Signal Transduction - Receptors

EVROPSKÁ UNIE
Evropské strukturální a investiční fondy
Operační program Výzkum, vývoj a vzdělávání

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Types of receptors

- G protein-coupled receptors (GPCR, 7H)

- ligand-gated ion channels

- voltage-gated ion channels

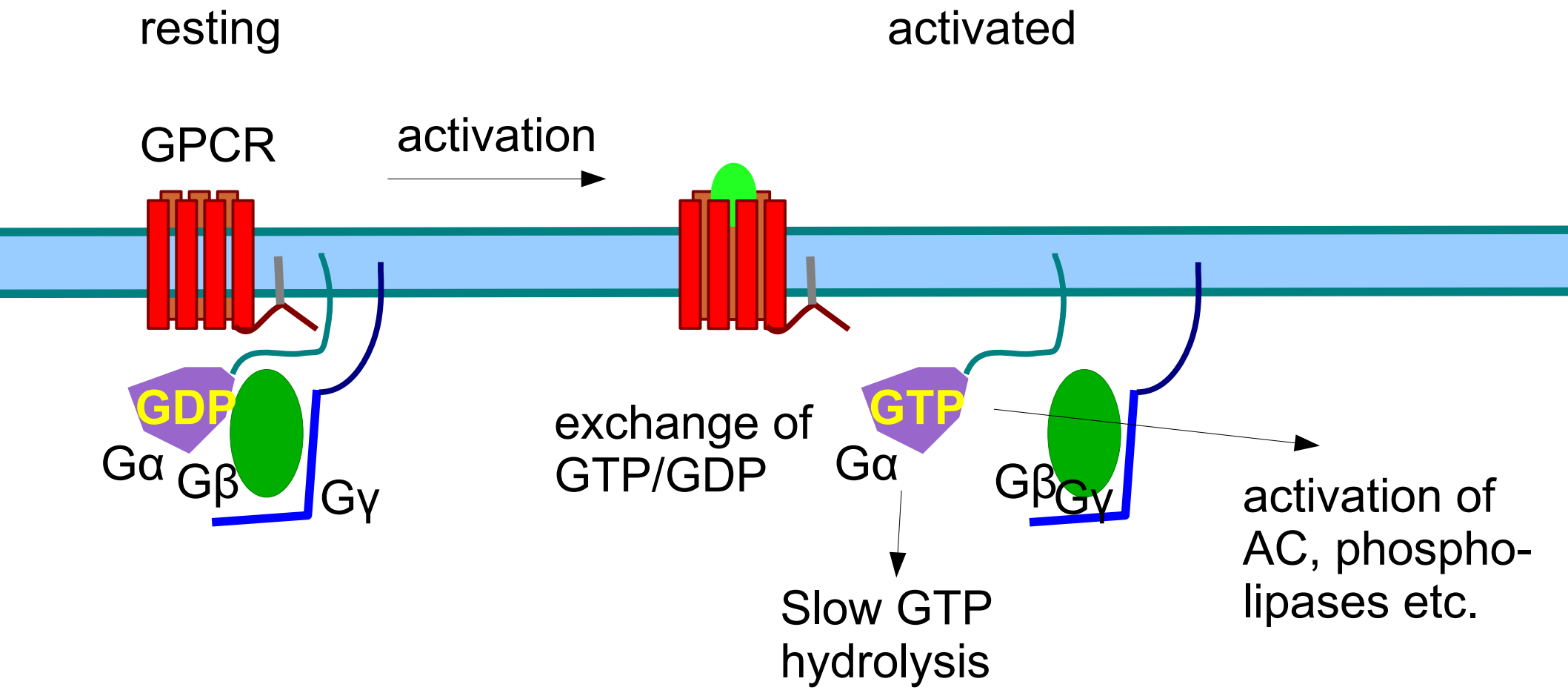
- receptor tyrosine kinases

- other kinase-associated receptors

- integrins, selectins, cadherins...

The term receptor was introduced for cellular elements that sense some signals, typically hormones. In this talk we will limit to receptors present on the surface of a cell that transduce the signal into the cell. Intracellular receptors, such as steroid hormone receptors, will not be discussed (steroids can pass the membrane and go to nucleus, where these receptors are present). There are several above mentioned types of receptors, many of them important drug targets.

G protein-coupled receptors (GPCR, 7H)



GPCRs are embedded in the membrane. Binding of a ligand (or a drug) triggers a conformational change in the receptor. This causes release of activated G proteins. G proteins exchange GDP for GTP. GTP is slowly hydrolysed and after hydrolysis the G protein is deactivated. During this period G proteins activate either adenylyl cyclase or phospholipase C. Activation of these enzymes triggers production of second messengers. Alternative mechanisms also exist.

G protein-coupled receptors (GPCR, 7H)

GPCR activation:
- biogenic amines (epinephrine, norepinephrine, dopamine, serotonin, GABA)
- amino acids (glutamate)
- nucleoside (adenosine)
- peptides (glucagon, angiotensins, oxytocin, vasopresin, ...)
- proteins (complement, interleukines, ...)
- odorants, flavours
- light (rhodopsin)
- other

GPCRs are important drug targets. Approximately 1/3 of drugs target GPCRs. They can act either as agonists, i.e. they bind to a receptor and activate it, or they can act as antagonists, i.e. they bind to receptors and block it from binding of an endogenous agonist, or they can act by alternative ways. Examples of drugs targetting GPCRs include beta-blockers (to treat hypertension), antihistaminics (to treat allergy), analgetic opioids and many others. It is believed that recent development in structural biology of GPCRs may open the door for new GPCR-based therapeutics.

G protein-coupled receptors (GPCR, 7H)

Structural biology of GPCRs:

Bacteriorhodopsin (1996)

Rhodopsin (2000)

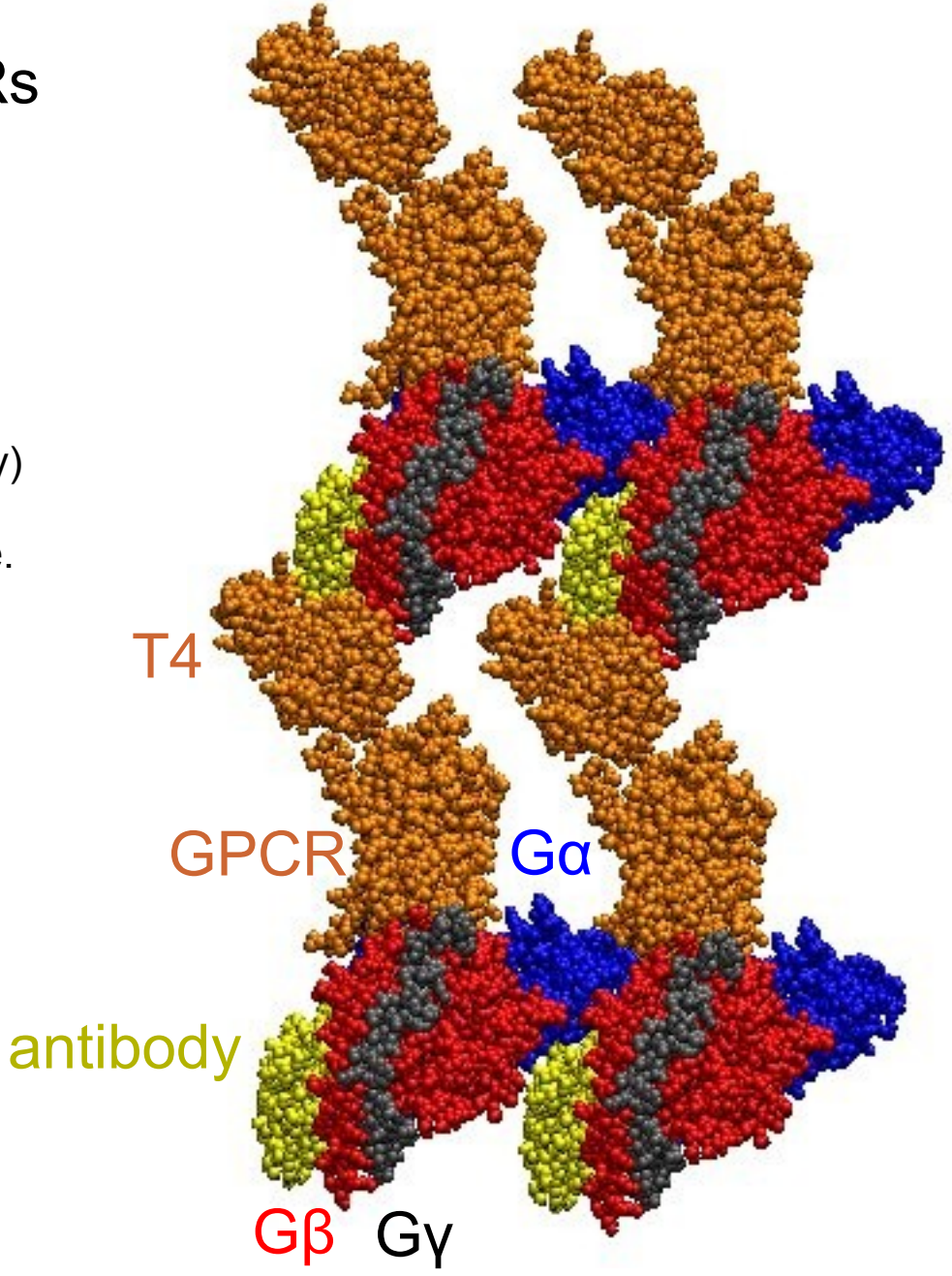
adrenergic receptor (2007)
GPCR-G-proteins complex (2011)
Nobel prize 2012 – B.K. Kobilka

Design of drugs acting on GPCR may benefit from the knowledge of GPCR structures. Determination of protein structure is extremely difficult for membrane proteins. Therefore, determination structures of GPCRs was impossible for many years. Bacteriorhodopsin – a distant homologue of GPCRs – was determined in 1996, rhodopsin in 2000 but major breakthrough was achieved by Brian Kobilka and others. Several “tricks” were developed to determine structures of GPCRs, namely using of chimeric proteins with easy to crystallize proteins, application of recombinant antibodies or preparation of thermostabilized GPCRs.

G protein-coupled receptors (GPCR, 7H)

Structural biology of GPCRs
β-adrenergic receptor

Complex of β-adrenergic receptor with G-proteins was determined by Kobilka group in 2011. It used a chimera “trick” (combined with a vital T4 lysozyme) and a nanobody (small recombinant antibody) trick. Membrane environment was mimicked by lipid cubic phase technique.



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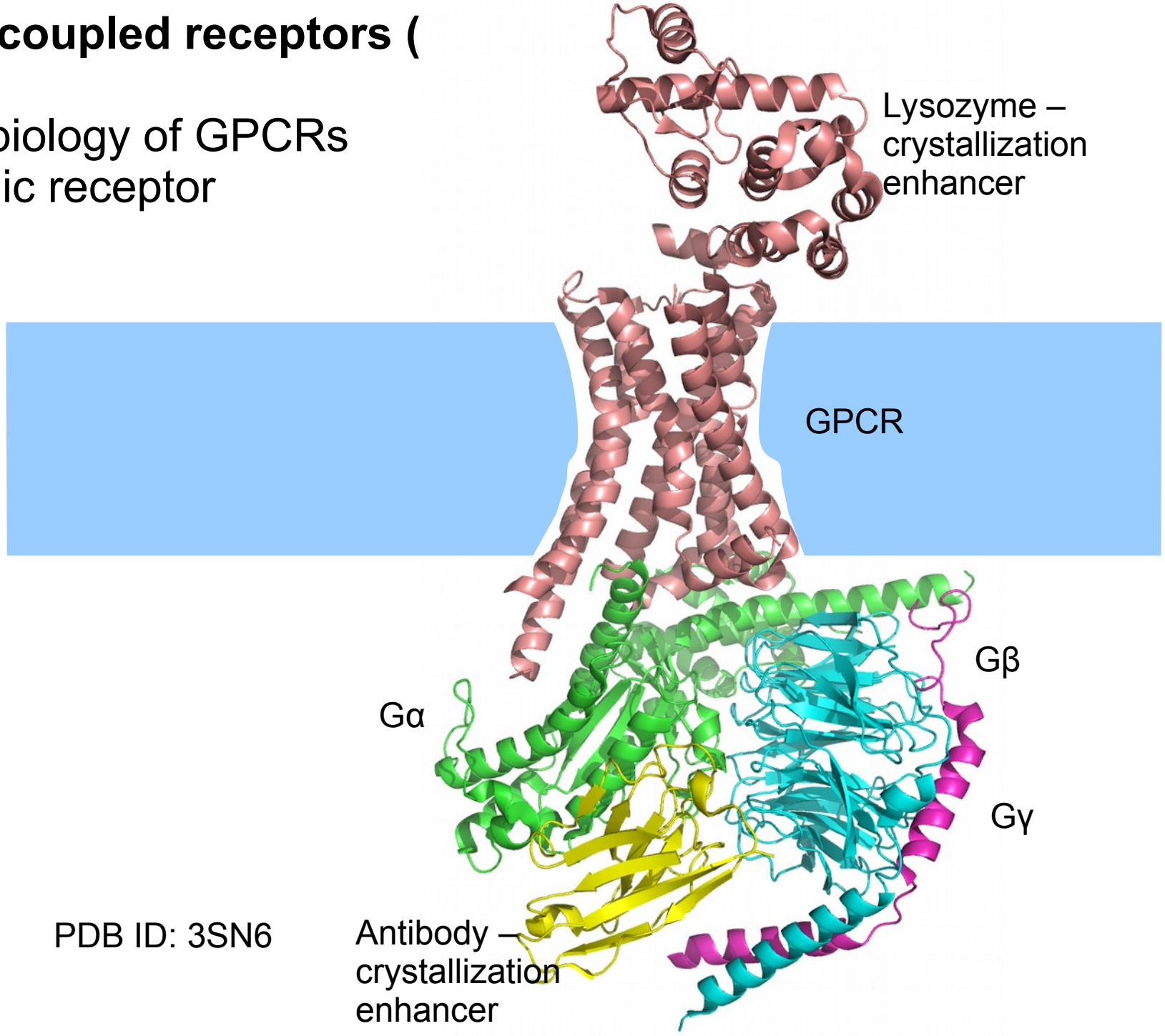
G protein-coupled receptors (

Structural biology of GPCRs
β-adrenergic receptor



G protein-coupled receptors (GPCR, 7H)

G proteins:

Gα – member of the family of small GTPases together with Ras, Rho, elongation factors etc., major activator of downstream processes, myristylated

Gβ – beta-propeller, in complex with Gγ also activates downstream processes

Gγ – anchors the Gβγ complex to a membrane

There are three G protein types and many other subtypes.

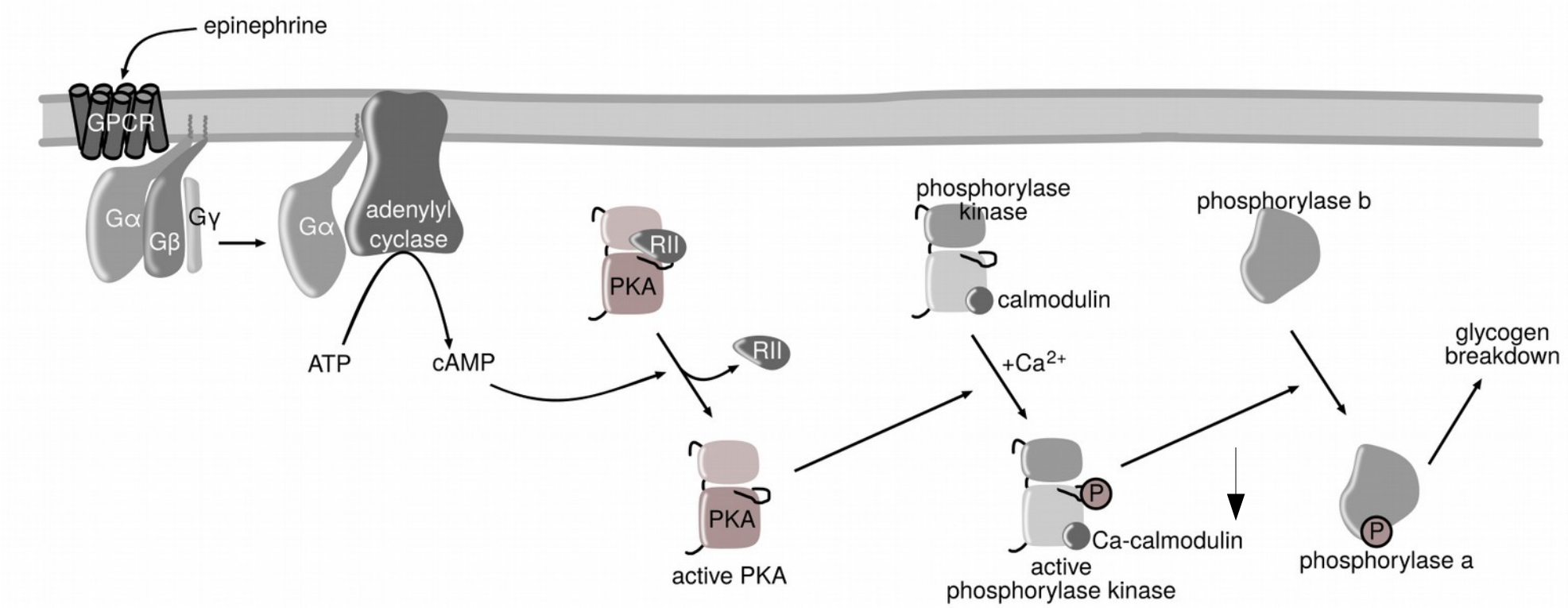
GTPase reaction follows first-order kinetics i.e. the concentration of the GTP-bound G-protein decreases exponentially. This controls the duration of activation of G-proteins.

G protein-coupled receptors (GPCR, 7H)

Downstream signaling:

Adenylyl cyclase (cAMP), guanylyl cyclase (cGMP)

Phospholipases



G protein-coupled receptors (GPCR, 7H)

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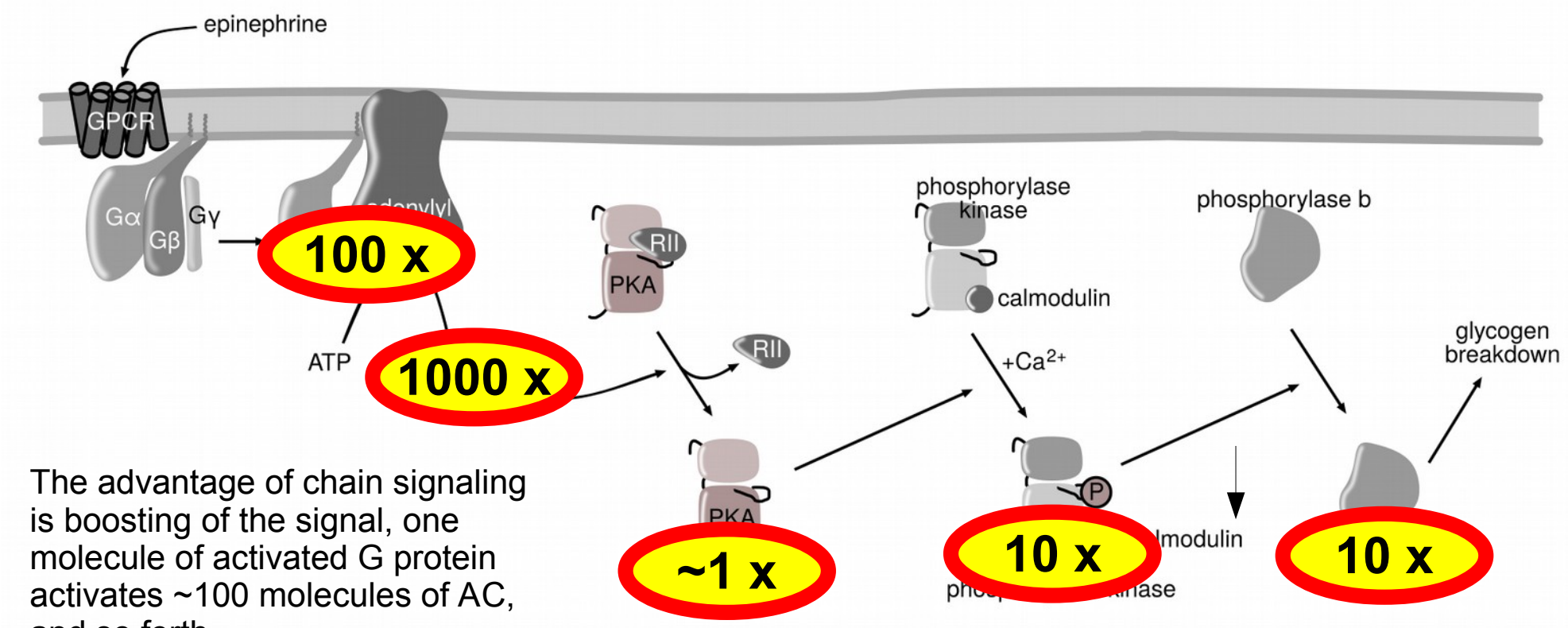
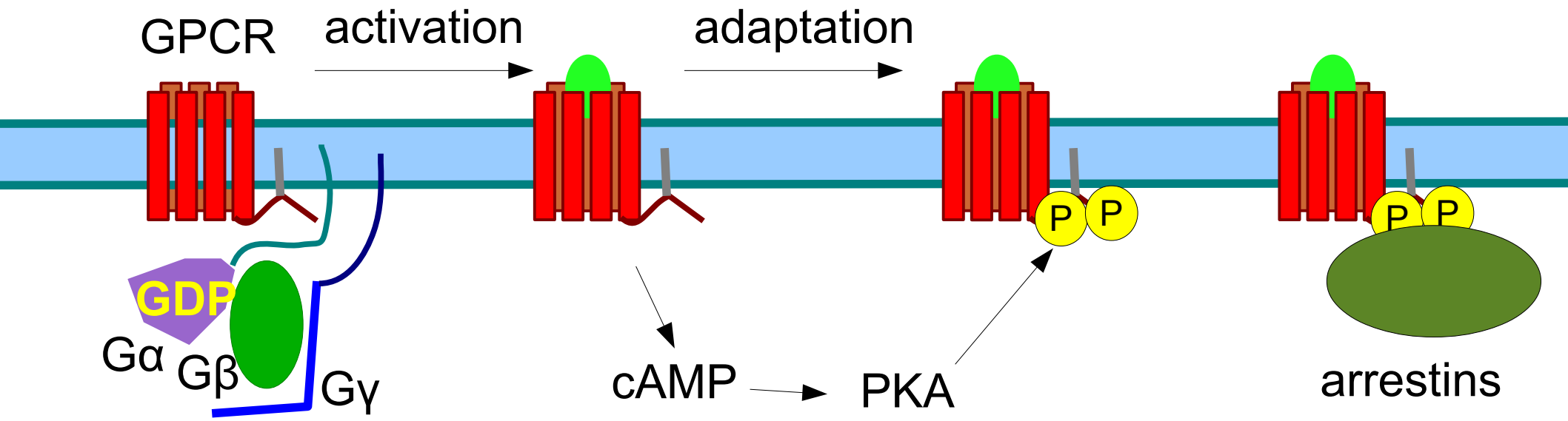


The advantage of chain signaling is boosting of the signal, one molecule of activated G protein activates ~100 molecules of AC, and so forth.

G protein-coupled receptors (GPCR, 7H)

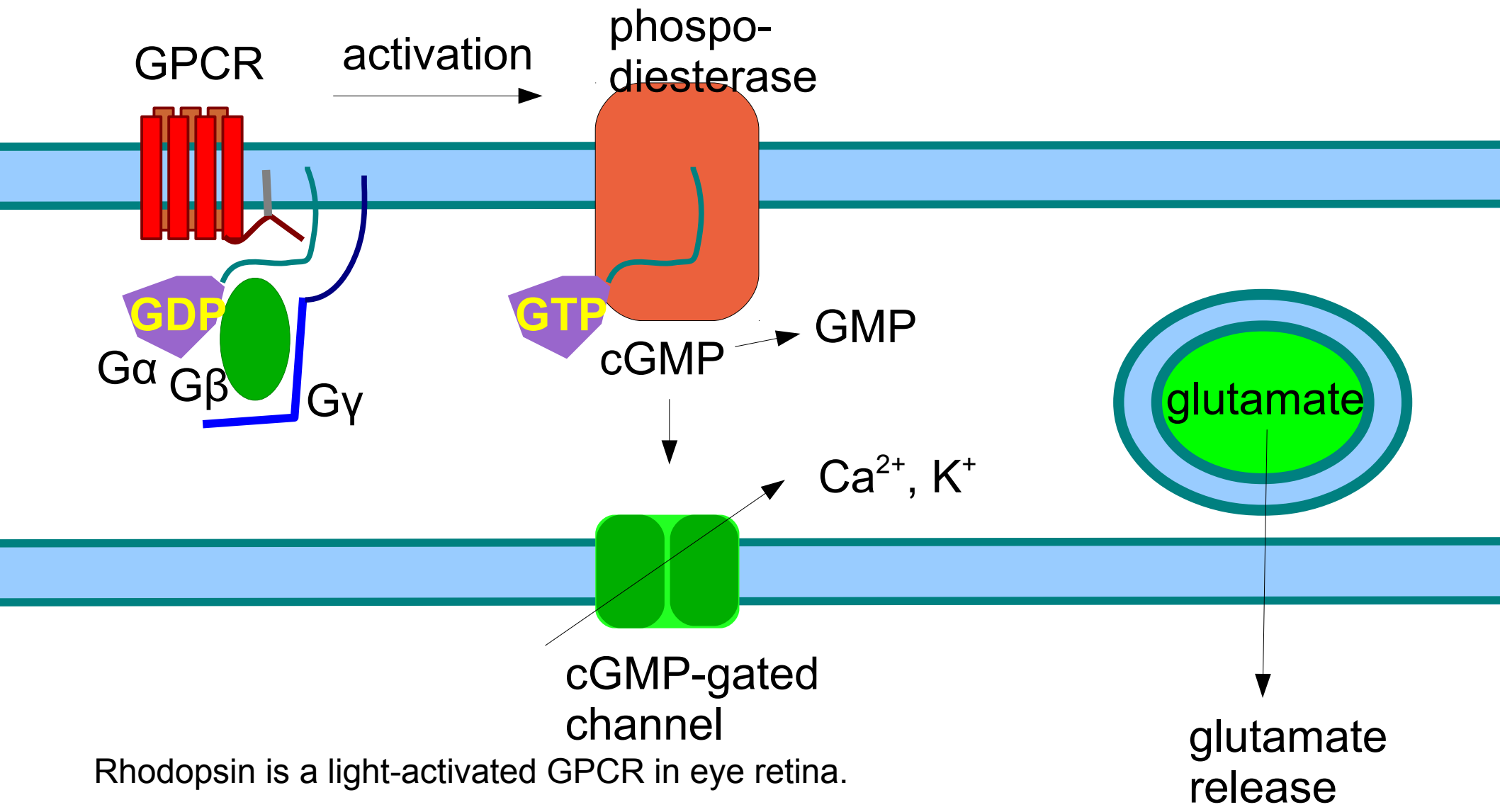
Adaptation



It is important to reduce sensitivity of GPCR activation in an excess of a ligand. When the GPCR is fully activated for a long time it activates PKA. PKA phosphorylates the receptor. A protein called arrestin binds to phosphorylated receptor and blocks it from function. It also triggers internalization of the GPCR (excision of membrane vesicle containing the receptor and its transport to lysosome). Arrestin also plays an important role in addiction to drugs of abuse.

G protein-coupled receptors (GPCR, 7H)

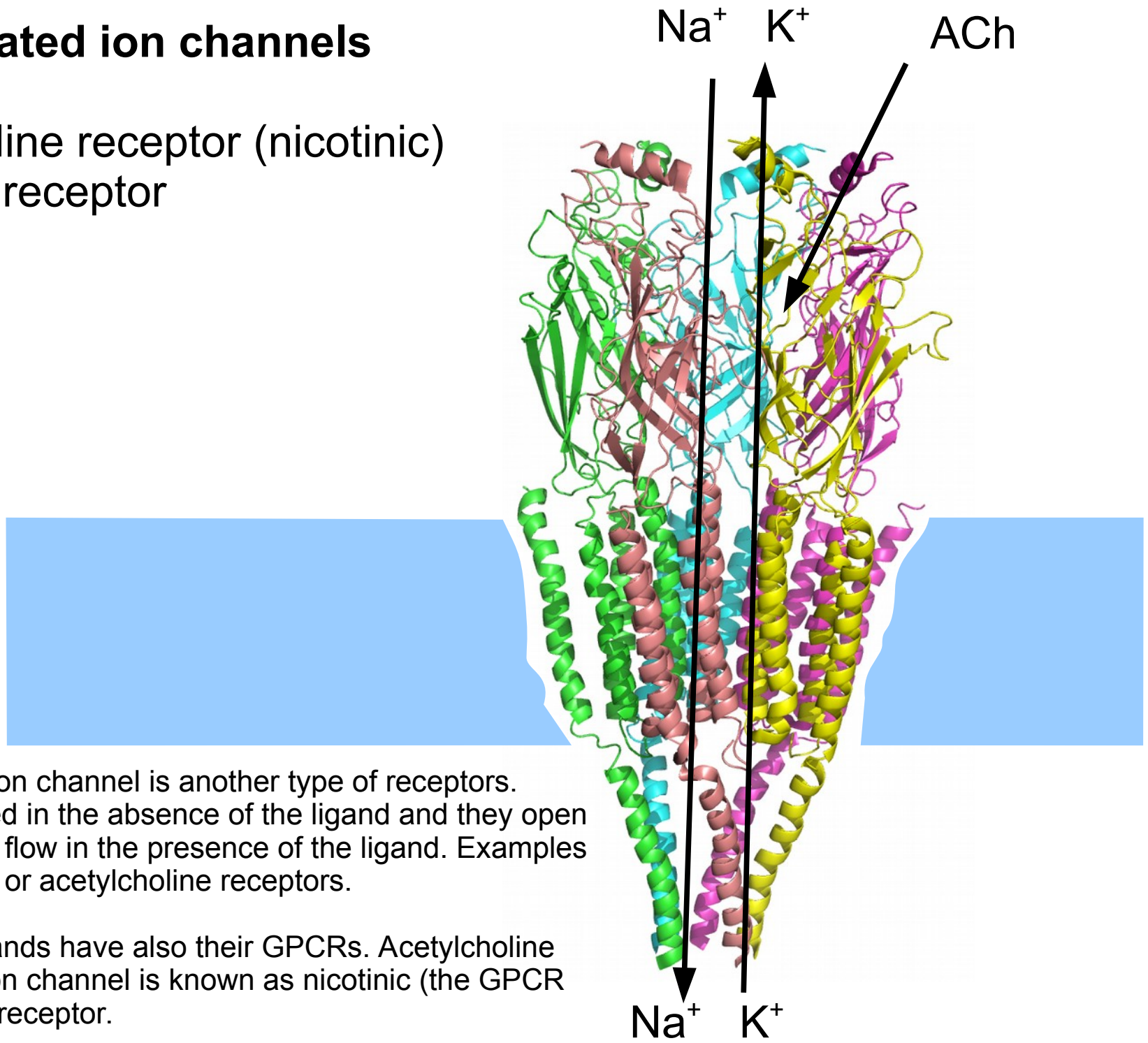
rhodopsin (activated by light)



Rhodopsin is a light-activated GPCR in eye retina.

Ligand-gated ion channels

acetylcholine receptor (nicotinic)
serotonin receptor

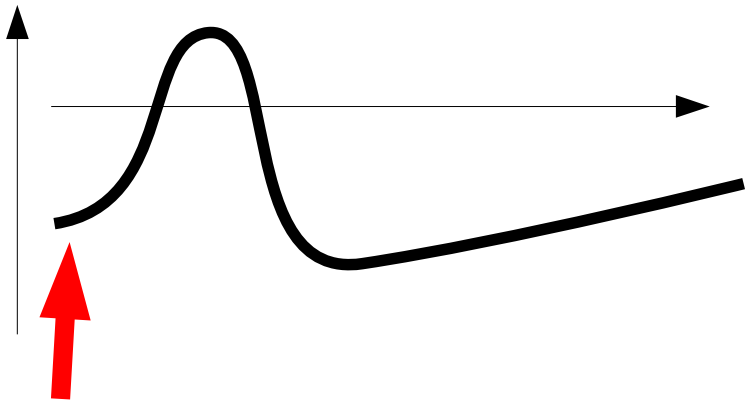


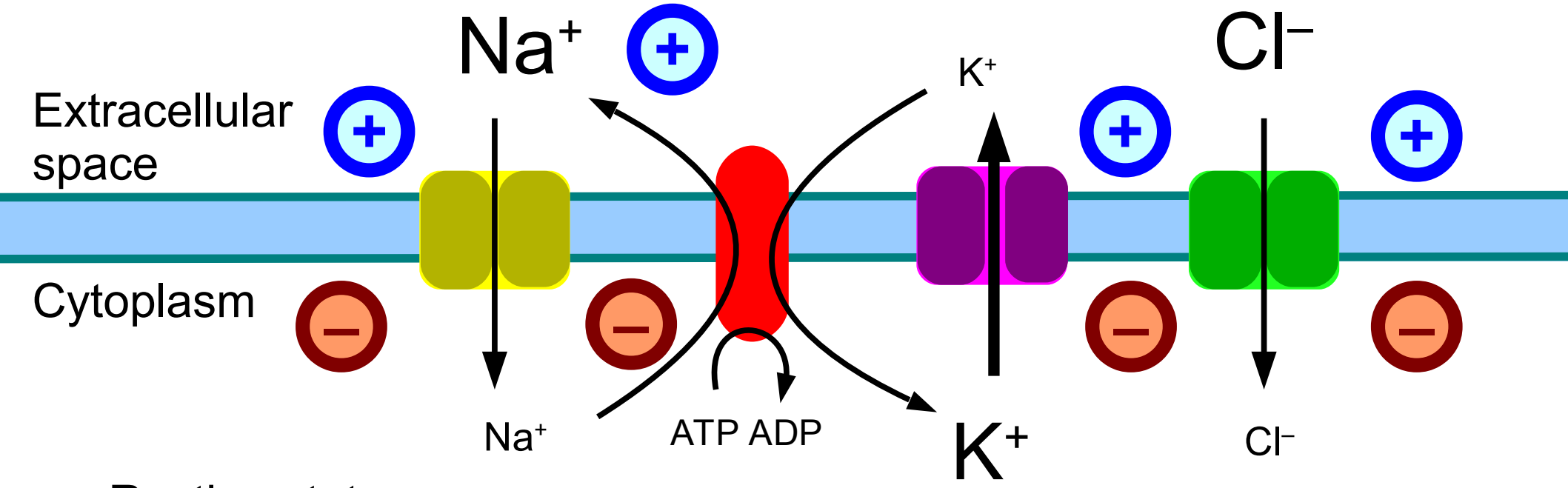
Ligand-gated ion channel is another type of receptors. They are closed in the absence of the ligand and they open and trigger ion flow in the presence of the ligand. Examples are serotonine or acetylcholine receptors.

Both these ligands have also their GPCRs. Acetylcholine ligand-gated ion channel is known as nicotinic (the GPCR is muscarinic) receptor.

Voltage-gated ion channels

voltage-gated potassium channel
voltage-gated sodium channel



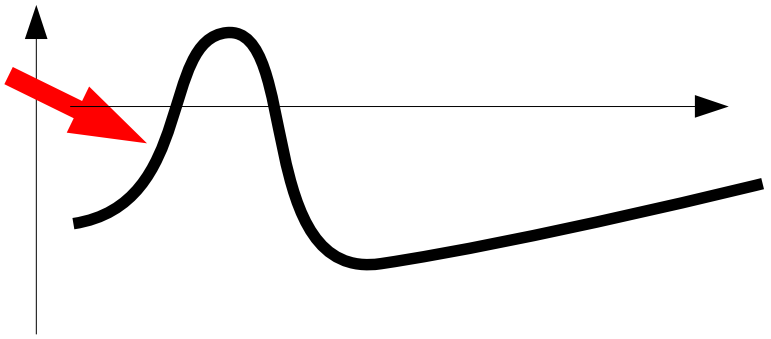


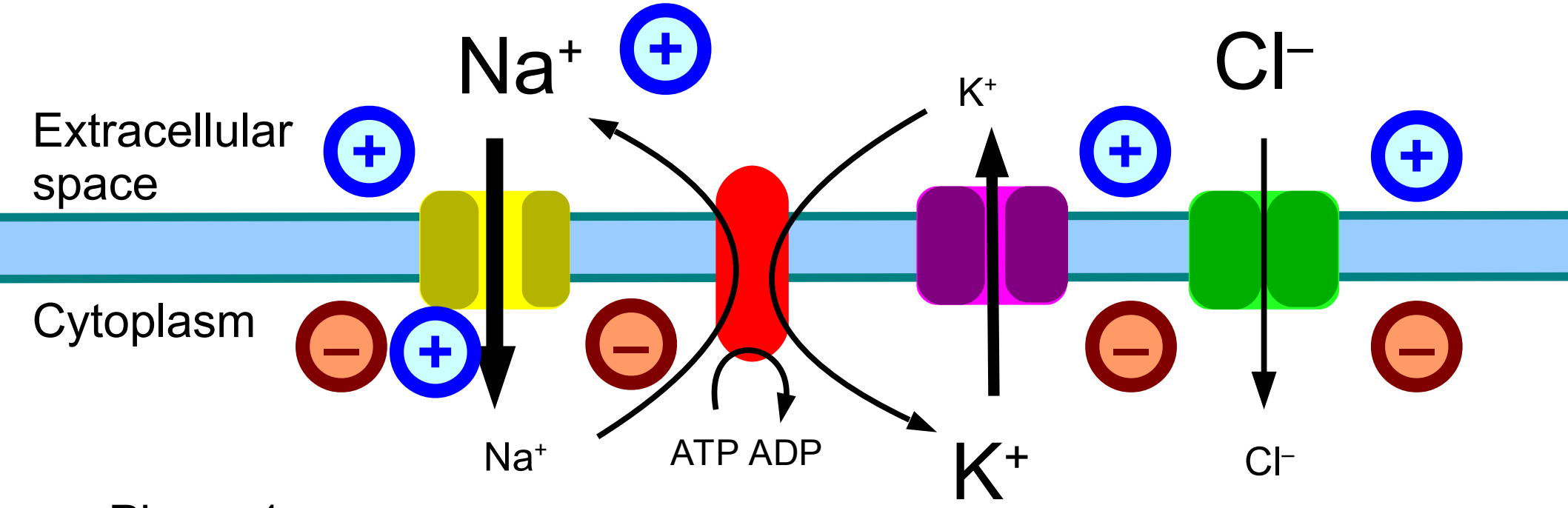
Resting state

In the resting state Na,K-ATPase creates a gradient of Na^+ and K^+ (there is high Na^+ concentration out and K^+ in). The K^+-channel leaks, which causes excess of charge out (negative membrane potential).

Voltage-gated ion channels

voltage-gated potassium channel
voltage-gated sodium channel



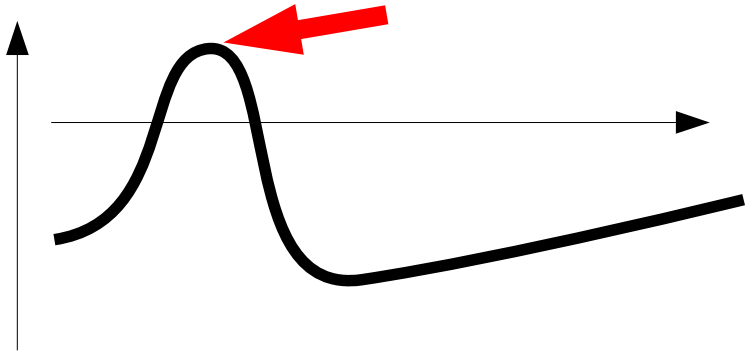


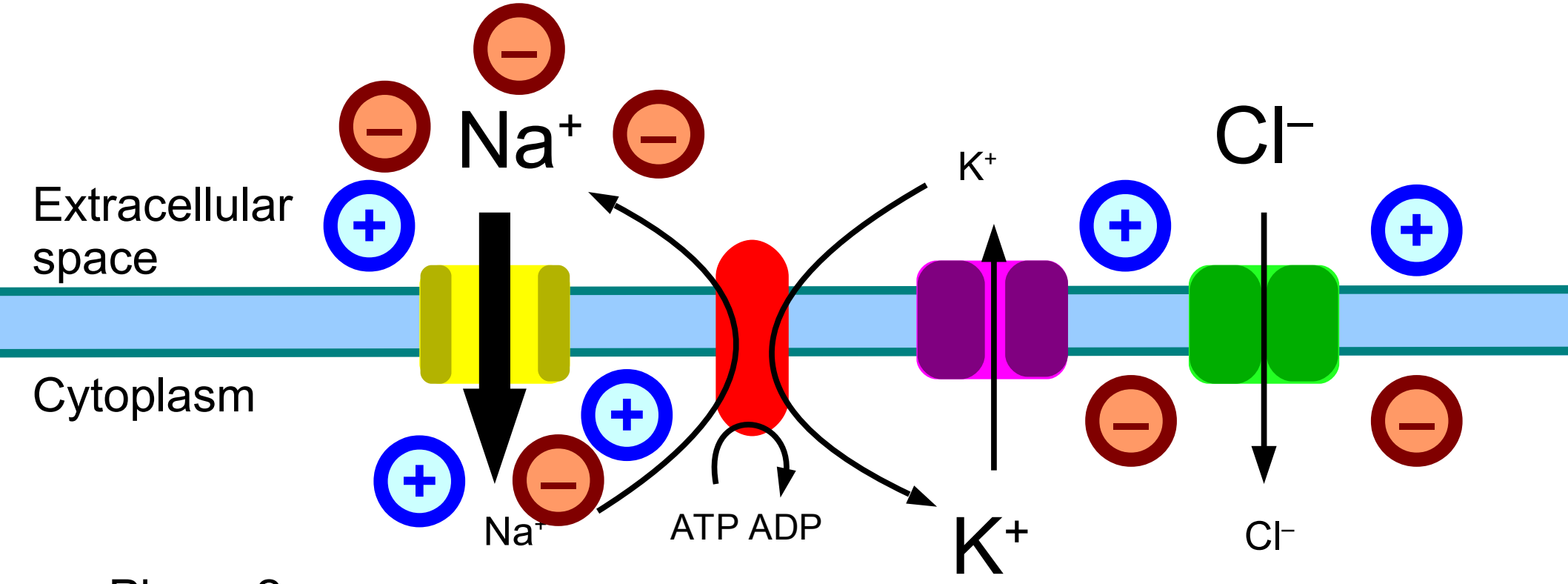
Phase 1

In the first phase of the signal transduction the Na^+ channel opens as a result of change in the potential. Na^+ goes in.

Voltage-gated ion channels

voltage-gated potassium channel
voltage-gated sodium channel



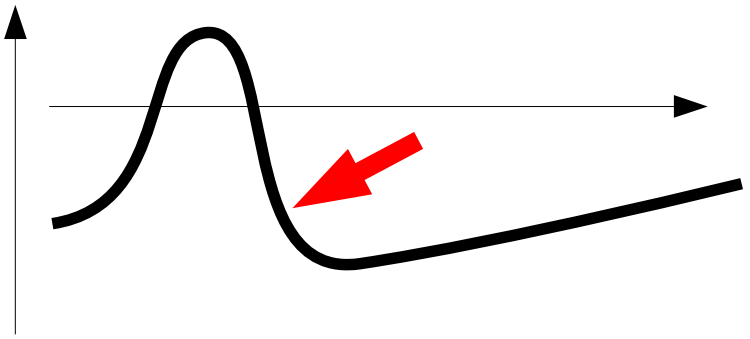


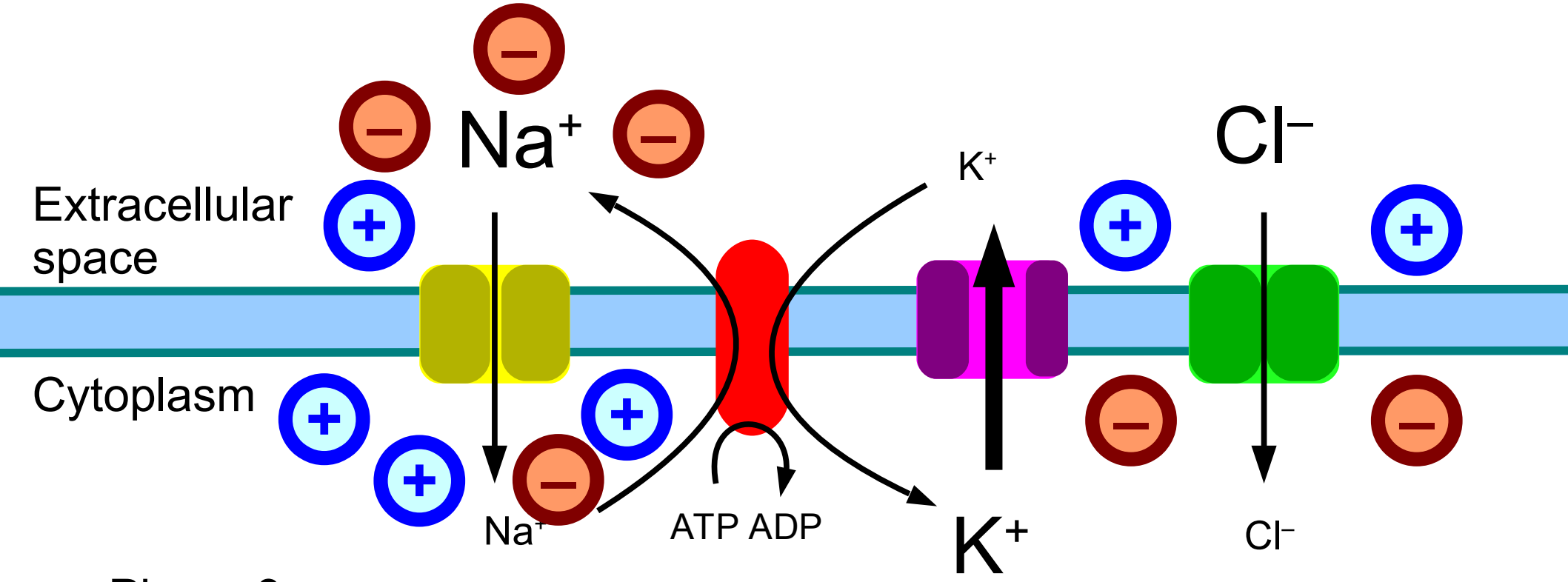
Phase 2

Transport of Na^+ switches the potential from negative to positive. Once the potential is positive, it opens the K^+-channel.

Voltage-gated ion channels

voltage-gated potassium channel
voltage-gated sodium channel



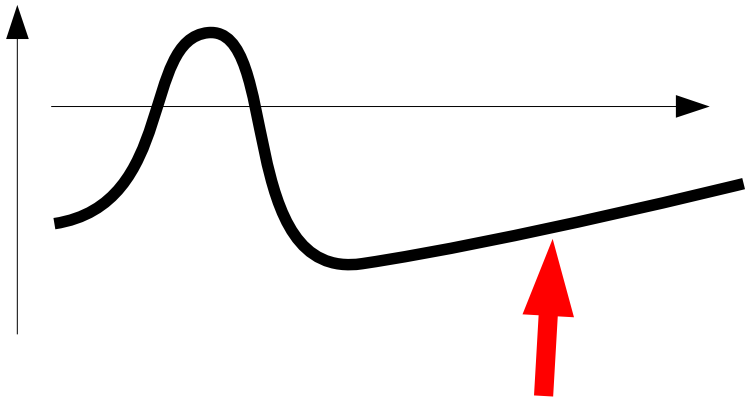


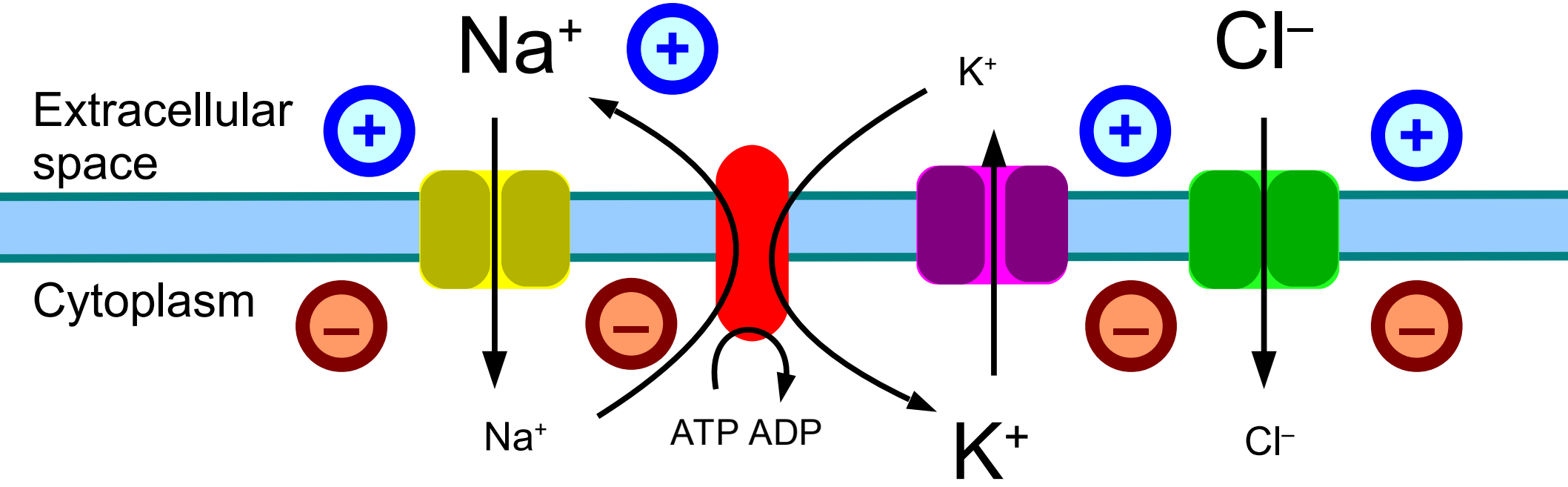
Phase 3

As K^+ goes out, the membrane potential returns towards the initial state.

Voltage-gated ion channels

voltage-gated potassium channel
voltage-gated sodium channel



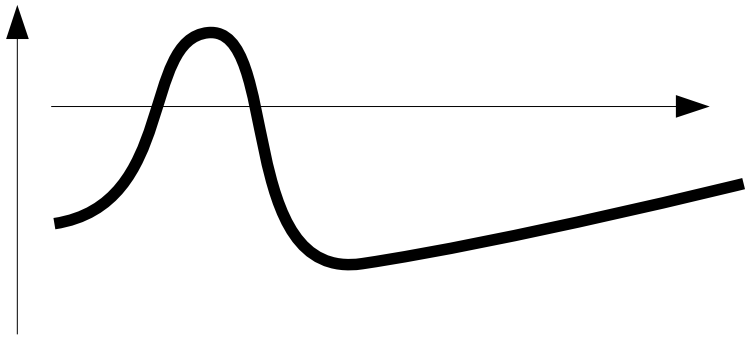


Phase 4

In the last phase the cell returns into the initial state. Since the neurons are long tubular cells, the process described on last 5 slides propagates linearly, as electric current, along the neuron.

Voltage-gated ion channels

voltage-gated potassium channel
voltage-gated sodium channel

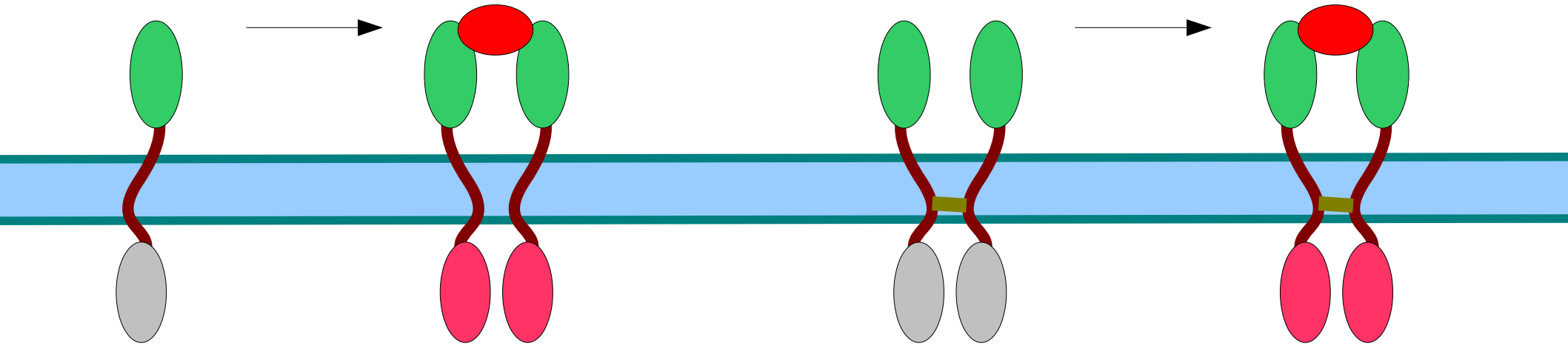


Goldman equation

$$E_{m,\mathrm{K}_x\mathrm{Na}_{1-x}\mathrm{Cl}} = \frac{RT}{F} \ln \left(\frac{P_{\mathrm{Na}}[\mathrm{Na}^+]_{\mathrm{out}} + P_{\mathrm{K}}[\mathrm{K}^+]_{\mathrm{out}} + P_{\mathrm{Cl}}[\mathrm{Cl}^-]_{\mathrm{in}}}{P_{\mathrm{Na}}[\mathrm{Na}^+]_{\mathrm{in}} + P_{\mathrm{K}}[\mathrm{K}^+]_{\mathrm{in}} + P_{\mathrm{Cl}}[\mathrm{Cl}^-]_{\mathrm{out}}} \right)$$

Goldman equation is a special case of Nernst equation for a neuron. It contains concentrations ([*X*]) and fluxes (P_X) of all relevant ions.

Receptor tyrosine kinases



EGFR

Insulin rec.

Other types of receptors are represented by receptor protein kinases. When a ligand (epidermal growth factor for EGFR or insulin for insulin receptor) binds to extracellular domain (this may or may not be associated with dimerization). This triggers protein kinase activity in the intracellular domain. Then the kinase domain can phosphorylate various intracellular proteins.

Similarly to GPCRs, also receptor protein kinases are examples of receptors controlled by allostery.

Other types of receptors

- integrins, selectins, cadherins...

Integrins mediate interactions of the extracellular matrix and the cell. They also sense signals from the extracellular matrix. They influence cell growth, differentiation and programmed cell death (apoptosis).

Selectins bind sugars on cell surfaces.

Cadherins are calcium-dependent cell adhesion molecules.