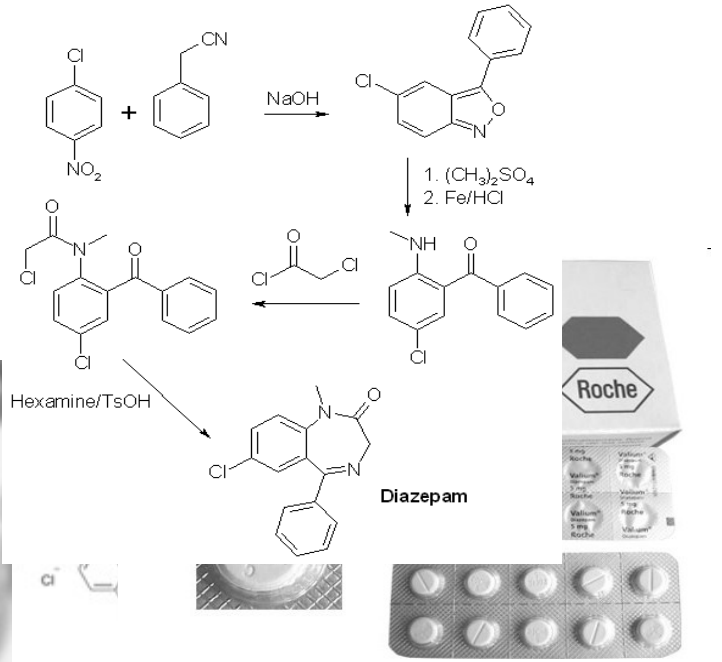
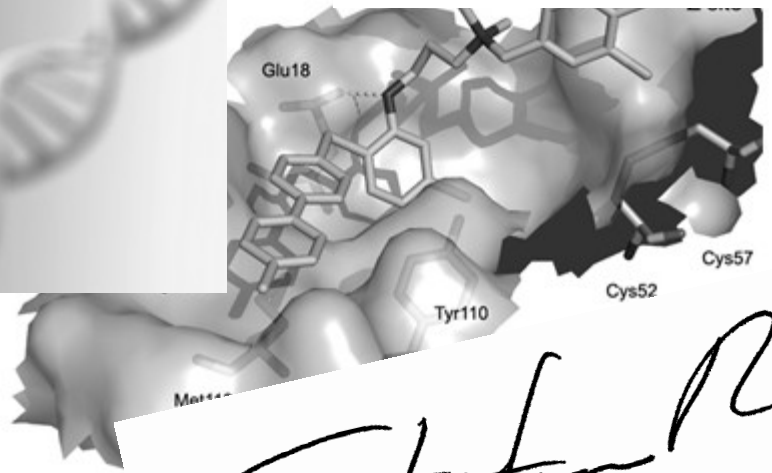
Chimica Farmaceutica e Tossicologica – Parte II





Menù (turistico) del trimestre di CFTII

- **Parte prima: generale**

Le basi chimico-fisiche dell'interazione farmaco-bersaglio molecolare;

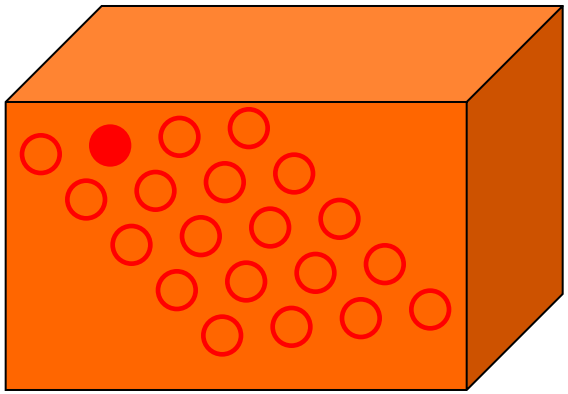
✓ ***Sistemi di comunicazione cellulare***

- **Parte seconda: sistematica**

Farmaci terapeuticamente rilevanti in patologie del SNC, dell'apparato cardiovascolare, del sistema immunitario e del sistema riproduttivo

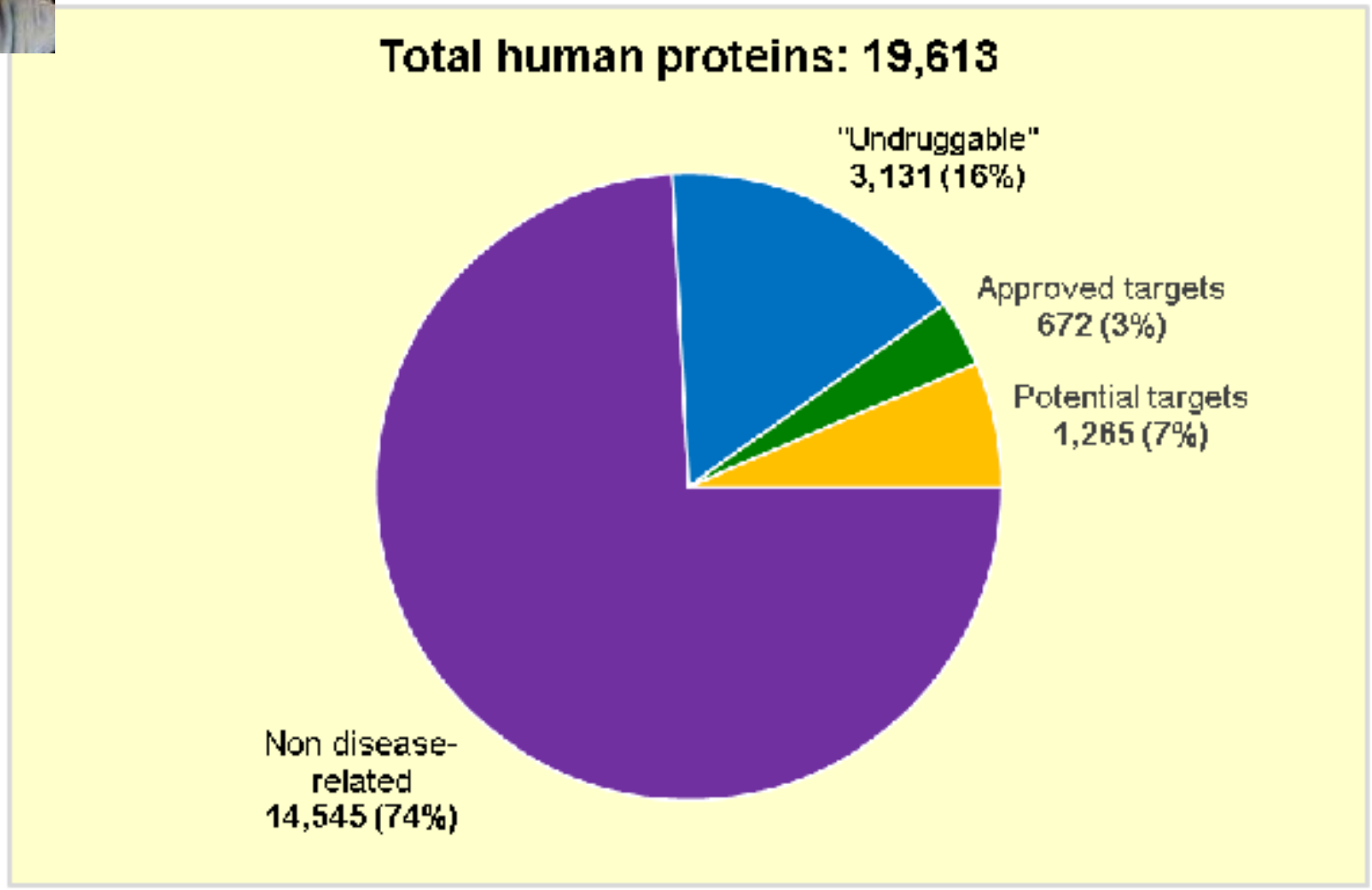
Be patient, I take off the jacket of pharmaceutical and chemical, undeservedly, I wear the pharmacologist:

Biological or "On" Target



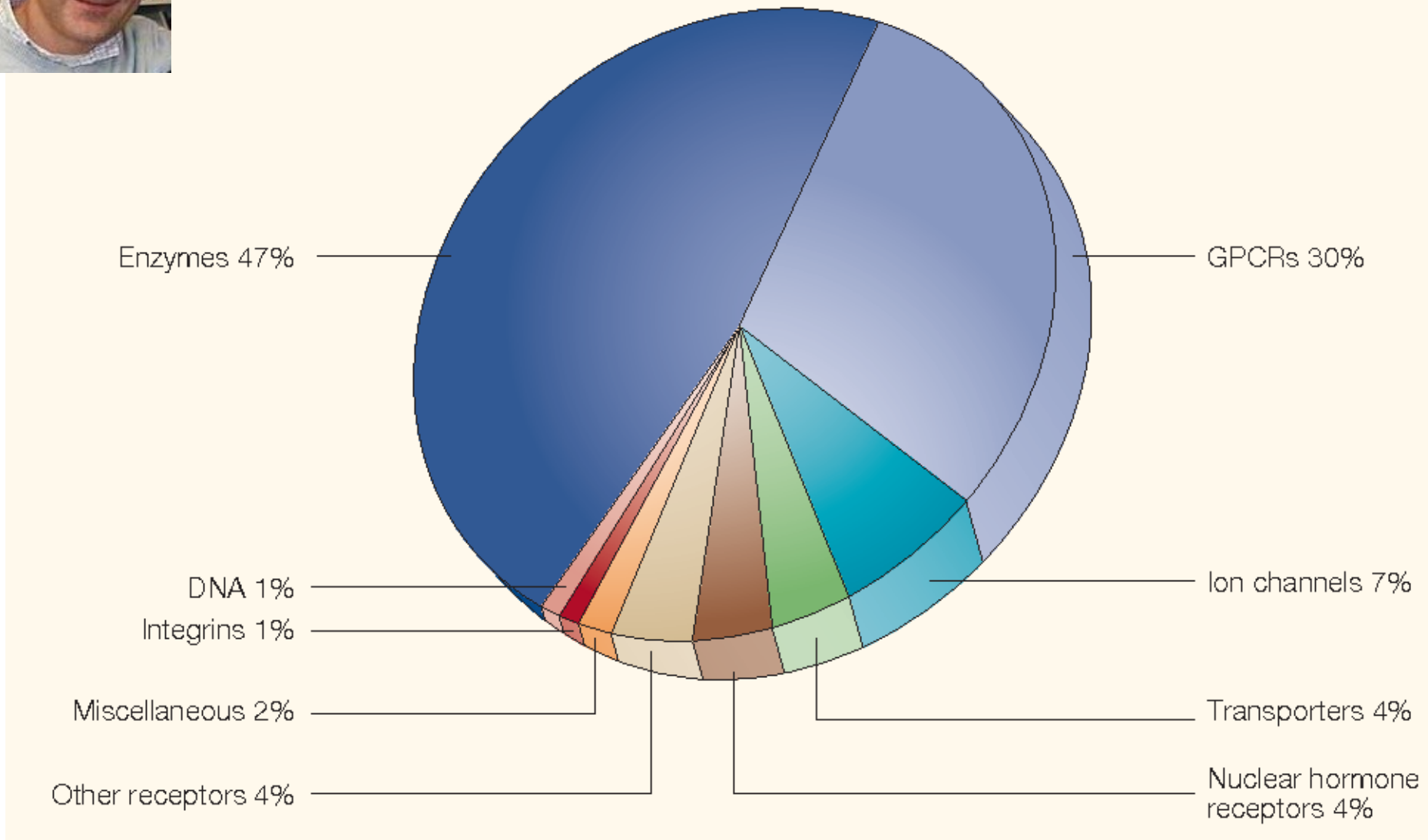
Biological Space

Really very interesting: the Human Protein Atlas…



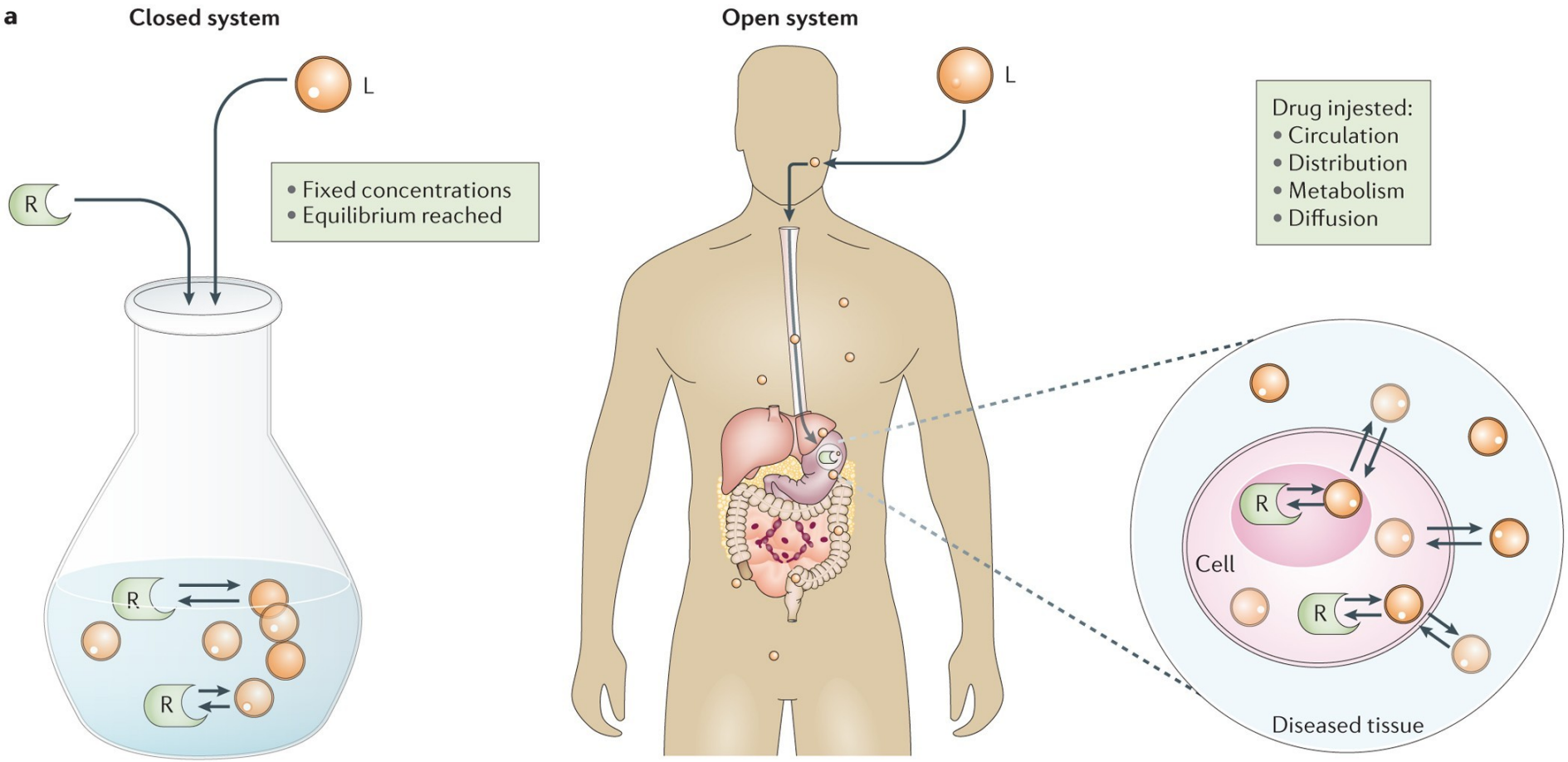
credits: https://www.proteinatlas.org/

Druggable targets…



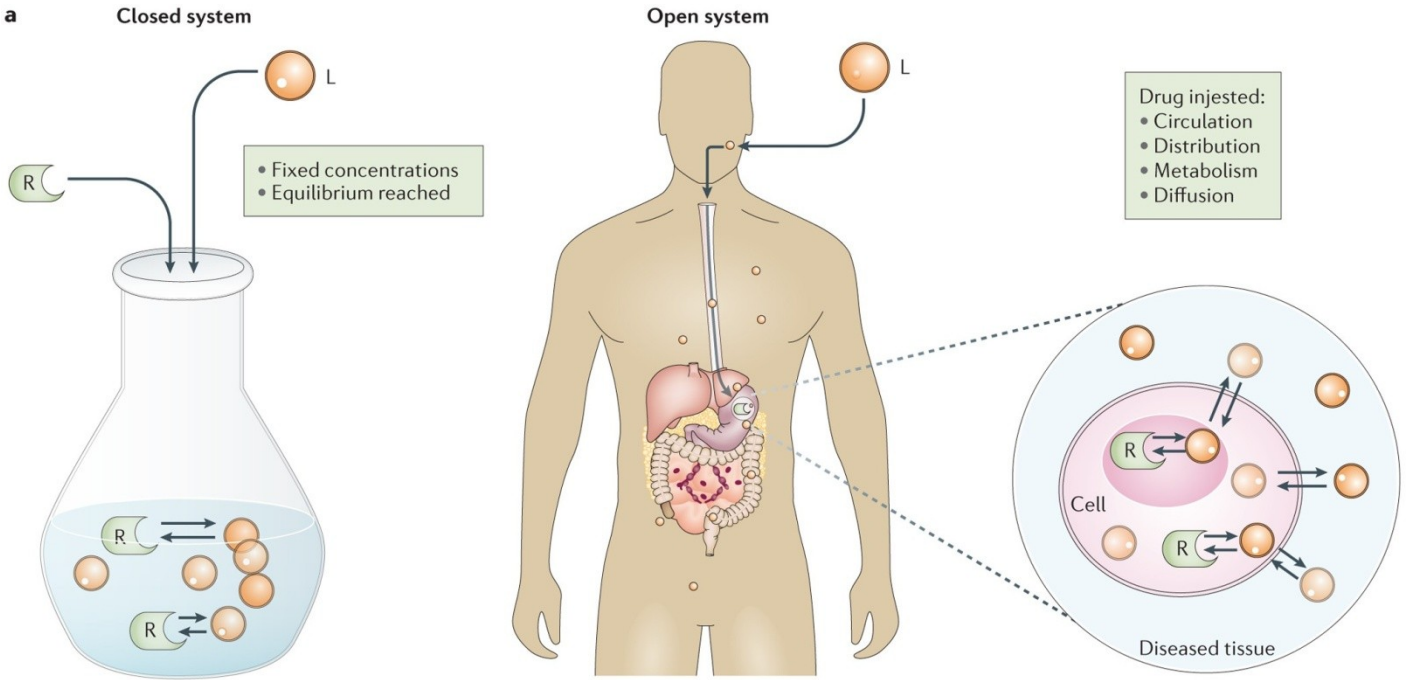
credits: https://www.semanticscholar.org/paper/The-druggable-genome-Hopkins-Groom/648a2ef46c4f7b335741111c445ee26adf2f0644/

A propedeutic concept: systems theory



credits: https://www.nature.com/articles/nrd.2015.18 /

A propedeutic concept: systems theory

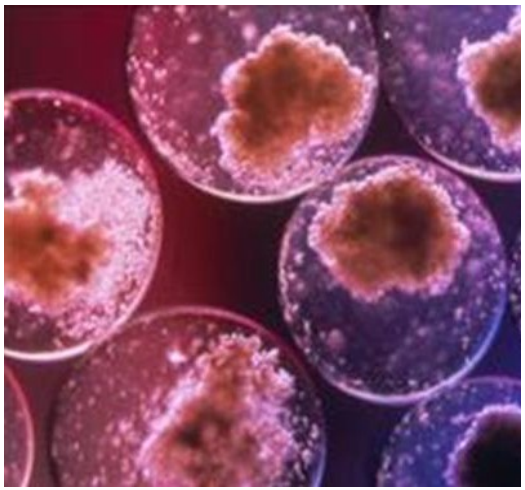


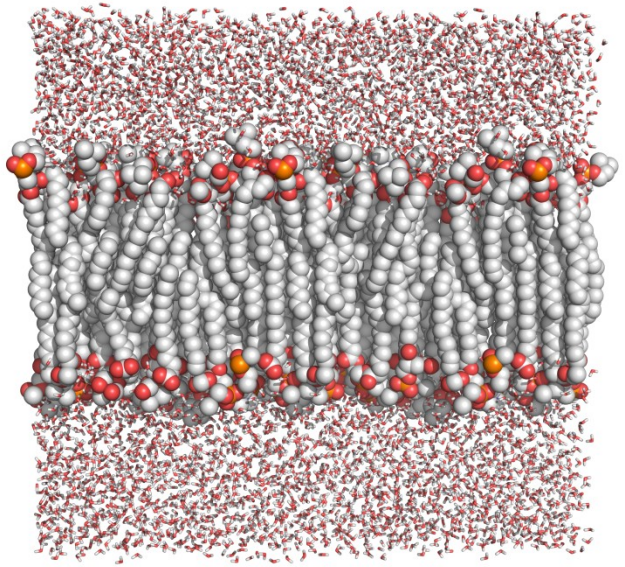
$$K_{eq} = \frac{k_{on}}{k_{off}} \qquad \text{residence time, } \tau = \frac{1}{k_{off}}$$

credits: https://www.nature.com/articles/nrd.2015.18/ ; https://www.nature.com/articles/nrd.2018.234/

Fundamentals of cellular communication theory:

Organization ⇔ Compartmentation



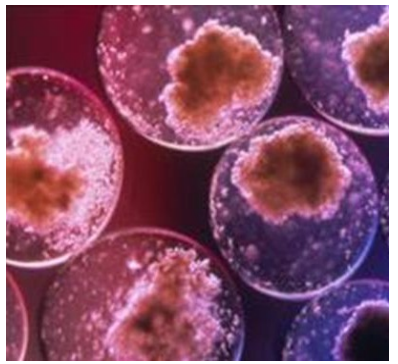


UT

N

A propedeutic concept: systems theory

Close system (thermodynamics)



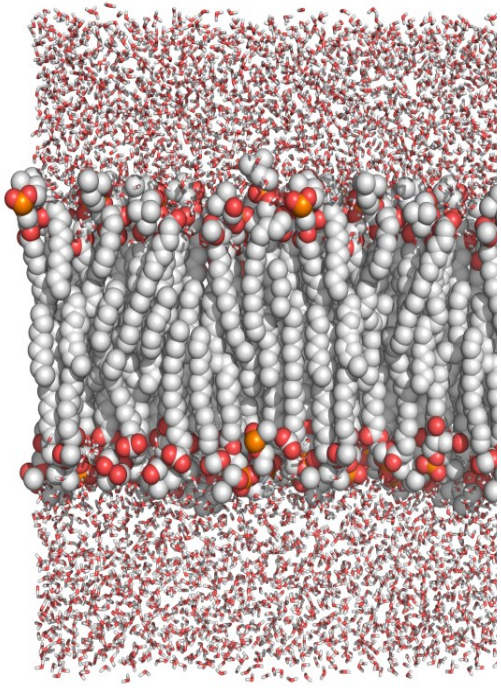
Open system (organized)

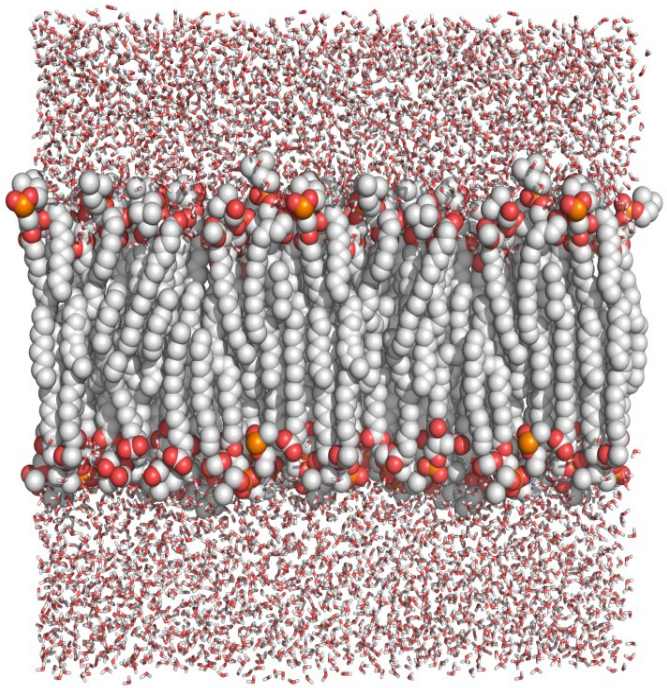
	Equilibrium state	Stationary state
Objectives:		
Operative tools:	ΔH e ΔS	ΔH e ΔS e ΔI
Operative process:	$\downarrow\Delta H$ e $\uparrow\Delta S$	$\downarrow\Delta H$ e $\downarrow\Delta S$ e $\uparrow\Delta I$

Fundamentals of cellular communication theory:

Fondamental problems of compartmentation

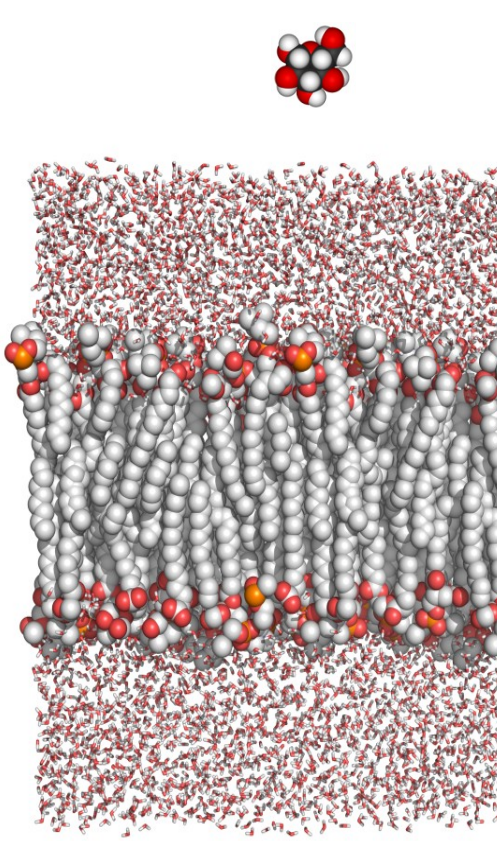
Through which mechanisms can it be possible to efficiently transfer mass and information between the two sides of the membrane?



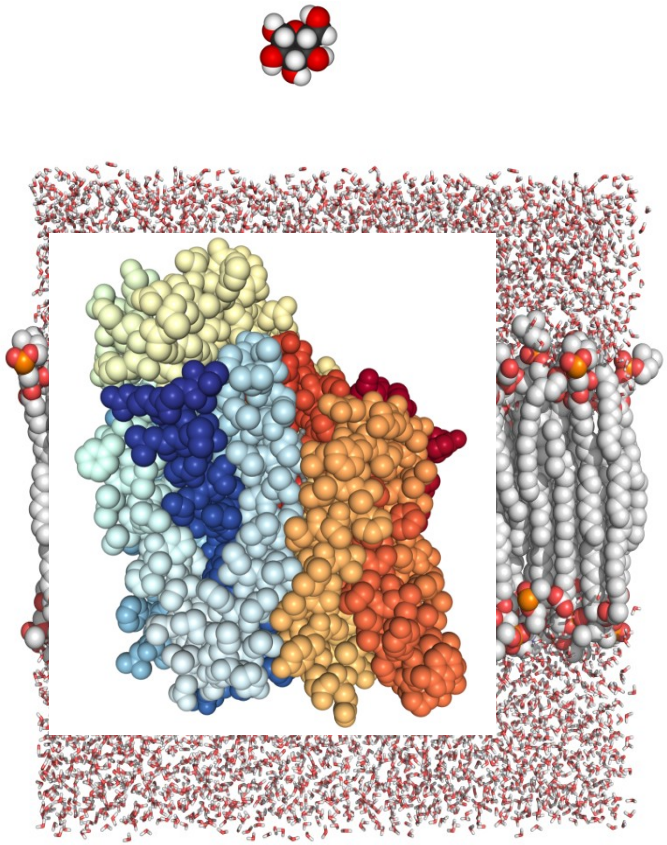


JT

Fundamentals of cellular communication theory: *transporters*

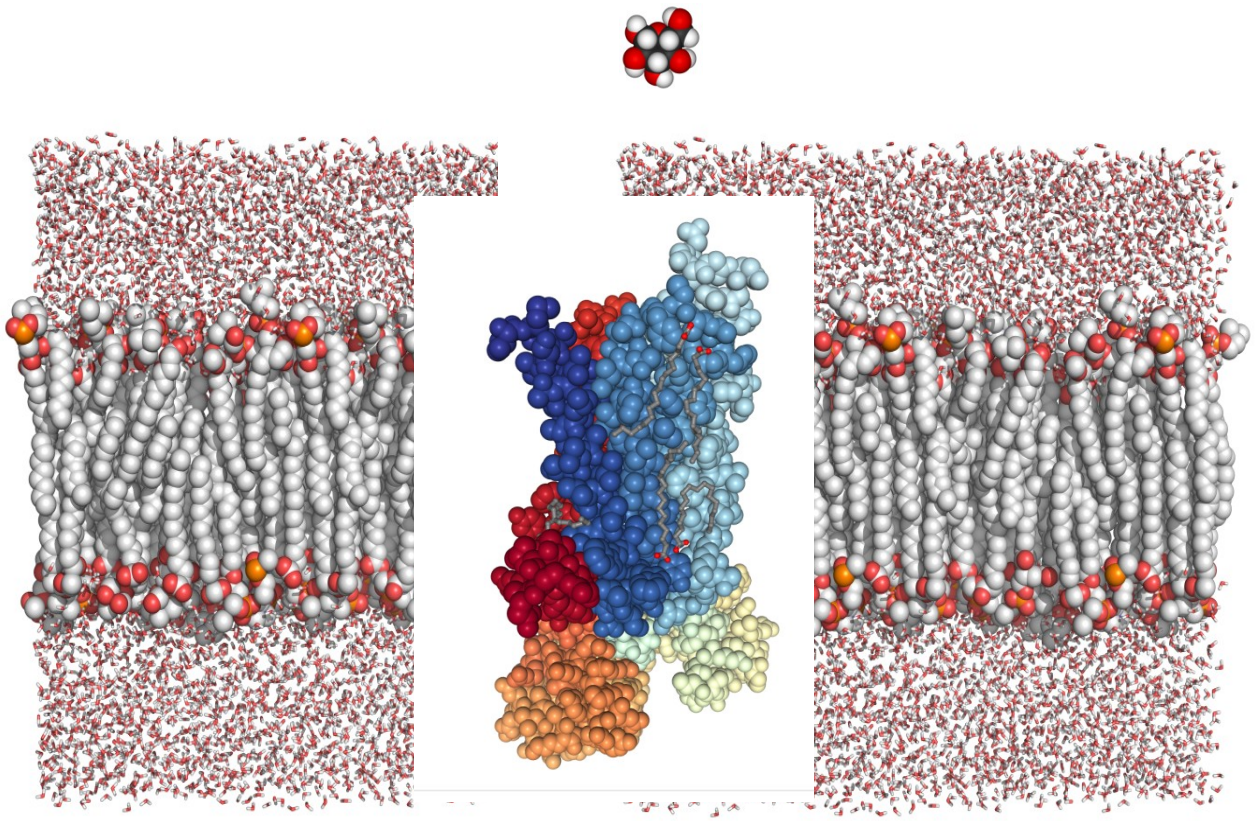


diffiusion



JT

Fundamentals of cellular communication theory: *receptors*



JT

CELLULAR RESPONCE

We can summarize as follows:

As it is true that a man does not live by bread alone, it is also true that an organized system does not live only of exchange of matter and energy but also of *information*.

What *receptor* is… from a molecular point of view:

Receptor as recognition machine

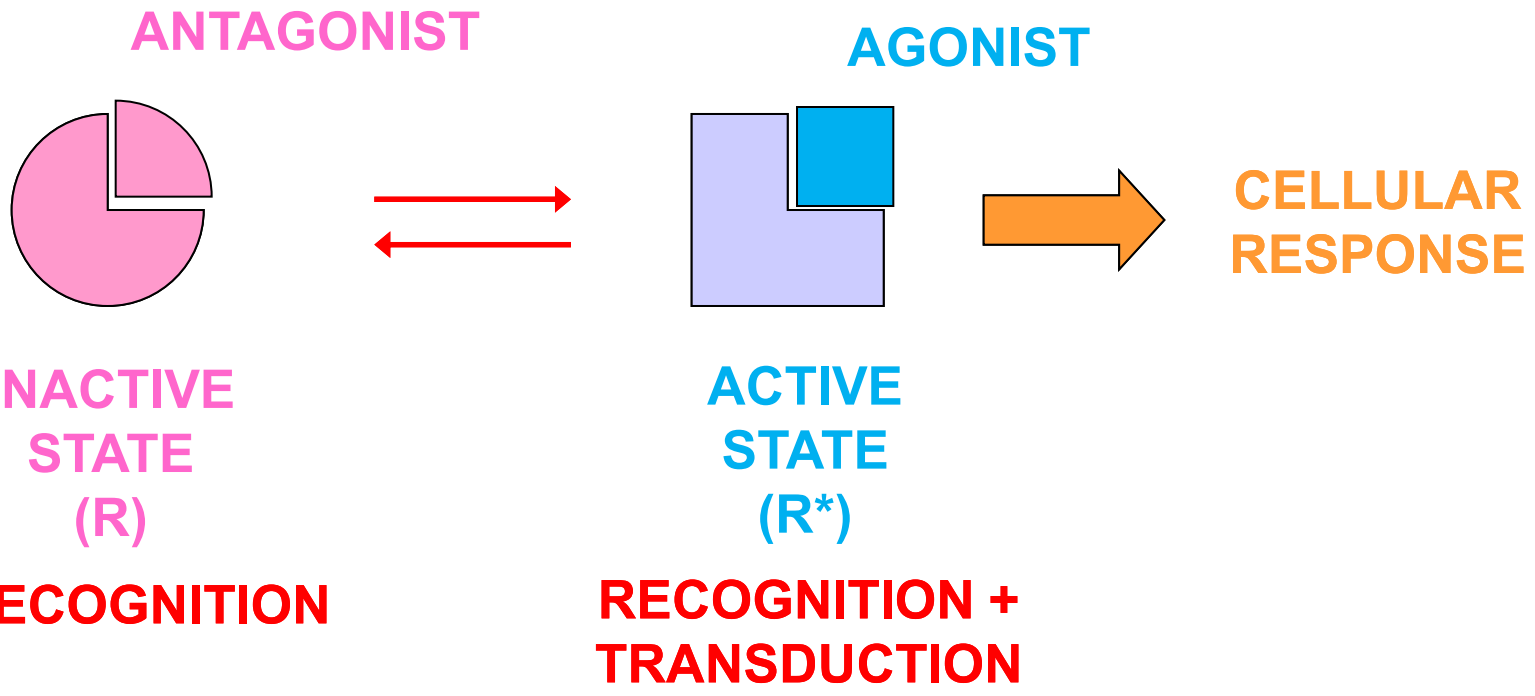
Receptor is a molecular machine, prevalently a protein-based object, able to measure at atomic level molecular complementarity. Molecular complementarity is a fundamental way to describe chemical information.

Receptor as trasduction machine

Recognition is a necessary, but not sufficient, characteristic of a receptor. Receptors are necessarily a molecular machines translating the chemical information of the interaction with the ligand into a cellular response.

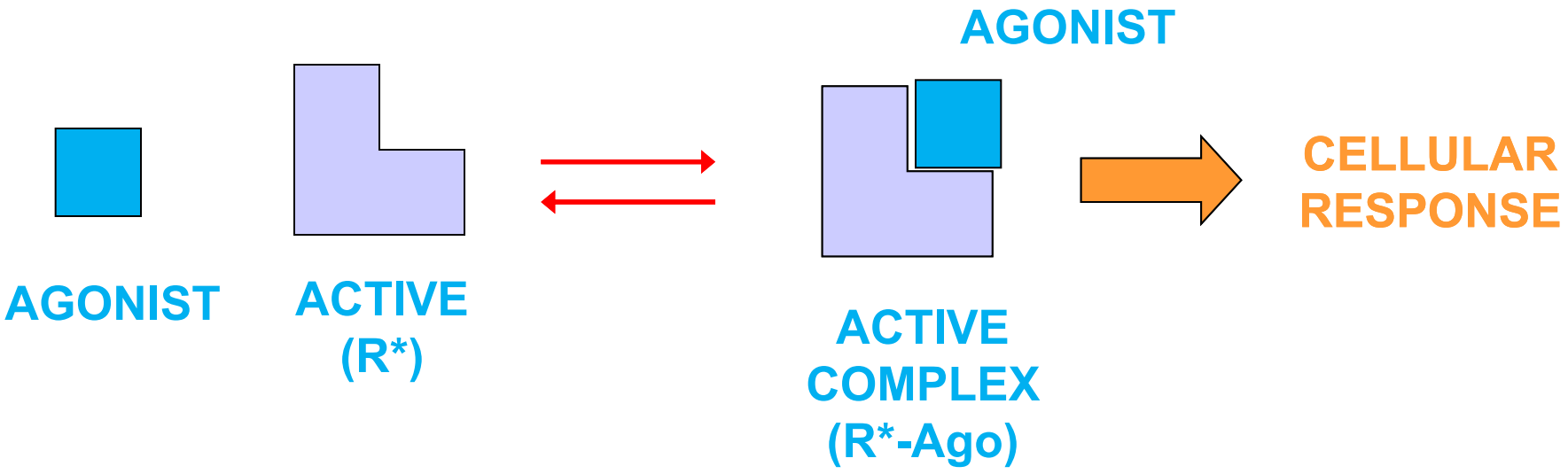
What *receptor* is… from a molecular point of view:

The simplest concept in receptor pharmacology



What *receptor* is… from a molecular point of view:

The simplest concept in receptor pharmacology



Mathematical model:

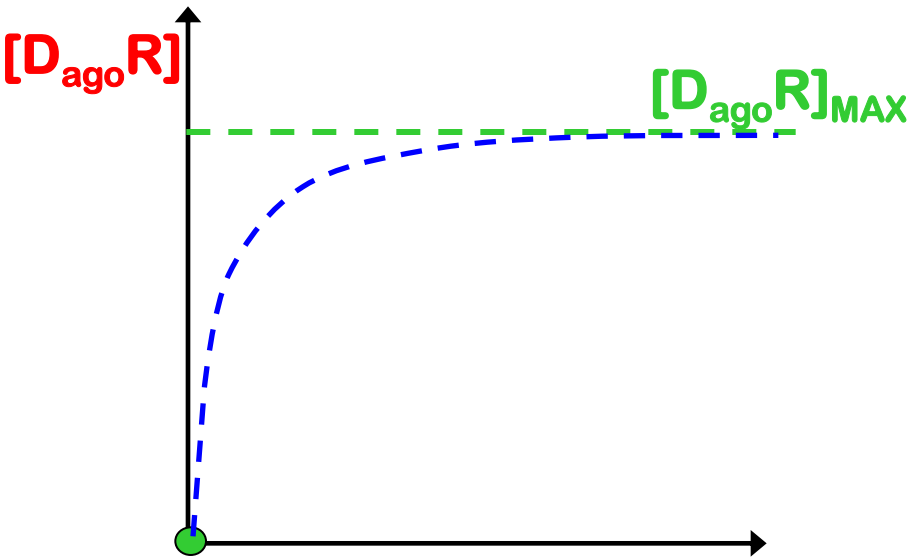
$$D_{ago} + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} [D_{ago}R] \xrightarrow{k_{eff}} Eff$$

Remember the differences between closed and open system!!!

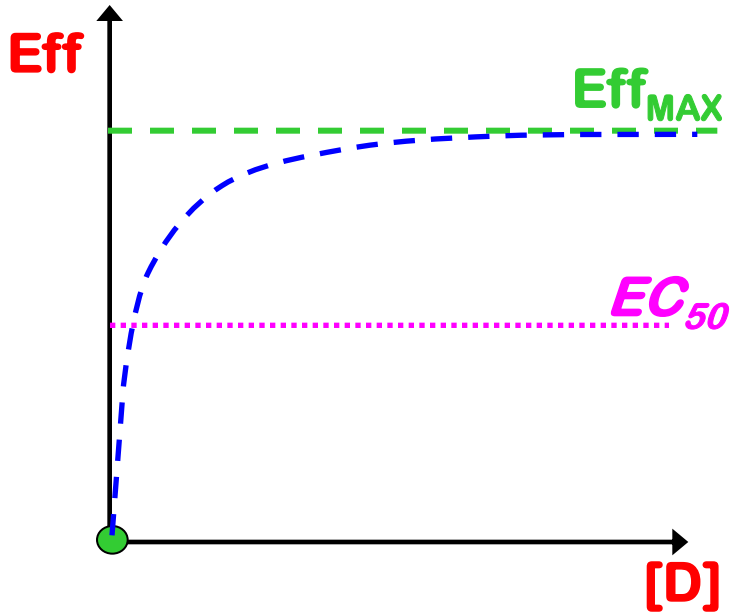
AGONISTA PIENO (FULL AGONIST): oggetti chimici che *RICONOSCONO* il recettore e sono in grado di *TRASFERIRE* l'informazione.

Modello Matematico:

$$D_{ago} + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} [D_{ago}R] \xrightarrow{k_{eff}} Eff$$



RICONOSCIMENTO (AFFINITY)

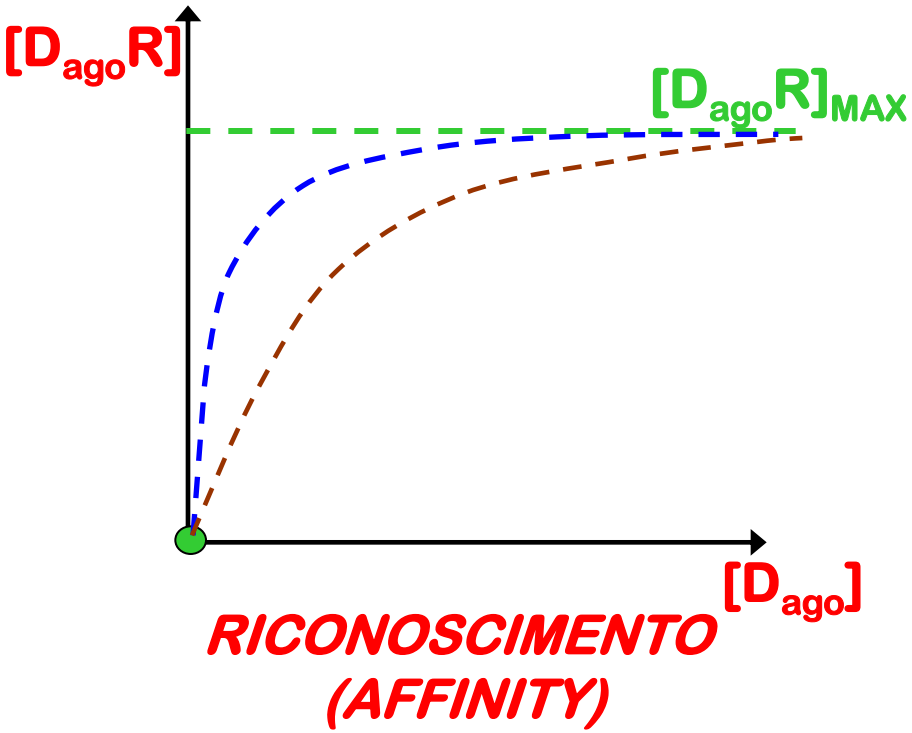


ATTIVAZIONE (INTRINSIC ATTIVITY)

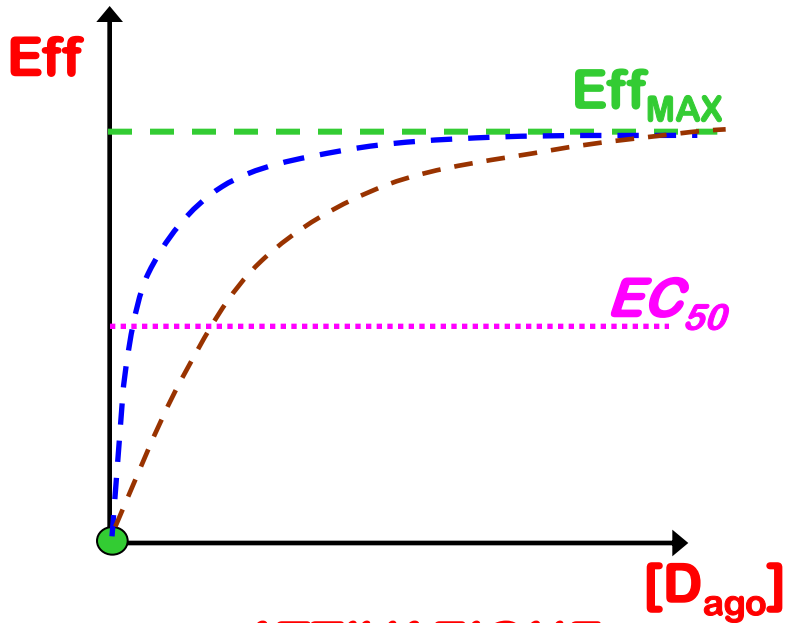
AGONISTI PIENI: EC_{50} un valutatore numerico dell'efficienza del trasferimento dell'informazione.

Modello Matematico:

$$D_{ago} + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} [D_{ago}R] \xrightarrow{k_{eff}} Eff$$



RICONOSCIMENTO (AFFINITY)

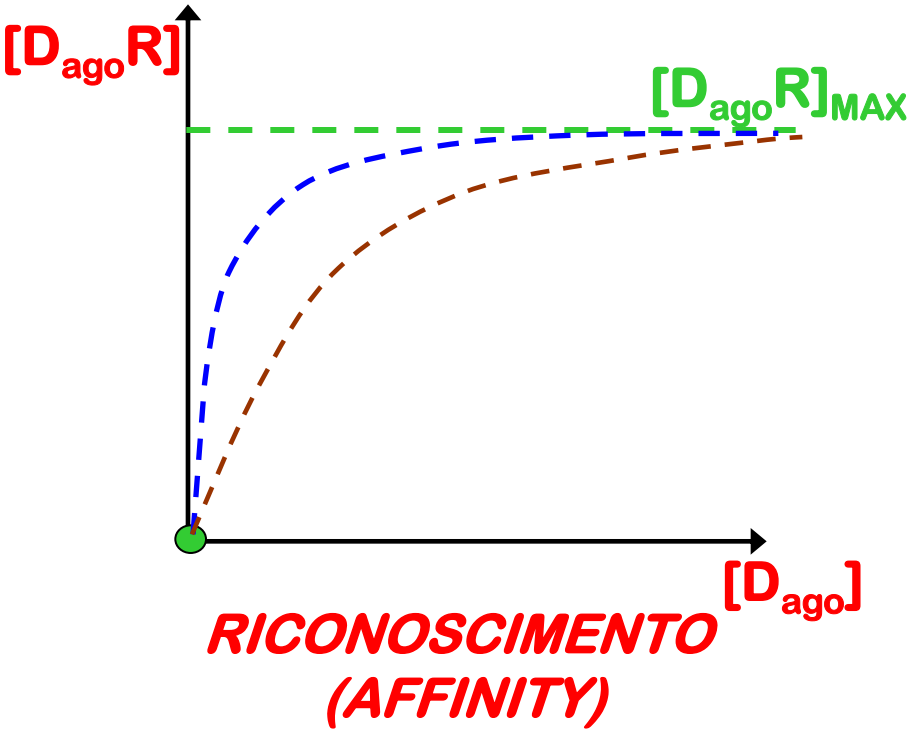


ATTIVAZIONE (INTRINSIC ATTIVITY)

AGONISTI PIENI: per la valutazione dell'efficienza del riconoscimento, siamo già pronti a definirla:

Modello Matematico:

$$D_{ago} + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} [D_{ago}R] \xrightarrow{k_{eff}} Eff$$

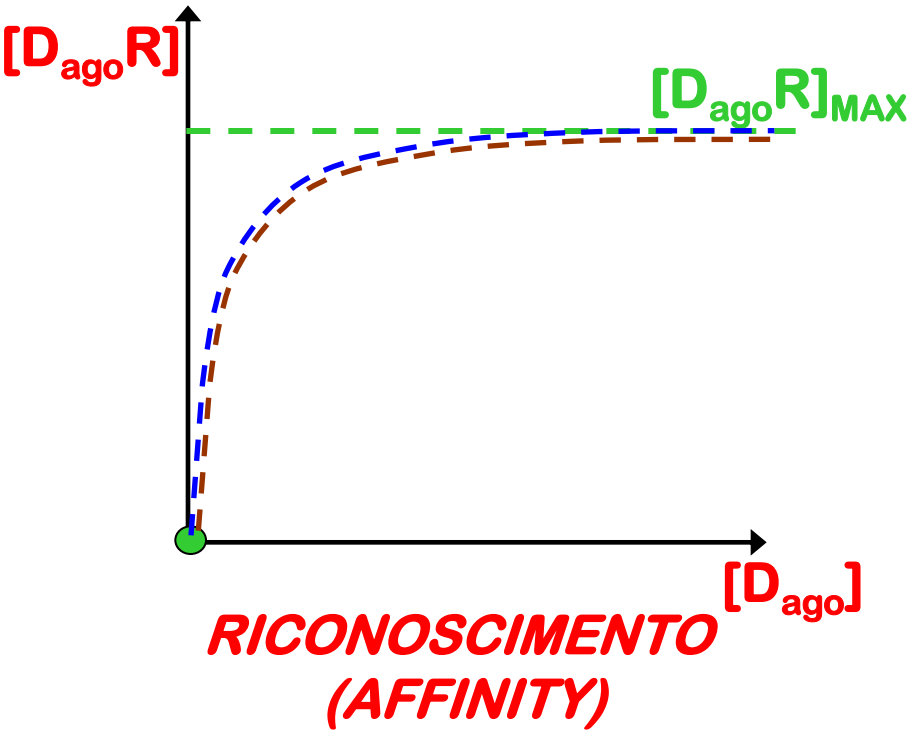


$$K_{bind} = \frac{k_1}{k_2} = \frac{\left[D_{ago}R\right]}{\left[D_{ago}\right] \cdot \left[R\right]}$$

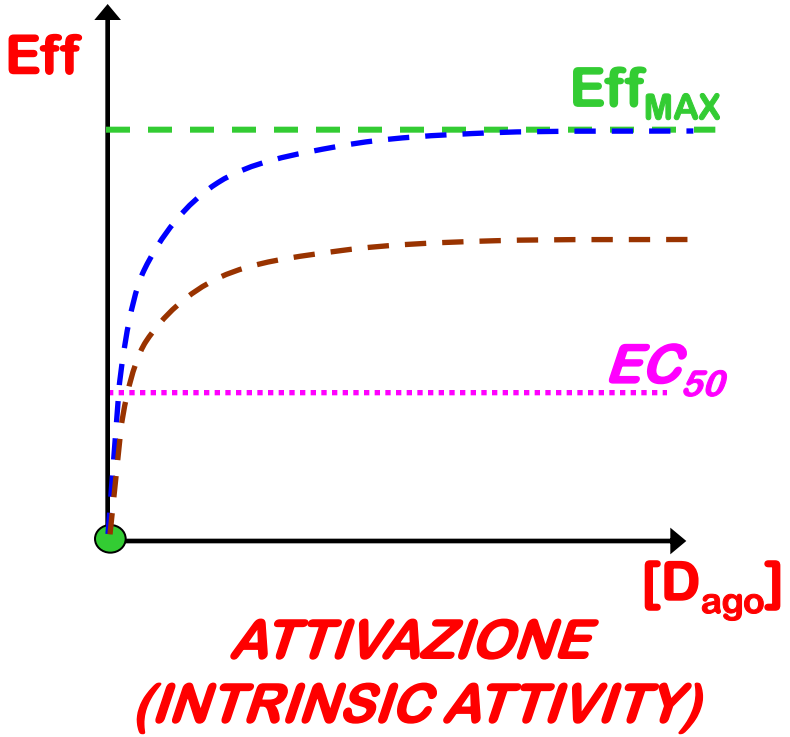
AGONISTI PARZIALI (PARTIAL AGONIST): il riconoscimento c'è ma l'efficienza del trasferimento dell'informazione non è la più quella ottimale.

Modello Matematico:

$$D_{ago} + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} [D_{ago}R] \xrightarrow{k_{eff}} Eff$$

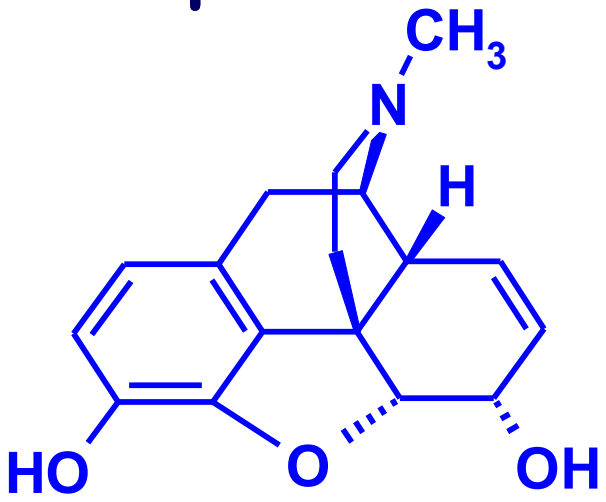


RICONOSCIMENTO (AFFINITY)



ATTIVAZIONE (INTRINSIC ATTIVITY)

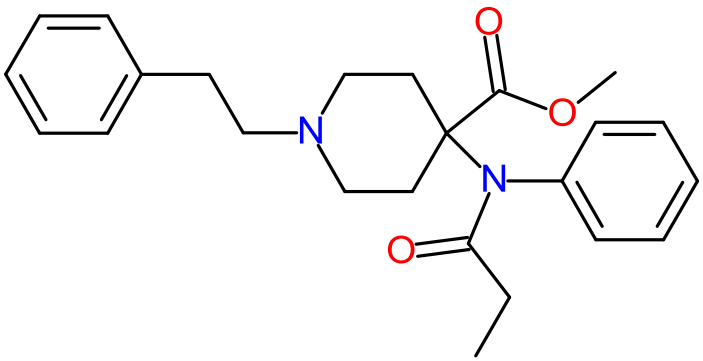
AGONISTI PARZIALI (PARTIAL AGONIST): un bellissimo esempio…



Morfina

Ki (μ) = 1.45 nM

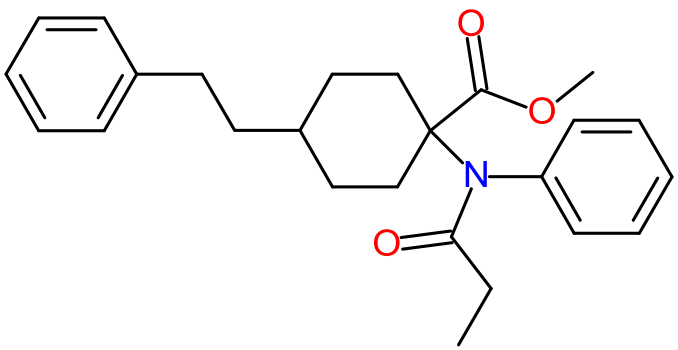
FULL AGONIST



Carfentanil

Ki (μ) = 0.024 nM

FULL AGONIST

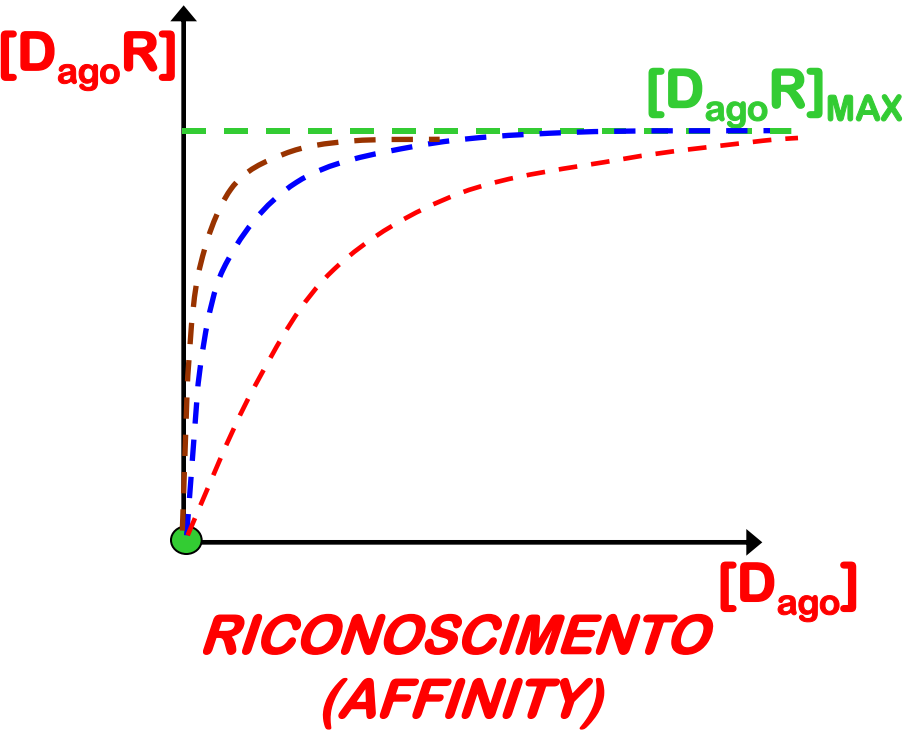


Carbo-carfentanil

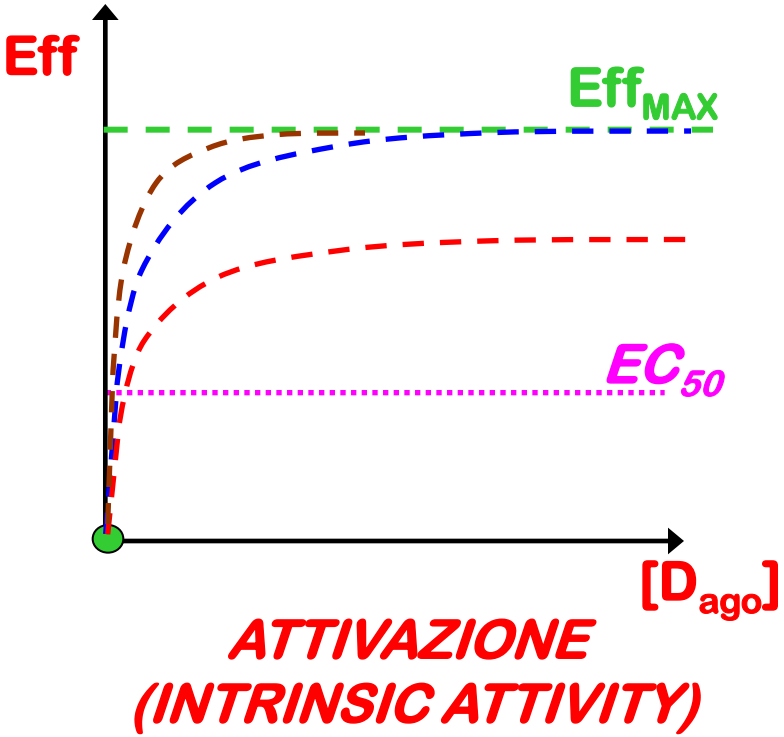
Ki (μ) = 95.2 nM

PARTIAL AGONIST

AGONISTI PARZIALI (PARTIAL AGONIST): un bellissimo esempio… indovinate un po'?



RICONOSCIMENTO (AFFINITY)

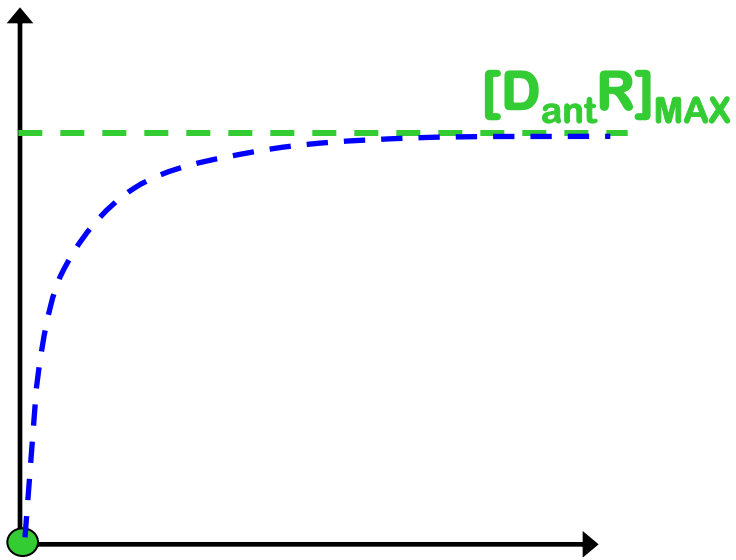


ATTIVAZIONE (INTRINSIC ATTIVITY)

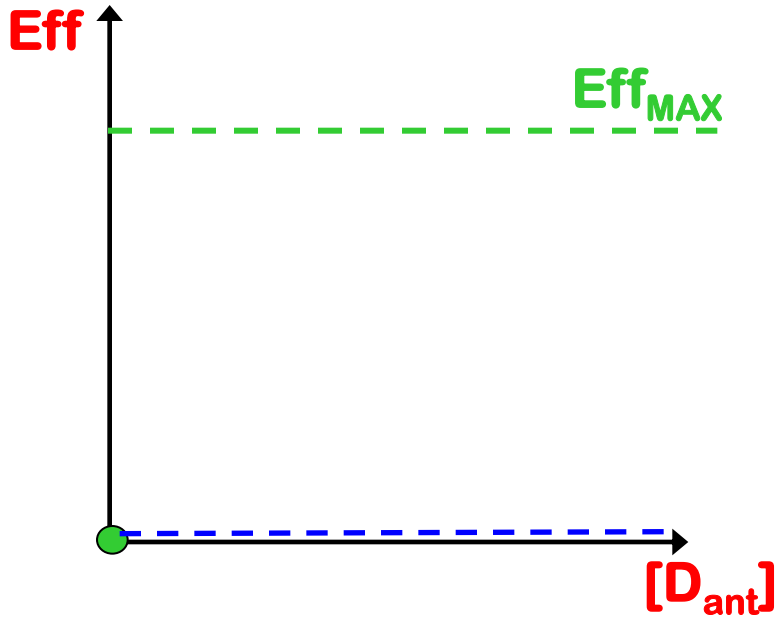
ANTAGONISTI (NEUTRAL ANTAGONIST): oggetti chimici che *RICONOSCONO* il recettore ma NON sono in grado di *TRASFERIRE* l'informazione.

Modello Matematico:

$$D_{ant} + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} [D_{ant}R] \not\rightarrow Eff$$



RICONOSCIMENTO (AFFINITY)

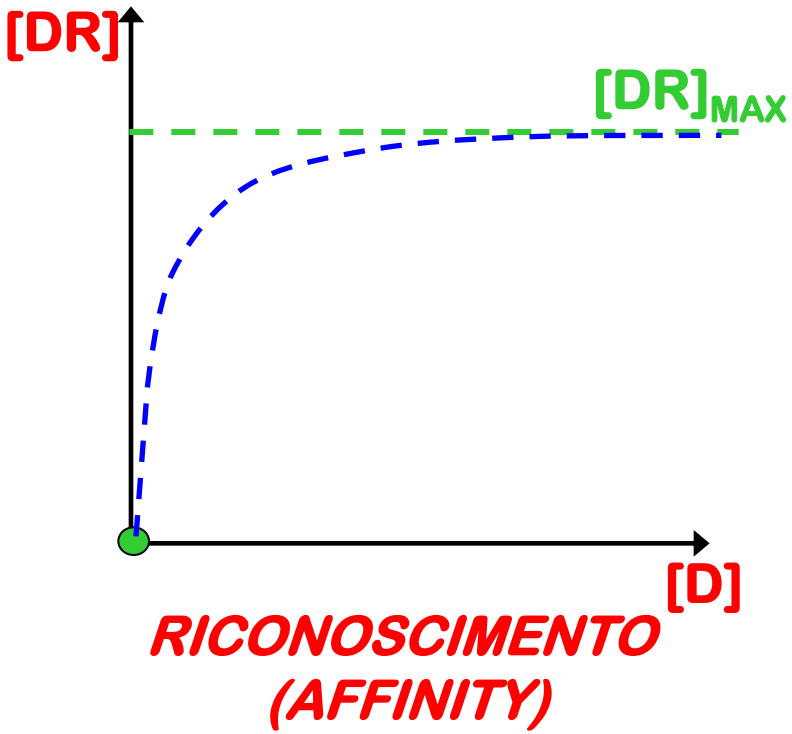


ATTIVAZIONE (INTRINSIC ATTIVITY)

ANTAGONISTI: come misuriamo la sua efficacia nell'antagonizzare l'azione di un agonista… o misurando la sua costante di affinità al recettore oppure..

Modello Matematico:

$$D_{ant} + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} [D_{ant}R] \not\rightarrow Eff$$



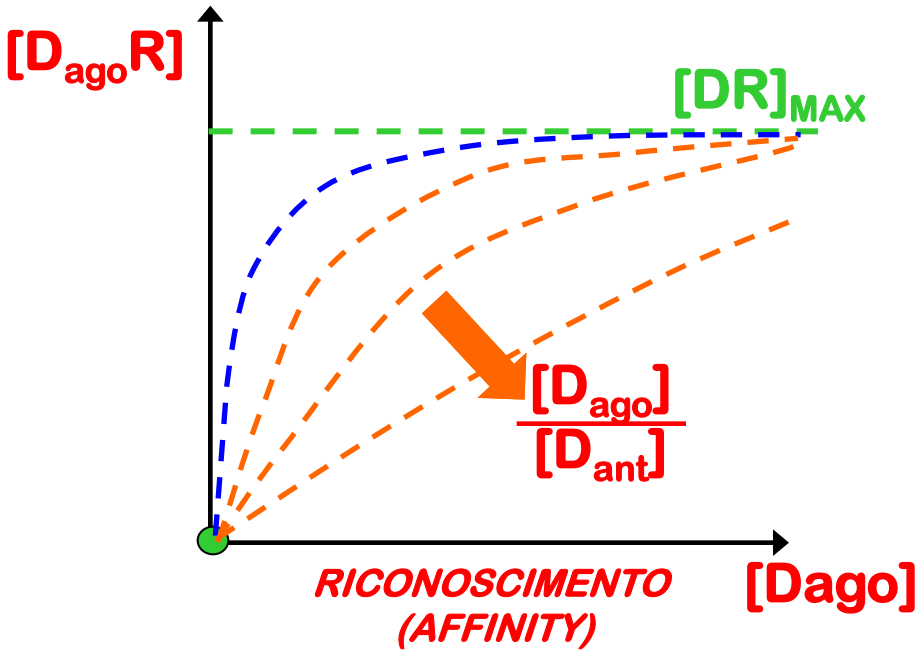
$$K_{bind} = \frac{k_1}{k_2} = \frac{[D_{ant}R]}{[D_{ant}] \cdot [R]}$$

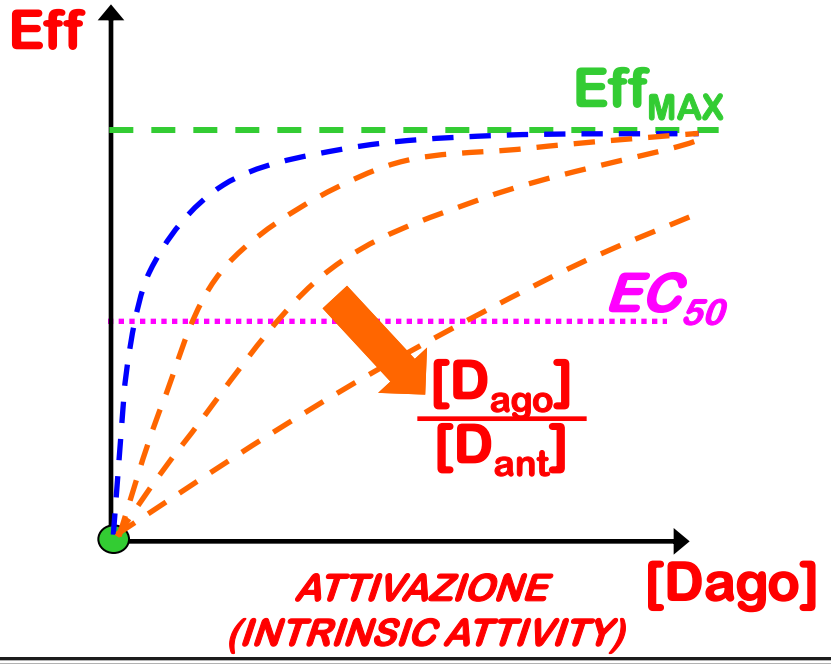
ANTAGONISTI COMPETITIVI: come misuriamo la sua efficacia nell'antagonizzare l'azione di un agonista… competendo con quest'ultimo!

Modello Matematico:

$$D_{ago} + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} [D_{ago}R] \xrightarrow{k_{eff}} Eff$$

$$D_{ant} + R \underset{k'_{off}}{\overset{k'_{on}}{\rightleftharpoons}} [D_{ant}R]$$

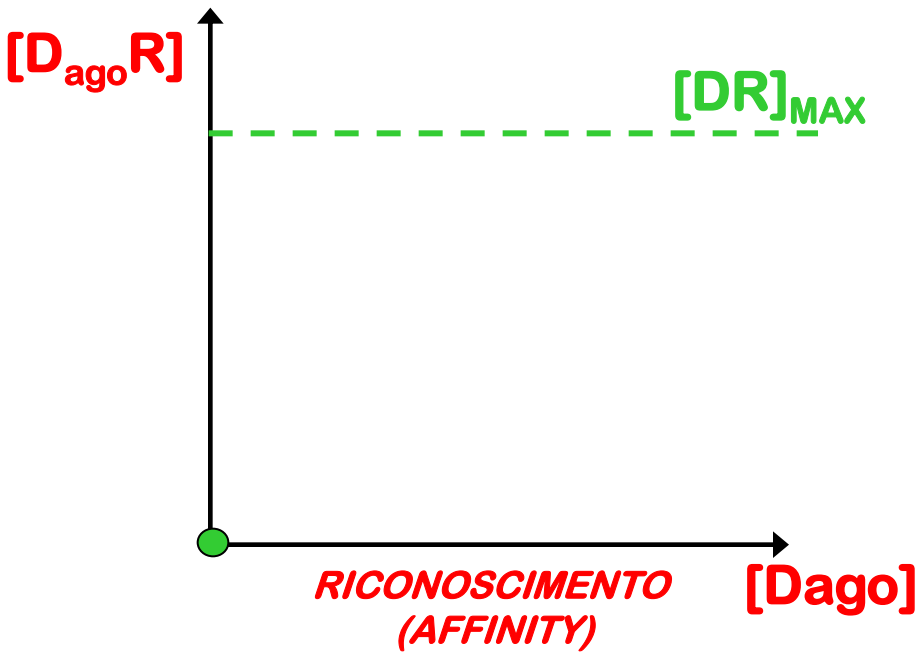


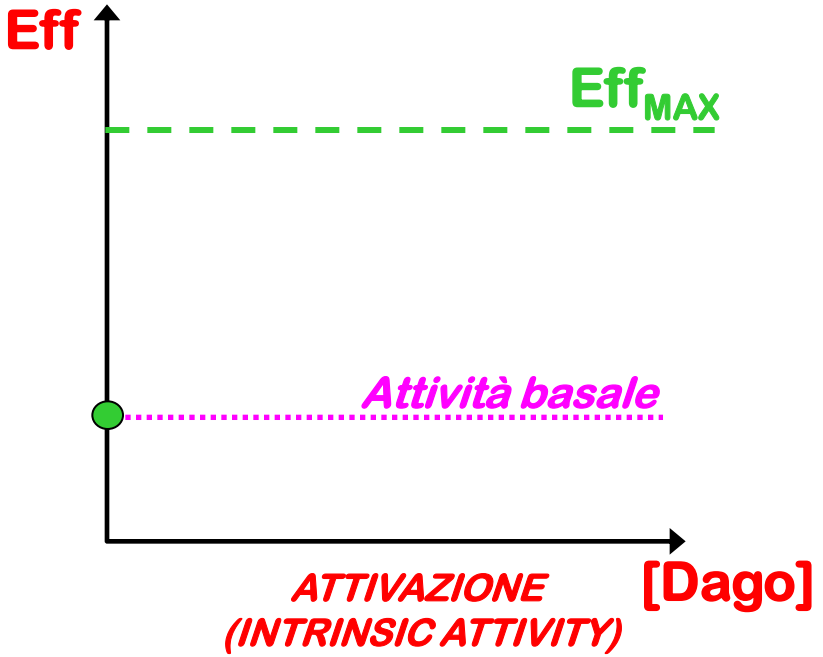


ATTIVITA' BASALE (BASAL ACTIVITY): esistono alcuni sistemi recettoriali che manifestano un *effetto* anche senza la presenza di un agonista.

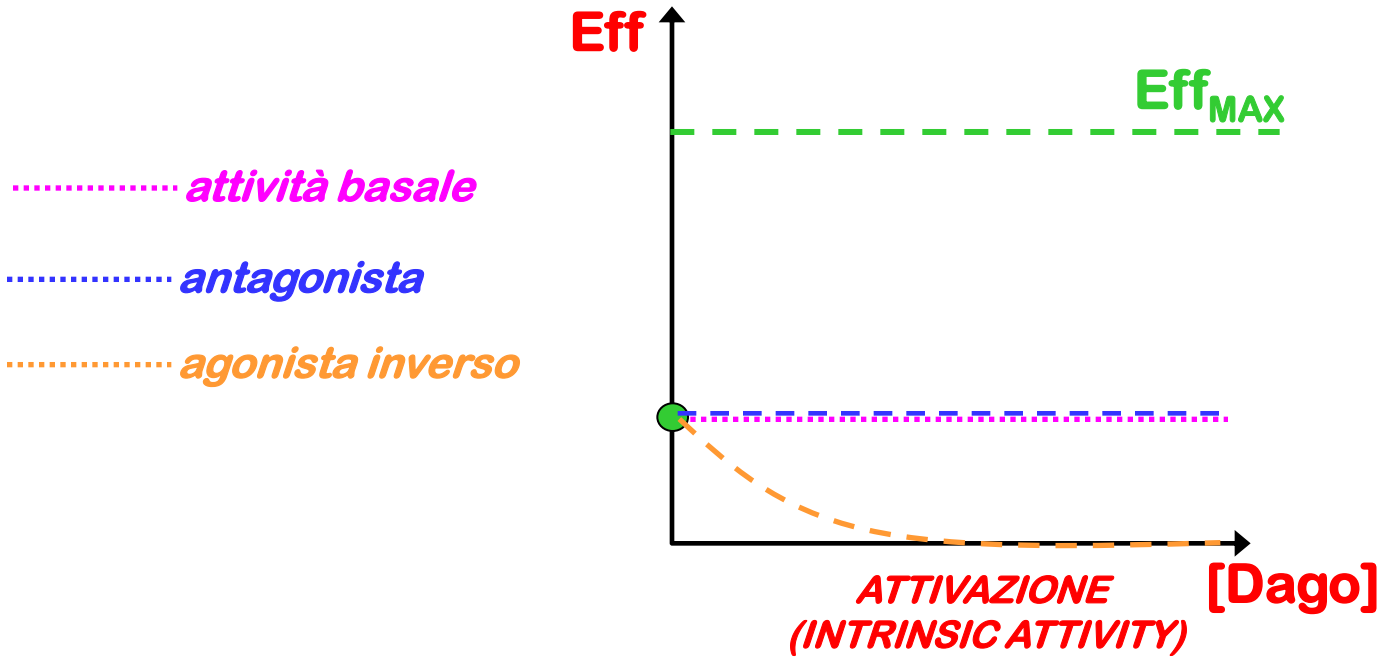
Modello Matematico:

$$R \underset{k^*_{off}}{\overset{k^*_{on}}{\rightleftharpoons}} [R^*] \xrightarrow{k_{eff}} Eff$$

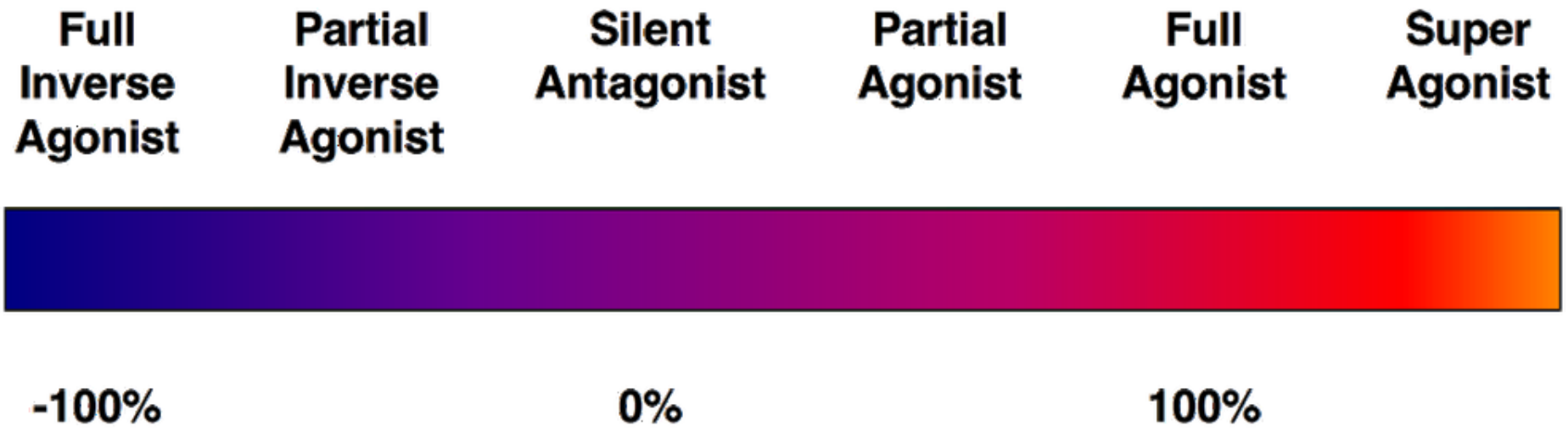




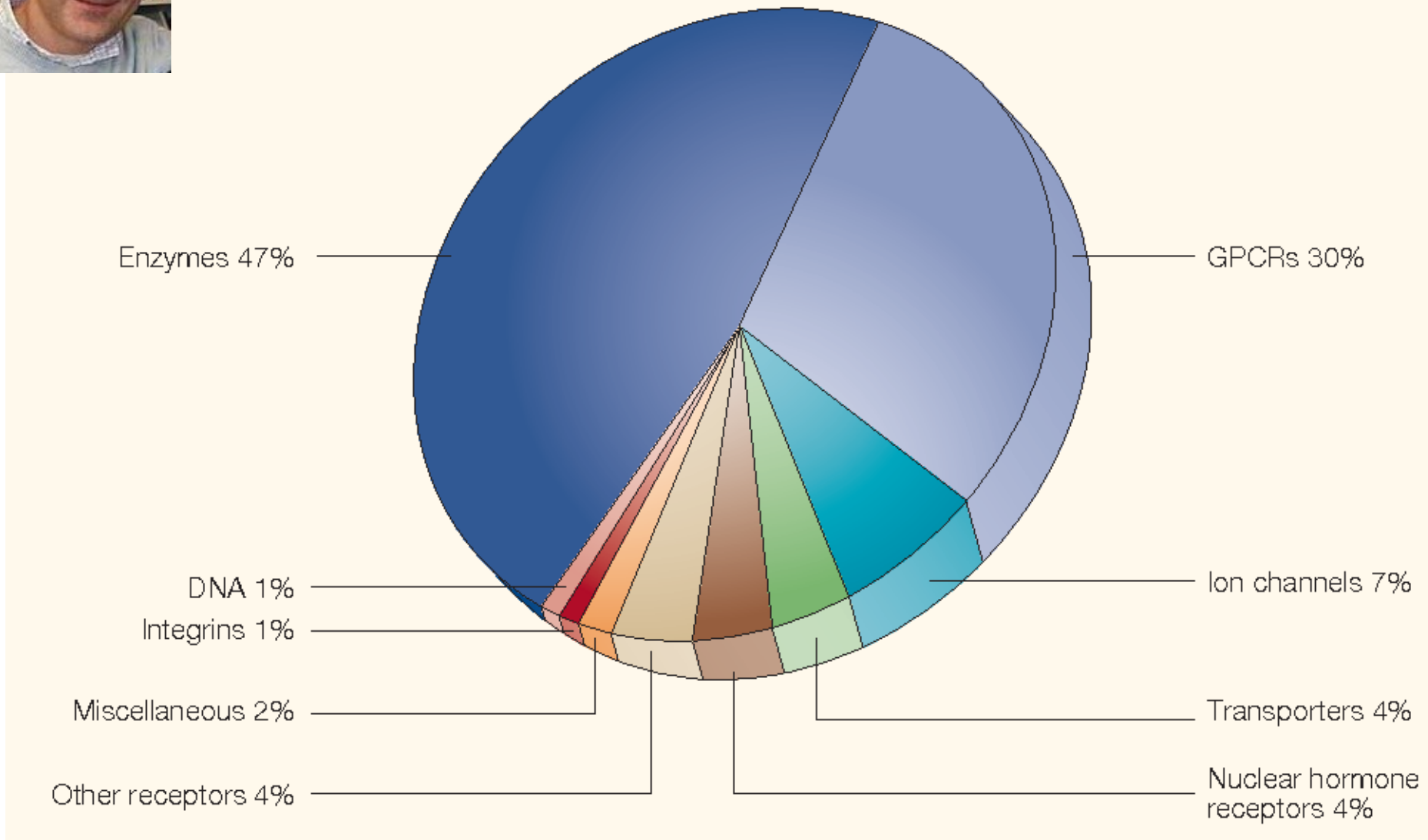
AGONISTA INVERSO (INVERS AGONIST): in presenza di una attività basale oltre agli antagonisti (*neutral antagonist*) possiamo osservare il fenomeno dell'agonismo inverso.



… riassumento oggi:



Back again to our pie chart:



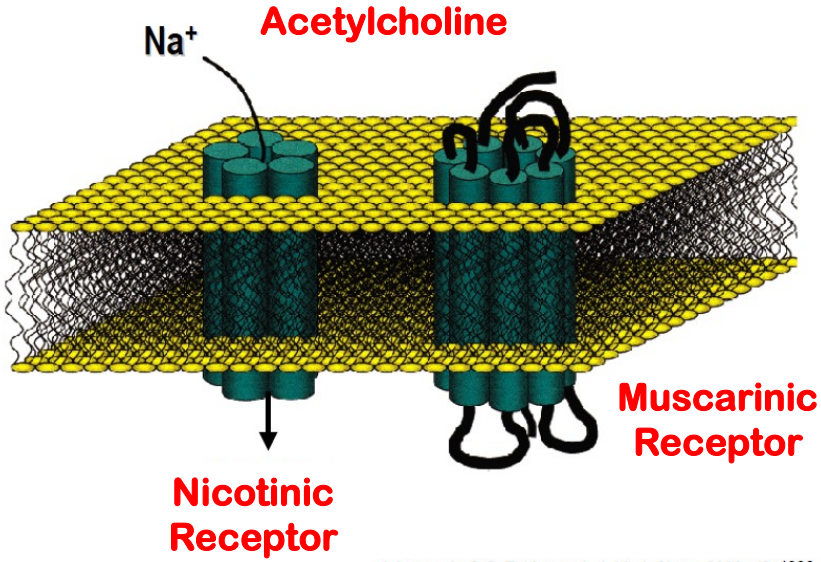
credits: https://www.semanticscholar.org/paper/The-druggable-genome-Hopkins-Groom/648a2ef46c4f7b335741111c445ee26adf2f0644/

Why two different molecular sensors?

Chemical Information: ***neurotransmitters, autacoids, hormones…***

Electrochemical Information: ***ionic gradients (in/out) and electrochemical potentials…***

Speed of signal transmission



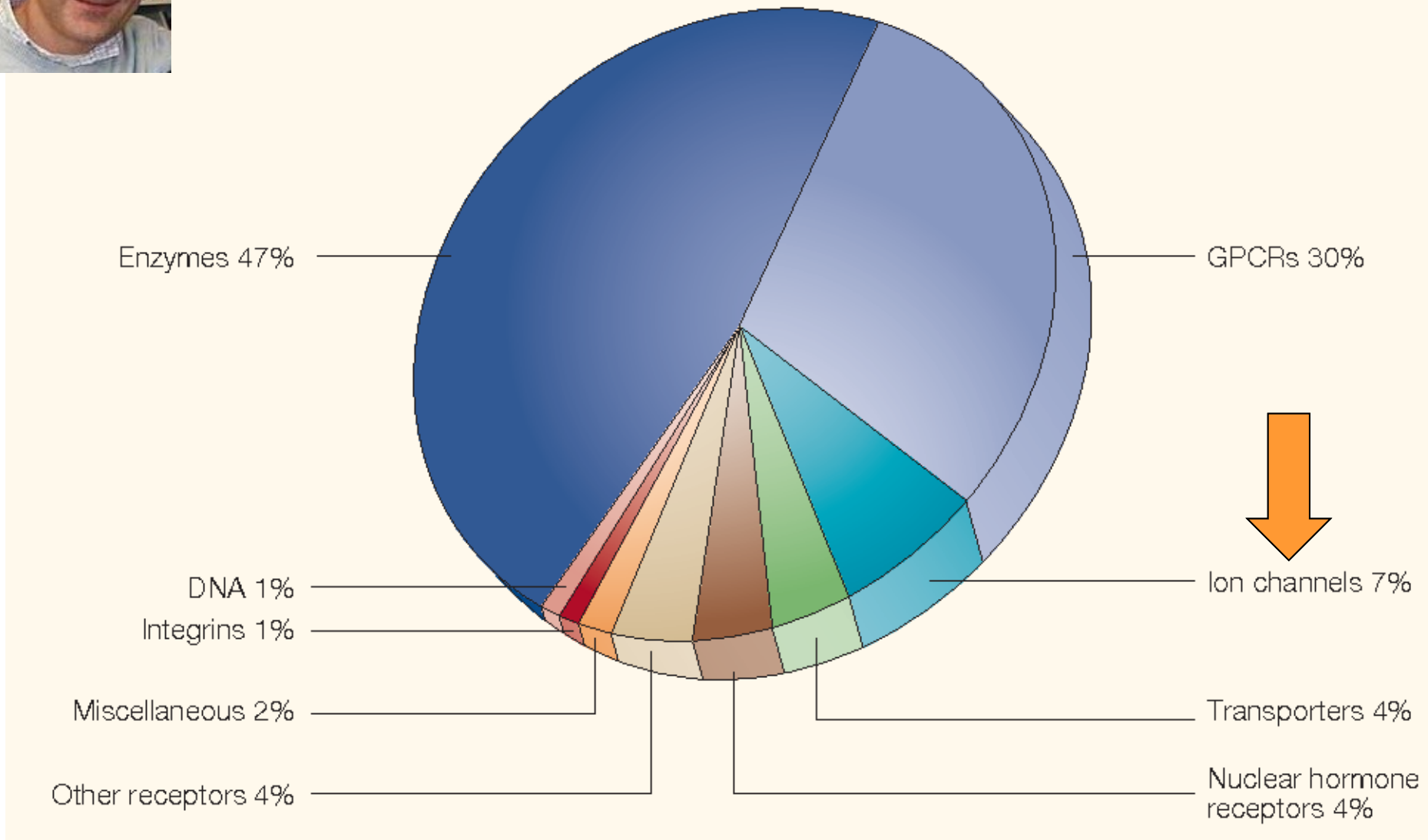
Adattato da C.C. Felder et al. *J. Med. Chem.* **2000**, *43*, 4333

Tuning of the intensity of signal transmission

Why two different molecular sensors?

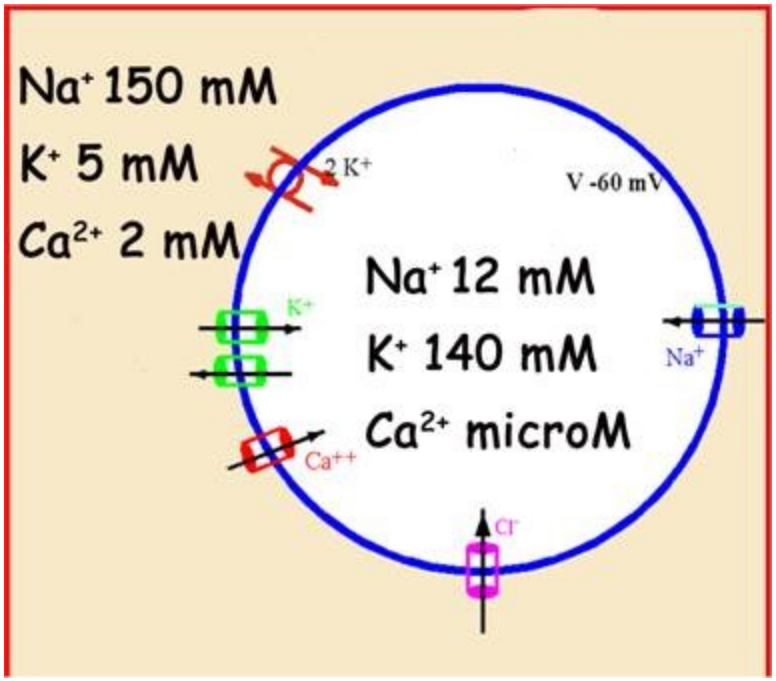
	Ionic channels	GPCRs
Speed of signal transmission	HIGH	MEDIUM/LOW
Tuning of the intensity of signal transmission	COARSE	ACCURATE

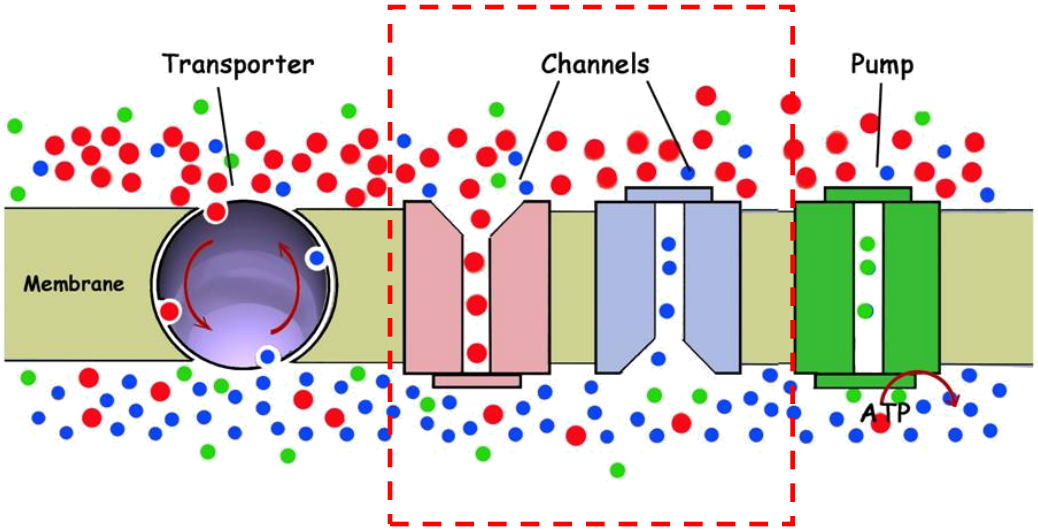
Back again to our pie chart:



credits: https://www.semanticscholar.org/paper/The-druggable-genome-Hopkins-Groom/648a2ef46c4f7b335741111c445ee26adf2f0644/

Do you remember the typical electrochemical information?

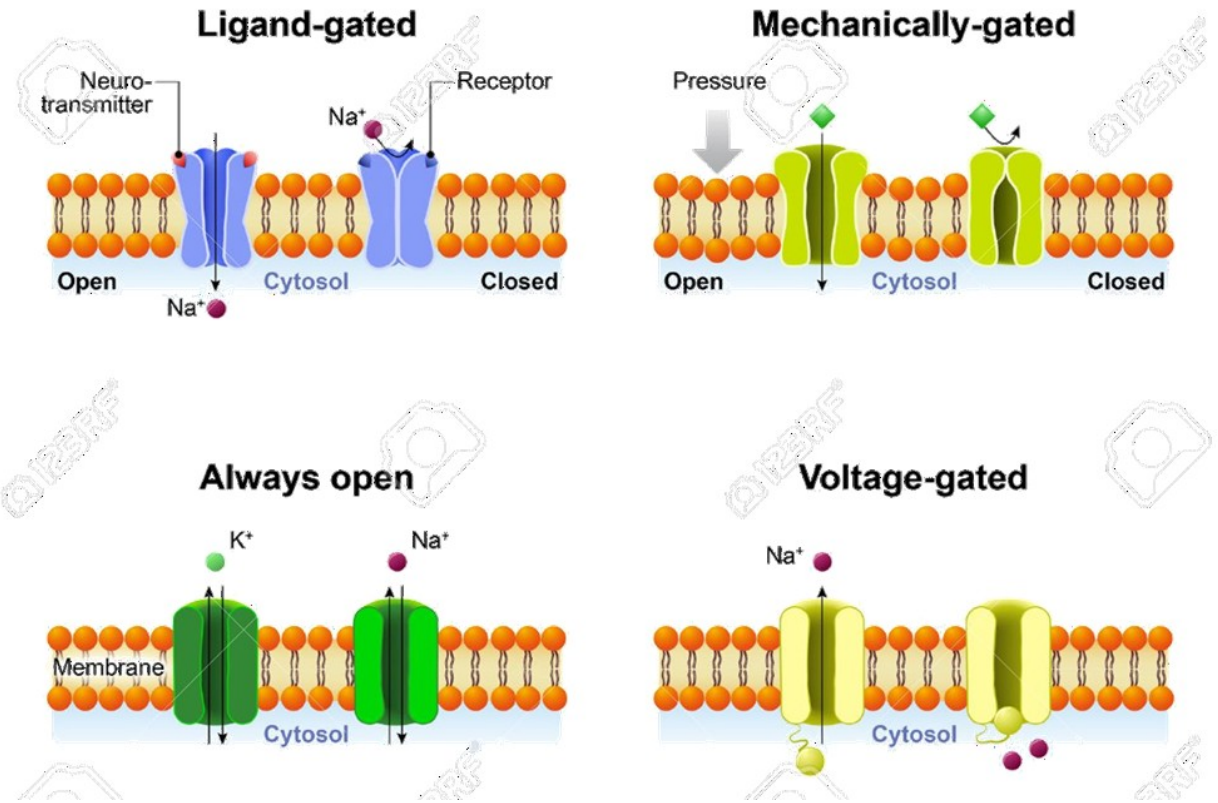




... in particular the correct ionic concentrations are essential for the survival of the cell and for communication between cells.

Do you remember the typical electrochemical information?

ION CHANNEL



credits: https://www.123rf.com/photo_63923652_stock-vector-types-of-ion-channel-classification-by-gating-mechanism-of-action-voltage-gated-ligand-gated-mechani.html

http://mms.dsfarm.unipd.it

Cerca

UNIVERSITÀ DEGLI STUDI DI PADOVA

MMS Molecular Modeling Section

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Via Marzolo 5, 35131 Padova (Italy) - phone: +39 049 8275704, fax: +39 049 8275366

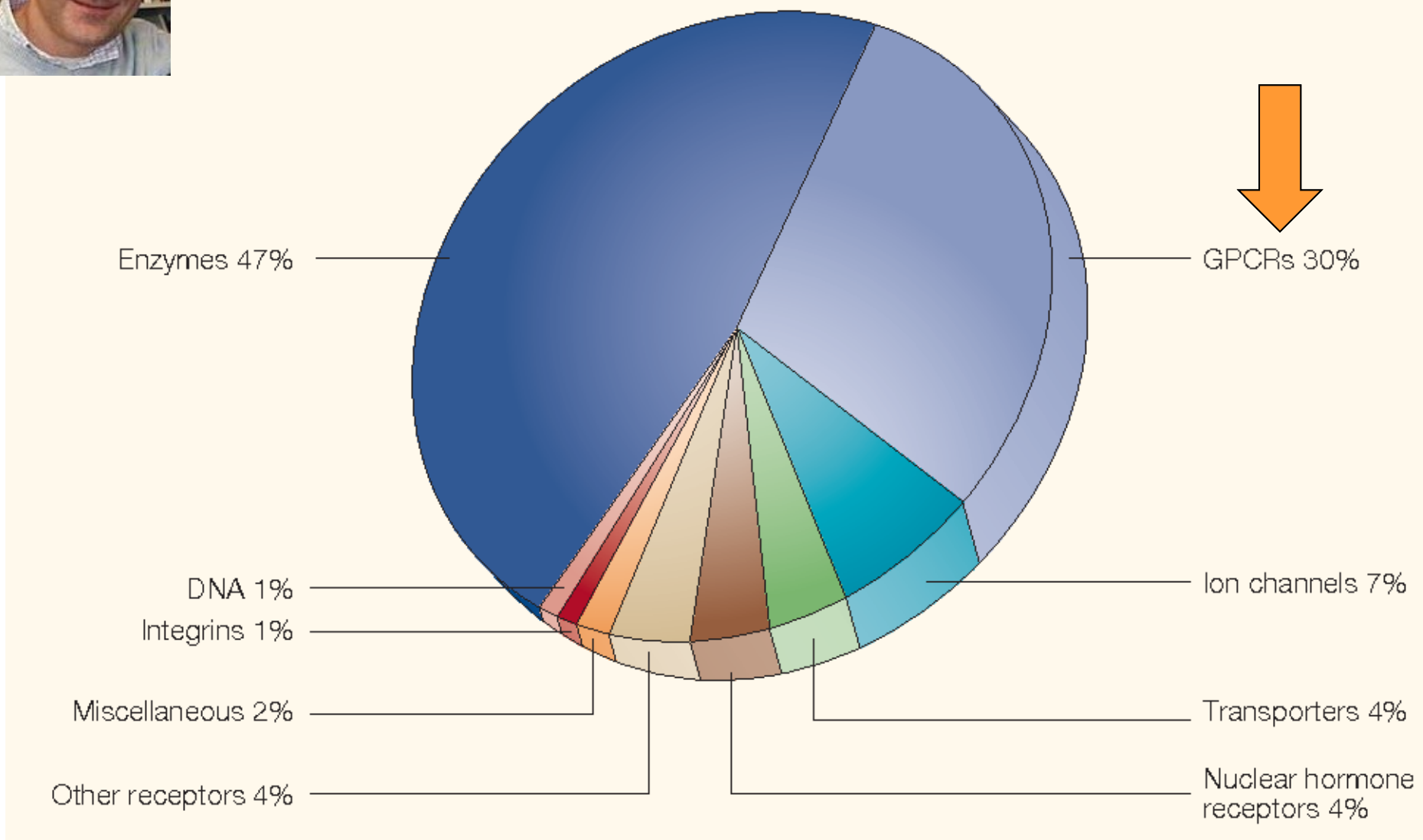
MMS Home | About us... | Lab Members | Projects | Pubblications | @learning | MMStools | MMS_Intranet

Obbiettivi del percorso formativo:

Gli obiettivi dell'insegnamento sono quelli di fornire allo studente le conoscenze fondamentali riguardanti le strategie generali di progettazione, la sintesi, lo studio dei meccanismi d'azione a livello molecolare e degli aspetti chimico-tossicologici e le relazioni fra struttura chimica e attività biologica di alcune classi di farmaci. In particolare saranno studiati i farmaci che agiscono sui sistemi nervosi centrali e periferici. Si prevede che lo studente acquisisca la conoscenza dei concetti fondamentali relativi allo studio chimico-molecolare dei farmaci e che sia in grado di discutere i meccanismi d'azione e le relazioni struttura-attività di farmaci sulla base delle caratteristiche chimiche delle molecole coinvolte. Si prevede inoltre che lo studente acquisisca gli elementi indispensabili per progettare farmaci su basi razionali.

1. Parte generale				
Basi termodinamiche del riconoscimento farmaco-recettore Sistemi di comunicazione cellulare				
	parte I			
	parte I			
2. Sistematica Farmaceutica				
Comunicazione colinergica				
	parte I			

Back again to our pie chart:



credits: https://www.semanticscholar.org/paper/The-druggable-genome-Hopkins-Groom/648a2ef46c4f7b335741111c445ee26adf2f0644/

G protein-coupled receptors (GPCRs):

- The largest family of cell-surface receptors involved in signal transmission (3% of human genome, $\sim$ 800 members in human)[1];
- Respond to various extracellular stimuli including hormones, neurotransmitters, odorant molecules and light;
- Target of more than 40% of drugs used today [2].

Nobel Prize in Chemistry 2012

Robert J. Lefkowitz Brian K. Kobilka

[1] Fredriksson R, Schiöth HB. The repertoire of G-protein-coupled receptors in fully sequenced genomes. Mol Pharmacol 2005;67:1414.
[2] Wise A, et al. Target validation of G-protein coupled receptors. Drug Discov Today 2002;7:235.

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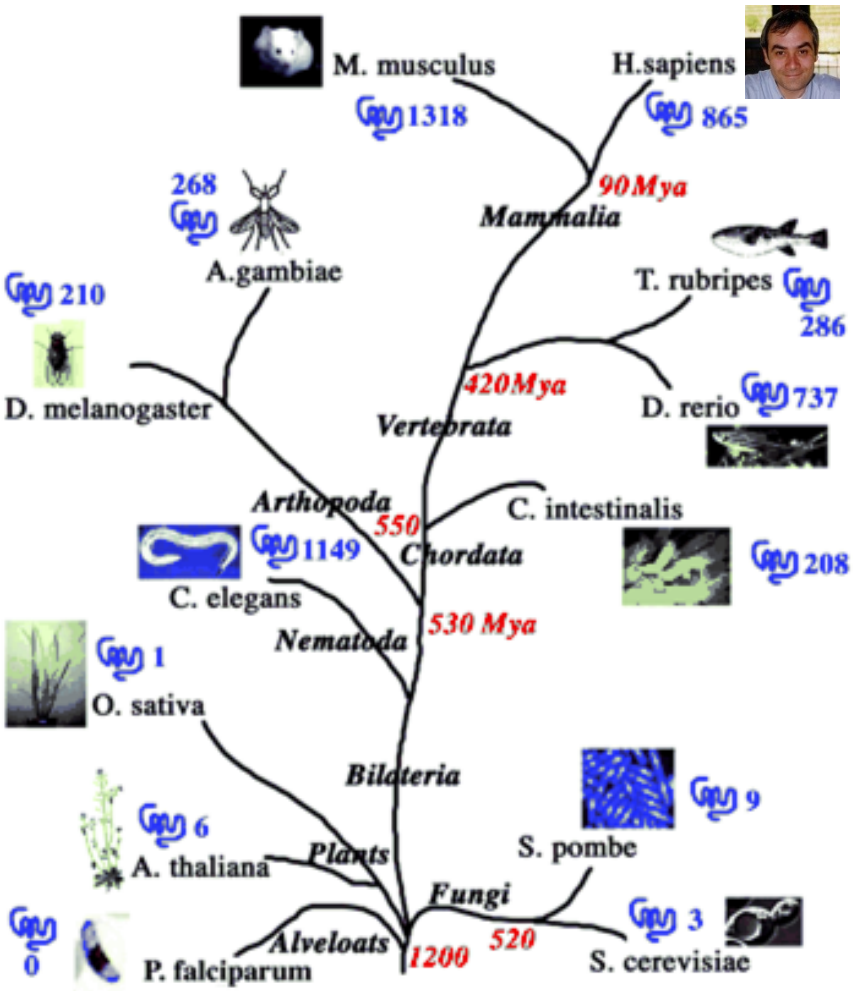
Basic GPCRs Principles

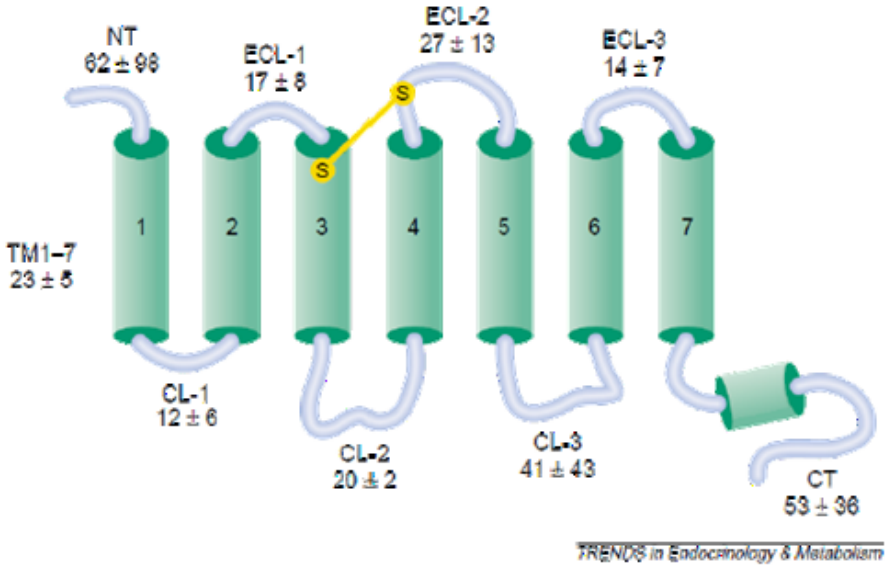
GPCR represents one of the biggest family of ancestrally related proteins.

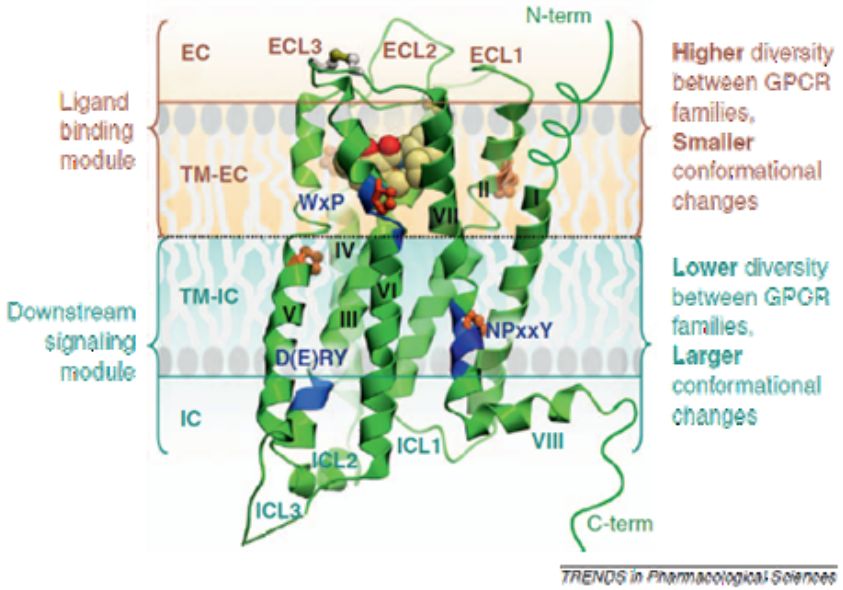


credits:Perez D.M. Mol Pharmacol 67, 1383-1384 (2005)

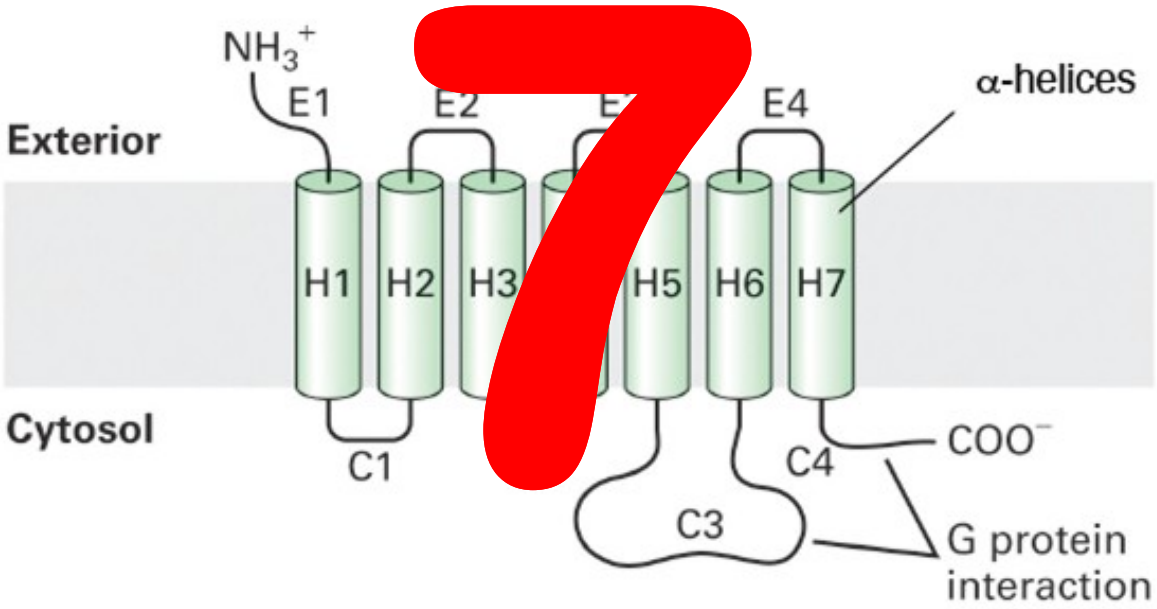
Basic GPCRs Principles

- Integral membrane proteins:
  - seven membrane-spanning α-helices (TM1-7, 25-35 hydrophobic residues);
  - three intracellular and extracellular loops (IL1-3 and EL1-3);
  - extracellular N-terminus;
  - intracellular C-terminus;
  - a S-S bond between TM3 and EL2 conserved in most GPCRs.

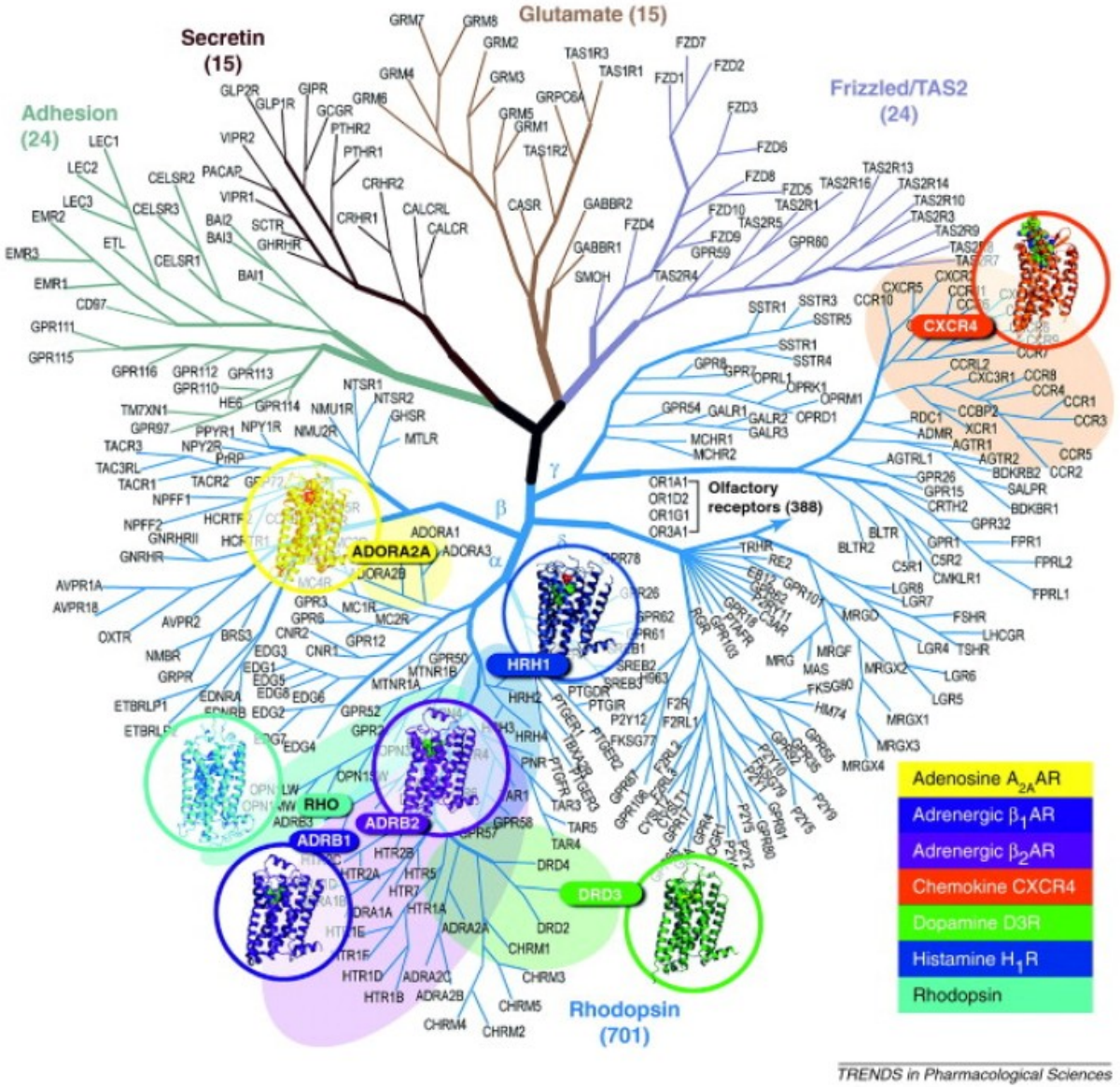


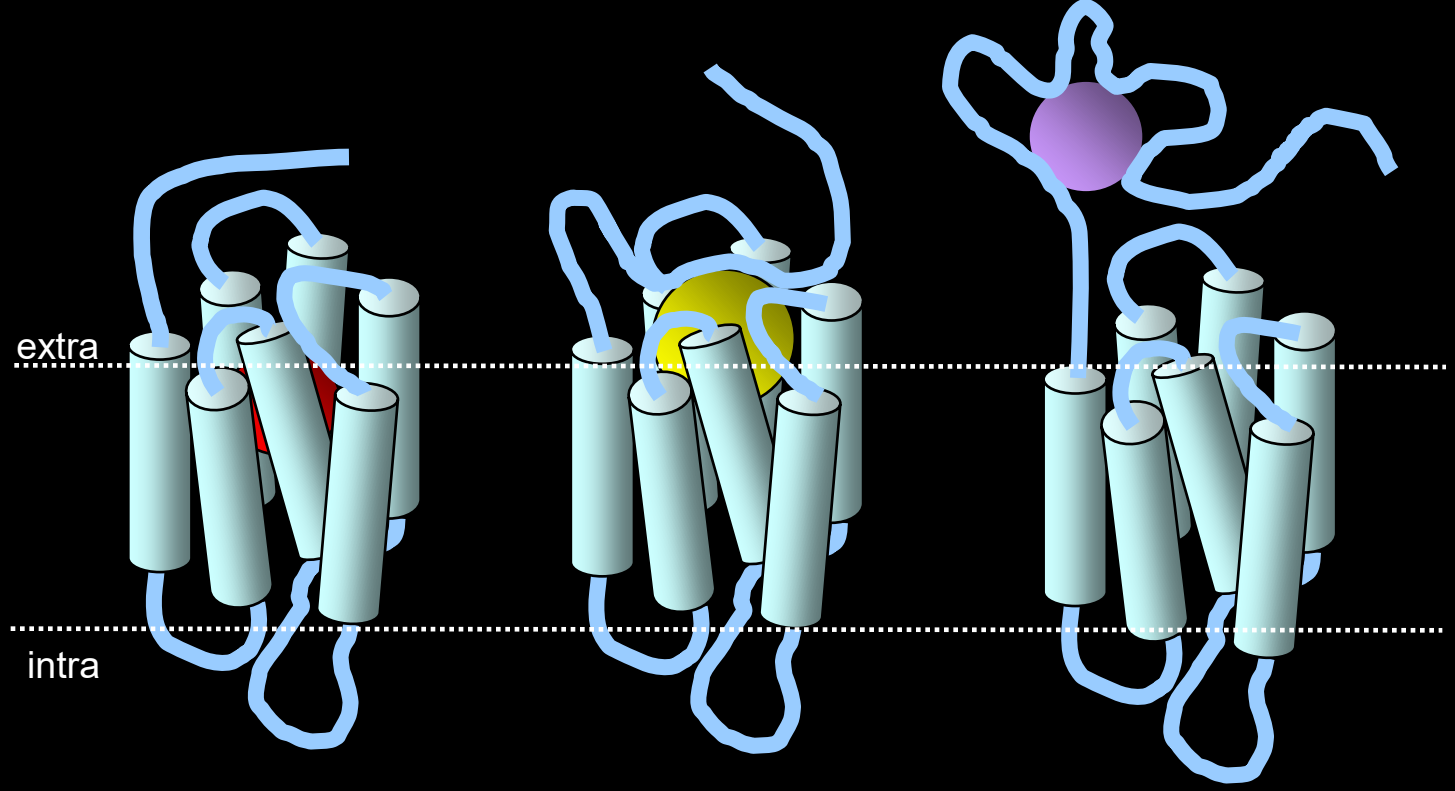


Why seven…


NH3+
E1
E2
E4
α-helices
Exterior
H1
H2
H3
H5
H6
H7
Cytosol
C1
C3
C4
COO−
G protein
interaction

GPCRs classification:





Family A

Peptide/Proteins
- Small peptides (38)
- Large peptides (19)
- Small Proteins (33)
- Large proteins (4)

Small molecules
- Biogenic amines (41)
- Lipids (16)
- Nucleosides (13)
- Others (3)

Olfactory and taste (51)
Glycoprotein's (5)
Viral (19)
Orphans (236)

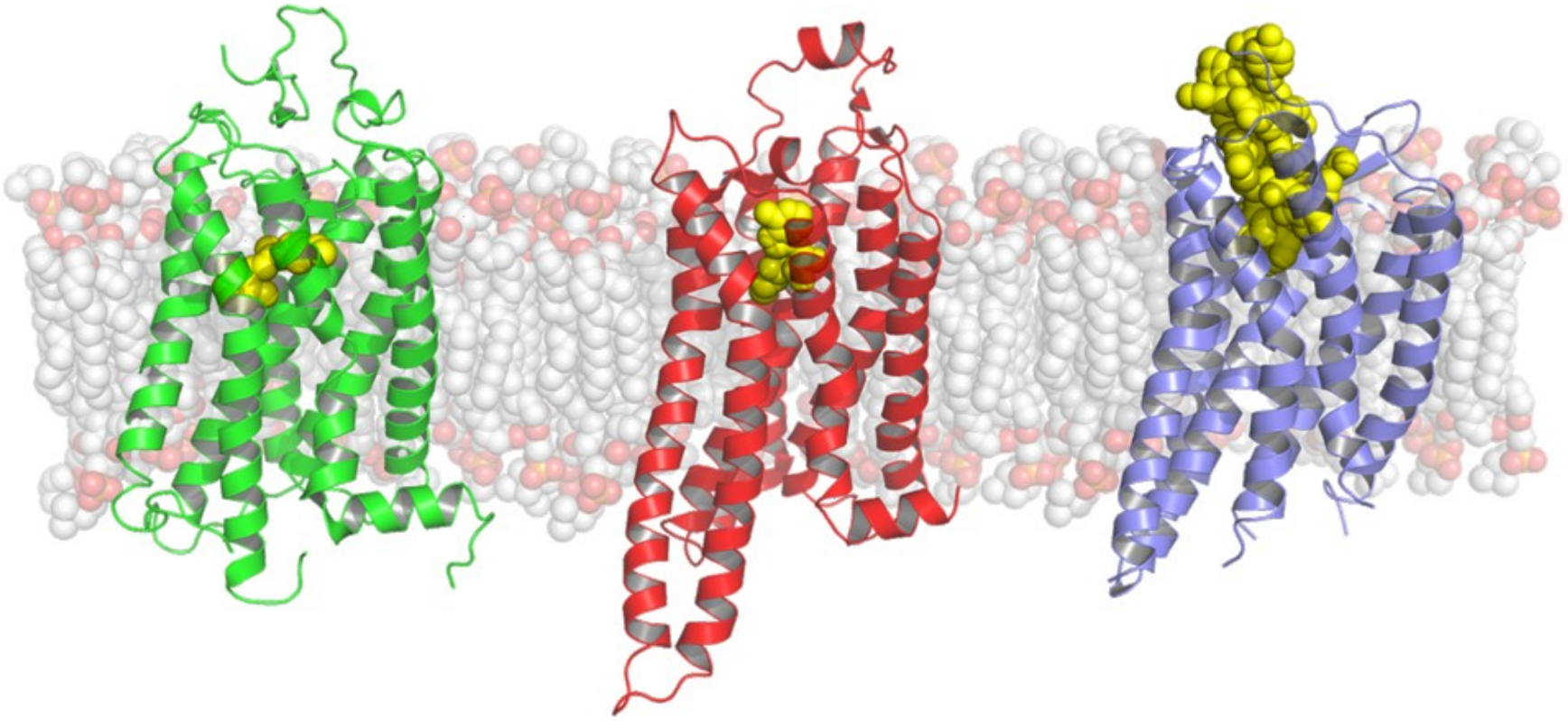
Family B

Peptide (3)
Protein (8)
Orphans (4)

Family C

Glutamate (8)
Ca^{2+} (1)

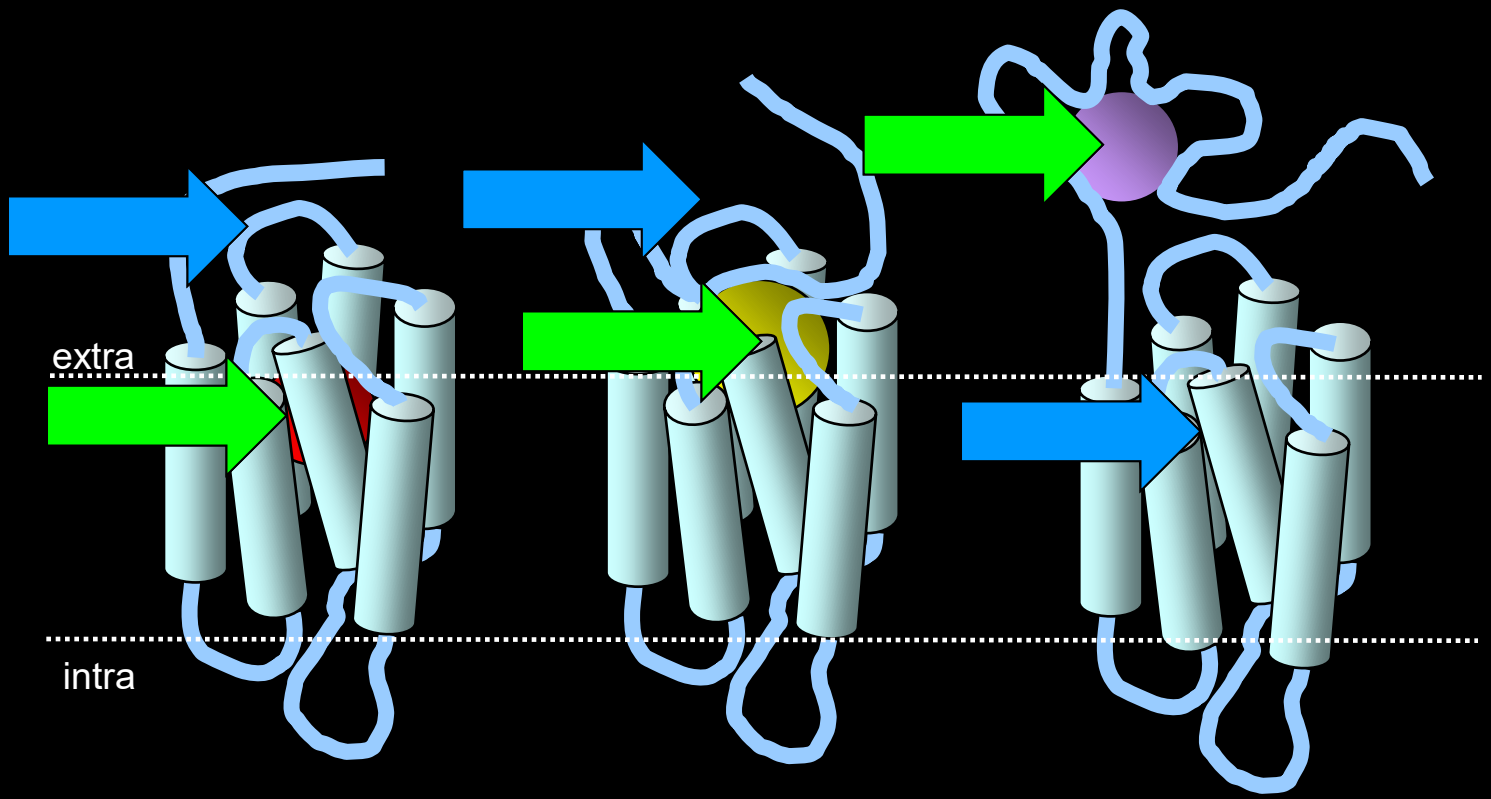
Families A and B orthosteric binding sites: what we learn from the crystallographic structures…



Small molecules

Peptides/Proteins/Lipids

Another important definition:



Family A

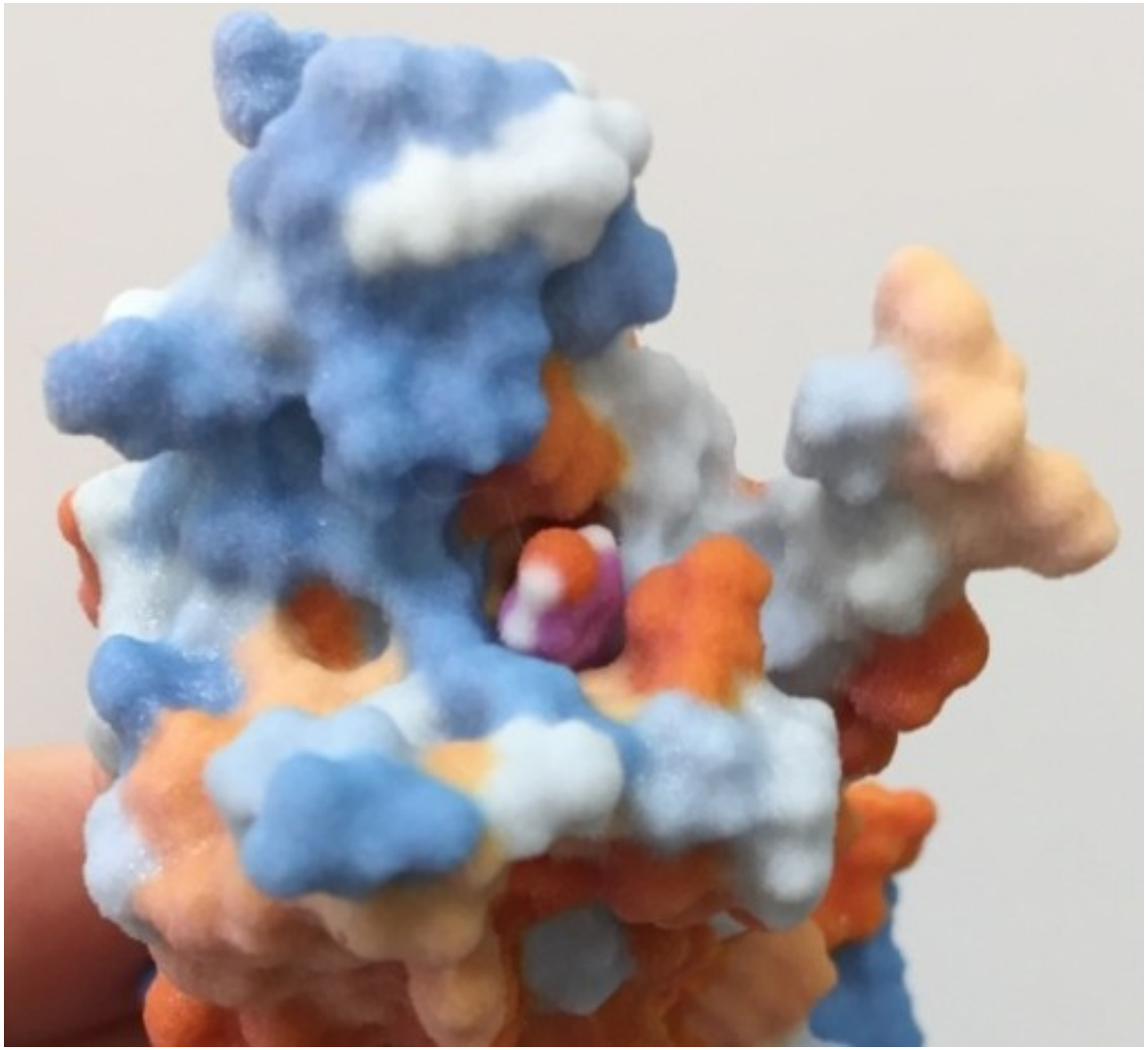
Family B

Family C

ORTHOSTERIC SITE

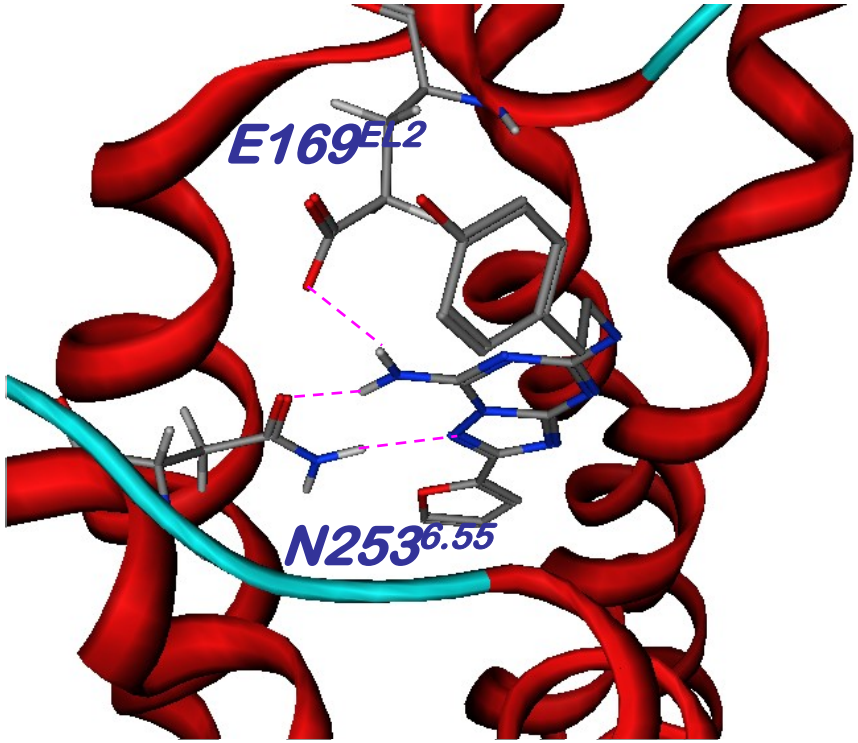
ALLOSTERIC SITE

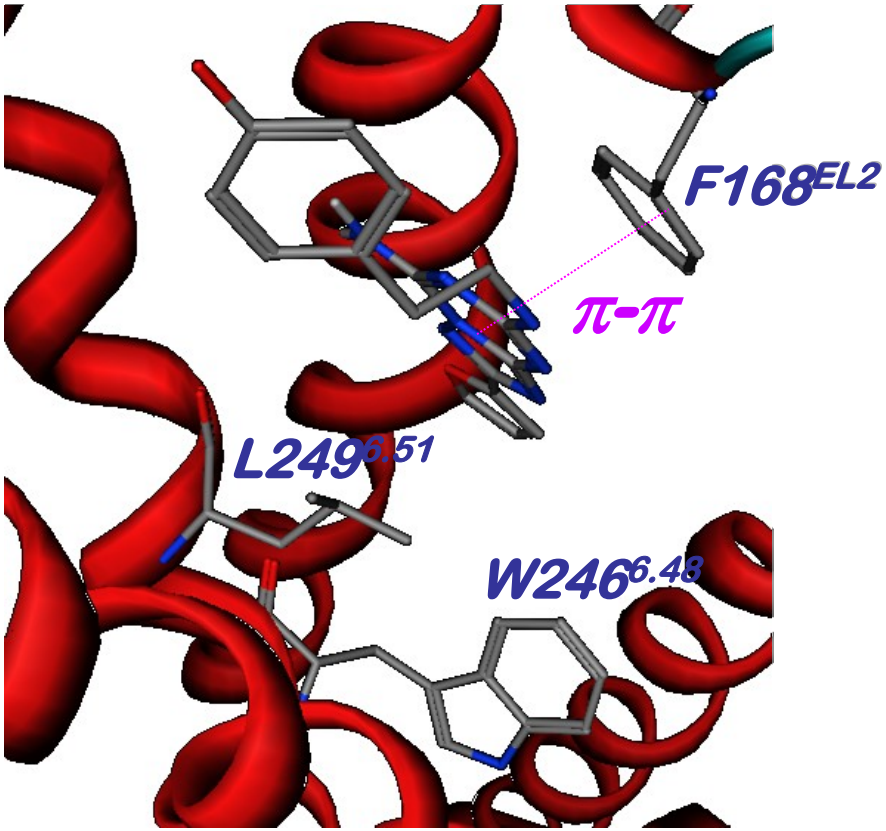
Families A and B orthosteric binding sites: what we learn from the crystallographic structures:



MMS Lab (2015): 3D printed model of the human A2A adenosine receptor co-crystallized its antagonist ZM 241385 (PDB entry: 4EIY)

Families A and B orthosteric binding sites: what we learn from the crystallographic structures:

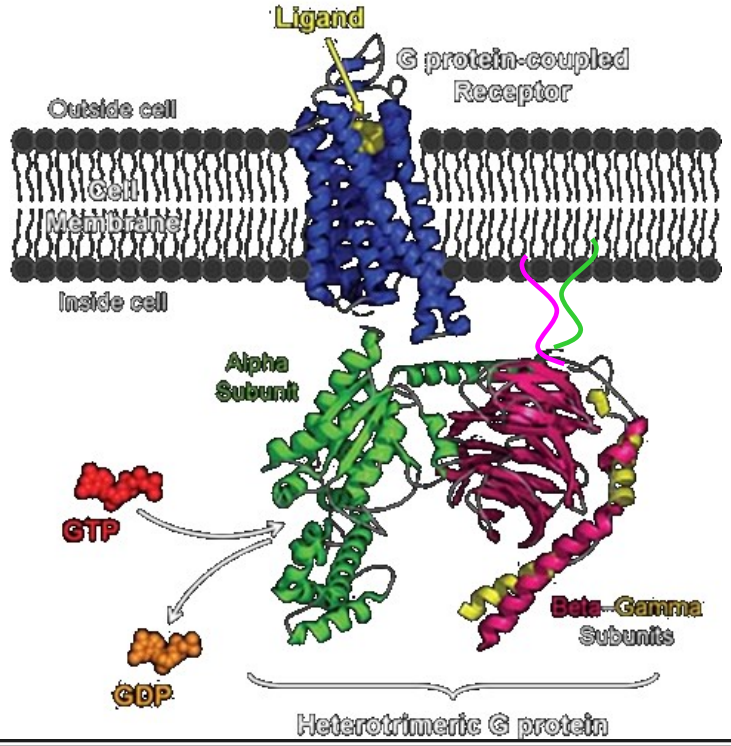




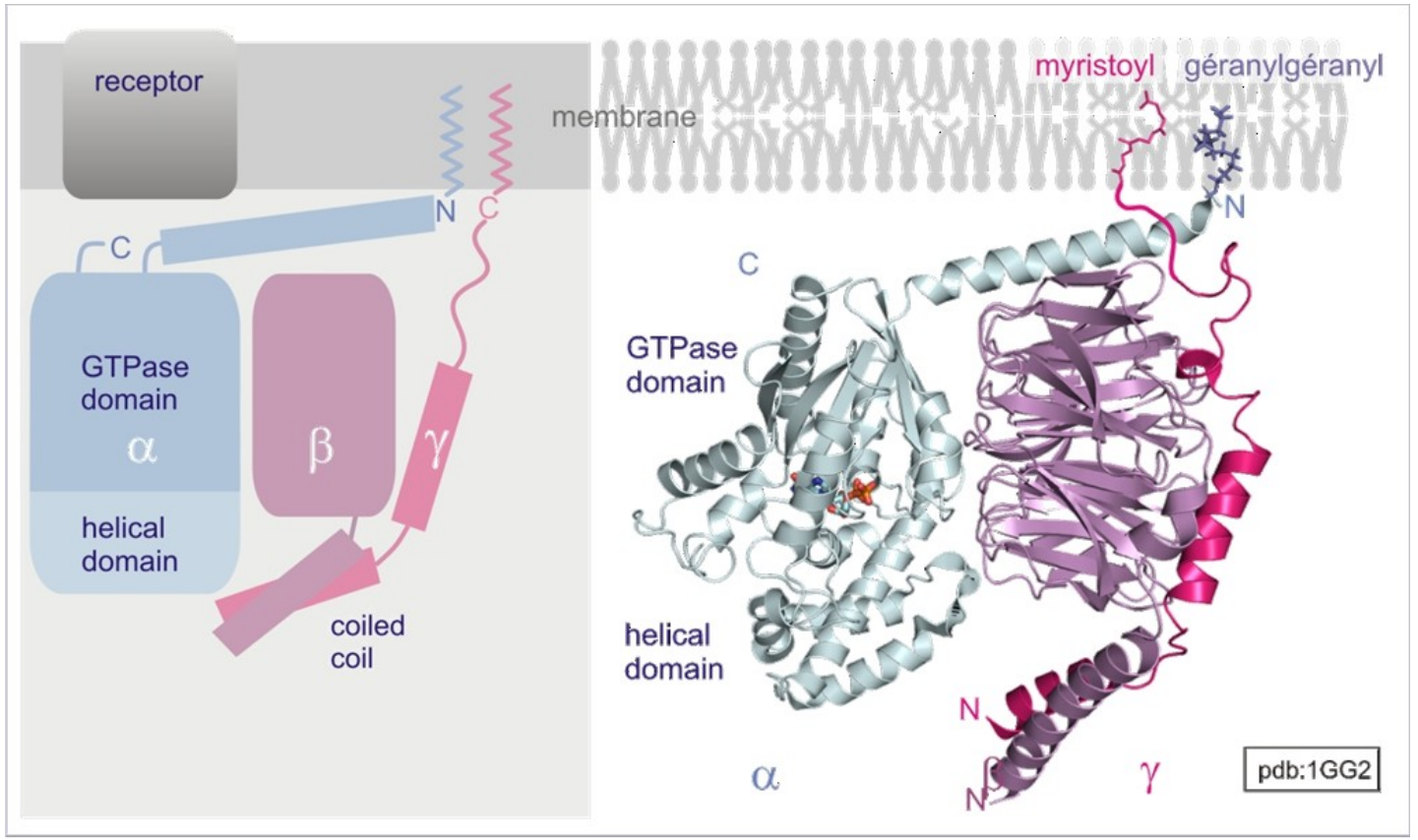
Basic GPCRs Principles

GPCRs have classically been assumed to exist and function as *monomeric* entities, and the paradigms of ligand binding and signal transduction were based on this hypothesis.

GPCR-driven signal transduction is based on G protein coupling from the intracellular environment!

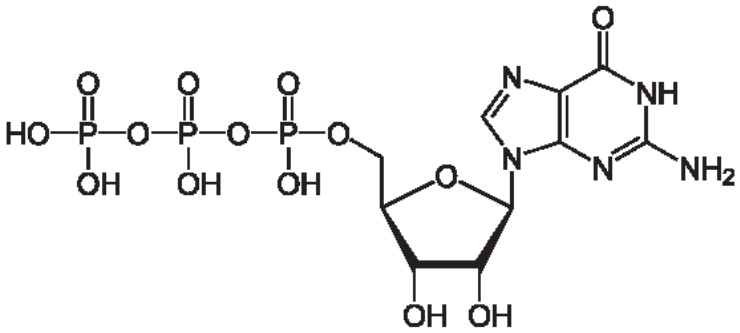


G protein: guanosine nucleotide-binding proteins.

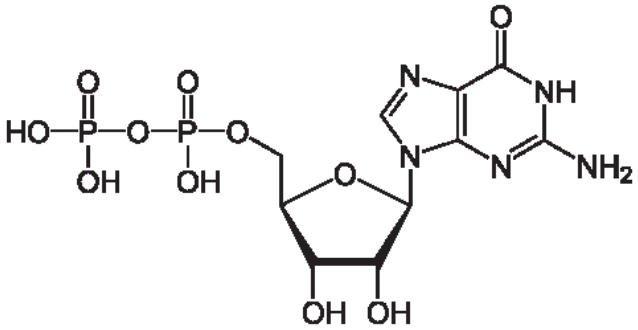


Heterotrimeric G protein… a GTPase

Heterotrimeric G proteins are composed of three subunits, α, β and γ, and their switching function depends on the ability of the G protein α-subunit (Gα) to cycle between an ***inactive GDP-bound*** conformation that is primed for interaction with an activated receptor, and an ***active GTP-bound*** conformation that can modulate the activity of downstream effector proteins.



Guanosine triphosphate, GTP



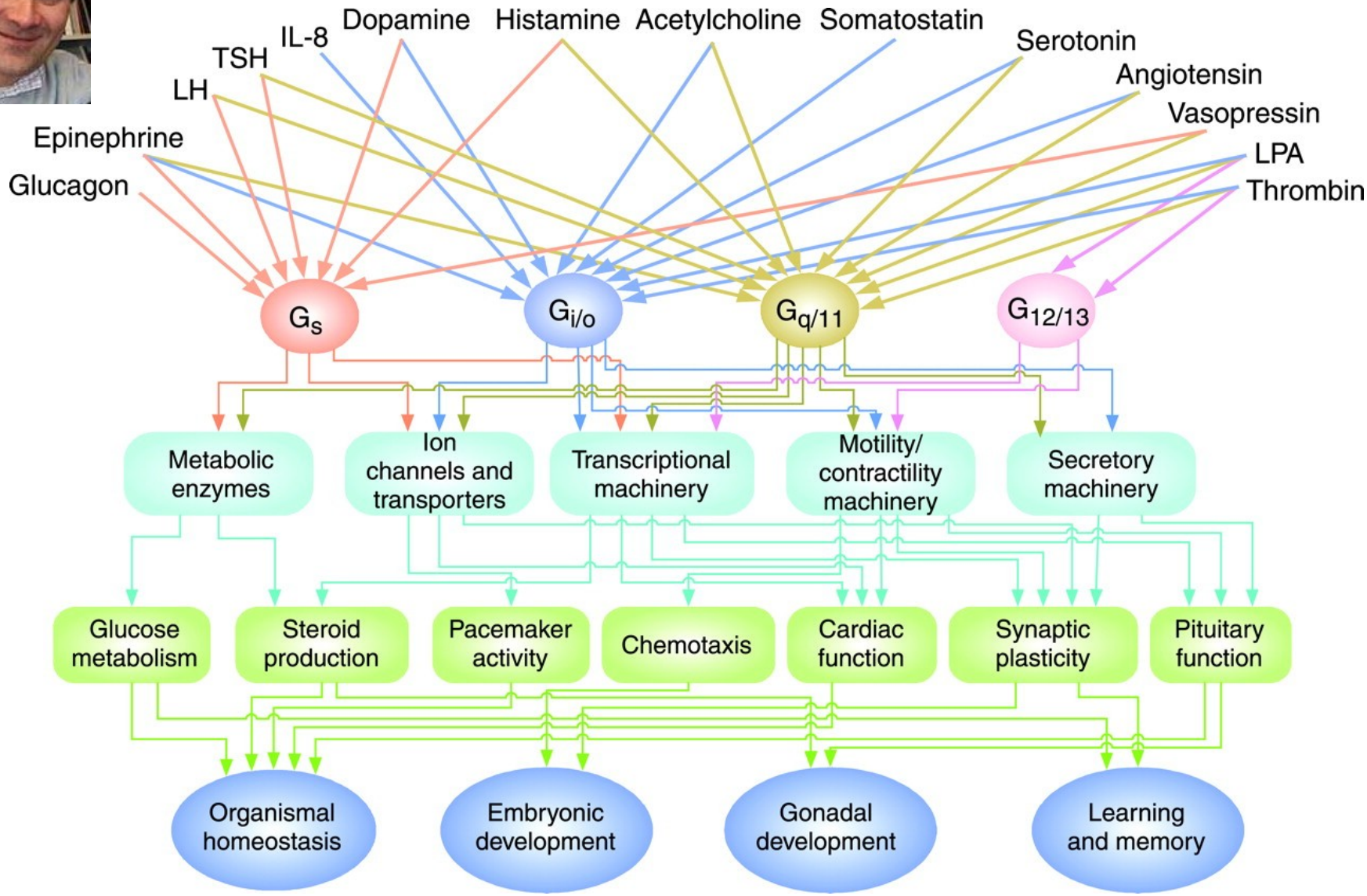
Guanosine diphosphate, GDP

Heterotrimeric G protein… a GTPase

Despite the size and diversity of the GPCR superfamily, these proteins interact with a relatively small number of G proteins to initiate intracellular signalling cascades. In humans, there are **21 Gα subunits, 6 Gβ subunits, and 12 Gγ subunits**.

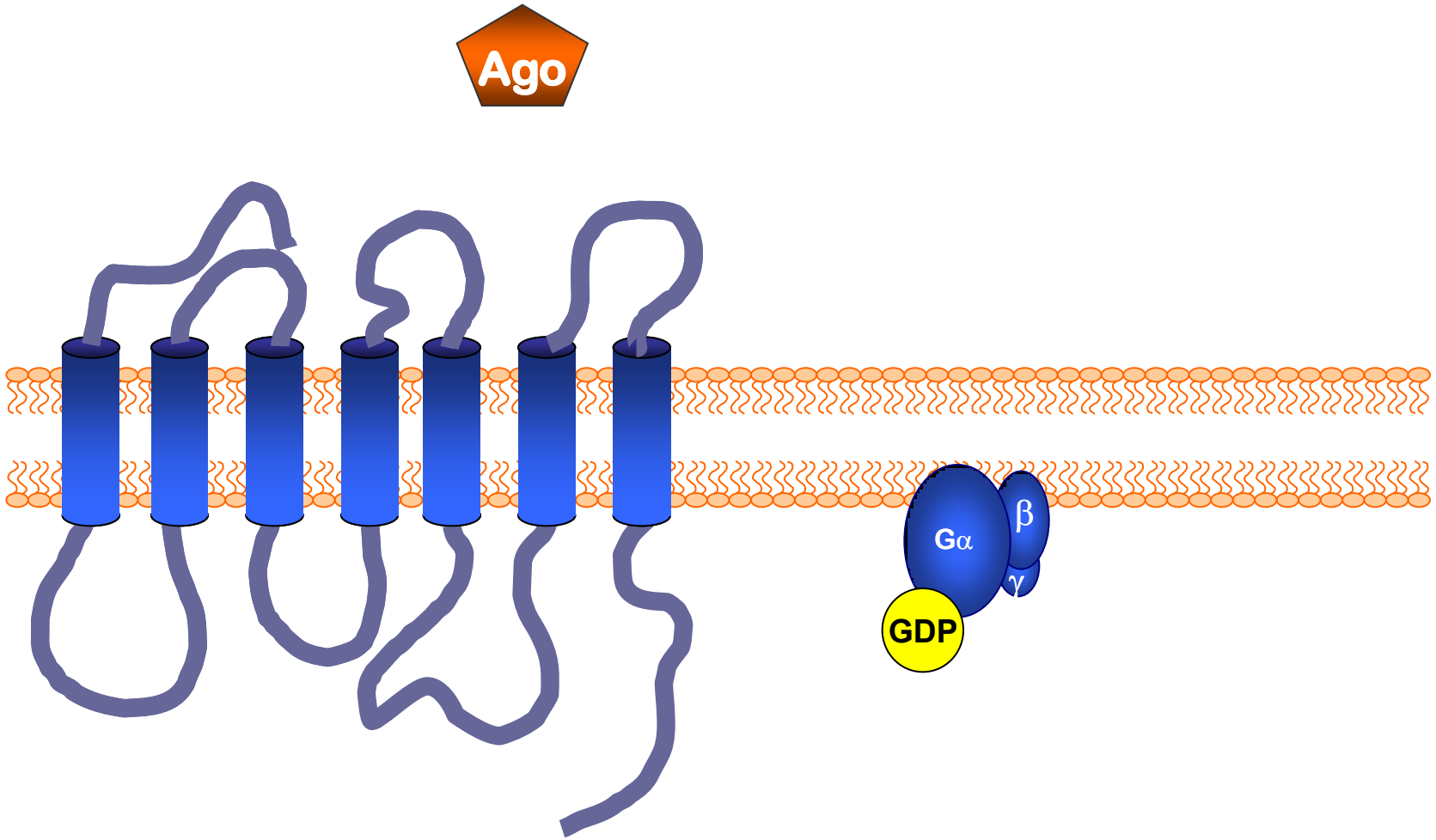
Heterotrimers are typically divided into four main classes based on the primary sequence similarity of the Gα subunit: **G_s, $G_{i/o}$, $G_{q/11}$ and $G_{12/13}$**

Heterotrimeric G protein… a GTPase

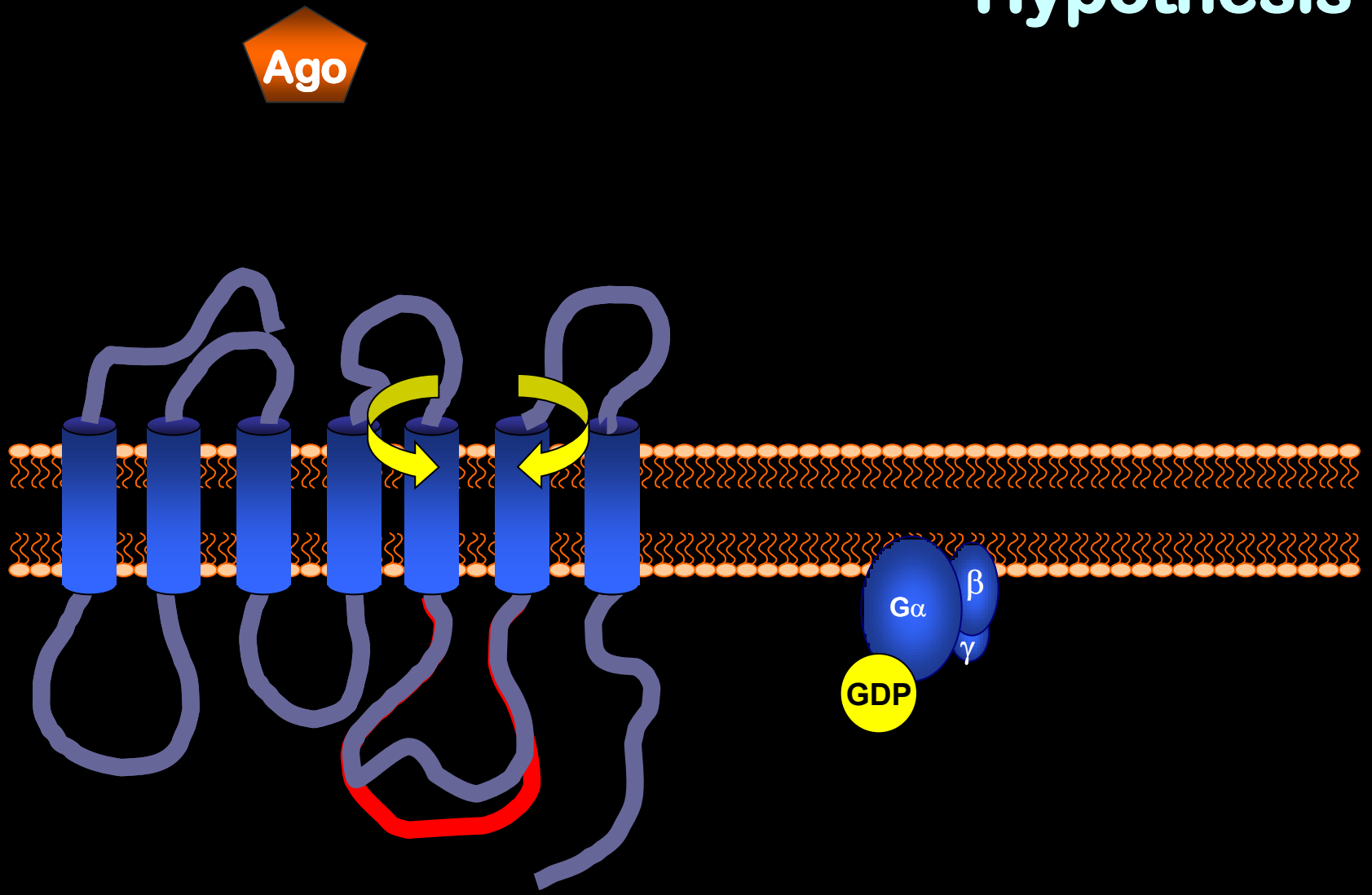


credits: http://science.sciencemag.org/content/296/5573/1636/tab-figures-data

G protein coupled to its receptor: a mechanicistic scenario.

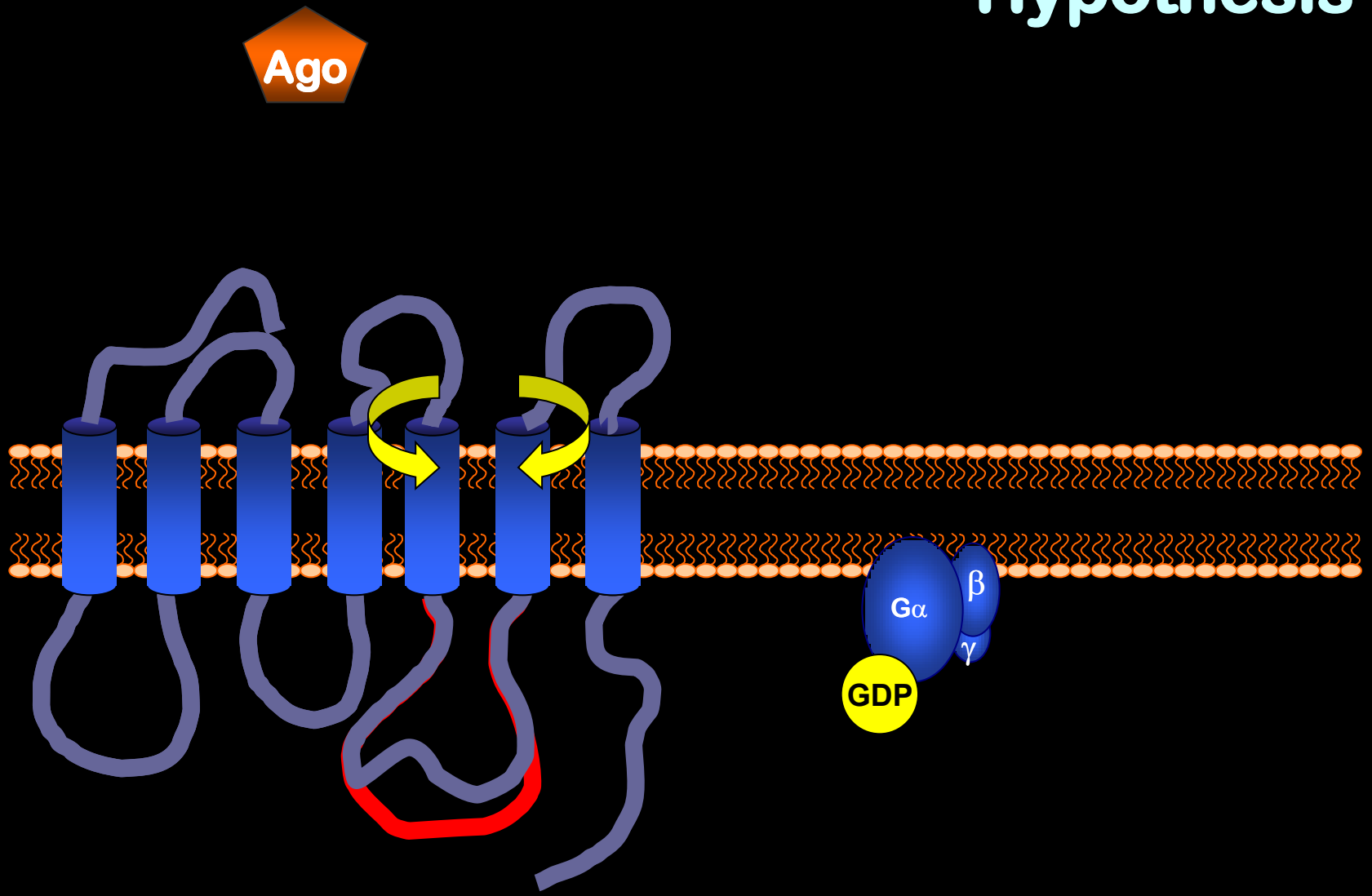


Hypothesis 1



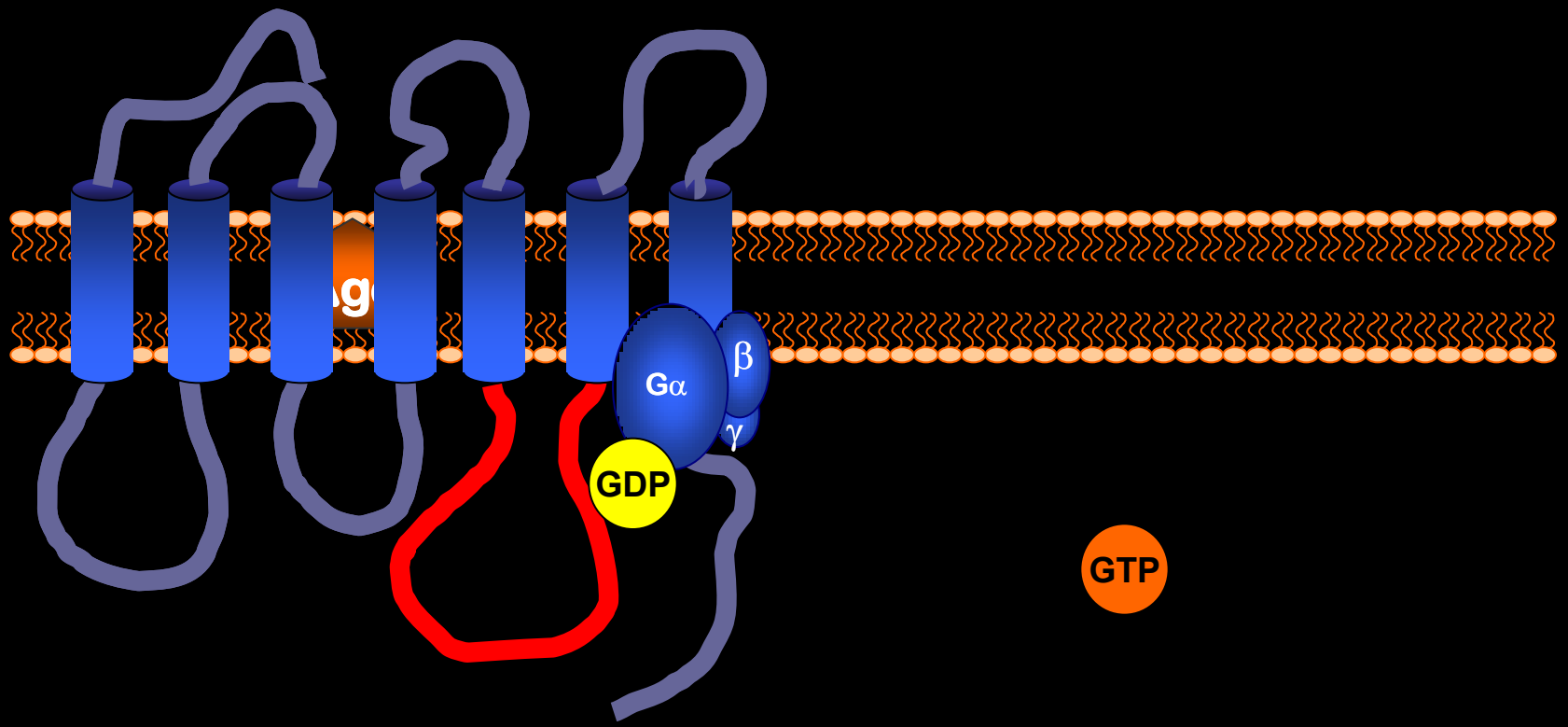
CLASSICAL HYPOTHESIS

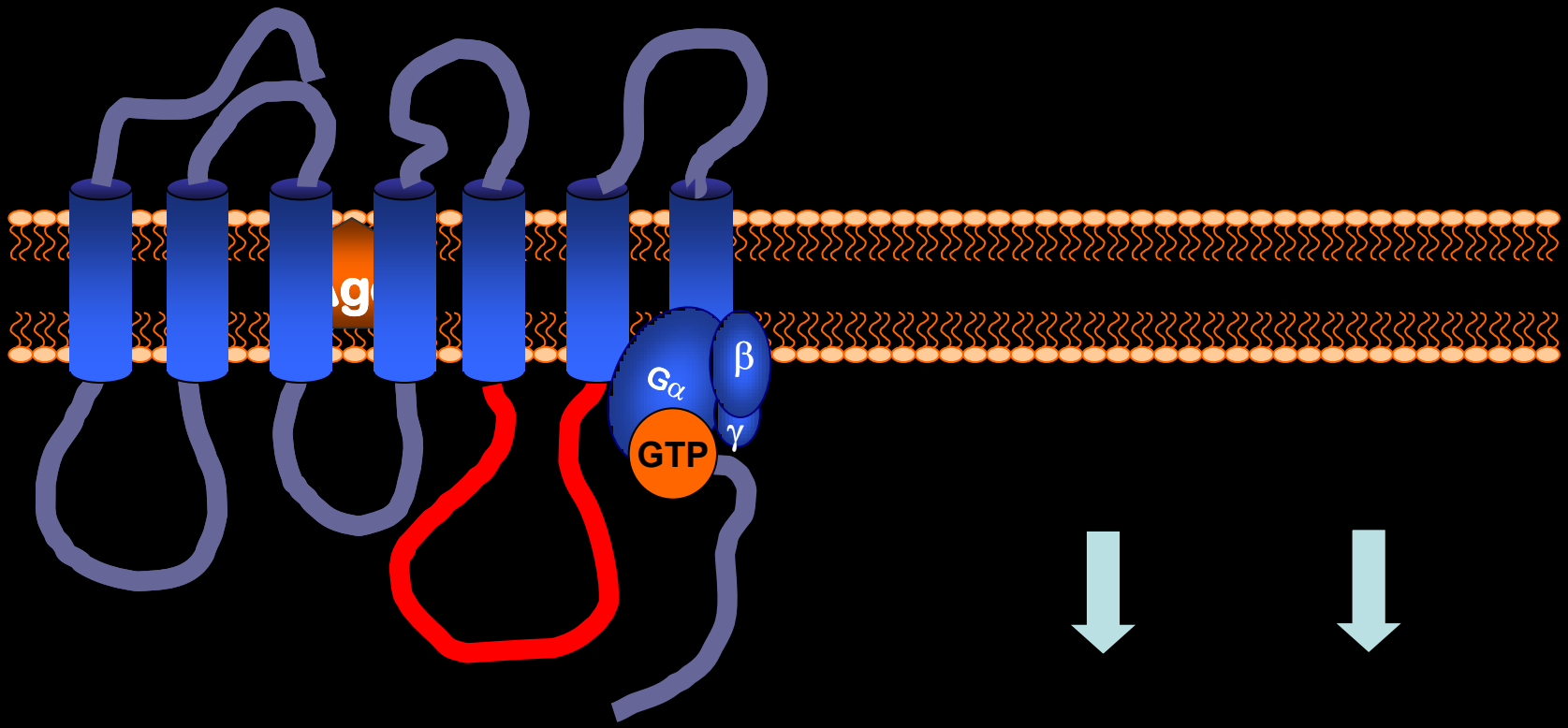
Hypothesis 2



BASAL ACTIVITY HYPOTHESIS

and the story continues …





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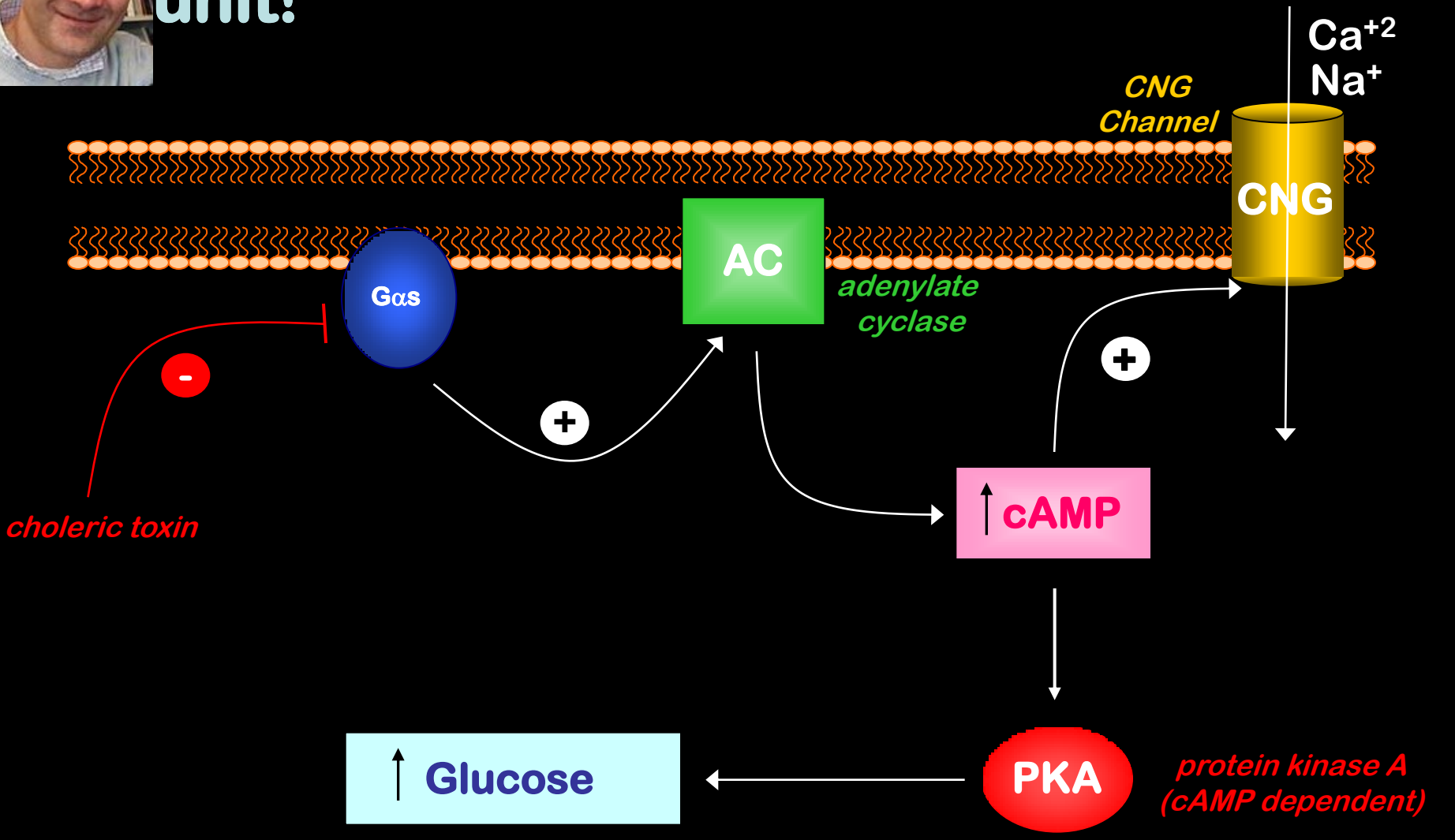
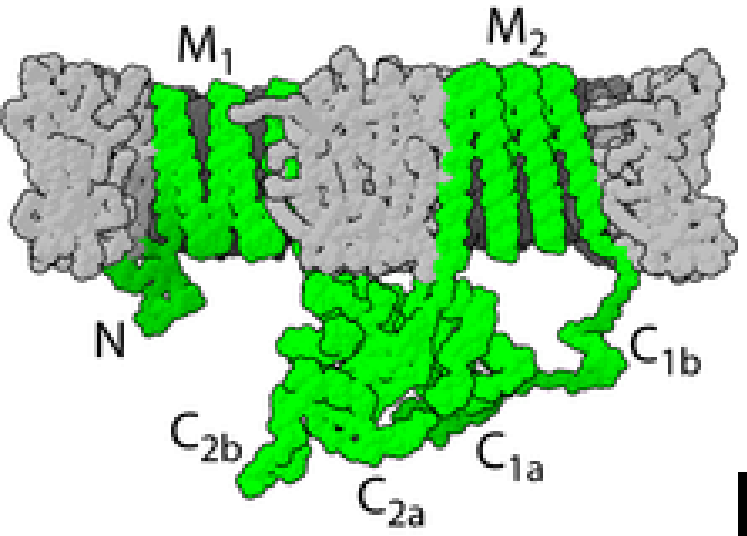
Heterotrimeric Gs protein… with a Gαs unit!

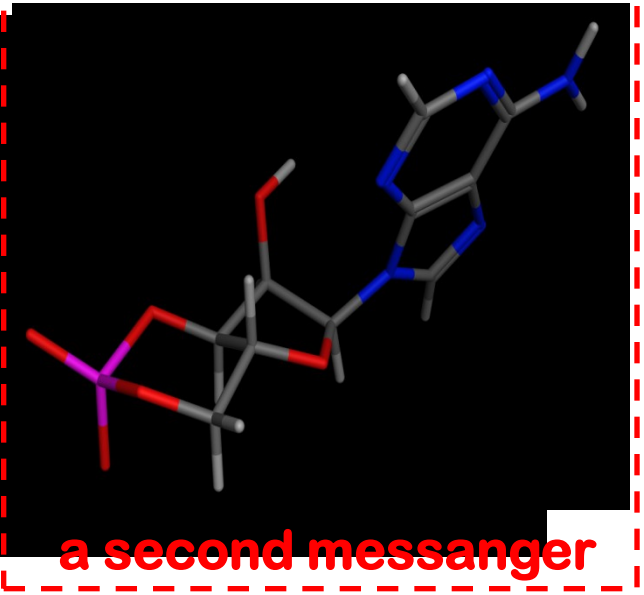


CNG: cyclic nucleotide-gated ion channel

Adenylate cyclase: an essenzial enzyme!



The nine cloned isoforms of mammalian adenylyl cyclase (AC) share a primary structure consisting of two transmembrane regions, M_1 and M_2, and two cytoplasmic regions, C_1 and C_2.



a second messanger

The crucial role of protein kinase A (PKA)

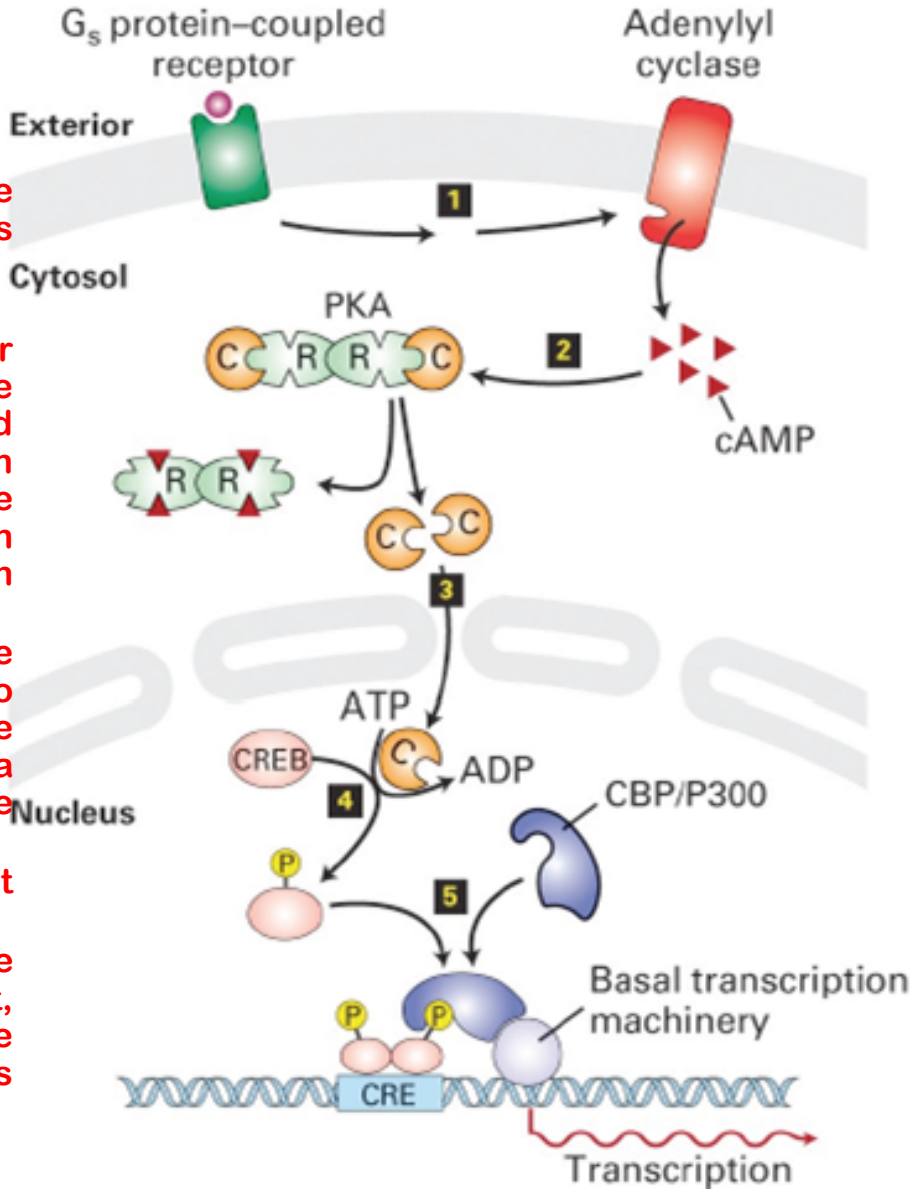
Protein kinase A phosphorylates and thereby changes the activity of a number of important molecules. Included in its target list are:

Enzymes: Phosphorylation is widely used as a molecular switching mechanism to activate or inactivate enzyme activity. In many cases, the enzyme being phosphorylated is itself a kinase. The classical example is that protein kinase A phosphorylates the enzyme phosphorylase kinase, which, in turn, phosphorylates glycogen phorphorylase, which leads to breakdown of glycogen in liver and muscle.

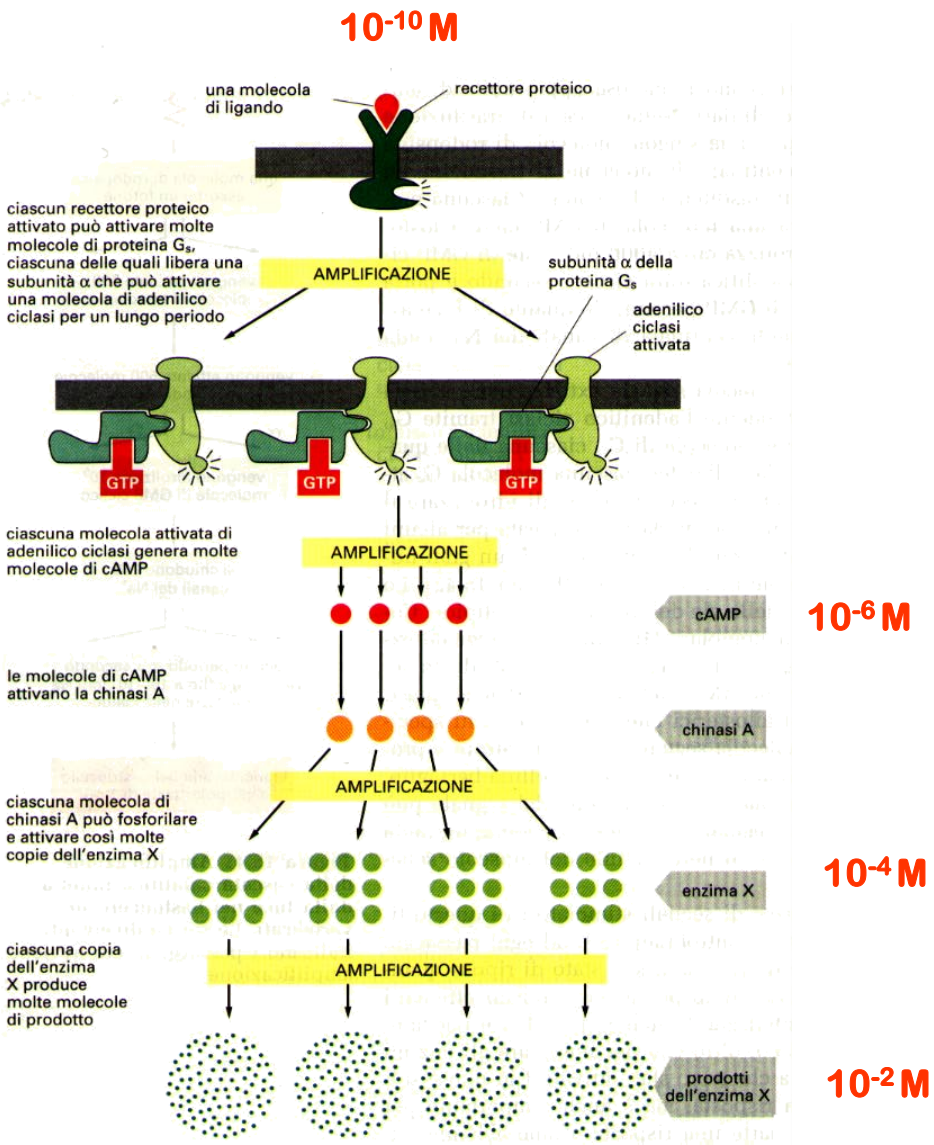
Ion channels: Certain calcium channels in cardiac muscle cells are activated by protein kinase A, ultimately leading to muscle contraction. Another medically important example is that protein kinase A phosphorylates and thus activates a chloride channel important in secretion of water in the small intestine.

Chromosomal proteins: Histone H1 was the first target identified for protein kinase A.

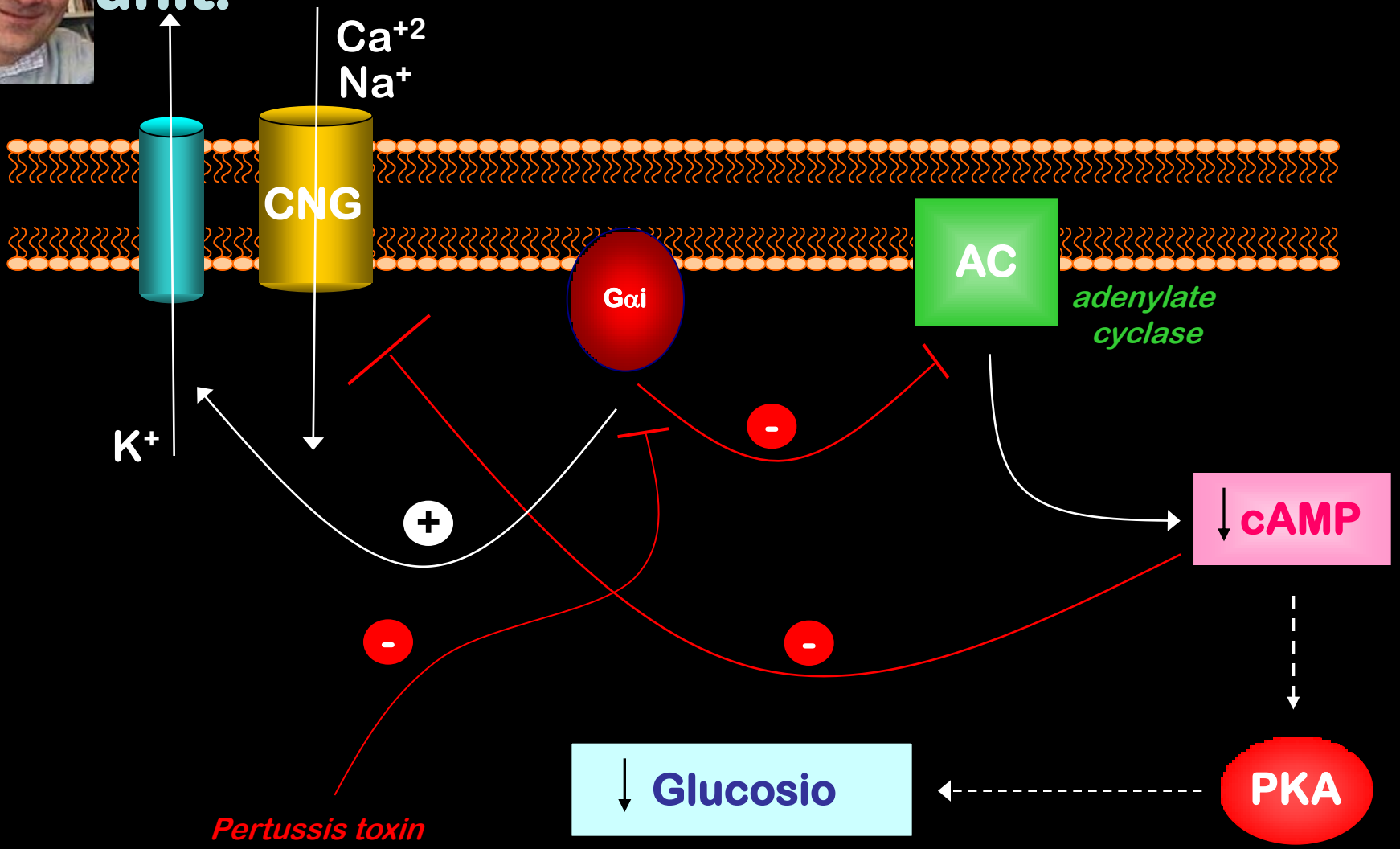
Transcription factors: CREB's (cyclic AMP response element binding proteins) are transcription factors that, when phosphorylated by protein kinase A, become competent to bind promoter regions of responsive genes and stimulate transcription.



Signal processing: amplification

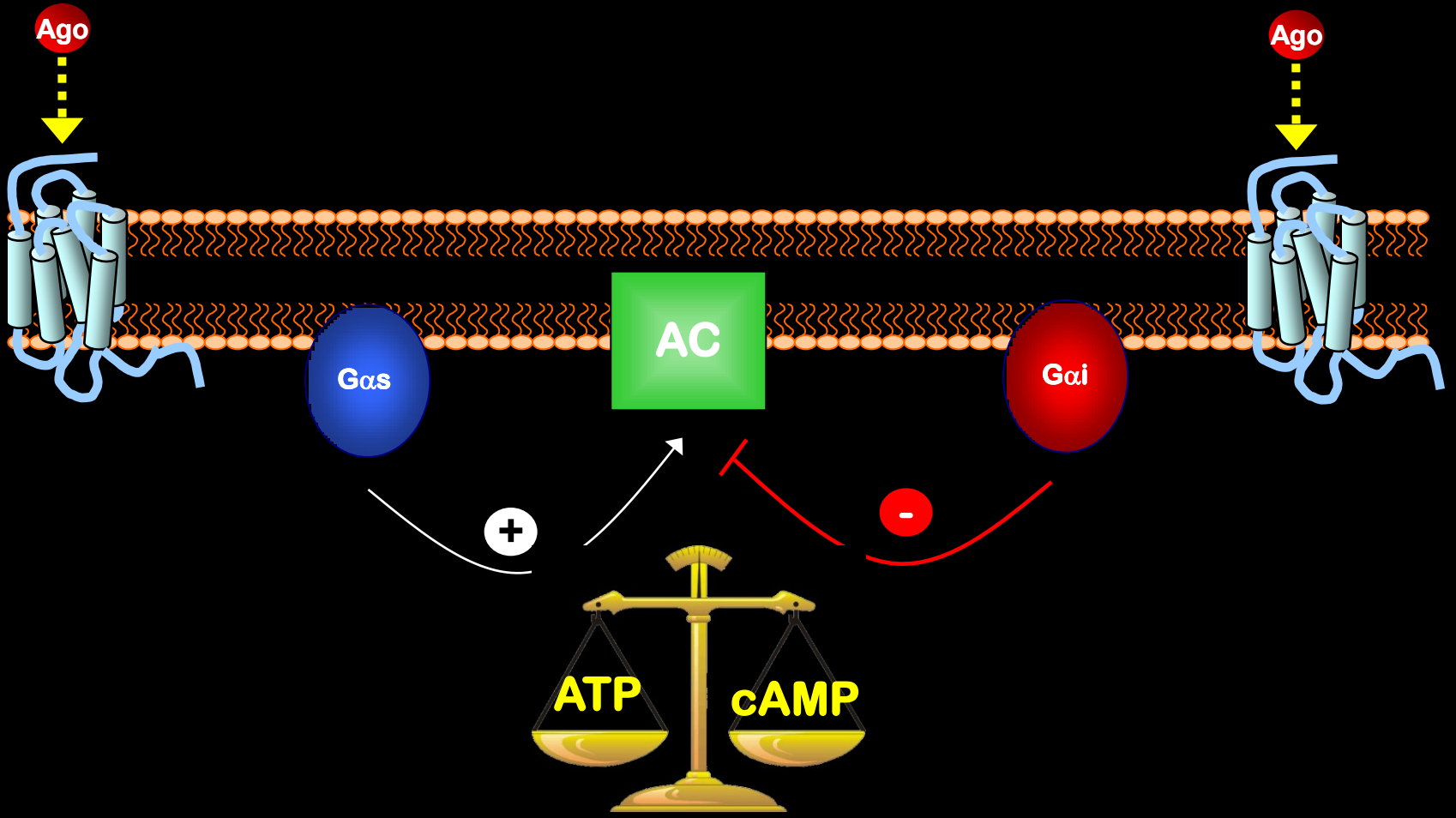


Heterotrimeric Gi/o protein... with a Gαi/o unit!

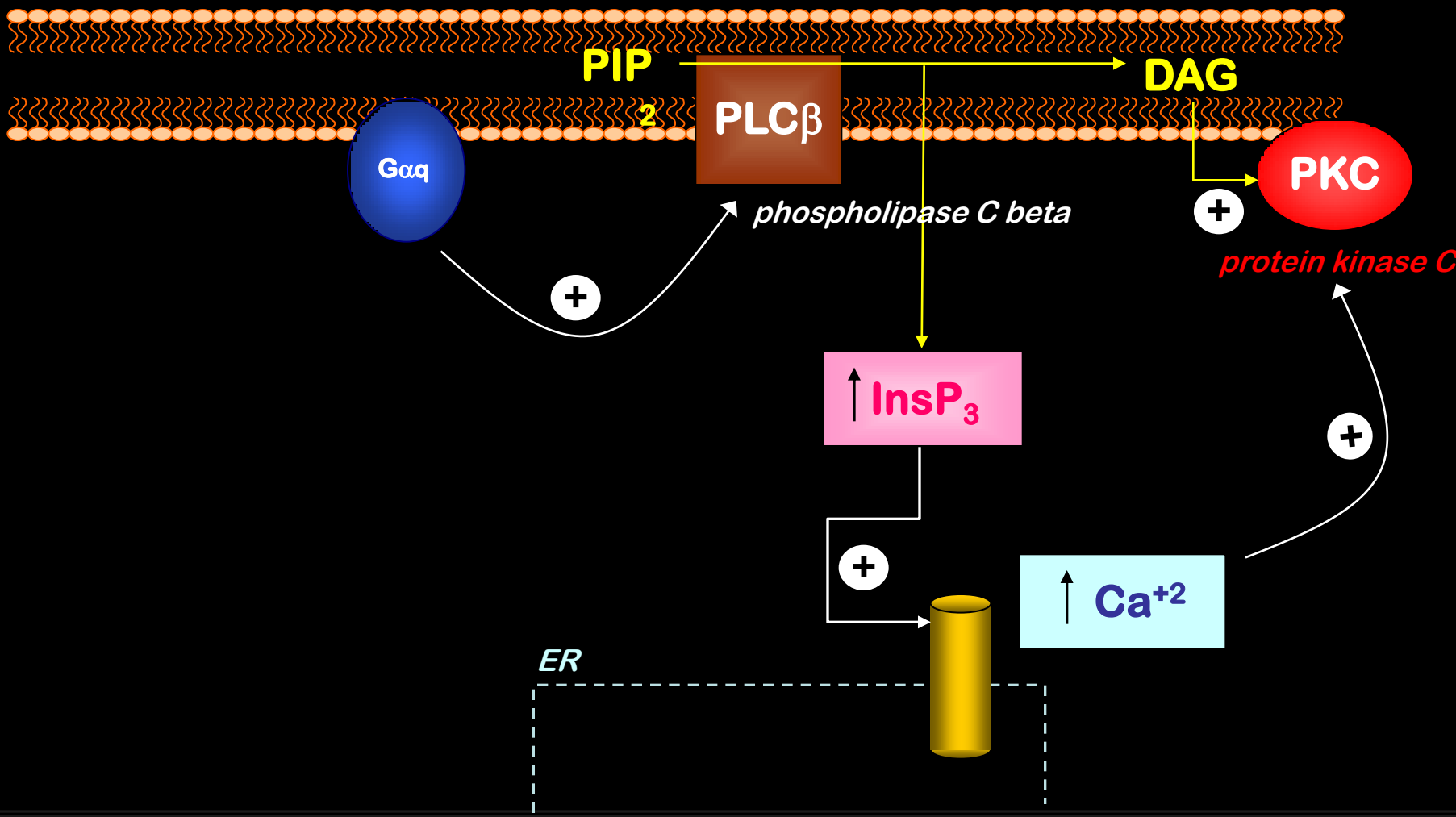


CNG: cyclic nucleotide-gated ion channel

Signal processing: integration

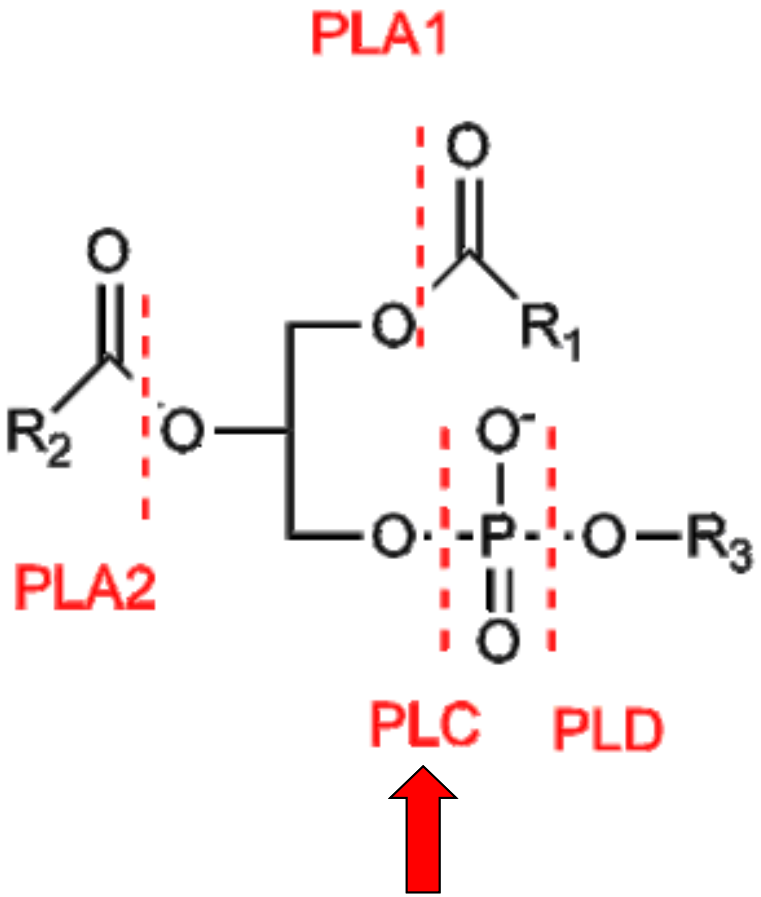


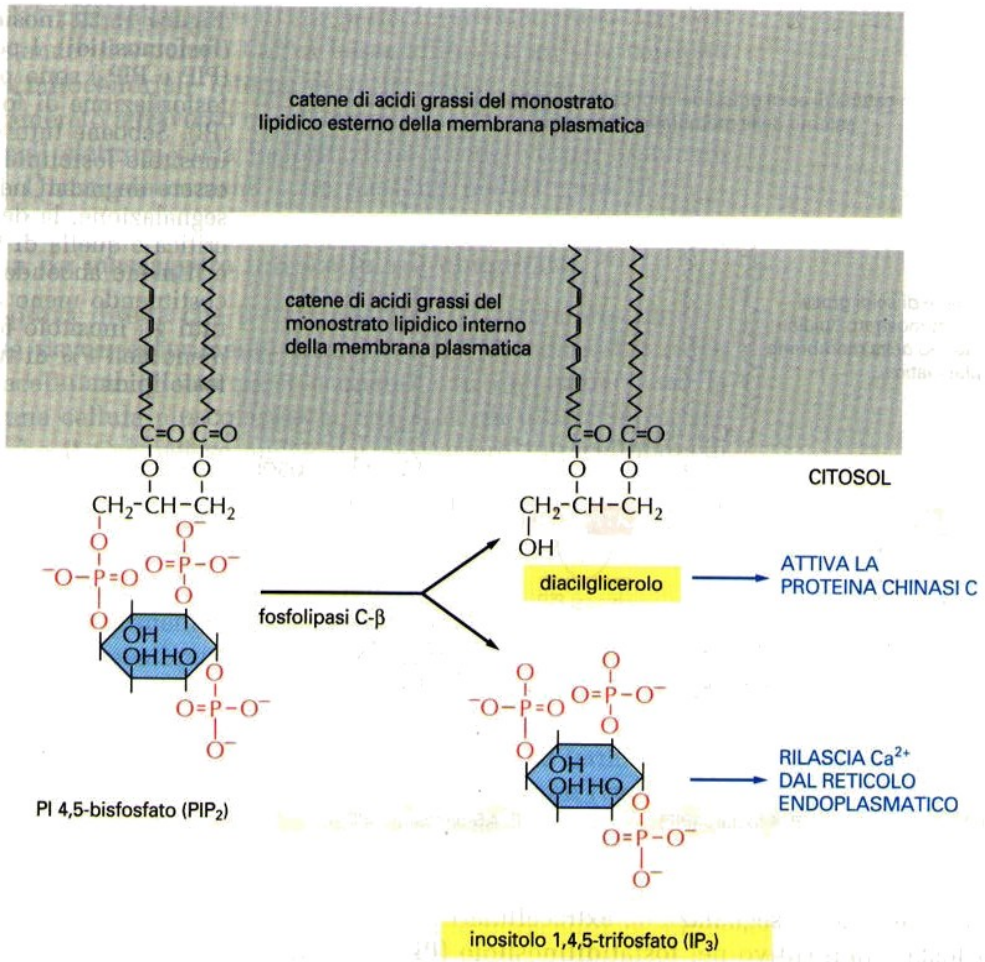
Heterotrimeric Gq/11 protein… with a Gαq/11 unit!



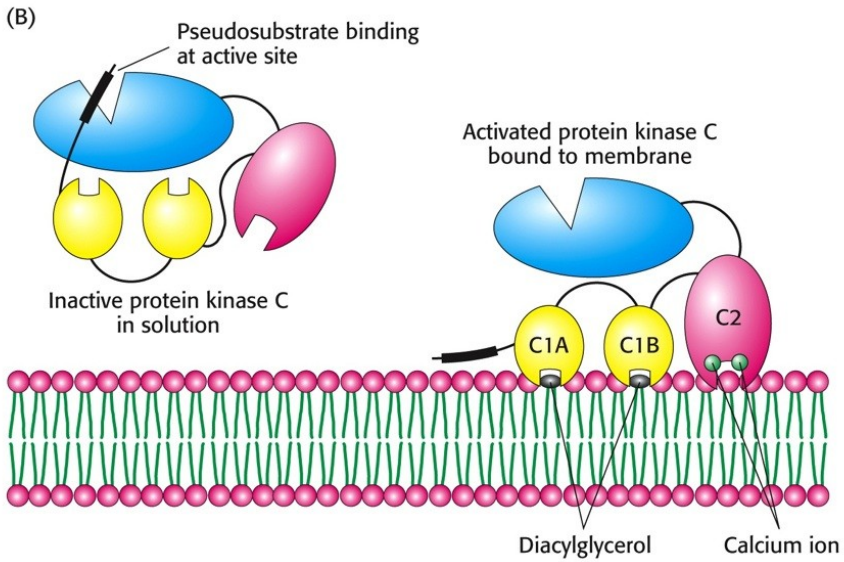
Do you remember?

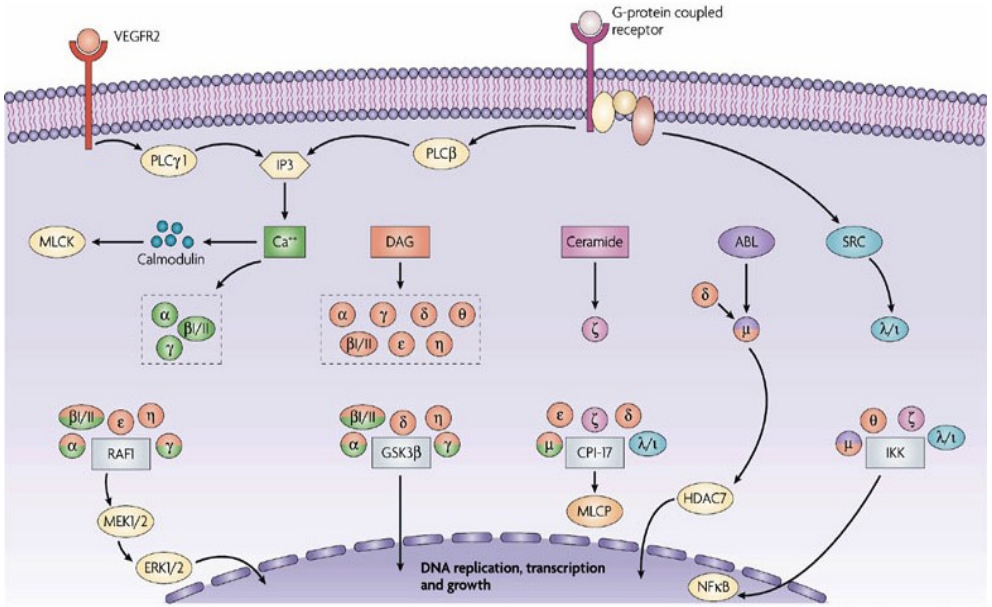
Cleavage sites of phospholipases.





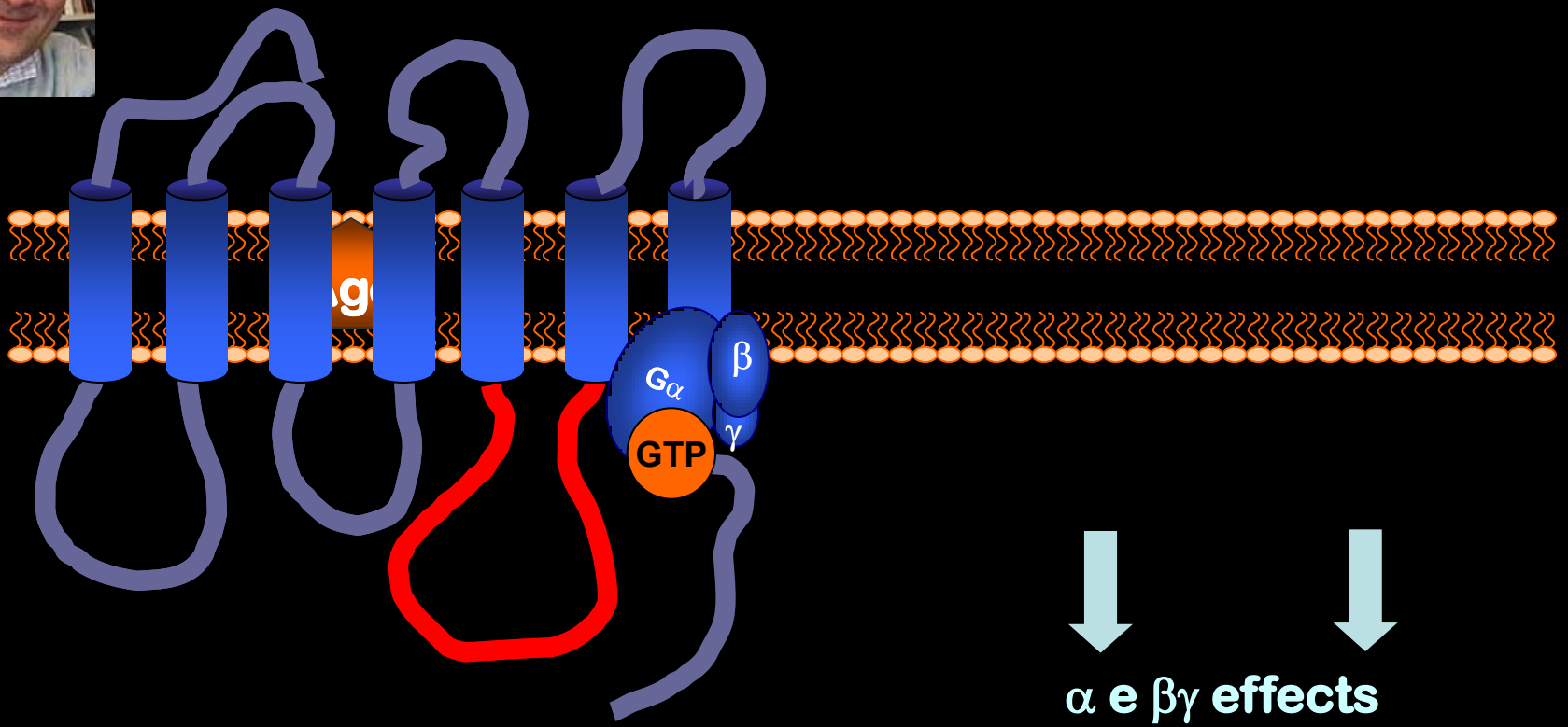
The crucial role of protein kinase C (PKC)





Protein kinase C serves many vital roles in the cell and overall system. However, it mainly serves as a specific kinase enzyme for Ca^{2+}, as designated by the C in PKC. While previously it was established that not all types of PKC use Ca^{2+} as a substrate for activation, it is known that PKC plays a pivitol role as a relay system in many Ca^{2+} dependent processes. Additionally, PKC serves as a promoter of lipid hydrolysis as well as an effective key in signal transductions throughout the cell. Generally, PKC's function is mediated by two mechanisms - 1) phosphorylations that align PKC for catalysis and localize it to the cytosol and 2) binding of substrates or ligands that free the PS from the core binding site thusly activating the enzyme.

Stopping the signal transmission:



α e βγ effects

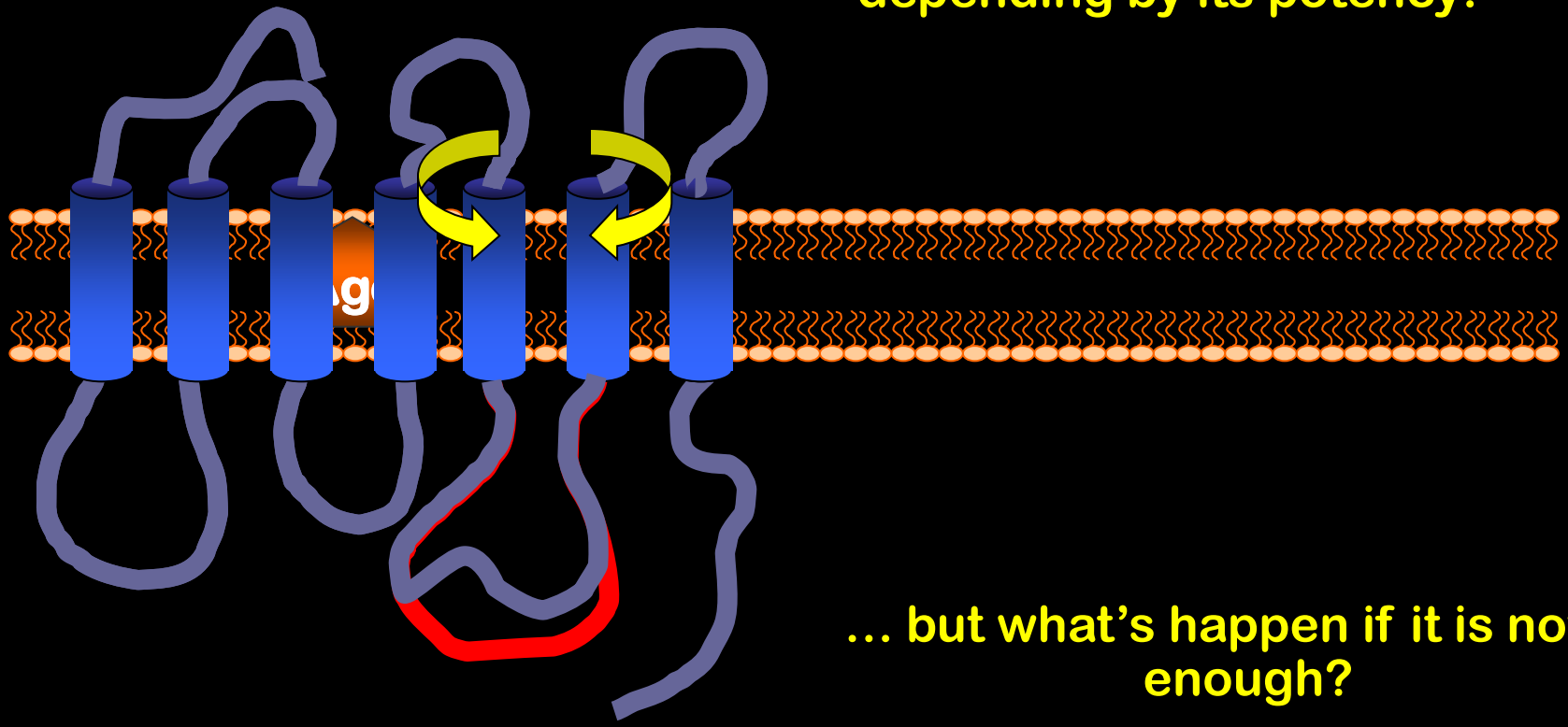
1 Desensitization

2 Recycling Gα-ATP→Gα–ADP

3 cAMP hydrolysis

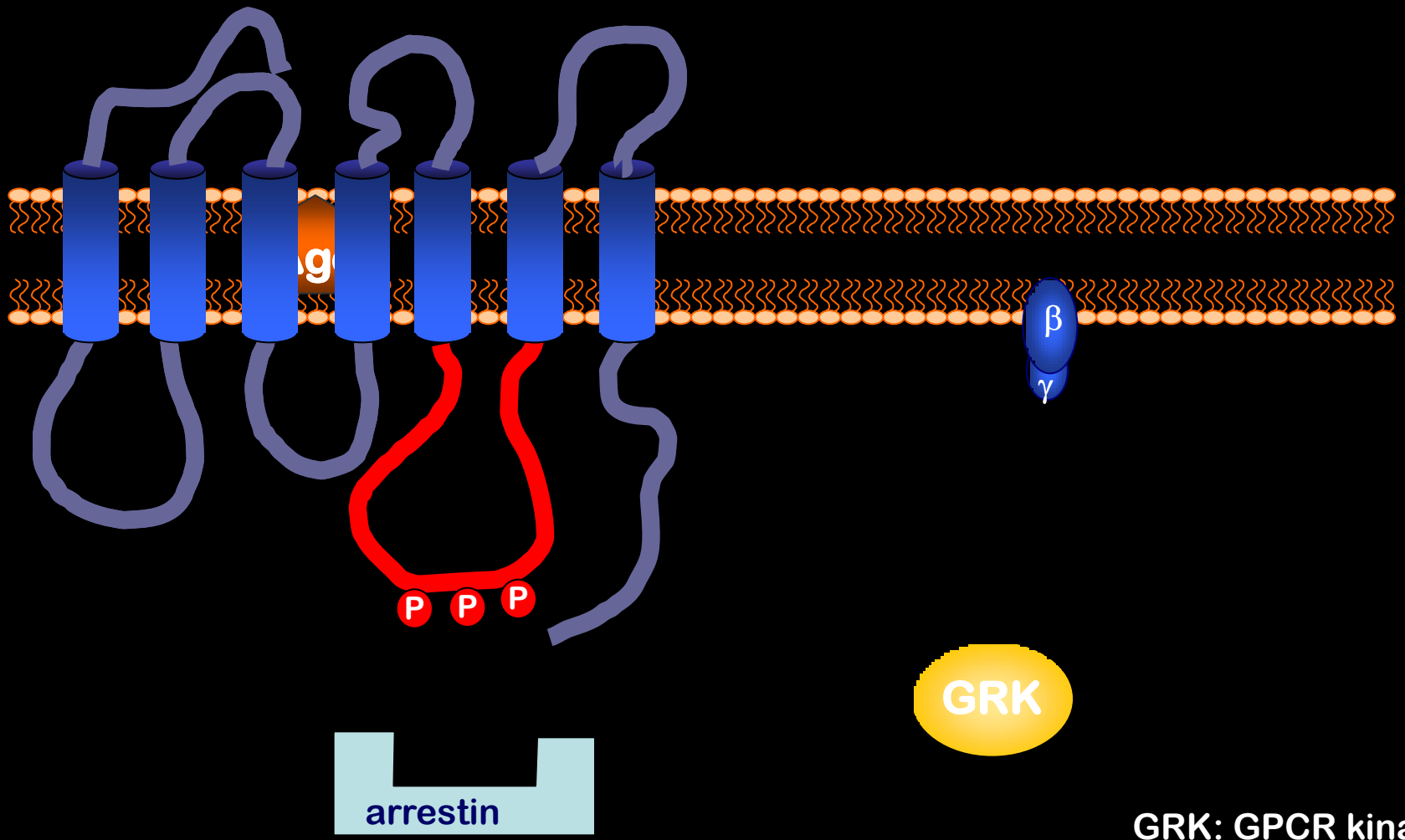
Desensitization: a fundamental *stop-step* in signal transmission

Agonist binding is a reversible process…
depending by its potency!



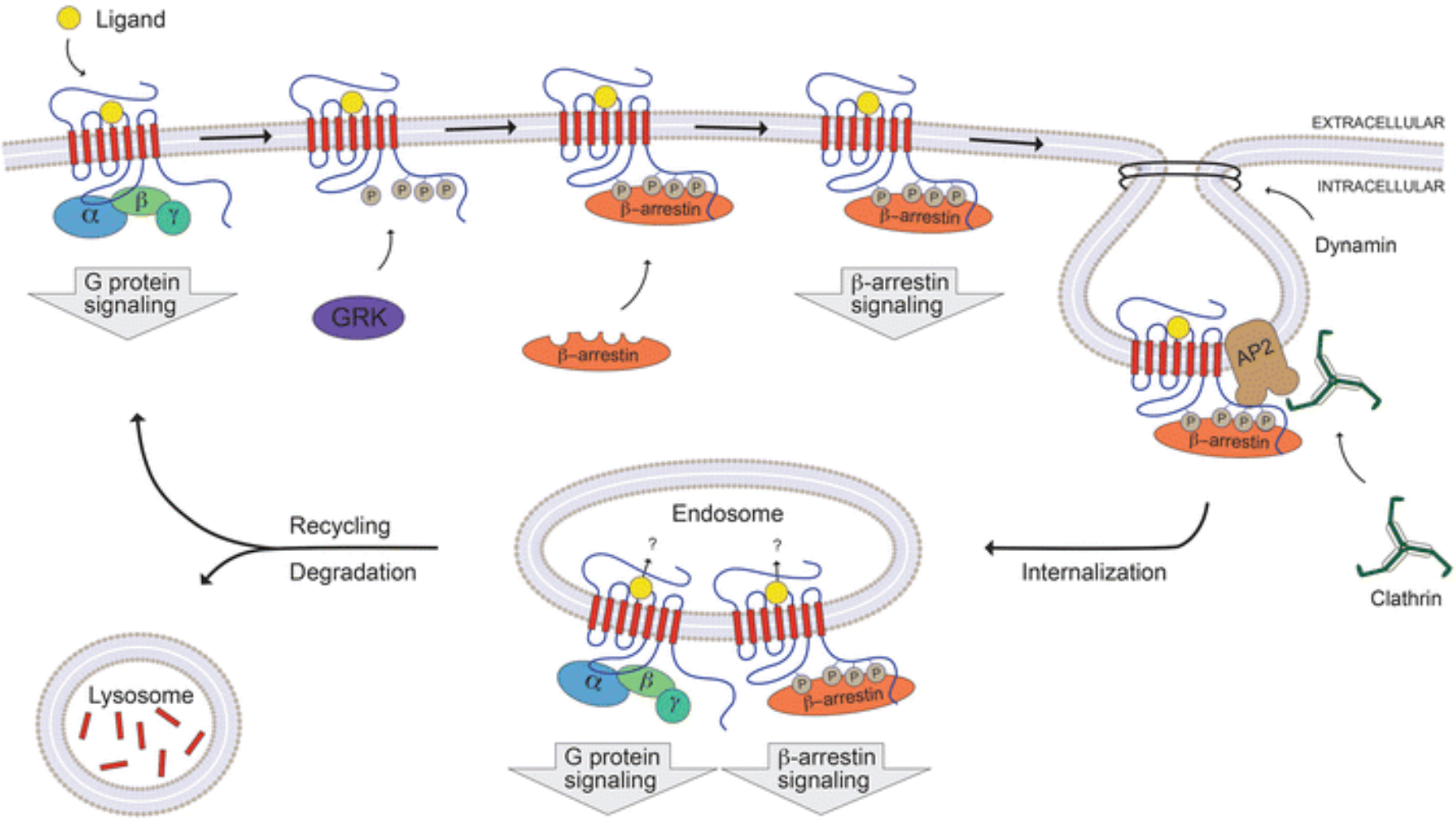
… but what's happen if it is not enough?

Here is a possible solution:



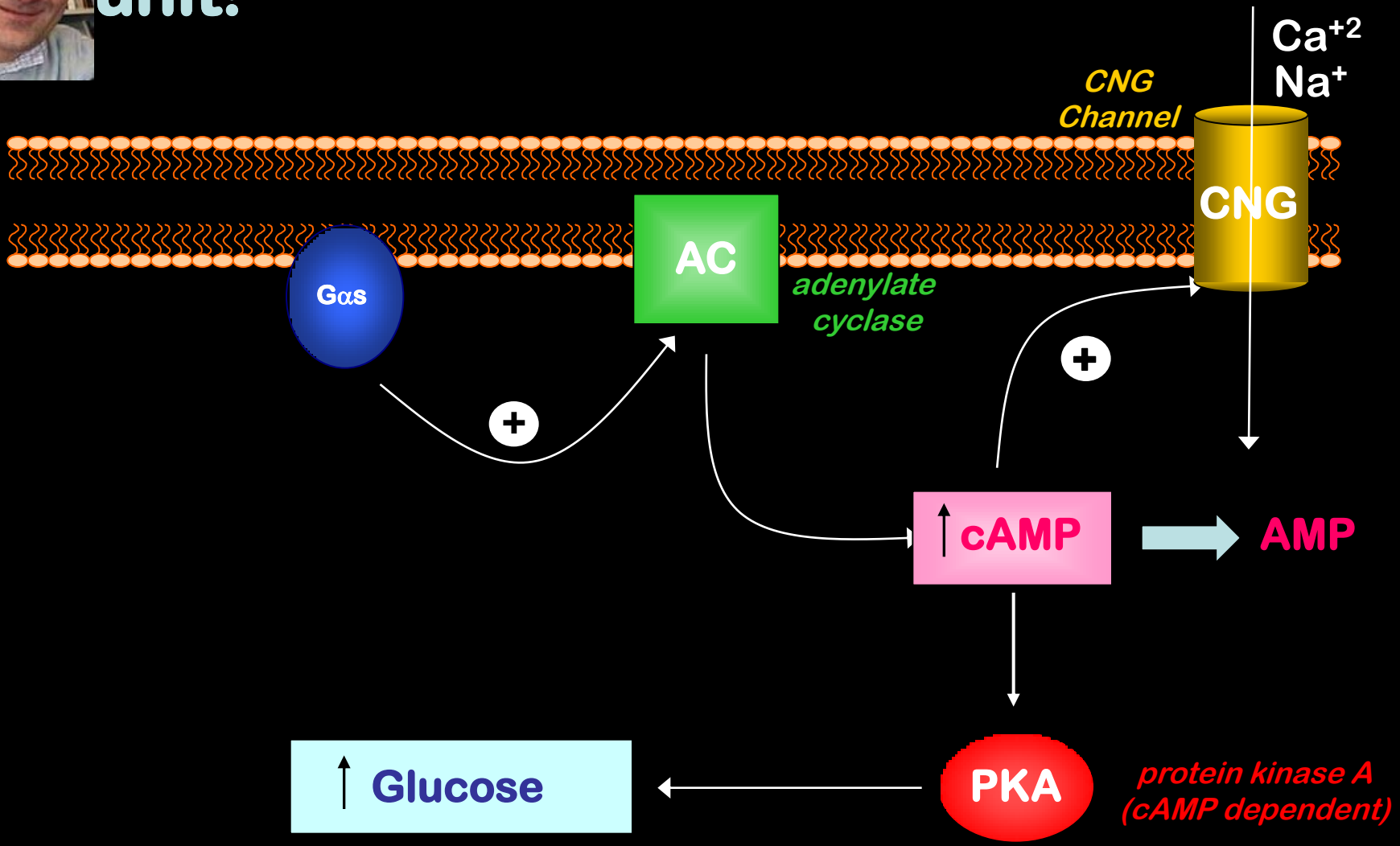
GRK: GPCR kinase

The end… or the beginning, again!



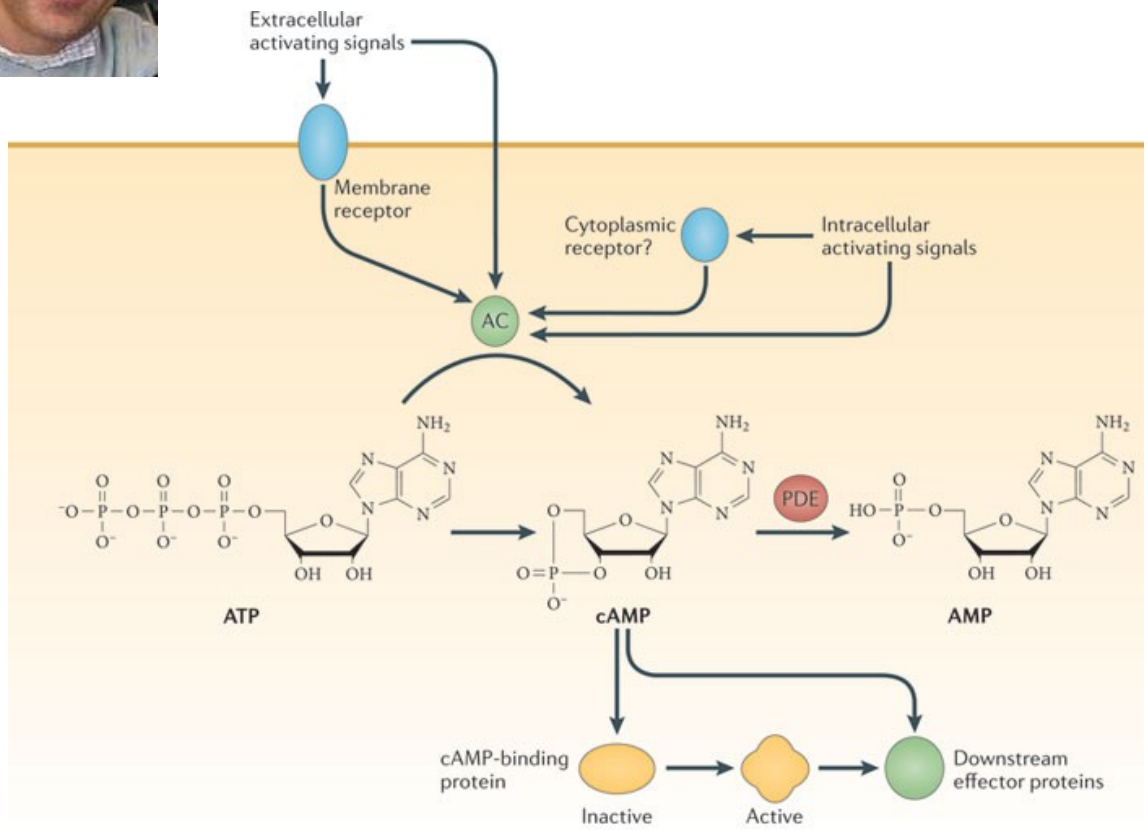
credits: https://link.springer.com/chapter/10.1007/164_2017_57

Heterotrimeric Gs protein… with a Gαs unit!



CNG: cyclic nucleotide-gated ion channel

And finally… cAMP hydrolysis by phosphodiesterases (PDE) :

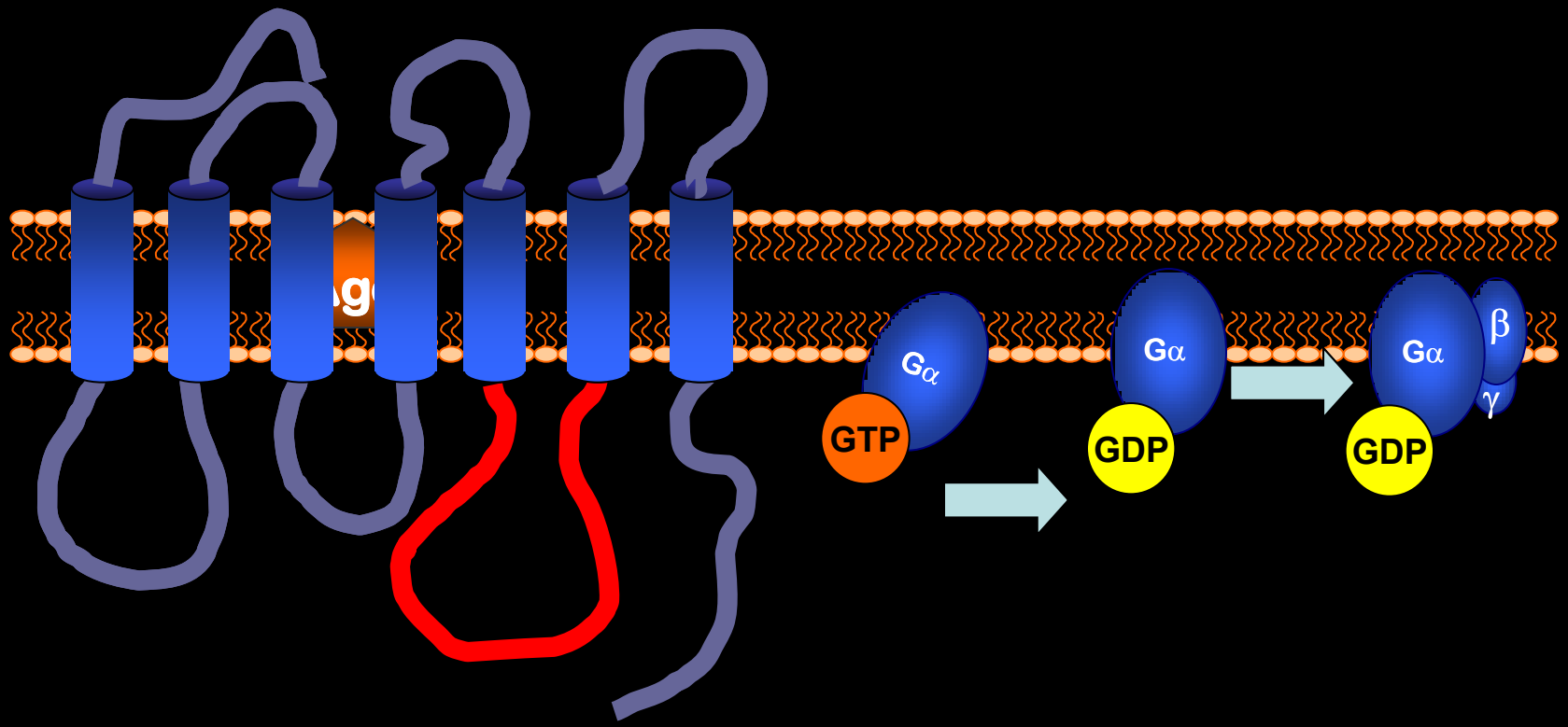


BOTH 1 2 3 10 11
cAMP 4 7 8
cGMP 5 6 9

Two imporant pharmaceutical applications:

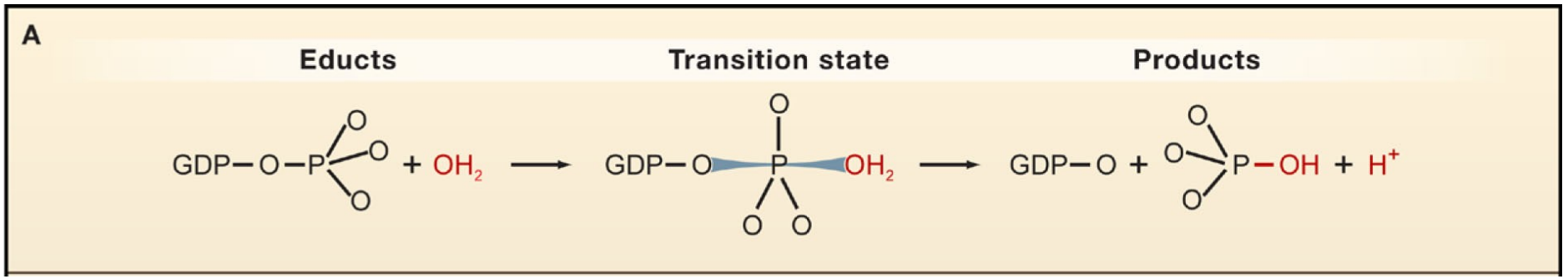
1. Sildenafil (Viagra®) as PDE5 inhibitor;
2. Diazepam (Valium®) as PDE4 inhibitor.

Last but not least the hydrolysis of GTP and the recycle of α subunit…



Last but not least the hydrolysis of GTP and the recycle of α subunit…

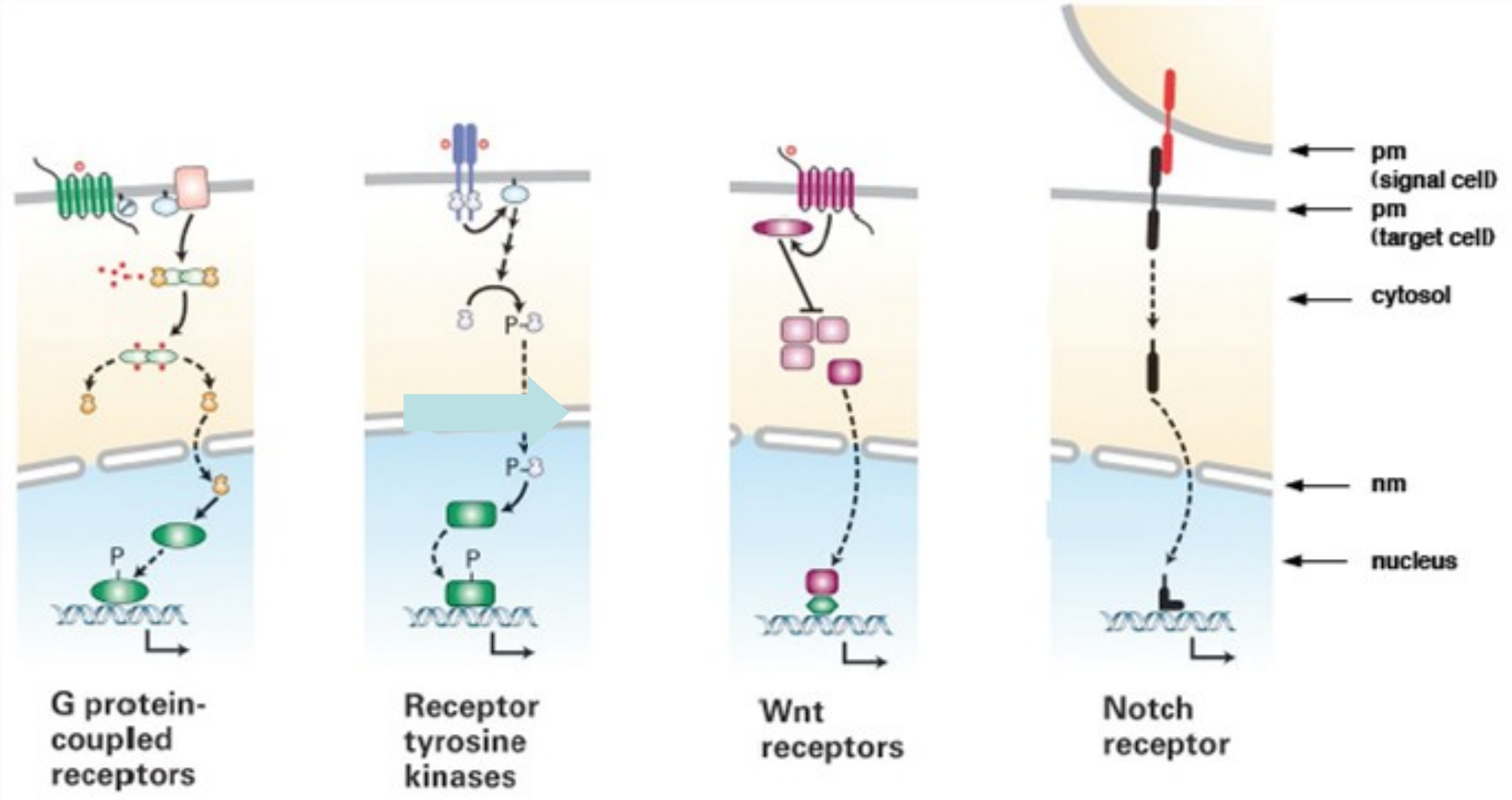
- Intrinsic GTPase rates of small G-proteins are slow (range: $k_{cat}=10^{-2}$ - 10^{-3} min^{-1})



- S_N2 nucleophilic attack with trigonal bipyramidal transition state
- Phosphate hydrolysis reaction is thermodynamically highly favourable but kinetically very slow

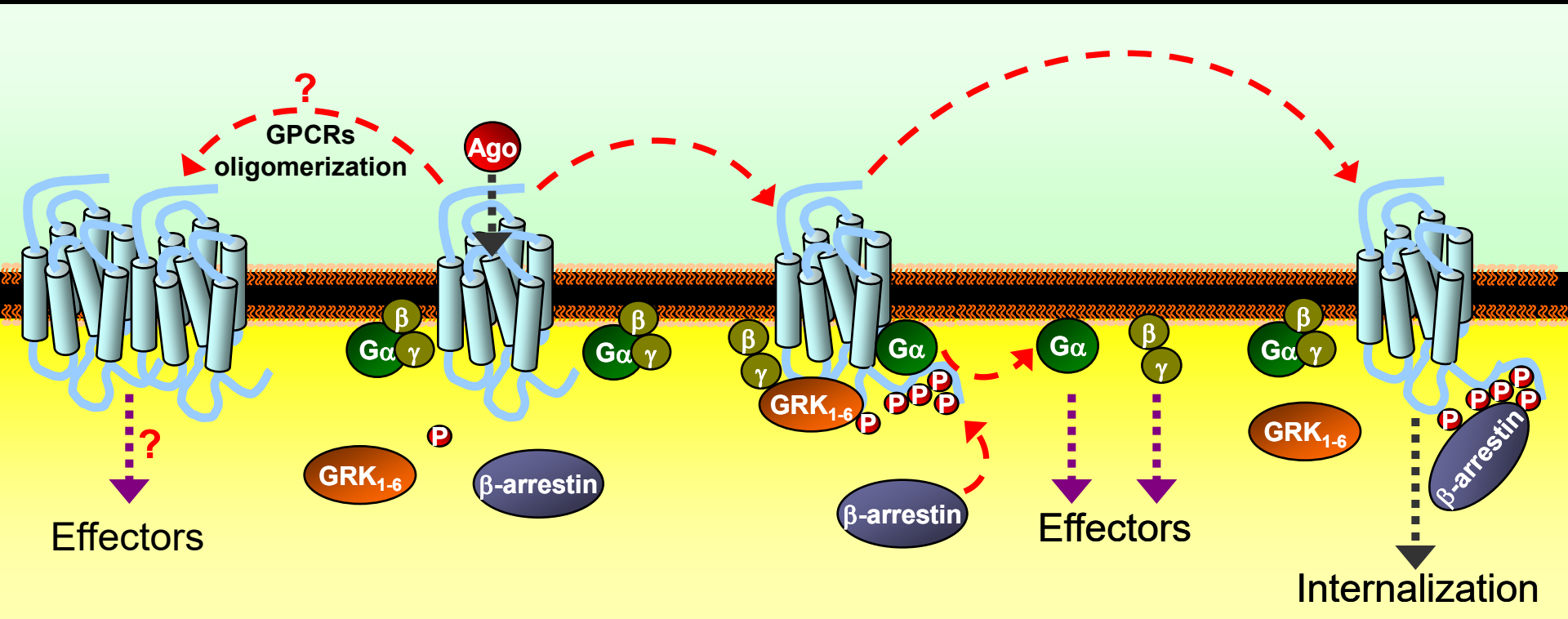
- Westheimer FH (1987), Why nature chose phosphates, Science 235, 1173-1178)

Signal transduction pathways: the story is much much more complex!!!



The wonderful world of GPCR-land:

G protein-coupled receptors (GPCRs) are the largest family of cell-surface receptors involved in signal transmission.



Moro S., Spalluto G., Jacobson K.A. *TIPS 26,* 44-51 (2005)