Российский Государственный Медицинский Университет
Московский Государственный Университет

Ю. А. Владимиров

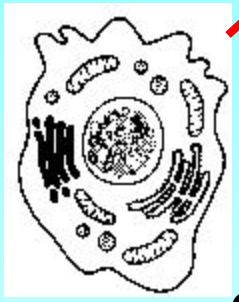
Физико-химические основы патологии клетки

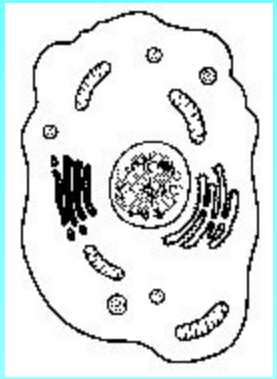
Митохондрии и апоптоз

Морфология клетки при некрозе и апоптозе

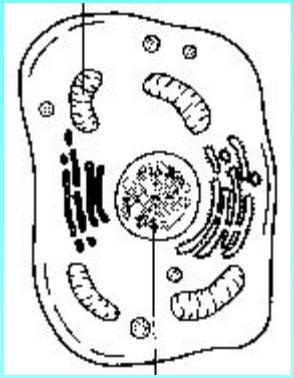
НЕКРОЗ

Норма





Митохондрии изменены



Разрыв мембран

Сохранена форма хроматина

Обратимое набухание

Необратимое набухание

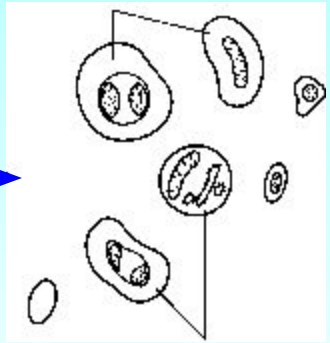
Дезинтеграция

АПОПТОЗ

Сохранена структура митохондрий

Ядро изменено

Intact membranes



Апоптотические тельца

Конденсация (cell blebbing)

Фрагментация

Вторичный некроз

Apoptosis in neutrophils

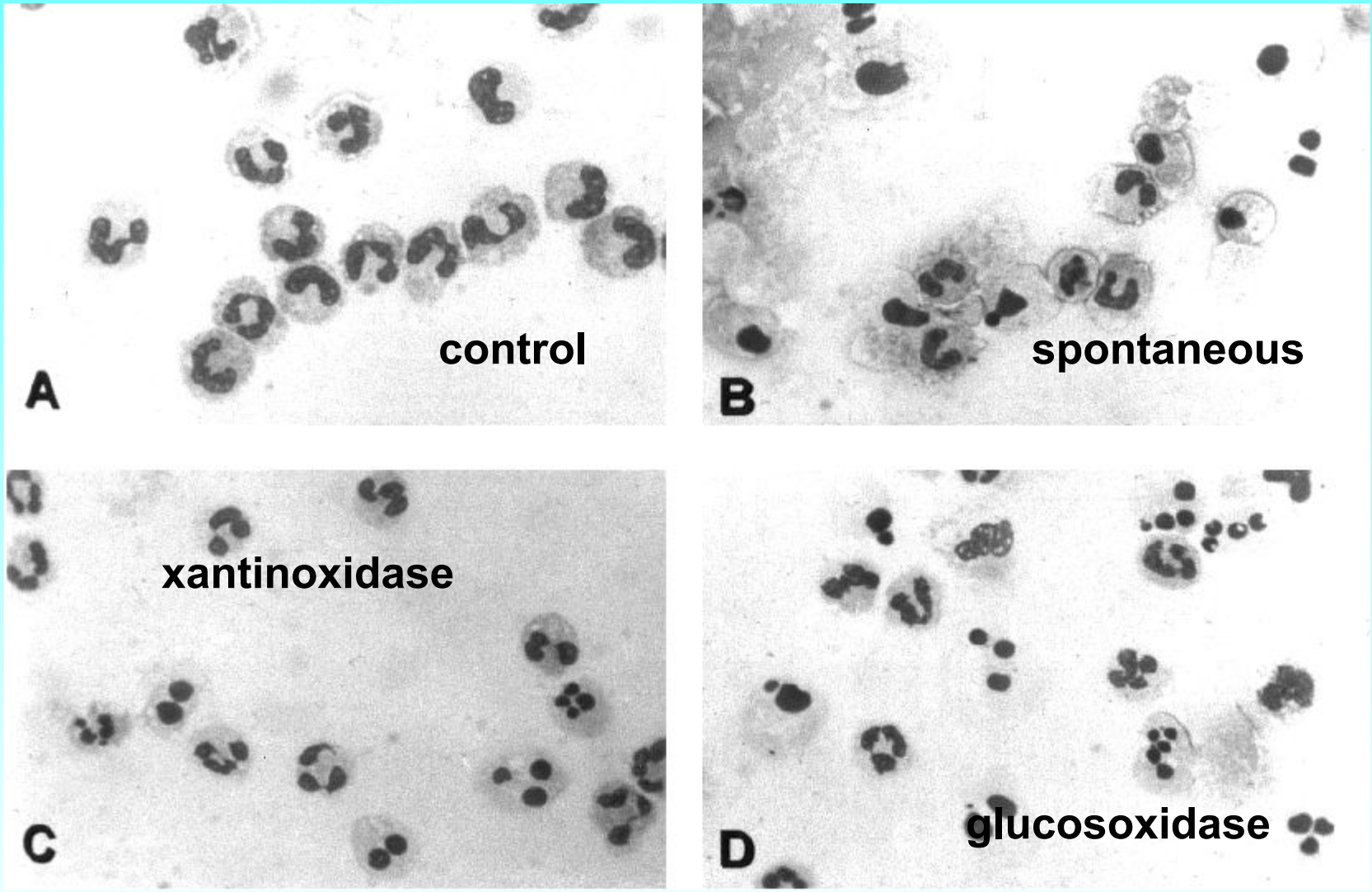
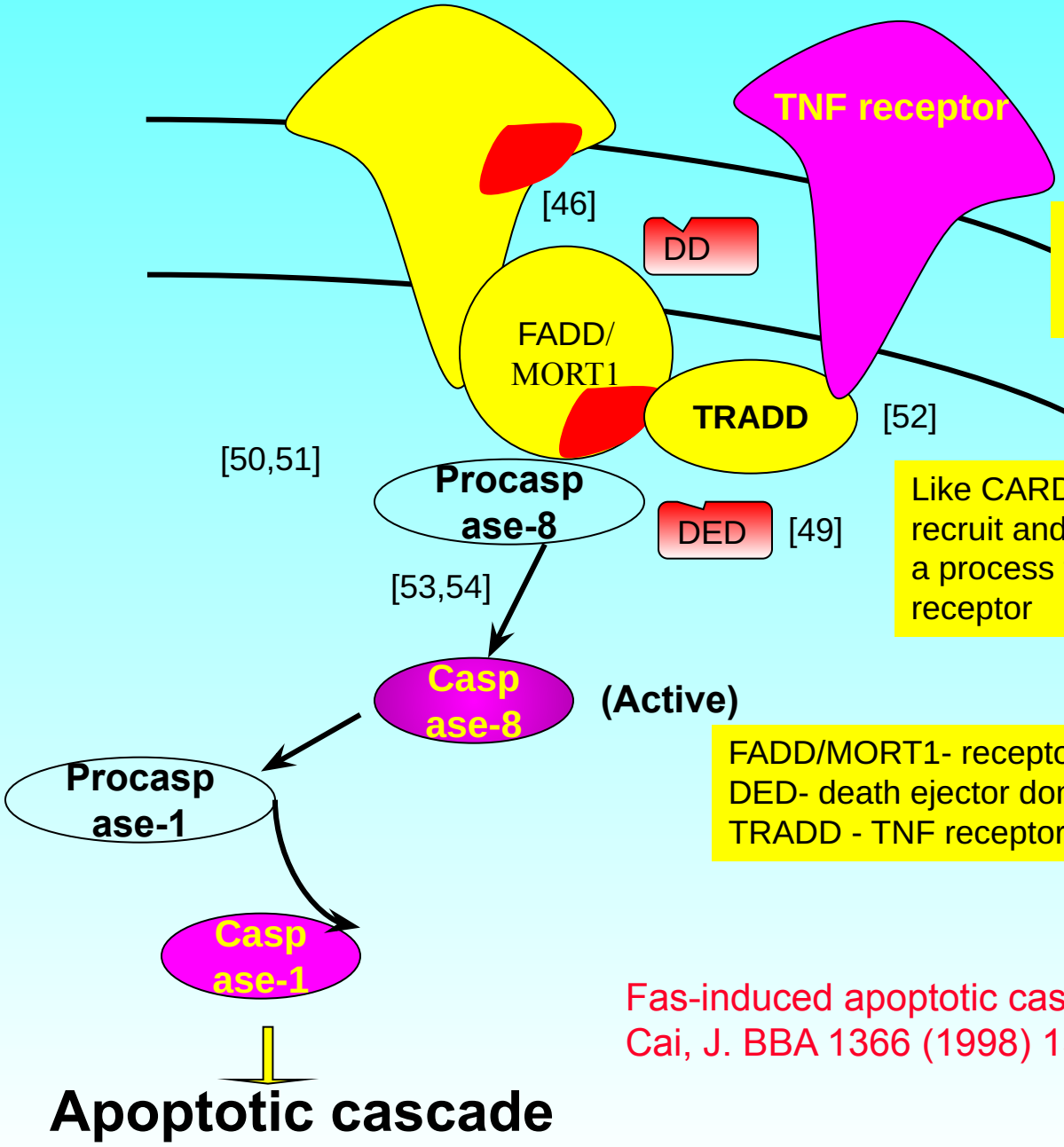


Fig. 4. Morphology of apoptotic neutrophils. 100,000 purified neutrophils were cytocentrifuged and the slides were then fixed and stained as indicated in Materials and Methods. (A) Freshly purified neutrophils; (B) purified neutrophils incubated in standard culture conditions for 22 h (spontaneous apoptosis); (C) purified neutrophils incubated with 27 mU/ml XO for 10 h; (D) purified neutrophils incubated with 5 mU/ml GO for 10 h.

Немитохондриальный путь активации апоптоза



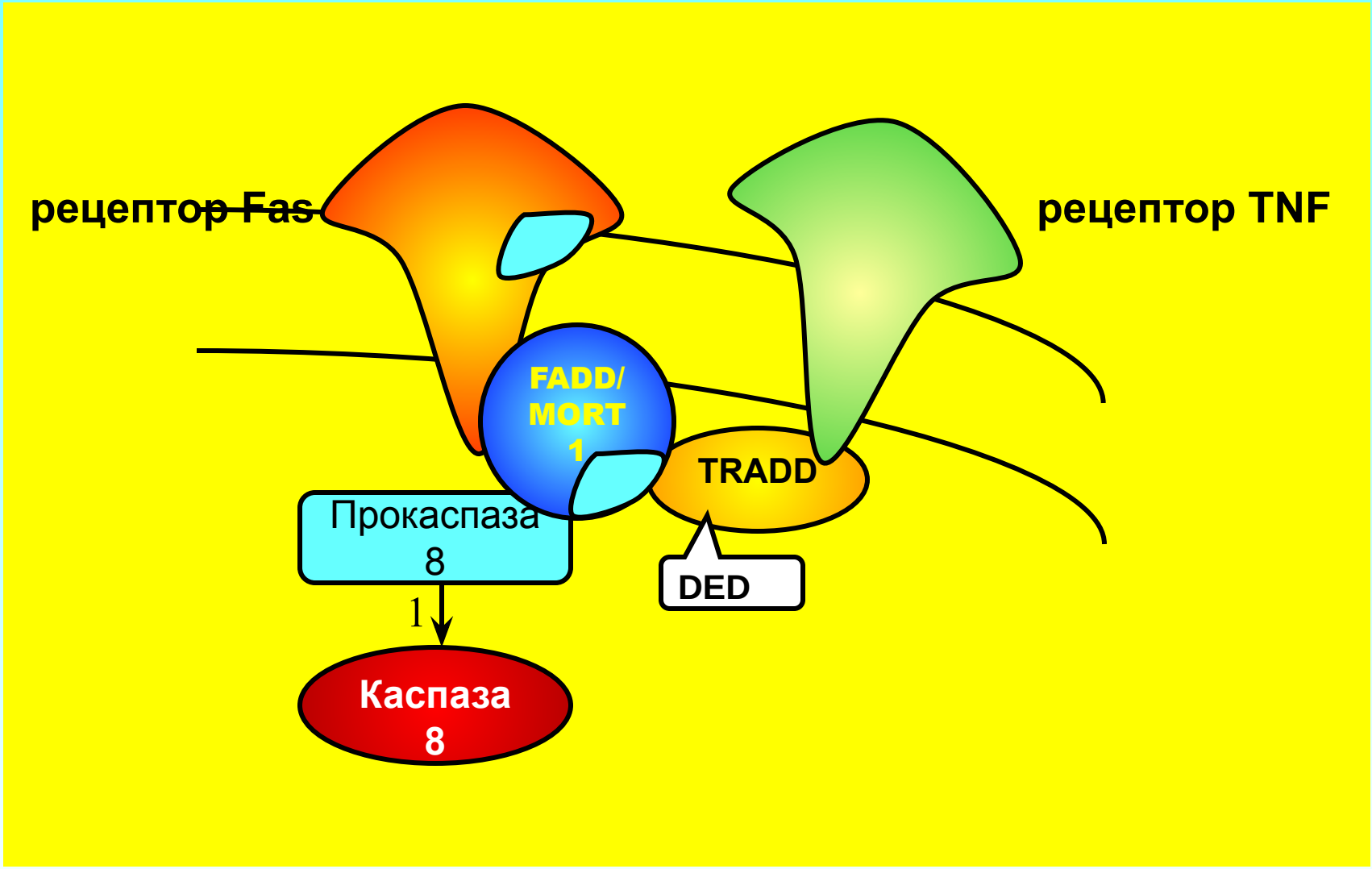
DED is shared by FADD/MORT1, caspase-8 and TRADD (TNF receptor associated protein) [52].

Like CARD, DED can be used by FADD to recruit and activate caspase-8 [53,54], in a process that occurs within minutes after receptor

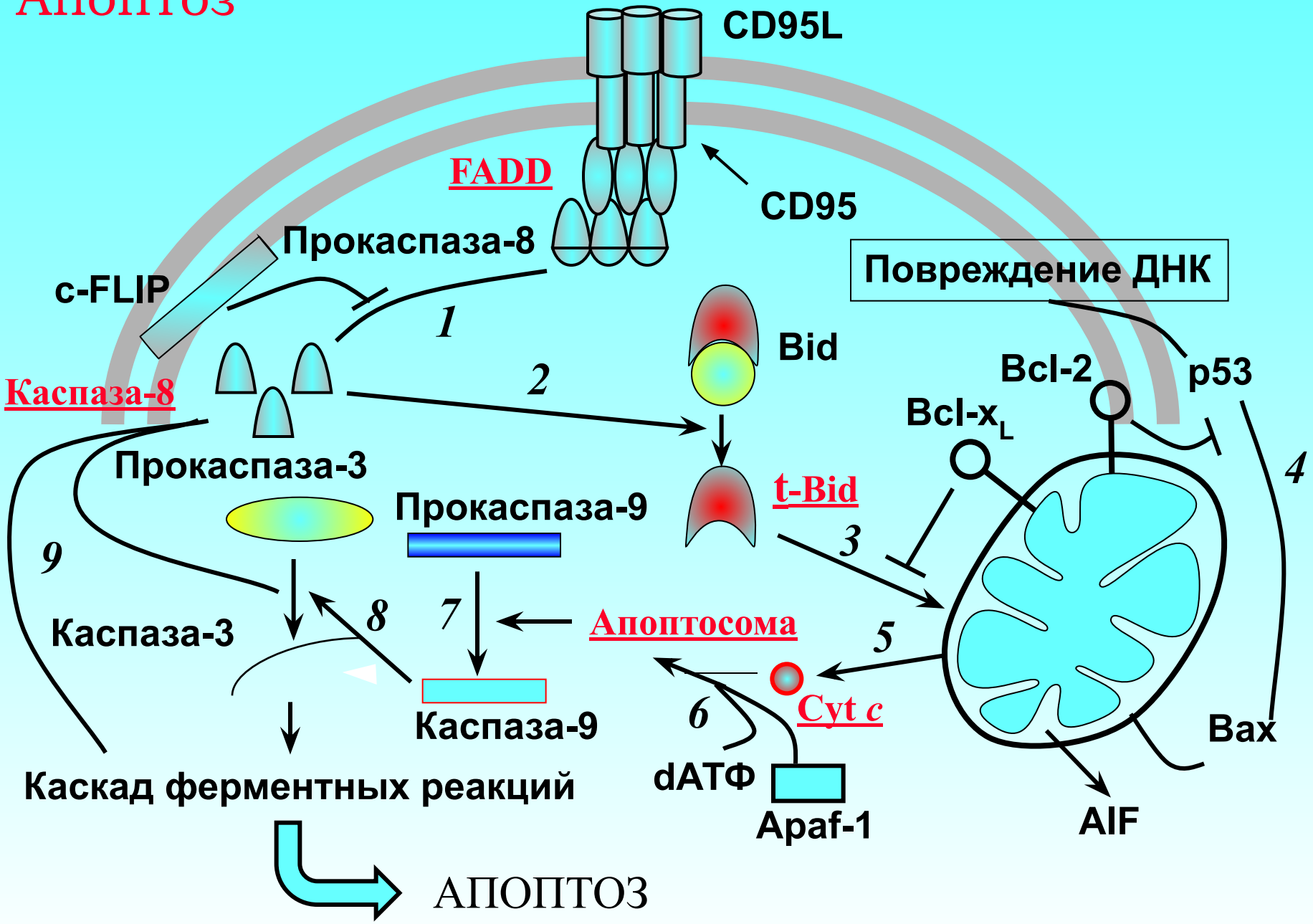
FADD/MORT1- receptor associated protein
DED- death ejector domain of FADD/MORT1
TRADD - TNF receptor associated protein

Fas-induced apoptotic cascade (on the basis of Cai, J. BBA 1366 (1998) 139-149)

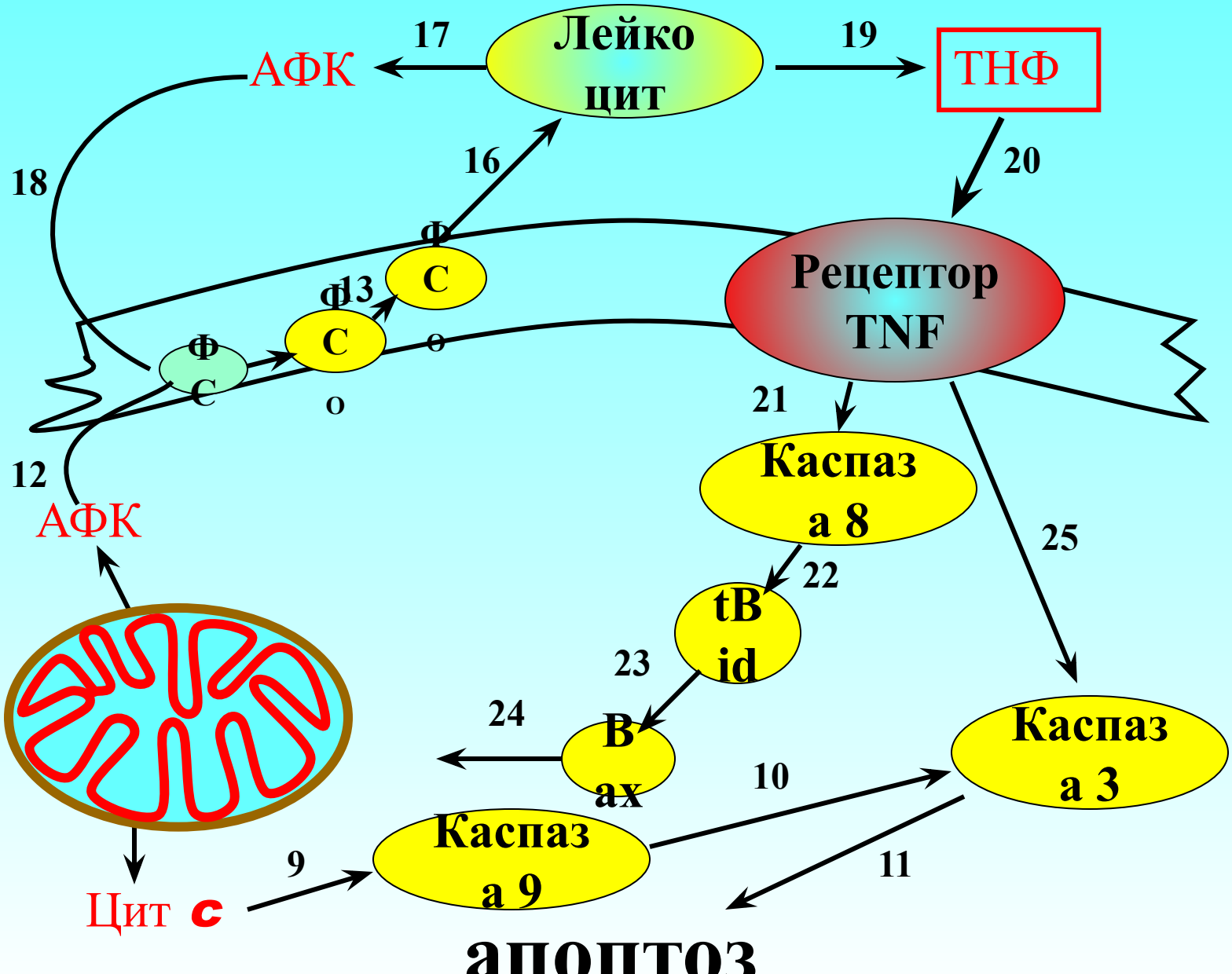
Активация каспазы 8 после связывания апоптогена с мембранным рецептором



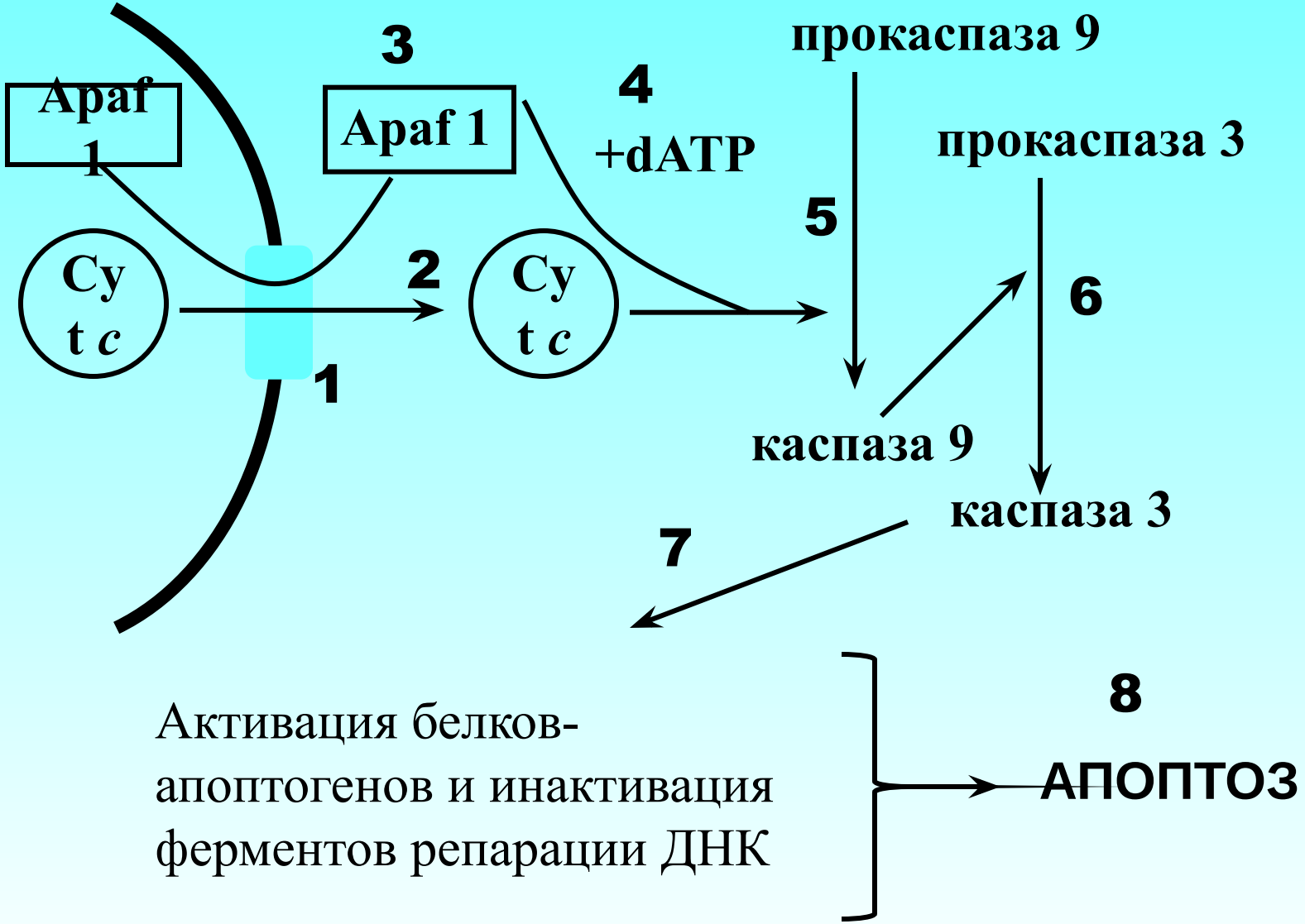
Апоптоз


CD95L
FADD
CD95
Прокаспаза-8
c-FLIP
Повреждение ДНК
1
Bid
2
Bcl-2
p53
Каспаза-8
Bcl-x$_L$
Прокаспаза-3
t-Bid
4
Прокаспаза-9
3
9
8
7
Апоптосома
5
Каспаза-3
Cyt c
6
Каспаза-9
Bax
dATФ
Каскад ферментных реакций
Apaf-1
AIF
АПОПТОЗ

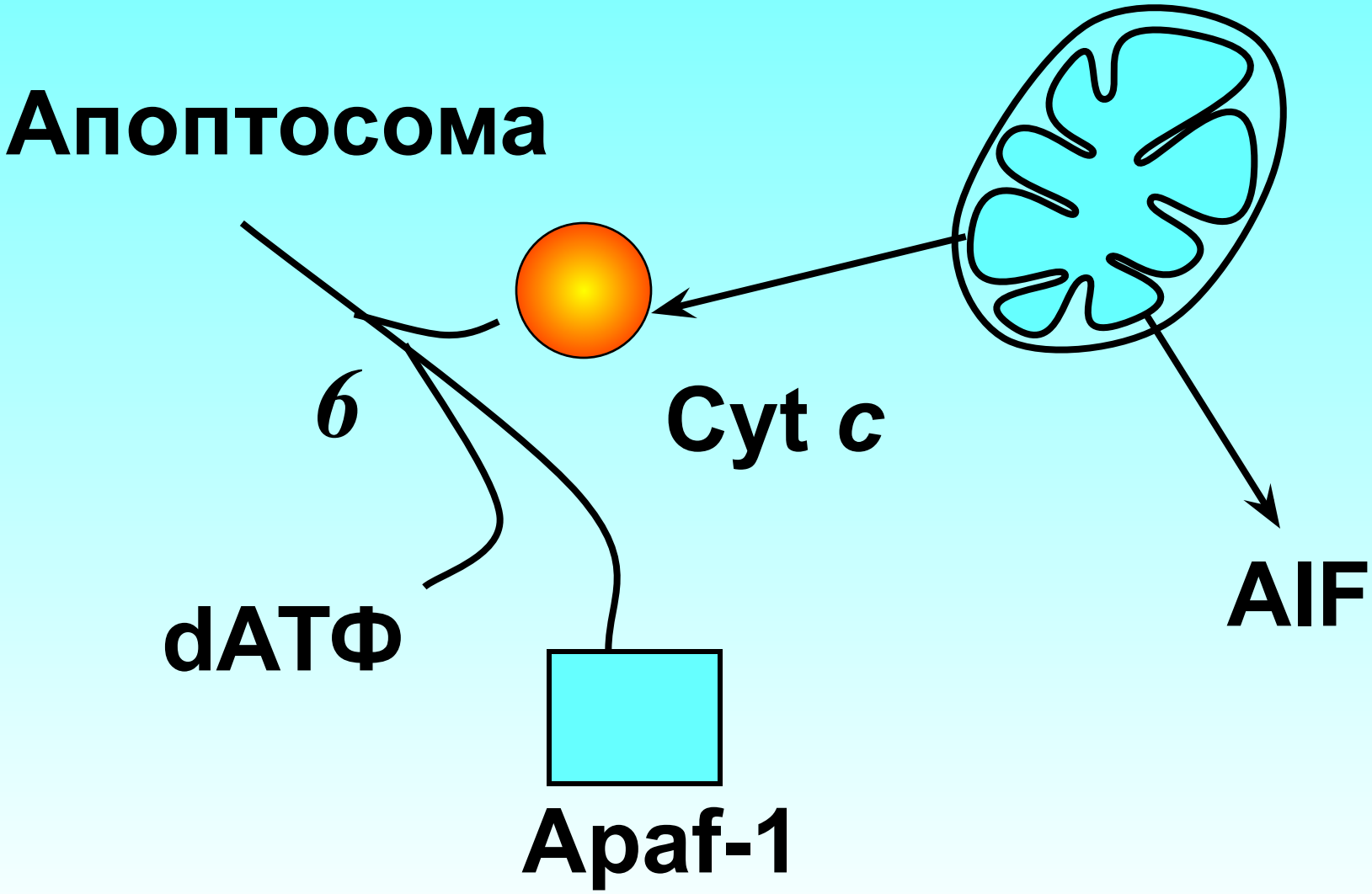
Передача апоптозных сигналов (по В. П. Скулачеву)



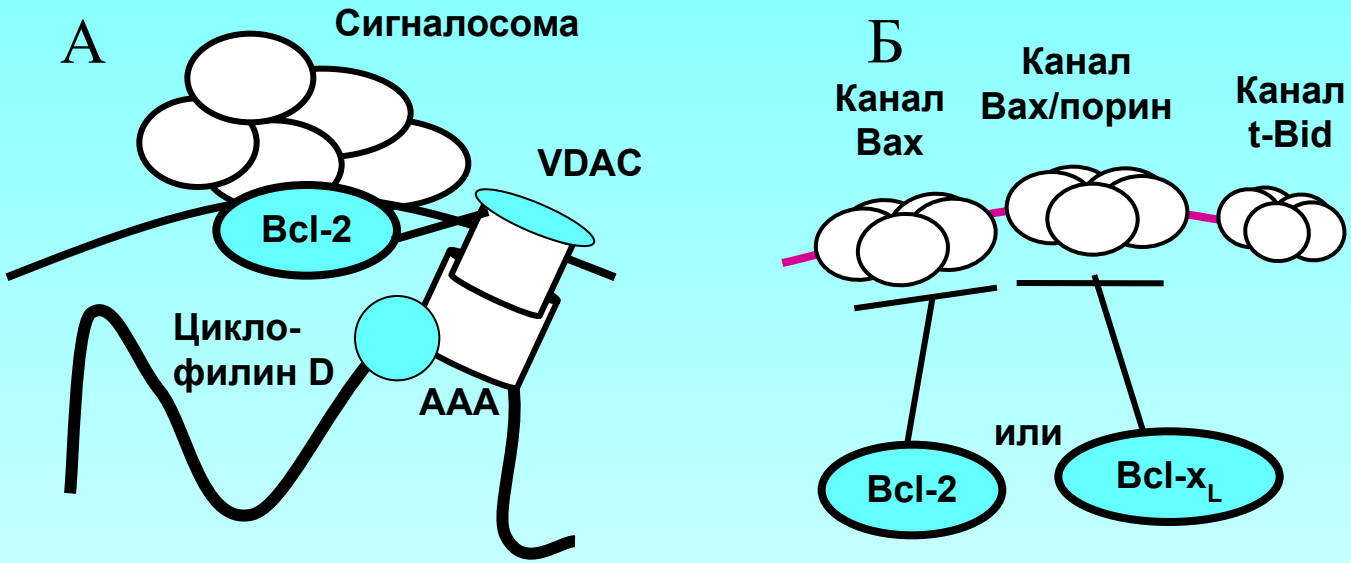
Каскад активации апоптоза, инициированный выходом цитохрома *c*



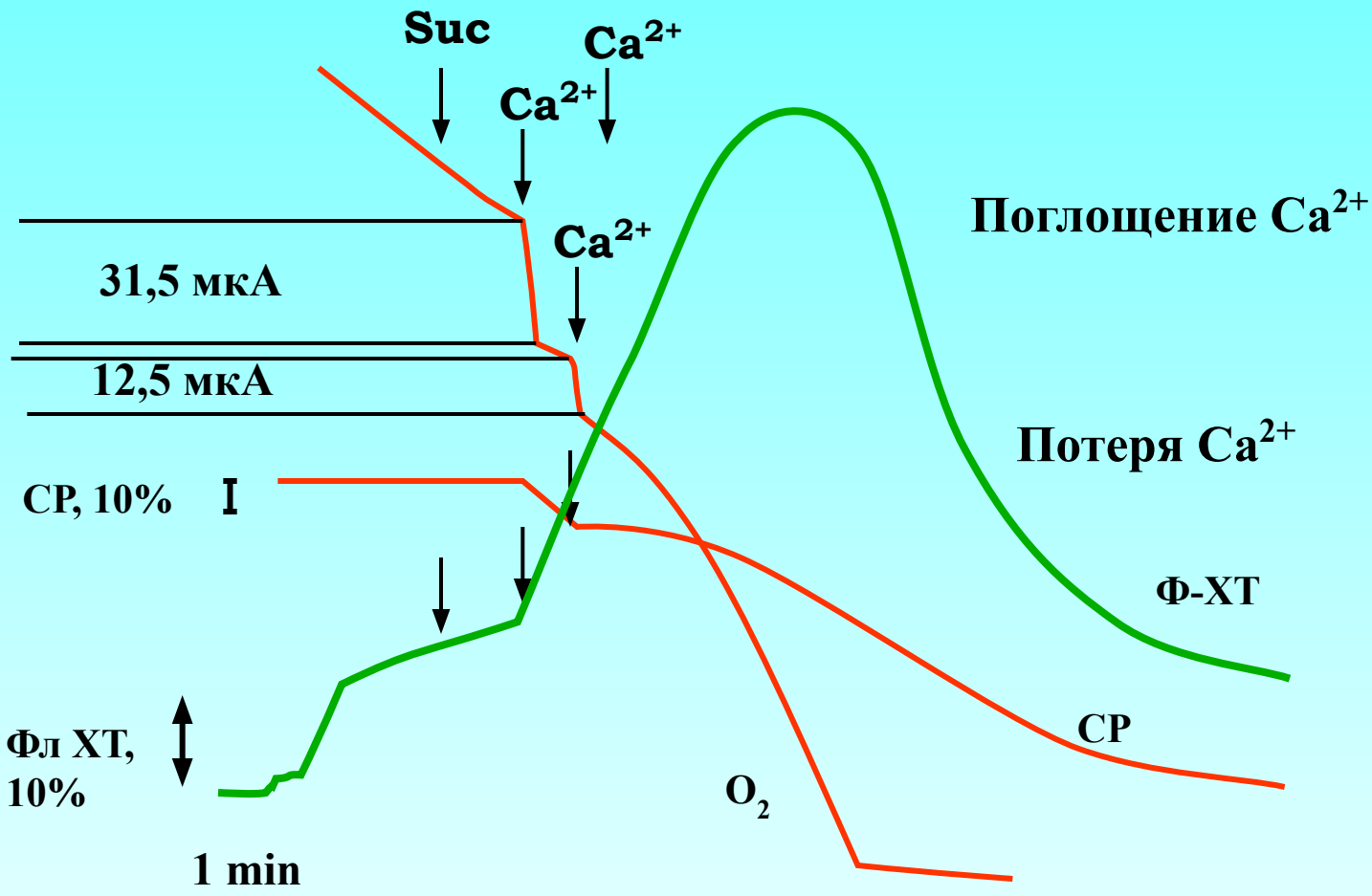
Образование апоптосомы



Каналы в мембранах митохондрий



Изменение флуоресценции митохондрий, окрашенных тетрациклином

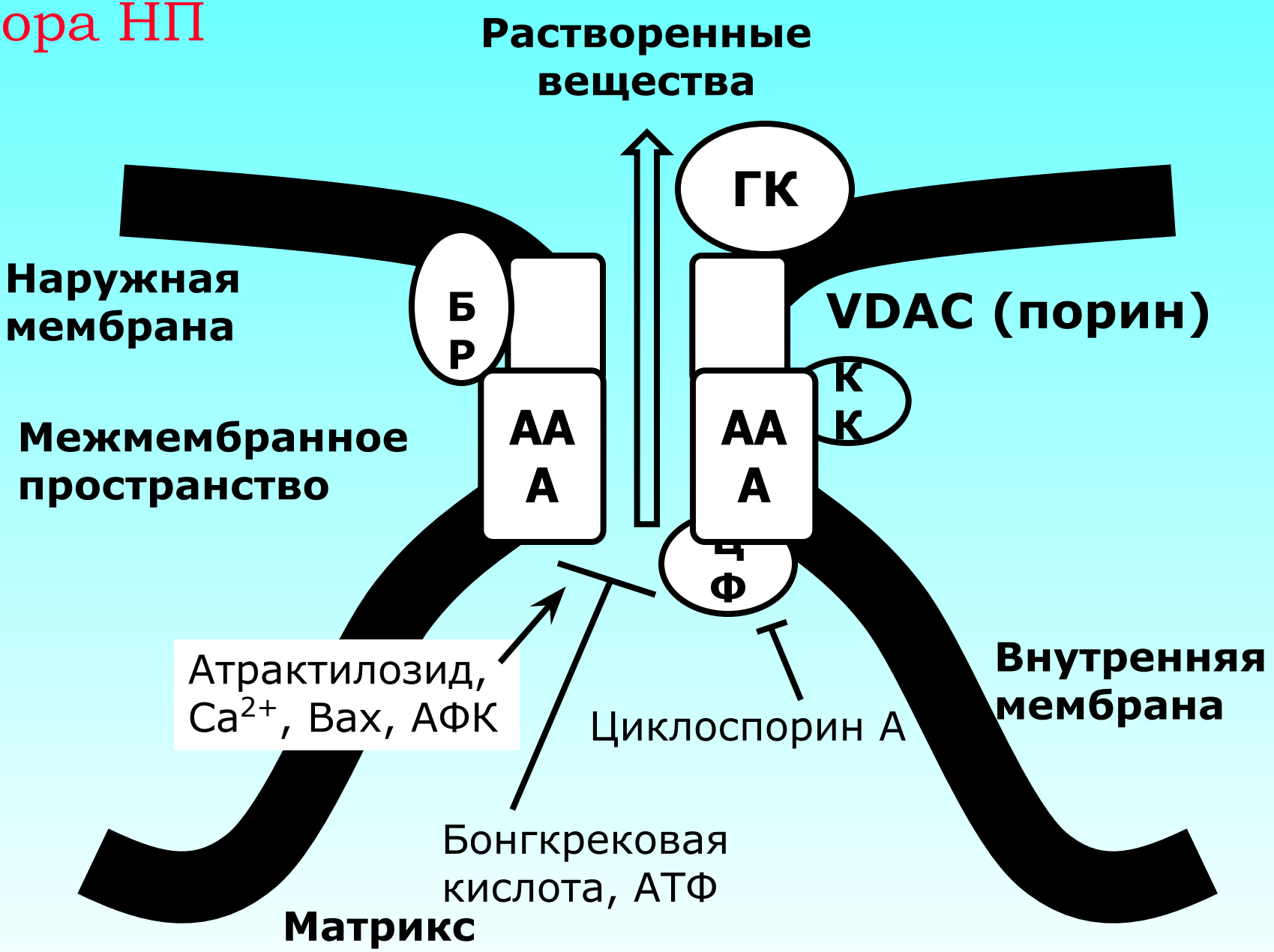


Ф-ХТ - флуоресценция хлортетрациклина

СР - светорассеяние

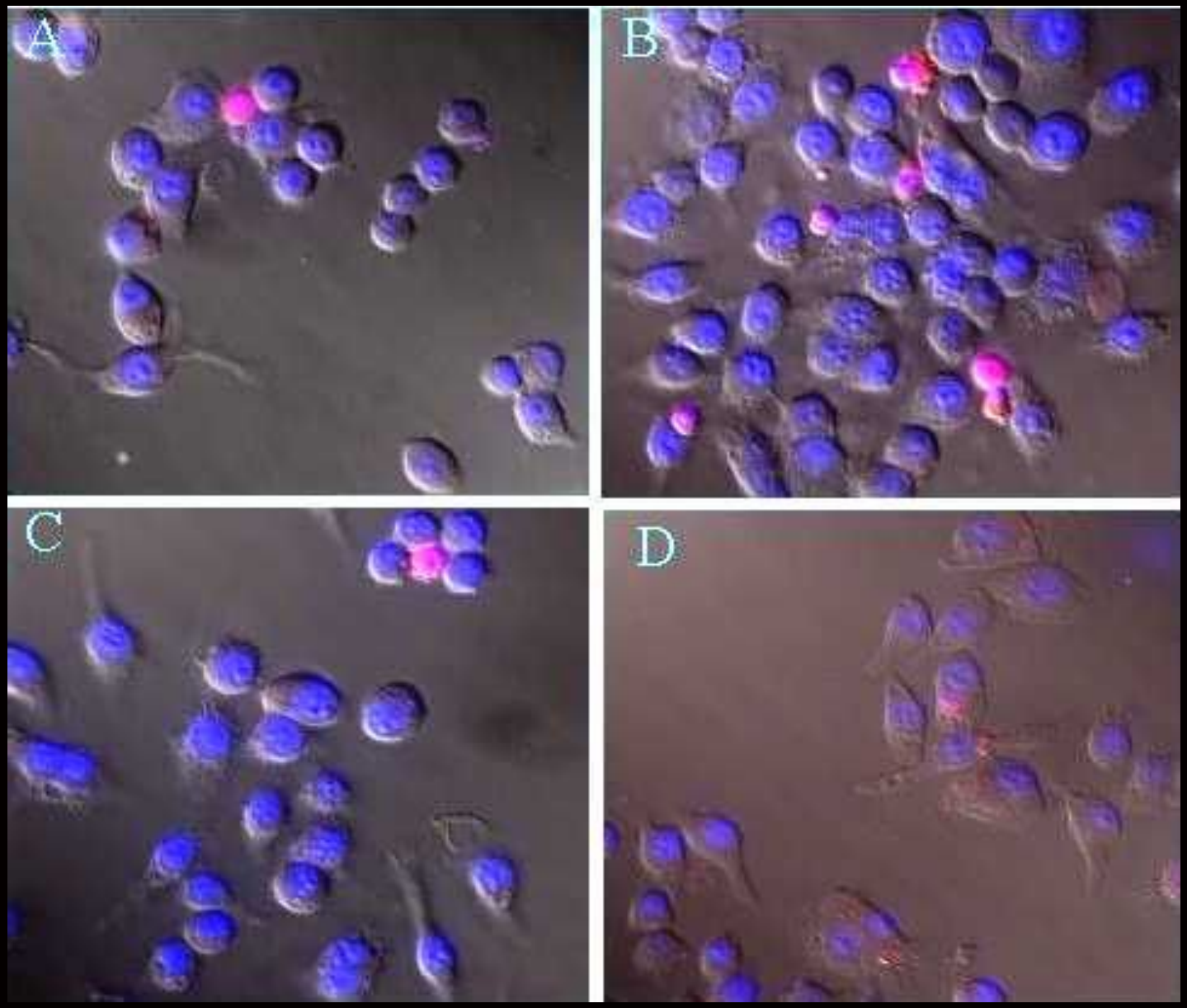
O_2 - концентрация кислорода

Пора НП

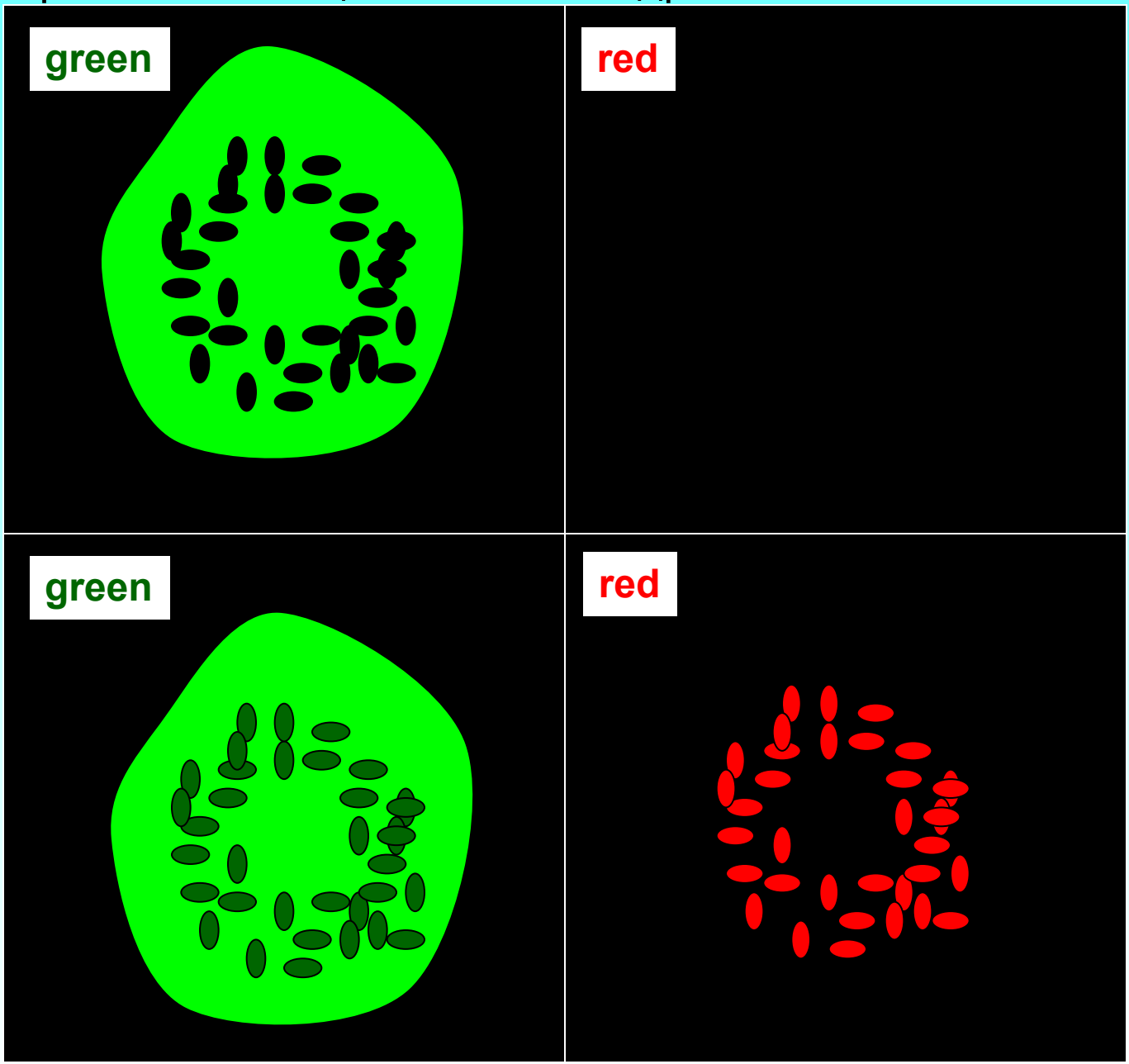

Растворенные вещества
ГК
Наружная мембрана
Б Р
VDAC (порин)
К К
Межмембранное пространство
ААА
ААА
Ц Ф
Атрактилозид, Ca2+, Bax, АФК
Циклоспорин А
Внутренняя мембрана
Бонгкрековая кислота, АТФ
Матрикс

Конфокальная лазерная микроскопия

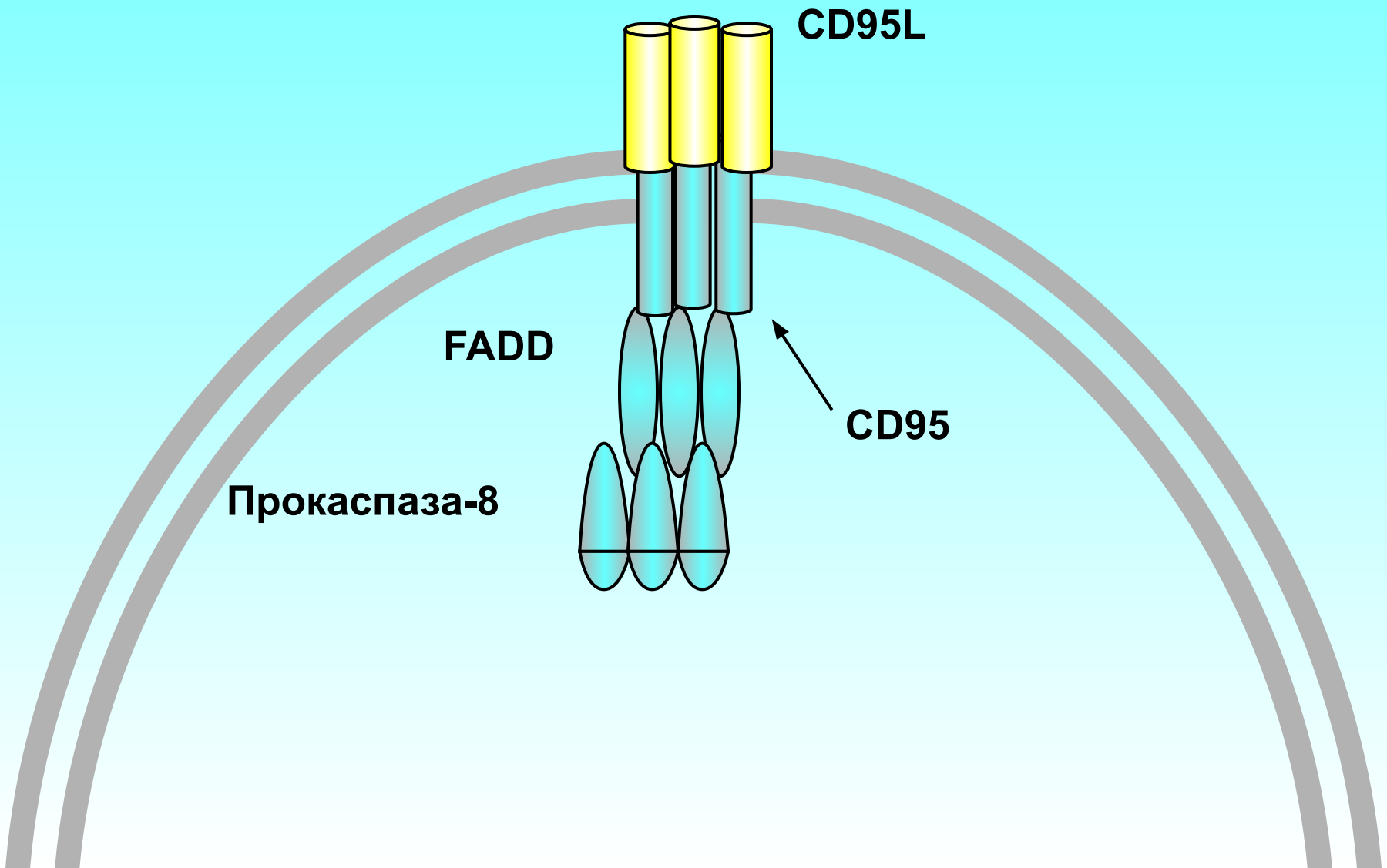
Фагоциты, вступающие в апоптоз



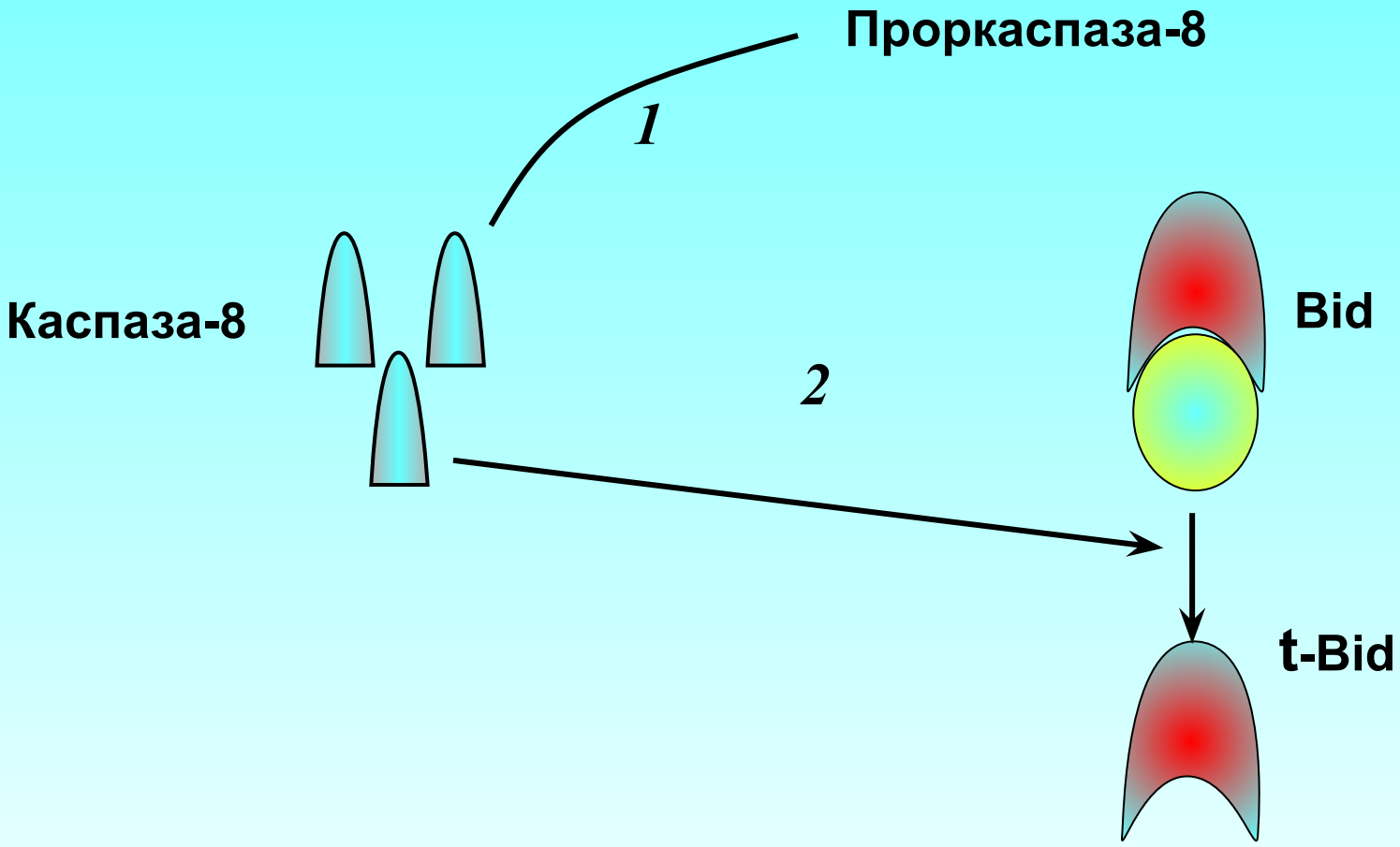
Мембранный потенциал в митохондриях живых клеток



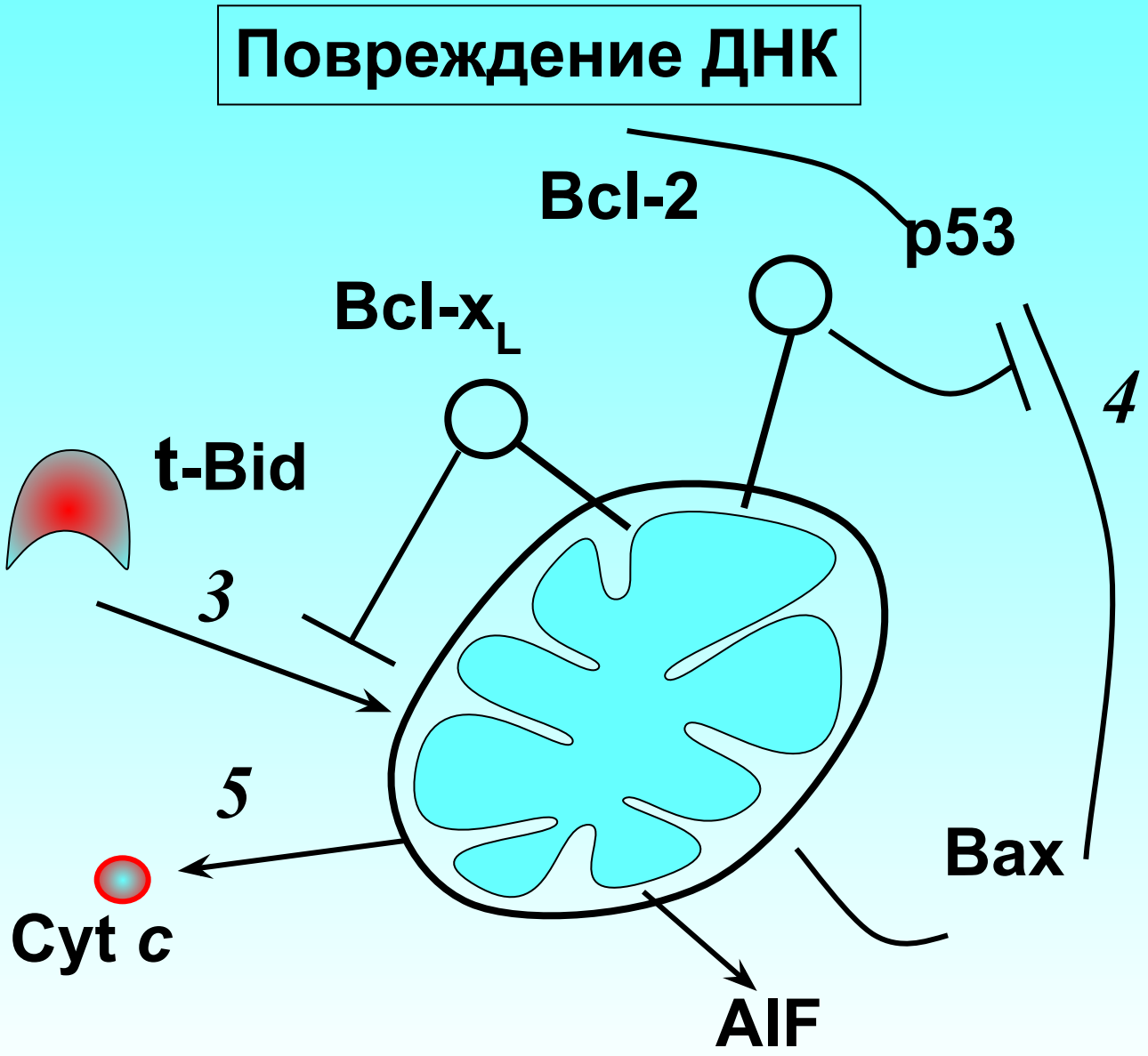
Мембранная фаза апоптоза: связывание прокаспазы 8



Подготовка к активации митохондрий

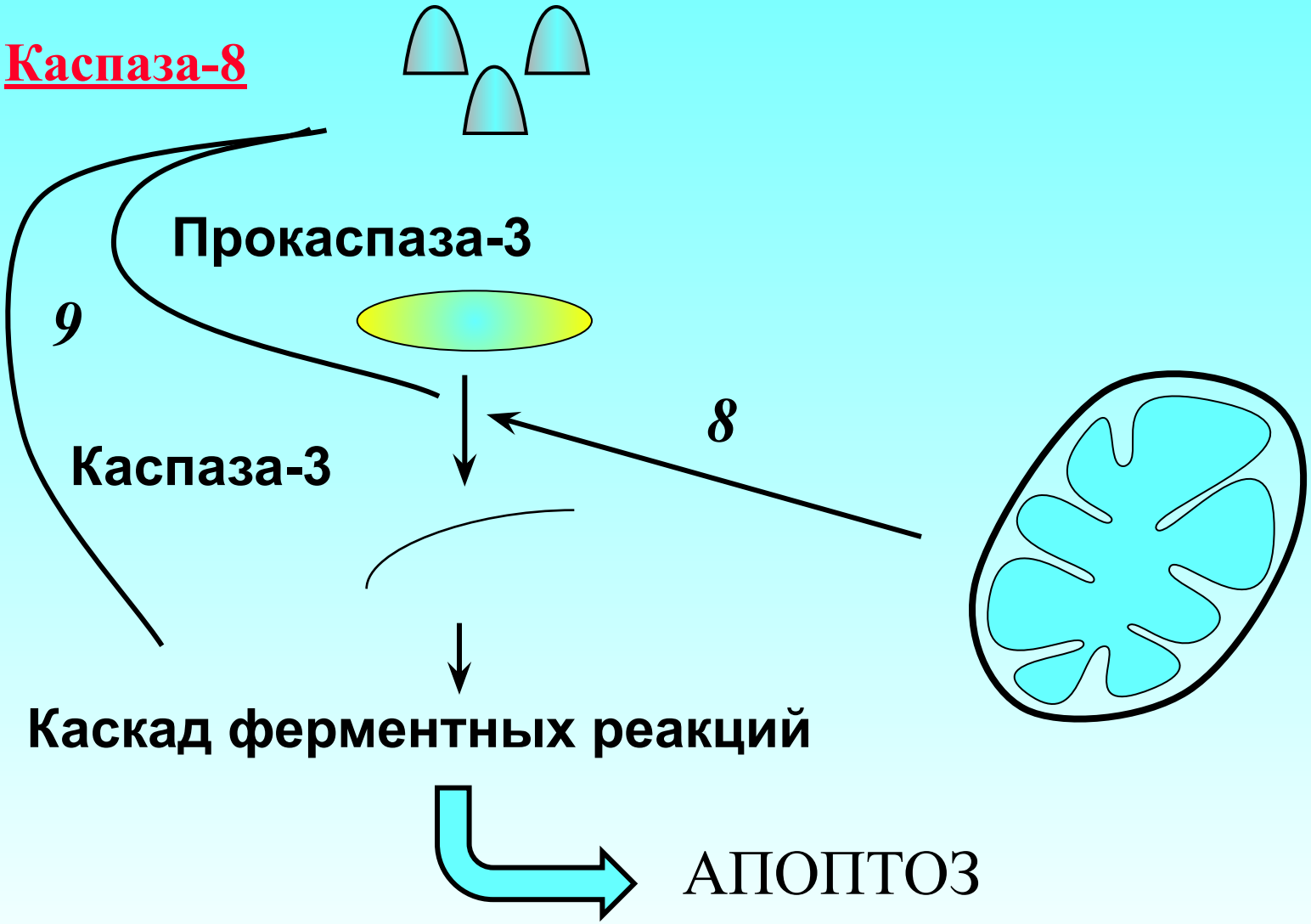


Выход цитохрома *c* из митохондрий

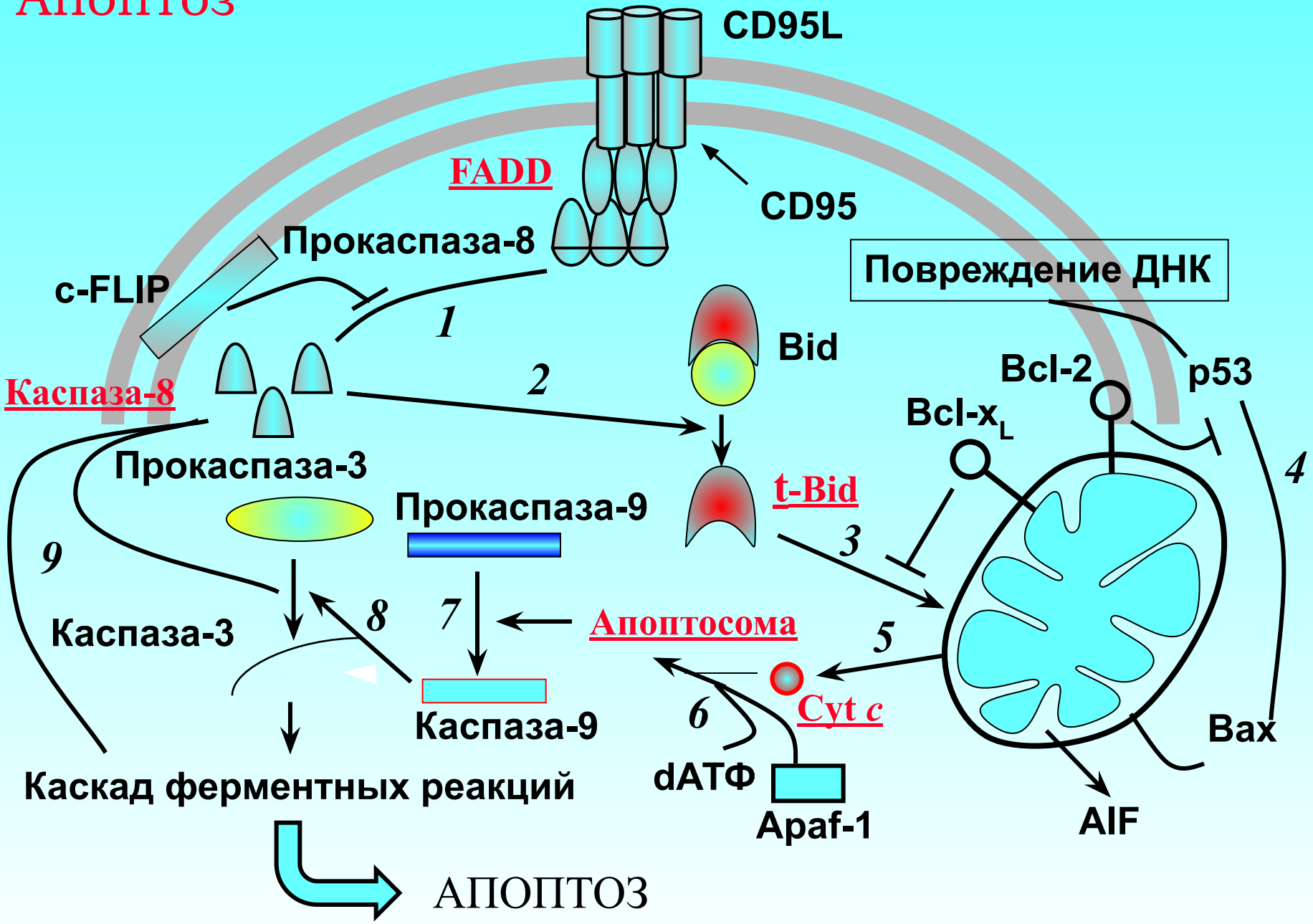


Апоптозный каскад реакций каспаз 3 → 9

Вне-митохондриальная активация апоптоза



Апоптоз


CD95L
FADD
CD95
Прокаспаза-8
c-FLIP
Повреждение ДНК
1
Bid
2
Bcl-2
p53
Каспаза-8
Bcl-x$_L$
Прокаспаза-3
t-Bid
4
Прокаспаза-9
3
9
8
7
Апоптосома
5
Каспаза-3
Cyt c
6
Каспаза-9
Bax
dATФ
Каскад ферментных реакций
Apaf-1
AIF
АПОПТОЗ

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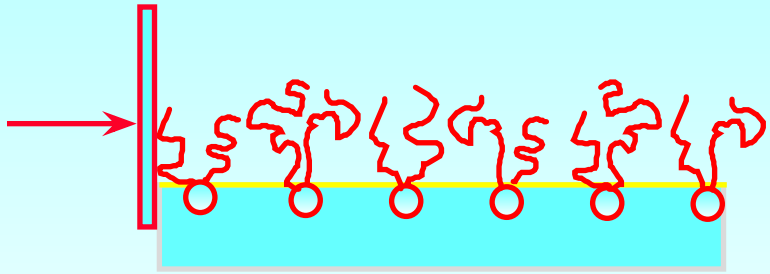
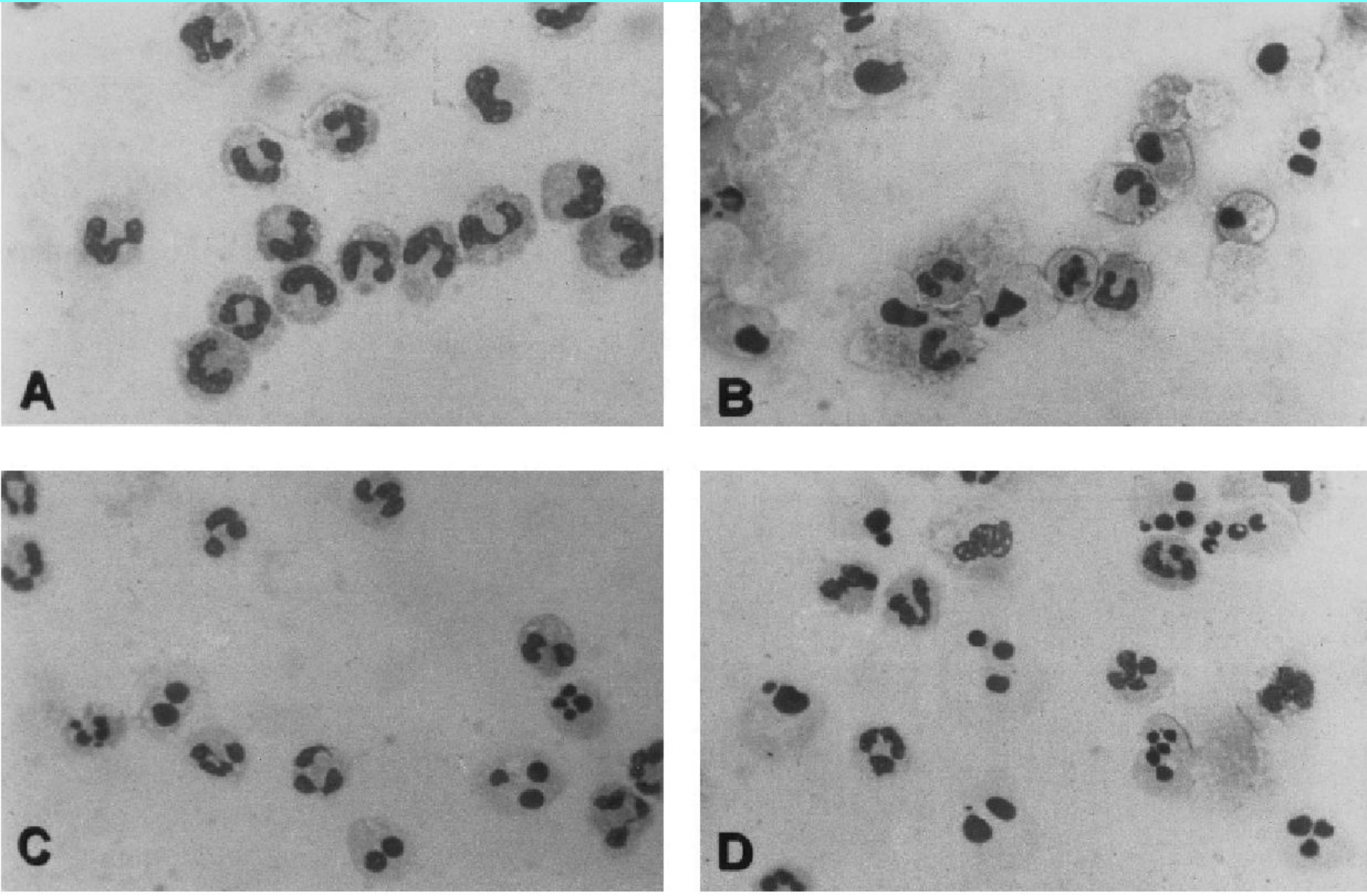
Физико-химические основы патологии клетки

АФК и апоптоз



Морфология нейтрофилов при апоптозе



Апоптоз, вызываемый активными формами кислорода

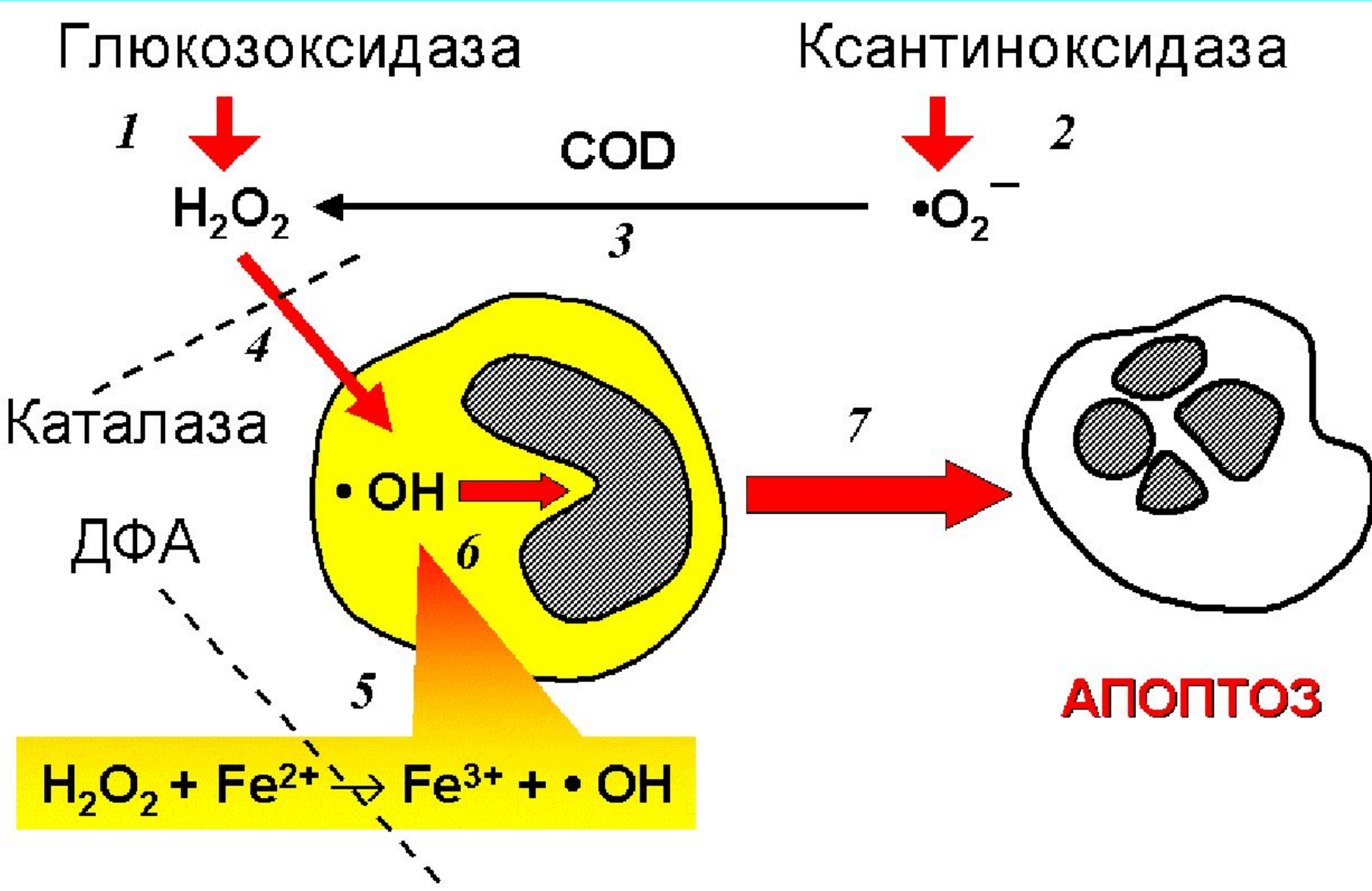
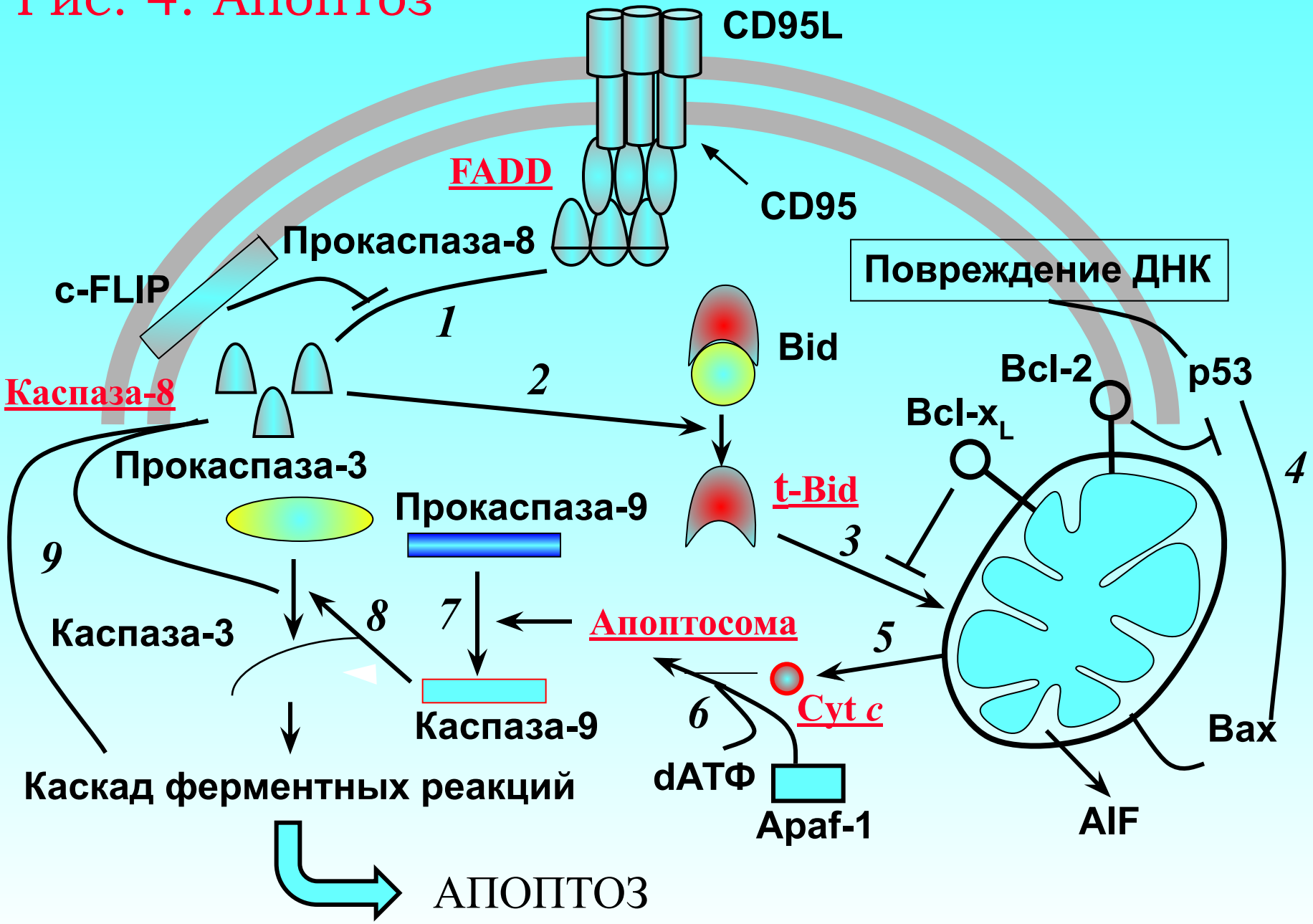
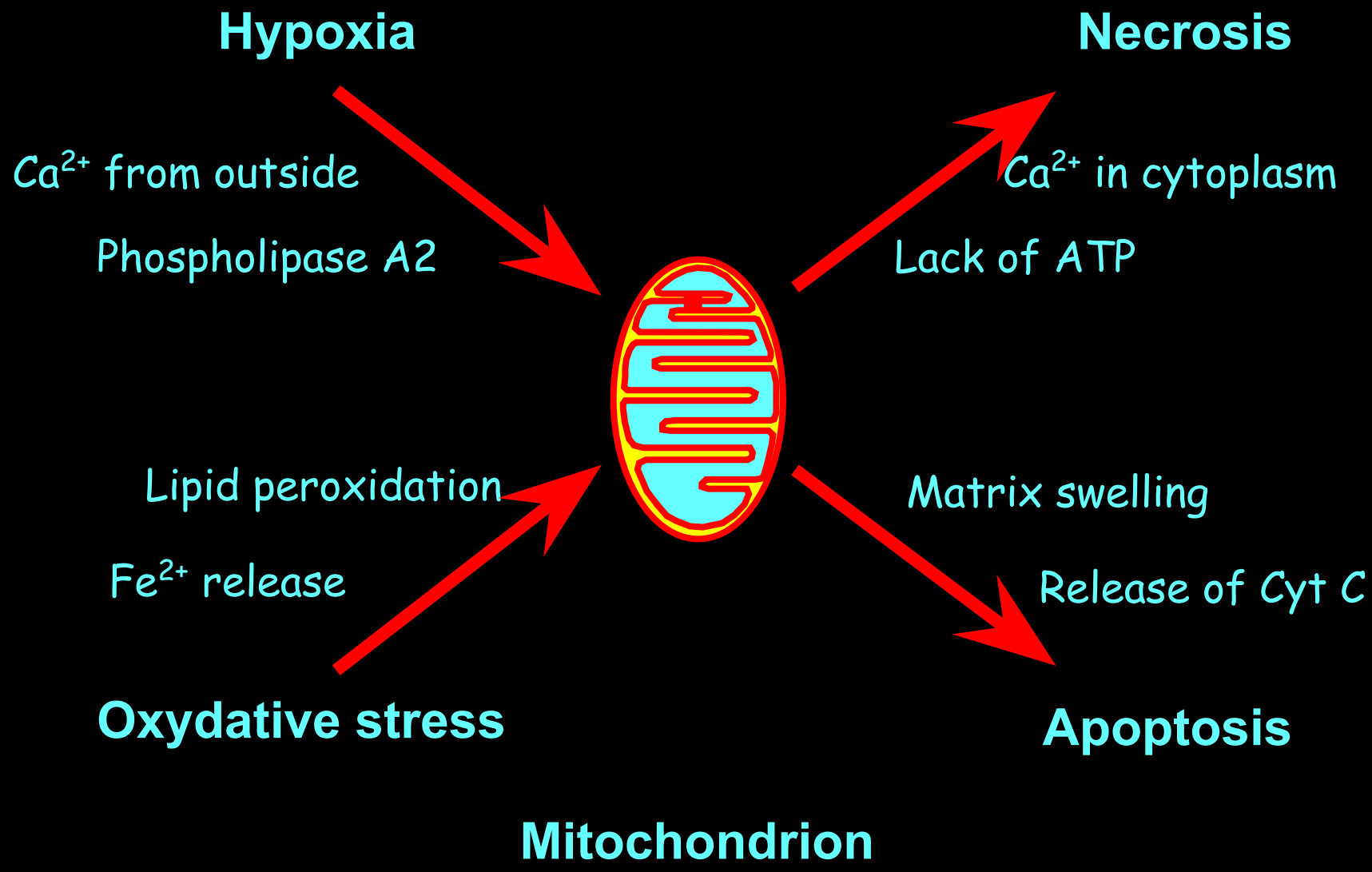


Рис. 4. Апоптоз

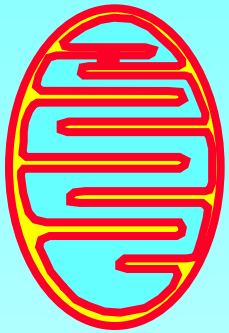


Mitochondrion is a target and a source of injury



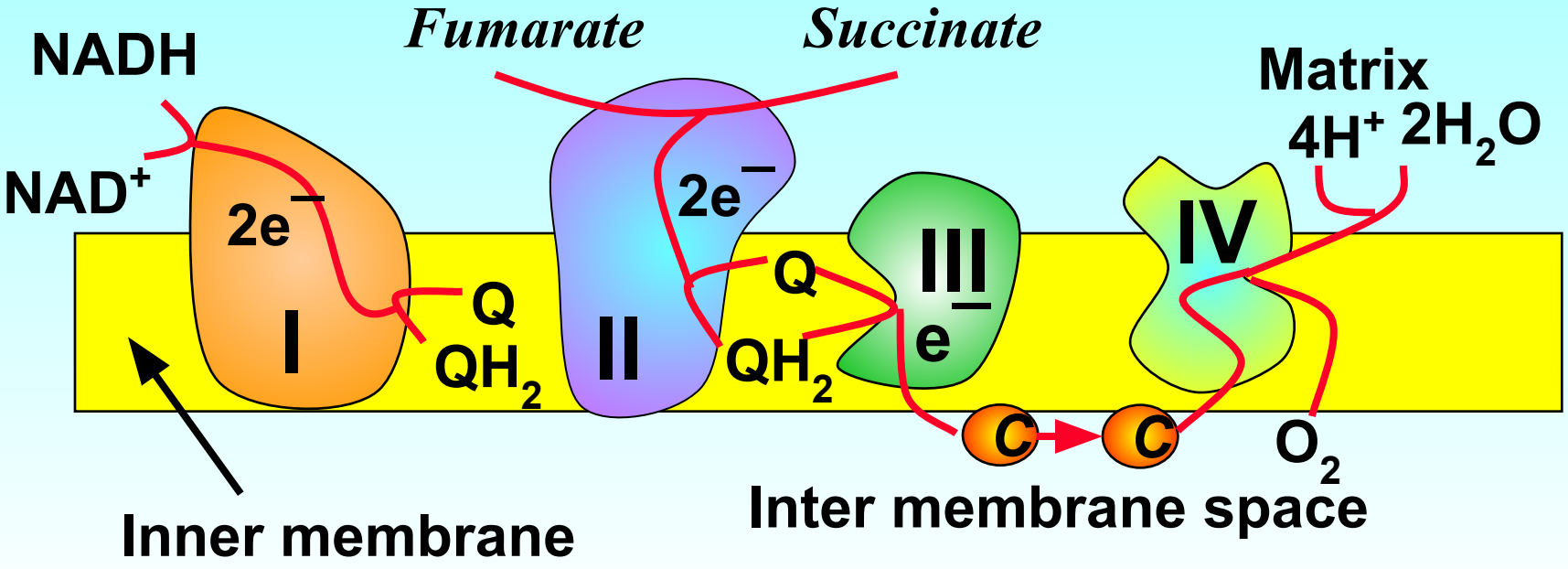
Superoxide manufacturers in the cell

1. NADPH oxidase in plasma membrane
2. Respiratory chain in mitochondria

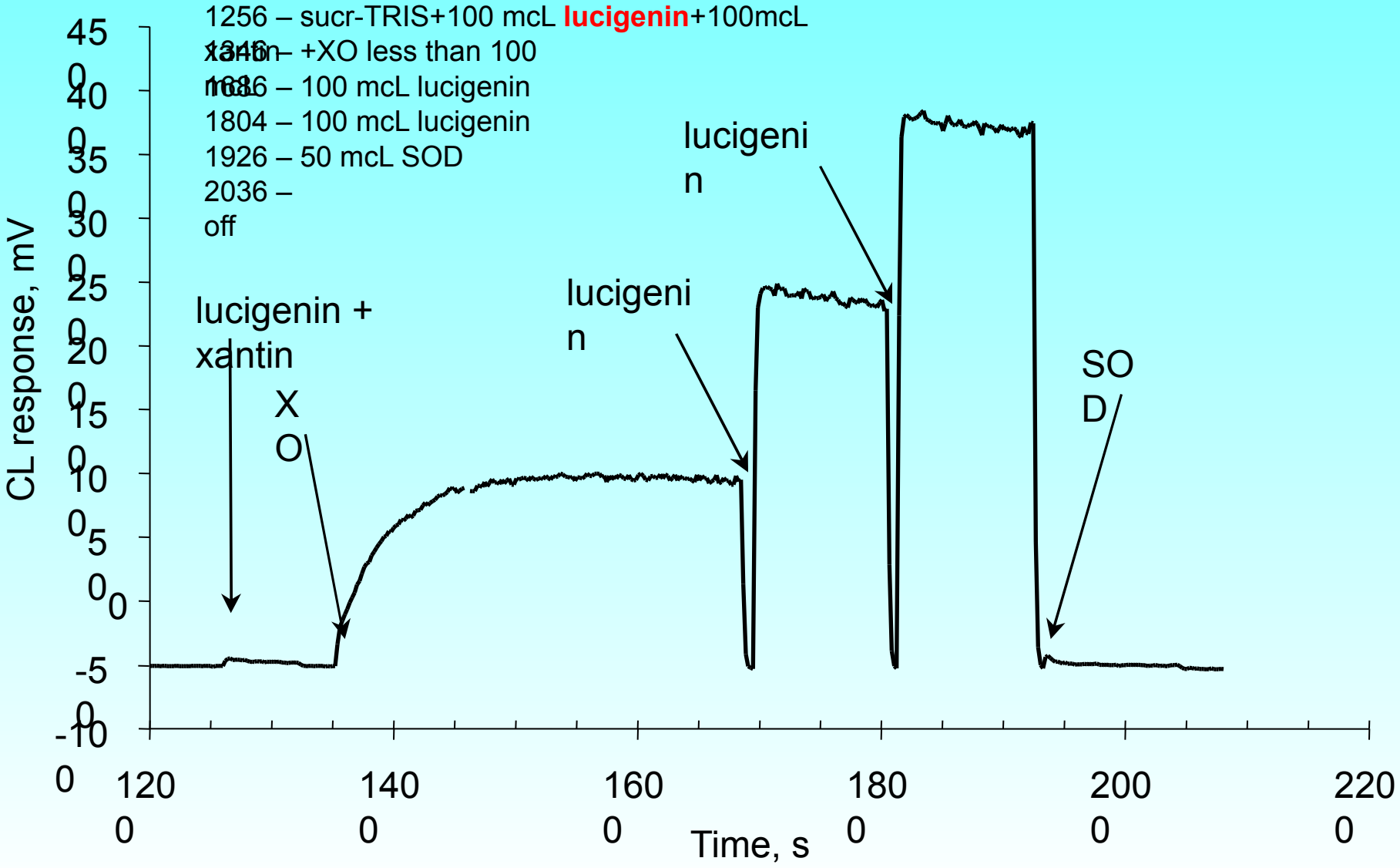


Mitochondrion

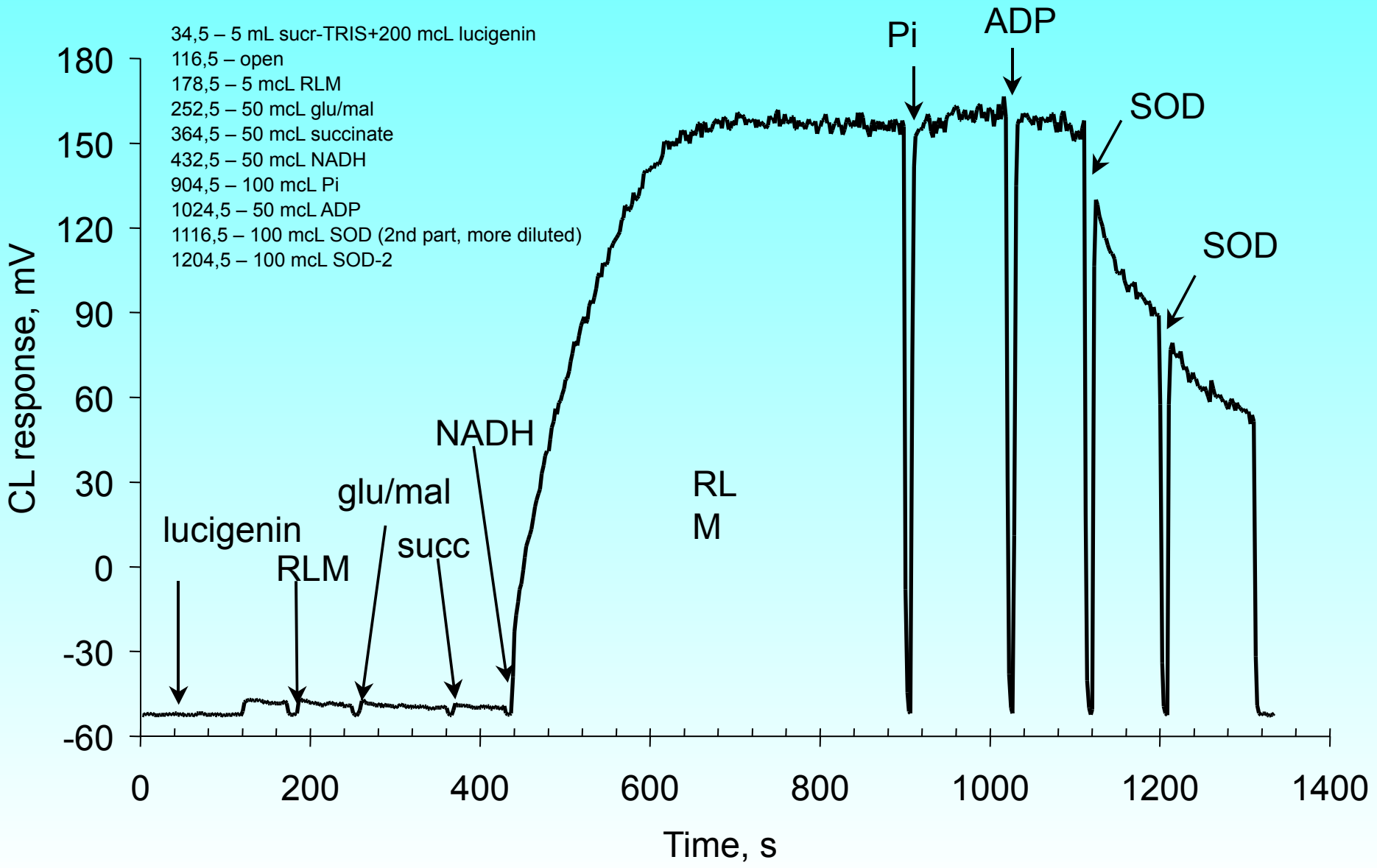
Phagocyte



Lucigenin is an adequate CL-probe for superoxide.

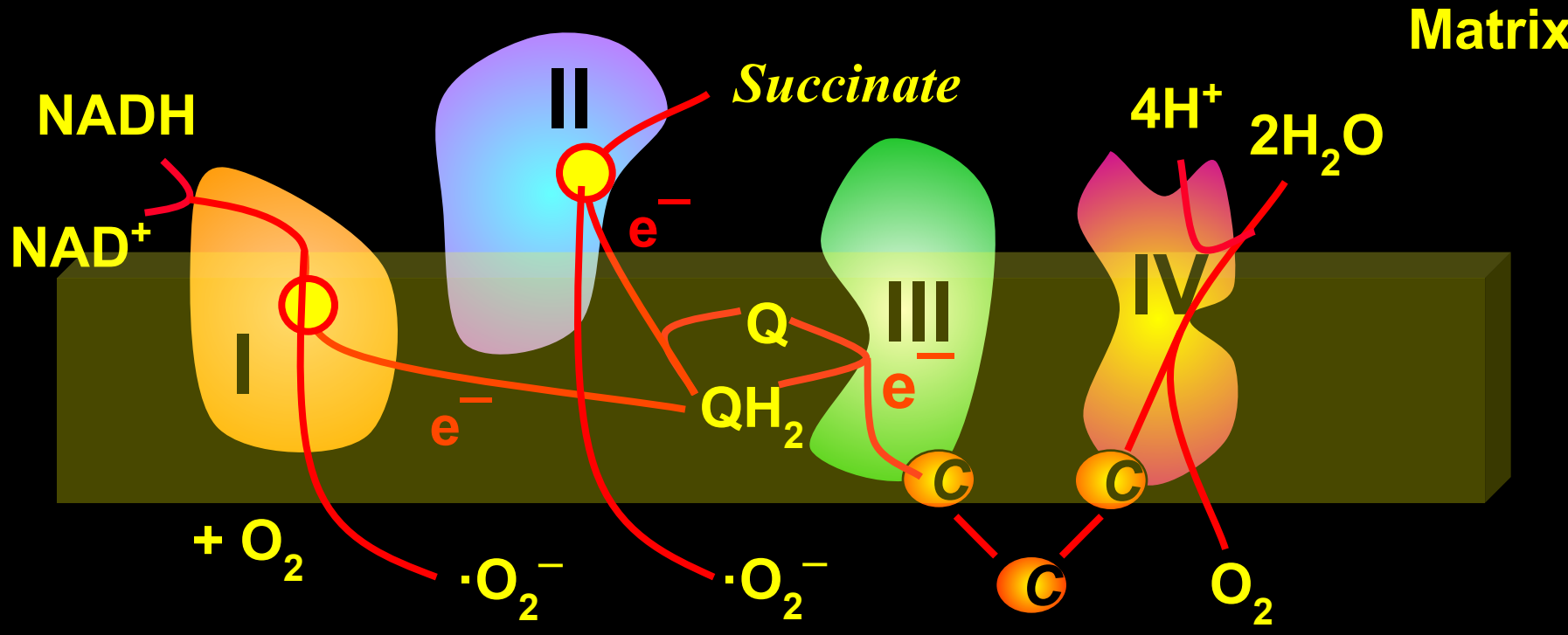


NADH is the best substrate for $\cdot O_2^-$ production.



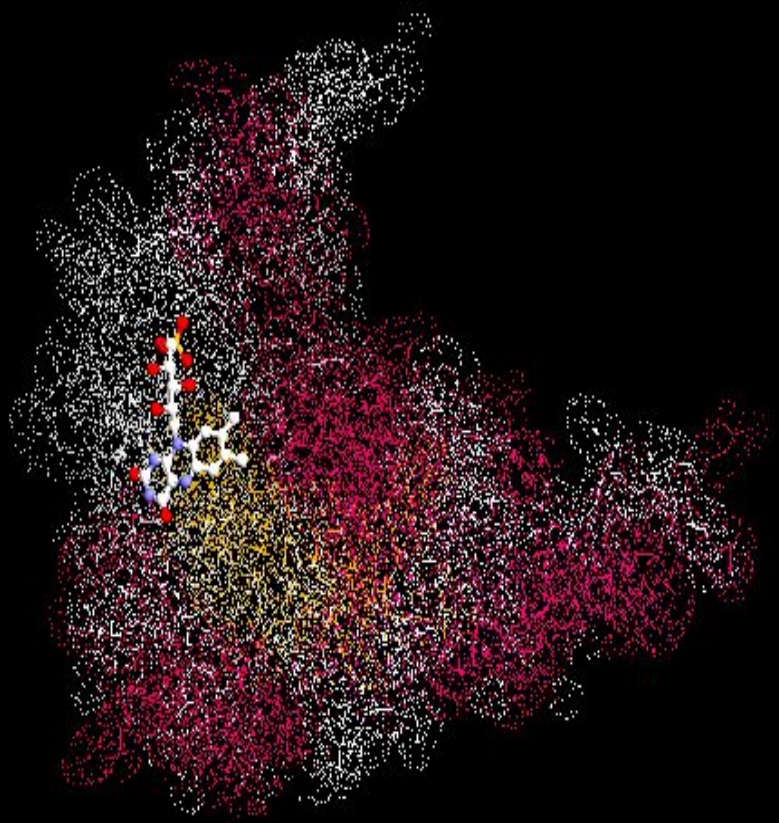
Kinetic control of superoxide production

There are at least two mechanisms regulating the bifurcation of electron fluxes: kinetic and structural.



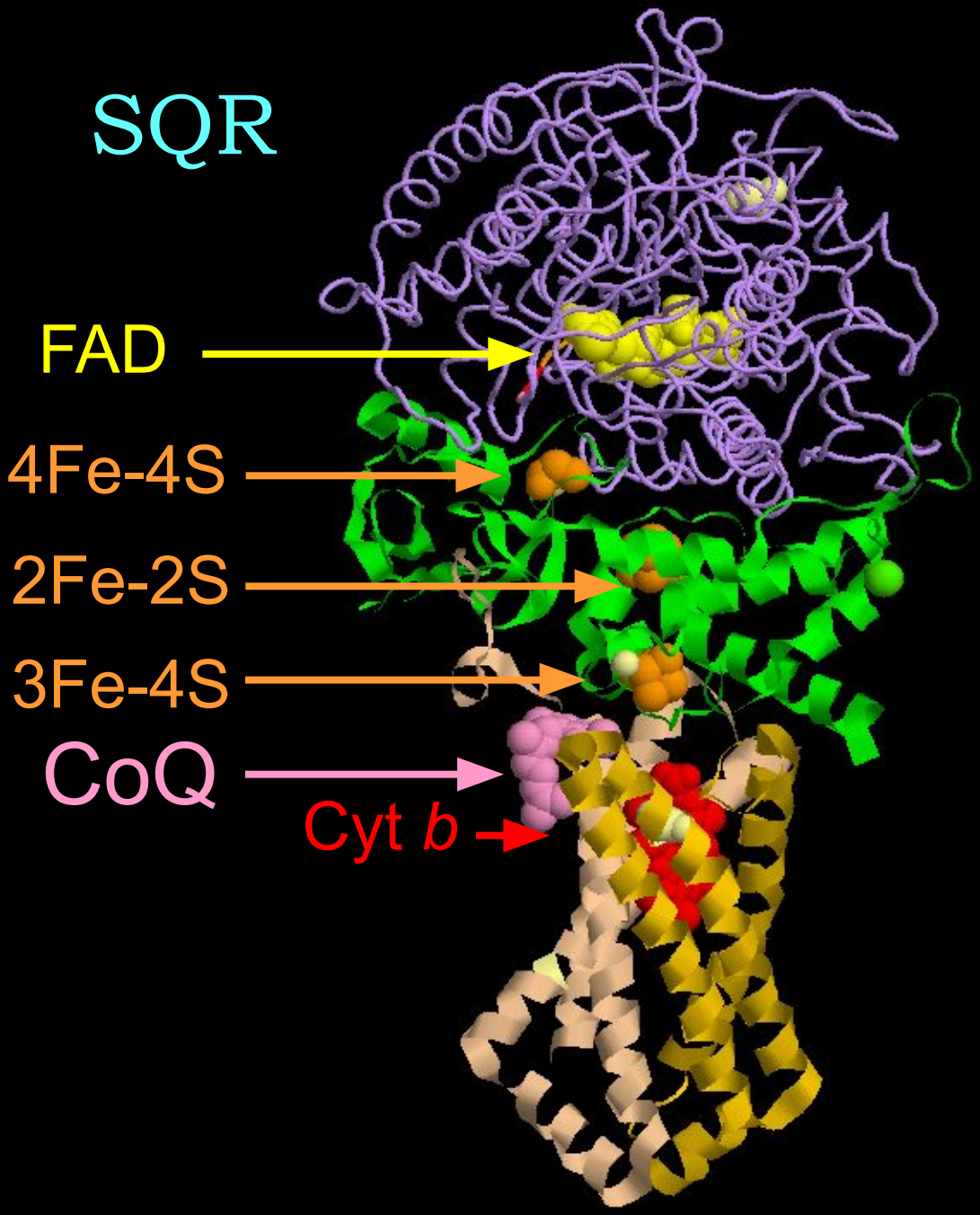
FAD is a probable bifurcation site

NADH oxidase



NAD(P)H – FMN oxidoreductase

E. Coli Succinate Dehydrogenase (SQR) is an analog of mammalian respiratory Complex II



Manifestations of mutations in SQR gene in eukaryotes

Clinical phenotypes:

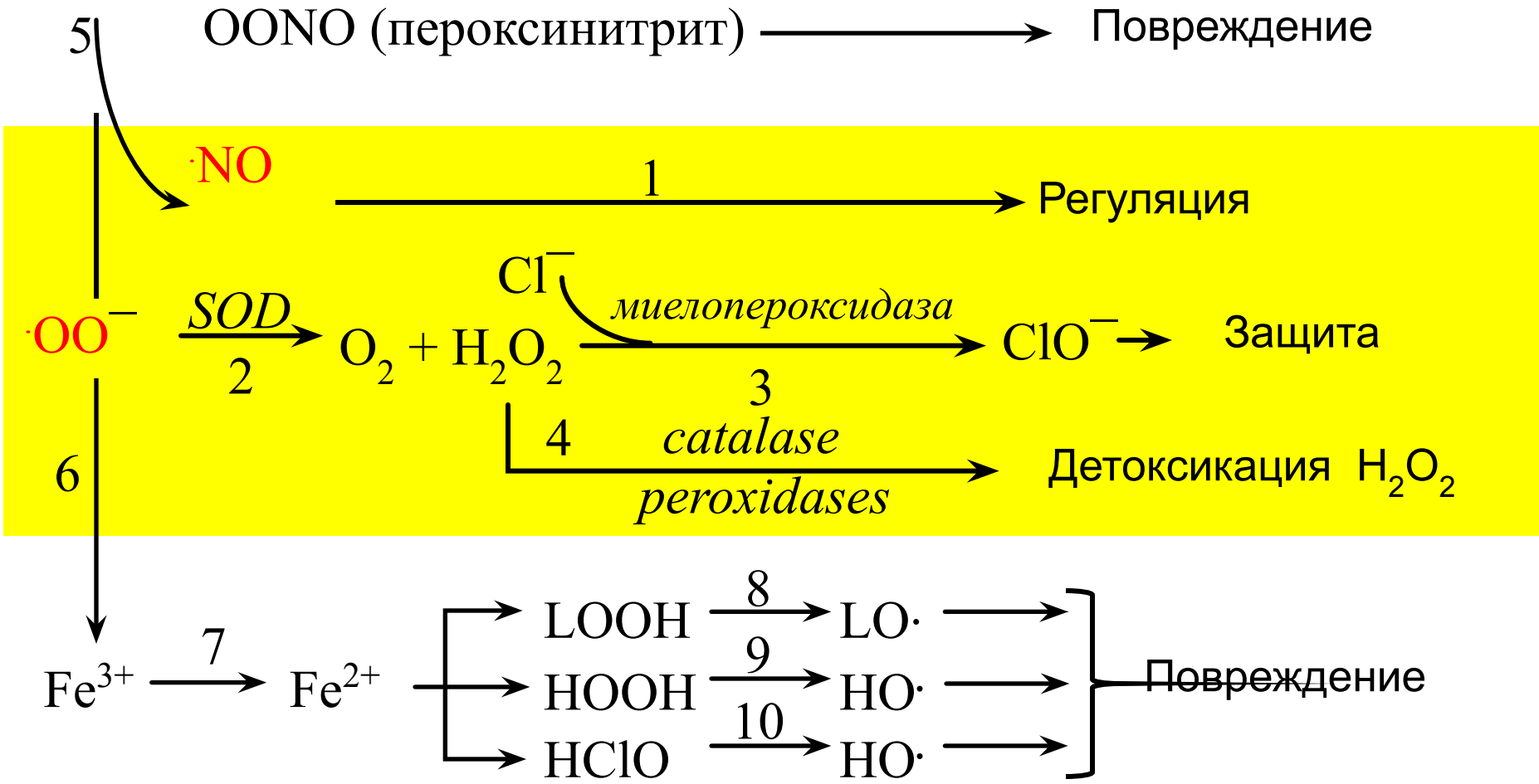
1. Optic atrophy
2. Tumor formation
   (paraganglioma, pheochromocytoma)
3. Myopathy
4. Encephalopathy

P. Rustin, A. Roetig, *Biochim. Biophys. Acta* **1553**, 117 (2002) – a review.
T. Bourgeron *et al.*, *Nature Genet.* **11,** 144 (1995).
B. E. Baysal *et al., Science* **287,** 848 (2000).
S. Niemann, U. MuËller, *Nature Genet.* **26,** 268(2000).

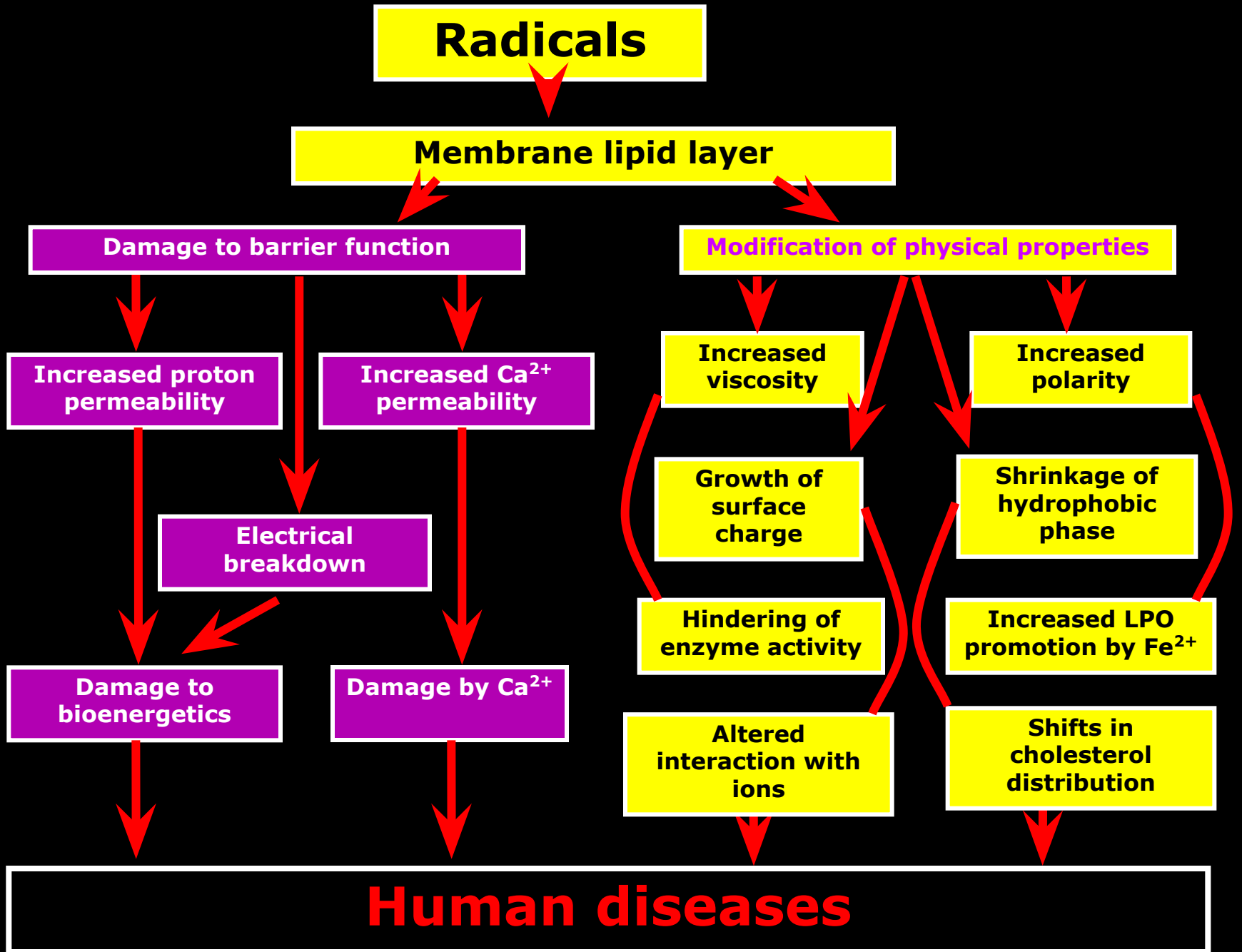
These disorders can be caused by oxidative stress produced by complex II

Метаболизм первичных радикалов

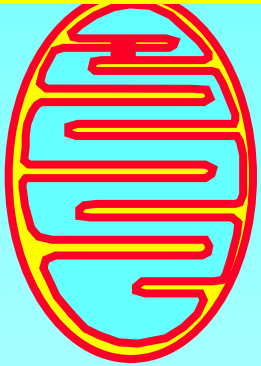
Владимиров, А., *Три гипотезы о механизме действия лазерного облучения на клетки и организм человека*, in *Эфферентная медицина*, С. Чикин (ред.), 1994, Институт Биомедицинской Химии РАМН: Москва. p. 51-66.



Damage to biomembranes resulting from lipid peroxidation



How we created and measured the membrane potential in mitochondria?

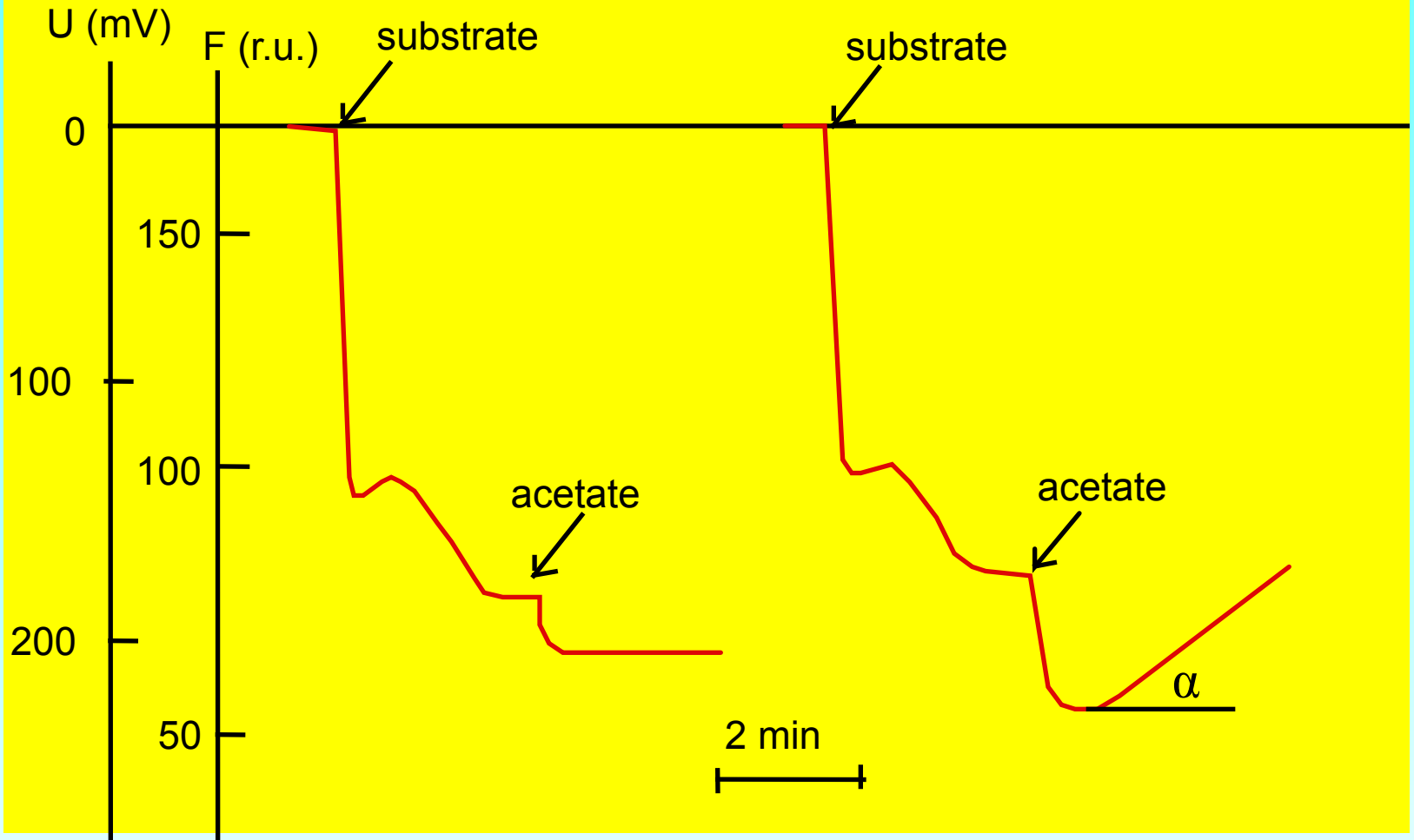


Inner mitochondrial membrane generates potential difference (**Δϕ**)and pH difference (**ΔpH**) between bathing solutions, in the presence of respiration substrates and oxygen.

- $\Delta\mu_{H}^{+} = RT\Delta\ln[H^{+}] + zF\Delta\phi$

Upon addition of permeable acid (e.g. acetic acid) **ΔpH** would decrease and hence **Δϕ** would increase.
The fluorescence probe was used to measure the membrane potential (**Δϕ**).

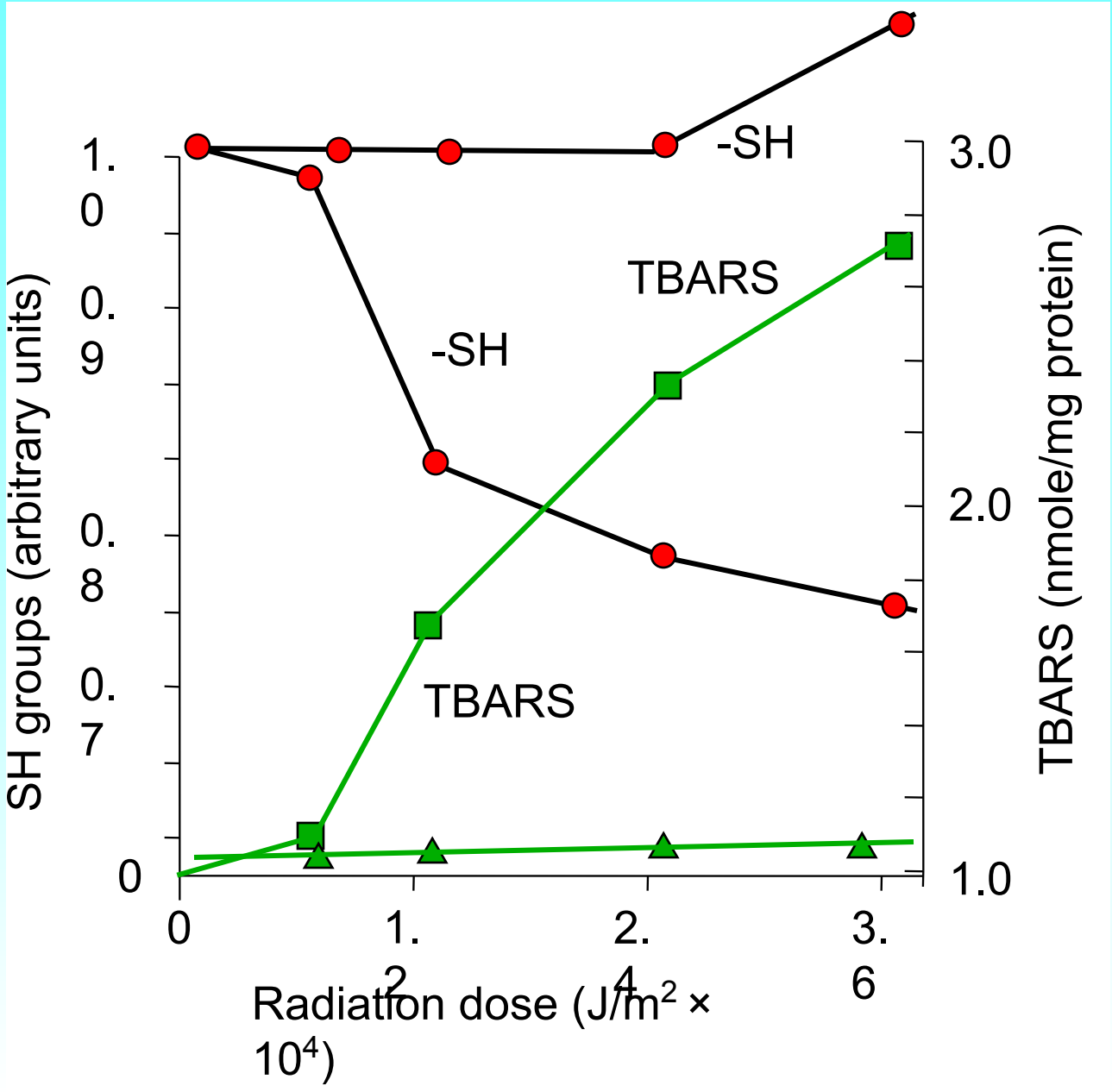
Electrical Breakdown in Mitochondria



Being penetrative, acetic acid neutralizes ΔpH on the membrane, so increasing Δϕ□ component of the proton-motive force. It is seen in the left part of the figure that potential is stable, as far as it does not exceed 200 mV.

If it does, the breakdown take place which leads to a gradual decrease of membrane potential; tan □ can serve as a measure of the membrane damage by the breakdown.

Dose-effect curves of SH group



Permeability transition pore

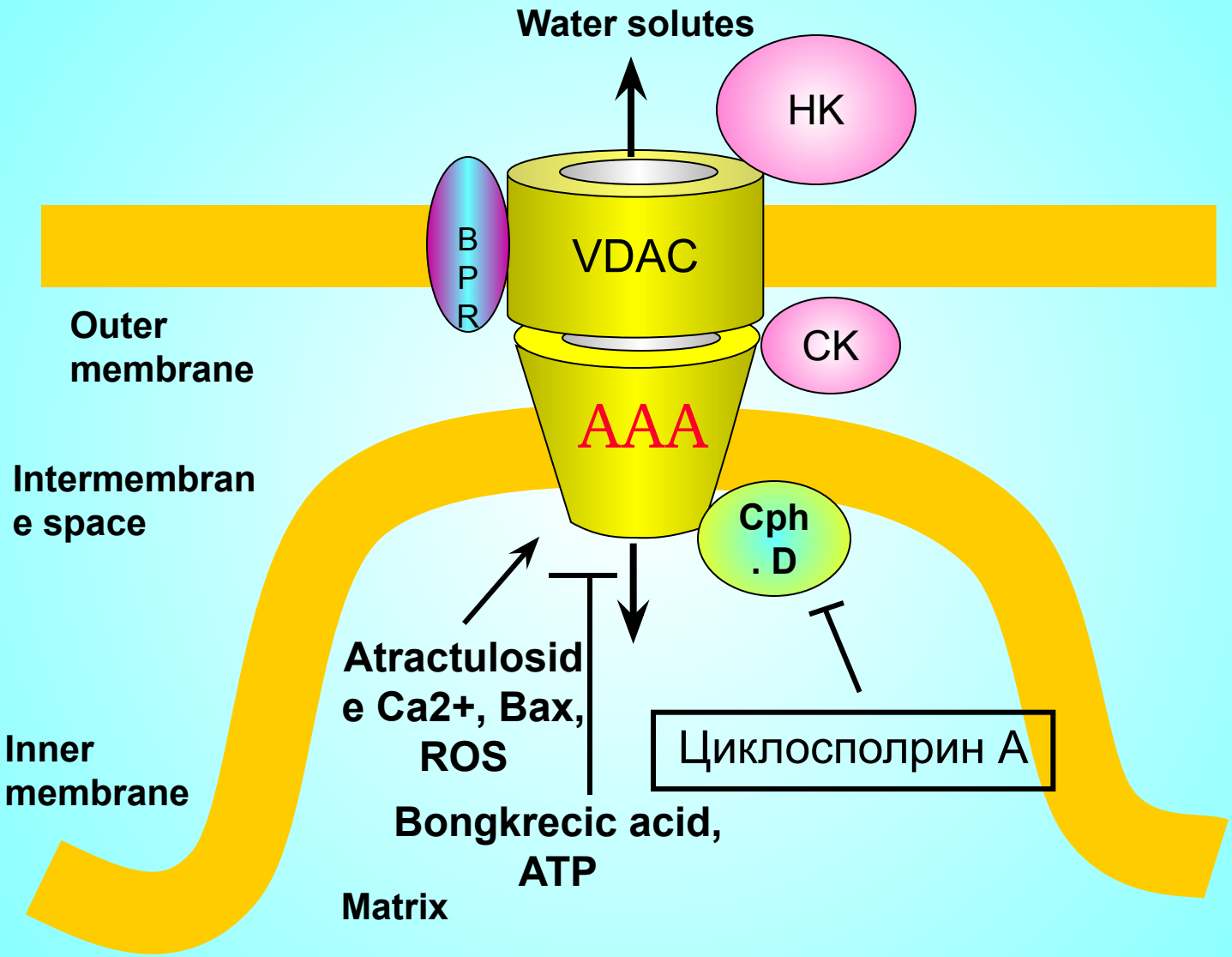
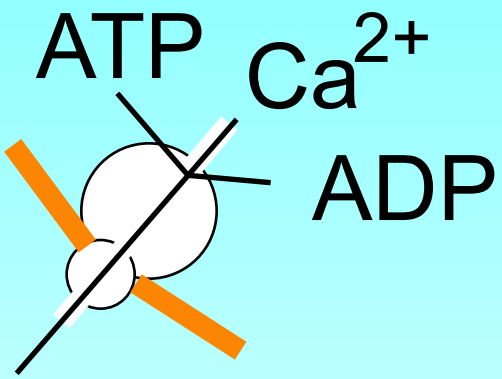
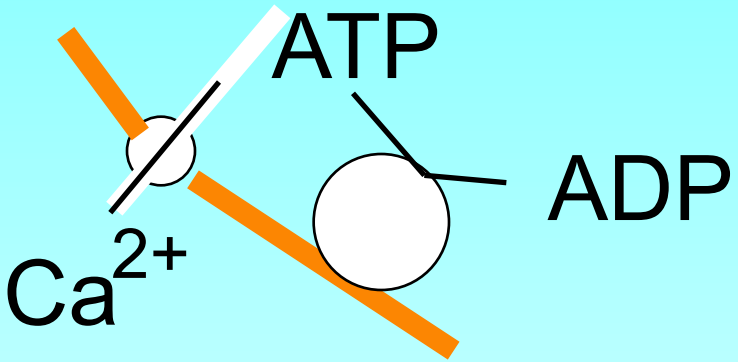


Fig. 6. Damage to Ca^{2+} ATPase under lipid peroxidation



Native
Ca-ATPase



Damaged
ATPase

Ca-АТФаза снизу (стерео)

Ca-АТФаза

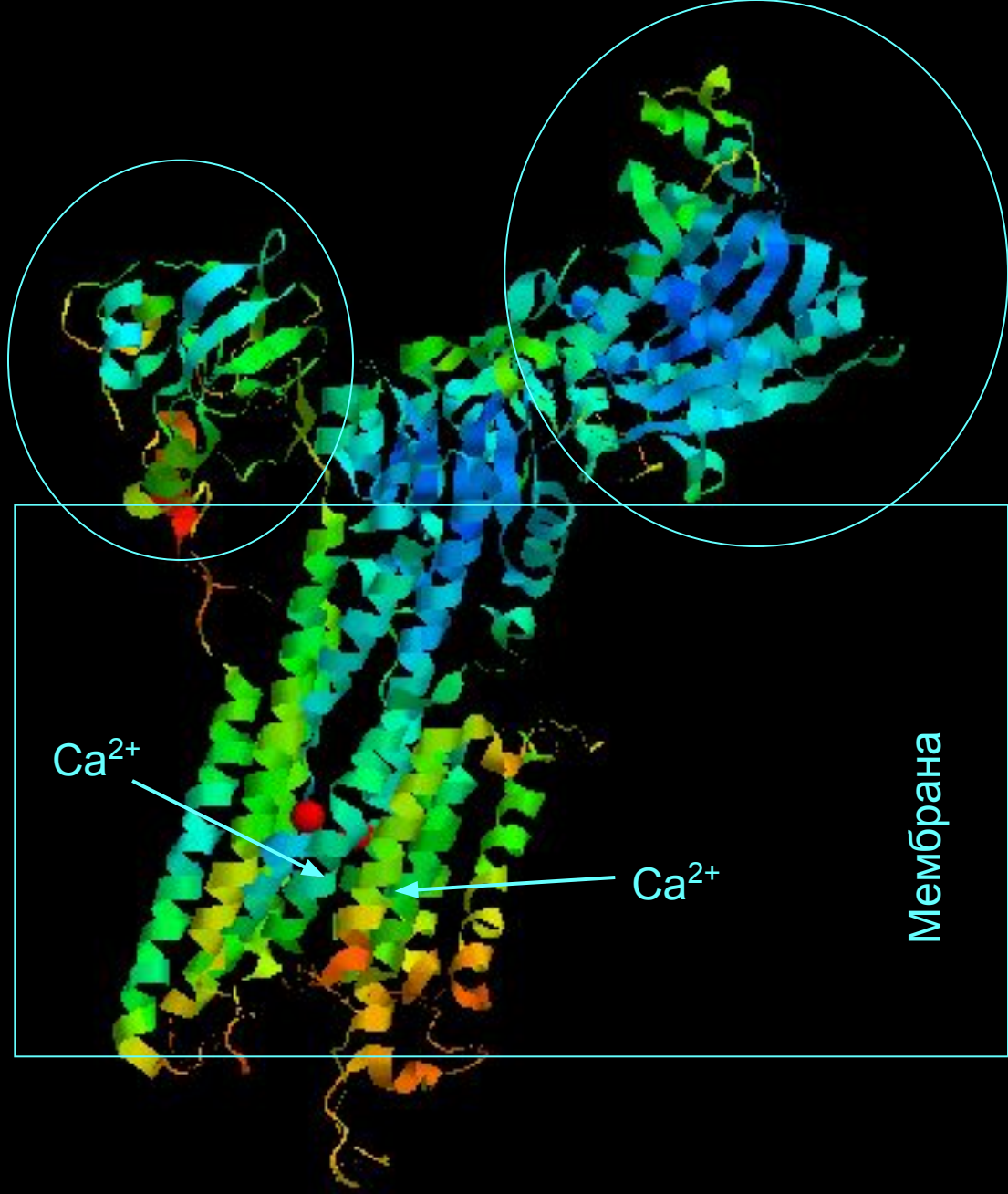
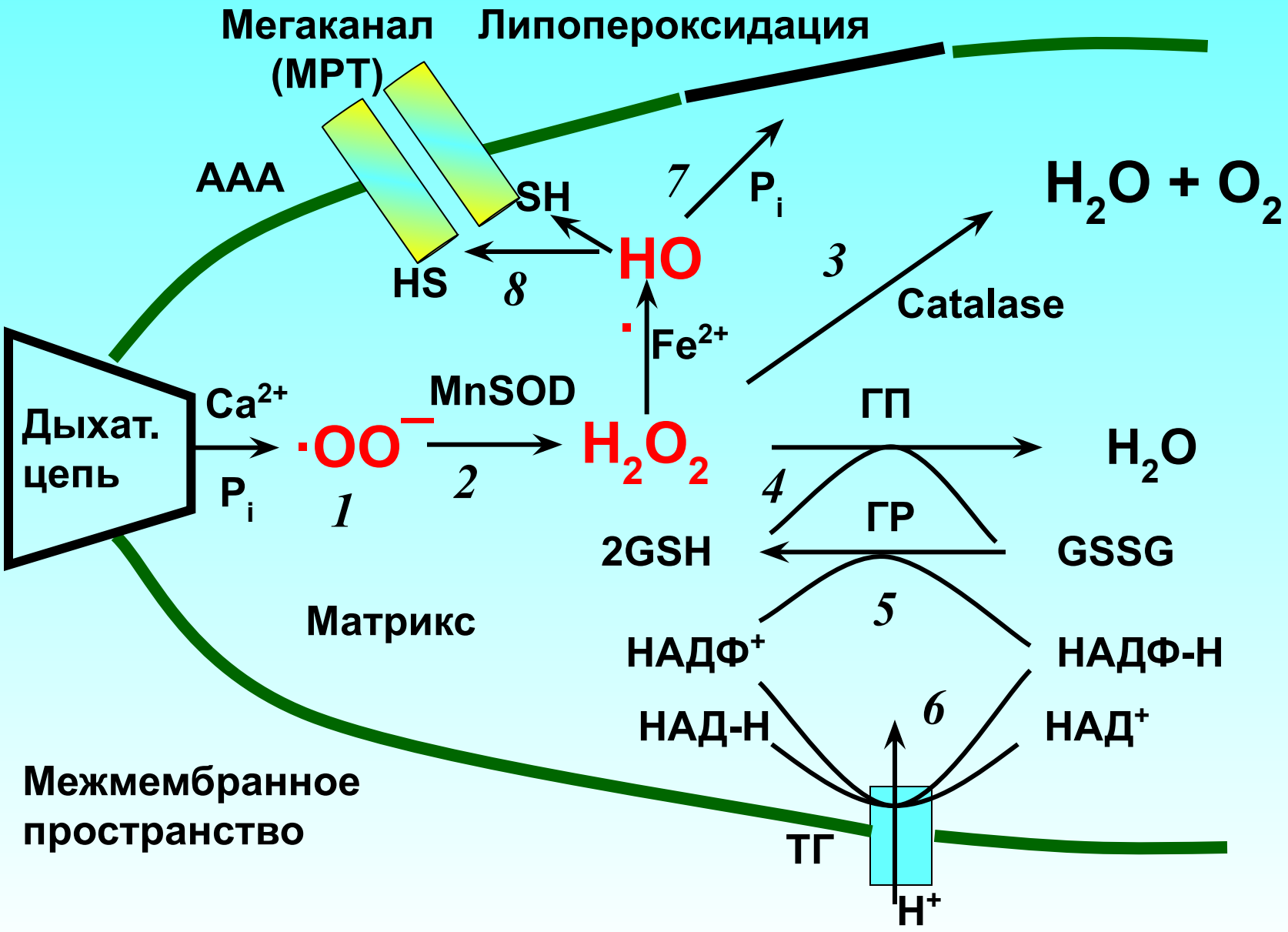
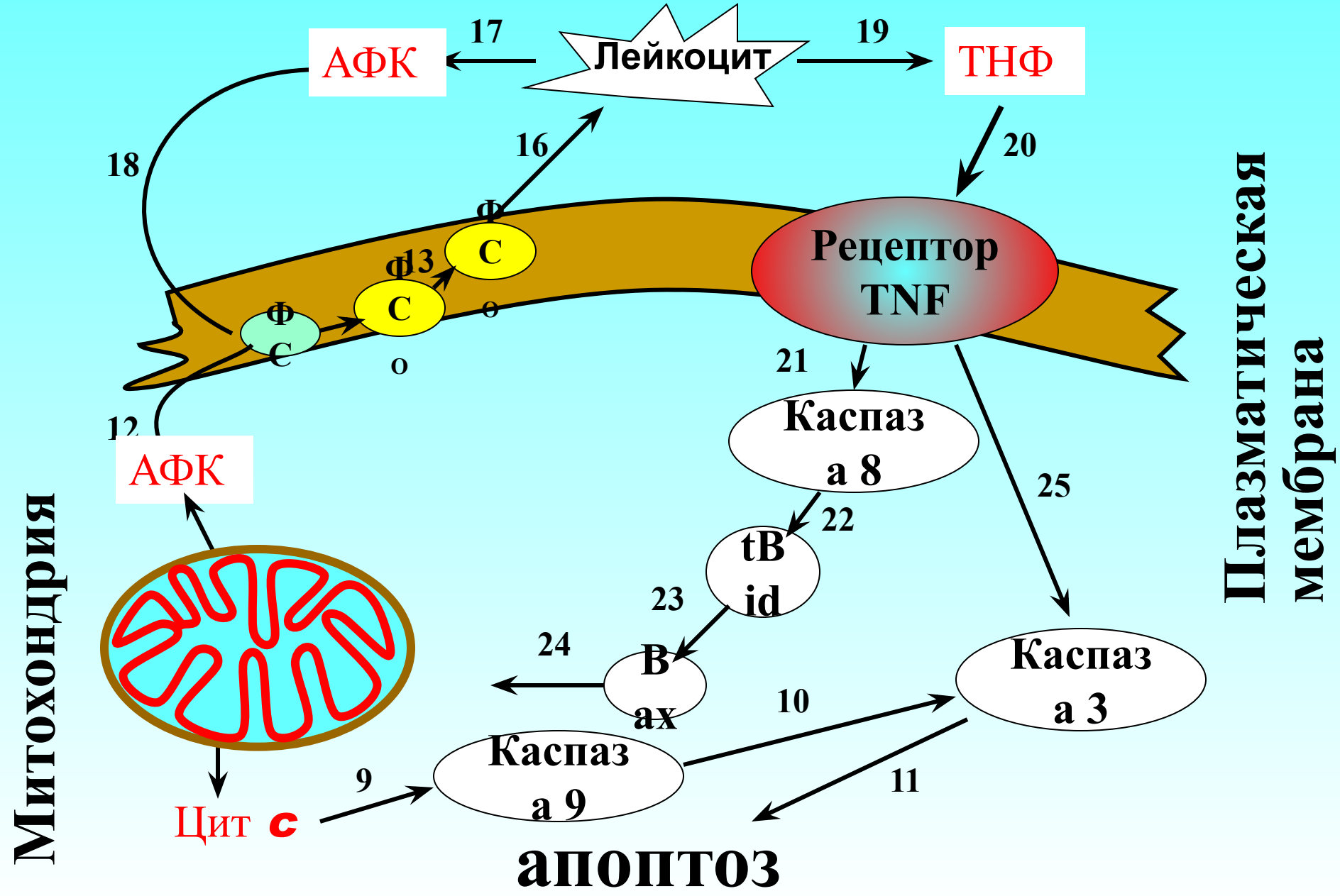


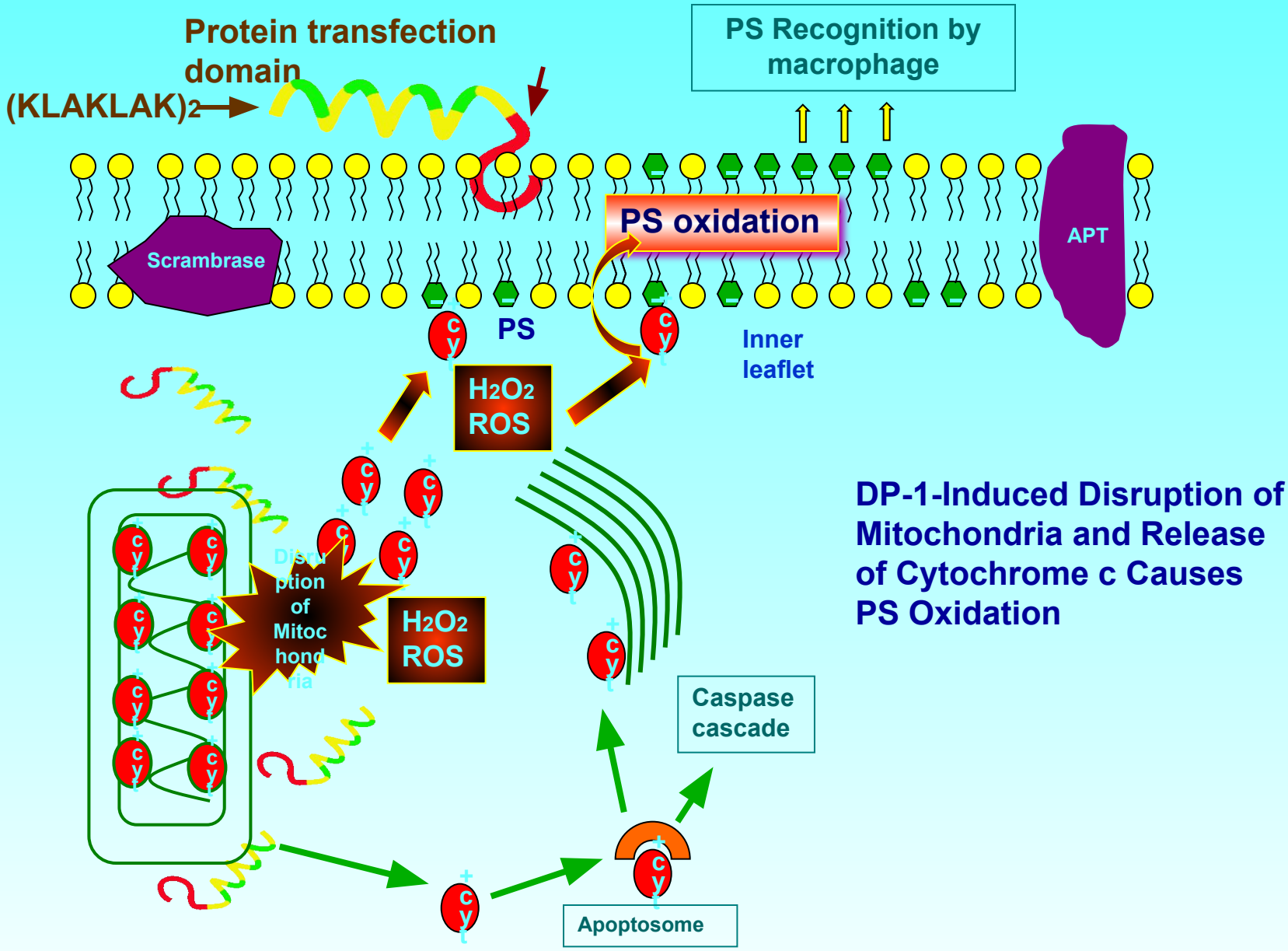
Рис. 5. Роль АФК в апоптозе



Радикалы, клеточная мембрана и апоптоз

Передача апоптозных сигналов (по В. П. Скулачеву)





DP-1-Induced Disruption of Mitochondria and Release of Cytochrome c Causes PS Oxidation

ble of Macrophages in Apoptosis