

Has dut mai unes ulleres atòmiques?
2014, Any Internacional de la Cristal·lografia

De com la vida d'ha protegit de l'oxidació: oxidoreductases

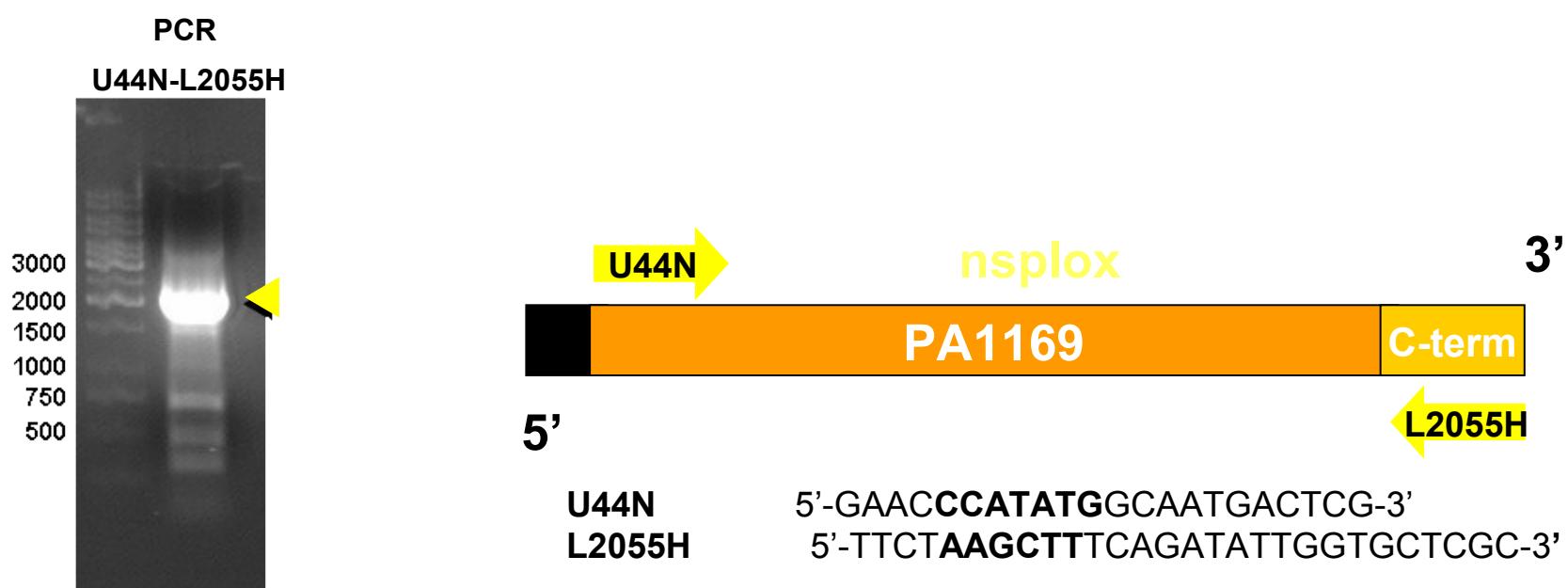
Xavi Carpena
UCE_2014
Prades

-ESCOLLIR TARGET --> UniProt

The screenshot shows the RCSB PDB homepage. At the top, there is a search bar with the placeholder "e.g., PDB ID, molecule name, author". Below the search bar, the main content area features a "Biological Macromolecular Resource" section with a "Molecule of the Month" feature on microtubules. To the right, there is a sidebar with "Latest release April 2014" and "RCSB PDB News". On the left, there are links for "Customize This Page", "Available on the App Store", "ANDROID APP ON Google play", and sections for "PDB-101", "MyPDB", "Home", and "News & Publications".

Esquema Procés Resolució Estructural per CRX

Clonatge del “target” d'interès

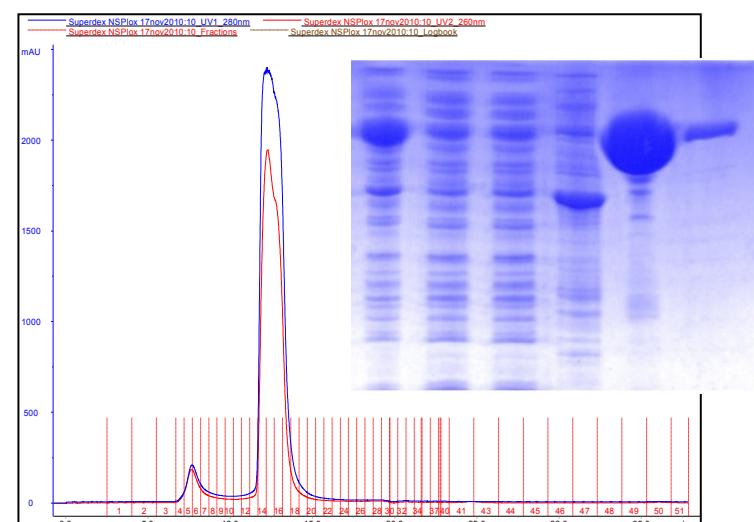
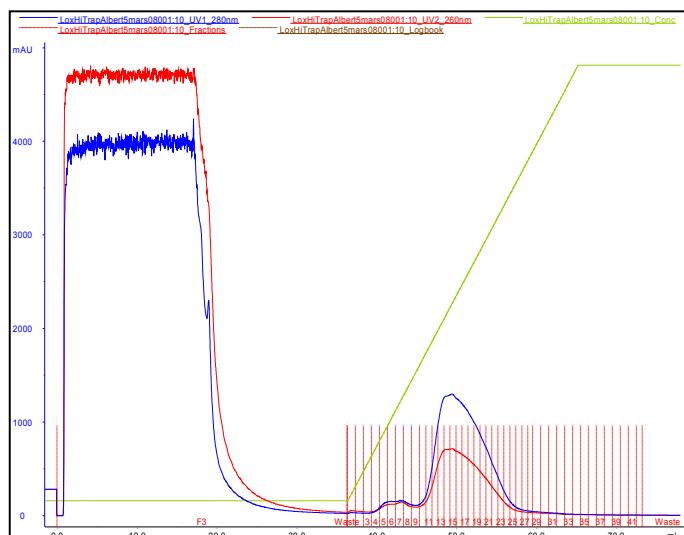


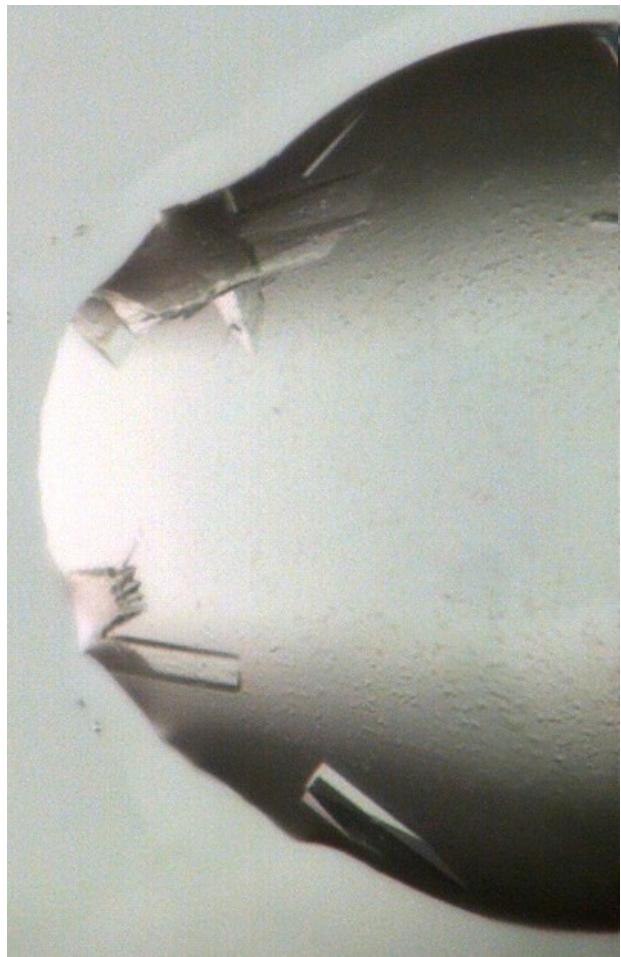
Clonatge: pET 28a

Producció: *E. coli* BL21 DE3

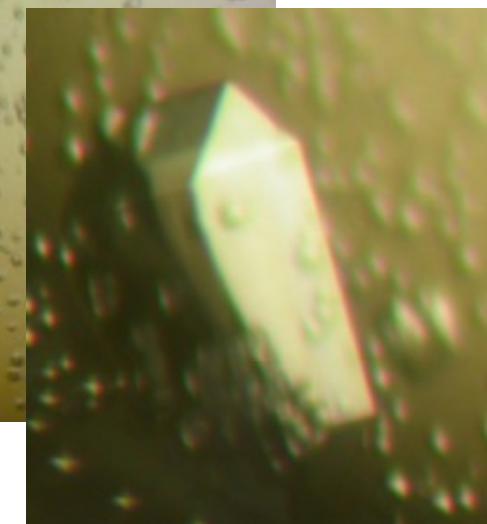
Sobreexpressió: IPTG 0.1 mM (OD: 0.3-0.5)
16 hours at 18°C

Purificació:
IMAC: (10-300mM Imid),
20mM Kpi, 0.5M NaCl, pH7.4
SEC: S200, 20mM Kpi,
150 mM NaCl, pH7



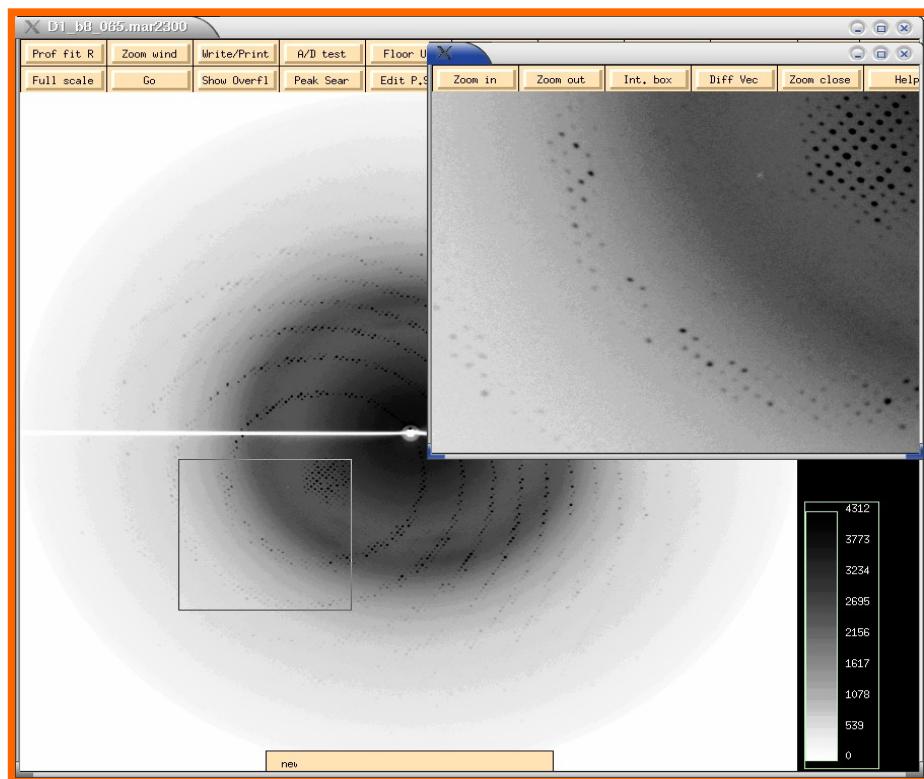


- LMB1_A5 (CS-I, 5)
- LMB1_F10 (CS-II, 22)
- LMB1_F11 (CS-II, 23)**
- LMB1_G5 (CS-II, 29)
- LMB4_B3 (GS PEG 6K, C3)
- LMB4_D2 (GS MPD, C2)
- LMB13_H1 (Index, 85)



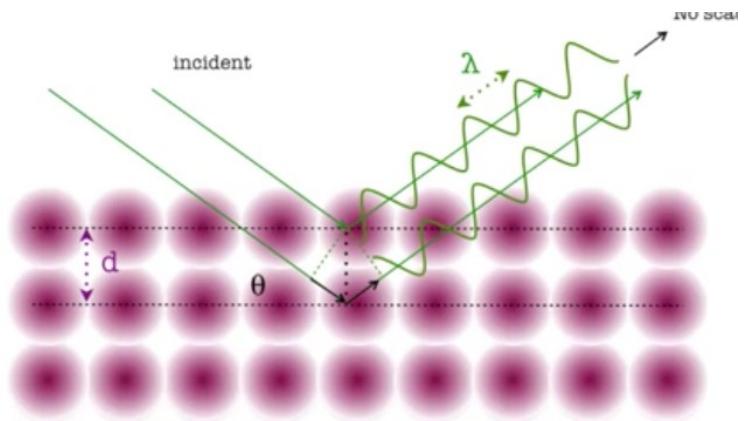
The conditions for the manual crystallization are:
 Cl_2Mg 0.05M
PEG 3350 10%
Hepes 7.5pH 0.1M
Purified protein (15mg/ml) 1drop
Time: 10 - 20 days
Cell parameters: $P(2/2_1) 2_{12_1}$,
a: 43.01 b: 116.51 c: 132.55
b:90 g:90

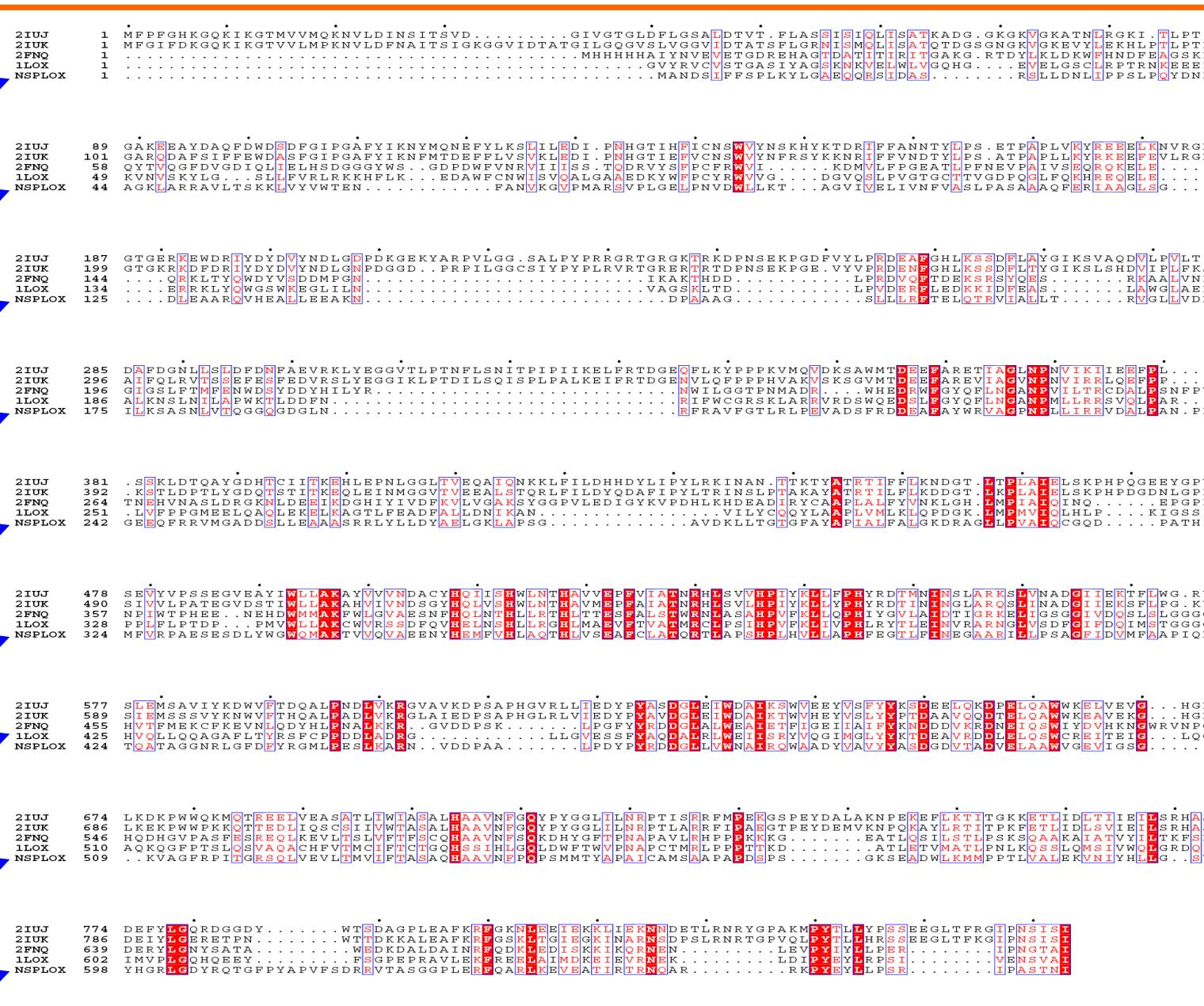
a:90

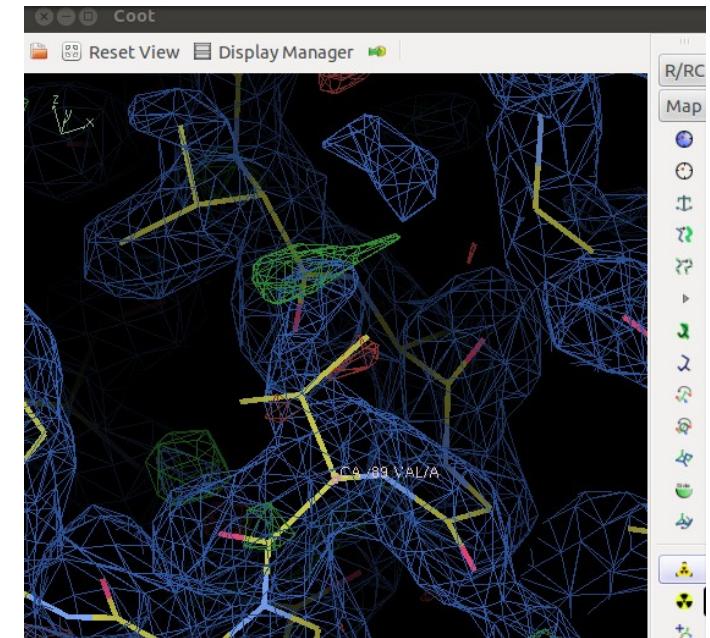
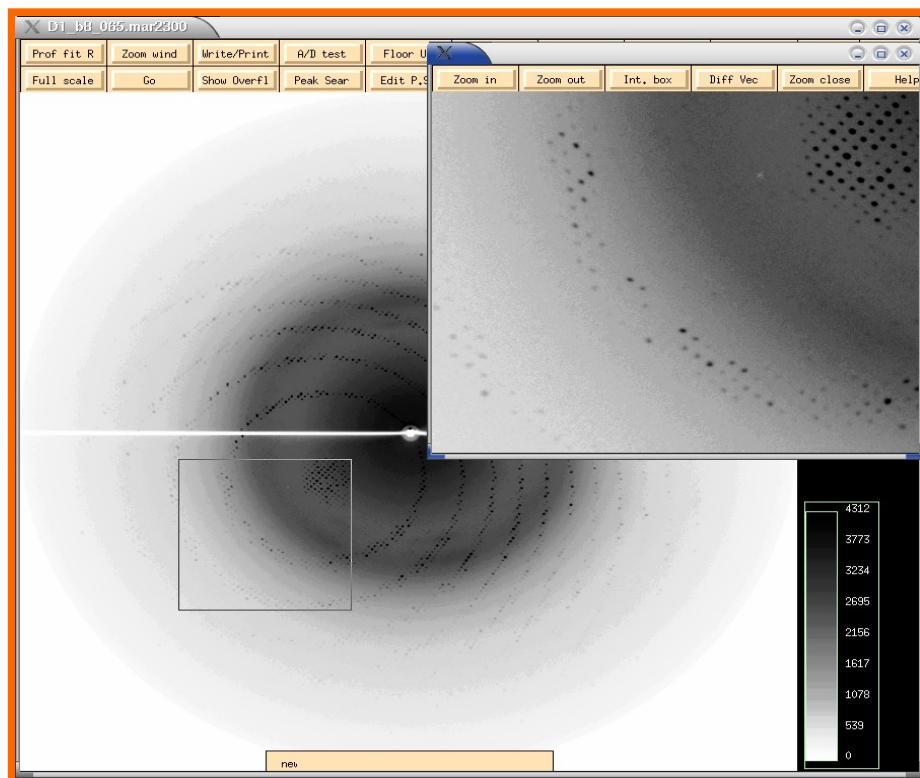


↓
Dades.mtz

$$(F_{hkl}, \sigma_{hkl})$$







↓
Dades.mtz

$$(F_{hkl}, \cancel{\theta}_{hkl}, \sigma_{hkl})$$

↑
Final.mtz

$$(F_{hkl}, \theta_{hkl}, \sigma_{hkl})$$

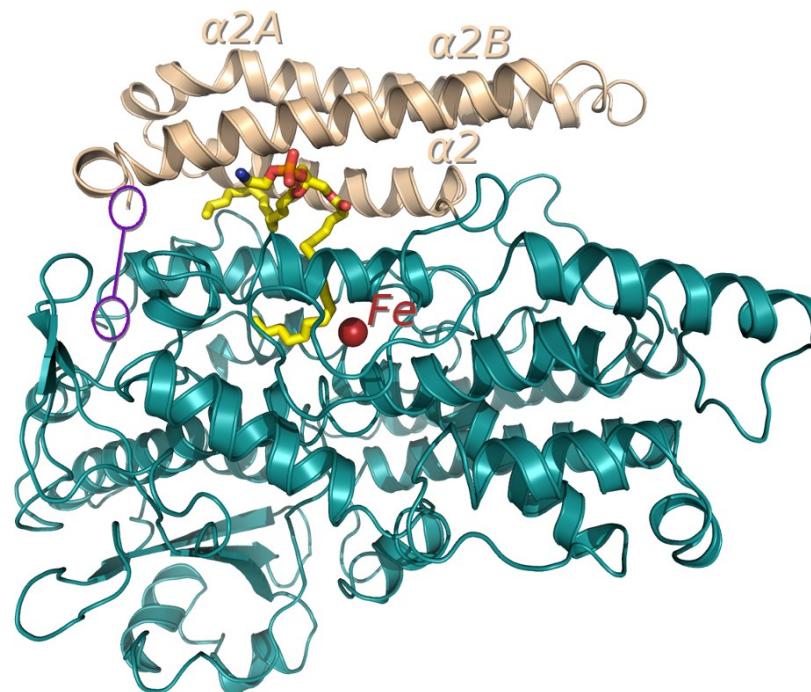
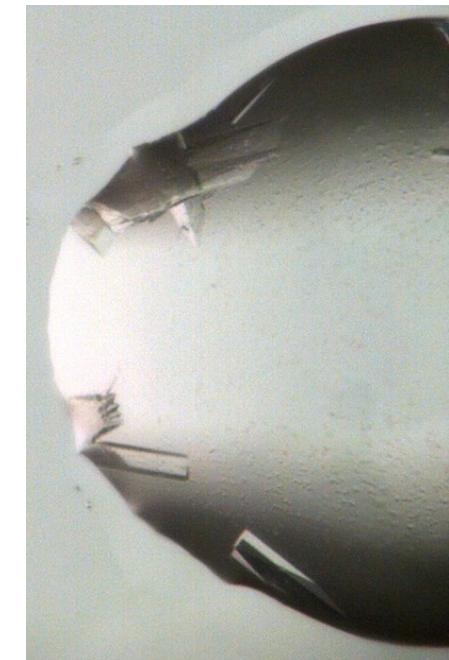
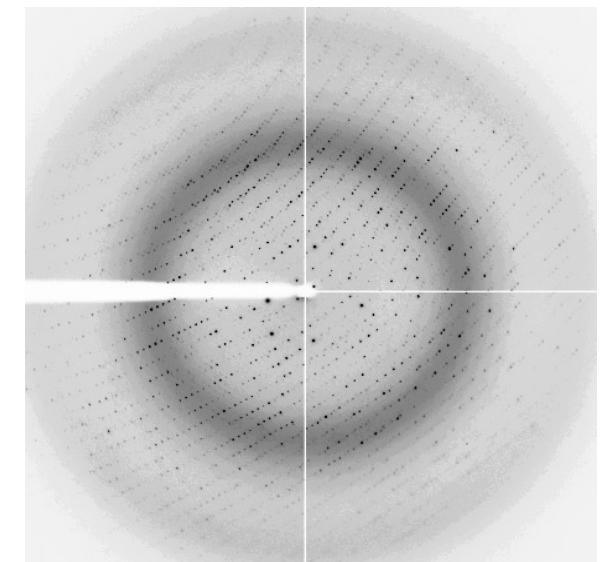


Table 1 Data collection and structural refinement statistics for LOXpa

<i>Data collection statistics</i>	
Space group	P2 ₁ 2 ₁ 2
Unit Cell parameters <i>a, b, c</i> (Å)	132.7, 116.0, 42.6
Resolution (Å)	20 – 1.75 (1.81 1.75)
Unique reflections	66015 (6460)
Completeness (%)	97.9 (97.0)
R _{sym} (%)	9.1 (63.0)
<I/σI>	10.7 (2.5)
Redundancy	3.9 (3.5)
<i>Model refinement statistics</i>	
Resolution	20 – 1.75 (1.79 1.75)
No. of reflections	62559 (4440)
Free reflections	3322 (243)
R _{cryst} (%)	19.2 (25.7)
R _{free} (%)	23.3 (29.3)
No. residues	636



10% PEG 3350
0.05M MgCl₂
0.1M Hepes pH 7.5

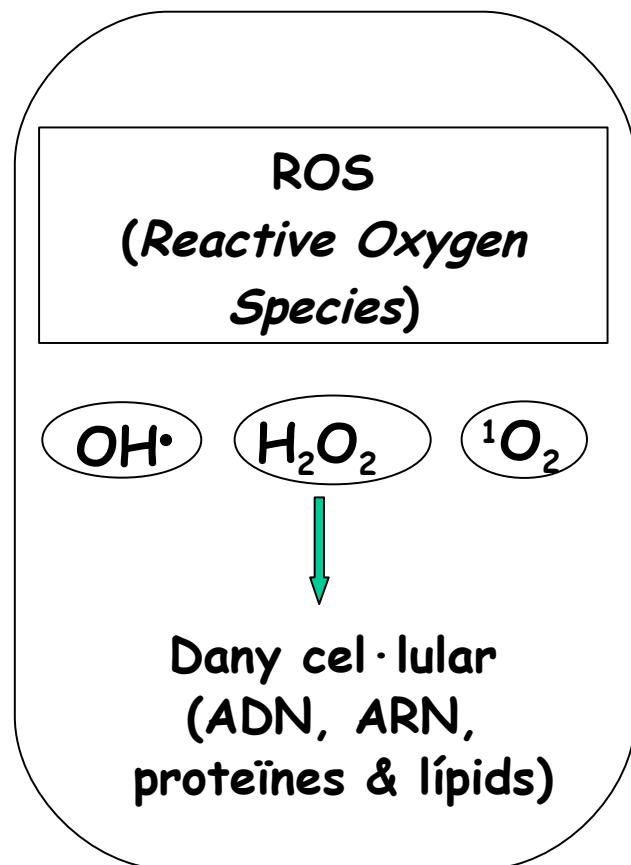


OXIDOREDUCTASES

- 1) Catalases
- 2) Peroxidasa KatG
- 3) Peroxisoma i MLYCD
- 4) Lipoxygenasa

ALTRES EXEMPLES

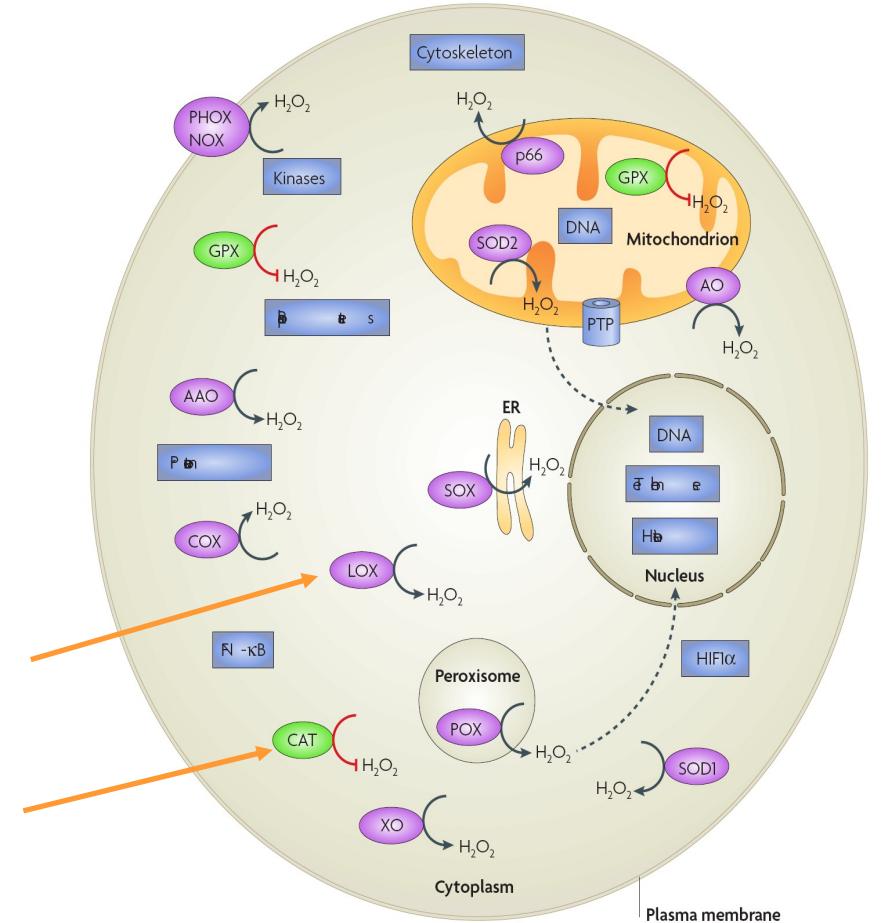
- 5) Transportador de membrana



H_2O_2 :

- { $t_{1/2} = 1\text{ms}$
- reactivitat $\downarrow\downarrow$
- $[\text{C}] = \text{M} \uparrow\uparrow$
- Permeabilitat $\uparrow\uparrow$

Els ROS tenen mala reputació

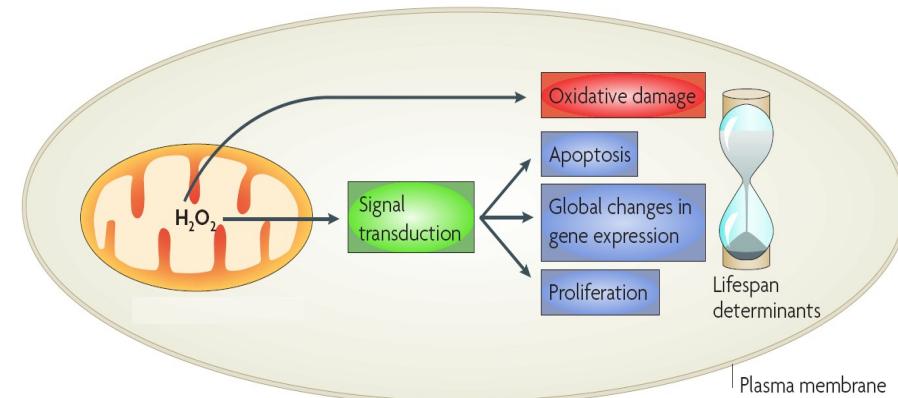


Els ROS són centrals en els processos metabòlics REDOX

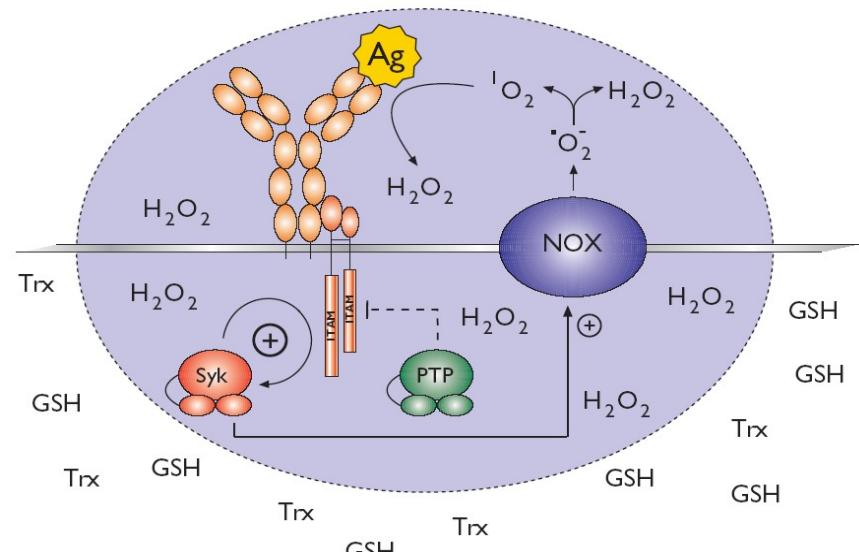
H_2O_2 producció endògena

Table 1. Examples of Redox-Regulated Proteins

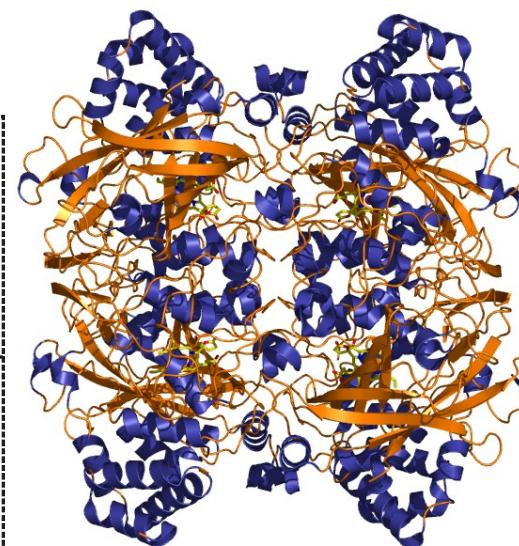
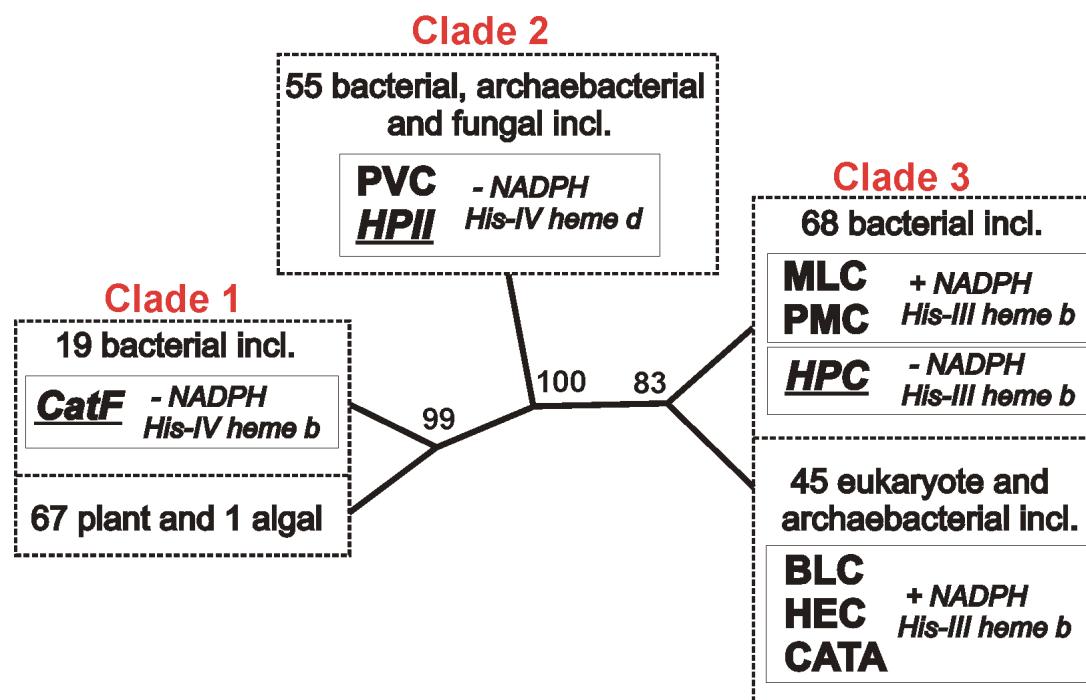
Type of Protein	Protein
Transcription Factor	c-Jun/c-Fos
	OxyR
	OhrR
	Hsf1
	Nrf-2/Keap-1
	Bach1
Kinase	JNK
	Sty1 (Spc1, Phh1)
	MEKK1 (MAPKKK)
	IKKbeta
	PKA RI (regulatory subunit)
	PKA C (catalytic subunit)
	Src tyrosine kinase
Phosphatases	LMW-PTPs
	PTEN
	Cdc25C
	PTP1B
Ion Channels	Ryanodine receptors
	K(ATP) channels (ATP-sensitive potassium channels)
Other	Serotonin N-acetyl transferase
	RNase H1
	GAPDH
	SUMO E1 subunit Uba2/ E2-conjugating enzyme Ubc9



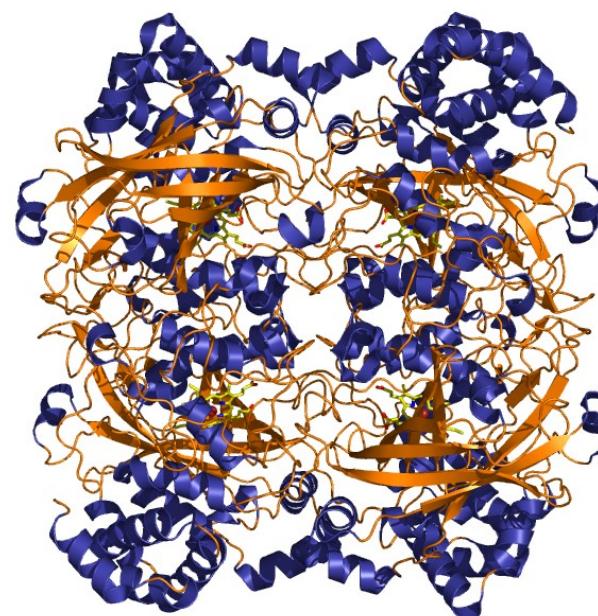
Extension of Murine Life Span by Overexpression of Catalase Targeted to Mitochondria
 Samuel E. Schriner, et al.
Science **308**, 1909 (2005);
 DOI: 10.1126/science.1106653



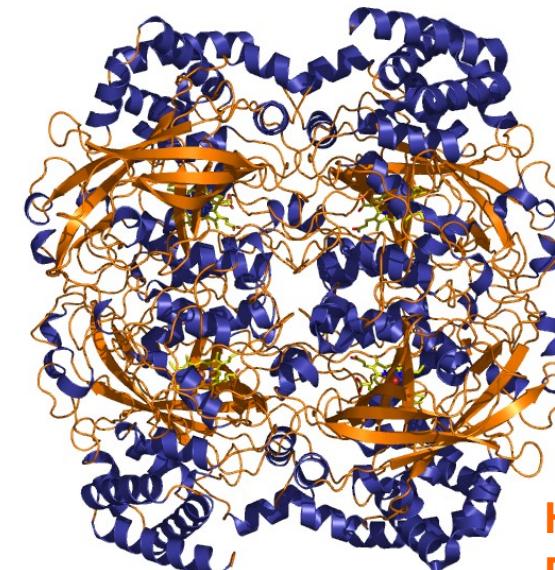
H_2O_2 actúa com a missatger 2^{ari} (i amplificador!) en el càncer de mama



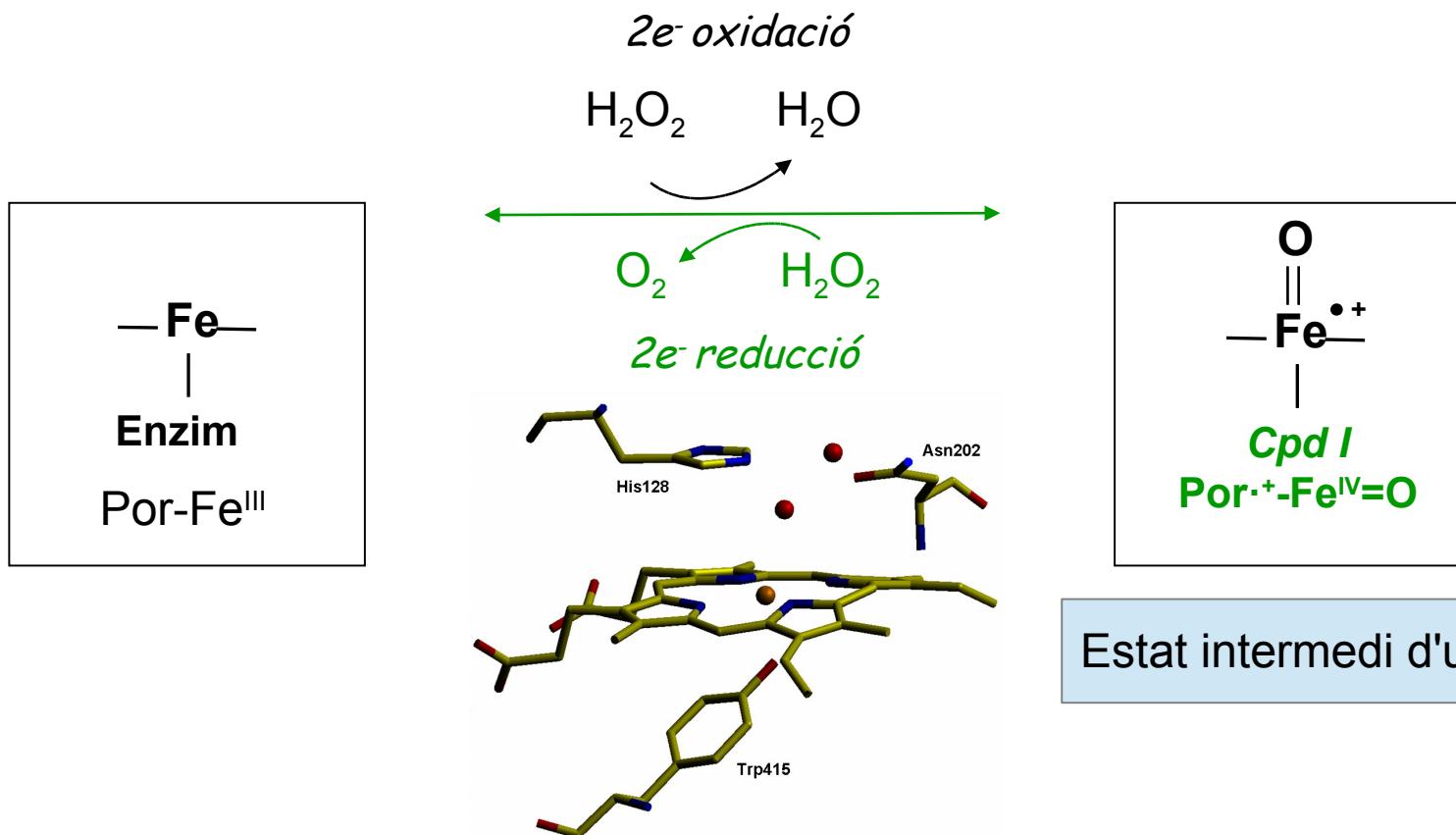
N,C-trunc₂₂
R= 2.8 Å



CatF₇
R= 1.8 Å



HPC₁₄
R= 1.6 Å



L'alien investigador



Zona d'investigació



Subjete a investigar



Estat intermedi d'un enzim?

Estat “A”



Estat “X”

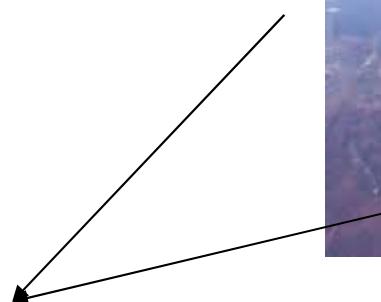
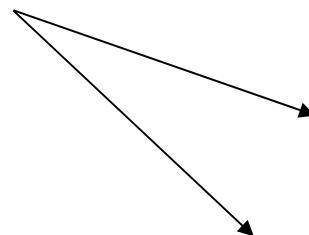


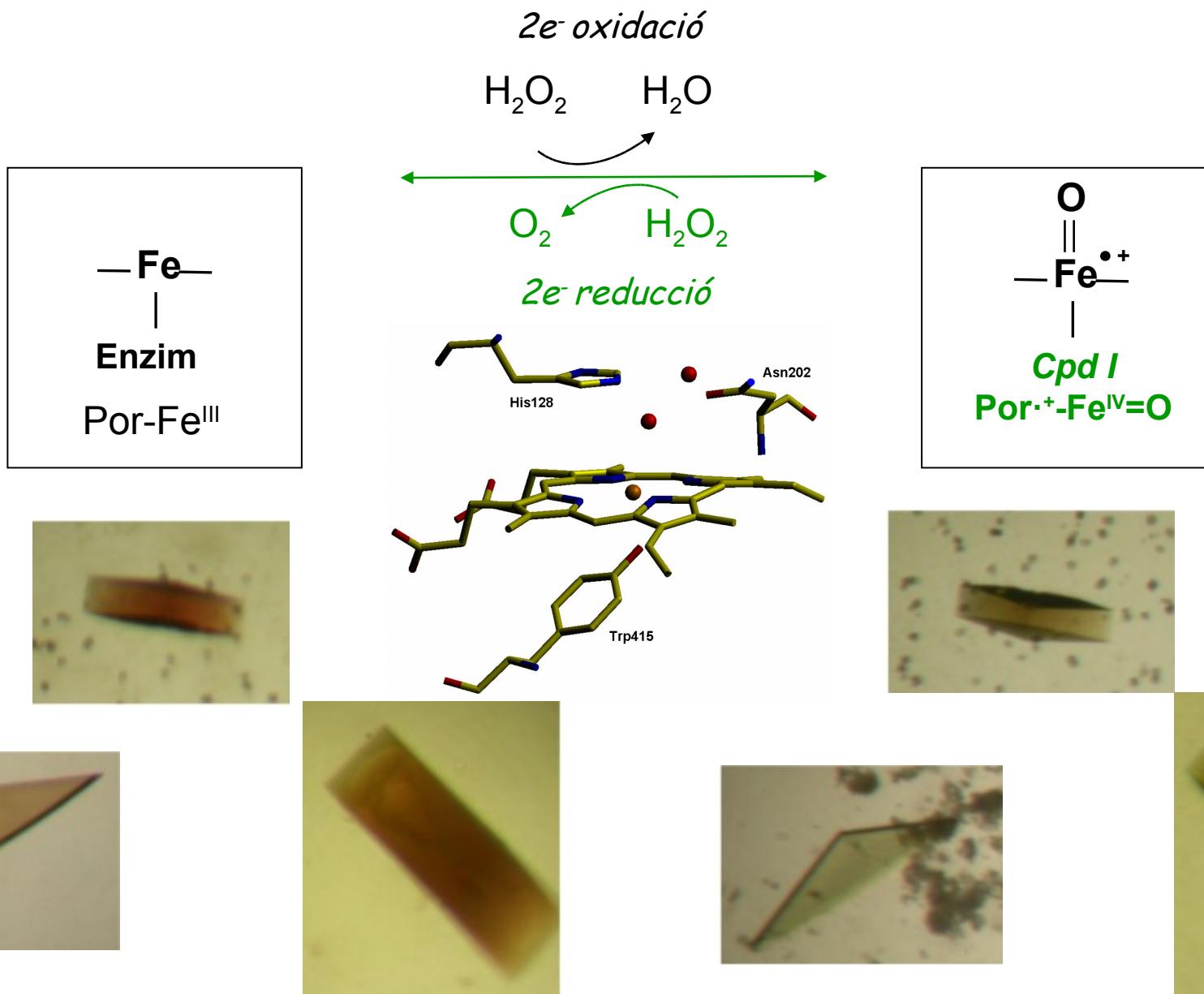
Estat “B”

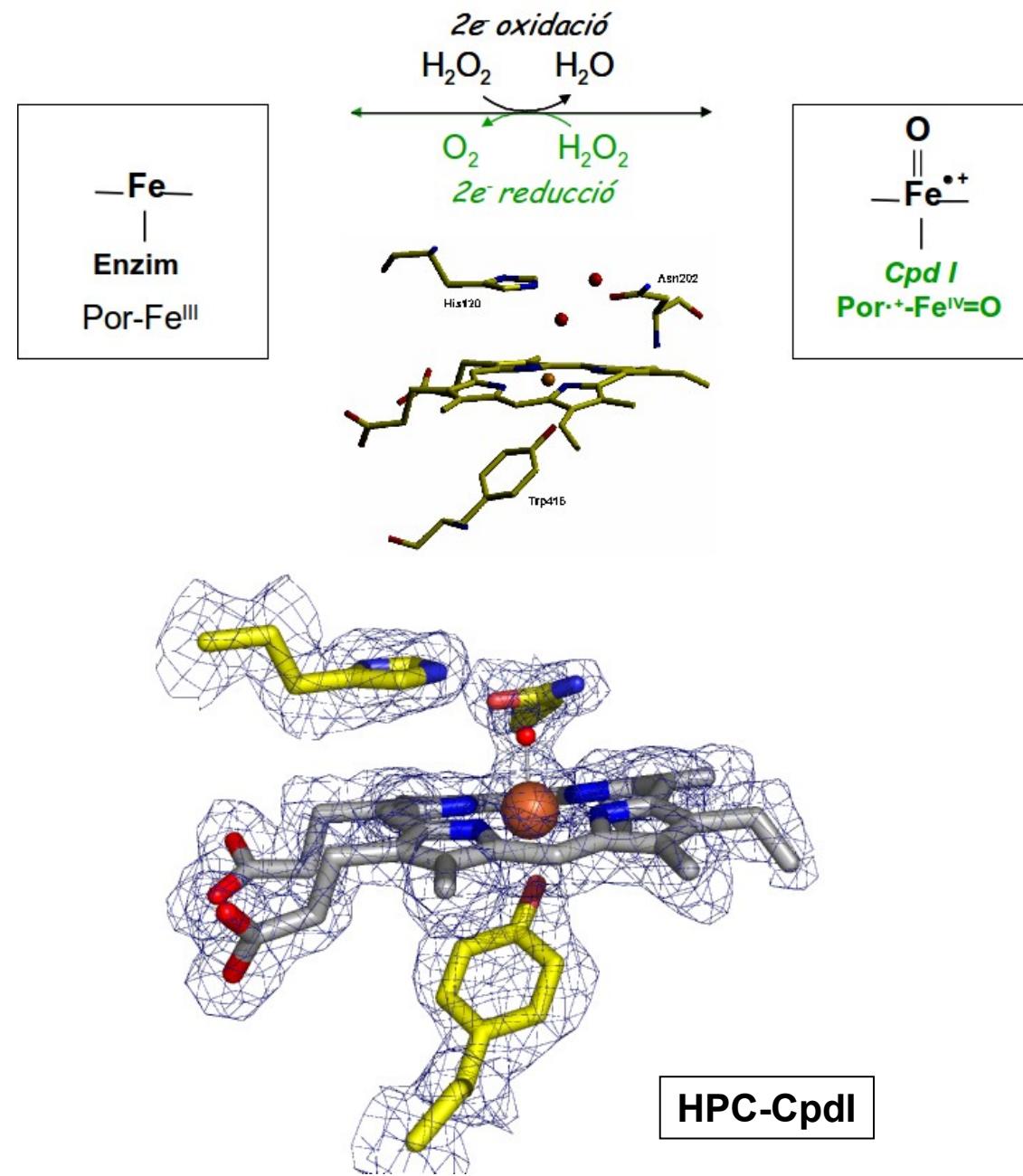


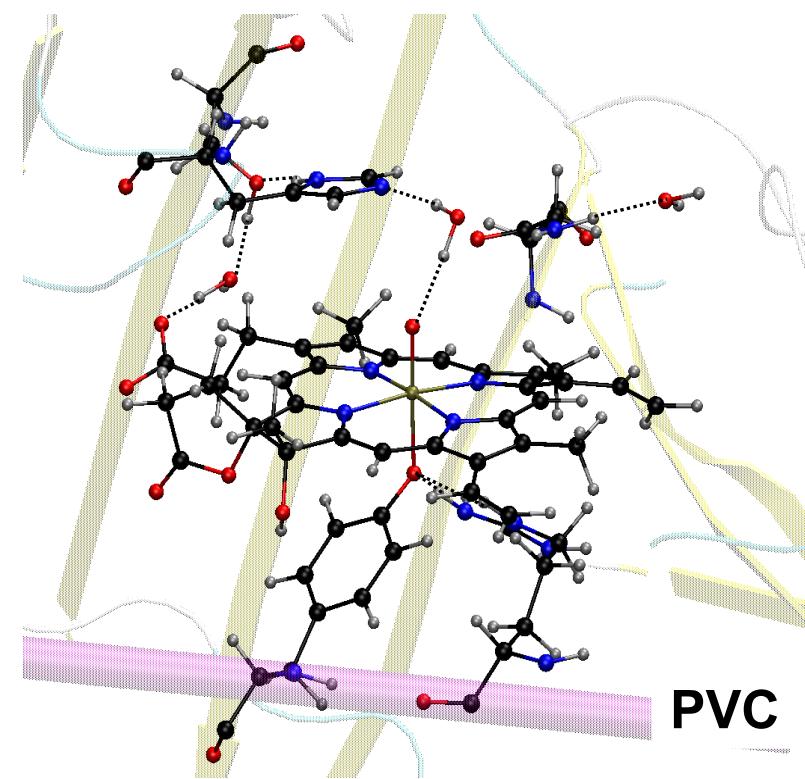
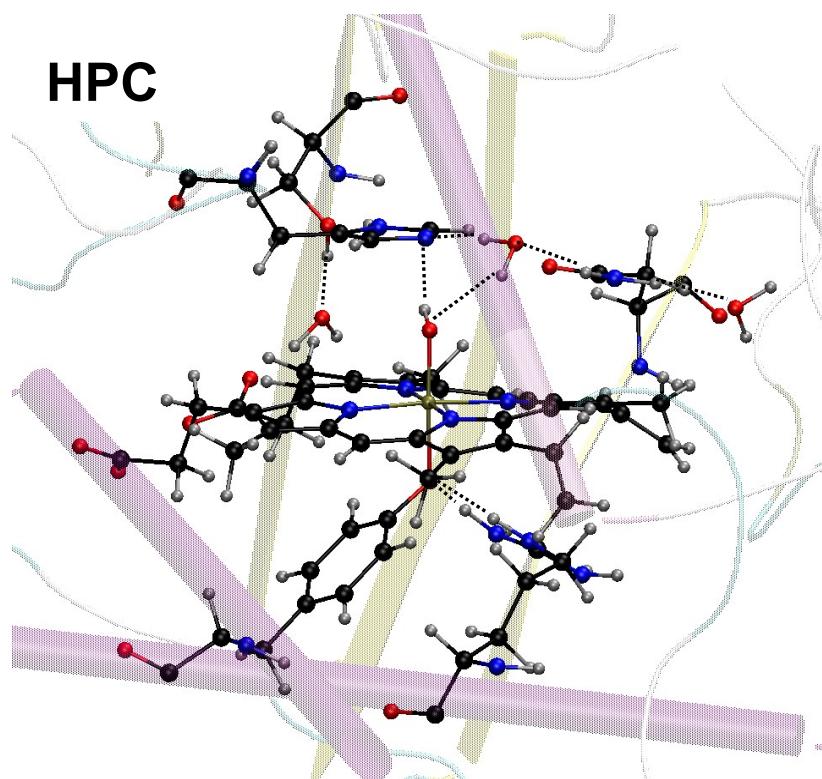
?

Estat intermedi d'un enzim?

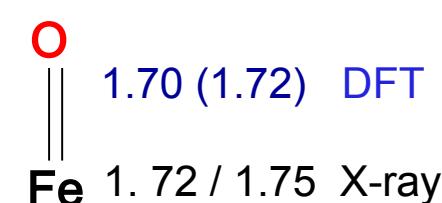




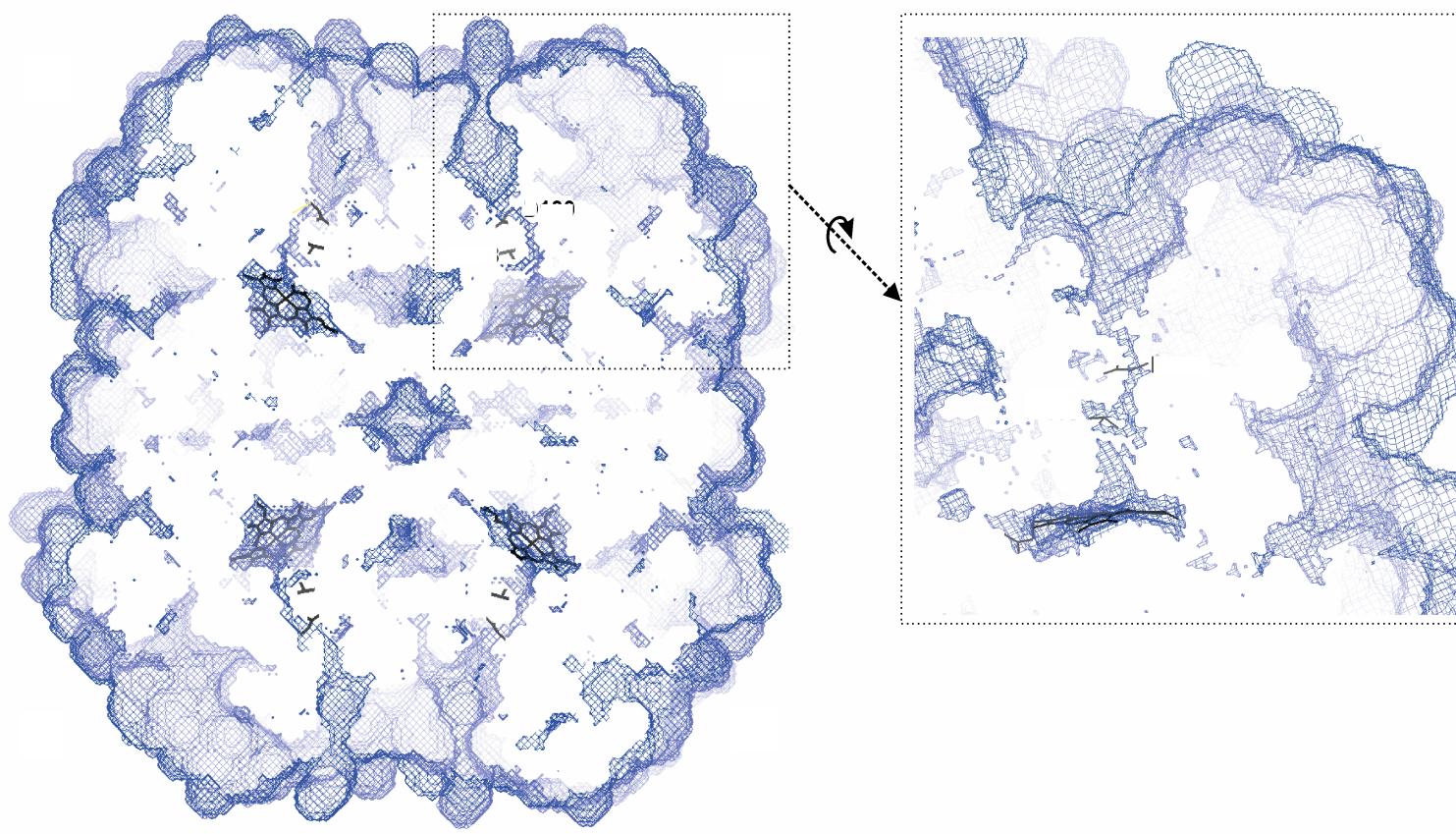




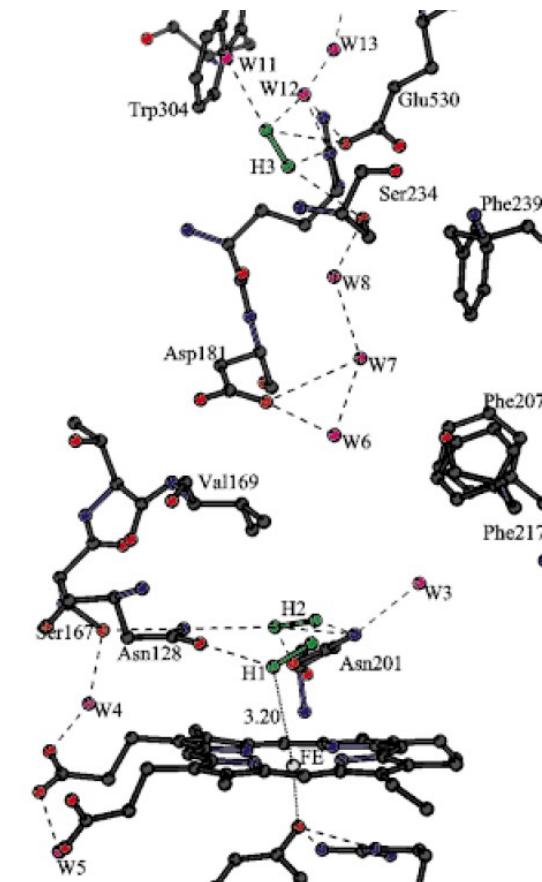
+ radical proteïna



+ radical porfirina



50.000 – 800.000
reaccions/s



Un mutant inactiu (H128N) de la catalasa HPII, ens va permetre obtenir un complexe amb H_2O_2 directament

L'alien investigador



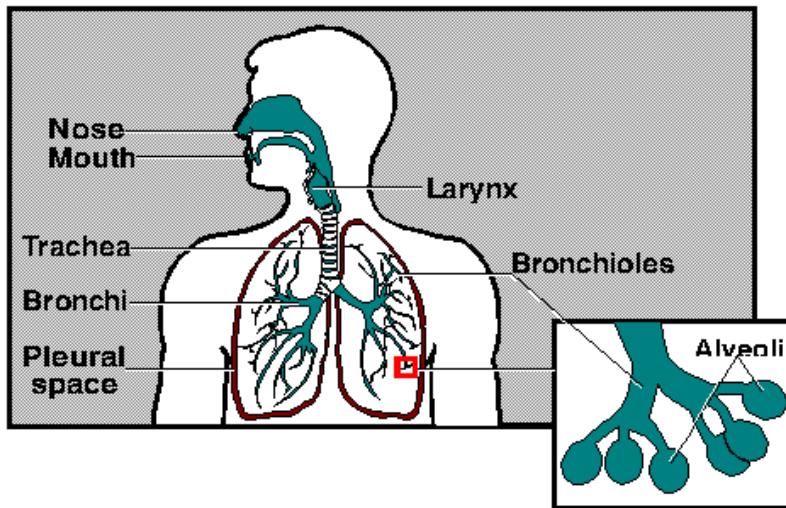
Zona d'investigació



Subjecte a investigar



Es creu que el H₂O₂ pot tenir un efecte activador del sistema inmunitari



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letters to nature

Nature 358, 591 - 593 (13 August 1992); doi:10.1038/358591a0

The catalase—peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*

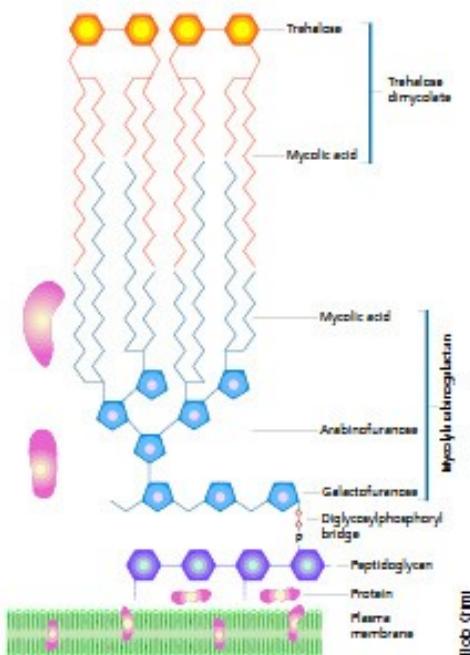
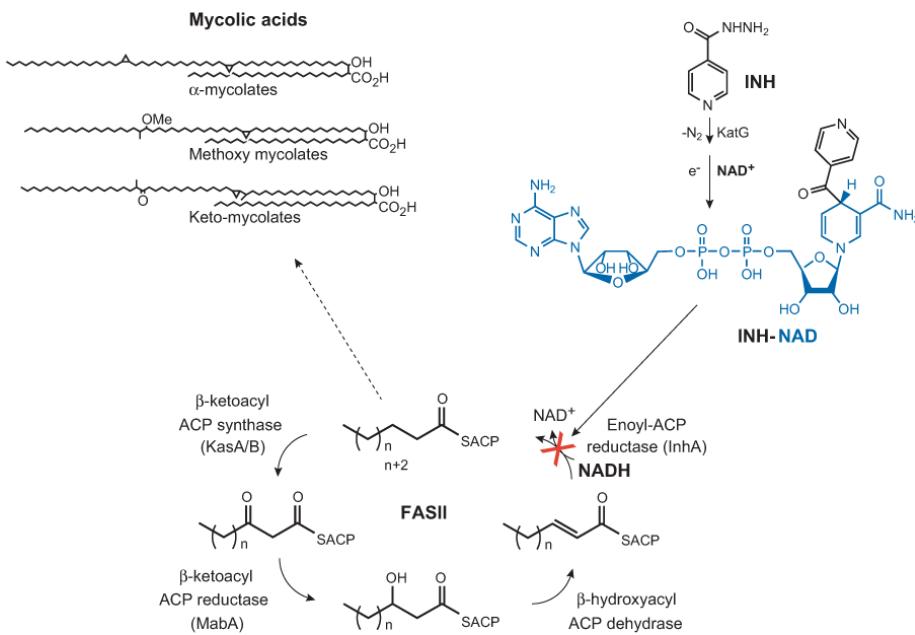
YING ZHANG¹, BEATE HEYM^{†‡}, BRYAN ALLEN[§], DOUGLAS YOUNG[†] & STEWART COLE[†]

¹ MRC Tuberculosis and Related Infections Unit, and [§] Department of Bacteriology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 OHS, UK

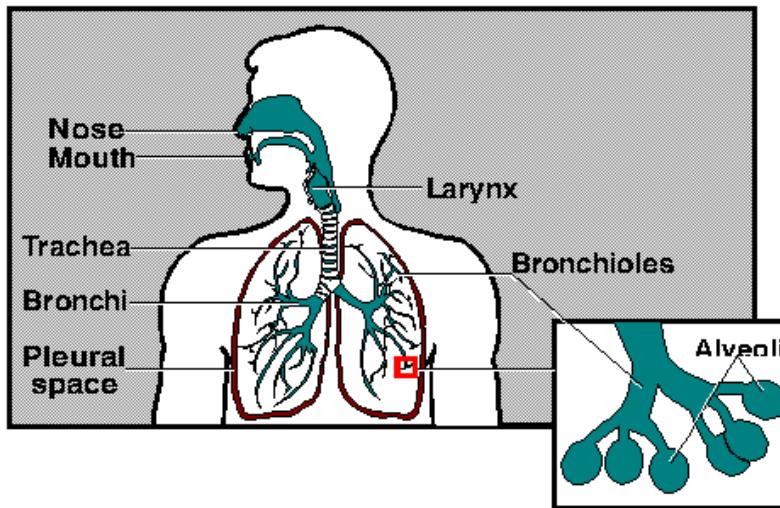
[†] Laboratoire de Génétique Moléculaire Bactérienne, Institut Pasteur, 75224 Paris 15, France

[‡] Service de Bactériologie-Virologie, CHU Pitié-Salpêtrière, 75634 Paris 13, France

TUBERCULOSIS is responsible for one in four of all avoidable adult deaths in developing countries¹. Increased frequency and accelerate among individuals infected with human immunodeficiency virus has raised worldwide concern that control programmes may be inadequate of multidrug-resistant strains of *Mycobacterium tuberculosis* has resulted in several recent fatal outbreaks in the United States². Isonicotin (isoniazid, INH) forms the core of antituberculosis regimens; however, clinical isolates that are resistant to INH show reduced catalase acti-



Es creu que el H₂O₂ pot tenir un efecte activador del sistema inmunitari



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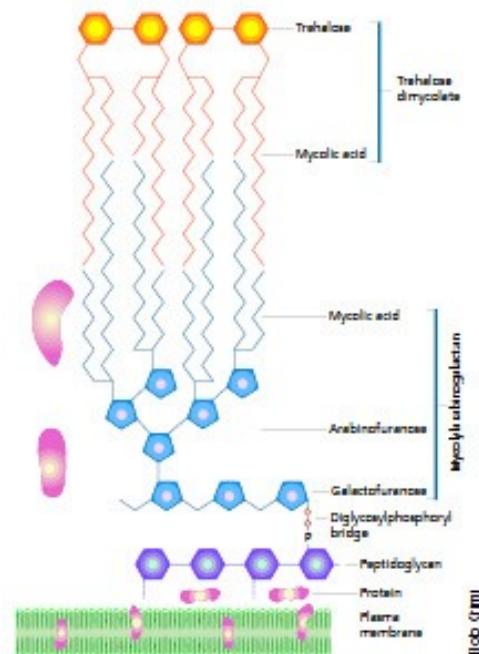
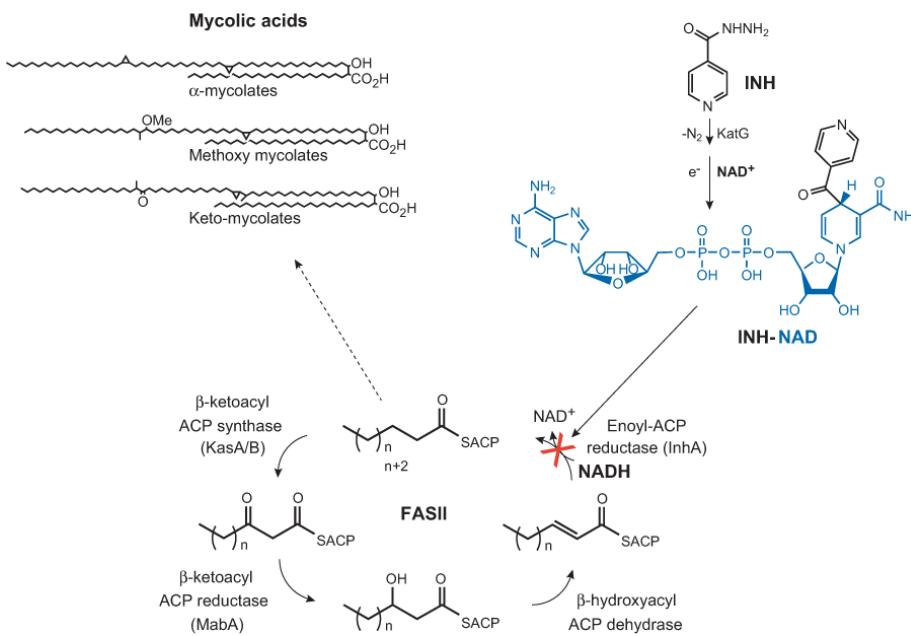
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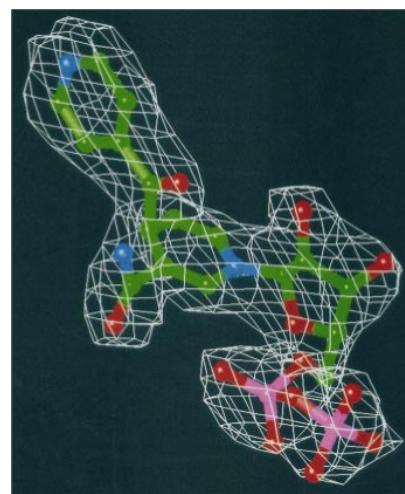
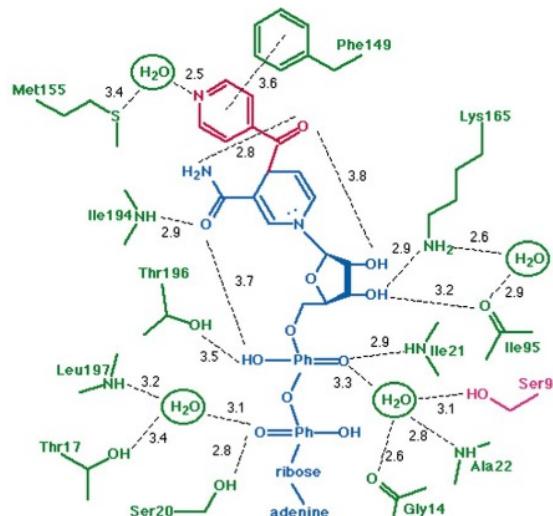
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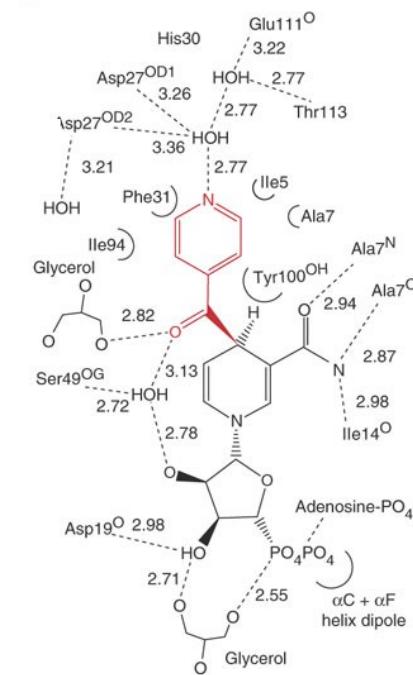
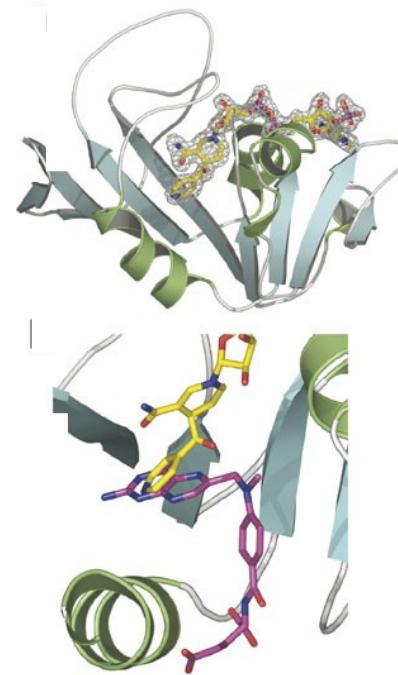
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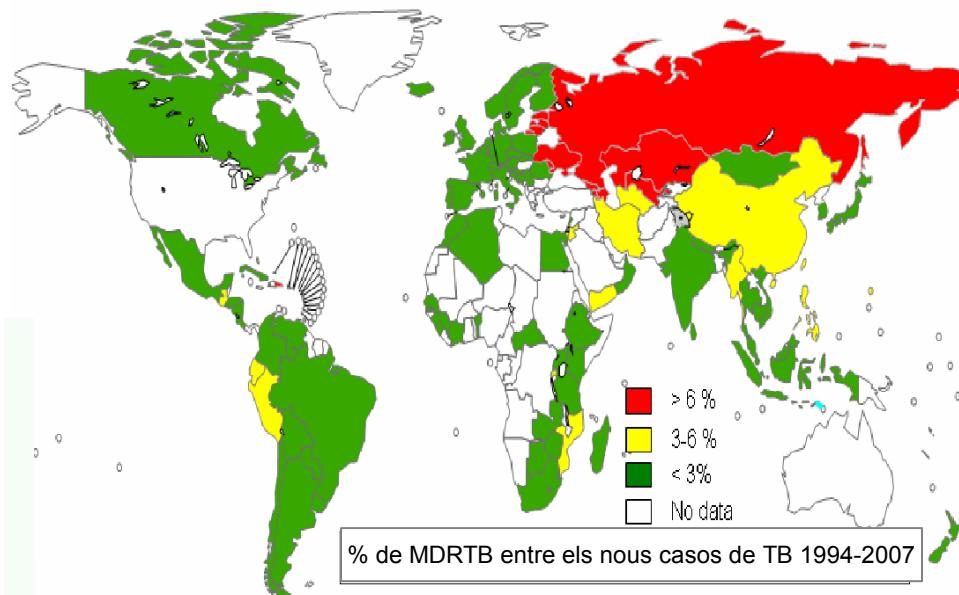
DIANES DE L'ISONIAZIDA



Rozwarski et al. *Science* 279, 98-102 (1998)



Argyrou et. al. *Nat Struct & Mol Biol* 13, 408 - 413 (2006)



> 2B infectat (1/3^{era} part de la població mundial)

2007 Nous MDRTB

India	131.000
China	112.000
Russian Fed.	43.000
S. Africa	16.000
Bangladesh	15.000

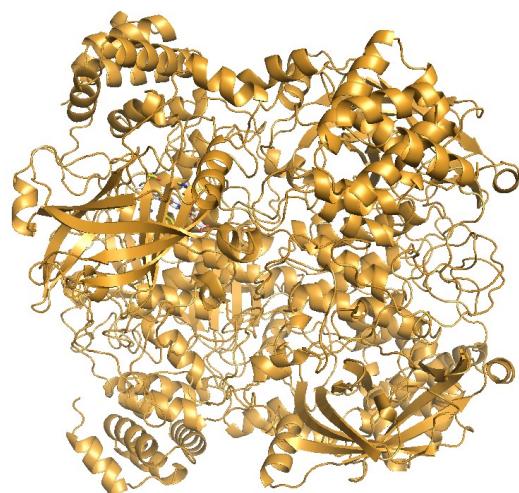
WHO, 2007

Gene	Mutation
<i>katG</i>	G->C pos 944, S315T
	G->A pos 944, S315N
	G->A pos 836, G279D
	G->A pos 946, R316Q
	A->C pos 970, T324P
<i>rpoB</i>	C->T pos 1350, S531L
	A->G pos 1335, H526D
	A->T pos 1305, D516V

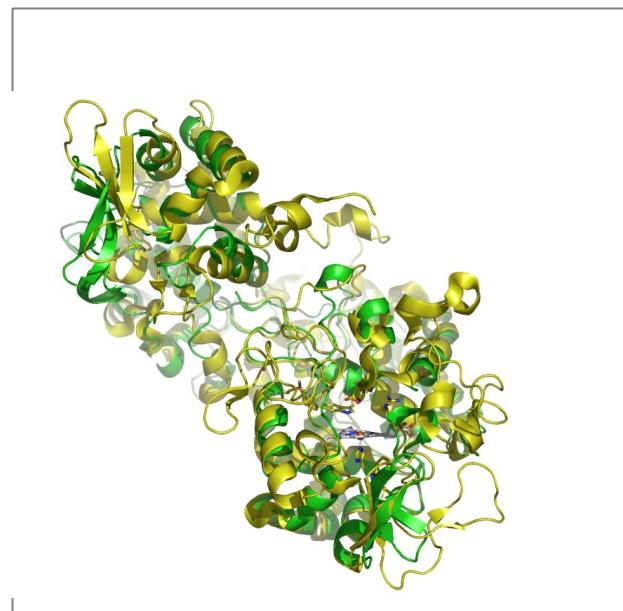
S315T es troba en ~50% de les soques resistentes a INH

C->G pos 1334, H526D	
C->G pos 1350, S531L	
C->A pos 1273, F505L	
T->C pos 1290, L511P	
C->A pos 1295, Q513K	
<i>embB</i>	A->G pos 917, M306I
	G->A pos 919, M306I
	A->C pos 917, M306L
	A->C pos 957, Y319S
<i>pncA</i>	G->A pos 949, M316I
	G->C pos 919, M306I
	C->T pos 195, S65S
	A->C pos 535, S178R
<i>gyrA</i> ⁹⁵	A->C pos 35, D12A
	Del C pos 344, STOP116
	Del G pos 166, STOP116
	Ins G pos 234, STOP126
<i>katG</i> ⁴⁶³	Ins G pos 394, Frameshift
	T->C pos 104, L34P
	G->C pos 357, W119C
	G->C pos 481, A160P
<i>gyrA</i> ⁹⁵	No mutations observed

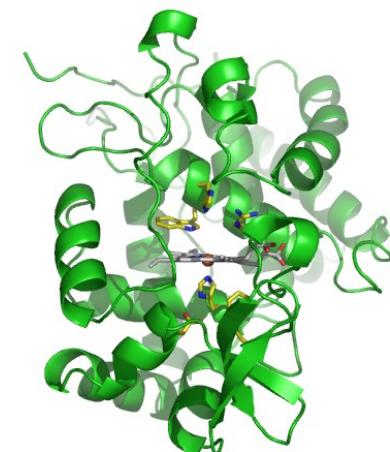
Stavrum R. et al. PLoS. V4, 2: e4540 (2009)



Catalasa HPII



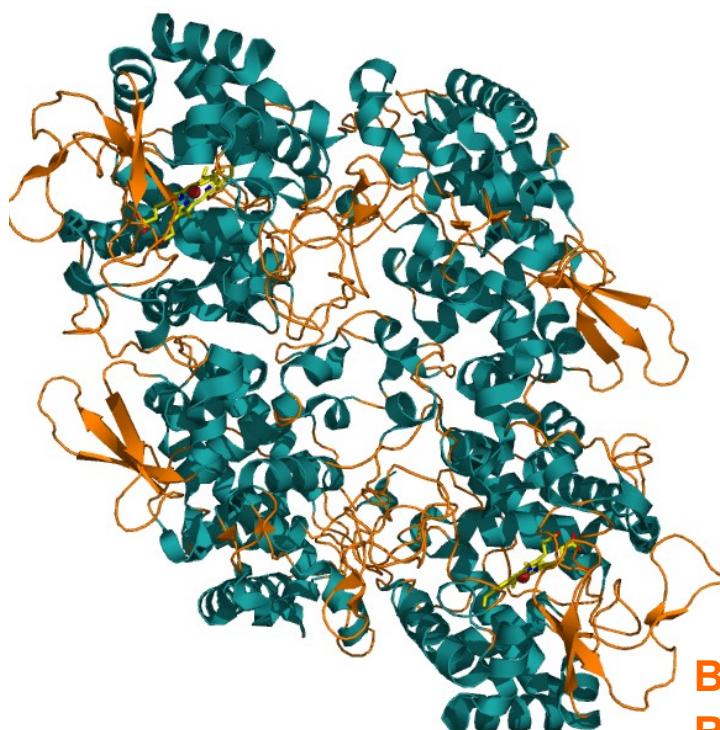
Cat-Px KatG



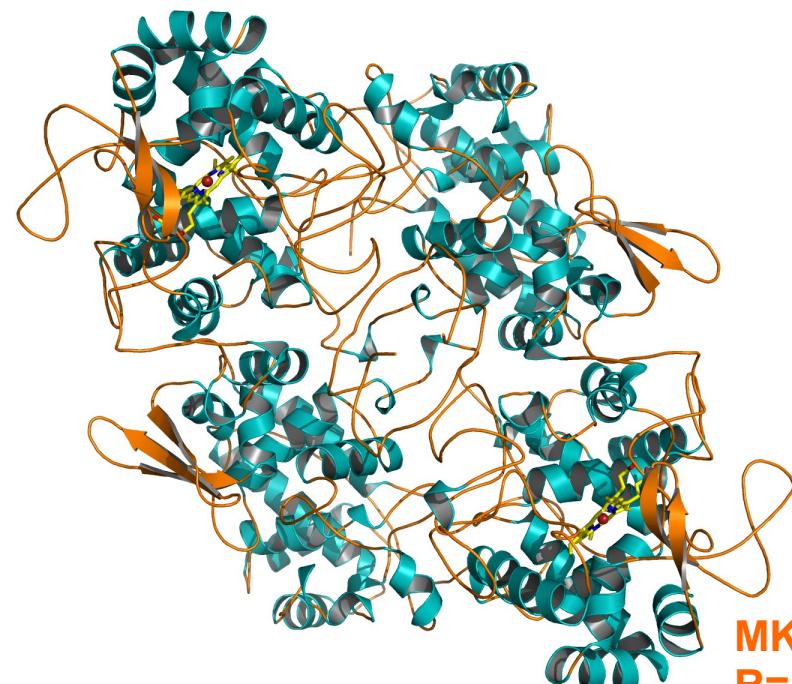
Peroxidasa CCP

Superfamília de les peroxidases de plantes

- { -Classe I: bacterial origin (CCP, APX, KatG)
- Classe II: fungal secreted (LiP, ARP)
- Classe III: plant secreted (HRP, PNP)



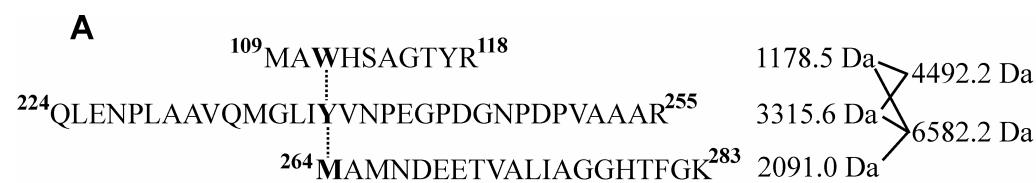
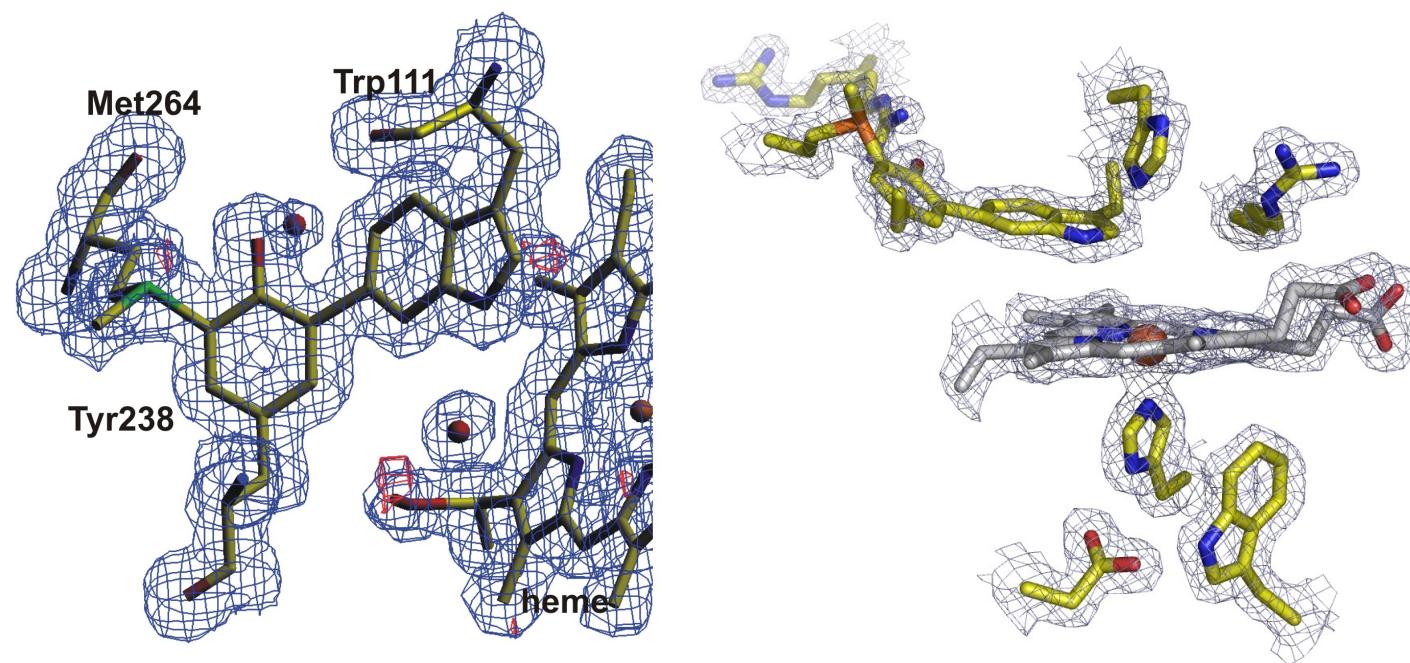
BKG₈
R= 1.7 Å

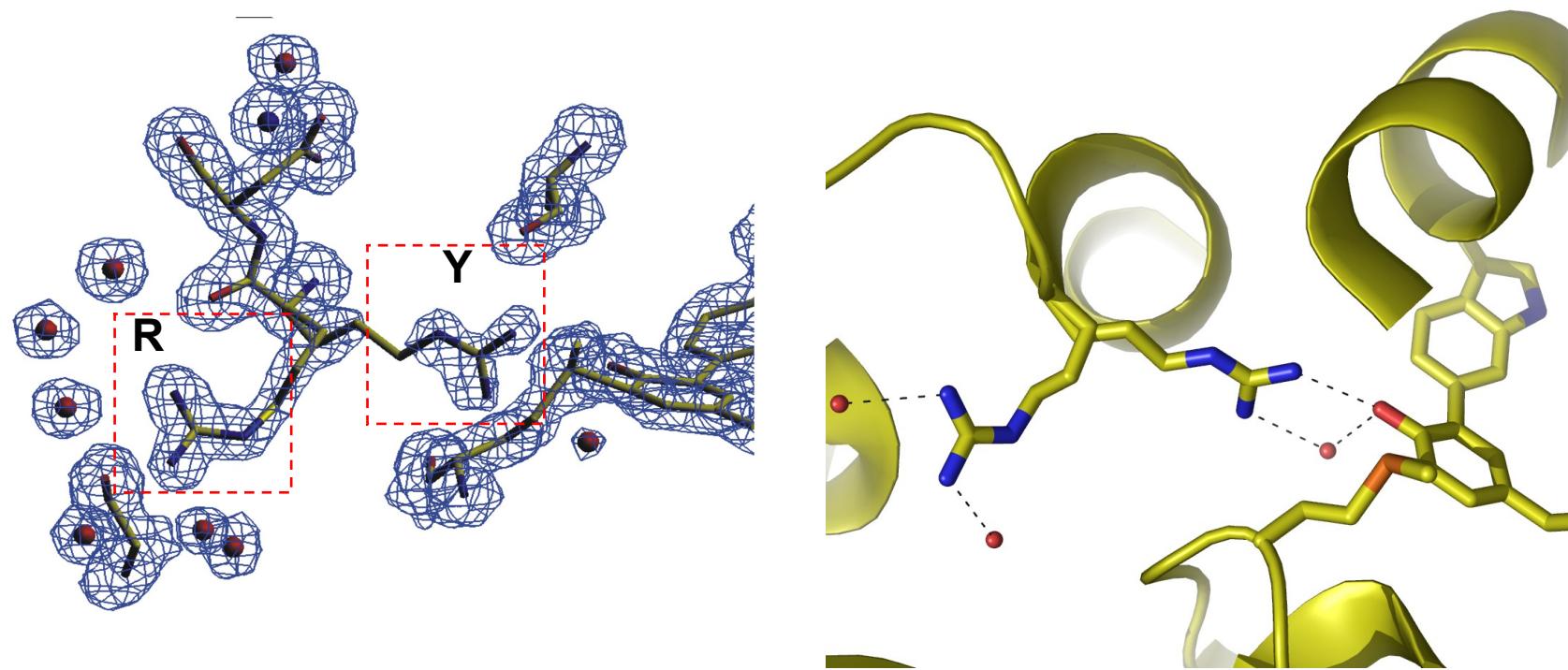


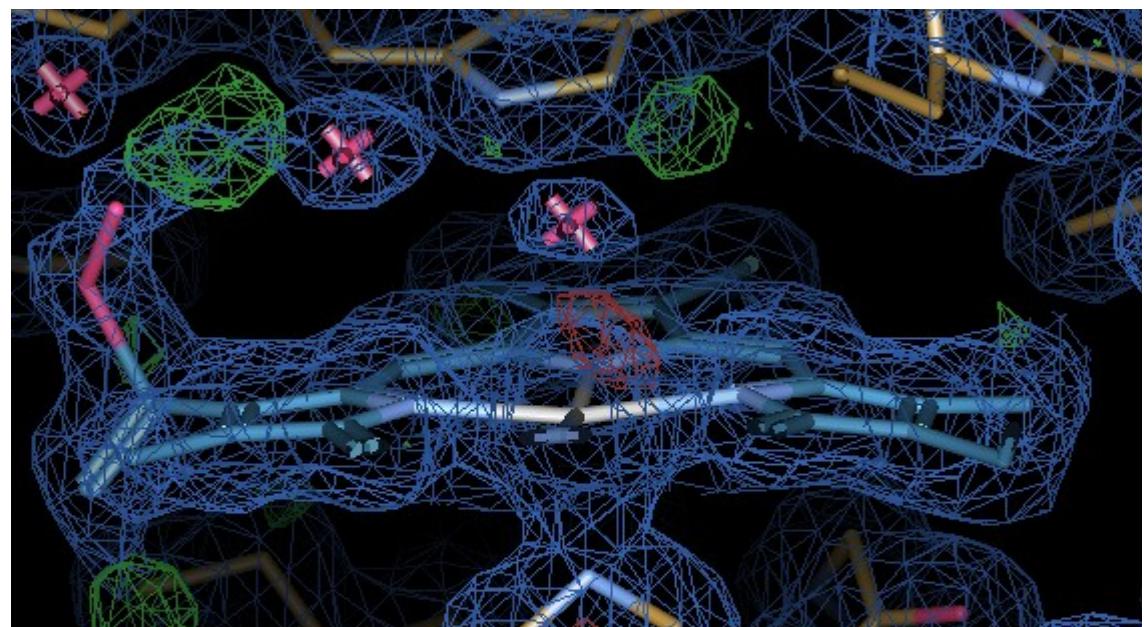
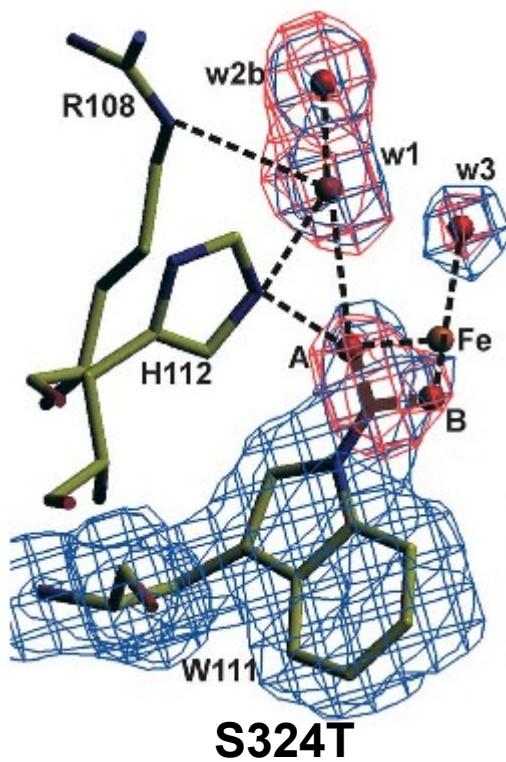
MKG
R= 1.5 Å

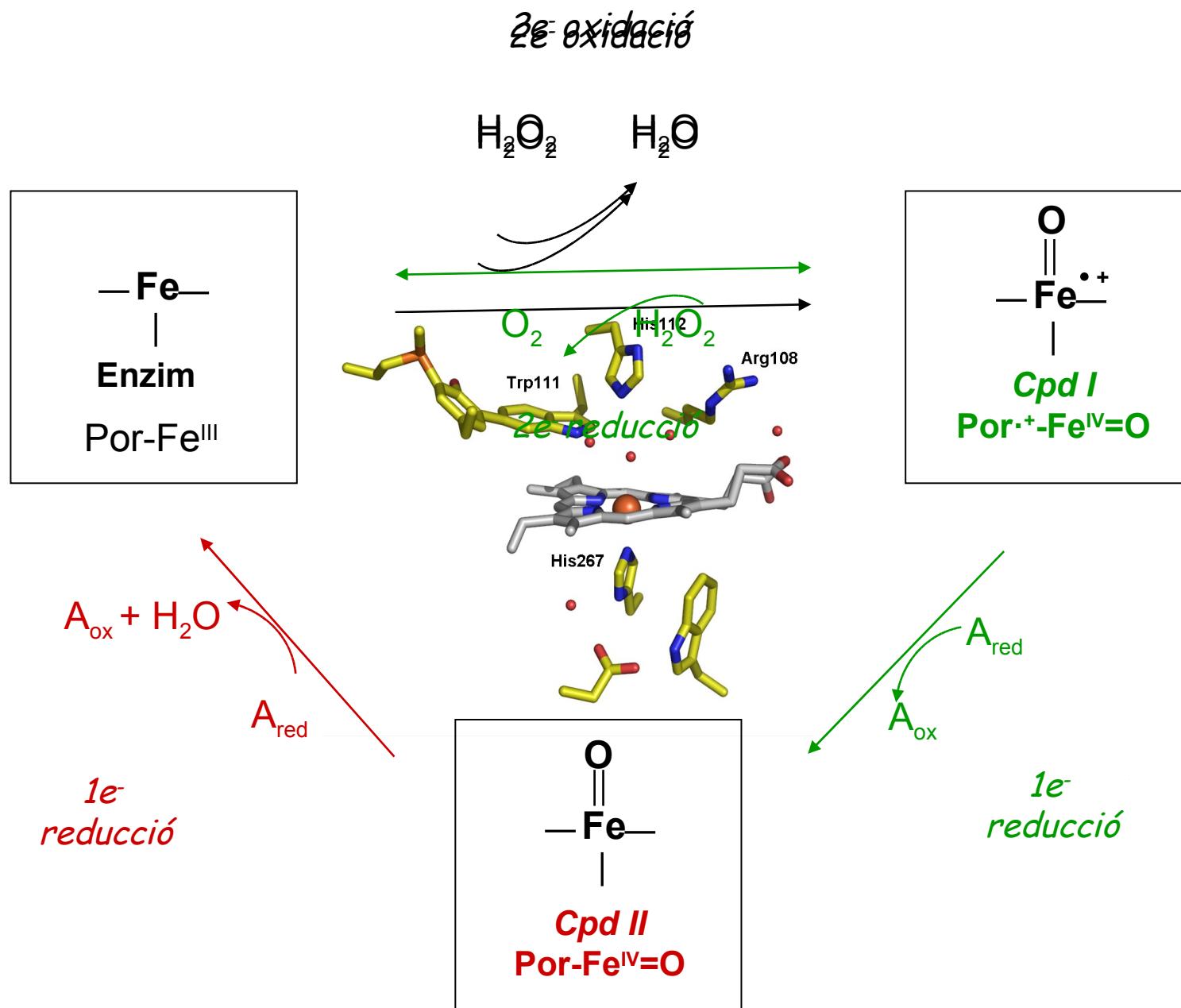


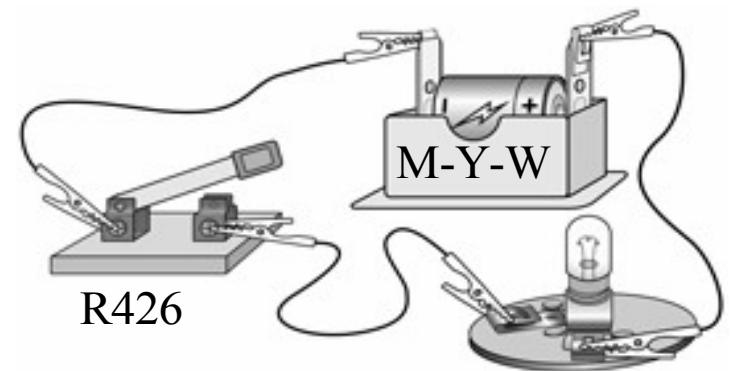
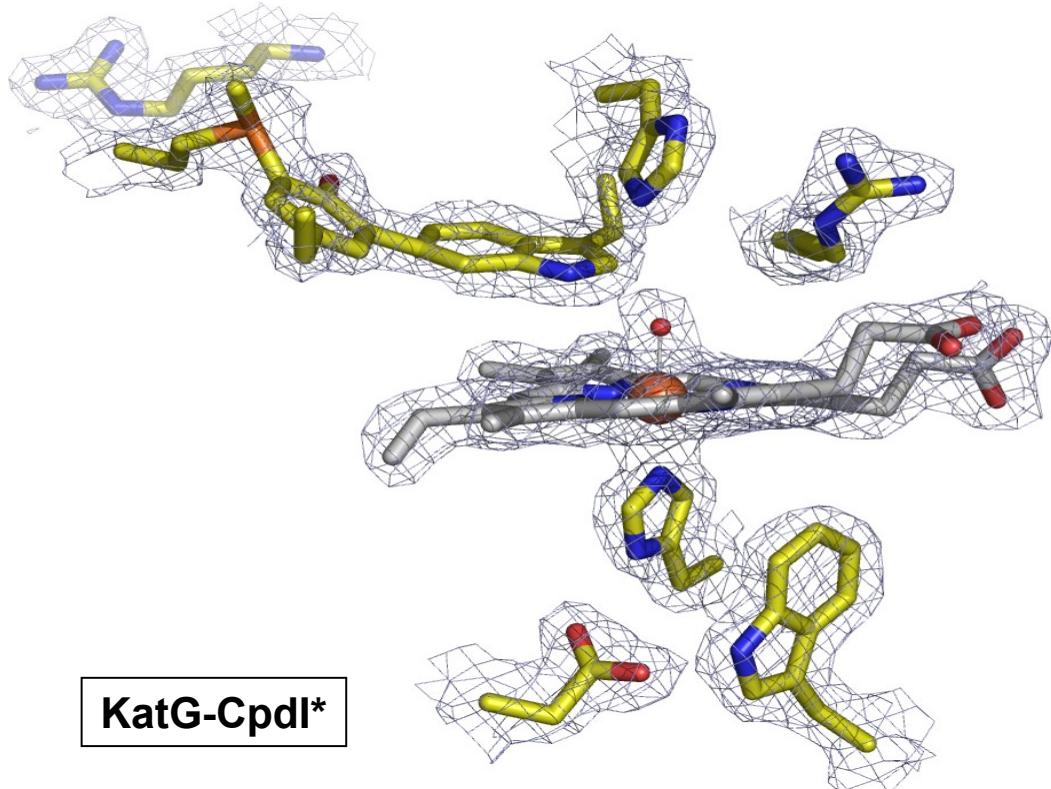
HPI_2D₁₈
R= 2.8 Å



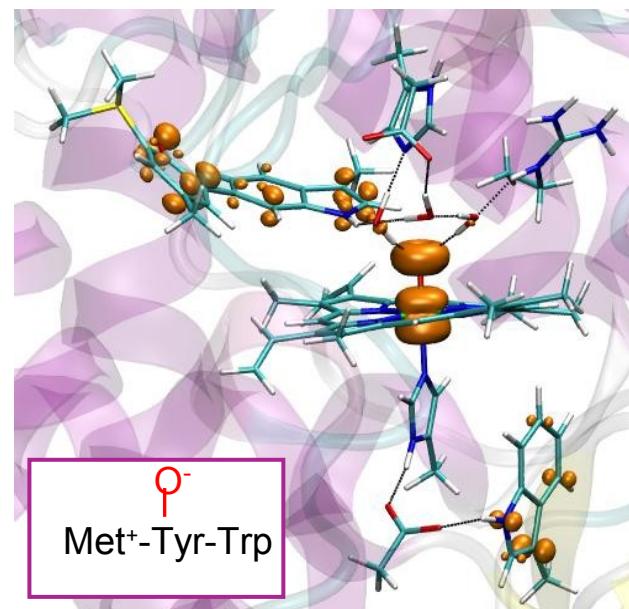
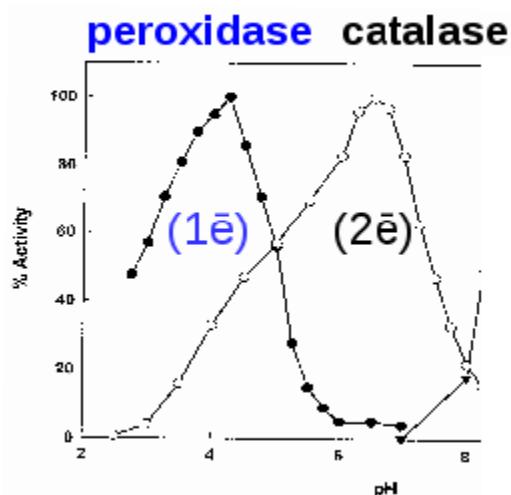






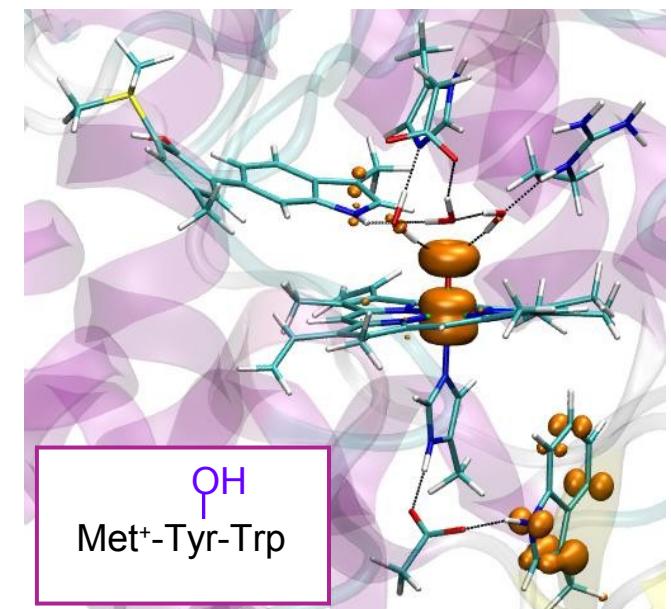


Carpena X, Wiseman B, Deemagarn T, Singh R, Switala J, Ivancich A, Fita I, Loewen PC. A molecular switch and electronic circuit modulate catalase activity in catalase-peroxidases. EMBO Rep. 2005 Dec;6(12):1156-62.



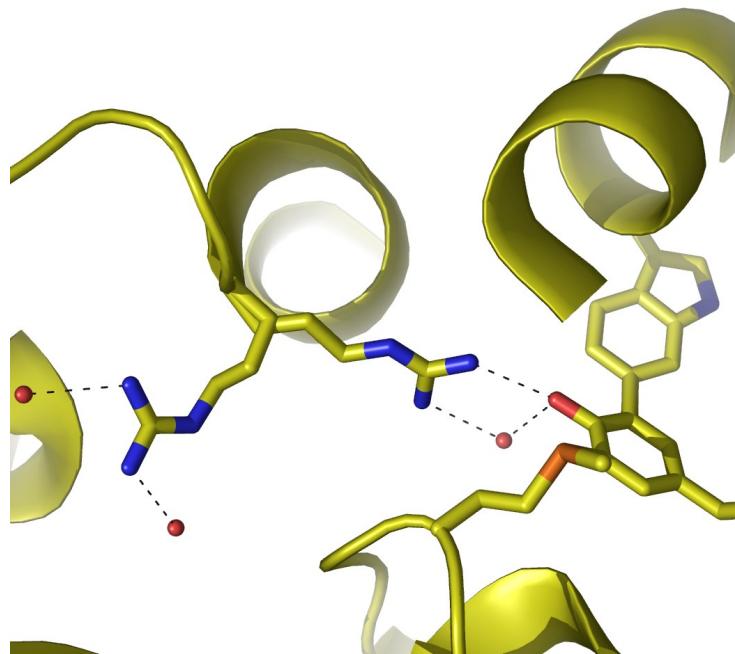
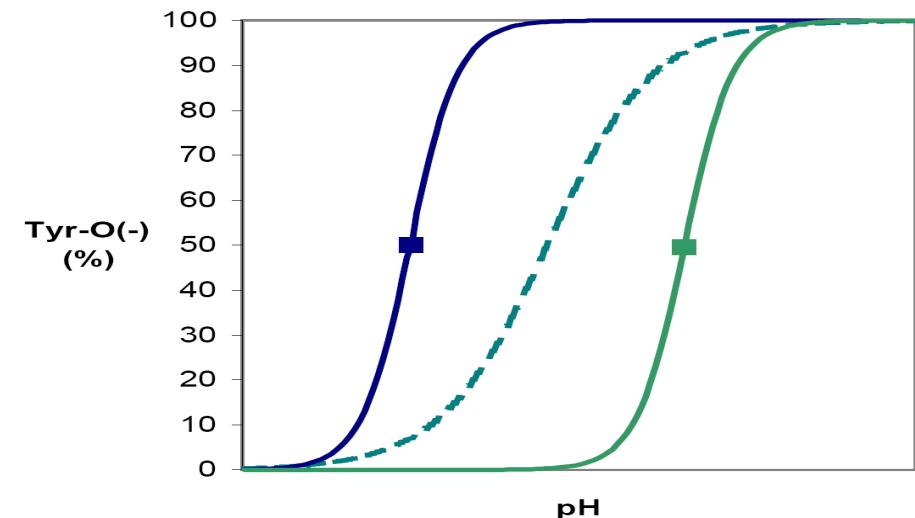
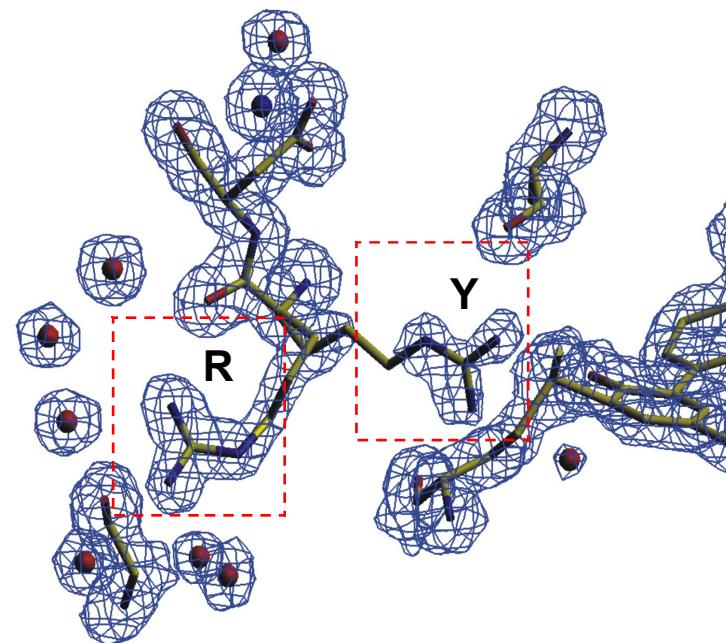
Cpd I

PEROX.

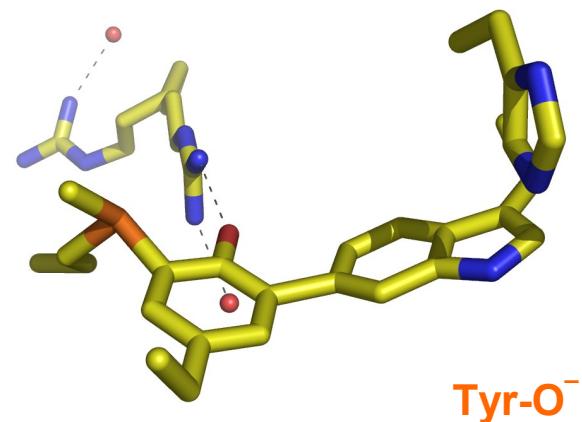


Cpd I

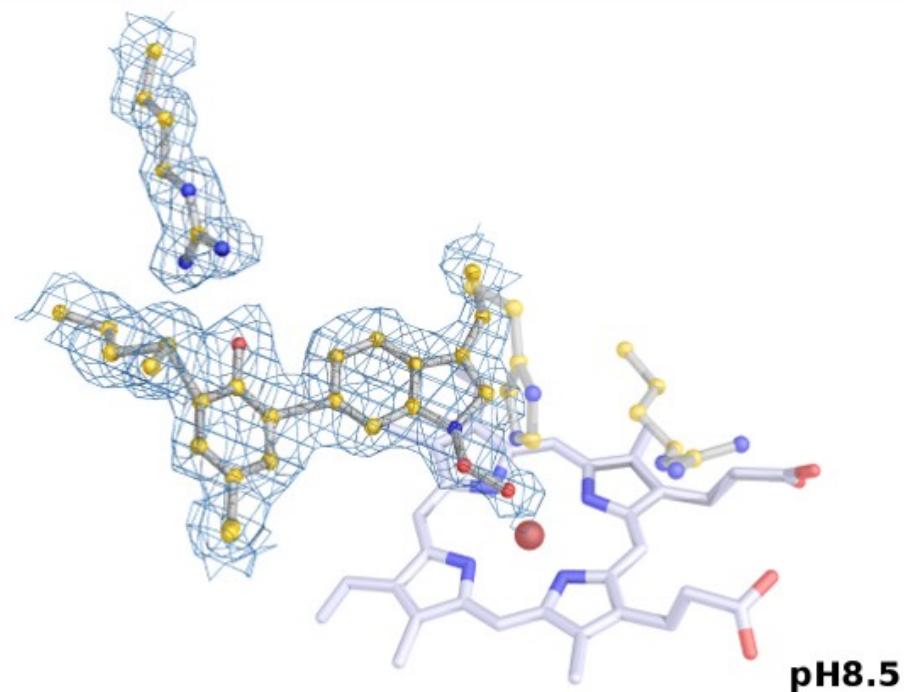
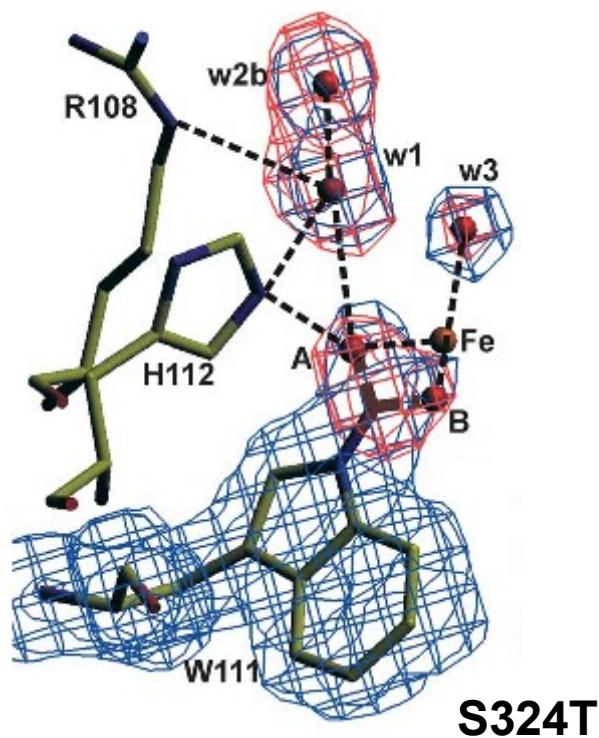
CATA.



pH distribution of BpKatG (%)				
	4.5	5.6	6.5	7.5
Arg-Y _{conf}	<10	30	50	75
Arg-R _{conf}	>90	70	50	25

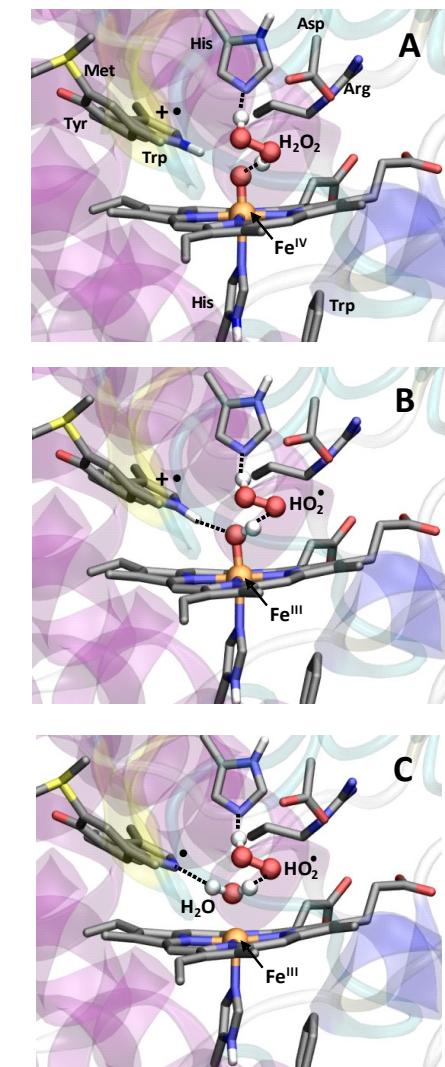
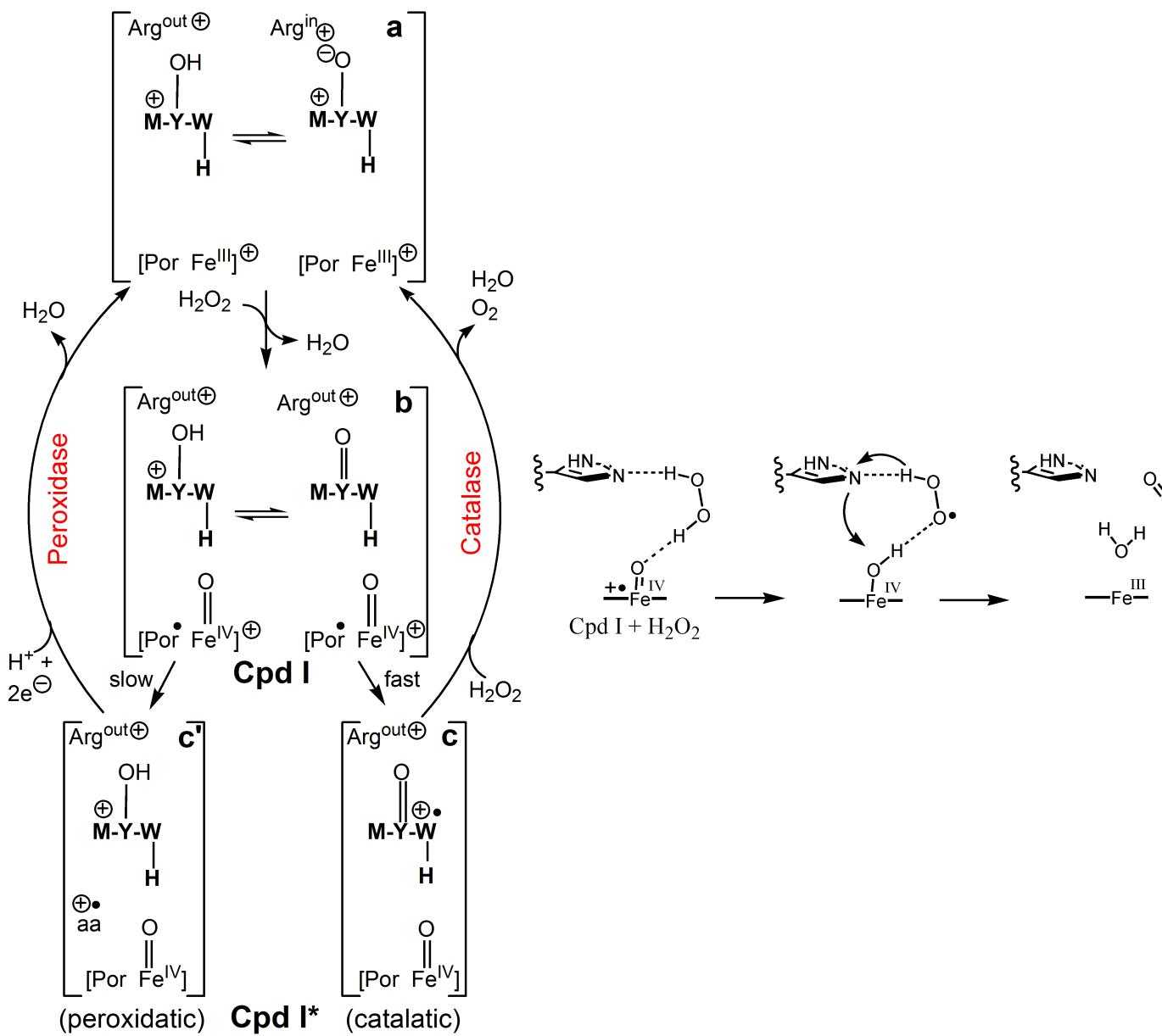


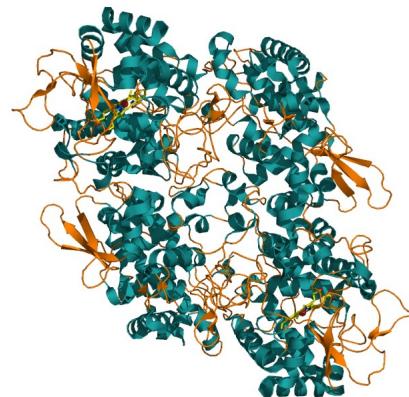
Carpeta X et al. Roles for Arg426 and Trp111 in the modulation of NADH oxidase activity of the catalase-peroxidase KatG from Burkholderia pseudomallei inferred from pH-induced structural changes. Biochemistry. 2006 Apr 25;45(16):5171-9.



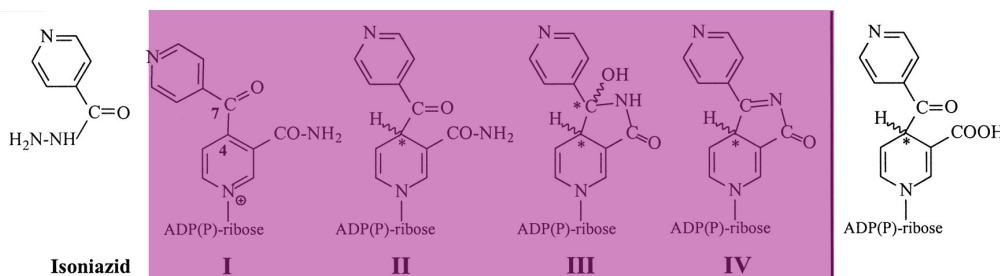
Catalasa-Peroxidases (KatG)

Cicle catalític de KatG

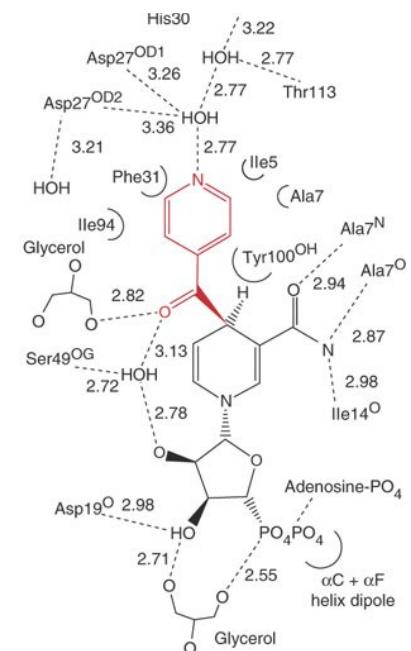
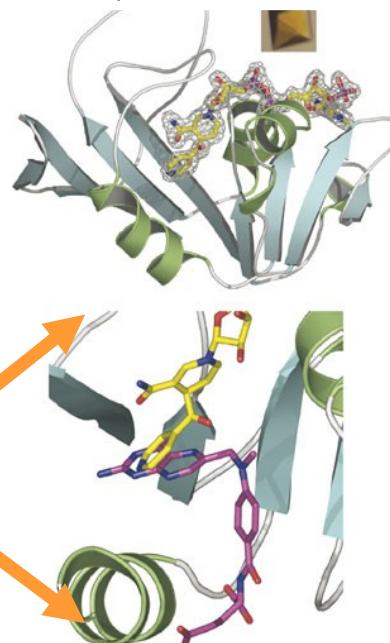




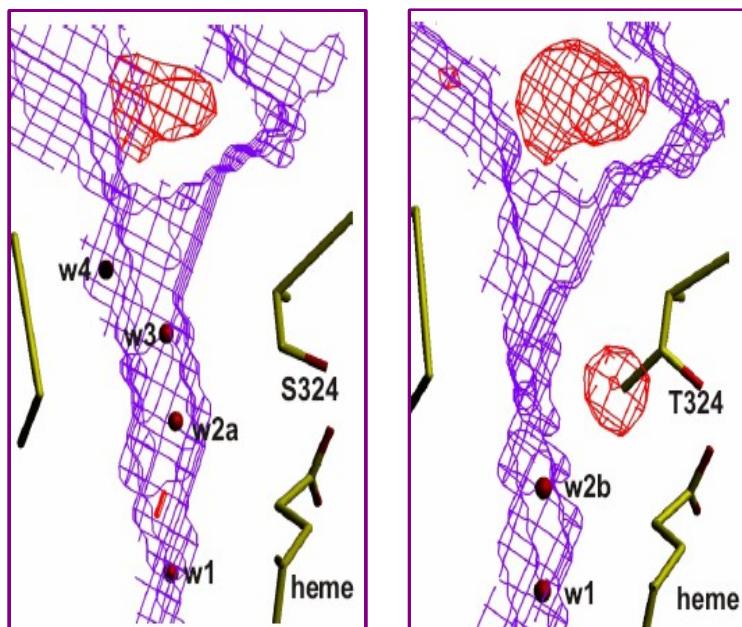
KatG actúa com a activador d'INH

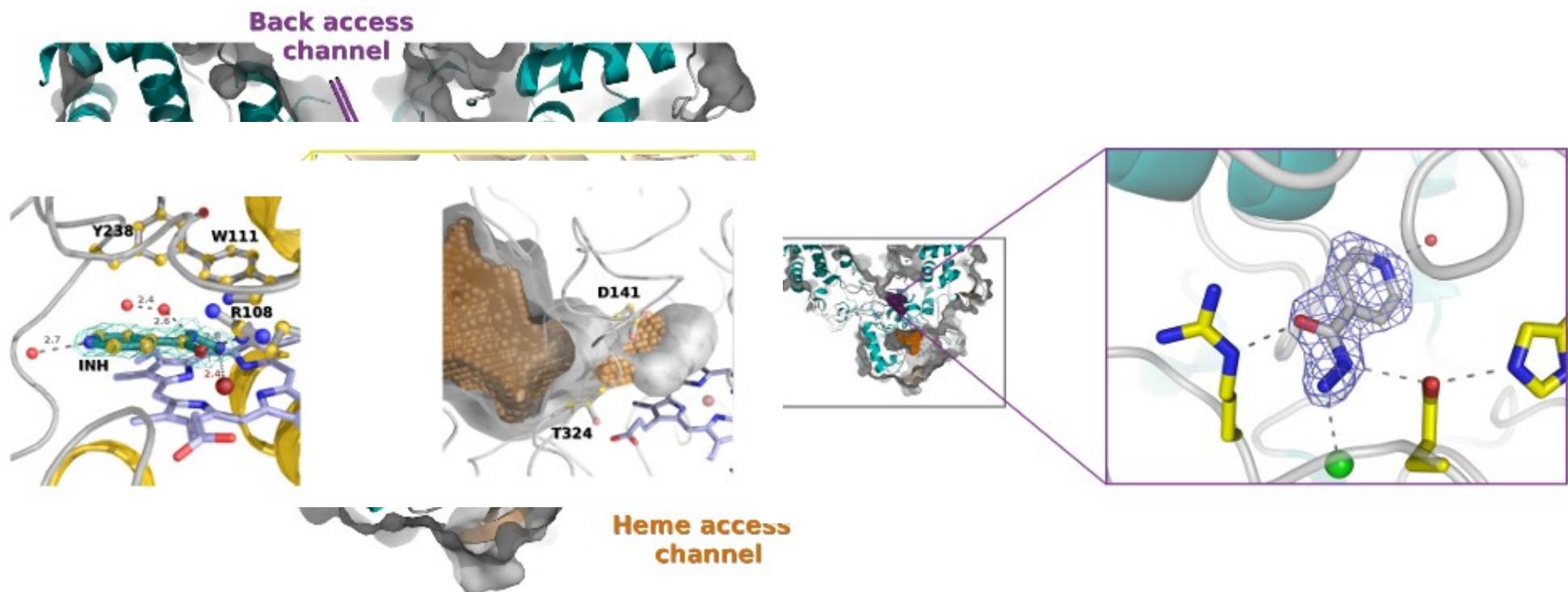


Argyrou et. al. *Nat Struct & Mol Biol* 13, 408 - 413 (2006)



INH-NADH és el compost veritablement efectiu





Vidossich P, Loewen PC, Carpenna X, Fiorin G, Fita I, Rovira C. *Binding of the Antitubercular Pro-Drug Isoniazid in the Heme Access Channel of Catalase-Peroxidase (KatG). A Combined Structural and Metadynamics Investigation.* J Phys Chem B. 2014 Mar 20;118(11):2924-31

L'alien investigador



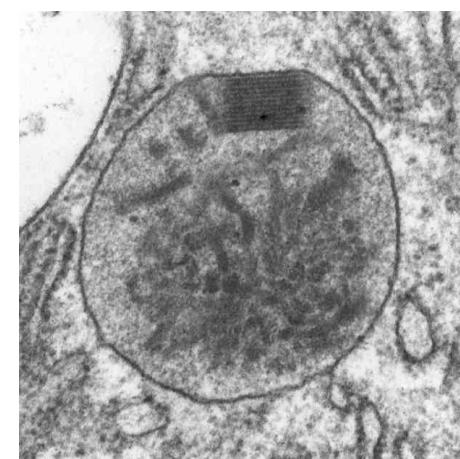
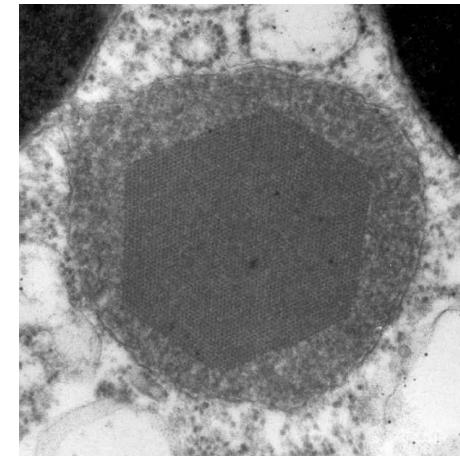
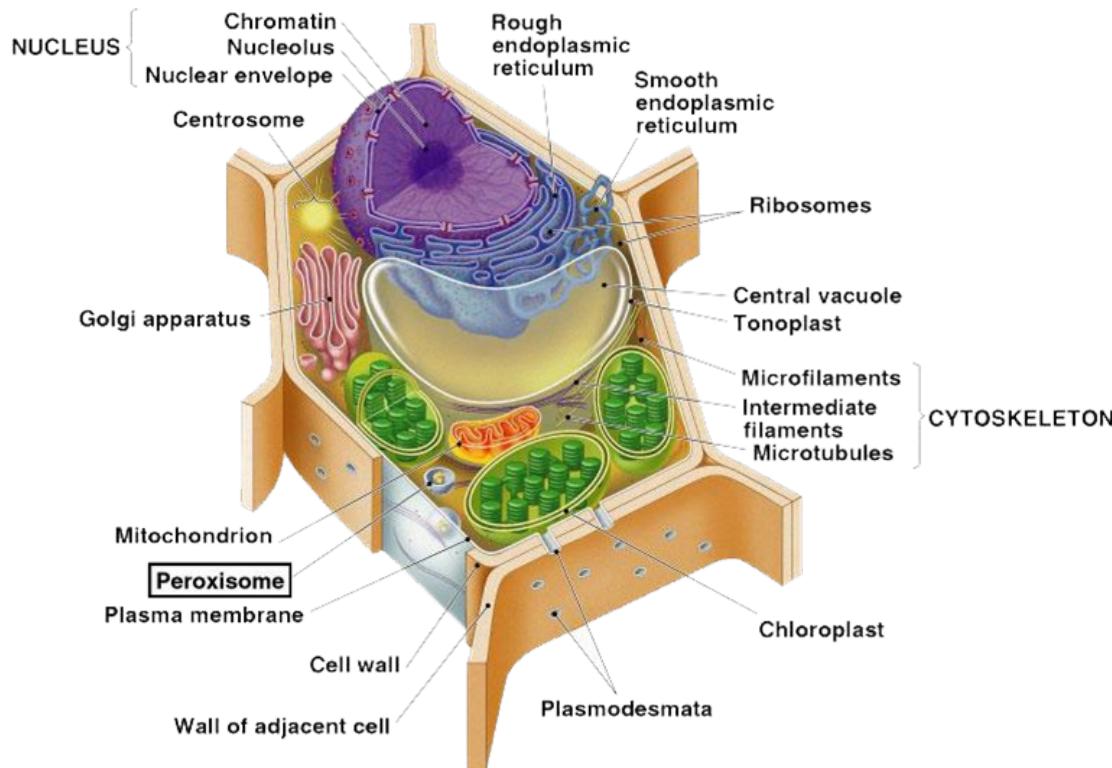
Zona d'investigació



Subjecte a investigar

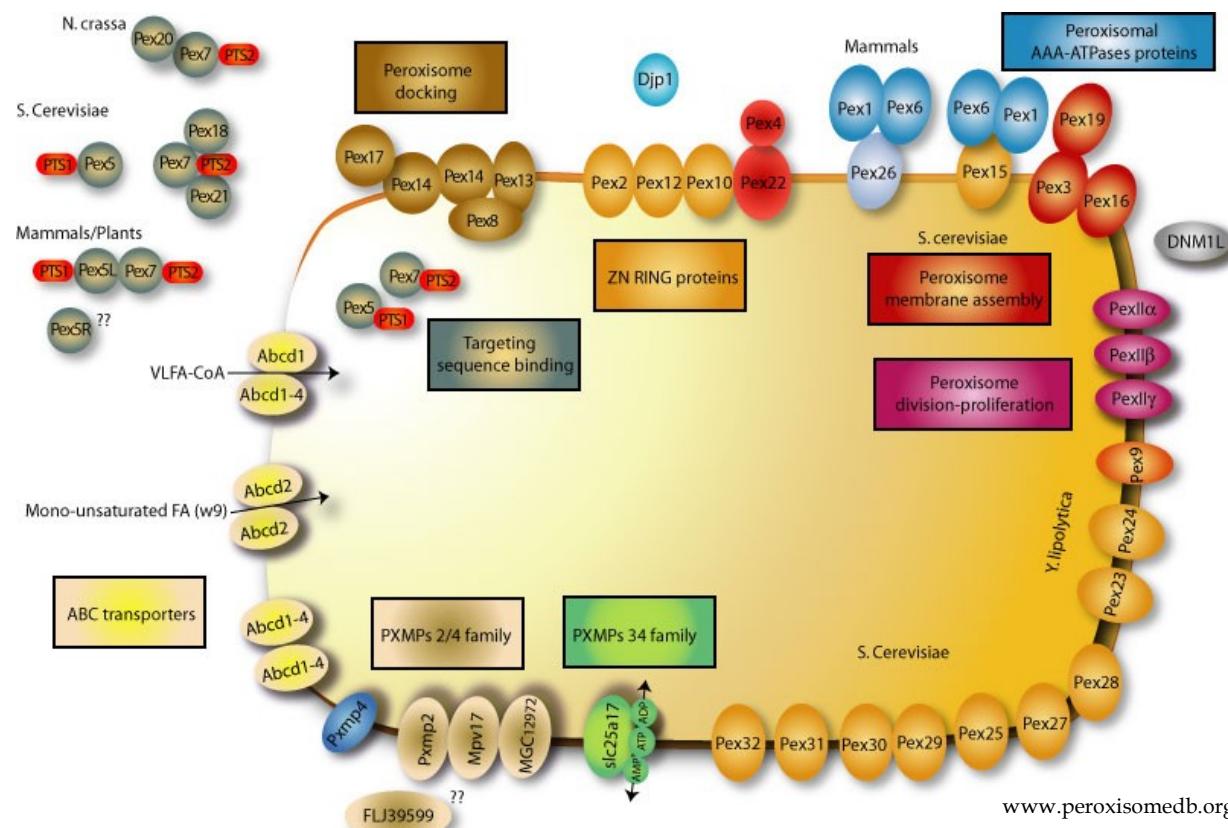


PEROXISOMA



Biology N.Campbell and J. Reece 2008 Pearson Education

- A dia d'avui, hi ha 32 peroxines conegeudes (**Gens Pex**)
 - 82 enzims (Catalasa, tiolasa, oxidases, SOD...)
- Biosíntesi: Colesterol, àcids biliars, àcids grassos insaturats, plasmalògens
- Degradació: Radicals liures (OH⁻, O₂⁻), etanol, oxidació d'àcids grassos, H₂O₂



➤ **ZS** (Síndrome de Zellweger) (12 gens Pex involucrats)

Dimorfisme facial

Endarreriment mental

Risc de mort a partir dels 6 mesos de naixement

➤ **NALD** (Adrenoleucodistrofia neonatal) (5 gens Pex)

Endarreriment mental

Atrofia muscular

➤ **RCDP** (Condrodisplàsia rizomèl·lica) (gen Pex7)

Problemas psicomotors

Dimorfismo facial

➤ **AM** (Acidúria malònica) (Malonil-coA descarboxilasa)

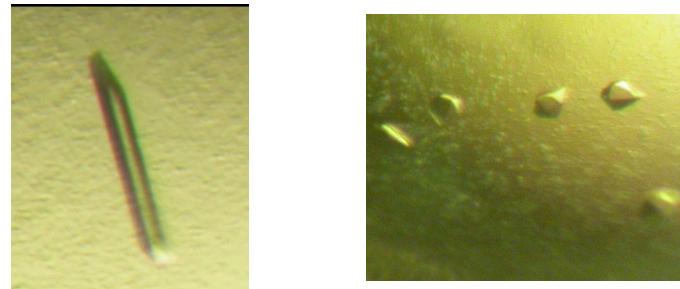
Convulsions

Endarreriment mental

Grau de severitat de les malalties

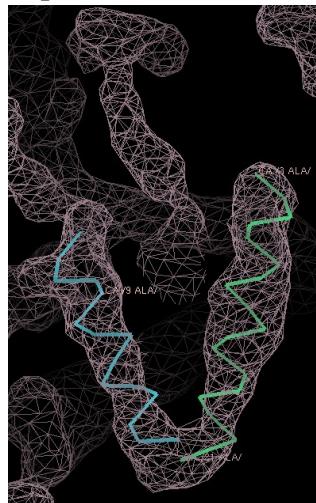
ZS > NALD > RCDP > AM

MCD SeMet **P1** 3.29 Å ESRF ID 23-2 SAD
 $a=79.55 \text{ \AA}$ $b=103.58 \text{ \AA}$ $c=134.23 \text{ \AA}$
 $\alpha=95.40$ $\beta=90.11$ $\gamma=94.82$



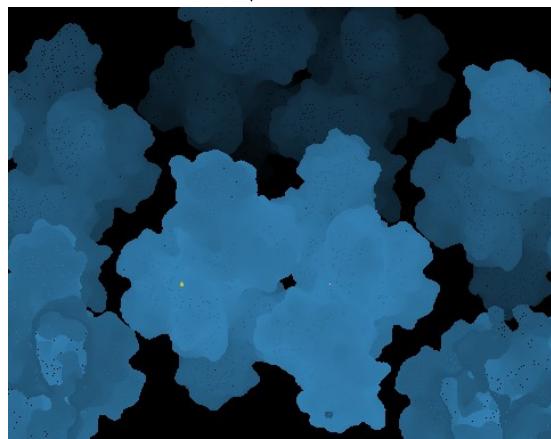
MCD SeMet 4.16 Å
Soleil Proxima1 SAD
P6₁22
 $a=b=144.7 \text{ \AA}$ $c=493.0 \text{ \AA}$ $\alpha=\beta=90$ $\gamma=120$

Mapa inicial a 4.2 Å P6₁22

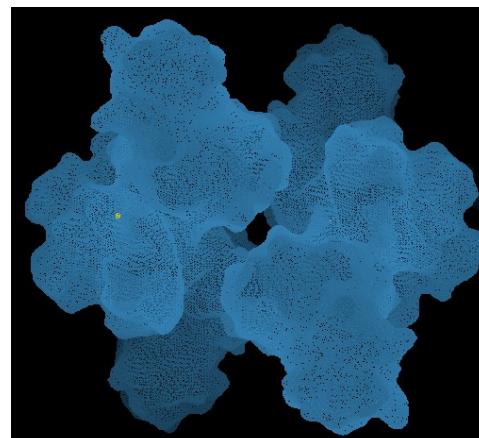


ncsmask

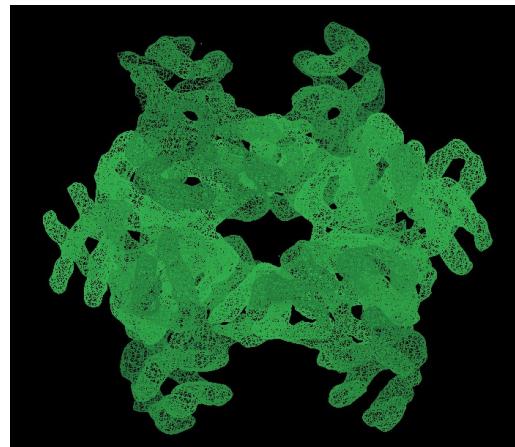
mapcutting



Màscara molecular en P6₁22

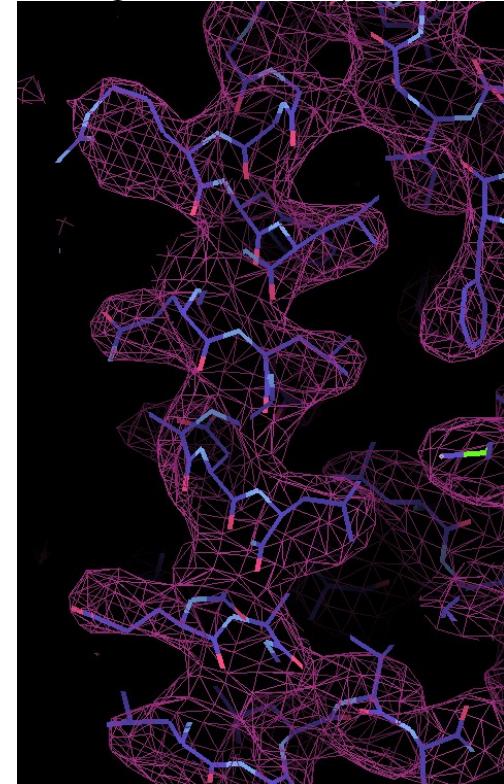


mapcutting



Mapa model x fer MR en P1

Mapa inicial en P1 (3.29 Å)

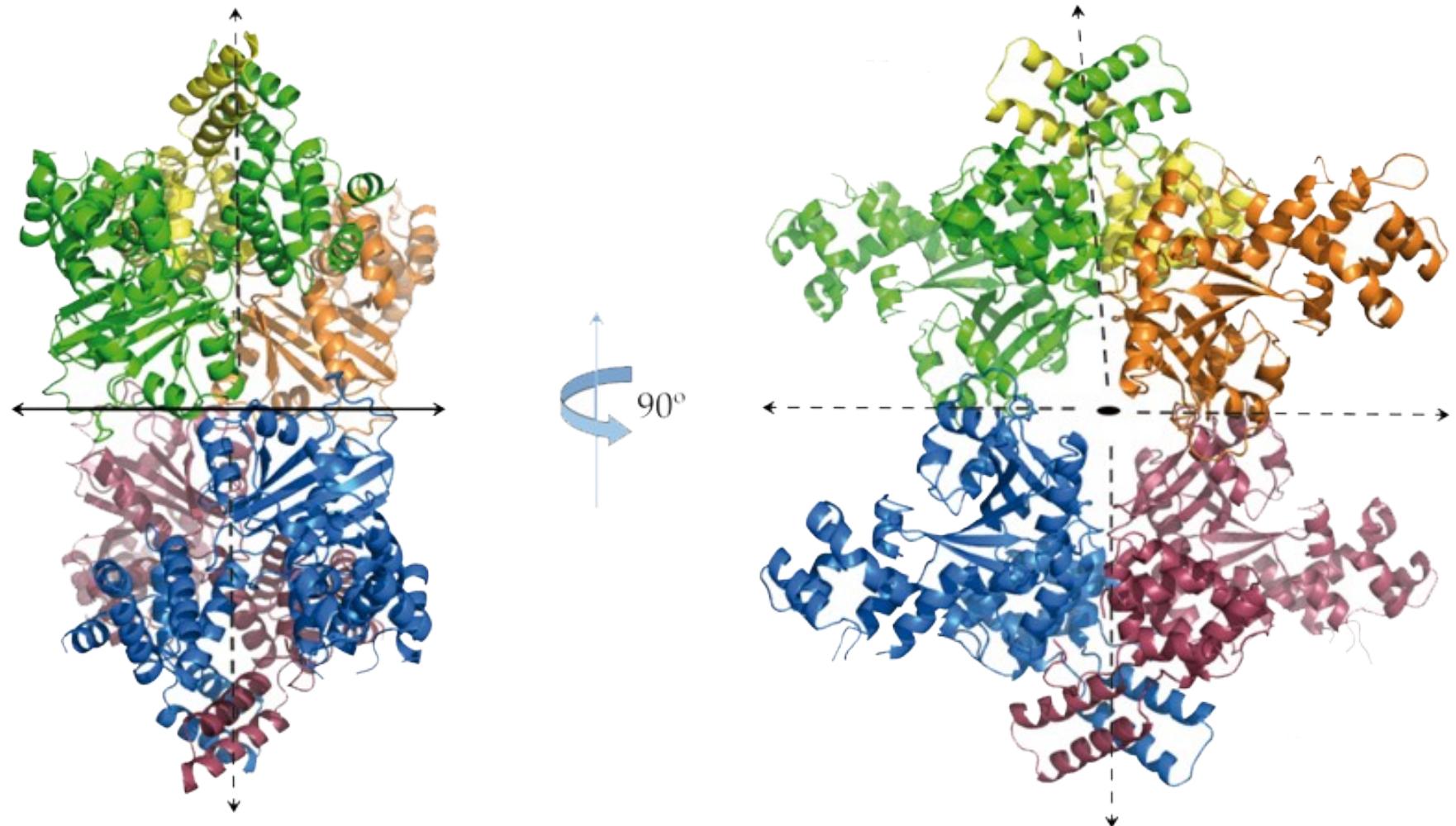


Promitjat densitat entre
P6₁22 i P1

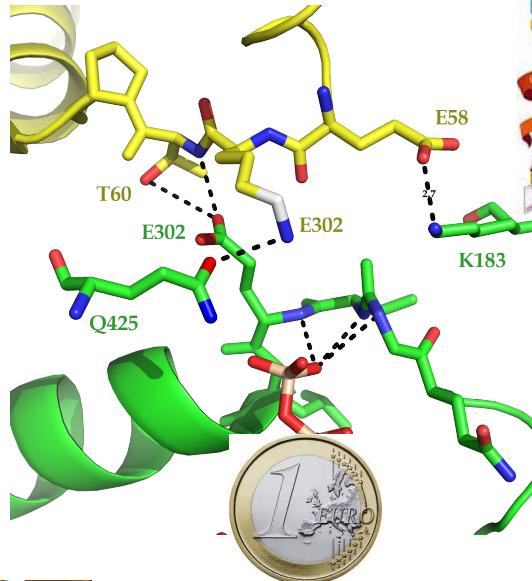
Phaser

Matrius de rotació i
traslació de P6₁22 a P1

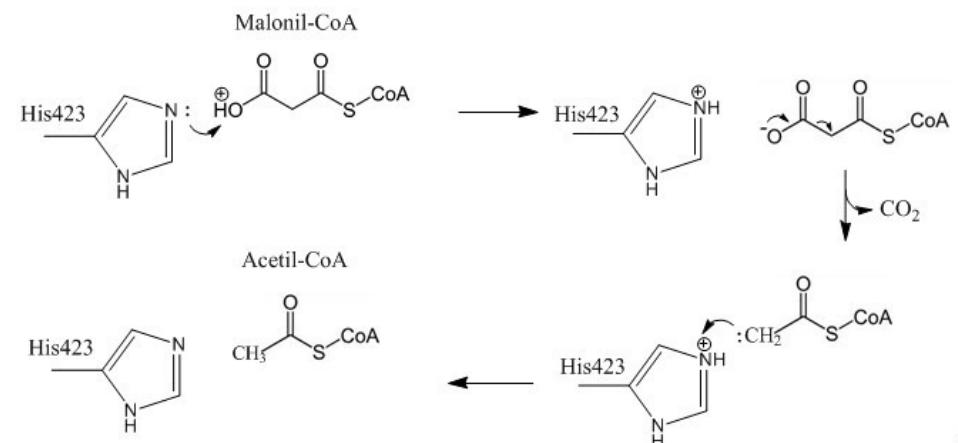
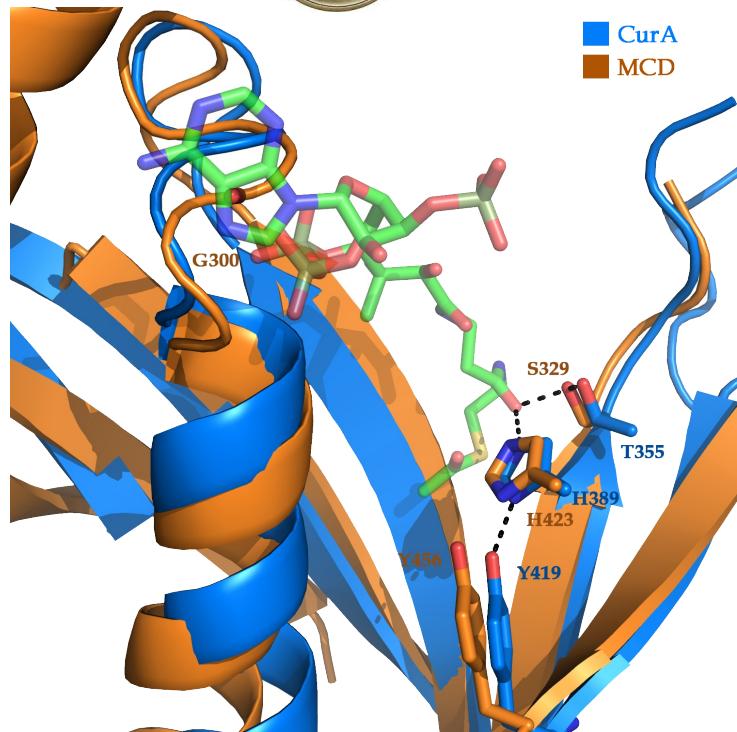
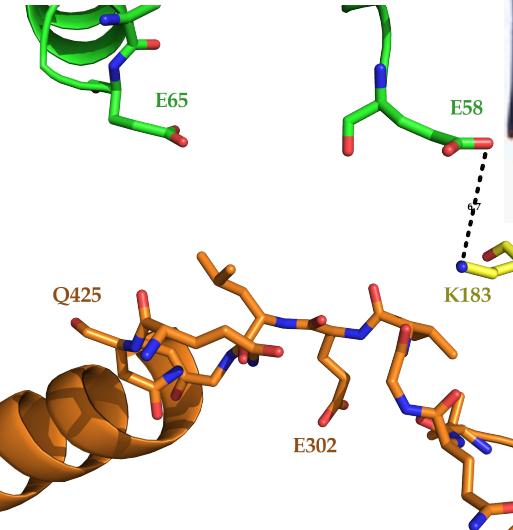
MCD no és un tetramer perfecte



Conformació UNIDA



Conformació LLIURE



L'alien investigador

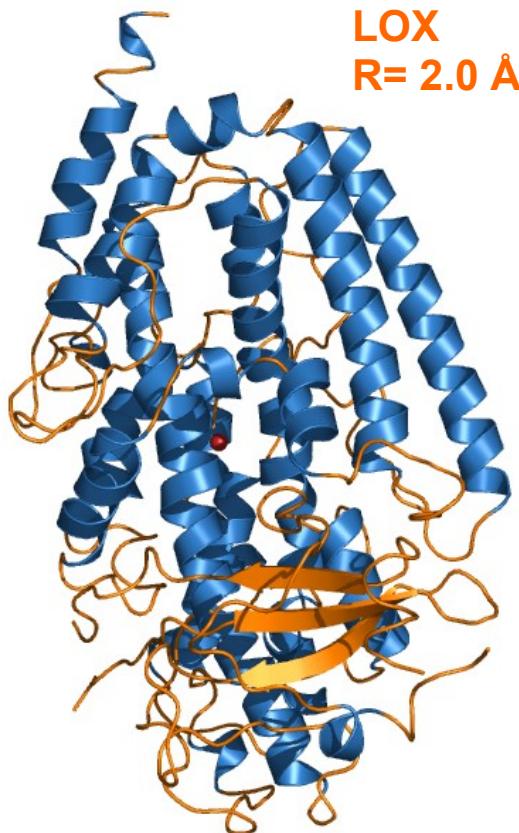


Zona d'investigació

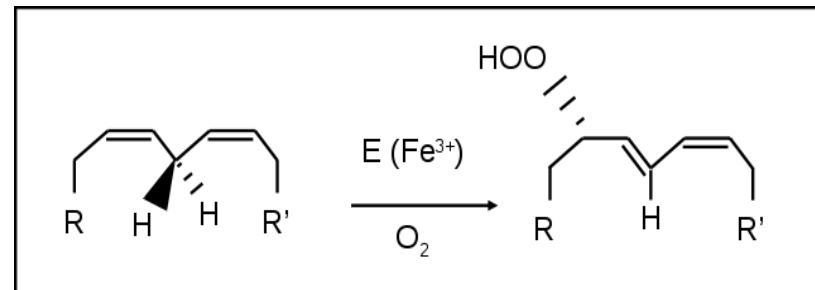


Subjecte a investigar

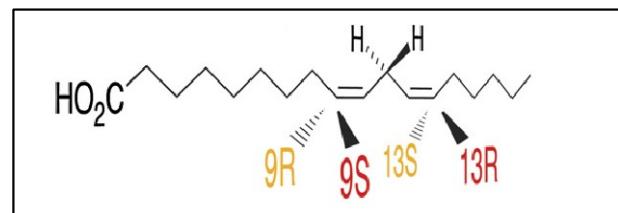




Dioxigenases de ferro que catalitzen l'addició d'un grup hidroperòxid en substrates 1,4 - Z-Z pentadienils.

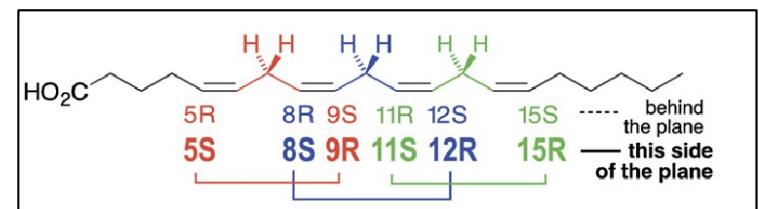


Àcid Linoleic

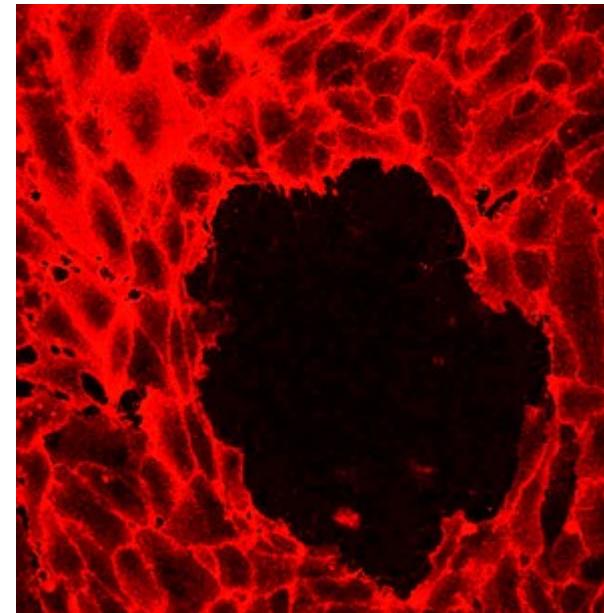
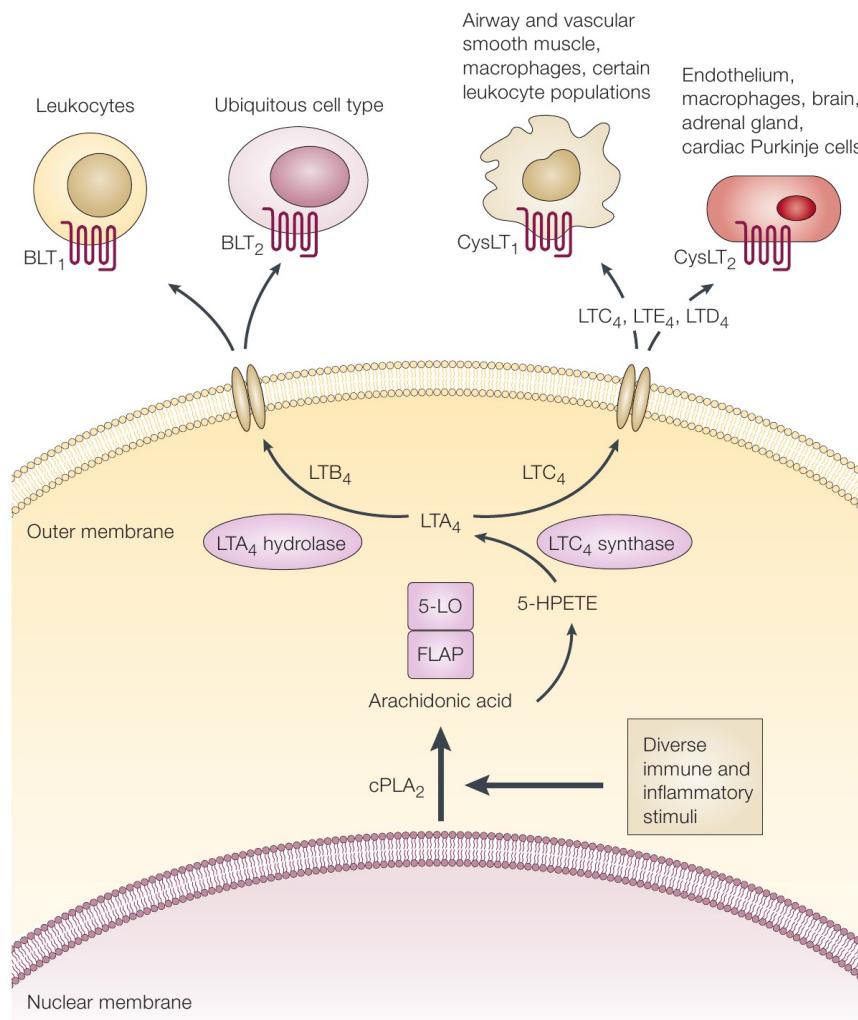


Vies de les oxilipines (plantes)

Àcid Araquidònic

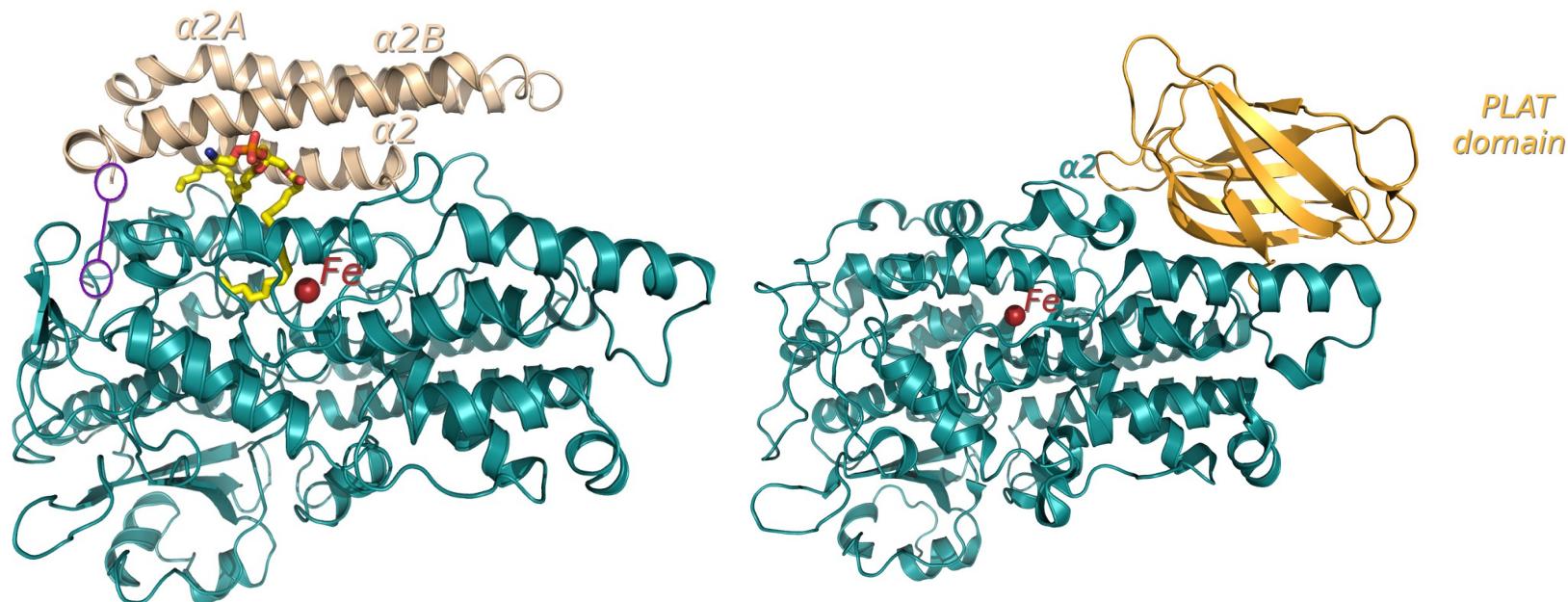
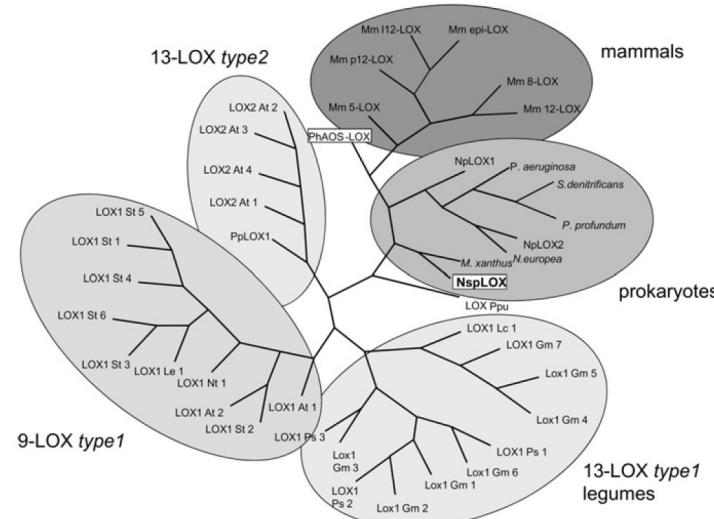


Biosintesi de Leucotriens (mamífers)



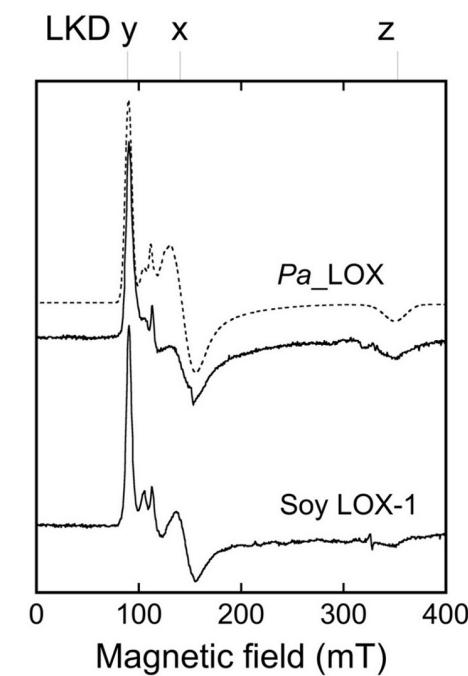
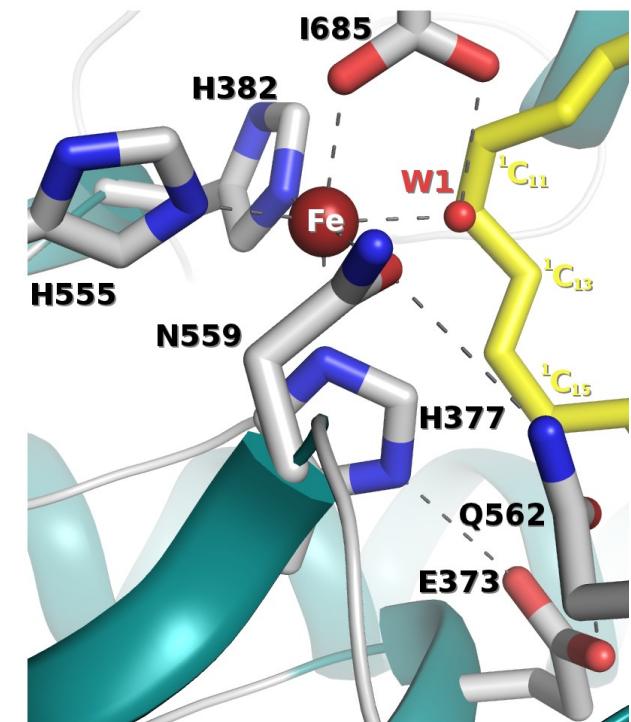
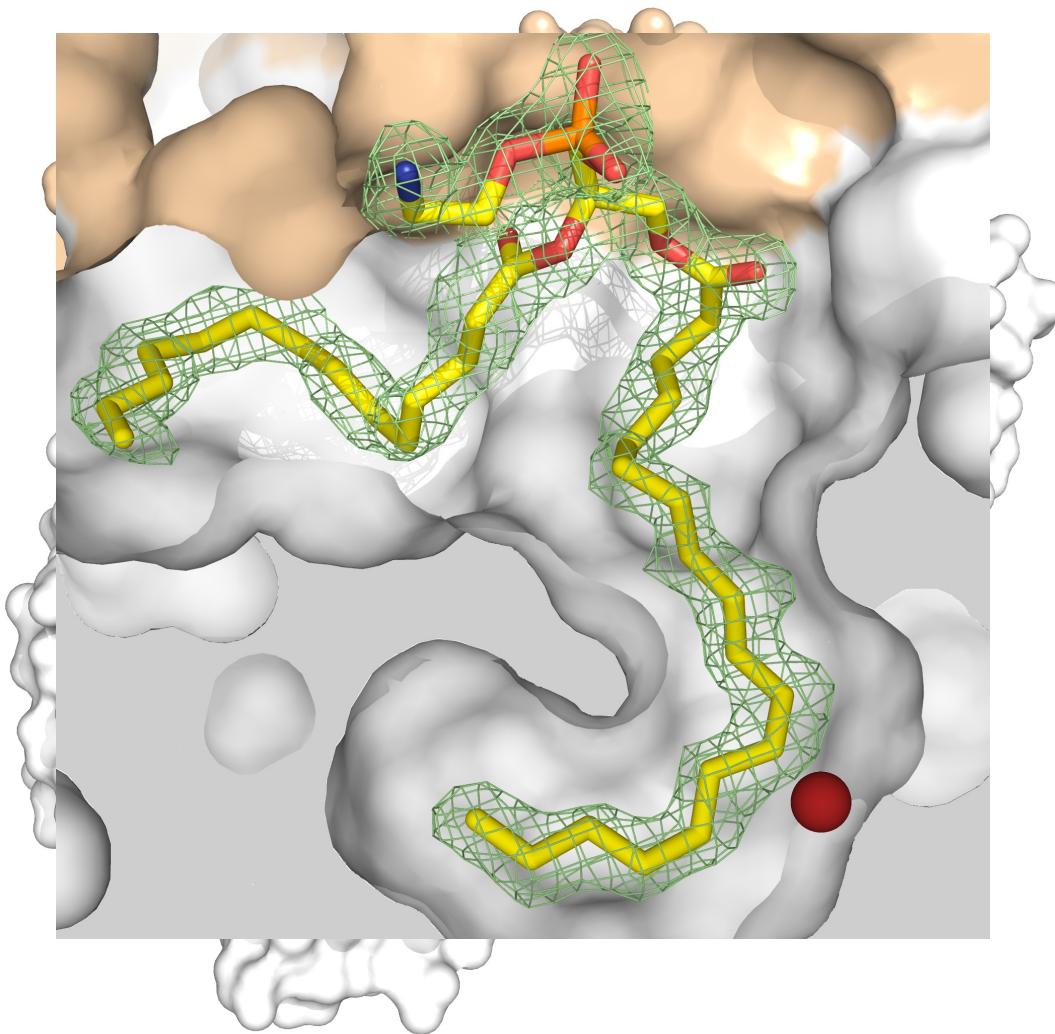
The spread of mammary carcinoma from the primary tumour to consecutive lymph nodes requires the lipoxygenase-derived eicosanoid 12(S)-HETE.

Kerjaschki D et al, J Clin Invest. 2011 (121): 2000-12

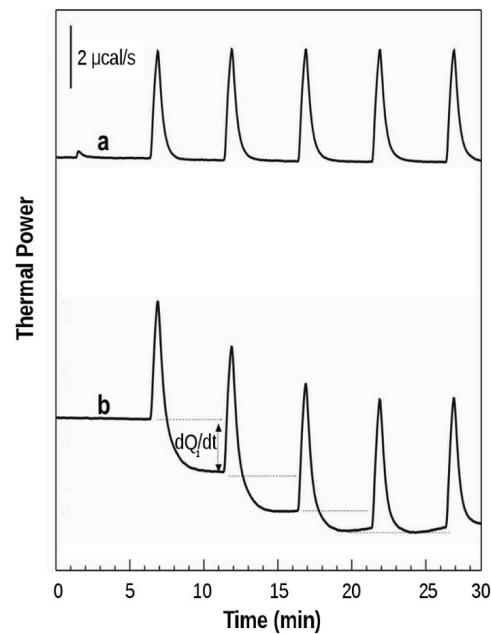
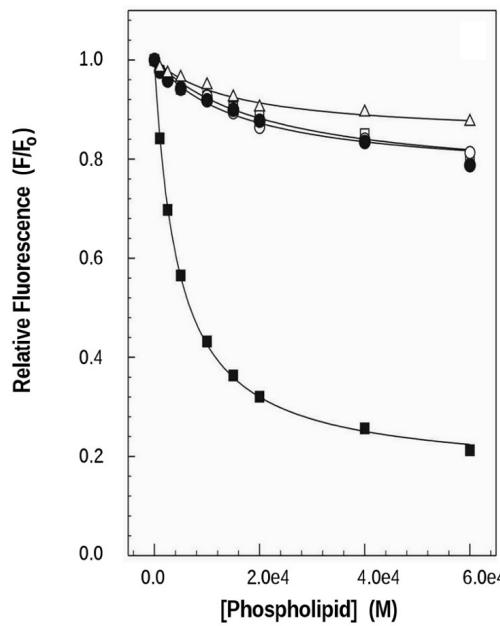
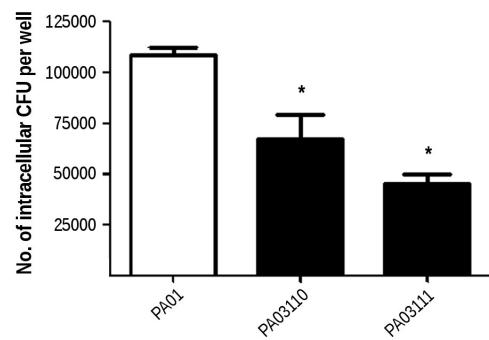
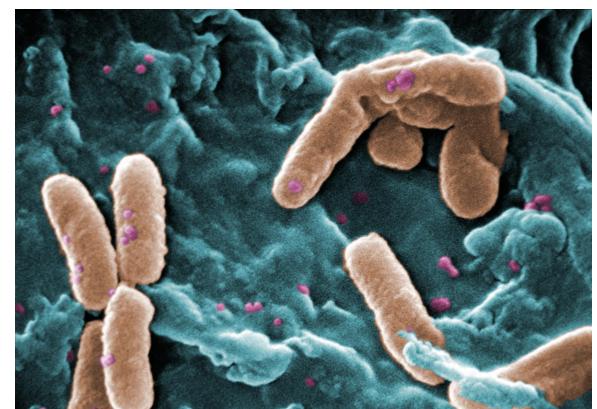


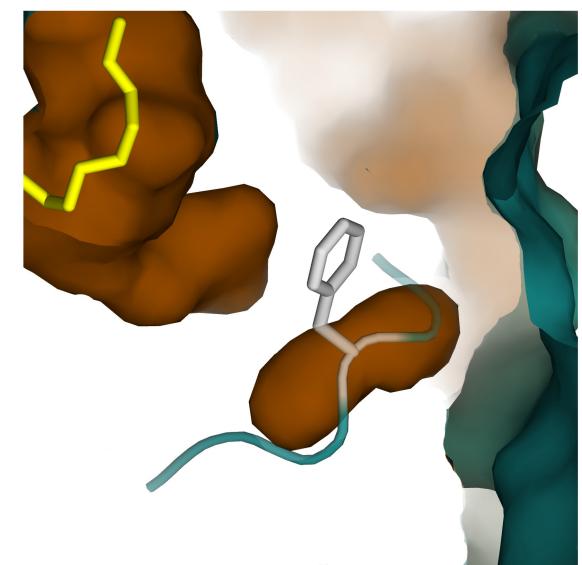
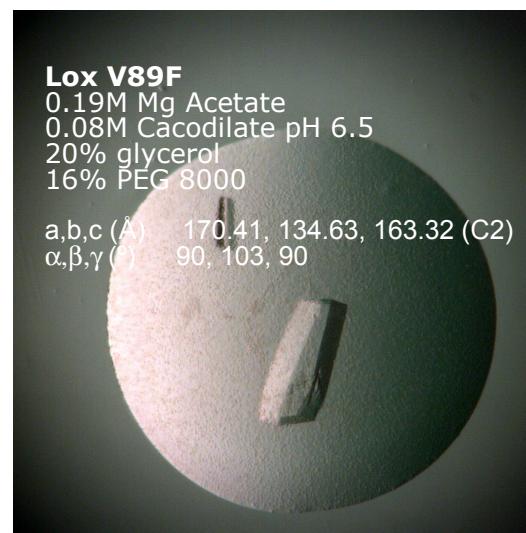
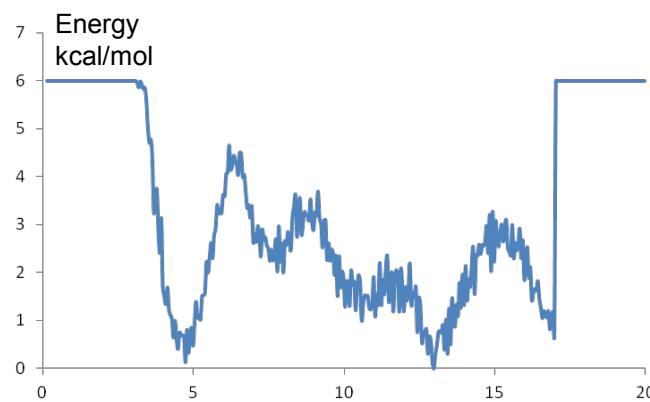
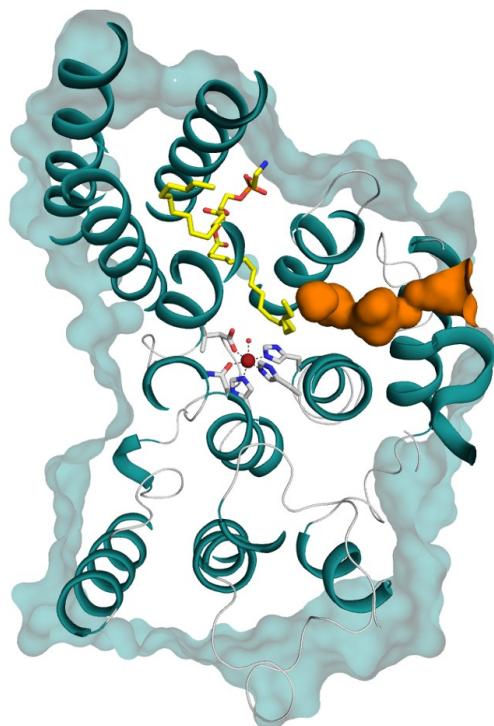
Lipoxygenasa Pa_{_}LOX

Complexe fosfolipídic

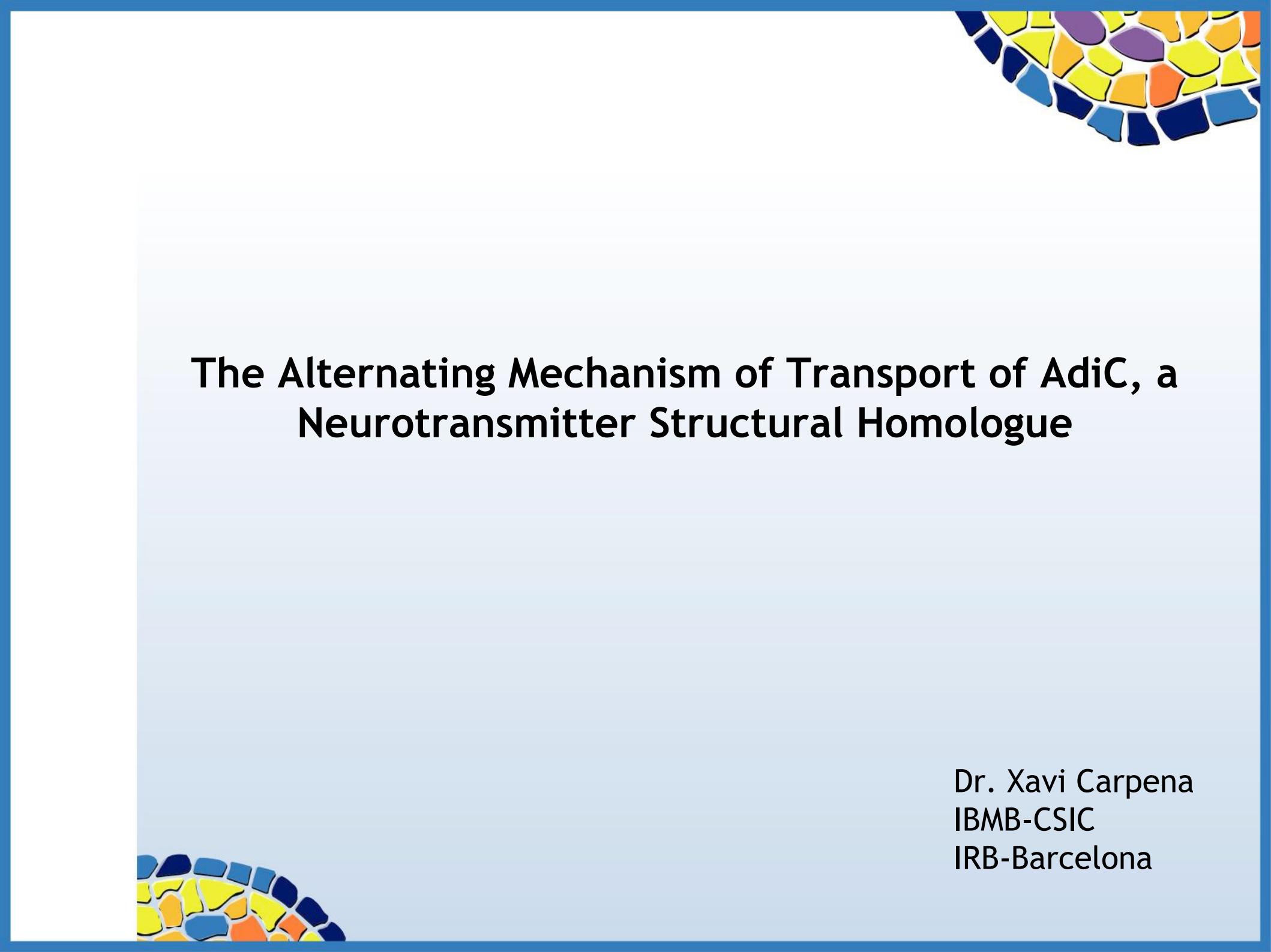


Val-Moraes SP, García-Fernández Q, Busquets M, Juan C, Oliver A, Ortiz A, Gaffney BJ, Fita I, Manresa A, Carpena X. *Structure and interaction with phospholipids of a prokaryotic lipoxygenase from Pseudomonas aeruginosa*. FASEB J. 2013 Dec;27(12):4811-21

(In vitro)**(In vivo)**

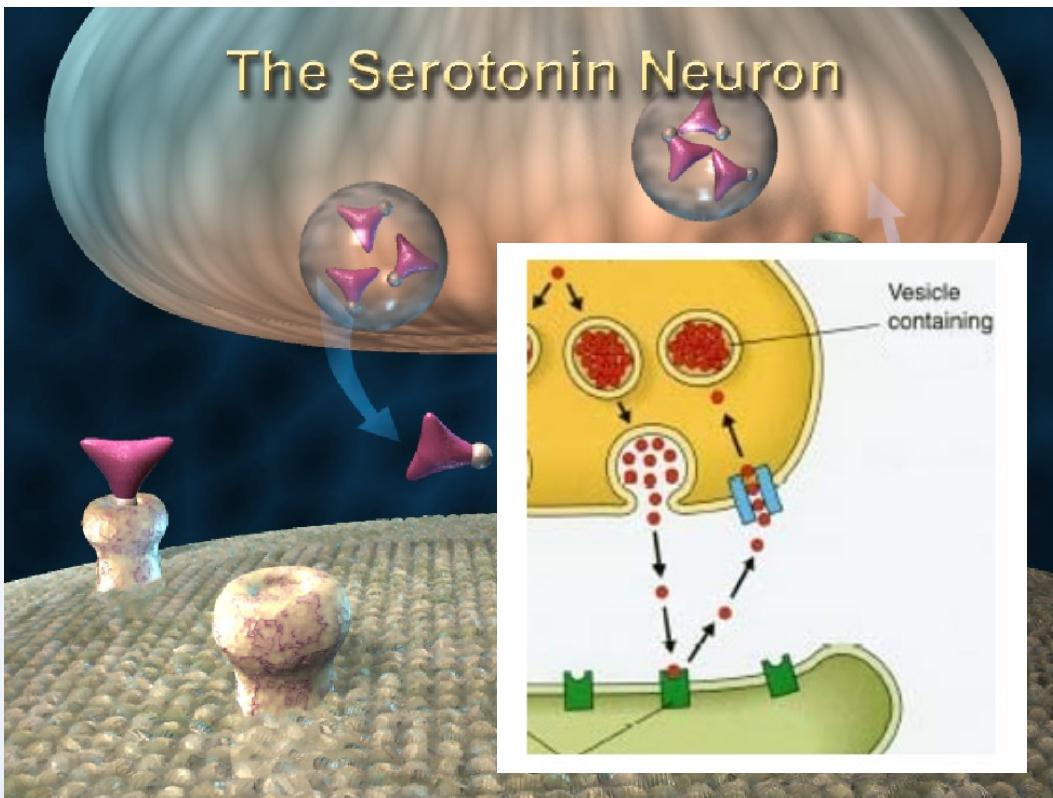


Mutants inactius cristal·litzats:
V89F → 0% actiu davant Àcids Grassos
V86F → <25% actiu davant Àcids Grassos



The Alternating Mechanism of Transport of AdiC, a Neurotransmitter Structural Homologue

Dr. Xavi Carpena
IBMB-CSIC
IRB-Barcelona

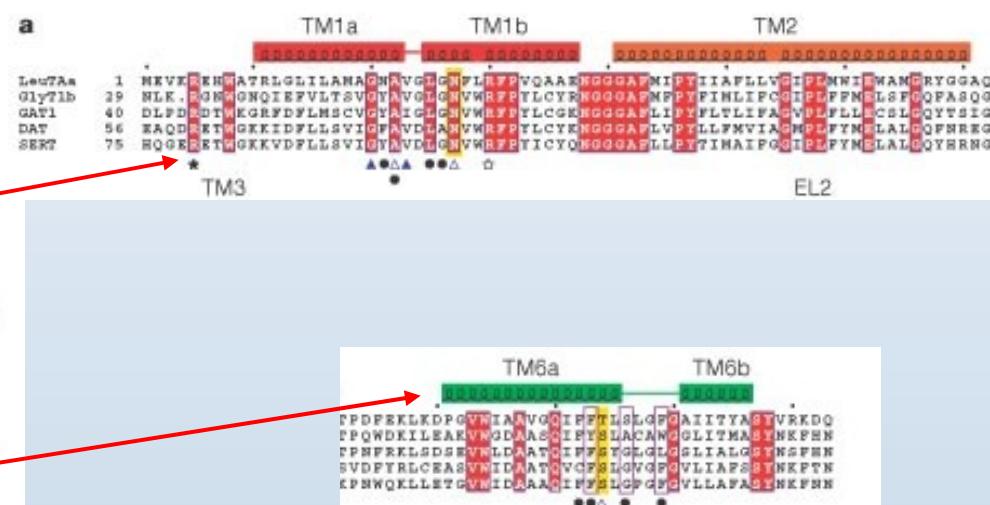
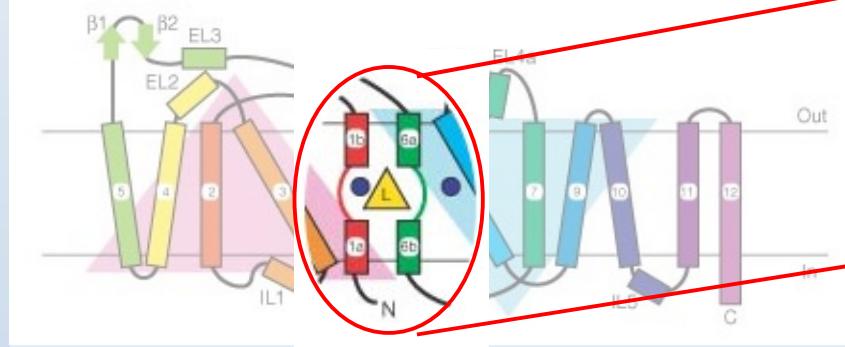


The Serotonin Neuron

The large Neurotransmitter: Na⁺ Symporter (NSS) family

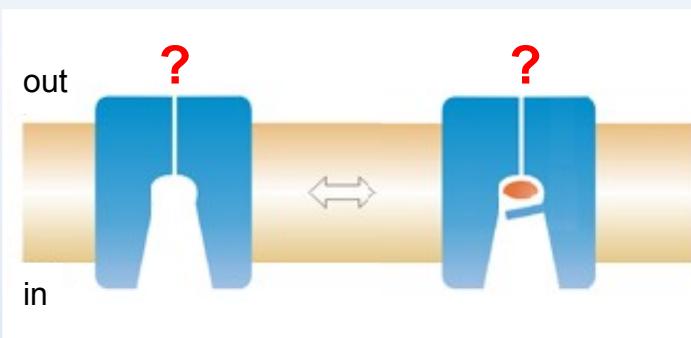
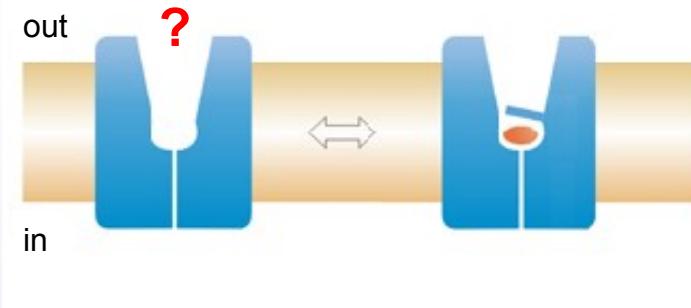
Monoamines (Dopamine (DAT), Norepinephrine (NET), Serotonin (SET))
Aminoacids (GABA, Gly, Pro, Tau)
Osmolytes (Betaine and creatine)

LeuT: a NSS structural homologue

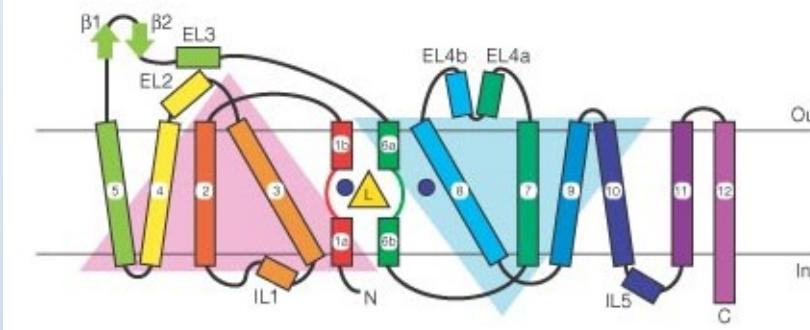


Yamashita A et al. (2005) Nature 437:215-23

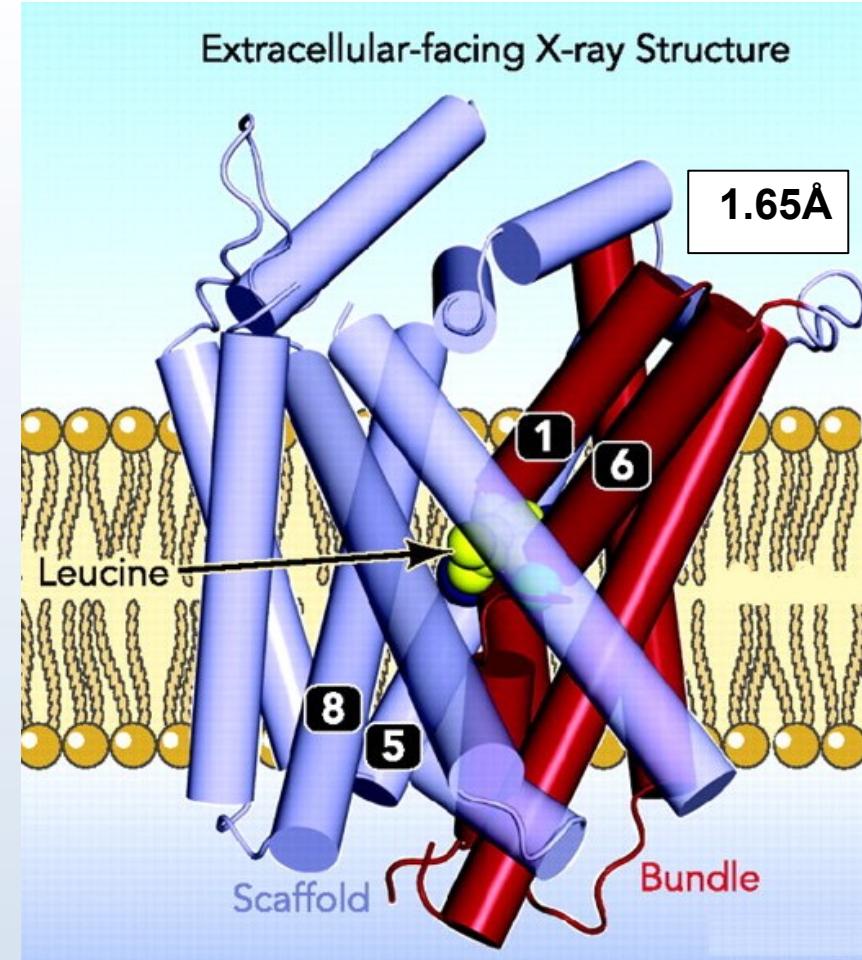
LeuT's crystal structure contribution: the alternating access transport mechanism



5+5 Inverted Repeats Transporters $(5+5)_{IR-T}$



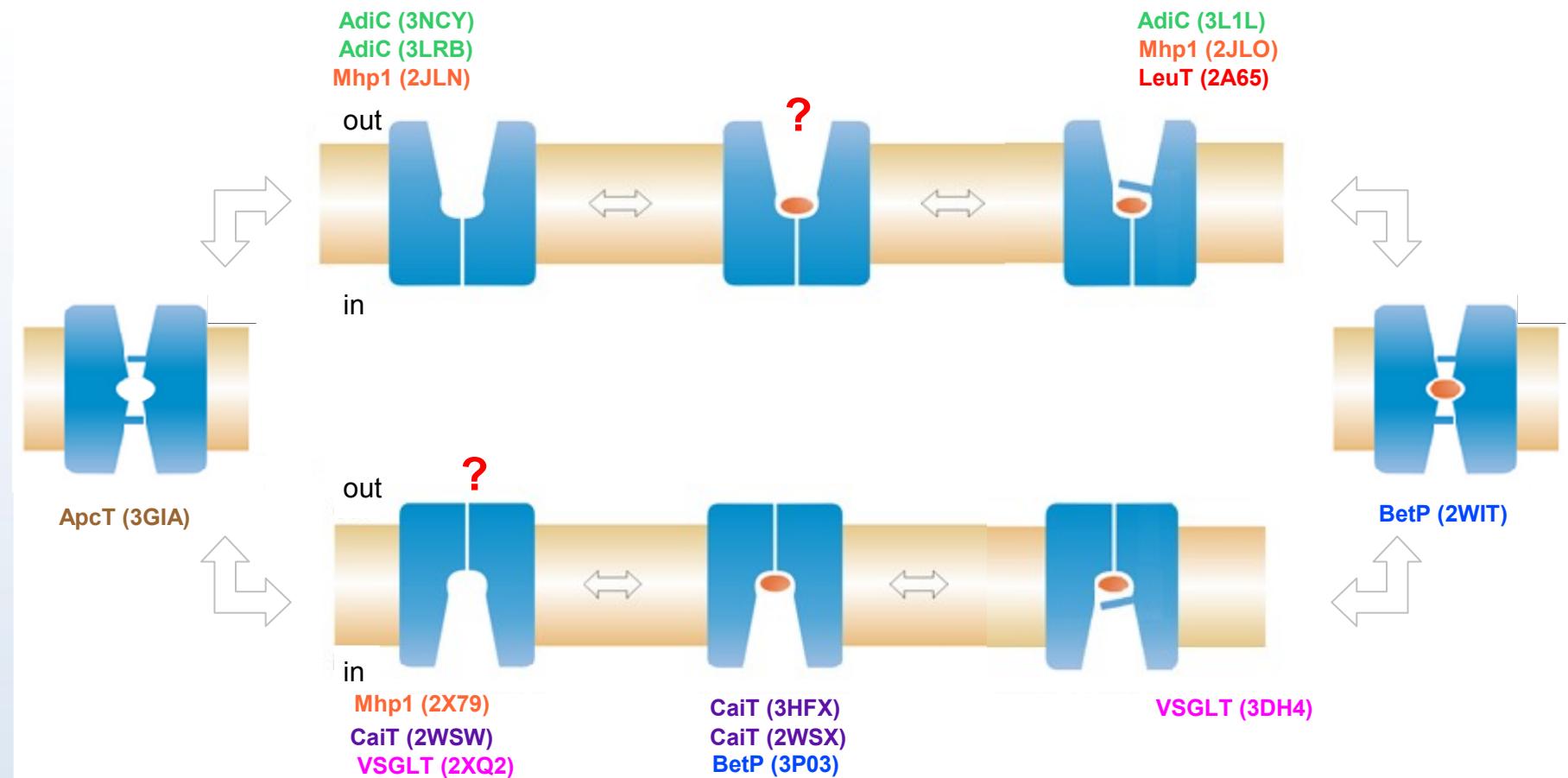
Yamashita A et al. (2005) Nature 437:215-23



but still many open questions:

- how did the substrate promoted the occlusion?
- what law translates outward into inward facing?
- do all $(5+5)_{IR-T}$ transits out <> in similarly?

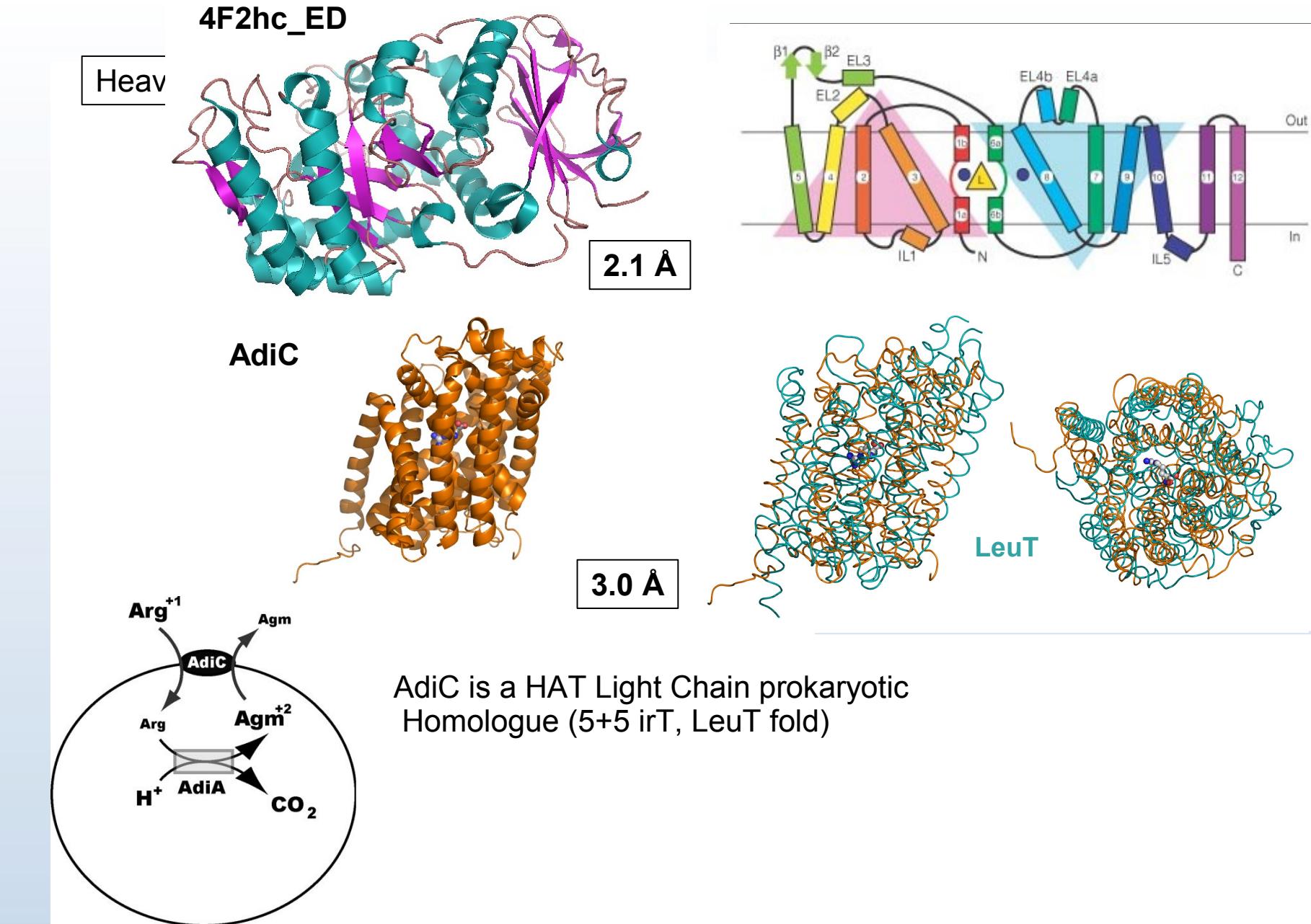
X-Ray Crystallography deepen our knowledge of (5+5)_{IR} Transporters



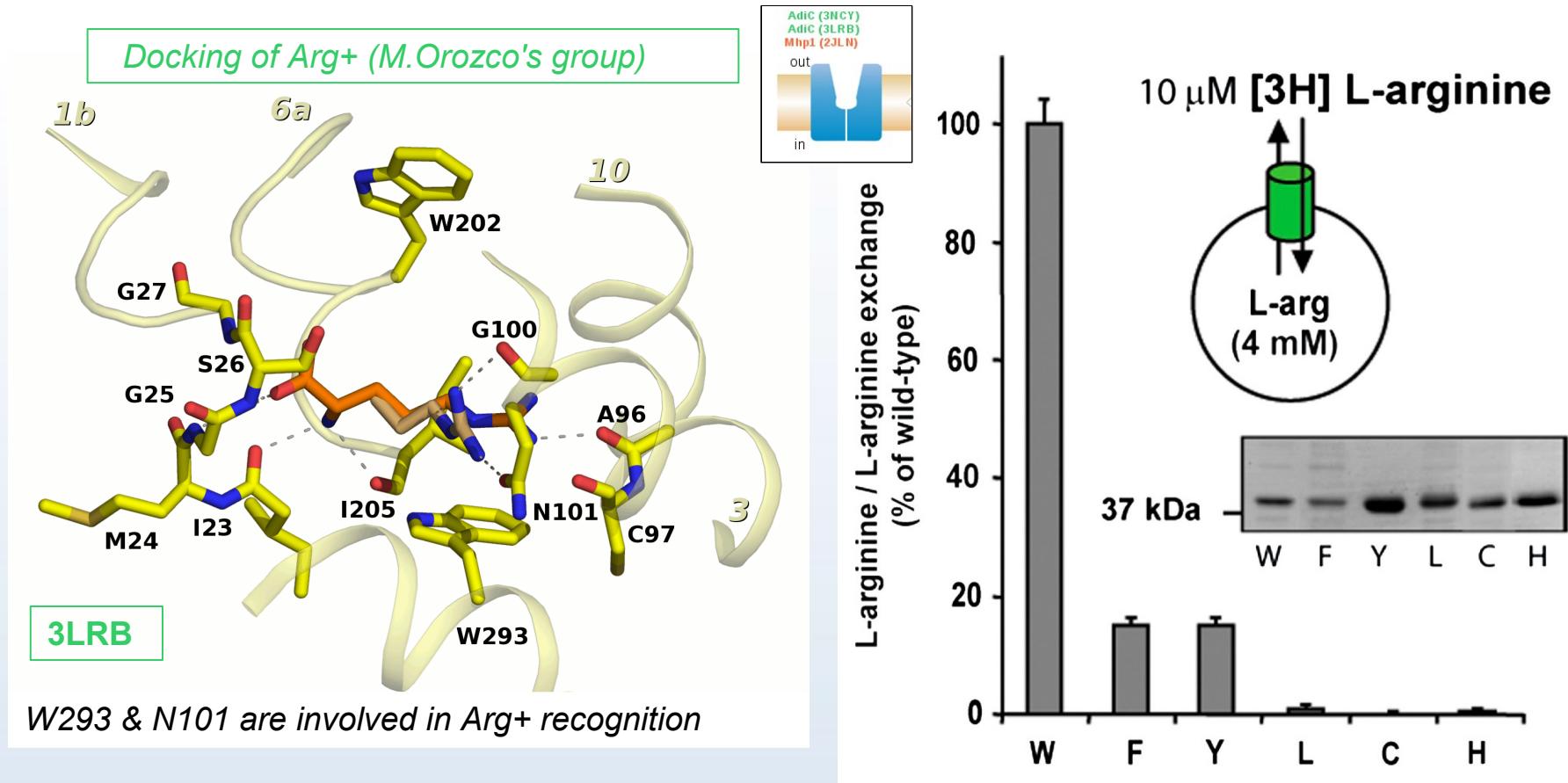
Ago 2005: LeuT, Out, Subs-Occ	2A65
Ago 2008: vSGLT, In, Subs-Occ	3DH4
Oct 2008: Mhp1, Out, Open	2JLN
Oct 2008: Mhp1, Out, Subs-Occ	2JLO
Feb 2009: BetP, Eq, Full-Subs-Occ	2WIT
Jun 2009: AdiC, Out, Open	3LRB
Jul 2009: AdiC, Out, Open	3NCY

Ago 2009: ApcT, Eq, Full-Occ	3GIA
Feb 2010: AdiC, Out, Subs-Occ	3L1L
Mar 2010: CaiT, In, Semi-Occ	3HFX
Mai 2010: Mhp1, In, Open	2X79
Set 2010: CaiT, In, Semi-Occ	2WSX
Set 2010: CaiT, In, Open	2WSW
Ago 2008: vSGLT, In, Subs-Occ	2XQ2
Mar 2011: BetP, In, Semi-Occ	3P03

LeuT-fold is also present in the light chain of Heteromeric Aminoacid Transporters (HATs)

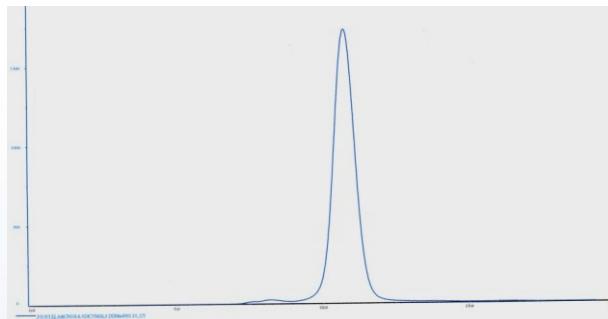


Structural determinants of Arg+ binding: N101 mutant

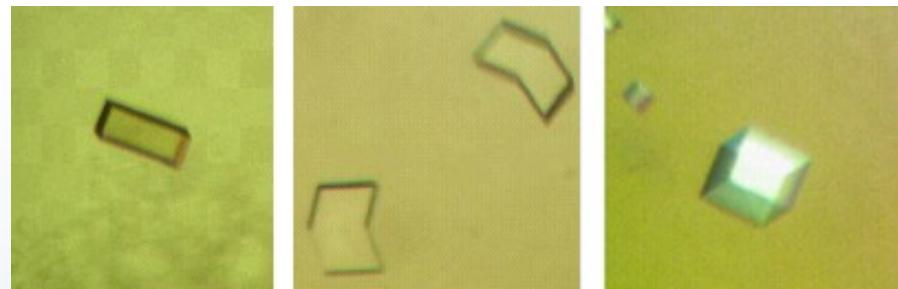


AdiC variant	Transport kinetics #		Binding (ITC) Apparent Kd (μ M)
	V_{MAX} (pmol/ μ g · min)	K_M (μ M)	
wild-type	36, 64	31, 42	95, 97, 100, 122
W293Y	5.1, 8.7	122, 133	no signal
N101A	0.7, 0.8	93, 111	no signal
N101D	89, 101	110, 100	112

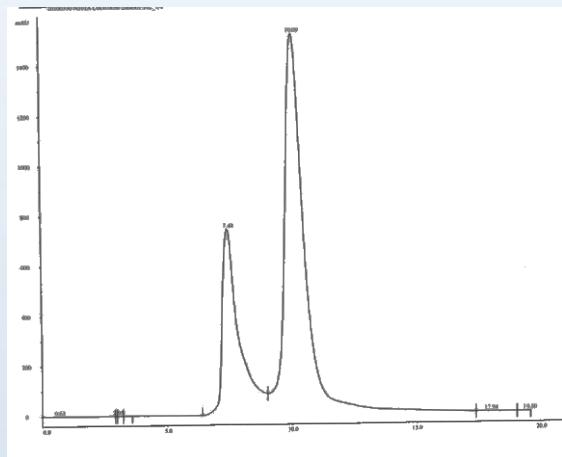
A Crystallographic Structural Project: AdiC



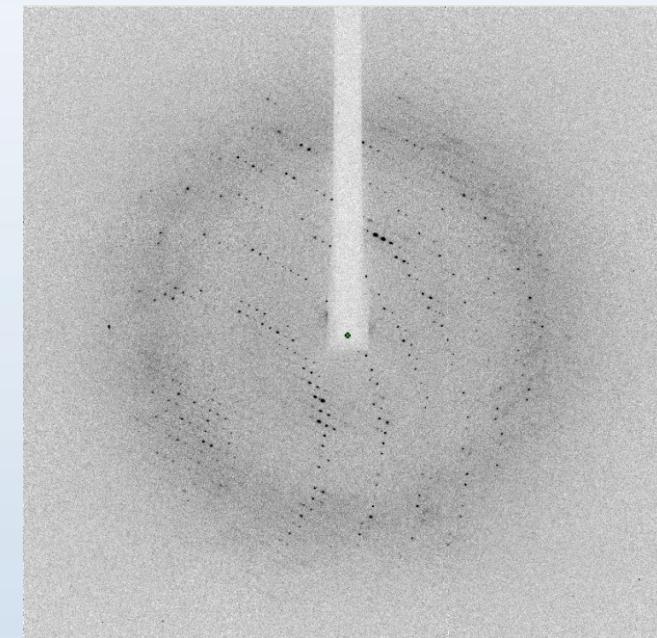
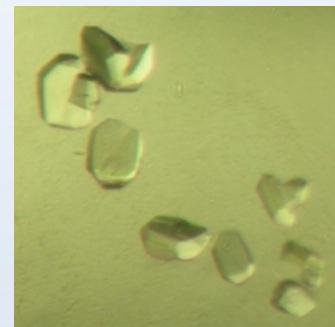
DDM (solubilized)/DM(SEC)



100 – 200 μ m

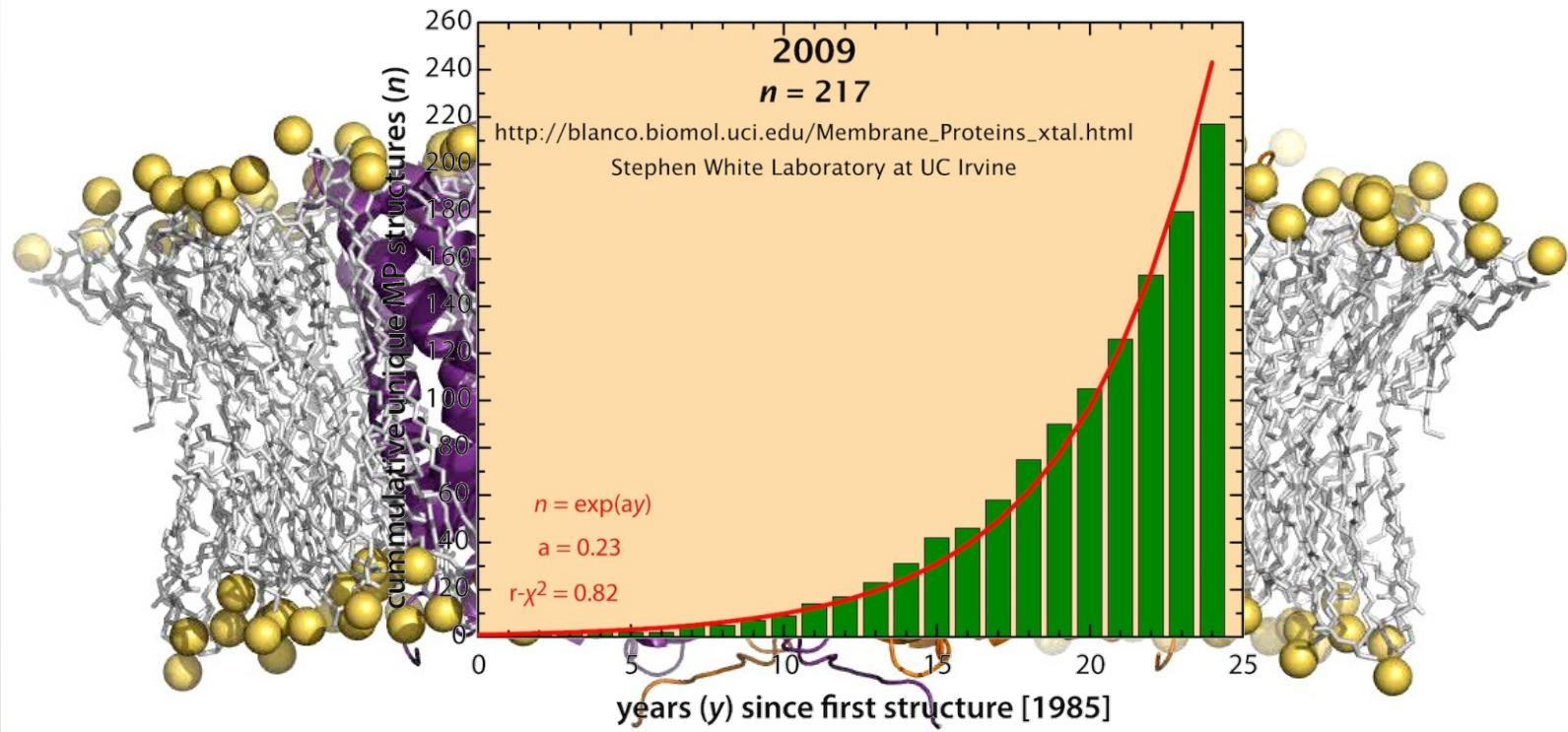


25-30% PEG400
0.1M Tris 8.2 – 8.8

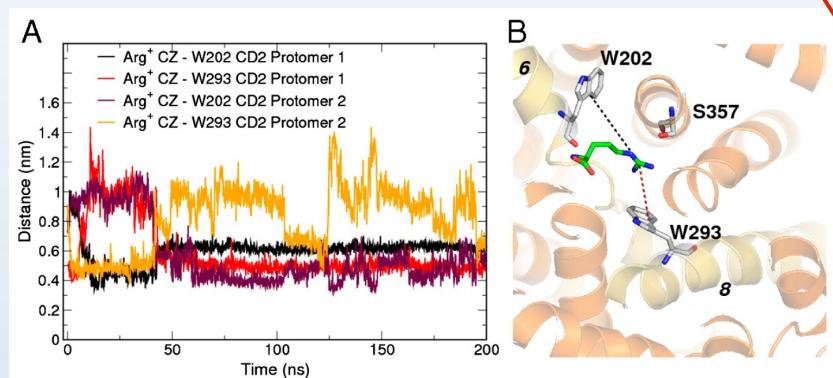
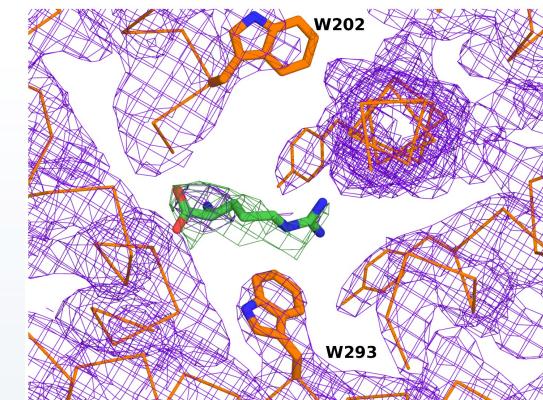
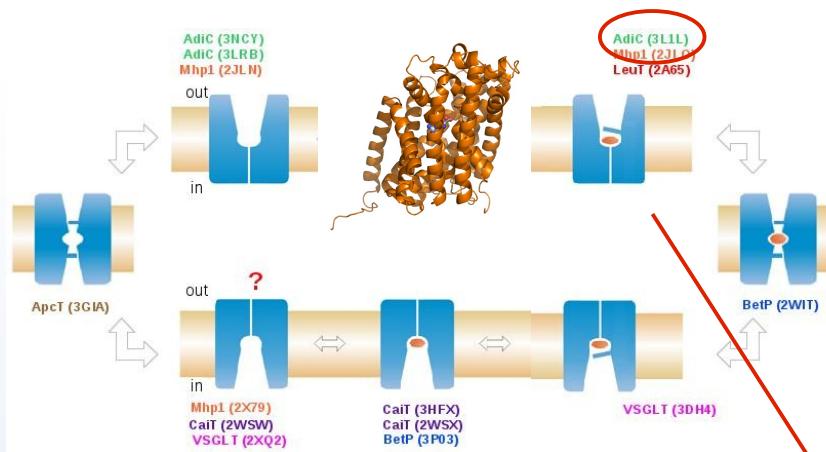


Solubilized: CYMAL6 (1.5%) + 2mM Arg

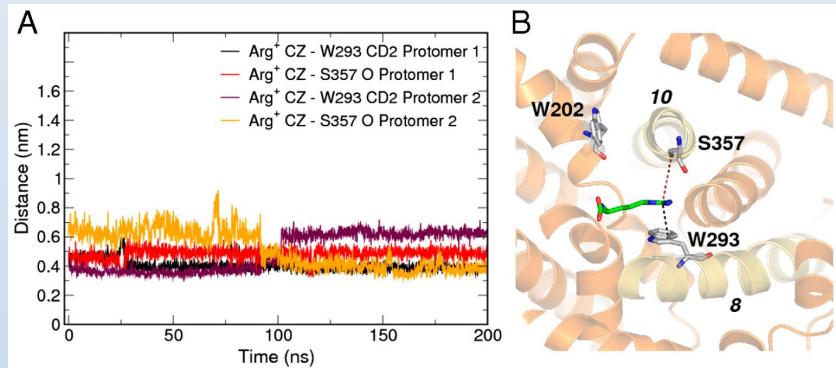
SEC: CYMAL6 (0.056%) + 2mM Arg



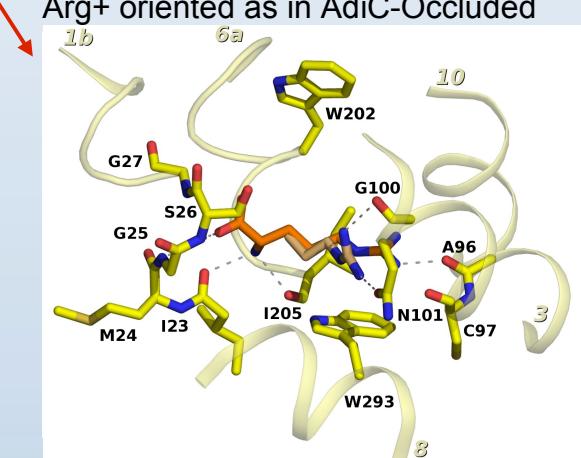
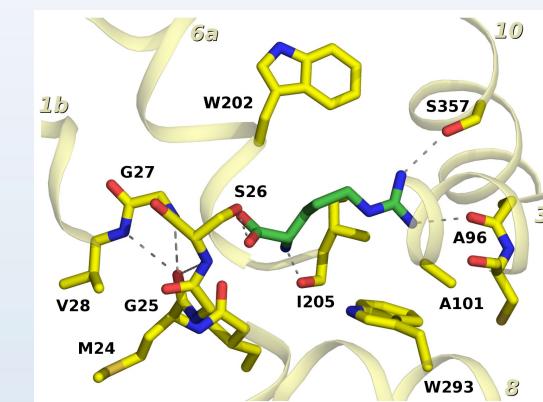
The Missing step of the transport cycle



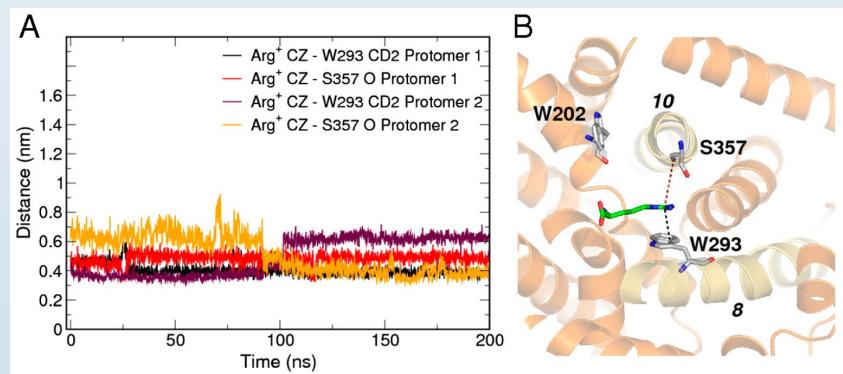
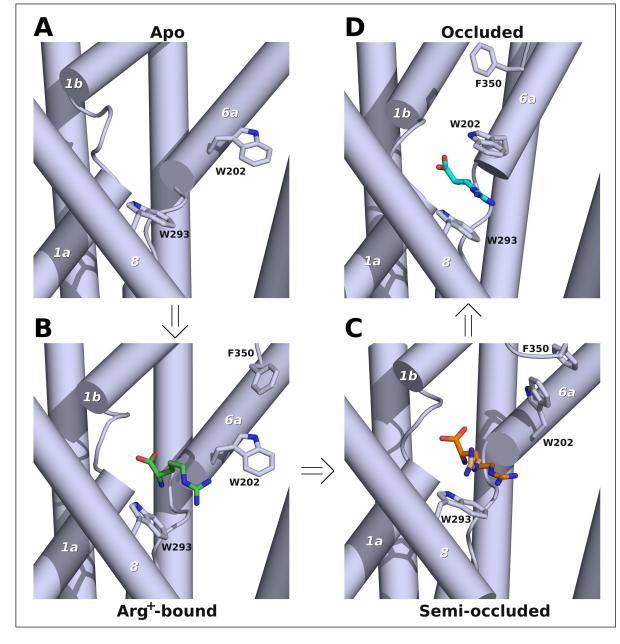
Fluctuates between two π environments



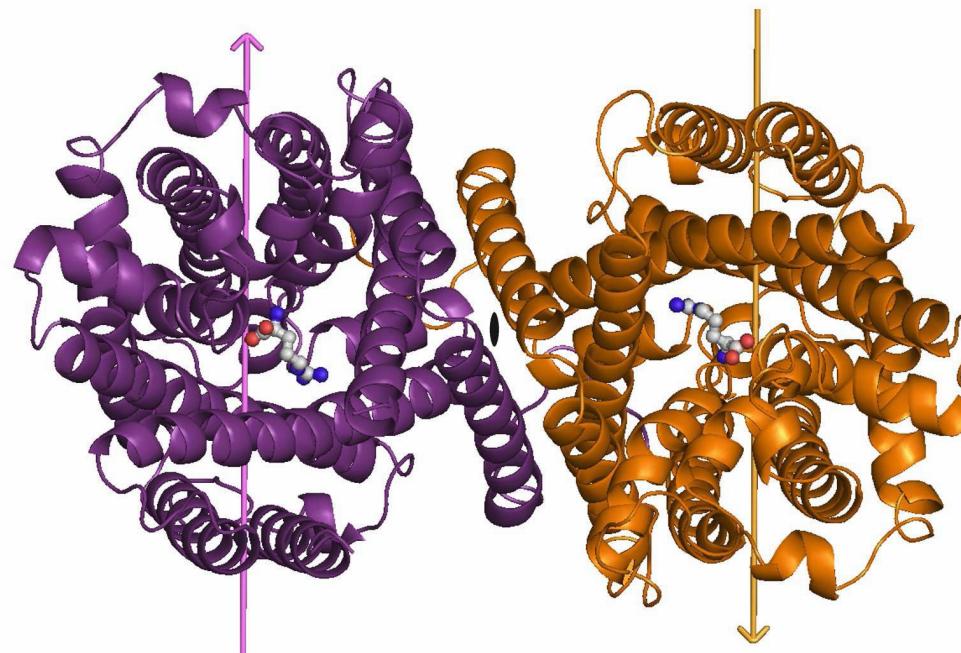
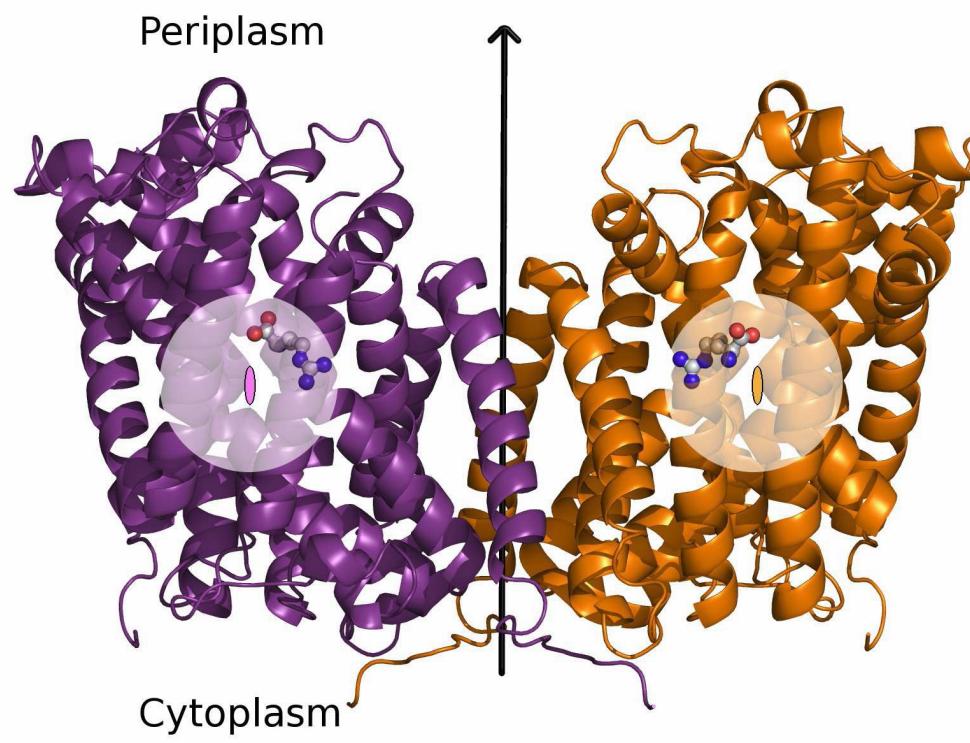
Docking on 3LRB structure represents a semi-occluded state



Arg⁺ induced fitting



A fluctuable Arg+ is present in its active site



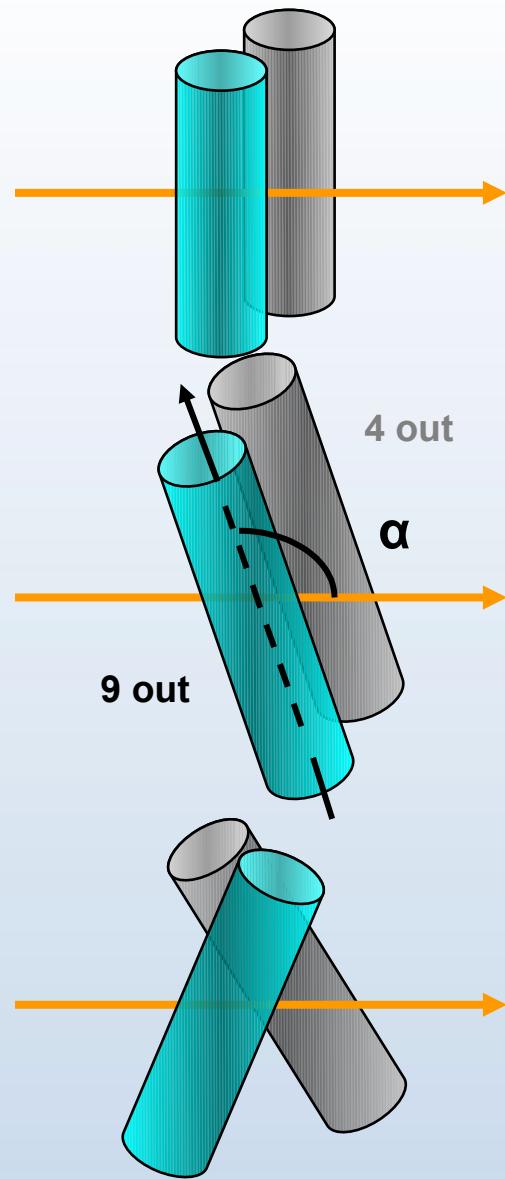
The existence of a two-fold axis is a common event in all (5+5) T



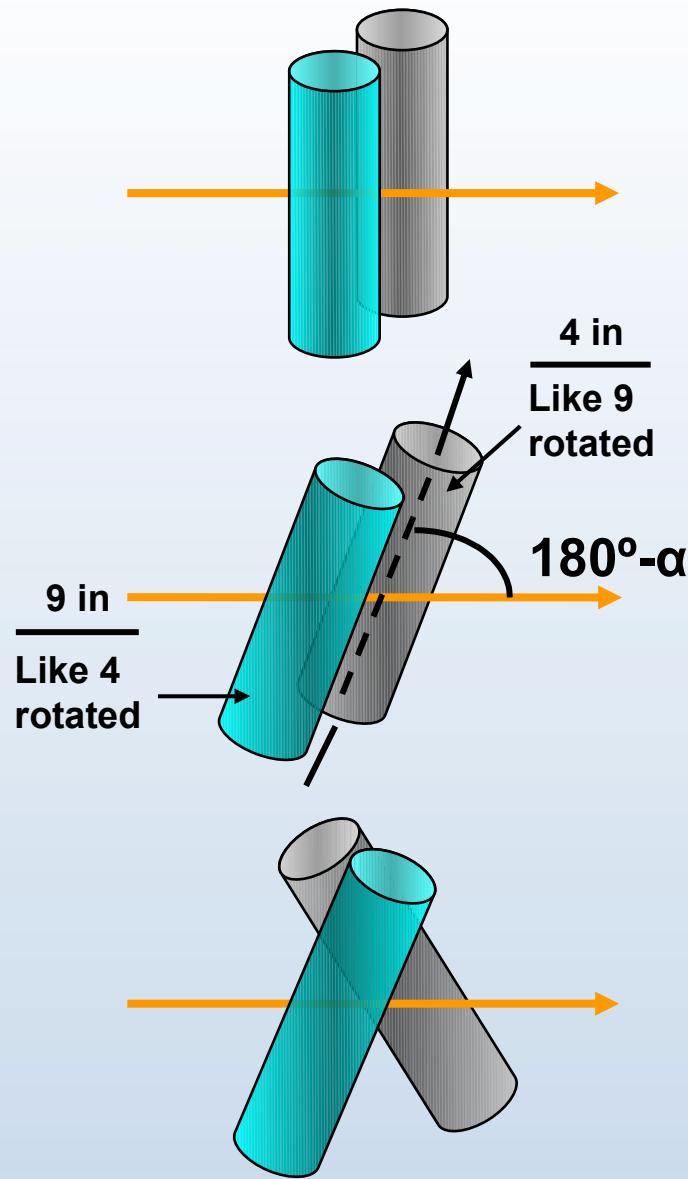
	Rot. 1	Rot. 2	Error Traslació	Dist. CM Subs.
AdiC_3L1L	177.2	91.24	0.97	3.09
AdiC_N101A	175.2	89.98	0.02	5.78
ApcT_3GIA	178.2	90.86	0.66	8.10 (wats)
BetP_2WIT	179.9	91.00	0.74	0.87
CaiT_2WSX	177.9	91.65	1.12	3.37
CaiT_3HFX	176.2	90.70	0.48	4.43 / 3.02
LeuT_2A65	172.7	89.89	0.09	1.63
Mhp1_2JLN	170.6	91.33	0.97	-----
Mhp1_2JLO	175.1	91.59	1.11	5.25
vSGLT_3DH4	178.9	90.27	0.22	5.02

Protein architecture (TM orientation versus 5+5 axis) dictates the conformational changes

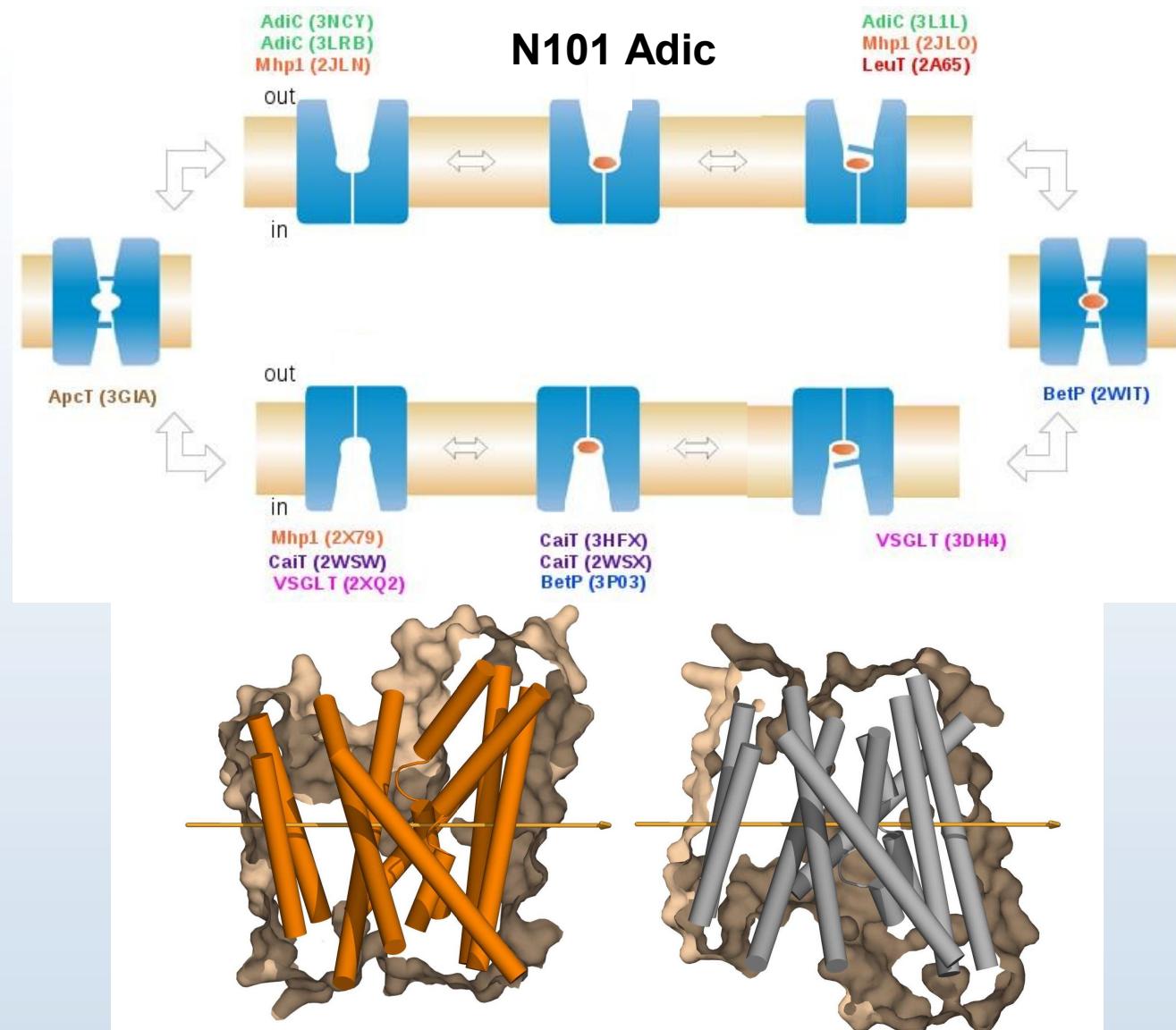
Original



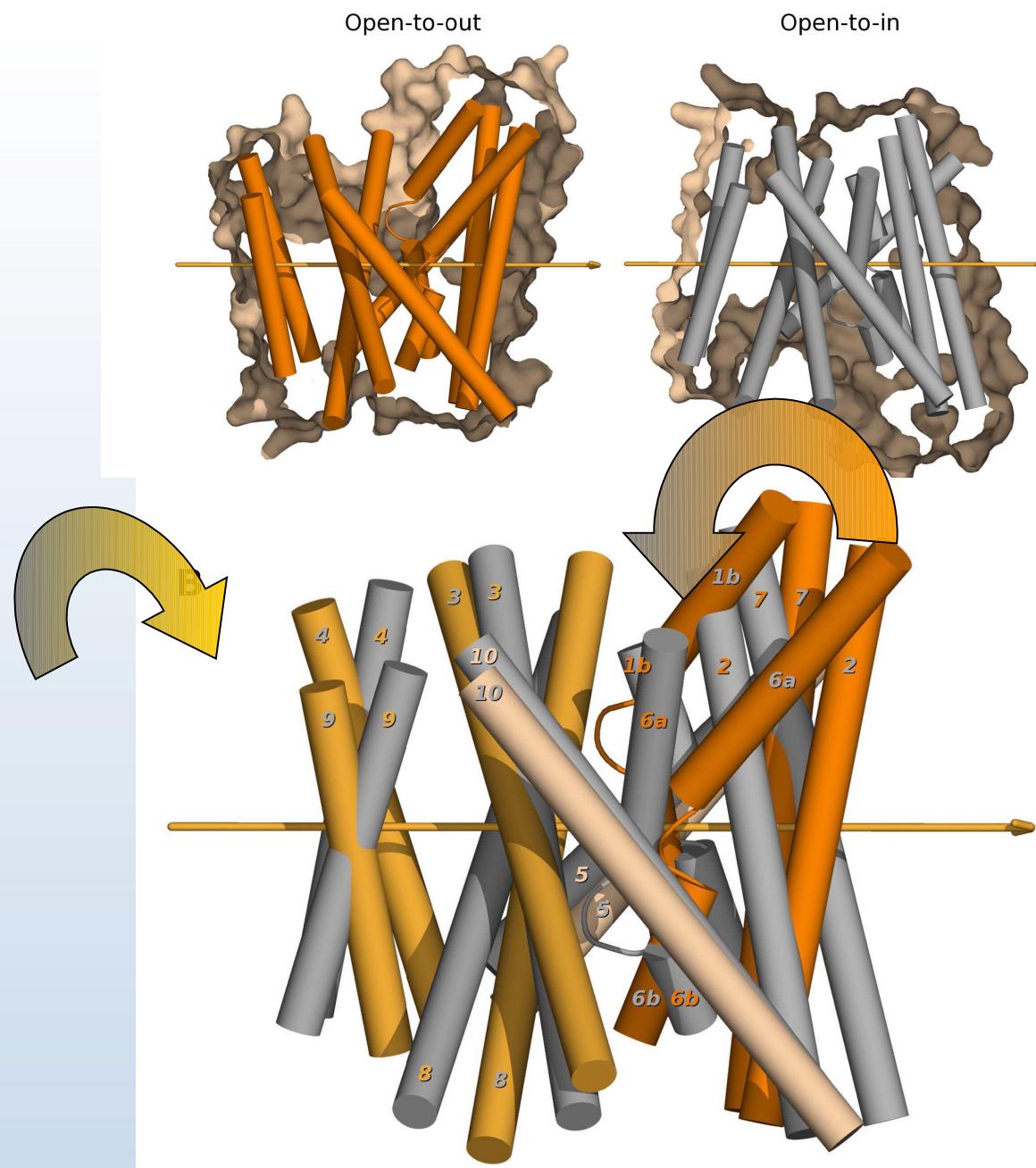
Symmetry fabrication



Is it possible to predict conformational changes of AdiC during transport?

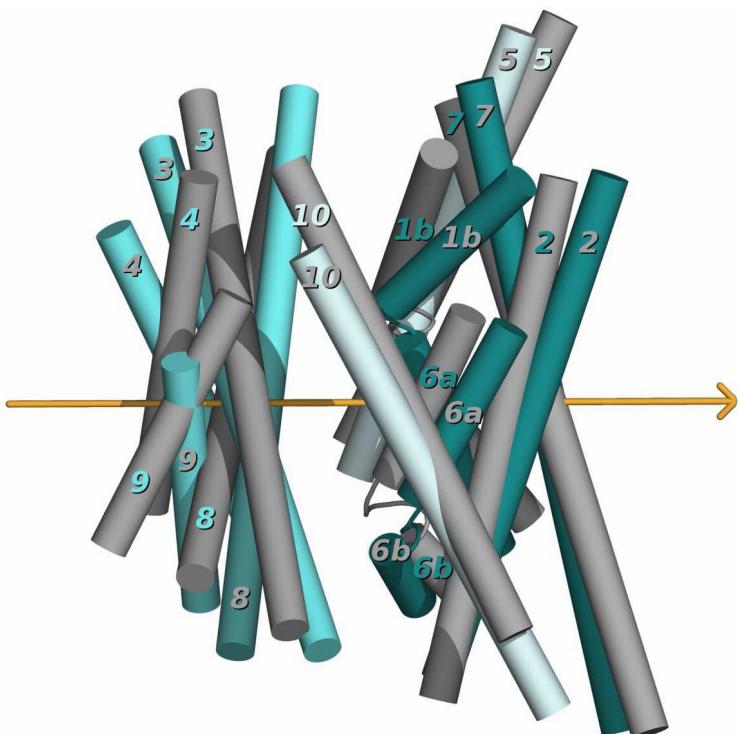


Unified vision of (5+5)_{IR}-T global movements



Symmetry model validation using Mhp1

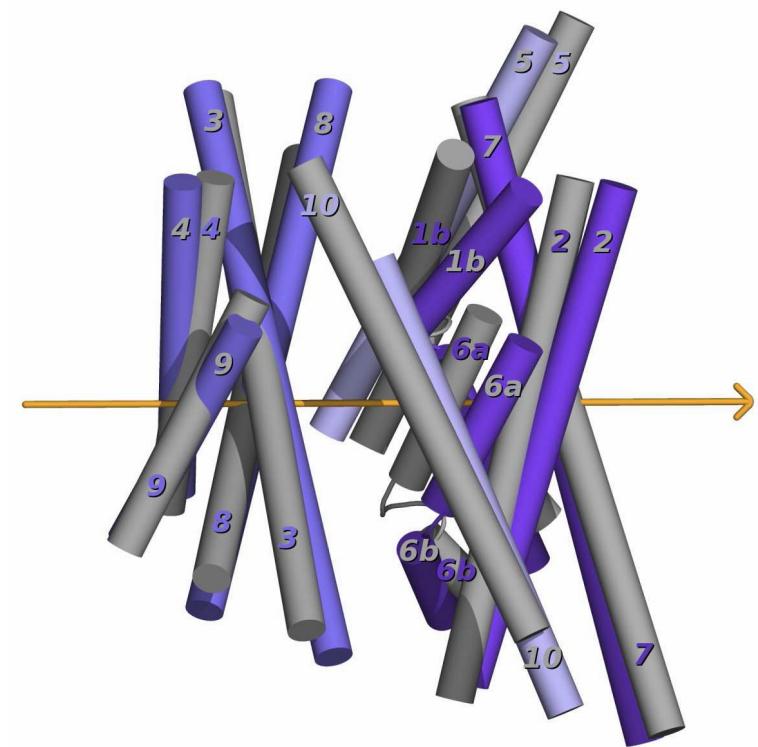
Open-to-out (MHP1)



2JLN

Weyand S et al (2008) *Science* 322:709-13

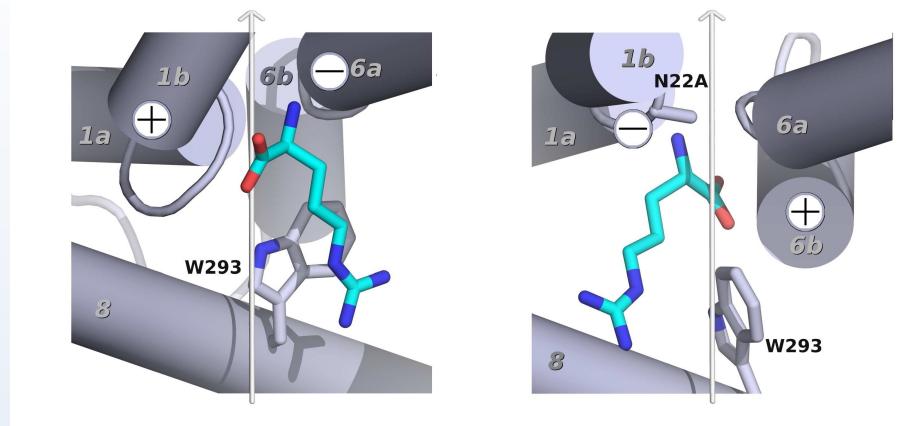
Open-to-in (MHP1)



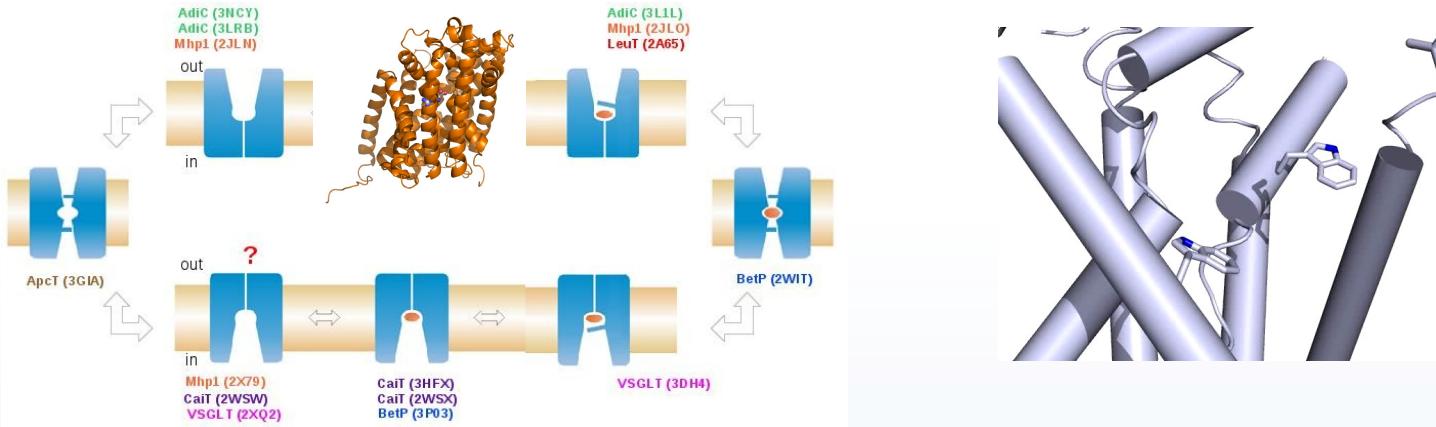
2X79

Shimamura T et al (2010) *Science* 328:470-3

And what happens to the substrate? A second likely binding site in AdiC

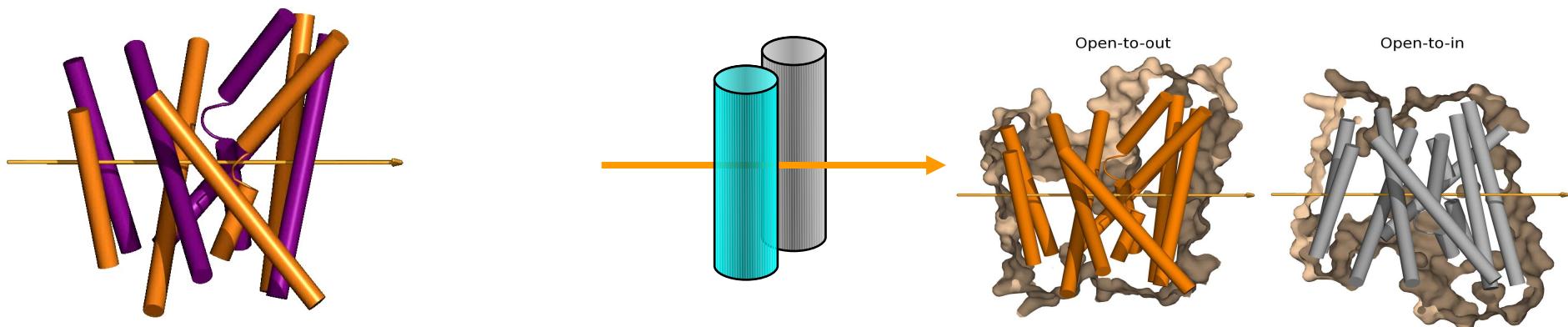


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BetP_2WIT	179.9	91.00	0.74	0.87
CaiT_2WSX	177.9	91.65	1.12	3.37
CaiT_3HFX	176.2	90.70	0.48	4.43 / 3.02
LeuT_2A65	172.7	89.89	0.09	1.63
Mhp1_2JLN	170.6	91.33	0.97	-----
Mhp1_2JLO	175.1	91.59	1.11	5.25
vSGLT_3DH4	178.9	90.27	0.22	5.02



CONCLUSIONS

- Last step of the alternating access model of transport
- Induced fitting of the substrate
- All (5+5)_{IR_T} are related by a pseudo-2-fold axis
- Each (5+5)_{IR_T}'s architecture (with respect to its internal symmetry axis), dictates its conformational changes
- All conformational states of (5+5)_{IR_T} are symmetrically related



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Prof. Ignasi Fita's group



Prof. Manuel Palacín's group

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B. Strandberg et al. 50 Years of Protein Structure Analysis.
JMB (2009). 392, 2-32

Biología Estructural 1986/2011. JL Campos, C. Abad-Zapatero, I. Fita
Crystallography across the sciences.