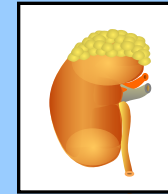


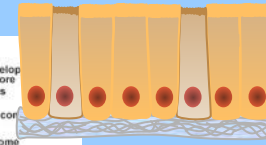
Hierarchies in Systems Biology



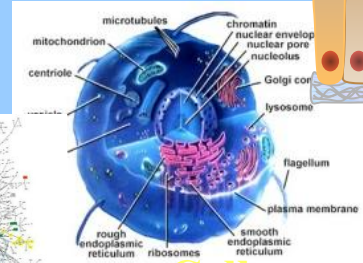
Organism



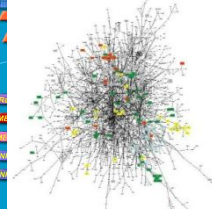
Organ



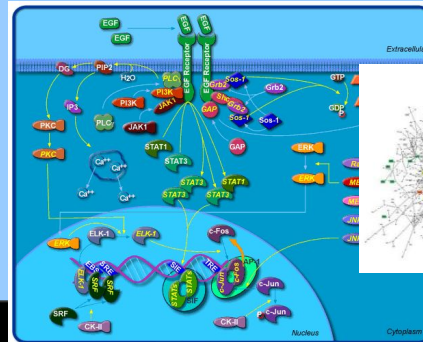
Tissue



Cell

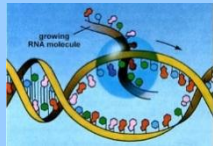


Network

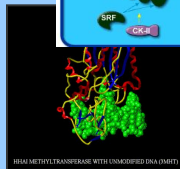


Pathway

Physical Laws of Nature



RNA



Protein



DNA



(Slide compliments of Joe Nadeau)

Systems biology involves

1. Data acquisition;
2. Data organization and integration;
3. Computation (mathematical modeling).

Mark A. Knepper, MD, PhD

**Laboratory of Kidney & Electrolyte Metabolism
National Heart, Lung, and Blood Institute
NIH**

Bethesda, Maryland, USA

Three Stages of Signal Transduction

1. **Reception** of extracellular signal by cell
2. **Transduction** of signal from outside of cell to inside of cell—often multi-stepped

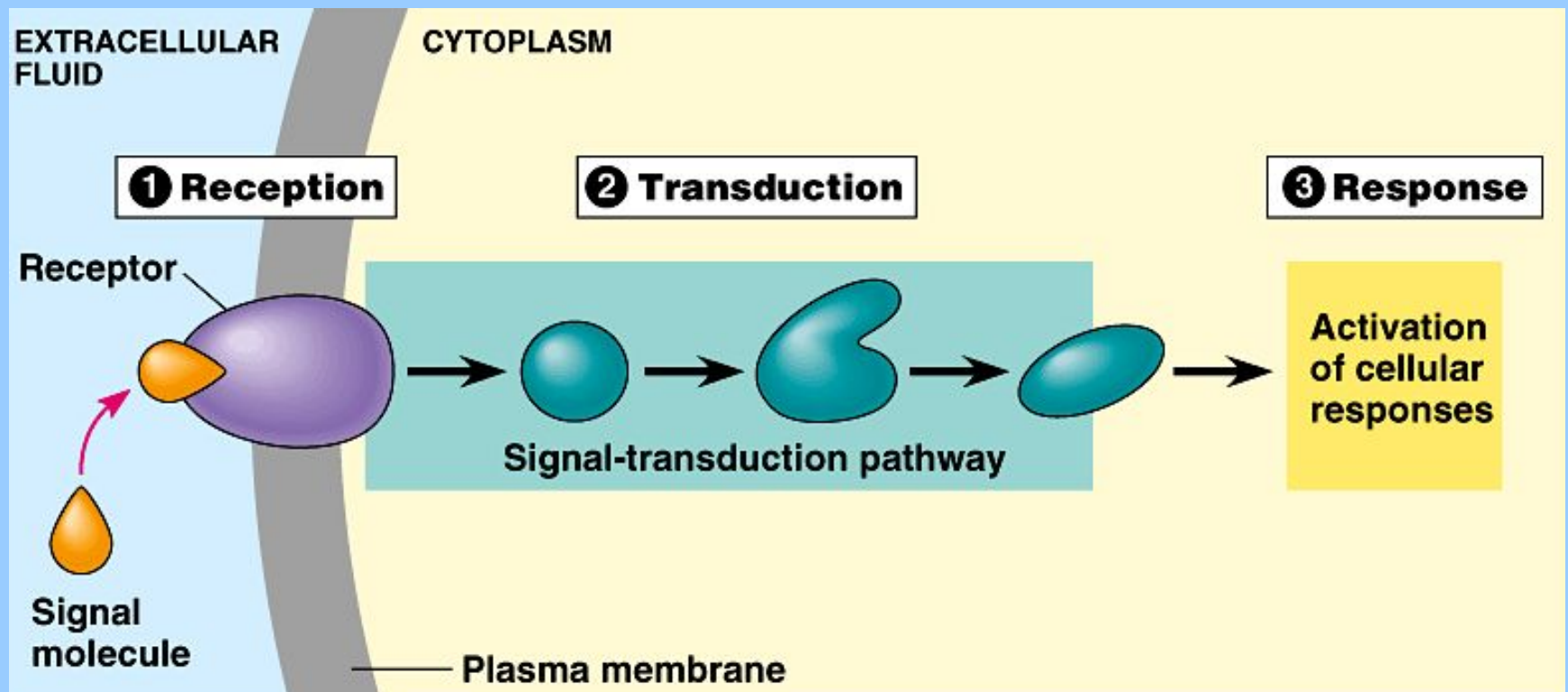
Note not necessarily transduction of ligand

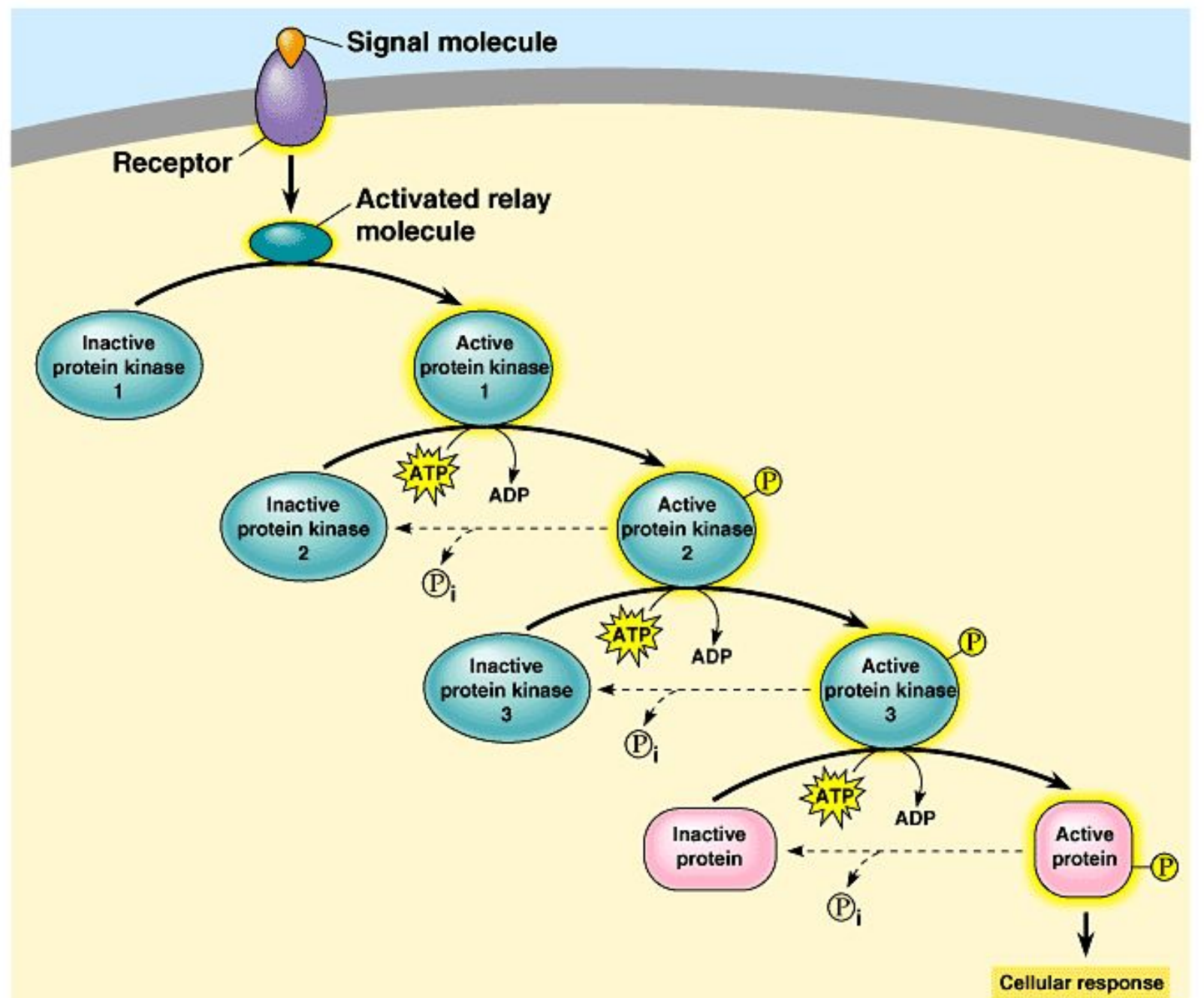
3. Cellular **Response**

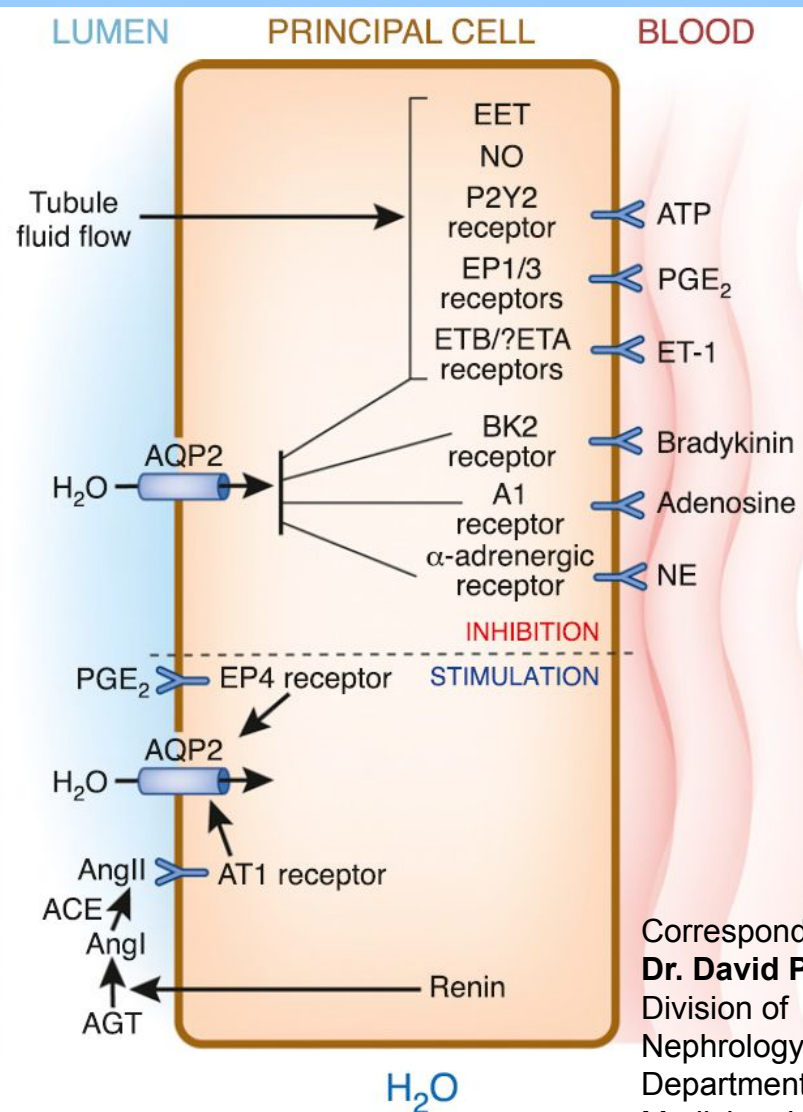
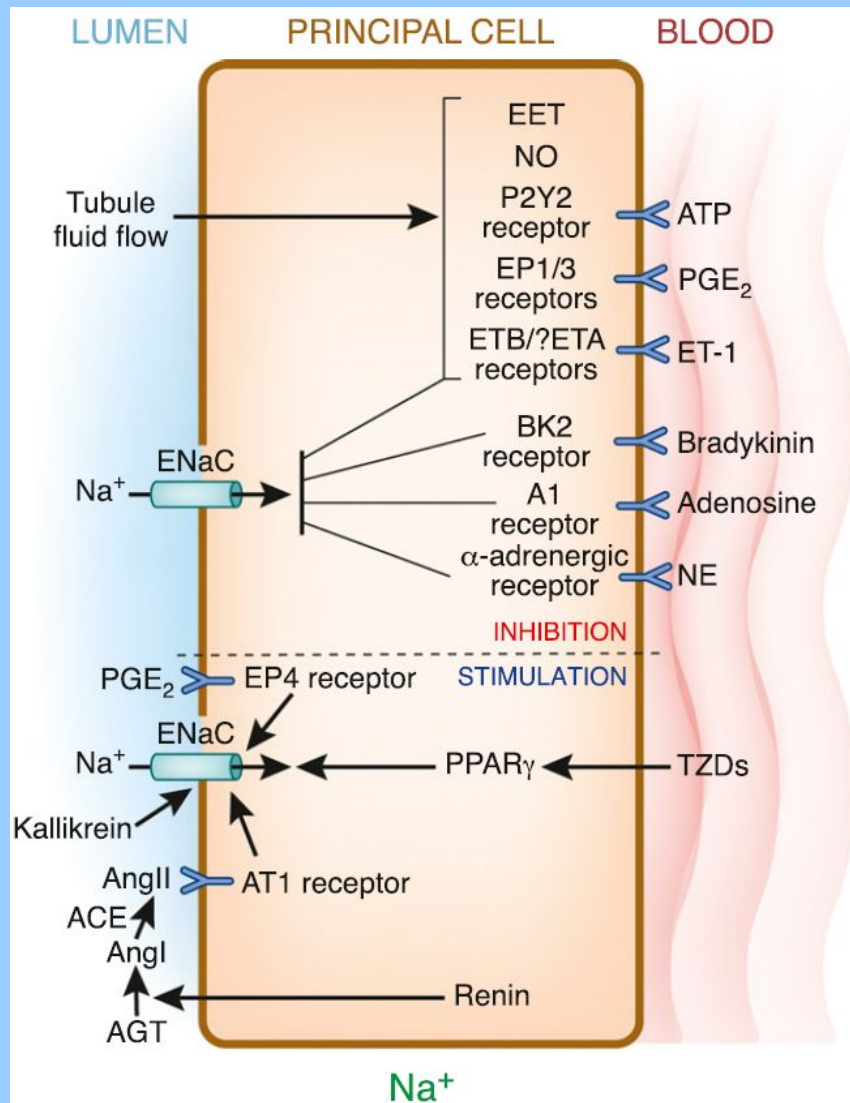
Response is initiated and/or occurs entirely within receiving cell

Transduction of signal from outside of cell to inside of cell—often multi-stepped

Note not necessarily transduction of ligand







Correspondence:
Dr. David Pearce,
 Division of Nephrology,
 Department of Medicine, University
 of California, San
 Francisco, HSE 672,
 San Francisco, CA
 94143. Email:
[dpearce@medsfgh.
 ucsf.edu](mailto:dpearce@medsfgh.ucsf.edu)

Chemical Signaling Between Cells

Three general categories of chemical signaling:

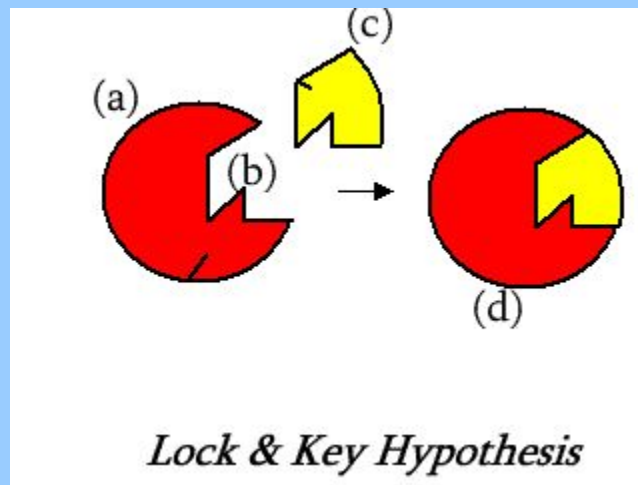
- Cytoplasmic connections between cells
- Cell-to-cell contact-mediated signaling
- Free diffusion between cells
 - Distant cells (hormones)
 - Adjacent cells (within interstitial space)

INTRODUCTION

- Signal Transducing Receptor Classes
 - Receptor Tyrosine Kinases
 - Receptor Serine/Threonine Kinases
 - Phospholipids and Phospholipases
 - G-Protein Coupled Receptors
 - G-Proteins
 - Intracellular Hormone Receptors

Природа биологических сигналов

Chemical Signaling Between Cells



Высокая аффинность рецептора и лиганда , по-видимому, достигается за счет конгруэнтности участка молекулы рецептора, непосредственно входящего в контакт, с лигандом. Впервые такая мысль была сформулирована нобелевскими лауреатами 1908 года **И.М. Мечниковым и П. Эрлихом.** Существование таких специализированных доменов в молекулах рецепторов подтверждено современными исследованиями.

Принципиальный момент:

Передача сигнала происходит в результате физического контакта лиганда с рецептором.

All of latter involves the physical movement of **Ligands**.

That is, **Ligand Reception by a Protein**

Note that **Reception** means
Molecule-to-Molecule Contact

Константа аффинности отражает энергию связи лиганд-рецептор.

The equilibrium constant
(association constant):

$$K_{eq} = K_A$$

Provides a qualitative measure of
the affinity

$$\Delta G = -RT \ln K_{eq}.$$

Энтропию термодинамической системы можно определить через множество возможных равновероятных (микро) конфигураций (Ω), соответствующих конкретному состоянию системы:

где k_b постоянная Больцмана (имеет размерность энергия/Т).

$$S = k_b \ln \Omega$$

Изменения энтропии в термодинамических обратимых процессах (ΔS). Макроскопическое определение:

$$\Delta S = \int \delta Q / T$$

Энтальпия или энергия расширенной системы E равна сумме внутренней энергии газа U

и

потенциальной энергии $E = PV$

$$H = U + PV$$

.

Свободная энергия (функция) Гиббса:

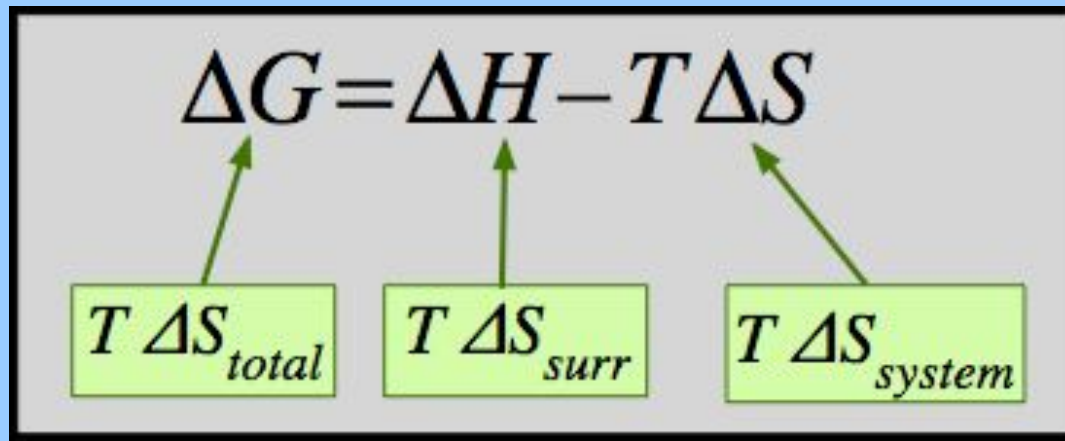
$$G = H - T S$$

$$\Delta G = \Delta H - T \Delta S$$

Must know this!

изменение энтропии мира в результате изменений в системе:

$$\Delta S_{total} = \Delta S_{surr} + \Delta S_{sys}$$



Классическим определением энергии Гиббса является выражение

$$G = U + P V - T S$$

Где:

U — внутренняя энергия,

P — давление,

V — объём,

T — абсолютная температура,

S — энтропия.

The free energy enables us to do changes that occur at a constant **temperature and pressure** (the *Gibbs free energy*) or constant temperature and volume (the *Helmholtz free energy*.)

The Helmholtz free energy is of interest mainly to chemical engineers

Clark's Theory

Условия применимости
классической модели
взаимодействия лиганд-
рецептор

- 1 обратимость связывания
- 2 все рецепторы эквивалентны и независимы
- 3 биологический ответ пропорционален количеству занятых рецепторов
- 4 параметры связывания и эффективность определяются в состоянии равновесия
- 5 лиганд не изменяется химически

SIGNAL-TRANSDUCTION EMPHASIS

signals that are released from one cell and allowed to freely diffuse to a second (or more) recipient cell(s)

We will consider in particular those events that follow the reception of chemical signals

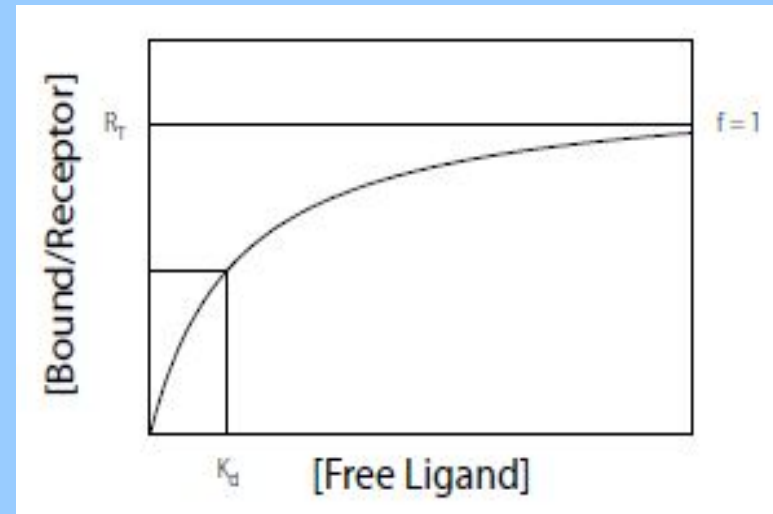
We will *not* dwell on the purpose of the signal

Взаимодействие молекулы лиганда (l) со связывающей молекулой (b) в условиях равновесия можно описать таким уравнением:

$$k_1 [b][l] - k_2 [bl] = 0;$$

$$k_1 / k_2 = K_a;$$

$$K_d = K_a^{-1}$$



$$K_a = \frac{[bl]}{[b][l]} = \frac{[bl]}{(B_0 - [bl])[l]}$$

$$K_a (B_0 - [bl]) = [bl] / [l] ;$$

$$\text{Если: } [bl] = \mathbf{B} ; [l] = \mathbf{F}$$

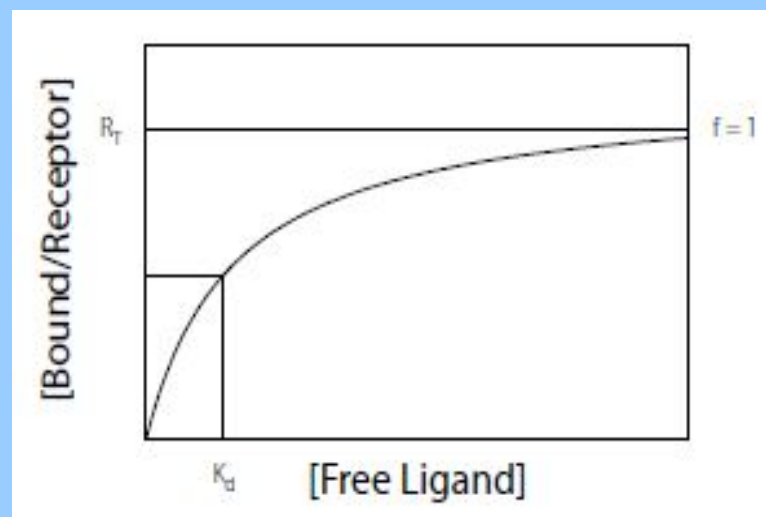
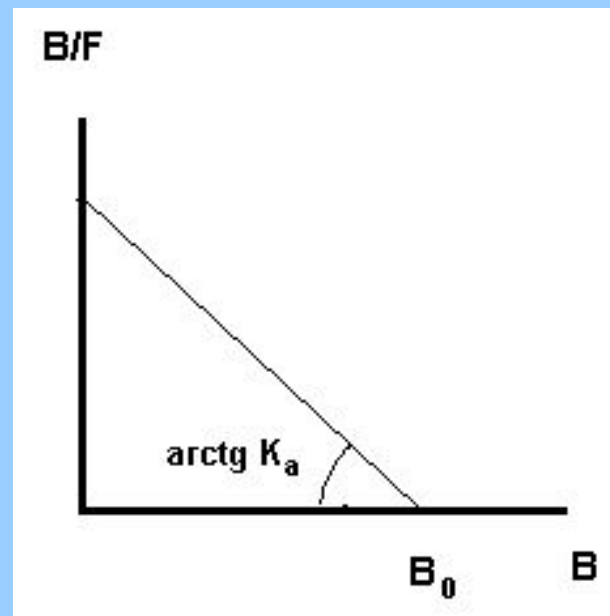
$$\text{То: } \mathbf{B} / \mathbf{F} = K_a (B_0 - \mathbf{B})$$

получаем уравнение Скетчарда

Размерность константы ассоциации – обратные моли (M^{-1}), но для характеристики аффинности чаще пользуются величиной константы диссоциации (M).

Что касается величин K_d наблюдаемых в природе, то для большинства гормонов значение этой константы лежит в области от 10^{-8} до $10^{-10} M$.

Для сердечного гликозида ouabain эта величина порядка $10^{-6} M$.



The equilibrium constant (association constant): $K_{eq} = K_A$
 Provides a qualitative measure of the affinity $\Delta G = -RT \ln K_{eq}$.

$$K_{eq} = \frac{[ML]}{[M][L]} K_D = \frac{[M][L]}{[ML]} \quad \begin{array}{l} [M] \text{ is the concentration of the protein,} \\ [L] \text{ is the concentration of the free ligand} \end{array}$$

The fractional saturation –Y:

$$Y = \frac{[ML]}{[M] + [ML]} = \frac{[M][L]K_{eq}}{[M] + [M][L]K_{eq}} = \frac{K_{eq}[L]}{1 + K_{eq}[L]} = \frac{[L]}{K_D + [L]}$$

When the protein is **half saturated** with the ligand the **ligand concentration is equal to K_D**

$$\frac{1}{2} = \frac{[L]_{1/2}}{K_D + [L]_{1/2}} \quad (K_D + [L]_{1/2}) = 2[L]_{1/2} \quad K_D = [L]_{1/2}$$

Multiple Independent Binding Sites

$v = nY$ v - the moles of bound ligand over the total protein concentration

$$v = n \sum_{i=1}^n Y_i = \frac{nK_{\text{ass}} [L]}{1 + K_{\text{ass}} [L]}$$

v - varies from 0 to n (instead of 0 to 1 for Y)

Scatchard plot

$$\frac{v}{[L]} = nK_{\text{ass}} - K_{\text{ass}} v = \frac{n}{K_D} - \frac{v}{K_D}$$

Конкурентное ингибирование

Ингибитор снижает K_{ass}

$$K'_{\text{ass}} = K_{\text{ass}} \frac{1}{(1 + K_I[I])}$$

[I] - inhibitor concentration,

K_I - association constant of the inhibitor.

The fractional saturation –Y:

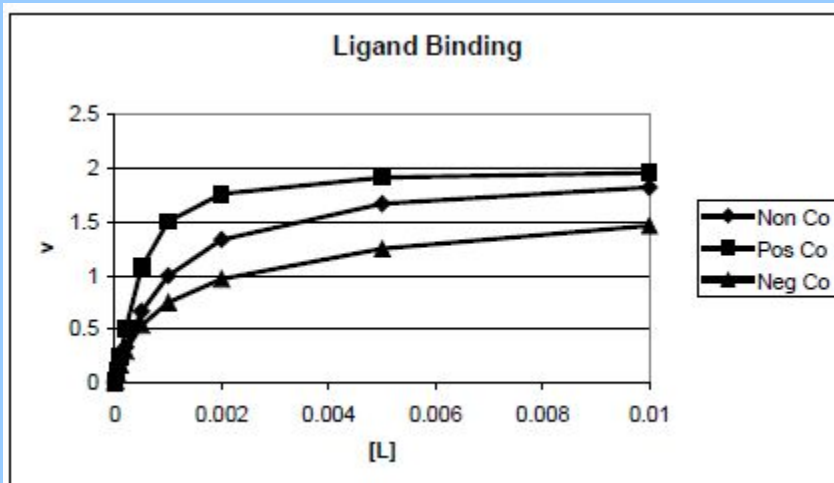
$$Y = \frac{K'_{\text{ass}}[L]}{1 + K'_{\text{ass}}[L]} \longrightarrow = \frac{K_{\text{ass}}[L]}{1 + K_{\text{ass}}[L] + K_I[I]}$$

Cooperative Binding

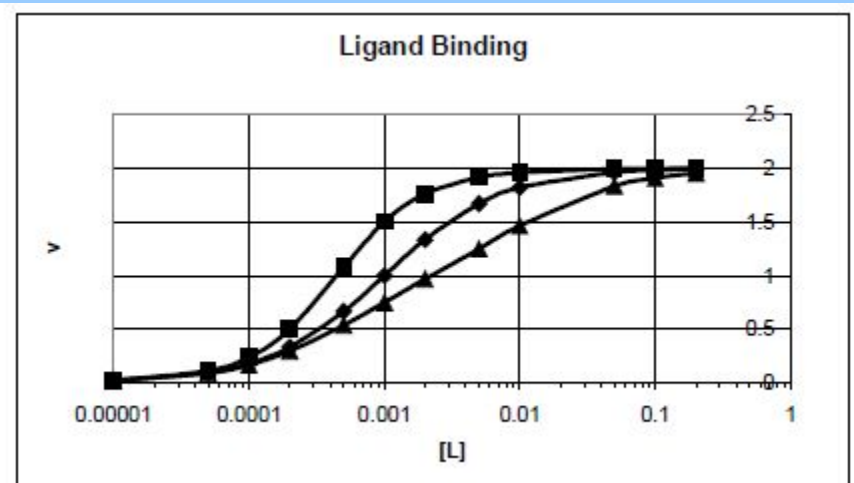
- Positive cooperativity is observed when K_2 is larger than $k_{on}/2k_{off}$.
- Negative cooperativity is observed when K_2 is less than $k_{on}/2k_{off}$

plots of $\bar{V}$ versus $[L]$

non-cooperative, positive cooperative, and negative cooperative



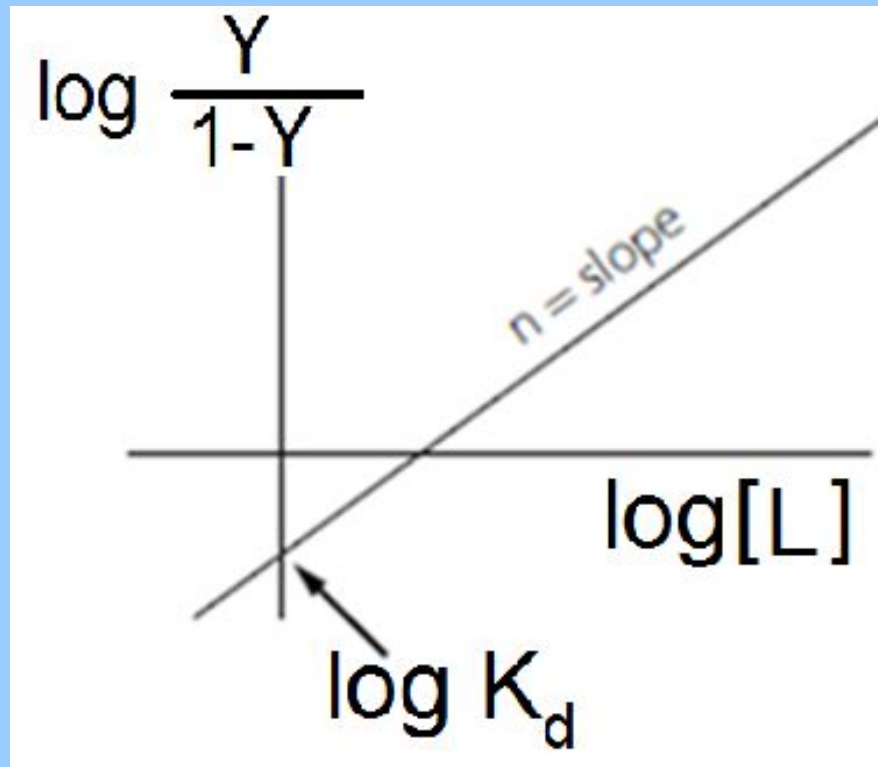
linear scale



semi-log scale

Hill plot

$$\log \frac{Y}{1-Y} = n \cdot \log[L] - n \cdot \log K_d$$

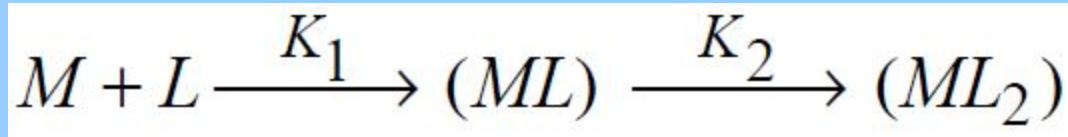


formulated by Archibald Hill in 1910

Hill Slope	B vs. L_f plot	Scatchard
If slope = 1, there is a single class of binding sites	hyperbola	straight line
If slope $\neq 1$ and is an integer, multiple non-interacting binding sites	hyperbola	concave-down
If slope $\neq 1$ and is fractional, you have cooperativity:		
If slope > 1 , Positive cooperativity	sigmoidal	concave-down
If slope < 1 , Negative cooperativity	no effect	concave-up

Independent sites - microscopic and macroscopic binding affinities

(binding of two ligands to an immunoglobulin)



$$K_1 = \frac{[ML]}{[M][L]}$$

$$K_2 = \frac{[ML_2]}{[ML][L]}$$

$$K_1 = \frac{2k_{on}}{k_{off}} \quad 2 \text{ -two ways to make } [ML].$$

K_1 equilibrium association constants

K_2 *macroscopic binding constants*

$$K_2 = \frac{k_{on}}{2k_{off}} \quad 2 \text{ -two ways of forming } [ML] \text{ starting from } [ML_2].$$

microscopic association constant

if only one ligand can bind

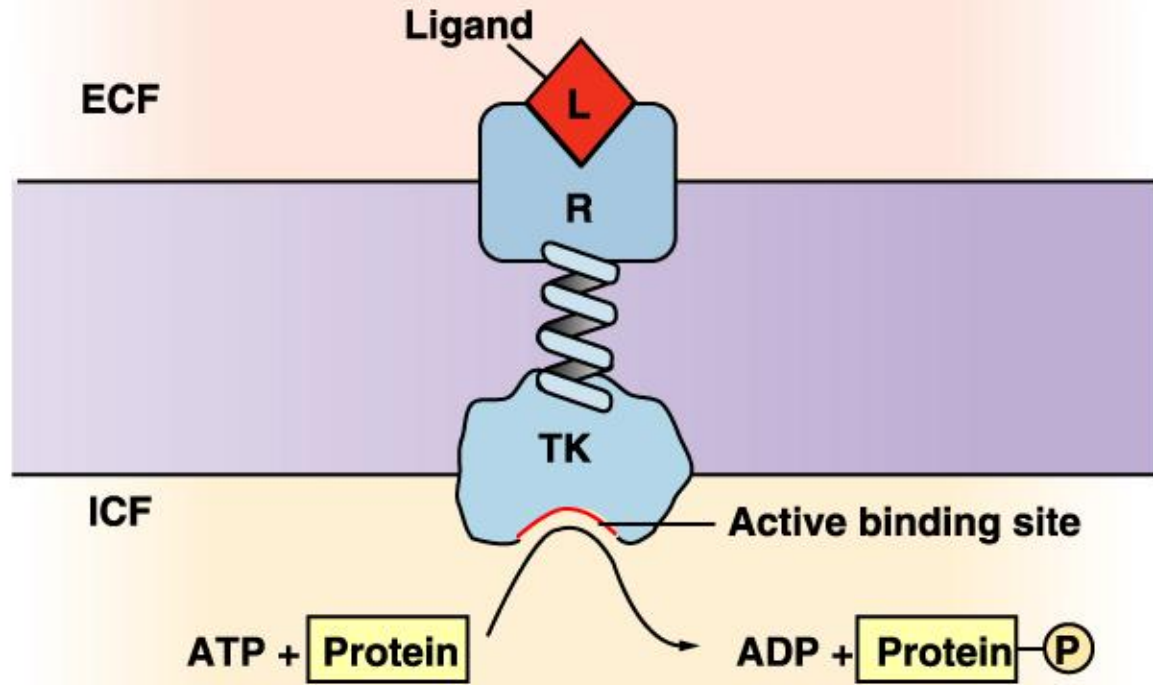
$$K_{micro} = \frac{k_{on}}{k_{off}}$$

kinetic rate constants

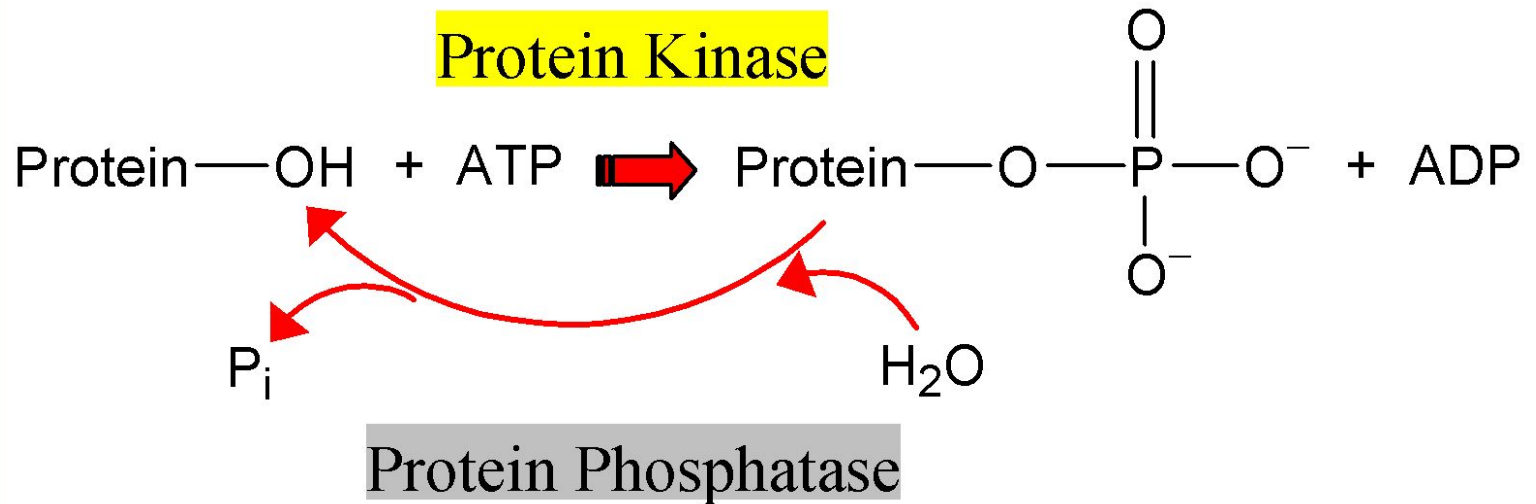
for ligand binding is k_{on}

for release of the ligand is k_{off}

Signal Amplification (Cascade)

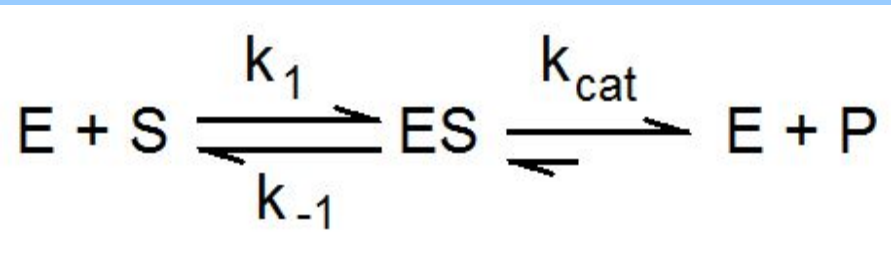


Phosphorylation Cascade



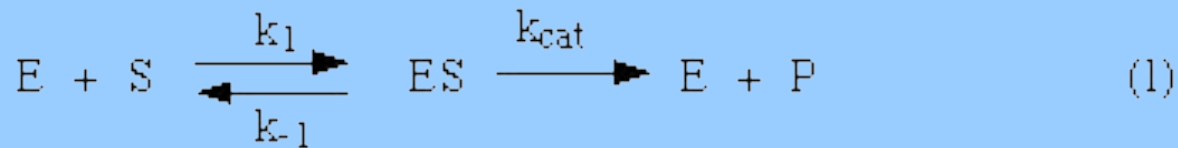
- Динамическое равновесие в клетке:
- Активация белка киназой, деактивация фосфатазой.
- В норме сигнал не может длиться вечно.

A simple model of enzyme action:



$$V_0 = k_{\text{cat}}[ES]$$

Начальная скорость реакции – концентрация продукта мала, субстрат в избытке, концентрация субстрата много больше чем продукта ($[P] \ll [S]$) и фермента ($[E] \ll [S]$).



Скорость образования: $[ES] = k_1[E][S]$.

Скорость убывания: $[ES] = k_{-1}[ES] + k_{cat}[ES]$.

Стационарное состояние: $k_{-1}[ES] + k_{cat}[ES] = k_1[E][S]$

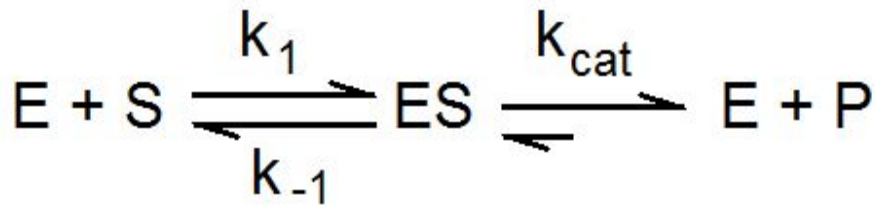
Условия ($[P] \ll [S]$) и ($[E] \ll [S]$).

обычно создаются в эксперименте и тогда согласно закону сохранения массы:

$$[S]_0 = [S]_{\text{free}} + [ES] + [P],$$

тогда приближенно - $[S]_0 = [S]$

для фермента - $[E]_{\text{total}} = [E]_{\text{free}} + [ES]$



Цель, определить [ES] через измеряемые величины.

Сгруппируем константы (кинетические) и переменные (концентрации):

$$(k_{-1} + k_{\text{cat}}) [ES] = k_1 [E][S],$$

$$(k_{-1} + k_{\text{cat}})/k_1 = [E][S]/[ES] ;$$

$$K_m = (k_{-1} + k_{\text{cat}})/k_1$$

Выразим $[E]$ через $[ES]$ и $[E]_{\text{total}}$:

$$[E] = [E]_{\text{total}} - [ES];$$

$$K_m = \frac{([E]_{\text{total}} - [ES]) [S]}{[ES]}$$

$$K_m = ([E]_{\text{total}} - [ES]) [S]/[ES]$$

$$[ES] K_m = [E]_{\text{total}} [S] - [ES][S]$$

$$[ES] K_m + [ES][S] = [E]_{\text{total}} [S]$$

$$[ES](K_m + [S]) = [E]_{\text{total}} [S]$$

$$[ES] = [E]_{\text{total}} [S]/(K_m + [S])$$

$$V_0 = k_{\text{cat}} [E]_{\text{total}} [S]/(K_m + [S]) \quad (\text{подставляя в } V_0 = k_{\text{cat}} [ES])$$

В условиях насыщения субстратом, когда $[ES] = [E]_{\text{total}}$

$$V_{\text{max}} = k_{\text{cat}} [E]_{\text{total}} \quad \text{подставляем в } V_0 = k_{\text{cat}} [E]_{\text{total}} [S] / (K_m + [S])$$

$$V_0 = V_{\text{max}} [S] / (K_m + [S])$$

Это выражение скорости реакции через концентрацию субстрата и K_m

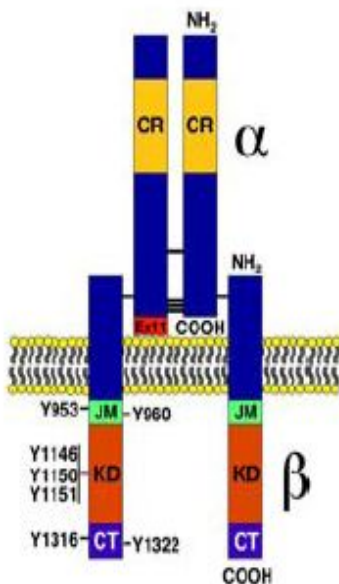
Физический смысл K_m

При $[S] = K_m$ $V_0 = 1/2 V_{\text{max}}$; при малых $[S]$

V_0 стремится к $V_{\text{max}} [S] / K_m$

V_{max} / K_m (или k_{cat} / K_m) - «каталитическая сила»

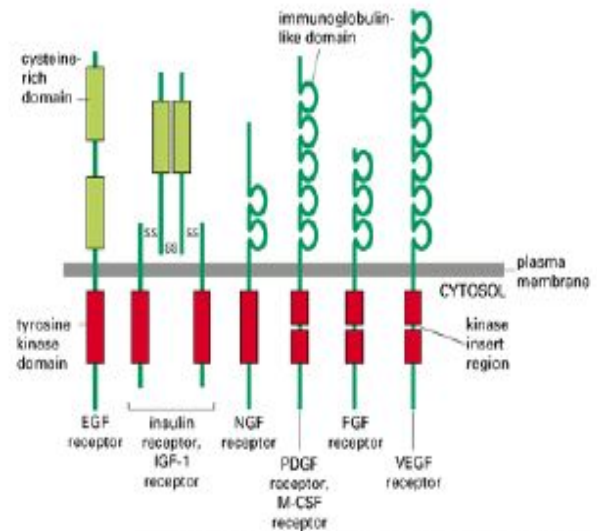
The insulin receptor is a covalent $(\alpha\beta)_2$ dimer



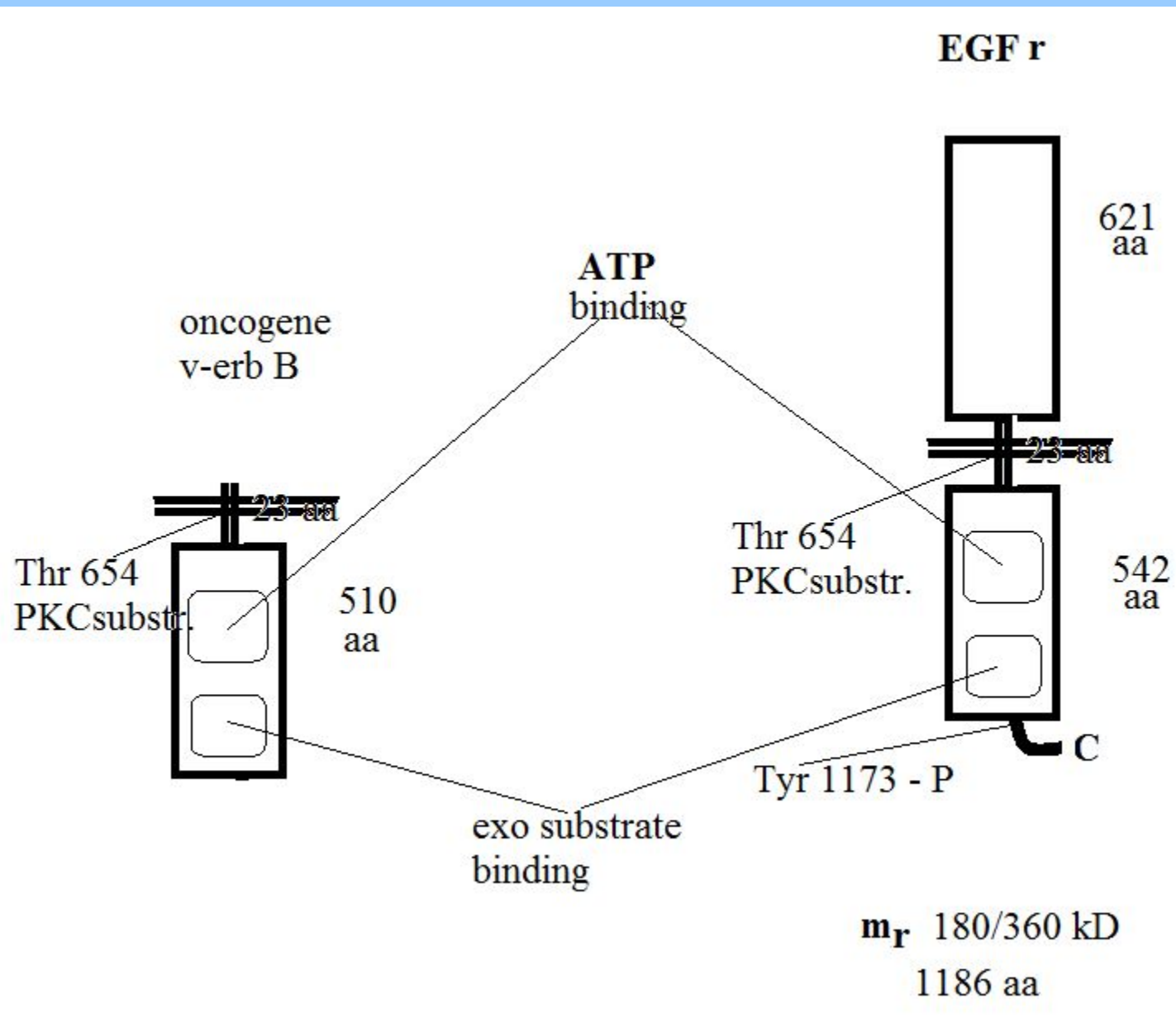
- α subunit – extracellular
- β subunit
- extracellular domain
- TM domain
- intracellular domain(TK)

Watson et al. 2004 Endocr Rev 25 177-204

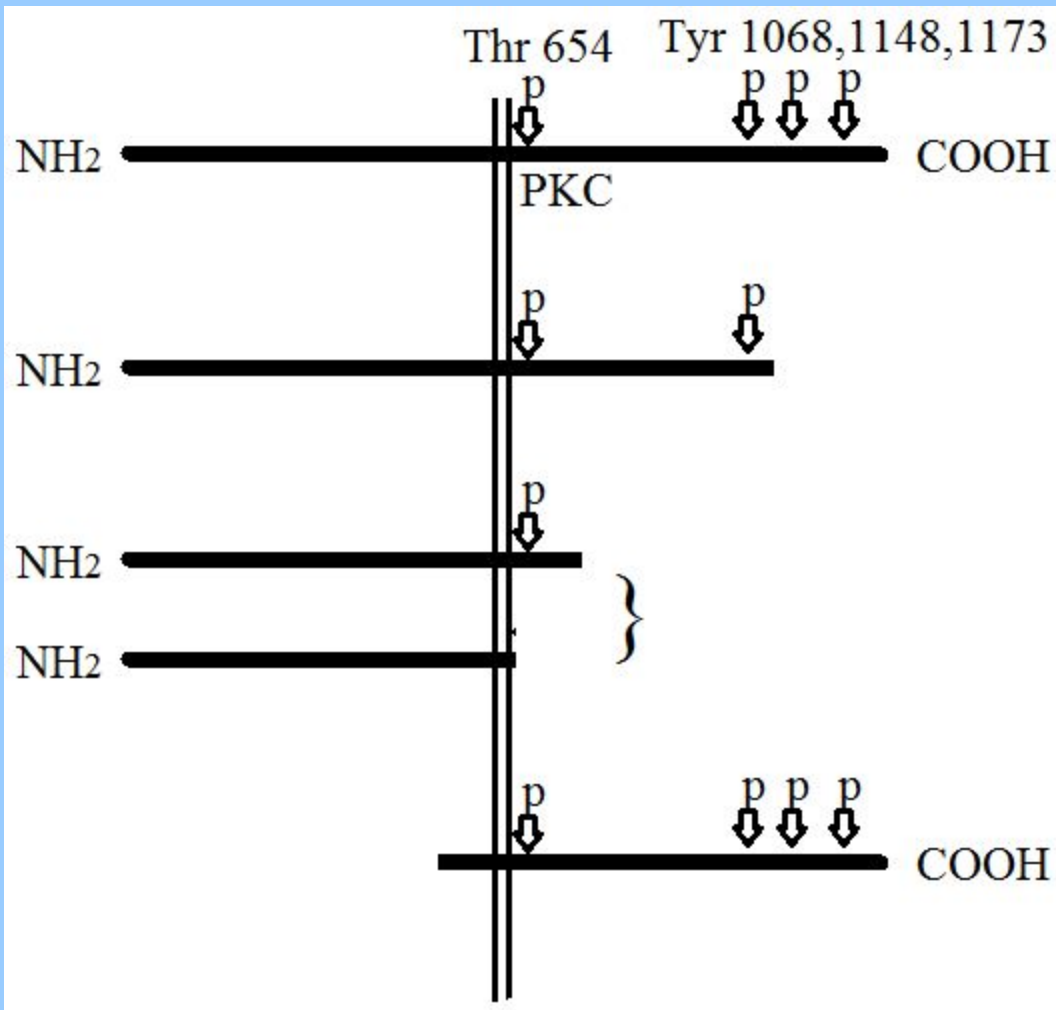
Insulin Receptor (IR) belongs to the family of tyrosine kinase receptors



Bray A, Lewis J, Raff M, Roberts K, and Watson JD (1994). The Cell



Мутанты рецептора EGF



affinity	Kinase activity	endocytosis	DNA synthesis	Transform. act.
High & Low	+	+	+	-
Low	+	+	+	-
Low	-	-	-	-
-	+	ND	ND	+

About 70 to 80 percent breast precancers have over expression of Her-2/neu oncogene. Although Her-2/neu oncogene was first identified in breast cancer



One of the fascinating things that have happened in recent years is that an antibody to counteract the Her-2/neu receptor, **can be given intravenously**. It has quite a unique mechanism of action. It attaches only to cells with too much Her-2/neu receptor, not the normal ones. Unlike chemotherapy, with which case most dividing cells are destroyed, it is a targeted therapy.

http://EzineArticles.com/?expert=Michael_Russell

Настоящий инженер
немедленно начнет писать
функцию с 7-ю
переменными и
минимизировать ее с
помощью системы
дифференциальных
уравнений...

Ингибитор снижает K_{ass}

$[I]$ - inhibitor concentration,

K_I - association constant of the inhibitor.

$$K'_{\text{ass}} = K_{\text{ass}} \frac{1}{(1 + K_I[I])}$$

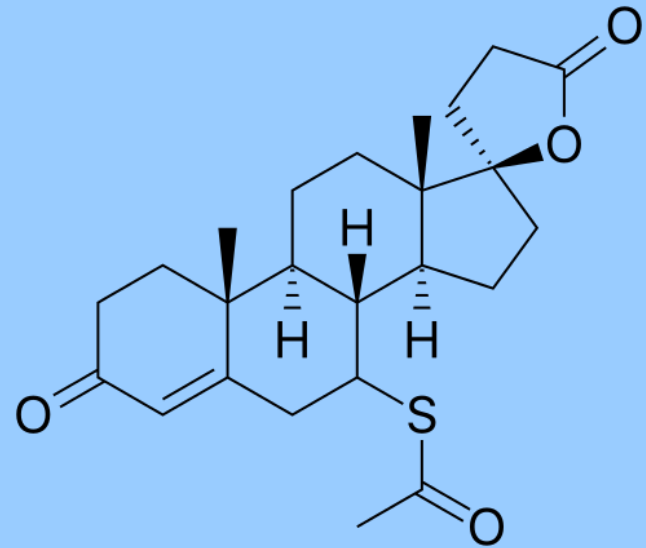
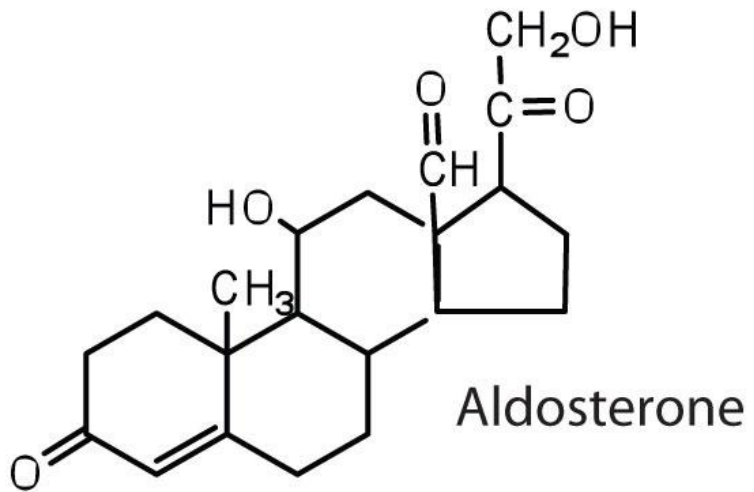
The fractional saturation –Y:

$$Y = \frac{K'_{\text{ass}}[L]}{1 + K'_{\text{ass}}[L]}$$



$$= \frac{K_{\text{ass}}[L]}{1 + K_{\text{ass}}[L] + K_I[I]}$$

Spironolactone MW 416.57



Spironolactone (верошпирон)

$IC_{50} \sim 2 \text{ nM}$

Aldosterone

$K_d \sim 0.14 \text{ nM}$

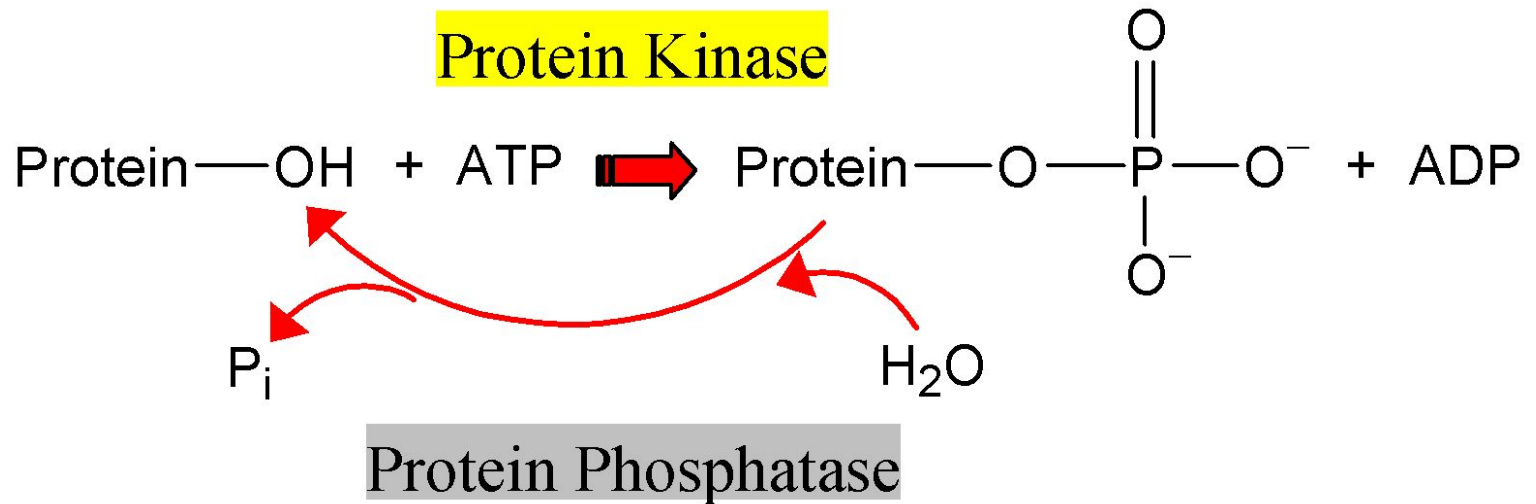
Aldosterone blood plasma concentration

«норма» $\sim 0.2 \text{ nM}$

«отеки, гипертония» $\sim 0.5 \text{ nM}$

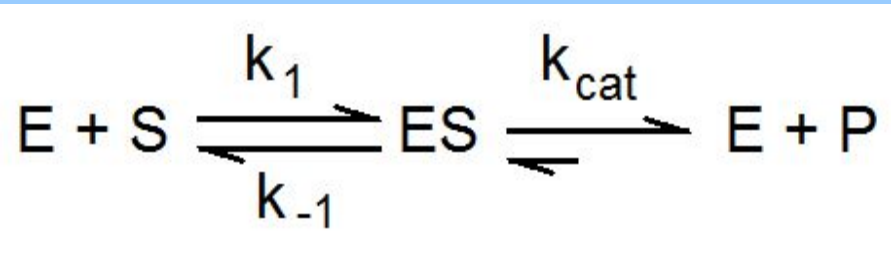
Доза верошпирона для нормализации - ?

Phosphorylation Cascade



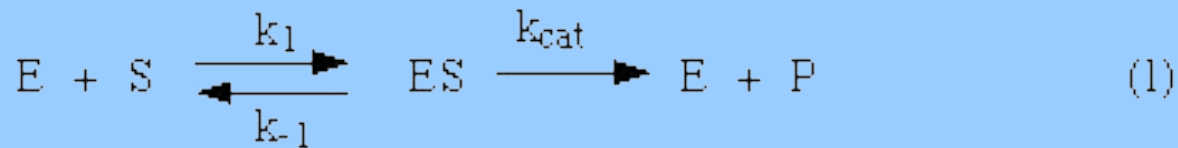
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Скорость убывания: $[ES] = k_{-1}[ES] + k_{cat}[ES]$.

Стационарное состояние: $k_{-1}[ES] + k_{cat}[ES] = k_1[E][S]$

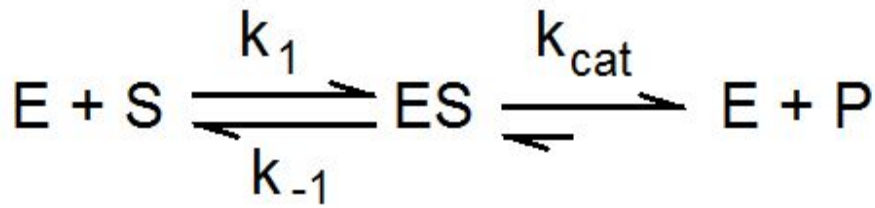
Условия ($[P] \ll [S]$) и ($[E] \ll [S]$).

обычно создаются в эксперименте и тогда согласно закону сохранения массы:

$$[S]_0 = [S]_{\text{free}} + [ES] + [P],$$

тогда приближенно - $[S]_0 = [S]$

для фермента - $[E]_{\text{total}} = [E]_{\text{free}} + [ES]$



Цель, определить [ES] через измеряемые величины.

Сгруппируем константы (кинетические) и переменные (концентрации):

$$(k_{-1} + k_{\text{cat}}) [ES] = k_1 [E][S],$$

$$(k_{-1} + k_{\text{cat}})/k_1 = [E][S]/[ES] ;$$

$$K_m = (k_{-1} + k_{\text{cat}})/k_1$$

Выразим $[E]$ через $[ES]$ и $[E]_{\text{total}}$:

$$[E] = [E]_{\text{total}} - [ES];$$

$$K_m = \frac{([E]_{\text{total}} - [ES]) [S]}{[ES]}$$

$$K_m = ([E]_{\text{total}} - [ES]) [S]/[ES]$$

$$[ES] K_m = [E]_{\text{total}} [S] - [ES][S]$$

$$[ES] K_m + [ES][S] = [E]_{\text{total}} [S]$$

$$[ES](K_m + [S]) = [E]_{\text{total}} [S]$$

$$[ES] = [E]_{\text{total}} [S]/(K_m + [S])$$

$$V_0 = k_{\text{cat}} [E]_{\text{total}} [S]/(K_m + [S]) \quad (\text{подставляя в } V_0 = k_{\text{cat}} [ES])$$

В условиях насыщения субстратом, когда $[ES] = [E]_{\text{total}}$

$$V_{\text{max}} = k_{\text{cat}} [E]_{\text{total}} \quad \text{подставляем в } V_0 = k_{\text{cat}} [E]_{\text{total}} [S] / (K_m + [S])$$

$$V_0 = V_{\text{max}} [S] / (K_m + [S])$$

Это выражение скорости реакции через концентрацию субстрата и K_m

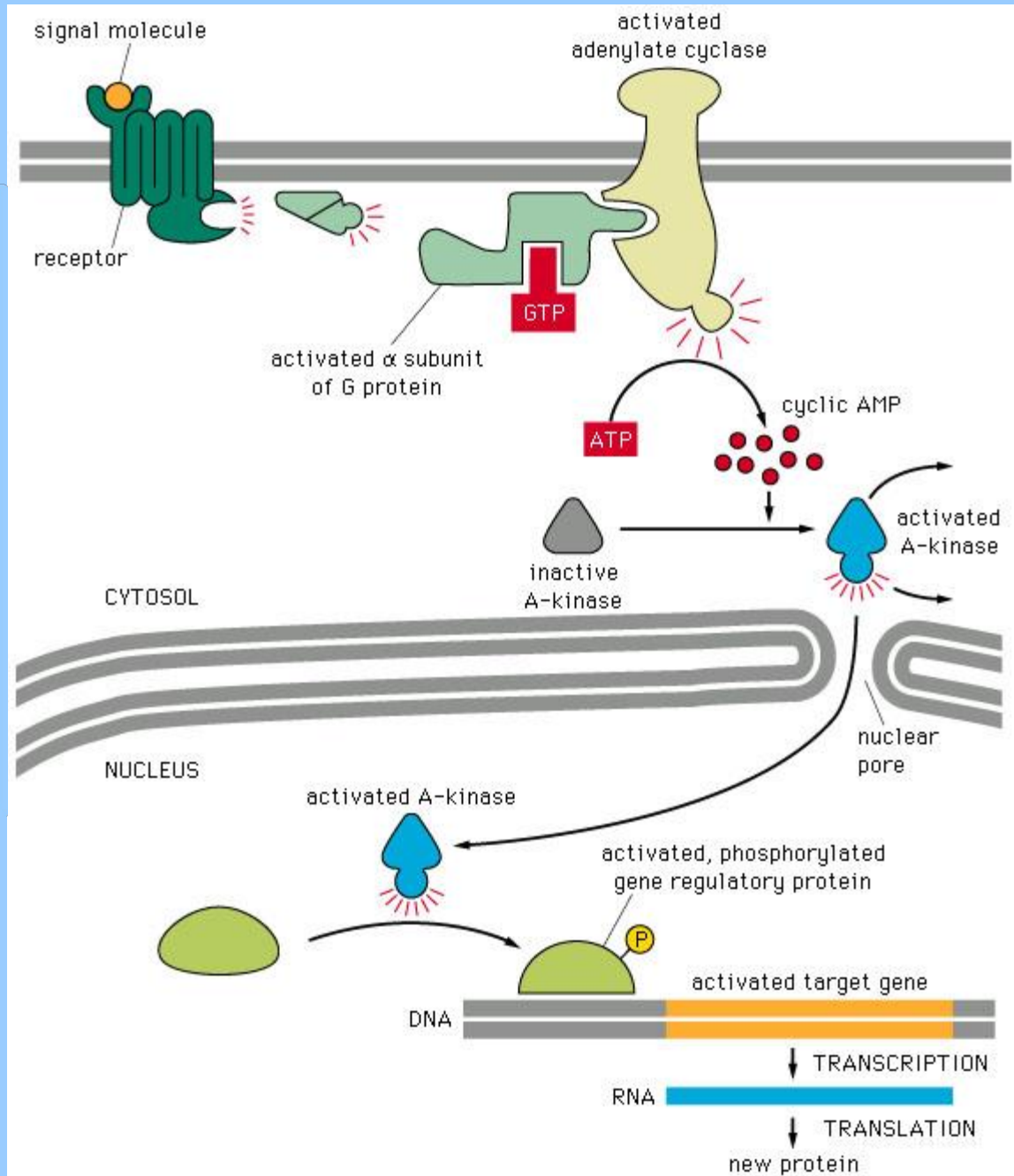
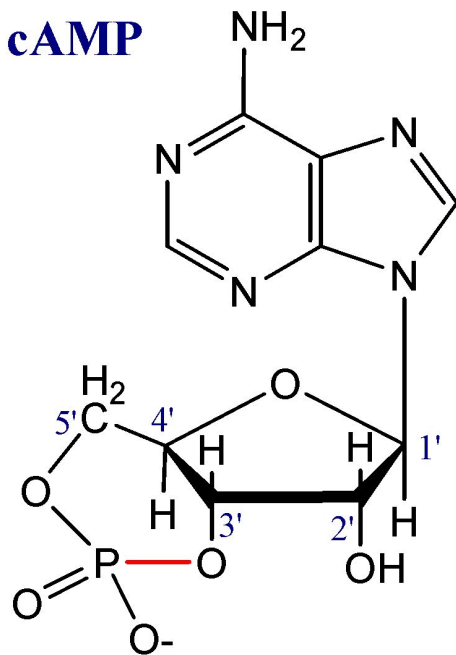
Физический смысл K_m

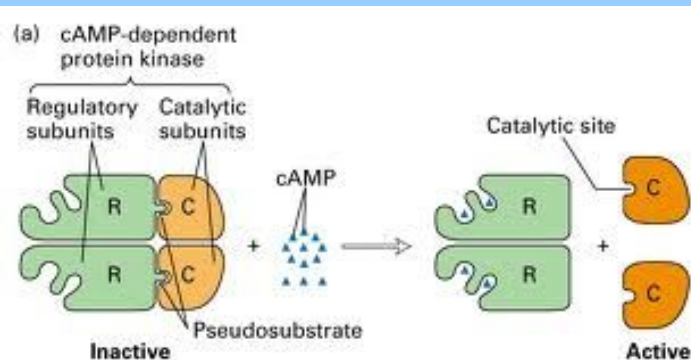
При $[S] = K_m$ $V_0 = 1/2 V_{\text{max}}$; при малых $[S]$

V_0 стремится к $V_{\text{max}} [S] / K_m$

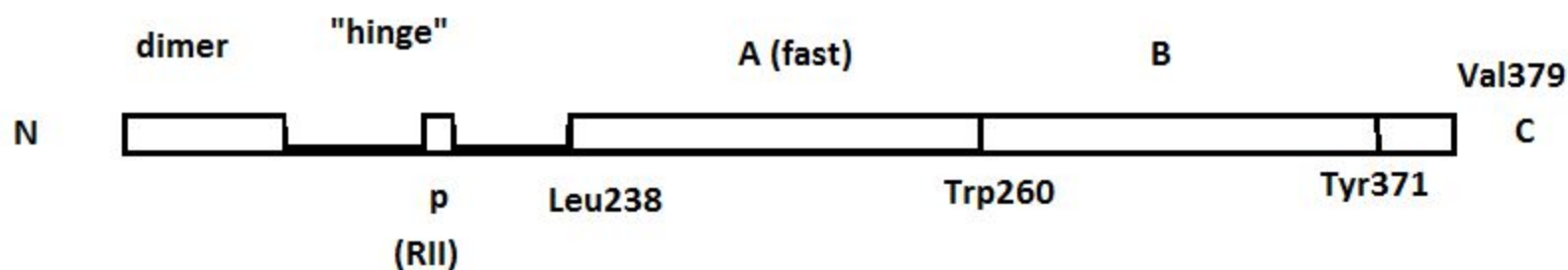
V_{max} / K_m (или k_{cat} / K_m) - «каталитическая сила»

cAMP

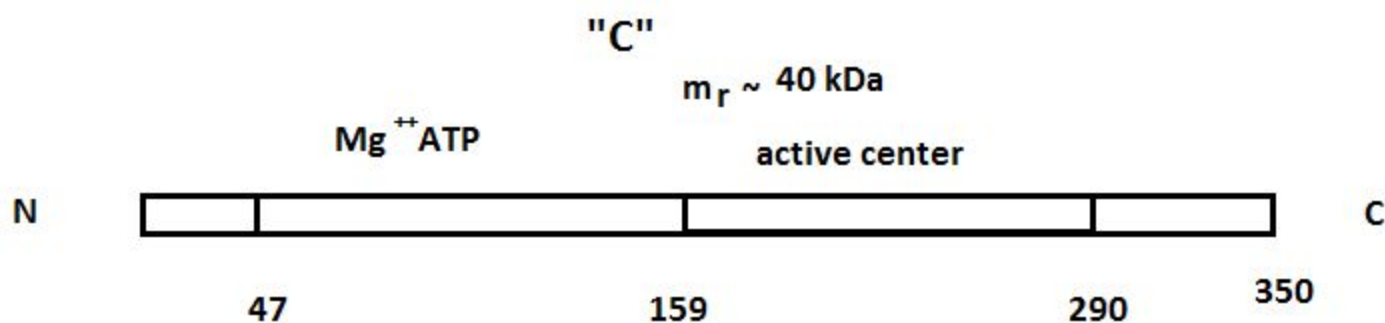




"R" RI, RII продукты разных генов



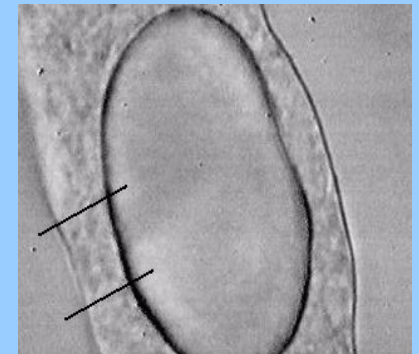
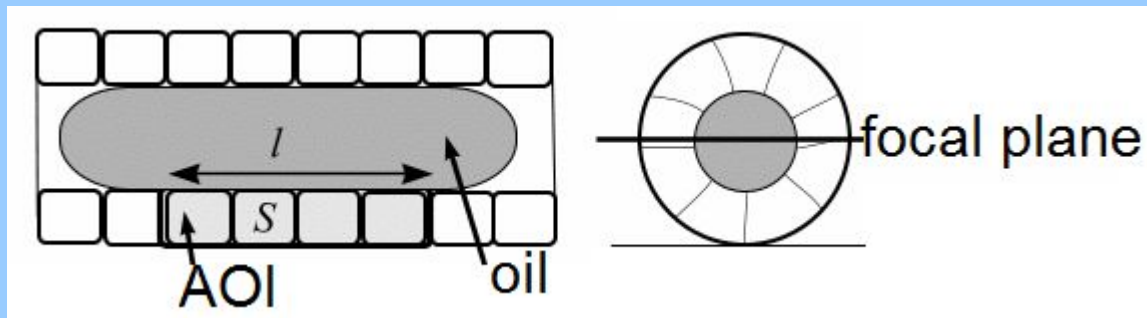
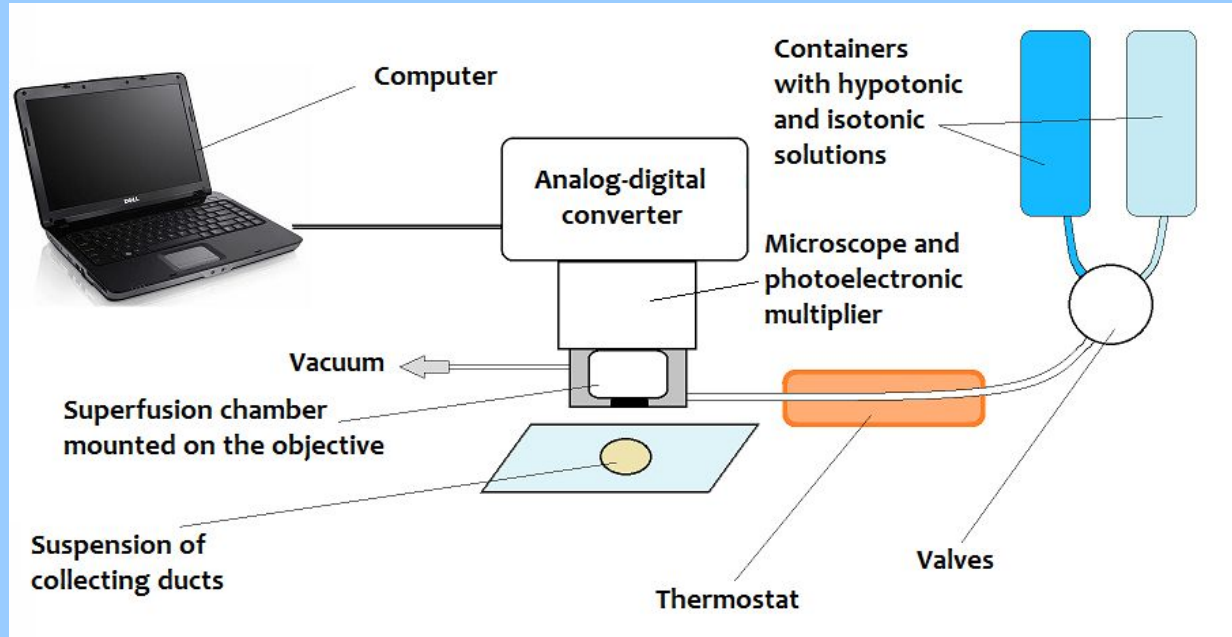
без cAMP
 $K_d \sim 0.2 \text{ nM}$



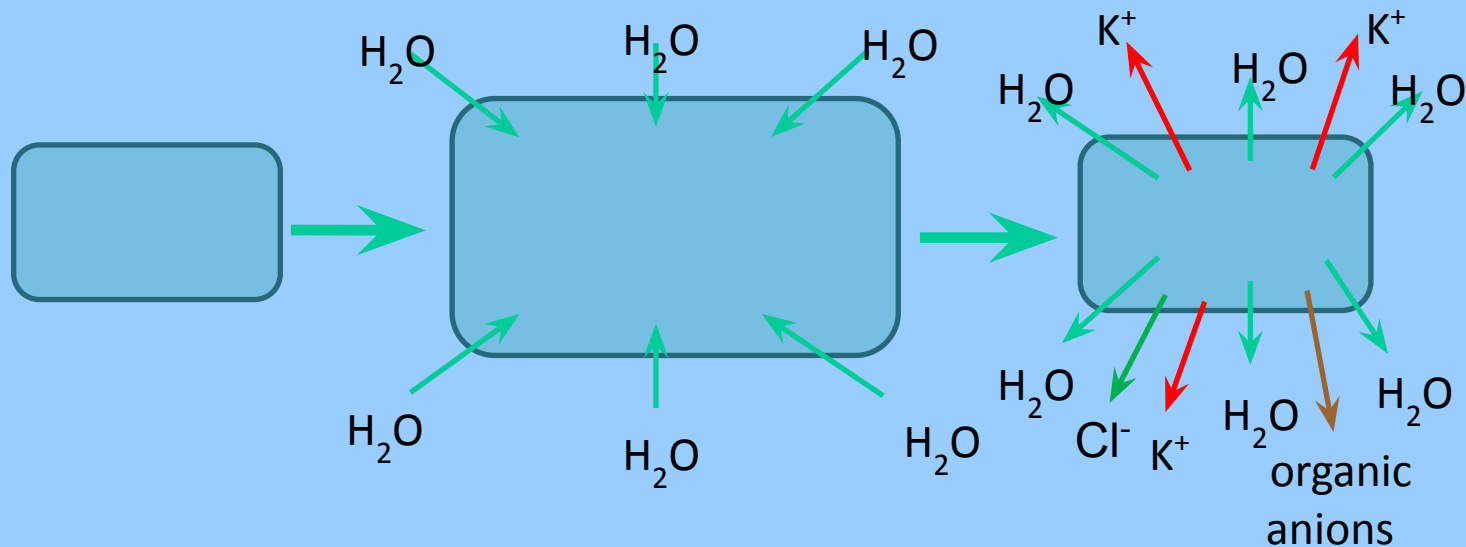
cAMP снижает $K_{acc} \sim 10^{-5}$ раз

Studies of water transport across cell plasma membrane.

Schematic diagram of the experimental setup



Hypotonic medium causes cell swelling and activates adaptive mechanism of recovery which is termed **Regulatory Volume Decrease (RVD)**.



To understand the adaptive reaction better one needs to use mathematical modeling of osmolytes fluxes.

Following processes were taken into account in the model of the cell response to the hypotonic shock :

The equations of mathematical model Cell ion content and ion fluxes

$$\frac{dn_{Na}}{dt} = A(-3J_{pump} + J_{Na} + J_{NKCC}),$$

Sodium

$$\frac{dn_K}{dt} = A(2J_{pump} + J_K + J_{KCC} + J_{NKCC}),$$

Potassium

$$\frac{dn_{Cl}}{dt} = A(J_{Cl} + J_{KCC} + 2J_{NKCC}),$$

Chloride

$$\frac{dn_X}{dt} = AJ_X,$$

Organic anions (X)

$$E_m = F (n_{Na^+} + n_{K^+} - n_{Cl^-} + zX) / C_m / A,$$

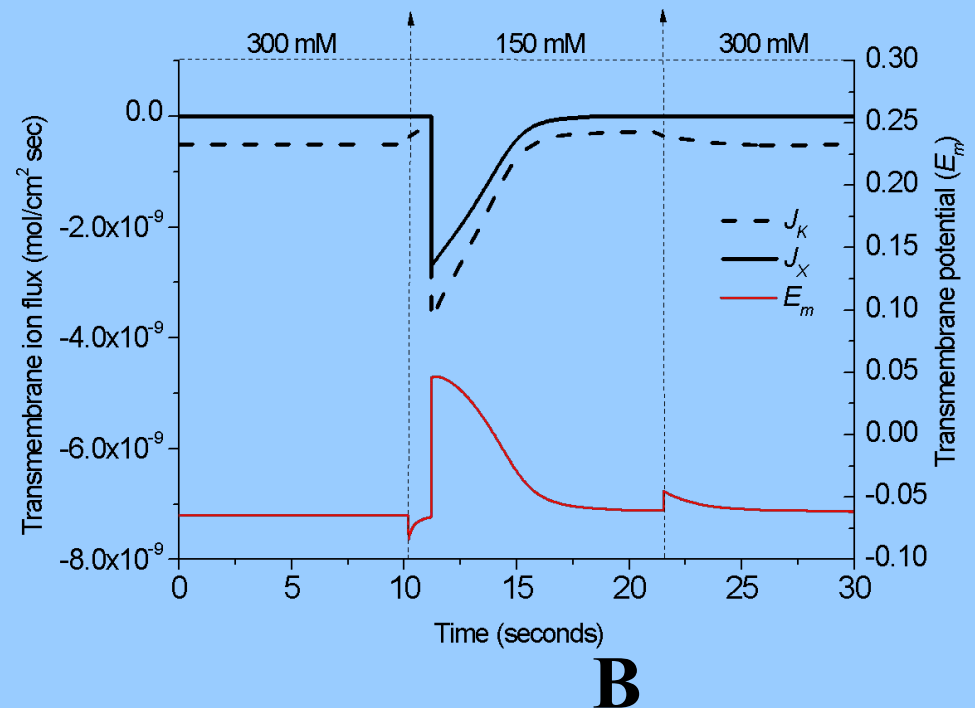
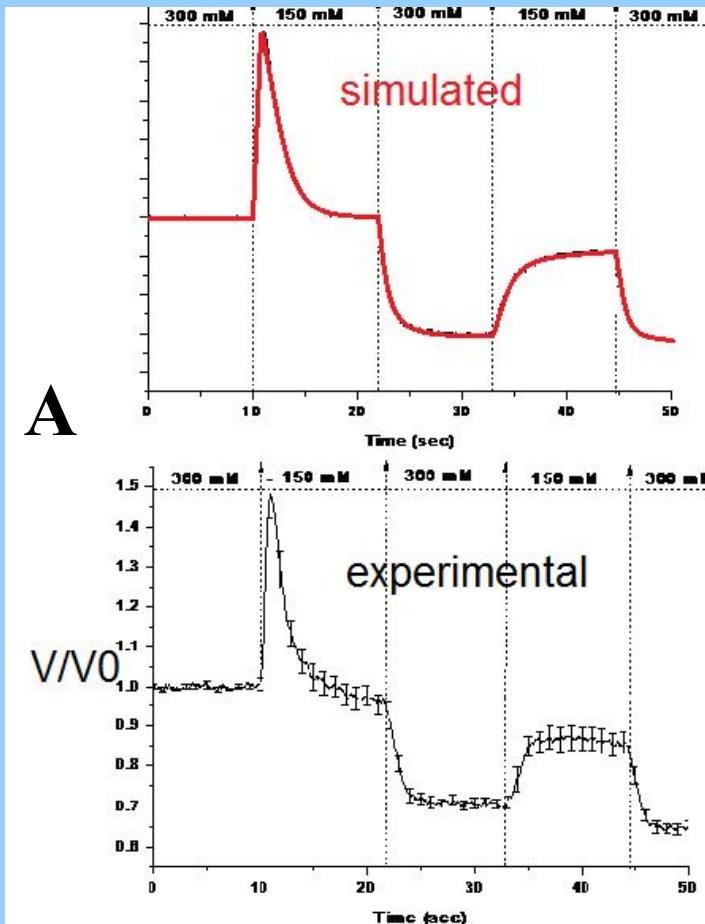
Transmembrane potential difference

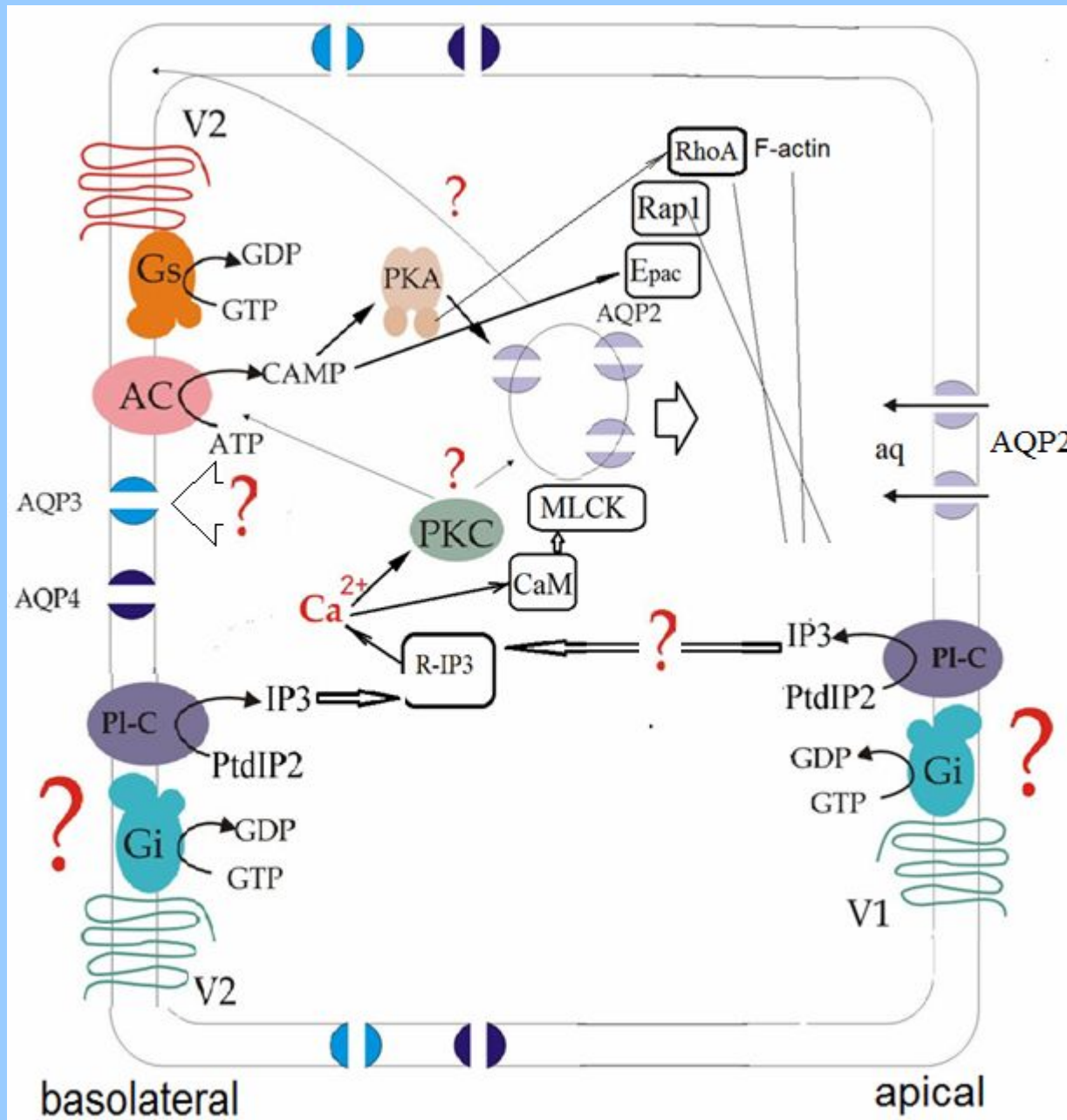
$$\frac{dV}{dt} = AV_w P_w \left(\frac{n_{Na} + n_K + n_{Cl} + n_X}{V} - \Pi_e \right).$$

Cell volume

Simulation of the RVD reaction in consequent shocks

Time dependence of relative cell volume in experiment (black) and in the model (red)(A). Osmolytes currents and electric transmembrane potential difference (B).





Principal cell

Some key agents
in the mechanism
of antidiuretic
action of
vasopressin