Ataxia de Friedreich

Bioquímica y estrategias terapéuticas

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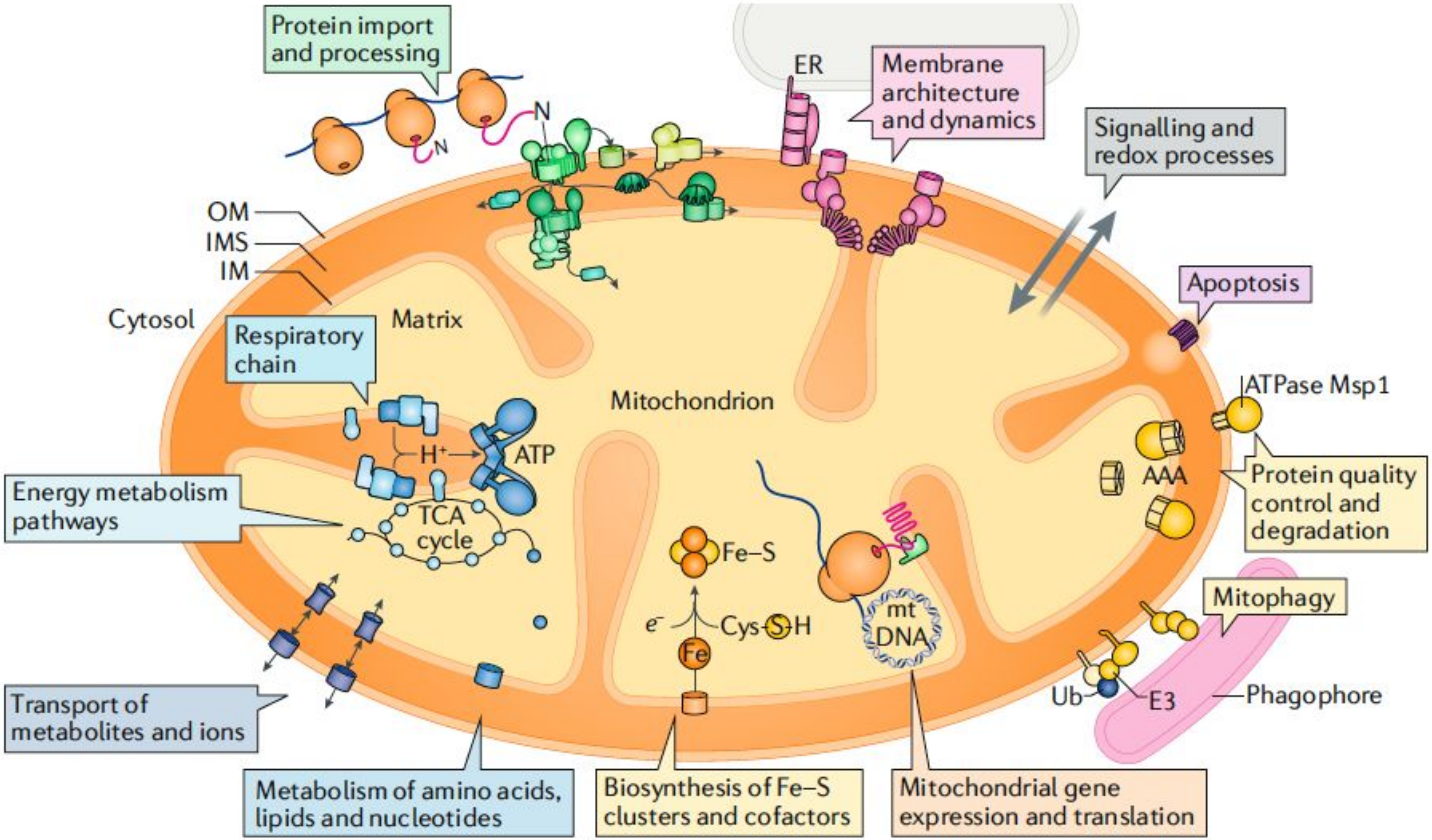
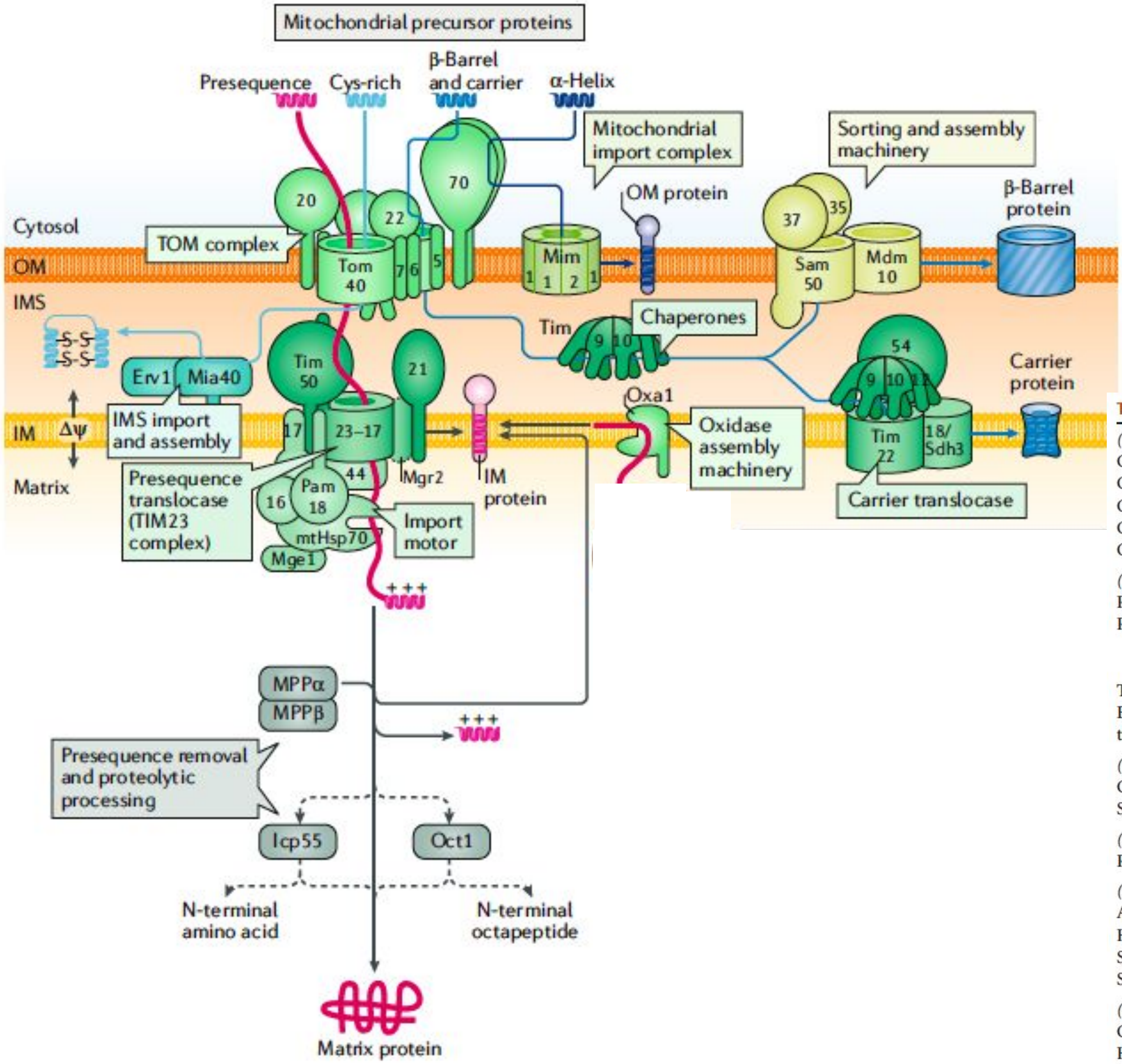


doi: 10.1038/s41580-018-0092-0



Proteínas mitocondriales

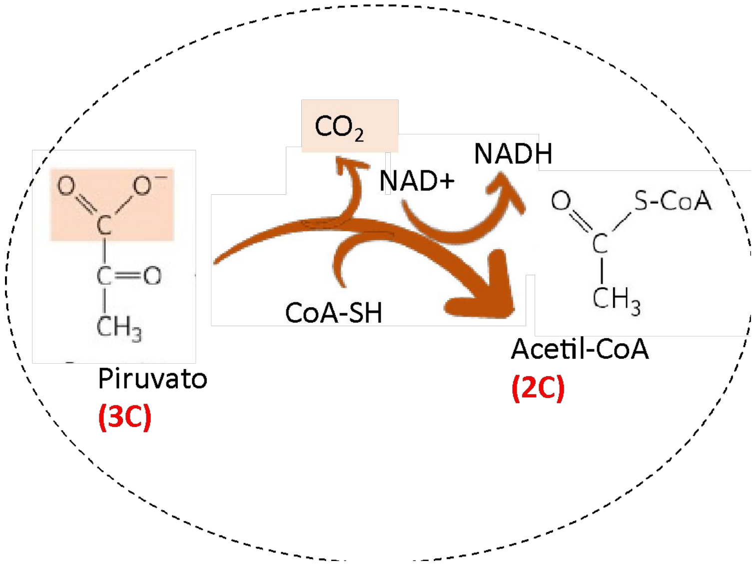
1% codificadas en el DNA mitochondrial

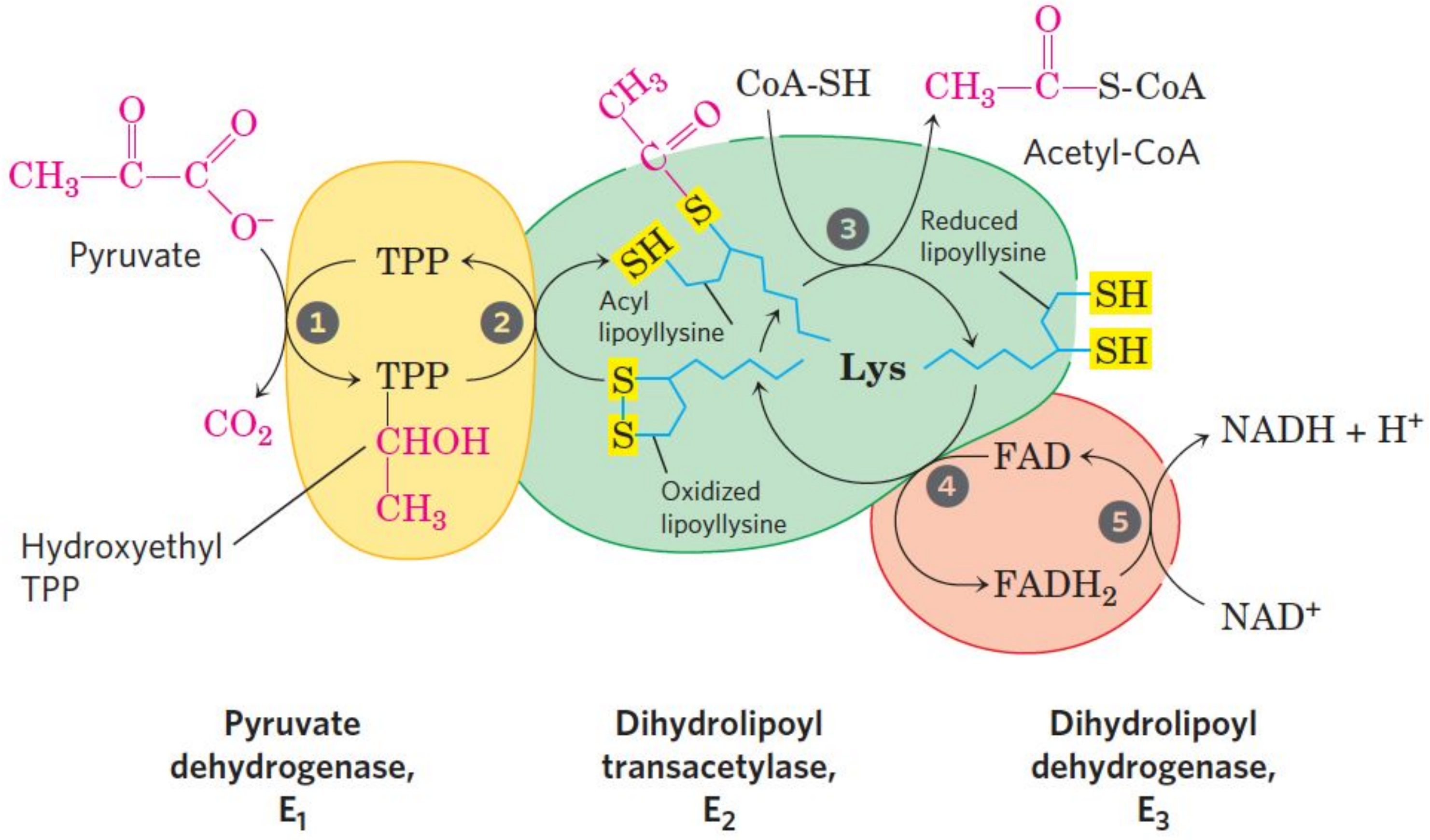
99% codificadas en el DNA nuclear

Table 1. Mitochondrial DNA-encoded genes and their functions

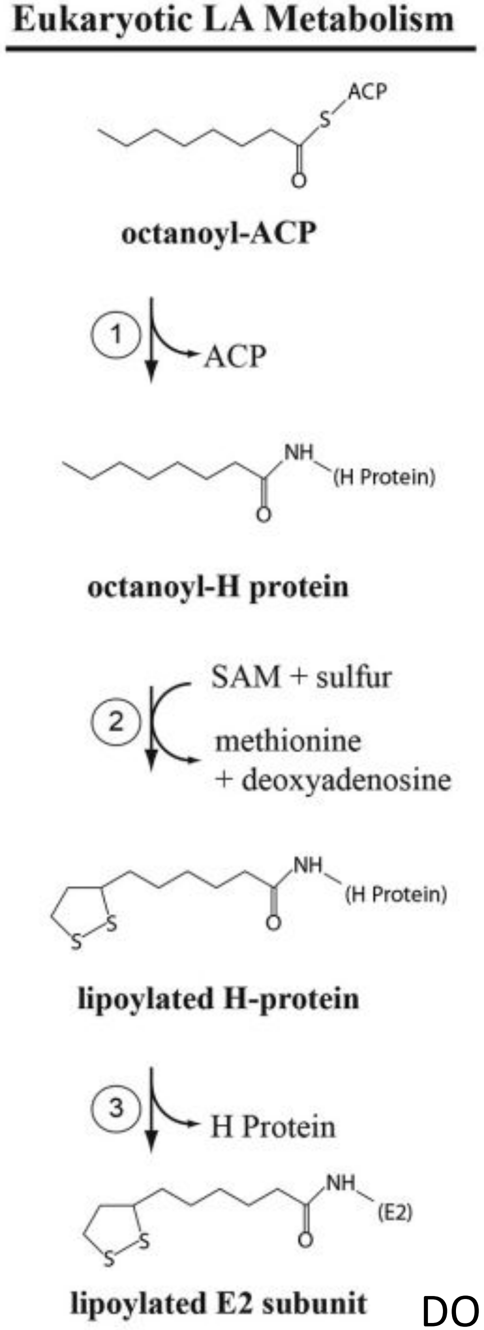
(1) Coupled electron transport–oxidative phosphorylation (ATP synthesis)	
Complex I (NADH:ubiquinone oxidoreductase)	***nad1*, 2, 3, 4, 4L, 5, 6, 7, 8, 9, 10, 11**
Complex II (succinate:ubiquinone oxidoreductase)	*sdh 2, 3, 4*
Complex III (ubiquinol:cytochrome *c* oxidoreductase)	***cob***
Complex IV (cytochrome *c*:O_2 oxidoreductase)	***cox1*, 2, 3**
Complex V (F_1F_0 ATP synthase)	*atp1, 3, 4,*[a] *6, 8,*[b] *9*
(2) Translation	
Ribosomal RNAs	***rnl* (LSU), *rns* (SSU)**, *rrr5* (5S)
Ribosomal proteins	
Small subunit (SSU)	*rps1, 2, 3, 4, 7, 8, 10, 11, 12, 13, 14, 19*
Large subunit (LSU)	*rpl1, 2, 5, 6, 10, 11, 14, 16, 18, 19, 20, 27, 31, 32, 34*
Transfer RNAs	***trnA*, C, . . . W, Y**
Elongation factor	*tufA*
tm RNA (unstalling of translation)	*ssrA*
(3) Transcription	
Core RNA polymerase	*rpoA, B, C*
Sigma factor	*rpoD*
(4) RNA processing	
RNase P RNA (5′ tRNA processing)	*rnpB*
(5) Protein import	
ABC transporter	*ccmA (yejV), ccmB (yejW)*
Heme delivery	*ccmC (yejU)*
SecY-type transporter	*secY*
Sec-independent transporter	*tatA (mttA)*[c], *tatC (mttB)*
(6) Protein maturation	
Cytochrome oxidase assembly	*cox11*
Heme *c* maturation	*ccmF (yejR)*

Piruvato deshidrogenasa

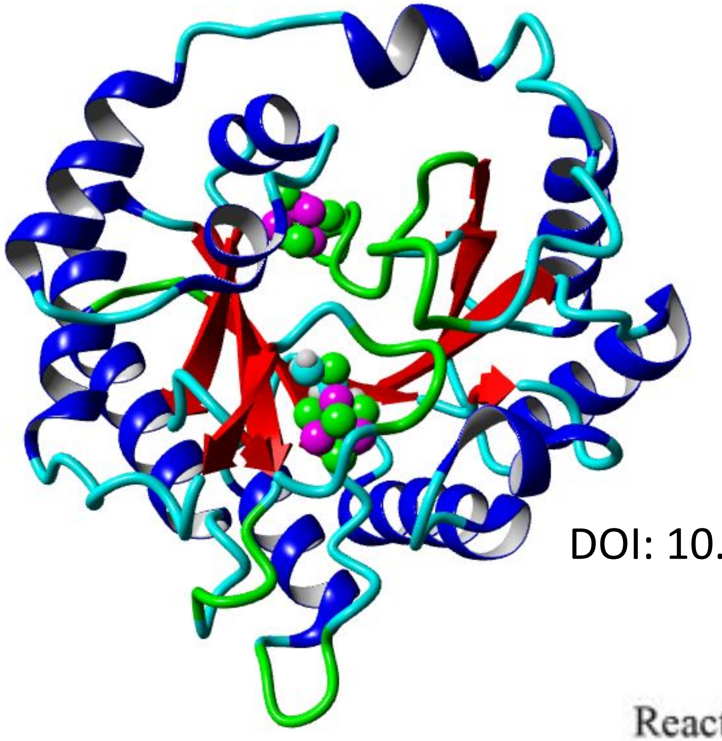




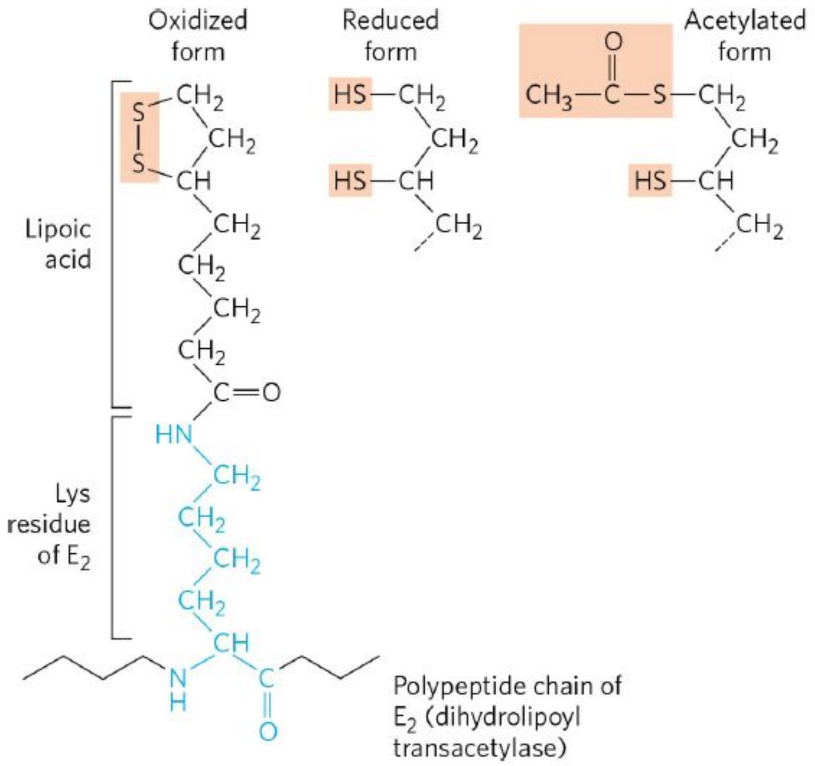
Biosíntesis de lipoamida



DOI 10.1074/jbc.TM117.000259

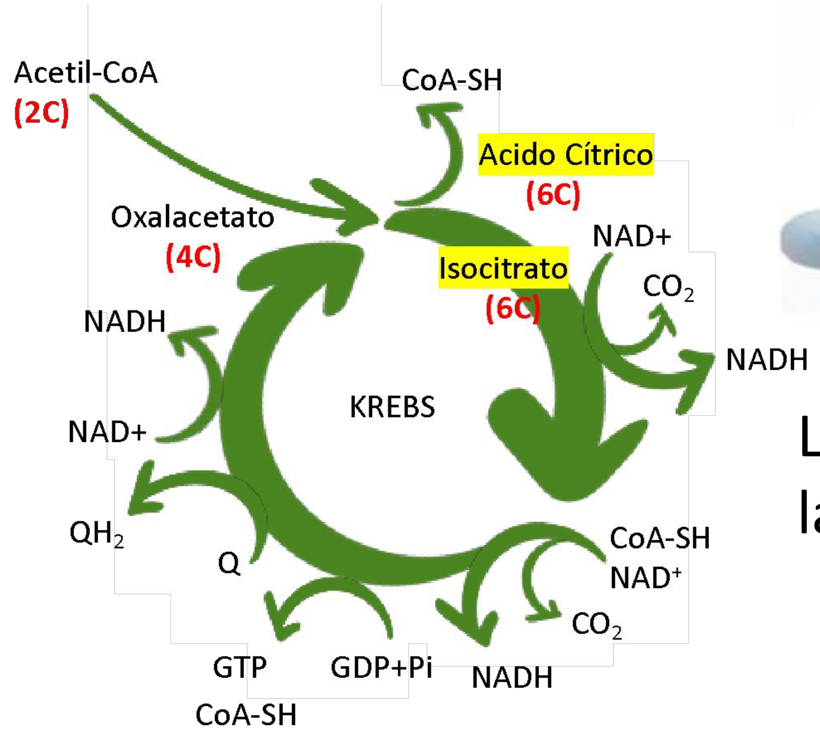


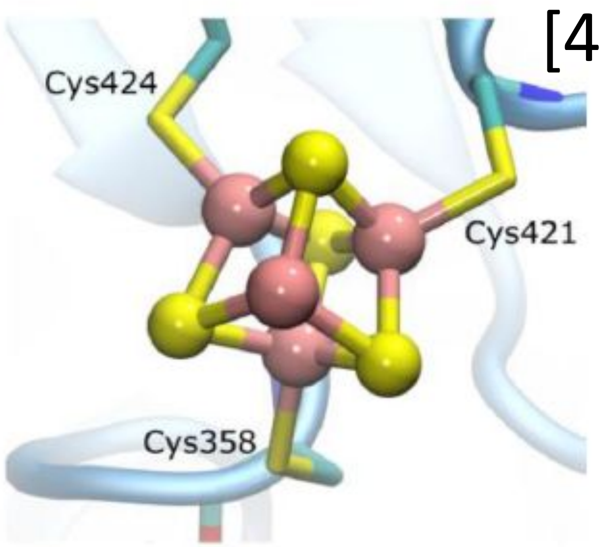
DOI: 10.2210/pdb4U0P/pdb



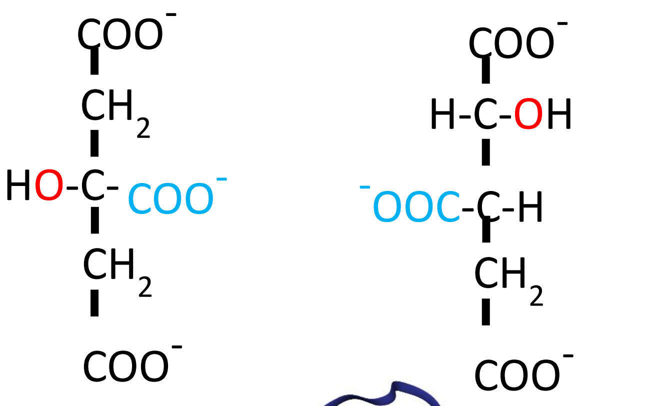
Eukaryotic LA Metabolism			
Reaction	1	2	3
Enzyme	octanoyltransferase	lipoate synthase	lipoyltransferase
S. cerevisiae	Lip2	Lip5	Lip3
H. sapiens	LIPT2	LIAS	LIPT1

Ciclo de Krebs

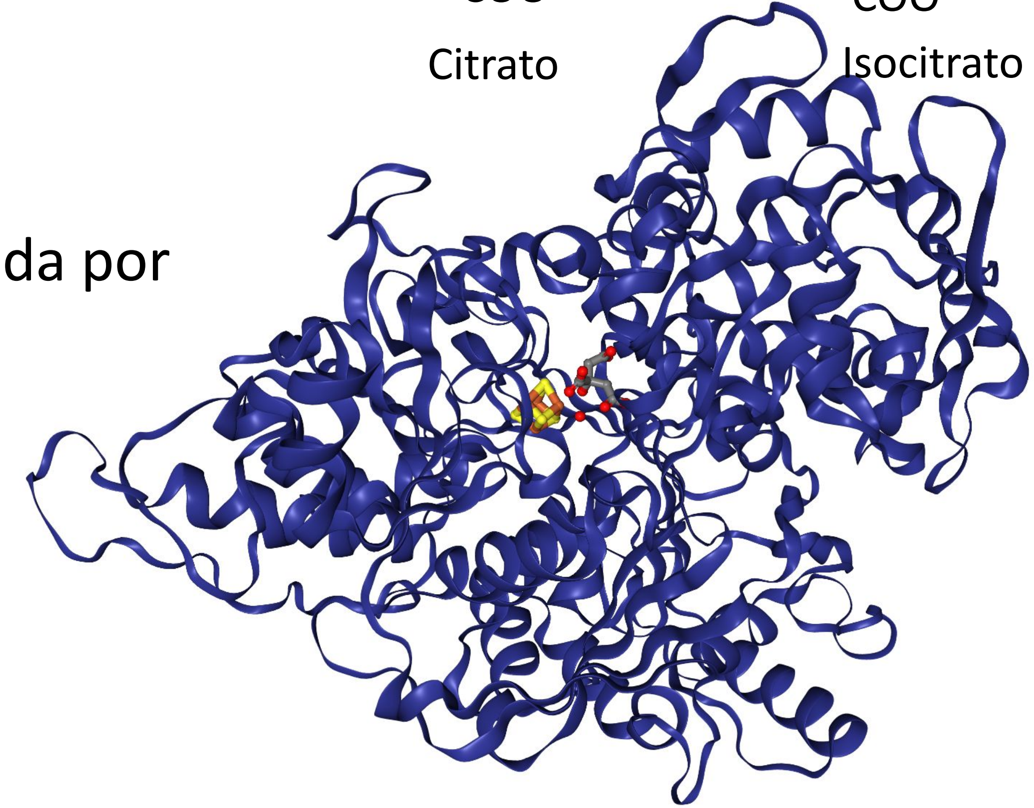




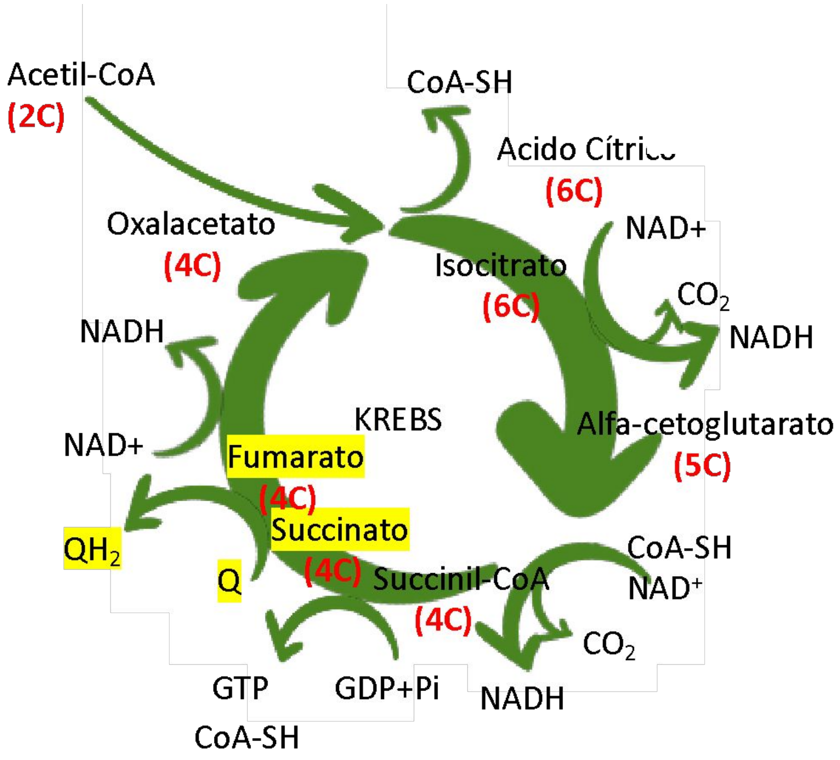
$[4Fe - 4S]^{2+}$

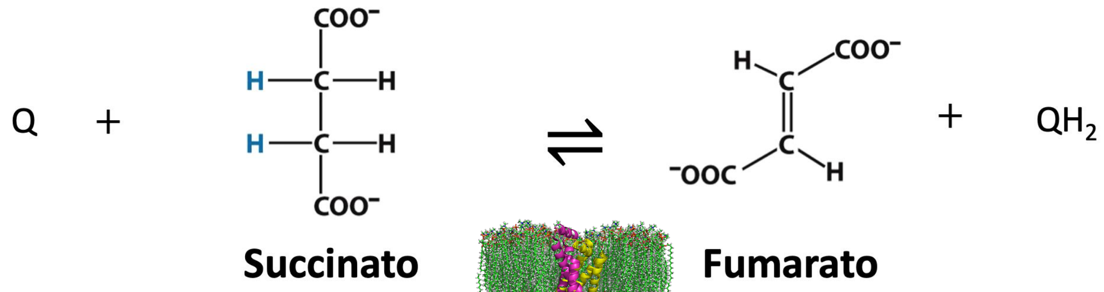


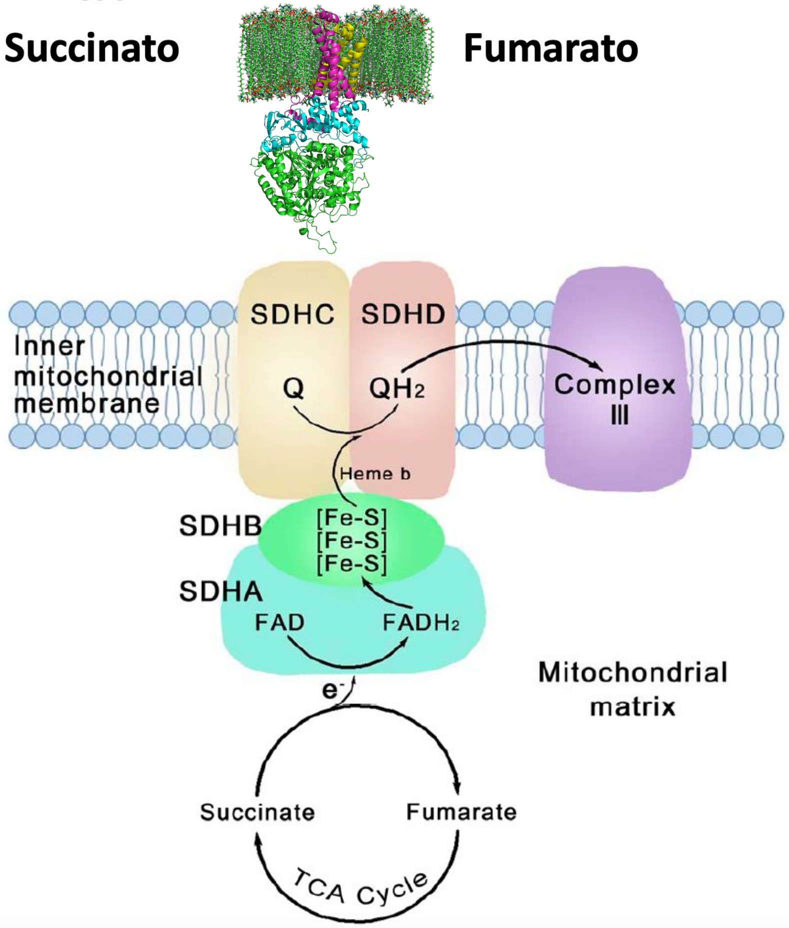
La reacción está catalizada por la aconitasa

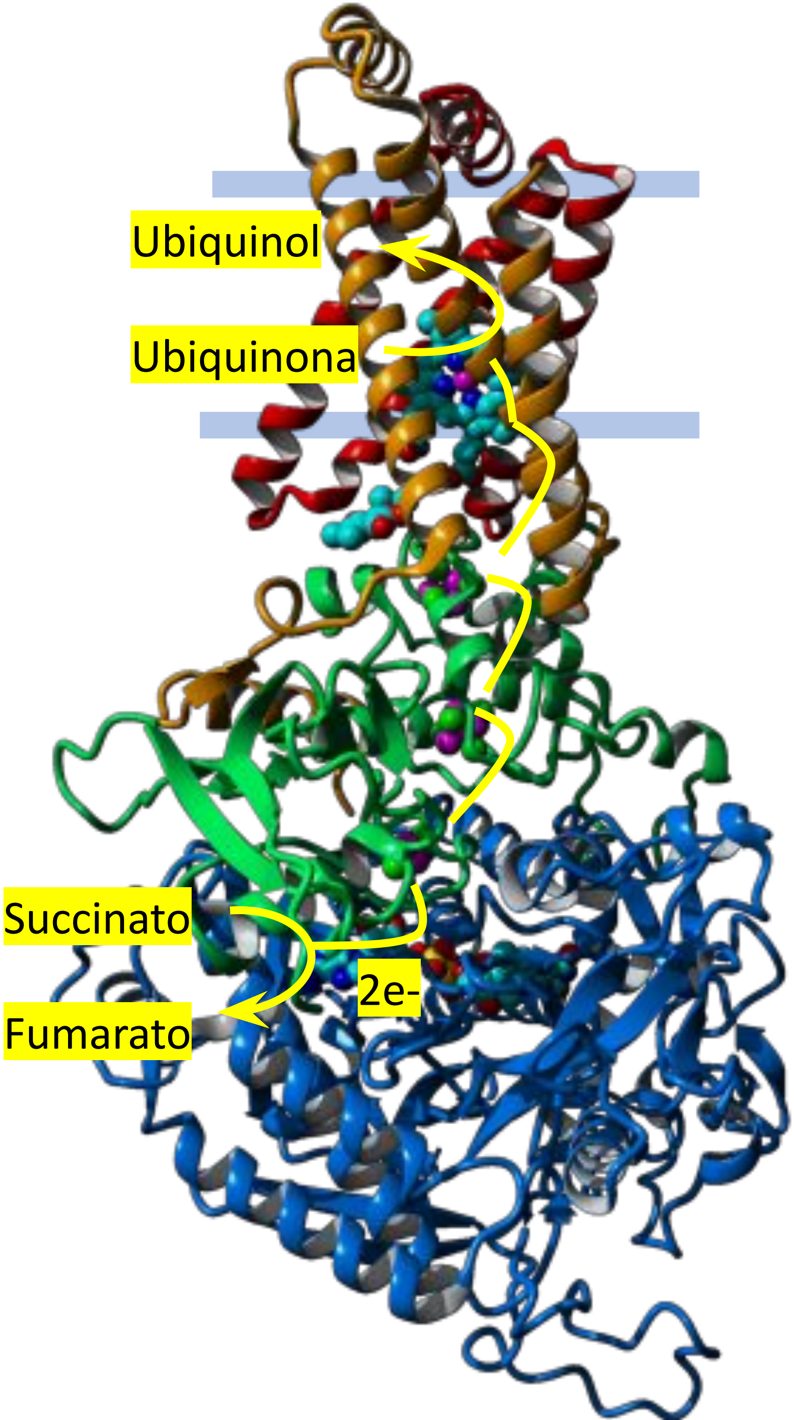


Ciclo de Krebs





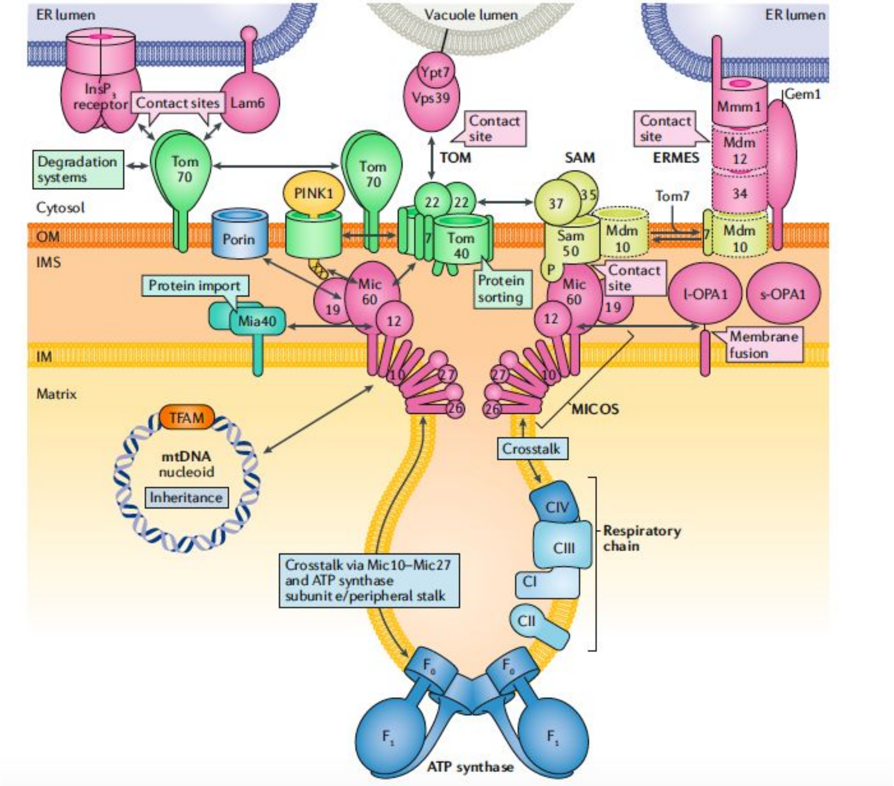


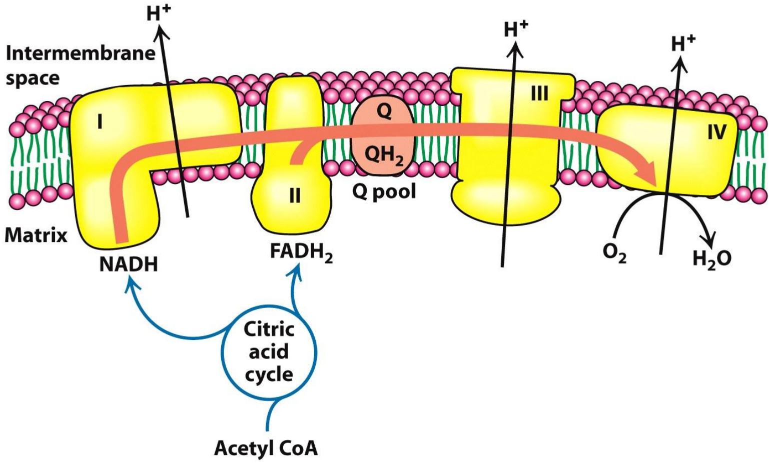


La reacción está catalizada por la enzima succinato deshidrogenasa

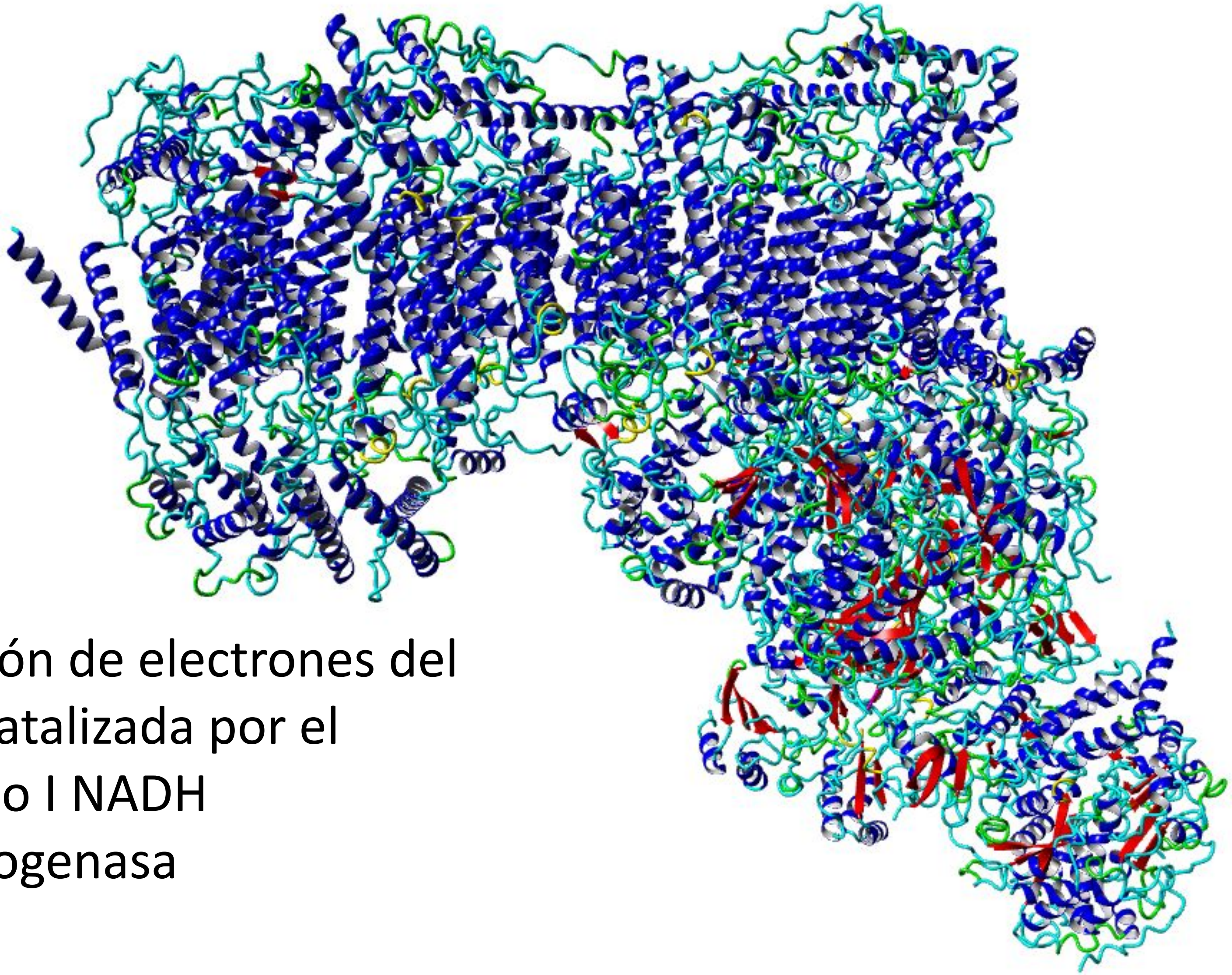
https://doi.org/10.1515/hsz-2019-0264

Cadena de transporte de electrones

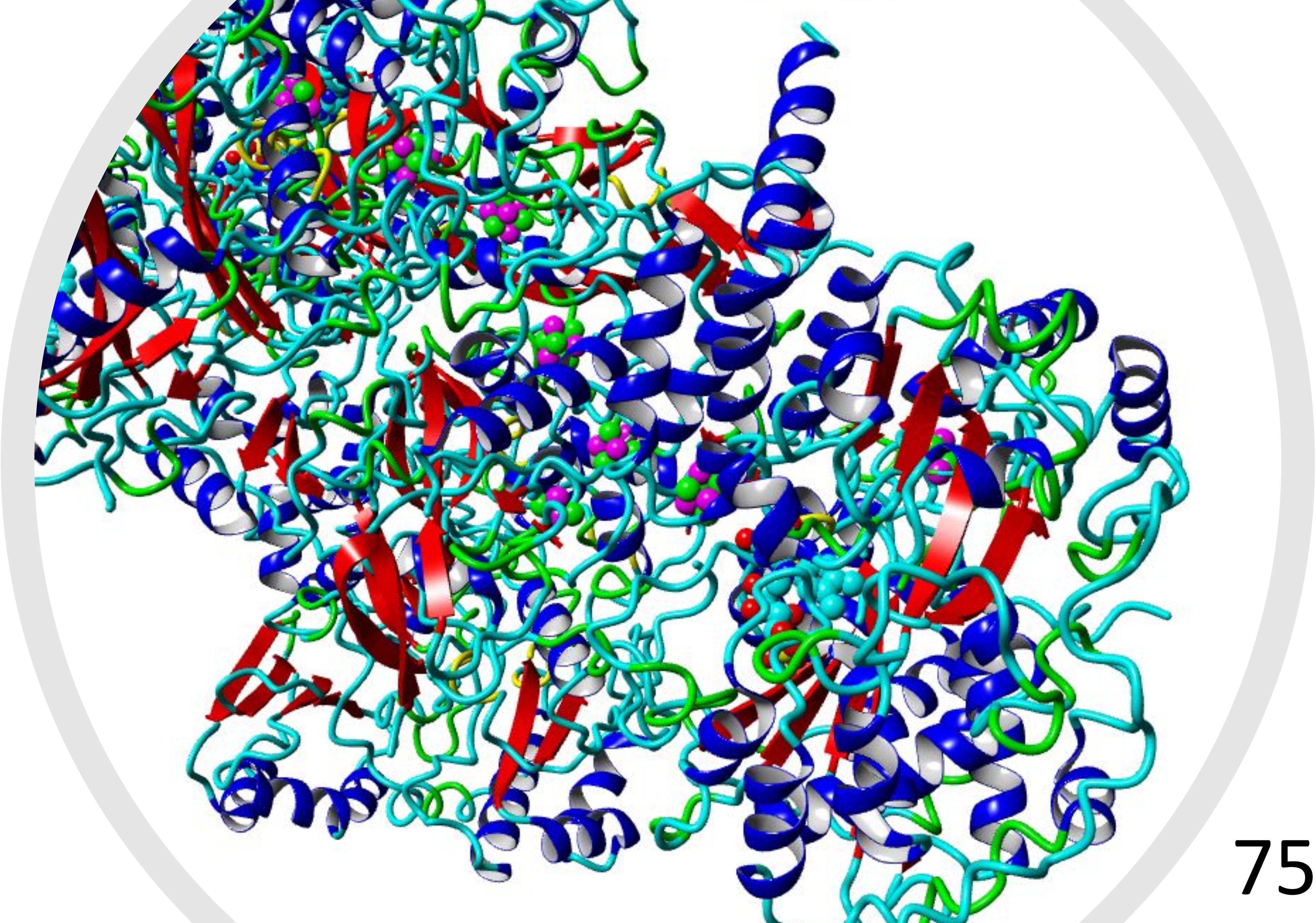




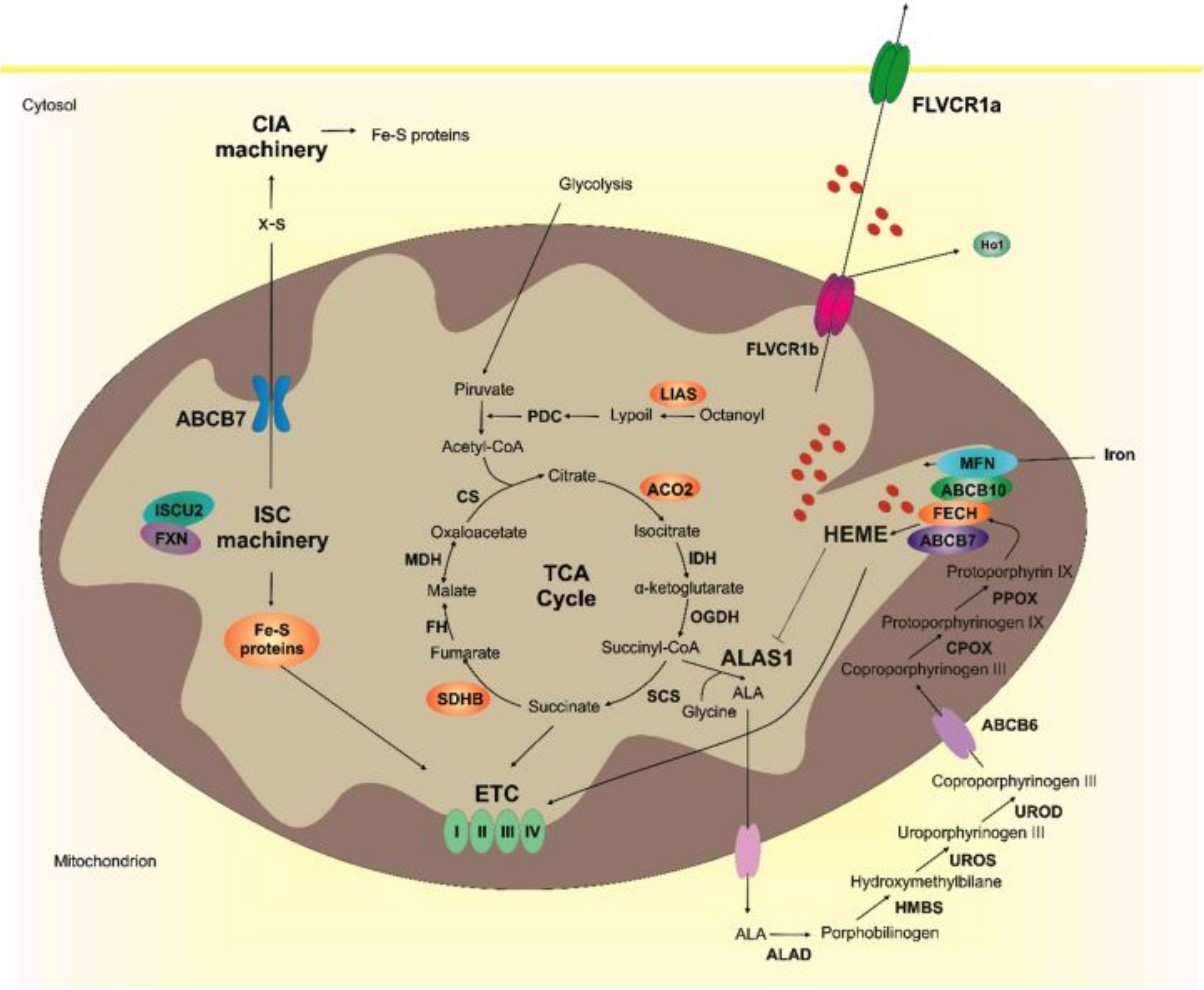
Extracción de electrones del NADH catalizada por el complejo I NADH deshidrogenasa



PDB ID 5XTD



75.5Å

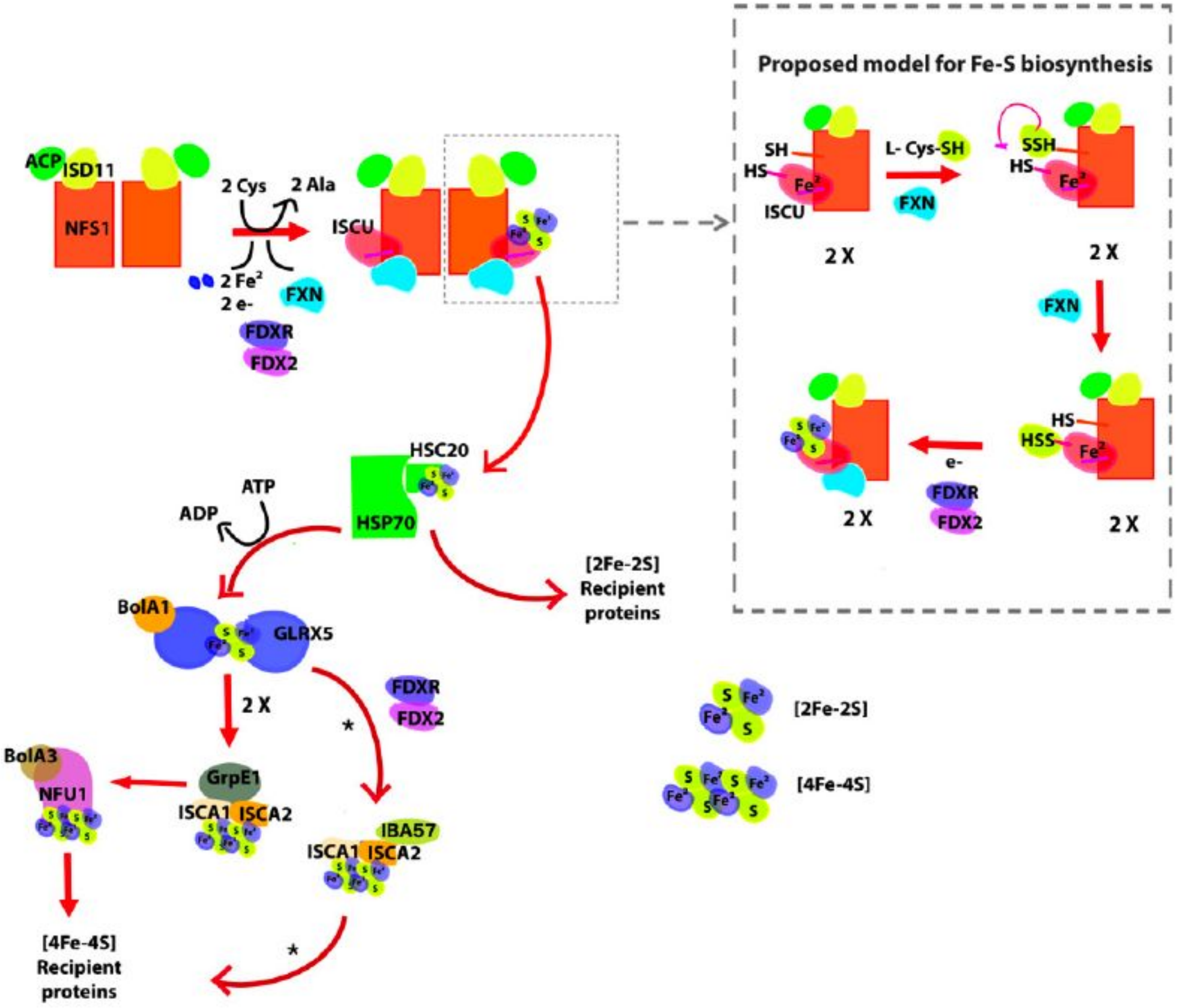


doi.org/10.3390/ijms21113760

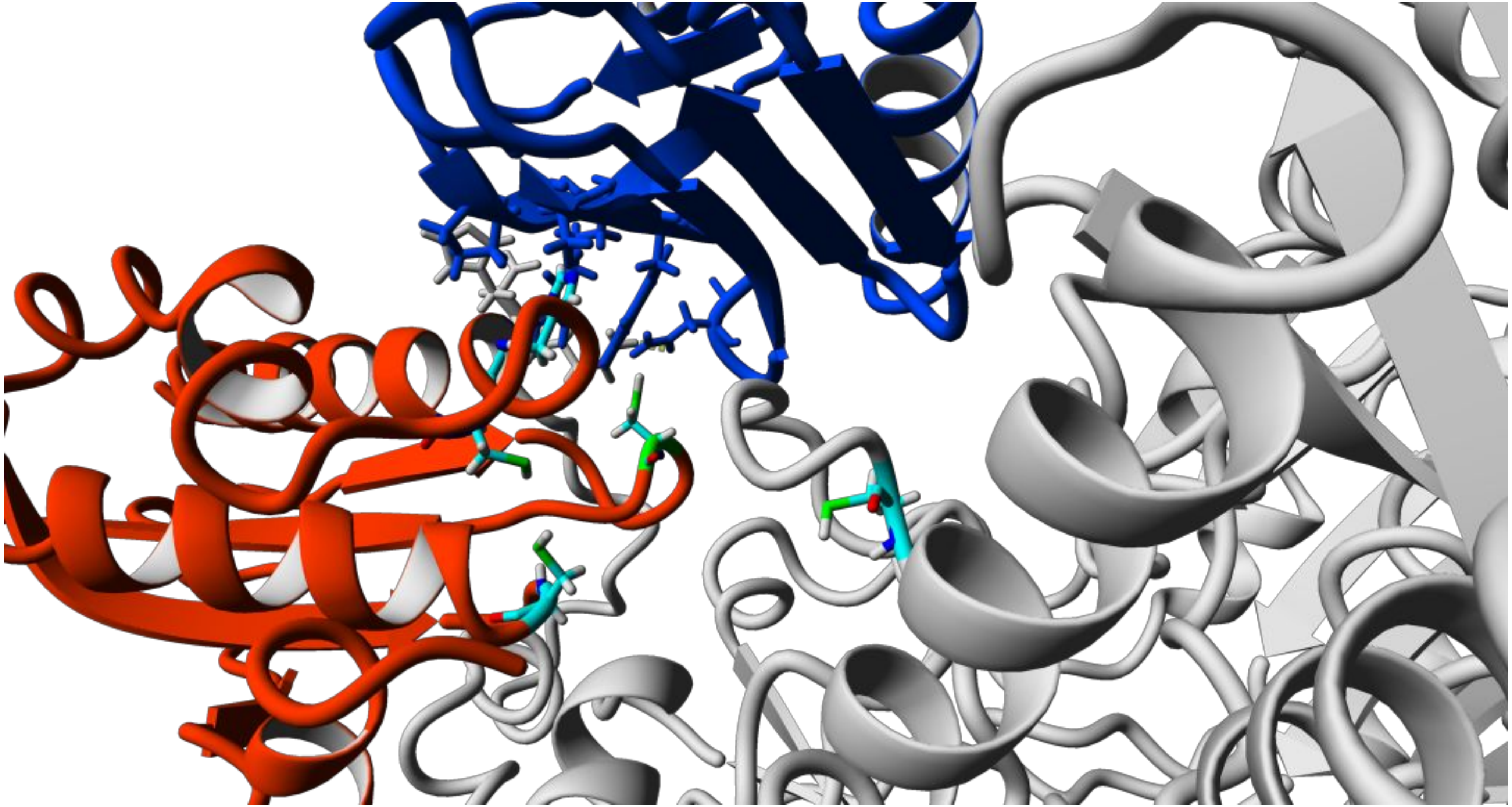
Síntesis de centros ferro-sulfurados

- ✔ NFS1 enzima desulfurasa
- ✔ ISCU andamiaje
- ✔ ACP-ISD11 tutora/estabilizadora
- ✔ Frataxina Activadora

Todas codificadas por genes nucleares.
Ubicación: Matriz mitocondr
Forman un Supercomplejo

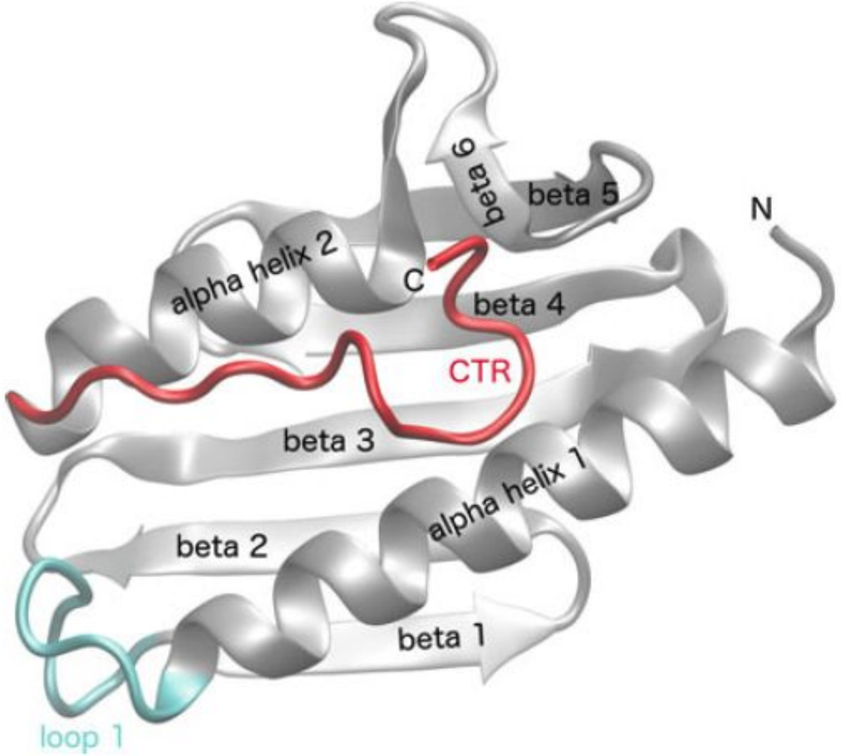


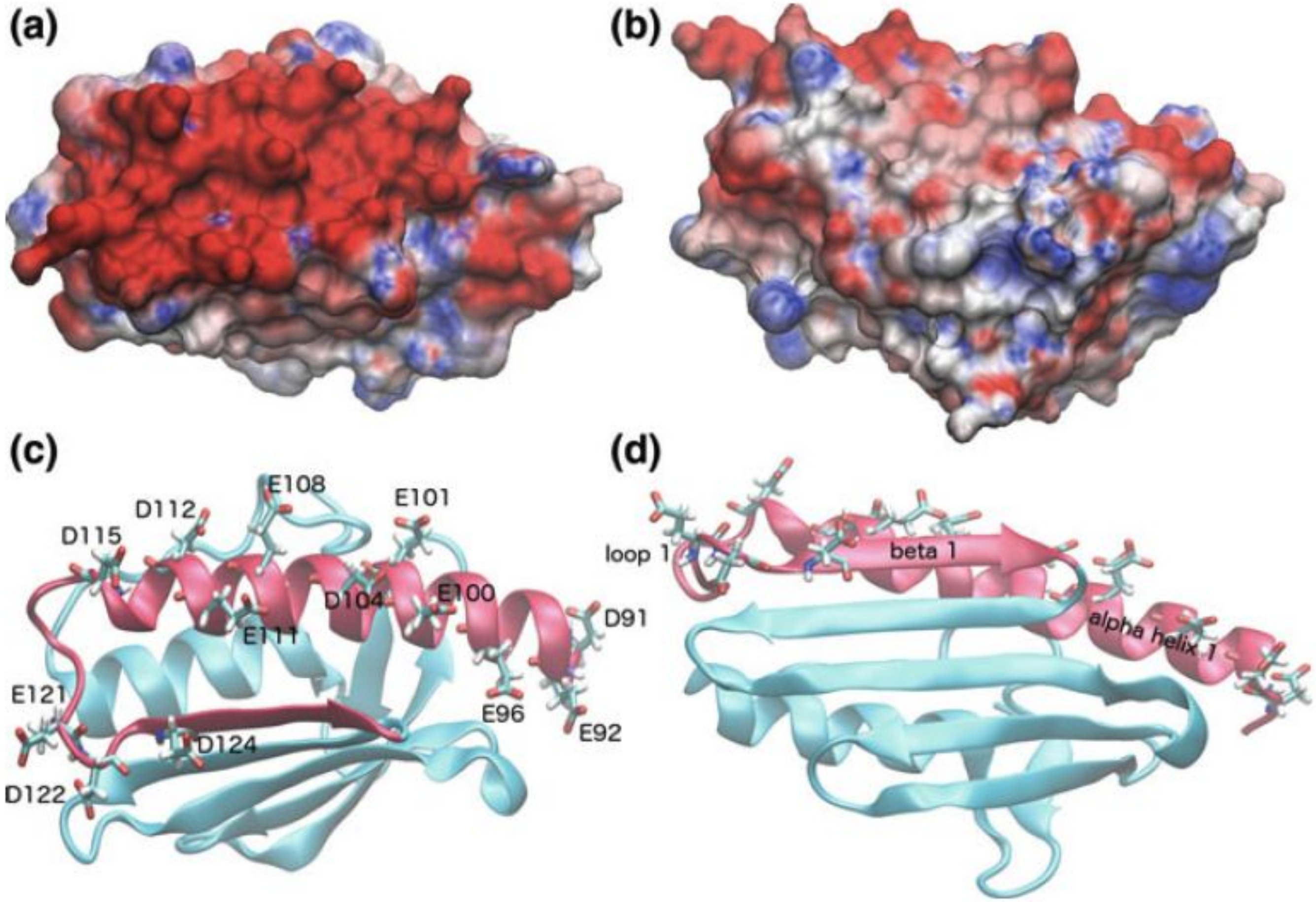
Rol de la frataxina



doi.org/10.1038/s41467-019-09989-y

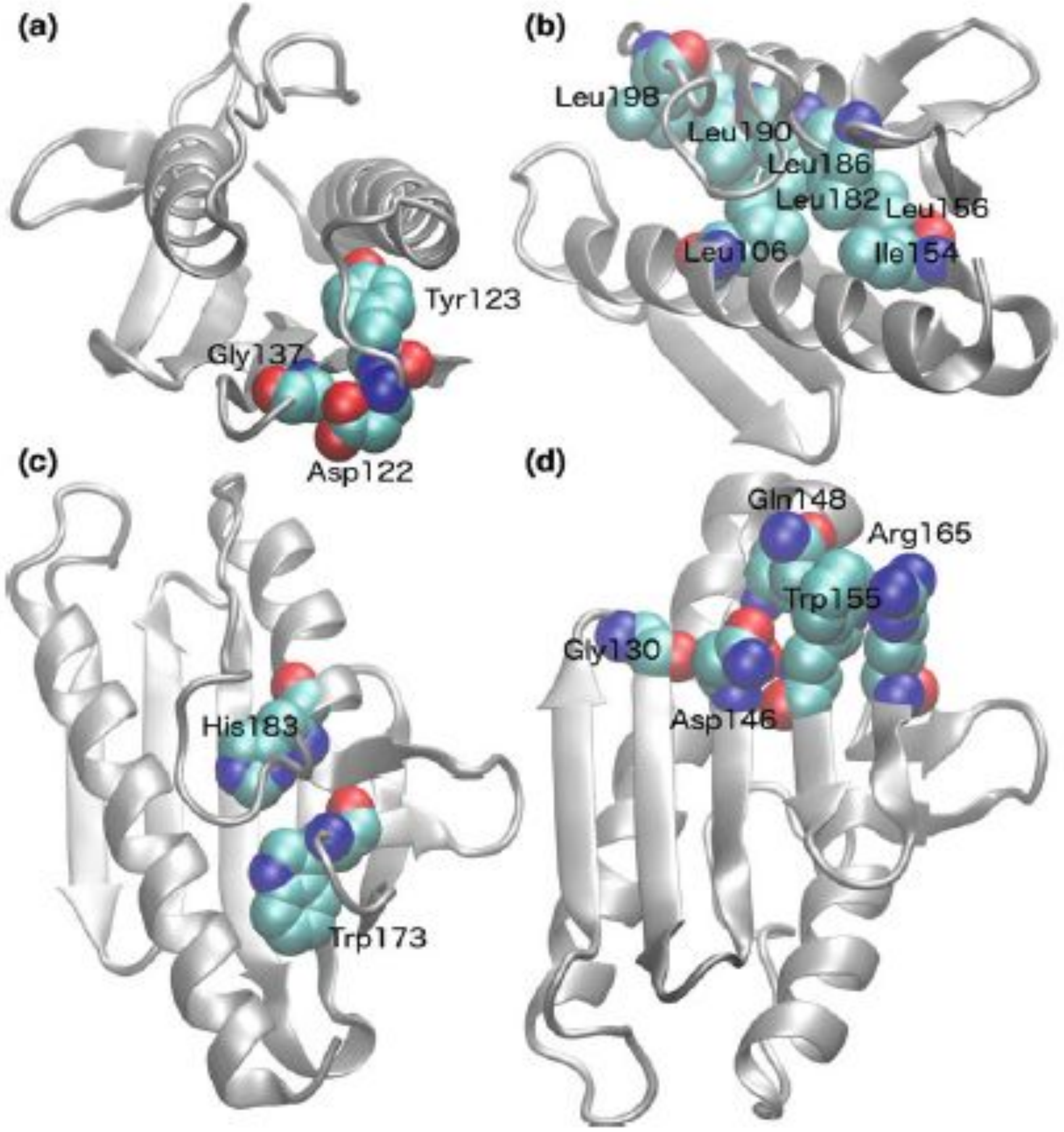
Estructura de la frataxina





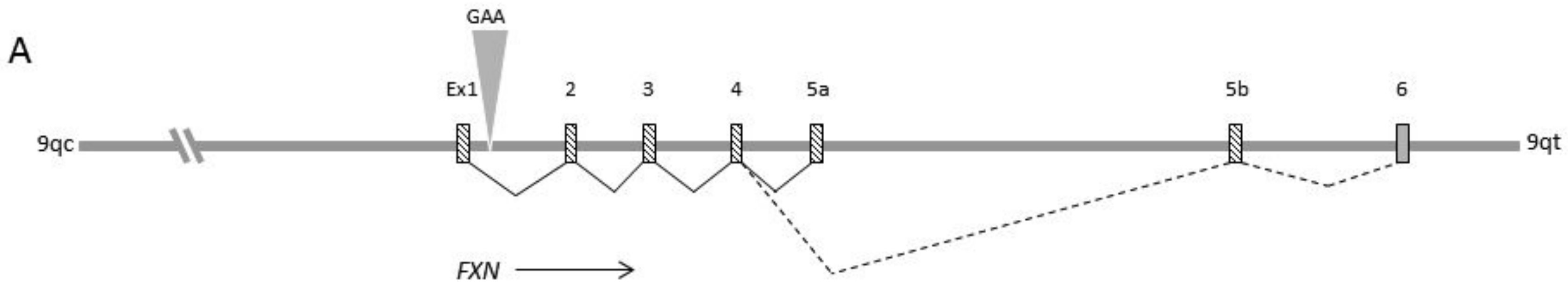
Variantes patogénicas de frataxina

- ✔ Estabilidad conformacional
- ✔ Interacciones intermoleculares
- ✔ Alteraciones en la dinámica molecular
- ✔ Alteraciones en la secuencia de tránsito mitocondrial
- ✔ Combinaciones de las anteriores
- ✔ Sabemos que solo en un 2-5% se observa una mutación en un alelo (qué pasa con el otro???)



El gen *fxn*

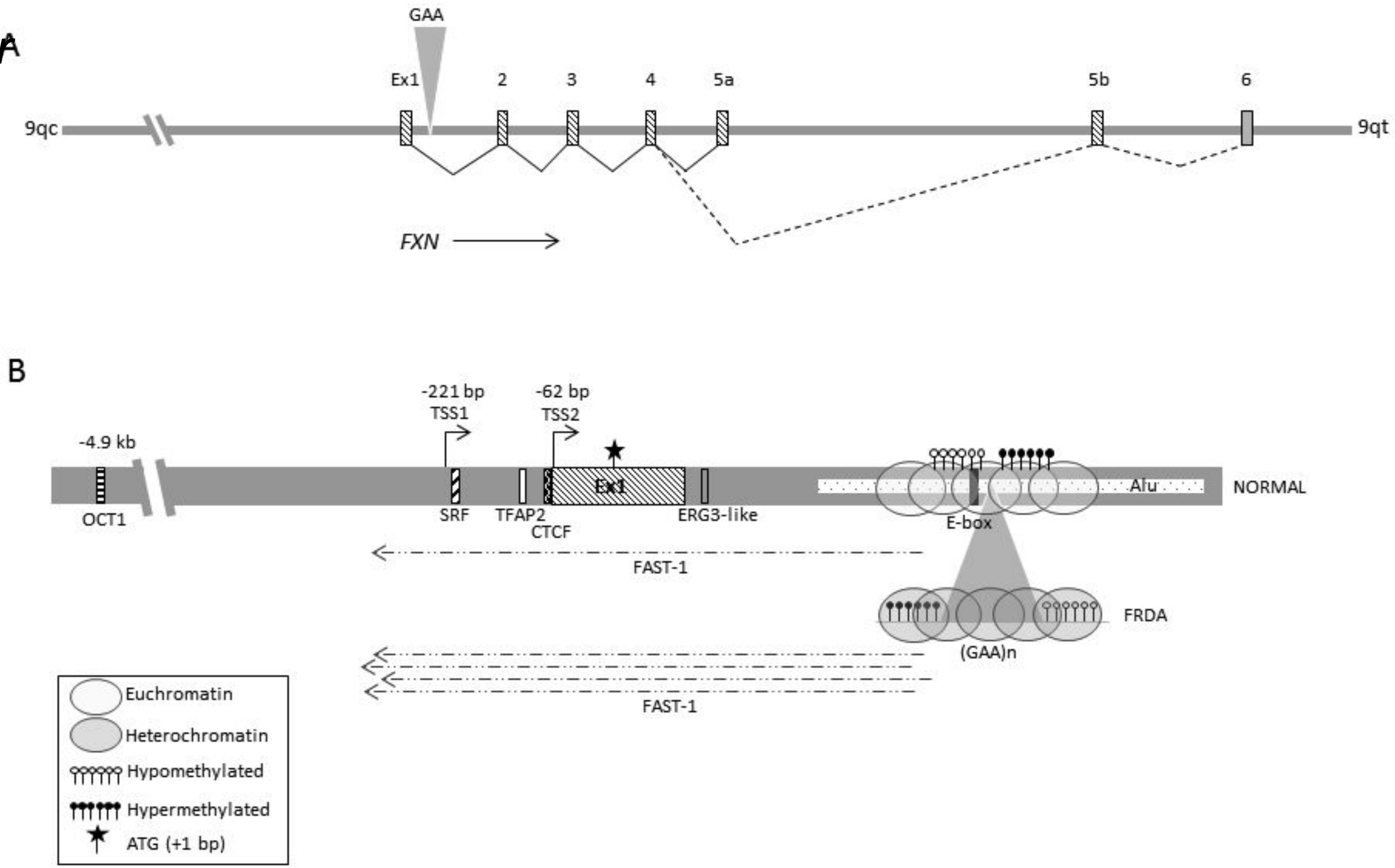
- ✔ El gen *fxn* abarca 95kb de DNA genómico (siete exones)
- ✔ Amplificación intrónica (95-98%) en ambos alelos >44 repeticiones GAA (600-900 hasta 1700)
- ✔ Autosómica recesiva



ISSN: 1539-6509; eISSN: 1944-7930

El gen *fxn*

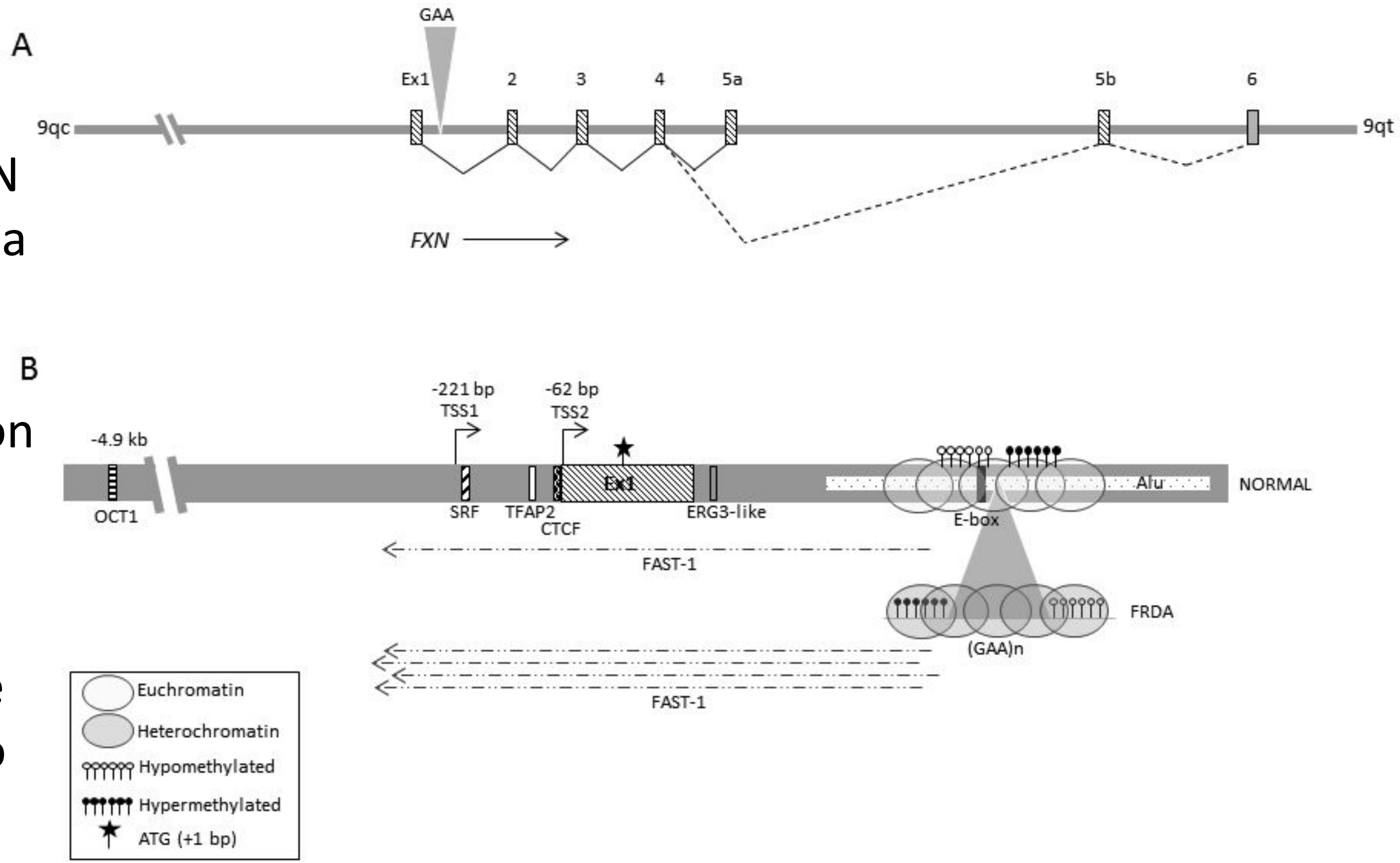
- ✔ El *serum response factor* (SRF) y el *transcription factor activator protein 2* (TFAP2) tiene sitios de unión entre TSS1 y TSS2.
- ✔ La sobreexpresión de SRF o TFAP2 lleva a un incremento de mRNA de FXN en linfoblastos de pacientes.
- ✔ Octamer Binding Protein es un factor de transcripción que se une específicamente al motivo octámero (ATTTGCAT)



ISSN: 1539-6509; eISSN: 1944-7930

Alteraciones en el gen *fxn*

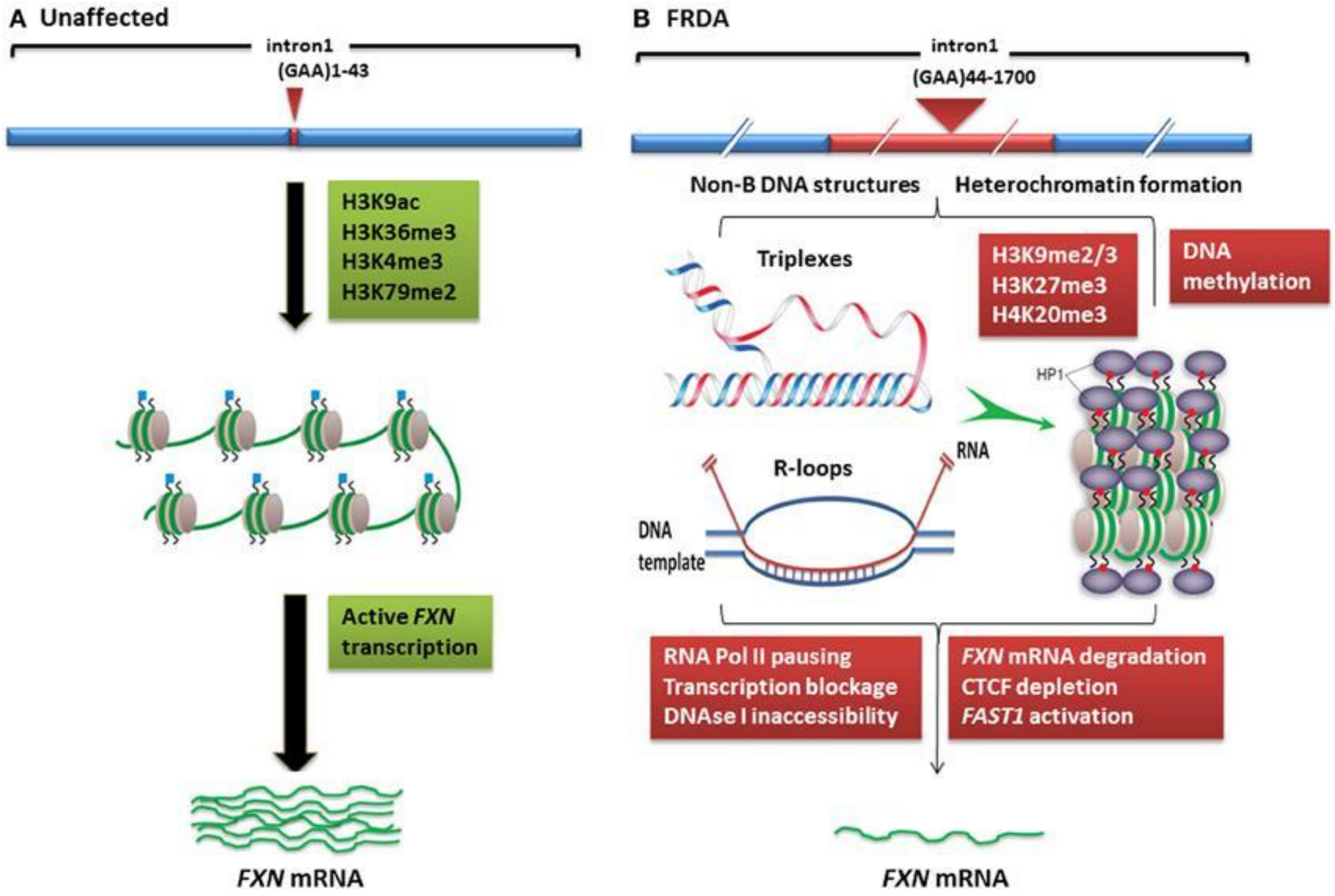
- ✔ En FRDA la expresión de FXN inversamente correlacionada con metilación del DNA.
- ✔ Sitio CpG específico en intrón 1, río arriba de la repetición GAA.
- ✔ Un aumento significativo de los niveles de un transcripto antisentido (FAST1) en células de fibroblastos FRDA.



ISSN: 1539-6509; eISSN: 1944-7930

Alteraciones en el gen *fxn*

- ✔ Disminución en la transcripción.
- ✔ Degradación del mRNA de FXN.
- ✔ La transcripción es inhibida por la formación de estructuras de DNA de tipo no B (bloquean físicamente la transcripción).
- ✔ *Carriers* asintomáticos (solo un alelo) 50% de expression.



Doi: 10.3389/fgene.2014.00165

Alteraciones en el gen *fxn*

- ✔ La transcripción de FXN también se ve afectada por alteraciones modificaciones de histonas.
- ✔ La heterocromatinización y el silenciamiento de genes se asocia con hipoacetilación de ciertos residuos de histonas, particularmente H3K9, junto con una mayor metilación de otros residuos (H3K9me2, H3K9me3, H3K27me3 y H4K20me3).

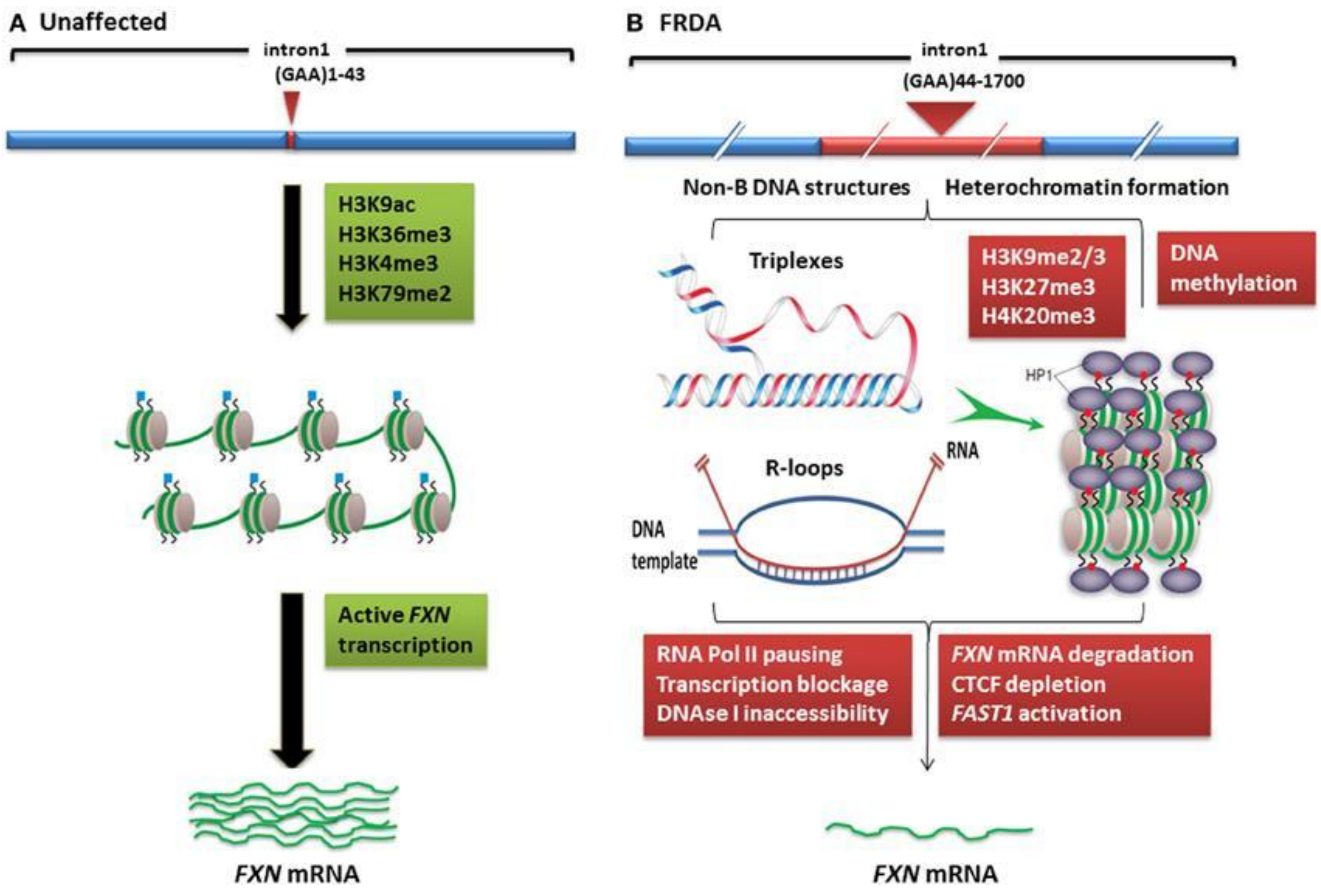
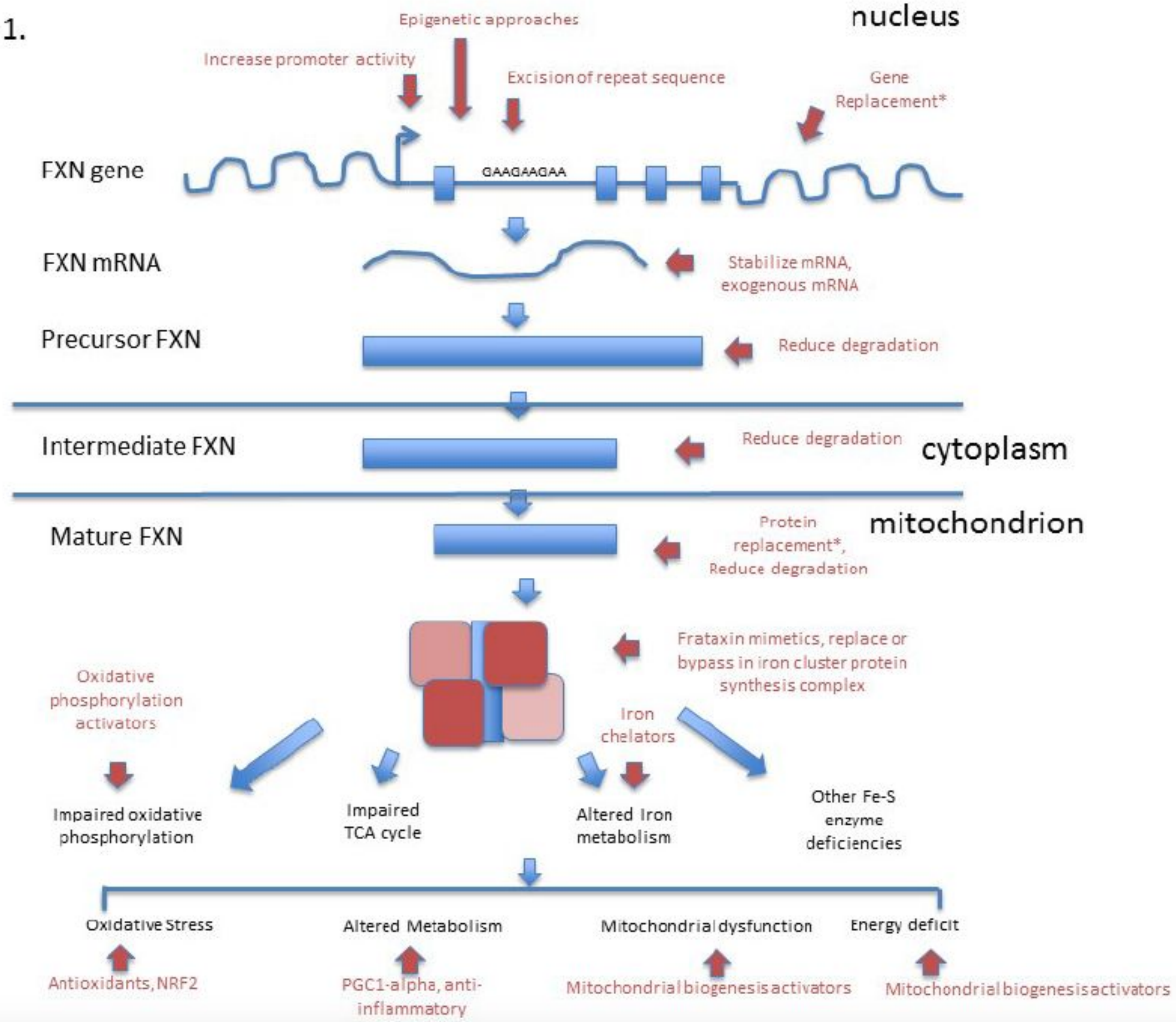
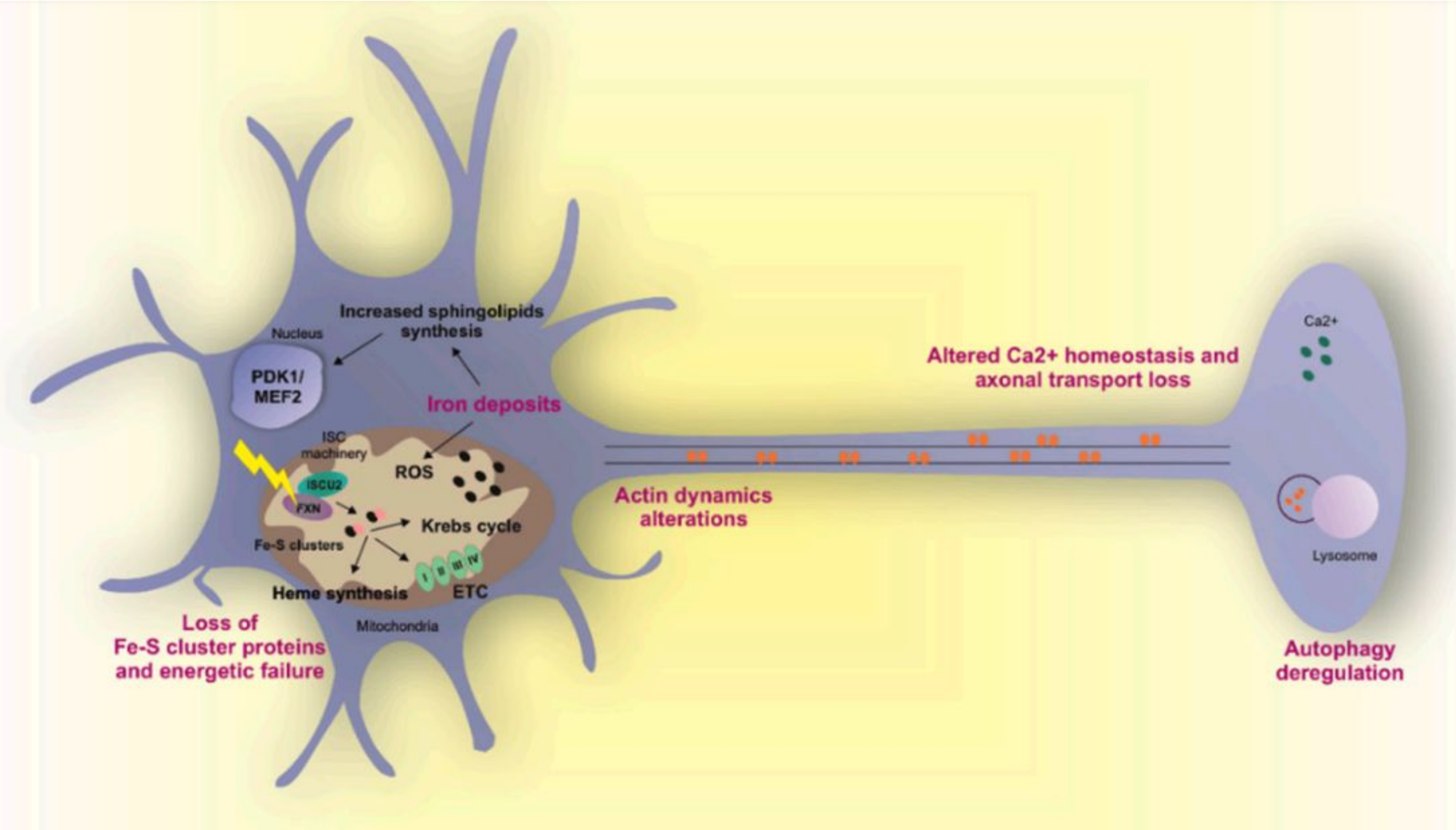


Figure 1.

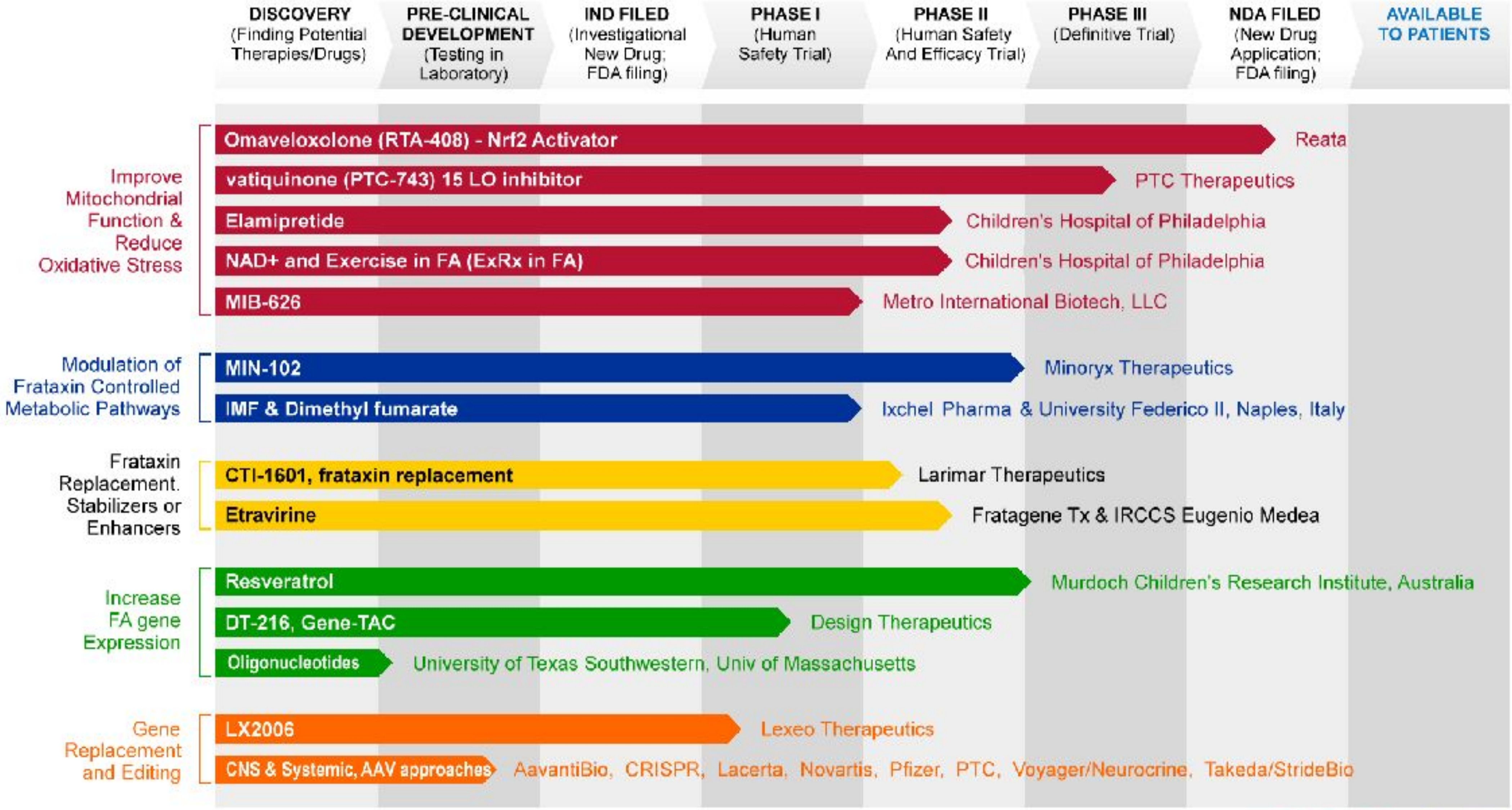


doi.org/10.1080/14737175.2017.1356721



doi:10.3390/ijms21113760

FRIEDREICH'S ATAXIA TREATMENT PIPELINE



FARA Friedreich's Ataxia Research Alliance

TABLE 1 | List of repurposed drugs in FRDA.

Drug	Research status	Mechanism of action in FRDA	Category
PPARγ agonists	Phase II placebo-controlled clinical trial (Leriglitazone, 36 patients) – Completed	Increases frataxin mRNA and protein.	Chemical drug
Dyclonine	Proof of concept trial in patients (8 patients) – Completed	Increases frataxin mRNA and protein. Activates Nrf2	Chemical drug
Src inhibitors	*In vitro* studies Patients' cells	Increases frataxin protein.	Chemical drug
Methylprednisolone	Phase II open-label clinical trial (11 patients) – Completed	Unknown.	Chemical drug
Diazoxide	Preclinical	Increases frataxin mRNA and protein. Activates Nrf2	Chemical drug
Dimethyl fumarate	Preclinical	Increases frataxin mRNA and protein. Activates Nrf2. Promotes mitochondrial biogenesis.	Chemical drug
Etravirine	Phase II open-label clinical trial (30 patients) – Ongoing	Increases frataxin protein. No effect on frataxin mRNA levels.	Chemical drug
Artesunate	Phase I-II open-label clinical trial (20 patients) – Ongoing	Decreases iron overload.	Chemical drug
Erythropoietin and derivatives	Phase II placebo-controlled clinical trials – Completed	Increases frataxin protein. No effect on frataxin mRNA levels.	Biological drug
Interferon-γ	Phase III placebo-controlled clinical trial (92 patients) – Completed	Increases frataxin mRNA and protein.	Biological drug
G-CSF	Phase II open-label clinical trial (7 patients) – Completed	Increases frataxin mRNA and protein.	Biological drug
Exenatide	Phase II open-label clinical trial (16 patients) – Completed	Increases frataxin protein. No effect on frataxin mRNA levels.	Biological drug
Nicotinamide	Phase II open-label clinical trial (10 patients) – Completed. Double-blind, placebo-controlled phase II trial (225 patients) – Ongoing	Increases frataxin mRNA and protein.	Natural product
NAD + precursor (Nicotinamide riboside)	Phase II placebo-controlled clinical trial (72 patients) – Ongoing	Enhances mitochondrial metabolism.	Natural product
NAD + precursor (MIB-626)	Phase II open-label clinical trial (10 patients) – Ongoing	Enhances mitochondrial metabolism.	Natural product
Acetyl-L-Carnitine	Phase II open-label clinical trial (20 patients) – Completed	Enhances mitochondrial metabolism.	Natural product
Resveratrol	Phase II open-label clinical trial (27 patients) – Completed. Double-blind, placebo-controlled phase II trial (40 patients) – Ongoing	Increases frataxin mRNA and protein.	Natural product
Thiamine	Phase II open-label (34 patients) – Completed	Unknown.	Natural product
Sulforaphane	*In vitro* studies Patients' cells	Increases frataxin mRNA and protein. Activates Nrf2.	Natural product

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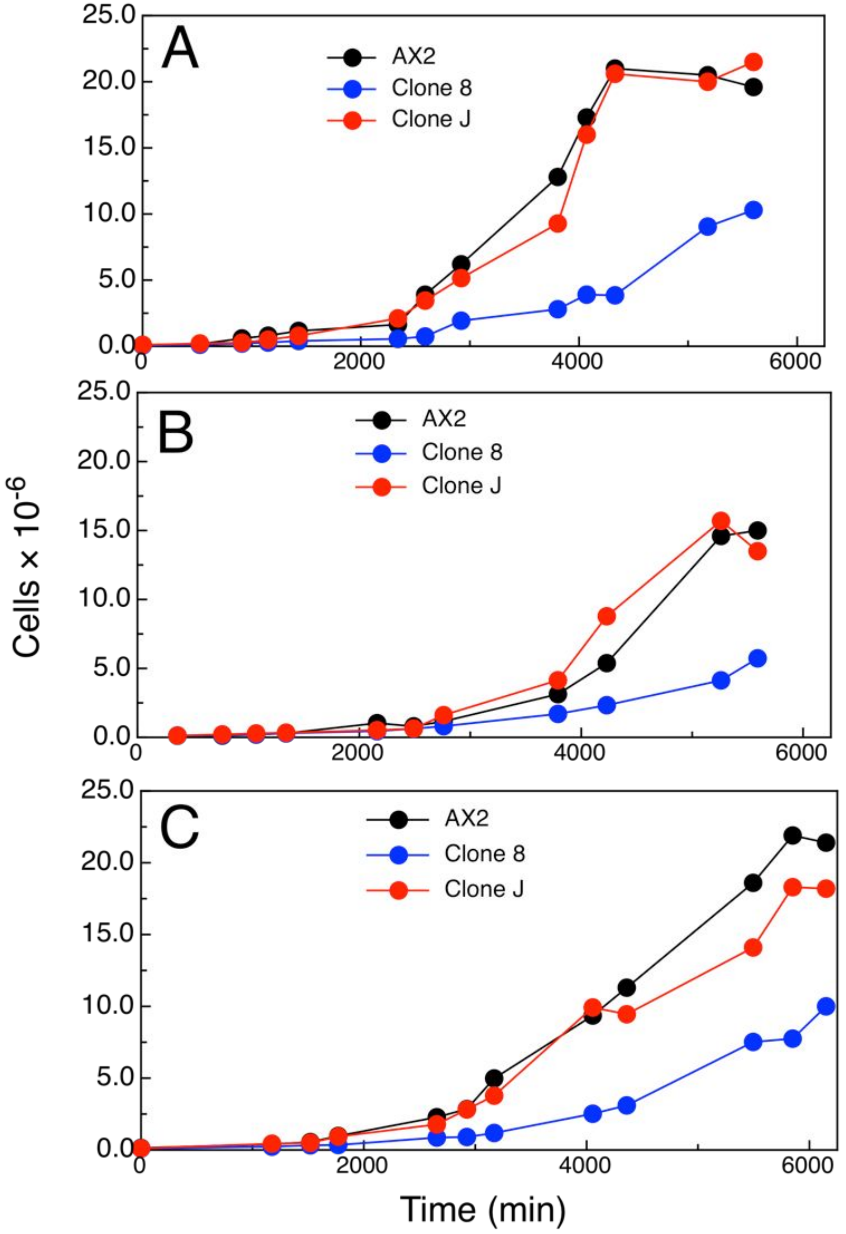
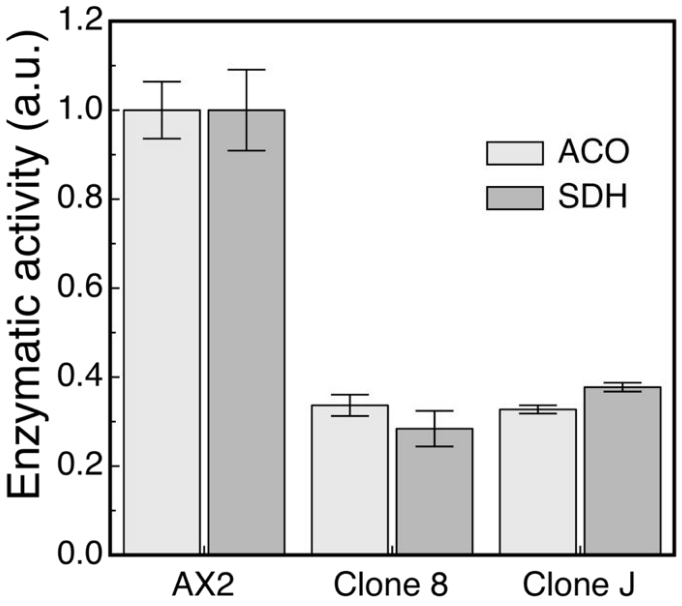
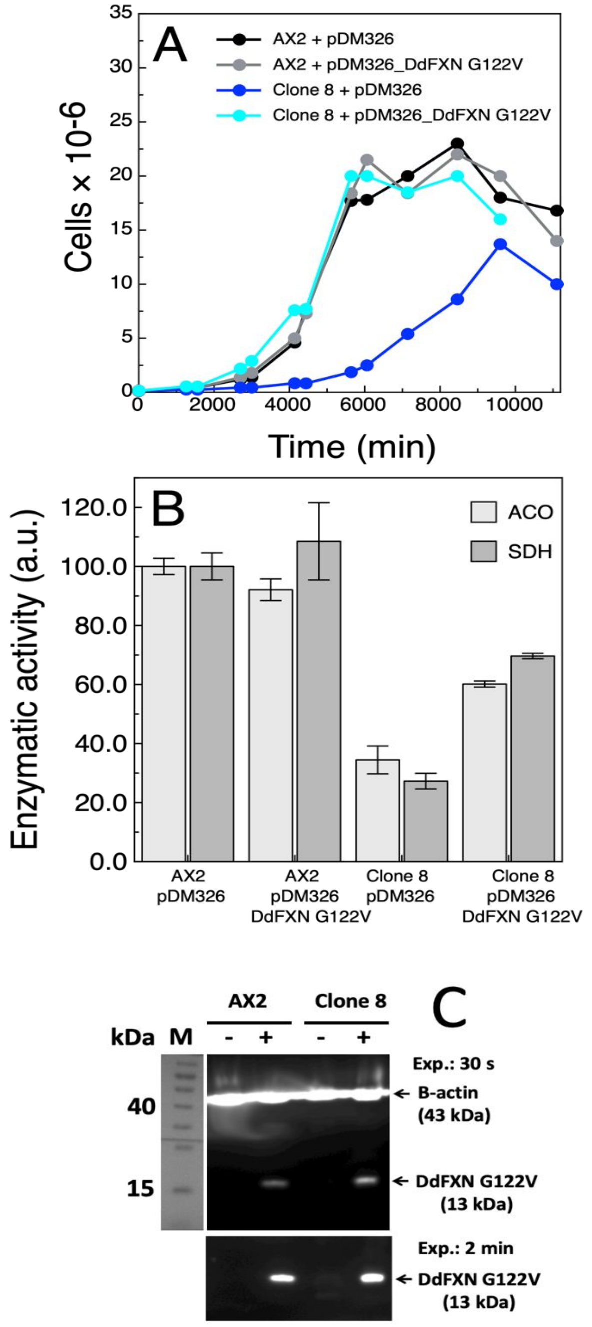
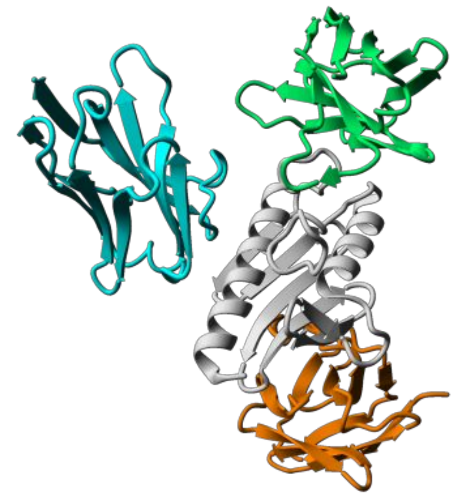
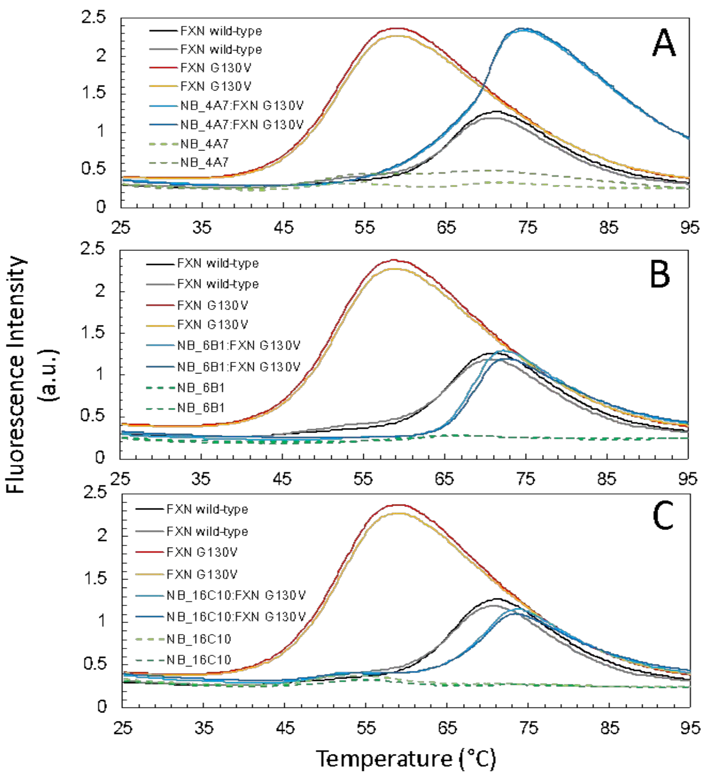
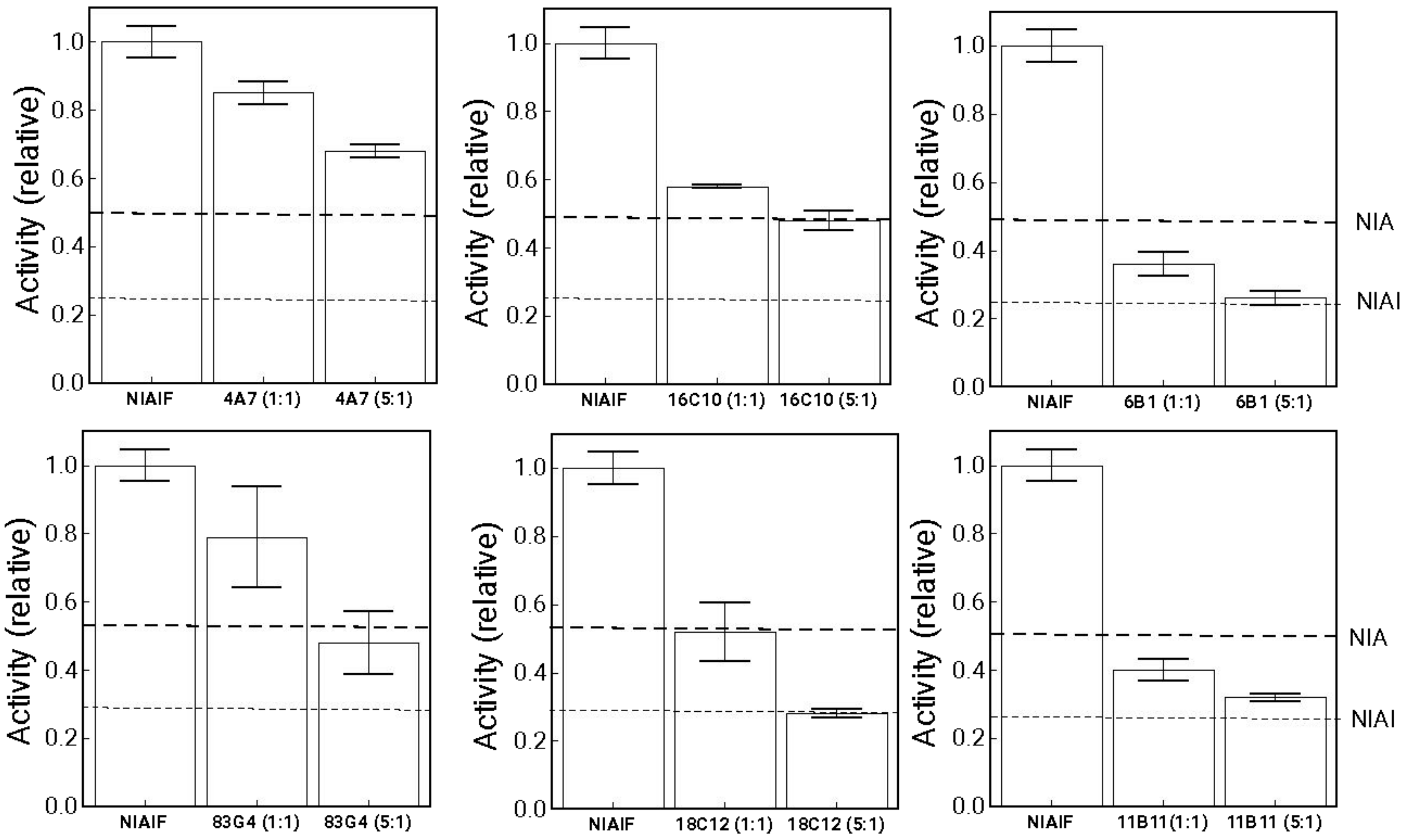

A
B
C
AX2
Clone 8
Clone J
Cells × 10-6
Time (min)
0.0
5.0
10.0
15.0
20.0
25.0
0
2000
4000
6000

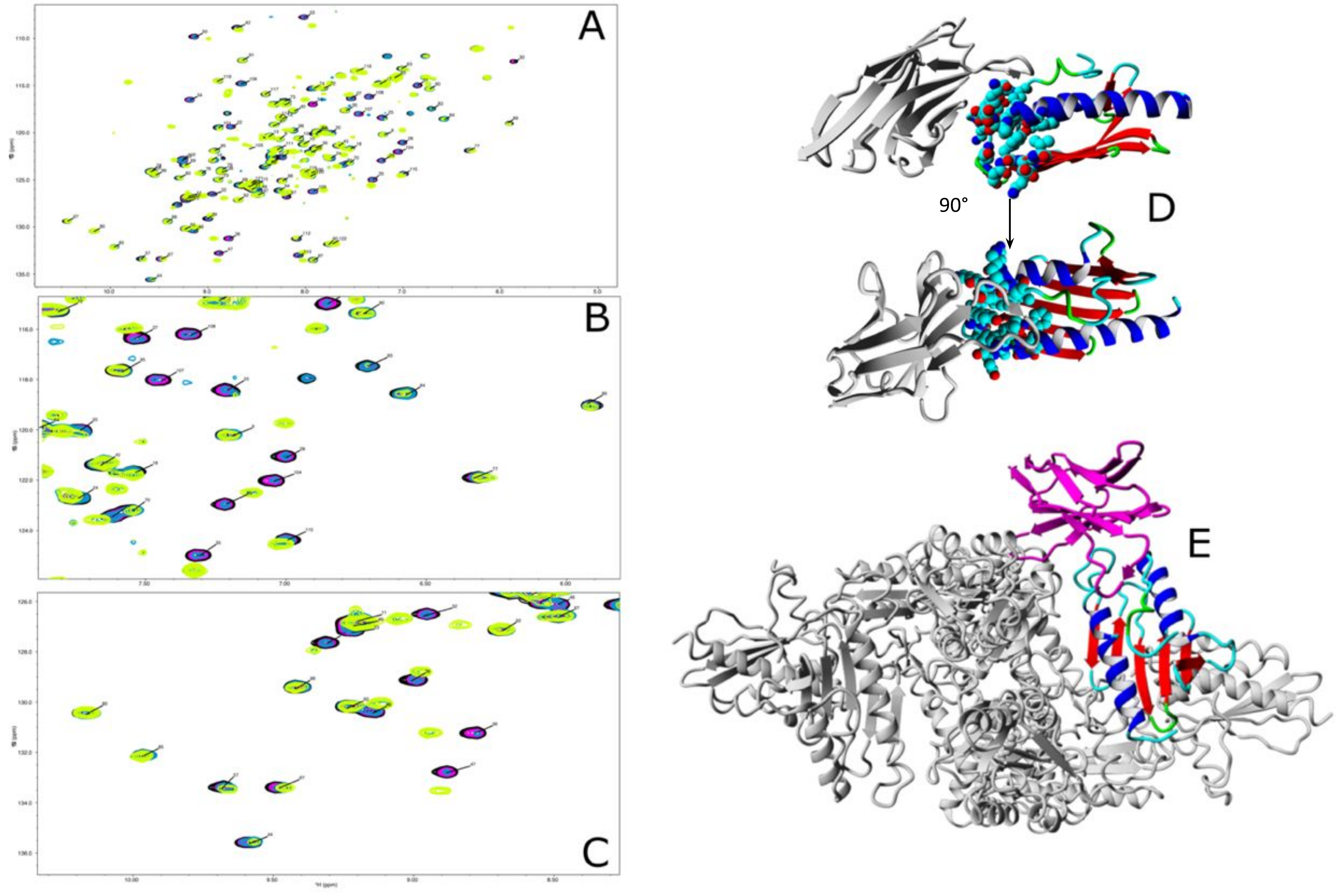

Enzymatic activity (a.u.)
ACO
SDH
AX2
Clone 8
Clone J
0.0
0.2
0.4
0.6
0.8
1.0
1.2


A
AX2 + pDM326
AX2 + pDM326_DdFXN G122V
Clone 8 + pDM326
Clone 8 + pDM326_DdFXN G122V
Cells × 10-6
Time (min)
B
ACO
SDH
Enzymatic activity (a.u.)
AX2
pDM326
AX2
pDM326
DdFXN G122V
Clone 8
pDM326
Clone 8
pDM326
DdFXN G122V
C
kDa
M
AX2
Clone 8
- +
- +
40
15
Exp.: 30 s
B-actin
(43 kDa)
DdFXN G122V
(13 kDa)
Exp.: 2 min
DdFXN G122V
(13 kDa)




A
FXN wild-type
FXN wild-type
FXN G130V
FXN G130V
NB_4A7:FXN G130V
NB_4A7:FXN G130V
NB_4A7
NB_4A7
B
FXN wild-type
FXN wild-type
FXN G130V
FXN G130V
NB_6B1:FXN G130V
NB_6B1:FXN G130V
NB_6B1
NB_6B1
C
FXN wild-type
FXN wild-type
FXN G130V
FXN G130V
NB_16C10:FXN G130V
NB_16C10:FXN G130V
NB_16C10
NB_16C10
Fluorescence Intensity (a.u.)
Temperature (°C)


Activity (relative)
NIAIF
4A7 (1:1)
4A7 (5:1)
16C10 (1:1)
16C10 (5:1)
6B1 (1:1)
6B1 (5:1)
83G4 (1:1)
83G4 (5:1)
18C12 (1:1)
18C12 (5:1)
11B11 (1:1)
11B11 (5:1)
NIA
NIAI


A
B
C
90°
D
E