<u>TEMA 6</u>: Transferencia electrónica en la fosforilación oxidativa: Rol de las metaloproteínas.

- **Glicólisis**
  - **Conversión de Glucosa a Piruvato**

- ***Transformación de piruvato a Acetil-CoA***
  - ***Funcionamiento del complejo piruvato desidrogenasa***
  - ***Cofactores involucrados***

- ***Ciclo de Krebs***
  - **Funcionamiento de algunas enzimas**

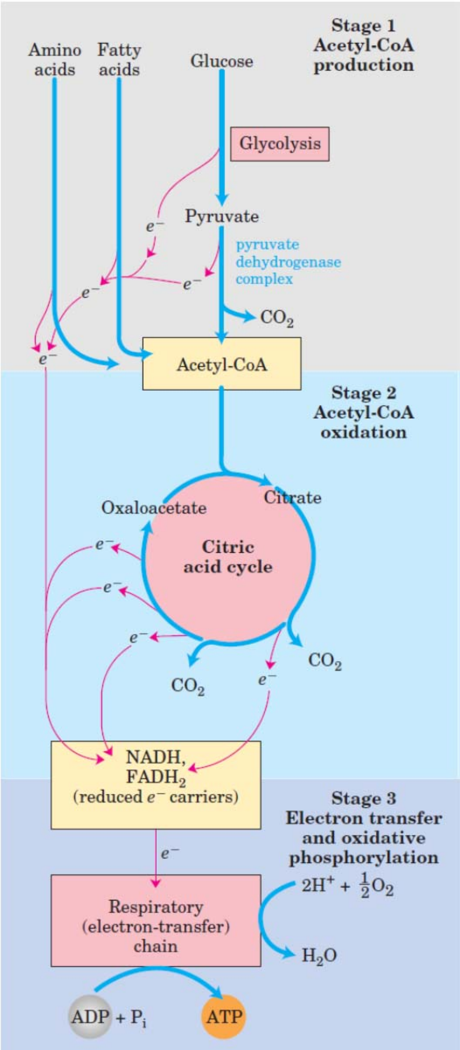
- ***Otros fuentes de energía***
  - **Catabolismo de lípidos**
  - **Catabolismo de aminoácidos**

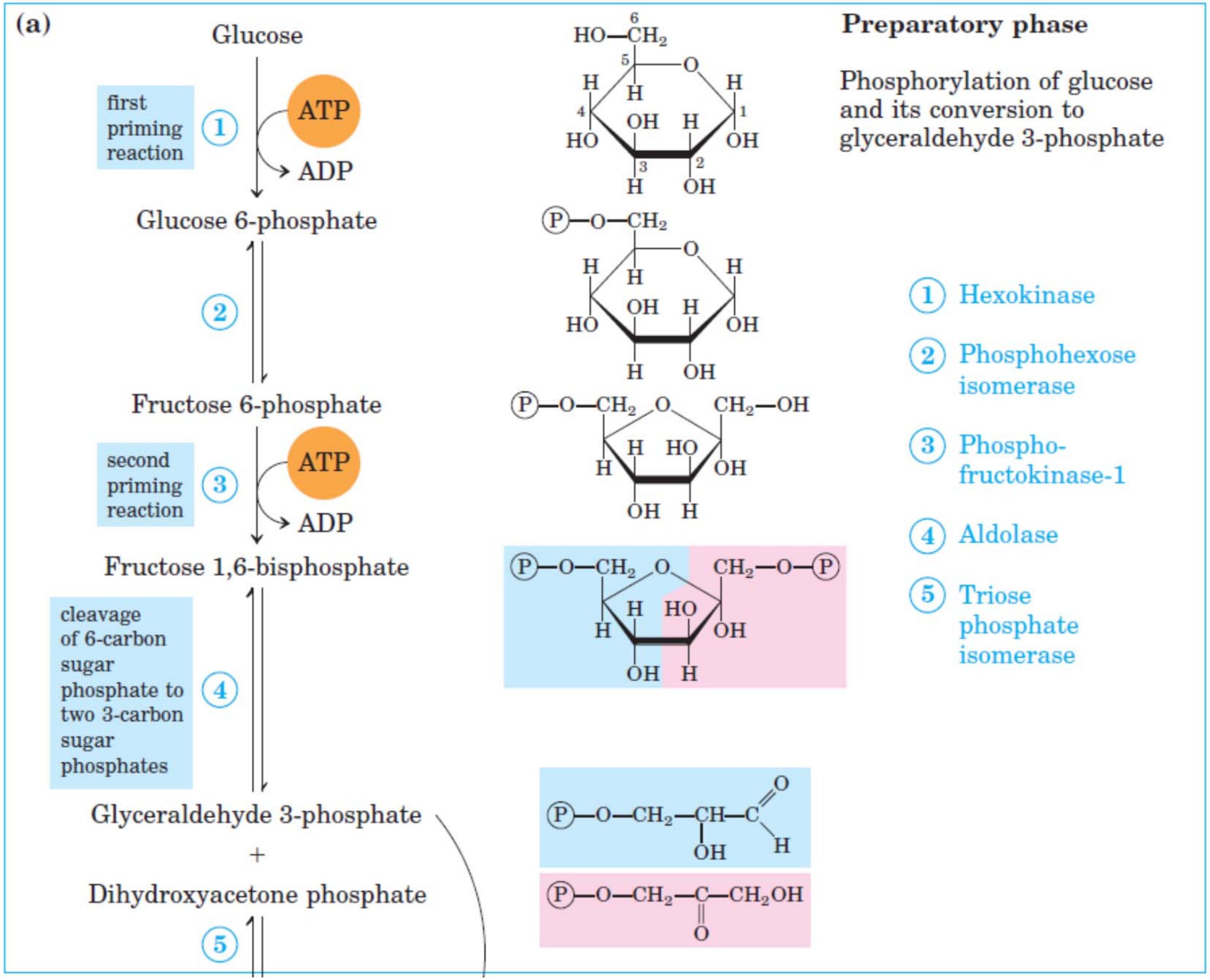
- ***Fosforilación oxidativa***
  - ***Complejo I***
  - ***Complejo II***
  - ***Complejo III***
  - ***Complejo IV***
  - ***Complejo V***

- ***Modelo de Mosser-Duton***
  - ***Cálculo de constantes de transferencia electrónica***

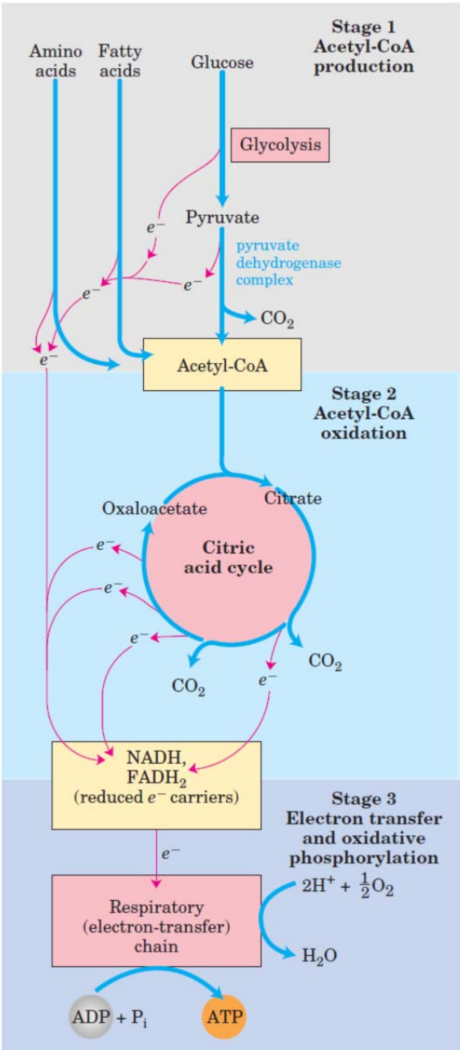
Lehninger
Principles of Biochemistry

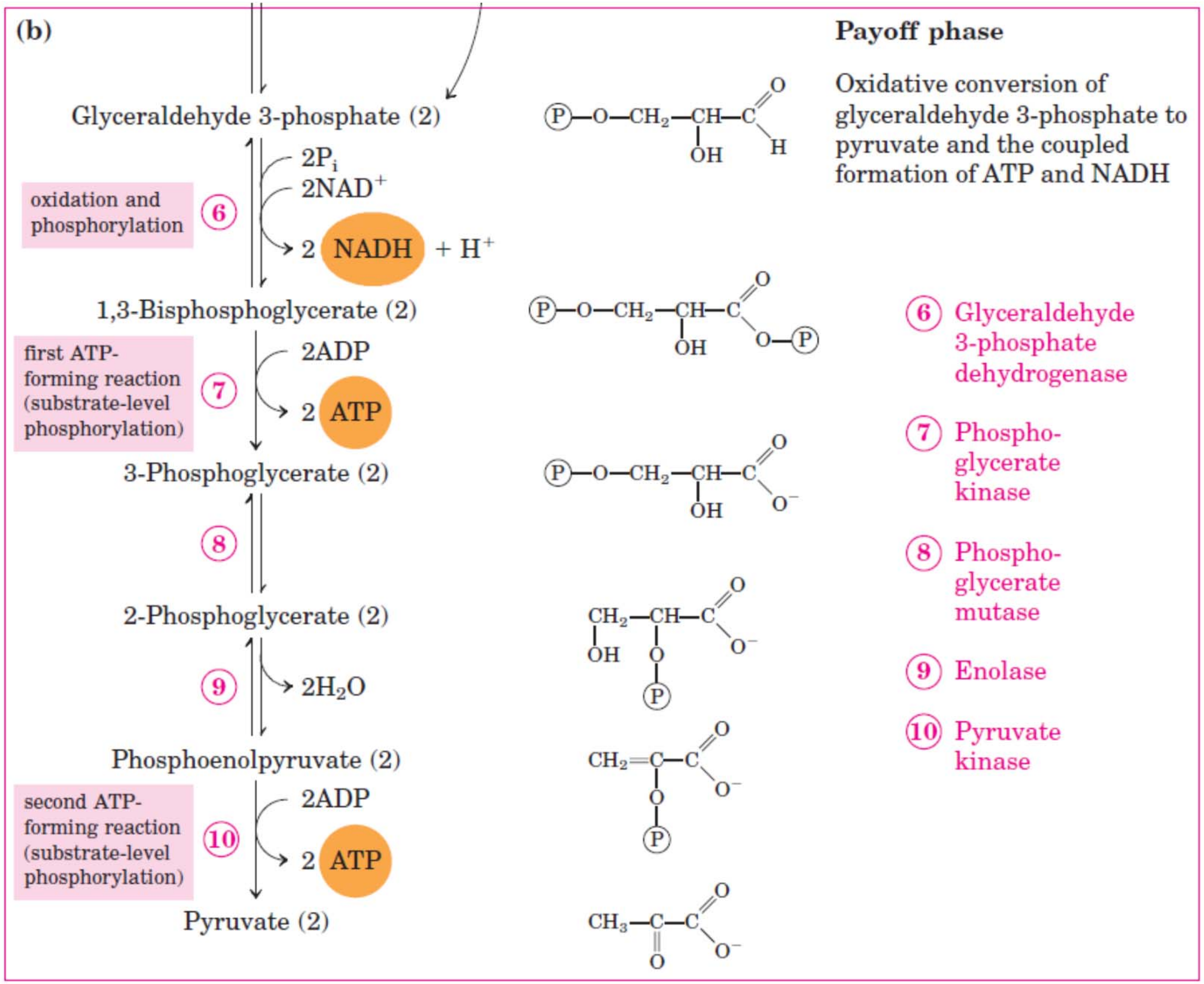
Glicólisis: Parte 1

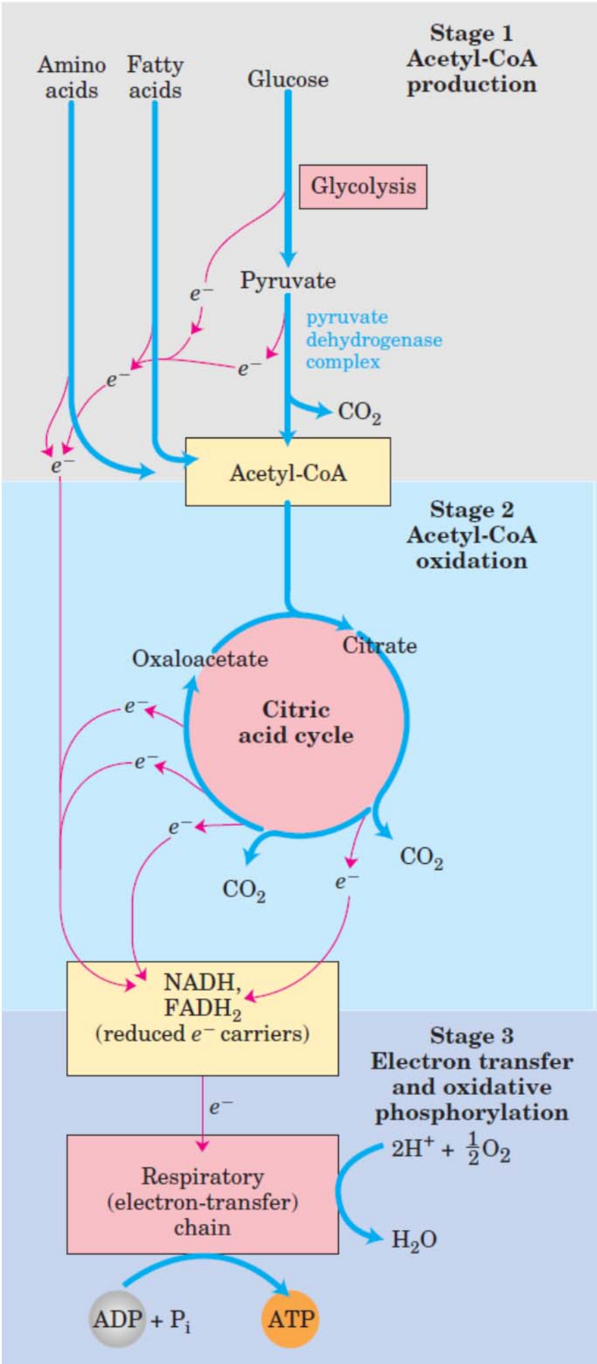




Glicólisis: Parte 2







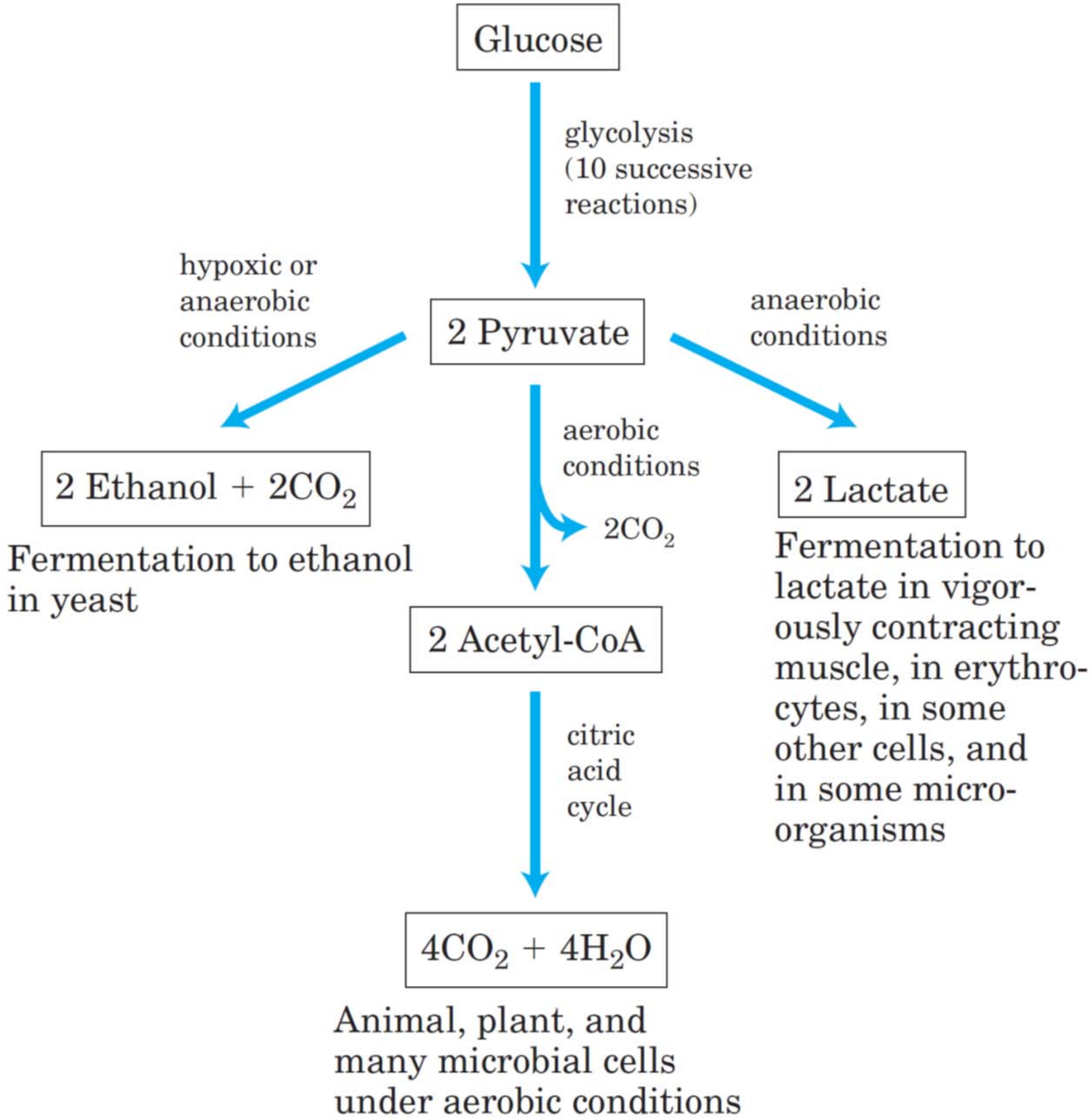
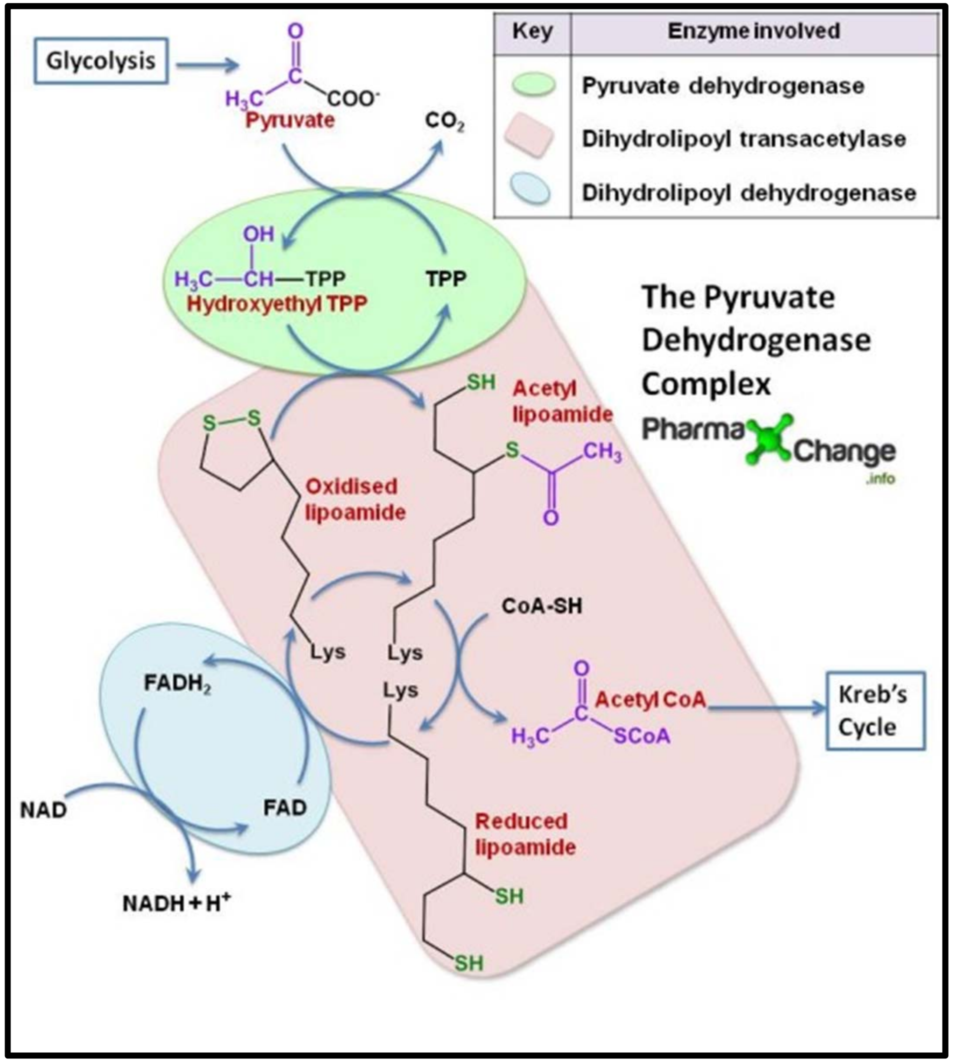
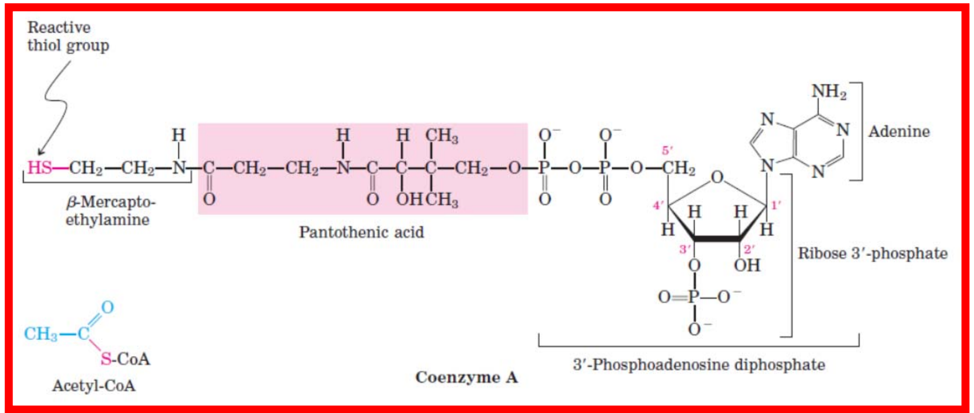


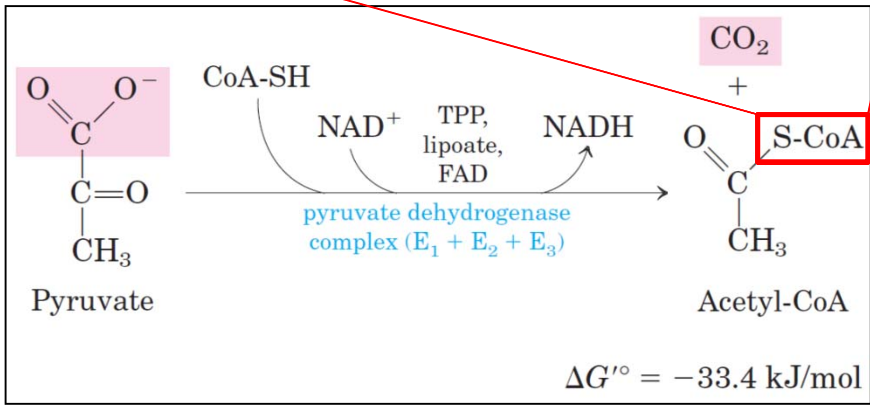
FIGURE 14–3 Three possible catabolic fates of the pyruvate formed in glycolysis. Pyruvate also serves as a precursor in many anabolic reactions, not shown here.

piruvato desidrogenasa

- Heterotramero complejo (αβγ)
- Sitio activo: Sin metales ☹ / TPP, FAD, CoA
- Reacción: Decarboxilación oxidativa (NAD^+)

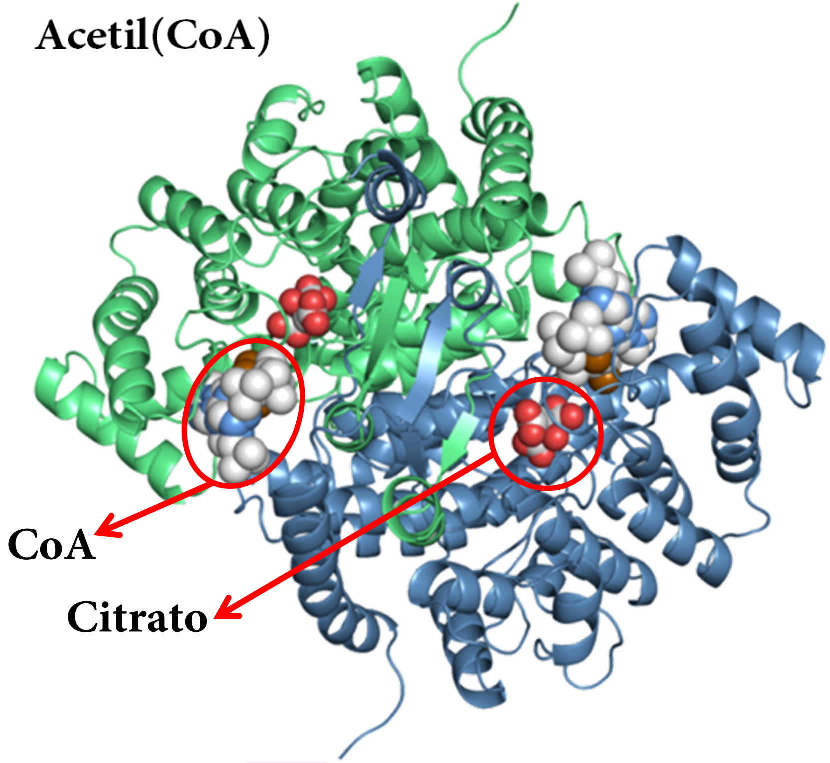


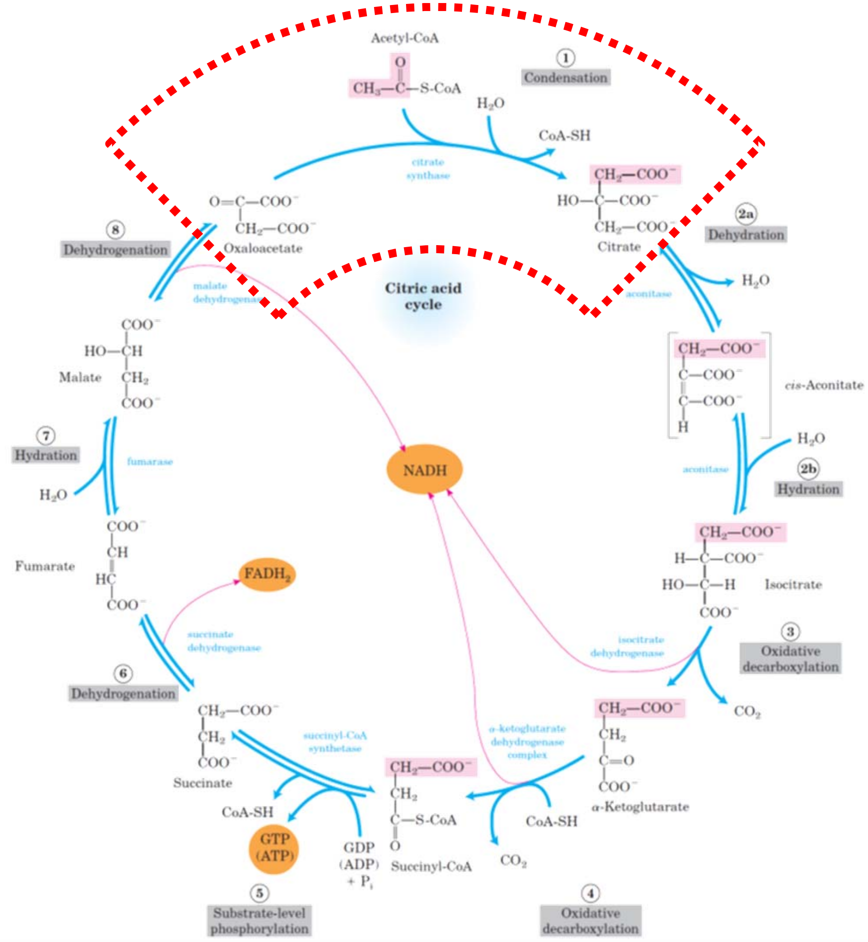




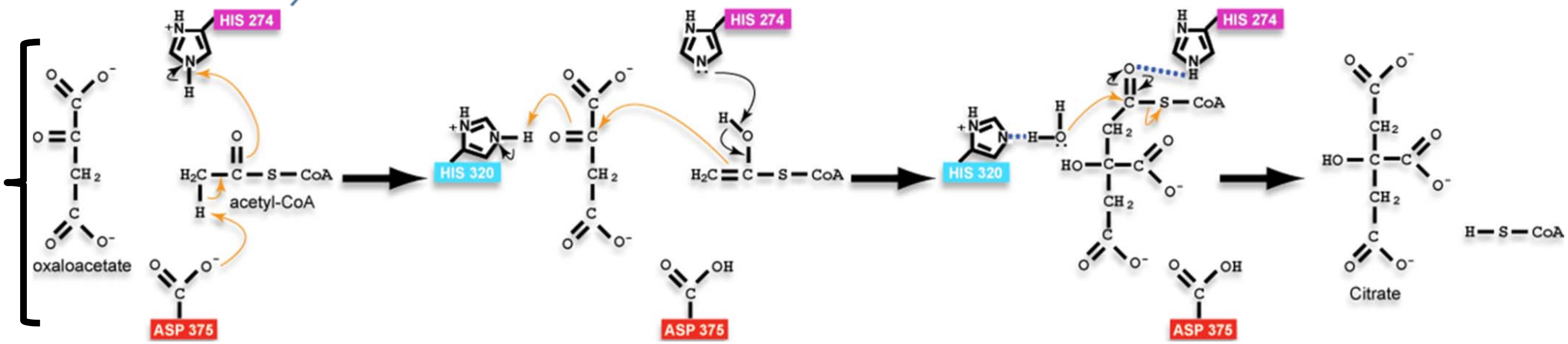
Citrato sintetasa

- Homodimero – Sin grupos prostéticos ☹
- His → ácido Lewis
- Reacción: condensación del Oxaloacetato + Acetil(CoA)



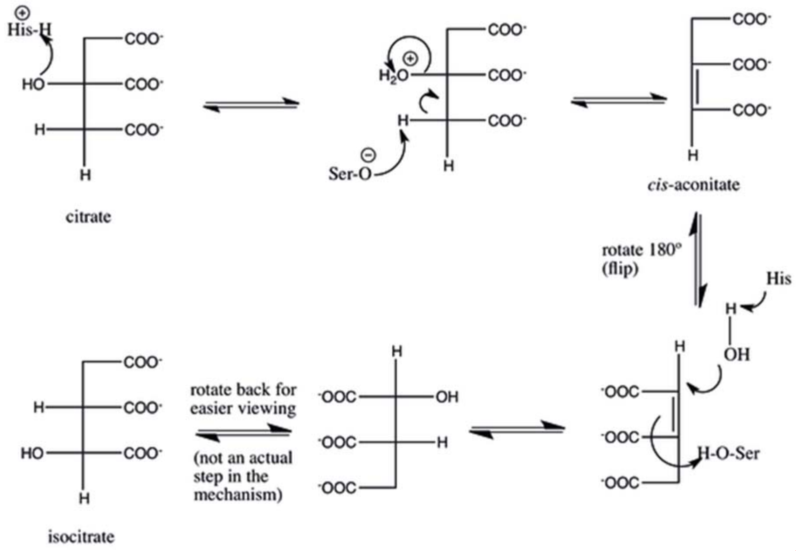


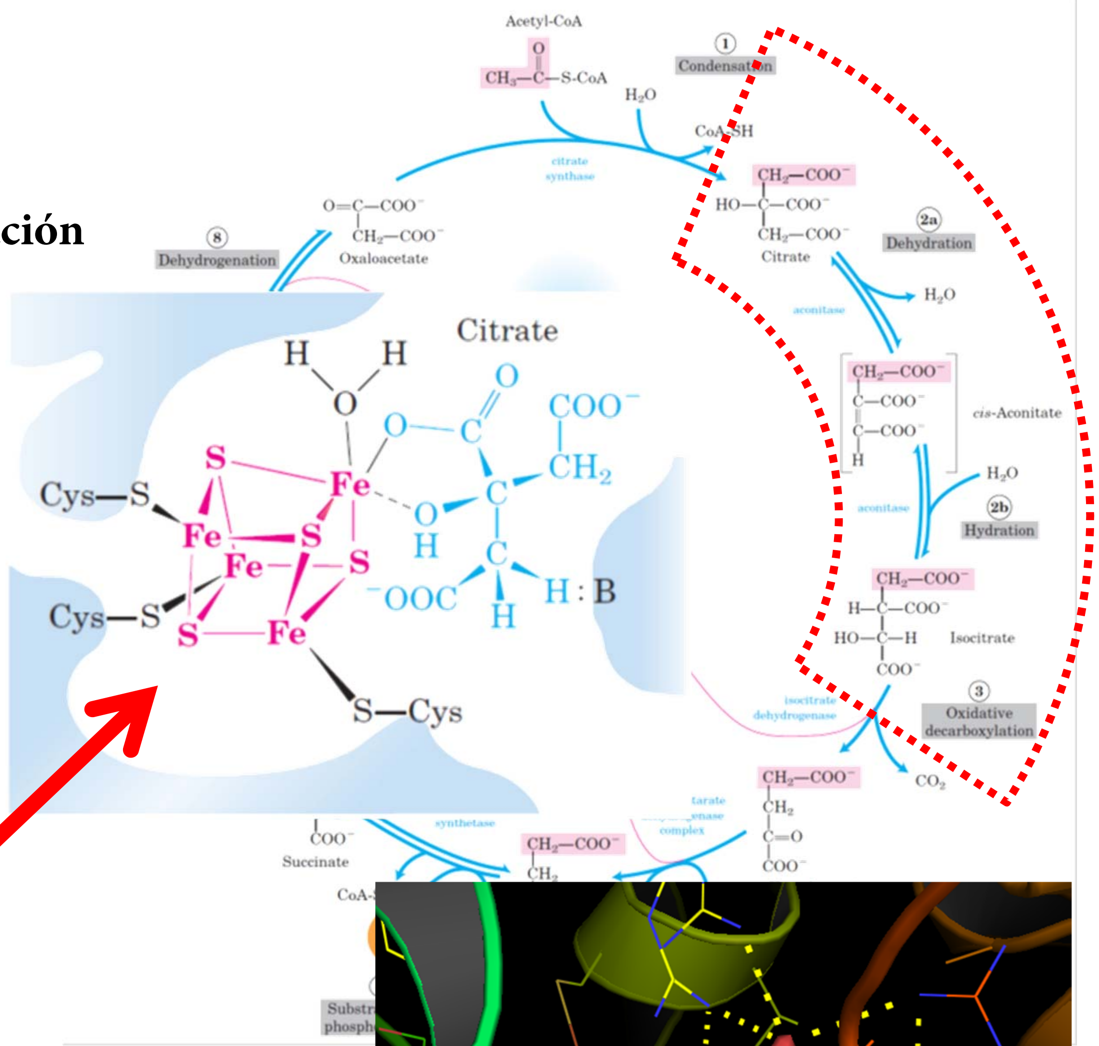
Mecanismo

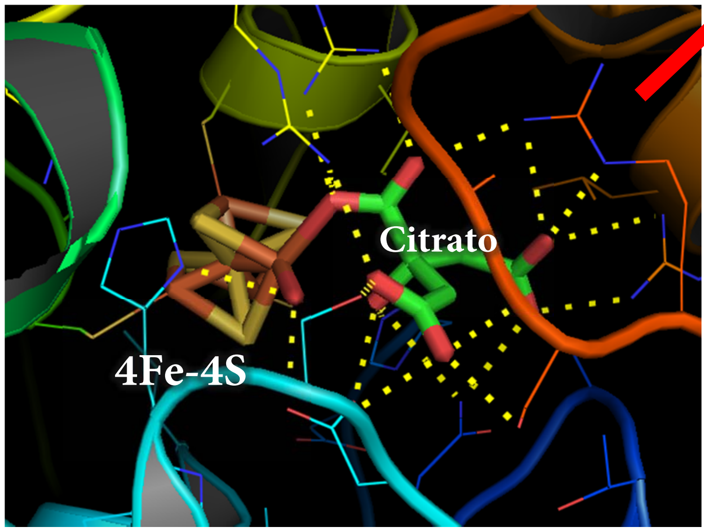


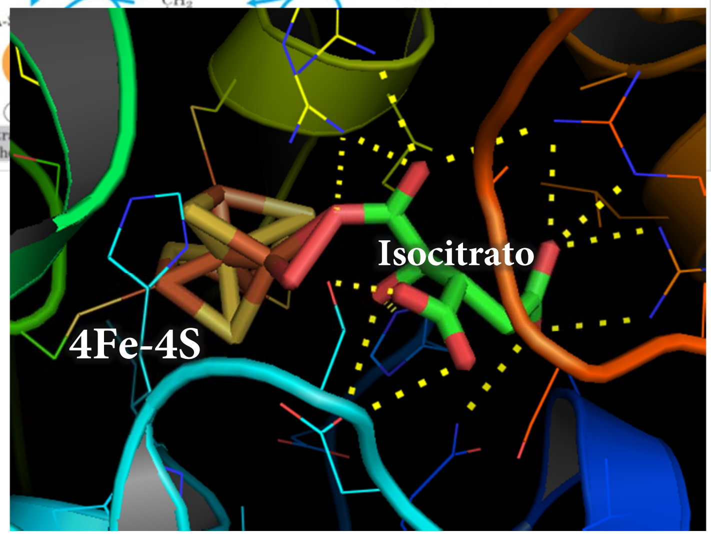
Aconitasa

- Monomero
- Sitio activo: [4Fe-4S] – No redox
- Reacción: Desidratación → Hidratación



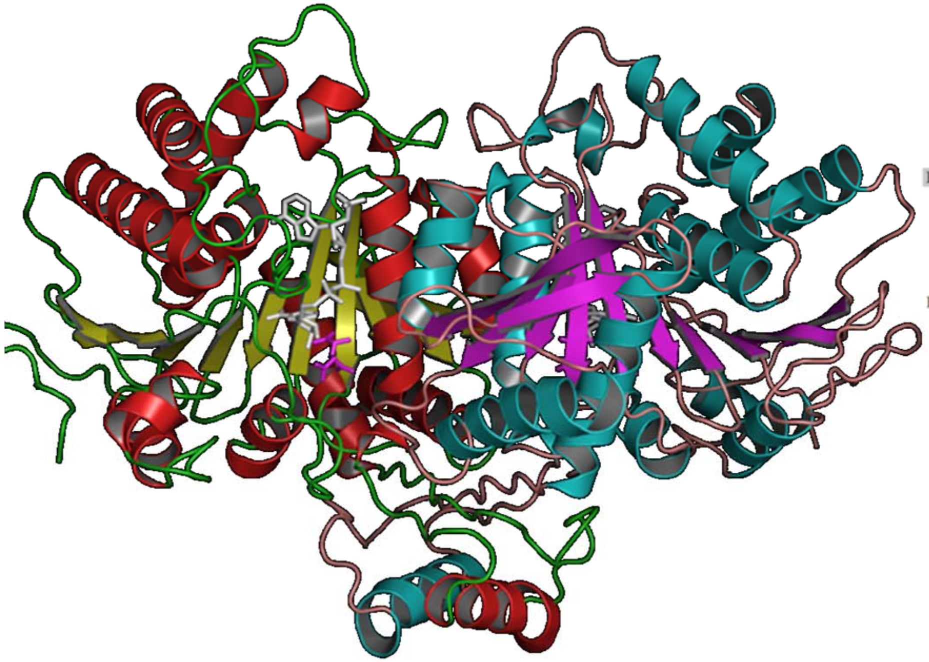


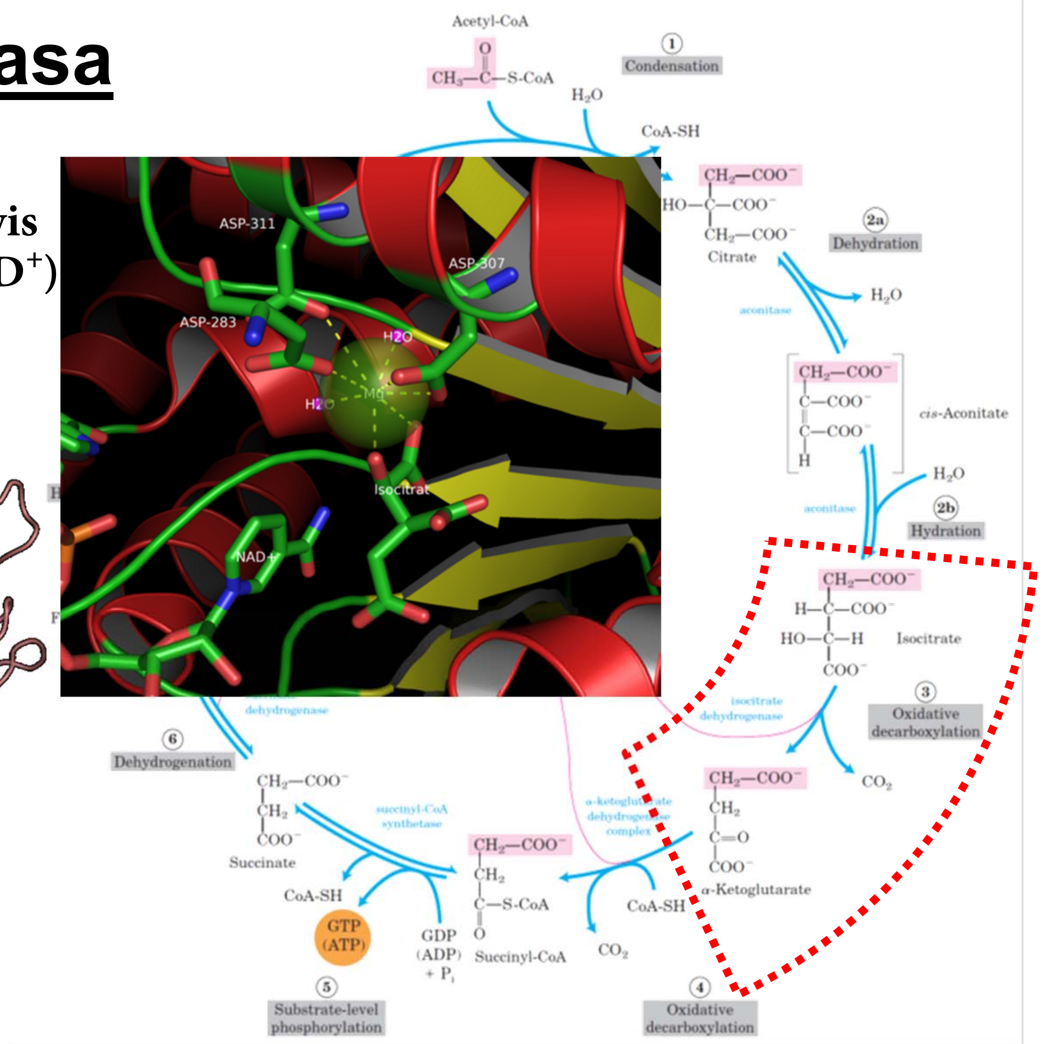




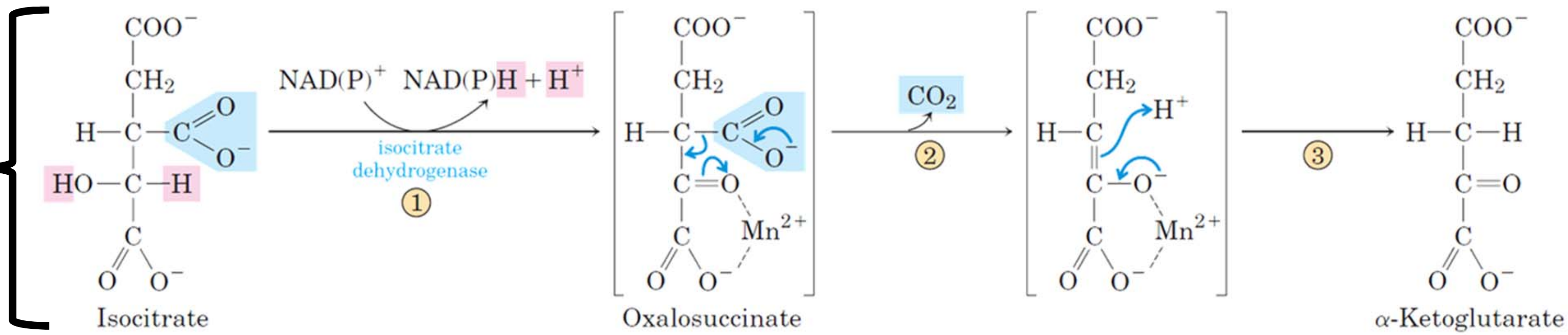
Isocitrato desidrogenasa

- Heterotramero ($\alpha_2\beta\gamma$)
- Sitio activo: Mn^{2+} → No redox/ácido Lewis
- Reacción: Decarboxilación oxidativa (NAD^+)



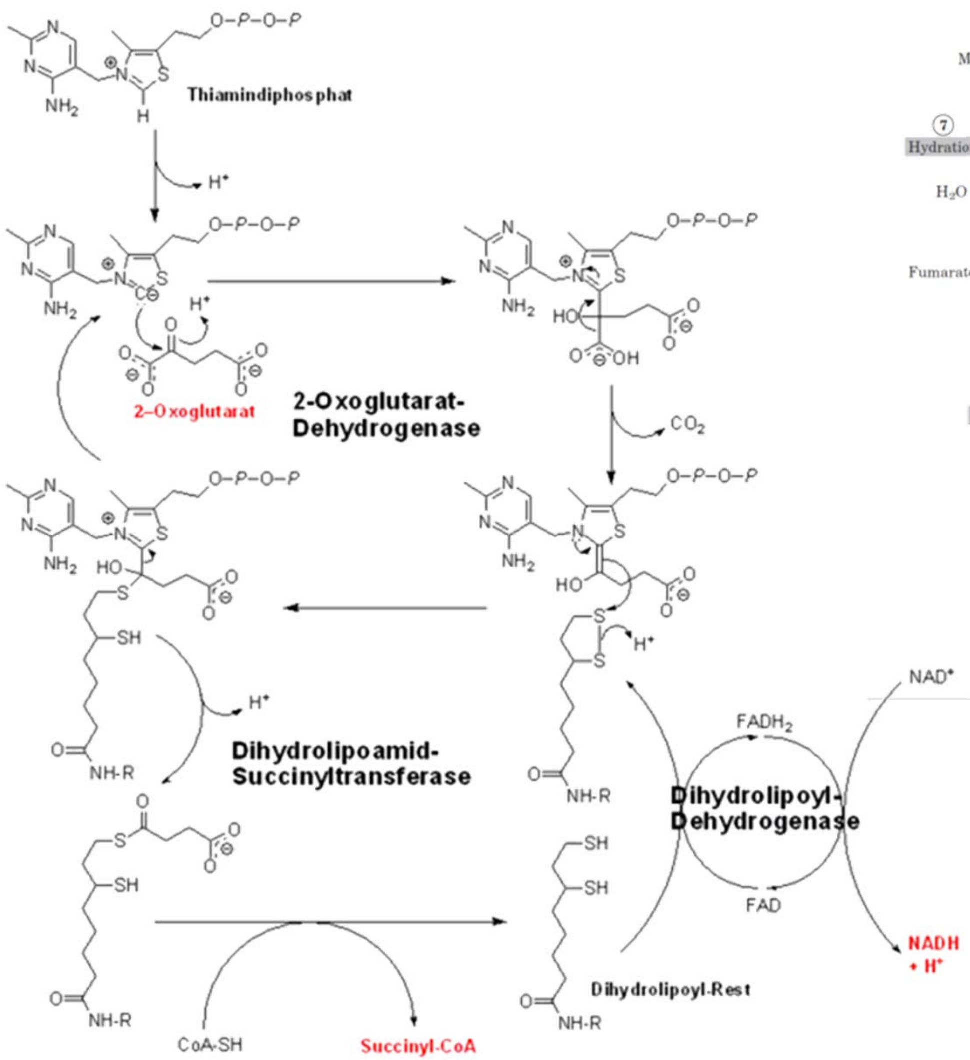


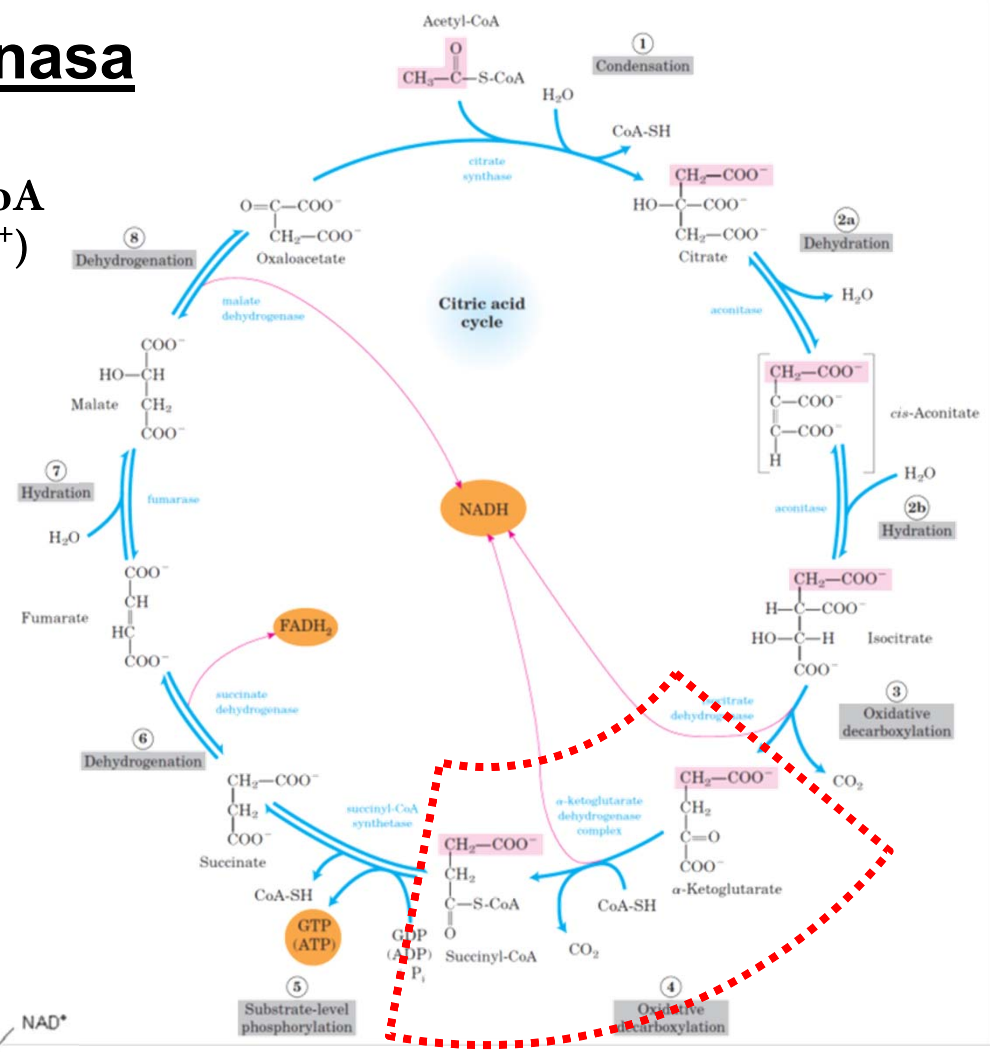
Mecanismo



α-cetoglutarato desidrogenasa

- Heterotrimero complejo (αβγ o $E_1E_2E_3$)
- Sitio activo: Sin metales ☹ / TPP, FAD, CoA
- Reacción: Decarboxilación oxidativa (NAD^+)





succinil-CoA sintetasa

- Succinato tioquinasa o succinato/CoA ligasa
- Heterodimero (αβ)
- Sitio activo: Sin metales ☹ - ADP o GDP
- Reacción: generación de ATP/GTP

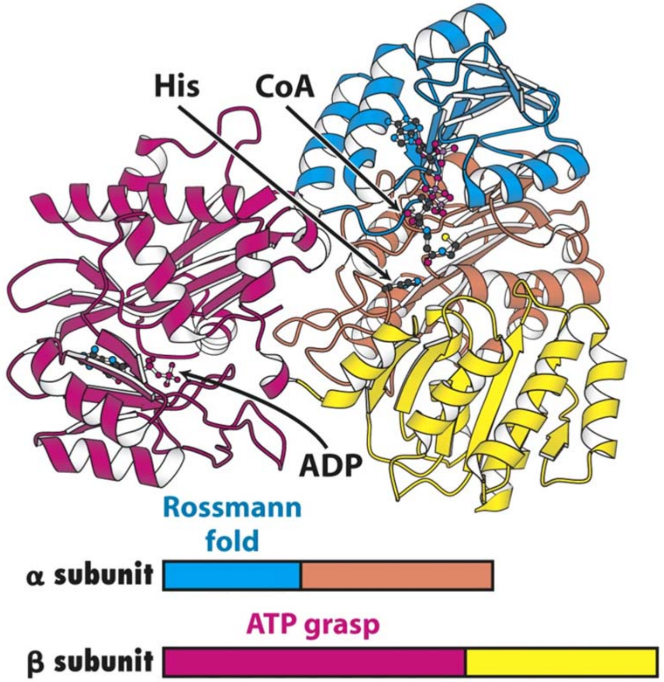
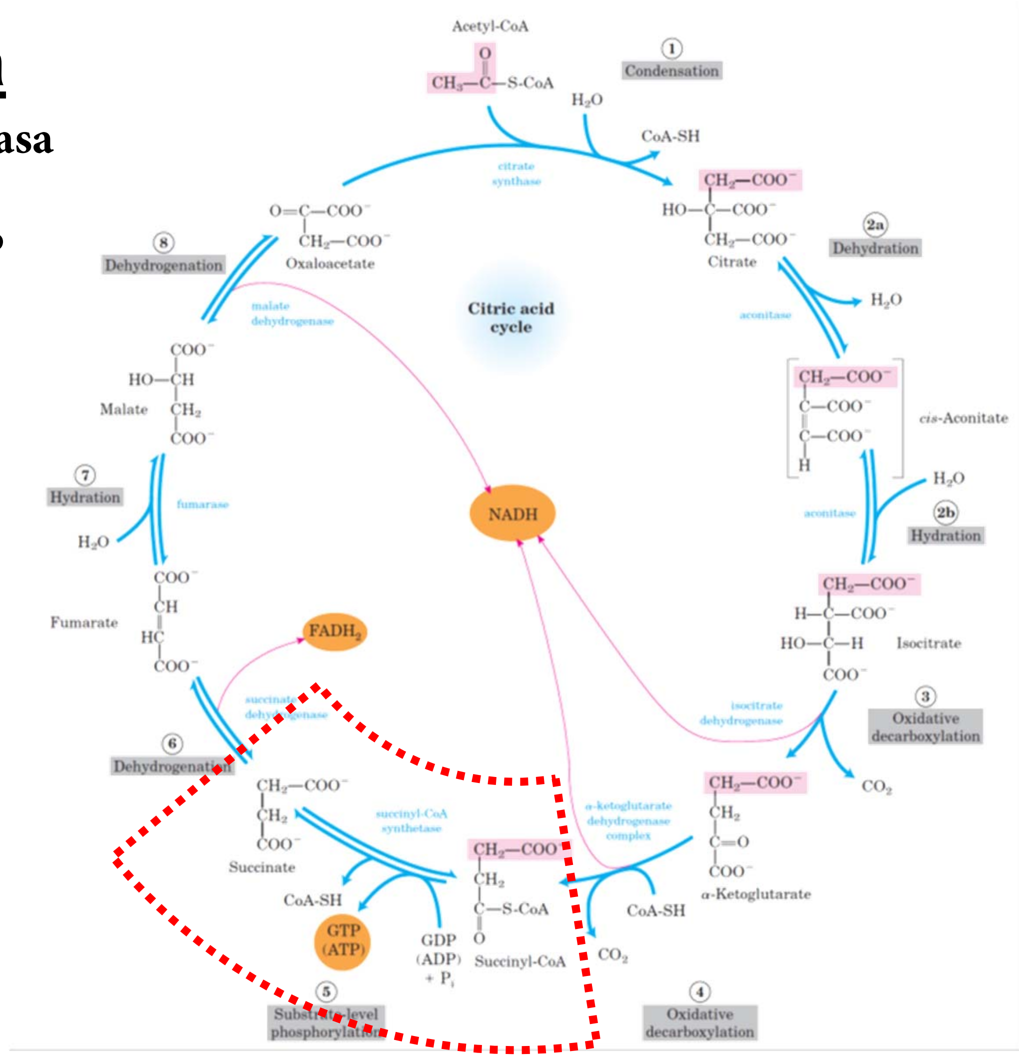
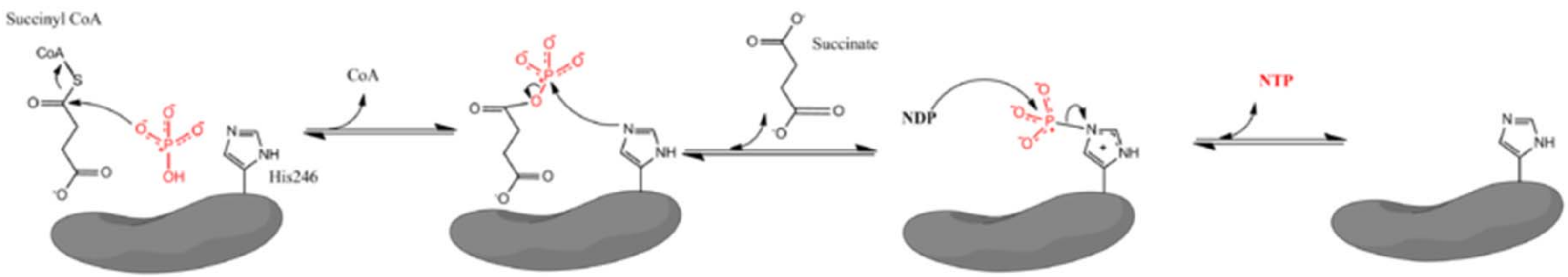


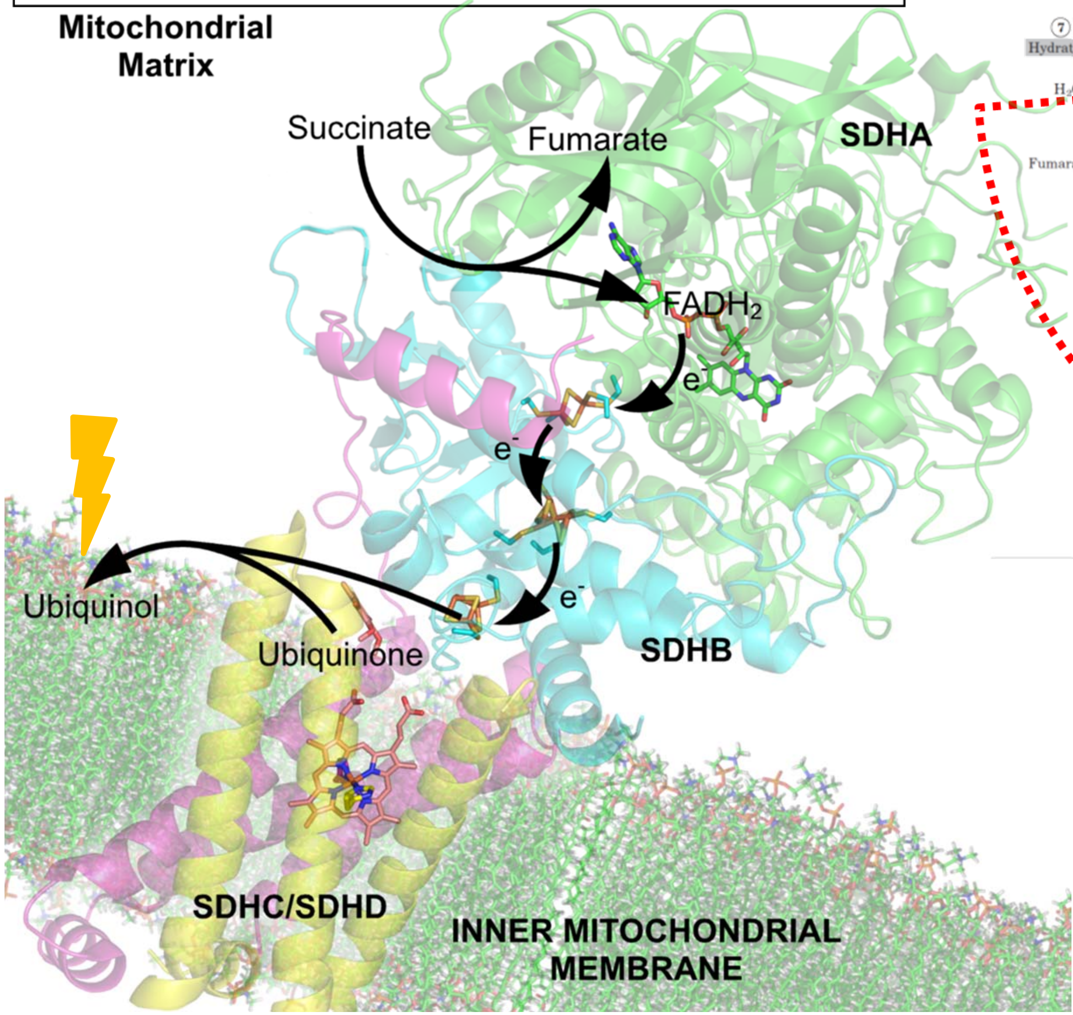
Figure 17-14
Biochemistry, Sixth Edition

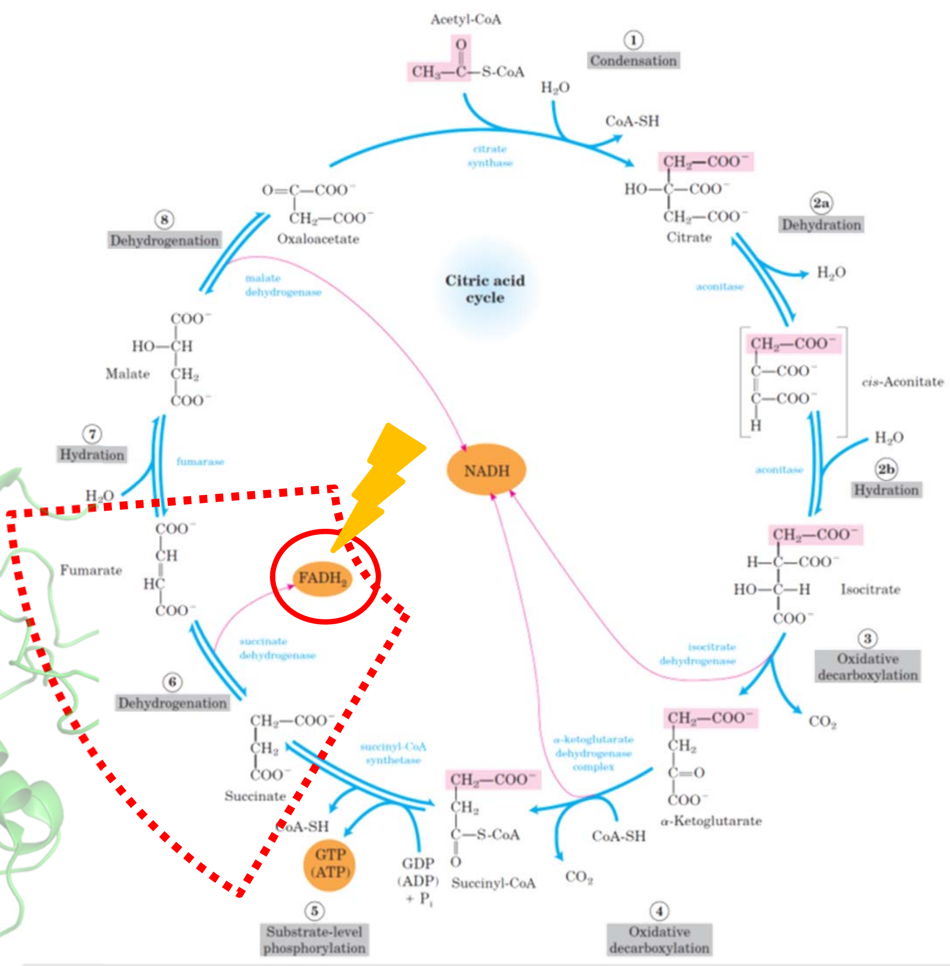




succinato deshidrogenasa

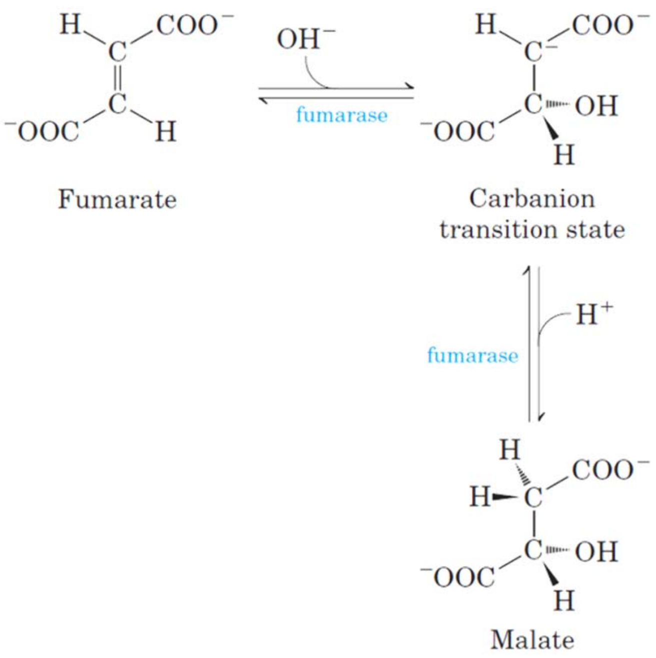
- succinato:CoQ oxidoreductasa o **Complejo II**
- Heterodimero (αβγδ)
- Sitio activo: FAD
- Transferencia e^-: [2Fe-2S], [4Fe-4S], and [3Fe-4S]
- Reacción: Redox
  (succinato → fumarato + $2e^-$)
  ($UQ + 2e^- \rightarrow UQH_2$)

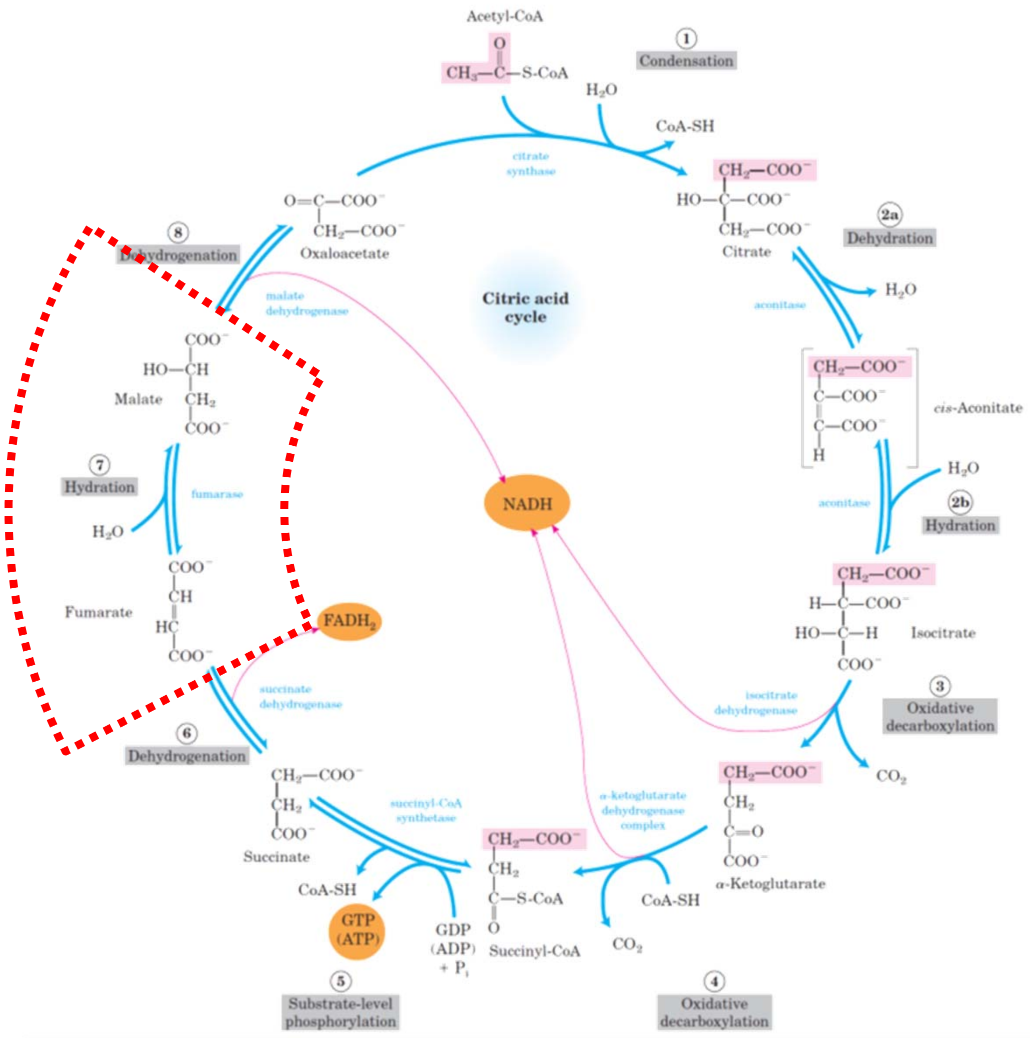




Fumarato hidratasa

- Fumarasa
- Homotetramero (α_4)
- Sitio activo: Sin metales ☹
- Reacción: Hidratación
  (fumarato → L-malato)



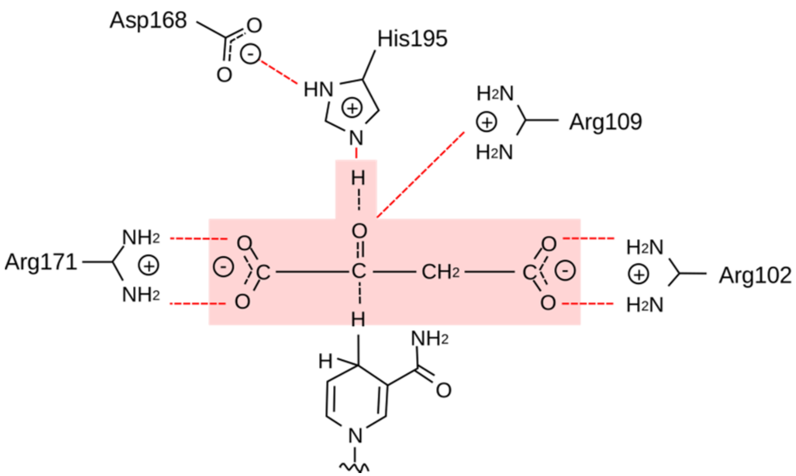


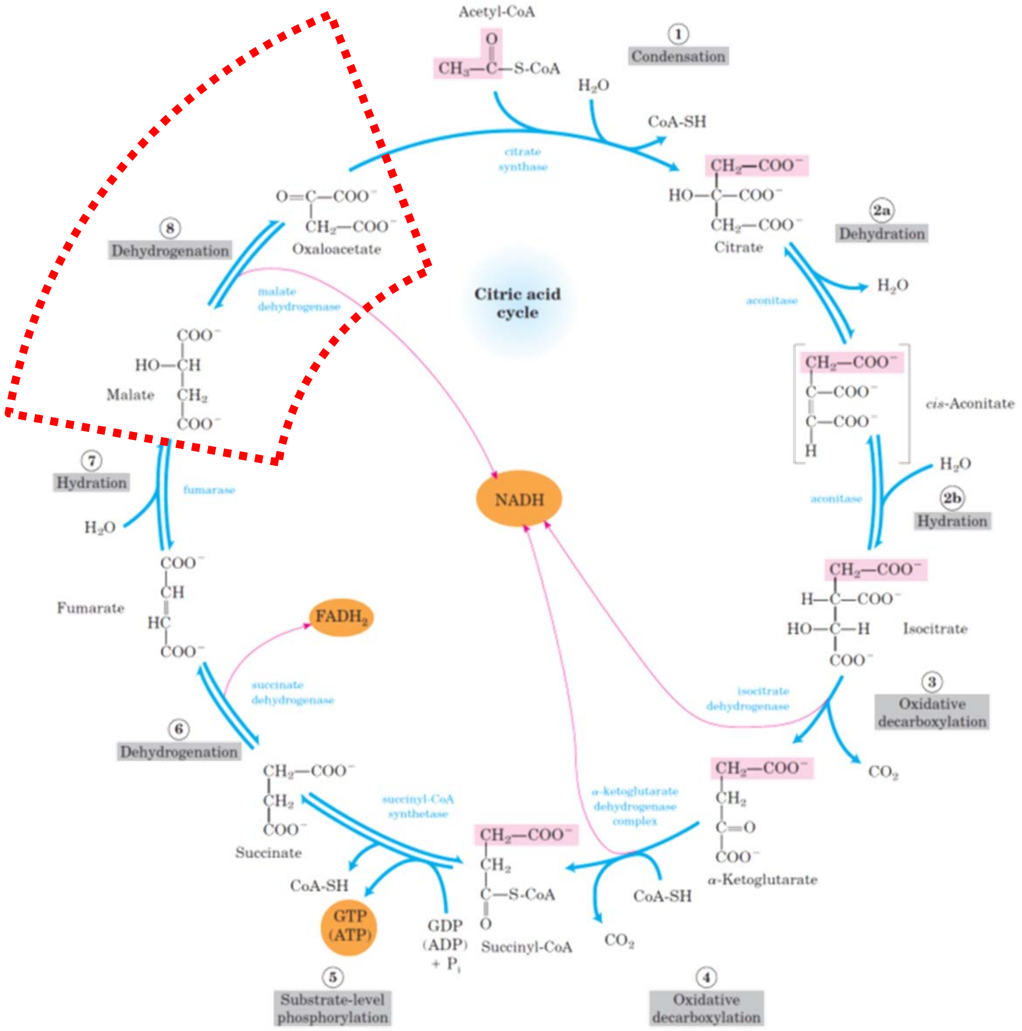
Malato desidrogenasa

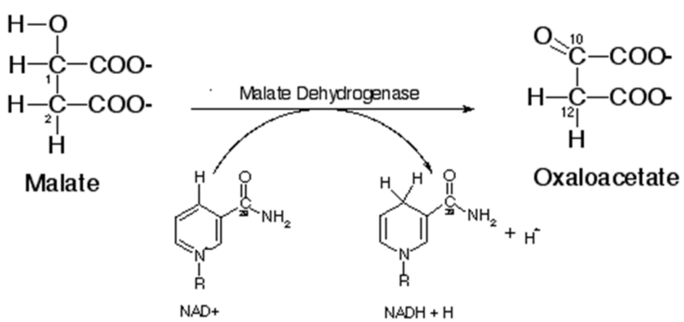
- Homodimero (α_2)
- Cercana filogenéticamente a LDH
- Sitio activo: Sin metales ☹
- Reacción: Redox

(malato → oxalato + $2e^-$)

($NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+$)







Balance energético

- → Glicólisis:
  - -2 ATP + 4 ATP = 2 ATP /glucosa
  - 2 NADH / glucosa
- → Piruvato desidrogenasa
  - 1 NADH / piruvato
- → Ciclo de Krebs
  - Sólo 1 ATP (o GTP) por ciclo ☹
  - 3 NADH / ciclo
  - 1 $FADH_2$ / ciclo (UQ + $2e^-$ → UQH_2)

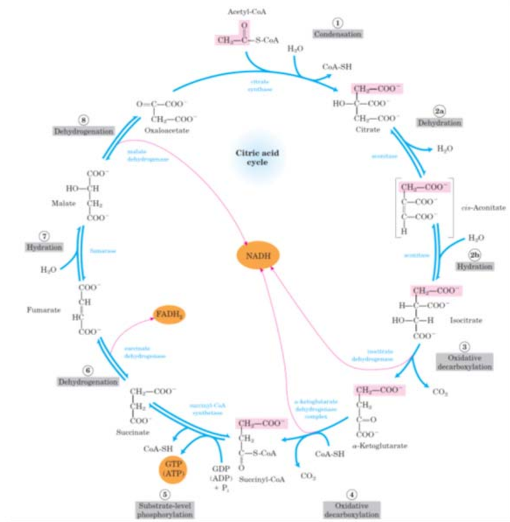


TABLE 16–1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	*Number of ATP or reduced coenzyme directly formed*	*Number of ATP ultimately formed**
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30–32

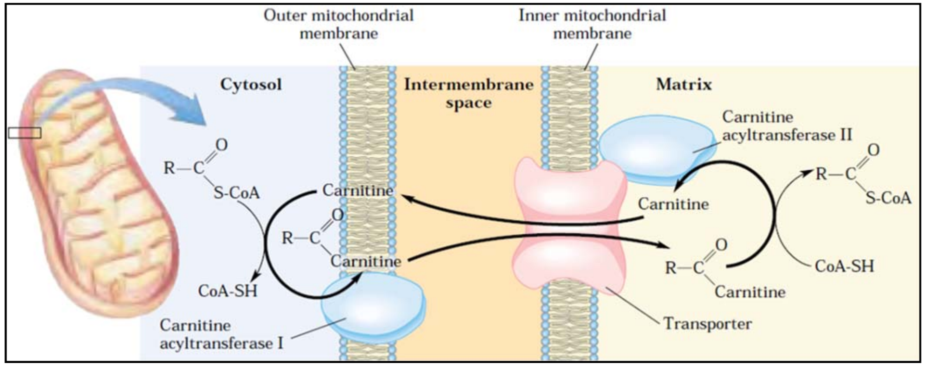
*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

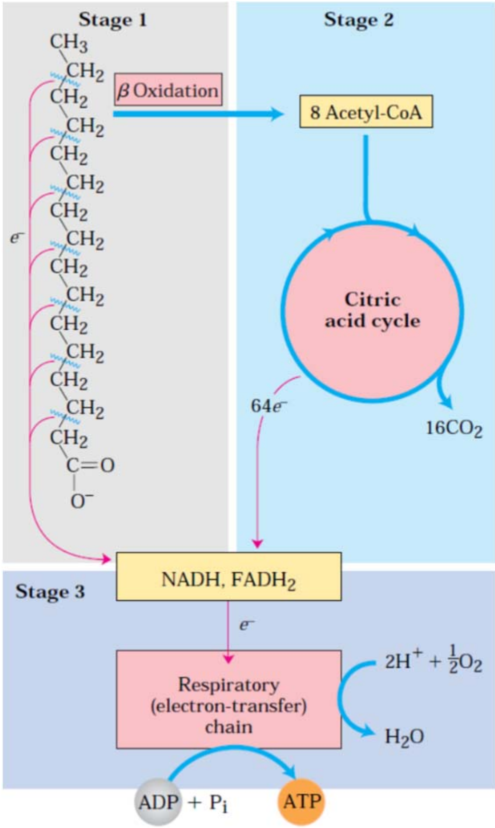
† This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19–27 and 19–28.

…no sólo los carbohidratos aportan energía…

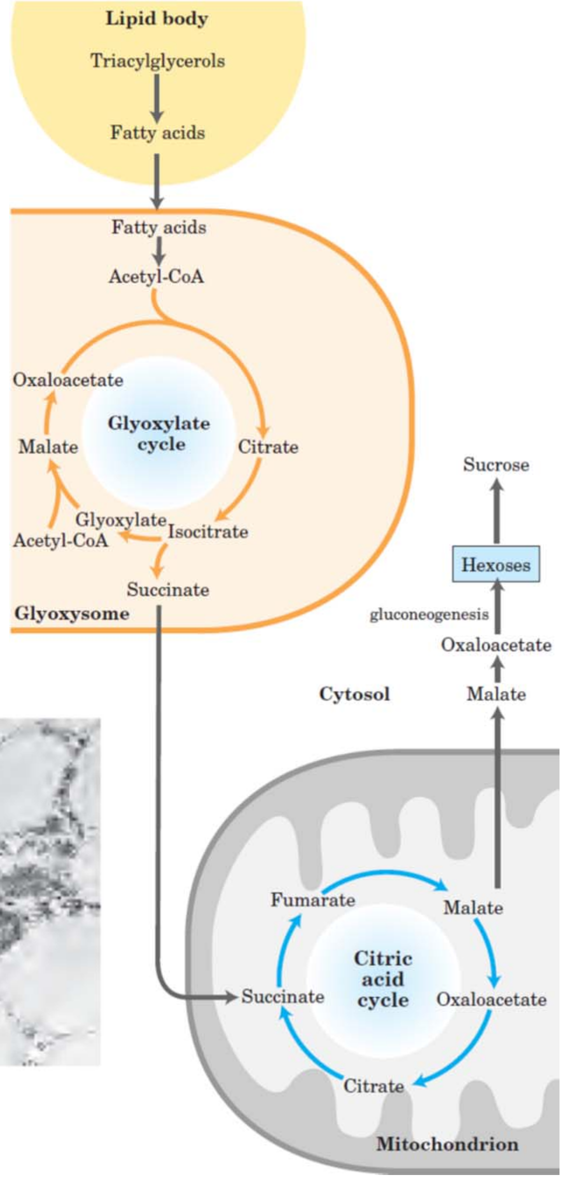
Catabolismo de lípidos

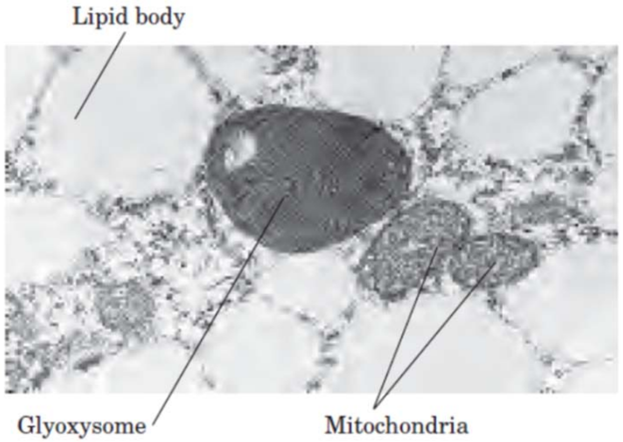
β-oxidación de lípidos en la mitocondria





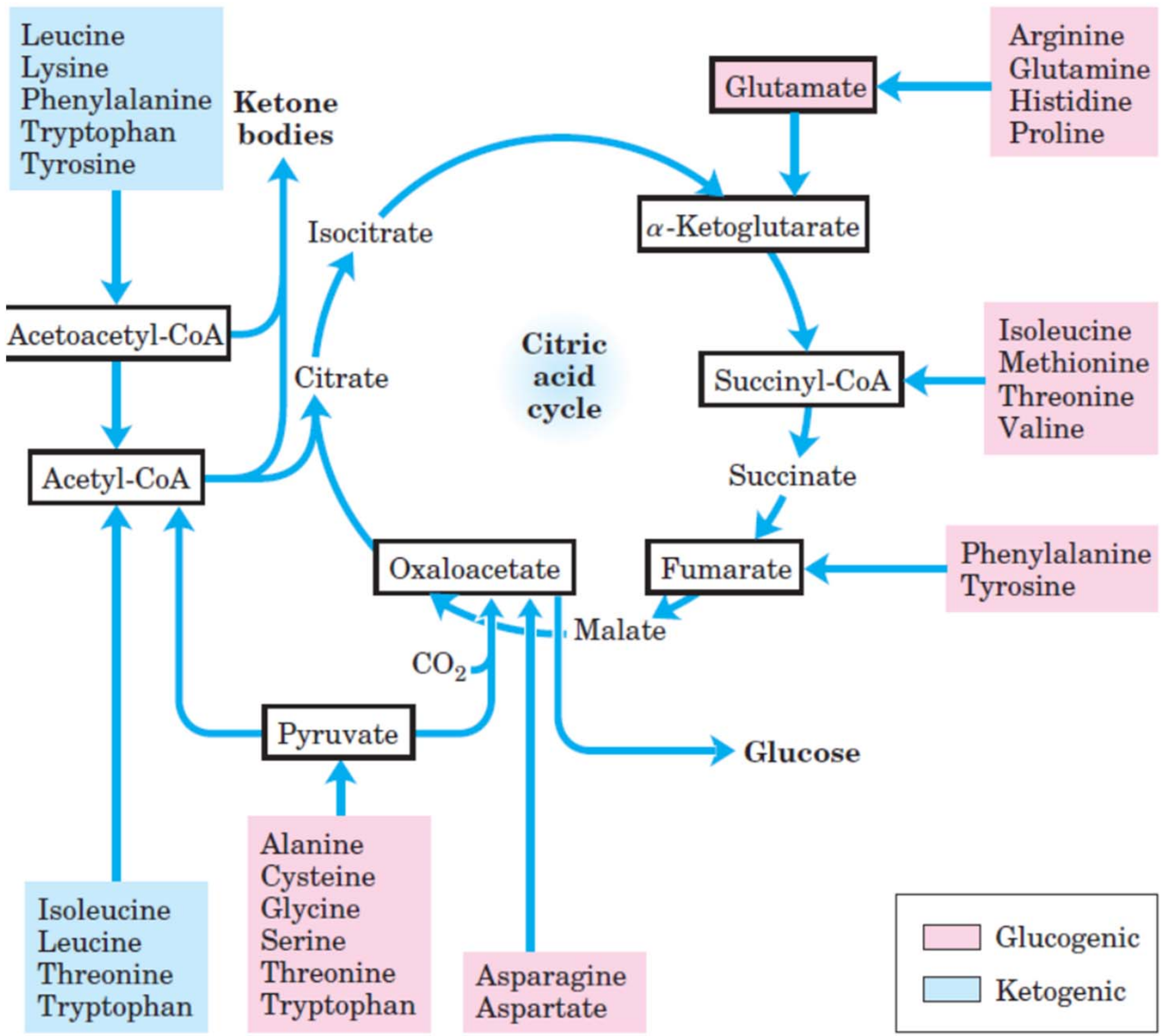
Oxidación en glioxisomas





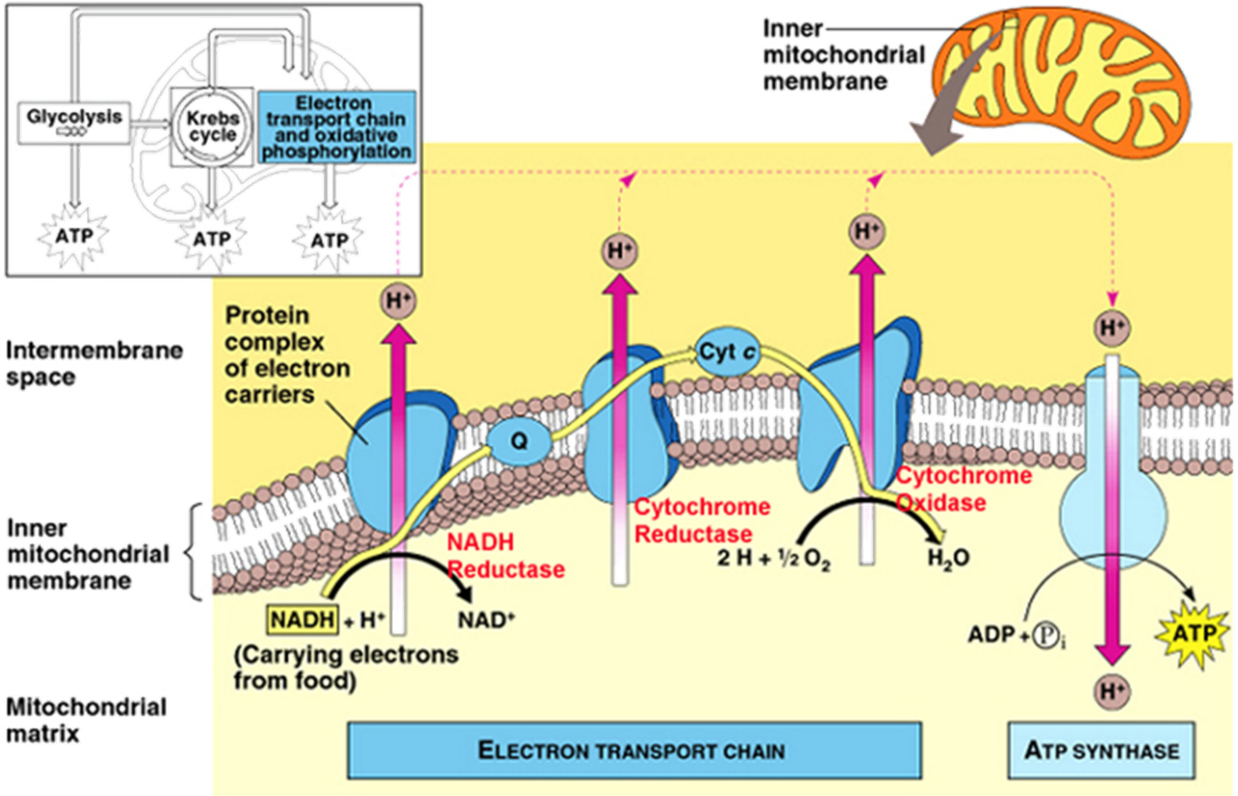
…no sólo los carbohidratos y lípidos aportan energía…

Catabolismo de aminoácidos



…todas las vías oxidativas (catabólicas) producen equivalentes de reducción en la forma de NADH (o $FADH_2$ → Coenzima Q)

→ Los equivalentes de reducción son usados para llevar a cabo la fosforilación oxidativa:



- La fosforilación oxidativa es el último paso de la respiración celular y ocurre enteramente en la mitocondria.
- En primer lugar, los compuestos de carbono se oxidan para producir equivalentes de reducción.
- Esta fuerza "electro-motriz" (EMF) se convierte en una fuerza "protón-motriz" (PMF).
- La conversión de EMF en PMF es llevada a cabo por tres bombas de protones: Complejos I, III y IV.
- La PMF se convierte en energía química (ATP) gracias al complejo V.

Fosforilación oxidativa

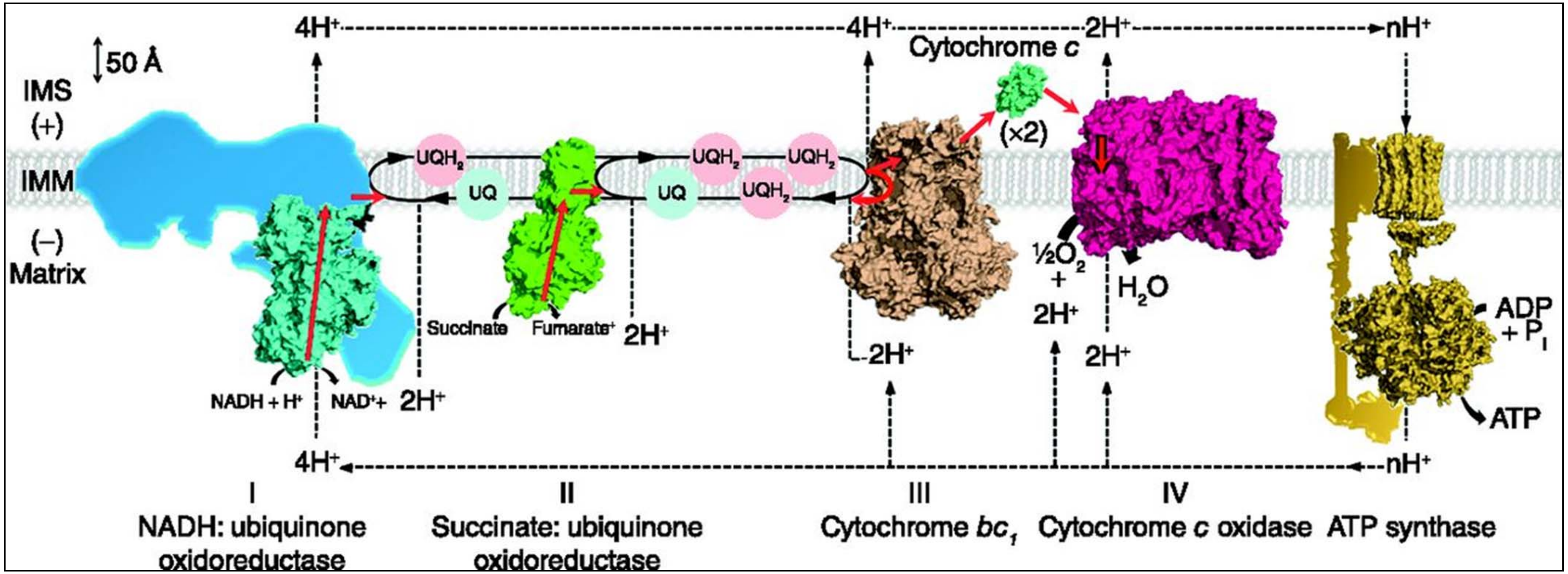
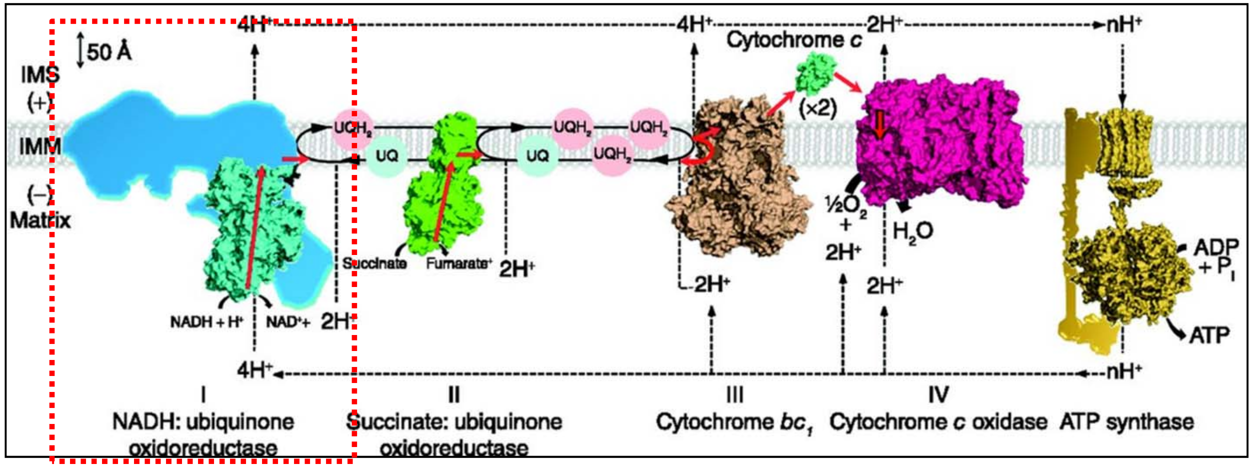
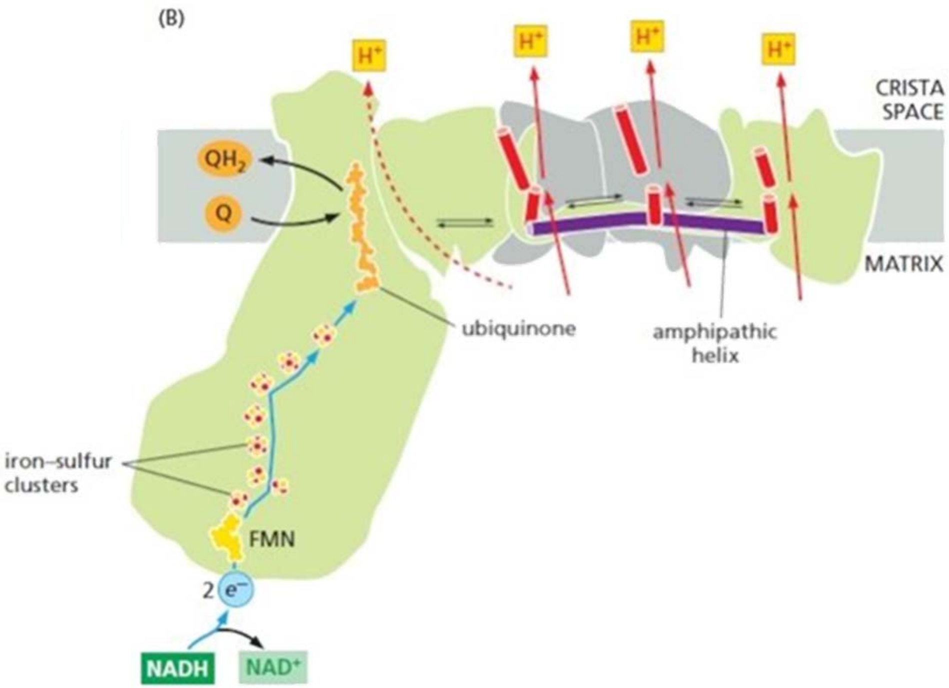


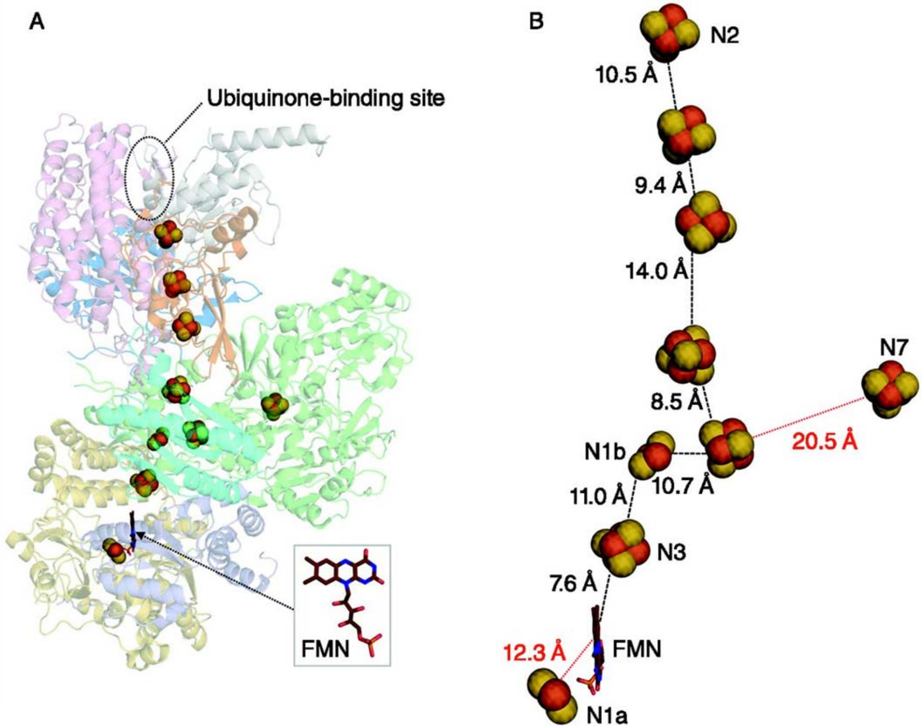
TABLE 19–3 The Protein Components of the Mitochondrial Electron-Transfer Chain

Enzyme complex/protein	*Mass (kDa)*	*Number of subunits**	*Prosthetic group(s)*
I NADH dehydrogenase	850	43 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone cytochrome *c* oxidoreductase	250	11	Hemes, Fe-S
Cytochrome c†	13	1	Heme
IV Cytochrome oxidase	160	13 (3–4)	Hemes; Cu_A, Cu_B

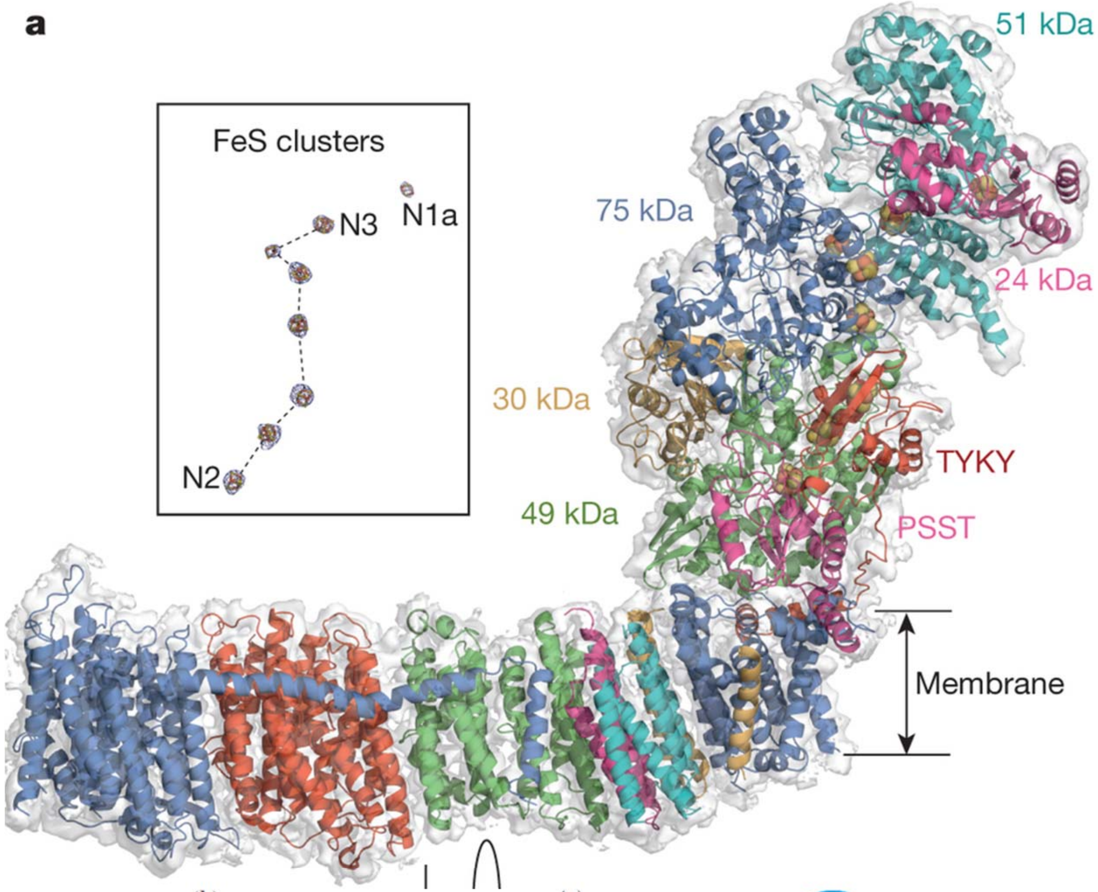
<u>Complejo I:</u> *NADH:UQ oxidoreductasa*

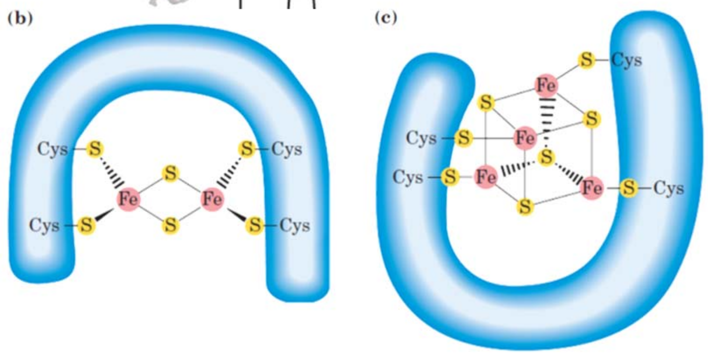


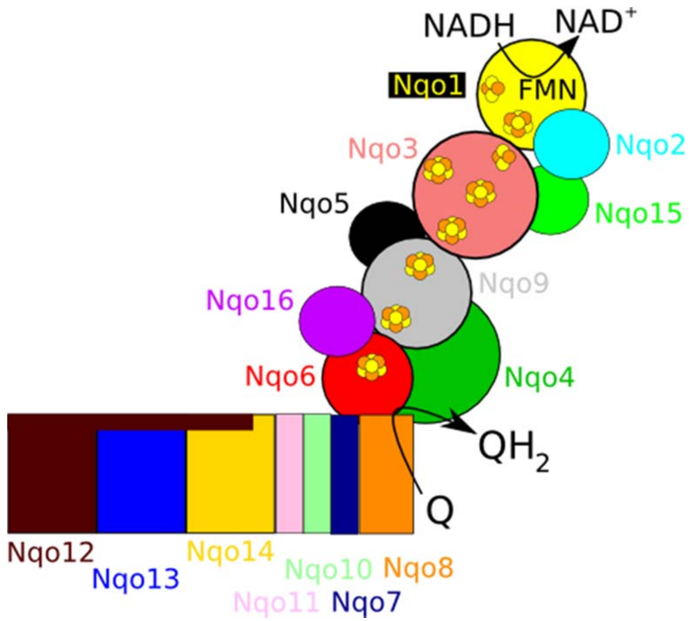




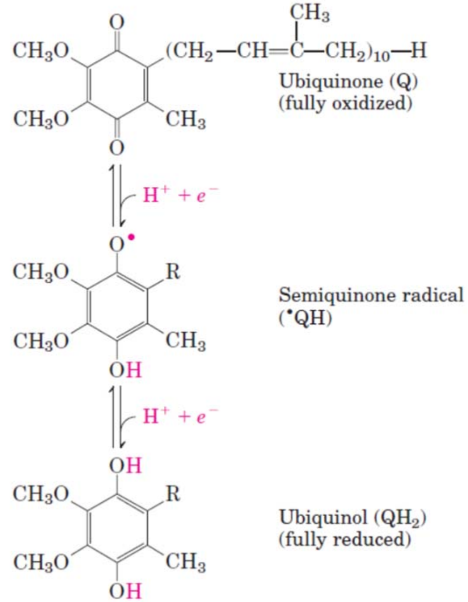
Complejo I: *NADH:UQ oxidoreductasa*





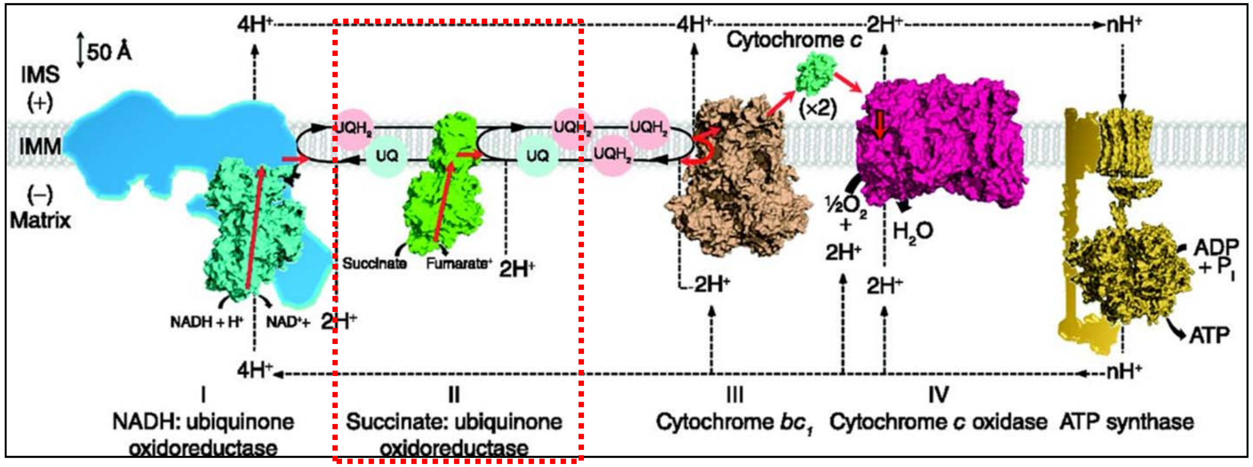


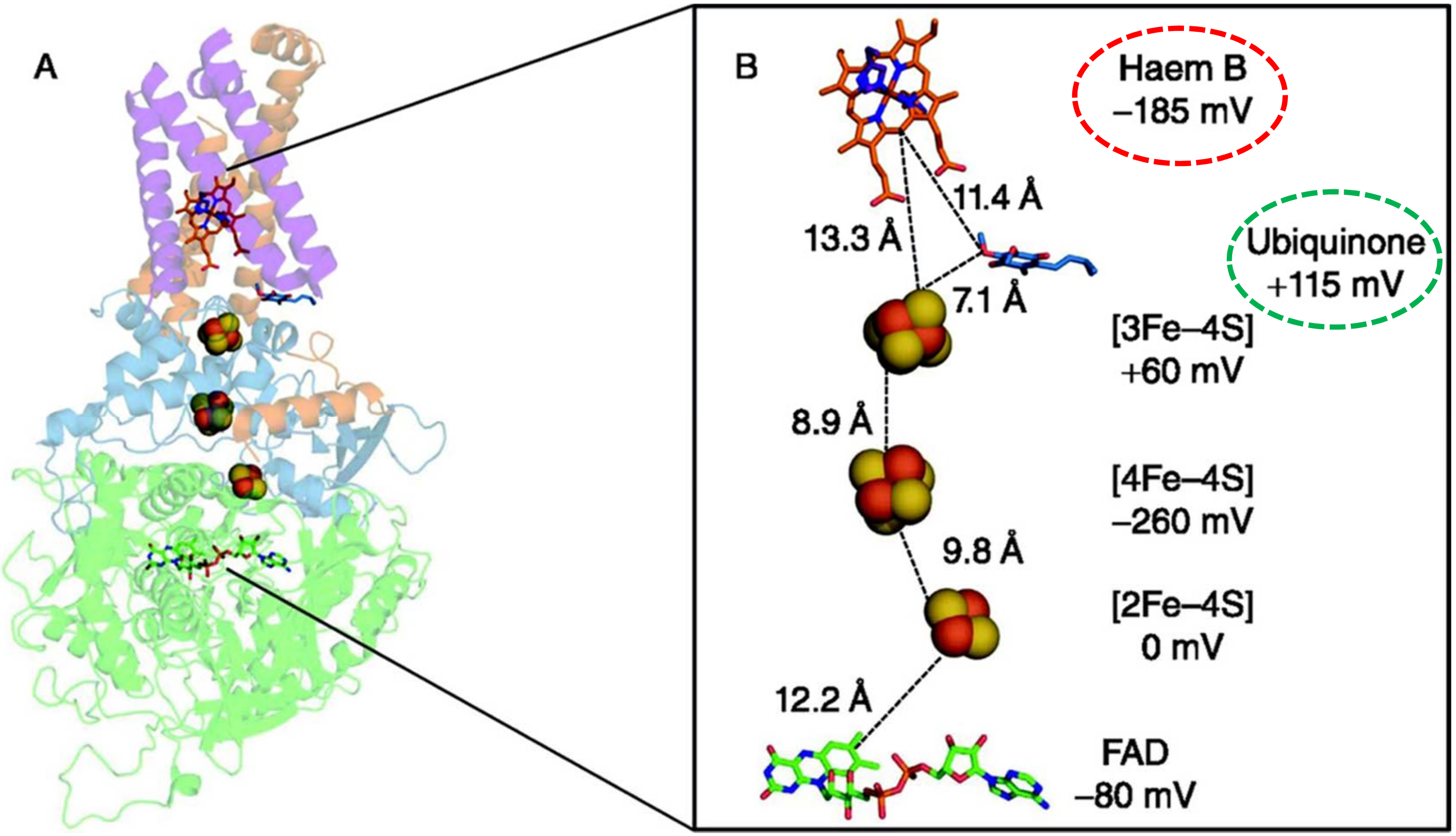
Reducción de UQ



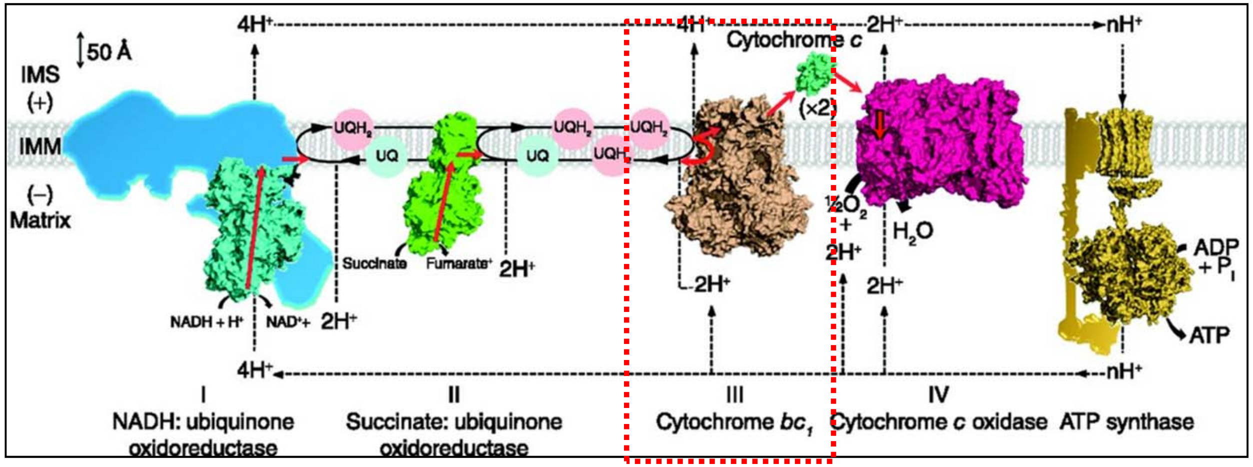
Ver → https://www.youtube.com/watch?v=S9wAFVQ1k7E

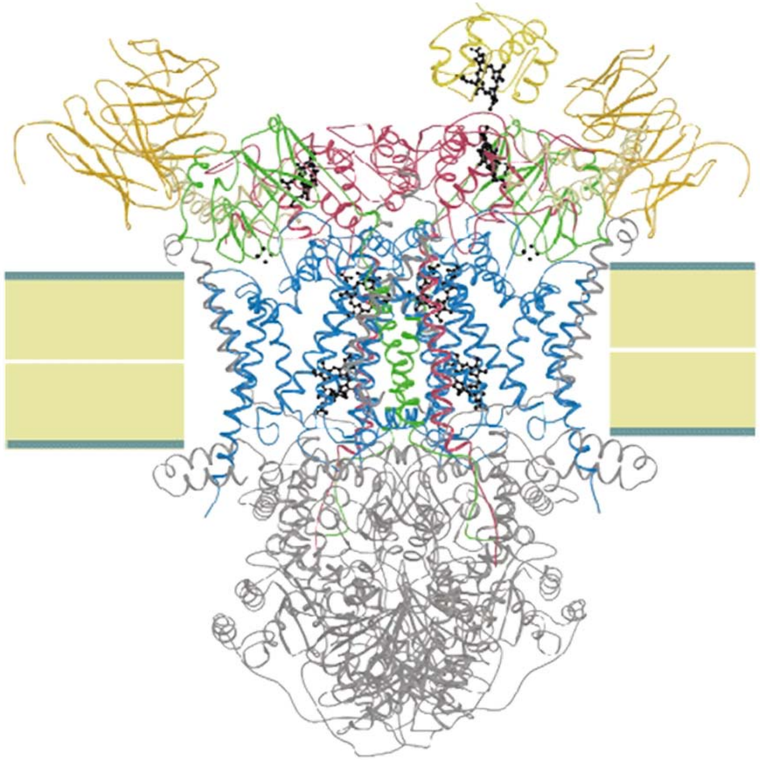
<u>Complejo II:</u> *succinato:UQ oxidoreductasa*

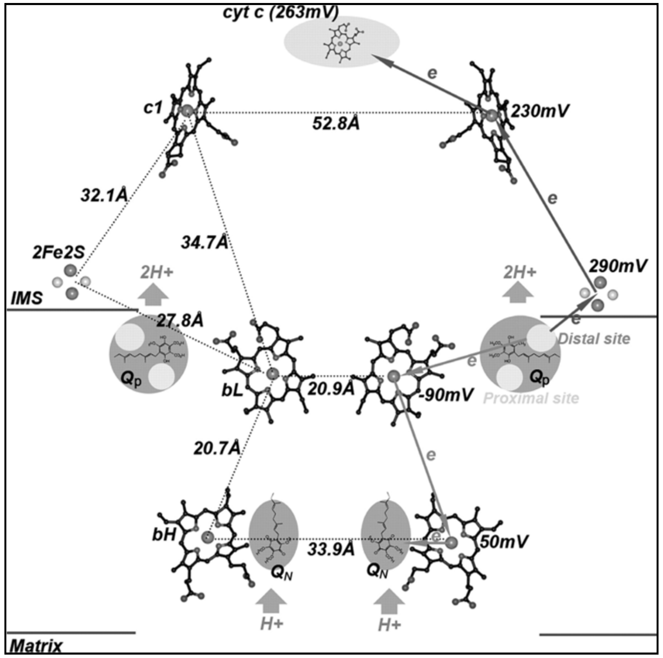




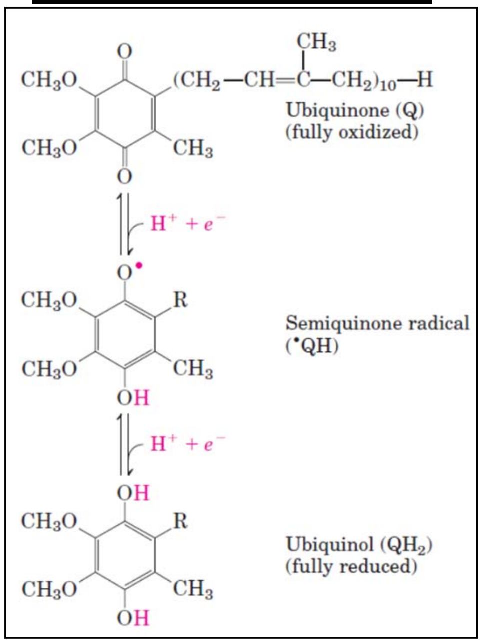
Complejo III: *UQ:citocromo C oxidoreductasa* → *Citocromo bc_1*





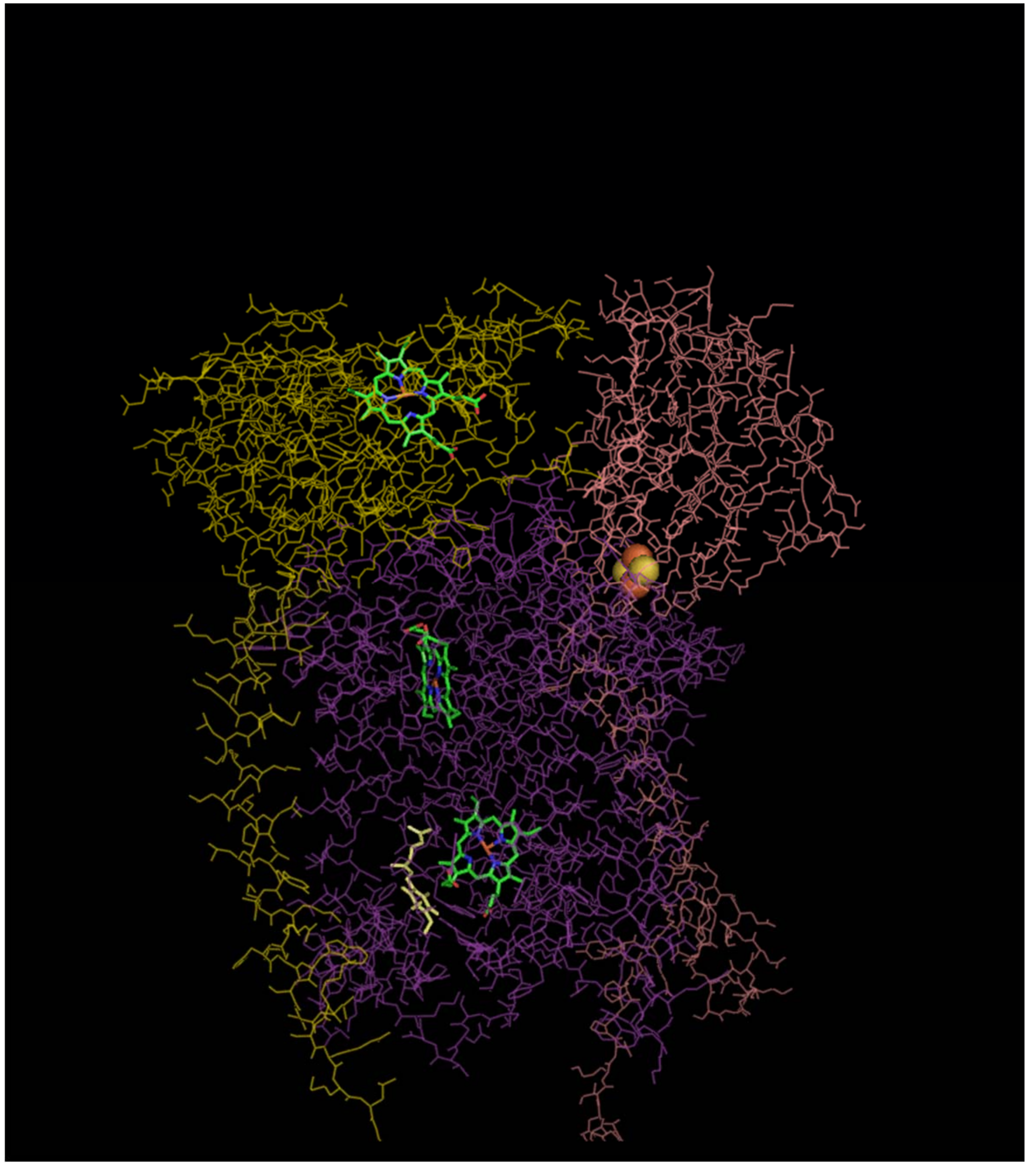


Reducción de UQ



<u>Complejo III:</u> *UQ:citocromo C oxidoreductasa* → Citocromo bc_1

El ciclo-Q



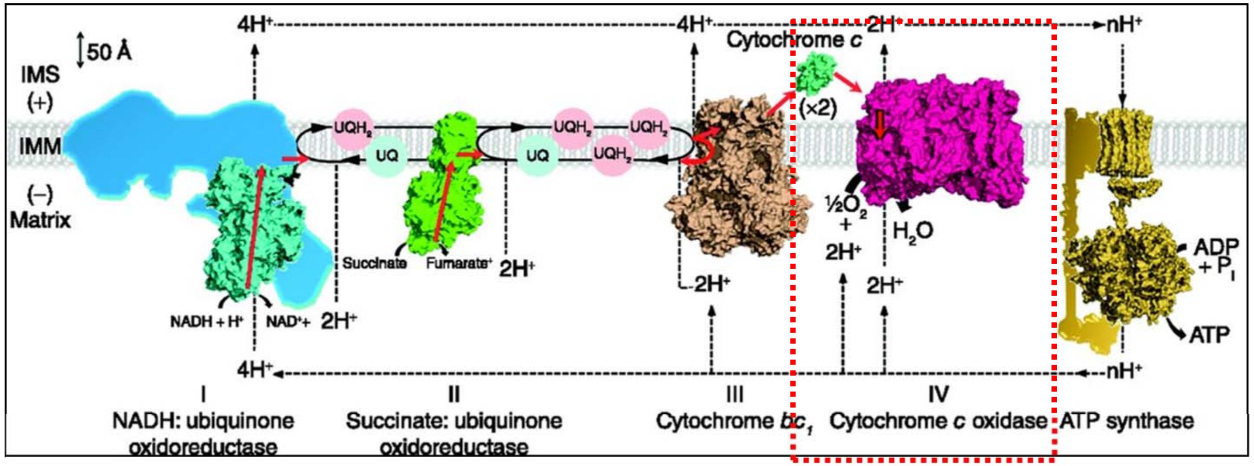
<u>Primera ronda</u>:

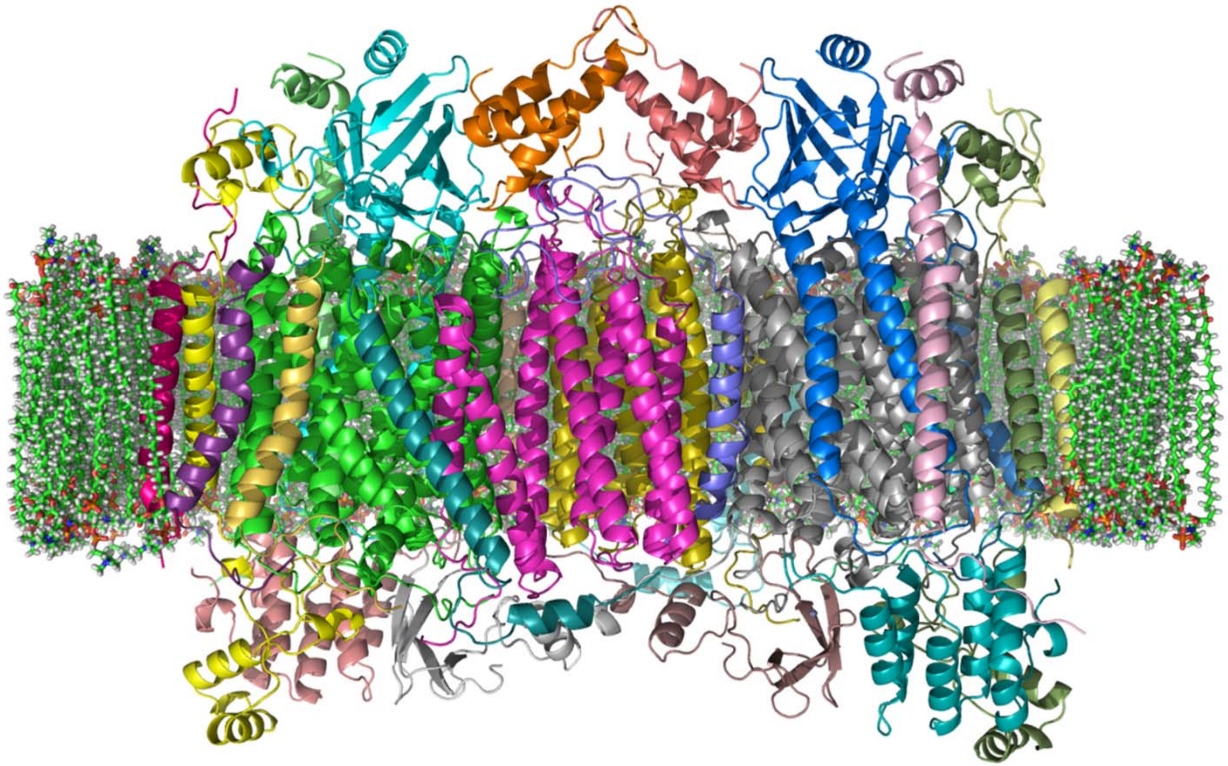
1. El citocromo *b* une una molécula de UQ y una de UQH_2.
2. El UQH_2 es completamente oxidado, 1 e^- para el 2Fe-2S y el otro para el hemo b_L, y $2H^+$ son translocados al espacio intermembrana.
3. El e^- del 2Fe-2S es transferido al citocromo c_1, mientras que el otro es transferido desde el grupo hemo b_L hacia el hemo b_H.
4. El citocromo c_1 transfiere su electrón hacia el citocromo c soluble (no confundir con el citocromo c1), y el heme b_H transfiere su electrón hacia la UQ, resultando en la formación de una ubisemiquinona.
5. El citocromo c soluble se separa del complejo III. La primer molécula de UQH_2 (que ahora se ha oxidado a UQ) se libera, mientras que la semiquinona permanece unida.

<u>Segunda ronda</u>:

1. Un segundo UQH_2 se une al citocromo b.
2. Otra vez, el UQH_2 es completamente oxidado, 1 e^- para el 2Fe-2S y el otro para el hemo b_L, y $2H^+$ son translocados al espacio intermembrana.
3. Otra vez, el e^- del 2Fe-2S es transferido al citocromo c_1, mientras que el otro es transferido desde el grupo hemo b_L hacia el hemo b_H.
4. El citocromo c_1 transfiere su electrón hacia el citocromo c soluble, mientras que la ubisemiquinona recoge el segundo electrón del hemo B_H, junto con $2H^+$ de la matriz mitocondrial.
5. El segundo citocromo c soluble se separa del complejo III. La segunda molécula de UQH_2 (que ahora se ha oxidado a UQ) se libera, y el UQH_2 (antes semiquinona) tambien se libera.

<u>Complejo IV:</u> *citocromo:O_2 oxidoreductasa* → *Citocromo c oxidasa*

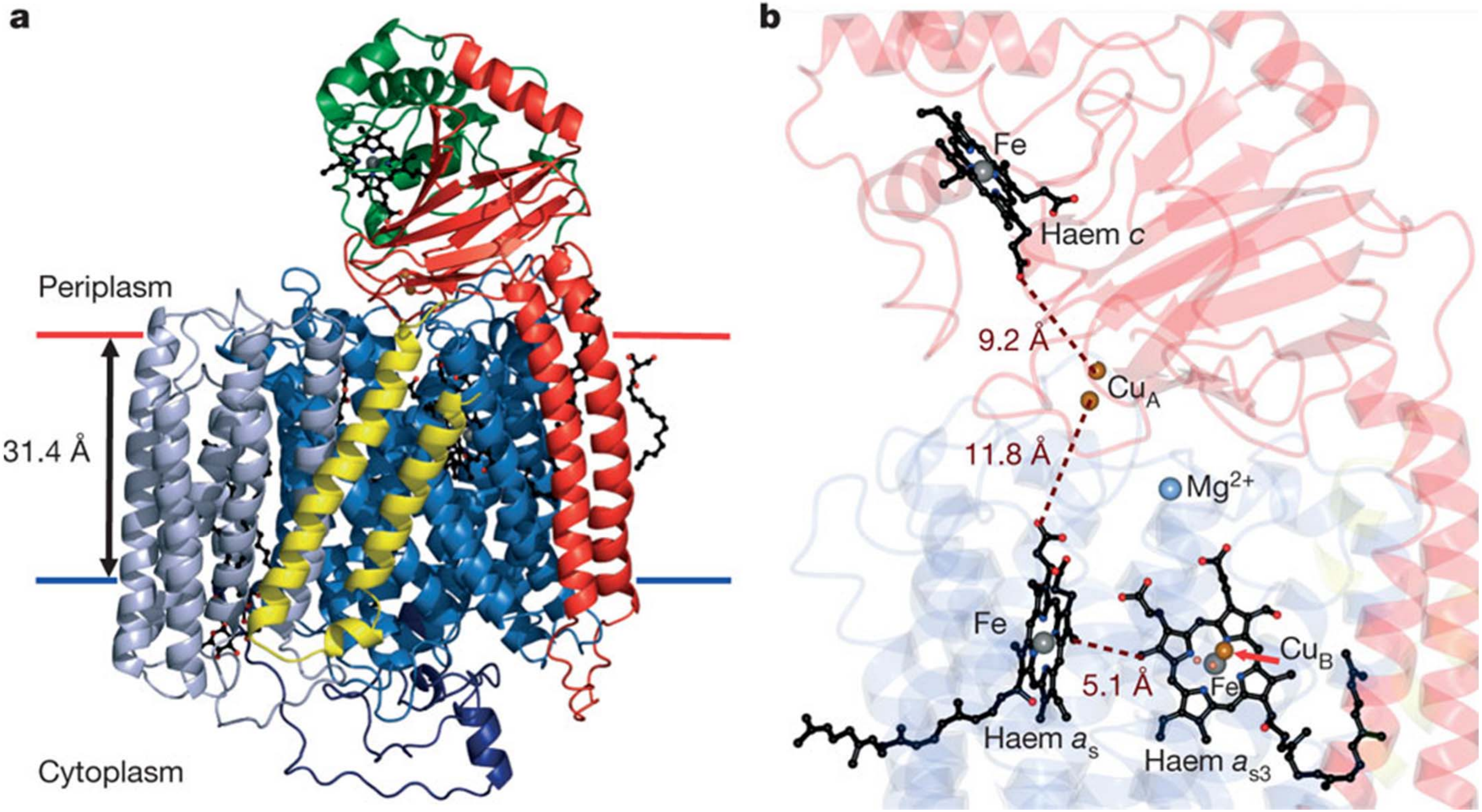




Citocromo c oxidasa (tipo aa_3) de corazón de vaca (*Bos taurus*)

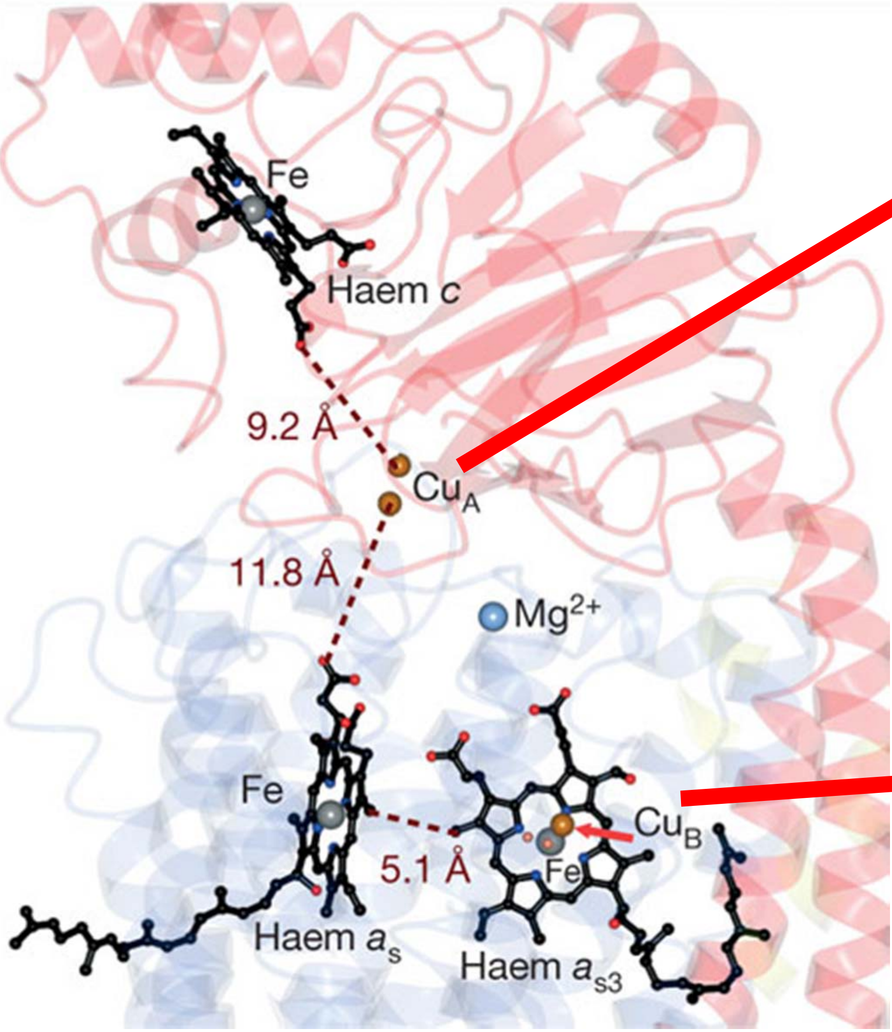
<u>Complejo IV:</u> ***citocromo:O_2 oxidoreductasa*** → ***Citocromo c oxidasa***

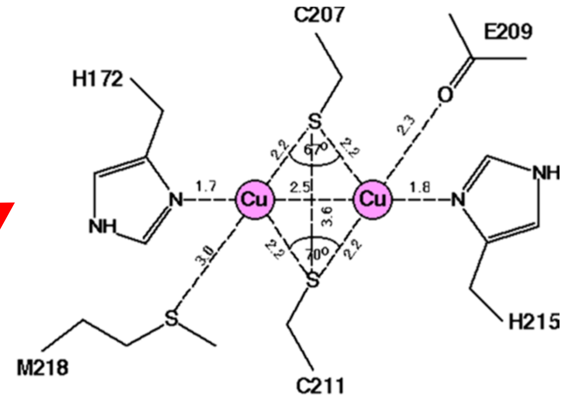
Citocromo c oxidasa (tipo aa_3) de *P. denitrificans*

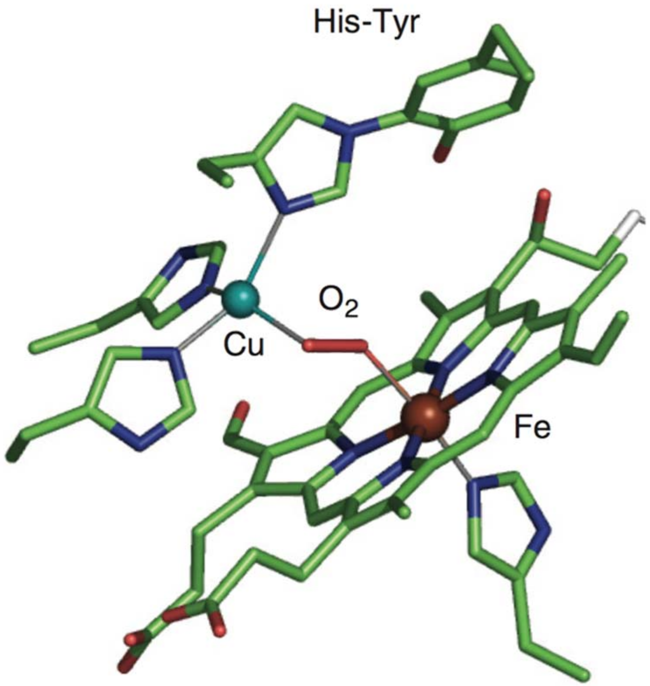


<u>Complejo IV:</u> *citocromo:O_2 oxidoreductasa* → *Citocromo c oxidasa*

Citocromo c oxidasa (tipo aa_3)

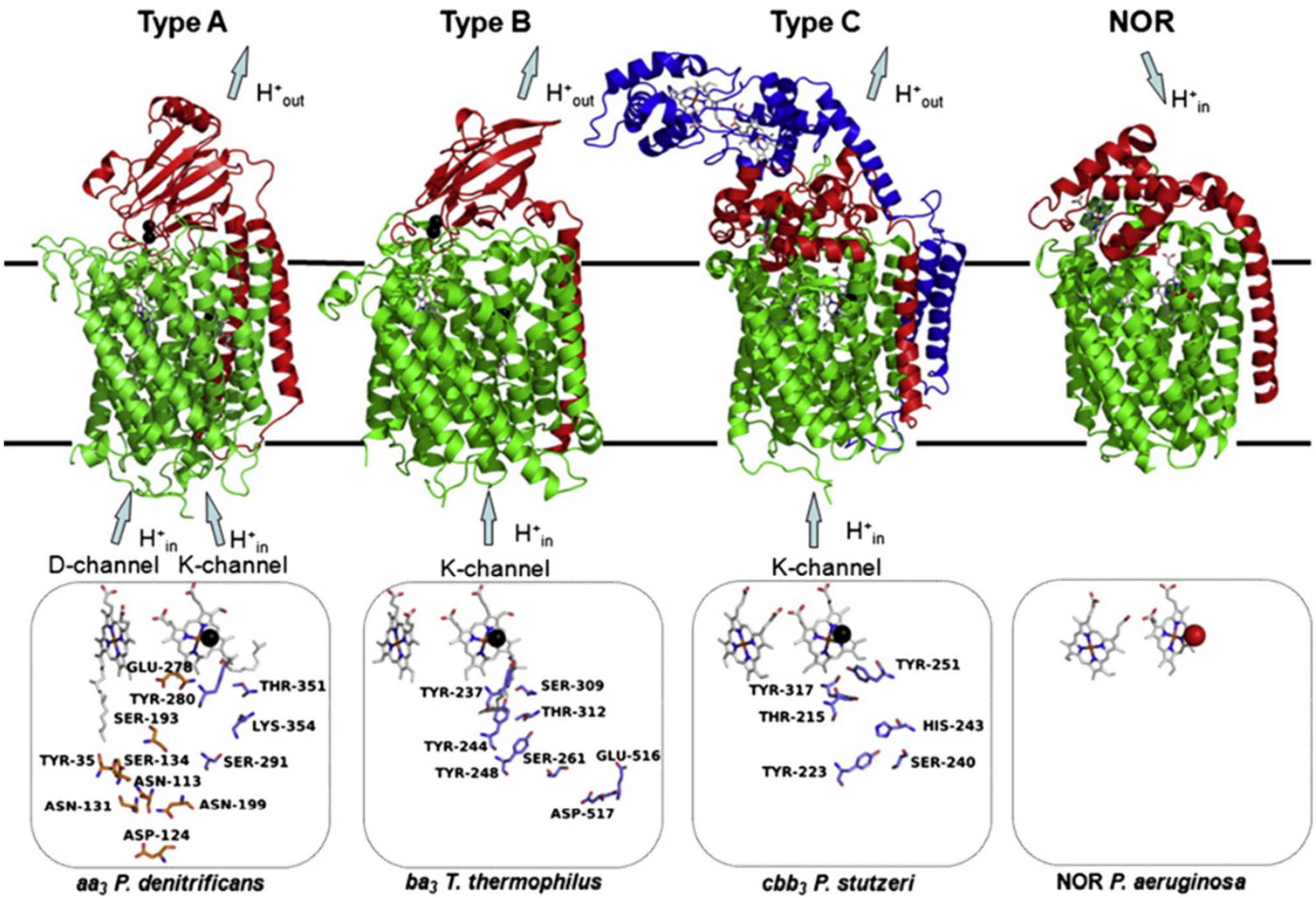




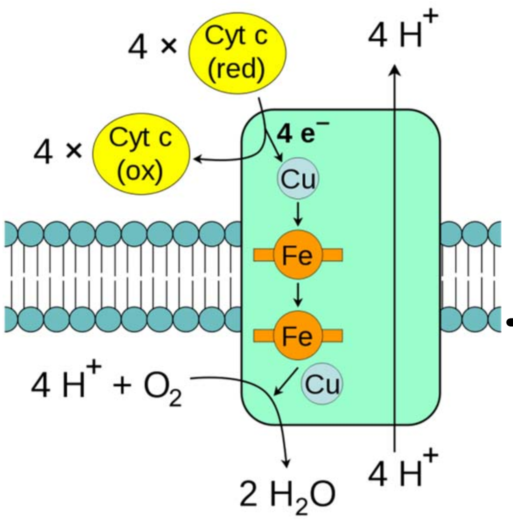


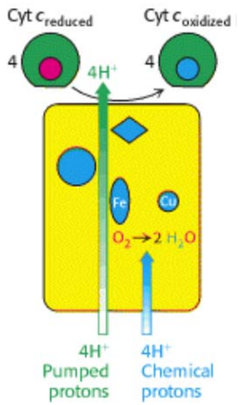
Complejo IV: *citocromo:O_2 oxidoreductasa* → *Citocromo c oxidasa*

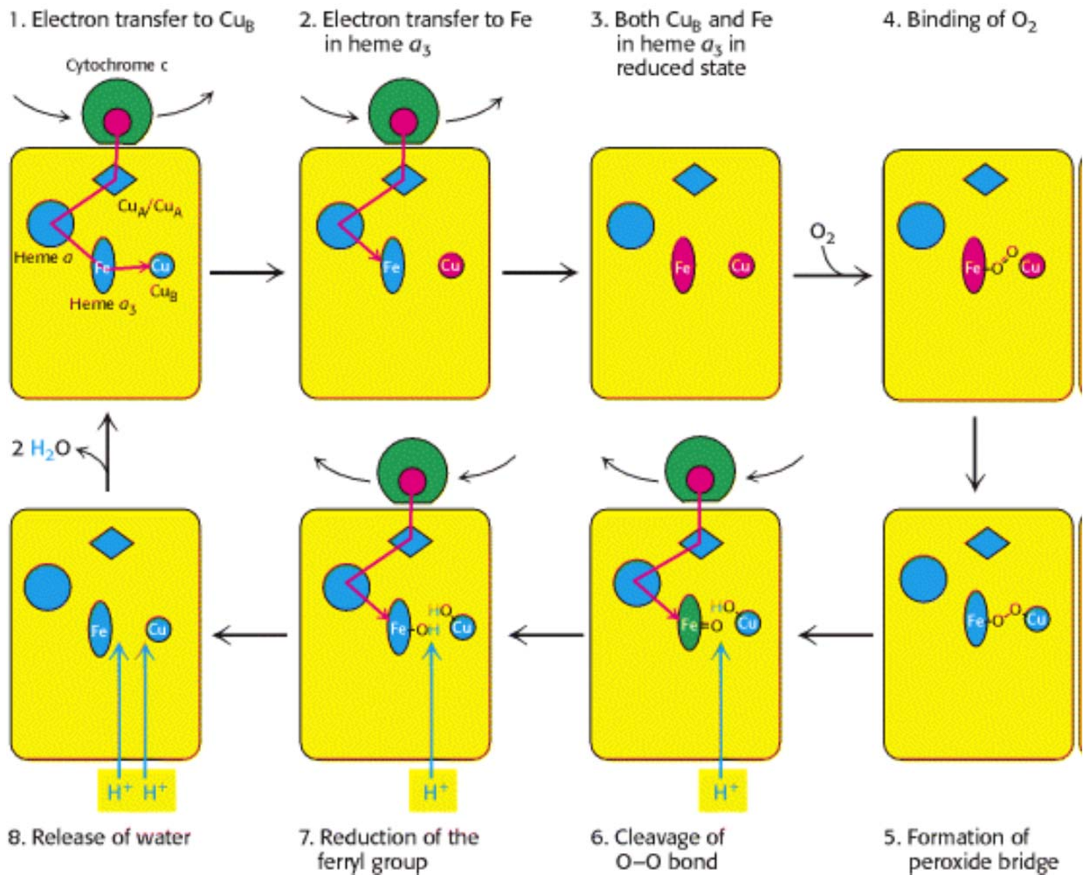
F.L. Sousa et al. / Biochimica et Biophysica Acta 1817 (2012) 629–637

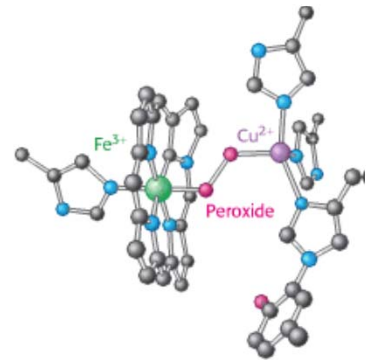


Complejo IV: *citocromo:O_2 oxidoreductasa* → *Citocromo c oxidasa*

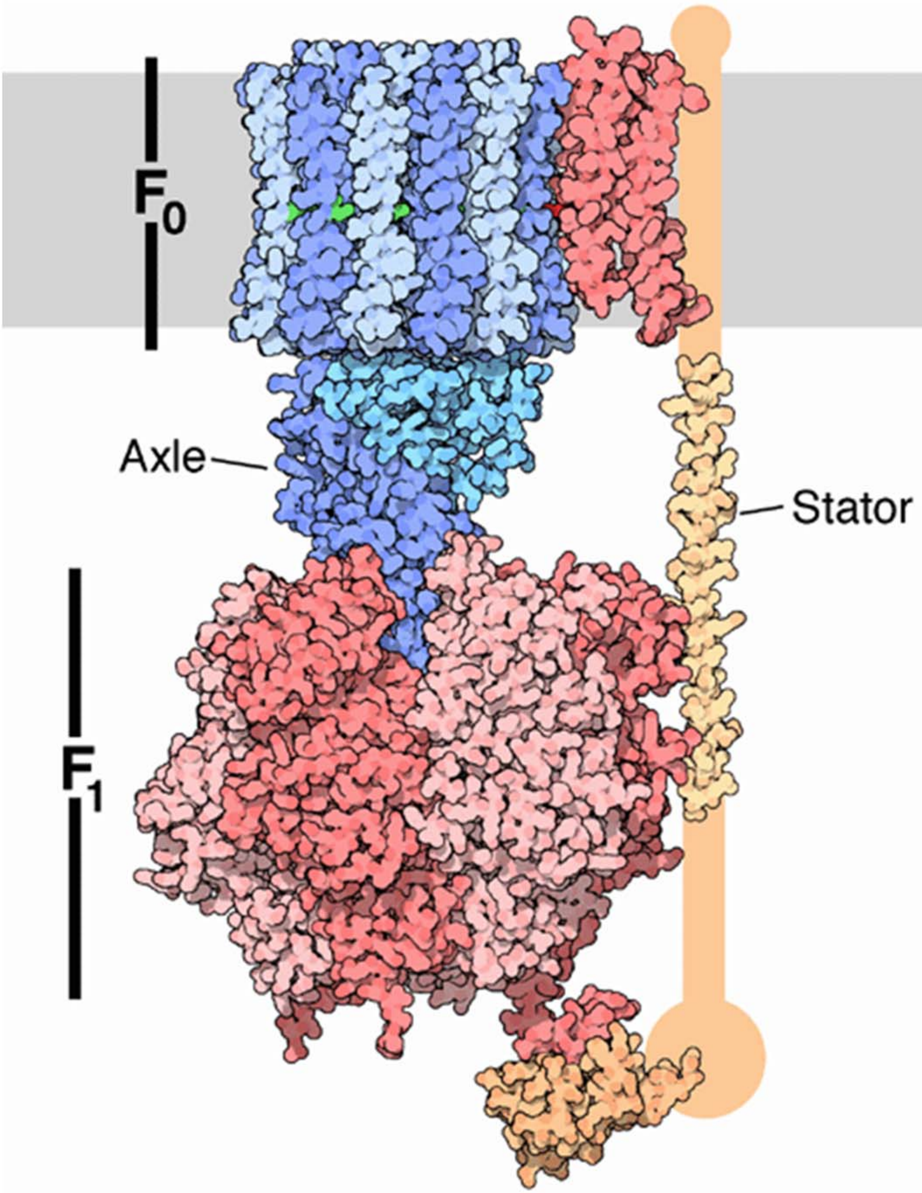


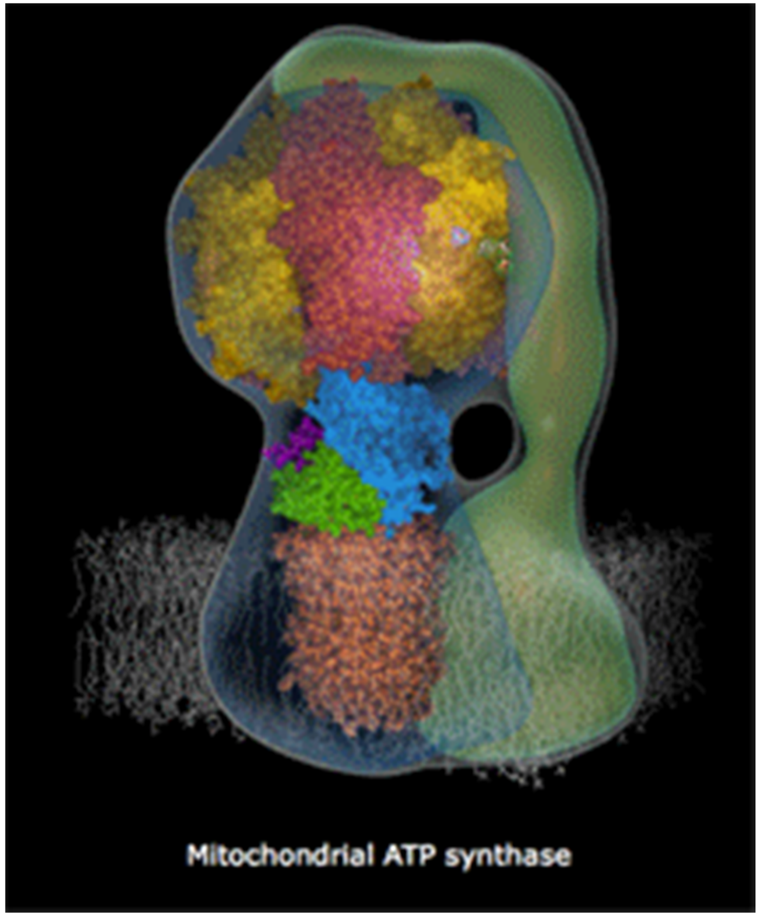




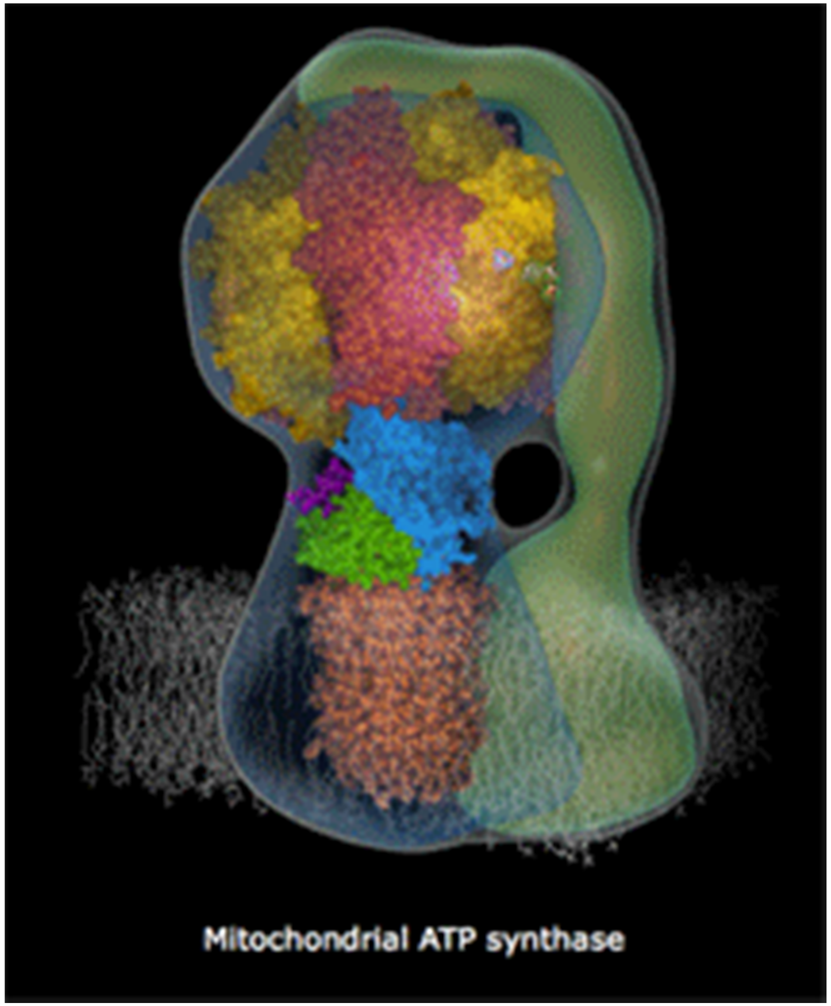


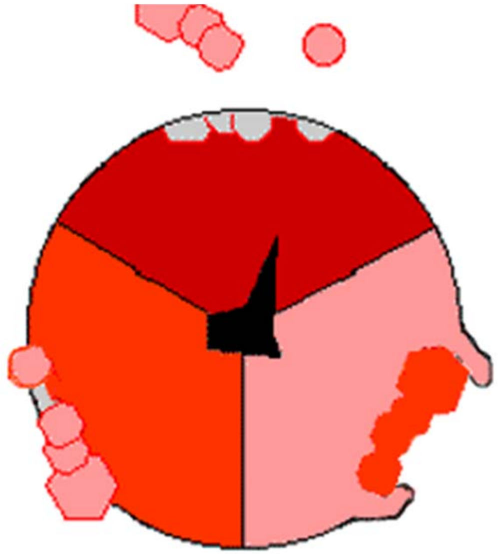
<u>Complejo V:</u> *ATP sintetasa*





<u>Complejo V:</u> *ATP sintetasa*

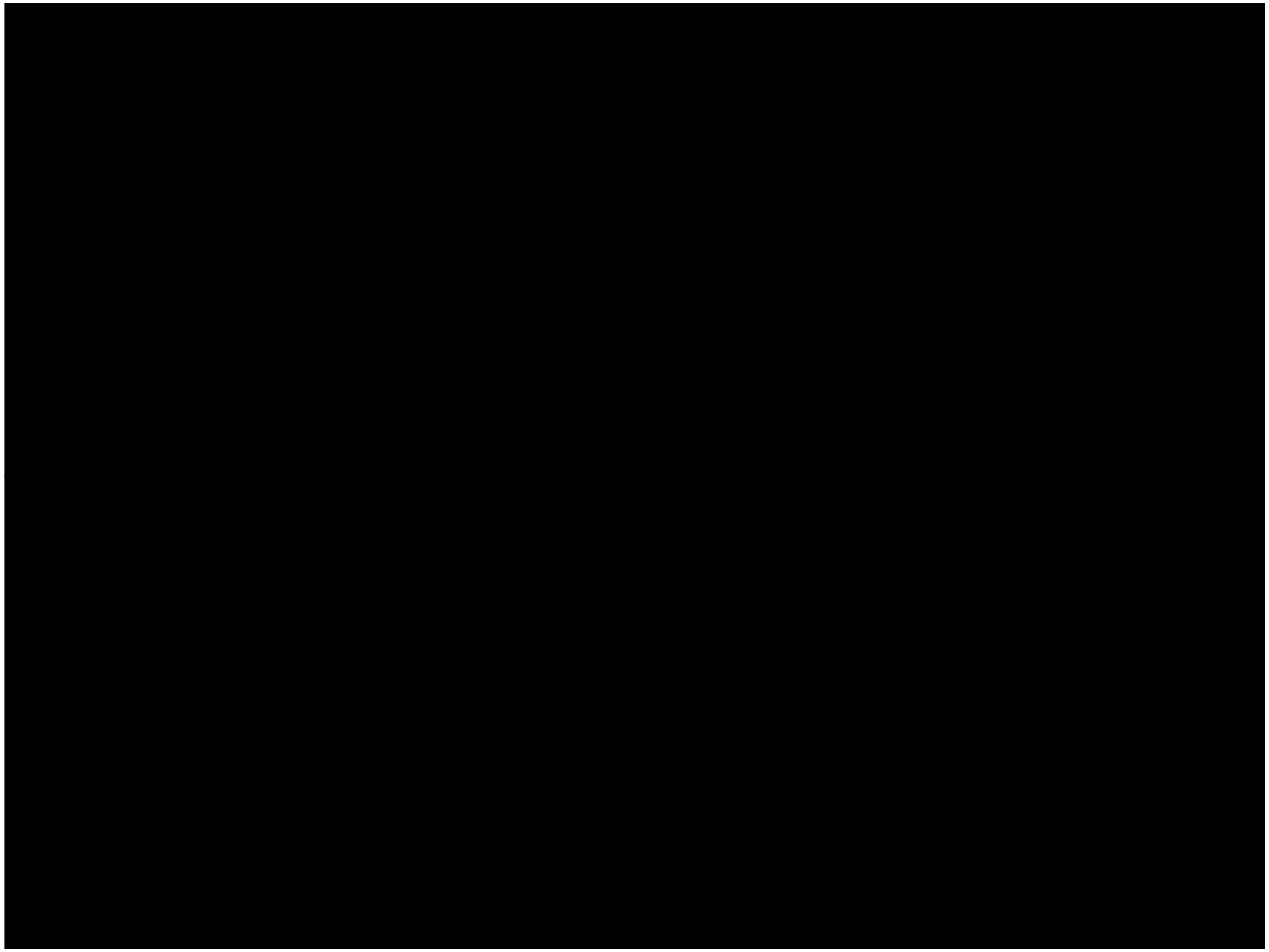




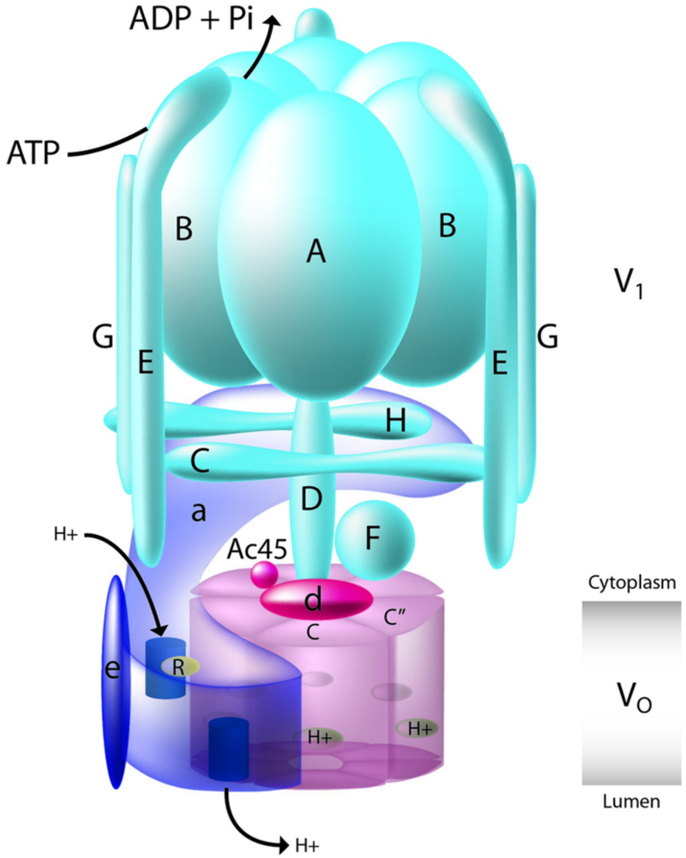
<u>Mecanismo</u>

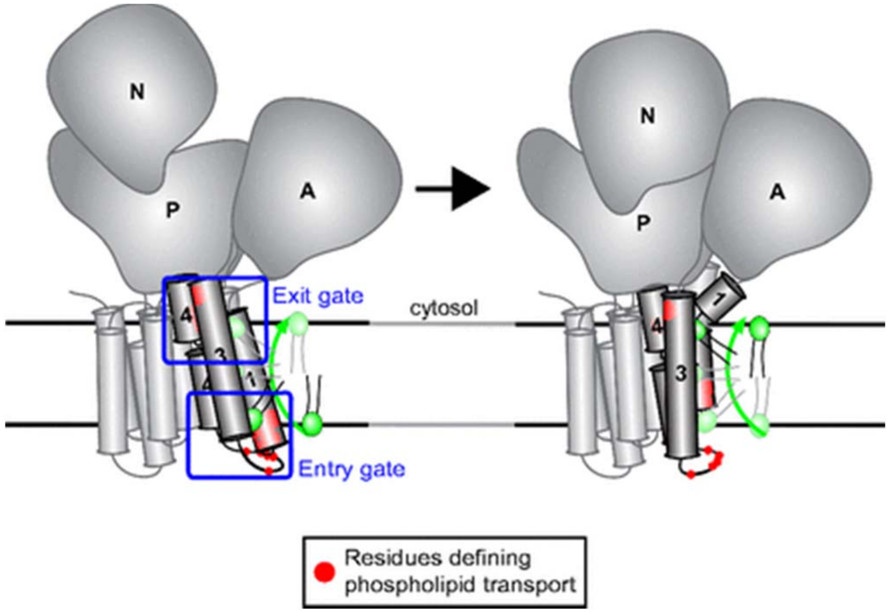
- Cada color es el dimero αβ de la unidad F1
- Rojo → abierto: ADP + Pi pueden entrar al sitio activo.
- Naranja → Se cierra el sitio activo y se forma ATP.
- Rosa → El ATP está listo para ser liberado.

Complejo V: ***ATP sintetasa***



→ otros tipos de ATPasas





ATPasas tipo vacuolar

- Rol de **V-ATPasas**: acidificar organelas intracelulares / Bombear H^+ a traves de la membrana citoplasmática de varias líneas celulares.
- Funcionamiento: hidrolizan ATP para bombear protones (opuesto a la ATP sintetasa).

ATPasas tipo P

- Rol de **P-ATPasas**: Bombear iones (H^+, Na^+, K^+) a traves de la membrana citoplasmática de varias líneas celulares / Tipo IV: invierten fosfolipidos para mantener la simetría/forma de la célula.
- Funcionamiento: hidrolizan ATP para bombear iones (opuesto a la ATP sintetasa).

Fosforilación oxidativa

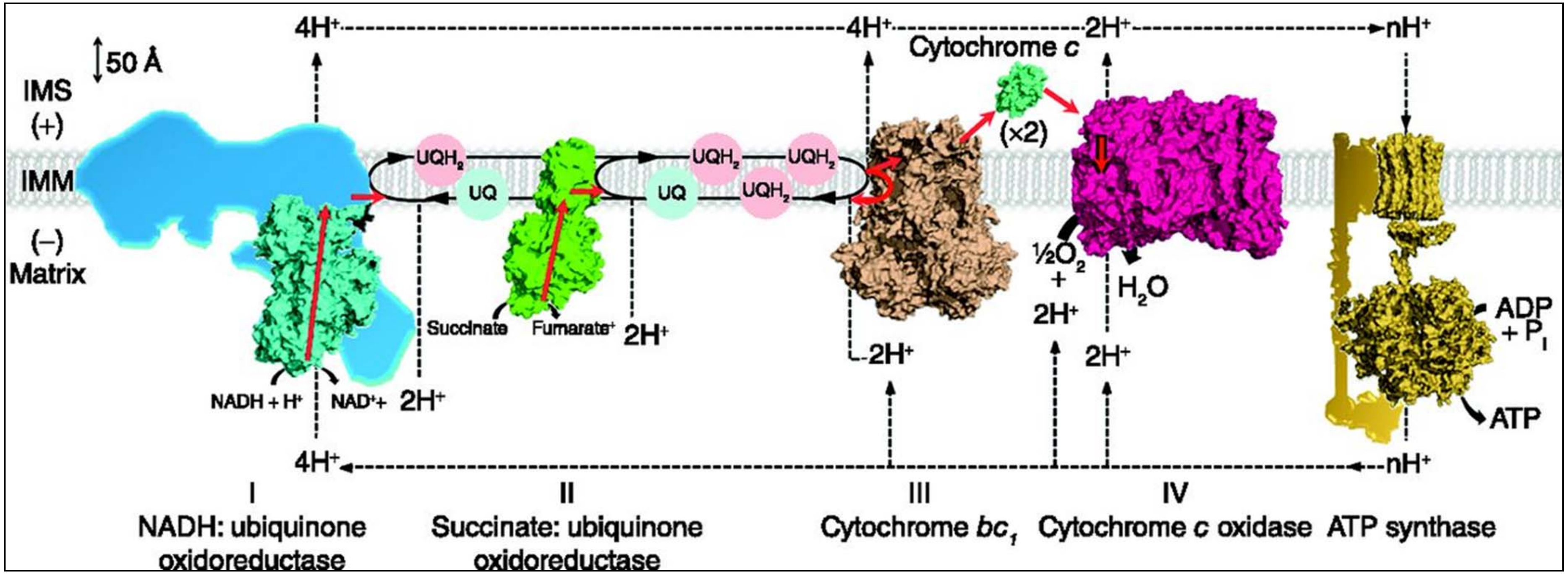
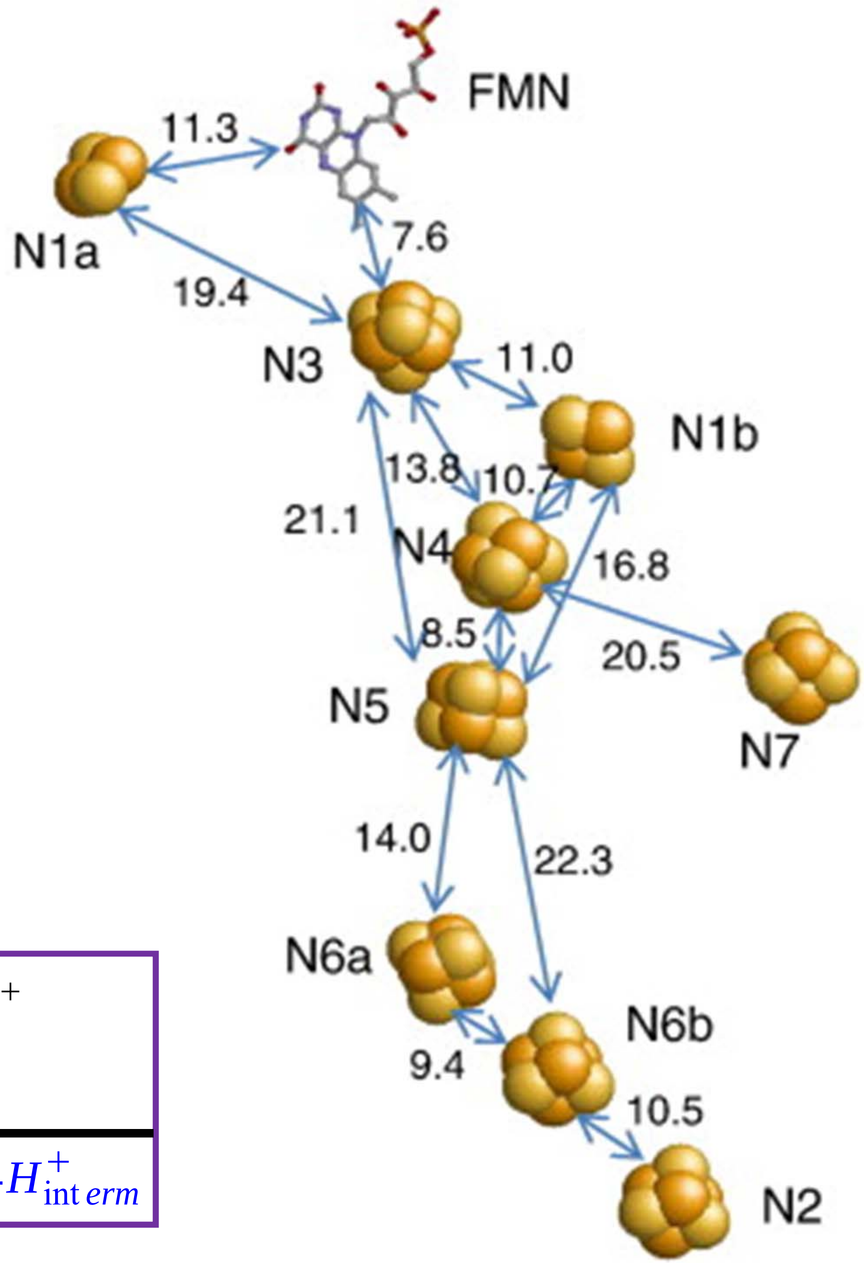


TABLE 19–3 The Protein Components of the Mitochondrial Electron-Transfer Chain

Enzyme complex/protein	Mass (kDa)	Number of subunits*	Prosthetic group(s)
I NADH dehydrogenase	850	43 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone cytochrome *c* oxidoreductase	250	11	Hemes, Fe-S
Cytochrome c†	13	1	Heme
IV Cytochrome oxidase	160	13 (3–4)	Hemes; Cu_A, Cu_B

Complejo I: *NADH:UQ oxidoreductasa*

Redox couple	E°' [mV] from ref [34]	E°' [mV] from ref [38]
NADH/NAD+	– 0.32	– 0.32
FMNH2/FMNH•	– 0.293	*– 0.293*
FMNH•/FMN	– 0.389	*– 0.389*
QH2/SQ•	0	*0*
SQ•/Q	– 0.12	*– 0.12*
N1a	– 0.38	– 0.235
N1b	– 0.25	– 0.282
N2	– 0.10	– 0.250
N3	– 0.25	– 0.315 (N7?)
N4	– 0.25	– 0.330 (N5?)
N5	– 0.25	– 0.330 (N4?)
N6a	– 0.25	*– 0.275*
N6b	– 0.25	– 0.275
N7	*– 0.25*	– 0.315 (N3?)



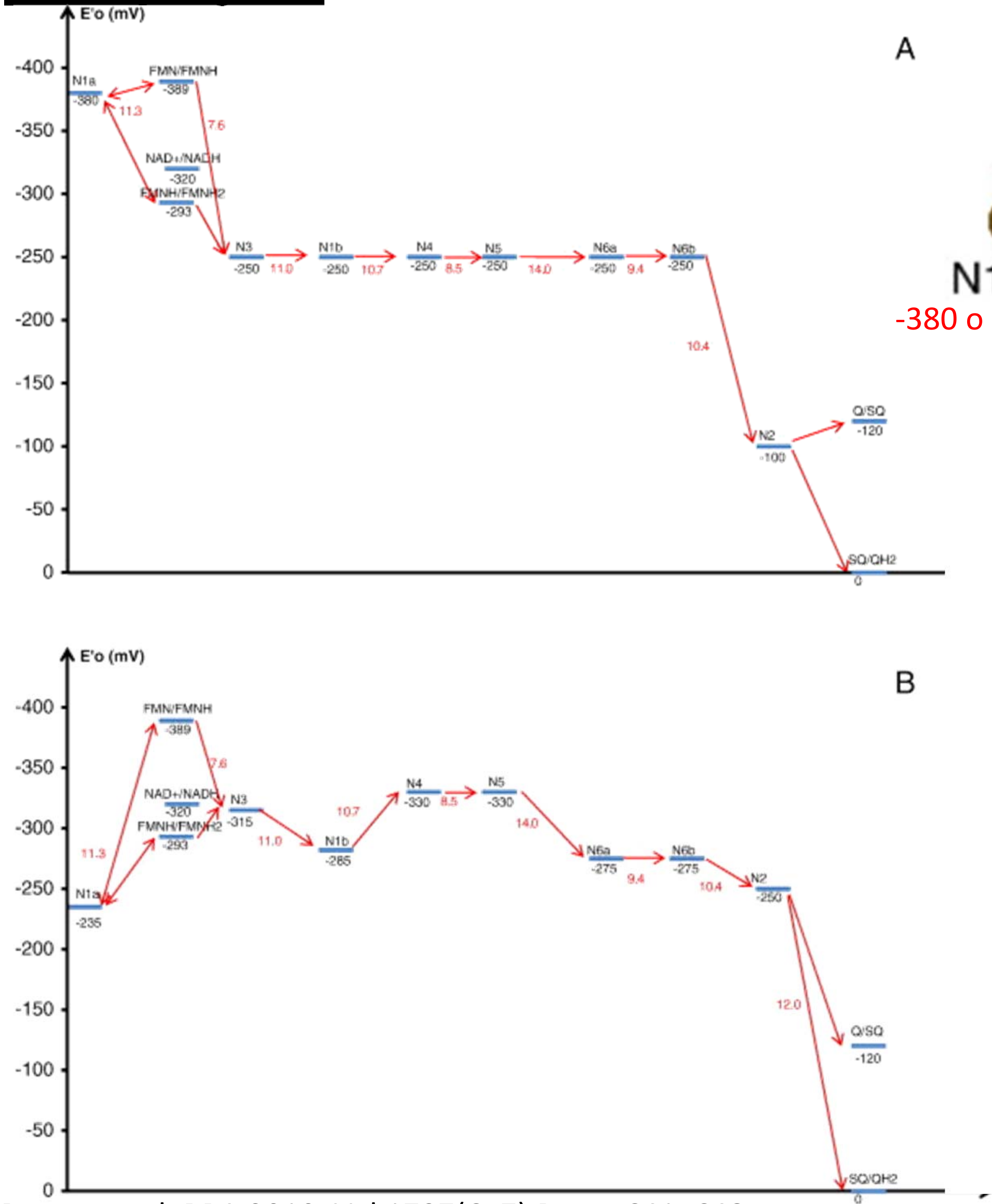
$$NADH \Leftrightarrow NAD^{+} + 2e^{-} + H^{+}$$

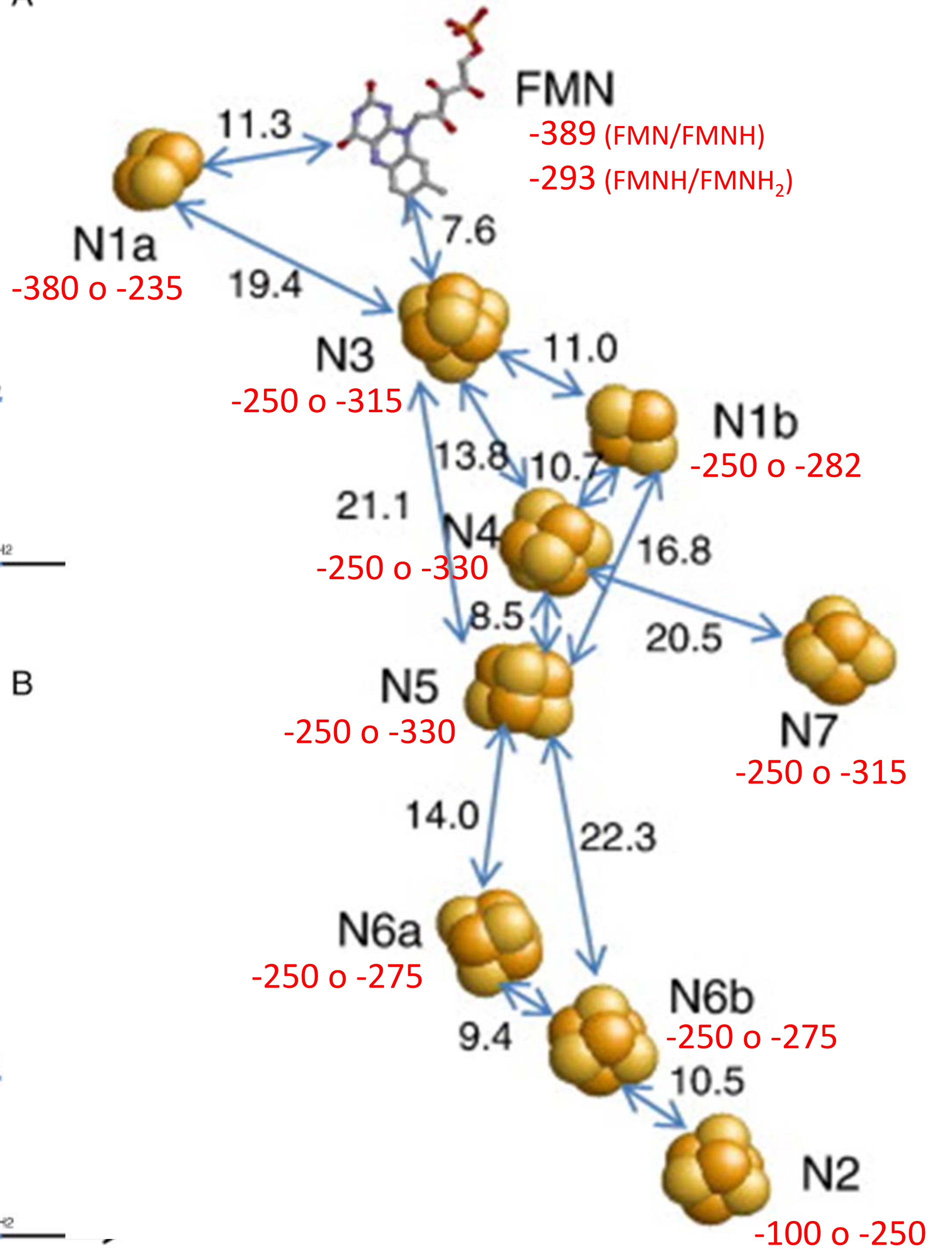
$$Q + 2e^{-} + 2H^{+} \Leftrightarrow QH_2$$

$$NADH + H^{+} + Q + 4H^{+}_{matriz} \Leftrightarrow NAD^{+} + QH_2 + 4H^{+}_{int\,erm}$$

Ransac et al. BBA 2010, Vol 1797(6–7) Pages 641–648

<u>Complejo I:</u> *NADH:UQ oxidoreductasa*

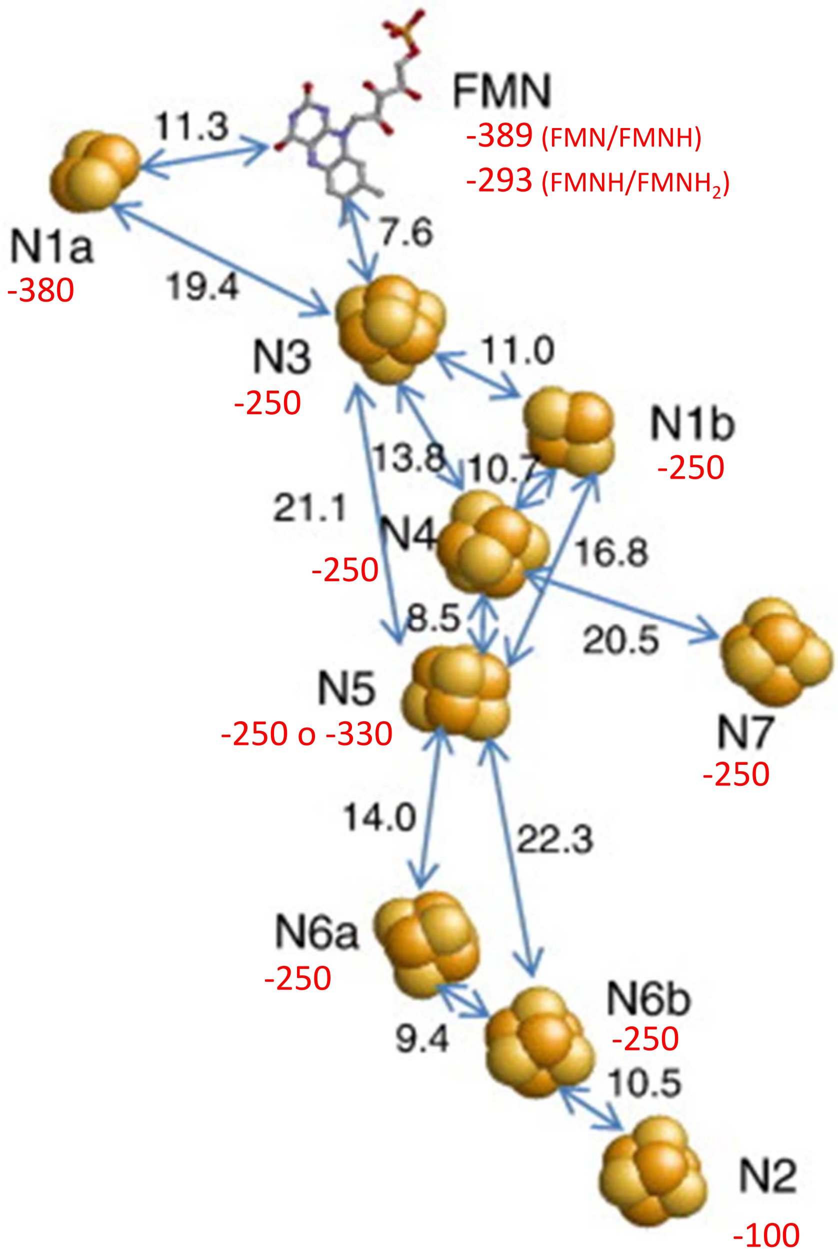




Ransac et al. BBA 2010, Vol 1797(6–7) Pages 641–648

Complejo I: ***NADH:UQ oxidoreductasa***

Reactions	Distance (Å)	$\Delta G°$ (eV)	$k_{forward}$ (s^{-1})	$k_{backward}$ (s^{-1})
NADH to FMN	3.2	0.02	$1.44\ 10^{12}$	$3.10\ 10^{12}$
FMNH2 to N1a	11.3	0.087	$2.13\ 10^{5}$	$5.99\ 10^{6}$
FMNH• to N1a	11.3	– 0.009	$2.12\ 10^{6}$	$1.50\ 10^{6}$
FMNH2 to N3	7.6	– 0.043	$5.25\ 10^{8}$	$1.01\ 10^{8}$
FMNH• to N3	7.6	– 0.139	$1.73\ 10^{9}$	$8.35\ 10^{6}$
N3 to N1b	11.0	0	$2.81\ 10^{6}$	$2.81\ 10^{6}$
N3 to N4	13.8	0	$6.19\ 10^{4}$	$6.19\ 10^{4}$
N1b to N4	10.7	0	$4.23\ 10^{6}$	$4.23\ 10^{6}$
N1b to N5	16.8	0	$1.04\ 10^{3}$	$1.04\ 10^{3}$
N4 to N5	8.5	0	$8.50\ 10^{7}$	$8.50\ 10^{7}$
N5 to N6a	14.0	0	$4.71\ 10^{4}$	$4.71\ 10^{4}$
N6a to N6b	9.4	0	$2.49\ 10^{7}$	$2.49\ 10^{7}$
N6b to N2	10.4	– 0.15	$4.31\ 10^{7}$	$1.36\ 10^{5}$
N1a to N3	19.4	– 0.13	161	1.10
N4 to N7	20.4	0	7.66	7.66
N3 to N5	21.1	0	2.95	2.95
N5 to N6b	22.3	0	0.575	0.575
N2 to Q	12	0.02	$4.43\ 10^{5}$	$9.54\ 10^{5}$
N2 to SQ•	12	– 0.10	$2.71\ 10^{6}$	$5.84\ 10^{4}$
NADH binding			$4.0\ 10^{8}\ M^{-1}$	100
NAD^{+} binding			$1.0\ 10^{7}\ M^{-1}$	1000
Q binding			$4.0\ 10^{7}\ M^{-1}$	100
SQ• binding			$2.0\ 10^{7}\ M^{-1}$	100
QH2 binding			$2.0\ 10^{7}\ M^{-1}$	1000



<u>Complejo I:</u> *NADH:UQ oxidoreductasa*

- **Completar para el set de valores #2:**

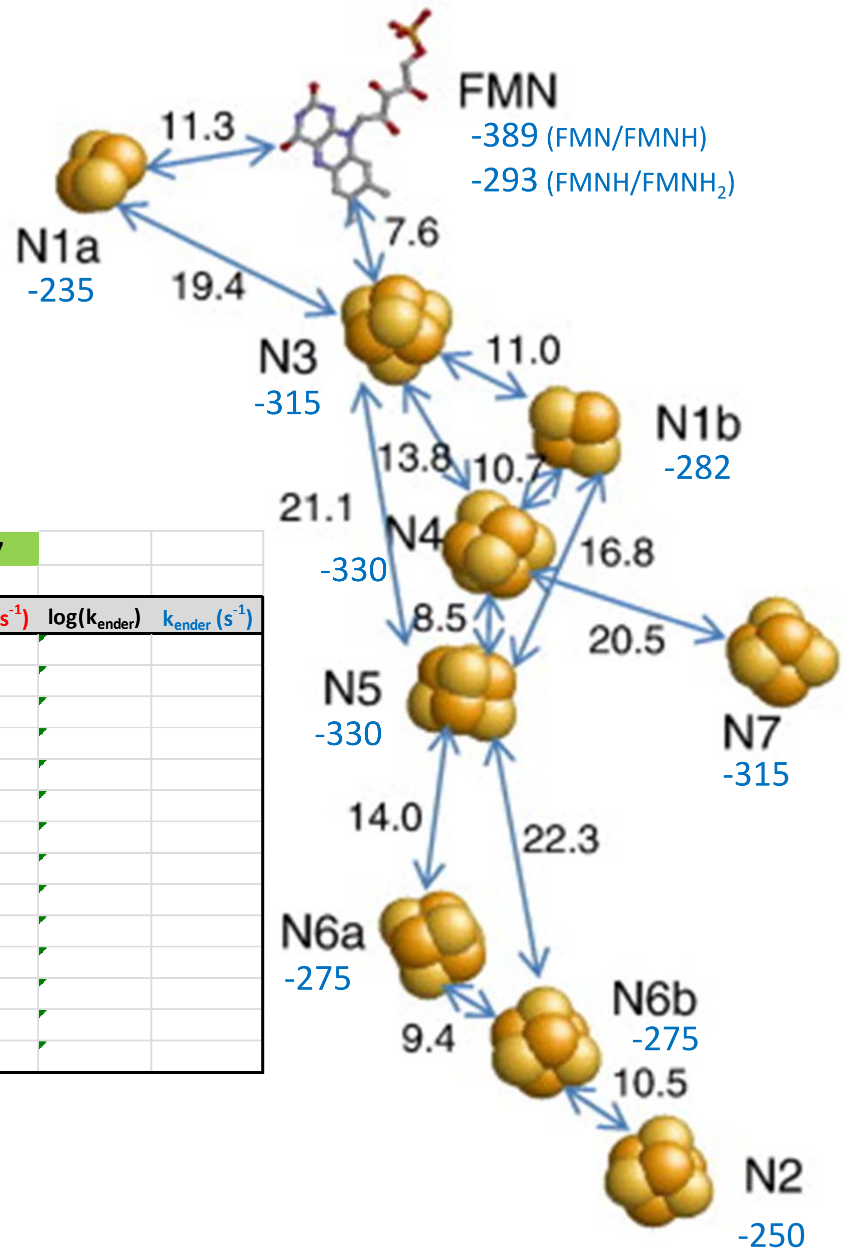
→ Tener en cuenta que:

$1.6x10^{-19}$ [J] = 1 [eV]

F = 96485 C/mol

$\Delta G = -nFE_{Cell}$

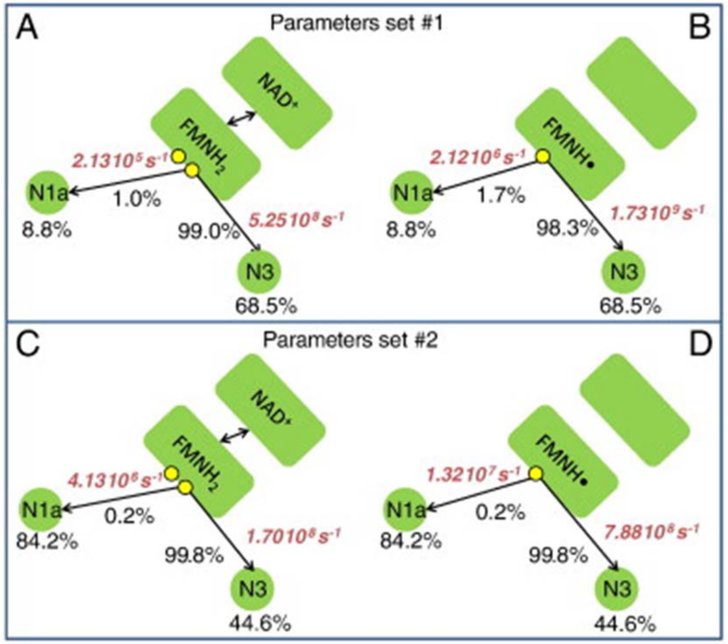
$$\Delta G[eV] = n.E_{Cell}[V]$$

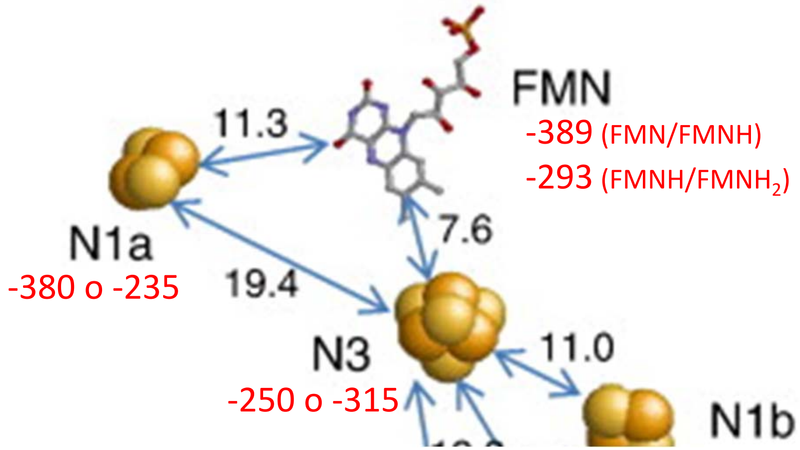


Reacciones invertidas para poder calcular correctamente la K_{exer} — lambda: **0.7**

	Tunneling between	$-E_m$ Donador (V)	E_m Aceptor (V)	deltaG (eV)	R (A)	log(k_{exer})	k_{exer} (s^{-1})	log(k_{ender})	k_{ender} (s^{-1})
COMPLEX I	FMNH2 / N1a	0.293	-0.235						
	FMNH· / N1a	0.389	-0.235						
	FMNH2 / N3	0.293	0.315						
	FMNH· / N3	0.389	-0.315						
	N3 / N1a	0.315	-0.235						
	N3 / N1b	0.315	-0.282						
	N4 / N3	0.33	-0.315						
	N4 / N1b	0.33	-0.282						
	N4 / N5	0.33	-0.33						
	N4 / N7	0.33	-0.315						
	N5 / N6a	0.33	-0.275						
	N5 / N6b	0.33	-0.275						
	N6a / N6b	0.275	-0.275						
	N6b / N2	0.275	-0.25						

Complejo I: *NADH:UQ oxidoreductasa*



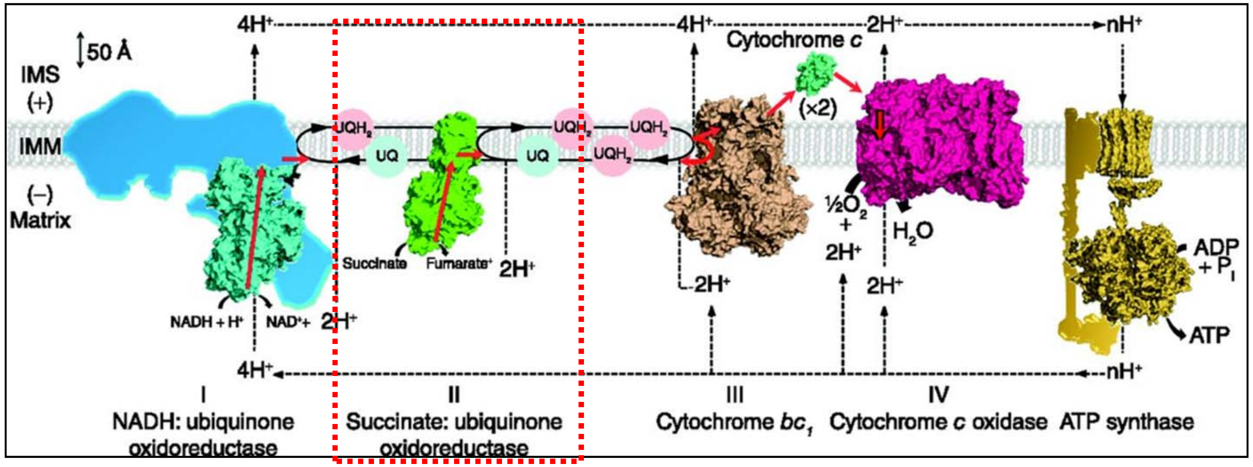


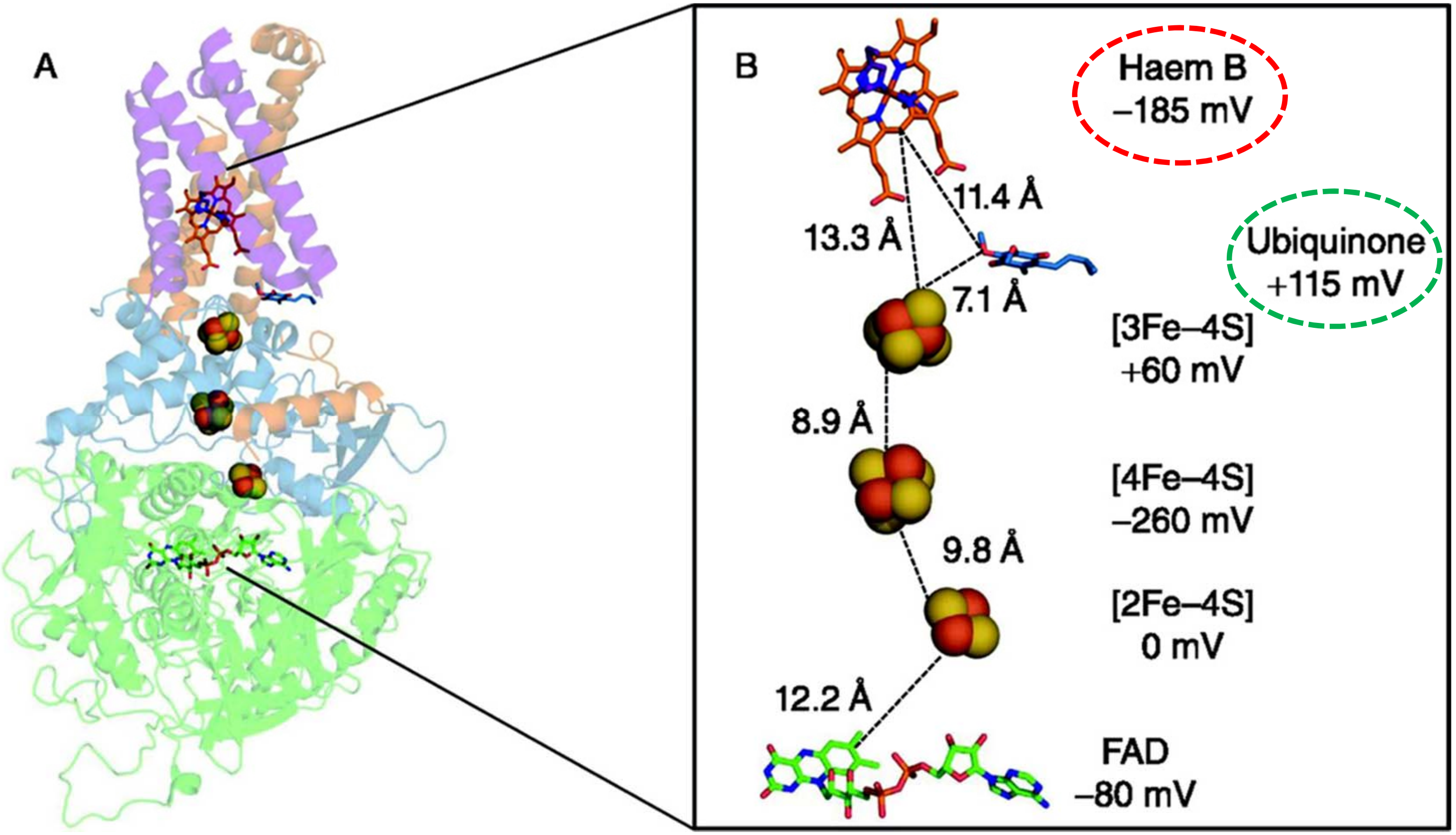
→ Los cálculos permiten ver que:

- $FMNH_2$ transfiere e- a N3.
- FMNH• no es estable →transfiere e- a N1a inmediatamente.
- El e- en N1a no está atrapado. Cuando la próxima molécula de $FMNH_2$ transfiera 1e- a N3, N1a la reduce de FMNH• a $FMNH_2$ al instante.
- Así se evitaría que el estado flavosemiquinona (FMNH•) reaccione con O_2 generando ROS.

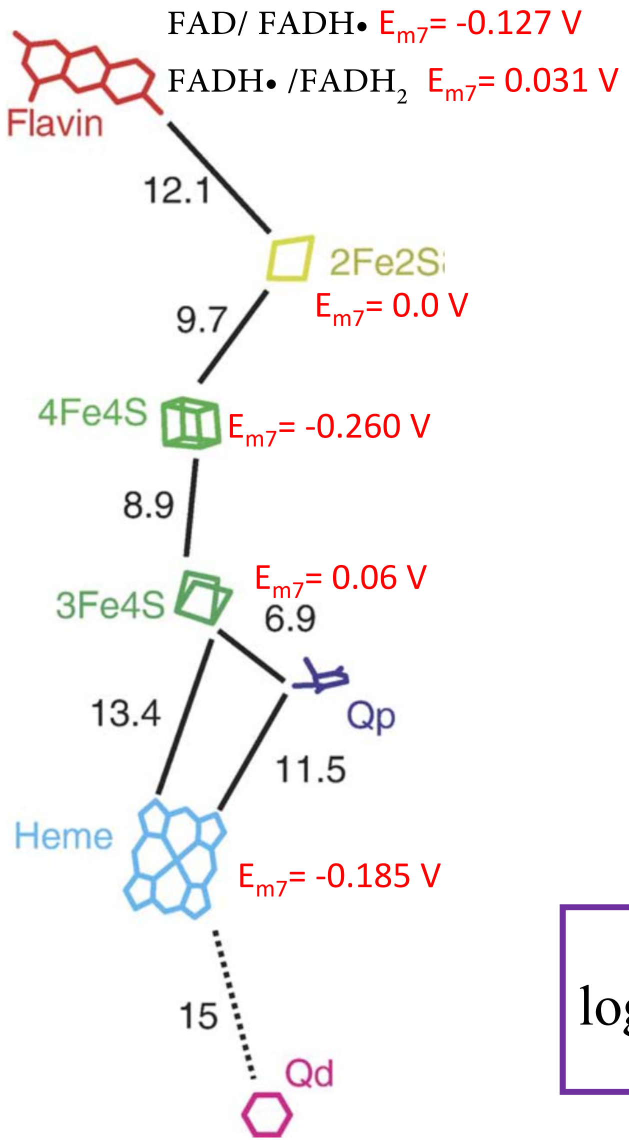
Ransac et al. BBA 2010, Vol 1797(6–7) Pages 641–648

Complejo II: ***succinato:UQ oxidoreductasa***





<u>Complejo II:</u> *succinato:UQ oxidoreductasa*



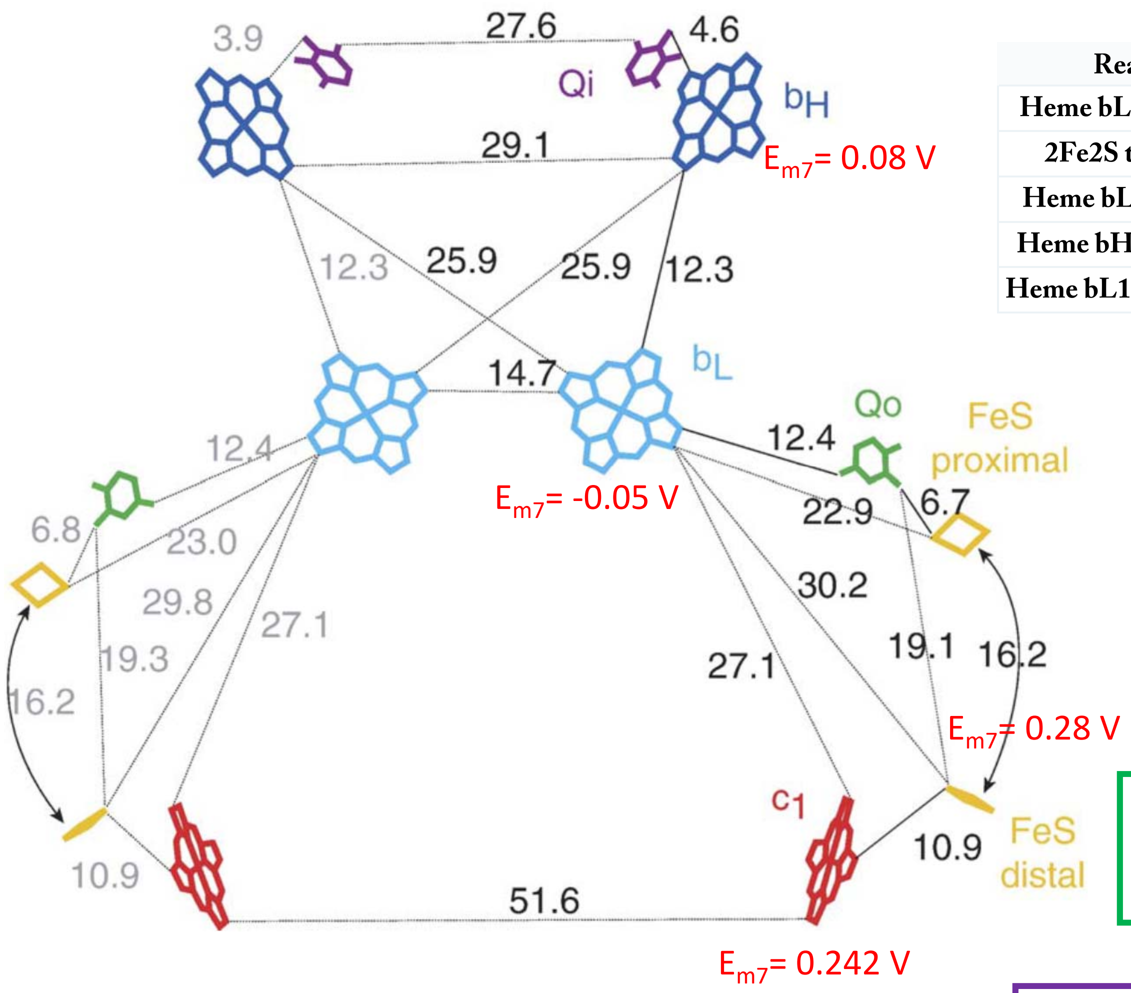
Reactions	Distance (Å)	$\Delta G°$ (eV)	$k$forward (s^{-1})	$k$backward (s^{-1})
FADH2 to 2Fe2S	12.1			
FADH• to 2Fe2S	12.1			
2Fe2S to 4Fe4S	9.7			
4Fe4S to 3Fe4S	8.9			
3Fe4S to Heme	13.4			

$$\Delta G[eV] = -n.E_{Cell}[V]$$

$$\log\left(k_{ET}^{exer}\right) = 15 - 0.6R - 3.1\frac{(\Delta G + 0.7)^2}{0.7}$$

$$\log\left(k_{ET}^{ender}\right) = 15 - 0.6R - 3.1\frac{(\Delta G + \lambda)^2}{\lambda} + \frac{\Delta G}{0.06}$$

Complejo III: *UQ:citocromo C oxidoreductasa* → Citocromo bc_1



Reactions	Distance (Å)	$\Delta G°$ (eV)	$k_{forward}$ (s^{-1})	$k_{backward}$ (s^{-1})
Heme bL to Heme bH				
2Fe2S to Heme c1				
Heme bL to Heme bL				
Heme bH to Heme bH				
Heme bL1 to Heme bH2				

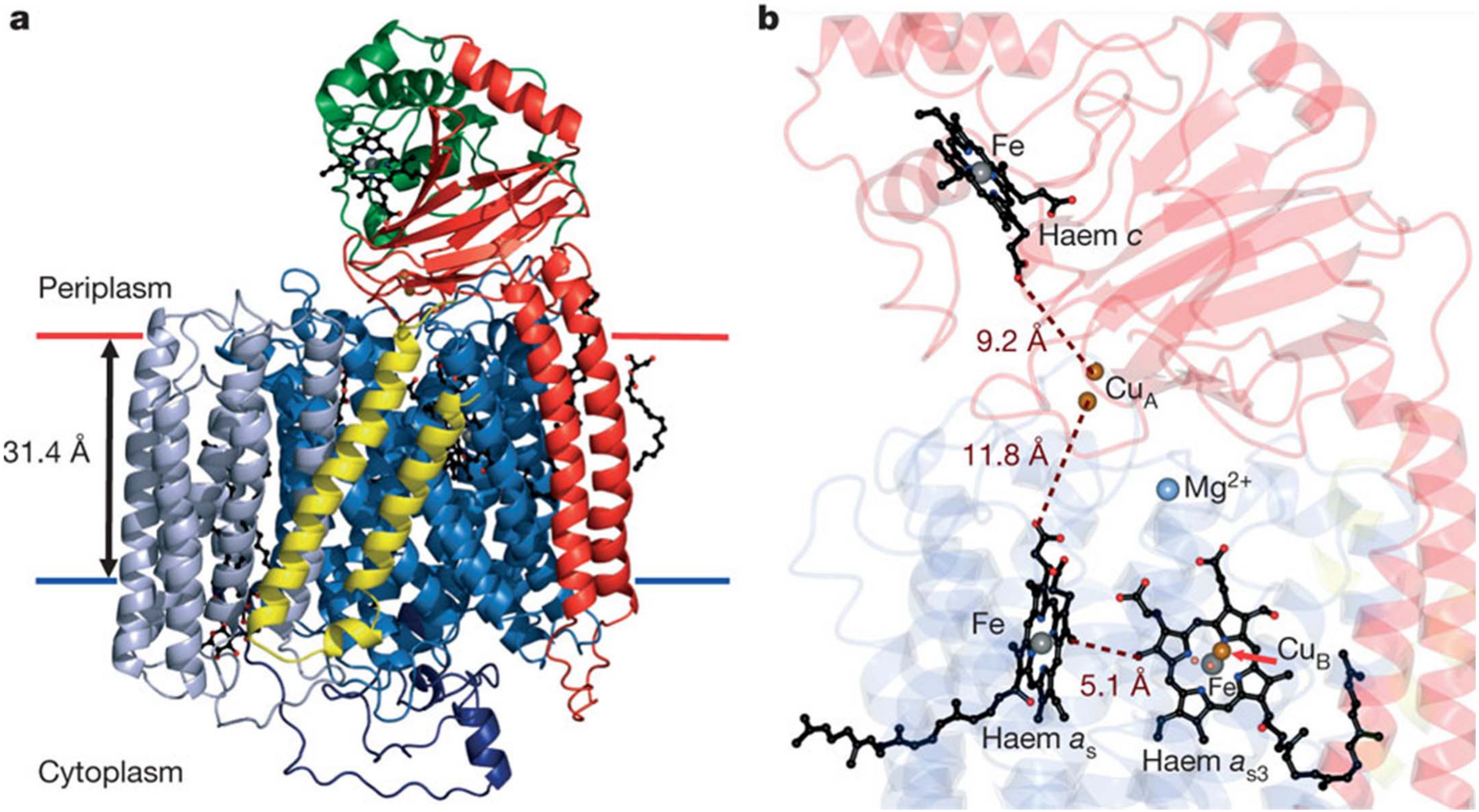
$$\Delta G\left[eV\right] = -n.E_{Cell}\left[\mathrm{V}\right]$$

$$\log\left(k_{ET}^{exer}\right) = 15 - 0.6R - 3.1\frac{(\Delta G + 0.7)^2}{0.7}$$

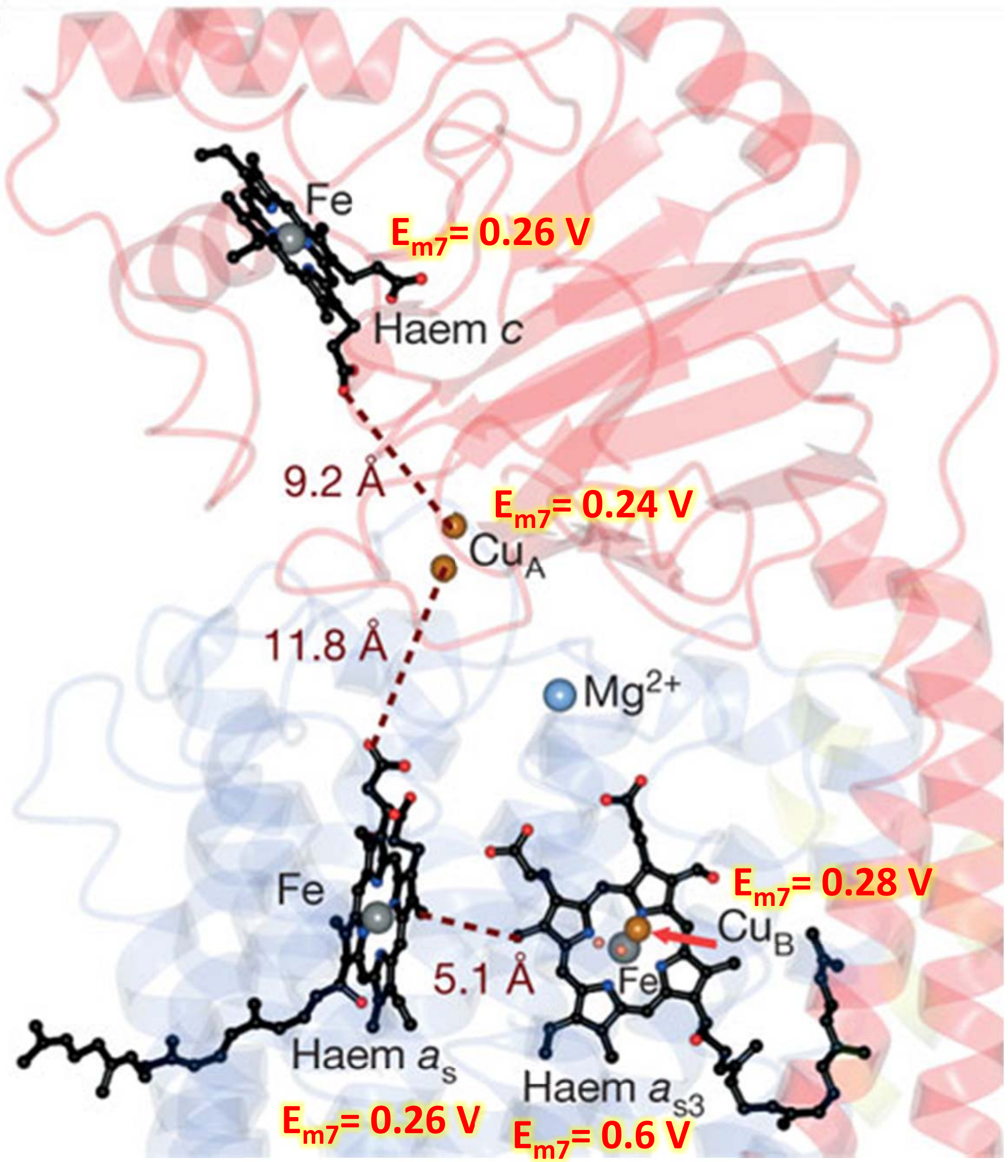
$$\log\left(k_{ET}^{ender}\right) = 15 - 0.6R - 3.1\frac{(\Delta G + \lambda)^2}{\lambda} + \frac{\Delta G}{0.06}$$

<u>Complejo IV:</u> *citocromo:O_2 oxidoreductasa* → *Citocromo c oxidasa*

Citocromo c oxidasa (tipo aa_3) de *P. denitrificans*



Complejo IV: *citocromo:O_2 oxidoreductasa* → *Citocromo c oxidasa*



Reactions	Distance (Å)	$\Delta G°$ (eV)	$k_{forward}$ (s^{-1})	$k_{backward}$ (s^{-1})
Heme c to CuA	9.2			
CuA to Heme a	11.8			
Heme a to Heme a3	5.1			
Heme a3 to CuB	2.5			

$$\Delta G[eV] = -n.E_{Cell}[V]$$

$$\log\left(k_{ET}^{exer}\right) = 15 - 0.6R - 3.1\frac{(\Delta G + 0.7)^2}{0.7}$$

$$\log\left(k_{ET}^{ender}\right) = 15 - 0.6R - 3.1\frac{(\Delta G + \lambda)^2}{\lambda} + \frac{\Delta G}{0.06}$$