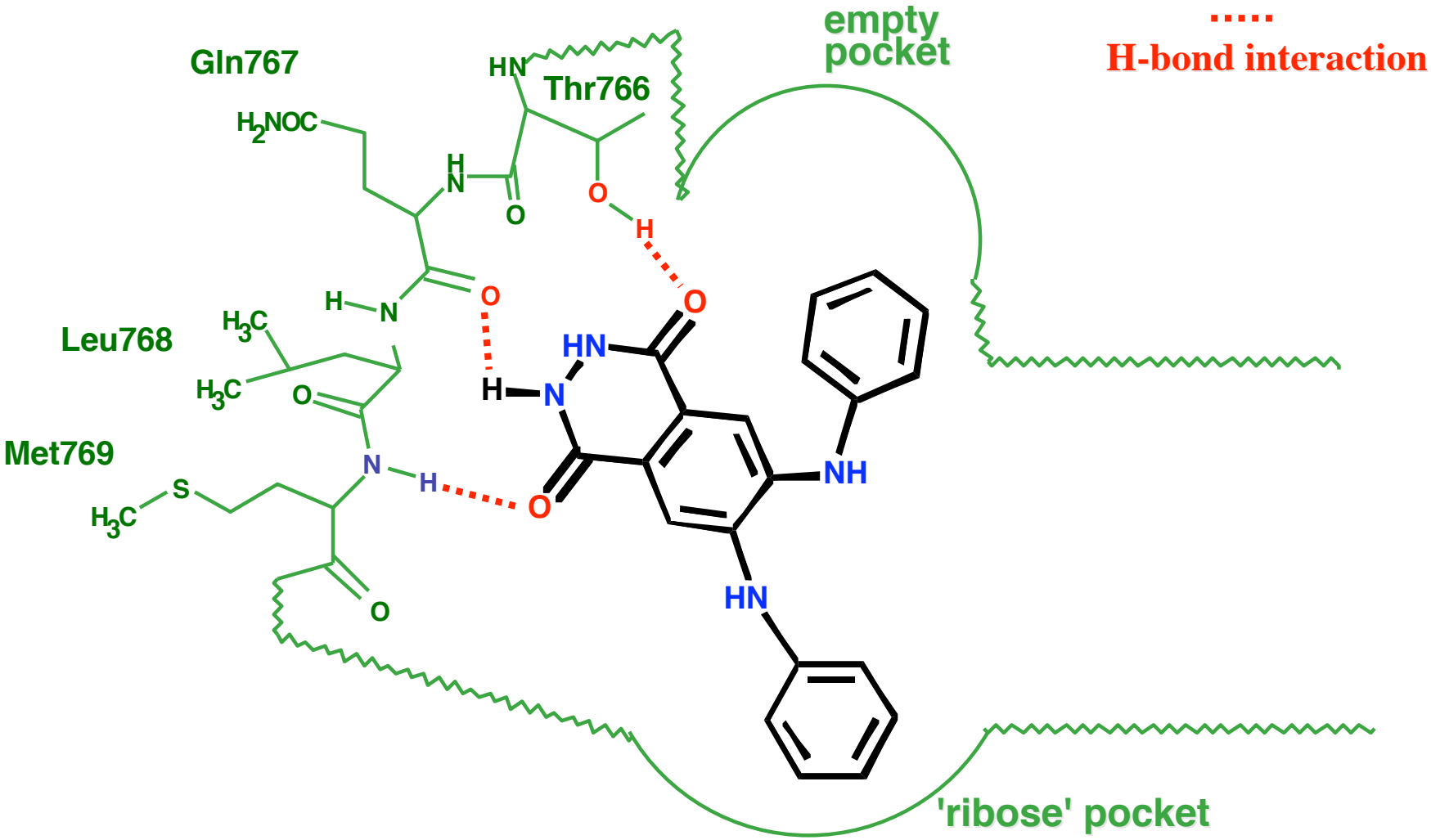
6.11 Model binding studies on Dianilinophthalimides



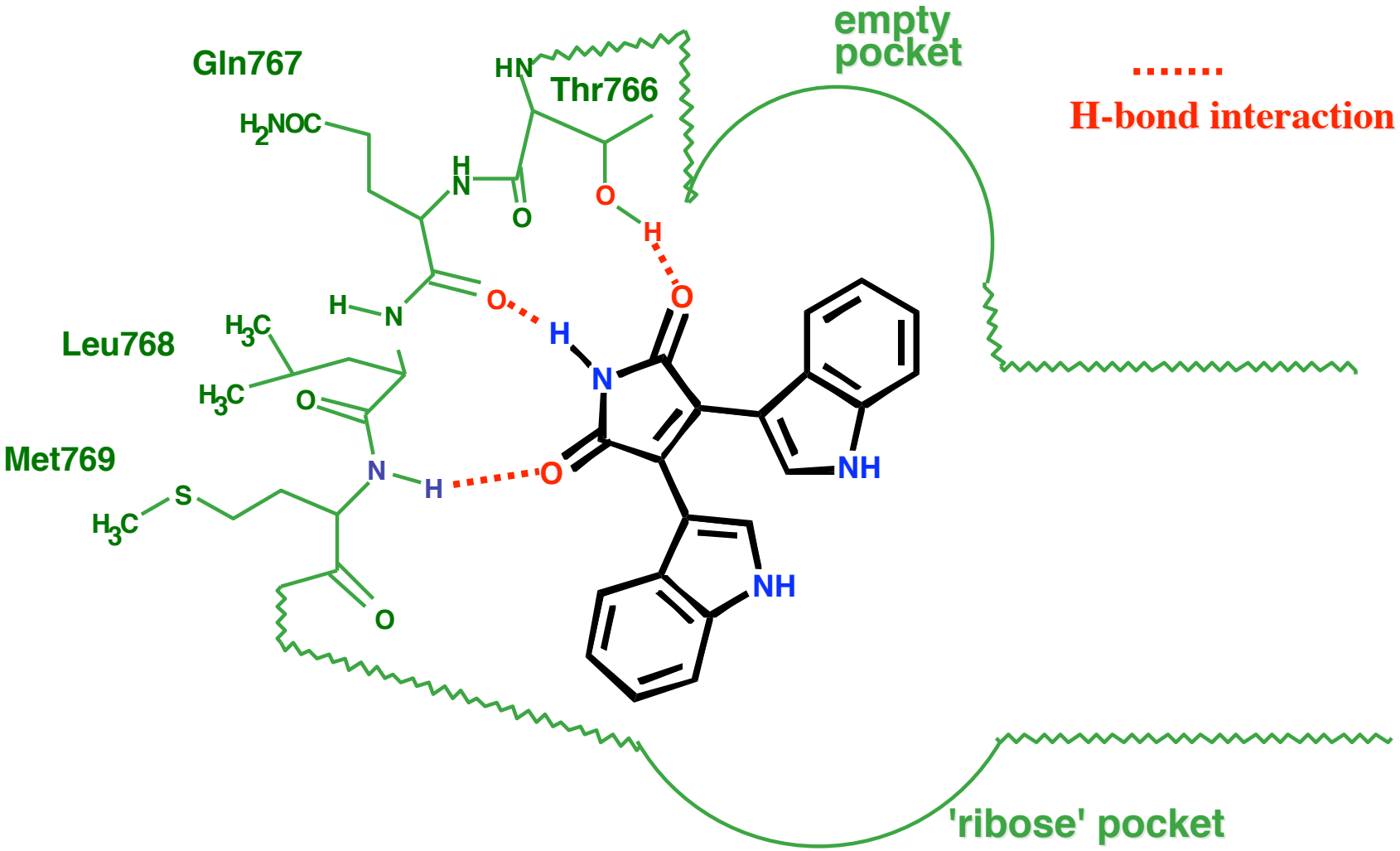
6.11 Model binding studies on Dianilinophthalimides

- **Both imide carbonyls act as H-bond acceptors (disrupted if carbonyl reduced)**
- **Imide NH acts as H bond donor (disrupted if N is substituted)**
- **Aniline aromatic ring fits small tight ribose pocket**
- **Substitution on aromatic ring or chain extension prevents aromatic ring fitting pocket**
- **Bisindolylmaleimides form H-bond interactions but cannot fit aromatic ring into ribose pocket.**
- **Implies ribose pocket interaction is crucial for selectivity**

6.11 Model binding studies on Dianilinophthalimides



6.11 Model binding studies on Dianilinophthalimides



6.12 Selectivity of action

POSERS ?

- **Ribose pocket normally accepts a polar ribose so why can it accept an aromatic ring?**

- **Why can't other kinases bind dianilinophthalimides in the same manner?**

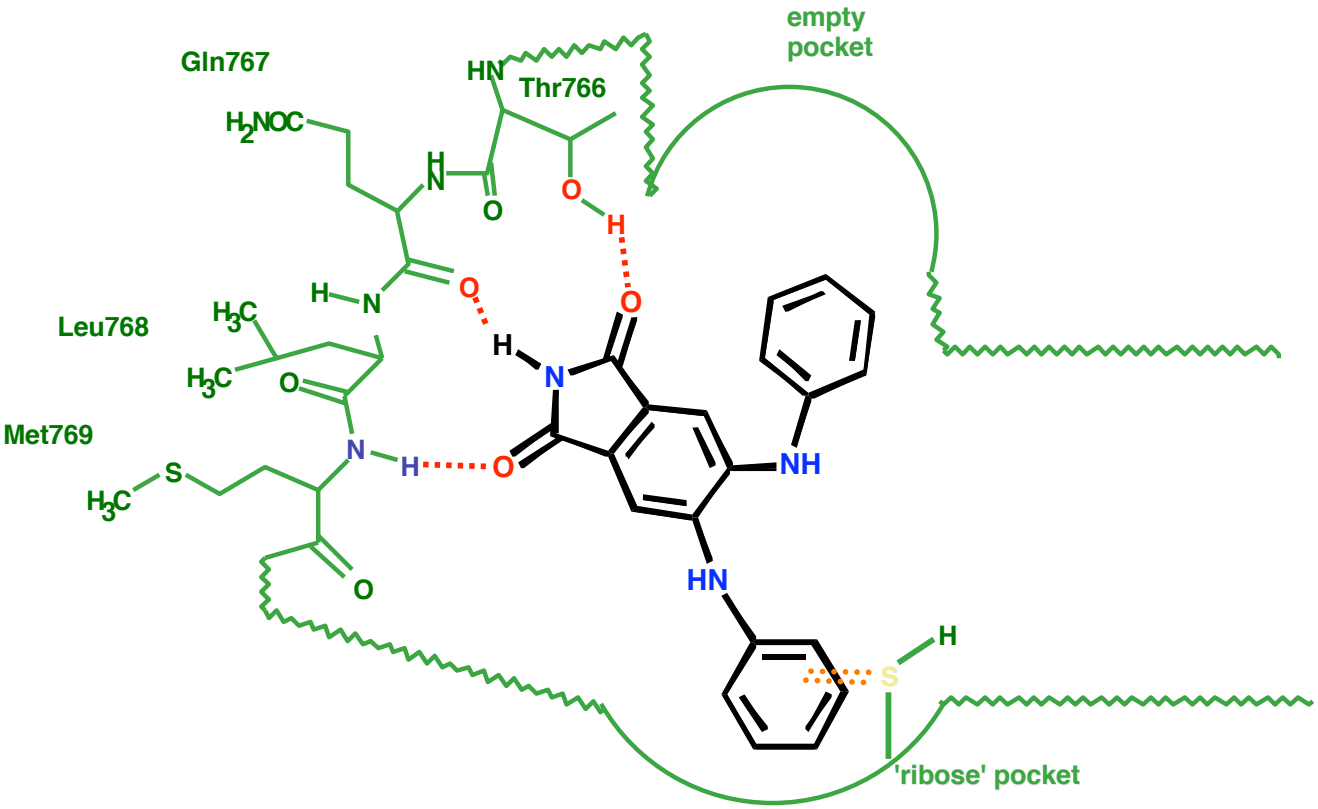
6.12 Selectivity of action

Amino Acids present in the ribose pocket

	Hydrophobic	Hydrophilic
Protein Kinase A	Leu,Gly,Val,Leu	Glu,Glu,Asn,Thr
EGF Receptor Kinase	Leu,Gly,Val,Leu,Cys	Arg,Asn,Thr

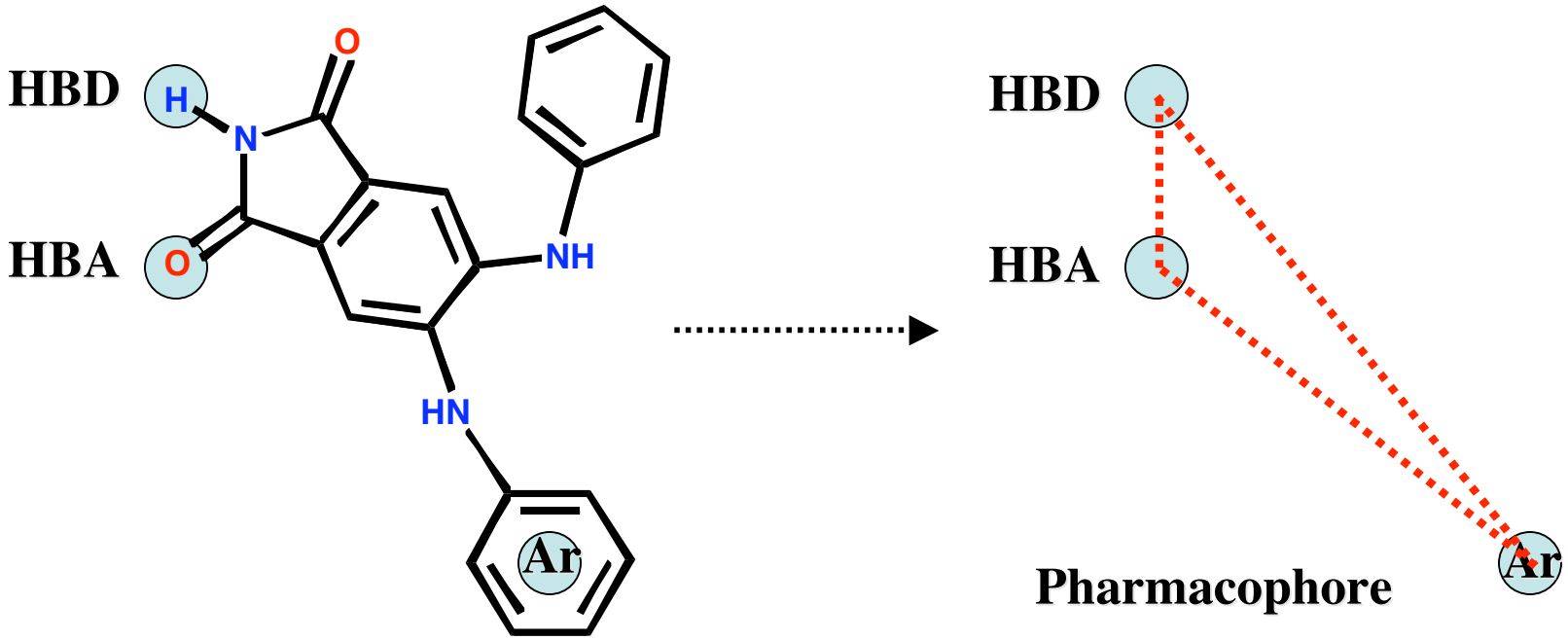
6.12 Selectivity of action

- **Ribose pocket is more hydrophobic in EGF-receptor kinase**
- **Cys can stabilise and bind to aromatic rings (S-Ar interaction)**



- **Stabilisation by S-Ar interaction not present in other kinases**
- **Leads to selectivity of action**

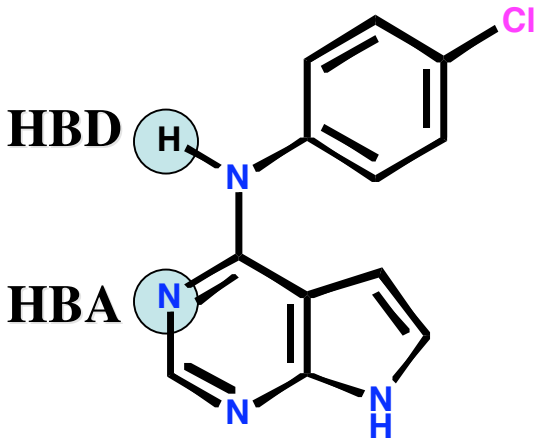
6.13 Pharmacophore for EGF-receptor kinase inhibitors



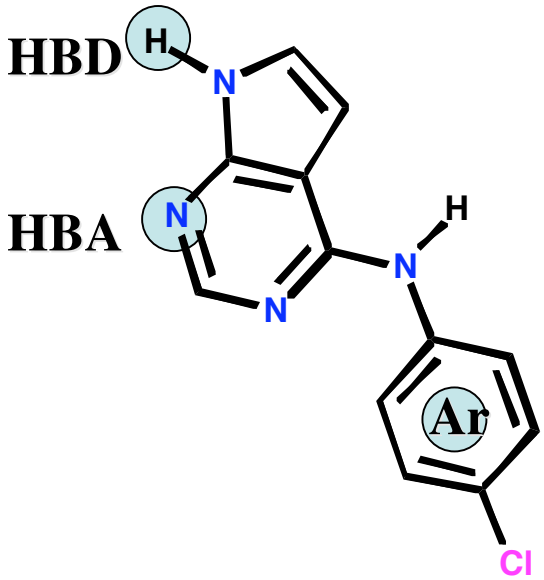
- **Pharmacophore allows identification of other potential inhibitors**
- **Search databases for structures containing same pharmacophore**
- **Can rationalise activity of different structural classes of inhibitor**

6.14 Phenylaminopyrrolopyrimidines

CGP 59326 - Two possible binding modes for H-bonding



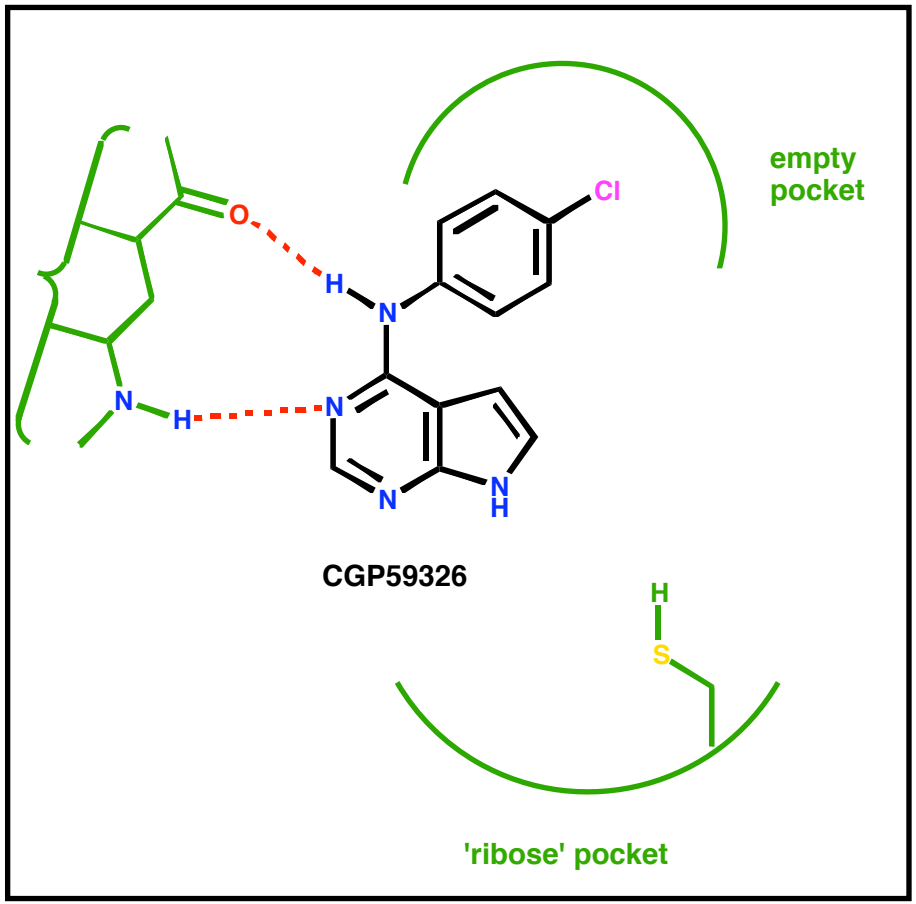
Mode I

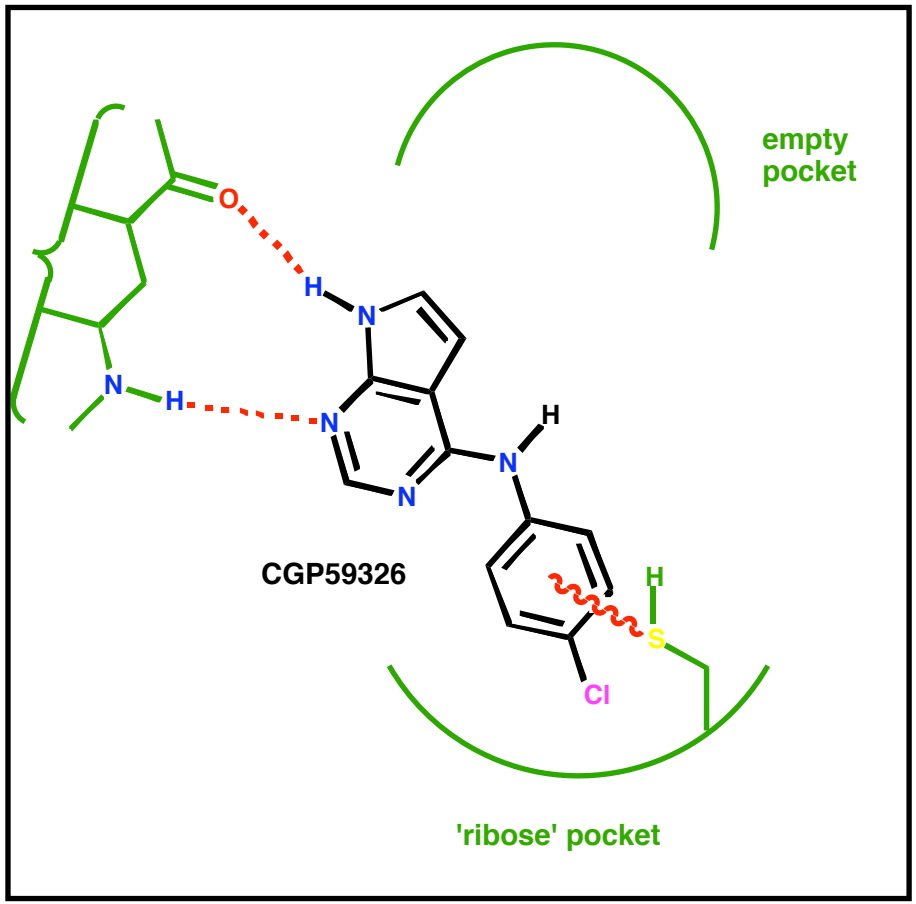


Mode II

Only mode II tallies with pharmacophore and explains activity and selectivity

6.14 Phenylaminopyrrolopyrimidines



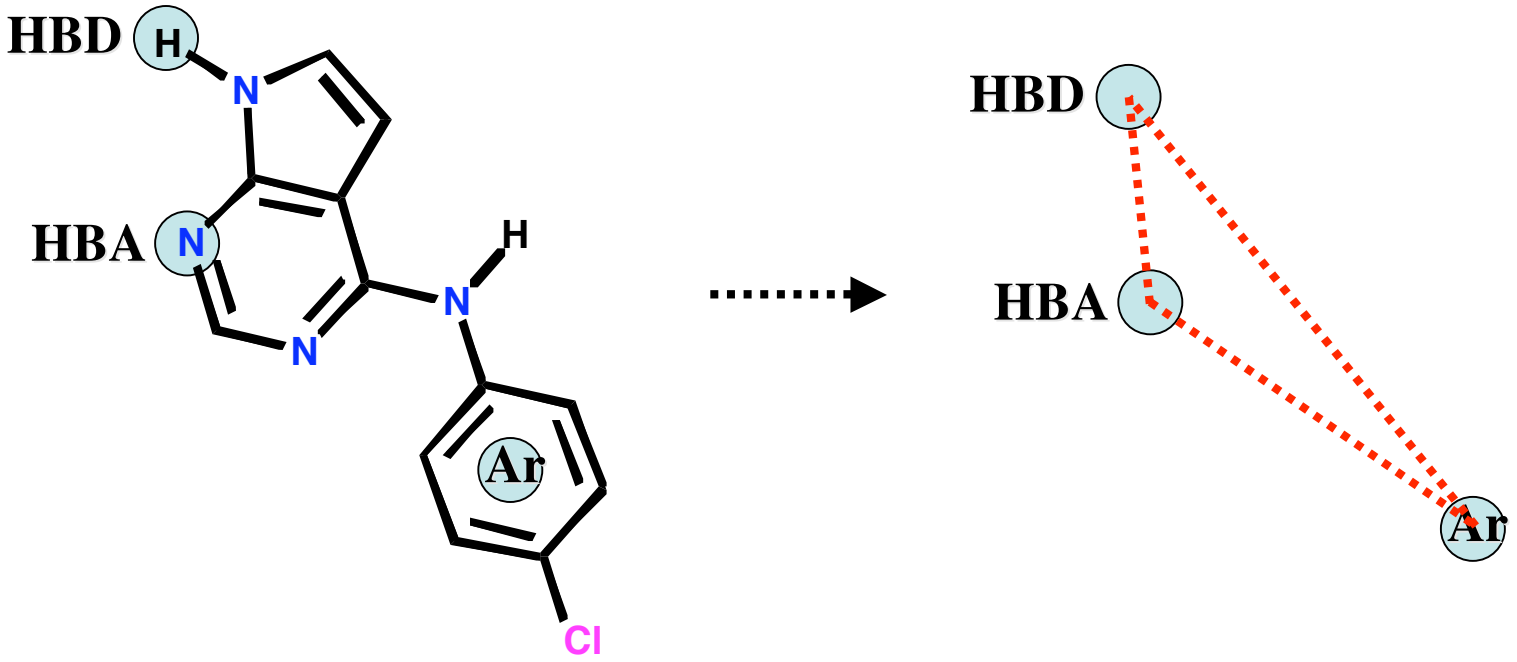


Binding Mode I like ATP (not favoured)

Binding mode II (favoured)

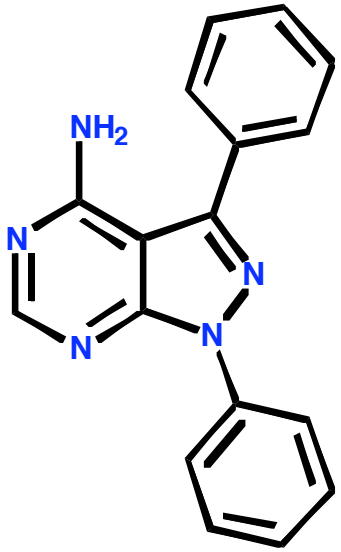
Illustrates dangers in comparing structures and assuming similar interactions (e.g. comparing CGP59326 with ATP)

6.14 Phenylaminopyrrolopyrimidines

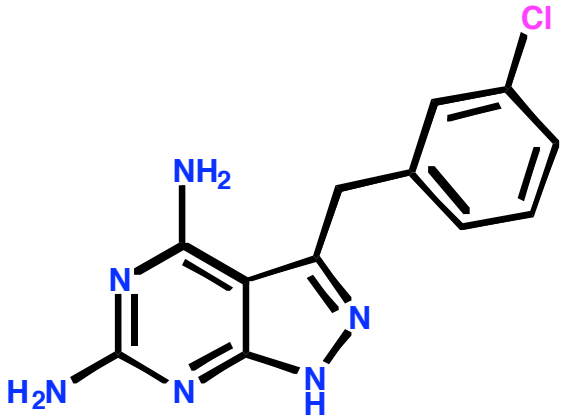


6.15 Pyrazolopyrimidines

i) Lead compounds



(I) EC50 0.80μM

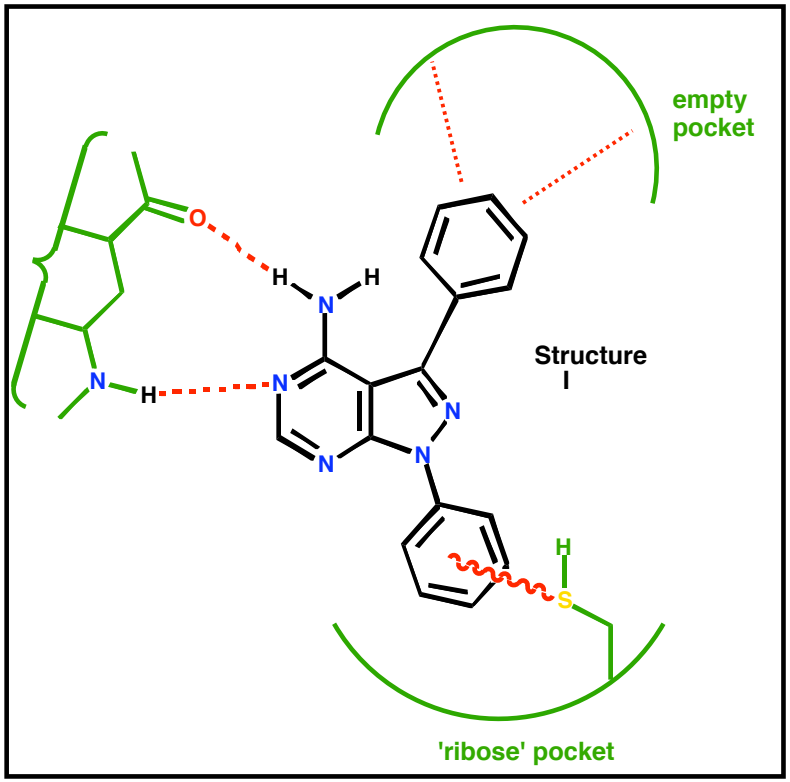


(II) EC50 0.22μM

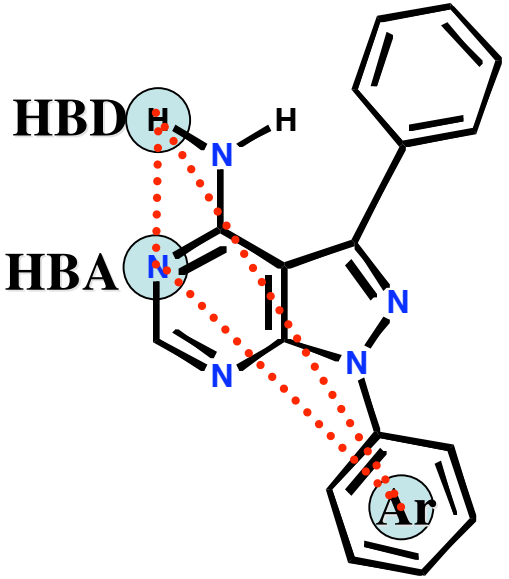
- **Both structures are selective EGF-receptor kinase inhibitors**
- **Both structures belong to same class of compounds**
- **Docking experiments reveal different binding modes to obey pharmacophore**

6.15 Pyrazolopyrimidines

ii) Structure I

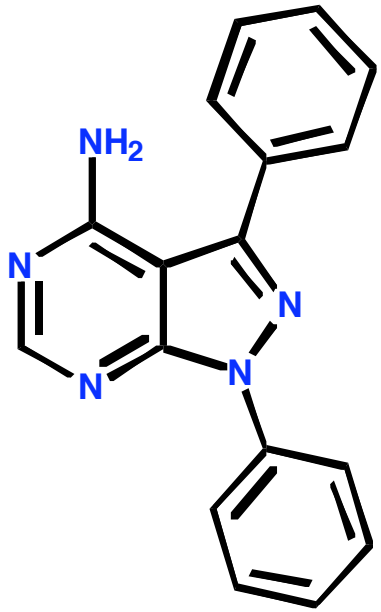


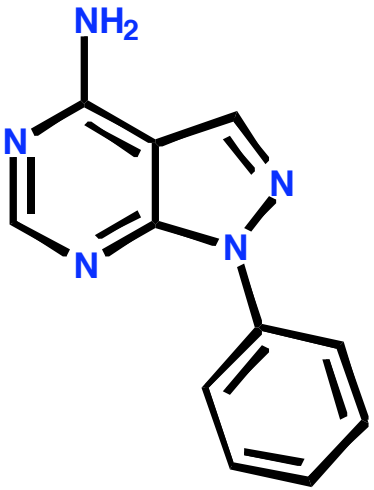
Extra binding interactions



6.15 Pyrazolopyrimidines

ii) Structure I



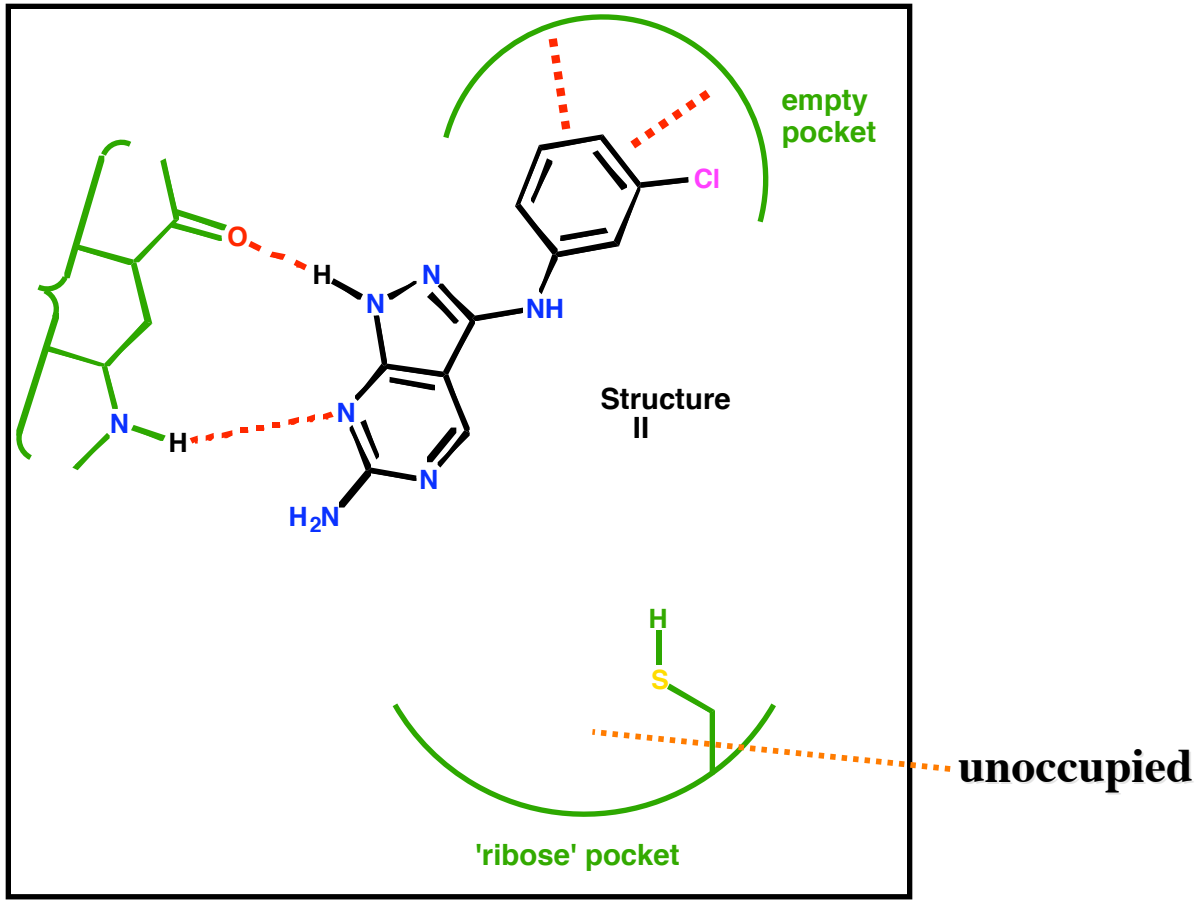


(I) EC_{50} 0.80μM

(III) EC_{50} 2.7μM

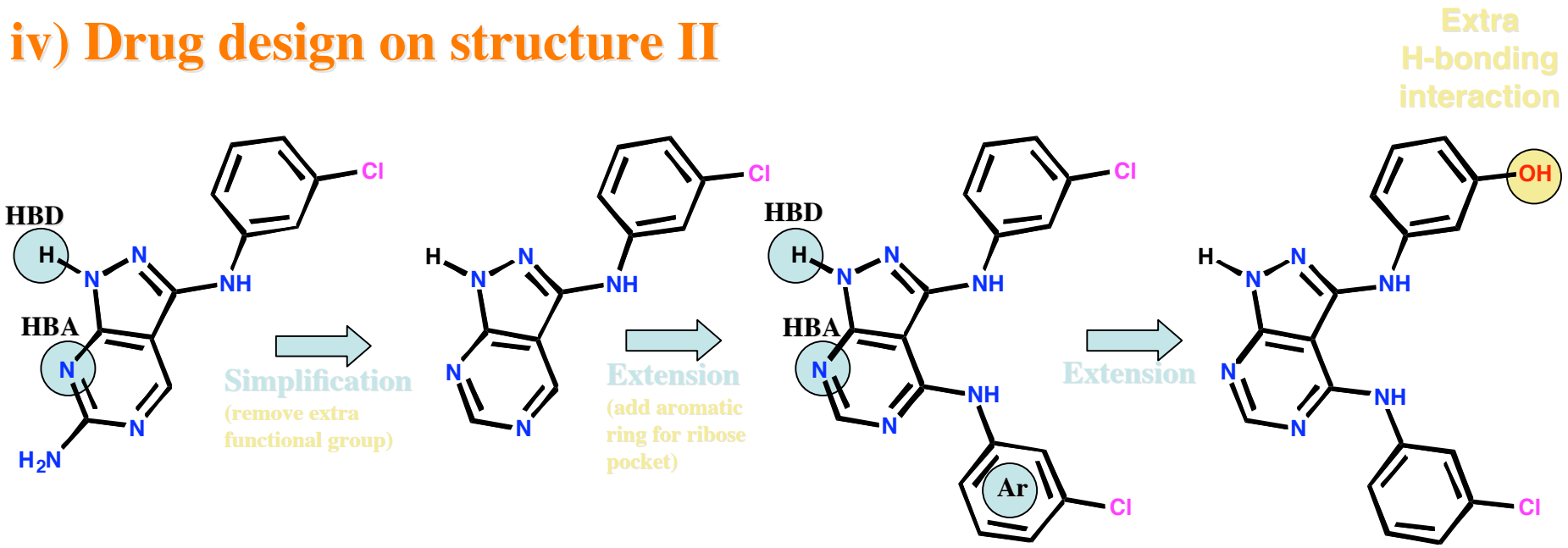
6.15 Pyrazolopyrimidines
iii) Structure II

- **Cannot bind in same mode since no fit to ribose pocket**
- **Binds in similar mode to phenylaminopyrrolopyrimidines**



6.15 Pyrazolopyrimidines

iv) Drug design on structure II



(II)
EC_{50} 0.22μM

(IV)
EC_{50} 0.16μM
Activity increases

(V)
EC_{50} 0.033μM
Activity increases
Ar fits ribose pocket

(VI)
EC_{50} 0.001μM
Activity increases

- **Upper binding pocket is larger than ribose pocket allowing greater variation of substituents on the 'upper' aromatic ring**