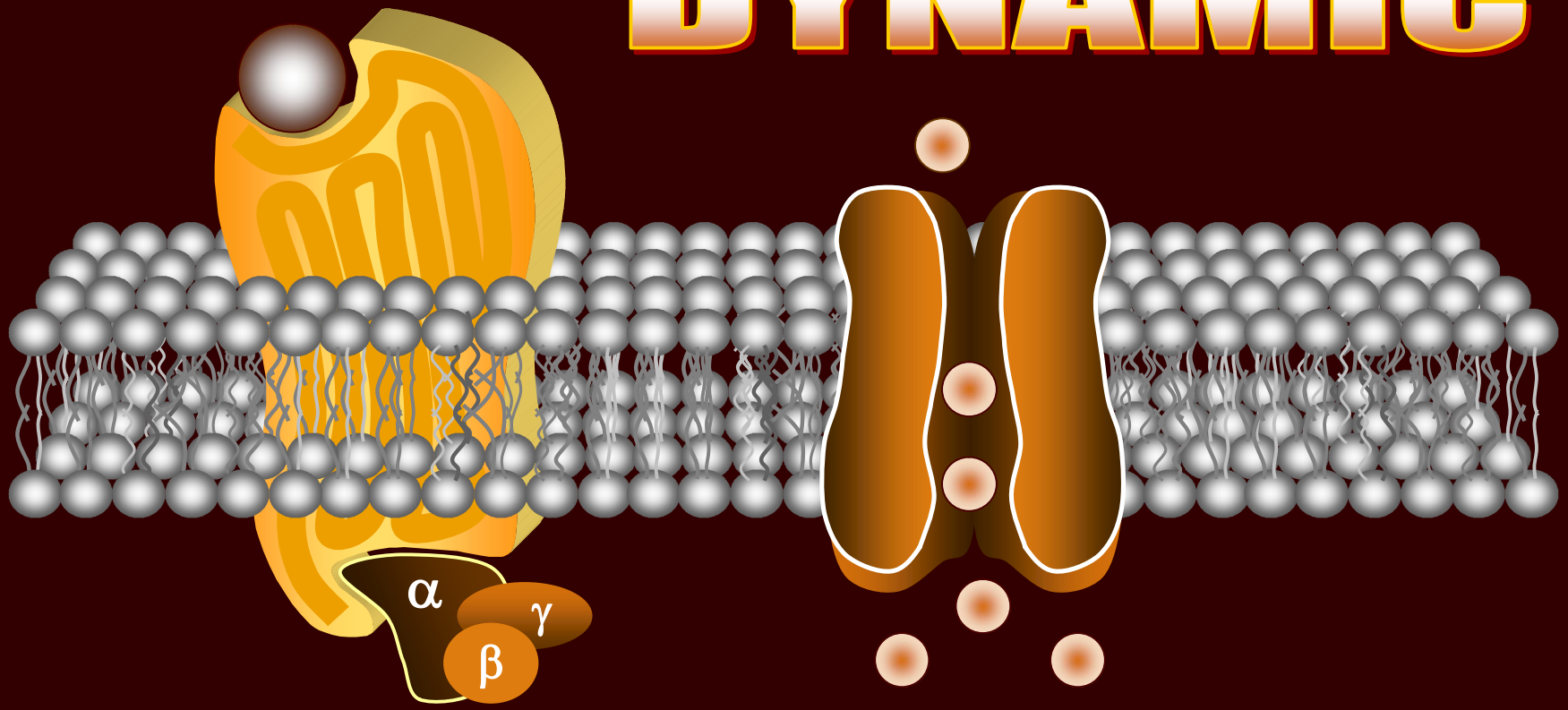
RNDr. Ivana Fellnerová, Ph.D.
Department of zoology, Faculty of Sciences, UP Olomouc

3. Plasma membrane: DYNAMIC



Cell membrane function - overview

Physical barrier

Separates the inside of the cell from the surrounding extracellular fluid
Separate cell organelles from cytosol
Allows selective communication between the intracellular and extracellular compartments

Protein transporters

Control selective transport of molecules across the cell membrane

Receptors

Recognize hormones, mediators or other regulatory molecules

Enzymes

Control of chemical reactions in the cell membrane

Cell markers

Identify the blood and tissue type of an individual

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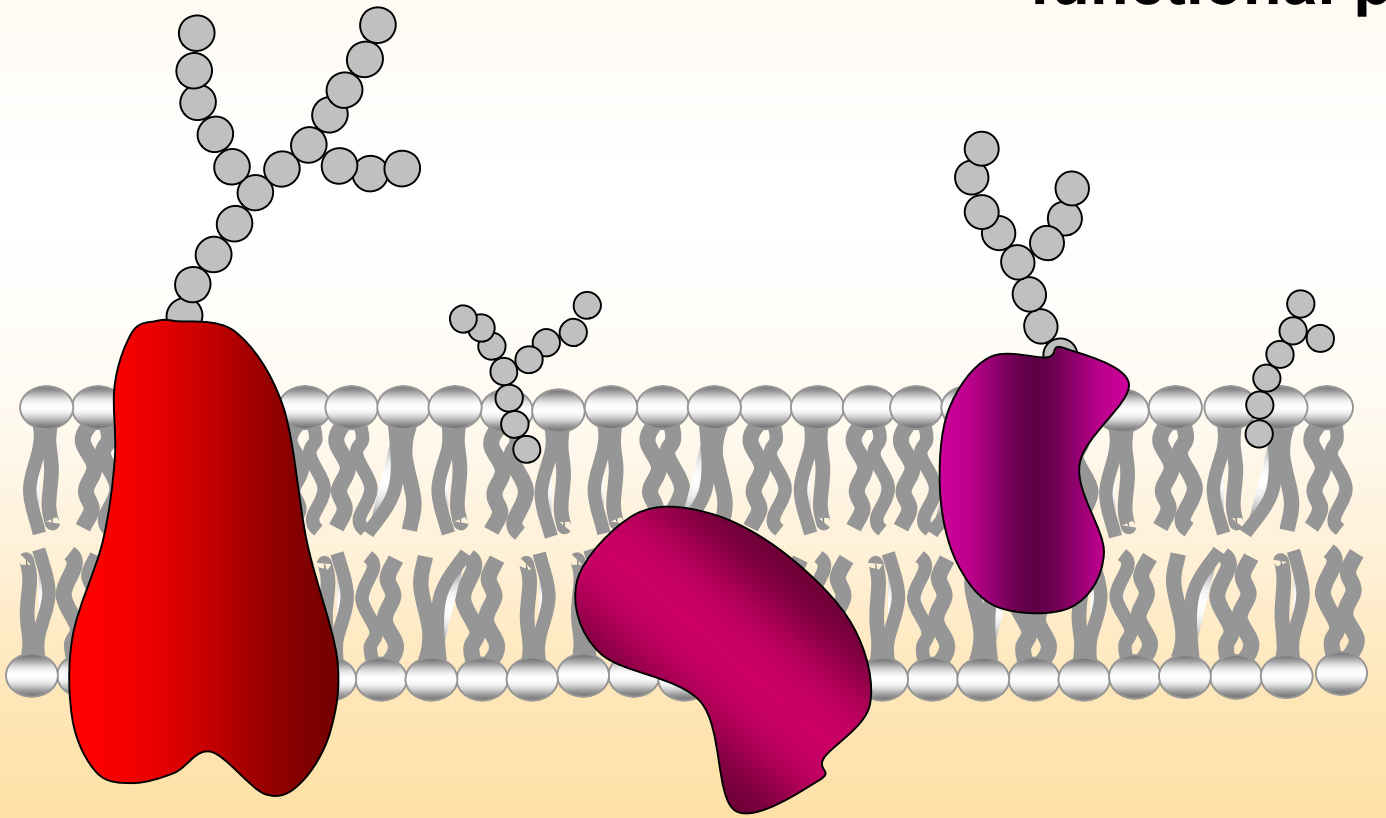
Protein in plasma membrane

Protein performs most of the specific functions of the membrane

Protein gives each type of membrane its characteristic functional properties



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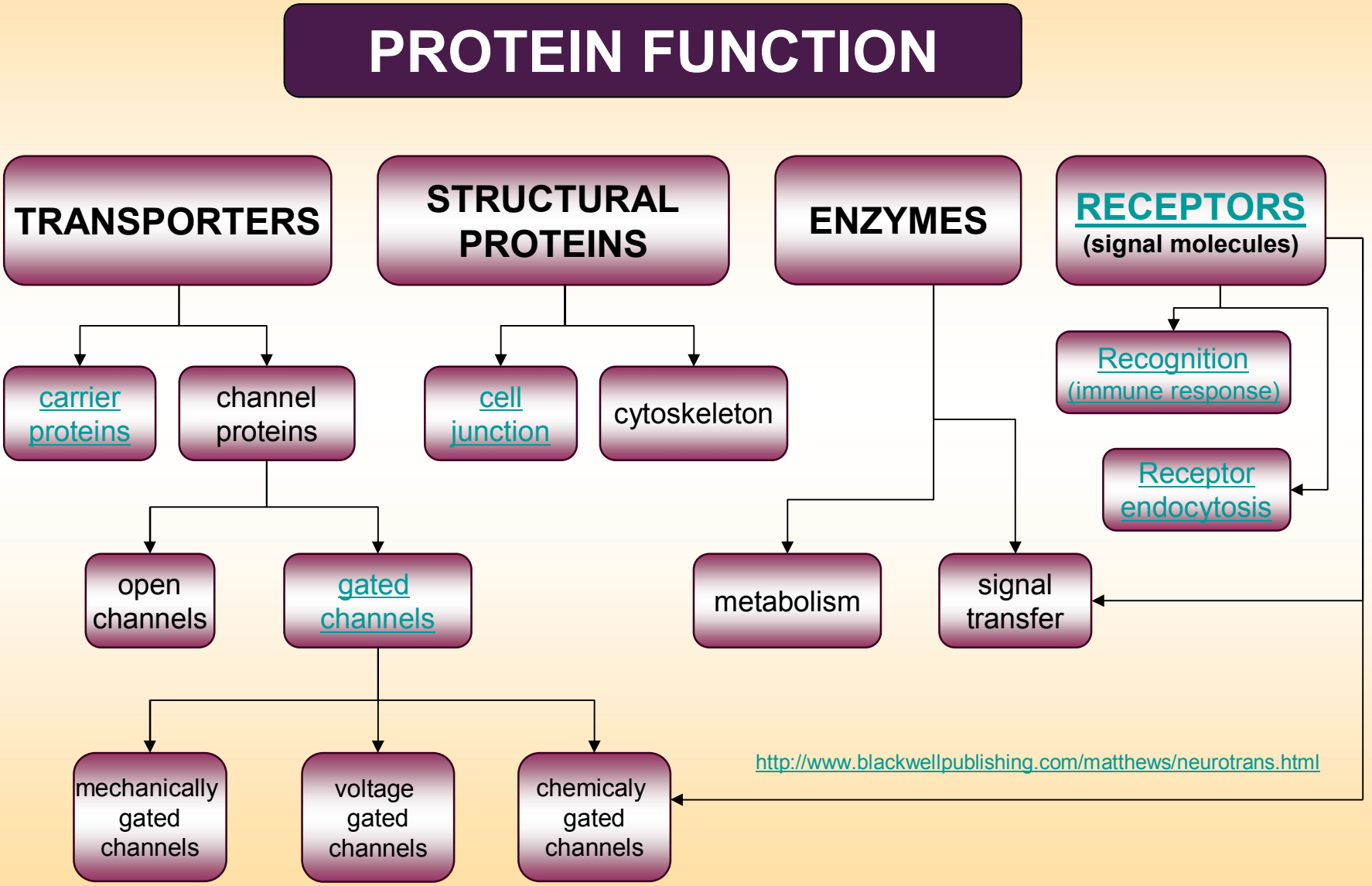
Functional types of the membrane proteins



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Channel proteins are made of membrane spanning proteins

CHANNELS

Channels play a key role in cell-cell comunication,
in regulating excitability of the cells.

Channels are primarily responsible for the initial depolarization phase of the

ACTION POTENTIAL

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CHANNELS: characteristic

Selectivity

Channels transport specifically one or few ions similar by size and charge

Selectivity is determined by:

- diameter of its central pore
- electrical charge of amino acids that line the channels

Ion channels: transport one or more similar ions
Aquaporins: water channels

Pasive transport

Transport does not required energy - **pasive transport**.
The direction of transport: **from side of higer concentration to place of lower concentration**

Open channels (leaking channels) – open most of the time
Gated channels: transport is regulated by gates; mostly clesed

Channel: structure

http://nobelprize.org/nobel_prizes/chemistry/laureates/2003/public.html

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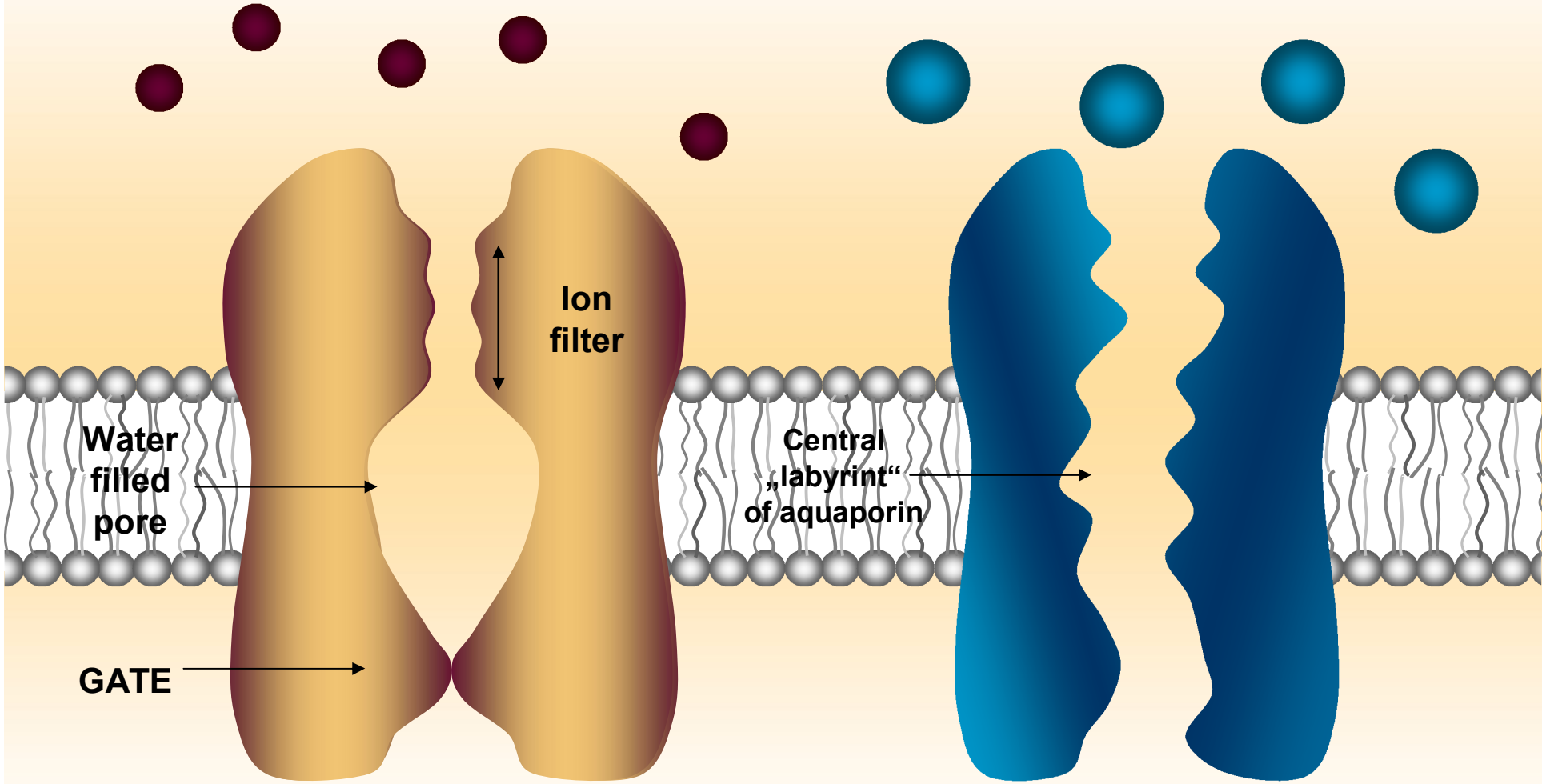


Ion channel

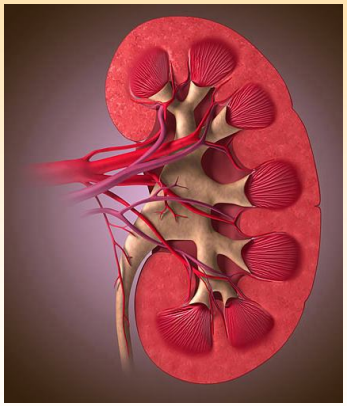
Aquaporin

Aquaporins – water pores

- Family of membrane channels that make membrane freely permeable to water
- There is known at least 10 isoforms in mammalian cells
- Cells usually have multiple isoforms of aquiporins

Aquaporins at the kidney cells

- Several isoforms of aquiporins
- Aquaporin-2 (AQP2) is located in a collecting duct (apical membrane and cytoplasmic storage vesicles)
- Aquaporin-2 (AQP2) is regulated by vasopresin



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Aquaporin-2 (AQP2) at the kidney cells

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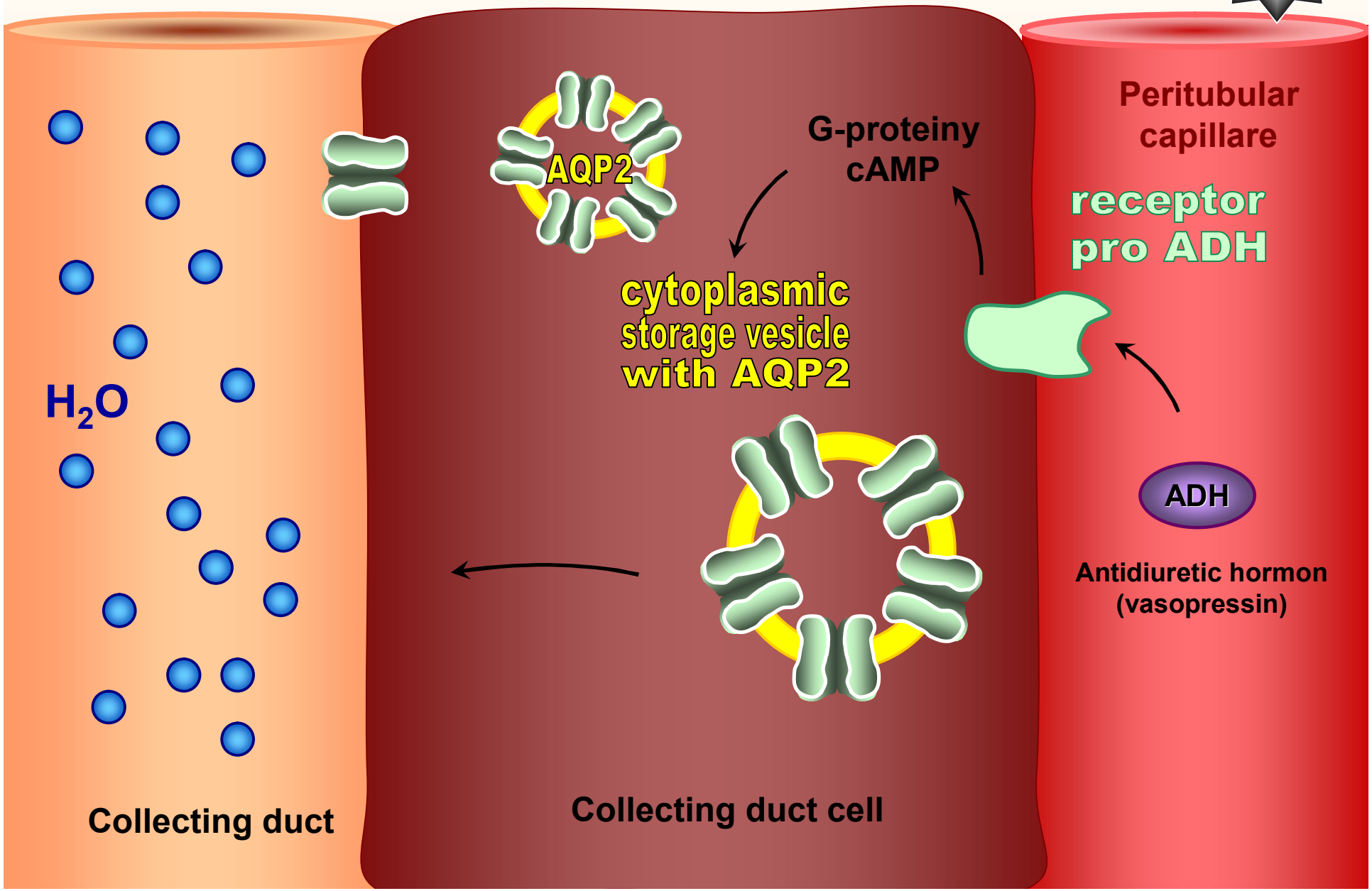

Aquaporin-2 (AQP2) at the kidney cells

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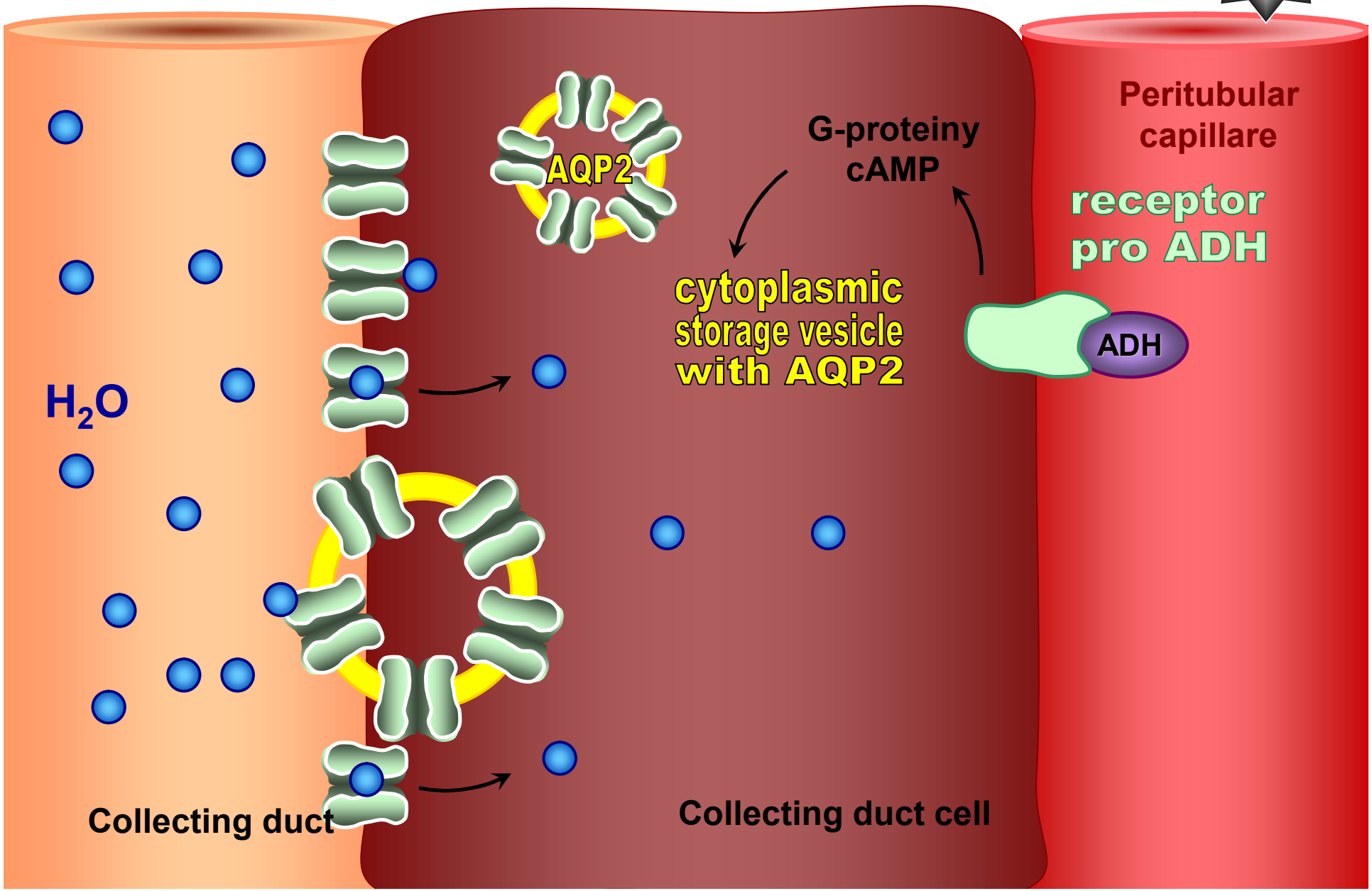
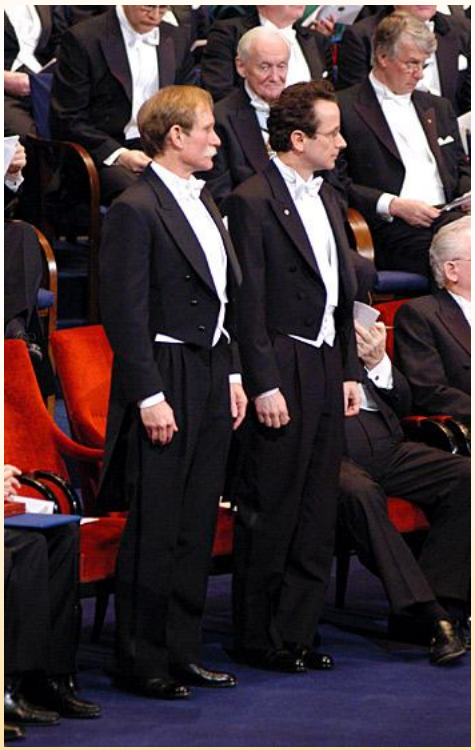


2003: NOBEL PRICE in CHEMISTRY:
Molecular channels through cell wall

Roderick MacKinnon
Showed the structure of **ion channels** and describe its function

Peter Agre
Studied transport of water through cell membrane
He discovered **aquaporins** – water pores



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GATED CHANNELS: overview

The cell can controle opening and closing of gated channels

Gating can be controlled by chemical, electrical or mechanical signal which stimulate a **molecullar sensor** situated close to the channel gate.

According to the signal to which channel (sensore) responses we can distinguish:

1. **VOLTAGE-GATED channels:**

   Sensor is stimulated by electrical stage of the cell (changes of ion concentratio)

2. **CHEMICALLY-GATED (ligand-gated) channels:**

   Sensor is stimulated by intracellular messenger molecules or by extracellular ligand.

3. **MECHANICALLY-GATED channels:**

   Sensor is stimulated by physical change, such as increased temperature, pressure

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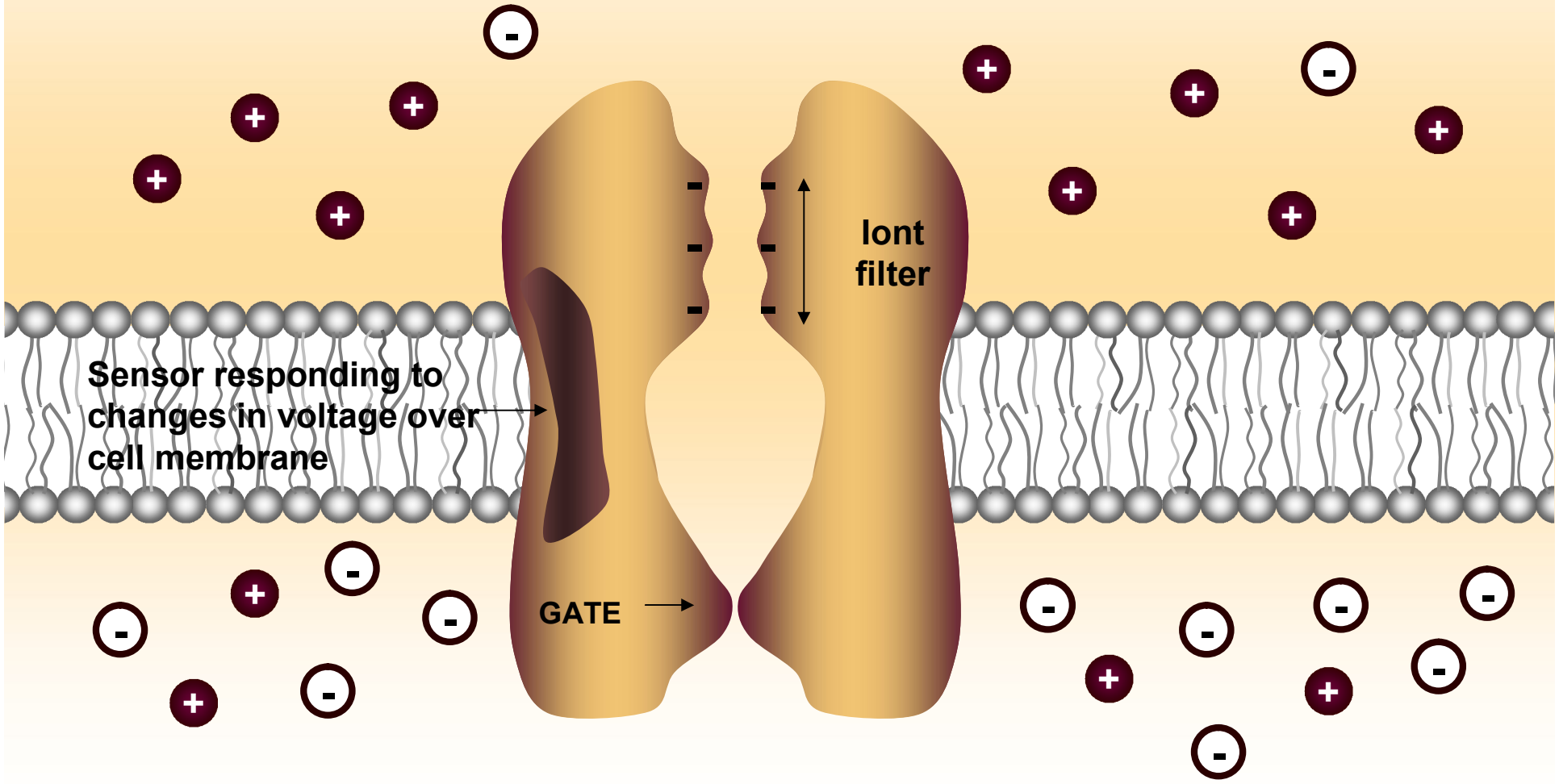
1. Voltage-gated channels

Channels are closed at the rest. Channel sensor responds to the cell membrane depolarization (change in voltage over the membrane. It causes channel opening for a short time. Typical Na+, K+, Ca+ channels in neurons and the heart.



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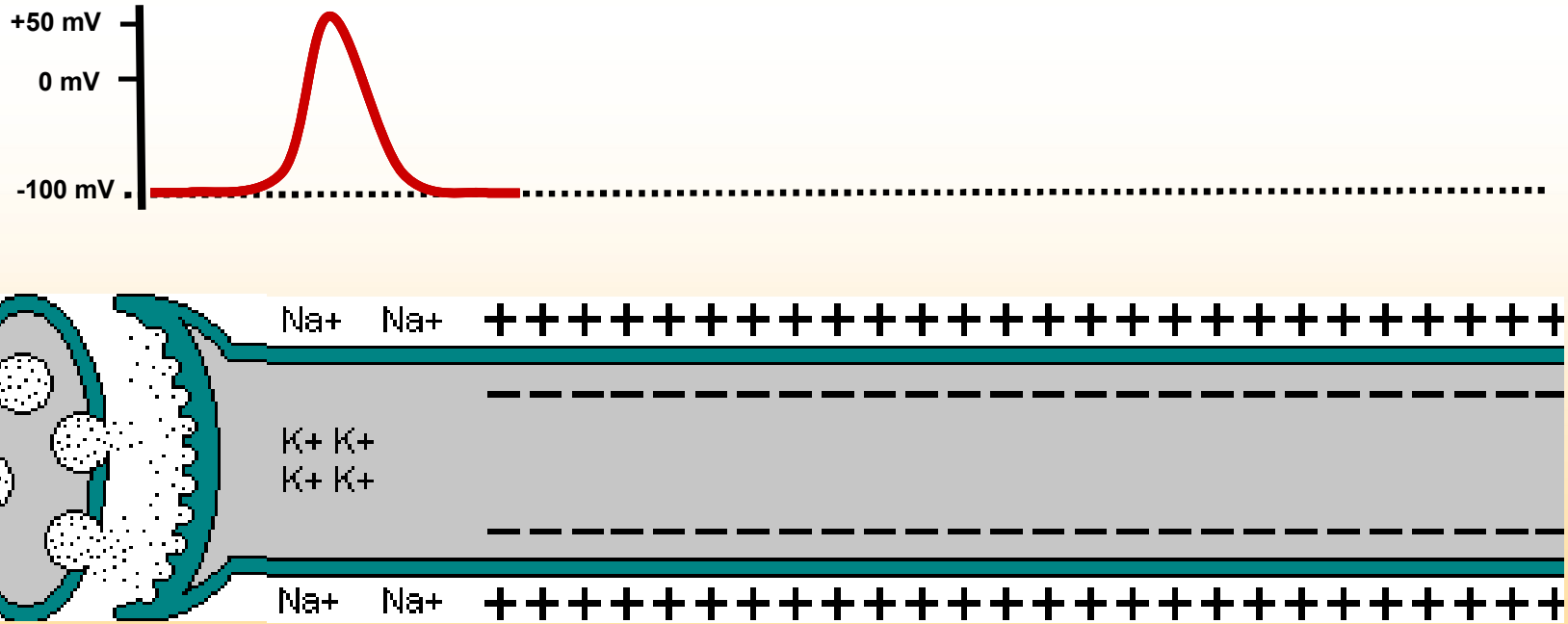
Na^+ and K^+ channels in nerve cells

Na^+ and K^+ channels are responsible for action potential (AP) at neurons

AP represents movement of Na^+ and K^+ across the membrane through
Voltage-gated Na^+ channels (Na^+ ions goes into the neuron axon)
Voltage-gated K^+ channels (K^+ ions goe out of the neuron axon)

It causes <u>depolarization</u> folowed by <u>repolarization</u> of the neuron cell membrane



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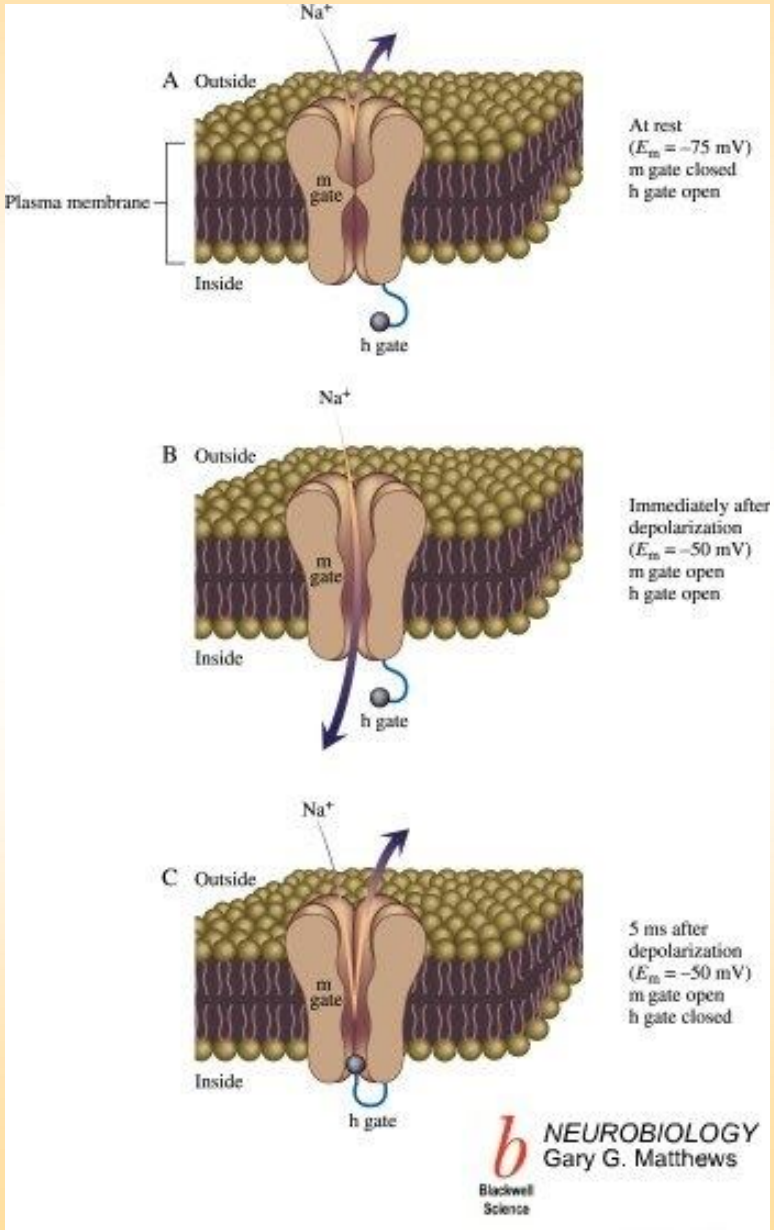
Na^+ channels are double gated



Voltage-gated Na^+ channels use a two-step process for opening and closing

ACTIVATION gate (m-gate)
INACTIVATION gate (h-gate)

The double gating of Na+ channels plays a major role in the phenomen known as the <u>refractory period</u>

AP cannot overlap and cannot travel backward because of refractory period

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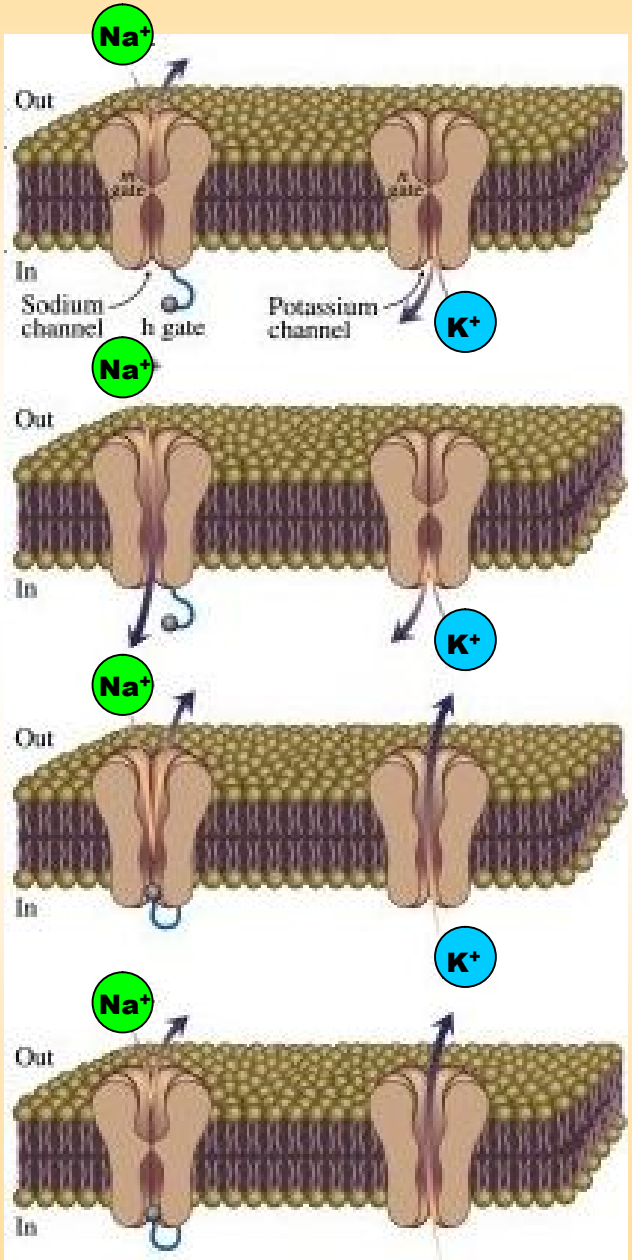
Na^+ and K^+ during AP



A: RESTING STATE

Na^+ channel: m-gate closed
h- gate **open**
K^+ channel: closed

B: DEPOLARIZING phase

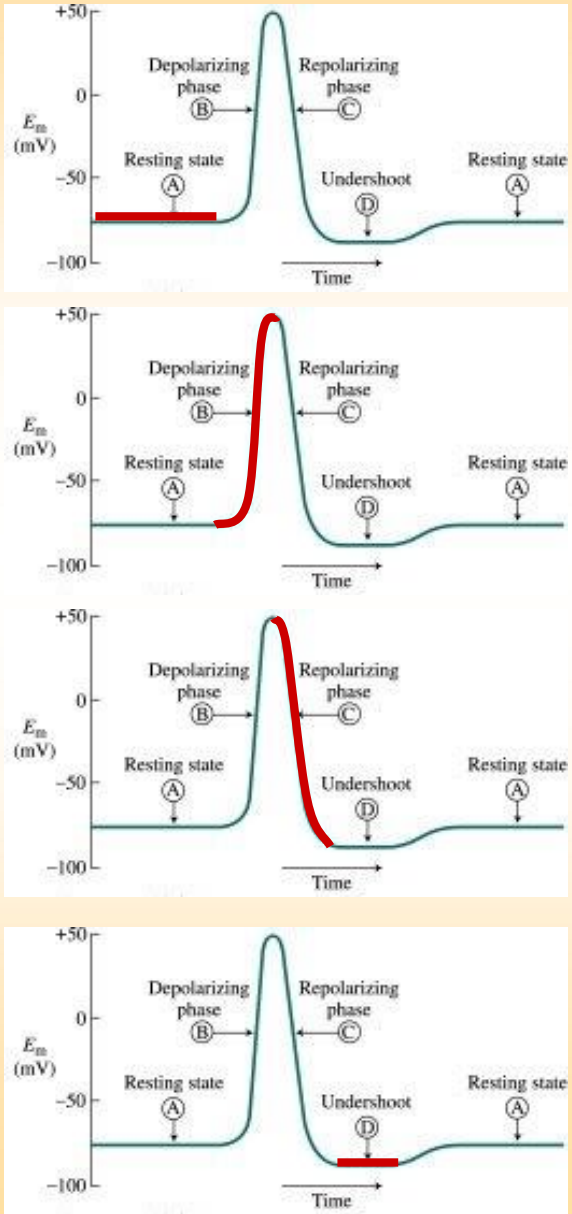
Na^+ channel: m-gate **open**
h- gate **open**
K^+ channel: closed

C: REPOLARIZING phase

Na^+ channel: m-gate open
h- gate closed
K^+ channel: **open**

D: UNDERSHOOT phase

Na^+ channel: m-gate open
h- gate closed
K^+ channel: permeability remains elevated because of slow closing h-gate of Na+ channels



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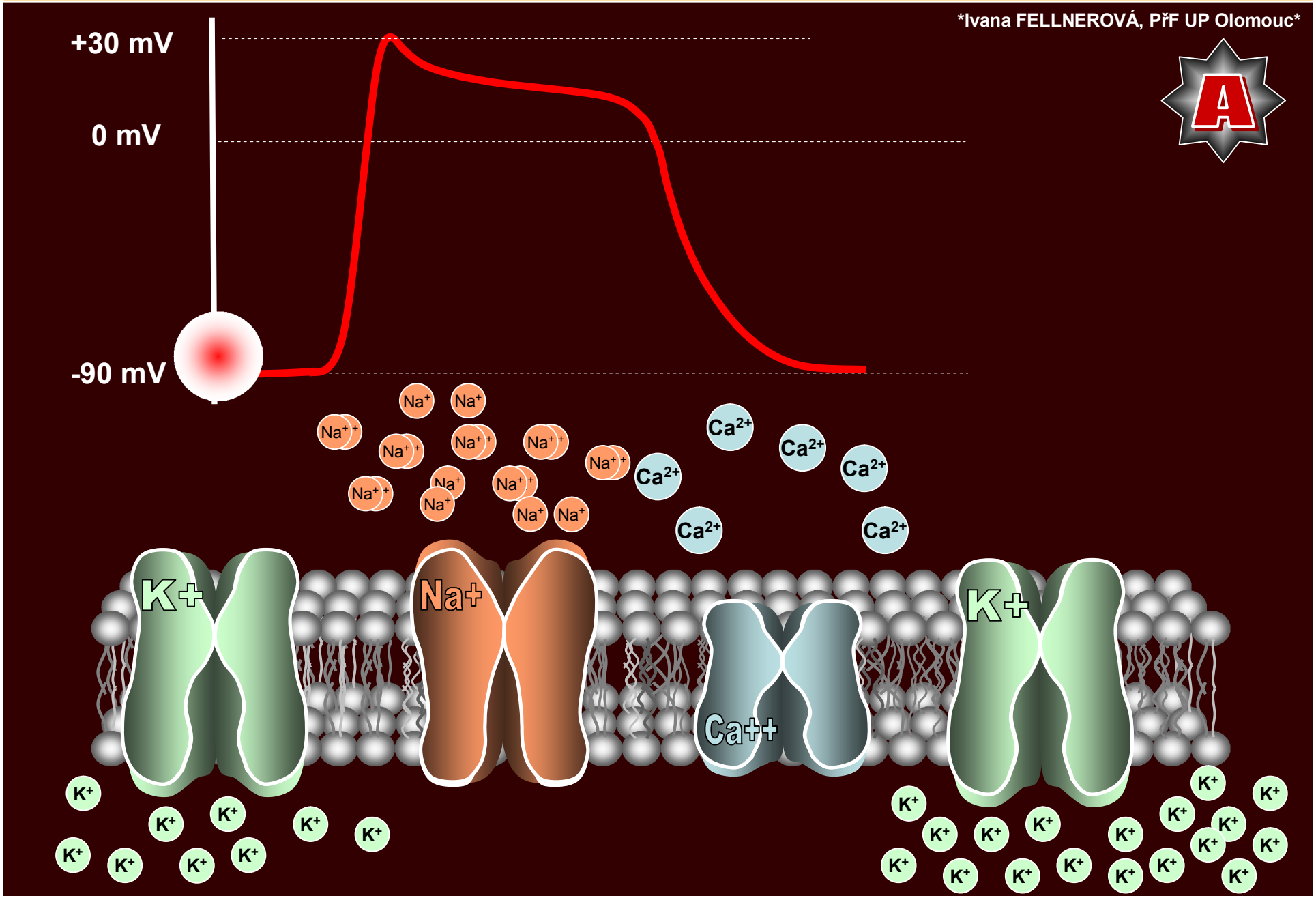
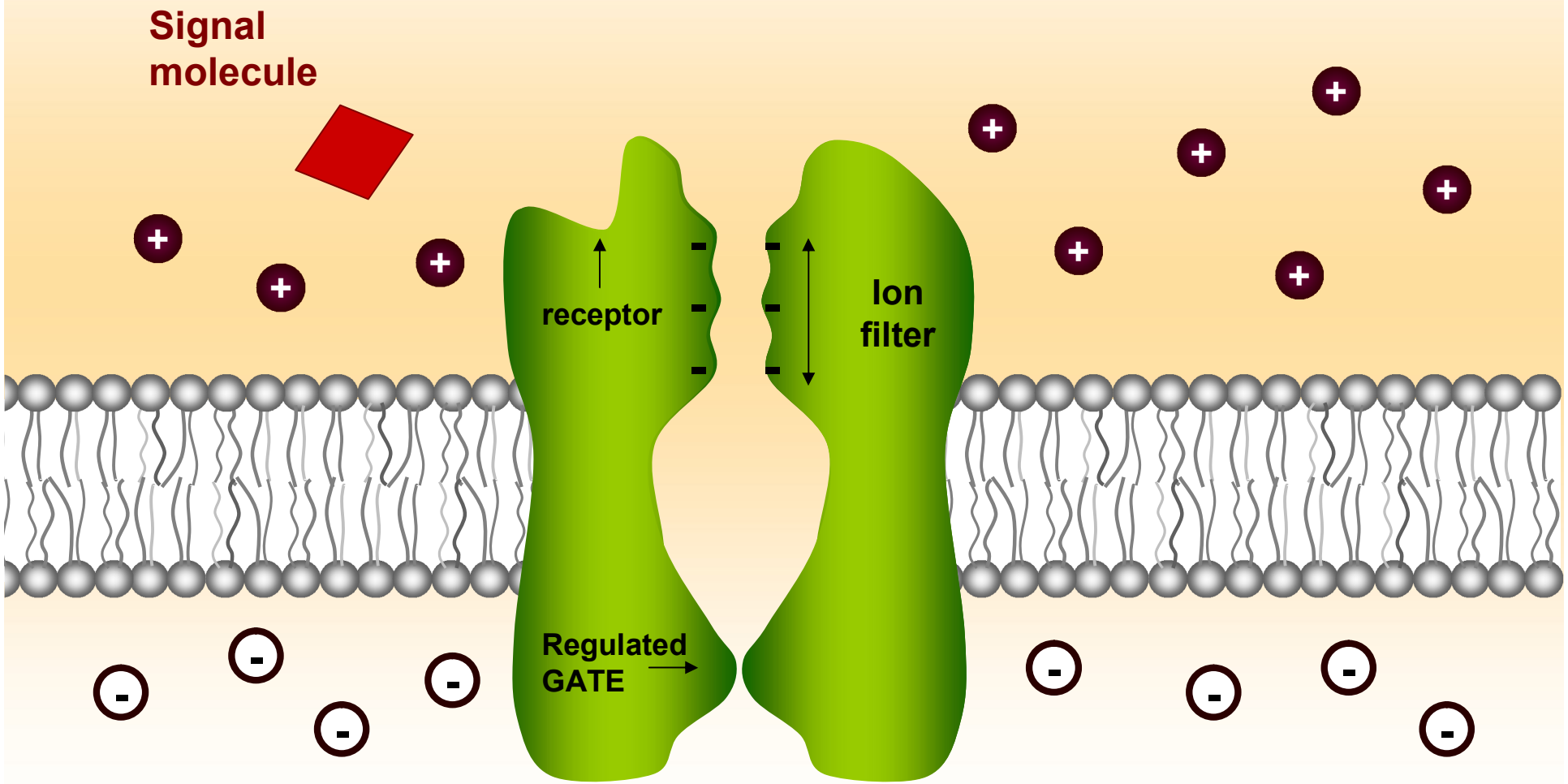
Voltage-gated ion channels in cardiac muscle cells



2a. Ligand gated channels - directly

Receptor is part of the channels.
Channel opens after binding extracellular ligand (neurotransmitter).
At the postsynaptic neurons at the neuromuscular junction



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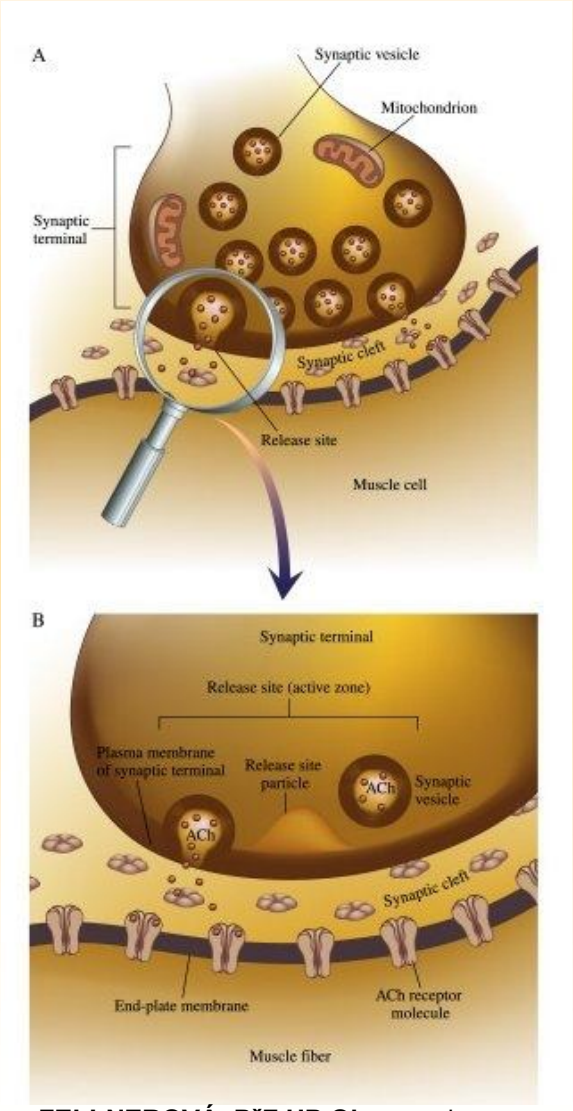
2a. Ligand gated channels - examples

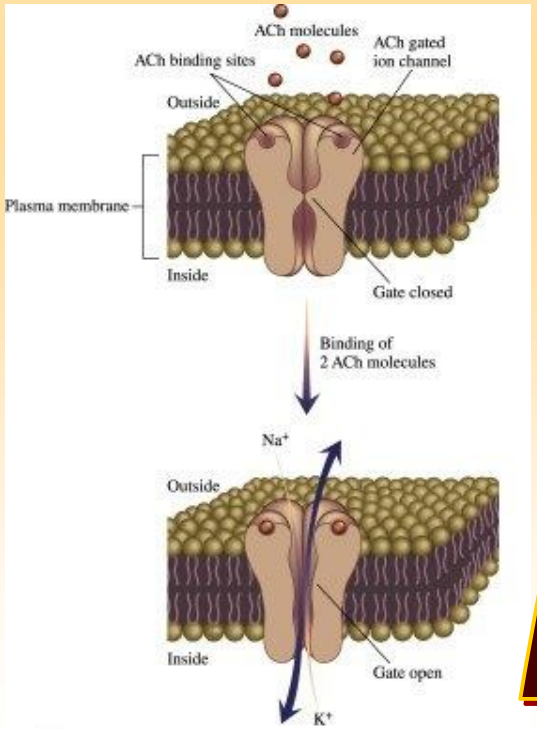
Nicotinic - Acetylcholine receptors nAchR:

Receptor on Na+ channels at neuromuscullar junction. Neurotransmiter acetylcholine end nicotine



Glutamate receptors:

Na+, K+ i Ca+ channels in the CNS and spinal cord. Glutamate signaling is essential for normal neurodevelopment, learning and memory. Overstimulation is associated with (Alzheimer‘s, Parkinson‘s disease or schizophrenia)



GABA receptors :

Stimulated by γ-aminobutyric Acid (GABA)

Glycine receptors:

Receptors of Cl- channels
Stimulated by glycine.
Inhibition of neurotransmition.

b NEUROBIOLOGY Gary G. Matthews Blackwell Science

Animace

http://www.blackwellpublishing.com/matthews/neurotrans.html

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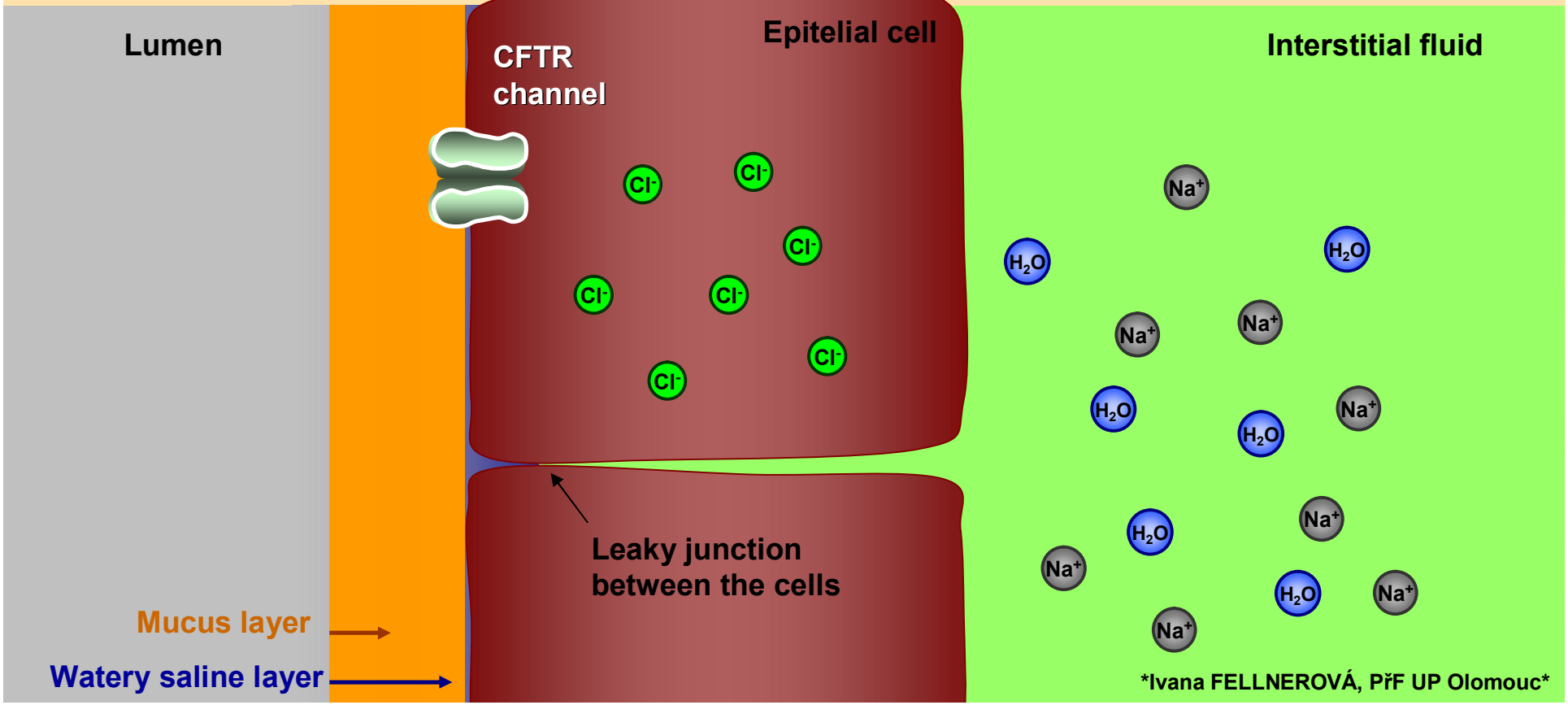
2a. Ligand gated channels – CFTR channel

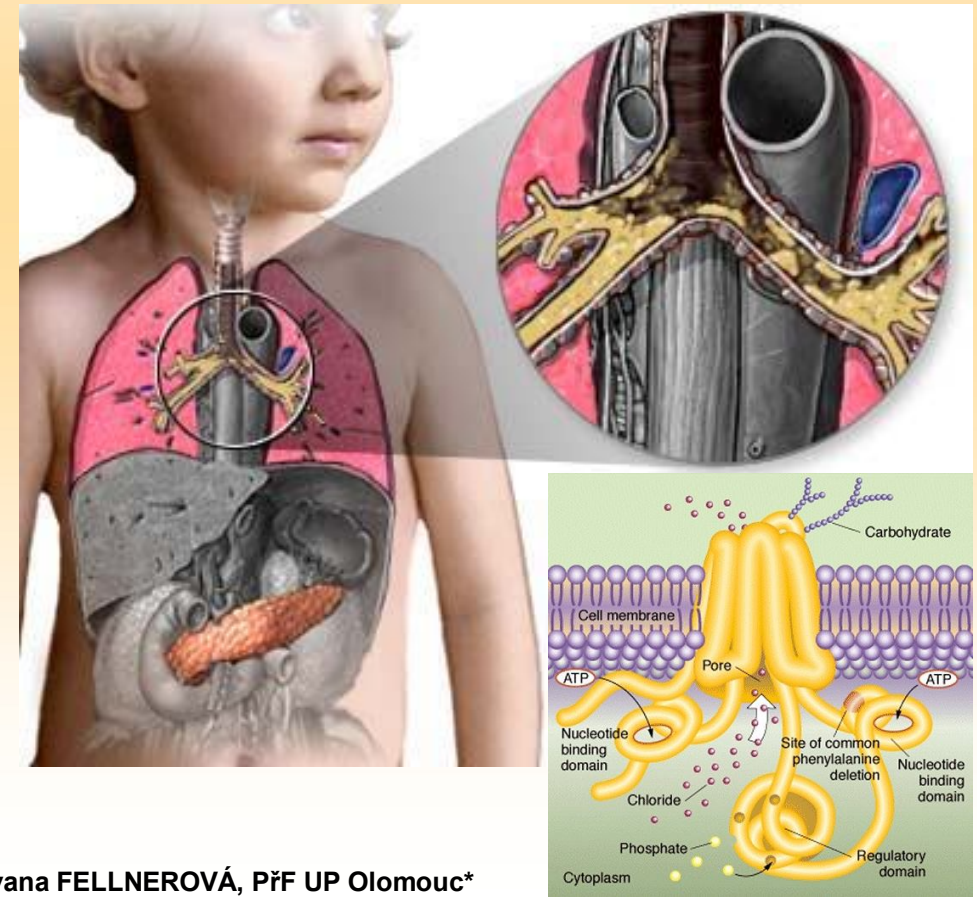
CFTR channel = cystisc fibrosis transmembrane conductance regulator

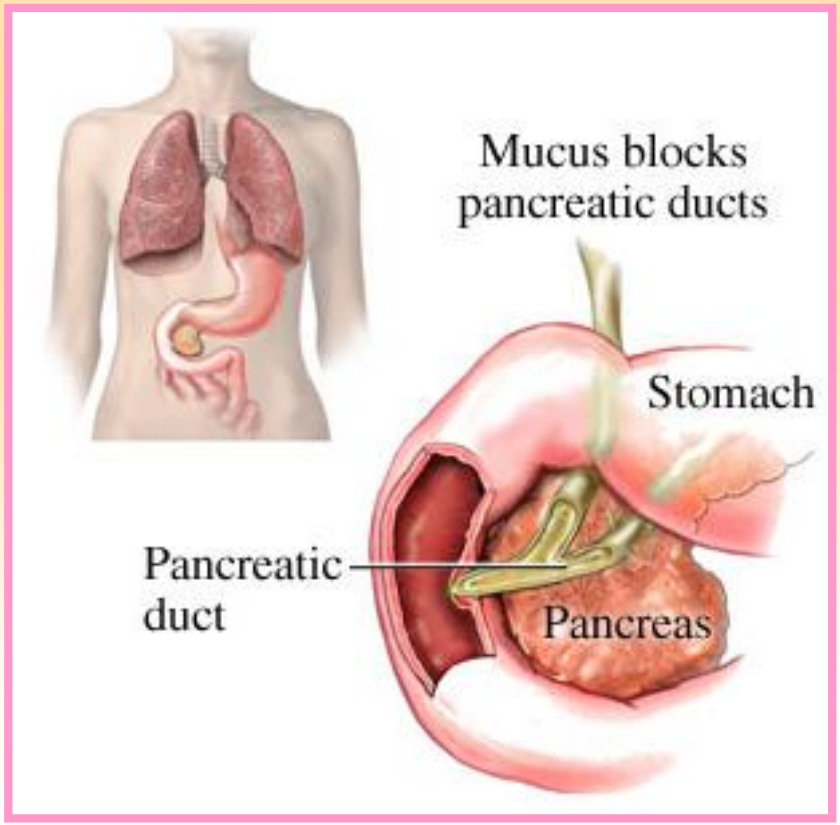
- CFTR is Cl^- gated channels (ligand ATP):
- At apical membrane of epitelial cells line the pancreatic ductules and small respiratore airways.
- CFTR transports Cl- outside of the epitelial cell to the lumen (Na+ and H_2O folow passively by paracellular transport)



Cystic fibrosis: Defect of CFTR channel

- **Cystisc fibrosis is an inherited disease**
- **People with cystic fibrosis has defected CFTR channels.**
- **Cystisc fibrosis affects multiple parts of the body including pancreas, the lung and the sweat glands.**





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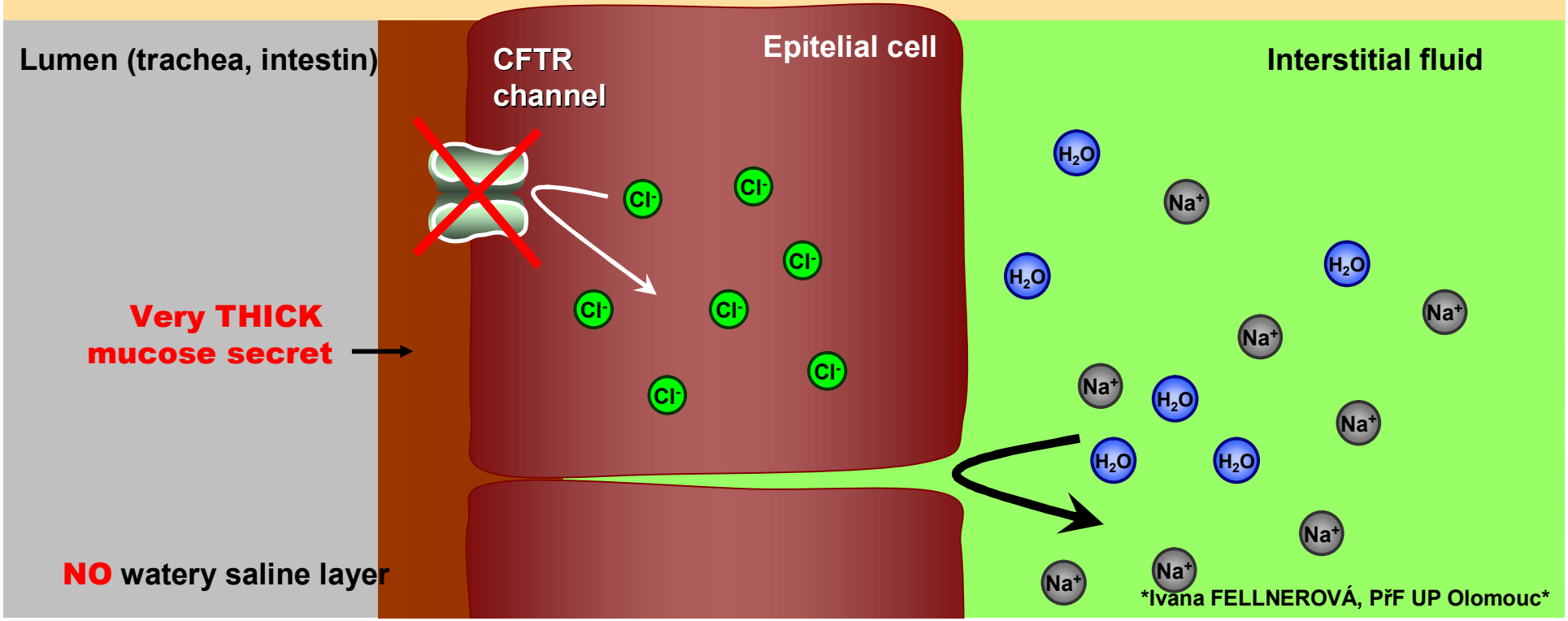
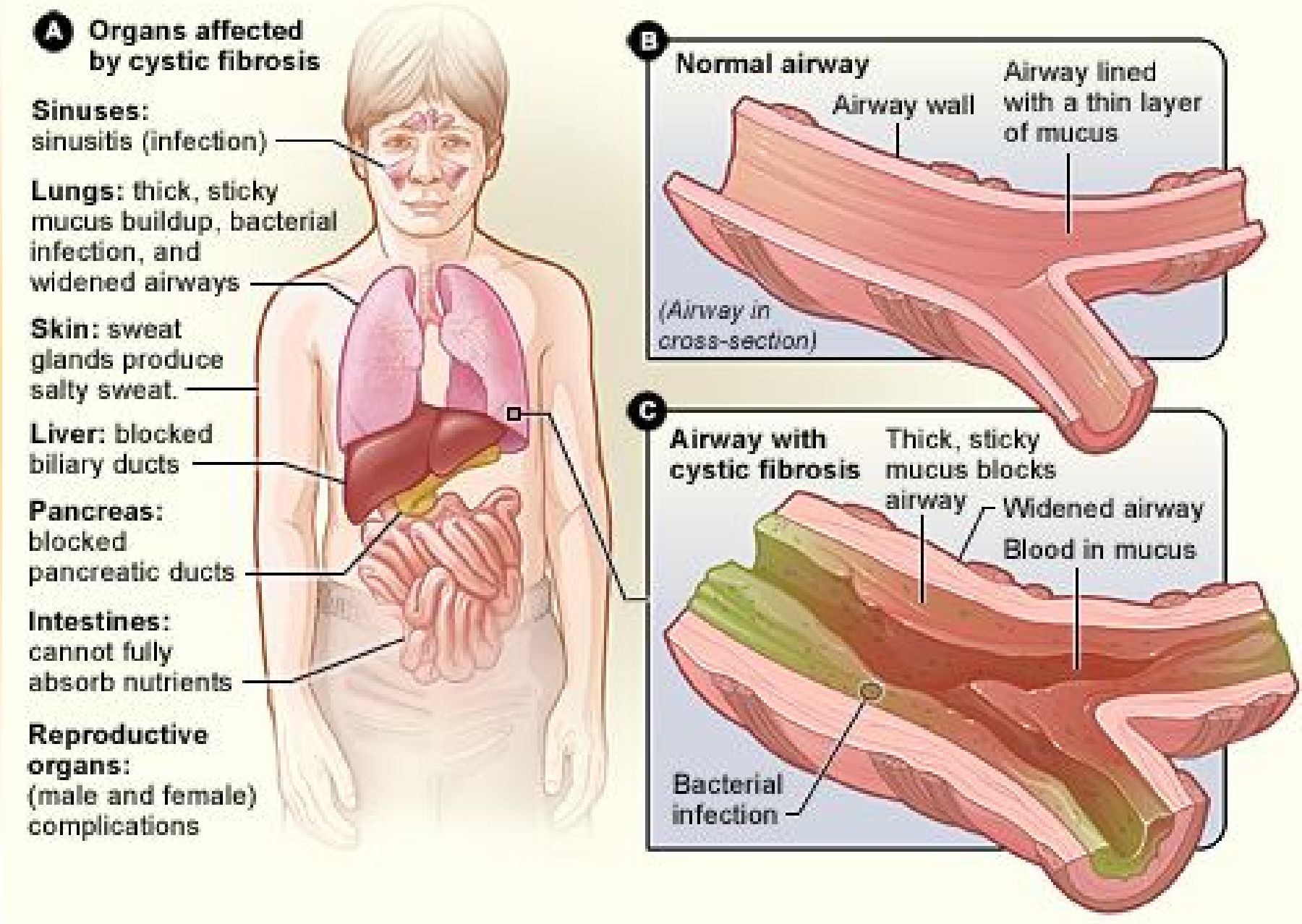
Cystic fibrosis: Defect of CFTR channel

People with cystic fibrosis have to much sticky mucus in the airways of their lungs and gets lots of lung infection

- no working CFTR means no salt (chloride) enters the air space
- water does not move by osmosis from; the mucus is very thick
- in the digestive system mucus clogs small pancreatic ducts and prevents digestive enzymes secretion into the intestin.
- in airways of the respiratory system sticky mucus cannot be easily moved to clear particles from the lungs (sticky mucus traps bacteria and cause more infection)

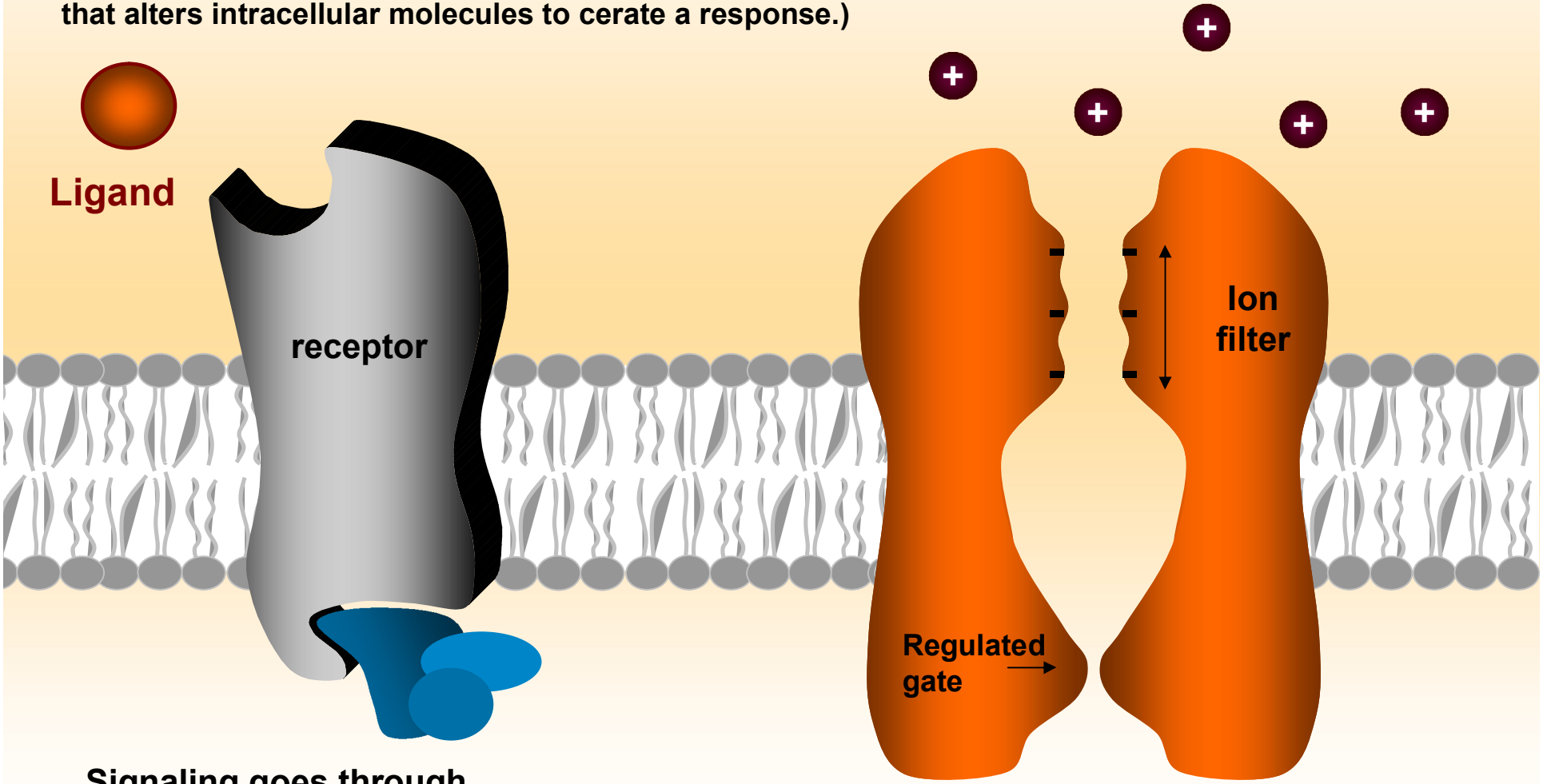


Cystic fibrosis - overview



2b. Ligand gated channels - indirectly

Receptor is situated outside of the channels.
Ligand binds to G-protein coupled receptor which facilitates **signal transduction**
(process, by which extracellular signal molecules activate a membrane receptor, that alters intracellular molecules to cerate a response.)



Signaling goes through second messenger **(G proteinu)**

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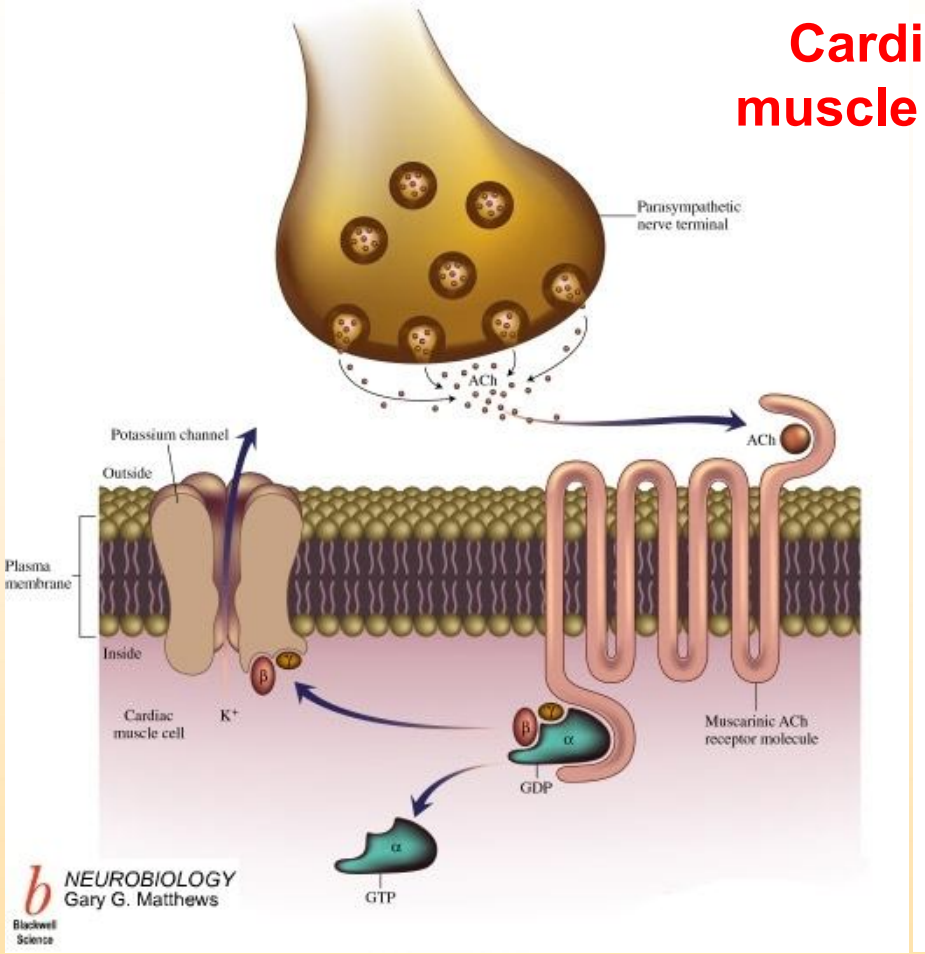
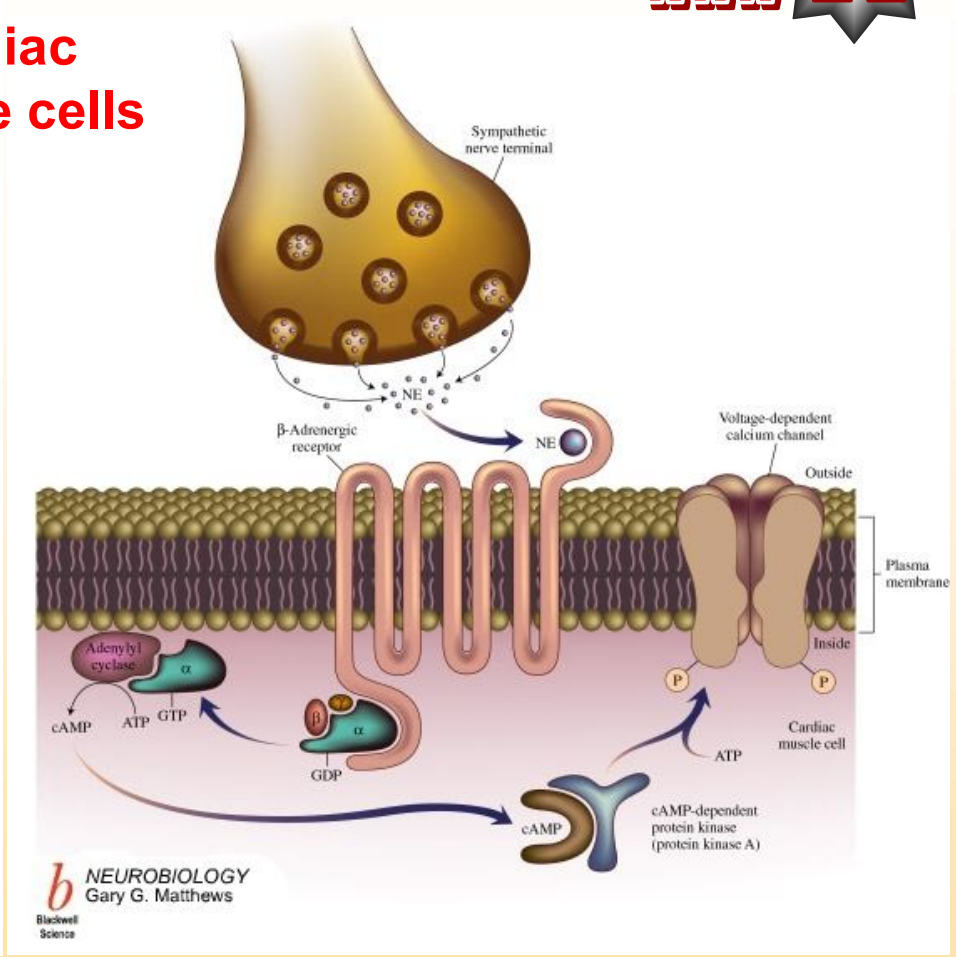
2b. Ligand gated channels - indirectly

Cardiac muscle cells



Parasympathetic synapse → acetylcholin → K+ channel



Symphathetic synapse → noradrenalin → Ca2+ channel

Animation

NEUROBIOLOGY
Gary G. Matthews
Blackwell Science

http://www.blackwellpublishing.com/matthews/neurotrans.html

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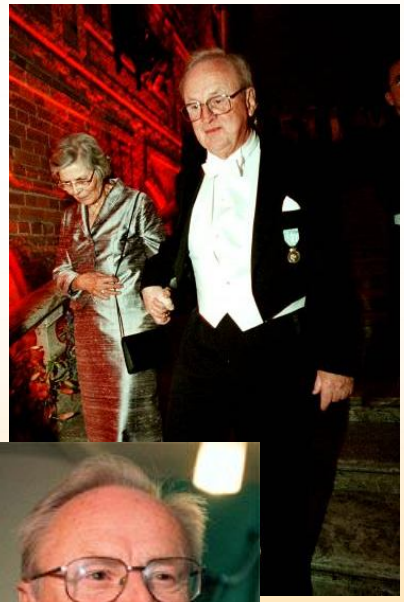
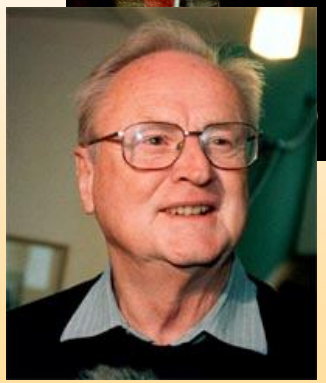
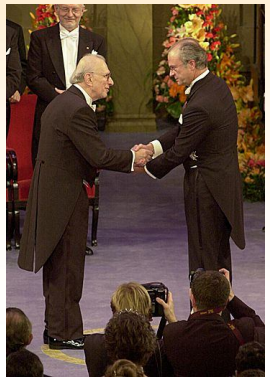
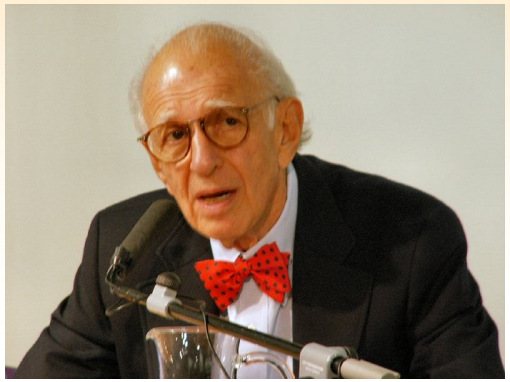
2000: NOBEL PRICE /Physiology,medicine
Signal transduction on the synapse

Arvid Carlsson
He discovered dopamin, as an neurotransmiter at the synapse



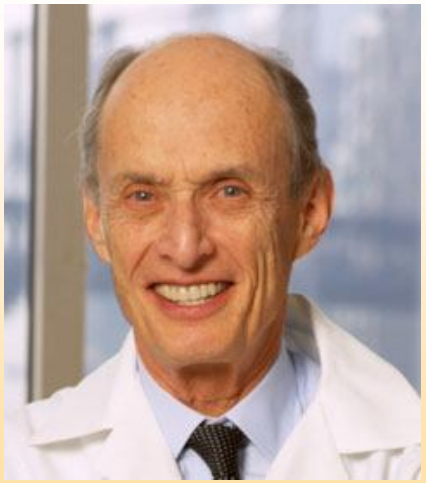






Paul Greengard
Stimulation membrain receptors change intracelular concentration of cAMP.

Eric Kandel
He showed that learning skils and memory dependent on cAMP concentration.



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3. Mechanically gated channels

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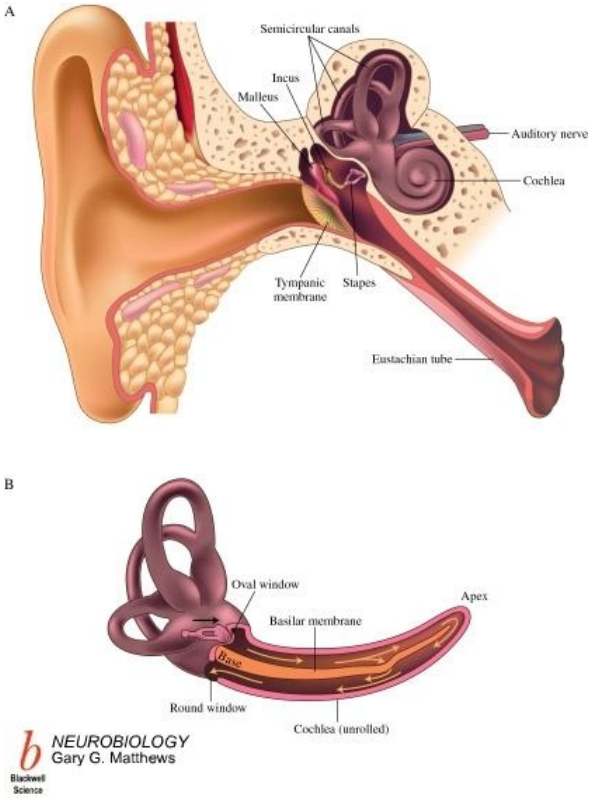
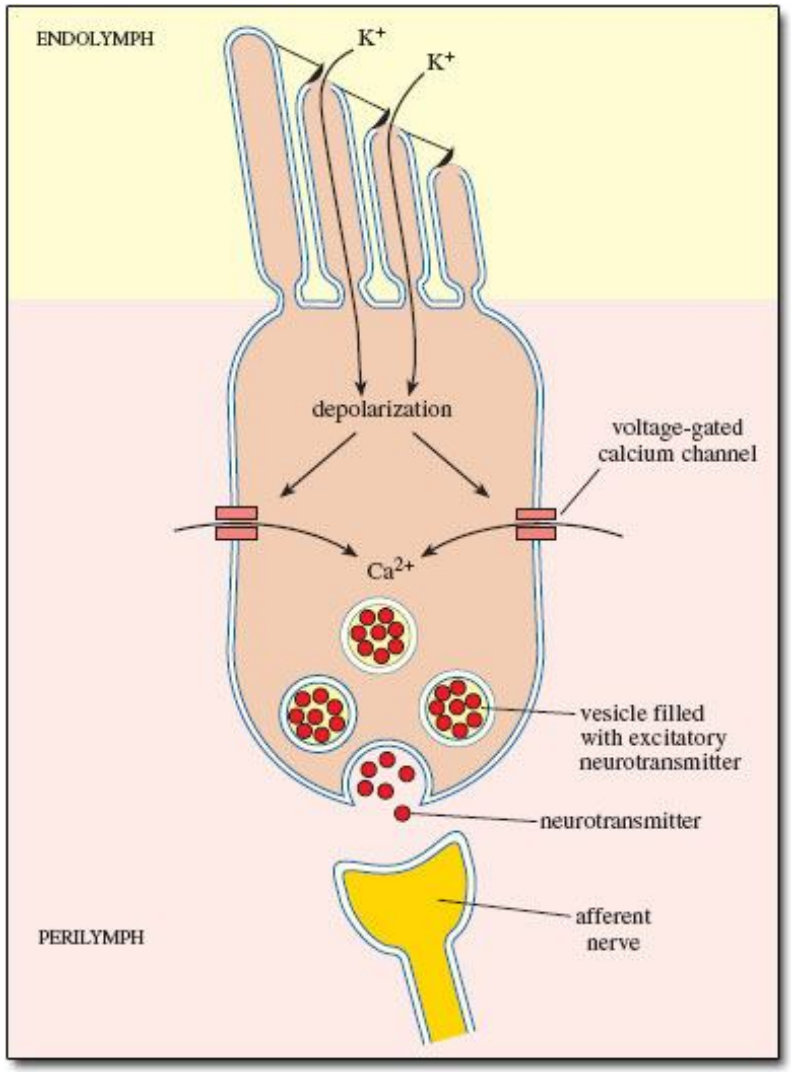
Hair cells in Cochlear duct (Organ of Corti)

The fluid waves push on the flexible membrane of the cochlear duct. The movement of the tectorial membrane moves the cilia on the hair cells. Hair cells develope action potential by opening mechanicly gated kalcium channels.



http://www.blackwellpublishing.com/matthews/haircell.html



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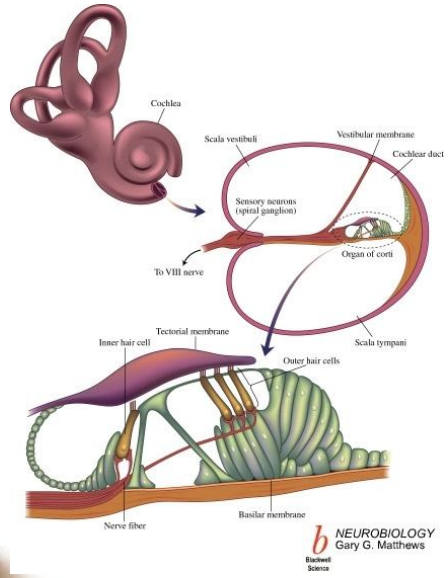
(b) The organ of Corti



Tectorial membrane

Stereocilia

Afferent axons

Basilar membrane

Inner hair cells

Tunnel of Corti

Efferent axons

Outer hair cells

Protein clasification

ANIMAL PHYSIOLOGY, Figure 13.30 (Part 2) © 2004 Sinauer Associates, Inc.

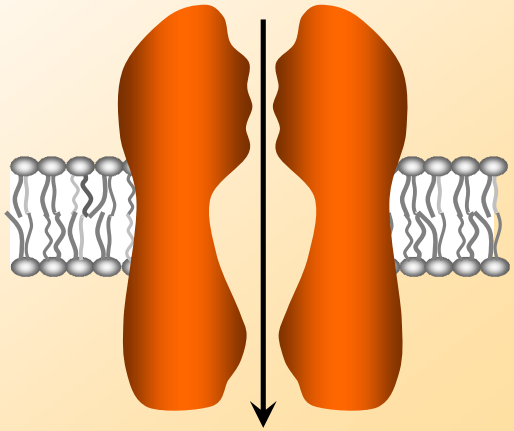
CARRIERS

- Transport membrane proteins
- Bind with specific substrates and carry them across the membrane by changing conformation
- Transport one or more molecules at one time
- Transport works with or without energy

COMPARISON:

Channels:

ATP Simple diffusion

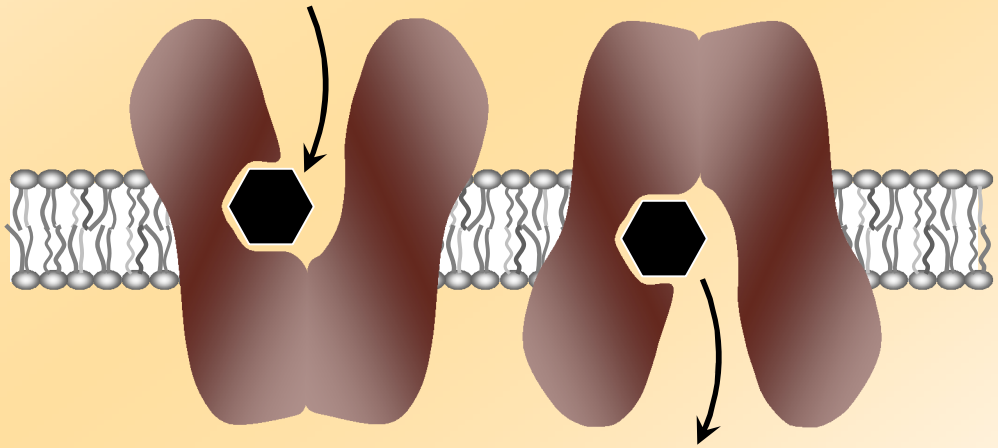


- Creates a **water-filled passageway directlylinking** the extracellular and intracellular environment
- Is like a **narrow doorways** into the cell.
- **Transports** small molecules, typically **ions** and **water**
- **Rapid transport** are not as selective about what they transport

Carriers:

ATP Simple diffusion

ATP Active transport



- **Never** forms a **direct connection** between the extracellular and intracellular environment
- Alternates between **two formations**. They bind to the substrates that they carry out.
- **Transports larger molecules** [glucose, nucleotides]
- **Transport** is slower but better distinguishes what they transport

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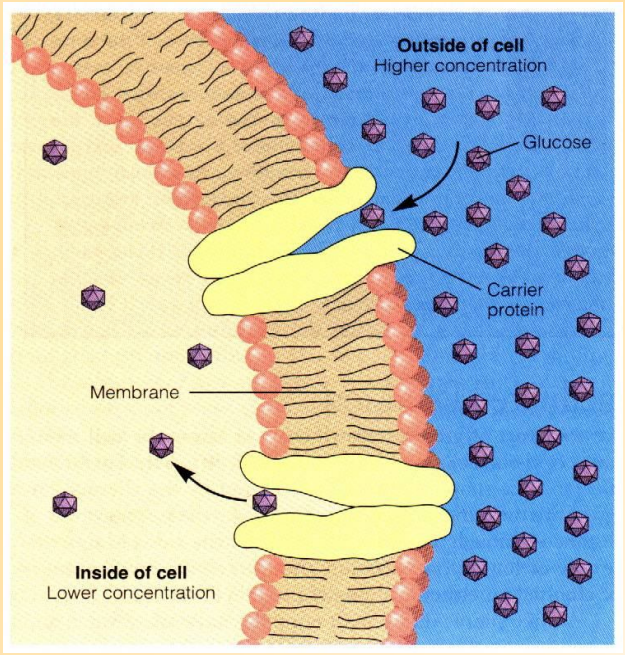
1. Carrier-mediated PASSIVE transport

GLUT

GLUcose **T**ransporter



GLUT allows the glucose to enter a cell withouth energy requirement.

The GLUT family of Glucose transporters

GLUT 1 is distributed in **most tissues** of the body ;
In adults more abundant in erythrocytes and brain endothelium . It is **very sensitive to glucose** concentration (adaptation to high requirement of brain cell) ;

GLUT 2 found primarily in **liver**, **pancreas** and transporting epitelium of **intestine** and **kidney**,

GLUT 3 found in **neurons**

GLUT 4 Is contained in the **Insulin-sensitive tissues** such as skeletal **muscle** and **adipose** tissue

GLUT 5 in **intestinal epitelium**; in fact transport **fructose**

(GLUT 6 through GLUT 12 remain under investigation)

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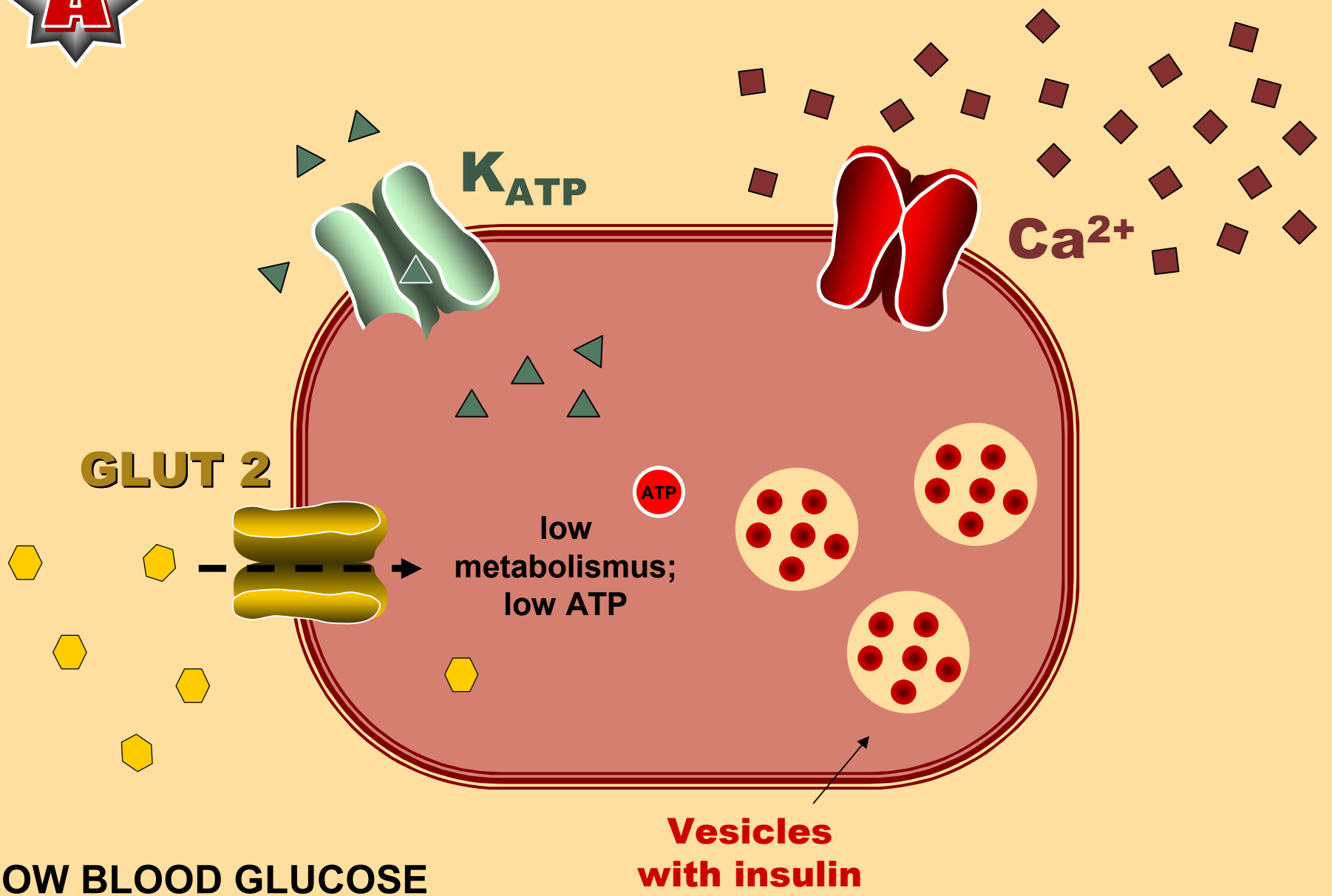
GLUT 2 in pancreatic β cell



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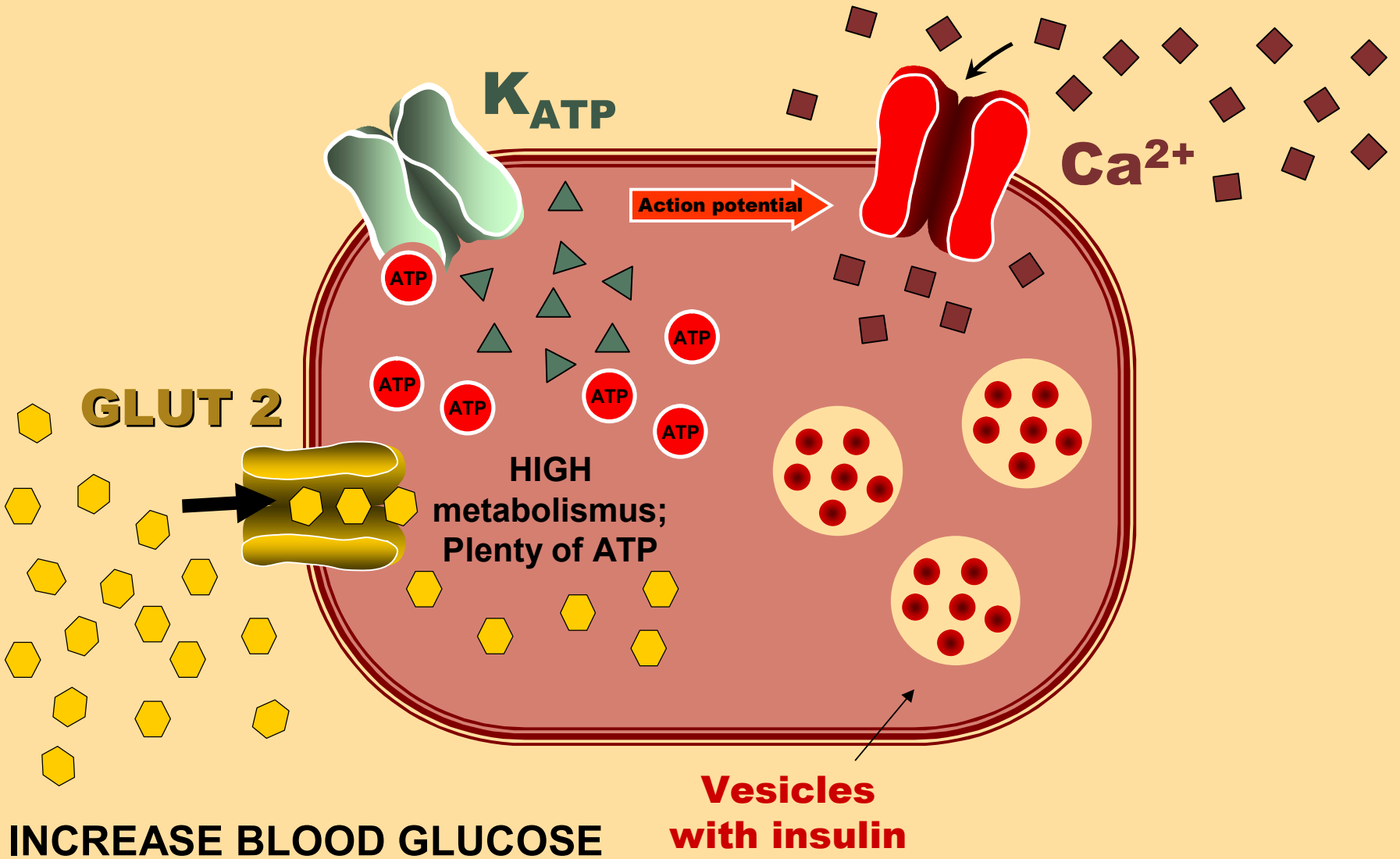
GLUT 2 in pancreatic β cell



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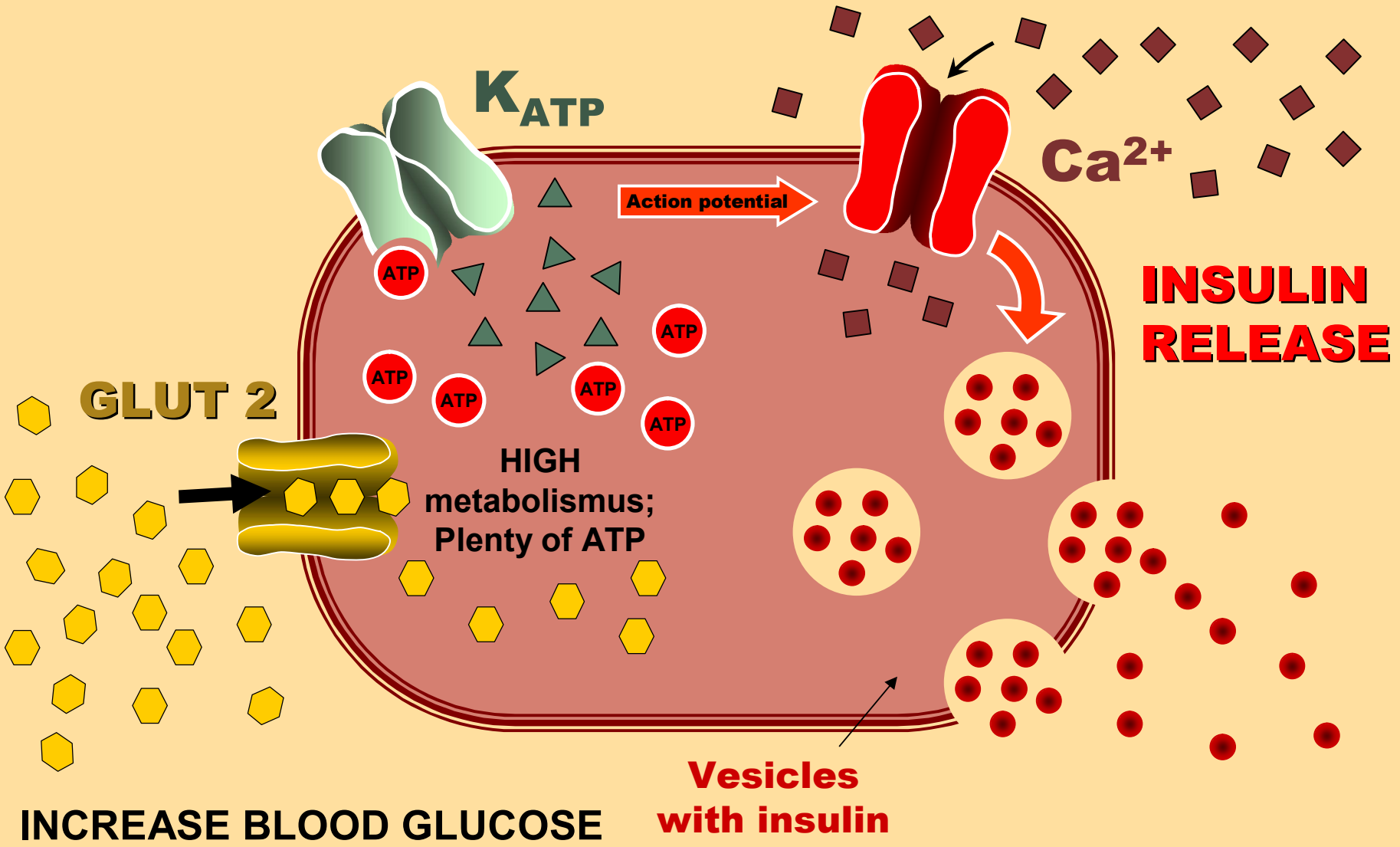
GLUT 2 in pancreatic β cell



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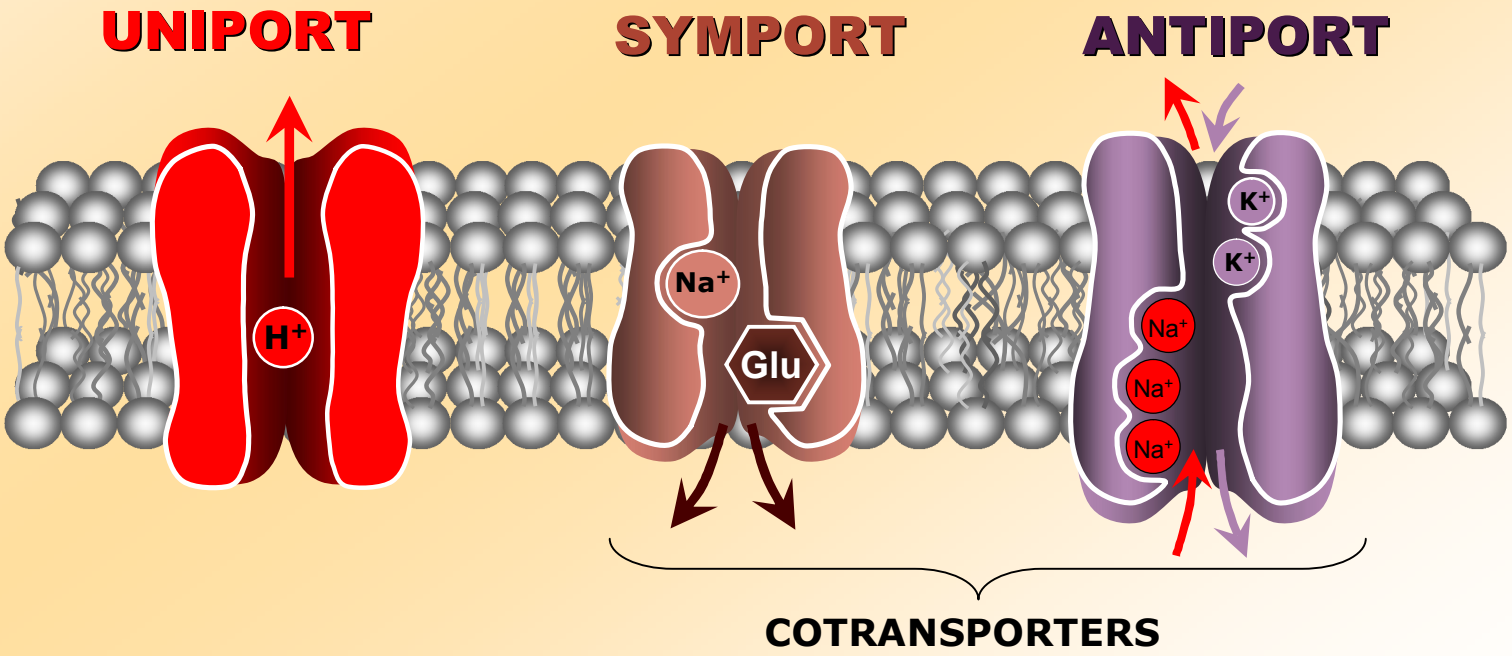
2. Carrier-mediated ACTIVE transport

ATP

- Active transport moves substances against their concentration gradient (the pumps)
- Moving molecules against their concentration gradient requires the input of outside energy
- The energy for active transport comes either **directly (primary)** or **indirectly (secondary)** from the high-energy bond of ATP



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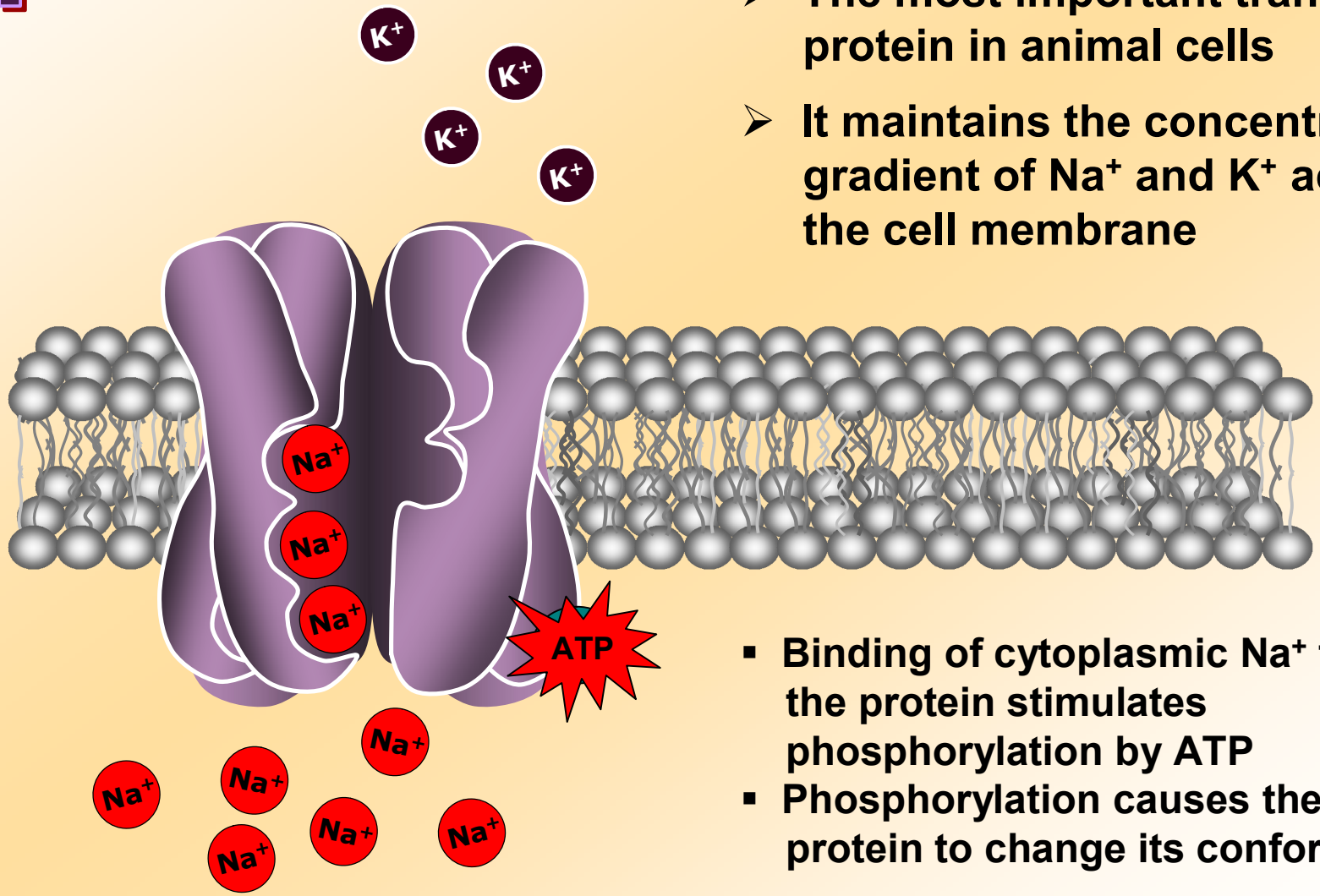
Sodium-potasium pump (Na^+ K^+ ATPase)

1.



- The most important transport protein in animal cells
- It maintains the concentration gradient of Na^+ and K^+ across the cell membrane

- Binding of cytoplasmic Na^+ to the protein stimulates phosphorylation by ATP
- Phosphorylation causes the protein to change its conformation

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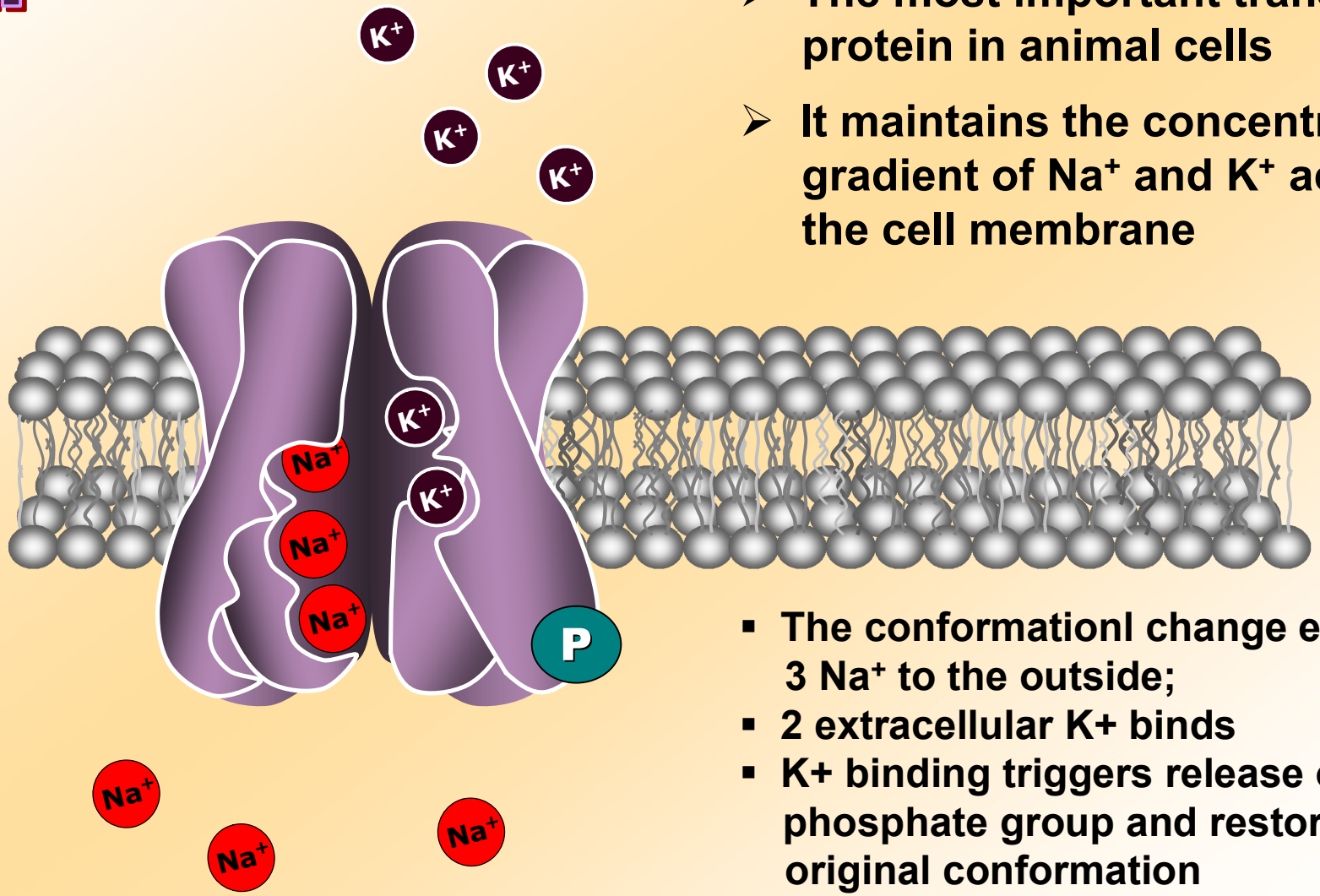
Sodium-potasium pump (Na^+ K^+ ATPase)

2.



- The most important transport protein in animal cells
- It maintains the concentration gradient of Na^+ and K^+ across the cell membrane

- The conformationl change expels 3 Na^+ to the outside;
- 2 extracellular K+ binds
- K+ binding triggers release of phosphate group and restore original conformation

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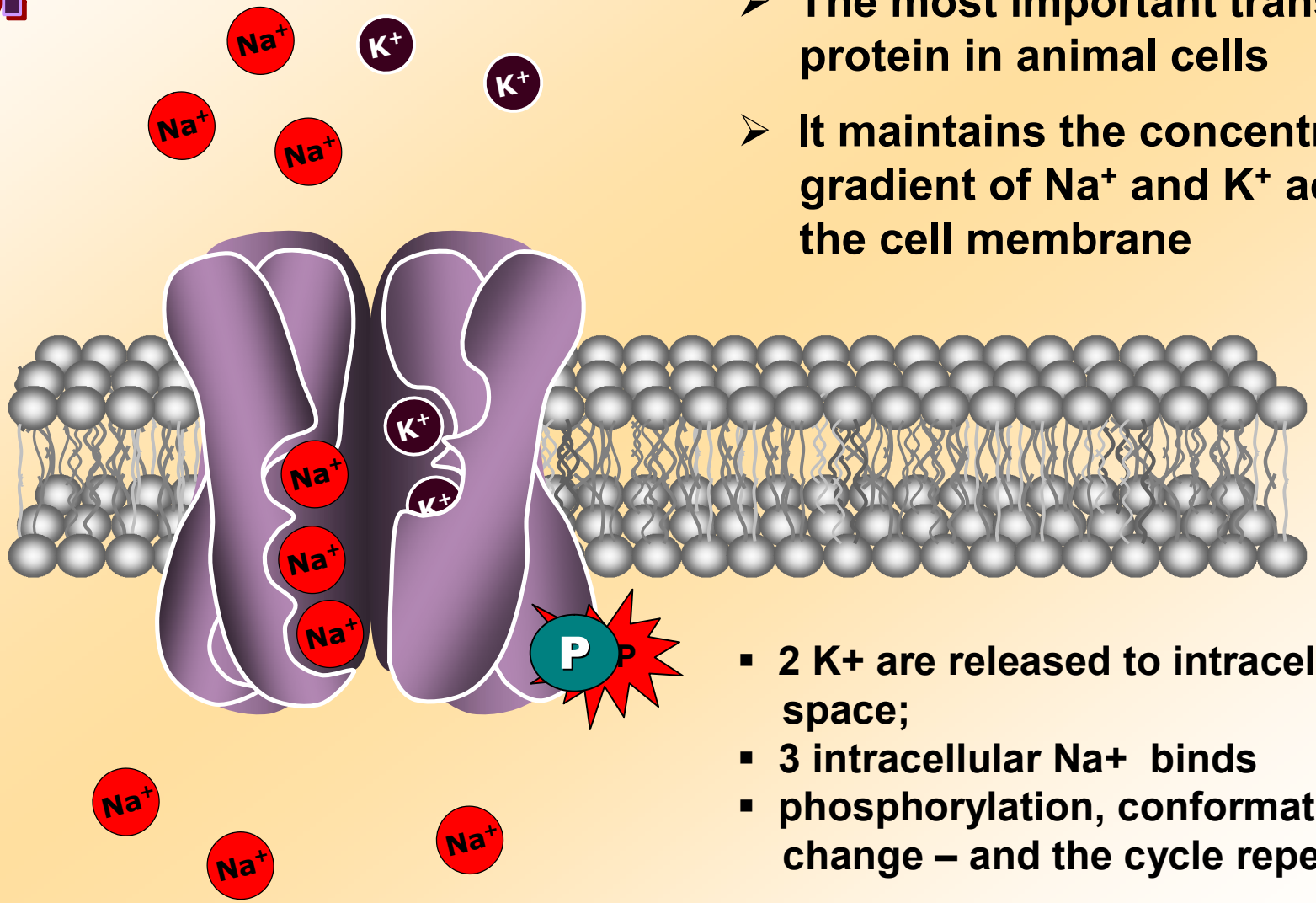
Sodium-potasium pump (Na^+ K^+ ATPase)

3.



- The most important transport protein in animal cells
- It maintains the concentration gradient of Na^+ and K^+ across the cell membrane

- 2 K+ are released to intracellular space;
- 3 intracellular Na+ binds
- phosphorylation, conformation change – and the cycle repeats

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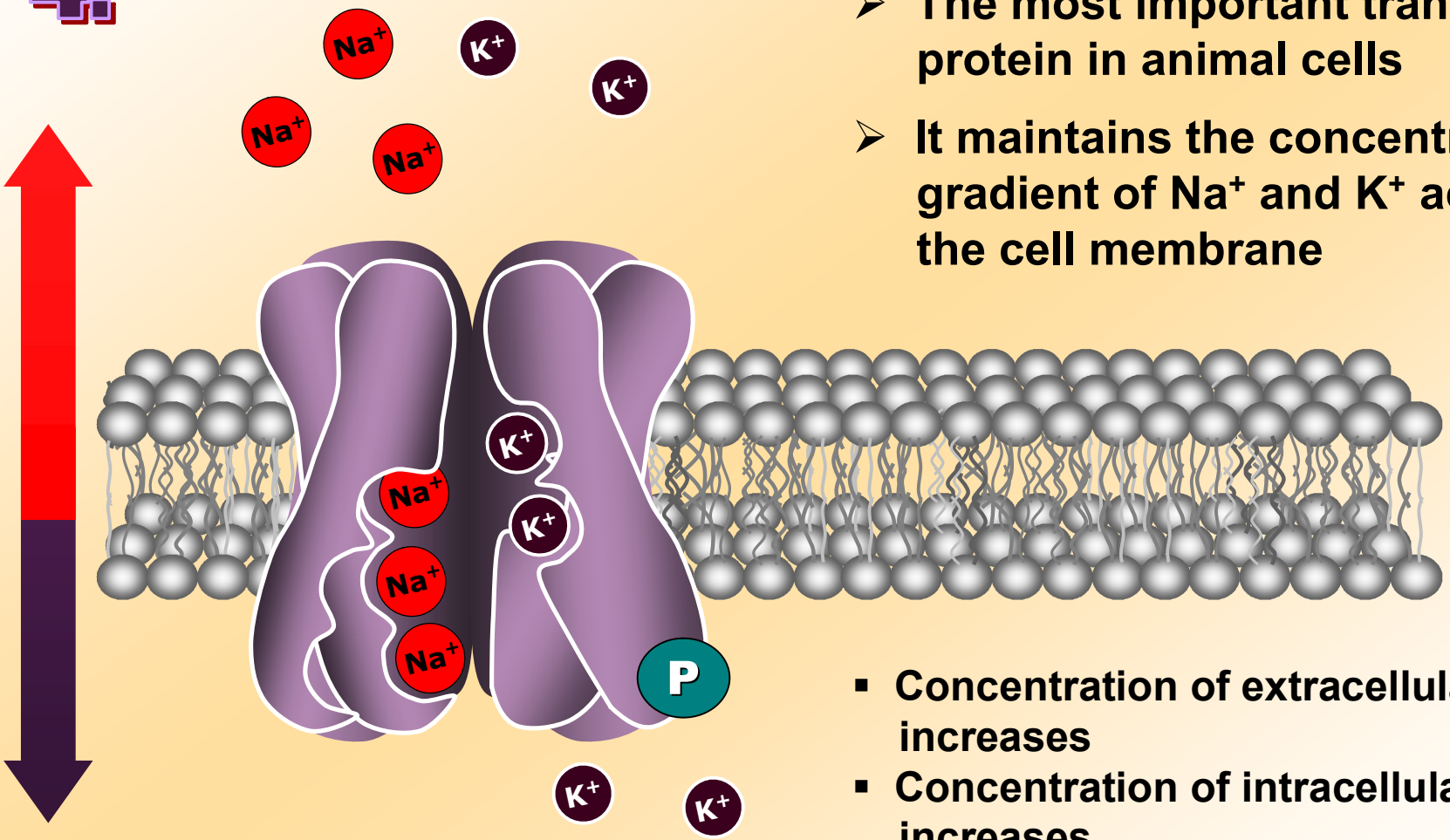
Sodium-potasium pump (Na^+ K^+ ATPase)

4.



- The most important transport protein in animal cells
- It maintains the concentration gradient of Na^+ and K^+ across the cell membrane

- Concentration of extracellular Na+ increases
- Concentration of intracellular K+ increases

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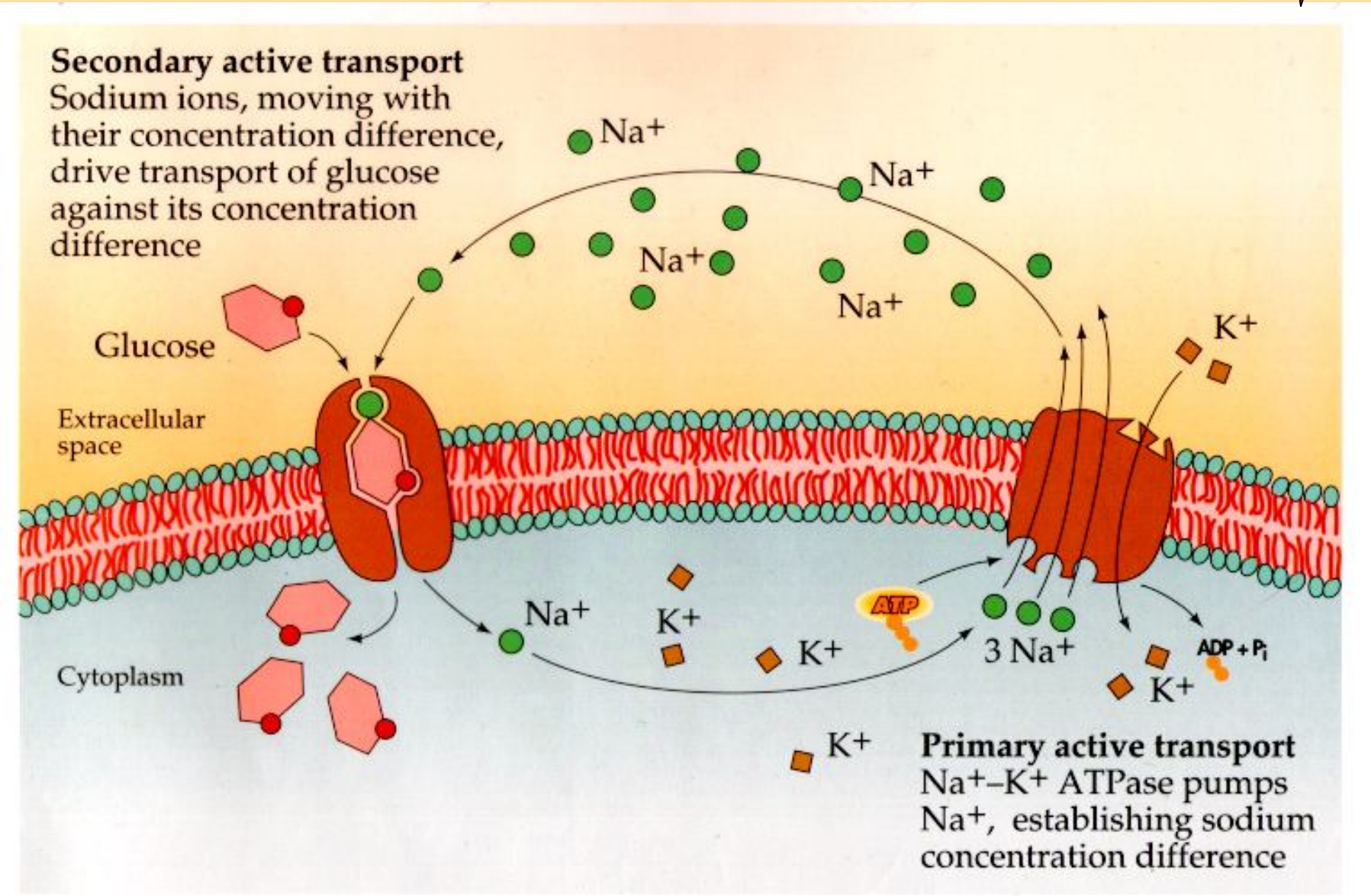
SGLT SECONDARY ACTIVE transport



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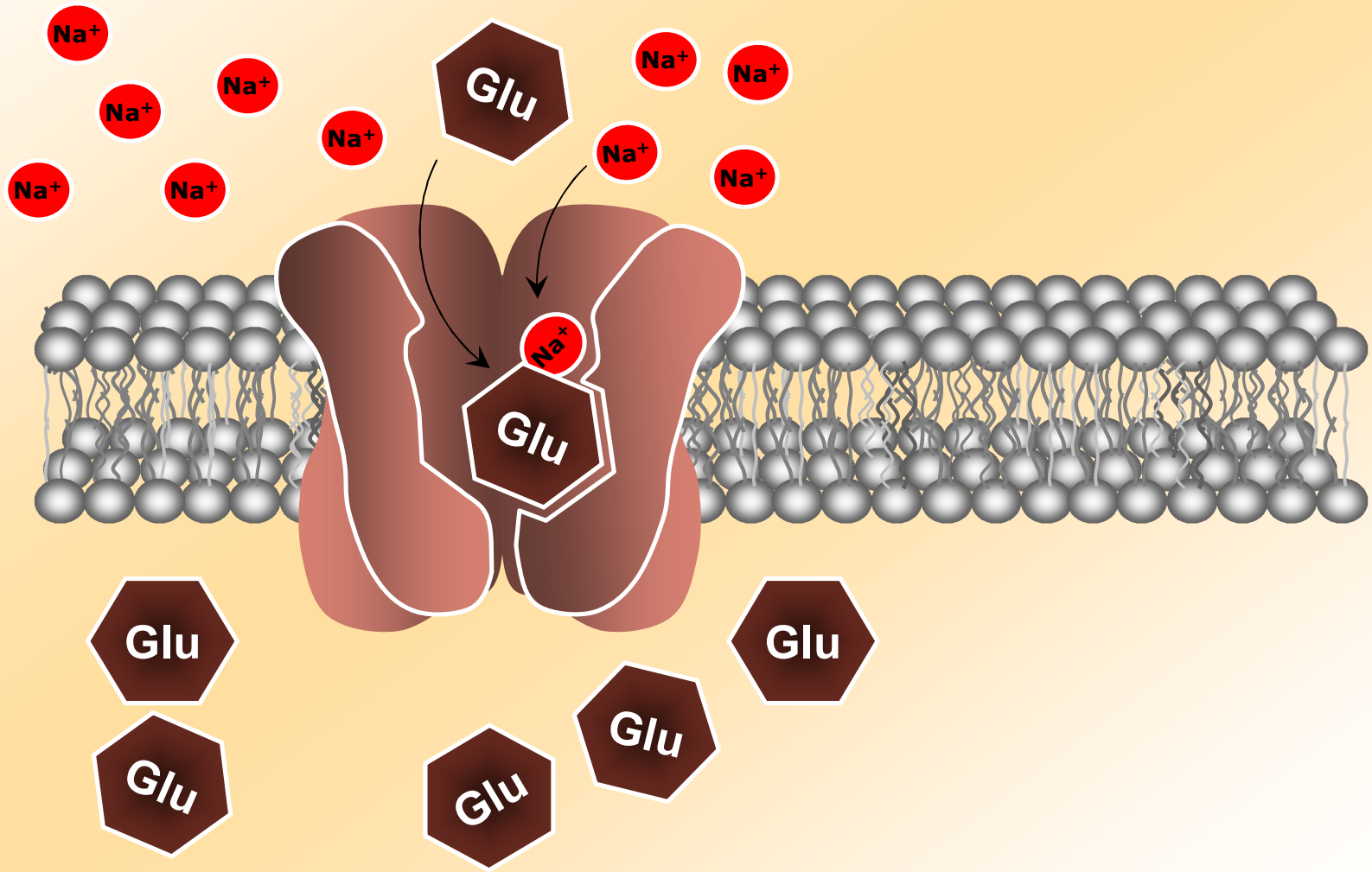
Na^+ glucose transporter (SGLT)

The concentration gradient of Na^+ and K^+ across the cell membrane maintained by Na+K+ pump is used by other transporters



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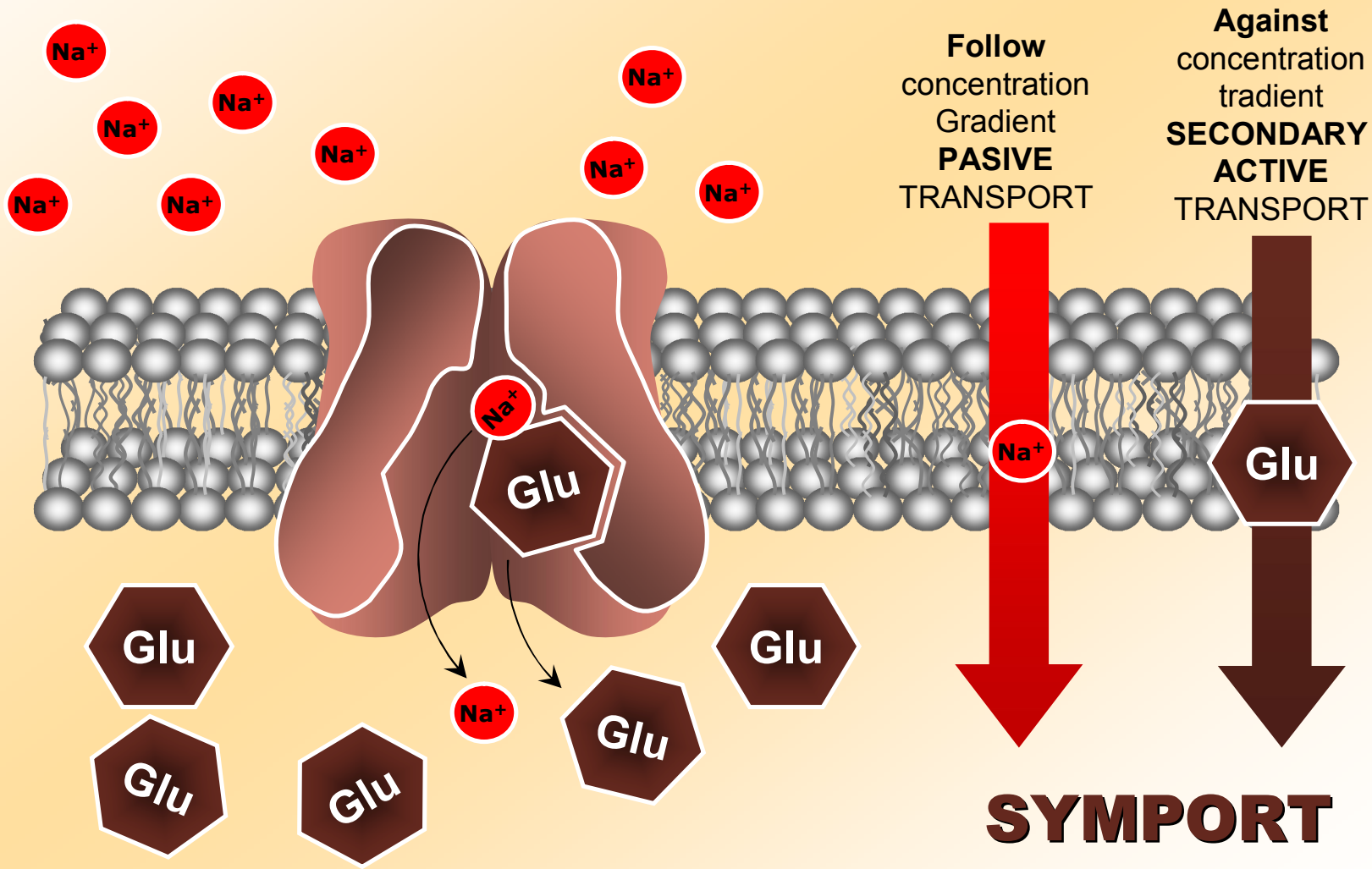

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Glucose transport : overview

PASIVE transport : GLUT

Most tissue of the body:	**GLUT 1**	into the cell
Sceletal muscle **Adipose tissue**	**GLUT 4**	into the cell (insulin dependent)
Liver cells	**GLUT 2**	into the cell (making glycogen) out of the cell (glycogenolysis)
Epitelial cell of intestin		
Outside membrane	**GLUT 5**	fructose into the cell
Inside membrane	**GLUT 2**	glucose out of the cell

SECONDARY ACTIVE transport : SGLT

Epitelial cell of intestin		
Outside membrane	Na+-glu transporter	active from outside ito the cell

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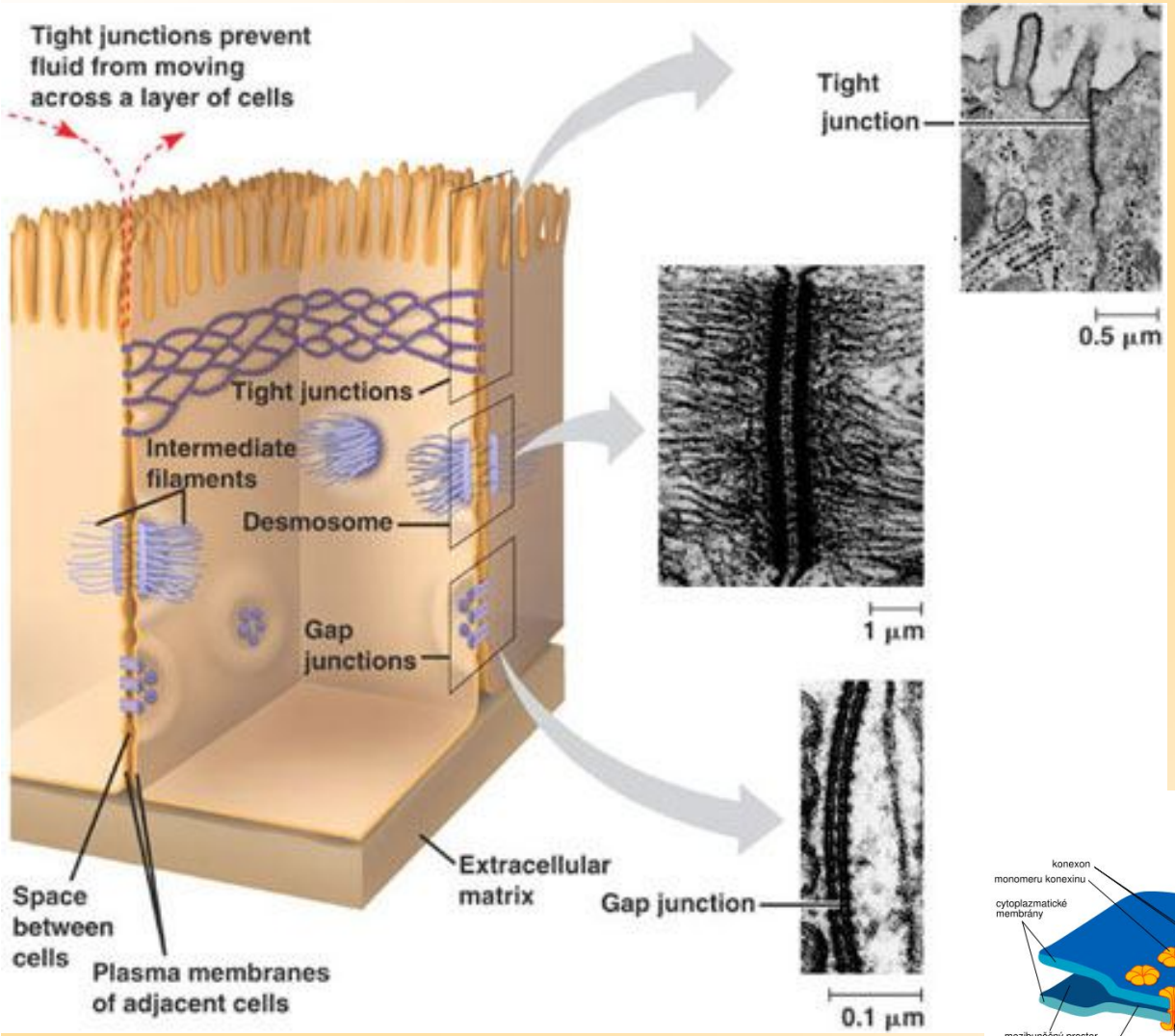
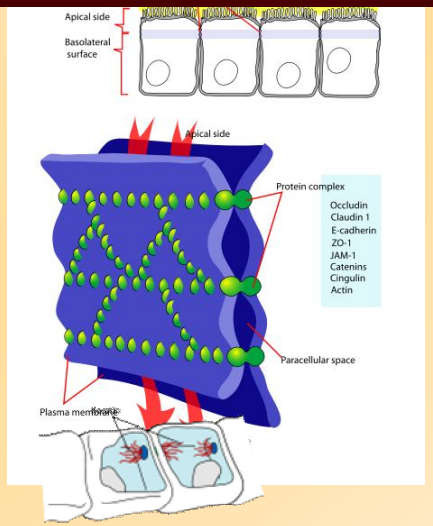
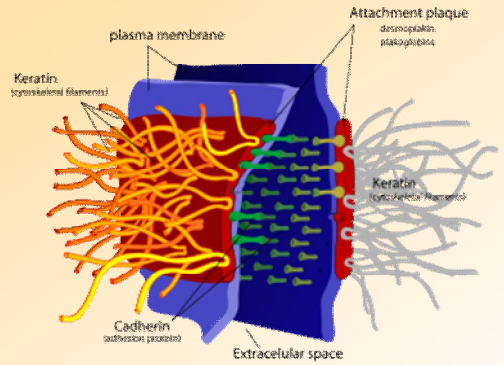
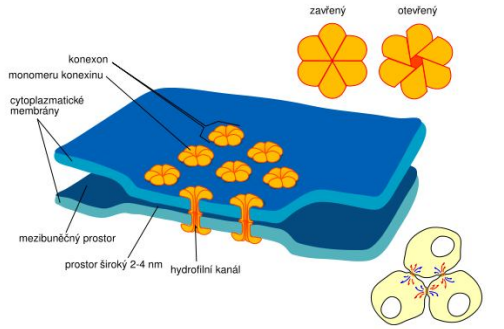
Protein clasification

Cell junction









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Protein clasification

Through receptors cells recognize and respond to molecules or changes in the external environment)

Receptors

Cell receptors activate after ligand binding.

Activated receptor triggers intracellular response.

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Target cell RECEPTORs

Cell receptores LOCATION

- At the **cell membrane** (receptor is an intrinsic membrane protein): bind lipophobic or lipophilic molecules on the cell surface
- In **cytosole**: bind lipophilic signal molecule which diffuse cell membrane
- In **nucleus**: bind lipophilic signal molecule which diffuse cell and nuclear membrane

Four categories of membrane receptors

1. **Ligand-gated channels**: ligand binding opens or closes channel
2. **G-protein coupled receptor**: ligand binding activates G-protein to open ion channel or start intracellular enzyme activity
3. **Enzyme linked receptor:** ligand binding activates intracellular catalytic sites of the receptor
4. **Integrin receptor:** ligand binding alters the cytockeleton

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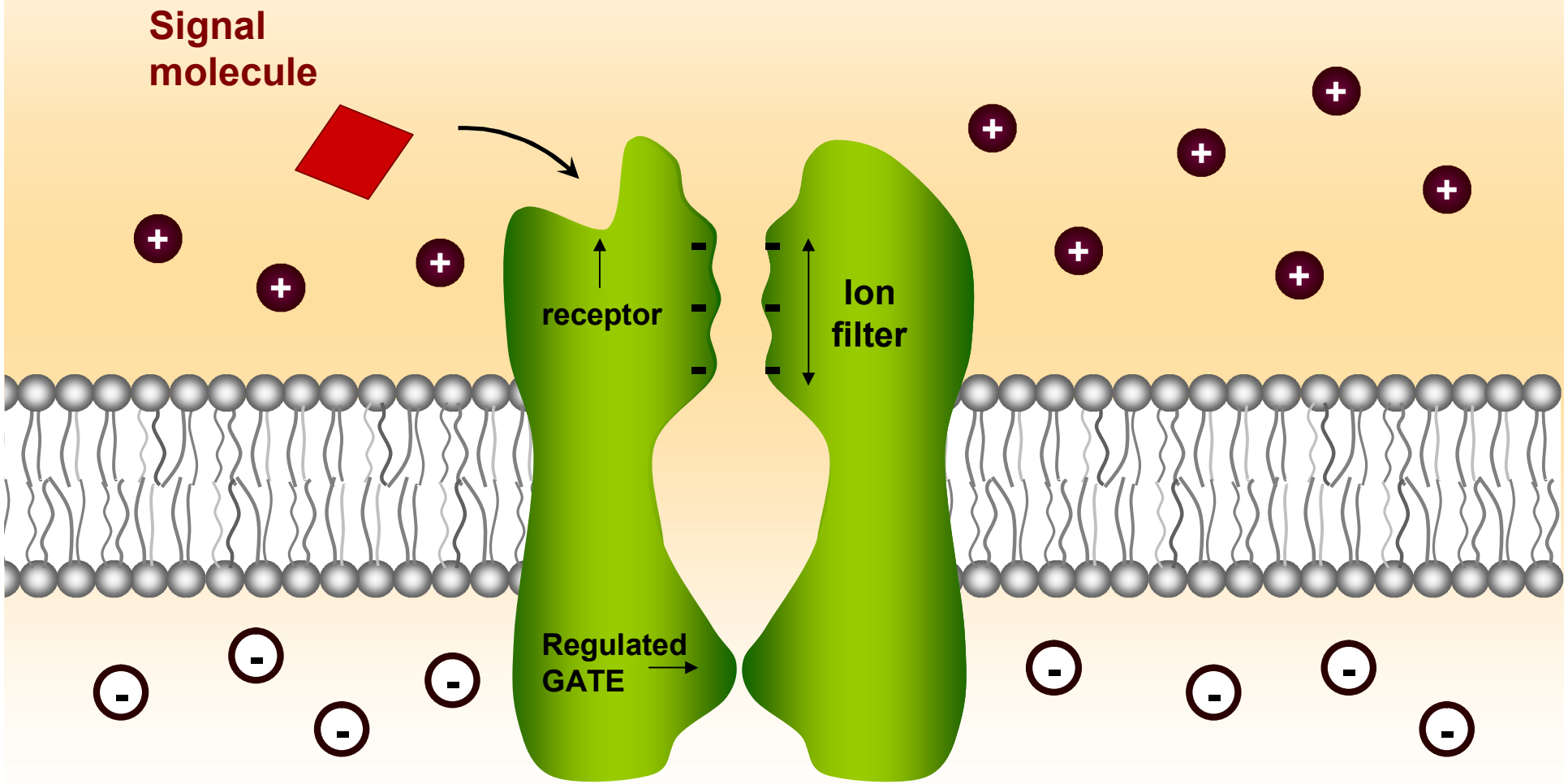
1. Ligand gated channel

http://www.blackwellpublishing.com/matthews/neurotrans.html

EXAMPLE: Neuromuscular junction

Acetylcholine directly opens sodium channels in muscle fiber.



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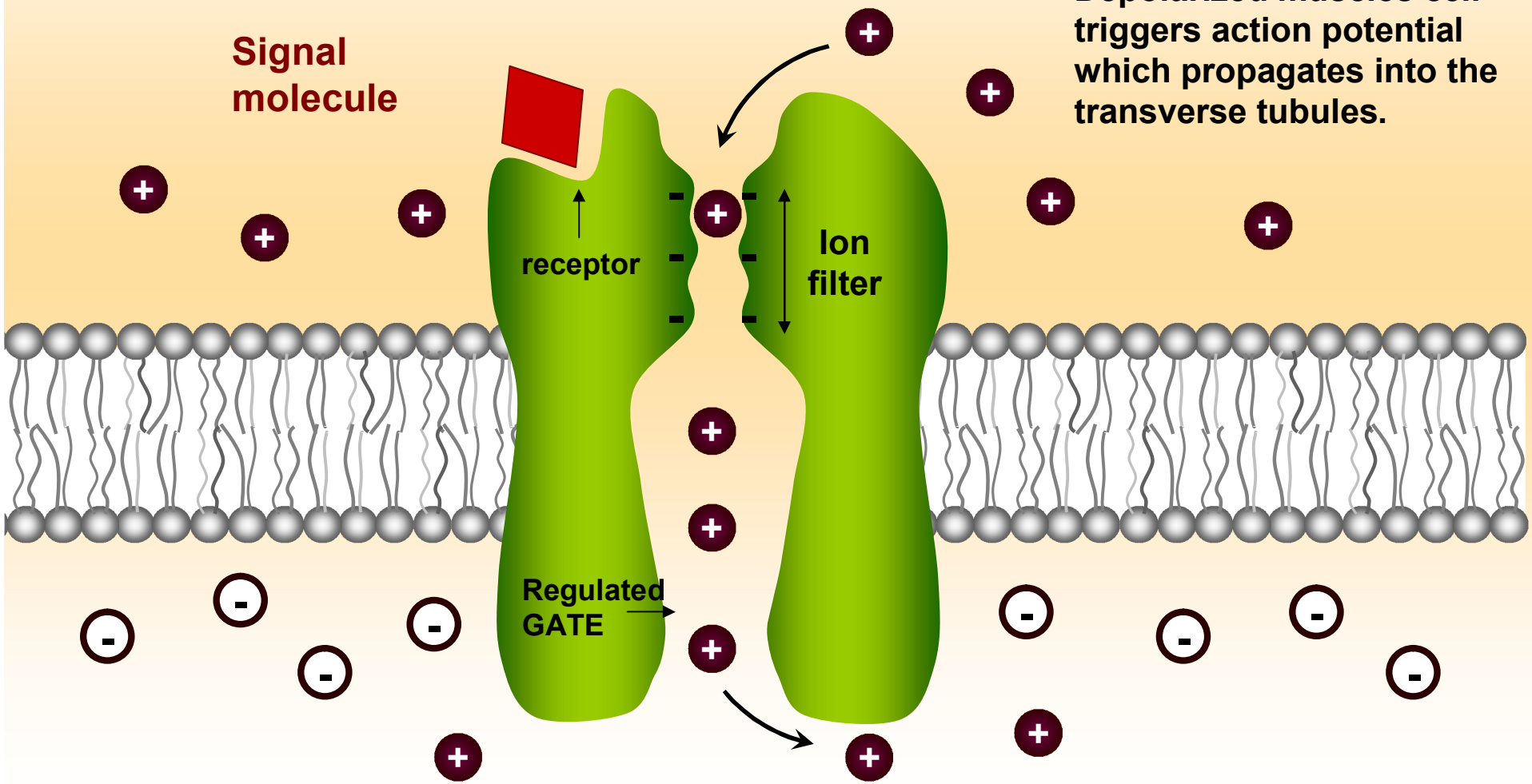

Depolarized muscles cell triggers action potential which propagates into the transverse tubules.

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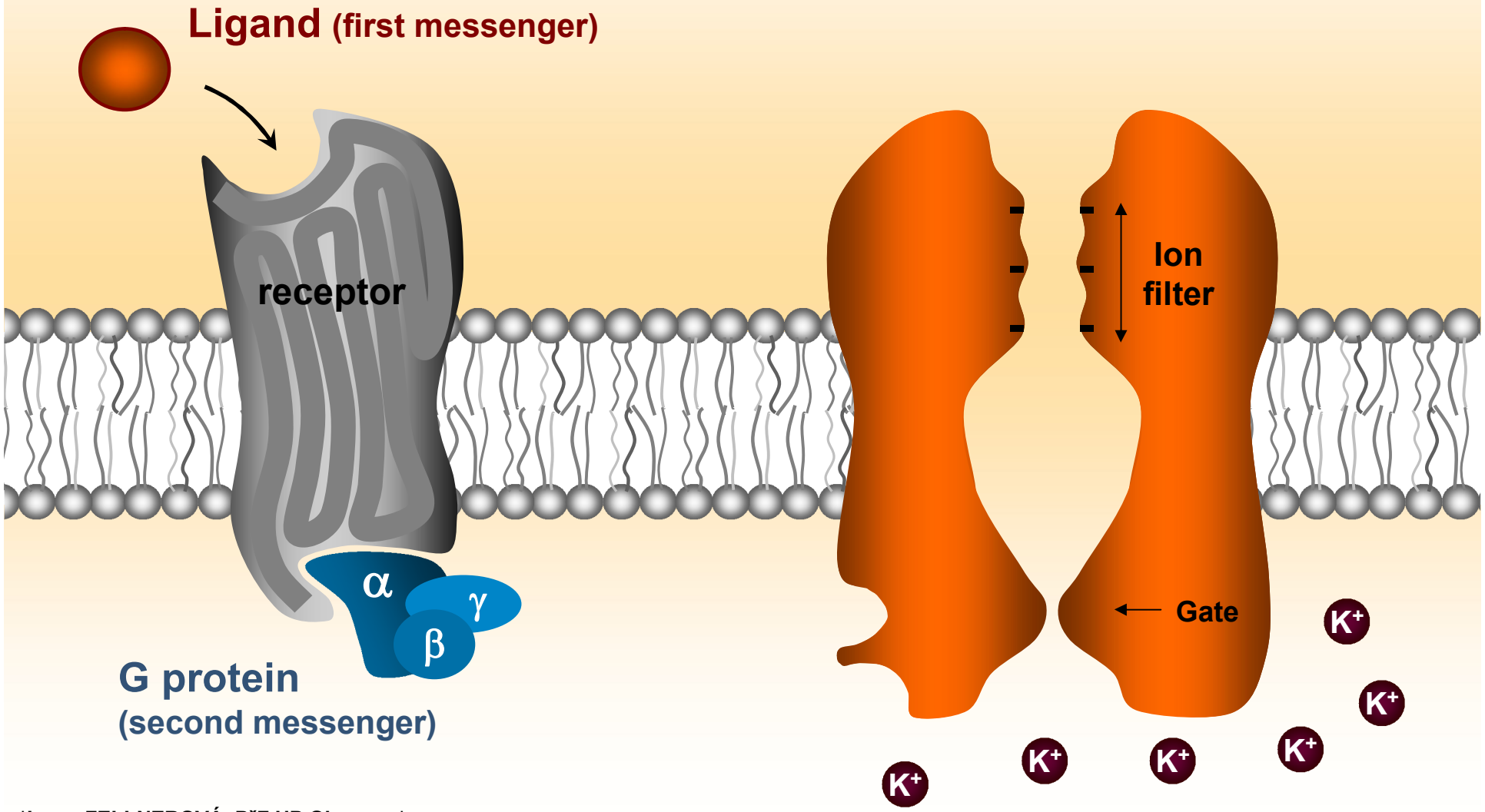
2a. G-protein coupled receptor

EXAMPLE: Muscarine Ach receptor

Acetylcholine (released from parasympathetic nerve terminal) indirectly opens potassium channels in cardiac muscle cells



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2a. G-protein coupled receptor

EXAMPLE: Muscarine Ach receptor

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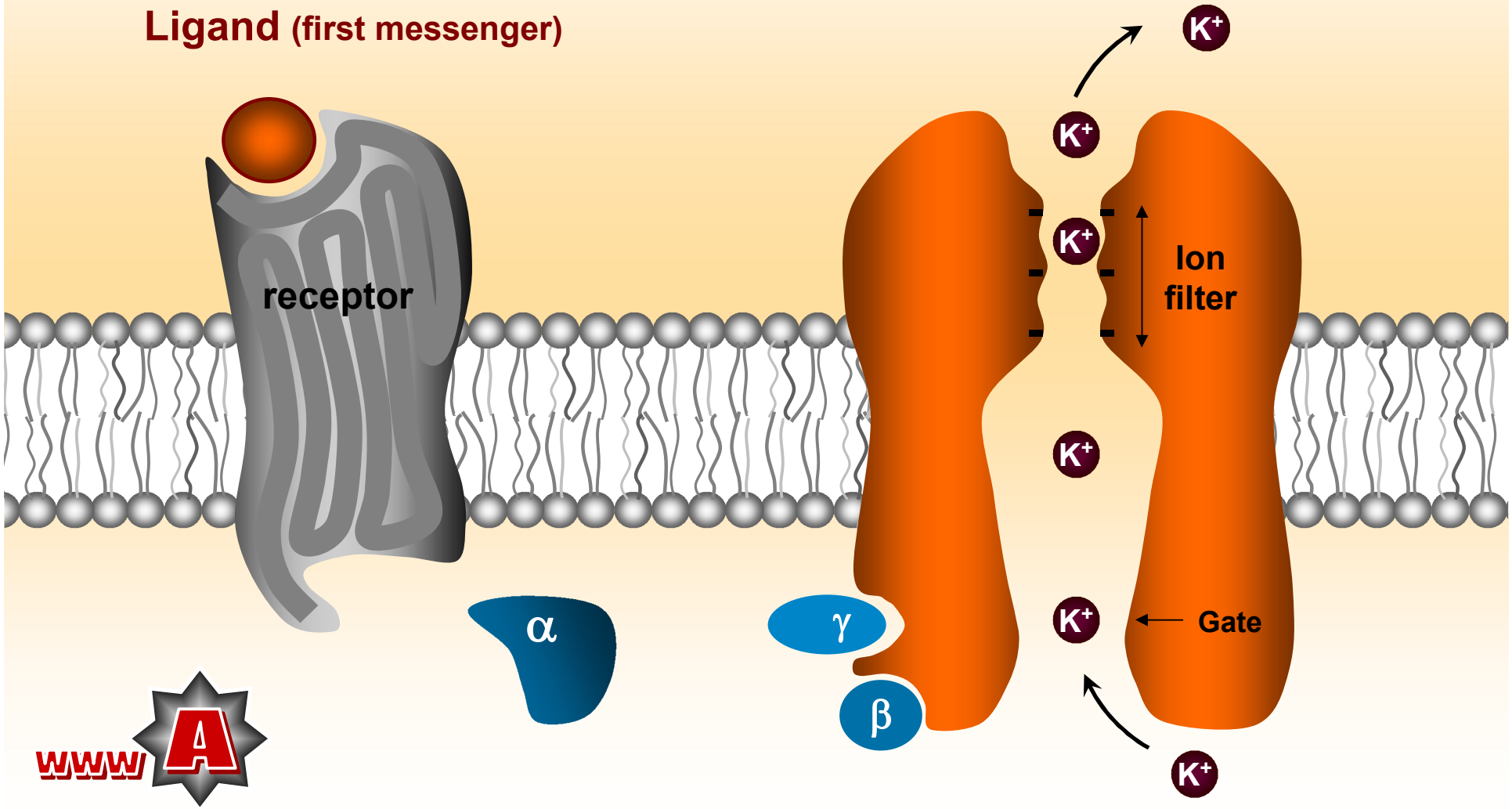

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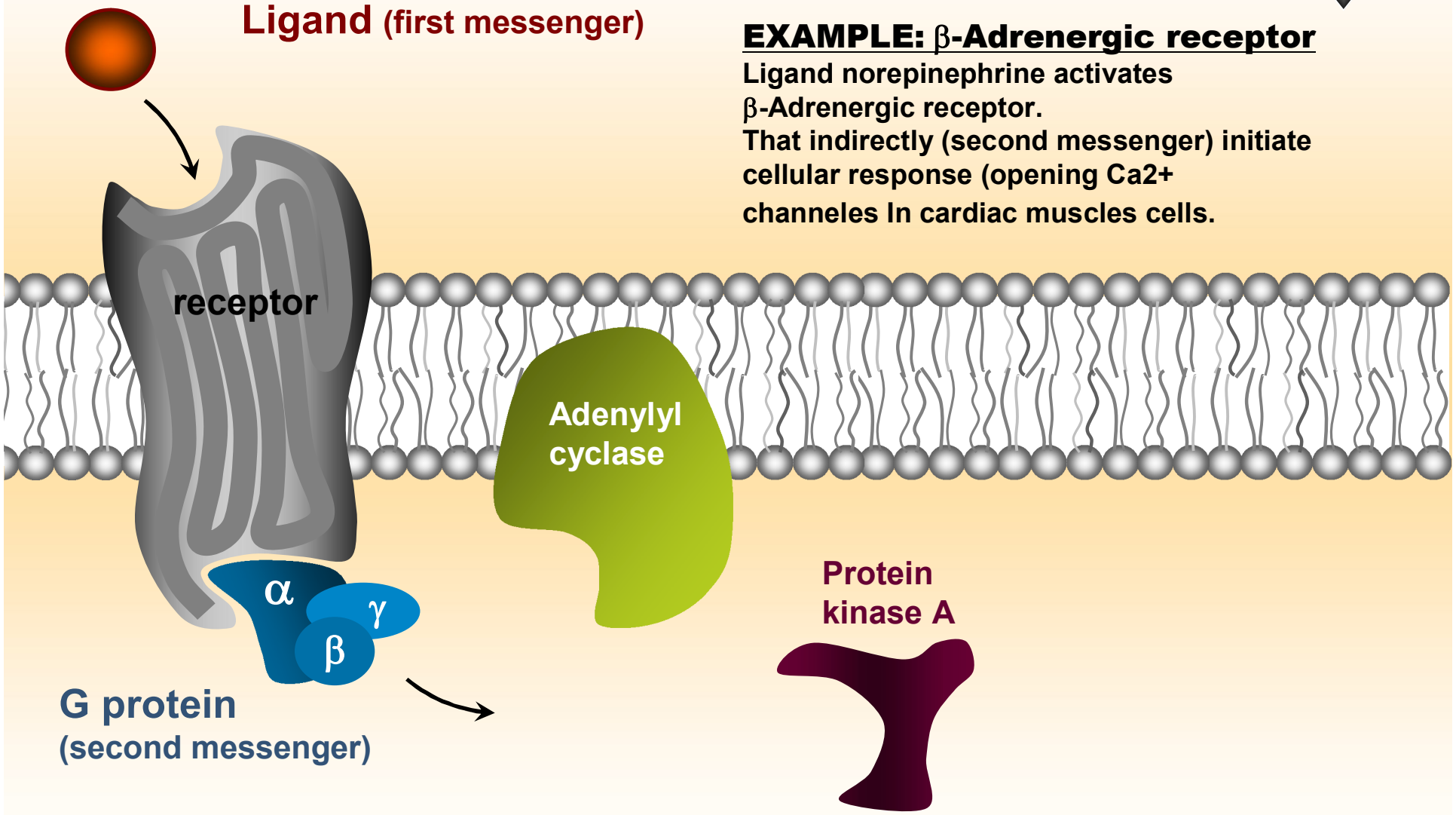
2b. G-protein coupled receptor



EXAMPLE: β-Adrenergic receptor

Ligand norepinephrine activates β-Adrenergic receptor.
That indirectly (second messenger) initiate cellular response (opening Ca2+ channeles In cardiac muscles cells.

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2b. G-protein coupled receptor

Ligand (first messenger)

EXAMPLE: β-Adrenergic receptor

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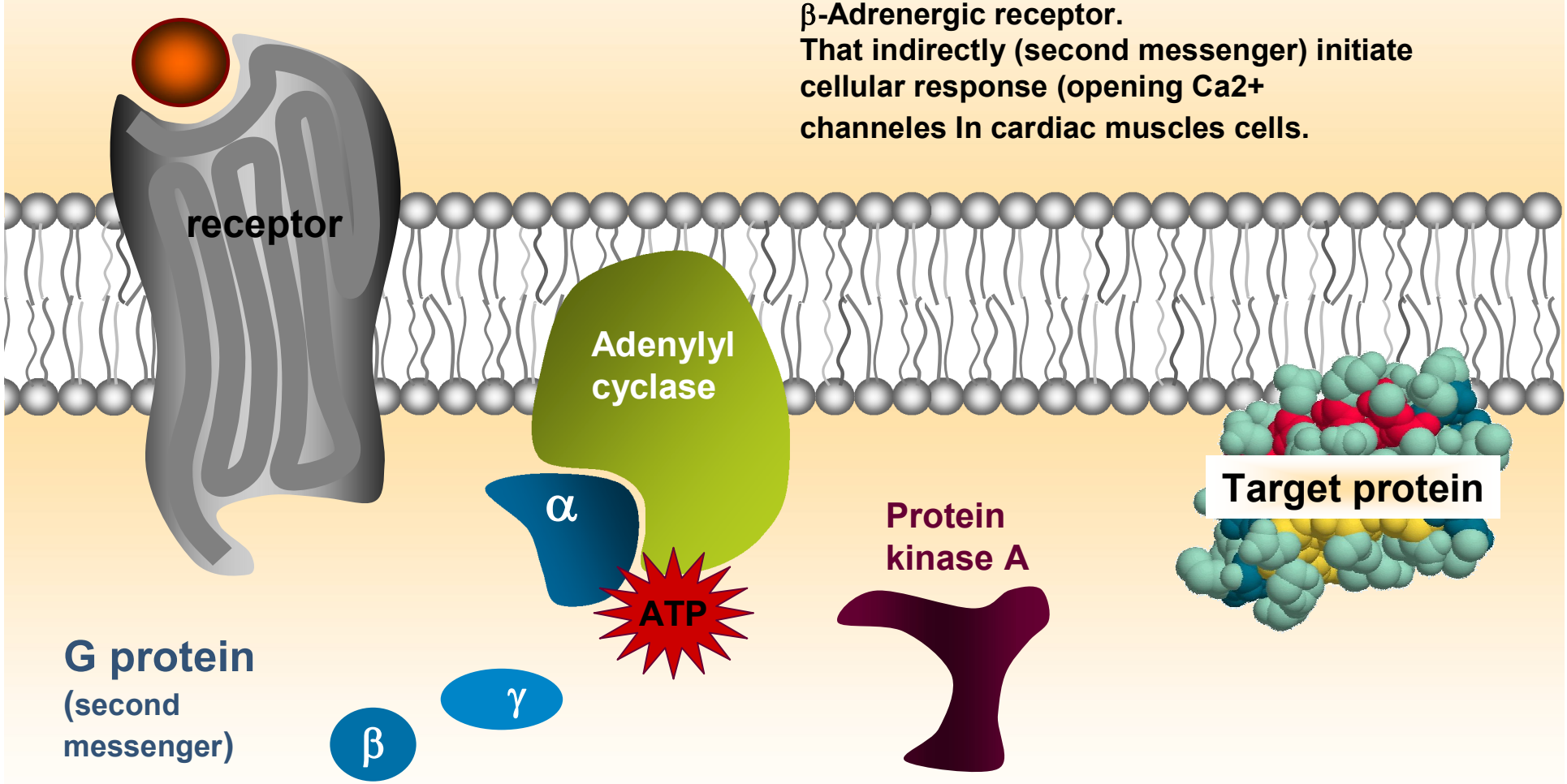

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2b. G-protein coupled receptor

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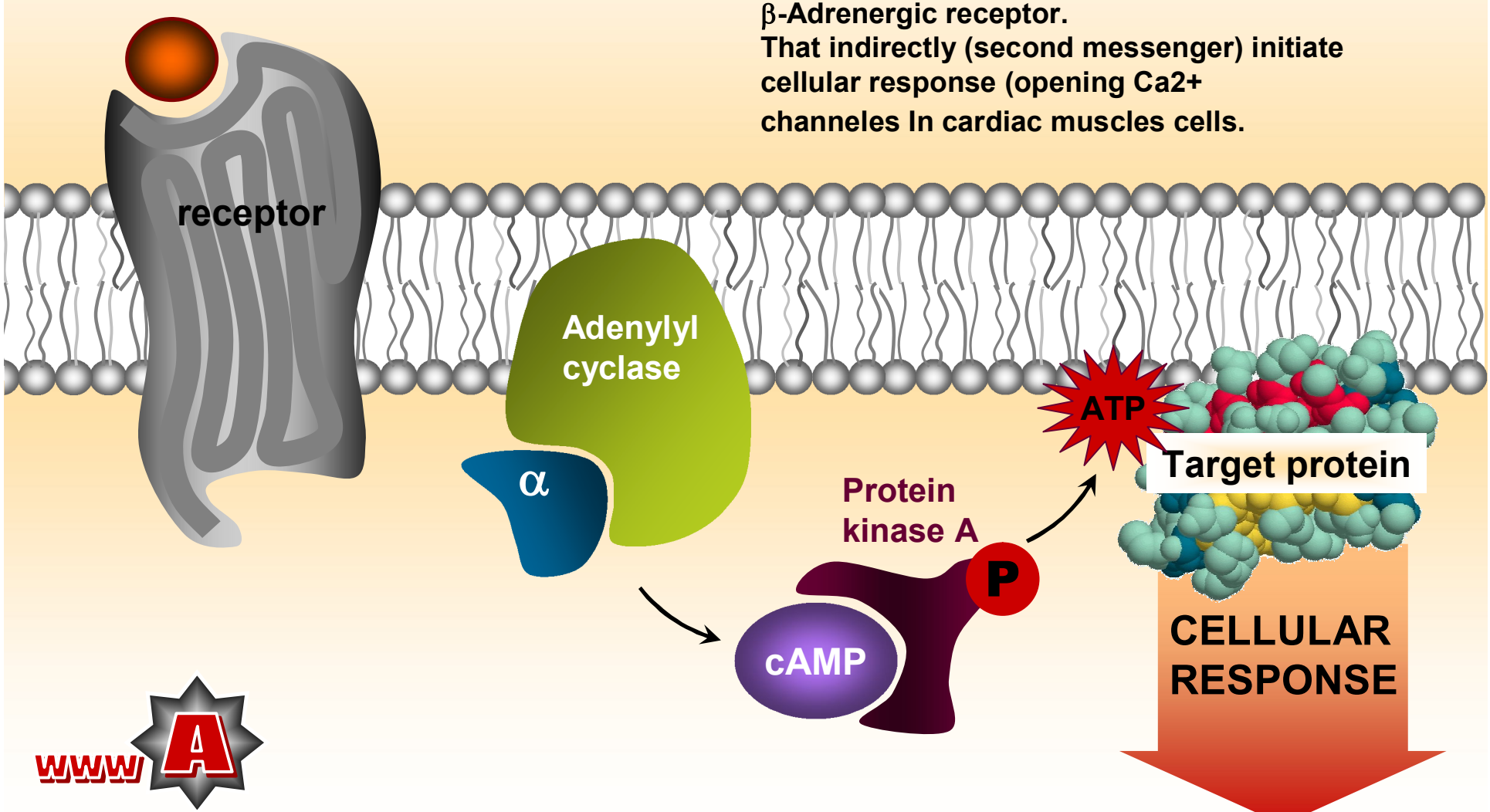

http://www.blackwellpublishing.com/matthews/neurotrans.html

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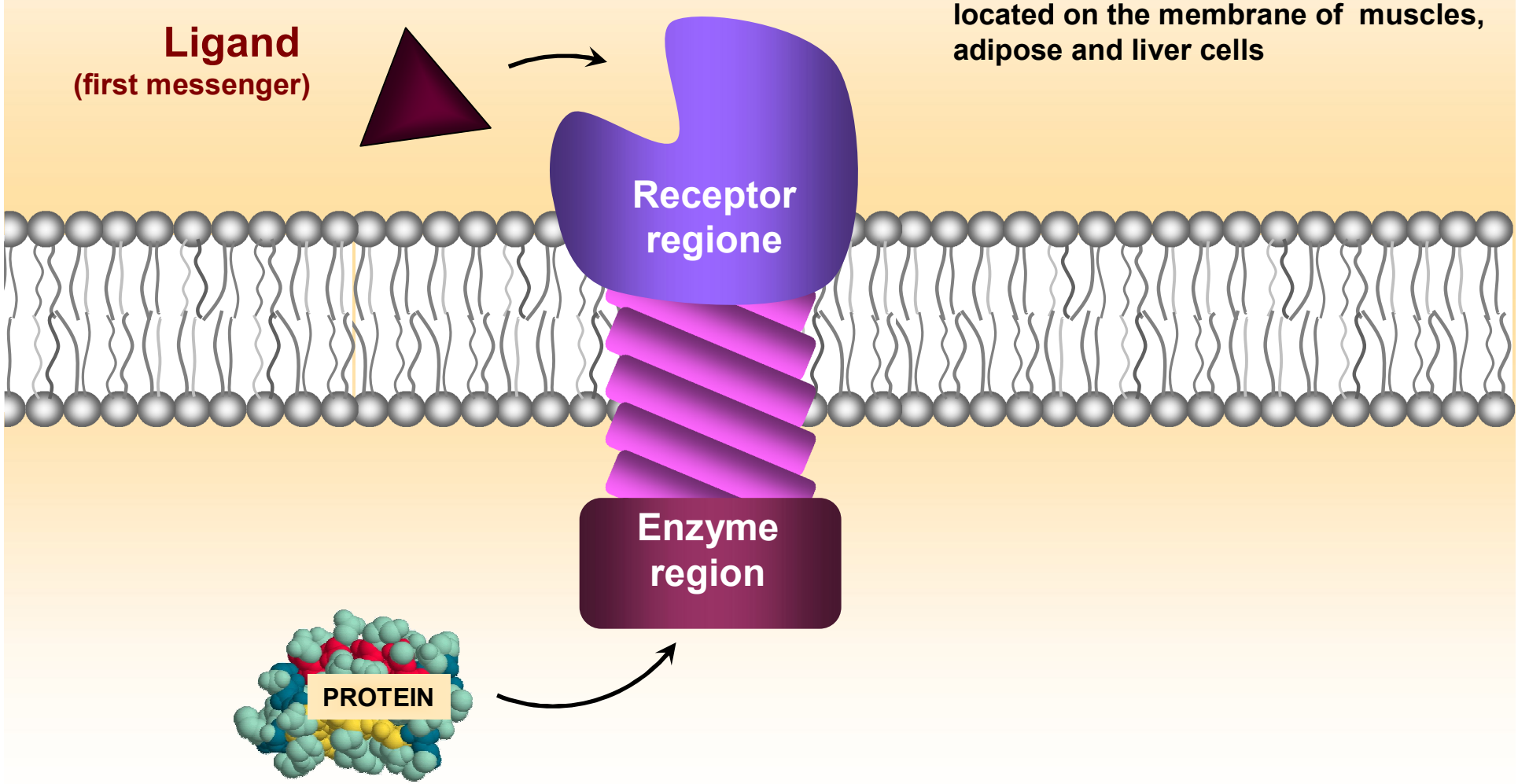
3. Enzyme-linked receptor

Receptor-Enzymes have two region:
Receptor region on the extracellular surface and Enzyme region on cytoplasmic side.

EXAMPLE: Insuline receptor
located on the membrane of muscles, adipose and liver cells



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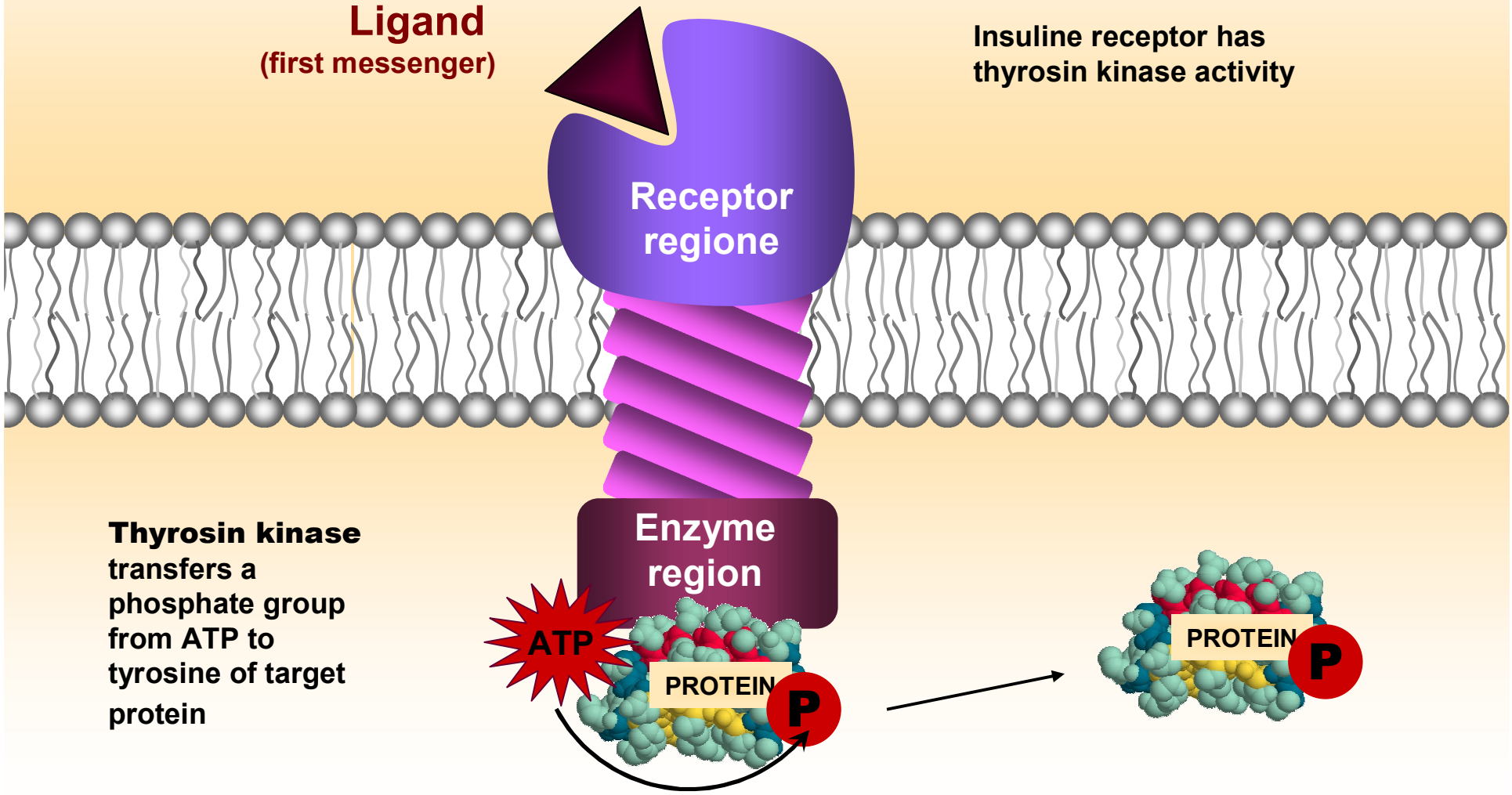
3. Enzyme-linked receptor

Ligand binding to receptor activates the enzyme (catalytic) region which has either thyrosin kinase or guanylyn cyclase activity



Thyrosin kinase transfers a phosphate group from ATP to tyrosine of target protein

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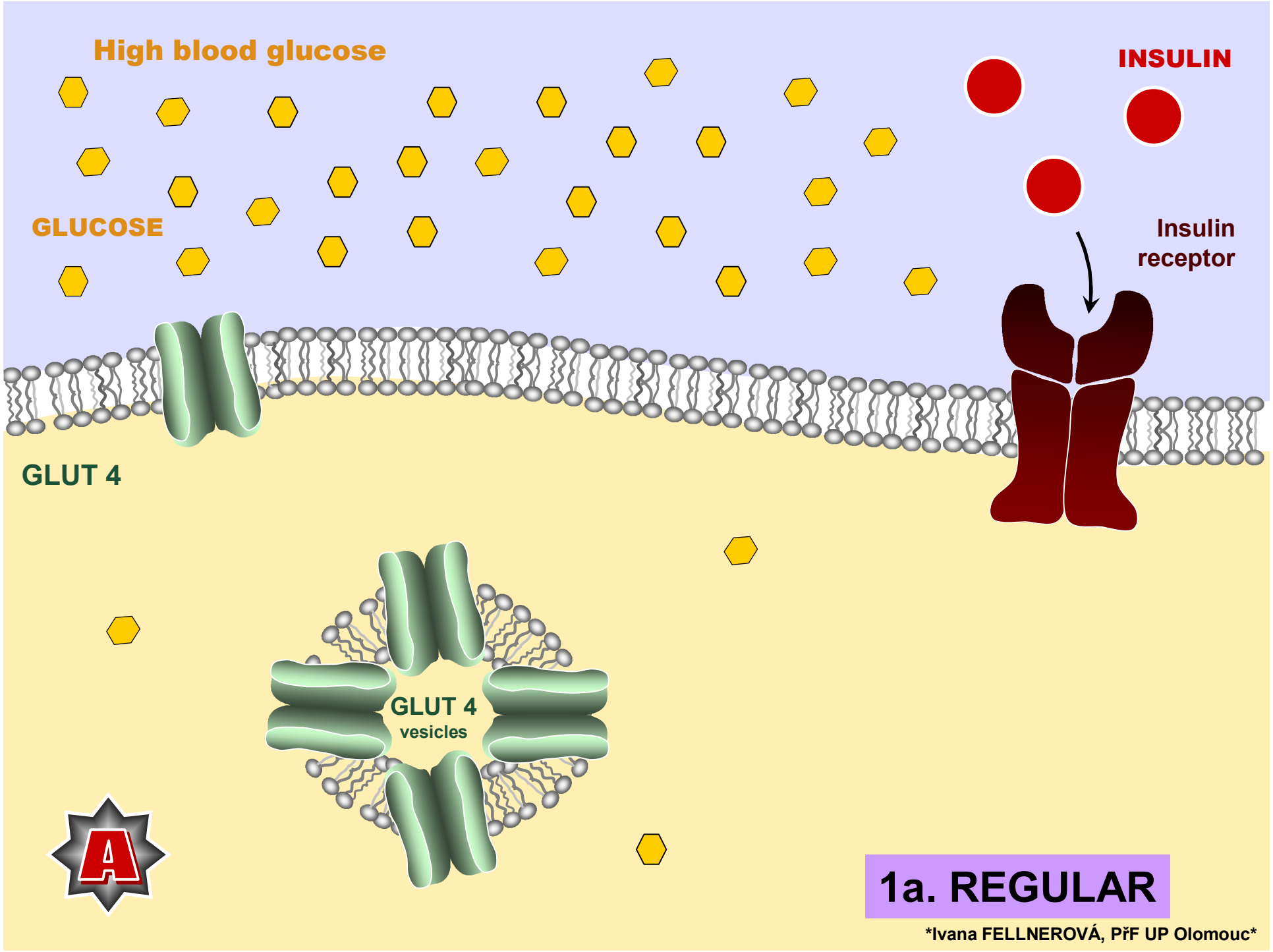
High blood glucose
INSULIN
GLUCOSE
Insulin receptor
GLUT 4
GLUT 4
vesicles
A
1a. REGULAR
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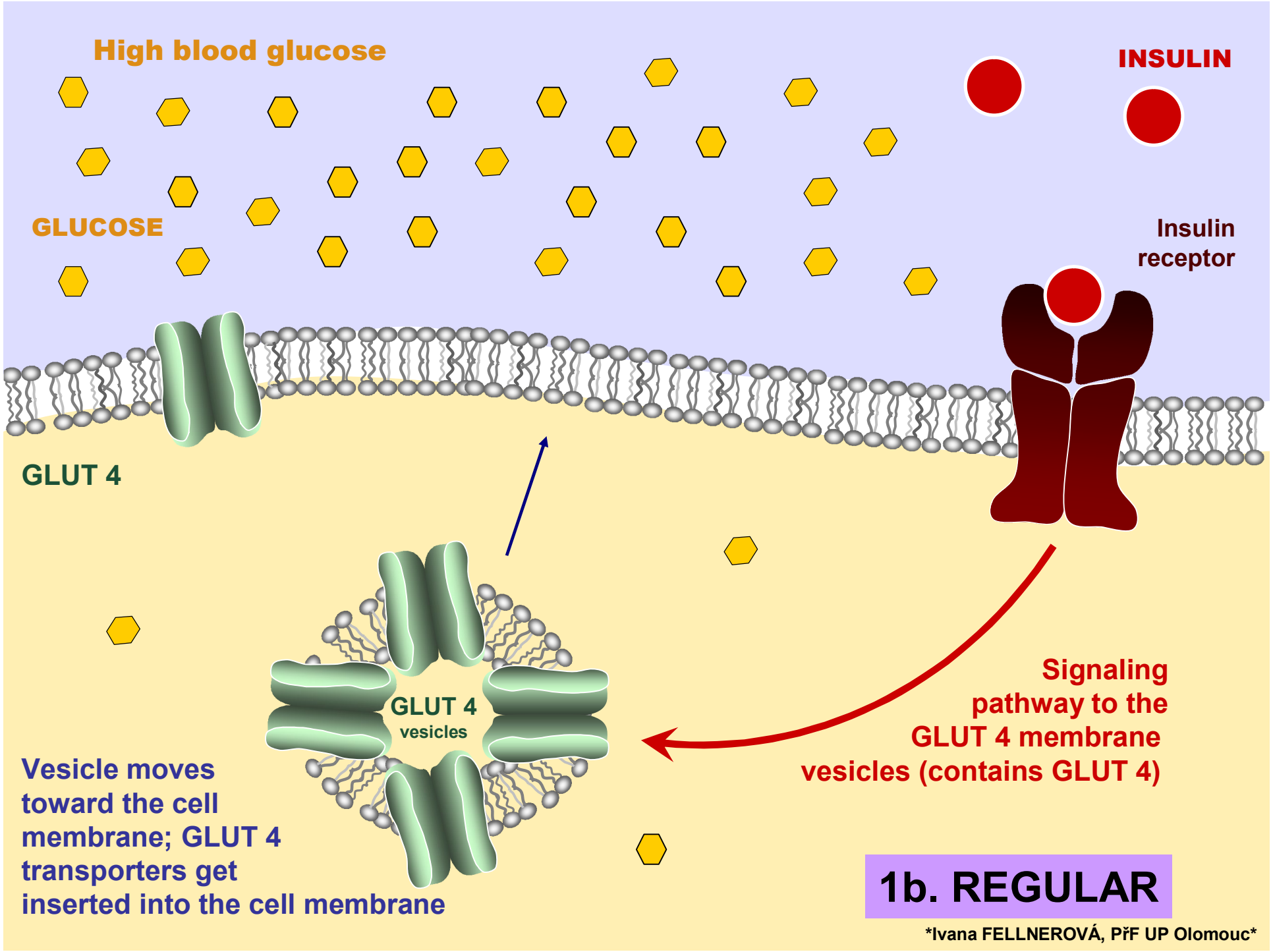

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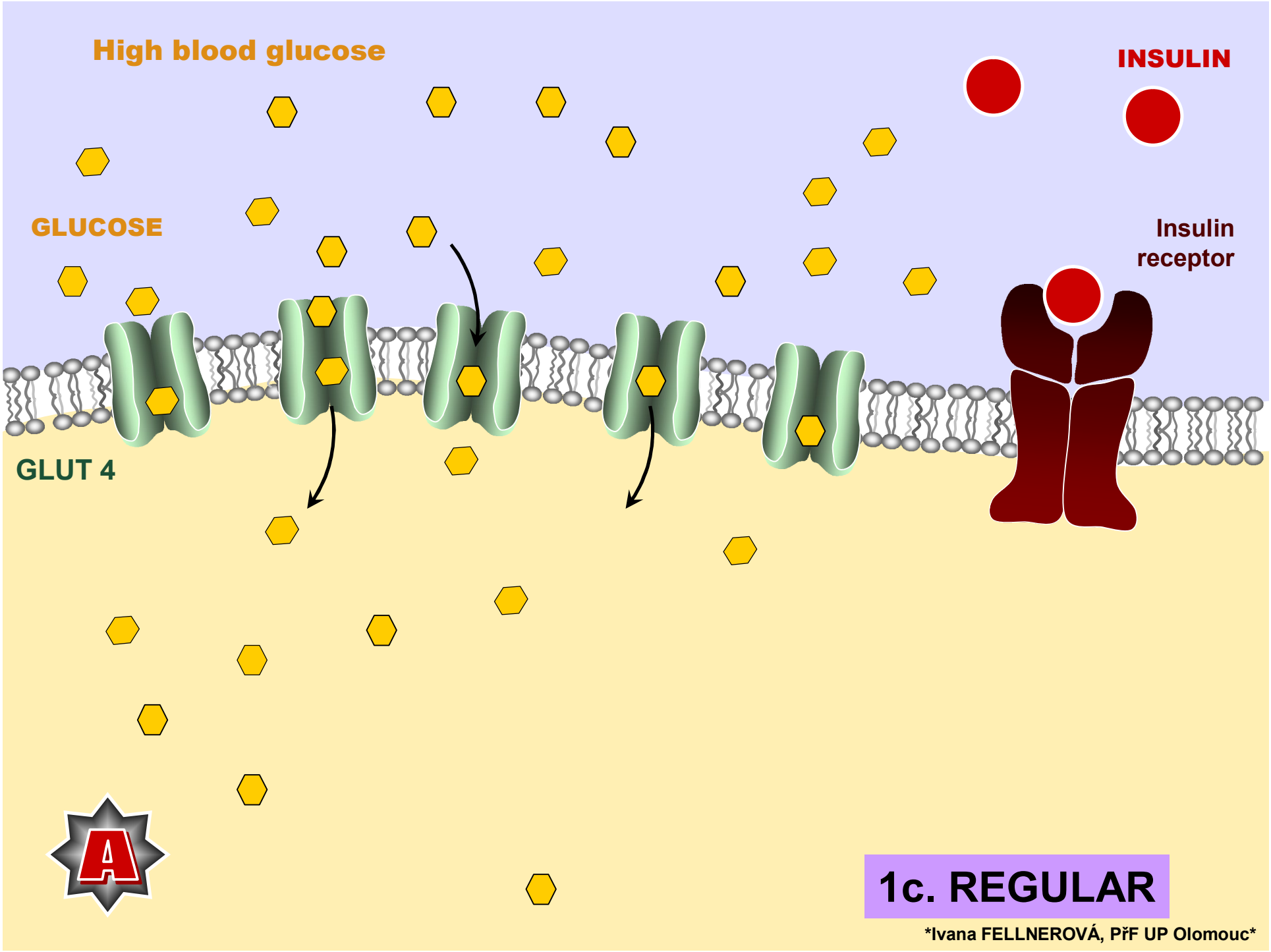
High blood glucose
INSULIN
GLUCOSE
Insulin receptor
GLUT 4
A
1c. REGULAR
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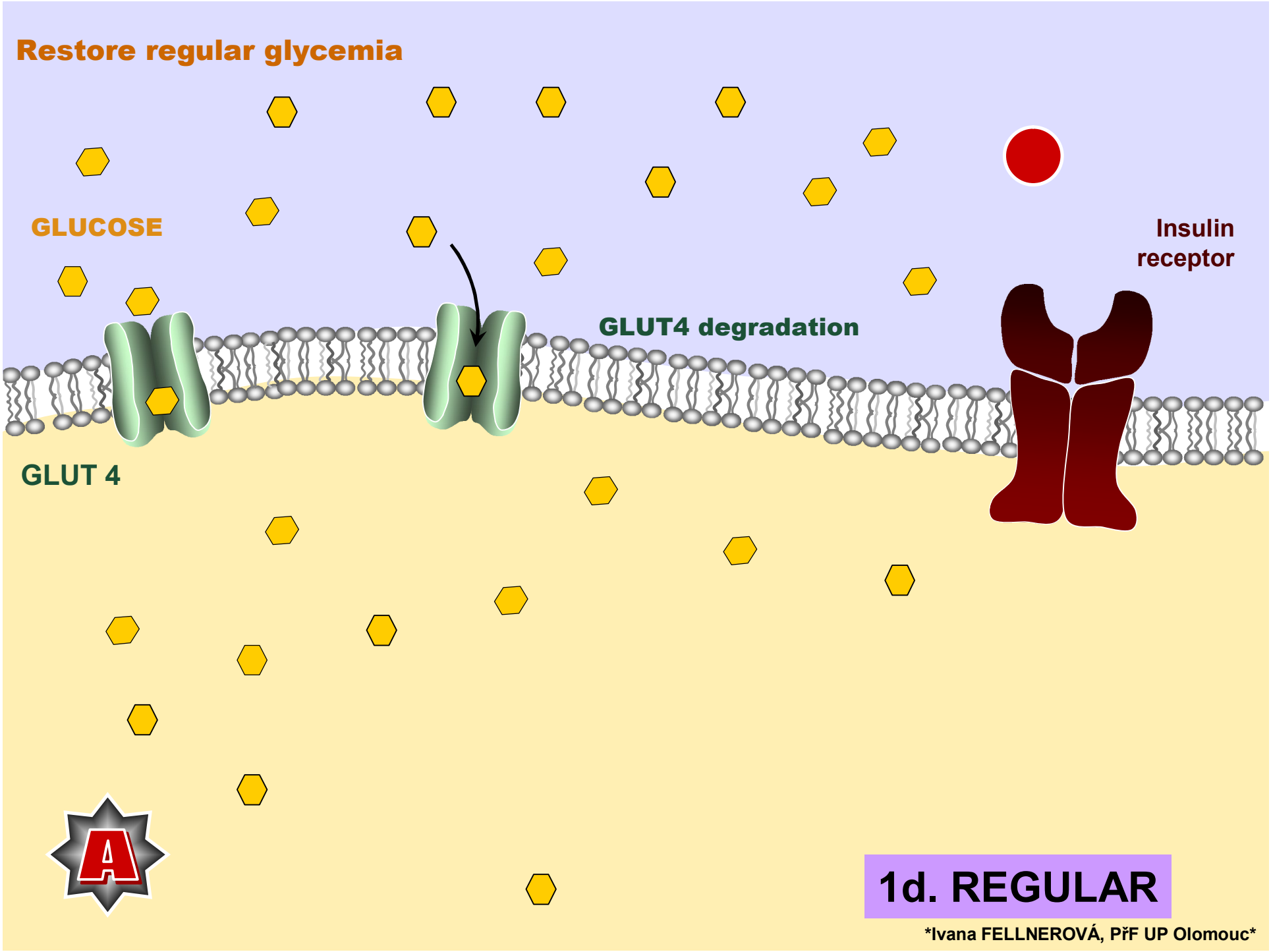
Restore regular glycemia
GLUCOSE
Insulin receptor
GLUT4 degradation
GLUT 4
A
1d. REGULAR
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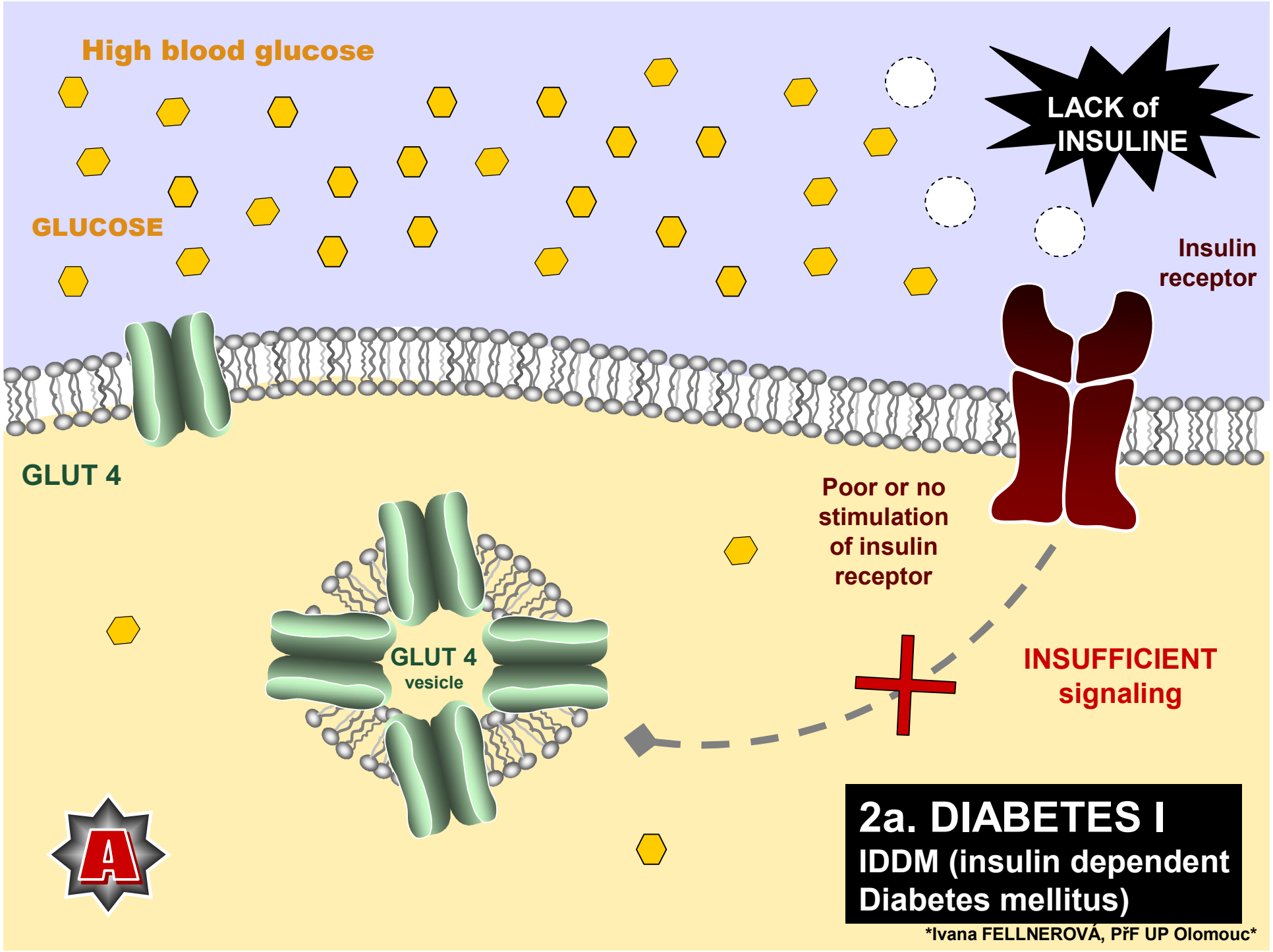

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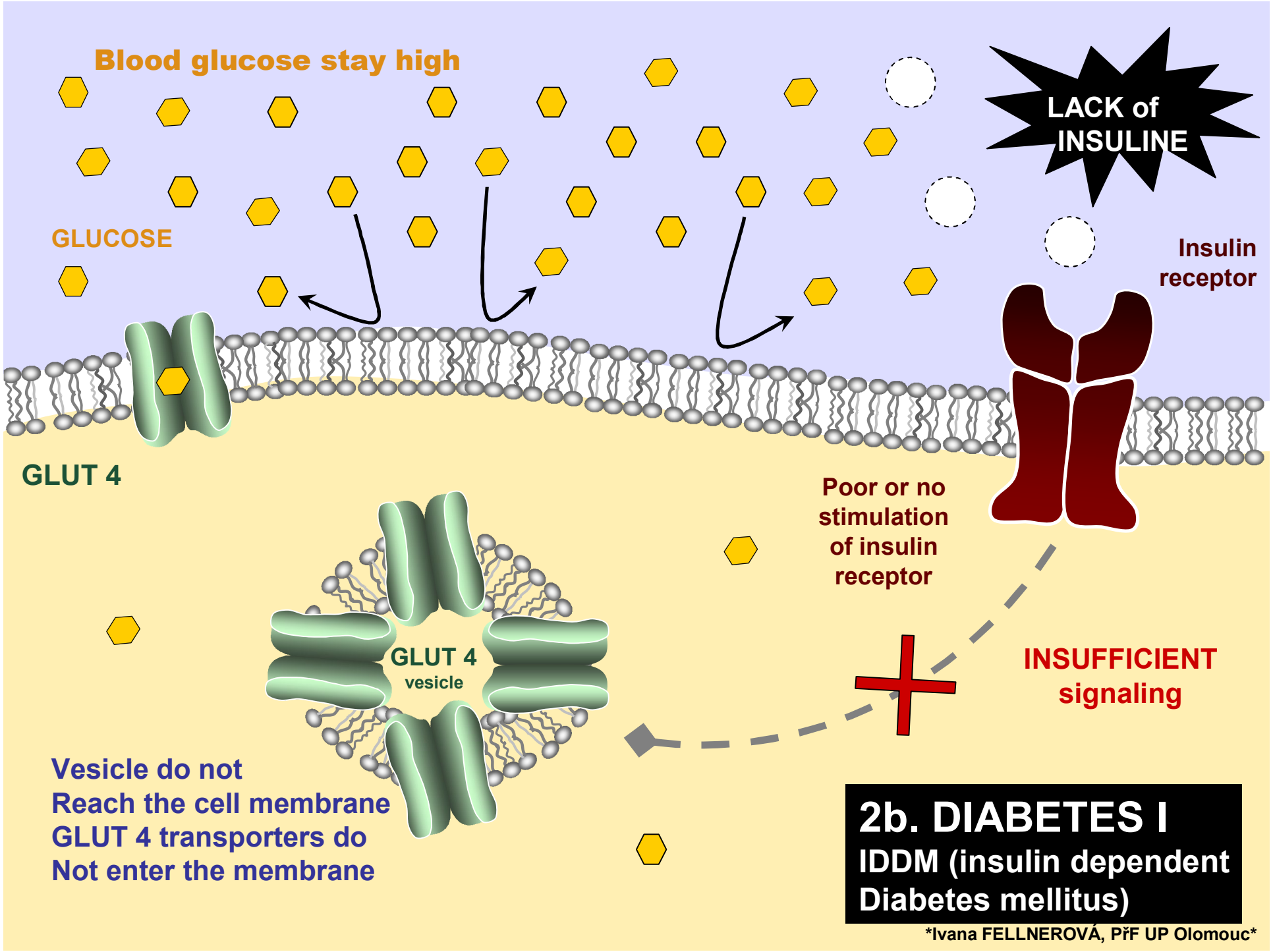
Blood glucose stay high
LACK of INSULINE
GLUCOSE
Insulin receptor
GLUT 4
Poor or no stimulation of insulin receptor
GLUT 4
vesicle
INSUFFICIENT signaling
Vesicle do not
Reach the cell membrane
GLUT 4 transporters do
Not enter the membrane
2b. DIABETES I
IDDM (insulin dependent Diabetes mellitus)
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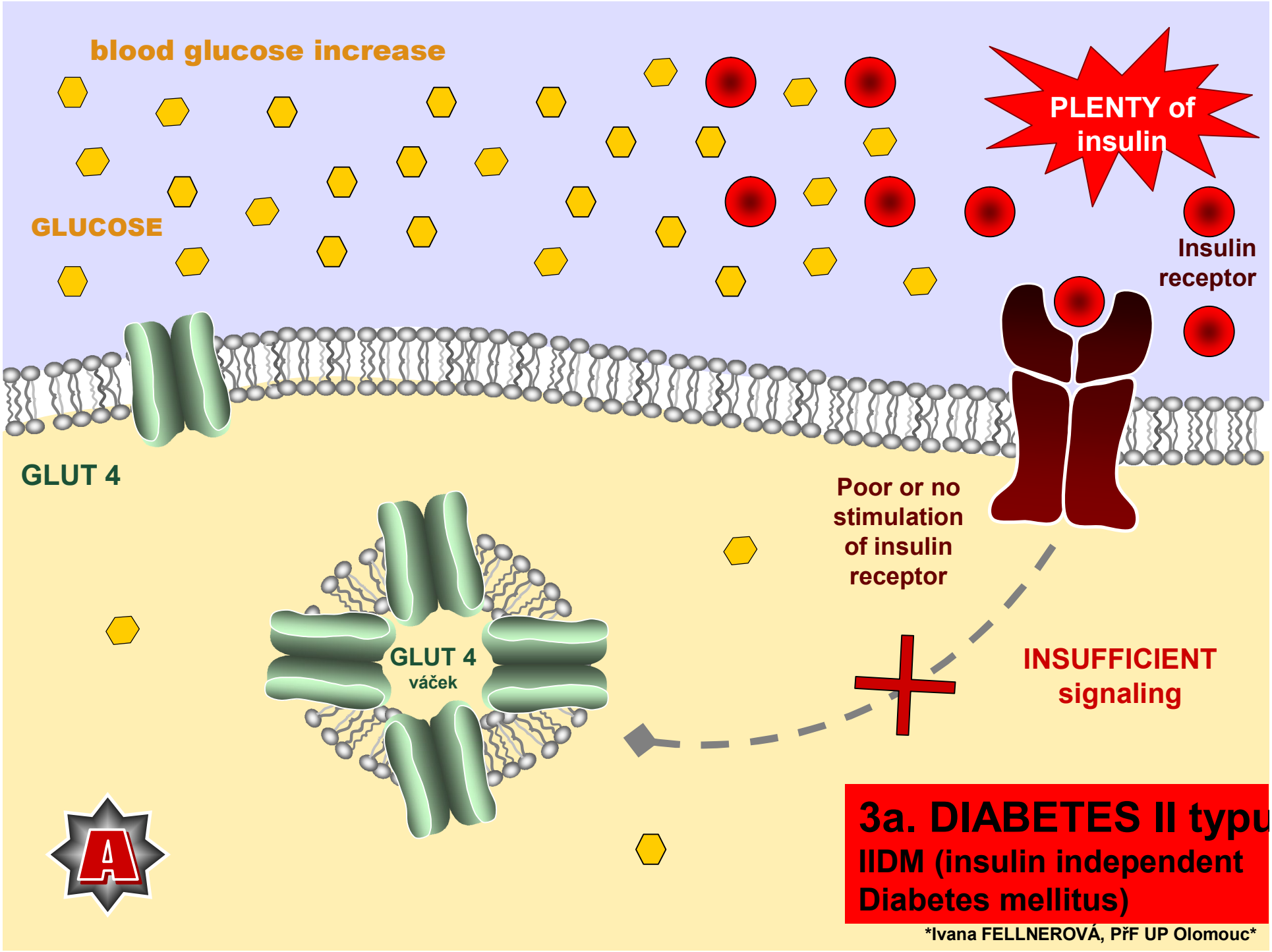
blood glucose increase
PLENTY of insulin
GLUCOSE
Insulin receptor
GLUT 4
Poor or no stimulation of insulin receptor
GLUT 4
váček
INSUFFICIENT signaling
A
3a. DIABETES II typu
IIDM (insulin independent Diabetes mellitus)
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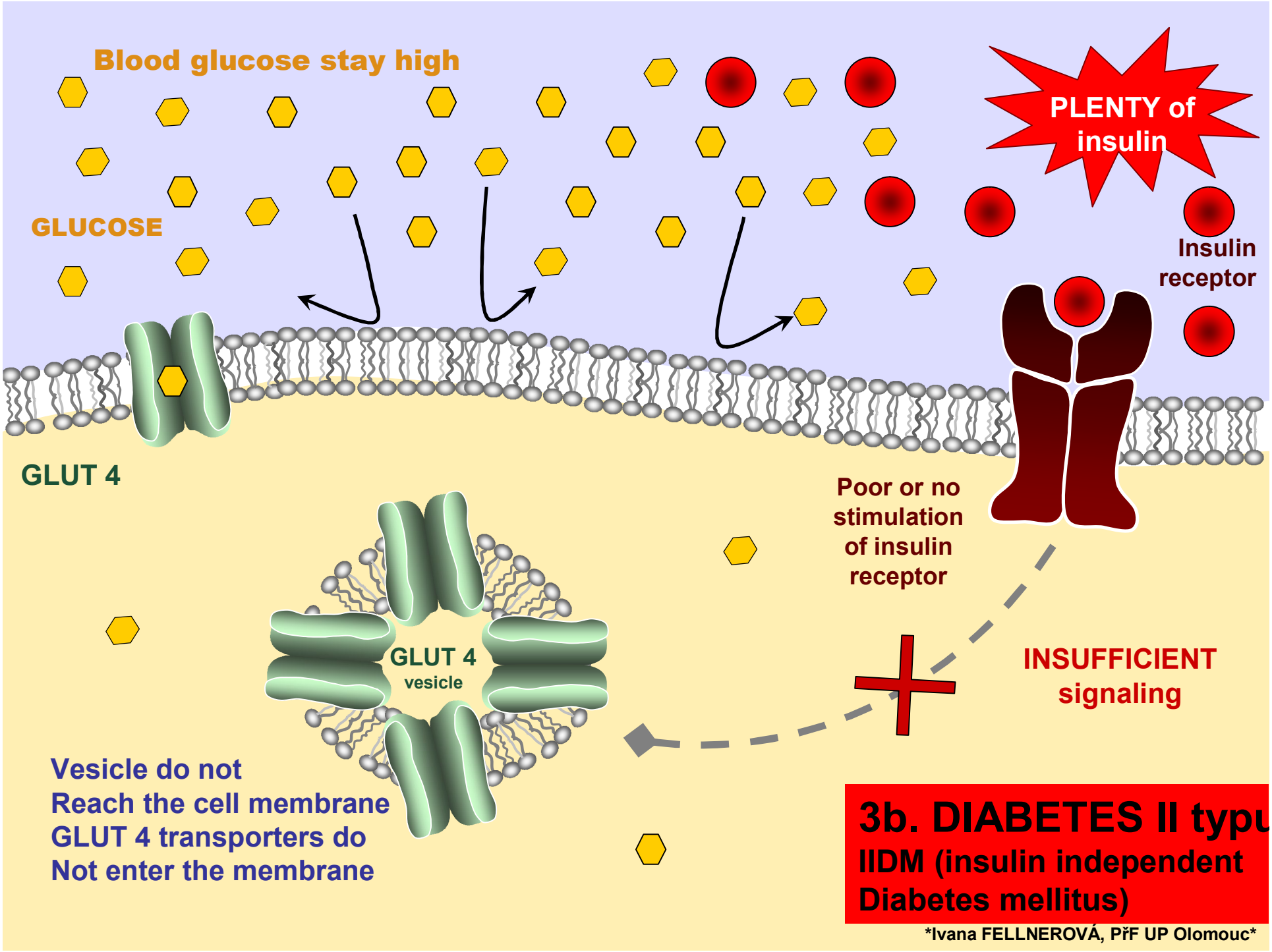
Blood glucose stay high
PLENTY of insulin
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GLUT 4
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GLUT 4
vesicle
INSUFFICIENT signaling
Vesicle do not
Reach the cell membrane
GLUT 4 transporters do
Not enter the membrane
3b. DIABETES II typu
IIDM (insulin independent
Diabetes mellitus)
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Antigens of red blood cells

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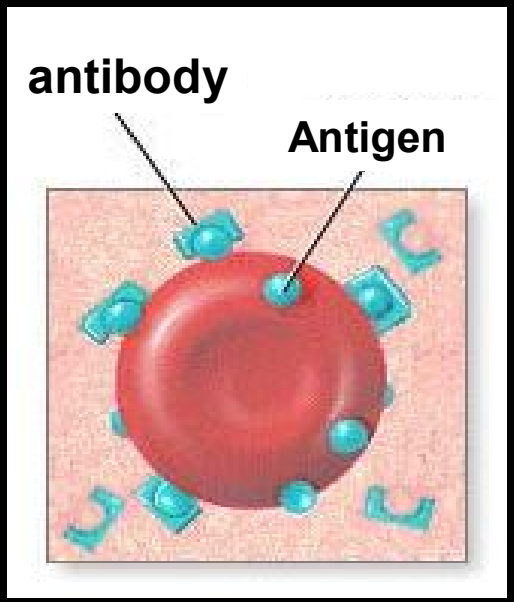
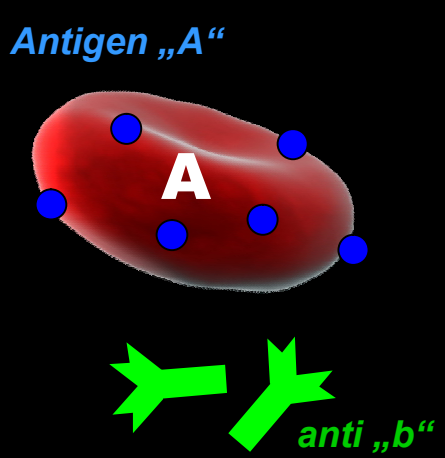
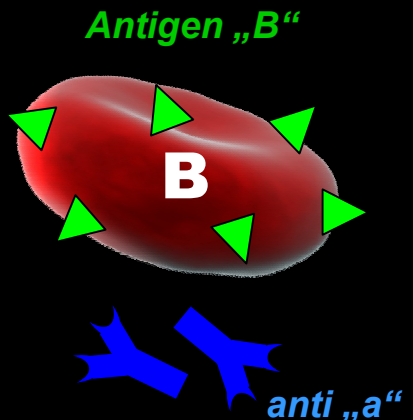
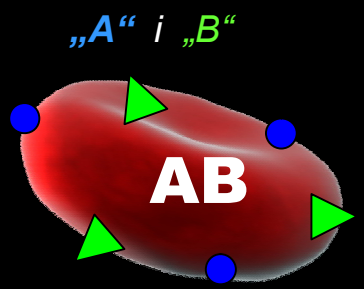
A and B antigens of erytrocytes are glycoproteins located on the red cells membrane









Membrane receptore of lymphocyte

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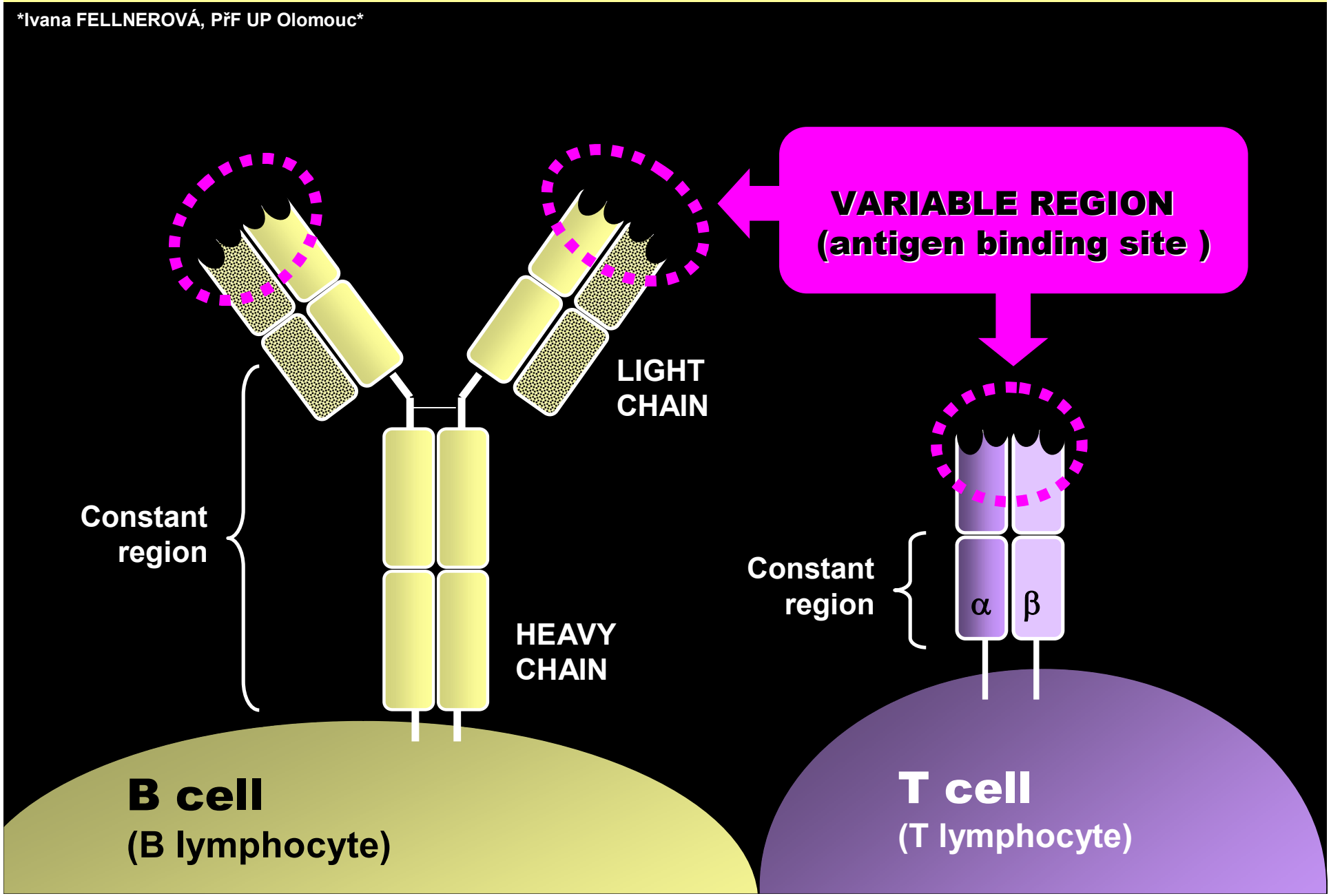

MHC molecules of immune cells