Como ‘calcular’ mitocôndrias?
Tópicos em bioenergética

Ariel M Silber; Mayke B Alencar, 22 de set 2020

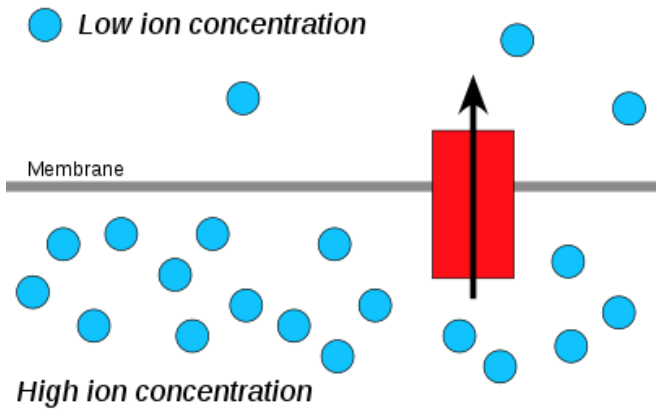
A teoria quimiosmótica e as membranas conservadoras de energia

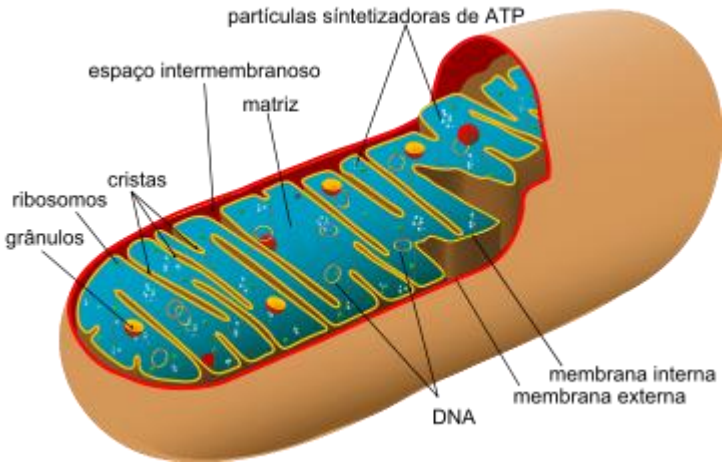
COUPLING OF PHOSPHORYLATION TO ELECTRON AND HYDROGEN TRANSFER BY A CHEMI-OSMOTIC TYPE OF MECHANISM

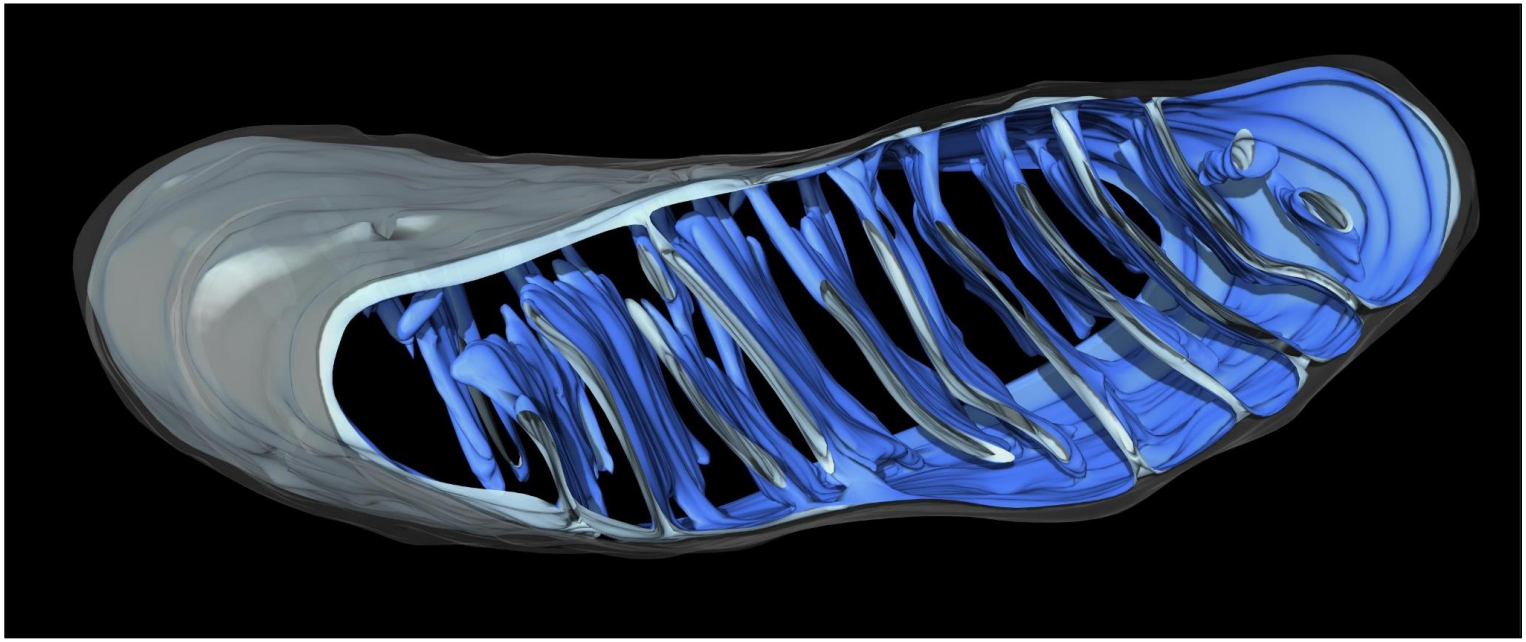
By Dr. PETER MITCHELL

Chemical Biology Unit, Zoology Department, University of Edinburgh

Peter D. Mitchell
Teoria quimiosmótica, 1961.
Nobel em Química, 1978.







(Stephan, T. et. Al, 2020)

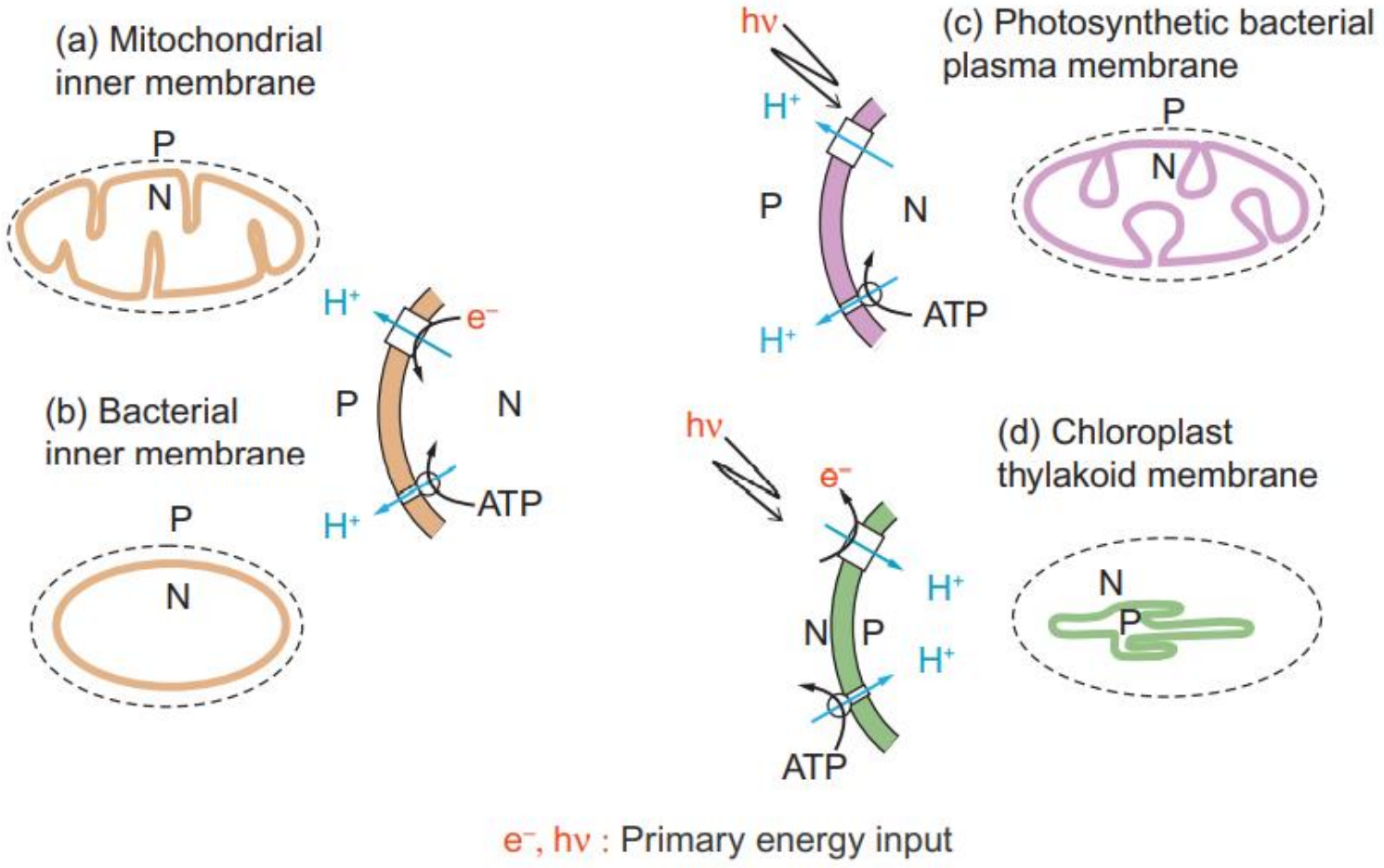
Istituto di Anatomia degli animali domestici,

Università di Torino, Italy

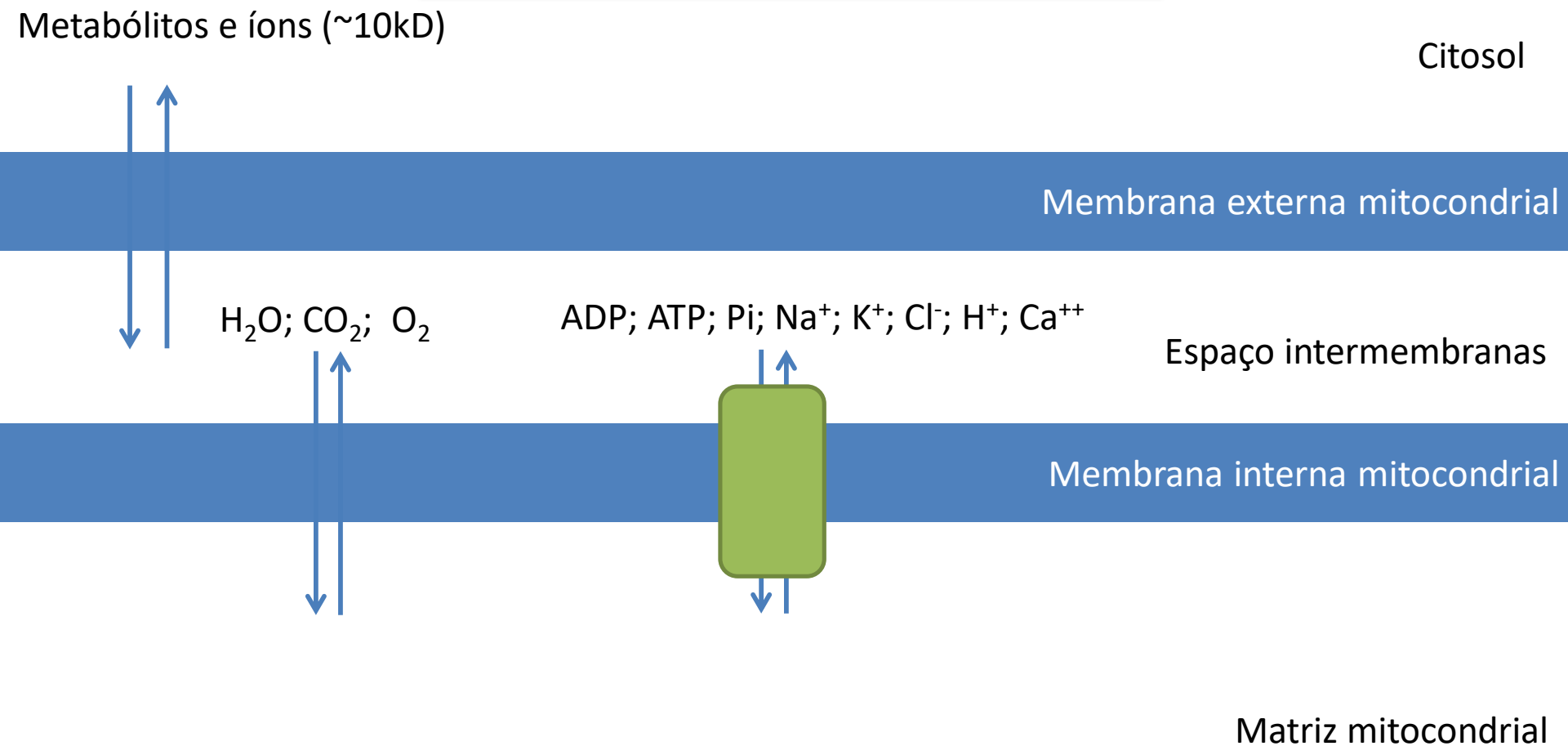
Istituto di Patologia generale,

Università di Modena, Italy

A teoria quimiósmótica e as membranas conservadoras de energia

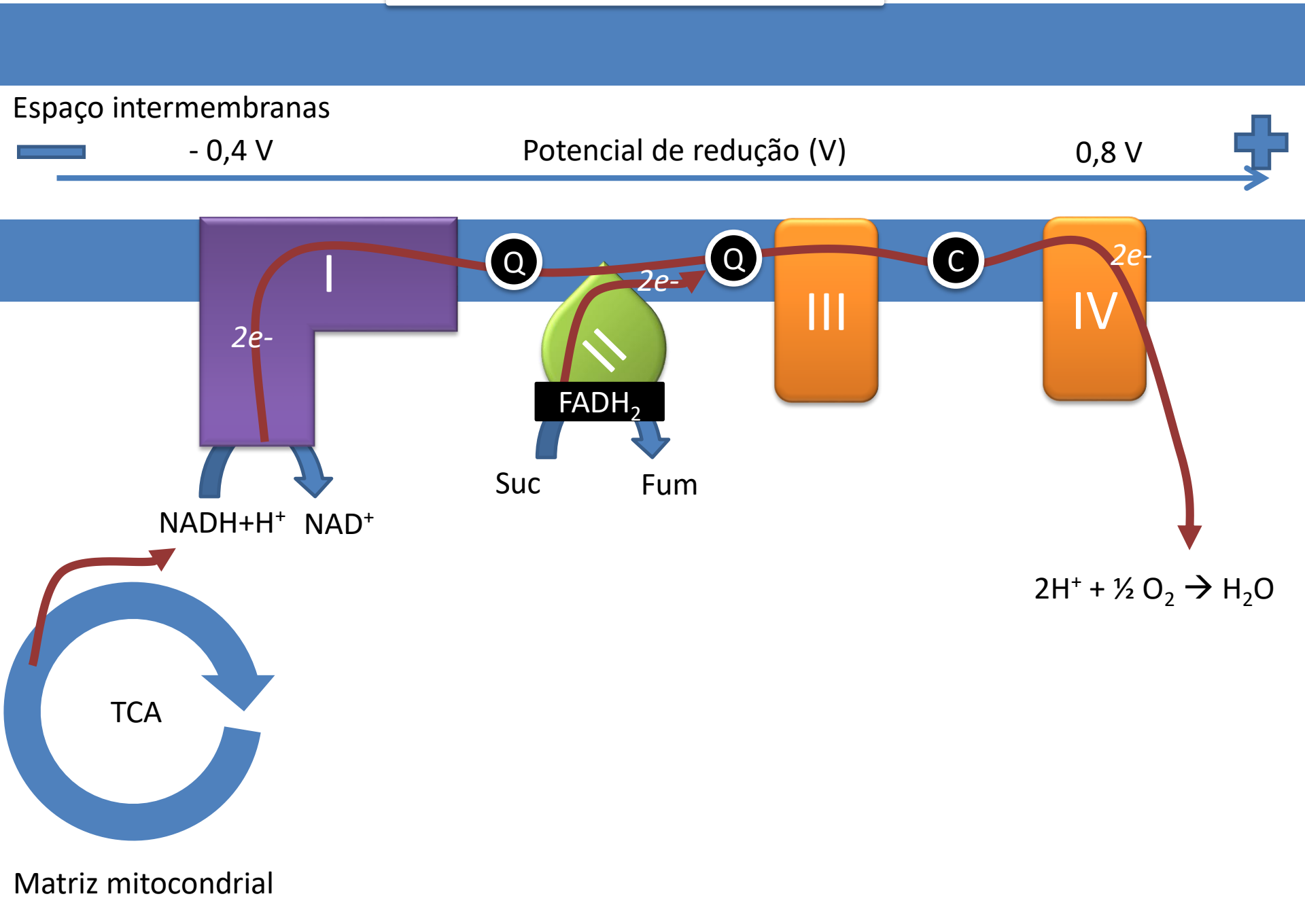


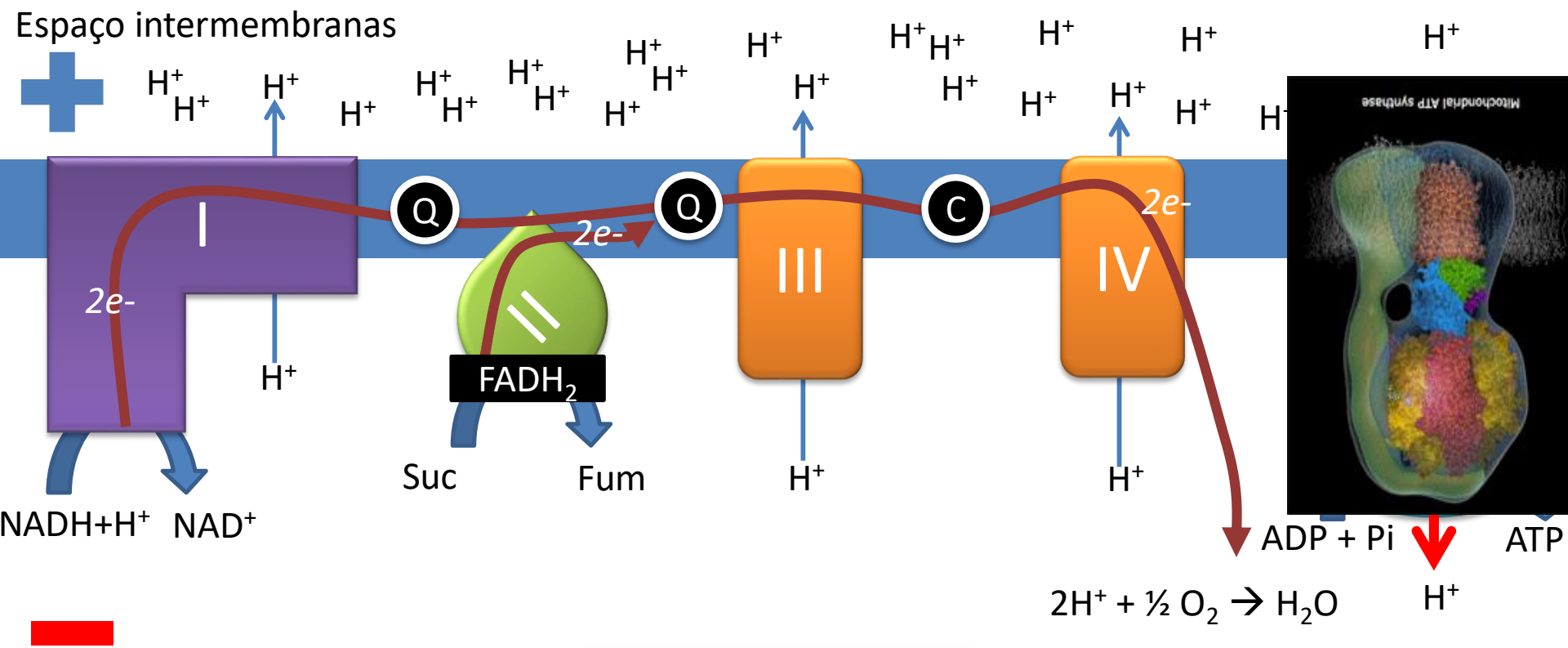
Propriedades das membranas mitocondriais



A membrana mitocondrial interna é seletivamente permeável!

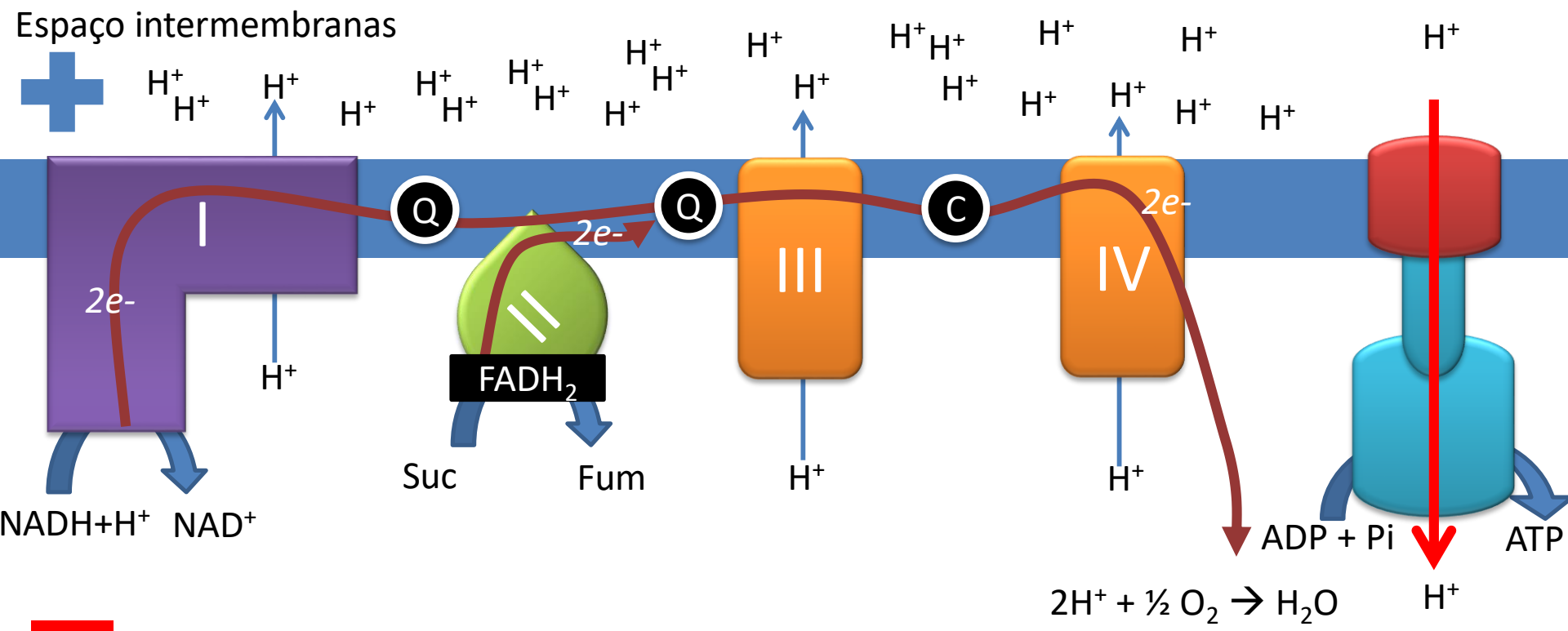
Sistema de Transporte de Elétrons!

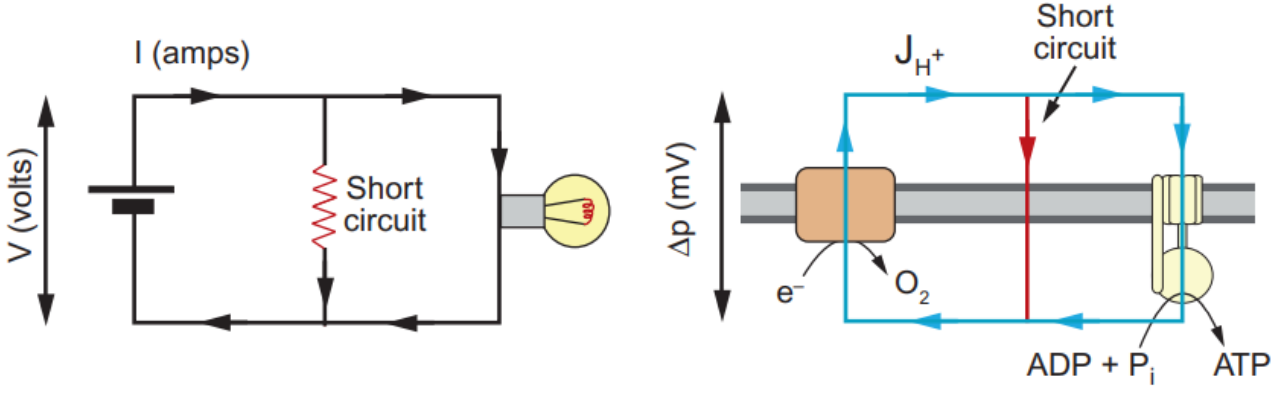


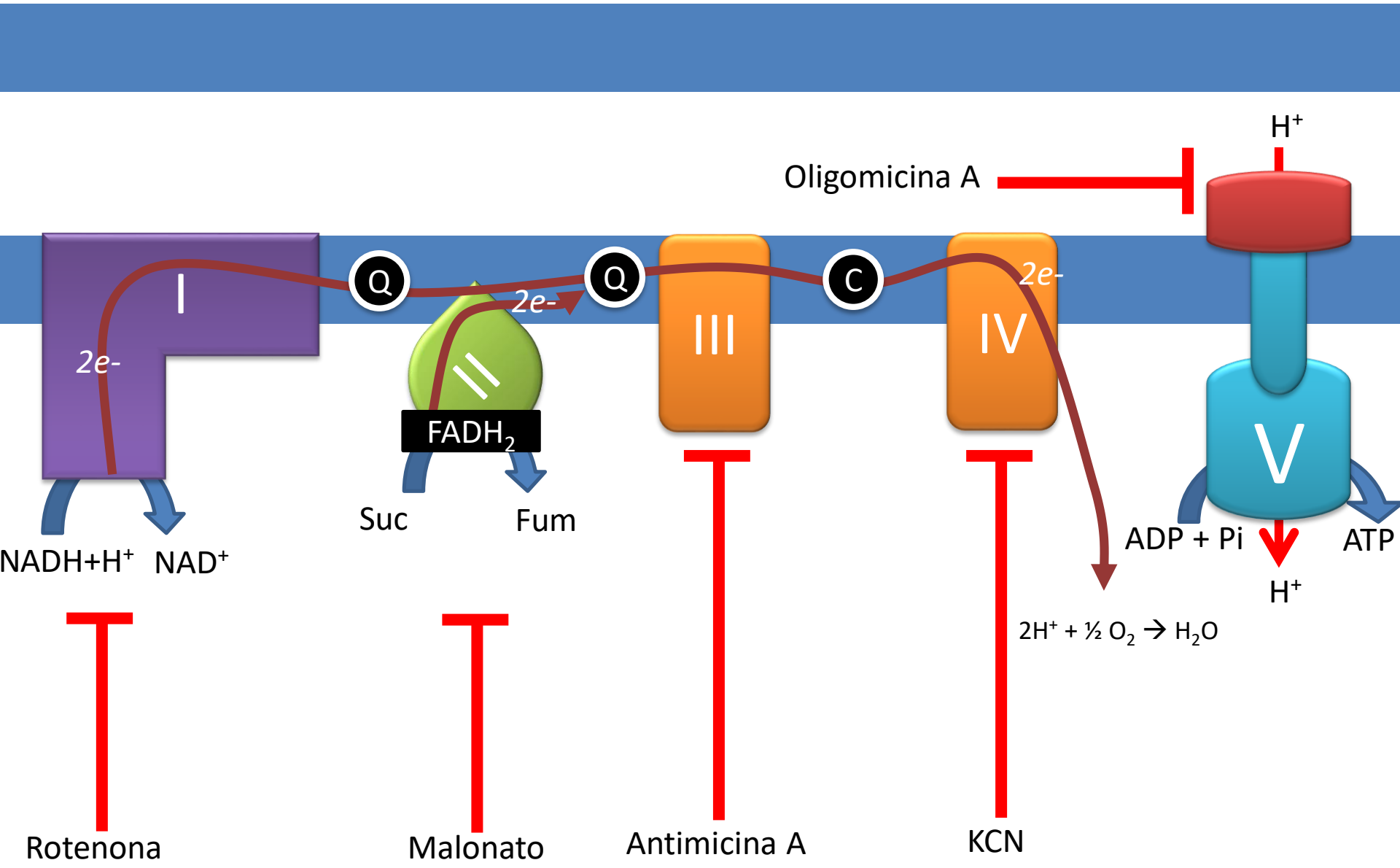


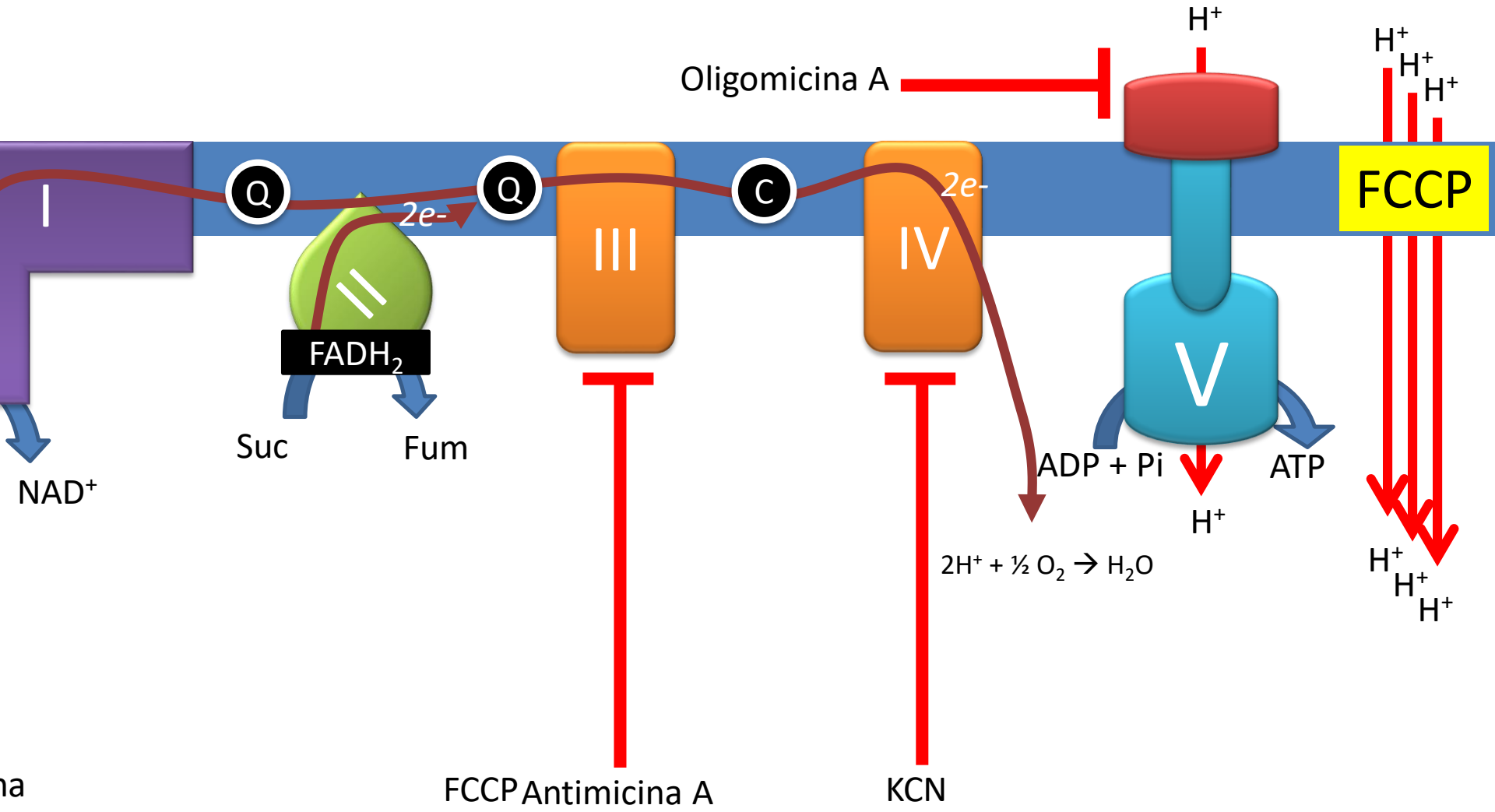
Fosforilação Oxidativa!

A taxa de consumo de O_2 por uma suspensão de células ou mitocôndrias é uma medida muito sensível da atividade da cadeia transportadora de elétrons!


Espaço intermembranas
H+
I
2e-
Q
II
FADH2
Suc
Fum
Q
2e-
III
C
IV
2e-
NADH+H+
NAD+
ADP + Pi
ATP
2H+ + ½ O2 → H2O

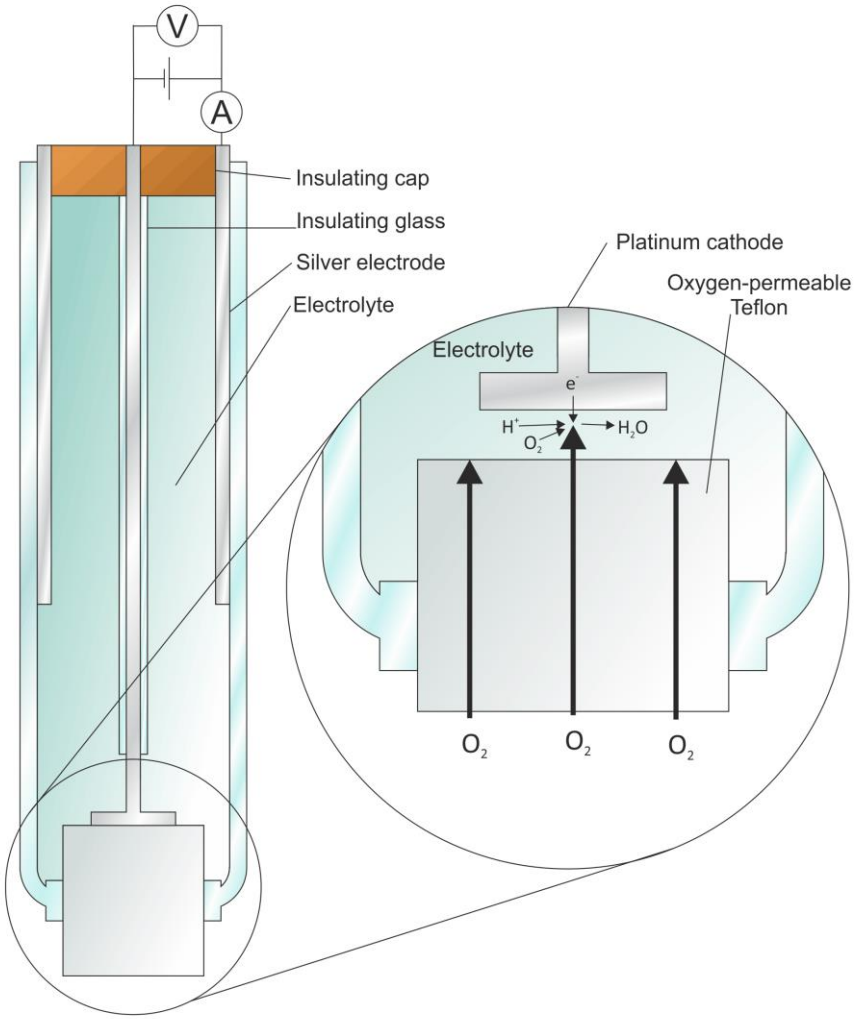

I (amps)
V (volts)
Short circuit
Short circuit
Δp (mV)
e−
O2
ADP + Pi
ATP


H+
Oligomicina A
I
2e-
Q
II
2e-
FADH2
Q
III
C
IV
2e-
V
NADH+H+
NAD+
Suc
Fum
ADP + Pi
ATP
H+
2H+ + ½ O2 → H2O
Rotenona
Malonato
Antimicina A
KCN


Oligomicina A
H+
H+
H+
H+
FCCP
I
Q
2e-
Q
II
FADH2
III
C
IV
2e-
V
NAD+
Suc
Fum
ADP + Pi
ATP
H+
2H+ + ½ O2 → H2O
H+
H+
H+
FCCP
Antimicina A
KCN

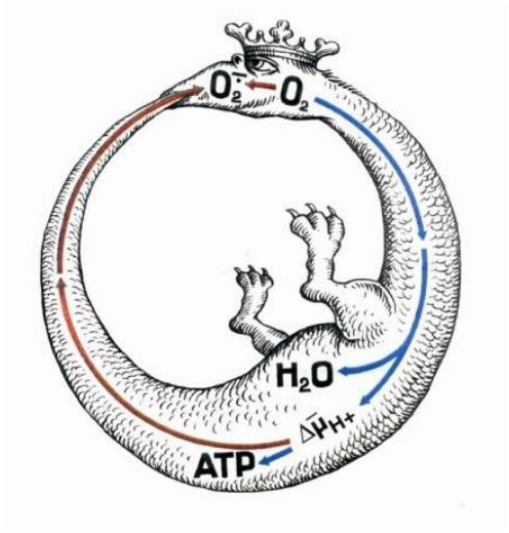
Eletrodo polarográfico (eletrodo de Clark)

Usado inicialmente para medir a concentração de O_2 no sangue durante cirurgias cardíacas



Leland C. Clark Jr. (1918-2005),

Respirometria de Alta Resolução (HRR)!



Parâmetros do Controle Respiratório

Células Intactas

Routine Respiration

Free Routine Activity

Leak Respiration

Electron Transfer Capacity

Residual **Ox**ygen Consumption

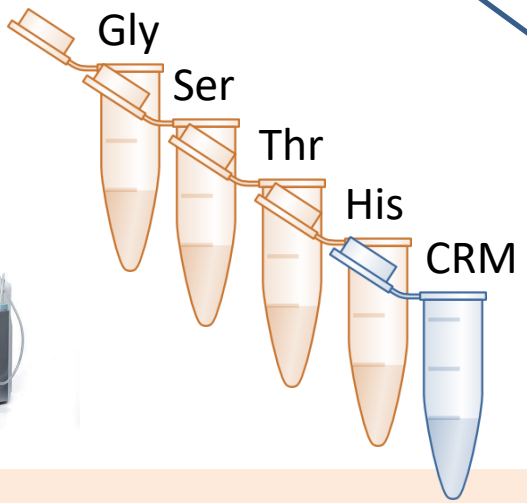
Modelo de estresse nutricional em epimastigotas

Epimastigotas em fase exponencial

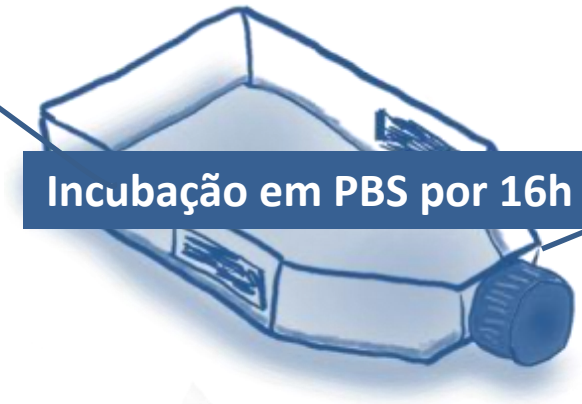
Clone 14, da cepa CL de *T. cruzi*

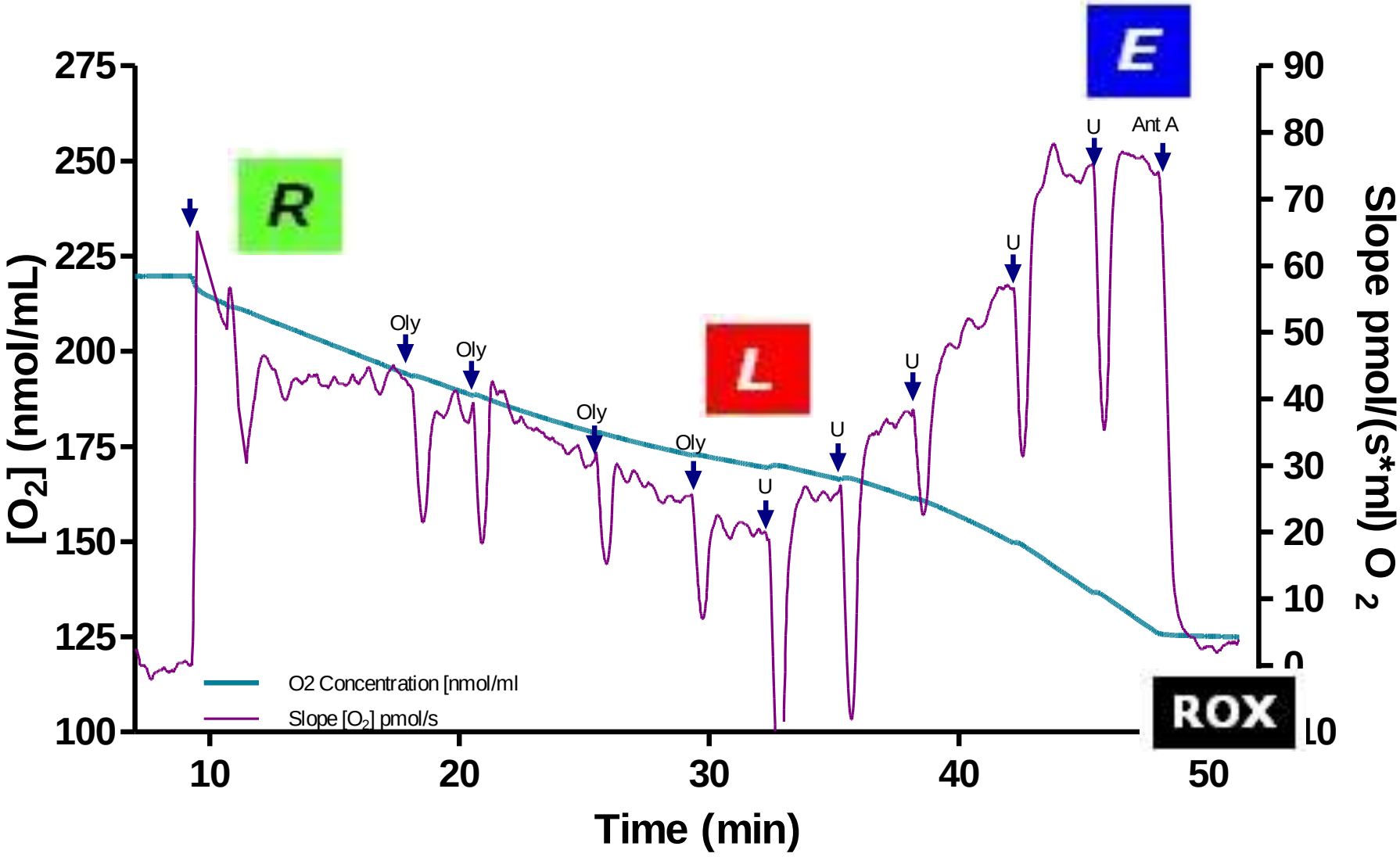
Lavagem em PBS (2x)

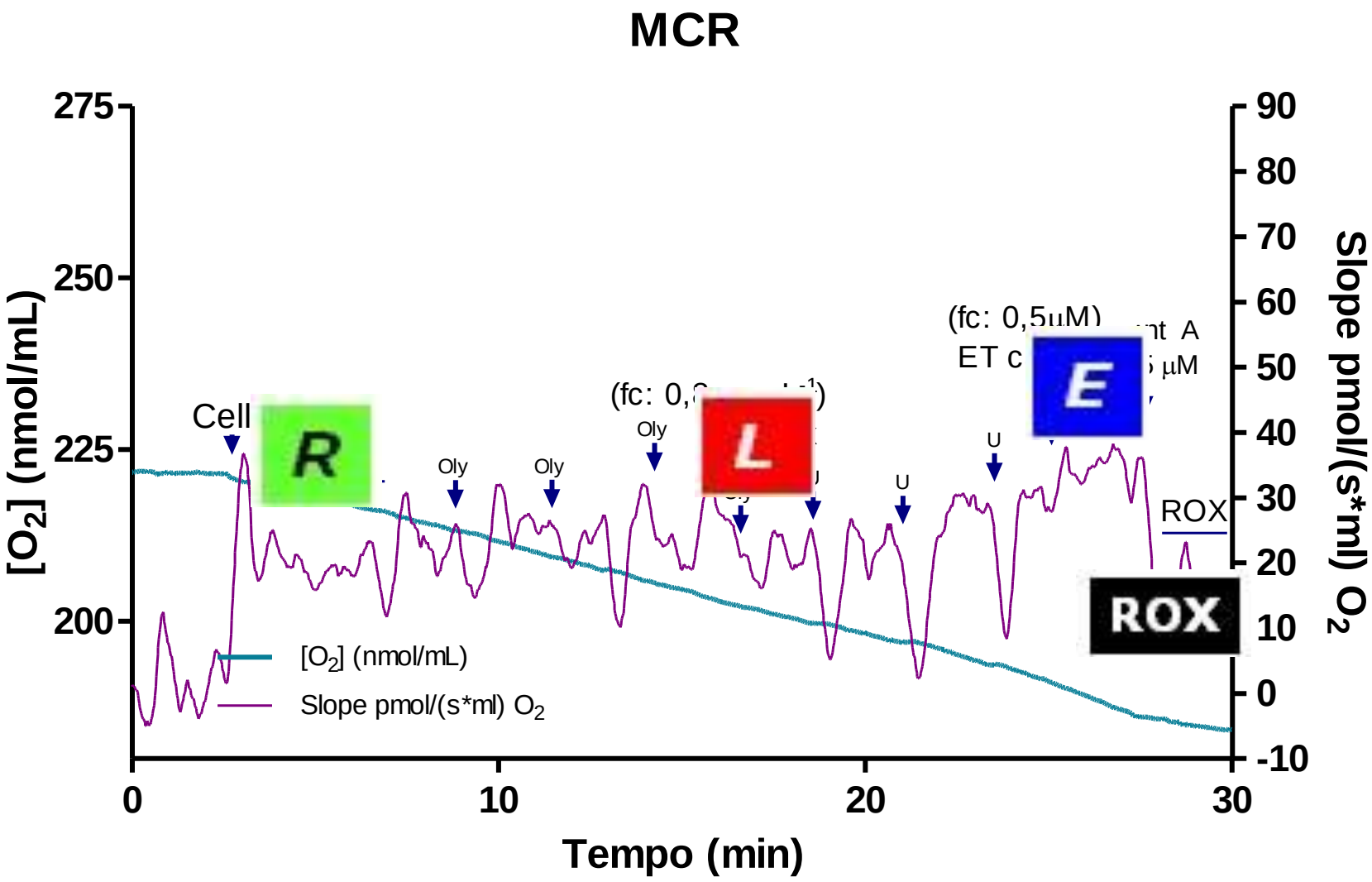
Recuperação por 120 min (5mM)

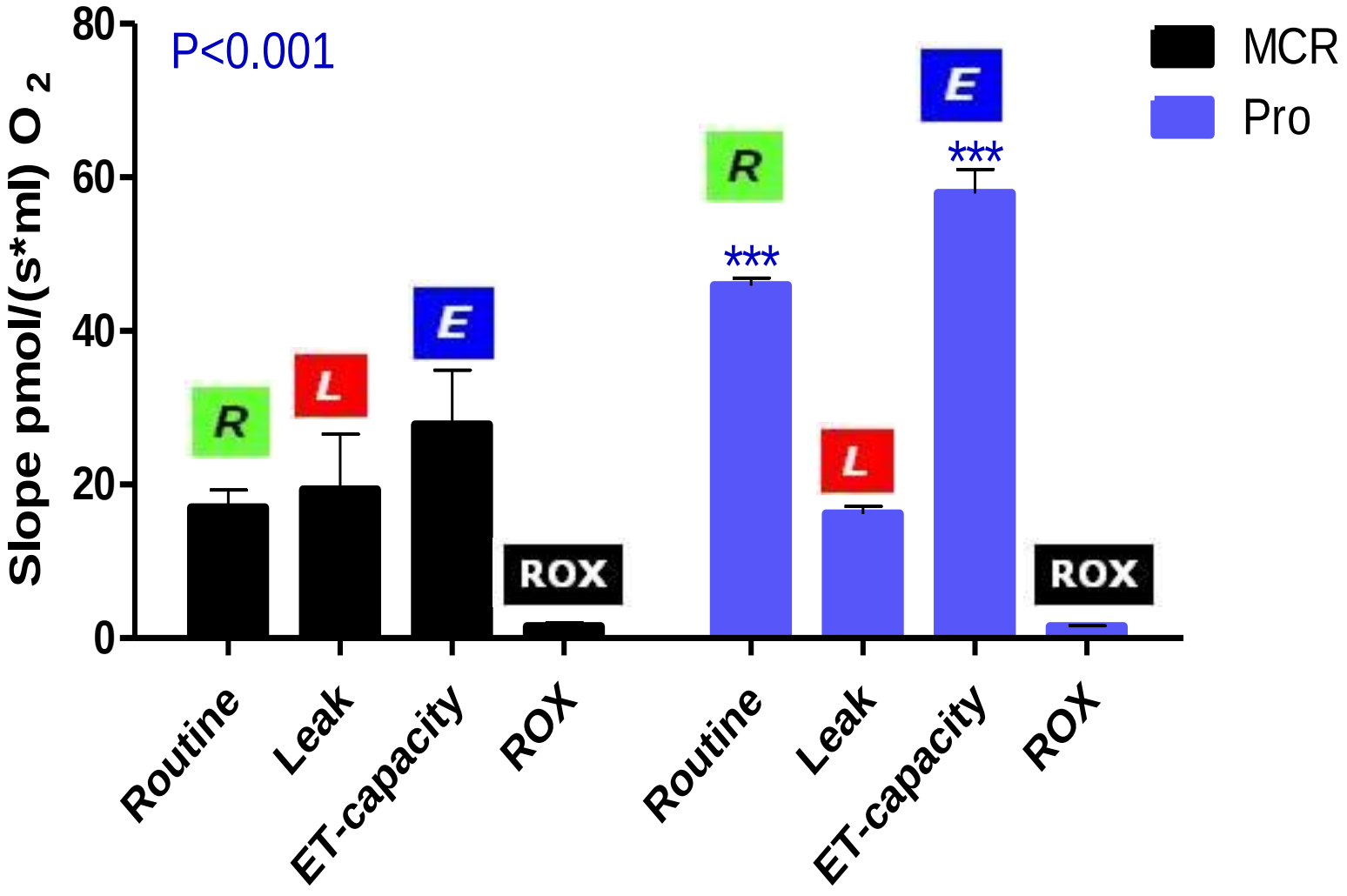


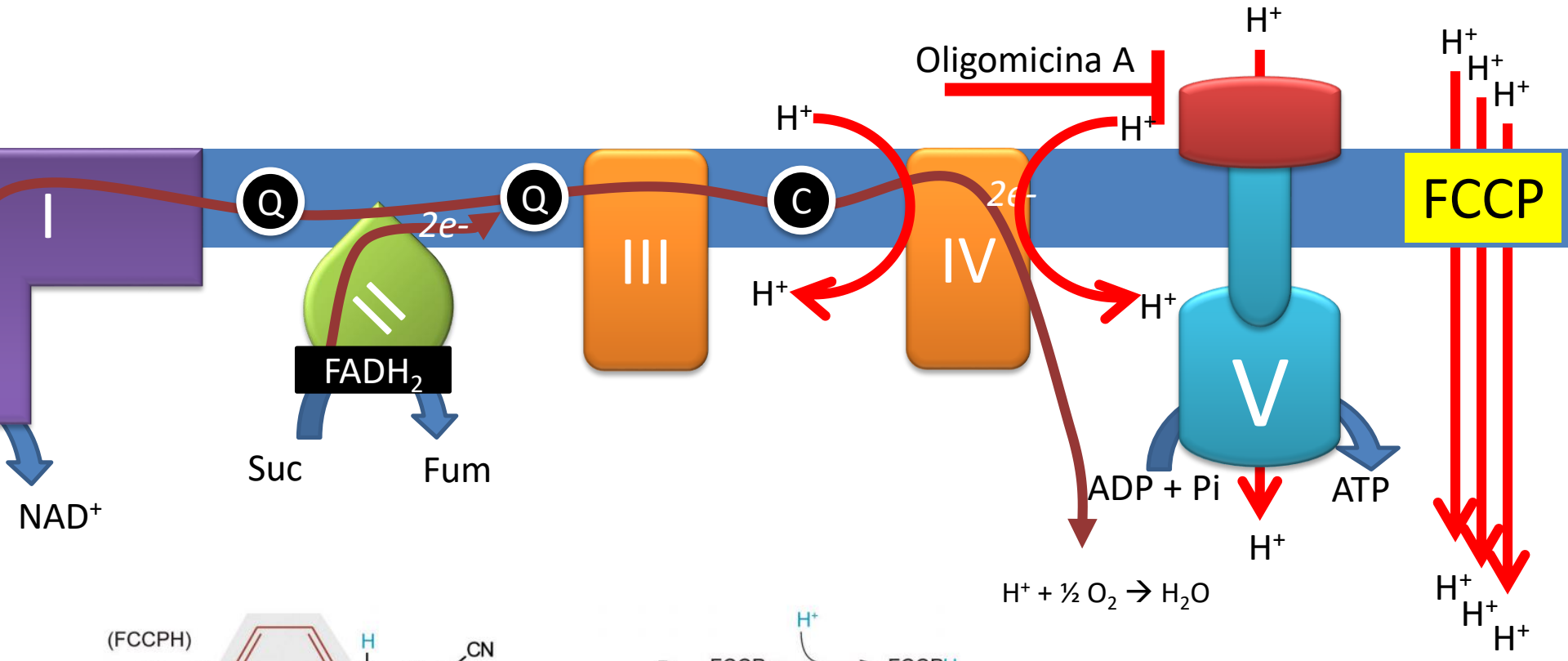
Incubação em PBS por 16h

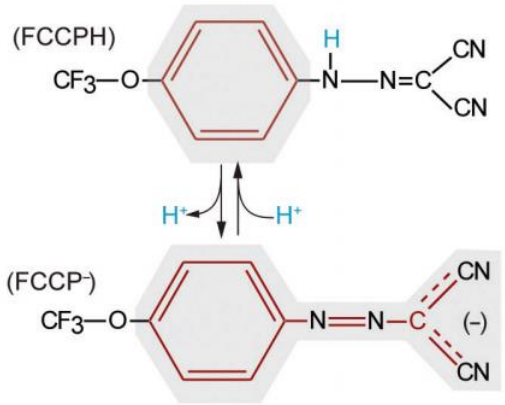


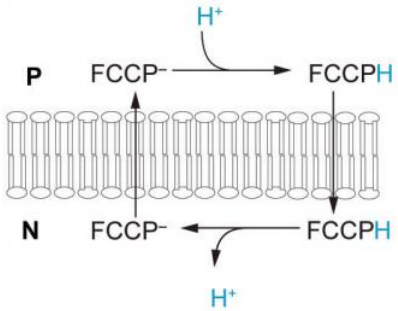

E
R
L
ROX
Oly
Oly
Oly
Oly
U
U
U
U
U
Ant A
O2 Concentration [nmol/ml
Slope [O2] pmol/s
[O2] (nmol/mL)
Slope pmol/(s*ml) O 2
Time (min)
275
250
225
200
175
150
125
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50

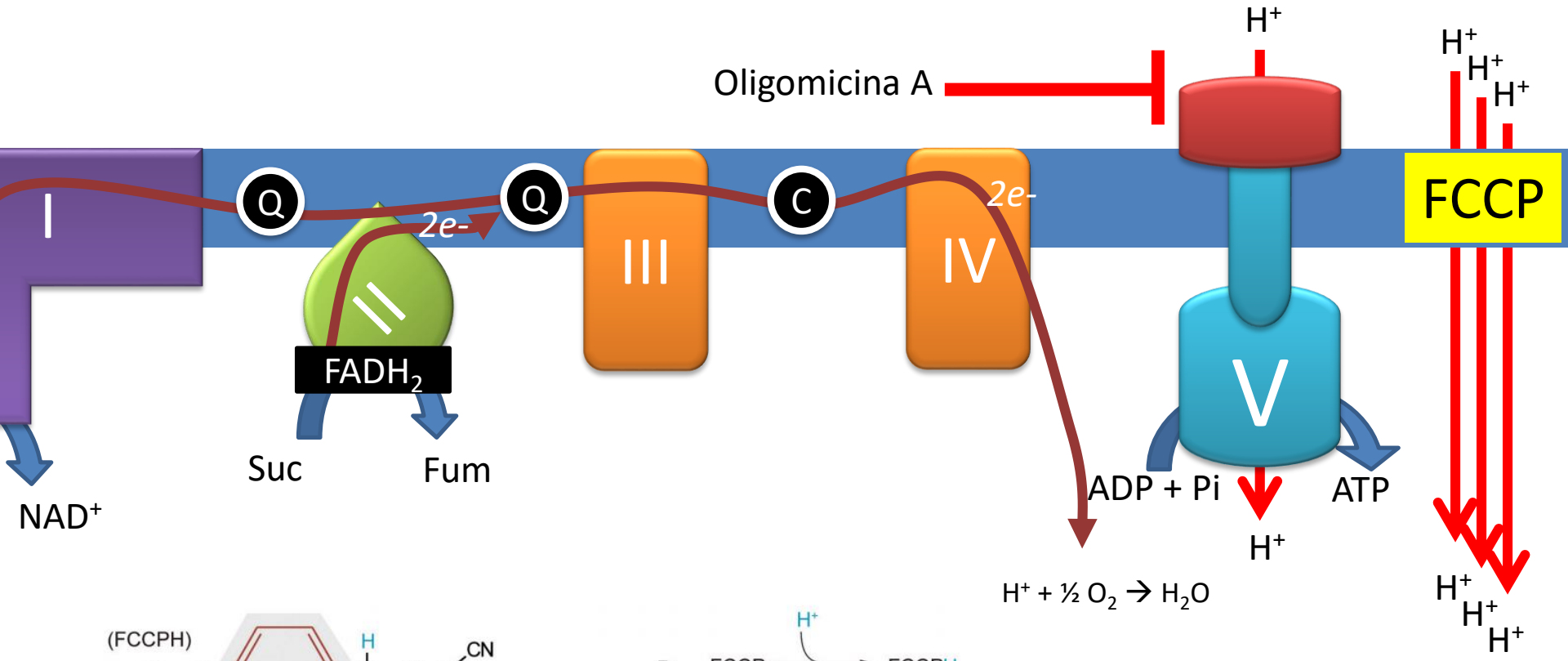

MCR
275
250
225
200
90
80
70
60
50
40
30
20
10
0
-10
0
10
20
30
[O2] (nmol/mL)
Slope pmol/(s*ml) O2
Tempo (min)
Cell
R
Oly
Oly
Oly
L
U
U
U
(fc: 0,5µM)
E
ROX
ROX
[O2] (nmol/mL)
Slope pmol/(s*ml) O2

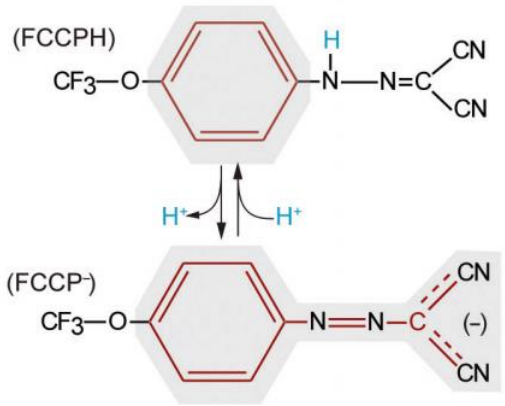

P<0.001
MCR
Pro
Slope pmol/(s*ml) O 2
80
60
40
20
0
R
L
E
ROX
***
Routine
Leak
ET-capacity
ROX

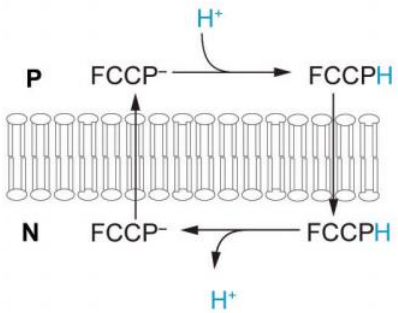

Oligomicina A
H+
H+
H+
H+
H+
H+
H+
H+
H+
H+
H+
I
Q
Q
2e-
II
FADH2
III
C
IV
2e-
V
FCCP
NAD+
Suc
Fum
ADP + Pi
ATP
H+ + ½ O2 → H2O

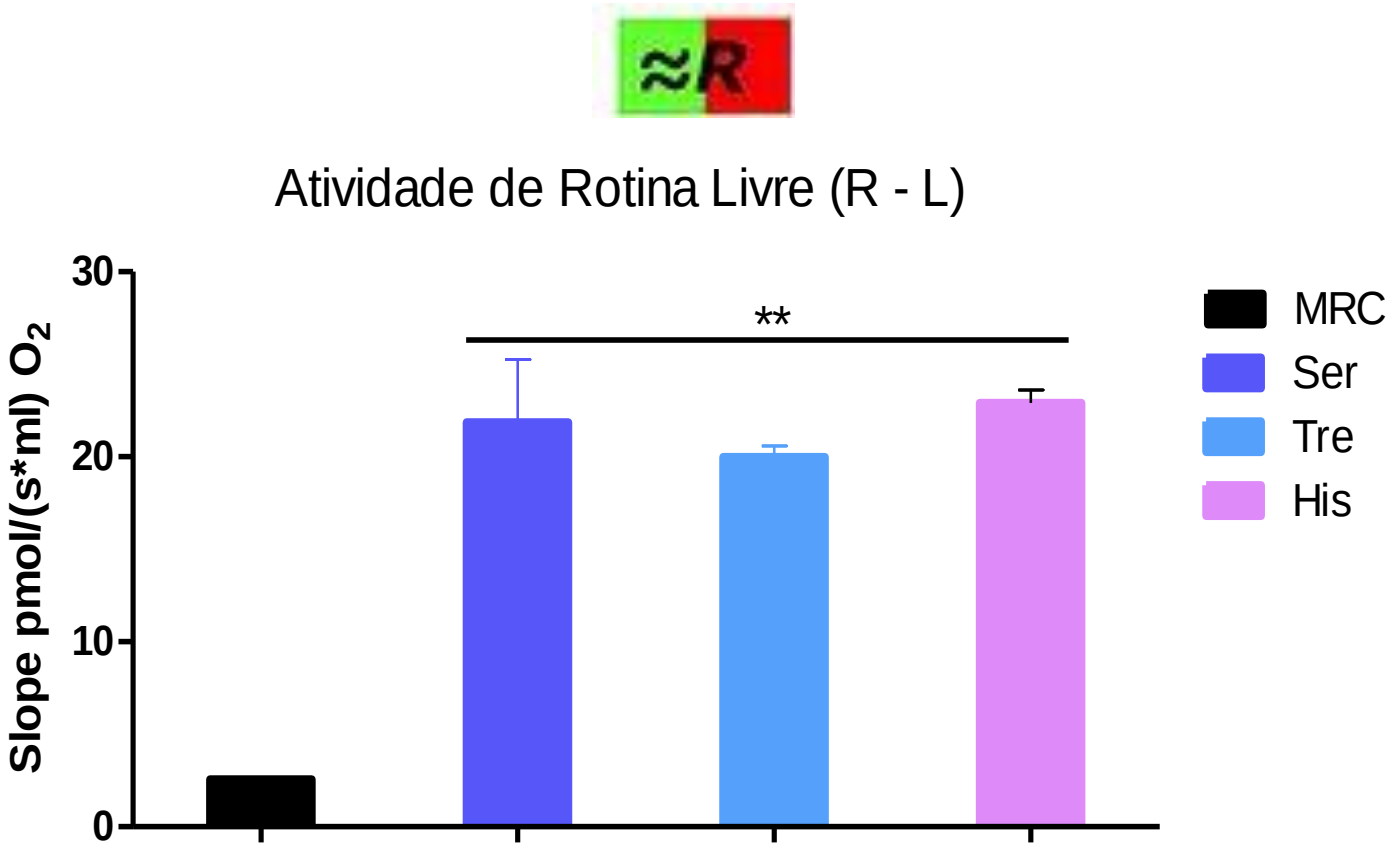

(FCCPH)
CF3—O
H
N—N=C
CN
CN
H+
H+
(FCCP-)
CF3—O
N=N—C
CN
(-)
CN


H+
P
FCCP-
FCCPH
N
FCCP-
FCCPH
H+


Oligomicina A
H+
H+
H+
H+
I
Q
2e-
Q
II
FADH2
III
C
IV
2e-
V
FCCP
NAD+
Suc
Fum
ADP + Pi
ATP
H+
H+ + ½ O2 → H2O
H+
H+
H+


(FCCPH)
CF3—O
H
N—N=C
CN
CN
H+
H+
(FCCP-)
CF3—O
N=N—C
CN
(-)
CN


H+
P
FCCP-
FCCPH
N
FCCP-
FCCPH
H+


Atividade de Rotina Livre (R - L)
Slope pmol/(s*ml) O2
30
20
10
0
**
MRC
Ser
Tre
His

Potencial de membrana interna mitocondrial ($\Delta\Psi_m$)

- Principal componente do Δp (Força próton-motriz)
- $\Delta\Psi_m$ Responsável por ~80 % (~150 mV) do Δp
- ΔpH Reponsável por ~20 % (~30 mV) do Δp

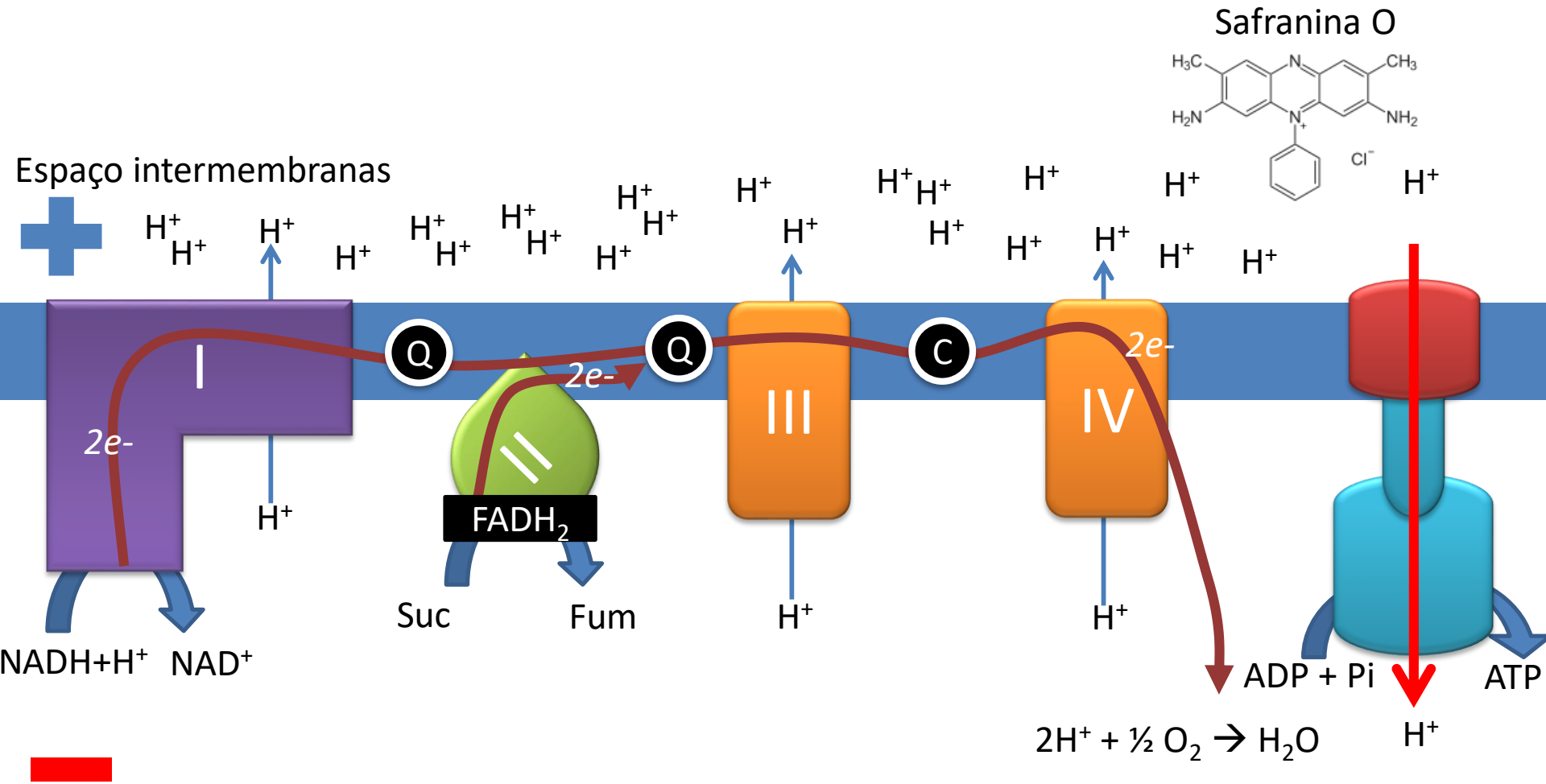
Como medir potencial de membrana interna mitocondrial ($\Delta\Psi_m$)

- Técnicas baseadas no gradiente de concentração de equilíbrio de membranas seletivas

Permeáveis a cátions monovalentes através da membrana interna e substituindo esses valores Na equação de Nerst. Ex: Safranina O

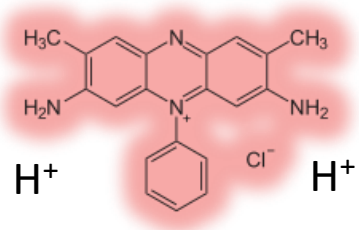
Nernst equation: $\Delta\Psi_m = 60 \times \log(K_{IN}^+ / K_{OUT}^+)$

Mitchell and Moyle (1969), Nicholls (1974)

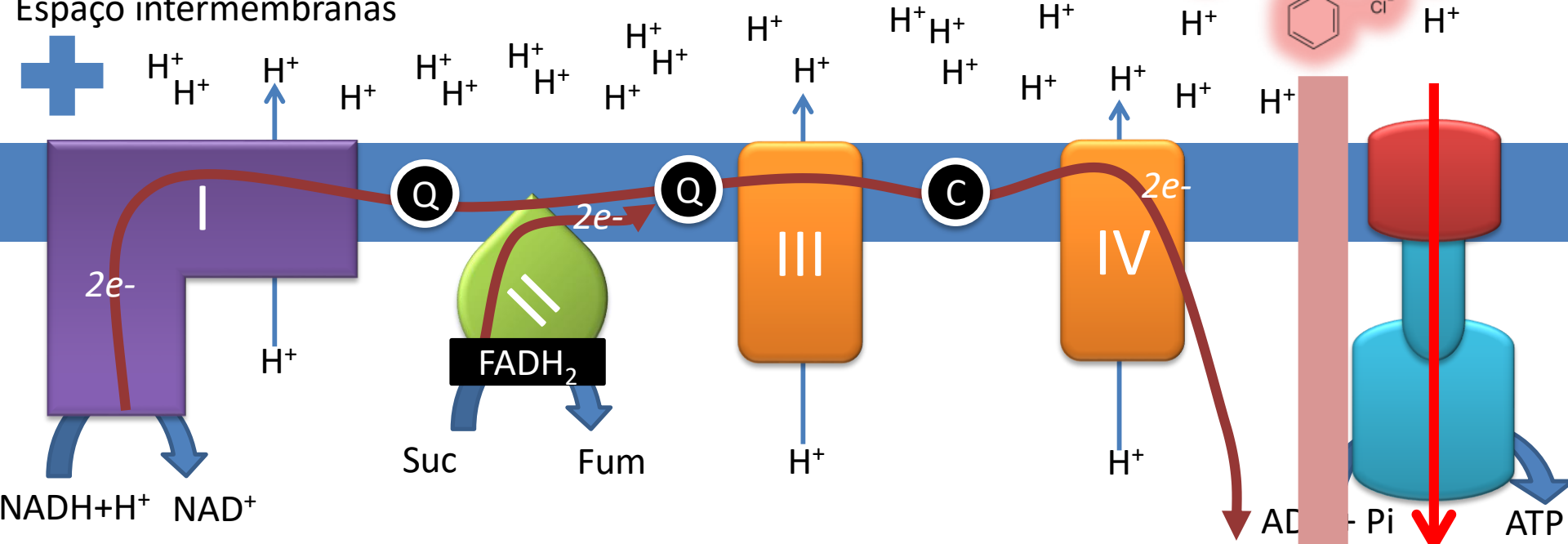

Safranina O
Espaço intermembranas
I
II
III
IV
Q
Q
C
2e-
2e-
FADH2
Suc
Fum
NADH+H+
NAD+
ADP + Pi
ATP
2H+ + ½ O2 → H2O

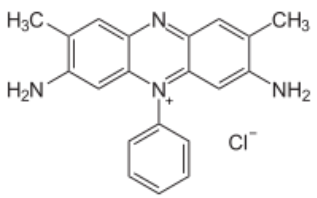
- Lipossolúvel e catiônico

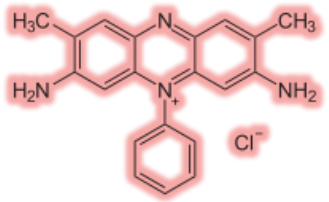
Safranina O

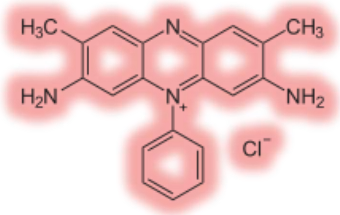


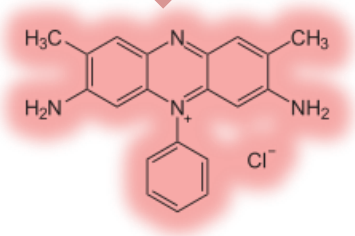
Espaço intermembranas





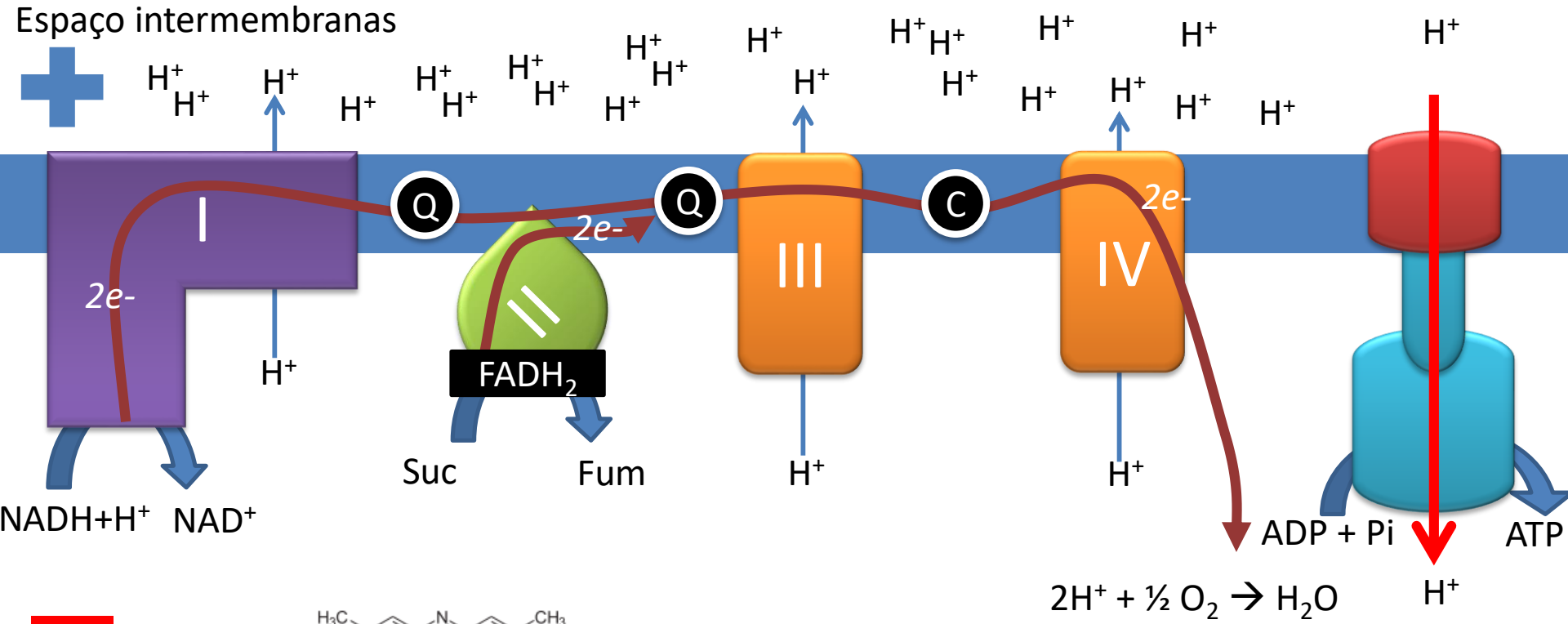


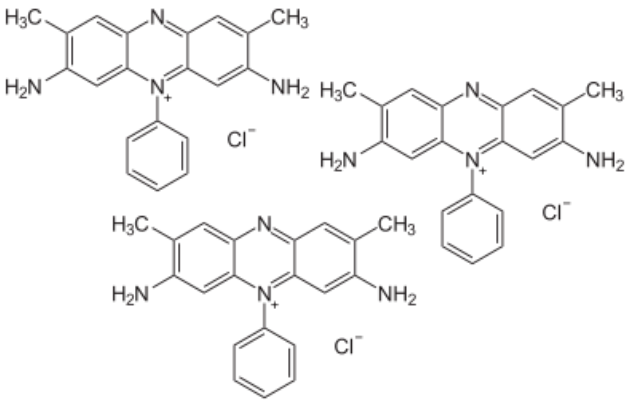




"quenching"

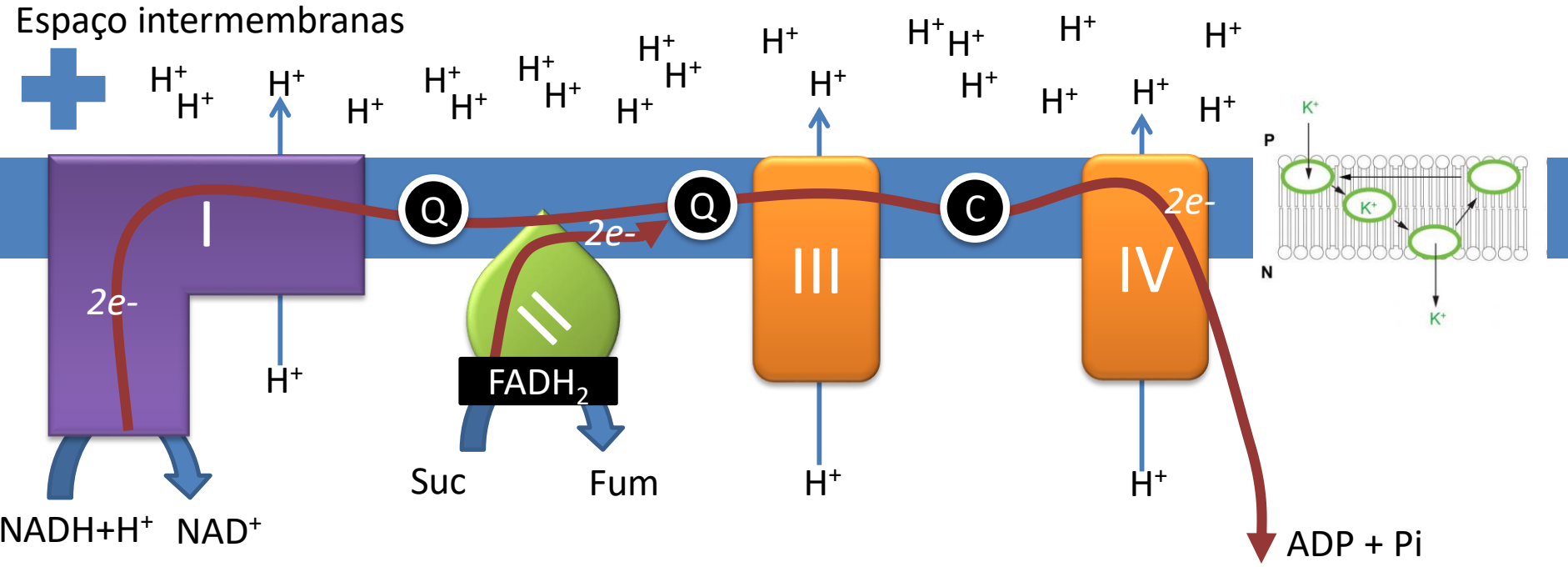
- Lipossolúvel e catiônico

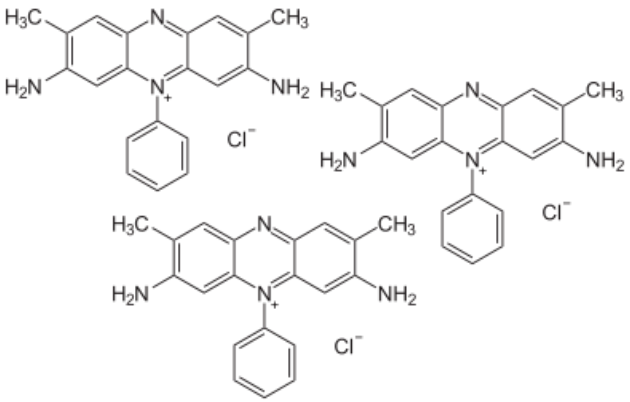




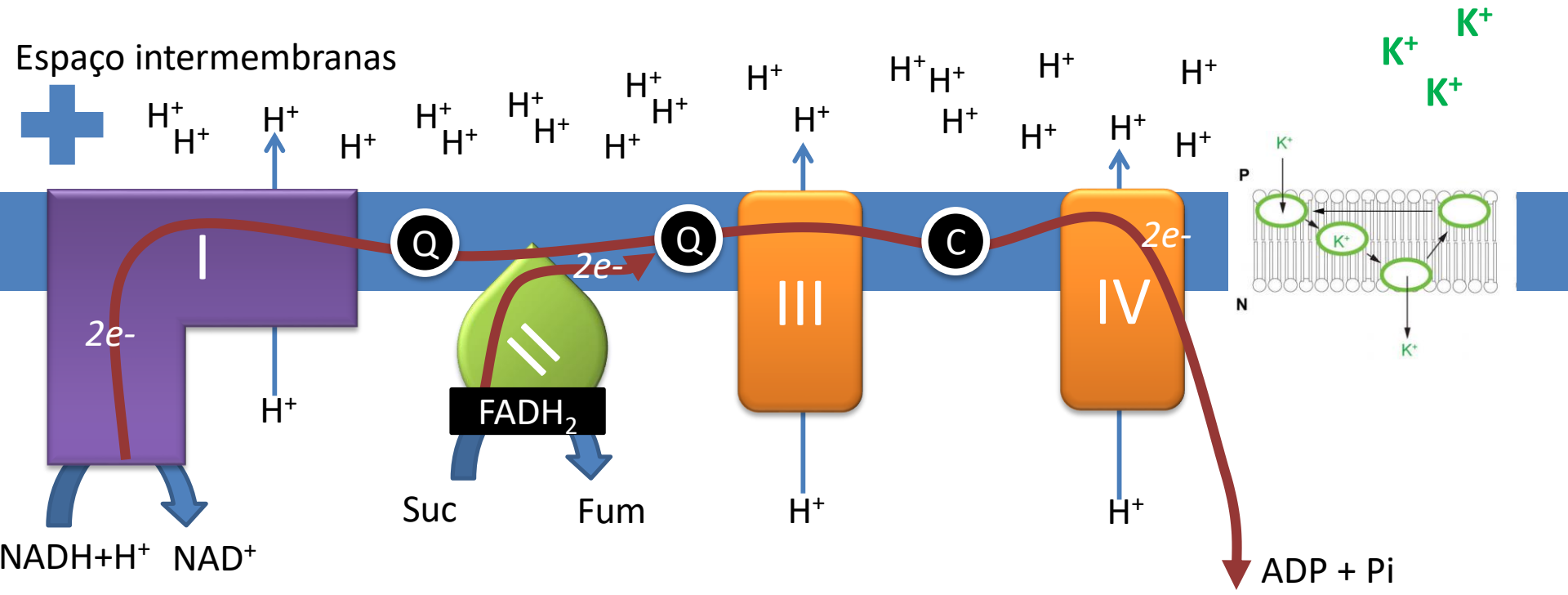
"quenching"

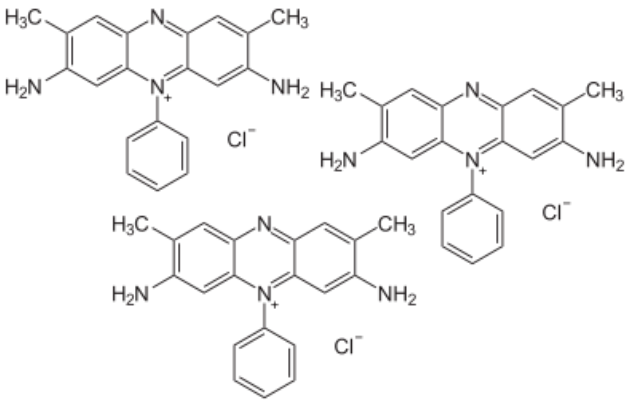
- Valinomicina (ionóforo)



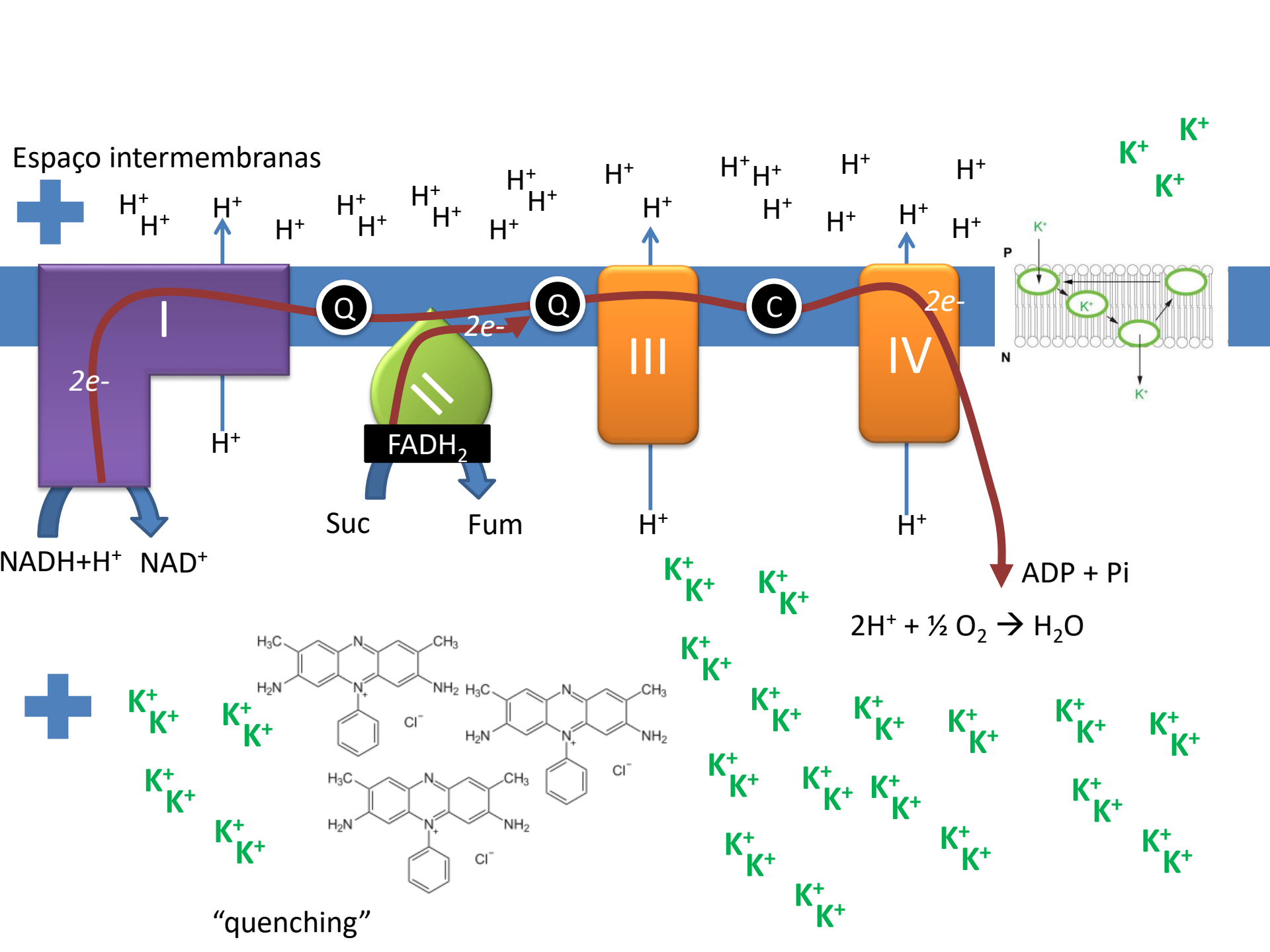


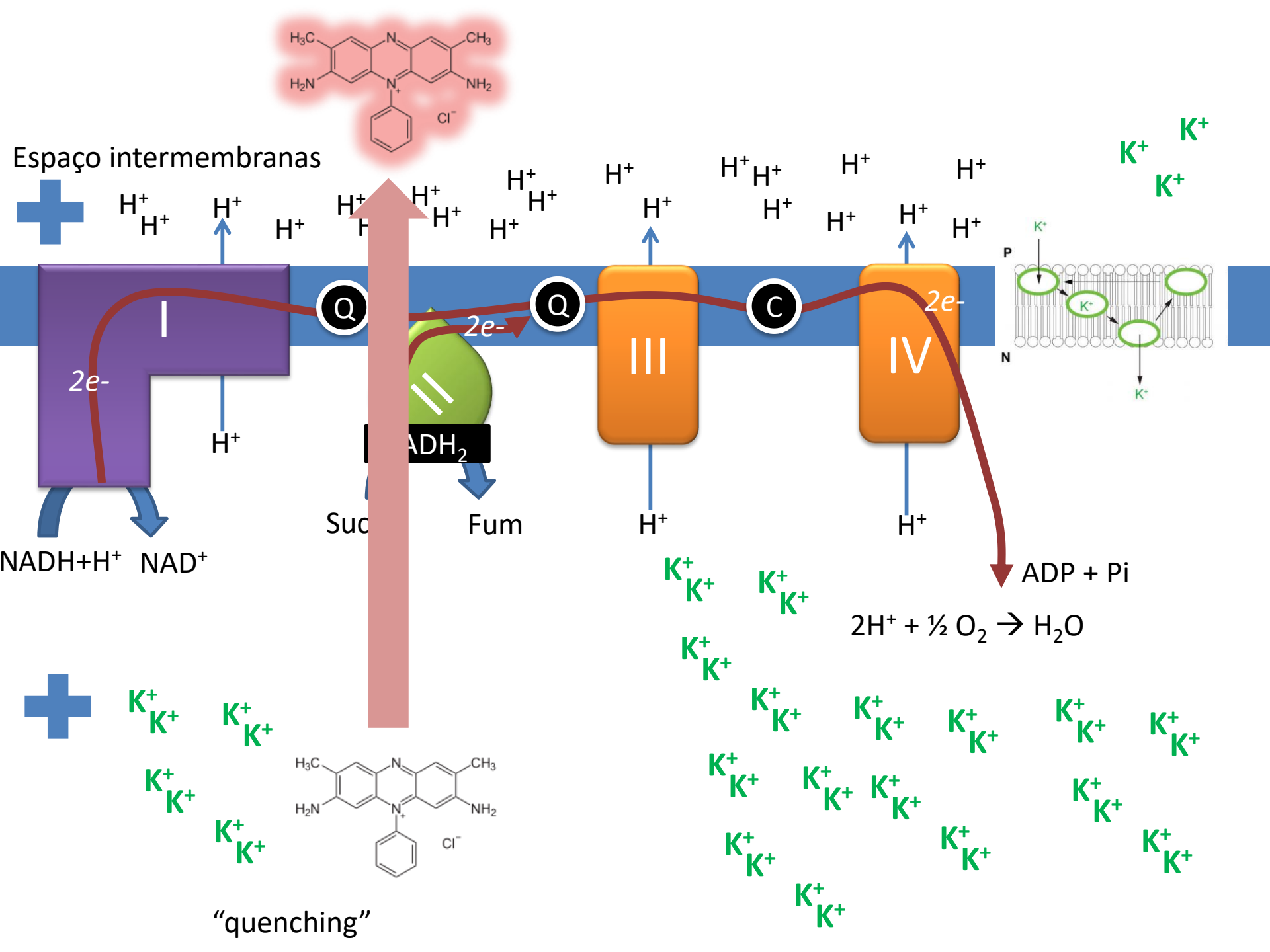
"quenching"


Espaço intermembranas
H+
I
2e-
Q
II
FADH2
Suc
Fum
Q
2e-
III
C
IV
2e-
P
N
K+
NADH+H+
NAD+
ADP + Pi
2H+ + ½ O2 → H2O

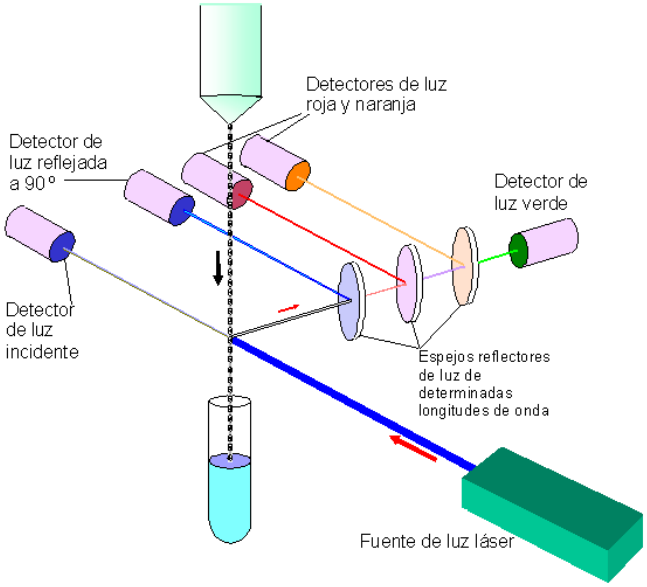

H3C
CH3
H2N
NH2
Cl-

"quenching"


Espaço intermembranas
I
II
III
IV
Q
Q
C
2e-
2e-
2e-
FADH2
Suc
Fum
NADH+H+
NAD+
ADP + Pi
2H+ + ½ O2 → H2O
P
N
"quenching"


Espaço intermembranas
I
II
III
IV
Q
Q
C
2e-
2e-
2e-
NADH+H+
NAD+
Suc
Fum
ADH2
ADP + Pi
2H+ + ½ O2 → H2O
P
N
"quenching"

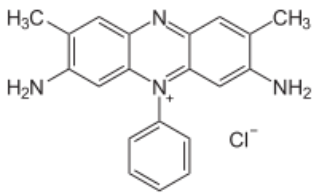
Potencial de membrana interna mitocondrial ($\Delta\Psi_m$)



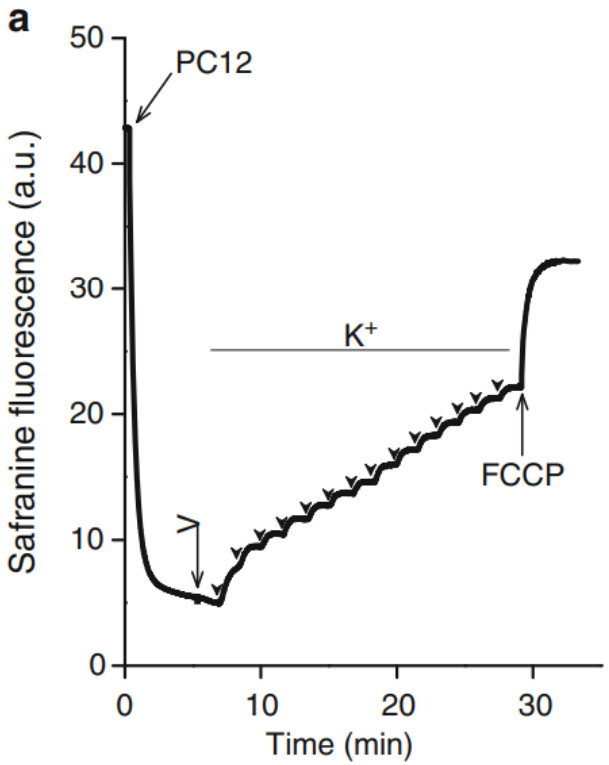
Nernst equation: $\Delta\Psi_m = 60 \times \log(K_{IN}^{+} / K_{OUT}^{+})$

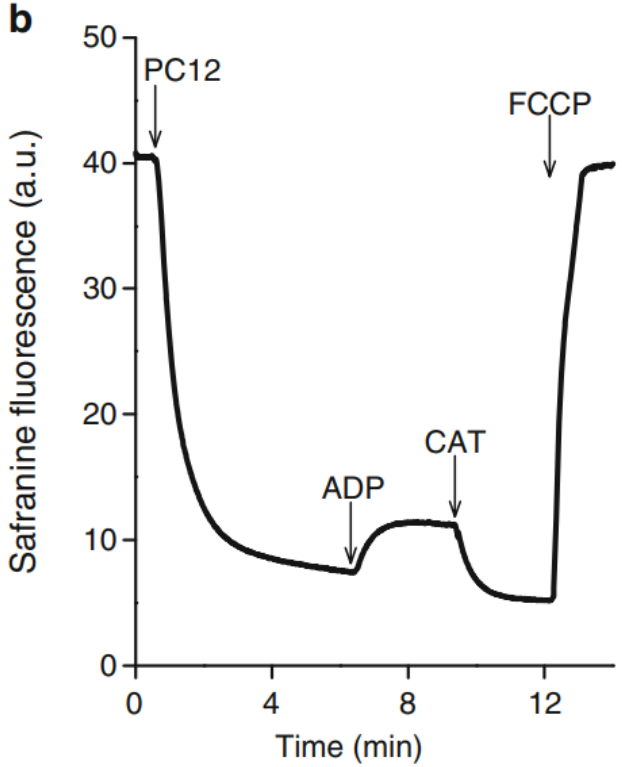
Potencial de membrana interna mitocondrial ($\Delta\Psi_m$)

Safranina O

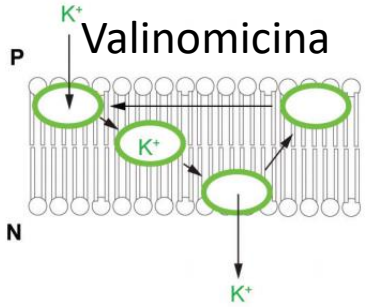


Safranina O



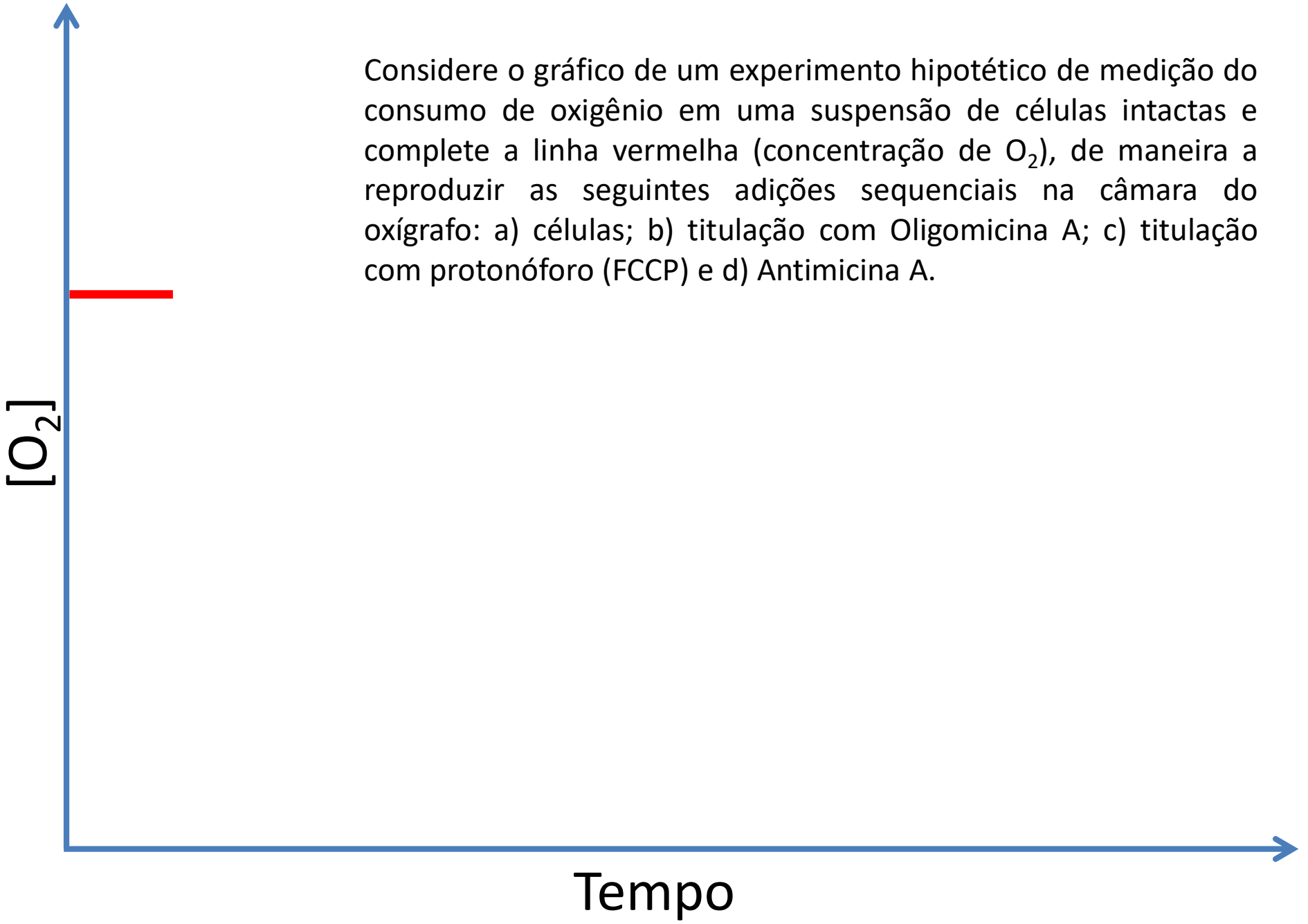


Valinomicina

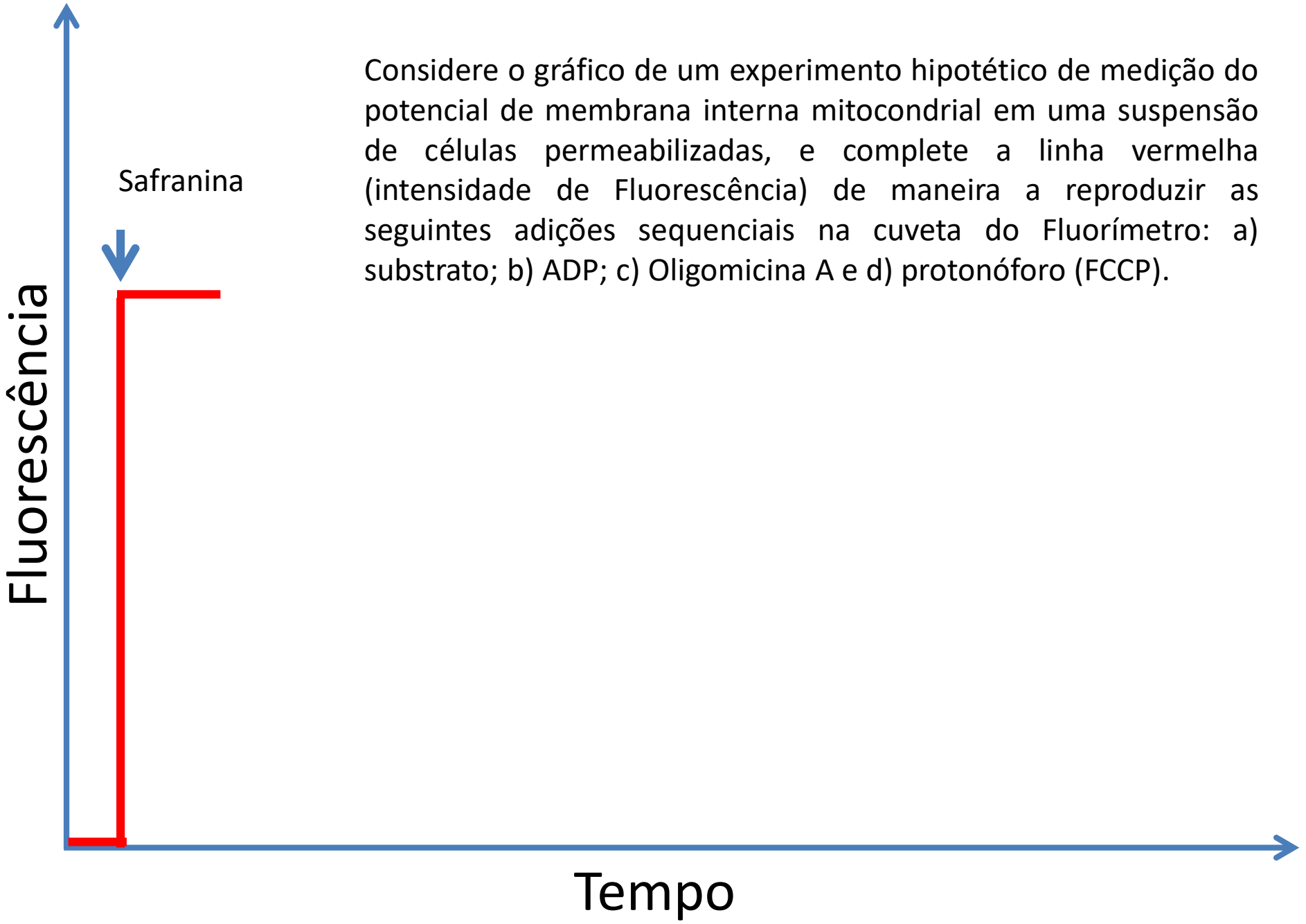


Nernst equation: $\Delta\Psi_m = 60 \times \log(K_{IN}^+ / K_{OUT}^+)$


MIGHTY
CHONDRION
Obrigado pela atenção!



Considere o gráfico de um experimento hipotético de medição do consumo de oxigênio em uma suspensão de células intactas e complete a linha vermelha (concentração de O_2), de maneira a reproduzir as seguintes adições sequenciais na câmara do oxígrafo: a) células; b) titulação com Oligomicina A; c) titulação com protonóforo (FCCP) e d) Antimicina A.



Considere o gráfico de um experimento hipotético de medição do potencial de membrana interna mitocondrial em uma suspensão de células permeabilizadas, e complete a linha vermelha (intensidade de Fluorescência) de maneira a reproduzir as seguintes adições sequenciais na cuveta do Fluorímetro: a) substrato; b) ADP; c) Oligomicina A e d) protonóforo (FCCP).