A Glance at Science at CERM-Florence

and at its links with Biomedical Research Infrastructures

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Magnetic Resonance Center (CERM)
University of Florence

CORBEL Final Meeting
2 March 2020

Main Topics of Cermians' Research

- **Bioinformatics and Computational Biology**
- ***To investigate the proteome and structure-function relationships at the whole cell level***
- **Cellular Structural Biology**

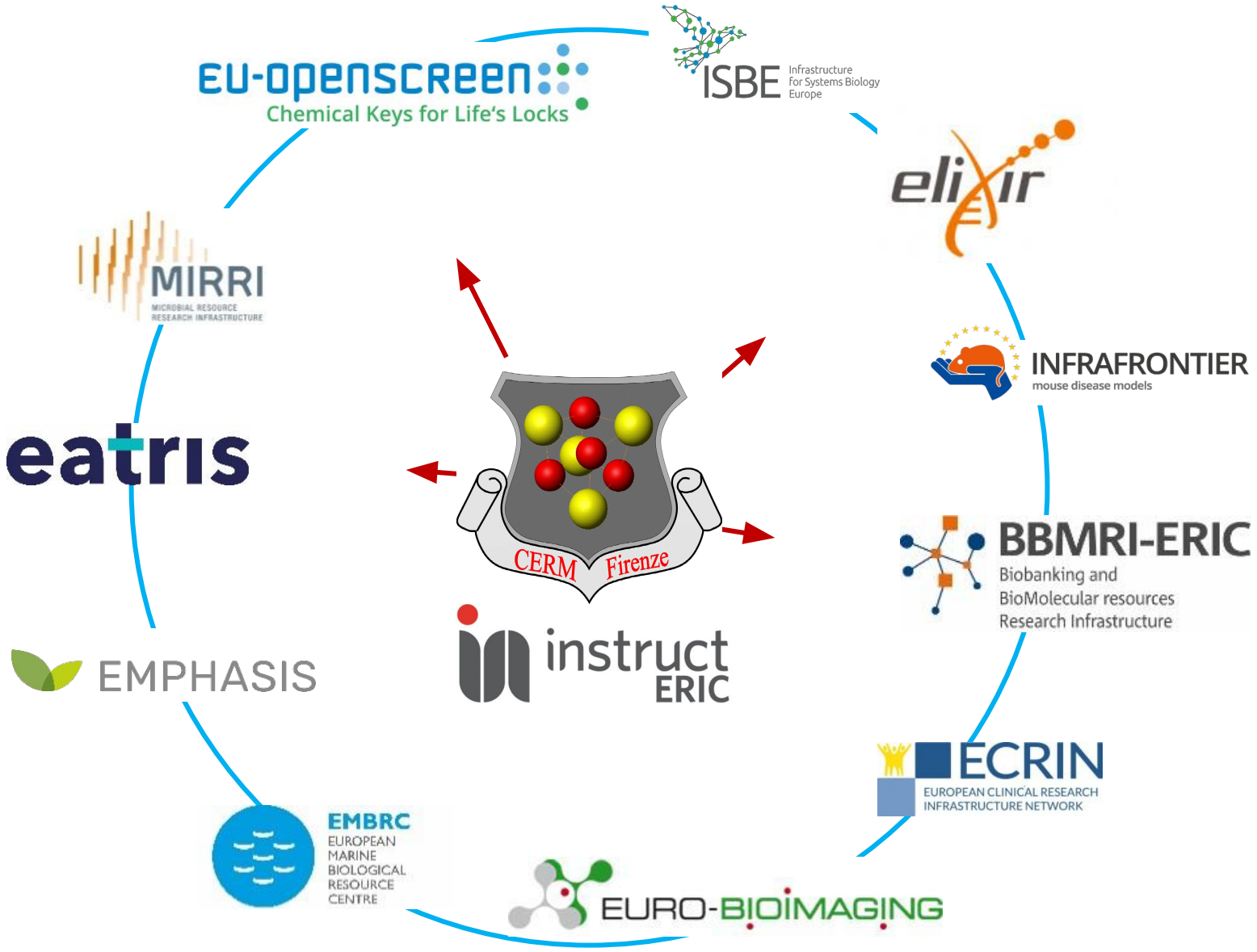
To describe and understand biological processes at molecular level in a cellular context

- **Structural Vaccinlogy**

Rational vaccine design based on the structural knowldge of the antigene

- **Metabolomics**

Analysis of all metabolites in biological fluids

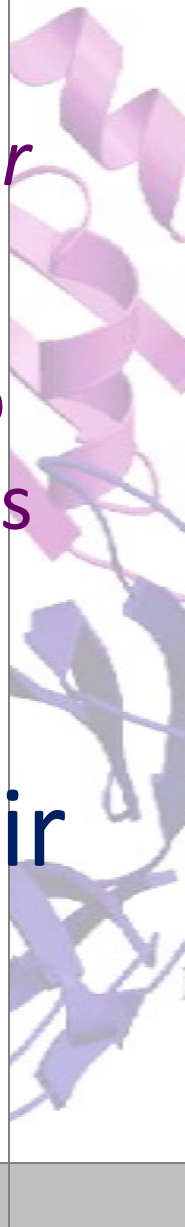

EU-OPENSCREEN
Chemical Keys for Life's Locks
ISBE
Infrastructure for Systems Biology Europe
elixir
MIRRI
MICROBIAL RESOURCE RESEARCH INFRASTRUCTURE
INFRAFRONTIER
mouse disease models
eatris
CERM
Firenze
BBMRI-ERIC
Biobanking and BioMolecular resources Research Infrastructure
instruct ERIC
EMPHASIS
ECRIN
EUROPEAN CLINICAL RESEARCH INFRASTRUCTURE NETWORK
EMBRC
EUROPEAN MARINE BIOLOGICAL RESOURCE CENTRE
EURO-BIOIMAGING

Integrating a Cellular Approach with Atomic Resolution

Living systems are complex: a mix of proteins, nucleic acids, other biomolecules, several cellular compartments,...etc

All the players involved in a given process have to be identified and their atomic-level description, as well as their dynamical interactions, determined

Proteins must be framed within their cellular context



Metal-binding proteins

…..constitute more than one third of our genome…

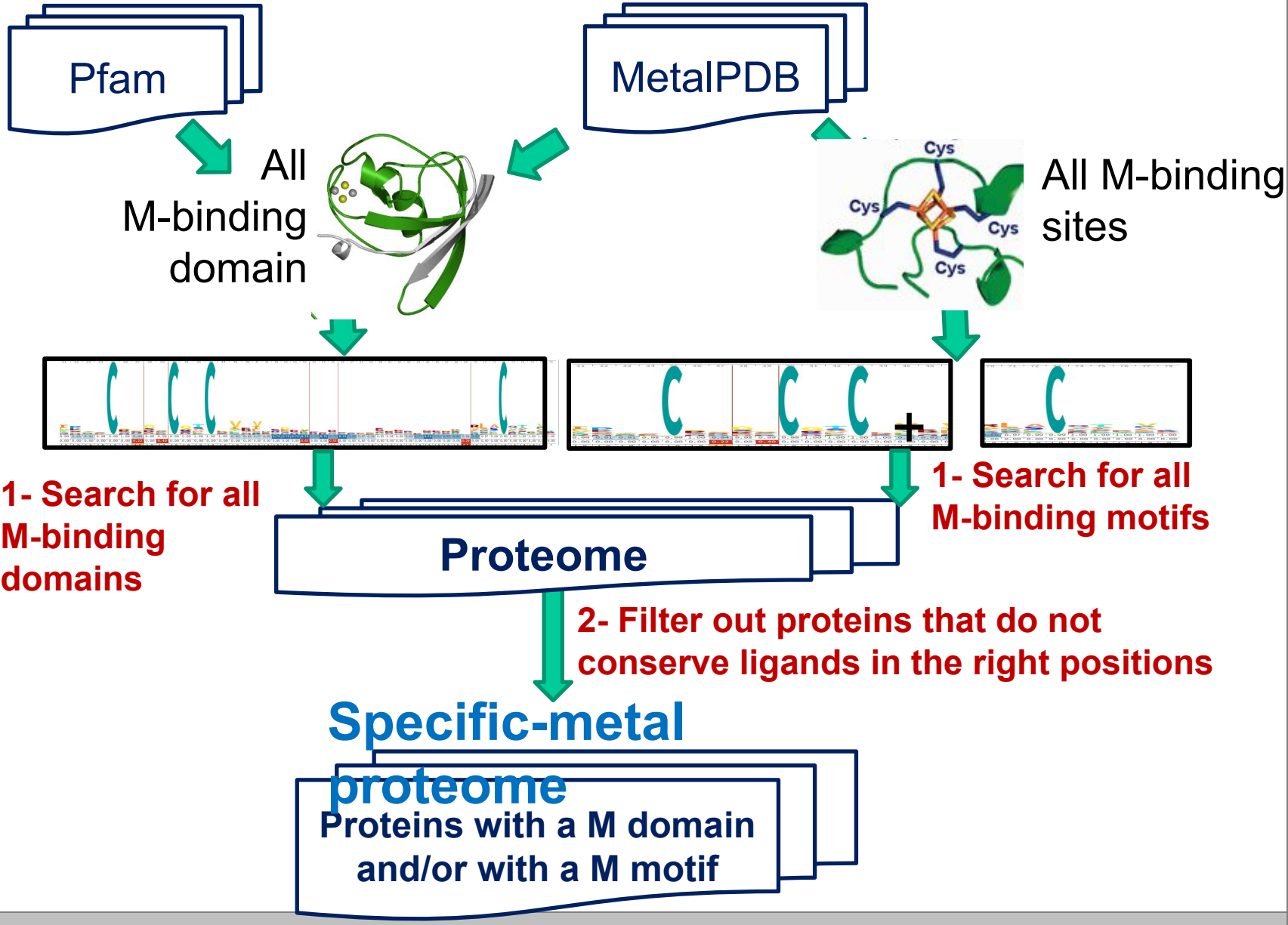
……responsible for essential functional processes….

still …….much less studied than other proteins

The first challenge is to identify them

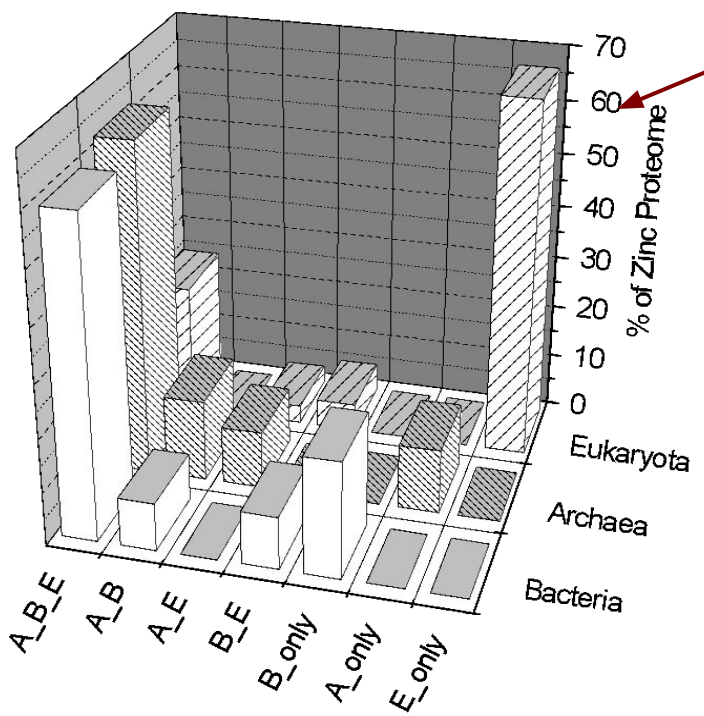
Bioinformatics tools are needed to annotate the genome sequences

Domain and motif search in the proteome



Valasatava Y, Rosato A, Banci L, Andreini C. ***Bioinformatics***. 2016

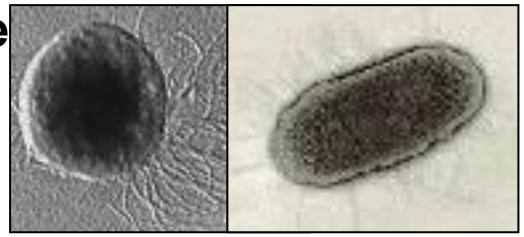
Specific and Common Zinc Proteins

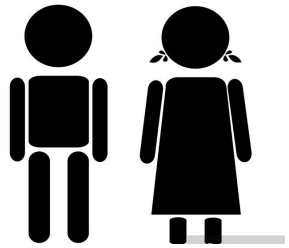


65% of the eukaryotic zinc-proteins do not have homologues in the other two domains of Life.

Evolution of zinc usage

Eukaryota have evolved many specific transcription regulators whereas Prokaryota use zinc only for enzymatic processes

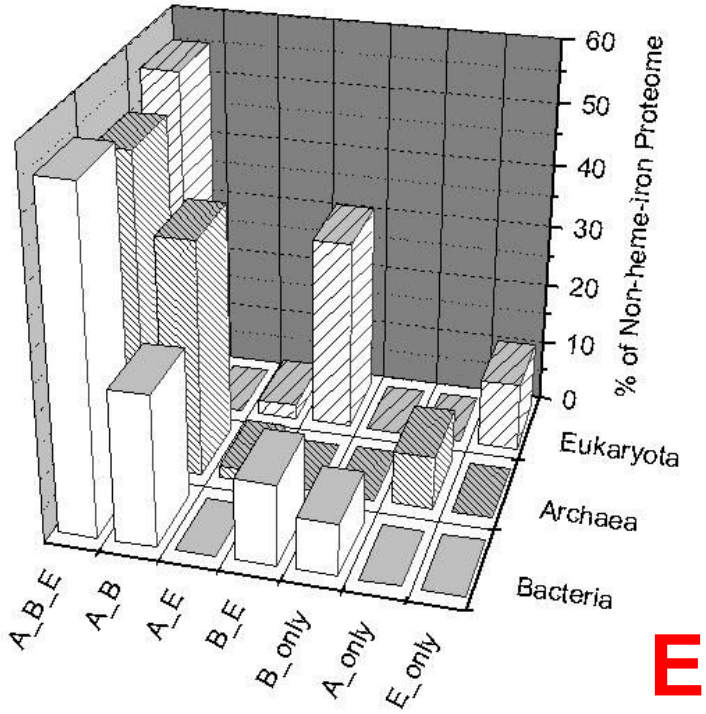




Prokary

Eukary

Specific and Common Iron-proteins

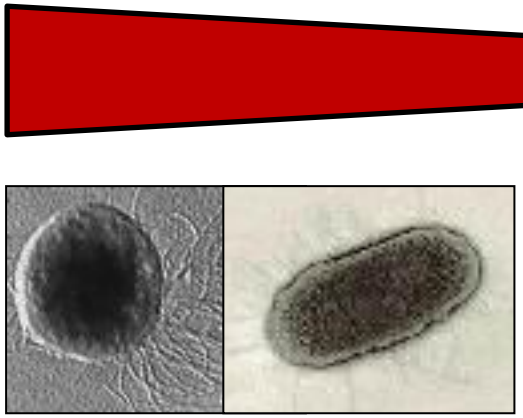


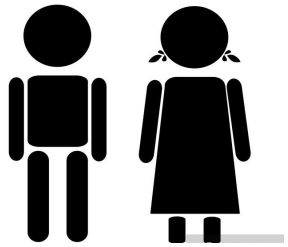
The majority of Fe-binding proteins have homologs in all the three domains of Life.

All the organisms share a set of non-heme Fe-proteins which possibly evolved early in the evolution

Evolution of iron usage

The increase of O_2 in the atmosphere reduced the number of iron-proteins in aerobic organisms

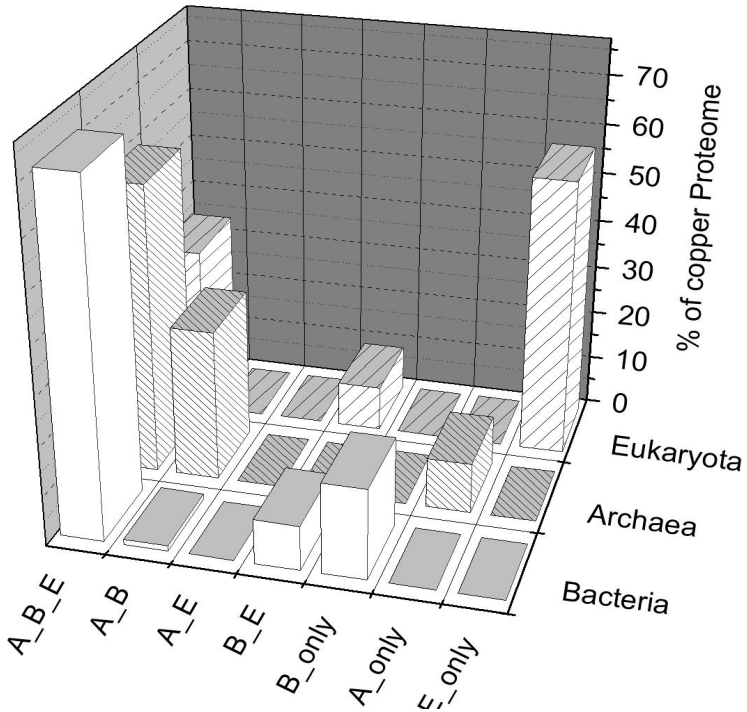




Prokary

Eukary

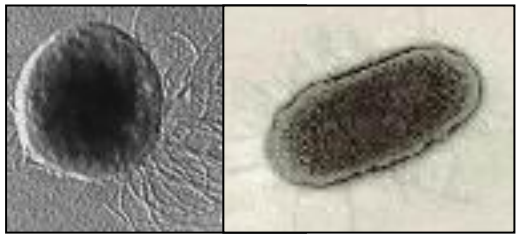
Specific and Common Copper proteins

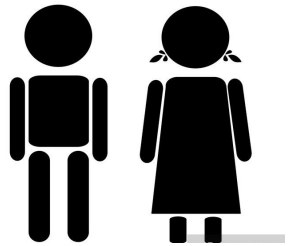


Eukaryota have evolved new specific copper proteins which were added to the common portfolio.
Copper proteins are involved either in copper homeostasis or in redox/electron transfer reactions

During evolution the number of copper proteins increased, but their share in the proteome remained similar

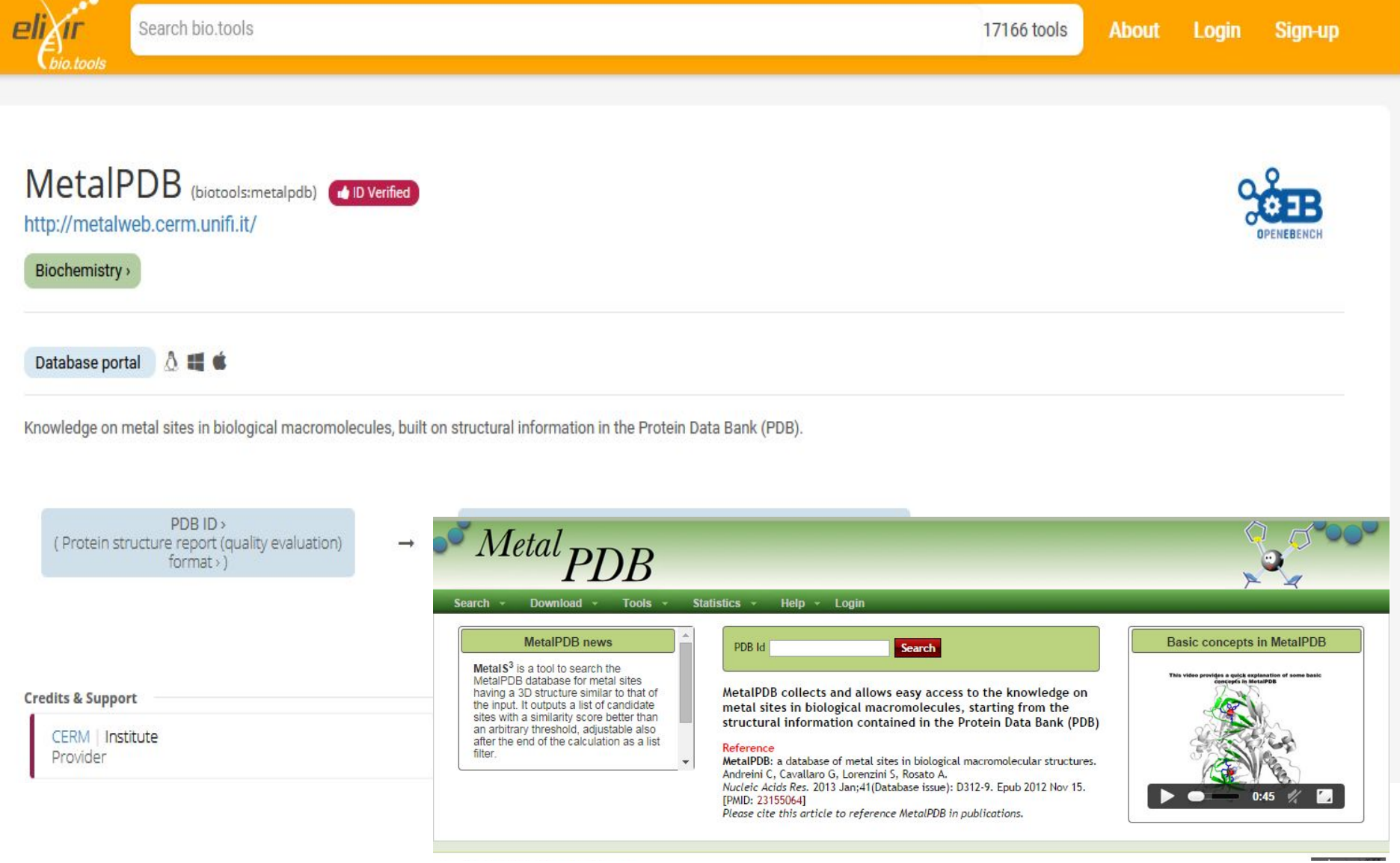
Eukaryota (0.3% ± 0.2%)
Archaea (0.4% ± 0.3%)
Bacteria (0.3% ± 0.2%)





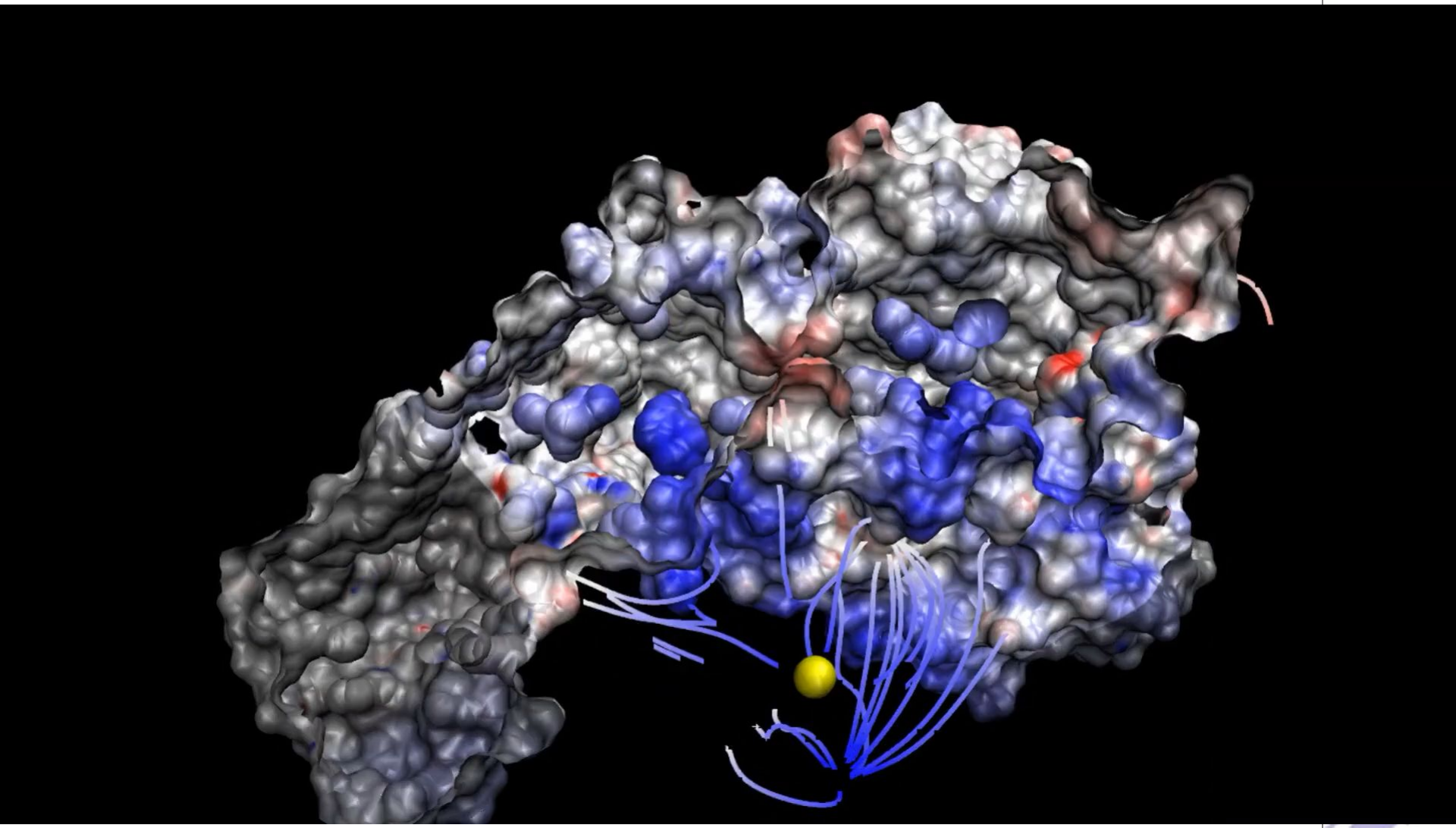
Prokary

Eukary



Currently working to push the information on physiological relevance to PDBe-KB

MD simulation of iron(II) escape from a protein nanocage



Sala, Ciambellotti, Giachetti, Turano, Rosato *J Chem Inf Model* 2017

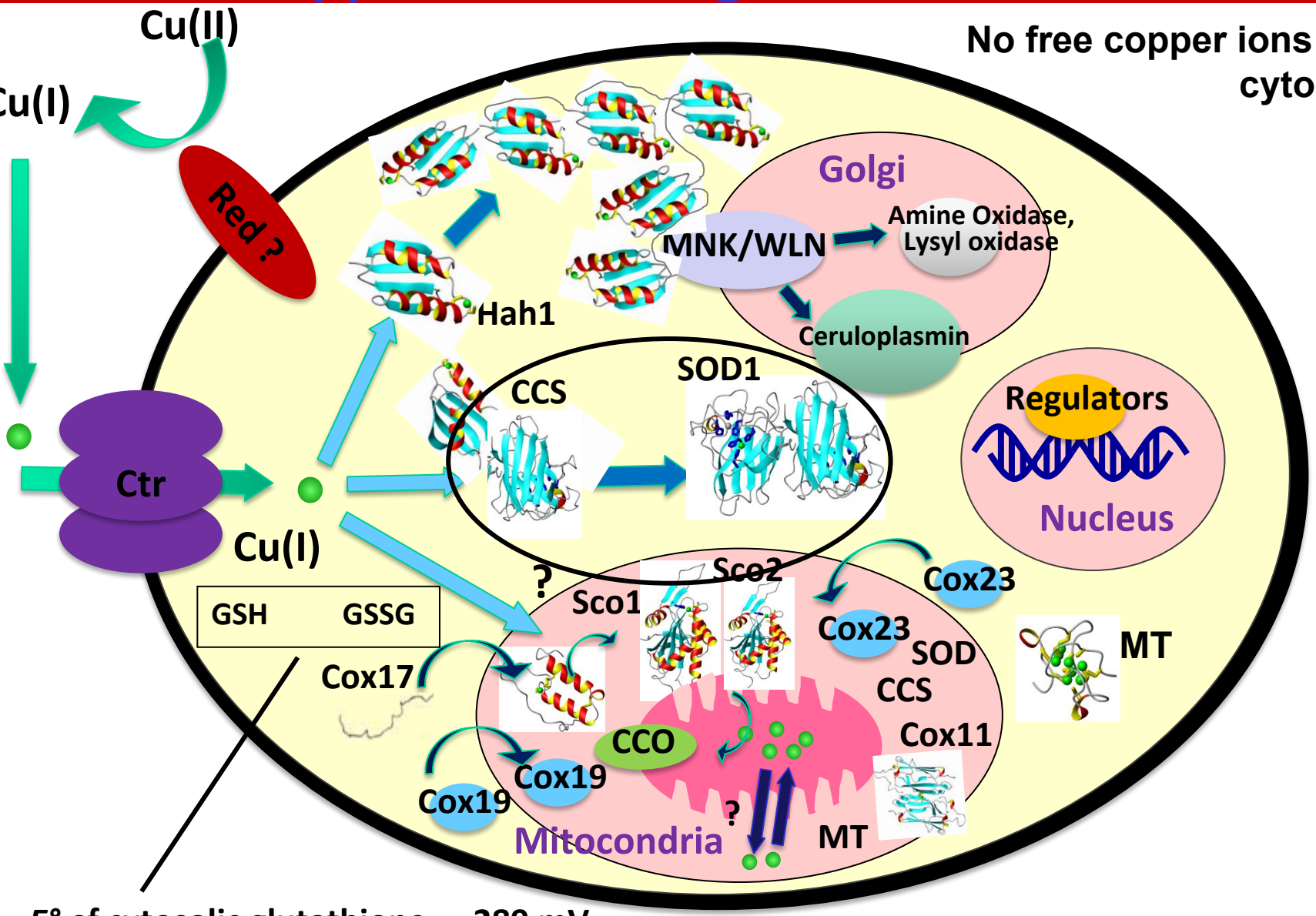
Characterization of metal-binding proteins

Functional processes involving metal ions then need to be experimentally characterized.

This requires the development of suitable techniques for the characterization at molecular level in a cellular context

Integrating Atomic Resolution with the Cellular Context

Copper trafficking in human cells

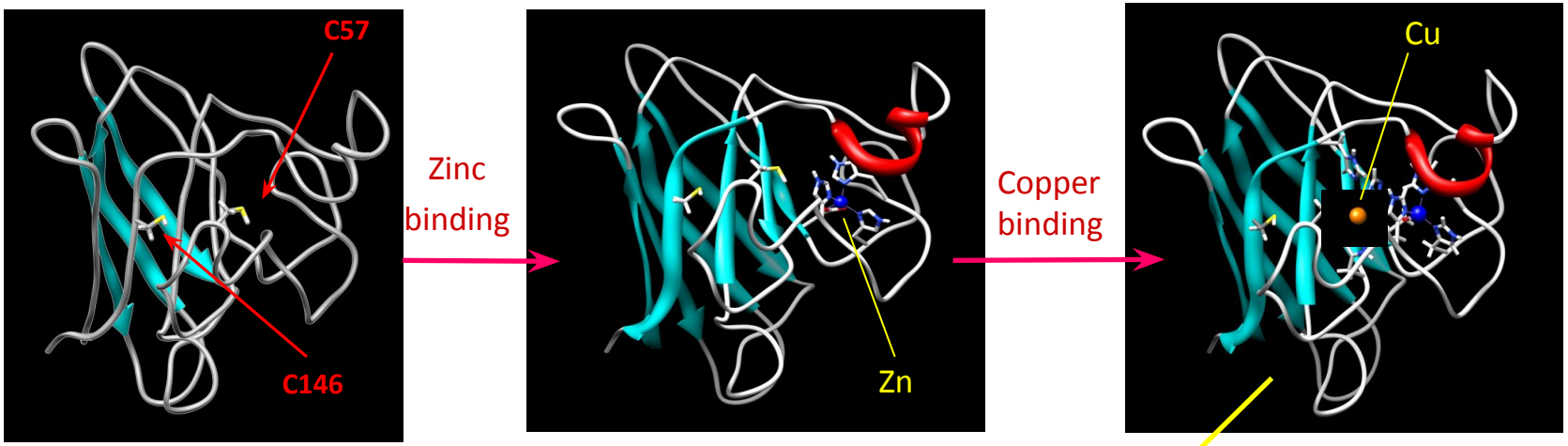


E° of cytosolic glutathione = -289 mV,

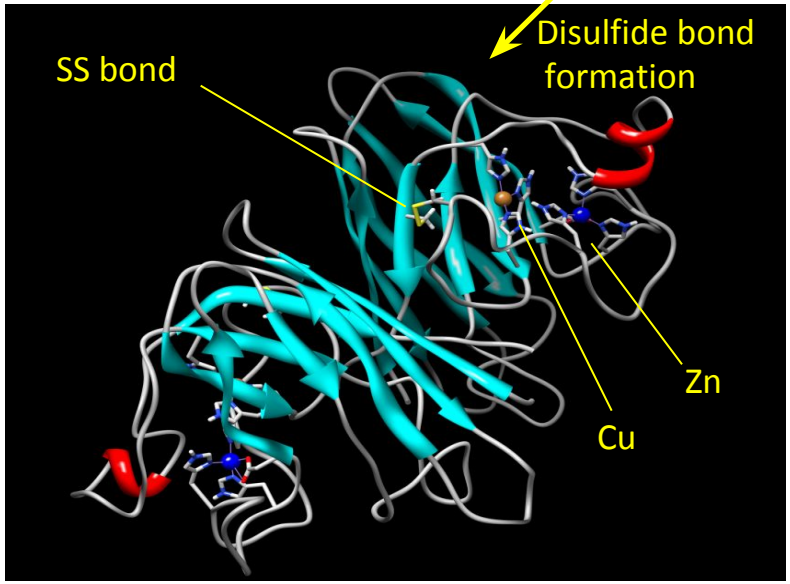
corresponding to GSH and GSSG *in vivo* concentrations of 13 mM and 0.7 mM

Let's start with a single process

Maturation of Cu,Zn-SOD1



monomeric
apo hSOD1^{SH-SH}



SOD1: present in cytoplasm, mitochondrial IMS, nucleus, peroxisomes

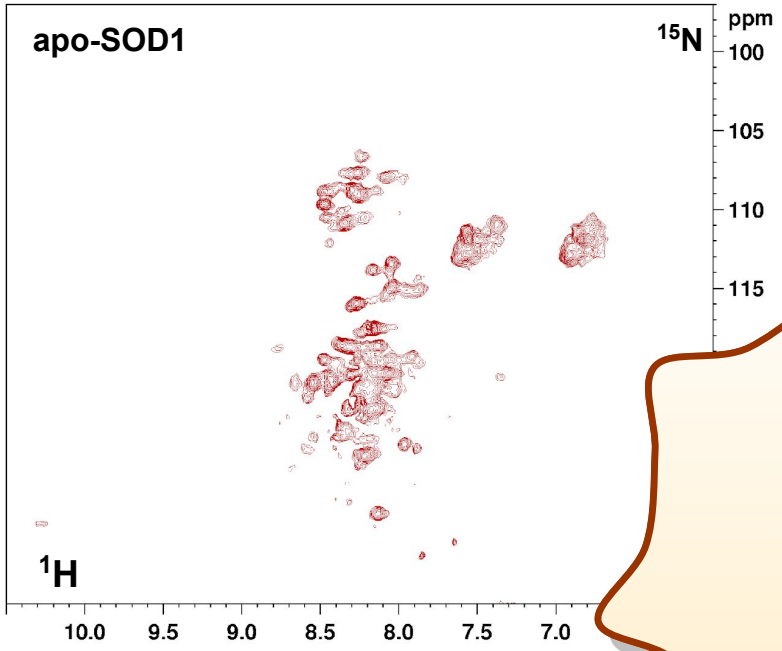
dimeric (Cu_2,Zn_2) hSOD1^{SS}
Active enzyme:
$(2O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2)$

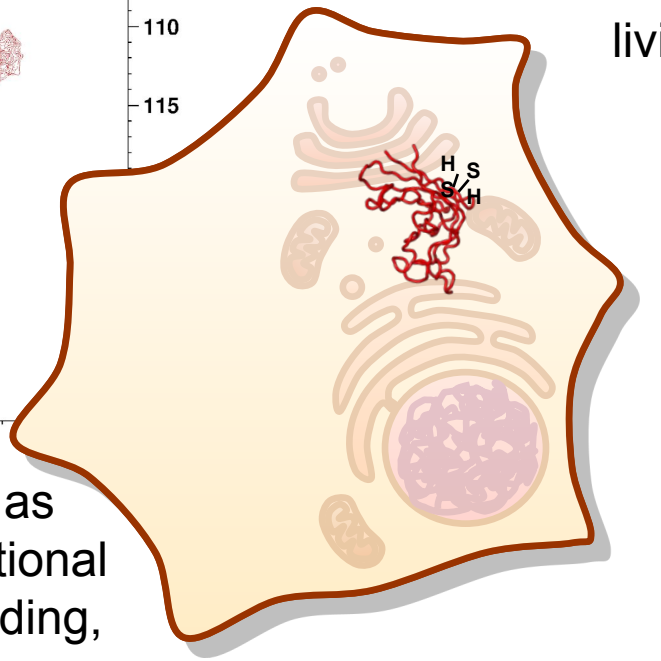
These post translational modifications affect the fold properties and monomer/dimer equilibrium

In-cell NMR can monitor functional processes in live human cells

Understanding intracellular processes at the molecular level requires a high resolution description. In-cell NMR provides atomic-level information on a protein in the cellular environment.

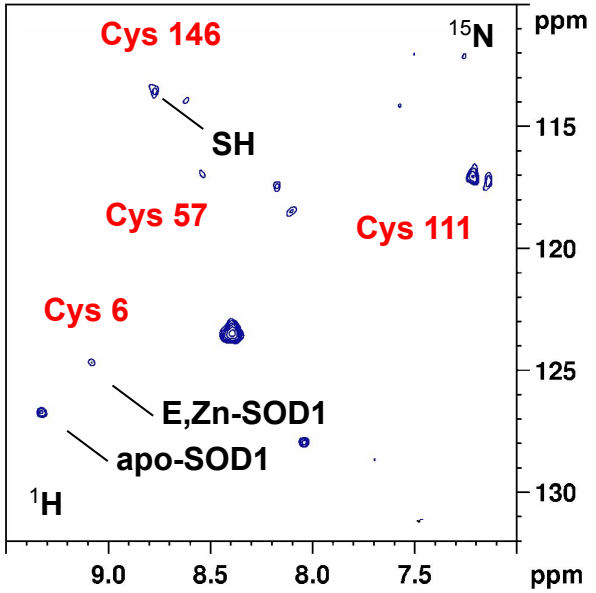
Transfected HEK293T cells are used as a model system for human cells





Maturation processes such as protein folding, post translational modifications (i.e. metal binding, disulfide bond formation) are followed at atomic resolution.

Isotopically labelled proteins are overexpressed and directly observed by hi-res NMR in living human cells.



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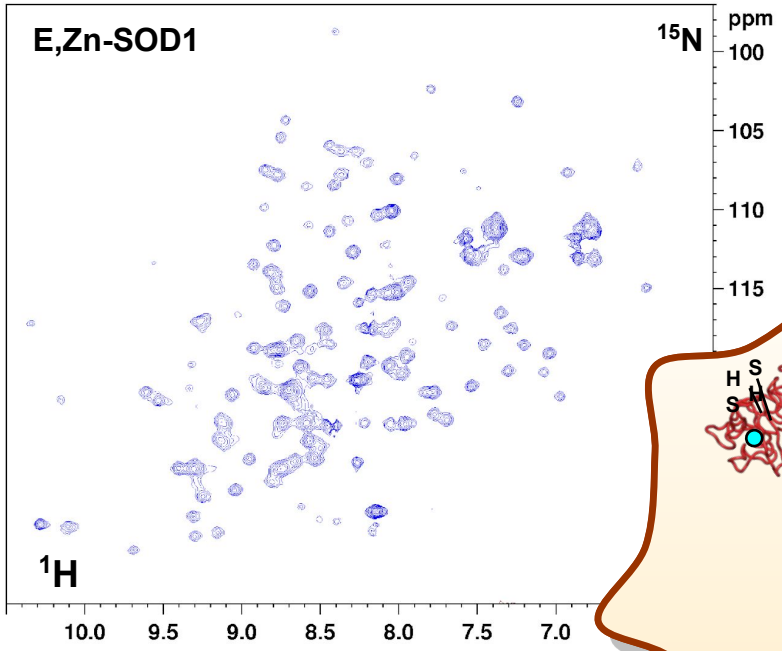

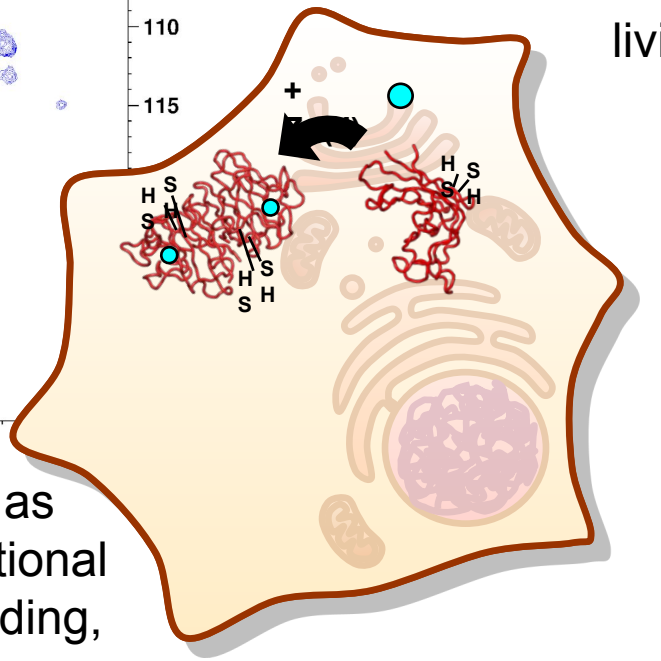

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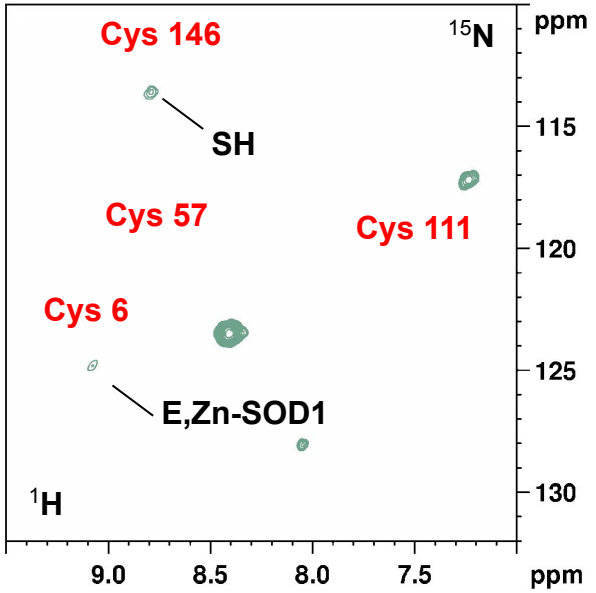

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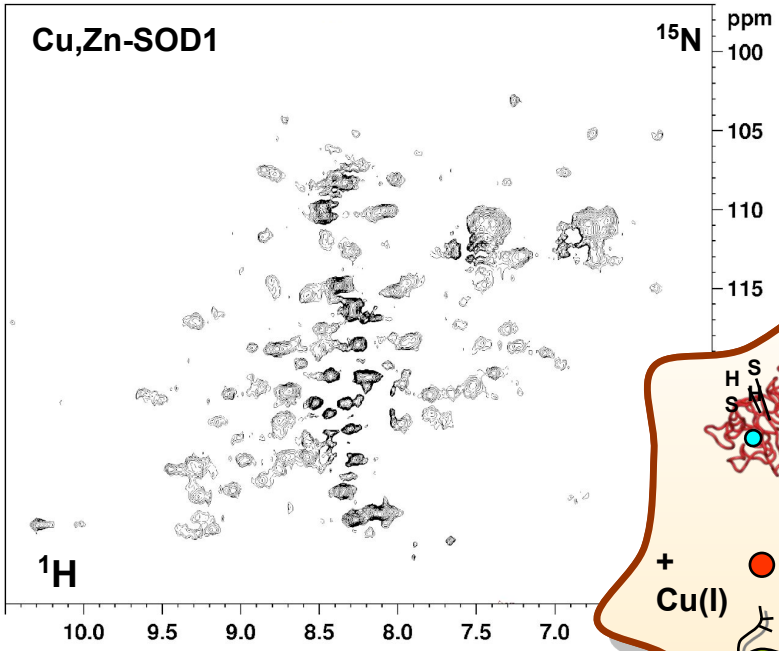

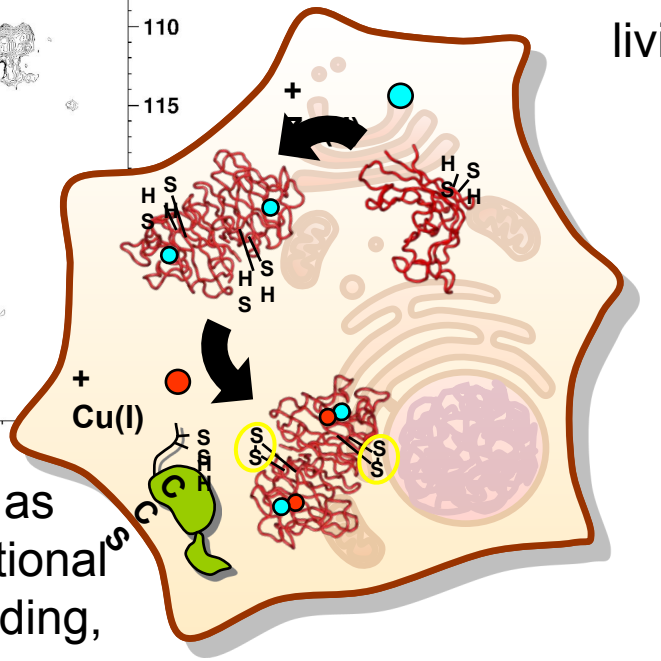

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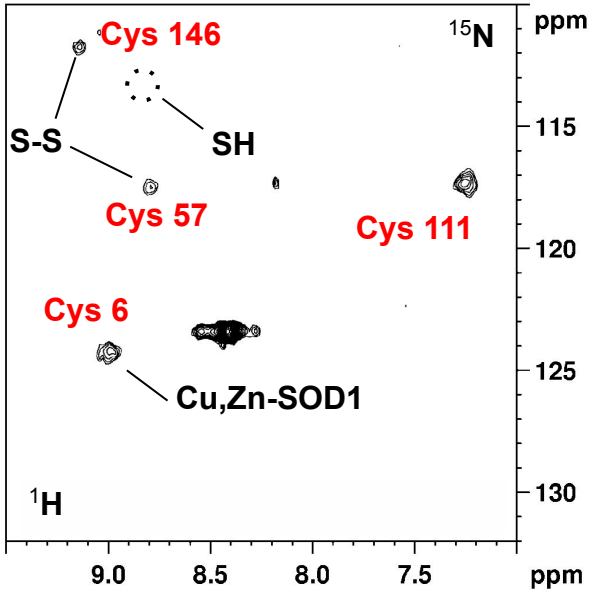

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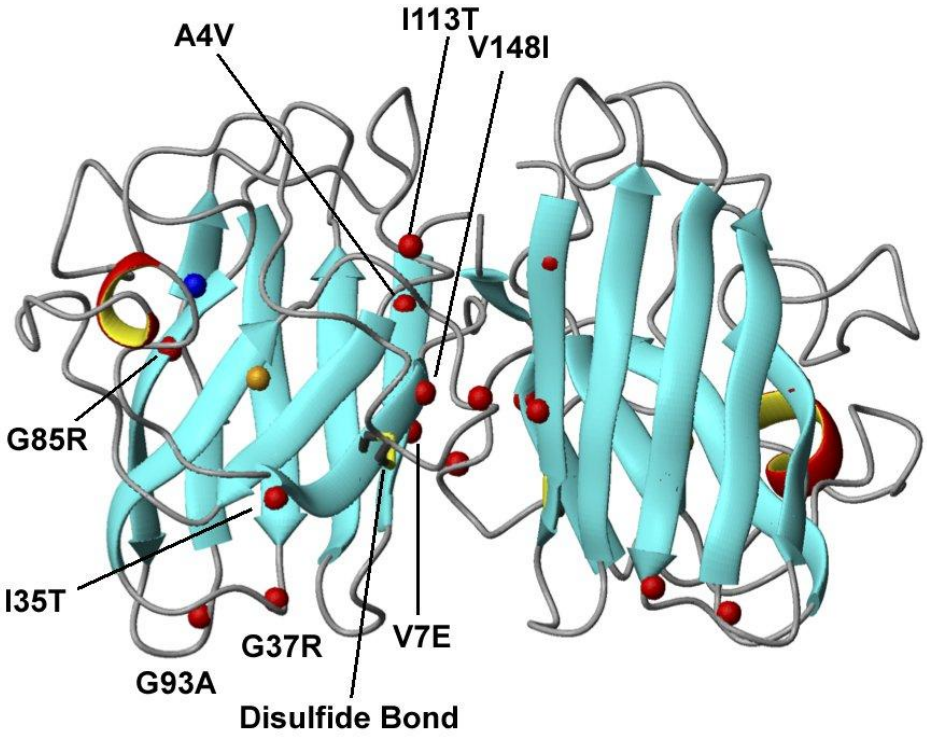
Banci L, Barbieri L, Bertini I, Luchinat E, Secci E, Zhao Y, Aricescu AR, *Nat Chem Biol*, 2013

Incomplete maturation of SOD1 fALS mutants



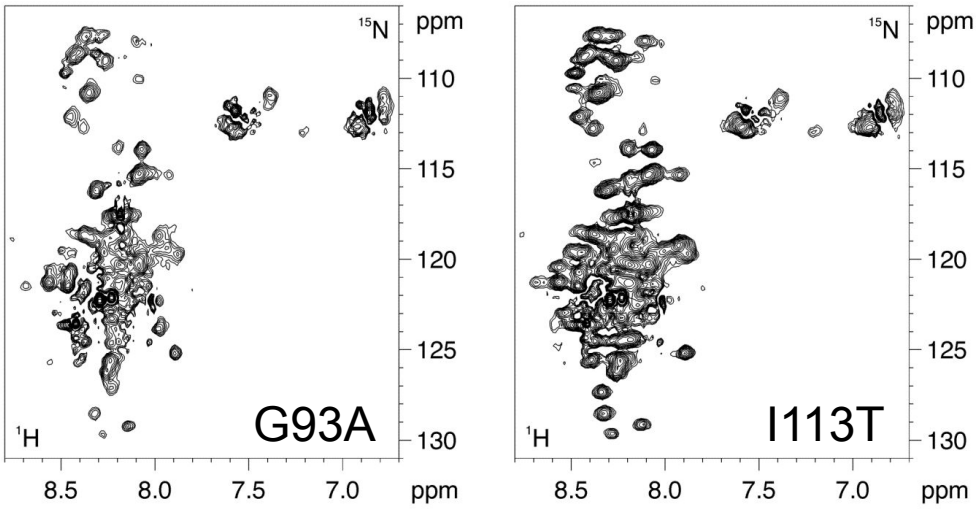
- ALS: a motor neuron disease
- 20% of familial cases is related to mutations of SOD1.
- 165 mutations identified so far, scattered throughout the sequence.

- Mutations are thought to cause defects in SOD1 maturation, promoting aggregation of the apo protein

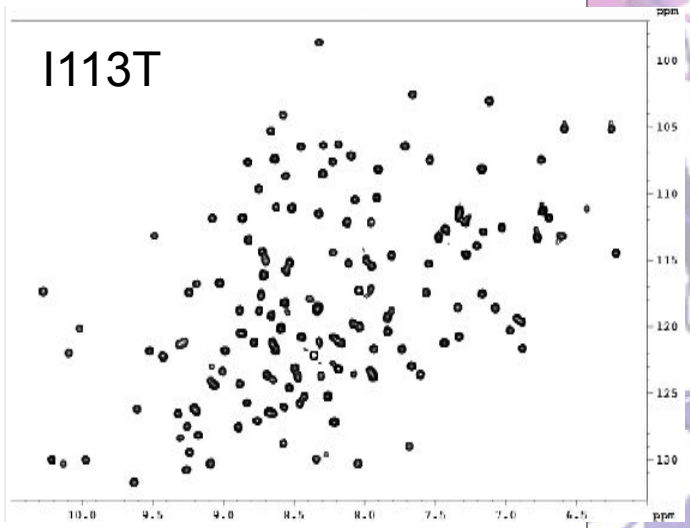
Maturation defects of fALS SOD1 mutants

Many SOD1 mutants do not bind zinc in the cell, and accumulate as an unstructured species, which does NOT evolve toward the native form

(A4V, I35T, G37R, G85R, G93A, I113T)



The mutations do not affect zinc binding *in vitro*



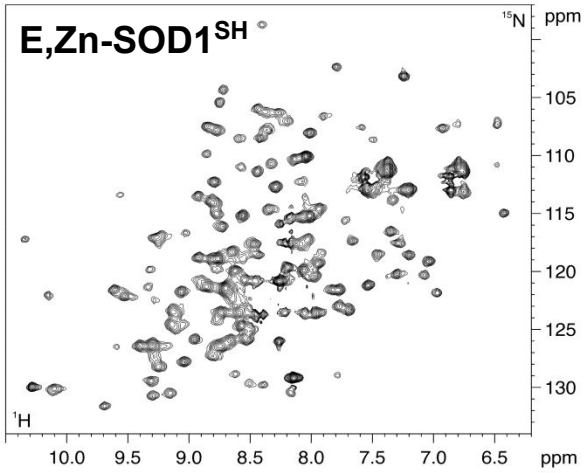
This unstructured species DOES NOT bind zinc

It could be a precursor of SOD1 aggregates

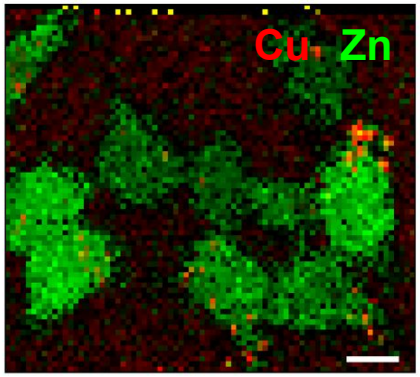
Luchinat E, Barbieri L, Rubino JT, Kozyreva T, Cantini F, Banci L, *Nat. Comm.*, 2014

Combining in-cell NMR with X-ray fluorescence microscopy

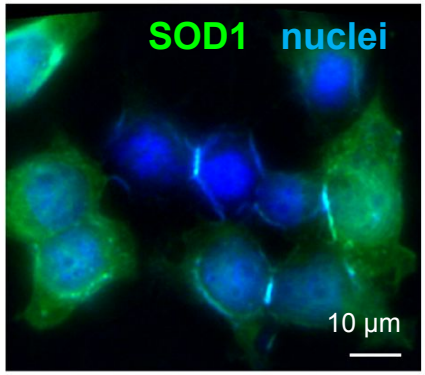
In-cell NMR



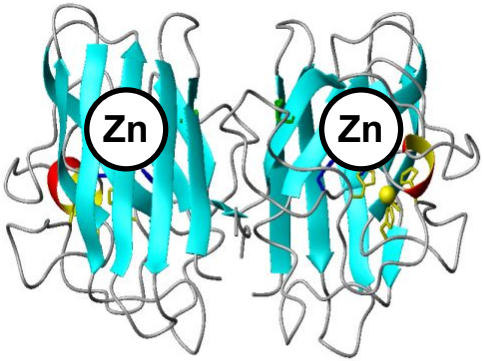
X-ray fluorescence

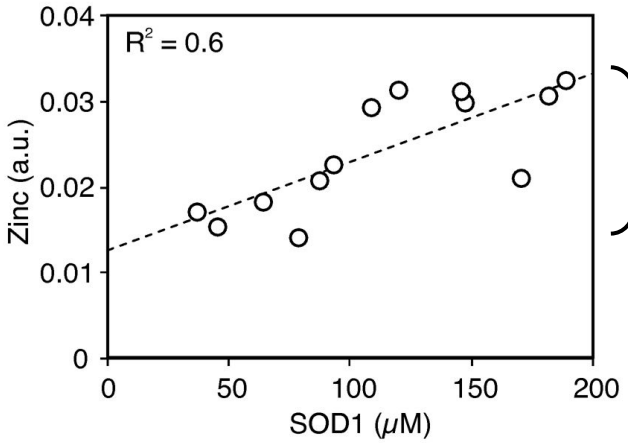


Optical fluorescence



Correlation between the intracellular levels of SOD1 and the content of zinc

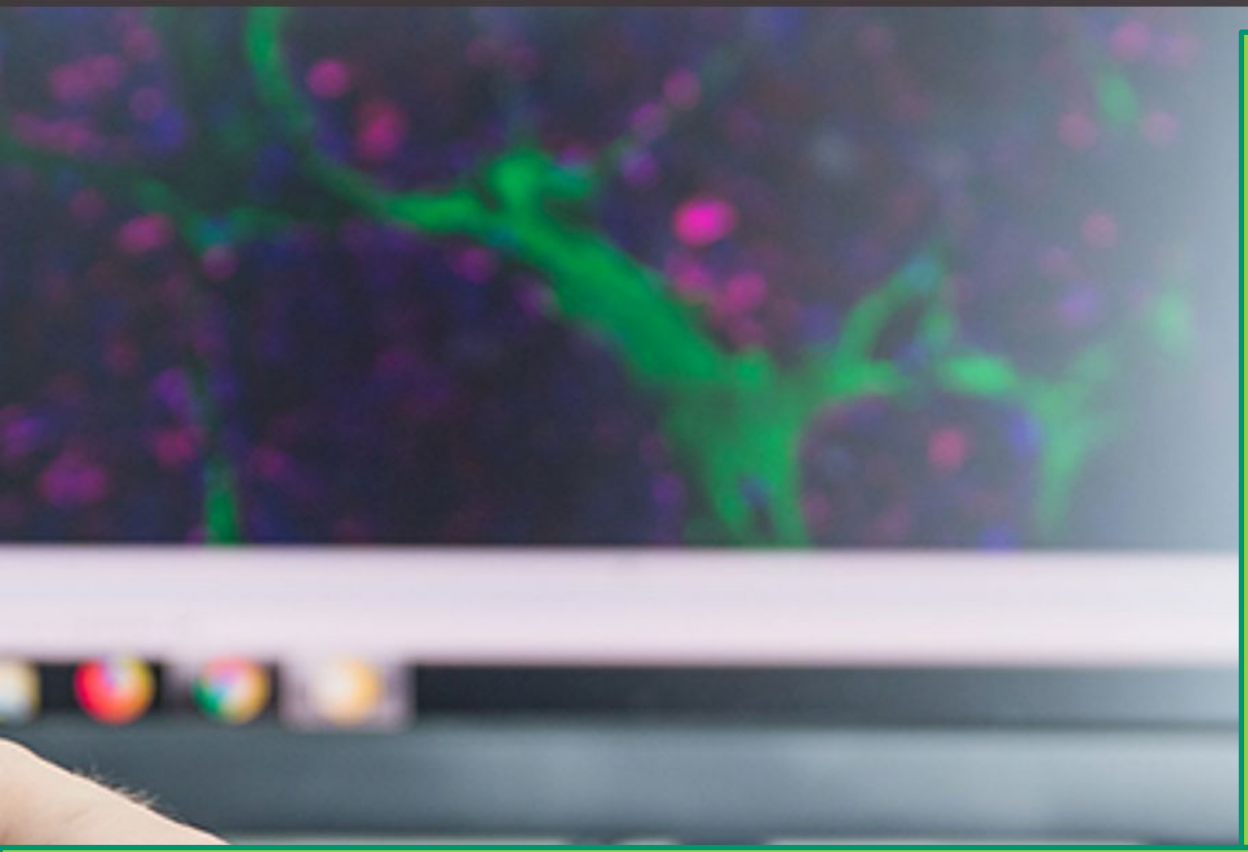




Zinc bound to E,Zn-SOD1

EURO BIOIMAGING

TECHNOLOGIES TRAINING DATA ABOUT US & MORE



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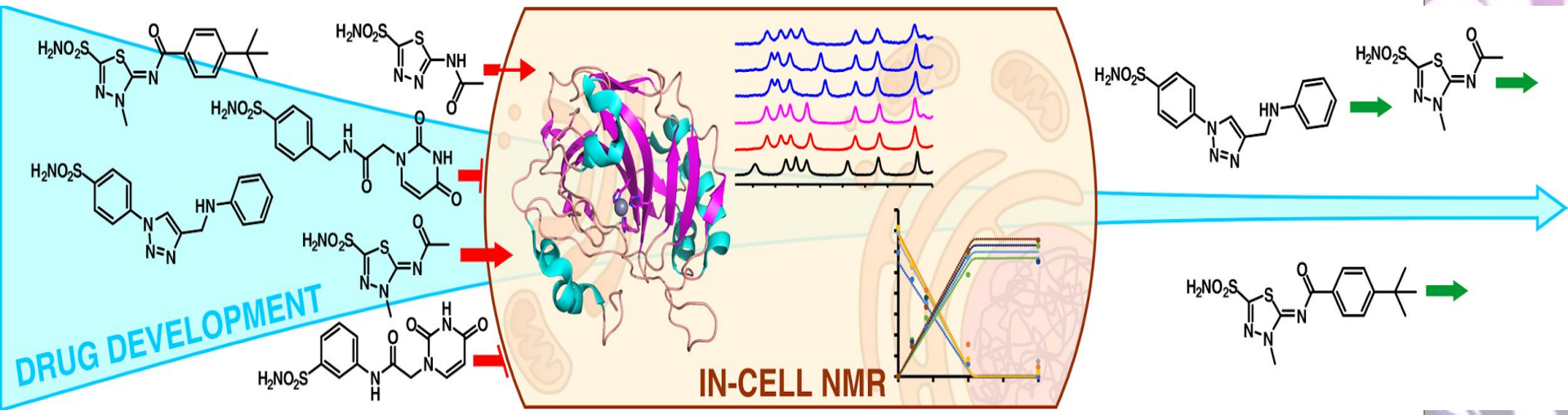
Drug screening by in-cell NMR

An in-cell NMR approach to perform drug screening in human cells

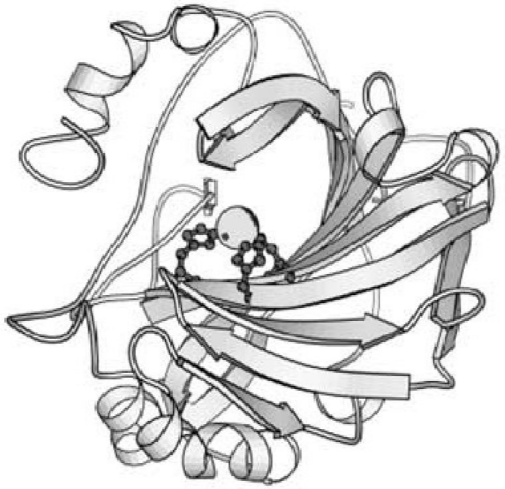


Binding of inhibitors to intracellular carbonic anhydrase 2 observed by 1D in-cell NMR allows dose- and time-dependent analysis, providing information related to cell permeability and target specificity.

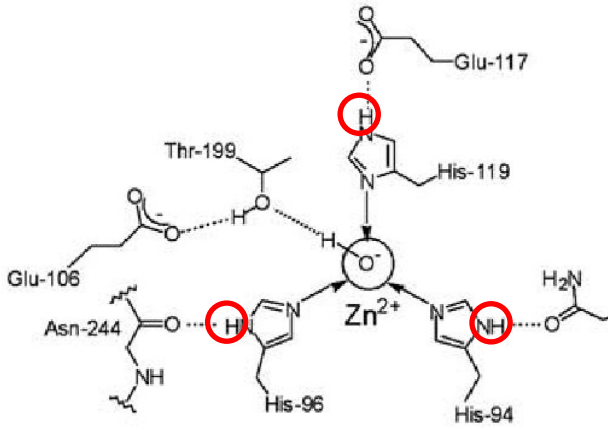
The method can be applied to assess drug potency at an early stage of drug development, without recurring to enzymatic assays.

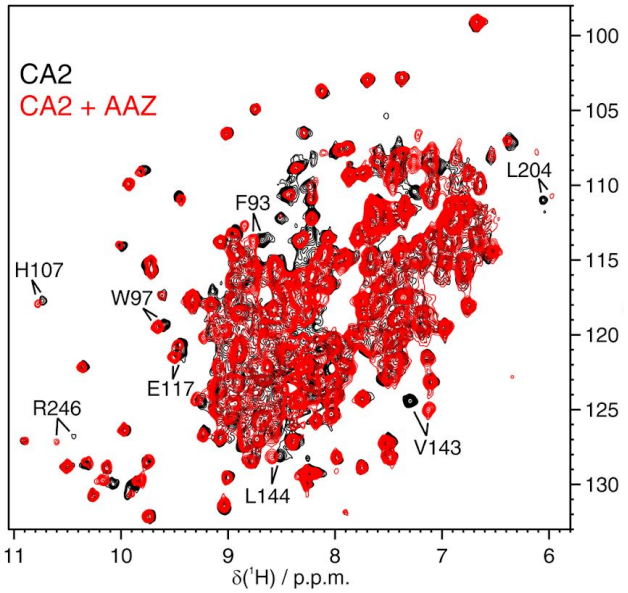
Luchinat E, Barbieri L, Cremonini M, Nocentini A, Supuran CT, Banci L, *Angew Chem Intl Ed. 2020*

Drug screening by in-cell NMR

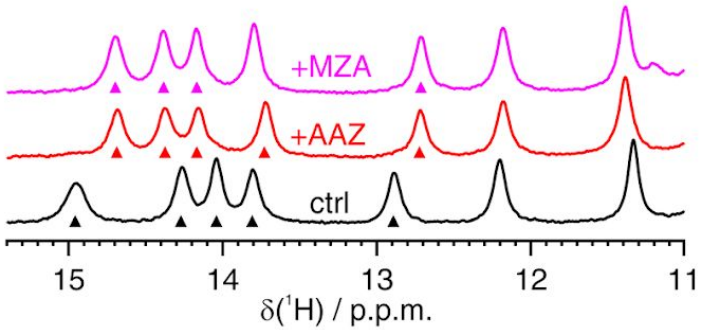


Human carbonic anhydrase 2 was chosen as a model target.



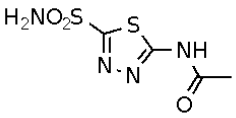


Intracellular ligand binding can be observed both by ^{1}H-^{15}N spectra and ^{1}H spectra in the histidine region

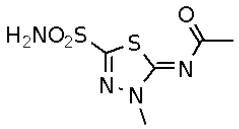


Luchinat E, Barbieri L, Cremonini M, Nocentini A, Supuran CT, Banci L, *Angew Chem Intl Ed. 2020*

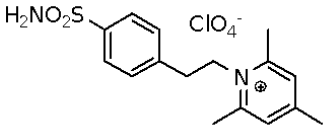
Intracellular ligand screening



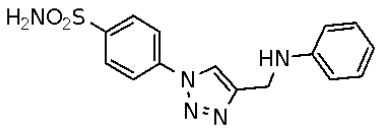
AAZ
K_I =12.5 ±1.0 nM



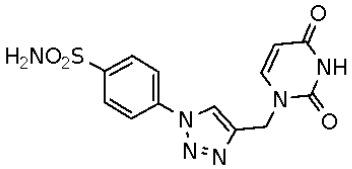
MZA
K_I =14.0 ±0.9 nM



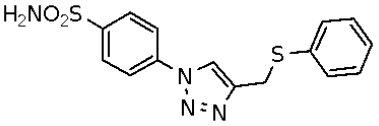
C18
K_I =21.0 ±1.5 nM



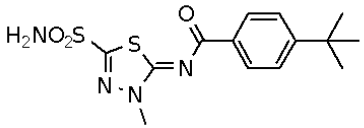
1
K_I =0.83 ±0.05 nM



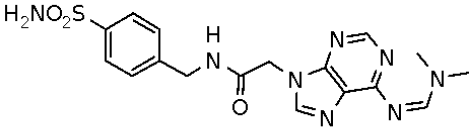
2
K_I =0.85 ±0.06 nM



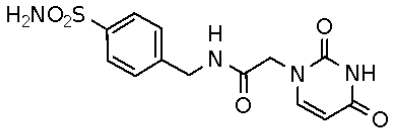
3
K_I =1.5 ±0.1 nM



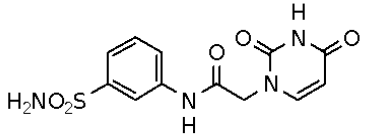
4
K_I =5.9 ±0.4 nM



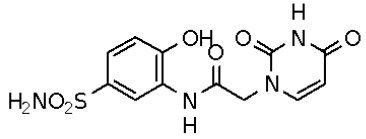
5
K_I =87.3 ±5.3 nM



6
K_I =495 ±35 nM

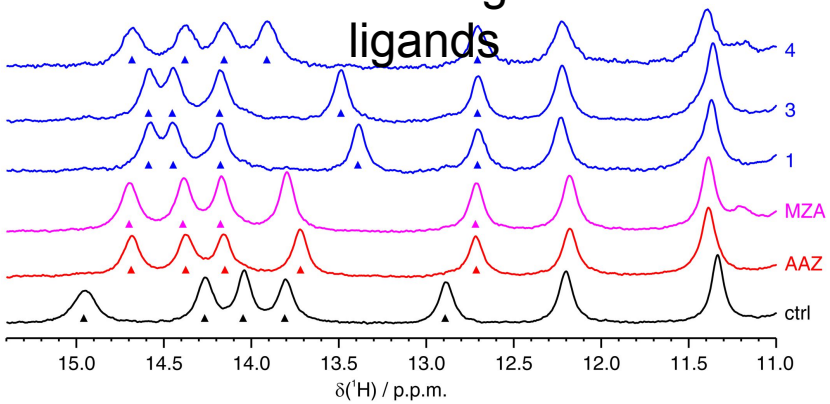


7
K_I =703 ±51 nM



8
K_I =4357 ±332 nM

Binding ligands

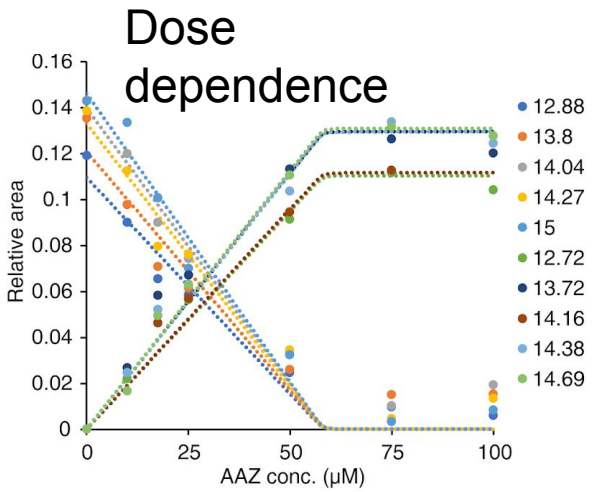


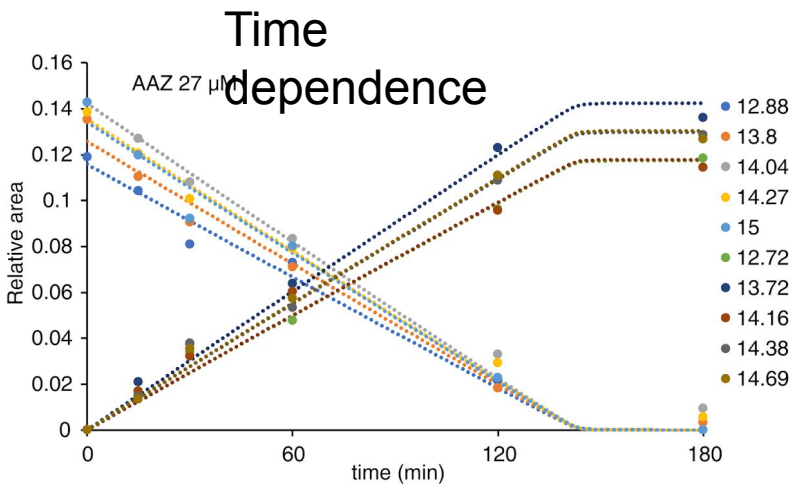
Non-binding ligands

No correlation with K_I

Luchinat E, Barbieri L, Cremonini M, Nocentini A, Supuran CT, Banci L, *Angew Chem Intl Ed. 2020*

Dose- and time-response analysis





Diffusion through the plasma membrane is the rate-limiting step

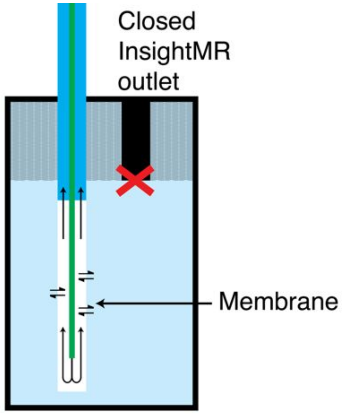
Membrane permeability coefficients (K_p) are obtained by fitting the curves with a kinetic model

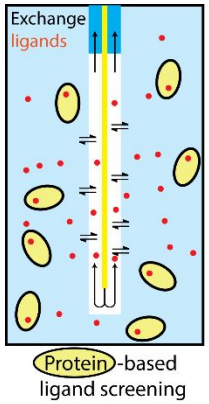
Striking correlation between
Permeability values and Doses of approved drugs

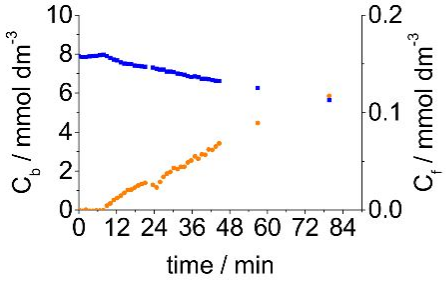
Luchinat E, Barbieri L, Cremonini M, Nocentini A, Supuran CT, Banci L, *Angew Chem Intl Ed. 2020*

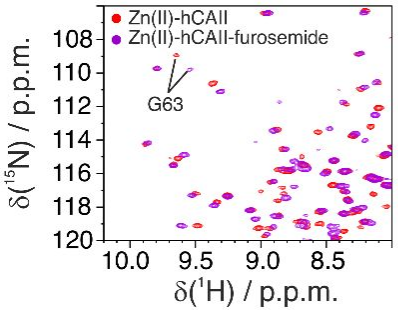
Confining proteins in the NMR tube for drug screening

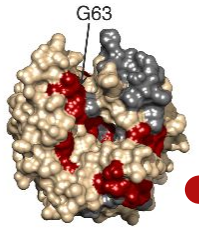
The interaction of a protein target with different ligands as they are flowing through a membrane can be monitored in a structure-based drug-screening



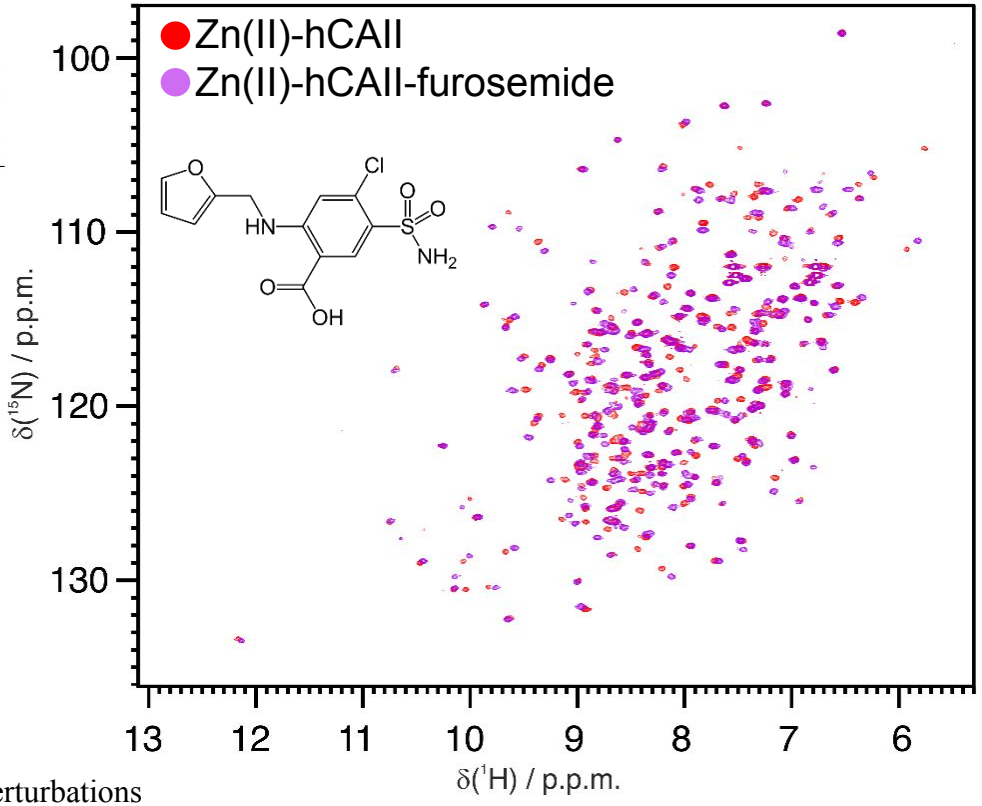








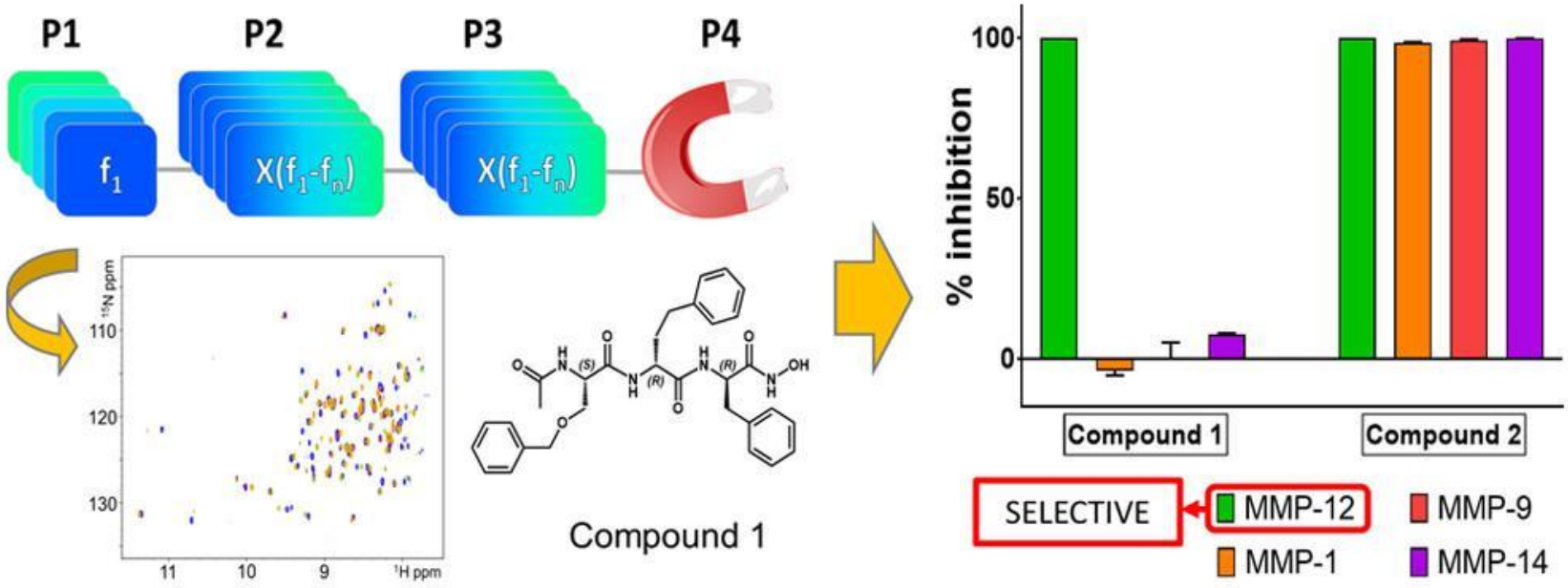
C.S. perturbations



L. Cerofolini, S. Giuntini, L. Barbieri, M. Pennestri, A. Codina, M. Fragai, L. Banci, E. Luchinat, E. Ravera, *Biophysical Journal, 2019*

Drug screening by NMR

HTS by NMR for the design of Potent and Selective Inhibitors of Metalloenzymes



Biophysical screening of fragment libraries led to the identification of fragments specific and selective for the given target.

Screening of a library by NMR spectroscopy in solution allowed the design of a novel and selective compound targeting MMP-12.

SERVICES

Screening

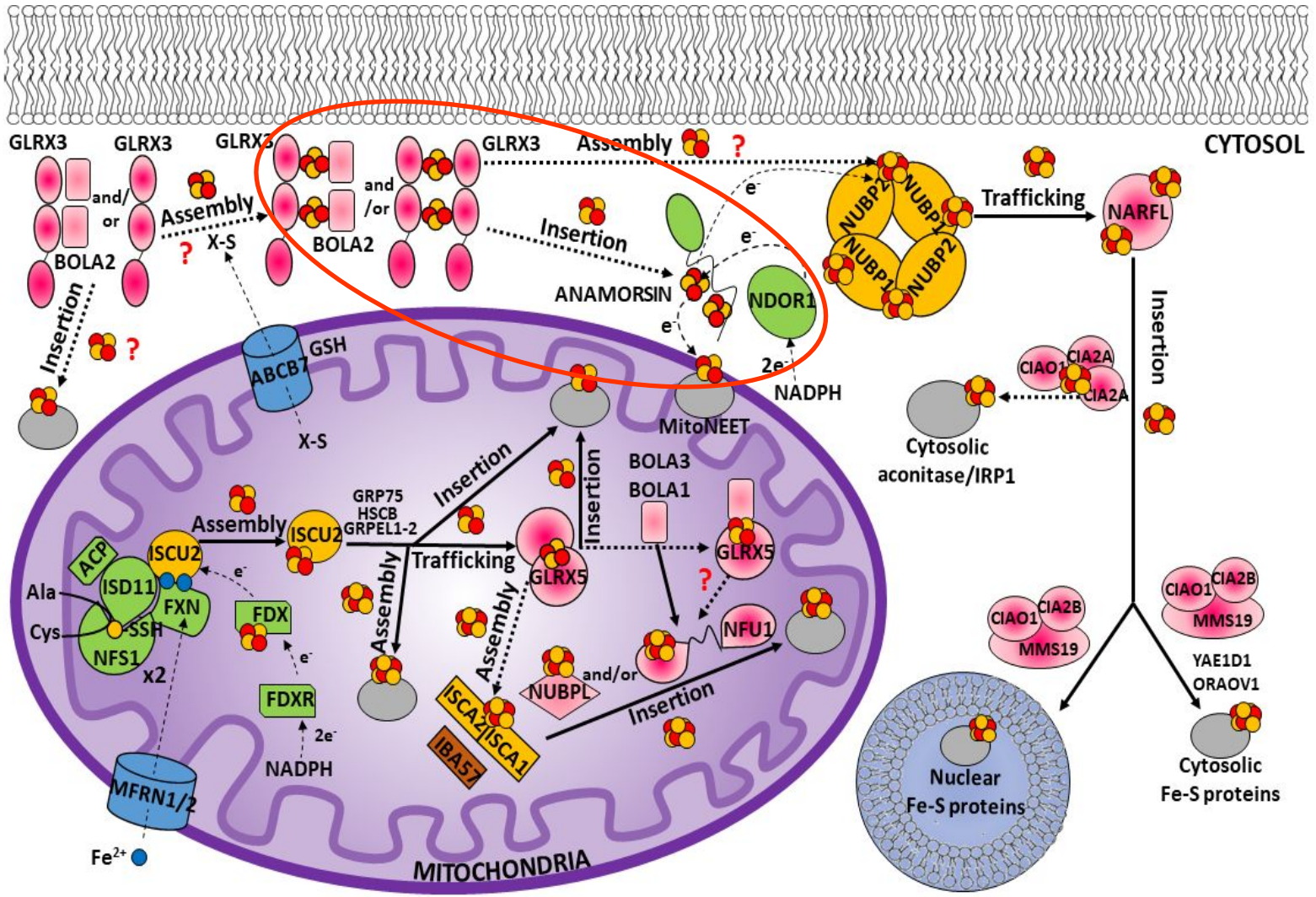
An EU-OPENSCREEN screening site will take the robust, miniaturised assay together with established orthogonal and counter screens through the high throughput screening process, followed by confirmatory (dose response) and counter screening (identical assay without the target or with non-functional target), until a final list of confirmed hit compounds is established.

EU-OPENSCREEN offers researchers from Europe and around the world open access to a uniquely broad range of high technologies and tools for the systematic screening of chemical substances for their biological effects.

EU-OPENSCREEN integrates high-capacity screening platforms throughout Europe, which jointly use a rationally selected compound collection, comprising up to 140.000 commercial and proprietary compounds collected from European chemists.

Iron Sulfur Biogenesis in human cells

*Humans have around **70** Fe-S proteins! They are **less than 0.5%** of the human proteome, but **absolutely essential**. And the Fe-S clusters need to be synthesized!*



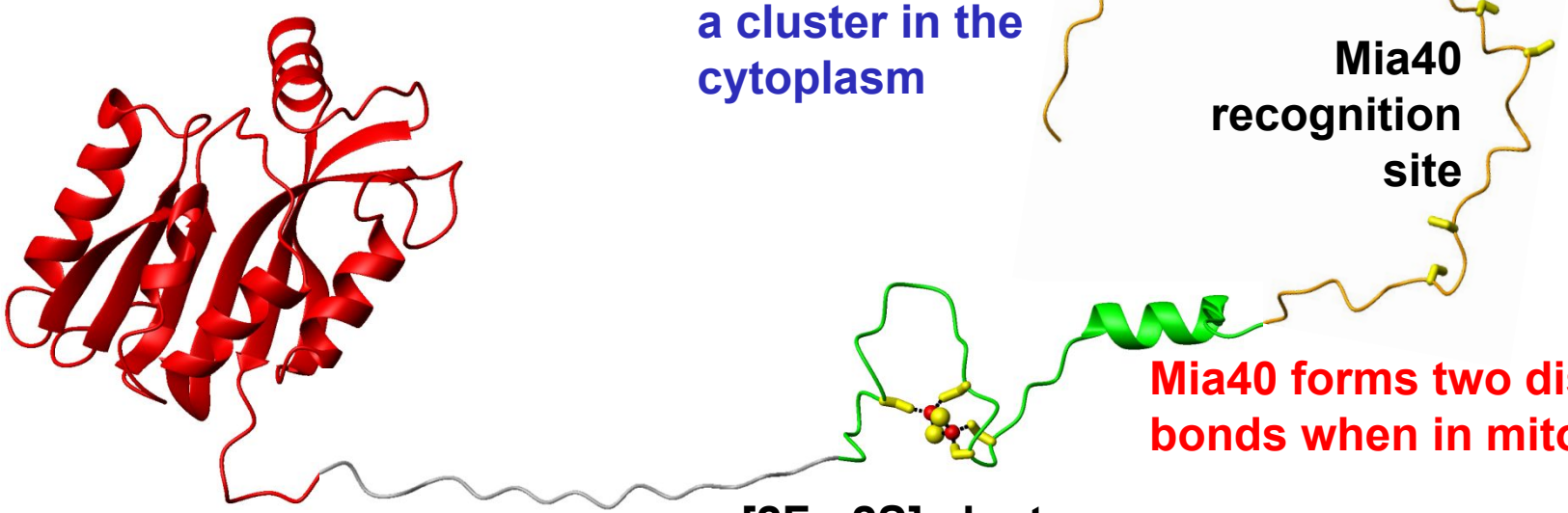
Ciofi-Baffoni S, Nasta V, Banci L., Metallomics. 2018, 49-72

Structural properties of Anamorsin
An essential protein for FeS cluster biosynthesis

Intrinsically Disordered Domain

N-terminal domain

This motif binds a cluster in the cytoplasm



Mia40 forms two disulfide bonds when in mitochondria

Linker

[2Fe-2S] cluster

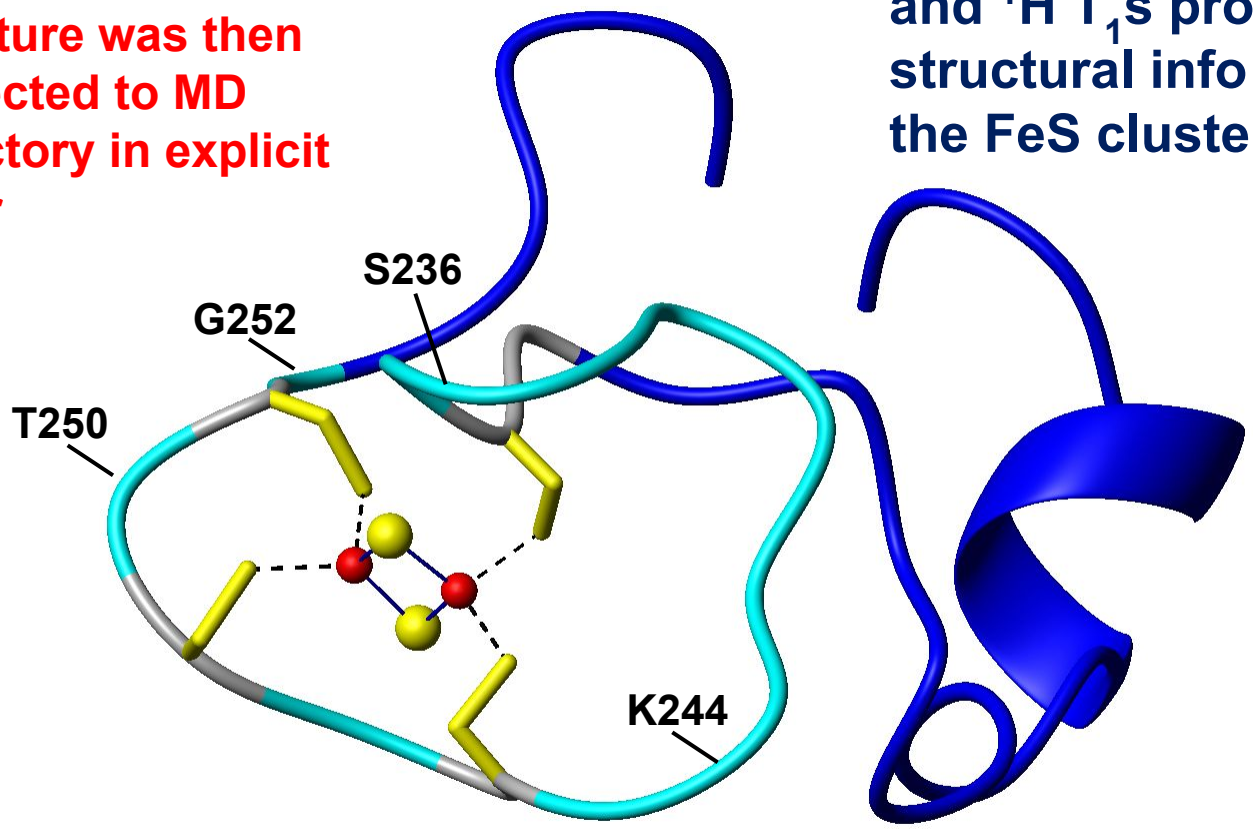
Folded domain
"standard" approach

Highly paramagnetic with fast relaxation and no PCS
The worst case!!

Structure of the [2Fe-2S] cluster binding region in anamorsin

The overall structure was then subjected to MD trajectory in explicit water

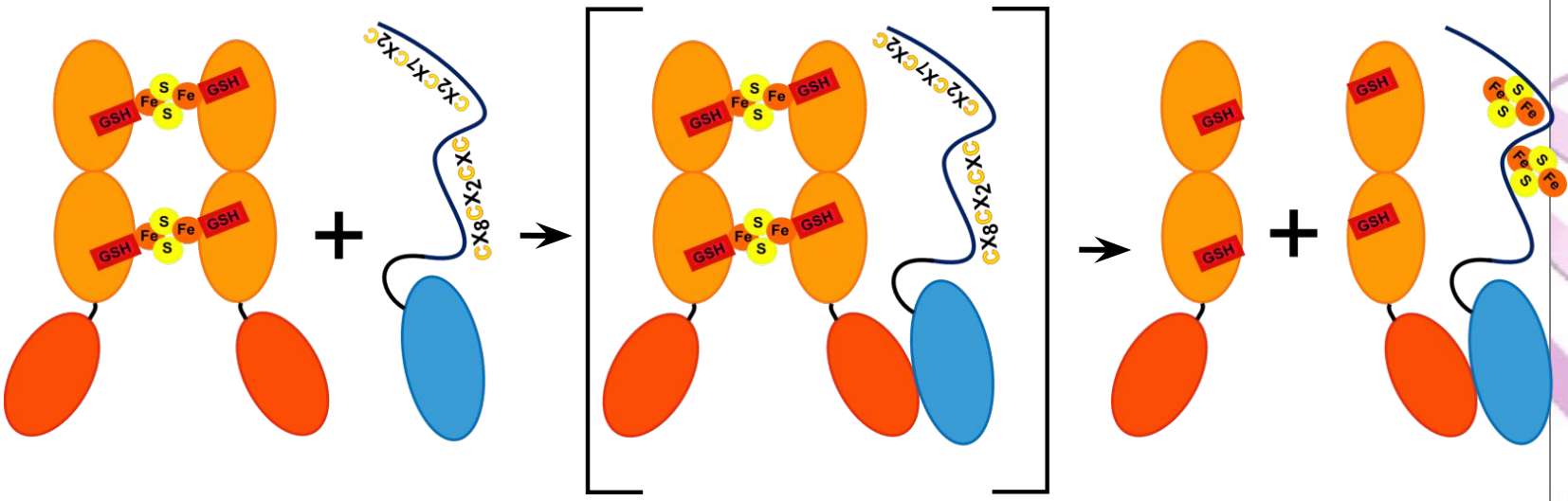
The "paramagnetic" ^{13}C and ^{1}H T_1s provide structural info around the FeS cluster

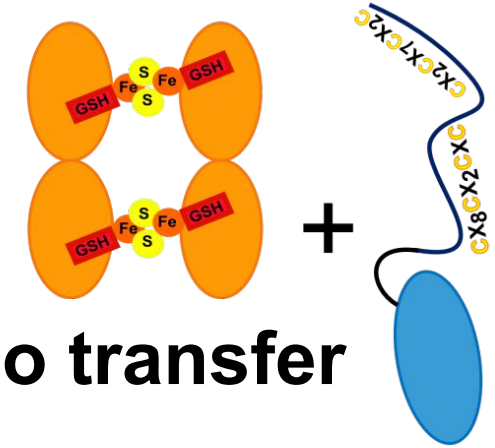


Blue - residues detected in the "diamagnetic" experiments

Cyano - residues whose ^{13}C or ^{15}N signals were detected in paramagnetic-tailored ^{13}C or ^{15}N experiments

Anamorsin receives the [2Fe-2S] clusters from GRX3 in the cytoplasm





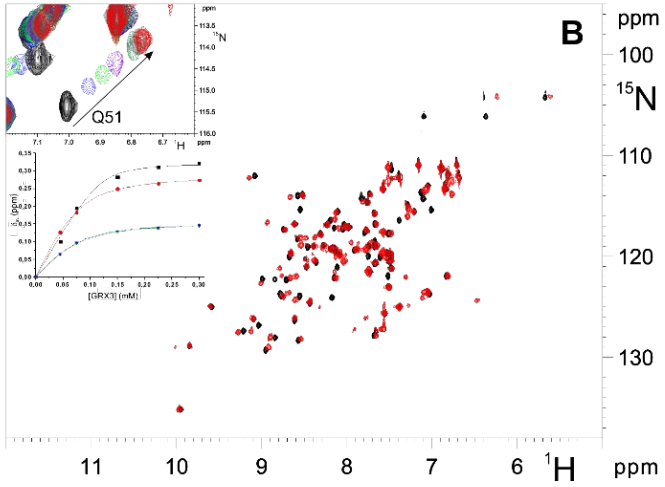
No transfer

The Trx domain of GRX3 is essential for protein-protein recognition

Cluster site characterization complemented with EPR and Moessbauer data

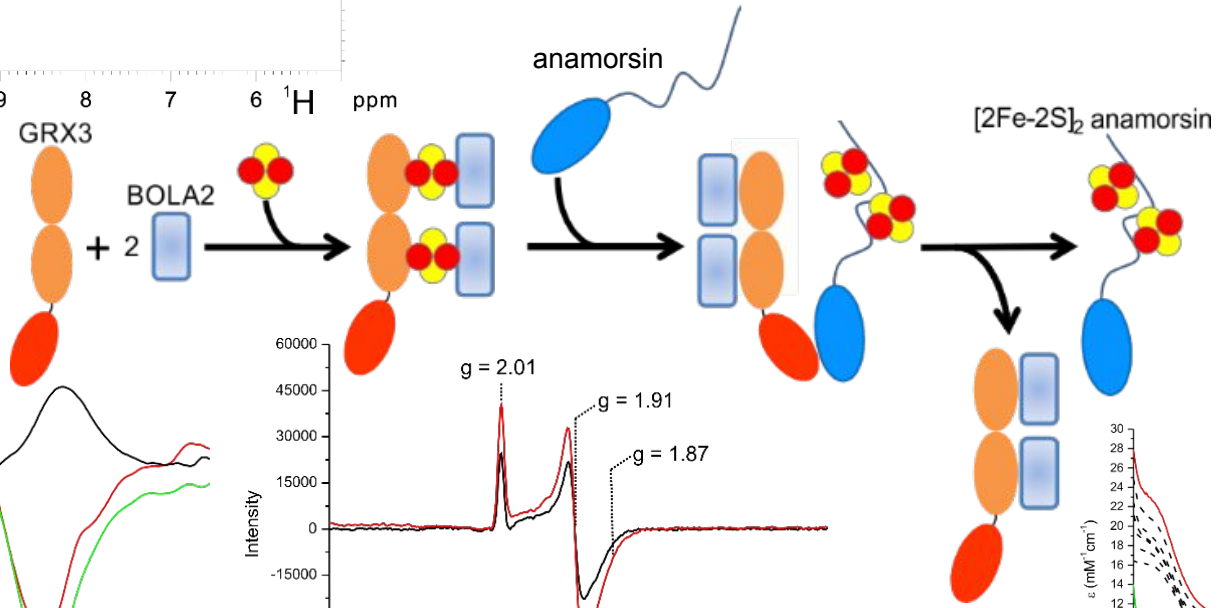
Banci, Ciofi-Baffoni, Gajda, Muzzioli, Peruzzini, Winkelmann, *Nat. Chem Biol. 2015*

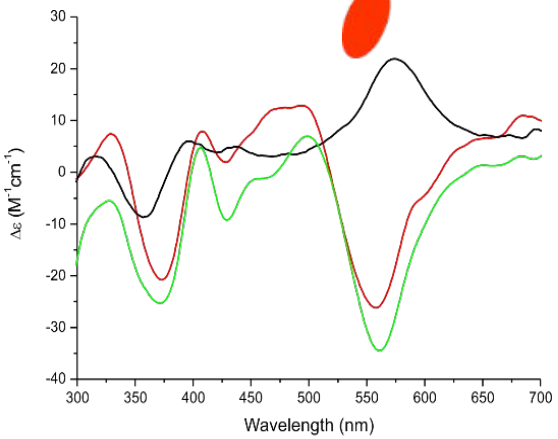
…but in oxidative conditions, the BolA2-GRX3 complex is more efficient in anamorsin maturation

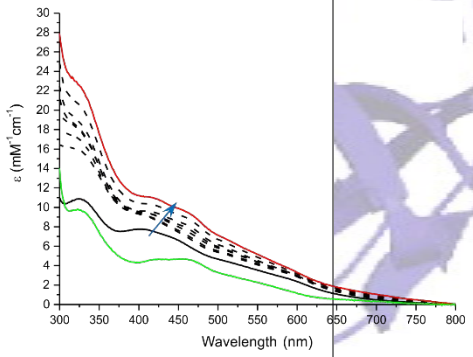


The GRX3/BolA2 heterocomplex is more stable in oxidative conditions

An alternative route to anamorsin maturation

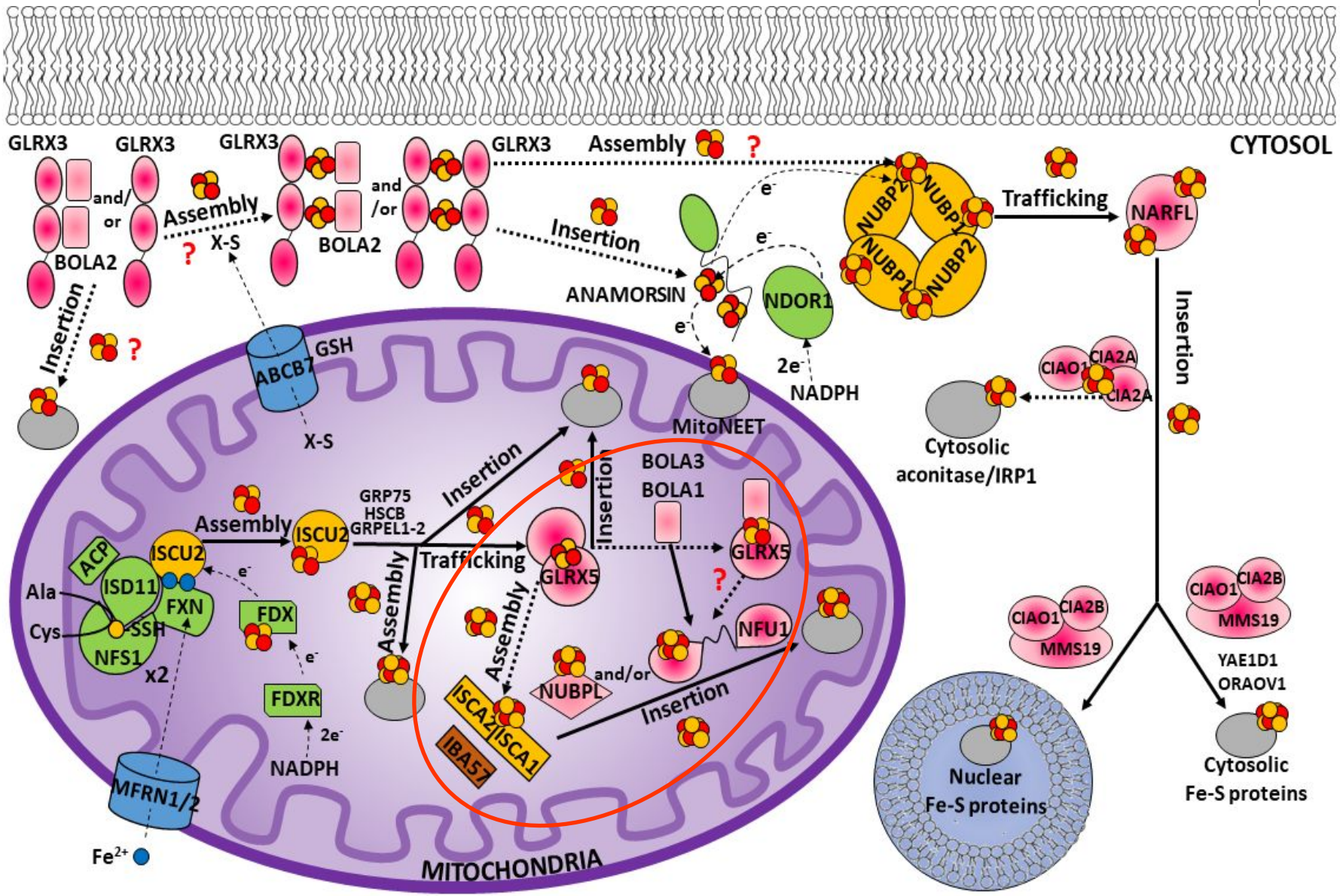






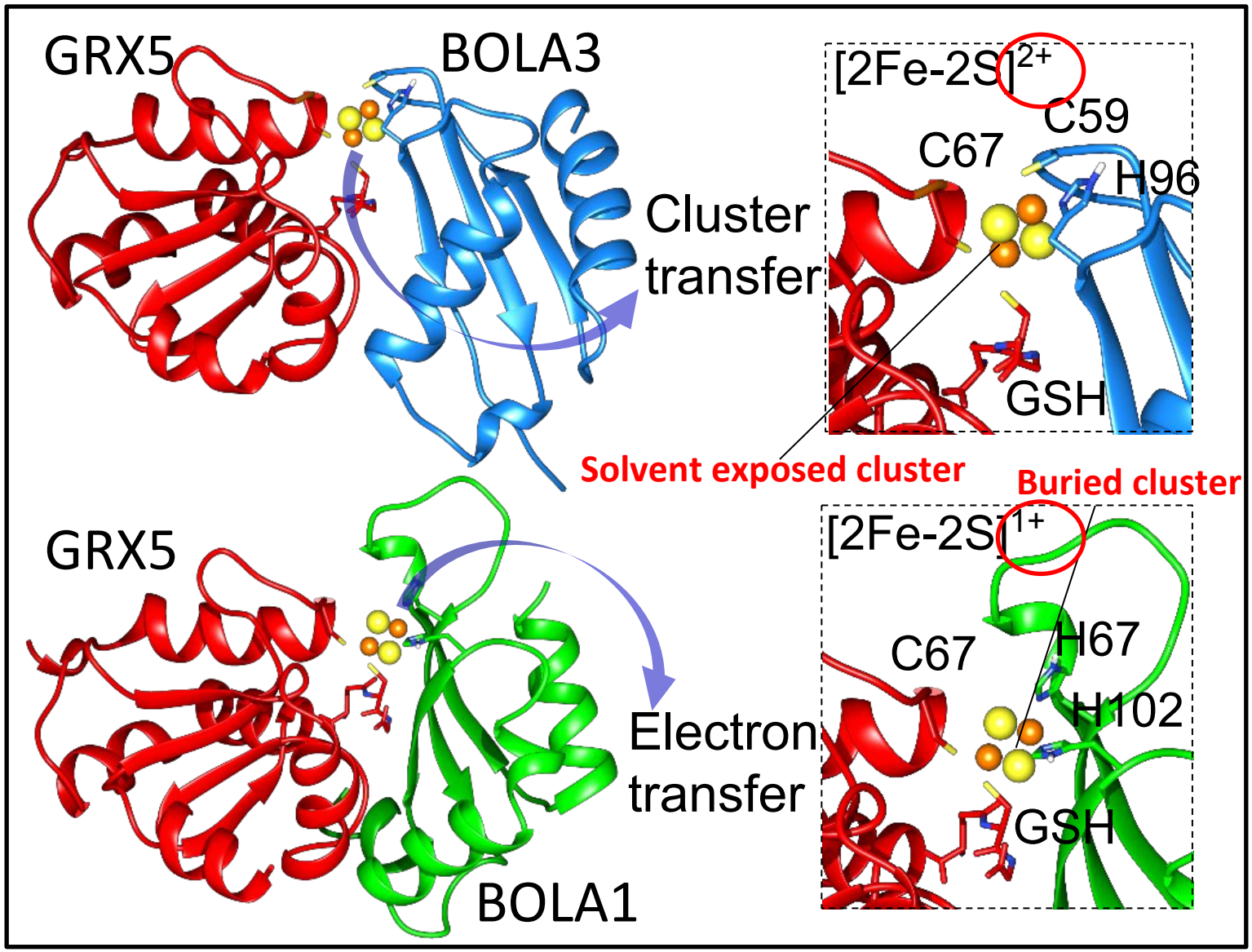
Banci L et al. *J Am Chem Soc.* 2015, Nuttle, X, Banci L, Eichler EE et al. *Nature* 2016

Iron Sulfur Biogenesis in human cells



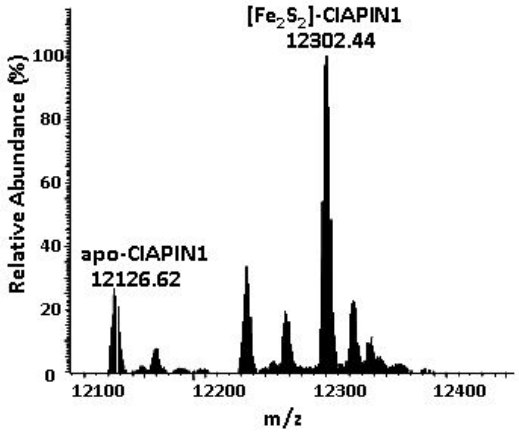
Ciofi-Baffoni S, Nasta V, Banci L., Metallomics. 2018, 49-72

The structures of [2Fe-2S] GRX5-BOLAs support two different functions for the two heterocomplexes



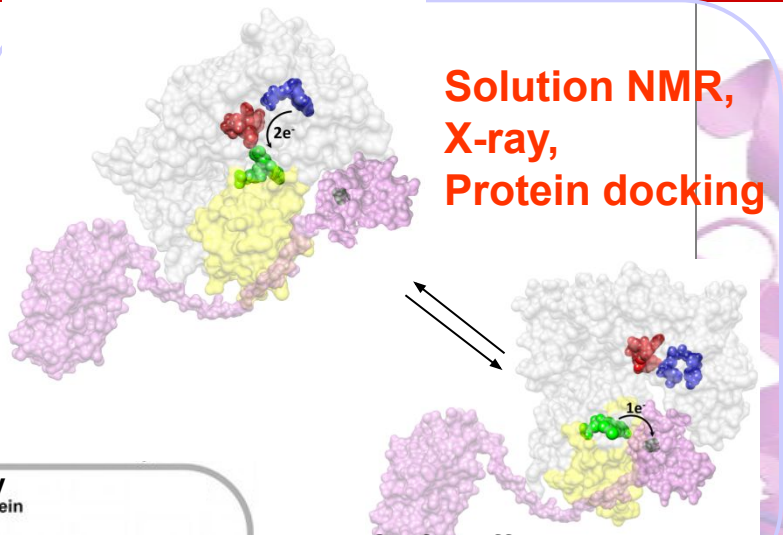
An integrated approach to investigate electrons and FeS cluster transfer processes

ESI-MS



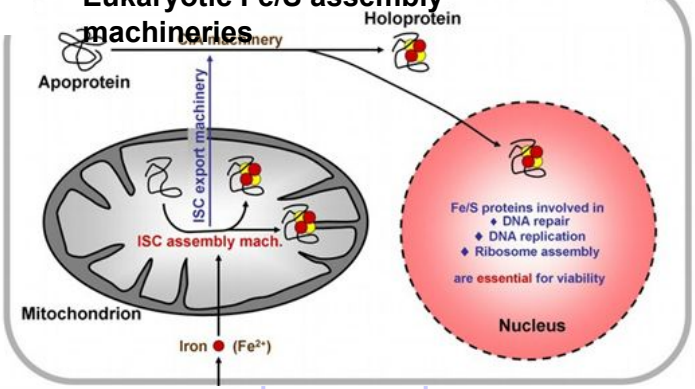
Banci, Ciofi-Baffoni *et al.*
Chem Biol. 2011

Solution NMR, X-ray, Protein docking

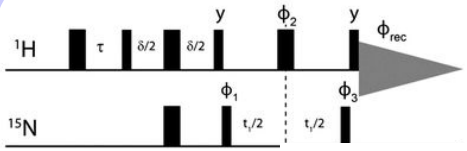


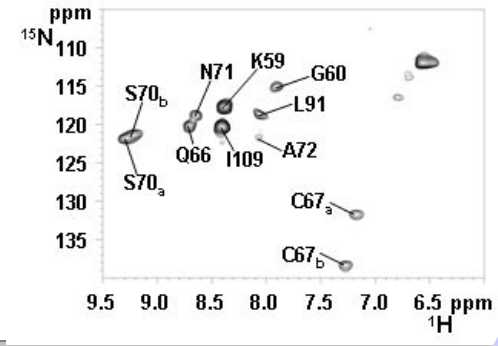
Banci, Ciofi-Baffoni *et al.*
PNAS 2013

Eukaryotic Fe/S assembly machineries



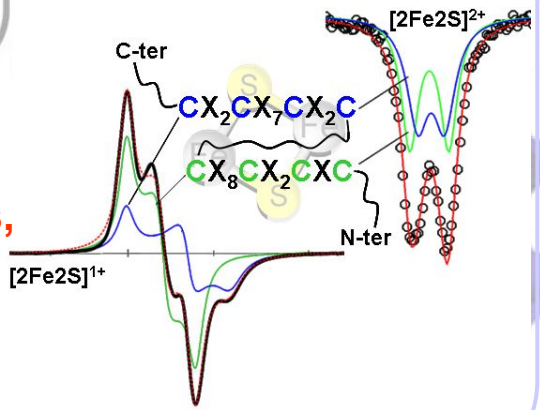
Para-NMR





Banci *et al.*
PNAS 2014

FF parameters determination



Biophysics: EPR, UV-vis, Mössbauer CD …

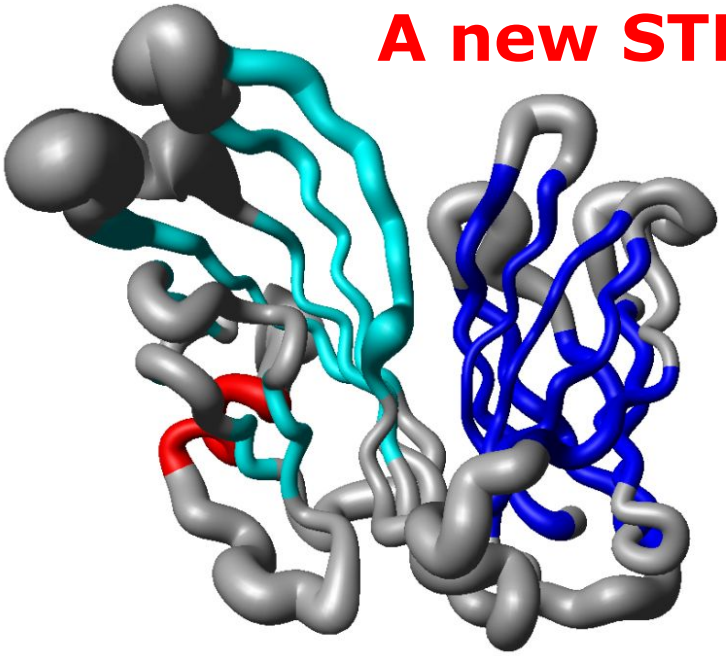
Banci, Ciofi-Baffoni, Bill *et al.* JBIC 2013

Structural Vaccinology:
the structure-based rational vaccine design

Meningococcal Infection — Unmet Medical Need

No suitable polysaccharide vaccine can be produced against MenB as its capsular polysaccharide is poorly immunogenic, making this type of vaccine ineffective.

A new STRATEGY needs to be developed



Determine the ANTIGEN STRUCTURE and study THE INTERACTIONS

between fHbp (variants 1, 2 and 3) and the antigen binding fragment (fAb) of various monoclonal antibodies (mAb)

TO CREATE

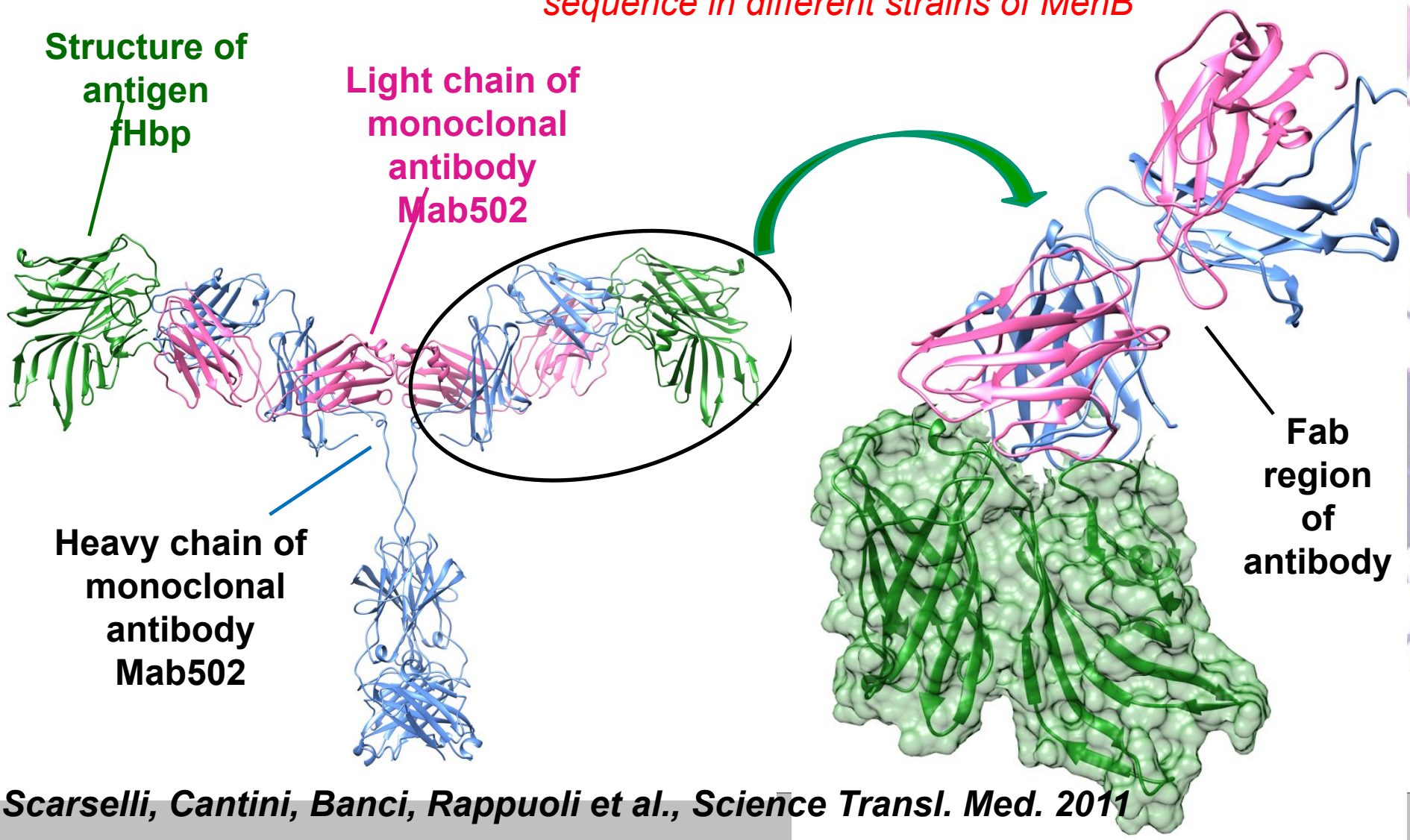
a ***chimeric protein*** in order to design a vaccine able to induce ***broad protective immunity*** against all antigenic variants of the pathogen

A collaboration with NOVARTIS gsk

The structure knowledge of potential antigens allowed us the identification of epitopes and the rational design of vaccine candidates.

Complex of a monoclonal antibody with a Meningococcus B antigen (Factor H binding protein)

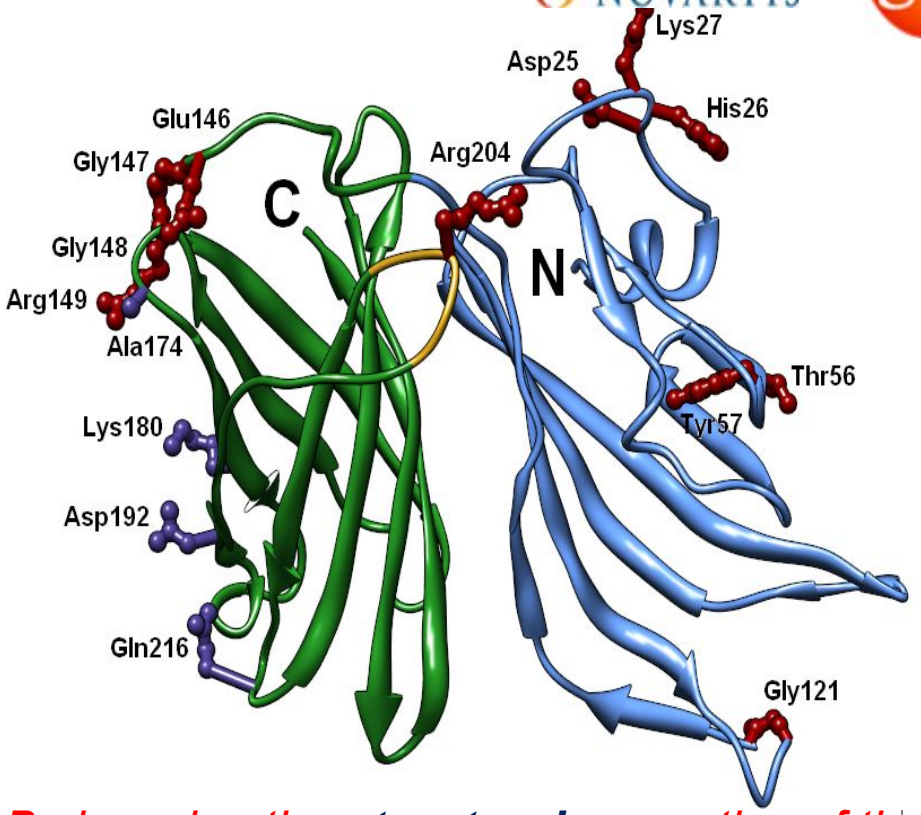
fHbp is very effective in inducing protective immunity eliciting antibodies but has different sequence in different strains of MenB



Scarselli, Cantini, Banci, Rappuoli et al., Science Transl. Med. 2011

Structure-based design of a vaccine against Mengingococcus B

A collaboration with



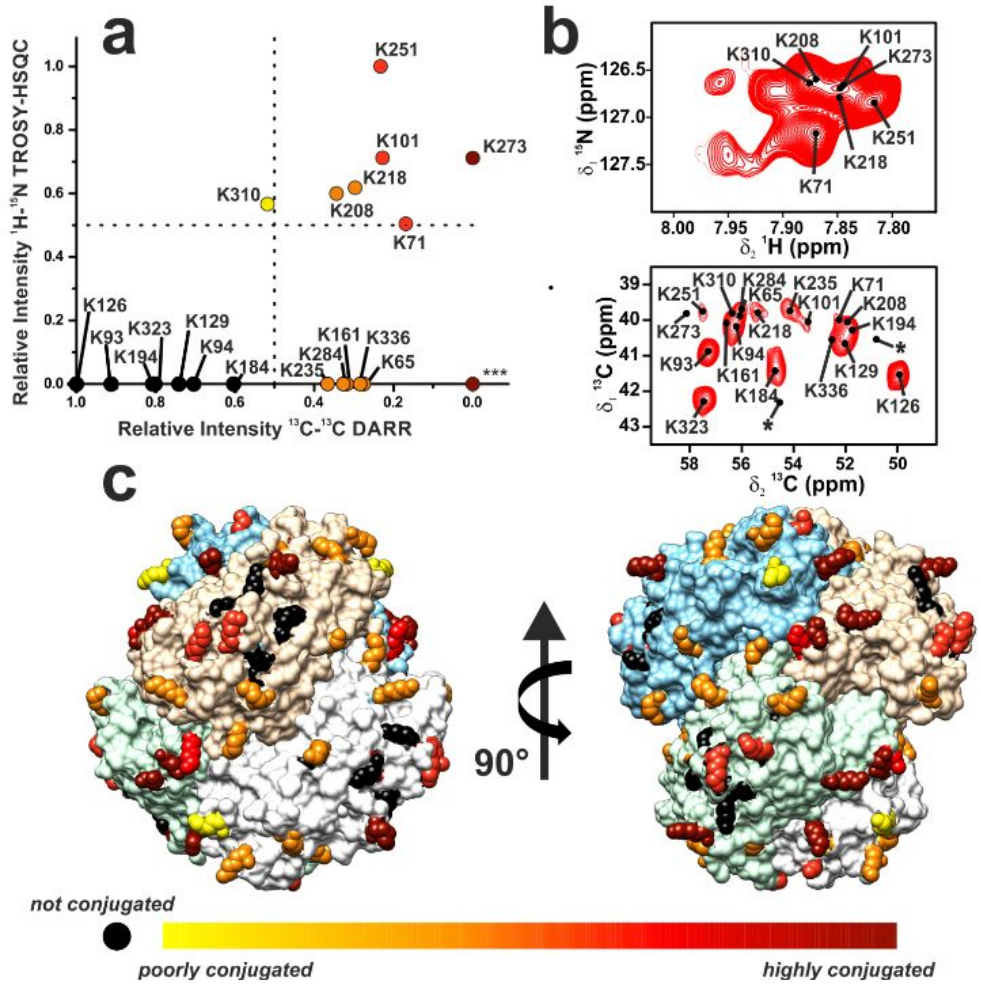
*By knowing the **structural** properties of the antigen and of the epitopes in all the variants, a **chimera antigen** was produced which elicits **complete protective immunity.***

Patent WO 2011051893 A1

Scarselli, Cantini, Banci, Rappuoli et al., Science Transl. Med. 2011

Vaccine control

A collaboration with NOVARTIS gsk



A novel approach exploiting solution and solid-state NMR methods was established as a control of polysaccharide-protein conjugated vaccines

NMR spectra and data analysis of L-asparaginase II (ANSII) conjugated to meningococcal serogroup C (MenC) polysaccharides. Lysine residues are color-coded according to the degree of conjugation, from dark red (highly conjugated) to yellow (poorly conjugated). The not conjugated lysines are represented as black spheres.

Giuntini, Balducci, Cerofolini, Ravera, Fragai, Berti, Luchinat, Angew. Chemie, 2017

STRUCTURAL VACCINOLOGY

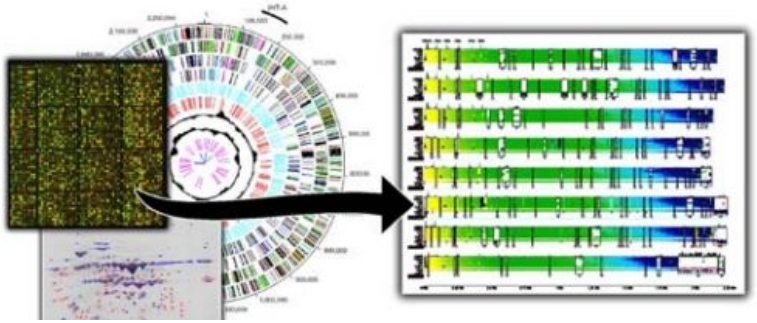
past → present

Conventional vaccinology

Cultivate microorganism

5-15 years

Reverse vaccinology

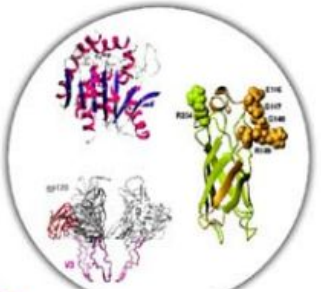


Genome-based approaches

Pan-genome approach

1-2 years

Structural vaccinology



Determination of antigens structure

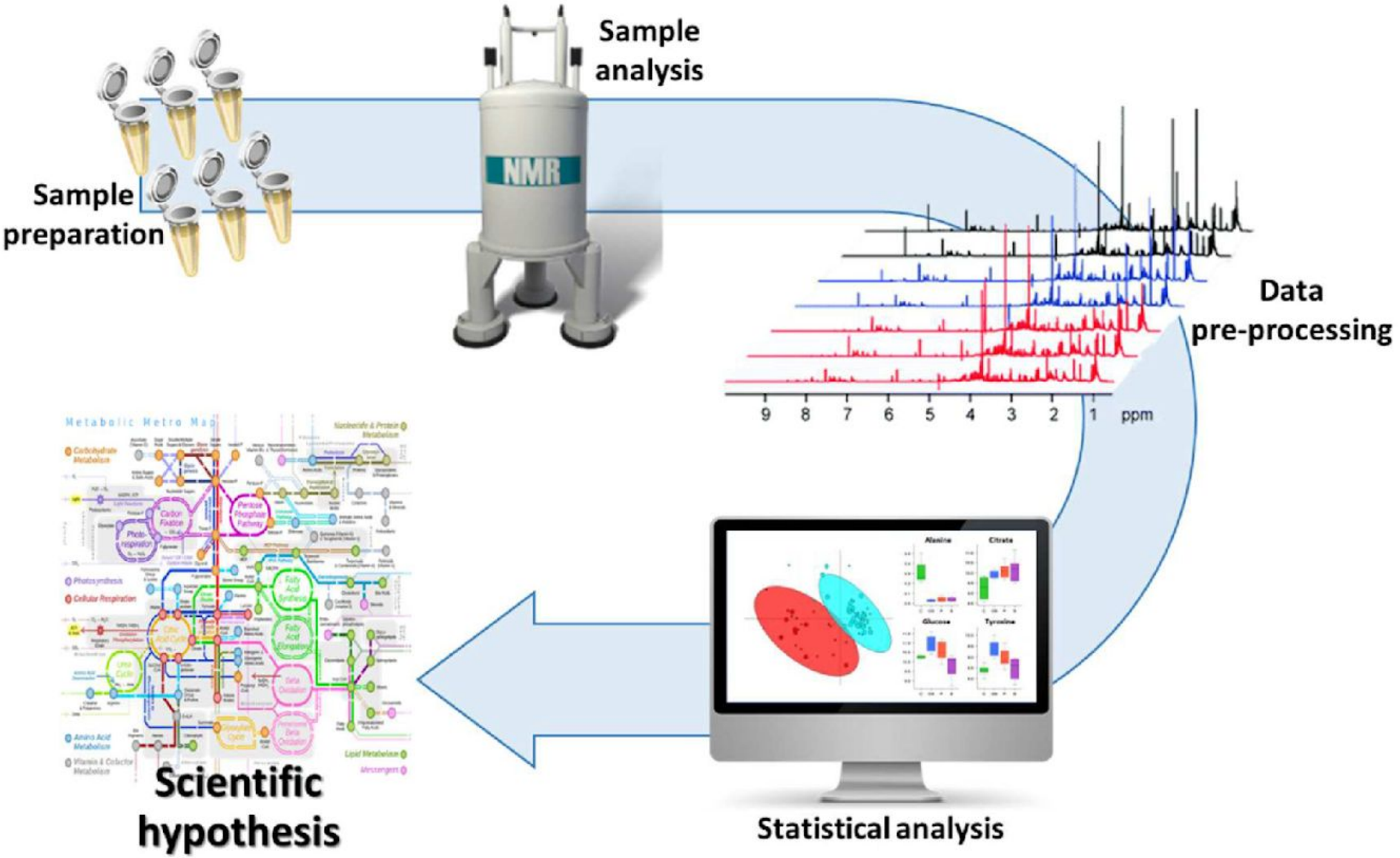
Antigen selection and validation

Rational design of target epitopes

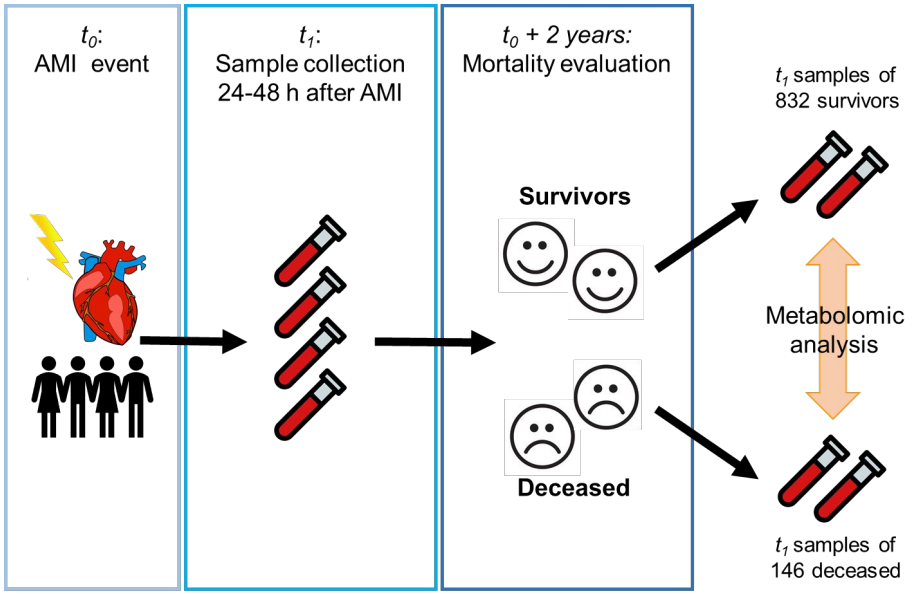
vaccine

The EATRIS Vaccines platform covers the entire vaccine development and production pipeline ranging from late-phase pre-clinical development to clinical trials. Partnering with 15 of Europe's most advanced development centres, the Vaccines platform offers proven state-of-the-art resources for all critical issues related to vaccine development.

Metabolomics by NMR



AMI-Florence II study



The goal: identification, through serum NMR-based metabolomics analysis, of **patients with high risk of death** within two years from Acute Myocardial Infarction

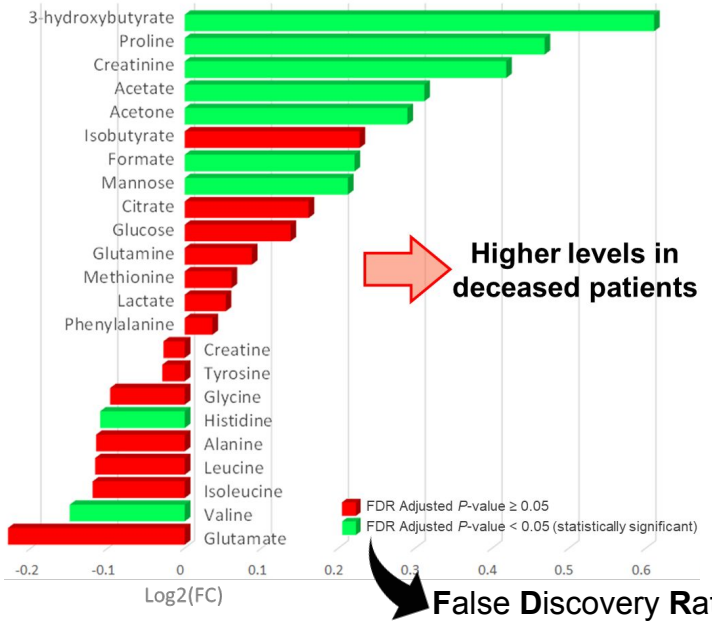
Training set:
80 Survivor patients
40 Deceased patients

Validation set:
752 Survivor patients
106 Deceased patients

The **G**lobal **R**egistry of **A**cute **C**oronary **E**vents (hospital score) calculated on clinical parameters.

	GRACE score	METABOLOMICS score	METABOLOMICS + GRACE scores
Training set,			
AUROC	0.815	0.859	0.875
Validation set,			
AUROC	0.756	0.801	0.823

Area **U**nder the **R**eceiver **O**perating **C**haracteristic curve



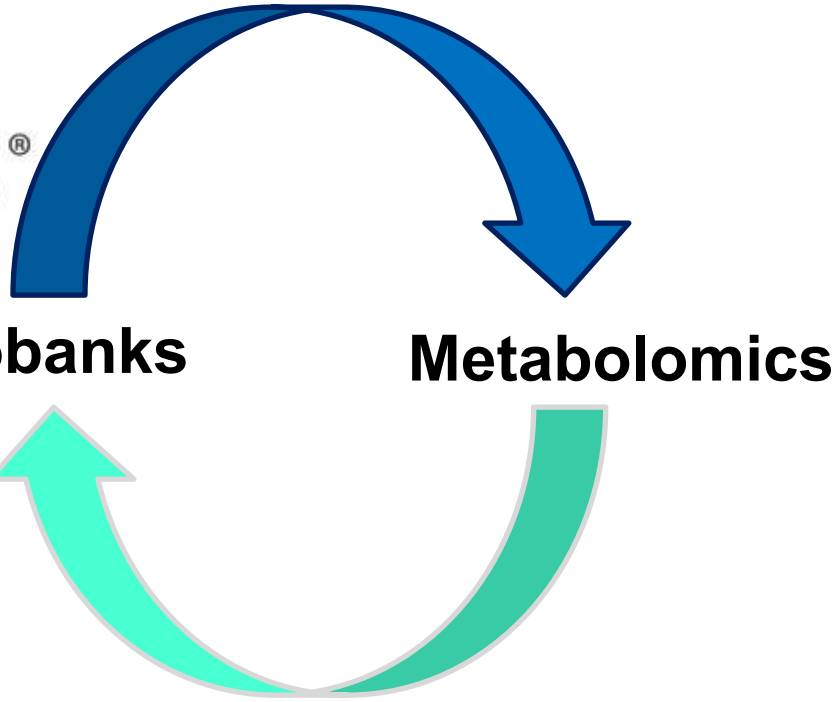
False **D**iscovery **R**ate

A. Vignoli, L. Tenori, B. Giusti, et al., *NMR-based metabolomics identifies patients at high risk of death within two years after acute myocardial infarction in the AMI-Florence II cohort*, BMC Med. 2019; 7;17(1):3.

Biobanks for metabolomics

Provision of large cohorts for metabolomics

BBMRI-ERIC®
gateway for health



Development of validated SOPs and quality control

Metabolomics for biobanks

Biobanks for metabolomics

cancers

MDPI

Article

Effect of Estrogen Receptor Status on Circulatory Immune and Metabolomics Profiles of HER2-Positive Breast Cancer Patients Enrolled for Neoadjuvant Targeted Chemotherapy

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	Visit 1 – Diagnosis	Visit 2 – 12° week treatment	Visit 3 – 24° week treatment	Visit 4 – 2 months after surgery	Visit 5 – 6 months after surgery	Visit 6 – 1 year after surgery	Visit 7 – 2 year after surgery
N of samples	43	34	32	27	22	21	21

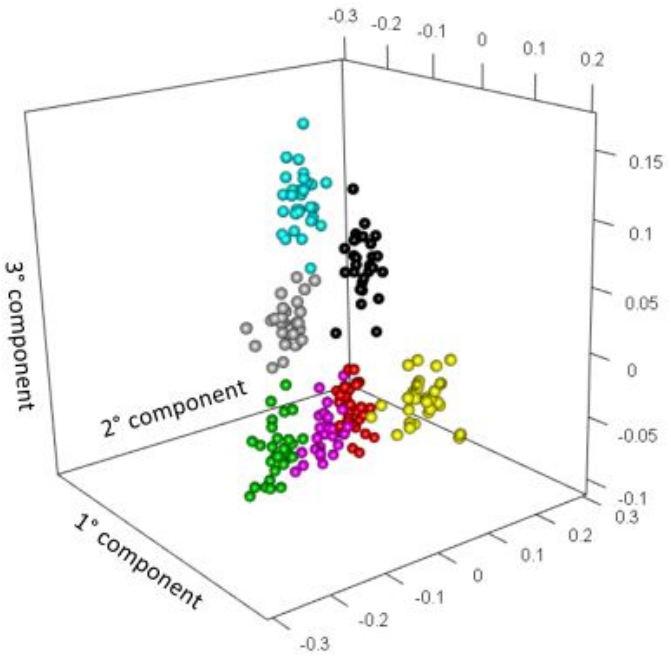
	Complete Responders	Partial Responders
N of patients	27	20

Available information:

- Age
- Type of surgery
- Pathological response
- Tumour size
- Lymph node involvement
- Stage
- Tumour histotype
- ER/PR status
- HER2
- Ki67
- Neoadjuvant CT toxicities
- Recurrence events
- Recurrence locations
- 3-year outcome (Alive/Dead)

Metabolomics for biobanks

8 BBMRI-ERIC biobanks

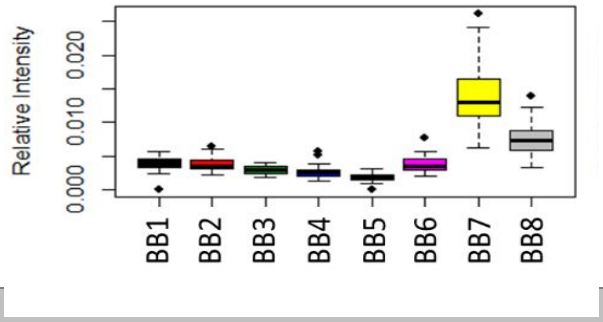


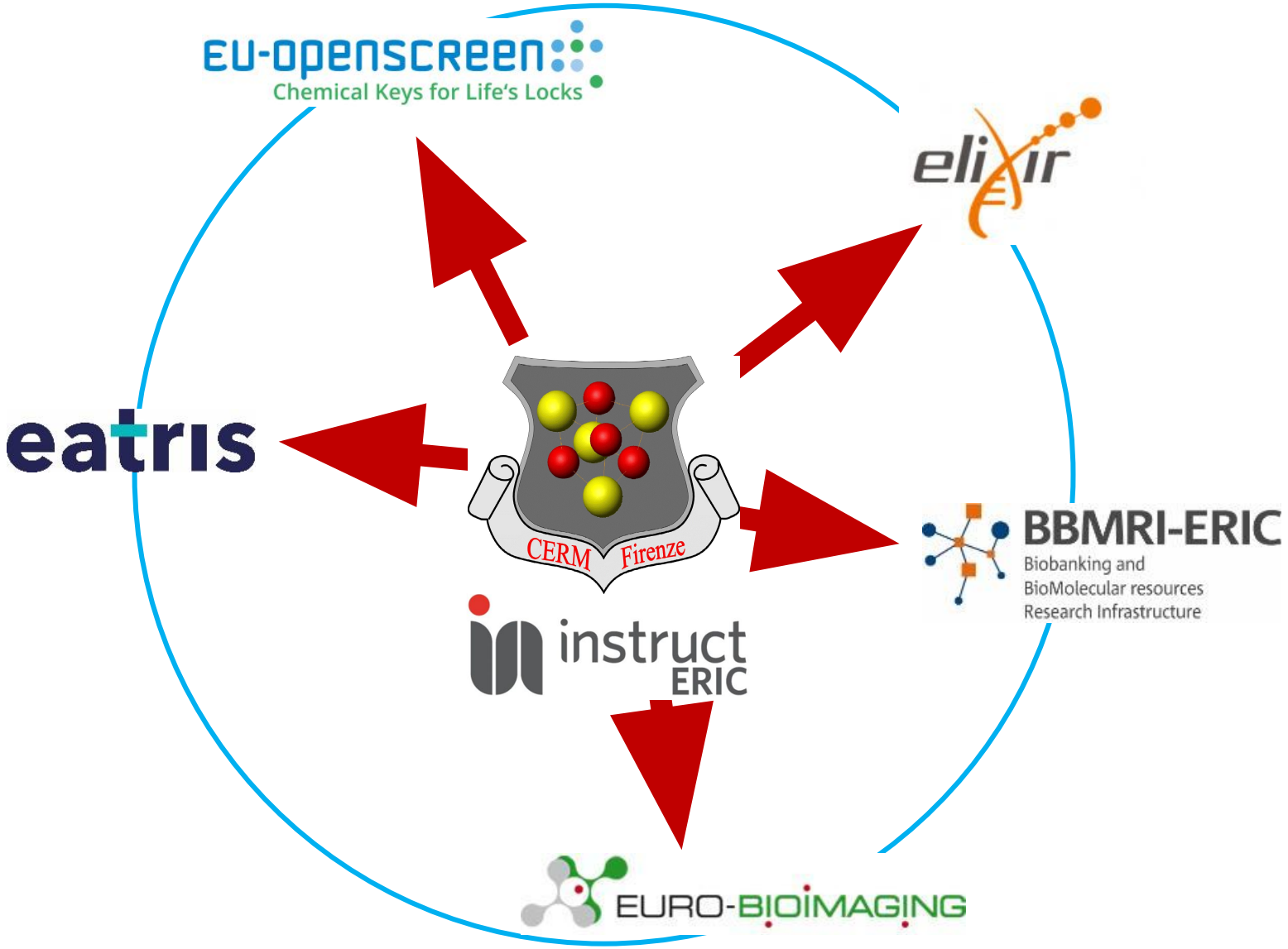
NMR fingerprinting as a function of the different SOPs

Development of validated SOPS to preserve the original metabolome of the sample

CEN Standard CEN/TS 16945:2016
"Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for metabolomics in urine, venous blood serum and plasma"

ISO standard
(under development, CD 23118)

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We may anticipate that the chemist of the future who is interested in biomolecules will come to rely upon a new structural chemistry, and that great progress will be made, through this technique, in biology and medicine.

From the Nobel lecture of Linus Pauling, 1954

Thank you for your attention !!

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