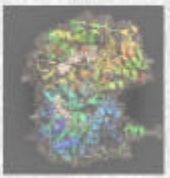
Home | Publications | Projects | Lab Members | Location | Links

Structural Biology Group

University of Pavia



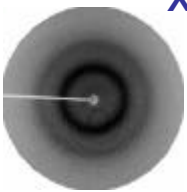
A common theme to the laboratory's research projects is the investigation of medically relevant enzymes with interesting chemical properties, such as complex multifunctional systems and proteins performing unusual catalytic functions. The core of the research activity is represented by X-ray crystallography, employed to study protein three-dimensional structures. This is complemented by other approaches such as site-directed mutagenesis, analysis of enzyme kinetics and computational chemistry. Current research is focusing on enzymes of the neurotransmitter metabolism, on a protein complex involved in chromatin remodeling, on an enzymatic system for the biosynthesis of a class of membrane phospholipids, on the structural genomics of viral replicative enzymes, and on the reaction of flavoenzymes with oxygen.

Vizier ACS PRF NATIONAL INSTITUTES OF HEALTH OXY GREEN SEVENTH FRAMEWORK PROGRAMME AIRC EMBO

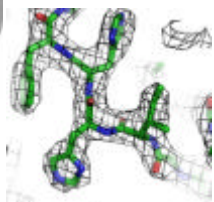
Department of Genetics and Microbiology "A. Buzzati-Traverso"
University of Pavia

www.unipv.it/biocry

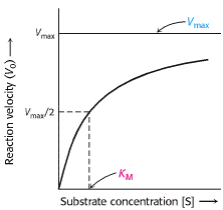
Structural Molecular Biology in Pavia



X-ray crystallography



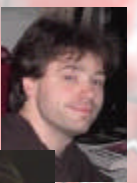
Biochemistry



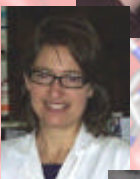
Computational chemistry

Biochemistry and Structure of Human Lysine-Specific Demethylase LSD1

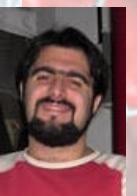
Federico Forneris

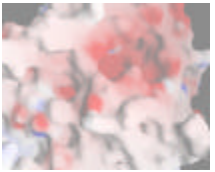


Claudia Binda

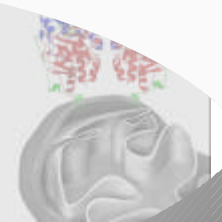


Aristotele Karytinos

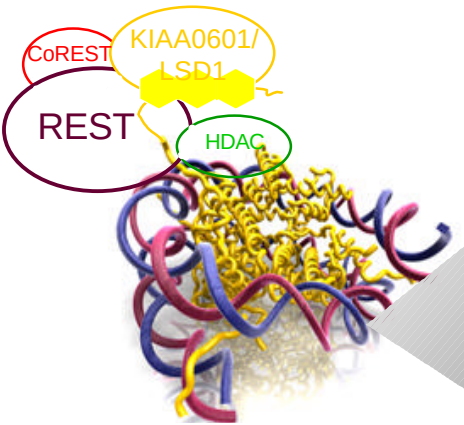




POLYAMINE OXIDASE



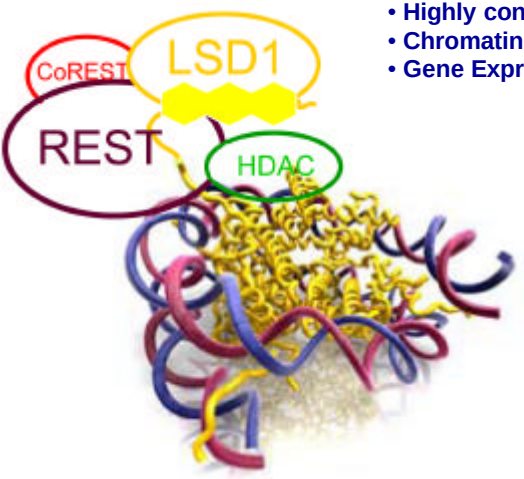
MONOAMI



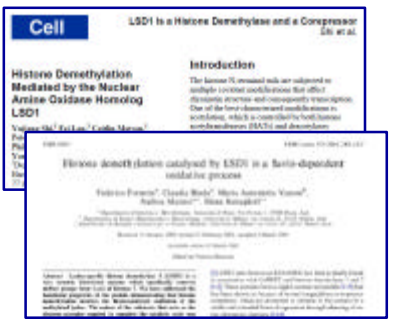
HISTONE DEMETHYLASE

Nat. Struct. Biol. 9, 22
(2005) *PNAS* 102, 12684
Binda et al. (2006) *Neurology* 67, S5

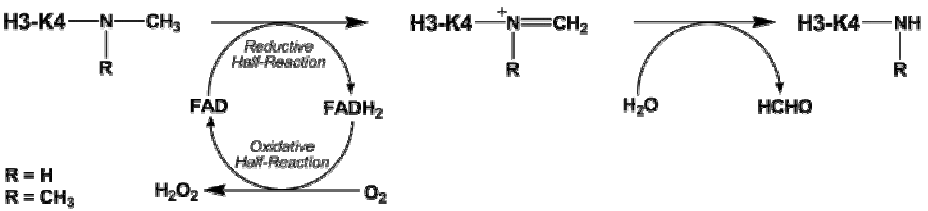
Human LSD1/KIAA0601: a nuclear flavin-dependent amine oxidase?



- Highly conserved in eukaryotes
- Chromatin Remodelling
- Gene Expression Regulation



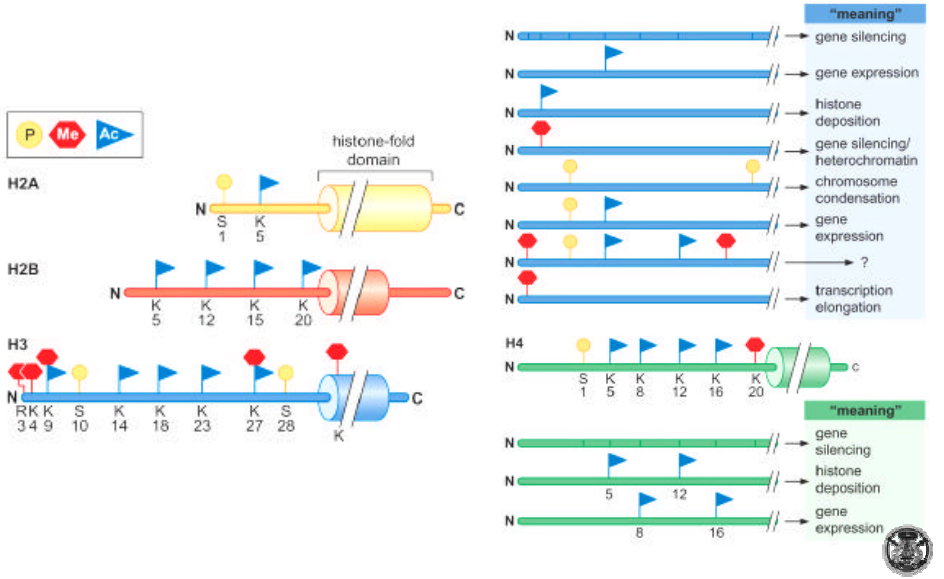
LSD1 catalyses histone demethylation through an oxidative process



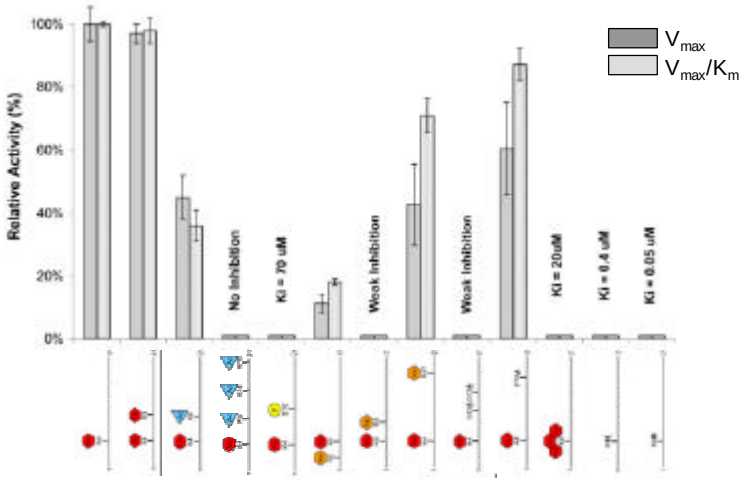
Highly specific for mono- and dimethylated H3-Lys4

Forneris et al., *FEBS Lett.* (*2005)* 579, 2203,2207

Histone Modifications and the Histone Code Hypothesis

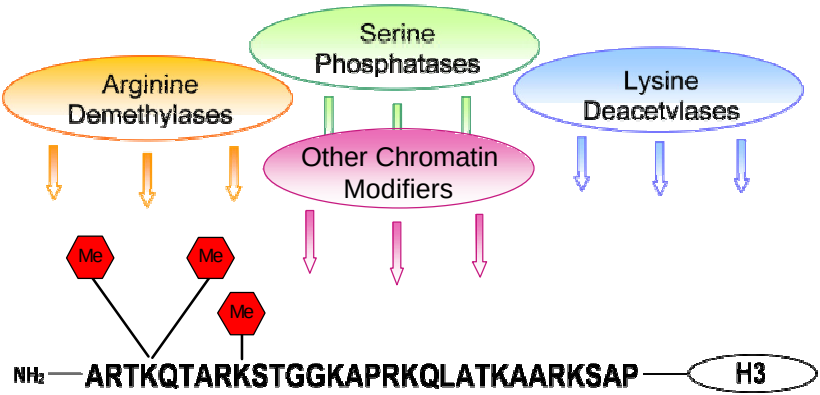


LSD1 reads the histone code



- Forneris et al., *J. Biol. Chem.* (2005) 280, 41360,41365
- Forneris et al., *J. Biol. Chem.* (2006) 281, 35289,35293

LSD1 as a switch between chromatin states

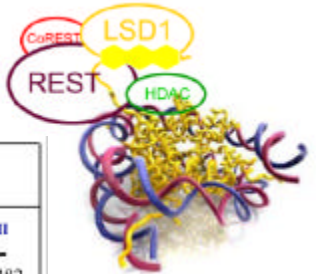


Heterochromatin Gene REPRESSION

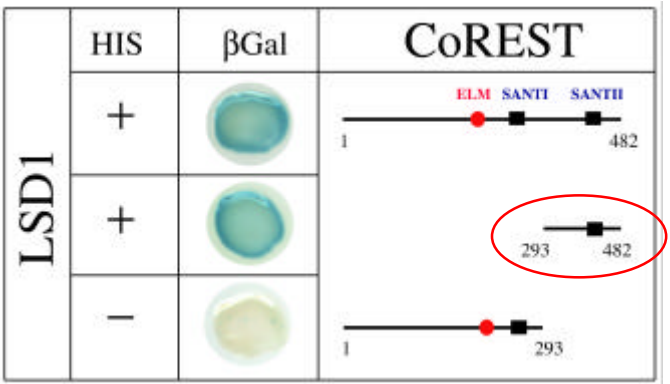
Euchromatin Gene ACTIVATION

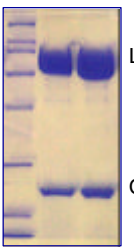
LSD1 in corepressor complex(es)

LSD1 and CoREST: Minimal domains required for interaction

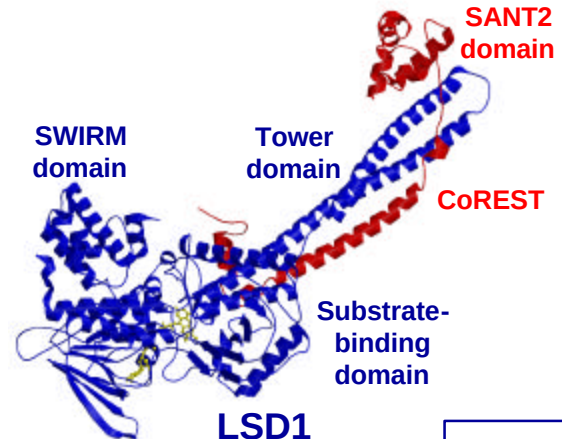


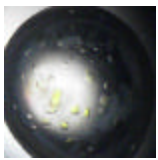
Yeast two-hybrid assay





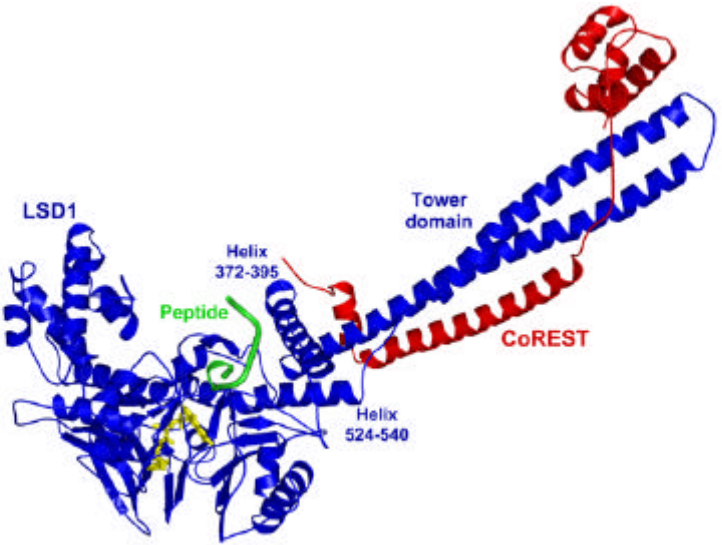
CoREST stabilizes LSD1 and increases its enzymatic activity





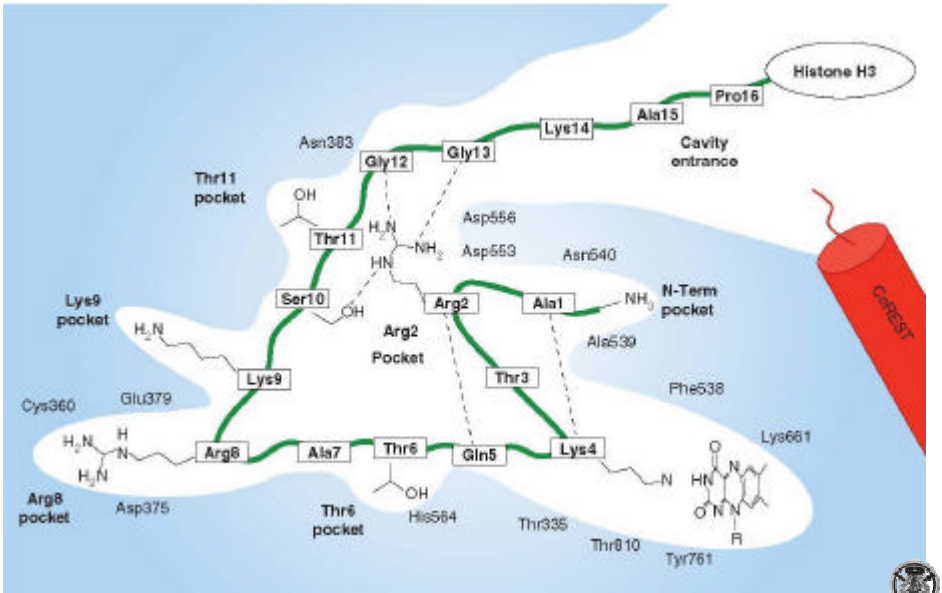
	v_{max} (min^{-1})	K_m (μM)
LSD1	3.4	3.4
LSD1+CoREST	**7.3**	**5.1**

Histone H3 recognition by LSD1-CoREST

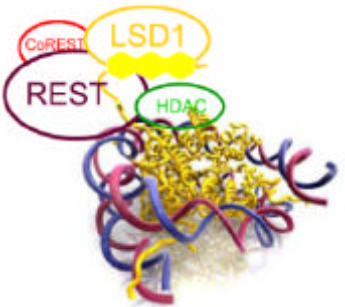


- Forneris et al., *J. Biol. Chem.* (2007) 282, 20070-20074
- Forneris et al., *Trends Biochem. Sci.* (2008) in press

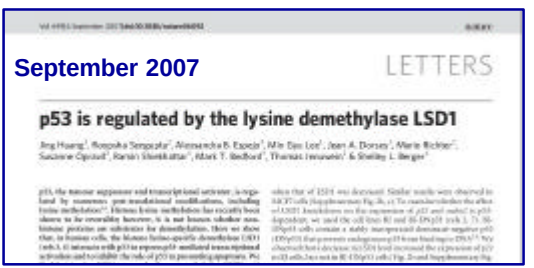
The histone peptide adopts a folded conformation



Perspectives



- LSD1/HDAC complex
- Complex with Nucleosomes
- Inhibition Studies
- LSD1/p53 interactions?

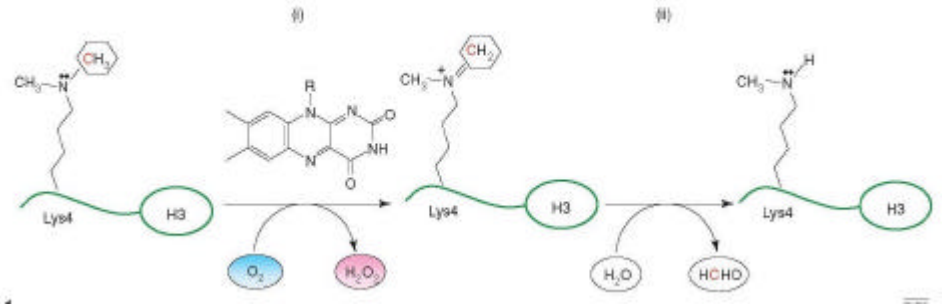


September 2007

LETTERS

p53 is regulated by the lysine demethylase LSD1

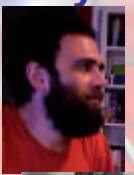
What about Oxygen and Hydrogen Peroxide?



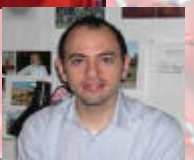
•Mattevi, *Trends Biochem. Sci.* (2006) 31, 276-283

Understanding Oxygen Reactivity using Flavin-containing Monooxygenases as a model system

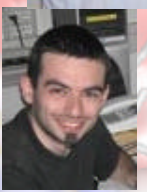
Enrico Malito



Andrea Alfieri



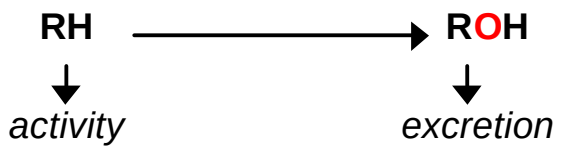
Roberto Orrù



Flavoprotein Monooxygenases

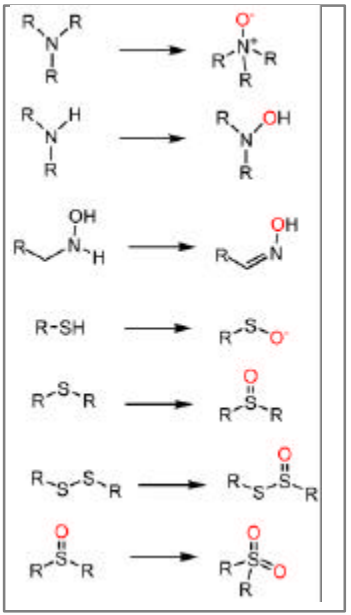
All **xenobiotic** compounds need to be combined with O_2 or other molecules in order to be made more soluble and more readily excreted.

Drugs do not escape this rule:



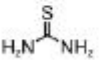
Production of **reactive oxygen species**

Flavin-containing Monooxygenases (FMOs)

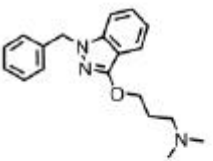


trimethylamine

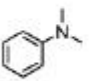
methimazole



thiourea



benzydamine



N,N-dimethylaniline

N-octylamine

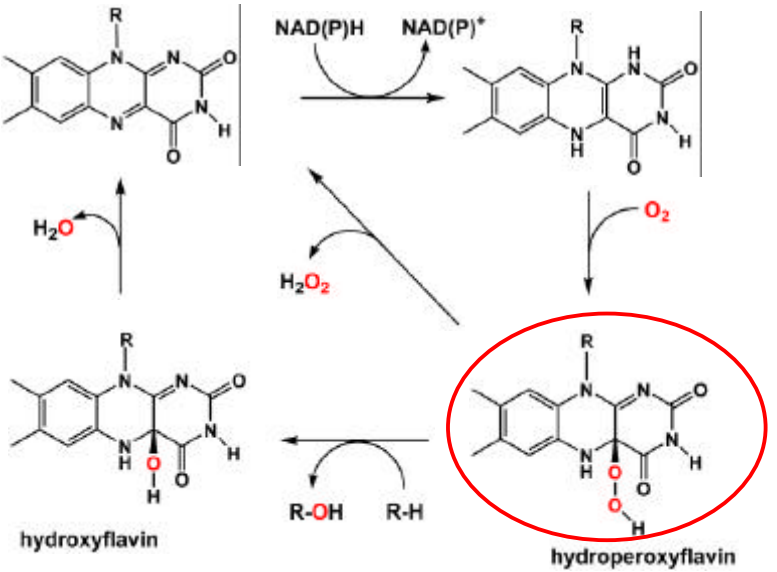
Crucial issues

Understanding the activity and

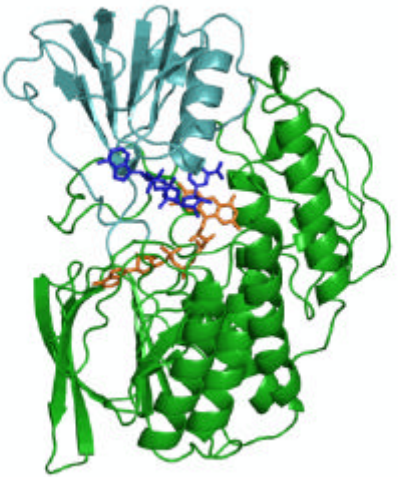
molecular pharmacology of FMOs

Understanding chemical/mechanistic details of flavin-mediated activation of ***molecular oxygen***

Flavoprotein Monooxygenases



Bacterial FMO three-dimensional structure



2.6 Å

FAD and $NADP^+$ bound

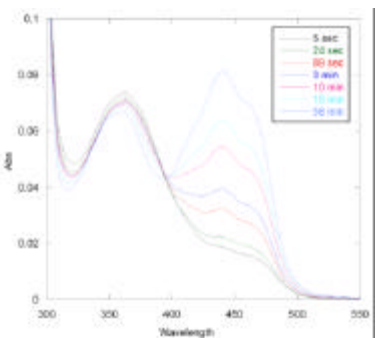
closest sequence homolog of hFMO3 in the Protein Data Bank

Alfieri et al., *Proc. Natl. Acad. USA* (2008) in press

FMO biochemistry

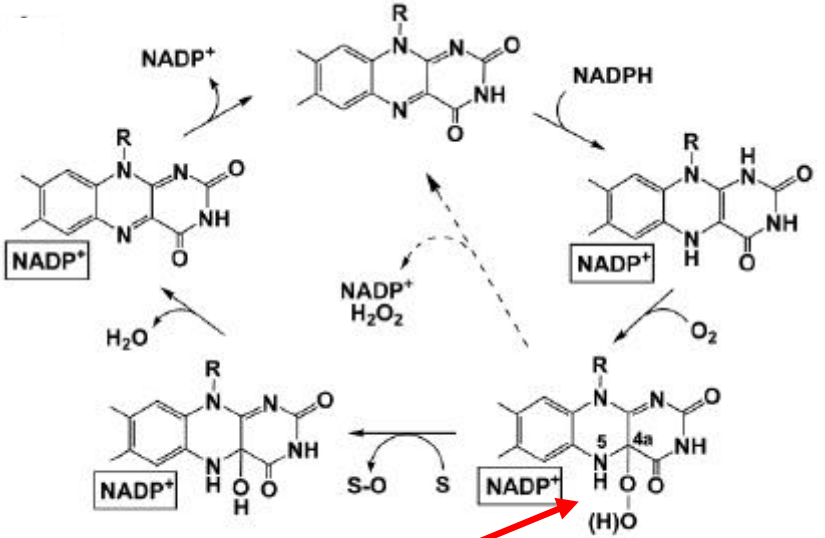
Substrate	Km (μM)	kcat (s^{-1})	kcat/Km ($M^{-1}\ s^{-1}$)
Trimethylamine	7	6.0	8.6×10^5
Nicotine	90	2.5	2.7×10^4
Methimazole	70	0.9	1.3×10^4
N,N Dimethylaniline	260	1.8	6.9×10^3
Indole	100	0.6	6.0×10^3

substrate specificity overlapping with hFMO3



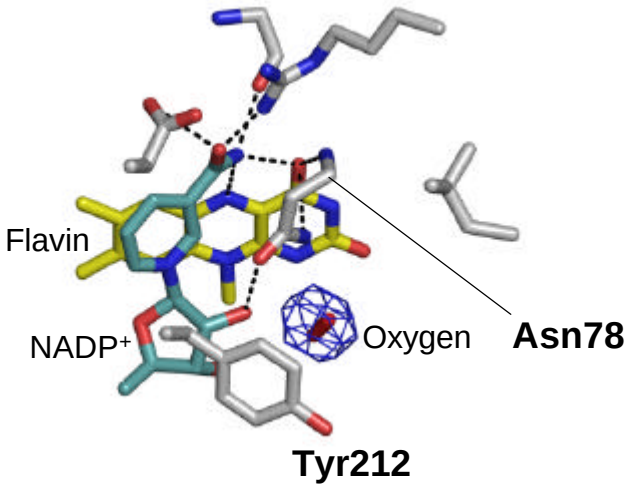
evidence for the stabilization of C4a-hydroperoxyflavin

Flavoprotein Monooxygenases

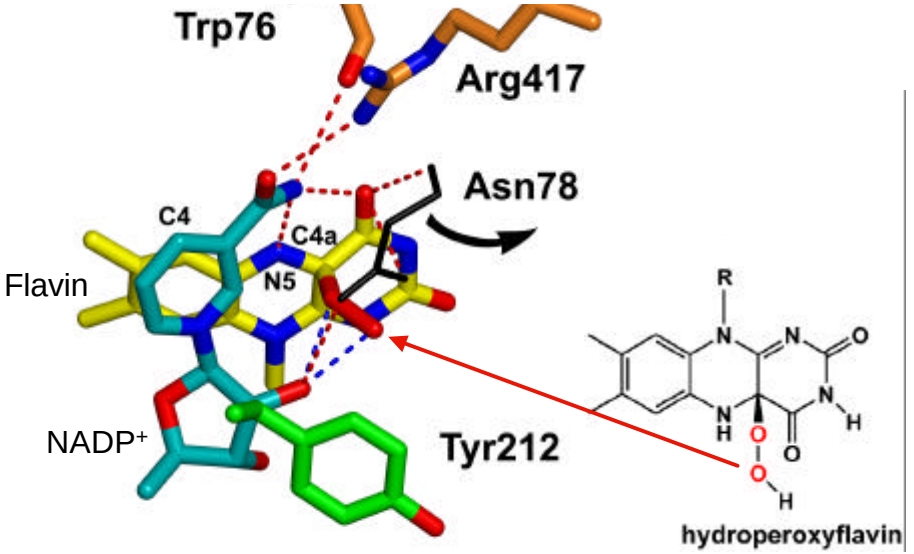


NADP+ is essential for intermediate stabilization

The dual role of NADP(H): electron donor and oxygen



The dual role of NADP(H): Intermediate stabilisation



How does oxygen bind?

A molecular dynamics study together with Riccardo Baron (UCSD)

•Many trajectories for oxygen binding

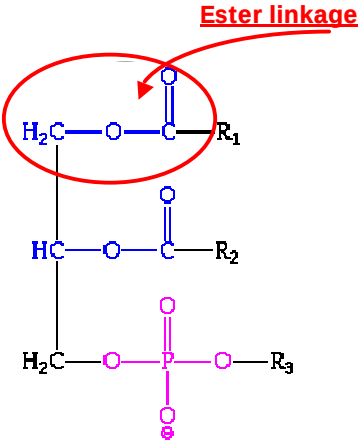
•A properly shaped cavity hosts the oxygen molecule that can thereby direct with the flavin

CHALLENGING UNUSUAL CATALYSIS IN PEROXISOMAL DISORDERS

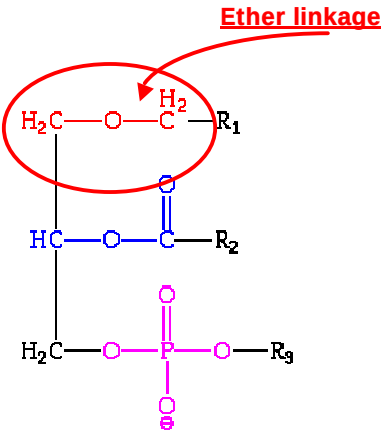
Adelia Razeto

Elena Carpanelli

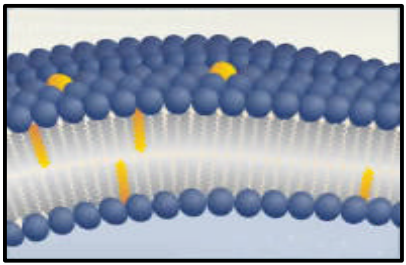
Phospholipids



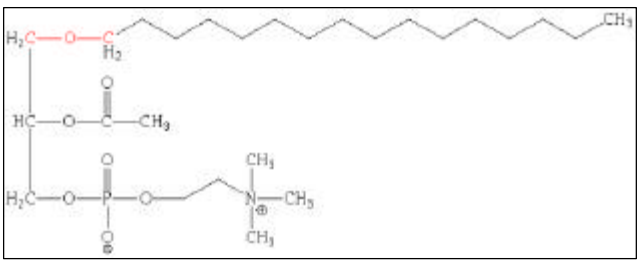
Etherphospholipids



Role of etherphospholipids



1. 18% of the total phospholipids
2. Components of the cellular membrane;
3. Platelet activating factor



Etherphospholipids in a perixosomal disorder

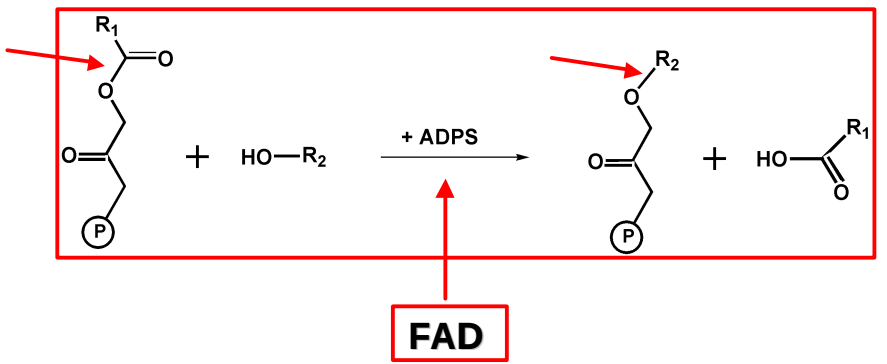
In Utrecht, Henk Van Den Bosch and colleagues found that

Rhizomelic chondrodysplasia punctata (RCDP) type III

is caused by a defect (R419H) in the functioning of

Alkyl-dihydroxyacetonephosphate synthase (**ADPS**)

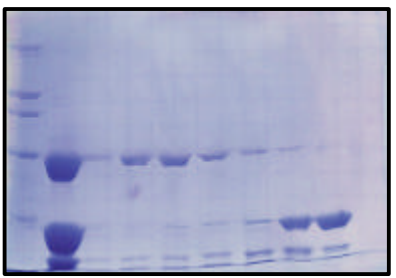
Etherphospholipids biosynthesis



Although it is not a net redox reaction, FAD is essential for catalysis !!

Cavia sp. ADPS

- We cloned ADPS (75-658) into a pMAL-c2x vector;
- We expressed the construct in *E.coli* BL21 (DE3) and we optimized a purification protocol;



Drosophila melanogaster ADPS

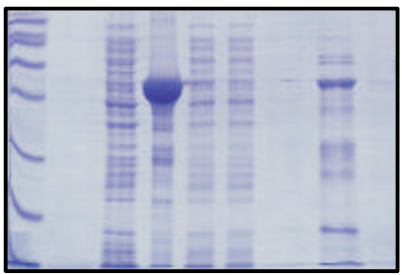
Drosophila ADPS has 52 % sequence identity with the Cavia sp. enzyme

It expressed in *E.coli* BL21 (DE3) RP+ as C-term maltose-binding protein fusion

- High level of degradation !!

ADPS di *Archaeoglobus fulgidus*

- Arch. Fulgidus ADPS has 30% sequence identity with the *Cavia sp.* enzyme



- It has been cloned in pET28bHT;
- Espression in *E.coli*
  - BL21 (DE3) RP+
  - C41 (DE3)
  - ROSETTA (DE3) pLysS Rare
  - ORIGAMI. (DE3) pLysS

INCLUSIONS BODIES !!!

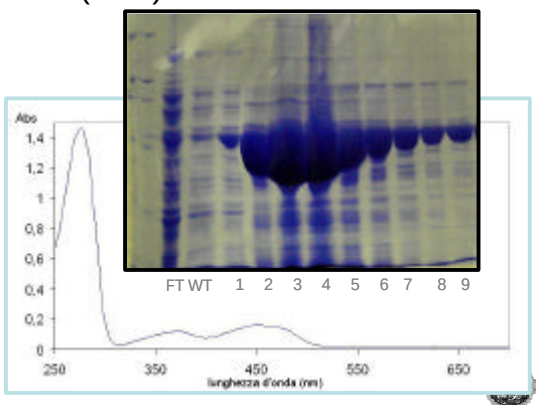
Dictyostelium discoideum ADPS

*Di*ADPS cDNA was cloned into a pET15b vector and expressed in *E.coli* BL21(DE3) and …..

*Di*ADPS is expressed as a

holo-enzyme

and it is very stable !!!



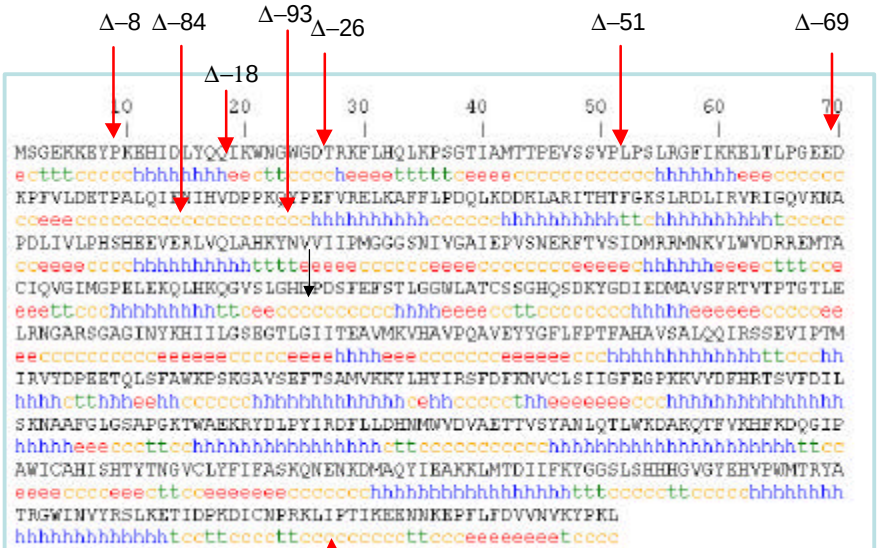
First DiADPS Xtals

DiADPS(1-587)
in 10 mM MES pH 6.0, 100 mM NaCl, 5 % glycerol,
1 mM DTT

- 25 % PEG 4K, 100 mM TrisCl pH 8.5, 200 mM $CaCl_2$
- 18 % PEG 8K, 100 mM MES pH 6.5, 200 mM $ZnAc_2$ *$2H_2O$

.....BUT THEY WERE NOT DIFFRACTING

Deletion mutants DiADPS



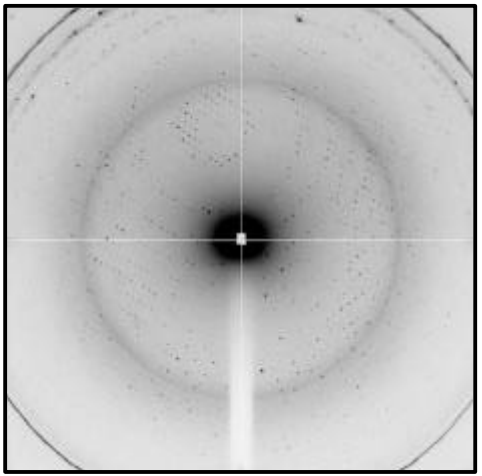
D-**38**

D-**24 C-term deletions**

Finally, DiADPS XTALS!

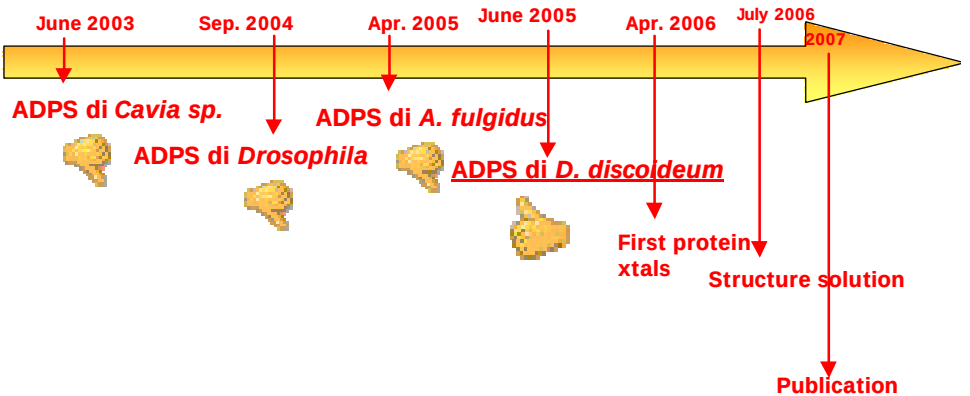
DiADPS(1-587)
in 20 mM MES pH 6.0, 100 mM NaCl, 50 mM NaH_2PO_4
1 mM DTT

16 % PEG 4K, 100 mM TrisCl pH 8.5, 200 mM Li_2SO_4



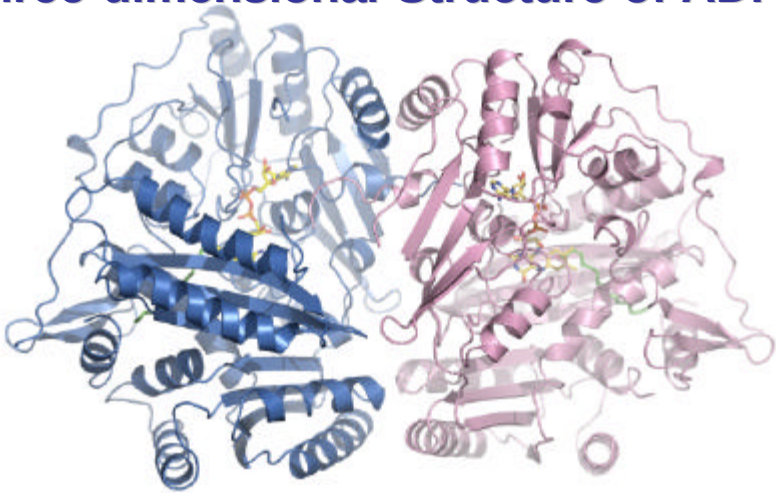
Risol.: 2.5 Å
Spacegroup: **P1**
a = 77Å b = 98Å c = 107Å a = 114° ß = 93° ? = 1[illegible]

In summary



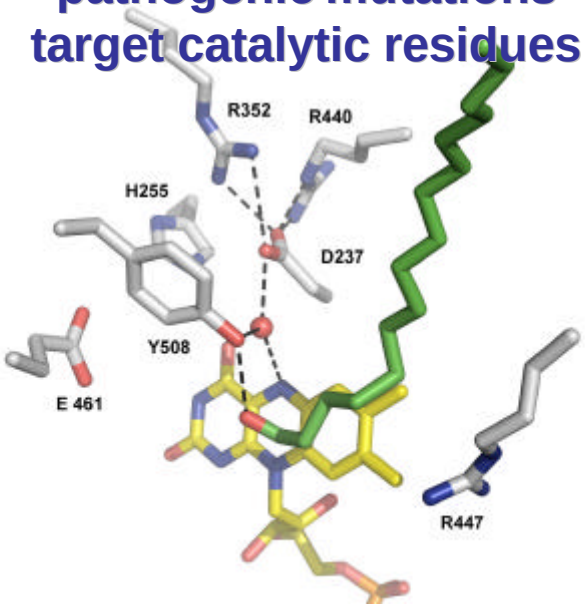
Razeto et al., *Protein Expr. Purif.* (2007) 55, 343-351

Three-dimensional Structure of ADPS

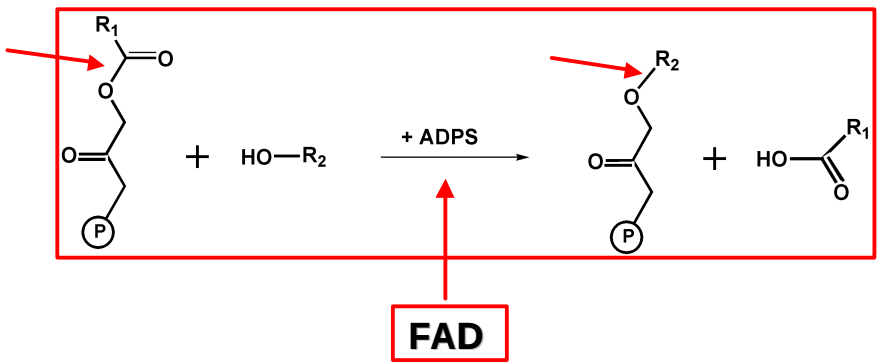


Razeto et al., *Structure* (2007) 15, 683-692

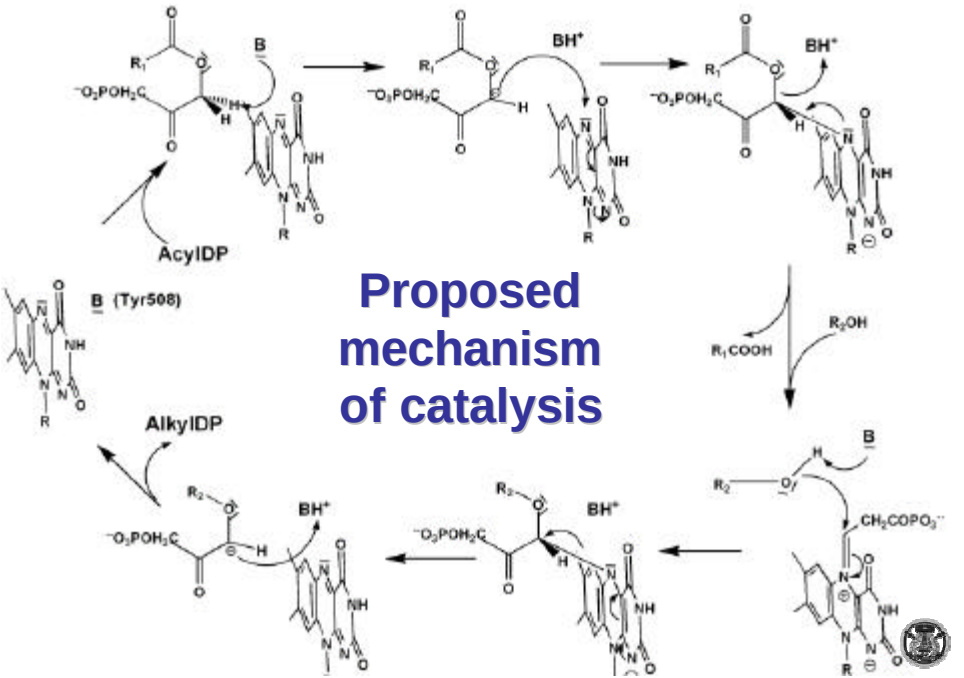
Active site of ADPS: pathogenic mutations target catalytic residues



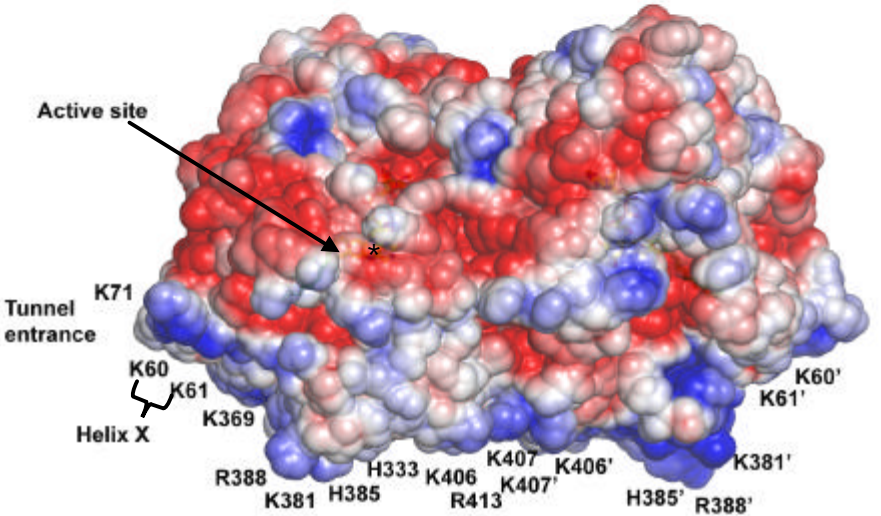
Etherphospholipids biosynthesis



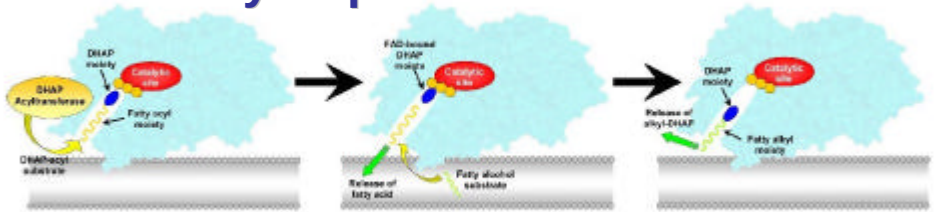
Although it is not a net redox reaction, FAD is essential for catalysis !!


B
BH+
BH+
AcylDP
B (Tyr508)
Proposed mechanism of catalysis
R1COOH
R2OH
AlkylDP
BH+
BH+
CH2COPO3--

Membrane association of ADPS



The unusual functional mechanism of ADPS: hydropophobic substrates and a hydrophilic intermediate



First substrate donated by preceding enzyme

First product released into the membrane, second substrate binds from the membrane

Ether product released

Forneris & Mattevi , *Science* (2008) submitted

1) Borsa di Dottorato 2008

2) Probabile borsa postdoc a fine 2008