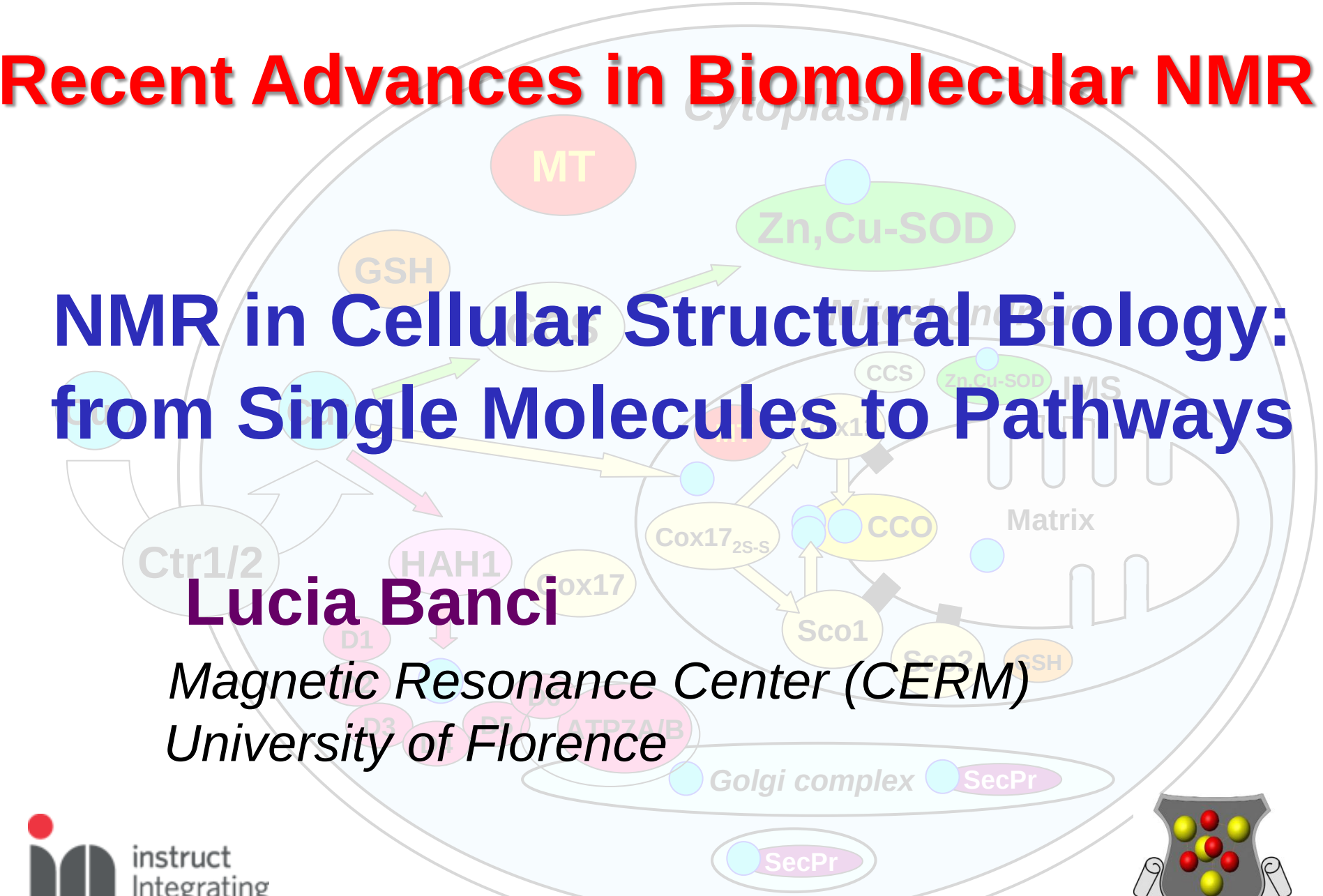
Recent Advances in Biomolecular NMR



NMR in Cellular Structural Biology: from Single Molecules to Pathways

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University of Florence

instruct
Integrating
Biology

CERM Firenze

Recent Advances in Biomolecular NMR

- **In cell NMR**

For studying biomolecules in a cellular context

- **Mechanistic Systems Biology**

To describe and understand biological processes at molecular level

- **Structural Vaccinlogy**

Rational vaccine design based on the structural knowldge of the antigene

Integrating a Cellular Approach with Atomic Resolution

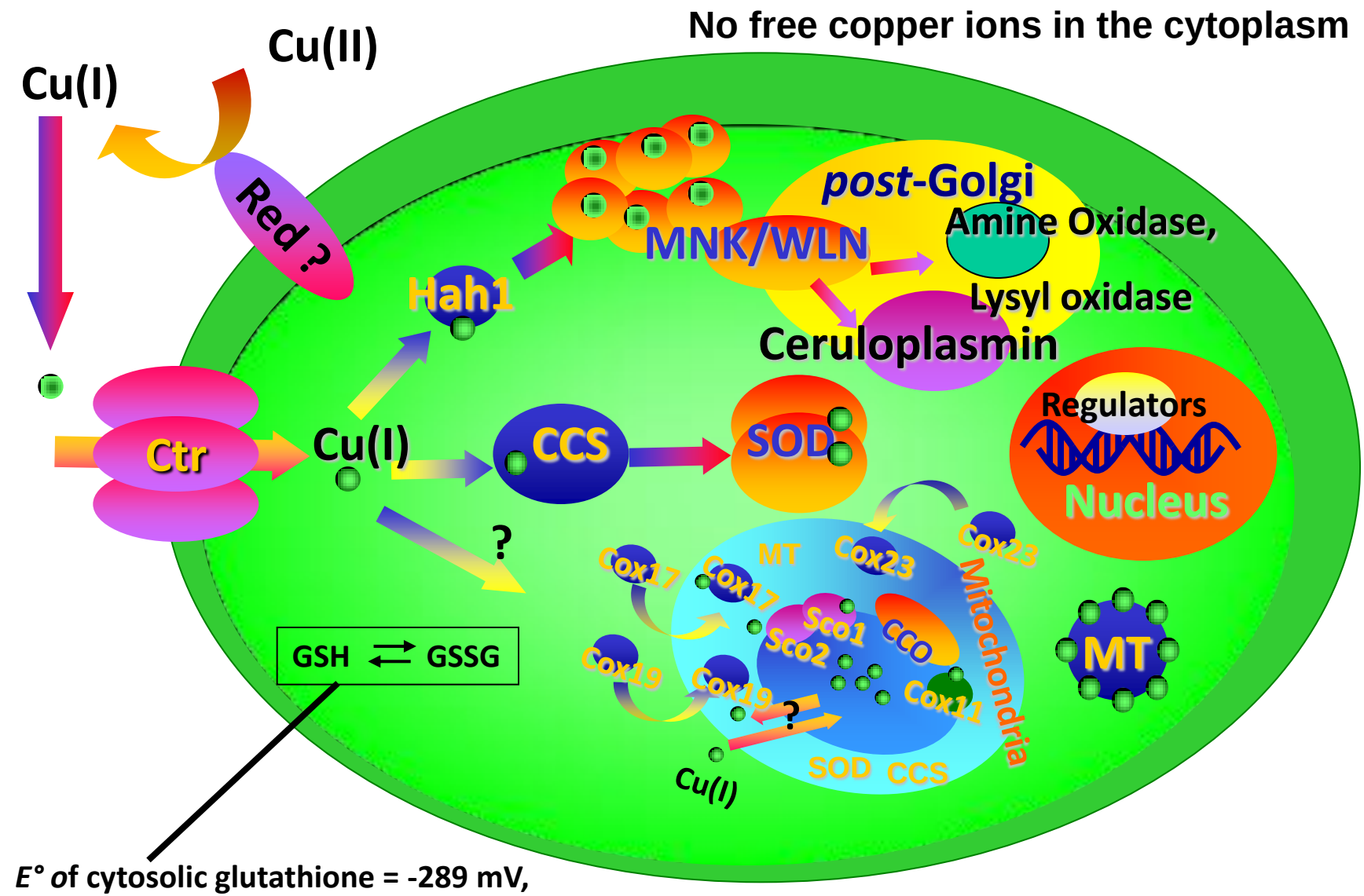
Living systems are complex: mixture of proteins, nucleic acids, other biomolecules, several cellular compartments,...etc

A **Systems Biology** approach is needed.

All the players involved in a given process have to be considered as well as their 3D structural and dynamical interactions determined.

Proteins must be framed within their cellular context

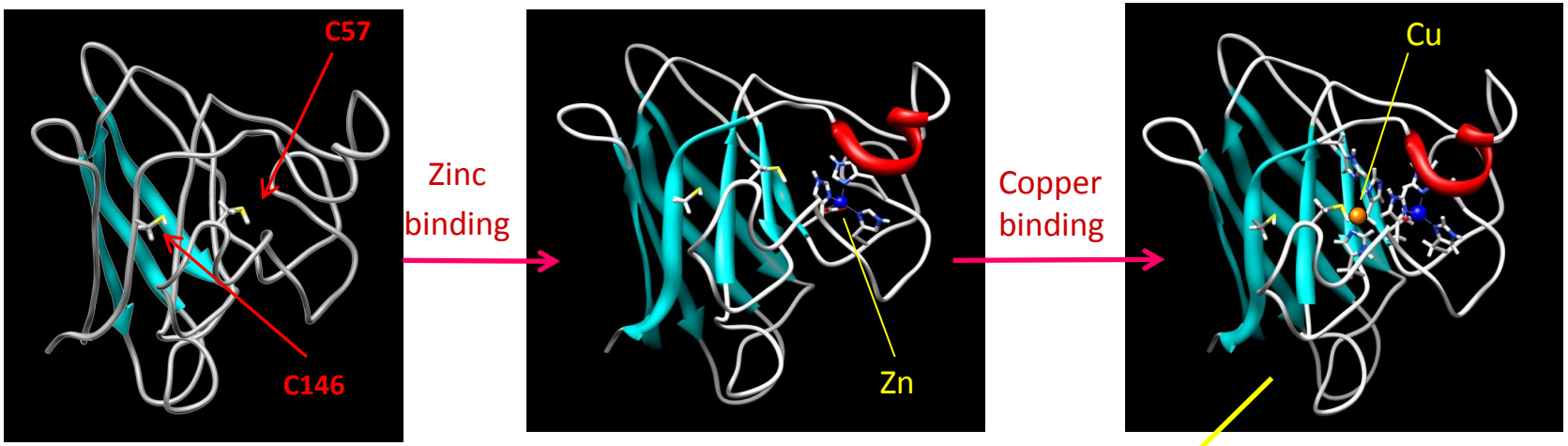
Copper trafficking in human cells



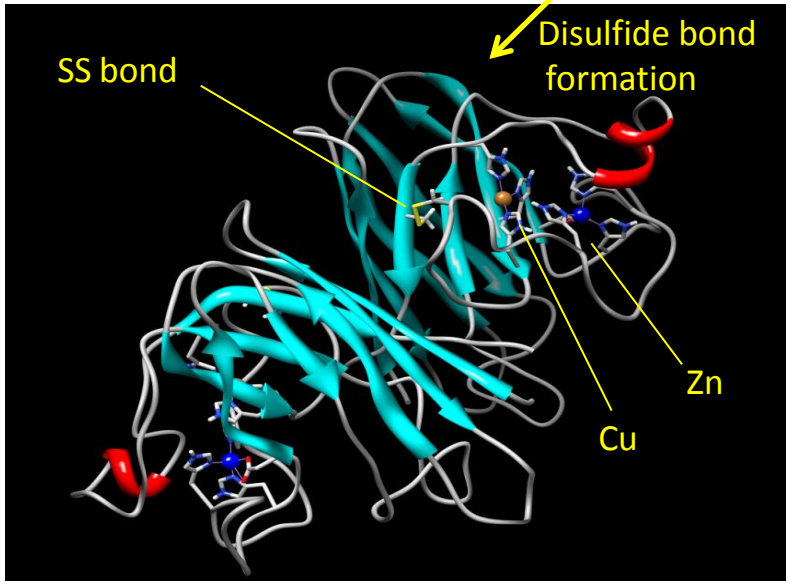
E° of cytosolic glutathione = -289 mV,
corresponding to GSH and GSSG *in vivo* concentrations of 13 mM and 0.7 mM

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:

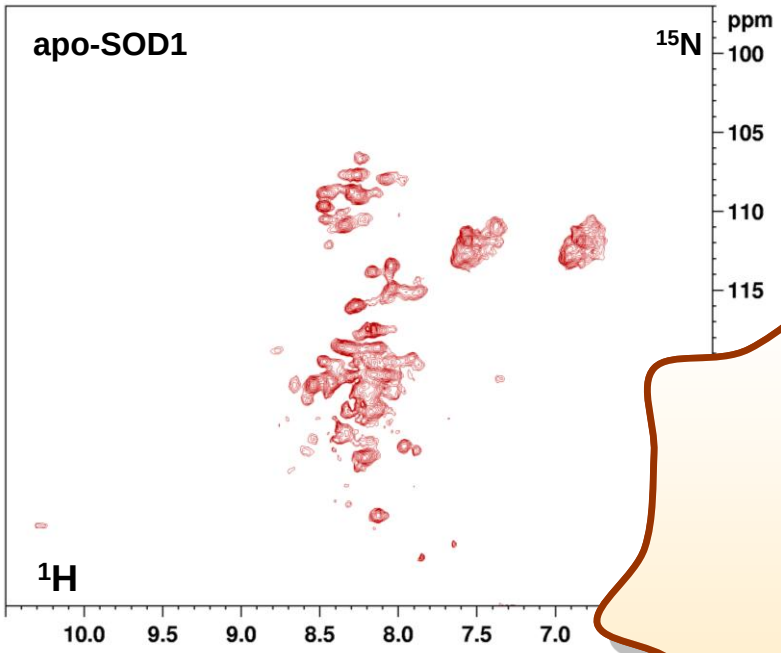
($2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$)

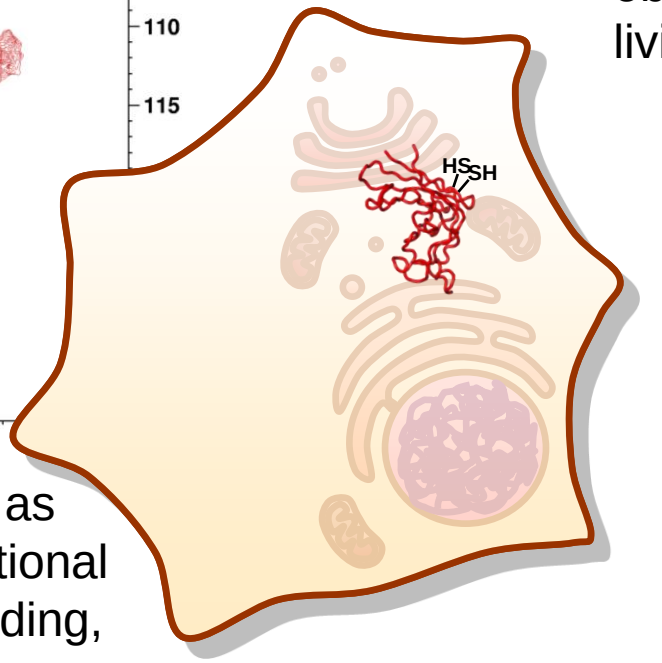
These post translational modifications affect the fold properties and monomer/dimer equilibrium

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

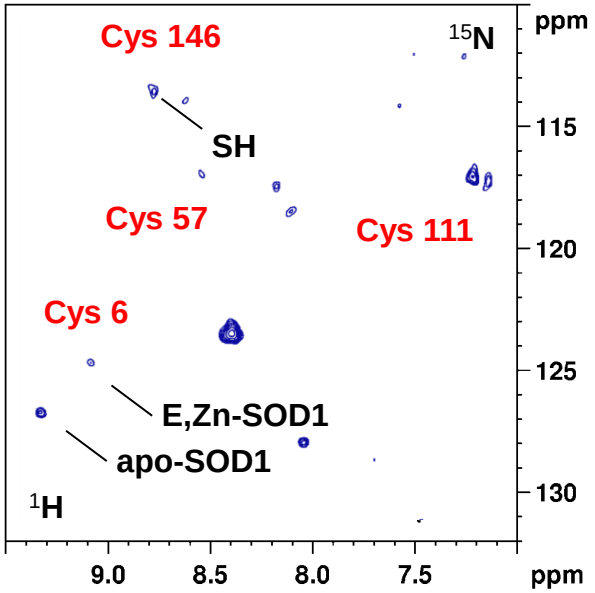
Understanding intracellular processes at the molecular level requires a high resolution description. In-cell NMR provides atomic-level information on a protein in the cellular environment.

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





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



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

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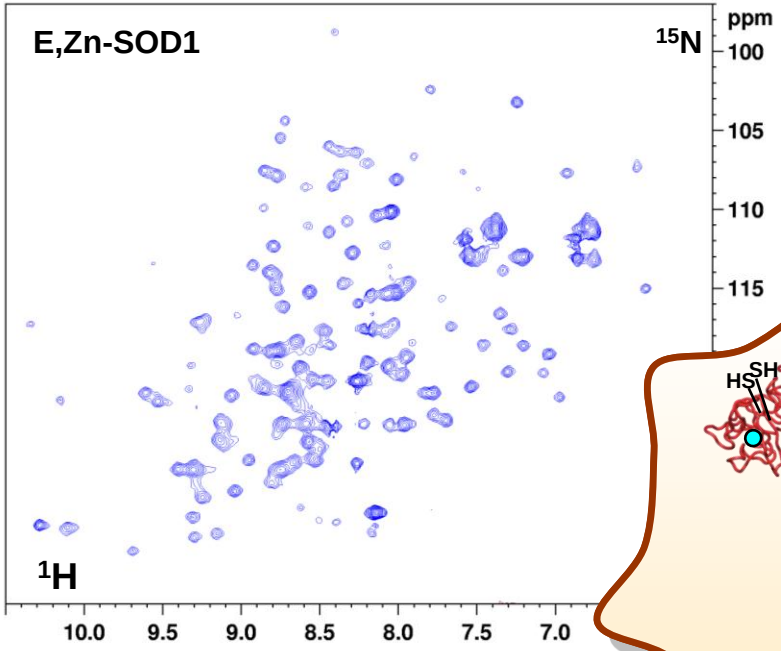

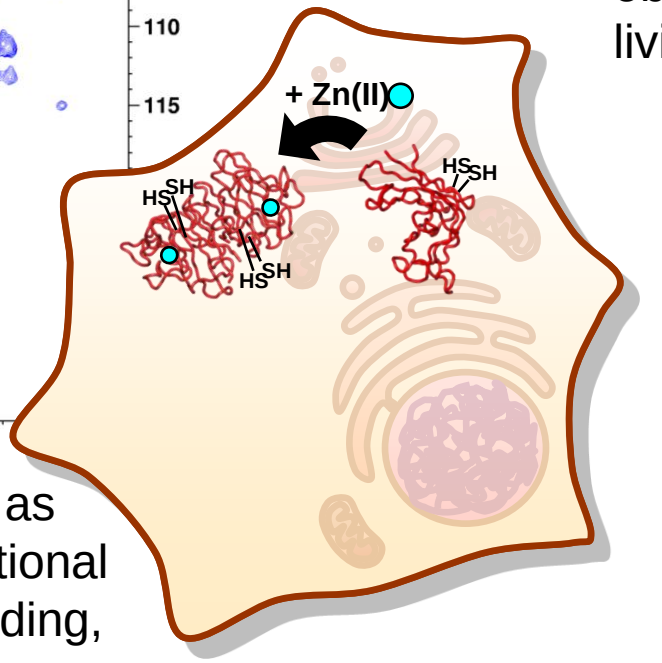

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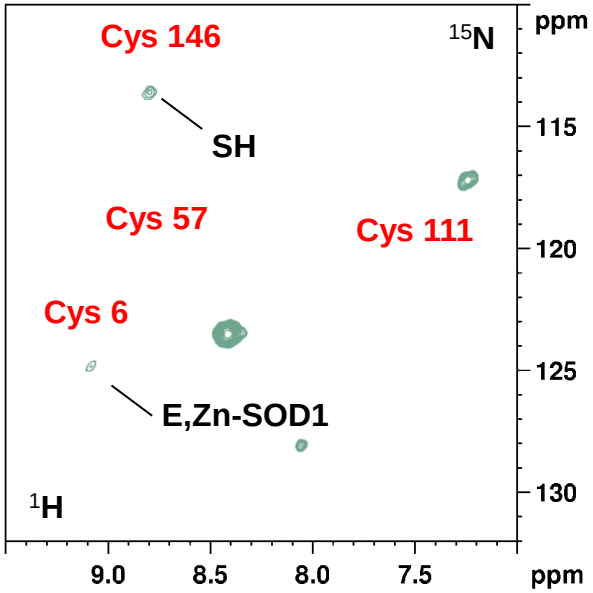

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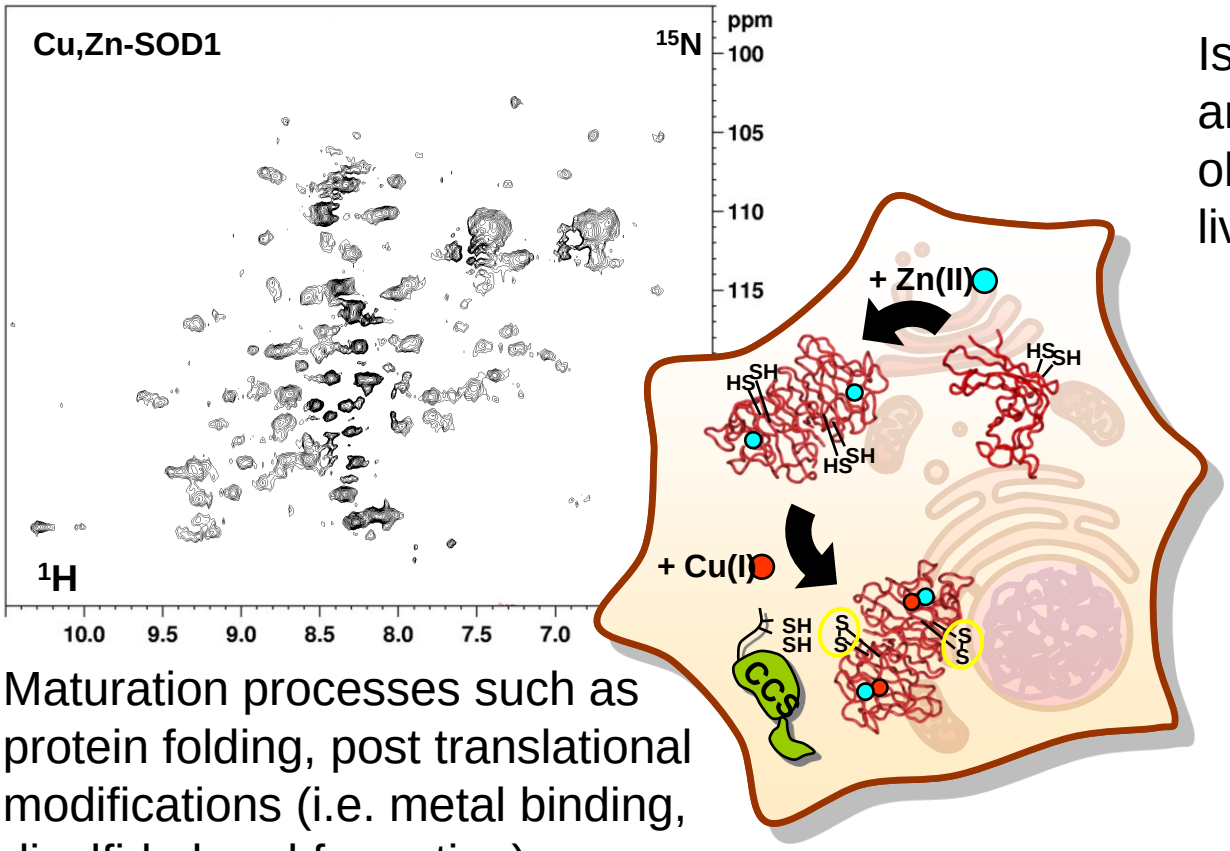

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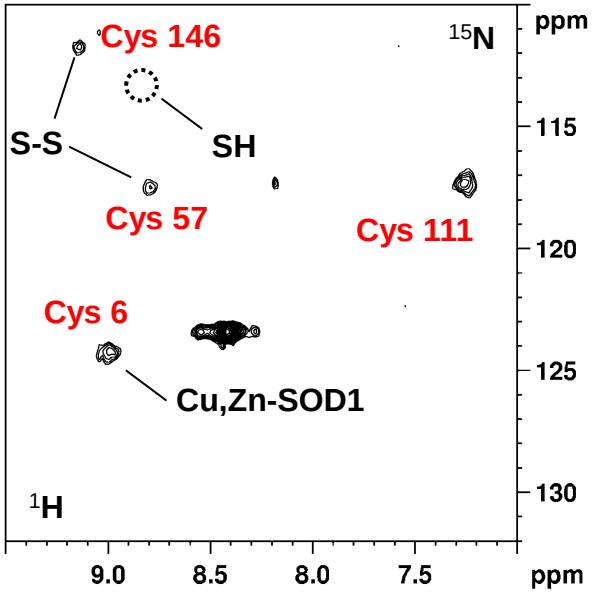


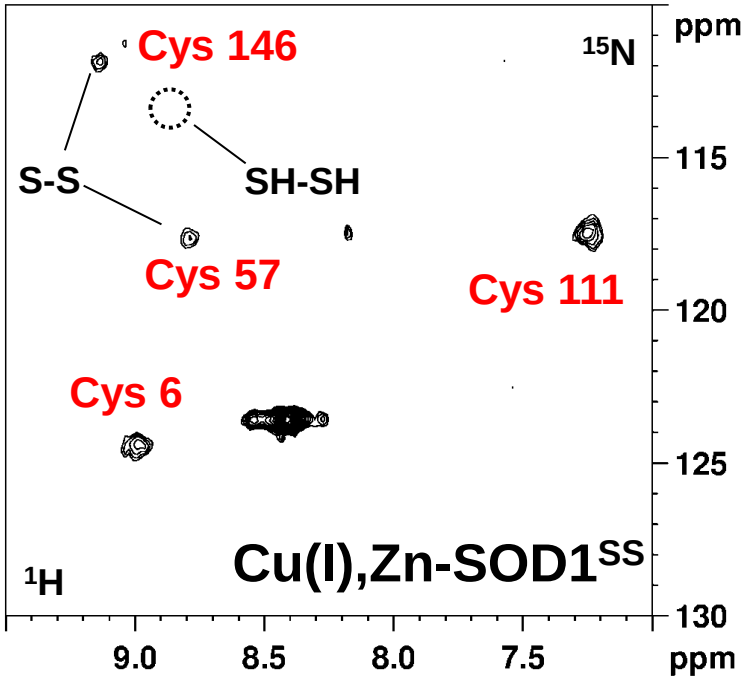
Maturation processes such as protein folding, post translational modifications (i.e. metal binding, disulfide bond formation) are followed at atomic resolution.

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

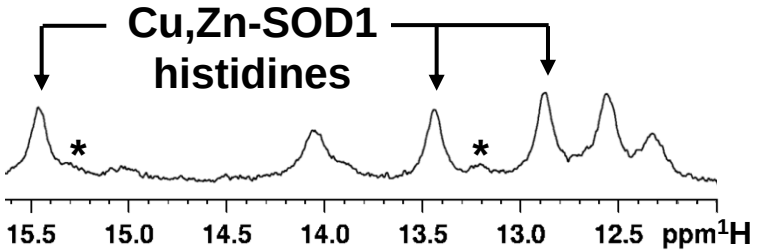
CCS role in copper uptake and disulfide formation

With CCS co-expressed with SOD1 and in absence of copper, the SOD1 disulfide bond is partially oxidized!

CCS catalyzes disulfide formation also without copper transfer



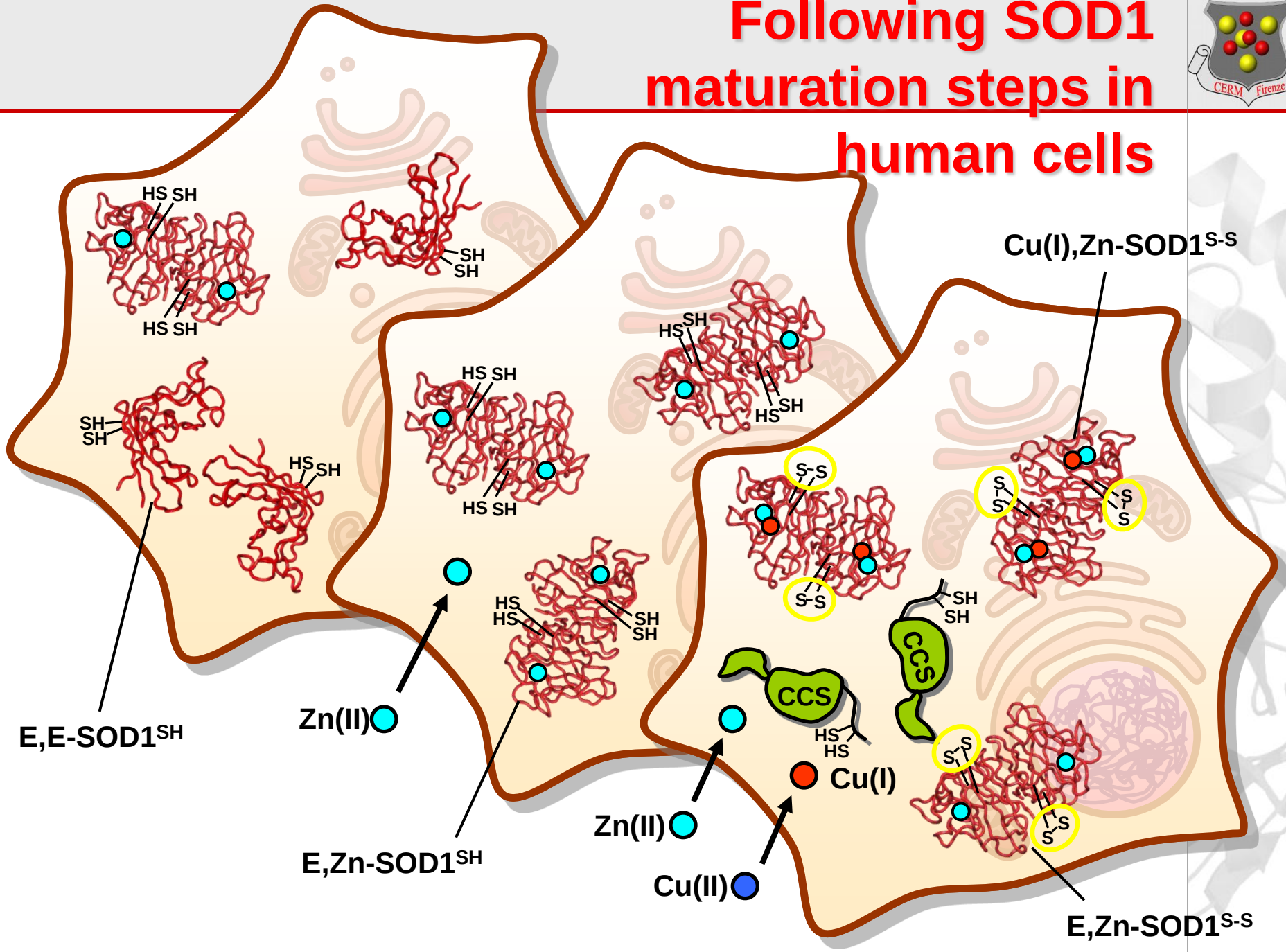
With both CCS and copper, SOD1 reaches the mature state!



(*) E,Zn-SOD1

Copper is added as Cu(II) but in the cell it is bound as Cu(I)

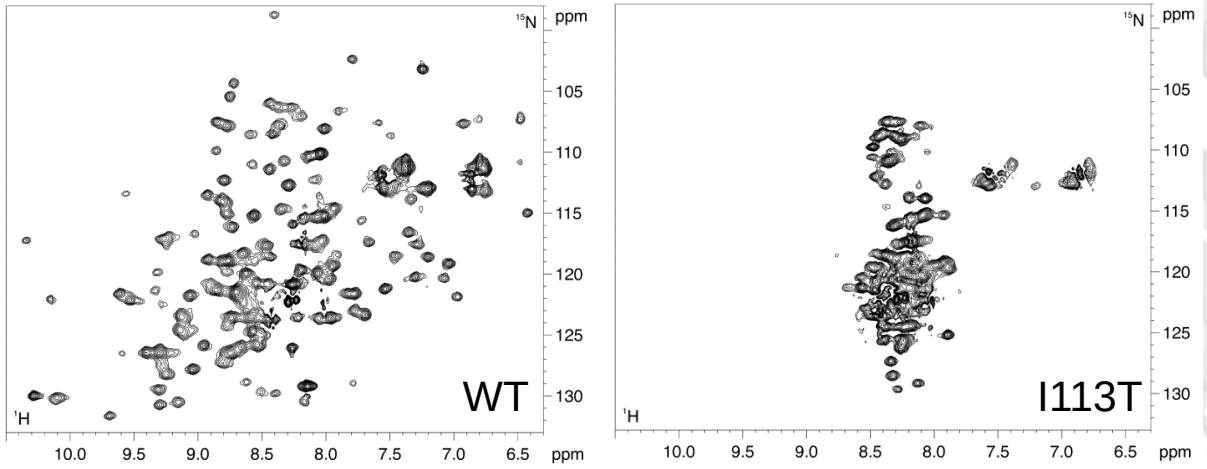
Following SOD1 maturation steps in human cells

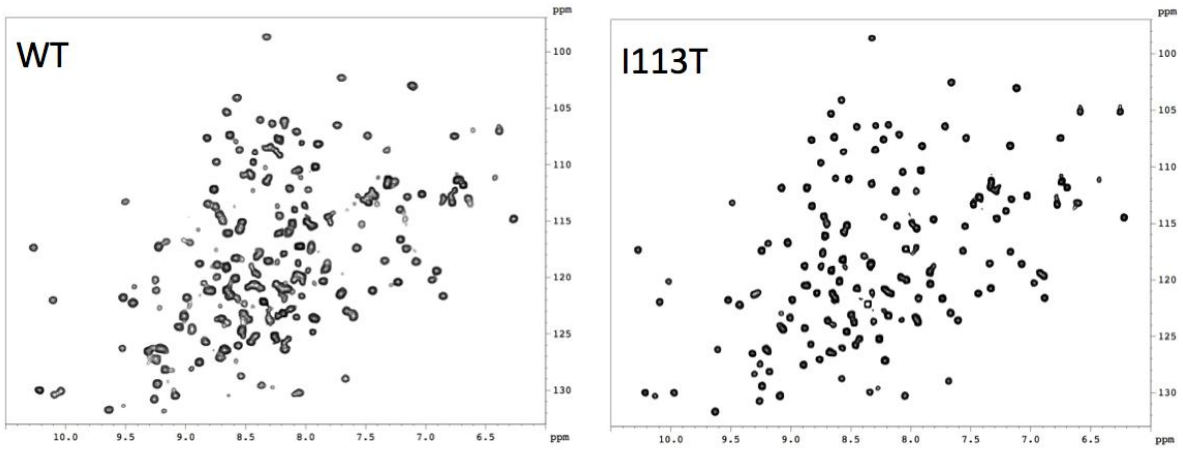


Banci L, Barbieri L, Bertini I, Luchinat E, Secci E, Zhao Y, Aricescu AR, *Nat Chem Biol*, 2013

In living cells, folding and metal binding of some SOD1 fALS mutants is impaired

Several SOD1 fALS mutants do not bind zinc in the cytoplasm (e.g. G93A, I113T) and remain in the unfolded apo state, which is prone to aggregation.





The same mutants *in vitro* behave like WT SOD1

Banci L, Barbieri L, Cantini F, Kozyreva T, Luchinat E, Rubino JT, *In preparation*

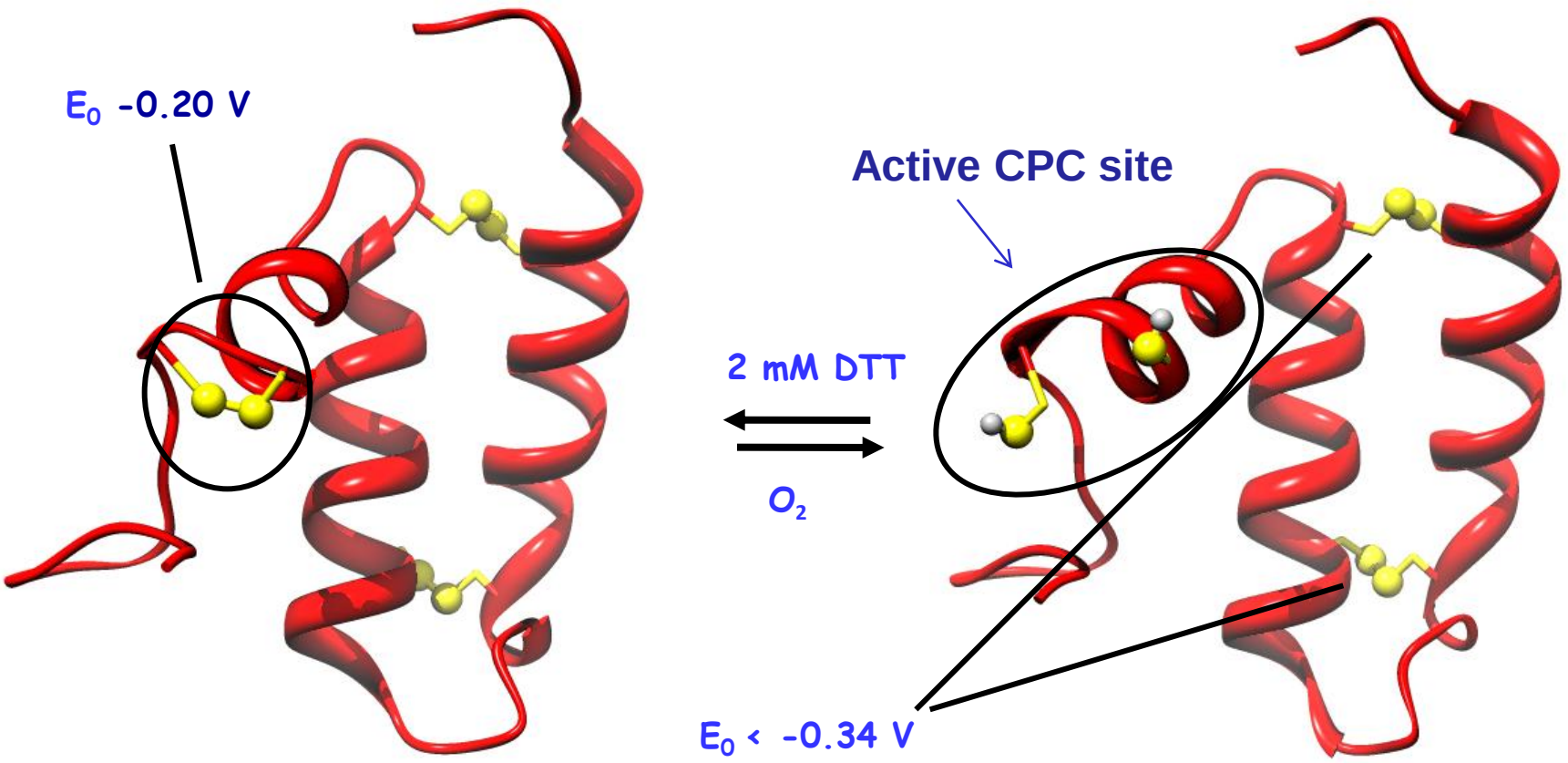
A relatively small-scale, physiologically central system for systems biology:

The mitochondrion

Mitochondria derive from **parassitic Gram-negative bacteria**: they contain 1000 proteins but only 15 are produced *in situ*

The large majority of mitochondrial proteins must be imported, including those involved in **copper trafficking**

A key protein for IMS protein import: Mia40, a hub for protein import



Oxidized Mia40

Partially Reduced Mia40

Solution structures

Banci, Bertini, Cefaro, Ciofi Baffoni, Gallo, Sideris, Tokatlidis *Nat Struct Mol Biol 2009*

Cox17 mitochondrial import

Reduced apoCox17
is unstructured

SH SH SH SH SH SH

TOM

IMS

SH SH SH SH SH SH

Cox 17 is transporting
Cu to CcO

+ Mia40

matrix

cytosol

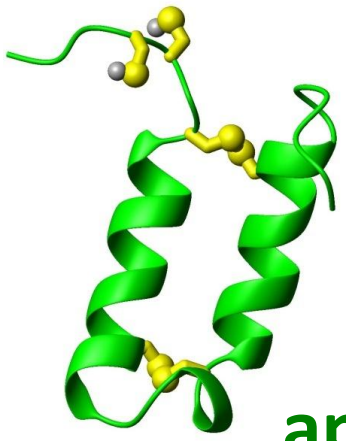
OM

TOM

IM

$Mia40_{3S\text{-}S}$ + $Cox17_{6SH}$

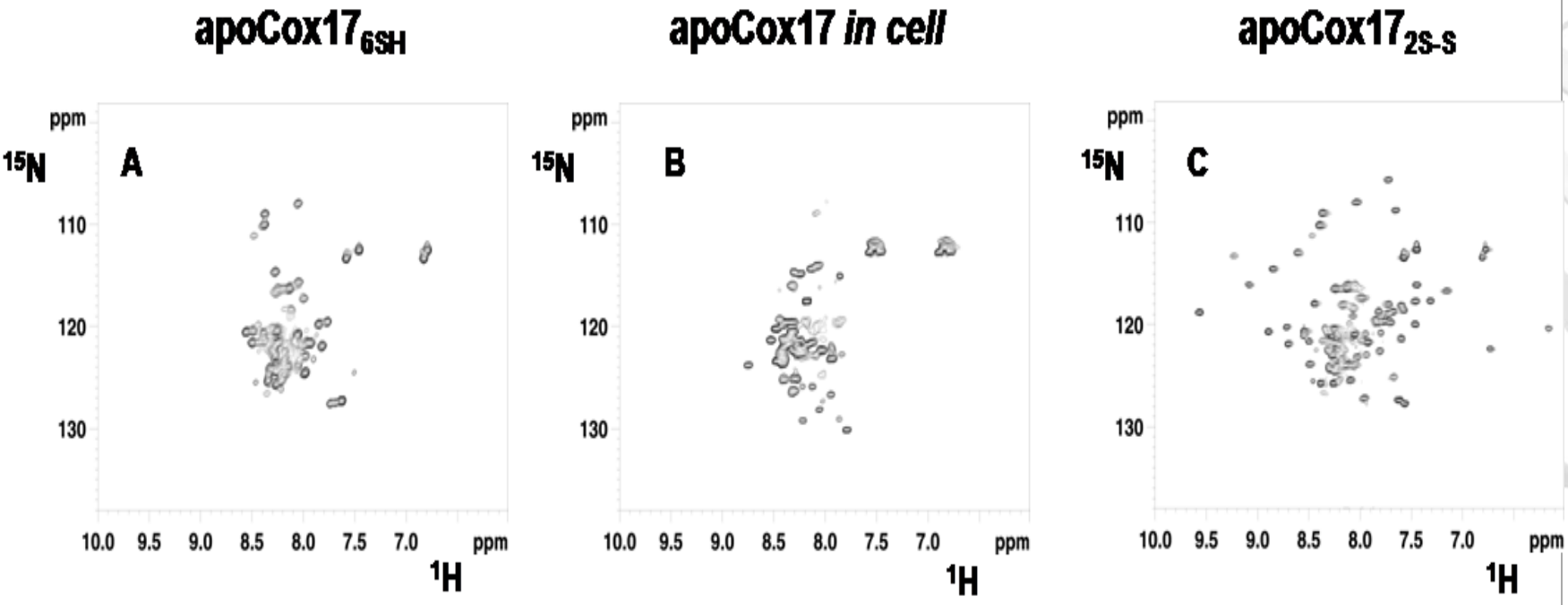
$Mia40_{2S\text{-}S/2SH}$ + $Cox17_{1S\text{-}S/4SH}$



$apoCox17_{2S\text{-}S}$

Cox17 is unfolded in the cytoplasm

detected in living cells

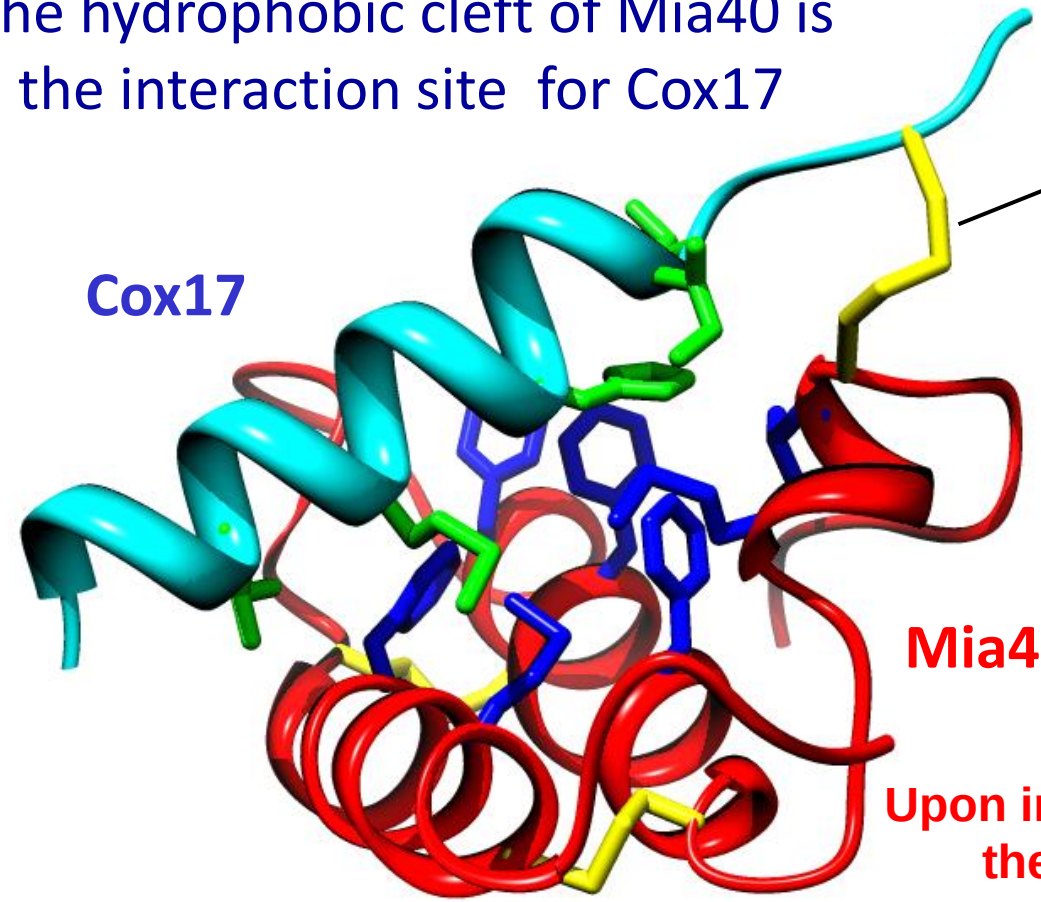


The protein folding state depends on the cellular compartment

Banci L, Bertini I, Cefaro C, Cenacchi L, Ciofi-Baffoni S, Felli I C, Gallo A, Gonnelli L, Luchinat E, Tokatlidis K, ***PNAS***, 2010

The first step in Cox17 folding

The hydrophobic cleft of Mia40 is the interaction site for Cox17



Intermolecular Mia40-Cox17 disulphide bond (detected by ^{13}C NMR on ^{13}C Cys)

Structure of Cox17-Mia40 intermediate

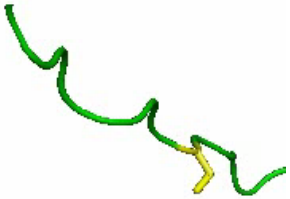
Upon intermolecular S-S bond formation, the first helix of Cox17 is formed

Then the first intramolecular S-S bond and the second helix are formed

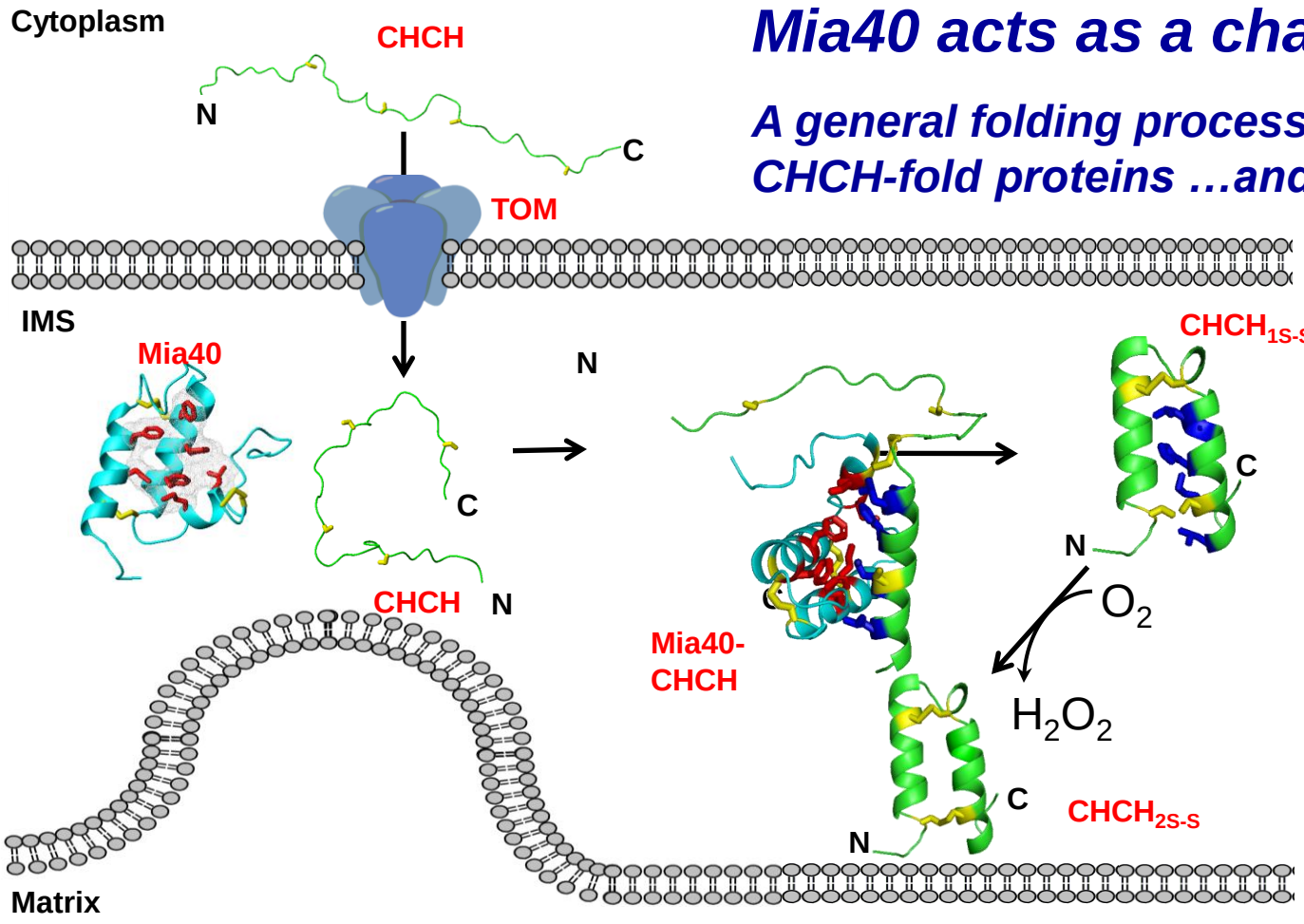
O_2 or glutathione can now rapidly form the second disulphide bond

Banci, Bertini I, Cefaro C, Cenacchi L, Ciofi-Baffoni S, Felli I C, Gallo A, Gonnelli L, Eluchinat, Tokatlidis K, PNAS, 2010

Oxidative folding reaction between Mia40 and Cox17



Oxidative folding processes in IMS



Mia40 acts as a chaperon

A general folding process for CHCH-fold proteins …and many more

Protein fold state depends on the cellular compartment and is modulated by the protein redox state

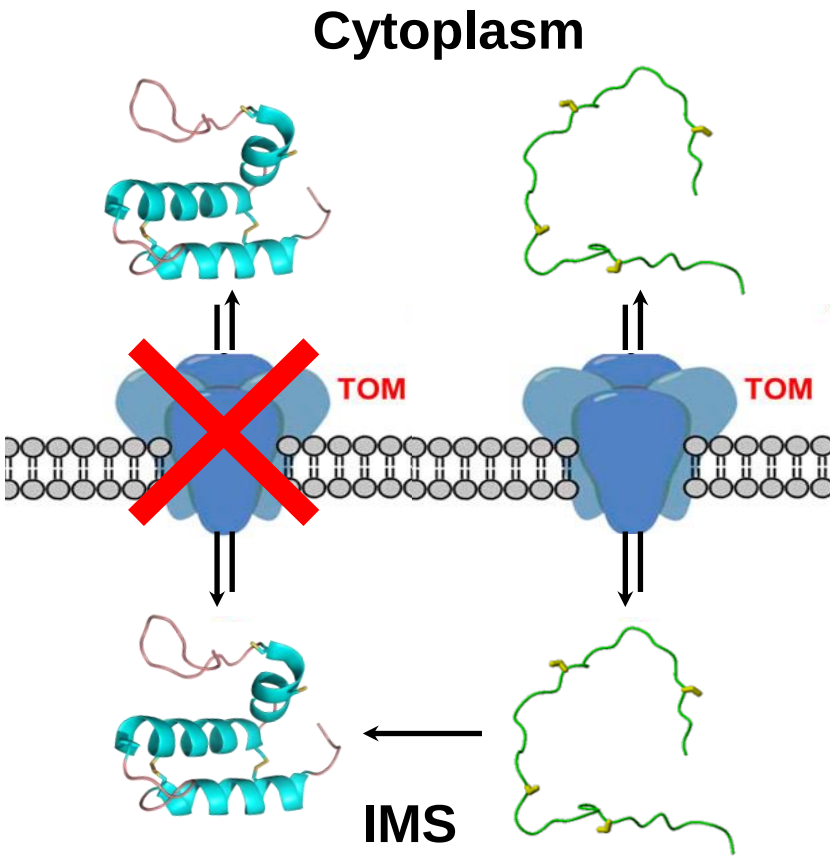
Banci L, Bertini I, Cefaro C, Cenacchi L, Ciofi S, Felli I C, Gallo A, Gonnelli L, Luchinat E, Tokatlidis K, PNAS, 2010

Redox-dependent folding of Mia40

Mia40 itself is imported in the IMS through the oxidative folding pathway

It has to cross the TOM channel in the unfolded state

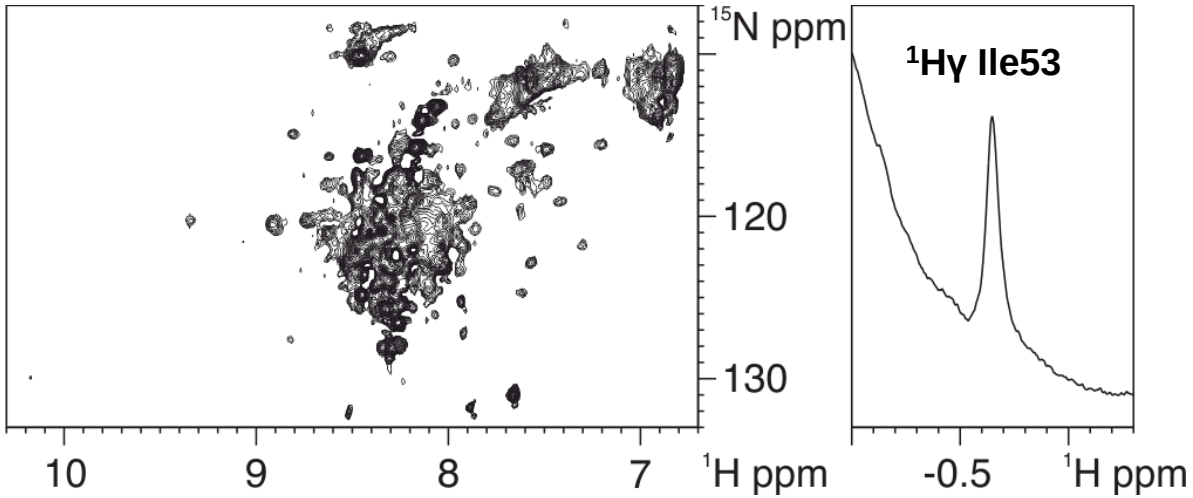
However, Mia40 when overexpressed in human cells accumulates in the cytoplasm as oxidized and folded

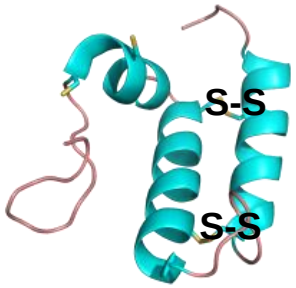


A thiol-disulfide regulation mechanism is necessary to keep Mia40 unfolded and import-competent in the cytoplasm

Redox-dependent folding of Mia40

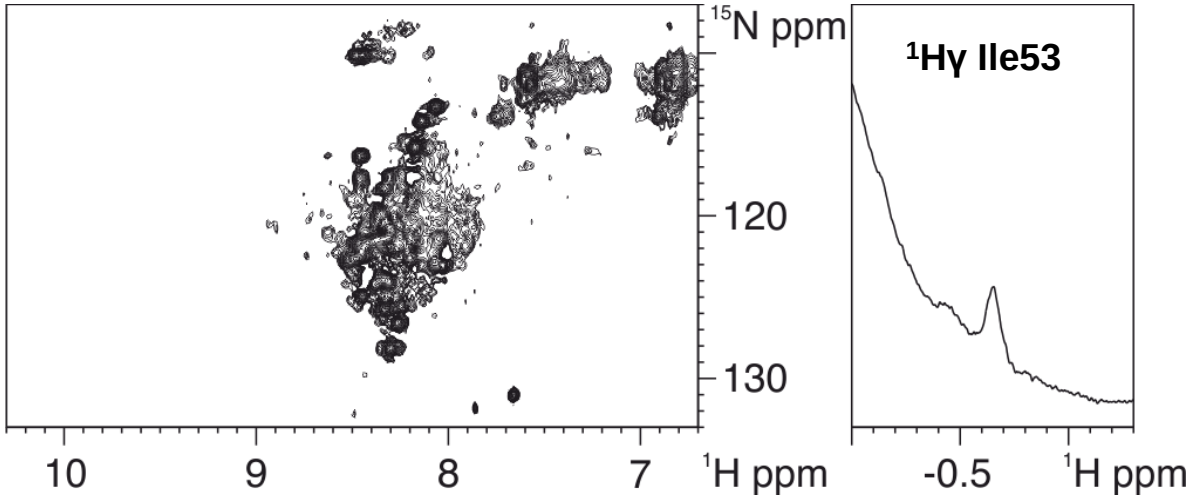
Cytoplasmic Mia40

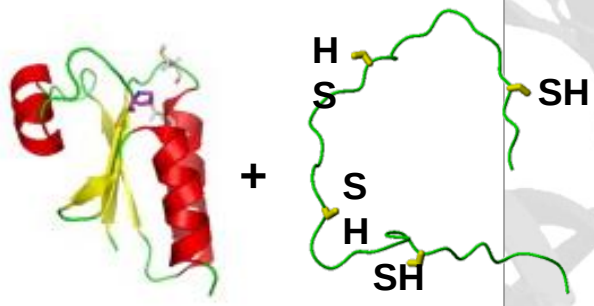




Oxidized, folded Mia40

Mia40 + Glutaredoxin 1





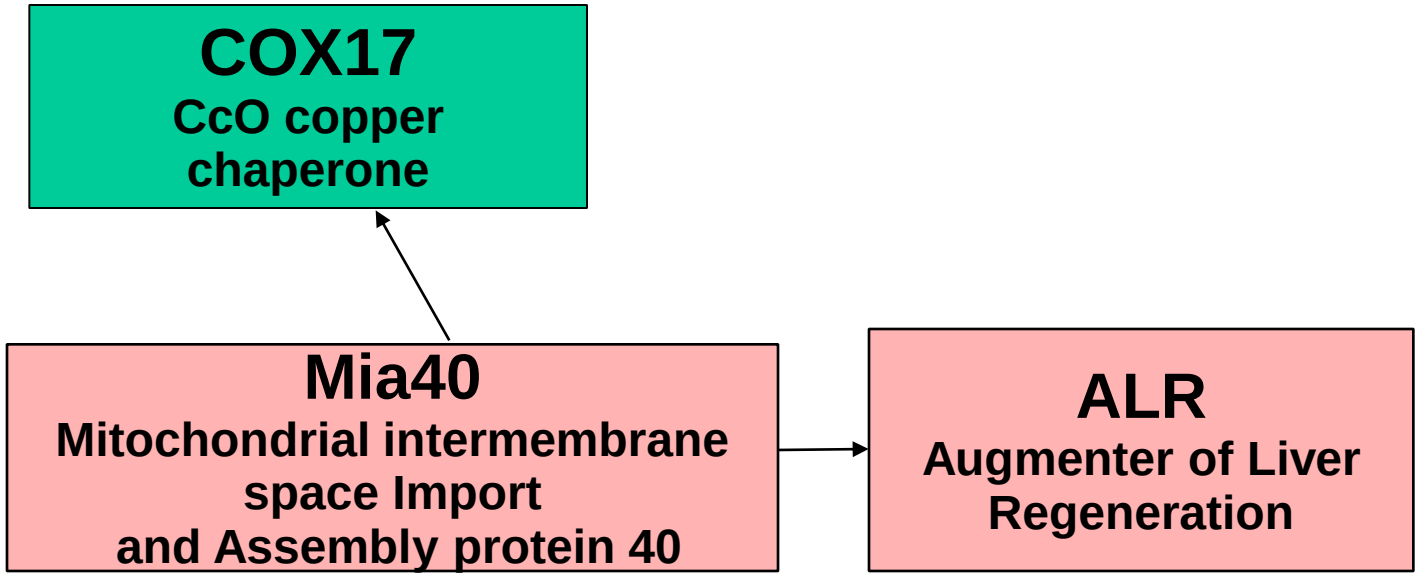
Glutaredoxin 1 **Unfolded Mia40**

Banci, L., Barbieri, Luchinat, E., Secci E., *Chemistry & Biology, 2013*

A general feature

Protein fold state depends on the cellular compartment and is modulated by the protein redox state

Steps in a mitochondrial pathway

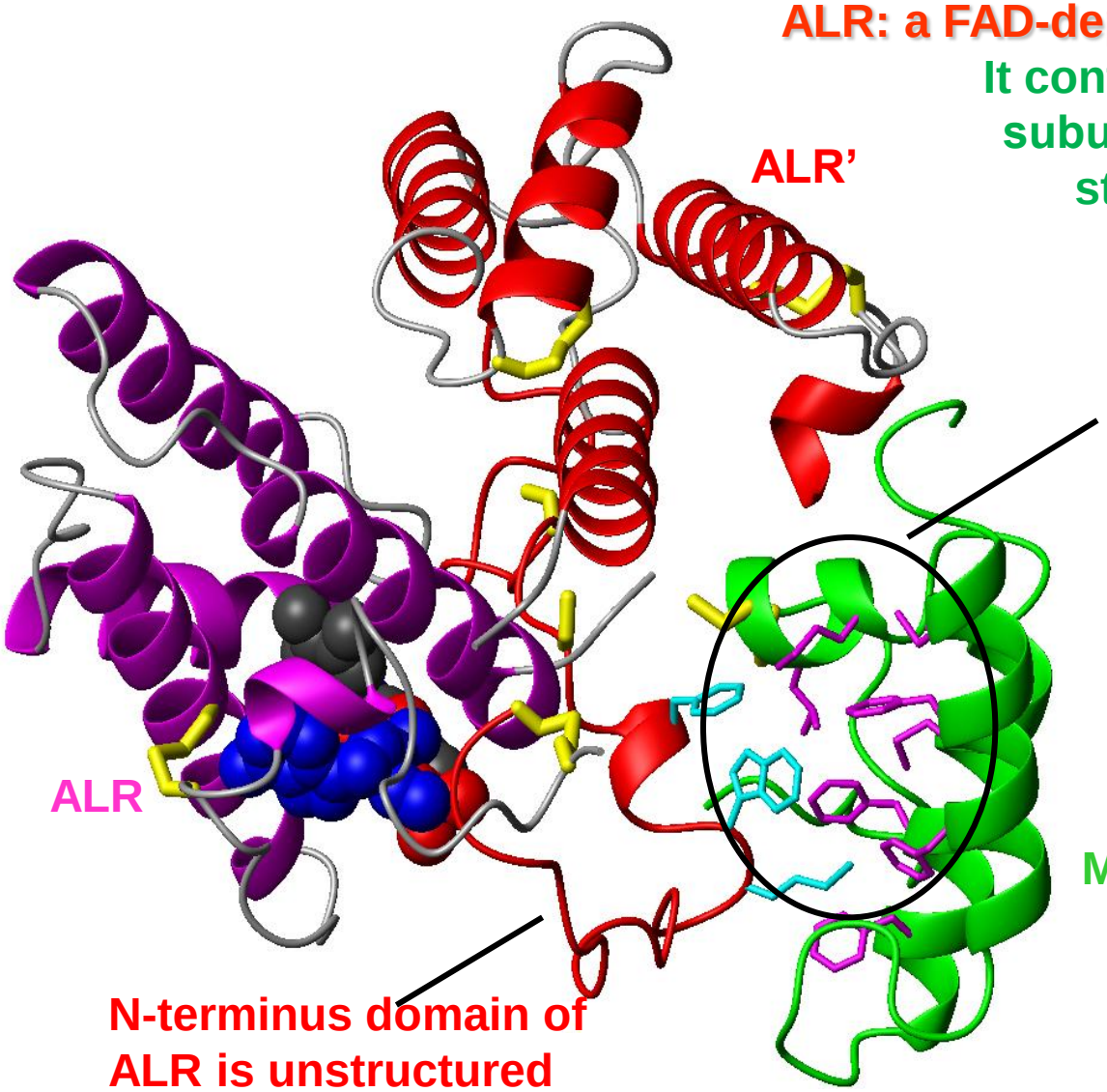


ALR regenerates the active import redox state of Mia40, i.e. with a disulfide bond in the CPC site

Structural model of the ALR/Mia40 complex based on NMR interaction data

ALR: a FAD-dependent thiol oxidase

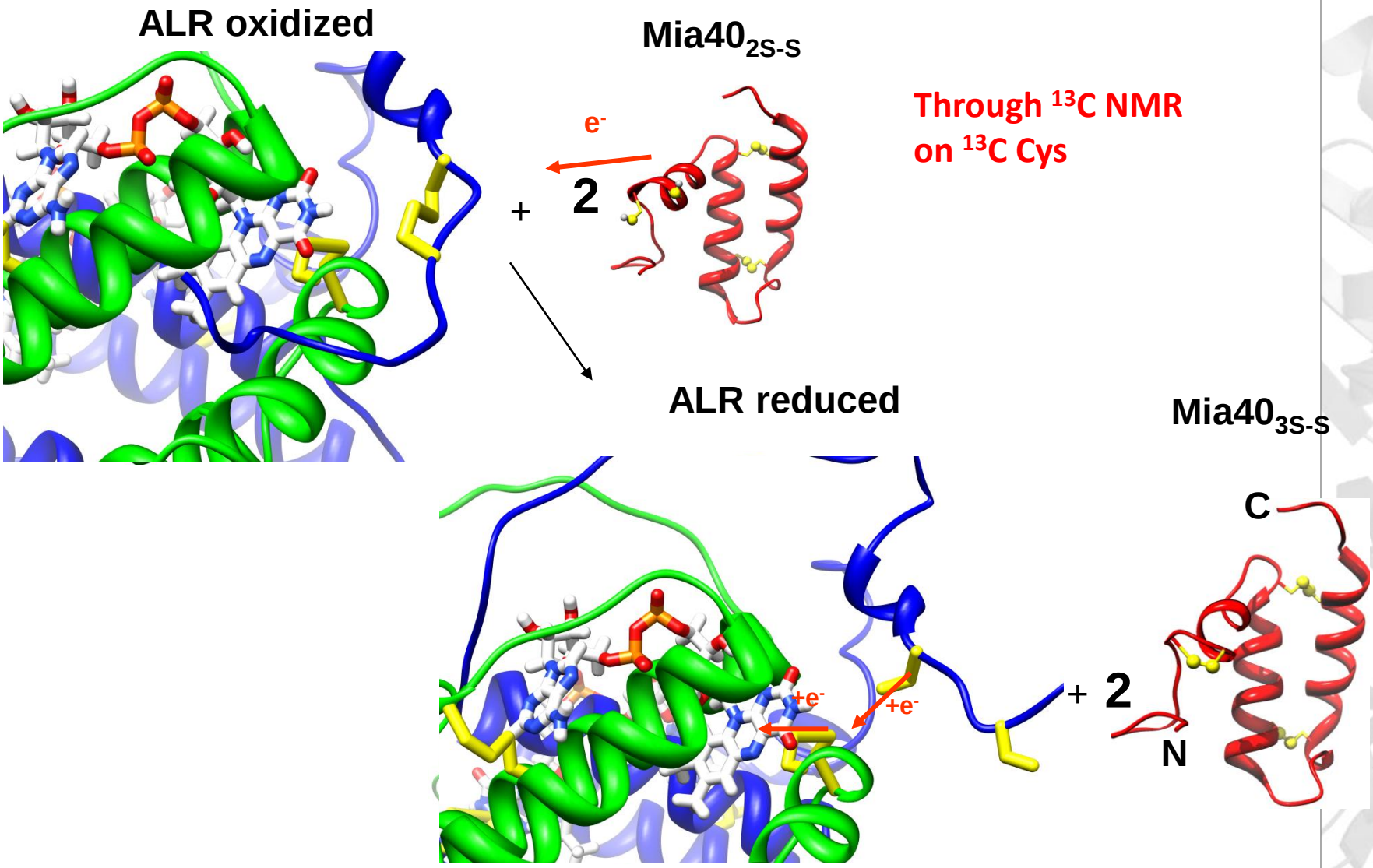
It contains 4 SS bonds per subunit, 2 "active" and 2 structural



Hydrophobic interactions between Mia40 and the N-ter domain of ALR mediate efficient electron transfer from Mia40 to FAD in ALR

Mia40

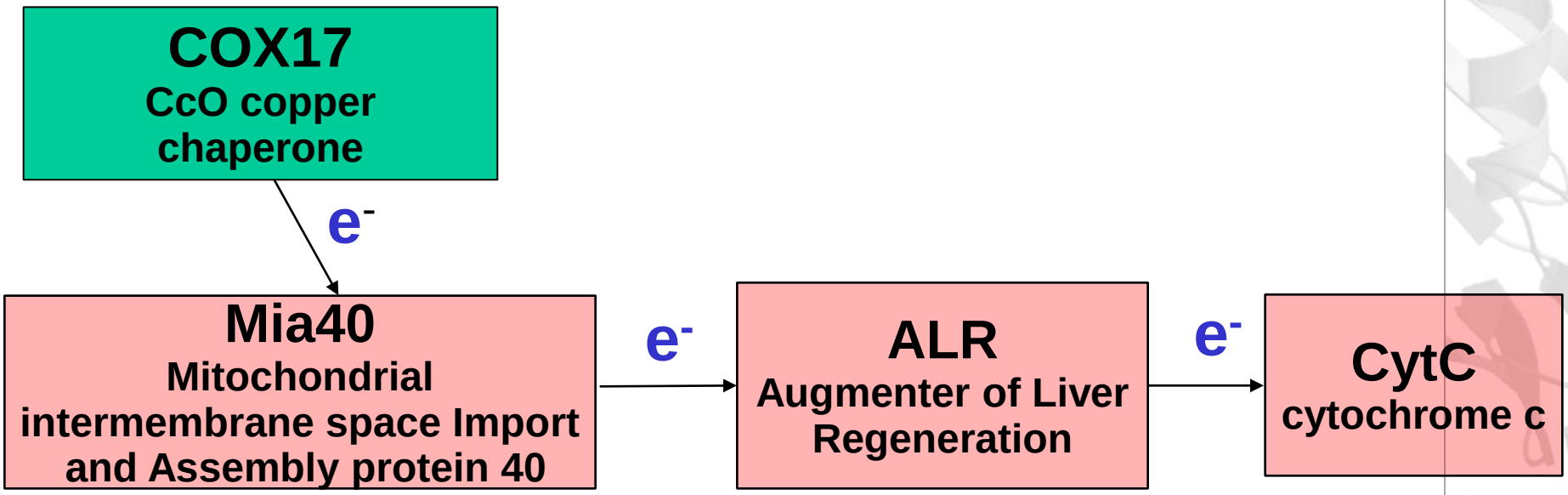
Electron shuttling mechanism



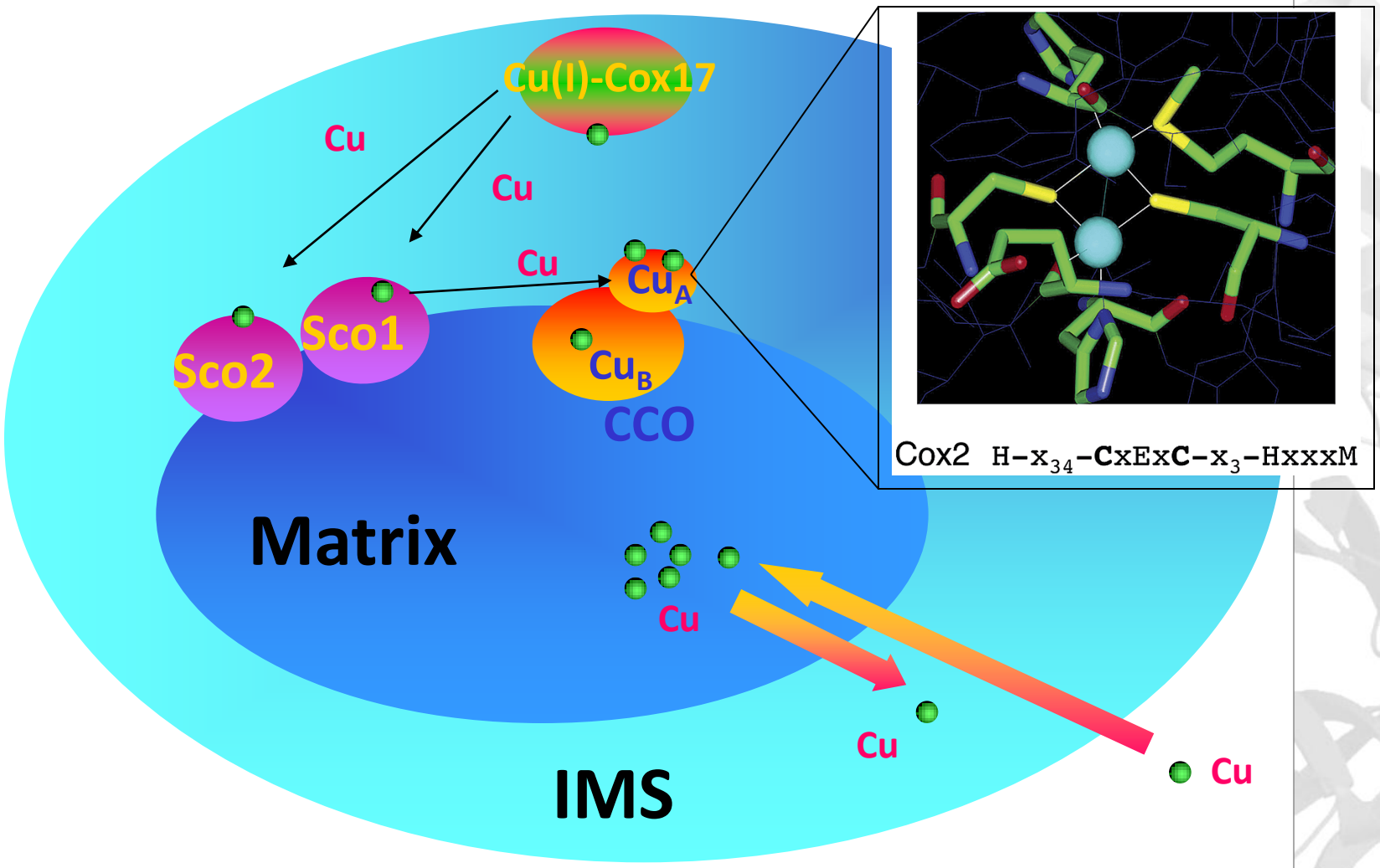
ALR then transfers electrons to Cyt c

Banci L, Bertini I, Calderone V, Cefaro C, Ciofi-Baffoni S, Gallo A, Tokatlidis K *PNAS 2011*

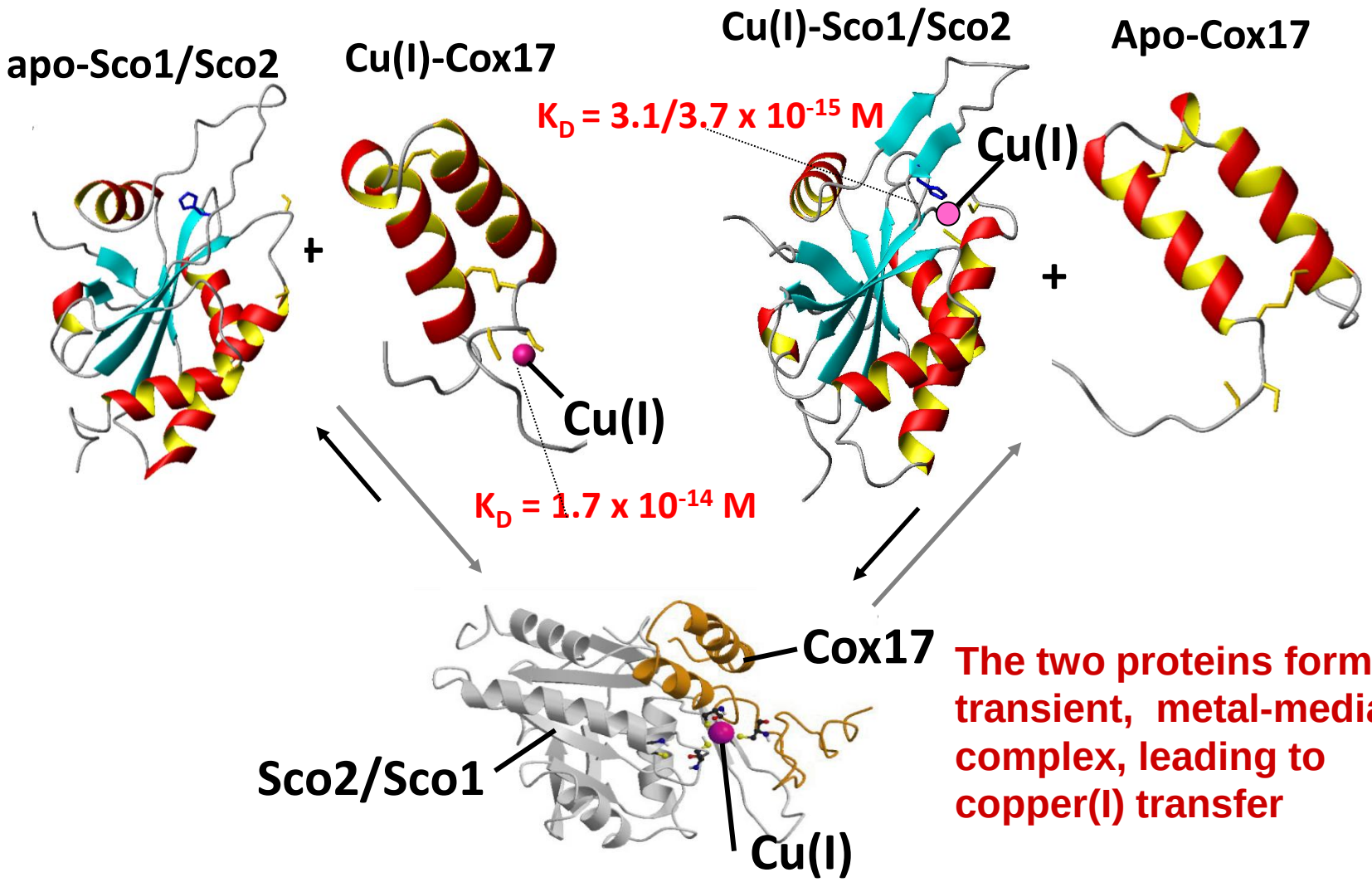
A parallel short respiratory chain



Cu_A assembly in the mitochondrion



Cox17 binds Cu(I) and transfers it to apo-Sco1/Sco2



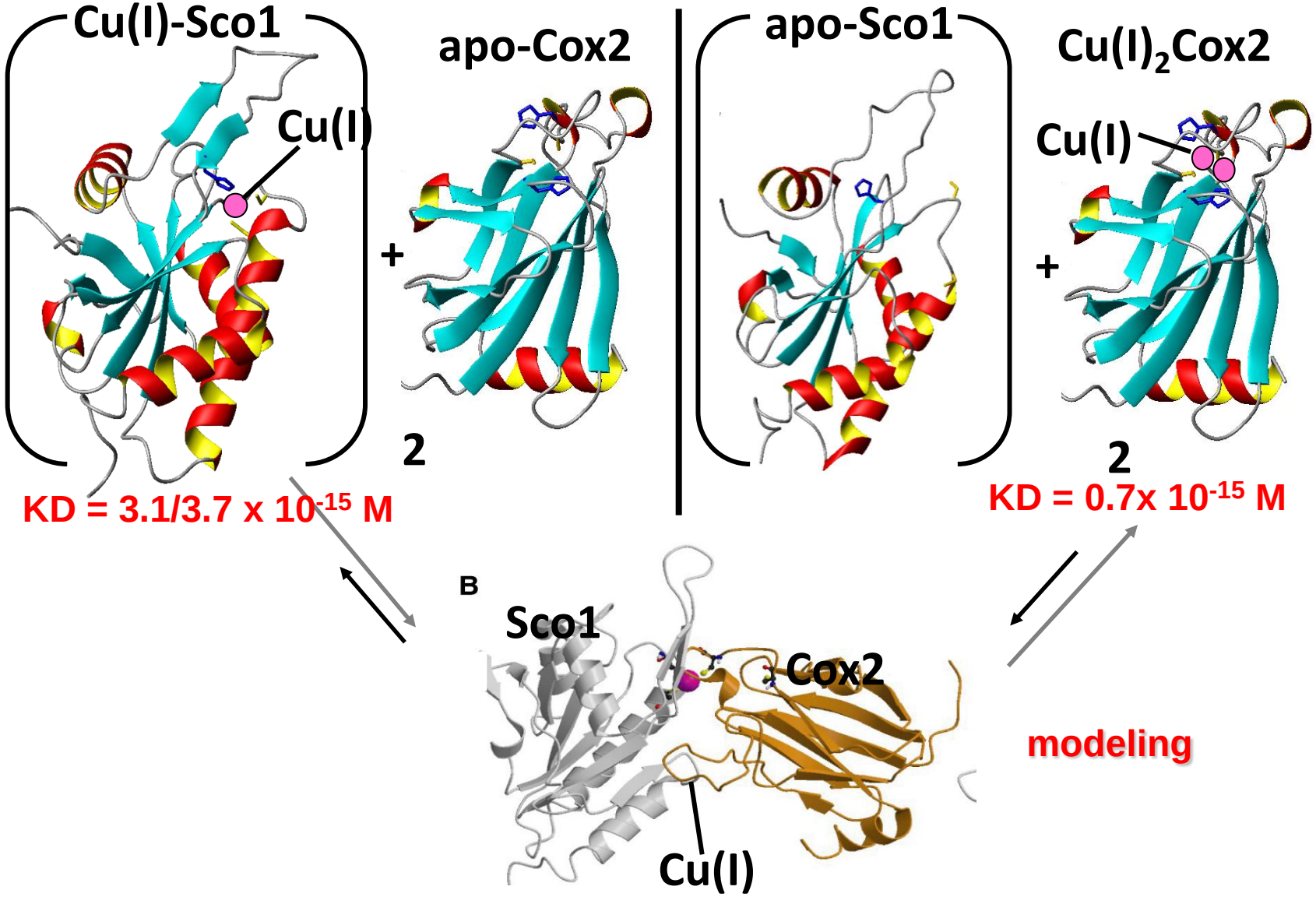
The two proteins form a transient, metal-mediated complex, leading to copper(I) transfer

Banci, Bertini, Ciofi-Baffoni, Martinelli, Palumaa, Wang *PNAS* 2006

Banci, Bertini, Ciofi-Baffoni, Martinelli, Palumaa, Hadjiloi, *PNAS* 2008

Banci, Bertini, Ciofi-Baffoni, Karit, Kozyreva, Palumaa, *Nature,* 2010

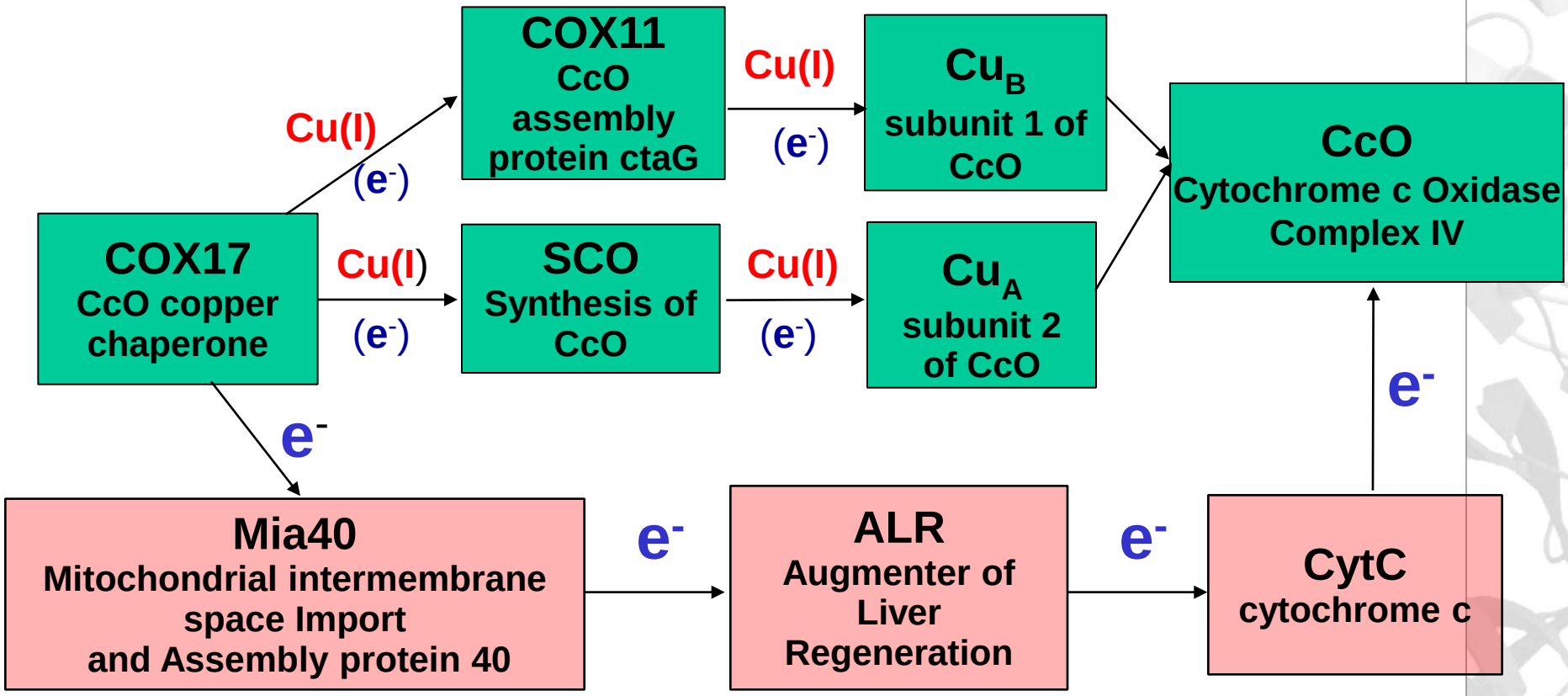
Sco1 transfers Cu(I) to apo-CuA



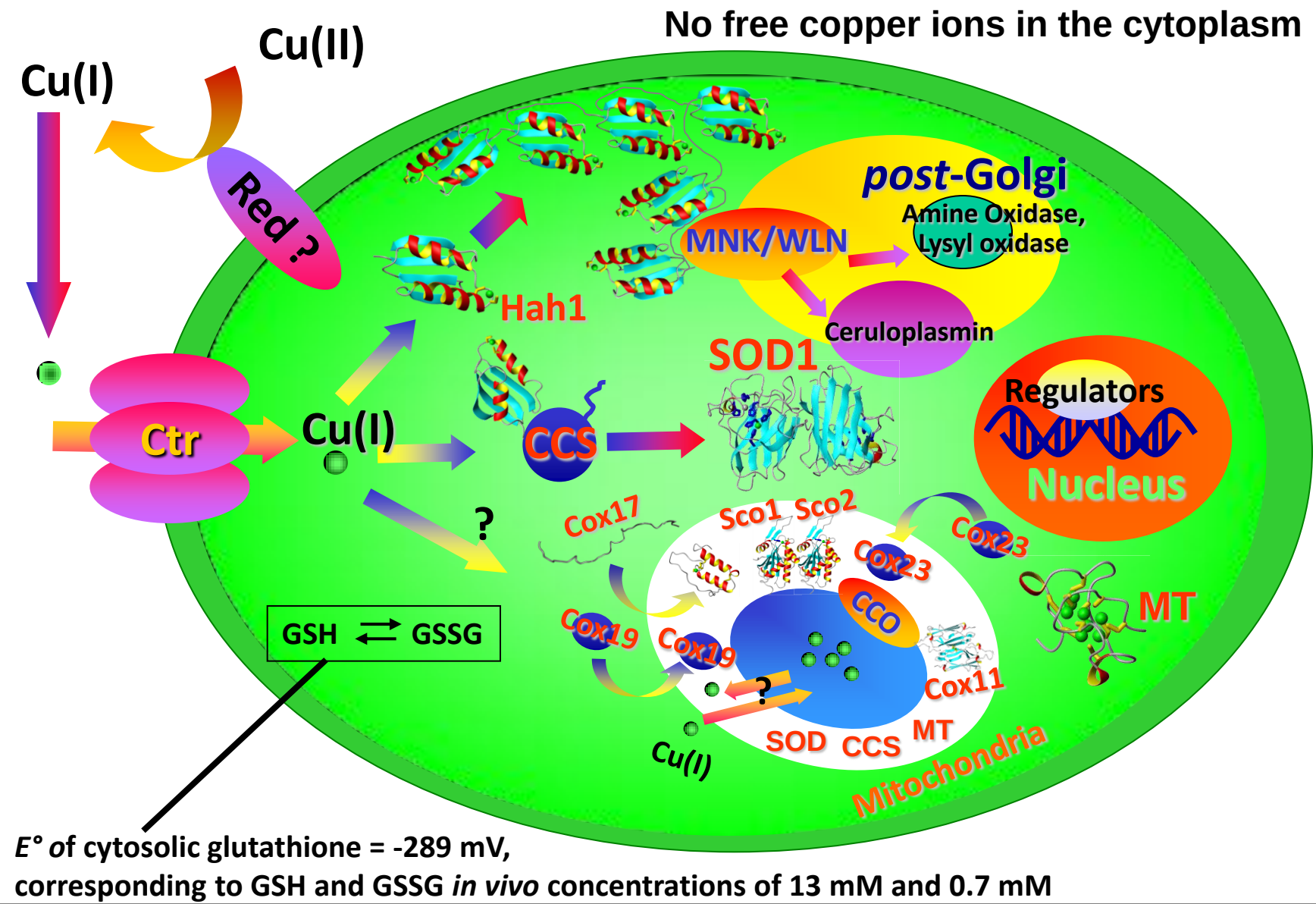
Banci, Bertini, Ciofi-Baffoni, Karit, Kozyreva, Palumaa, *Nature,* 2010

van Dijk, Ciofi-Baffoni, Banci, Bertini, Boelens, Bonvin *J. Proteome Res.* 2007

IMS protein import is linked to the respiratory chain through electron shuttling reactions and through copper transfer processes



Copper trafficking in human cells

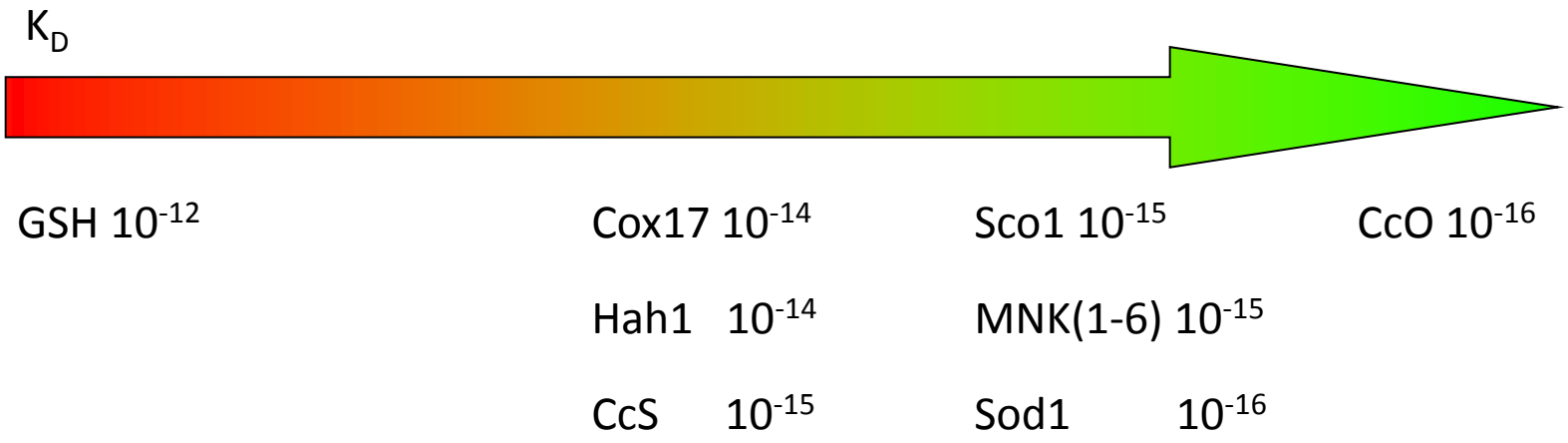


E° of cytosolic glutathione = -289 mV,
corresponding to GSH and GSSG *in vivo* concentrations of 13 mM and 0.7 mM

Copper cellular redistribution

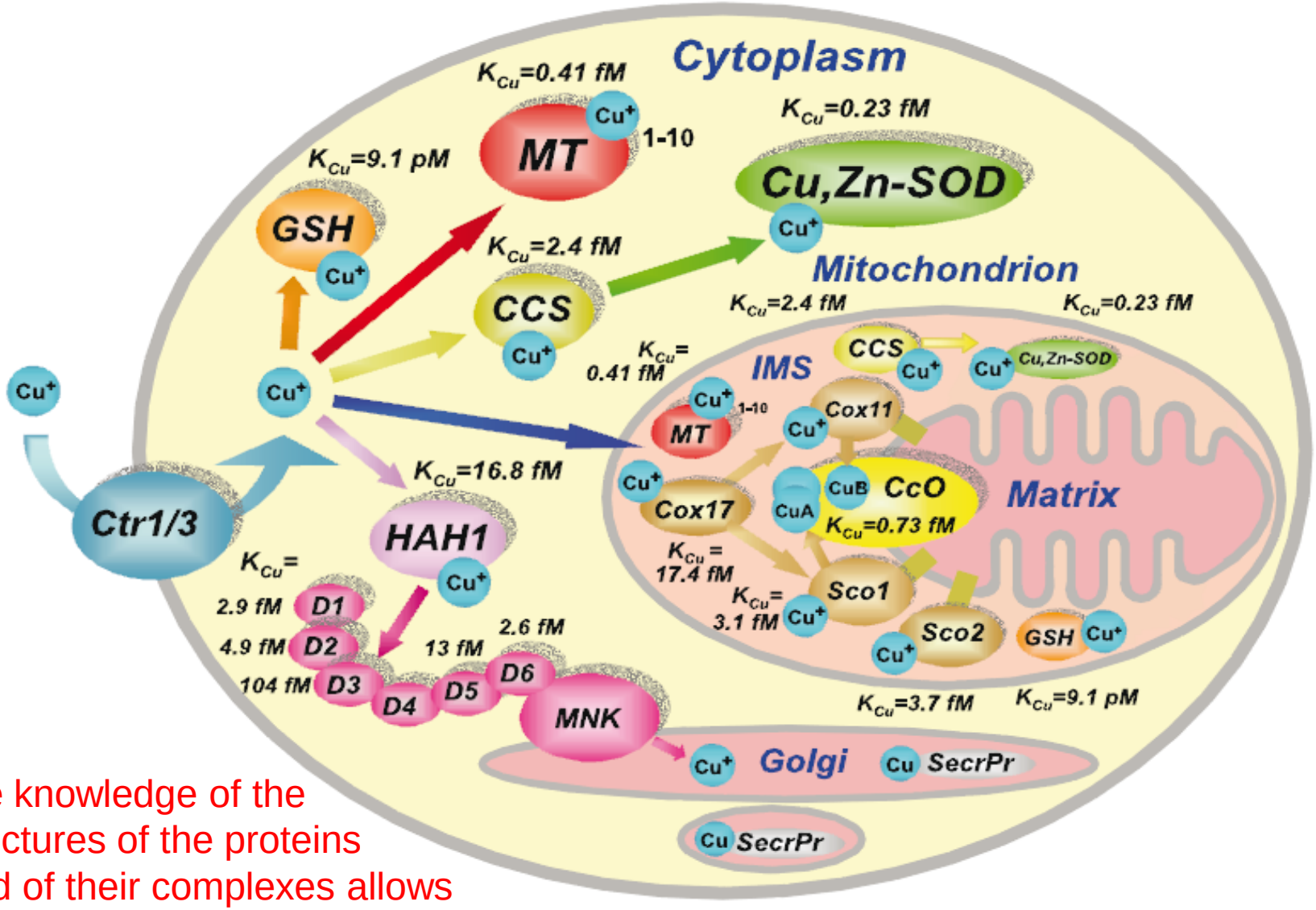
The cellular routes for copper delivery obey a Cu(I)-thermodynamic binding hierarchy among Cu(I)-binding proteins, i.e. from chaperones to intermediate copper transport proteins and finally to enzymes
Molecular recognition prevents the cross of pathways

Copper affinity in mitochondrial and cytoplasmic routes:



Kinetic factors contribute to the selectivity of the processes

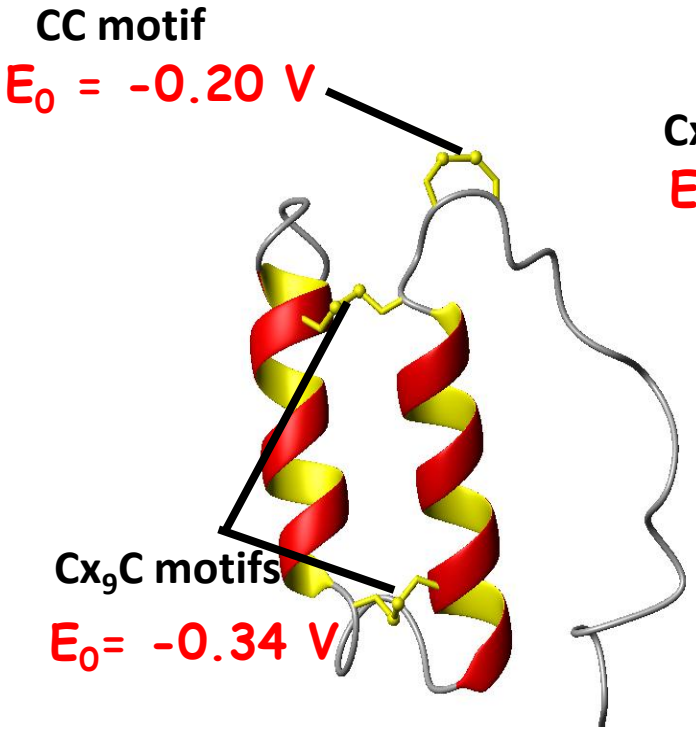
Towards systems biology of copper



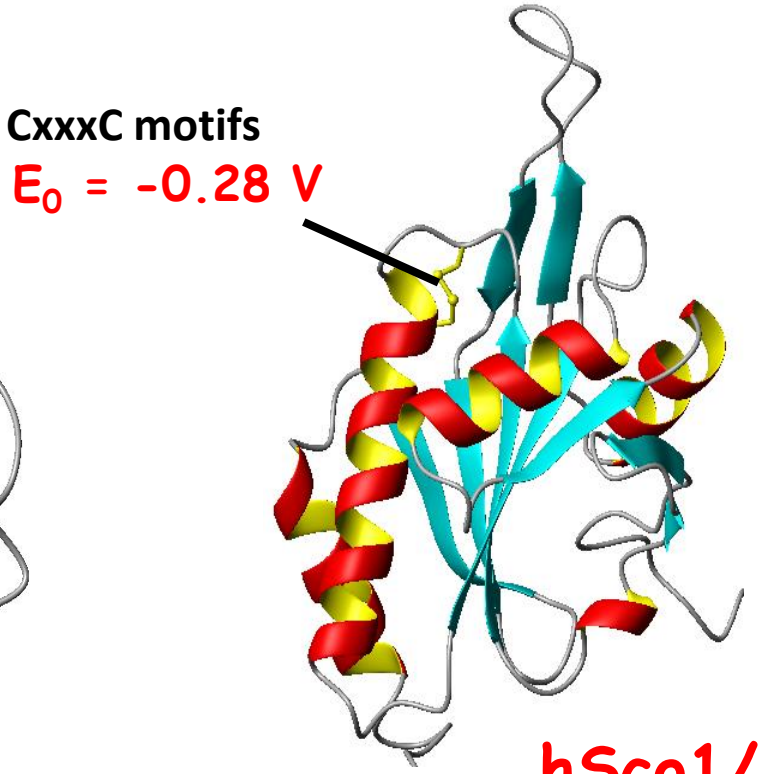
The knowledge of the structures of the proteins and of their complexes allows the atomic level description of the transfer processes

Banci, Bertini, Ciofi-Baffoni, Karit, Kozyreva, Palumaa, Nature, 2010

The redox state of a protein depends on the redox potential of the cellular compartment



hCox17



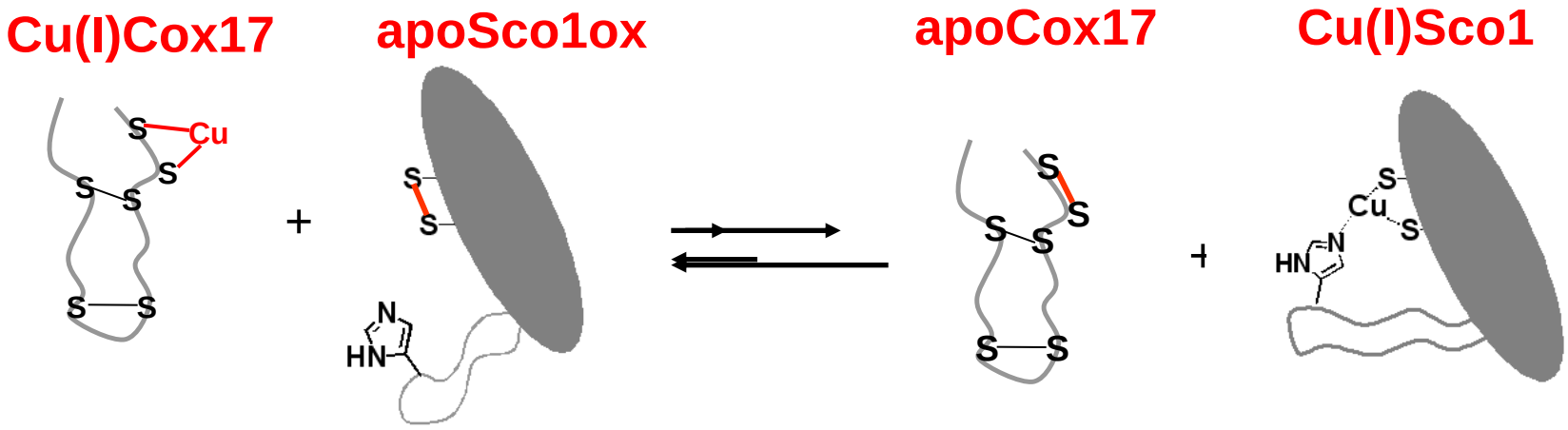
hSco1/hSco2

E_0 of IMS = -0.26 V

Hu, Dong, Outten CE *JBC* 2008

hSco1/hSco2	a mixture of ox/red thiols
hCox17 Cx_9C	100% ox
CC	98% red

If Sco1 were oxidized…
Copper transfer drives reduction, i.e. electron flow



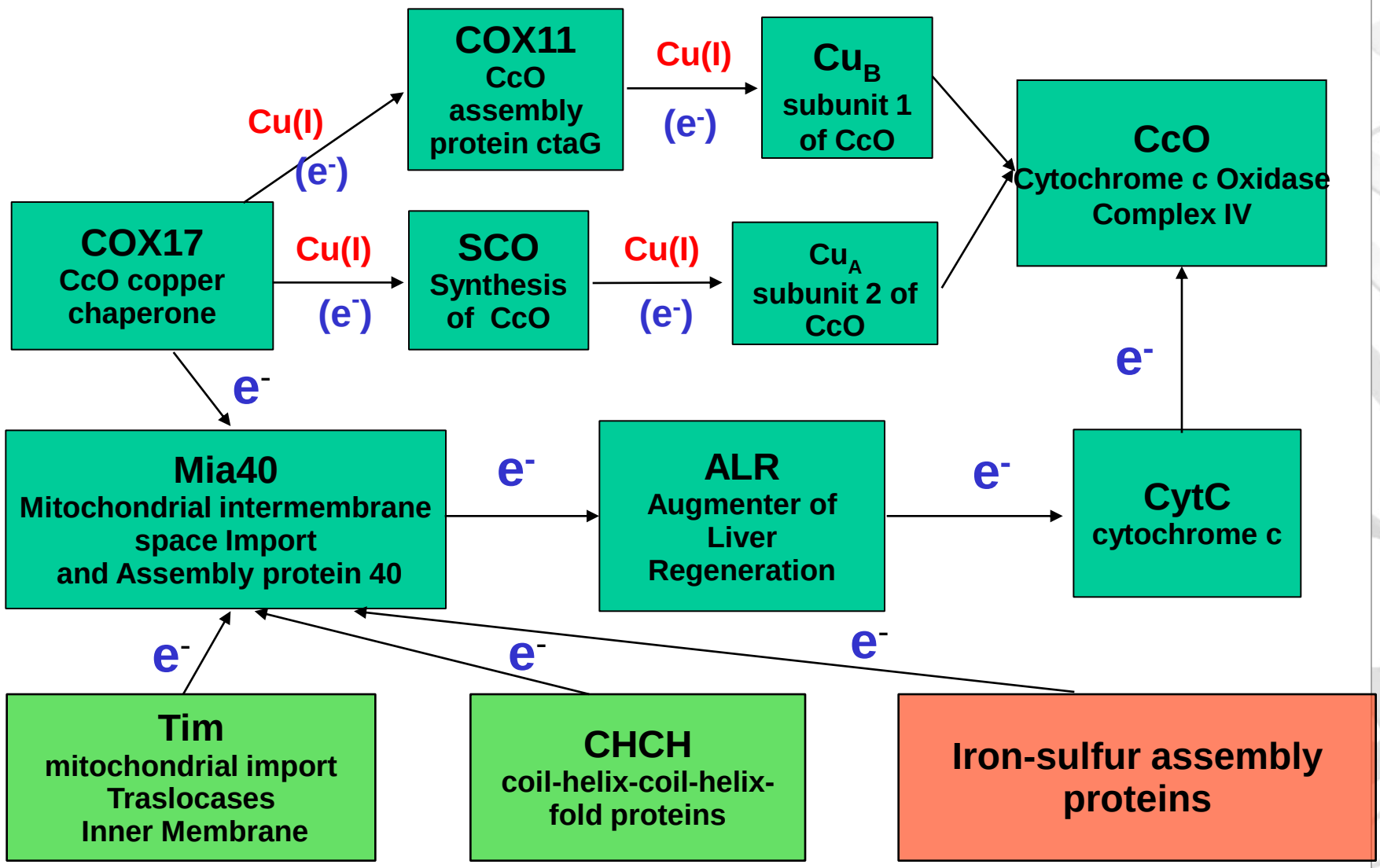
Redox potential of apoSco1 E_0 = - 0.28 V
Redox potential of apoCox17 E_0 = - 0.20 V

K_D of Cu(I)Sco1 = 2 x 10^{-15} M
K_D of Cu(I)Cox17 = 1.4 x 10^{-14} M

Cox17 is therefore also a redox protein

Banci, Bertini, Ciofi-Baffoni, Martinelli, Palumaa, Hadjiloi, *PNAS* 2008
Banci, Bertini, Ciofi-Baffoni, Karit, Kozyreva, Palumaa, *Nature*, 2010

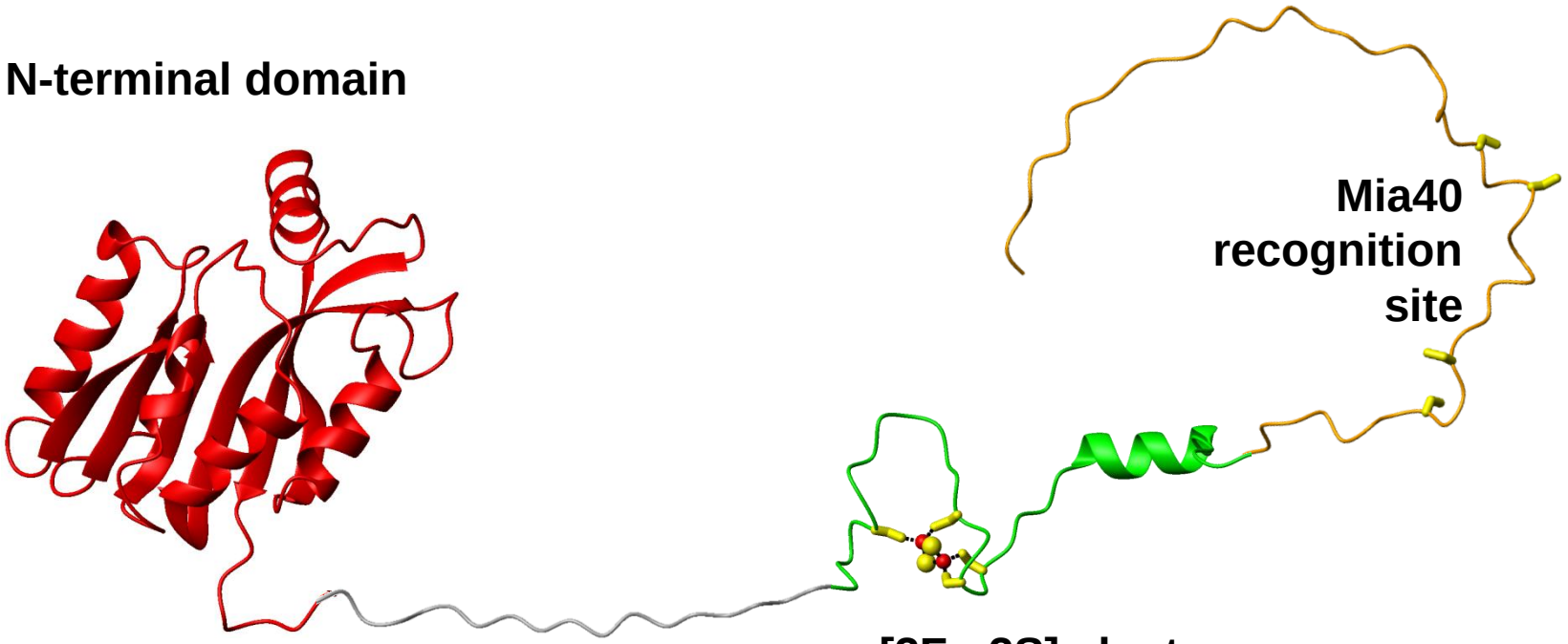
Schematic overview of (some) mitochondrial pathways



Structural properties of Anamorsin
An essential protein for FeS cluster biosynthesis

Intrinsically Disordered Domain
Mia40 forms two disulfide bonds

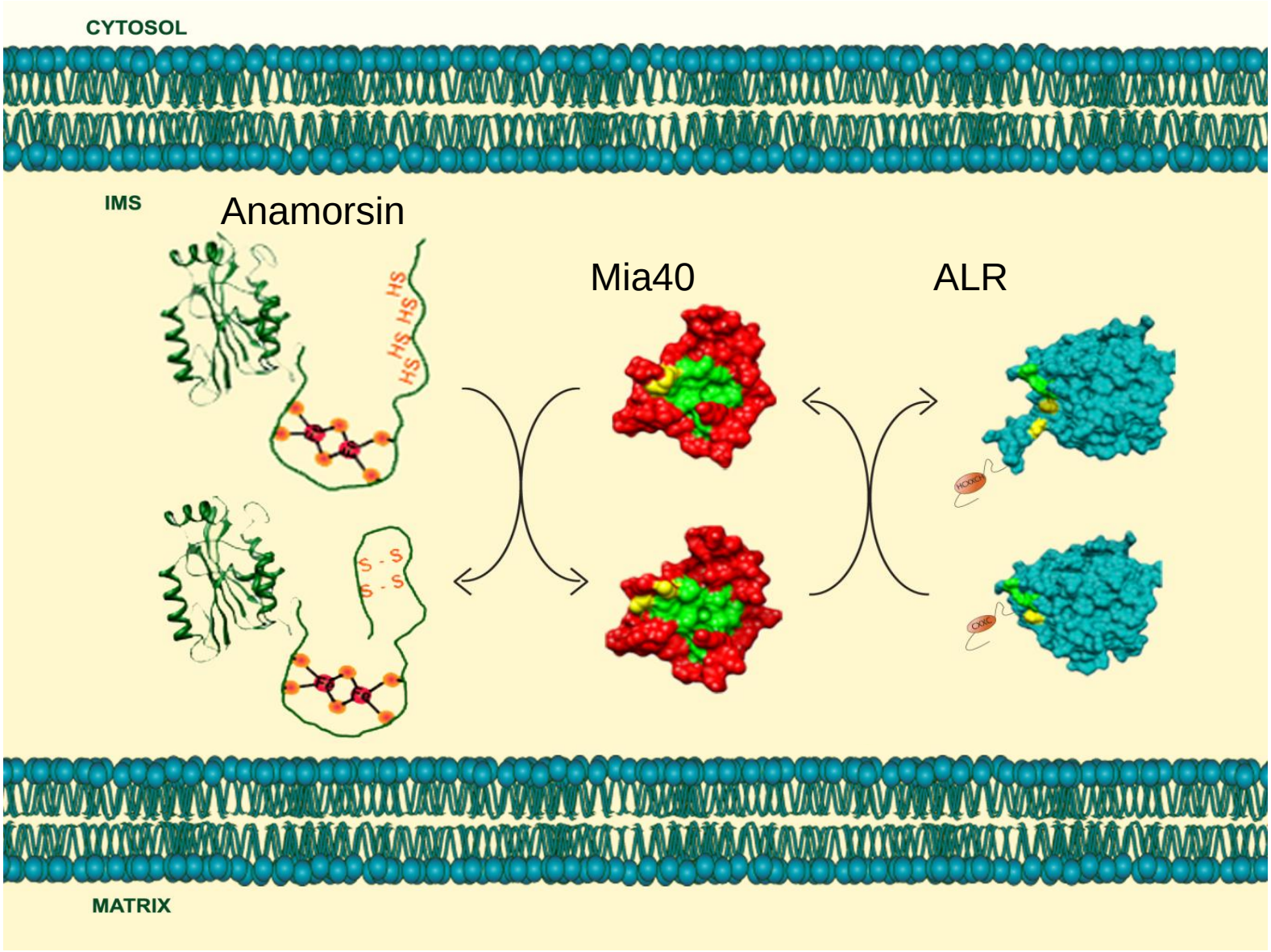
N-terminal domain



Folded domain
“standard” approach

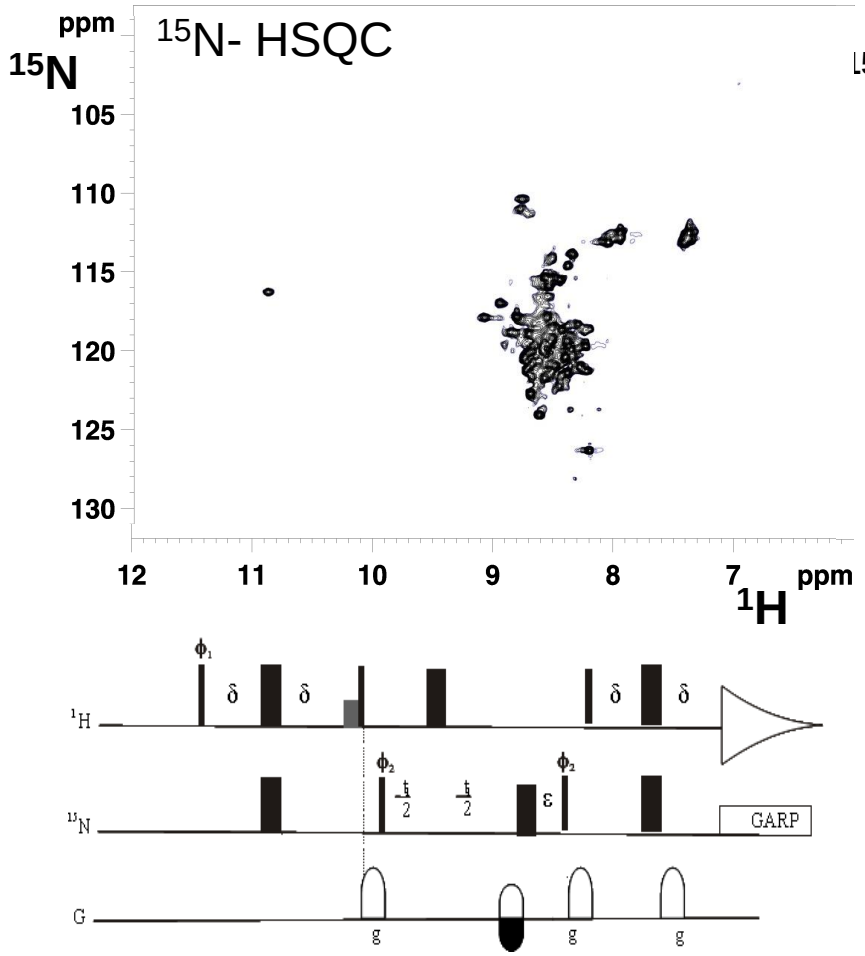
Highly paramagnetic

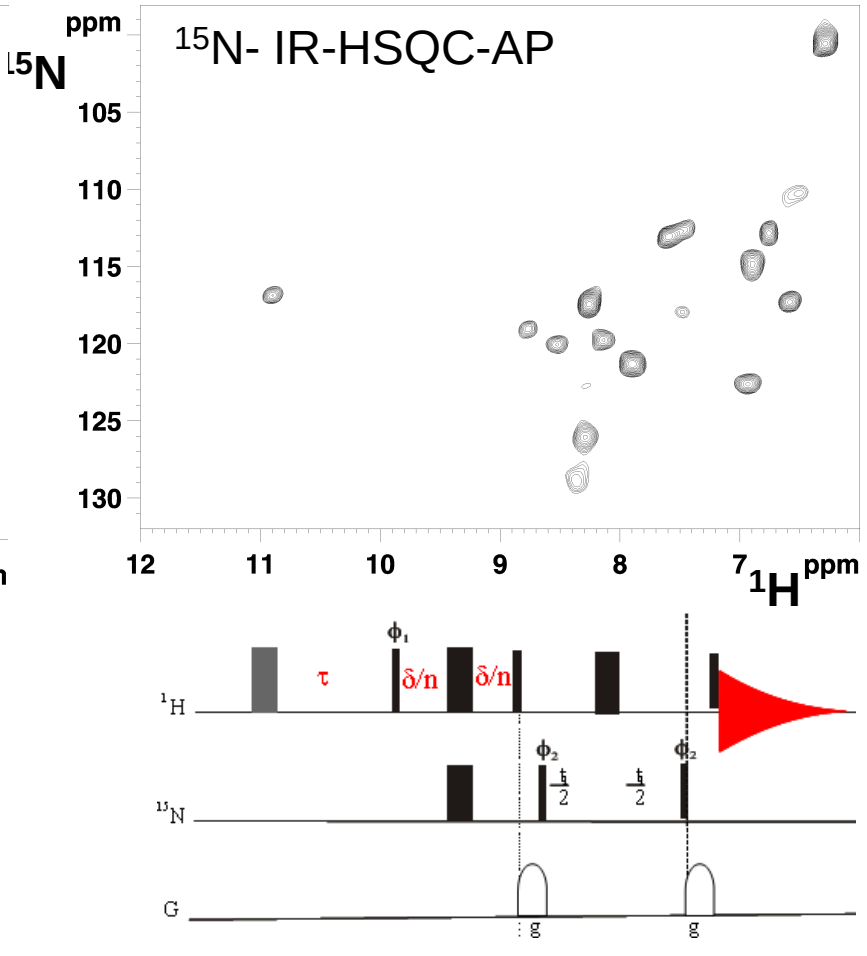
Anamorsin is a [2Fe-2S] cluster-containing substrate of Mia40



Banci, Bertini, Ciofi-Baffoni, Boscaro, Chatzi, Mikolajczyk, Tokatlidis, Winkelmann **Chem Biol**. 2011

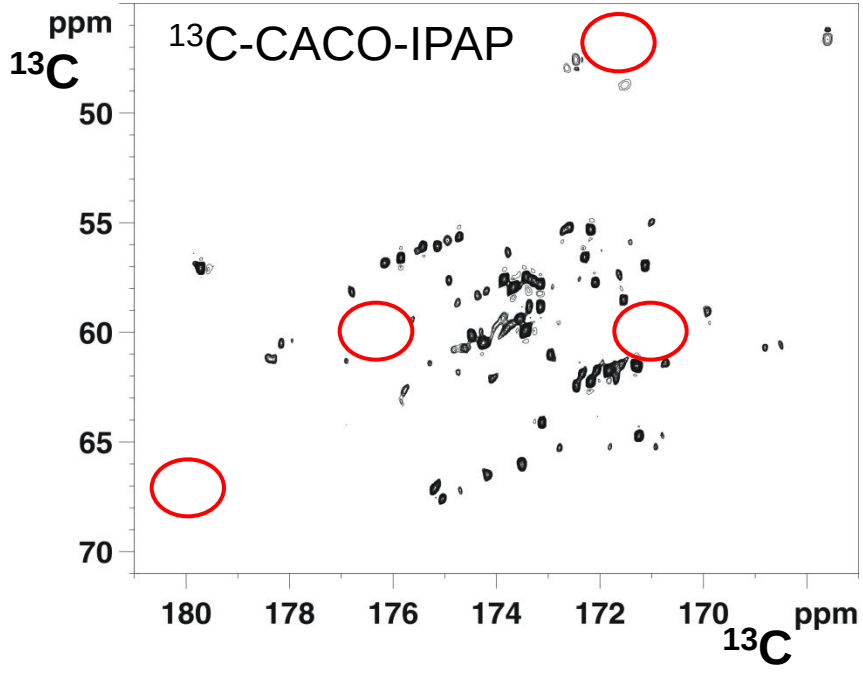
Paramagnetic Tailored ^{15}N HSQC of the [2Fe-2S]-domain of anamorsin

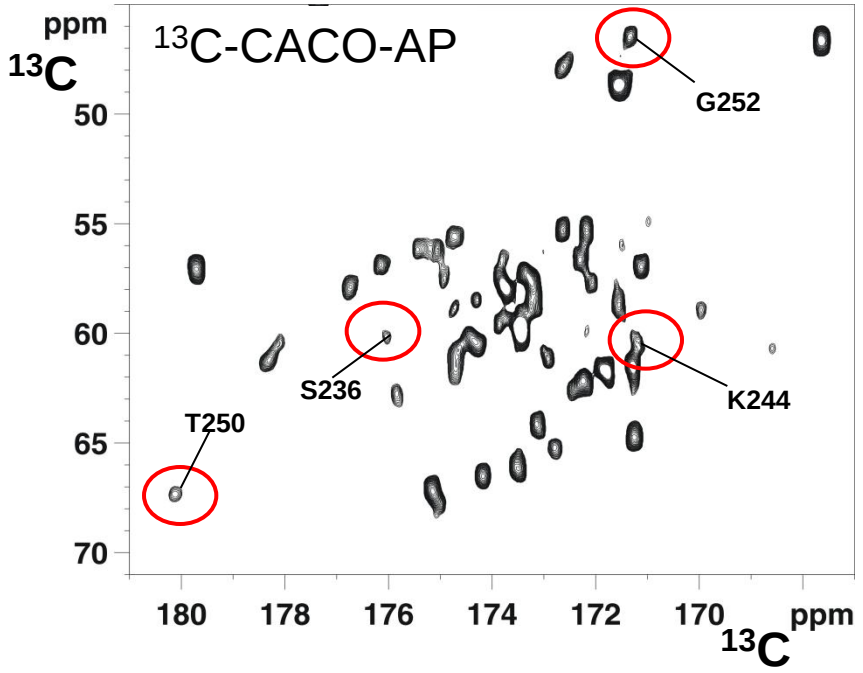




10 HN peaks, missing in standard ^{15}N HSQC, can be detected.
^{1}H T_1 values range from 5 to 30 ms

Paramagnetic-tailored ^{13}C-direct CACO of [2Fe-2S]-domain of anamorsin



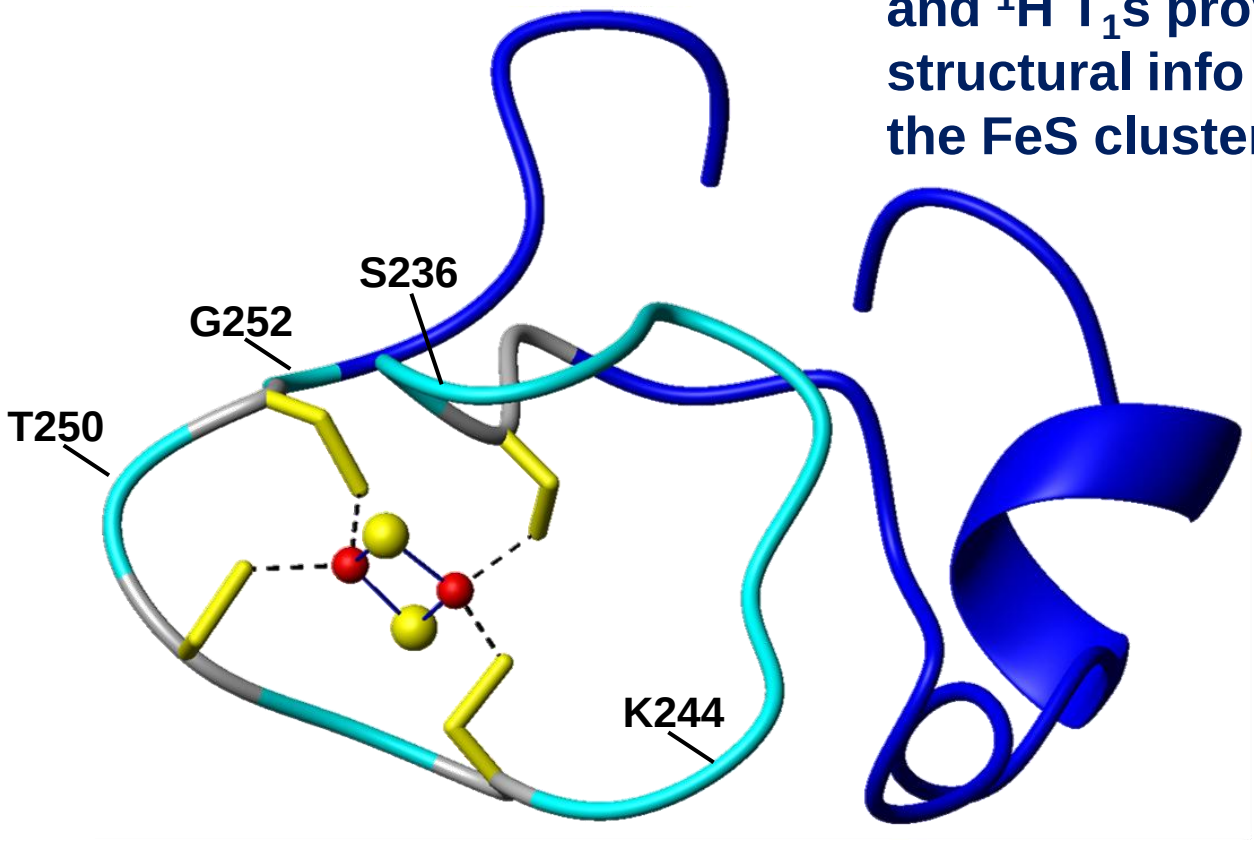


^{13}C signals, absent in standard ^{13}C-direct esperiments, are also observed via ^{13}C COSY and tailored CON

Overall, about 10 additional ^{13}C resonances are detected

Structural model of the [2Fe-2S] cluster in anamorsin

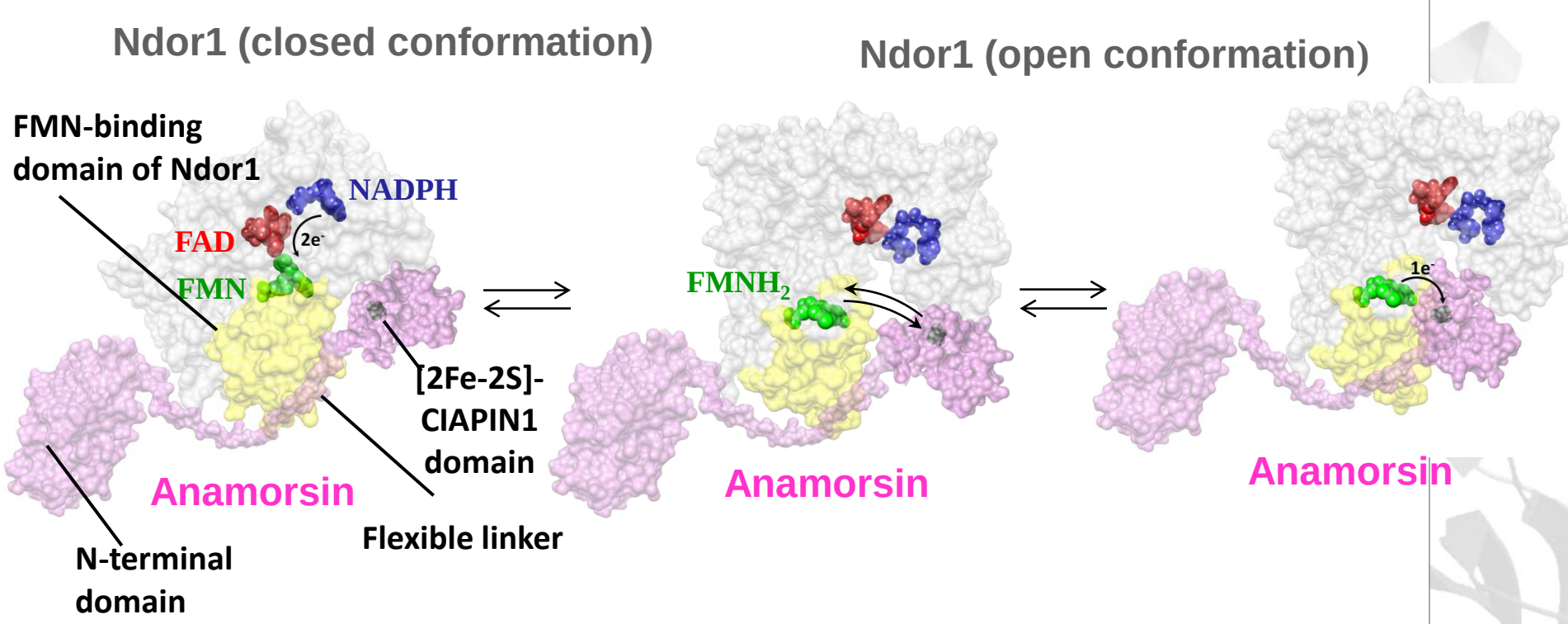
The "paramagnetic" ^{13}C and ^{1}H T_1s provide structural info around the FeS cluster



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

Electron transfer between Ndor1 and anamorsin

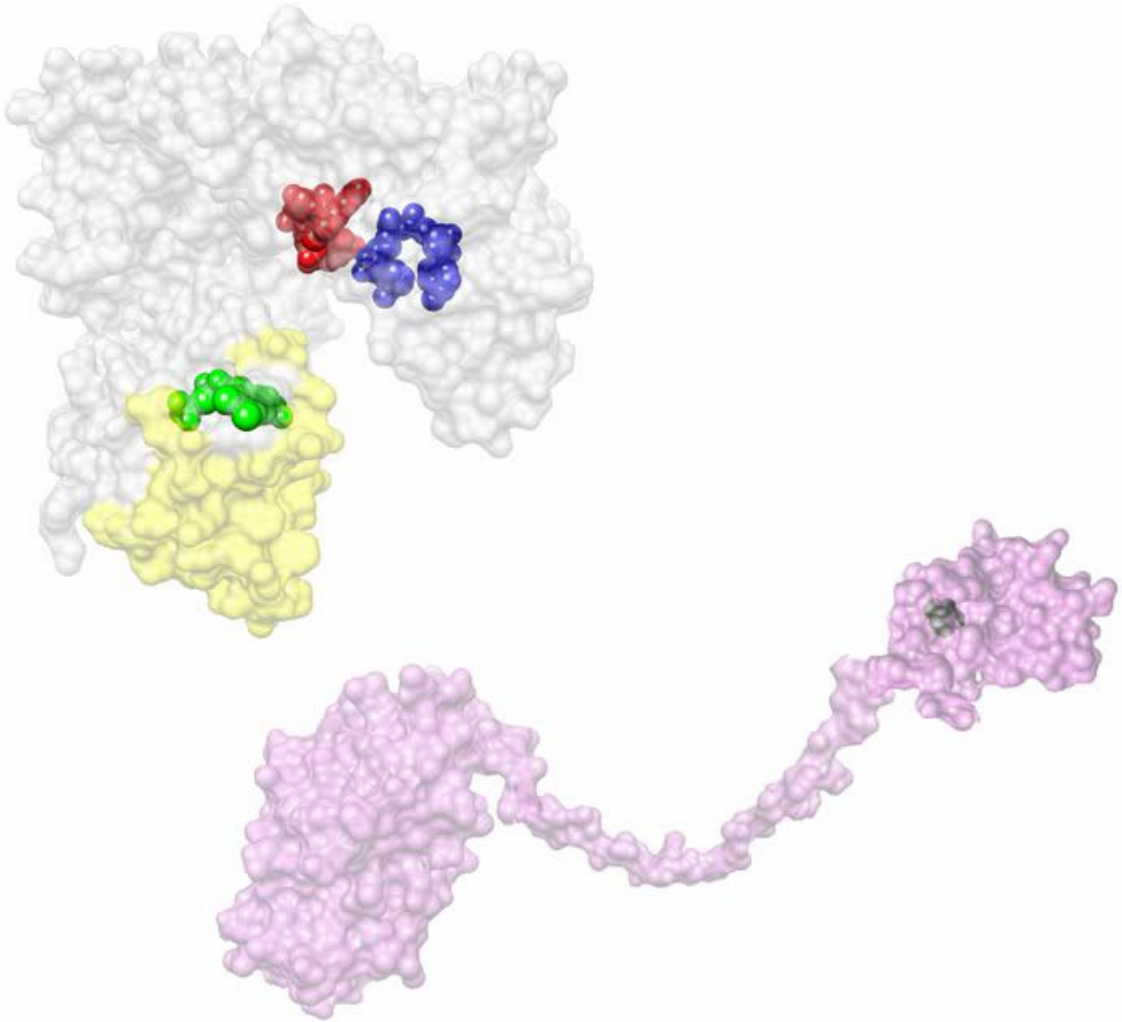


✓Anamorsin tightly interacts with Ndor1 through its flexible linker

✓ The N-terminal domain of anamorsin is not involved in the Ndor1 recognition

✓The [2Fe-2S]-CIAPIN1 domain of anamorsin transiently interacts with the FMN-binding domain of Ndor1 and transfers one electron from FMN to the [2Fe-2S] cluster.

Electron transfer between Ndor1 and anamorsin

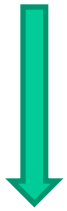


System-wide understanding of biological processes on a molecular basis and in a cellular context is critical to understand them and to discover the reasons for their impairment (diseases)

Structural Vaccinology:
the structure-based rational vaccine design

Structural Vaccinology

The strategy of vaccine design is based on the determination of the structure of the antigene and of its interactions with antibodies

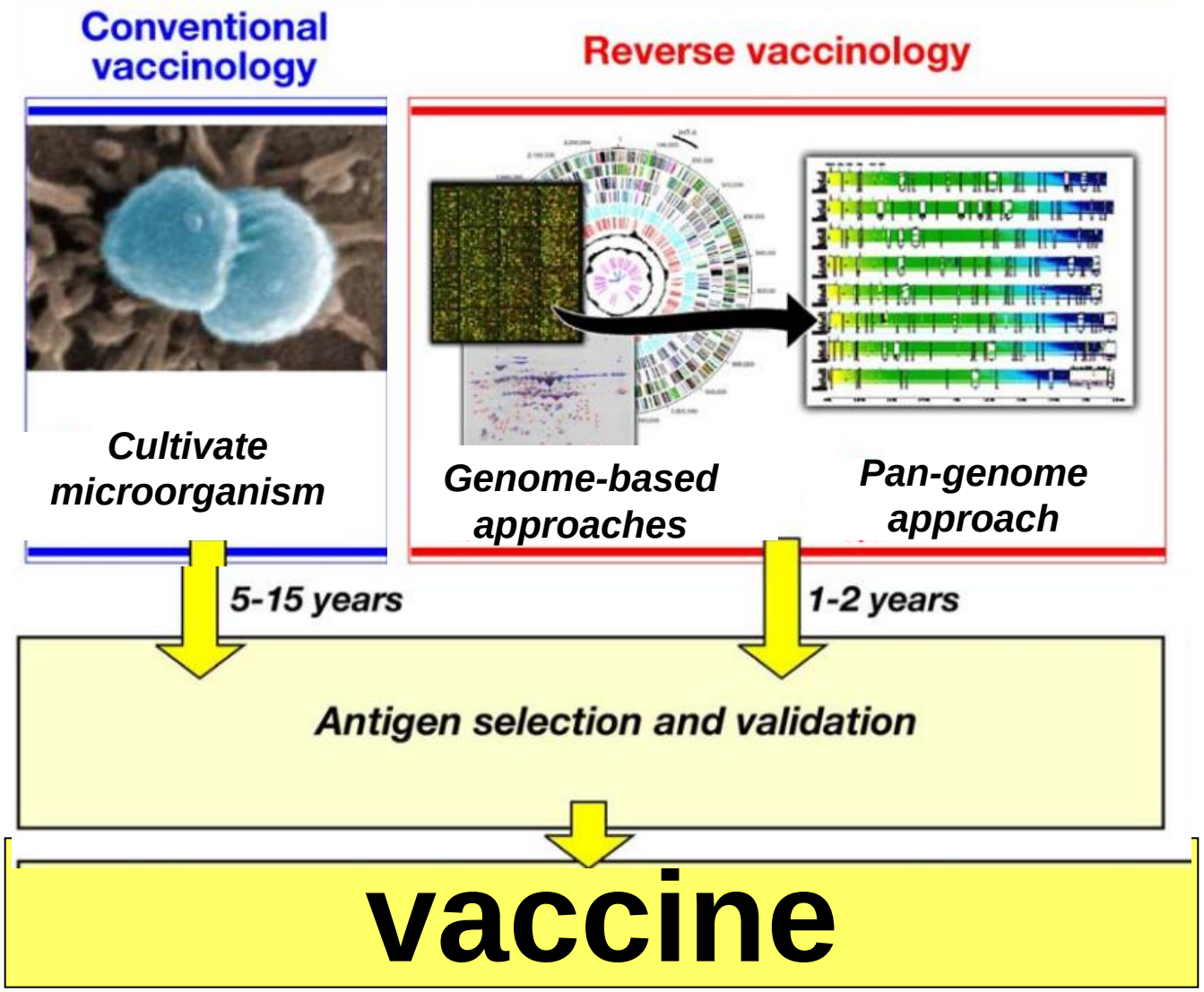


The application to Meningococcus B

Meningococcal Infection — Unmet Medical Need

- Major cause of septicemia and meningitis
- 13 serogroups according to the polysaccharides of the capsule. More than 95% of total cases of this invasive disease are caused by 5 major serogroups: A, B, C, Y and W.
- Meningococcal polysaccharide–related vaccines are available against A, C, Y and W serogroups
- No suitable polysaccharide vaccine is available against MenB as the capsular polysaccharide of serogroup B is poorly immunogenic, making this type of vaccine ineffective.

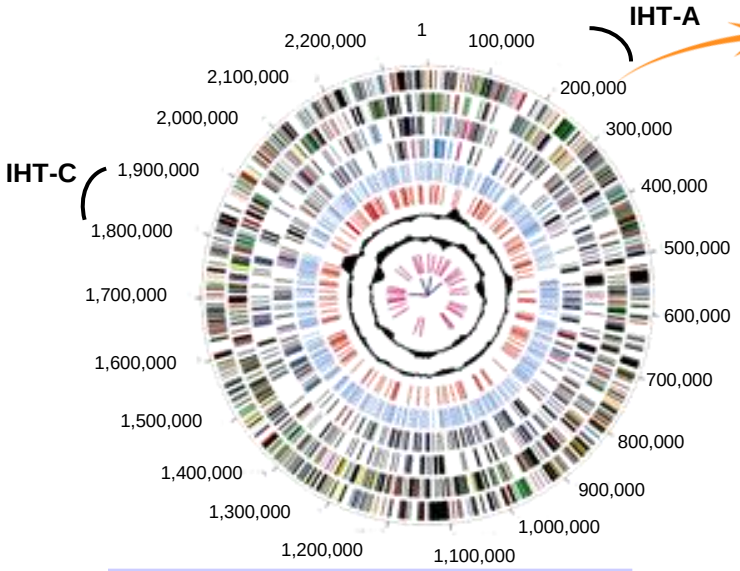
Reverse Vaccinology



From Davide Serruto, Rino Rappuoli, Post-genomic vaccine development
FEBS Letters 580 (2006) 2985–2992

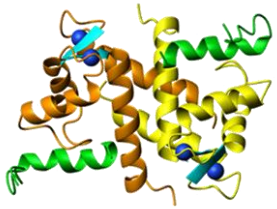
Reverse Vaccinology: MenB

Based on the genome sequence of MenB MC58 strain, 600 ORFs that potentially encode surface exposed or exported proteins were identified



~350 proteins successfully expressed in *E.coli*, purified, and used to immunize mice

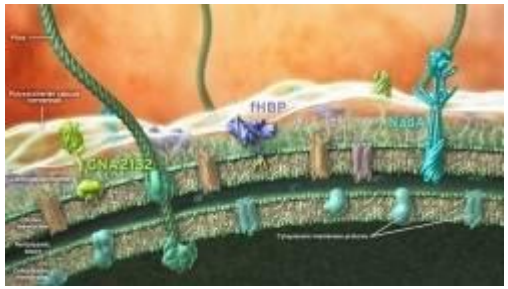
expression and purification



purified proteins

immunizations

91 novel surface-exposed proteins identified



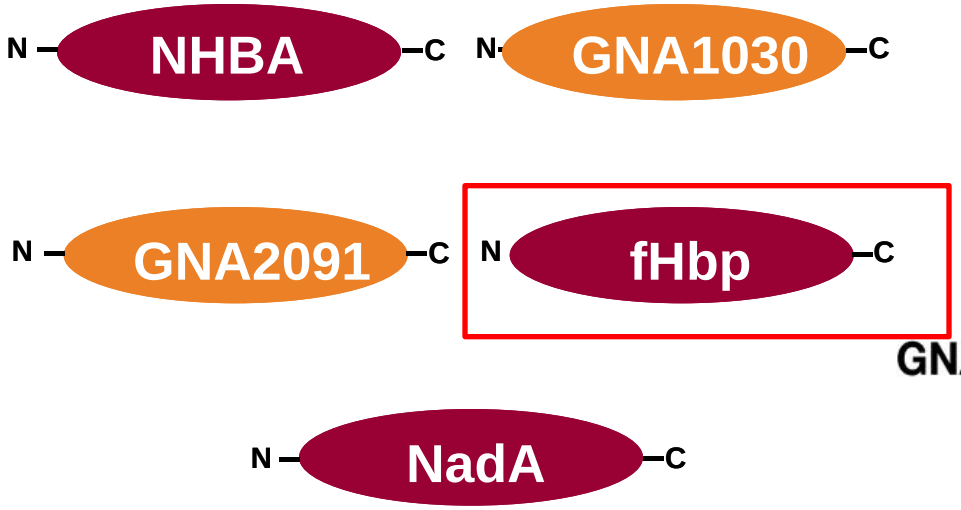
28 novel protein antigens with bactericidal activity were identified

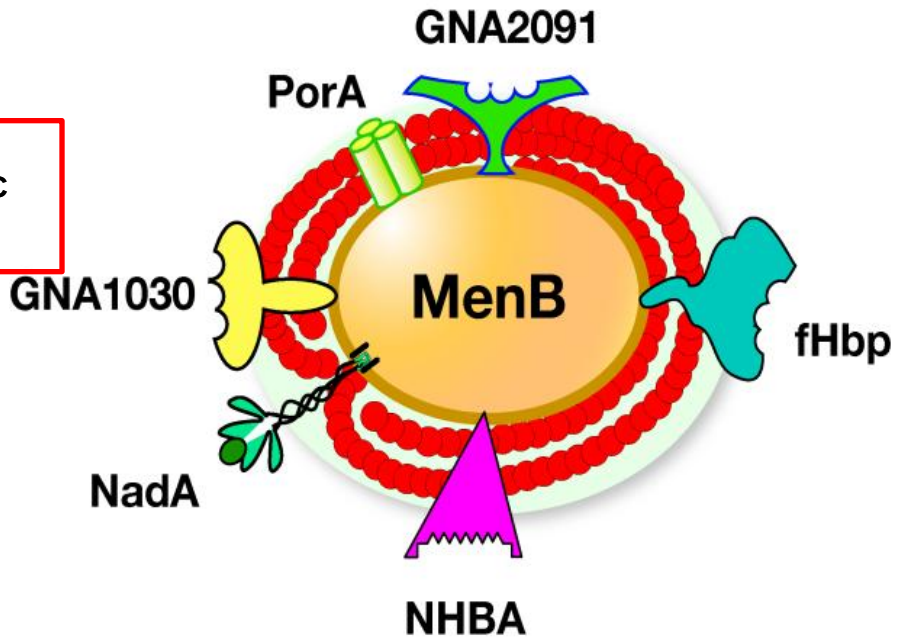
Pizza et al. Science 2000

NOVARTIS

New Antigens were selected through Reverse Vaccinology to develop a first-generation Vaccine against MenB

- Five surface proteins are the most effective to induce protective immunity eliciting antibodies, able to kill the bacterium





Factor H (fH) is an essential and abundant regulator of the human innate and acquired immune system, which kills pathogens. Many pathogens have evolved the ability to avoid immune-killing by sequestering fH to their surface

Factor H Binding Protein (fHbp)

- 28-kDa surface-exposed lipoprotein of 255 aminoacid

- fHbp is a promising vaccine candidate for MenB vaccine.

- fHbp deletion *N. meningitidis* mutant s are killed in human blood, indicating the essential role of this antigen for bacterial survival in its host

- Binding is specific for human fH (low for chimpanzee and not detected with fH from lower Primates). This is consitsent with *N. meningitidis* being a strictly human pathogen

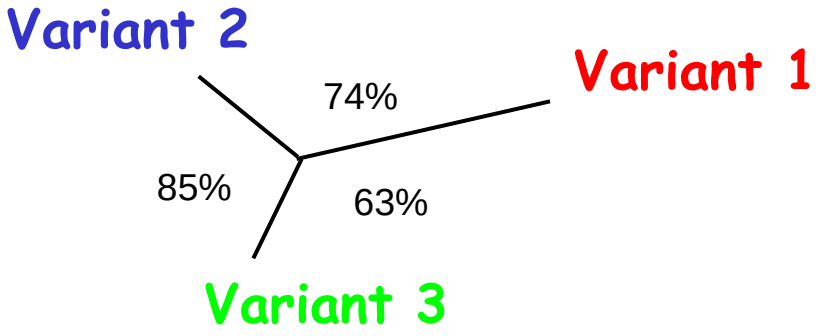
Strategy for vaccine design

Blocking the binding of MenB proteins (e.g. fHbP) to fH would induce the immune response which would be killing the bacteria

The vaccine should stimulate antibodies against fHbp

Factor H Binding Protein (fHbp)

- 448 different nucleotide sequences exist of fHbp in the **MenB** strain population, coding for 378 different polypeptides
- They can be grouped in three distinct genetic and immunogenic variants (1, 2, and 3).
- Amino acid identity is 91–100% within each variant , 63–85% between variants
- **The three variants do not induce cross protection!!**



Is it possible to engineer fHbp to collect into a single molecule the antigenic repertoire of all the three variants?

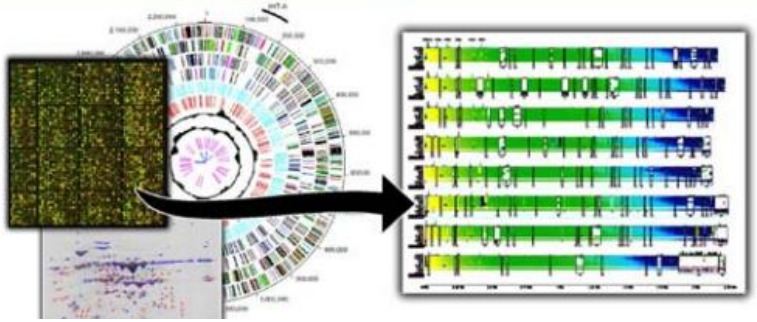
STRUCTURAL VACCINOLOGY

past → present

Conventional vaccinology

Cultivate microorganism

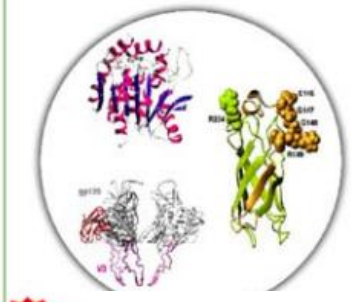
Reverse vaccinology



Genome-based approaches

Pan-genome approach

Structural vaccinology



Determination of antigens structure

5-15 years

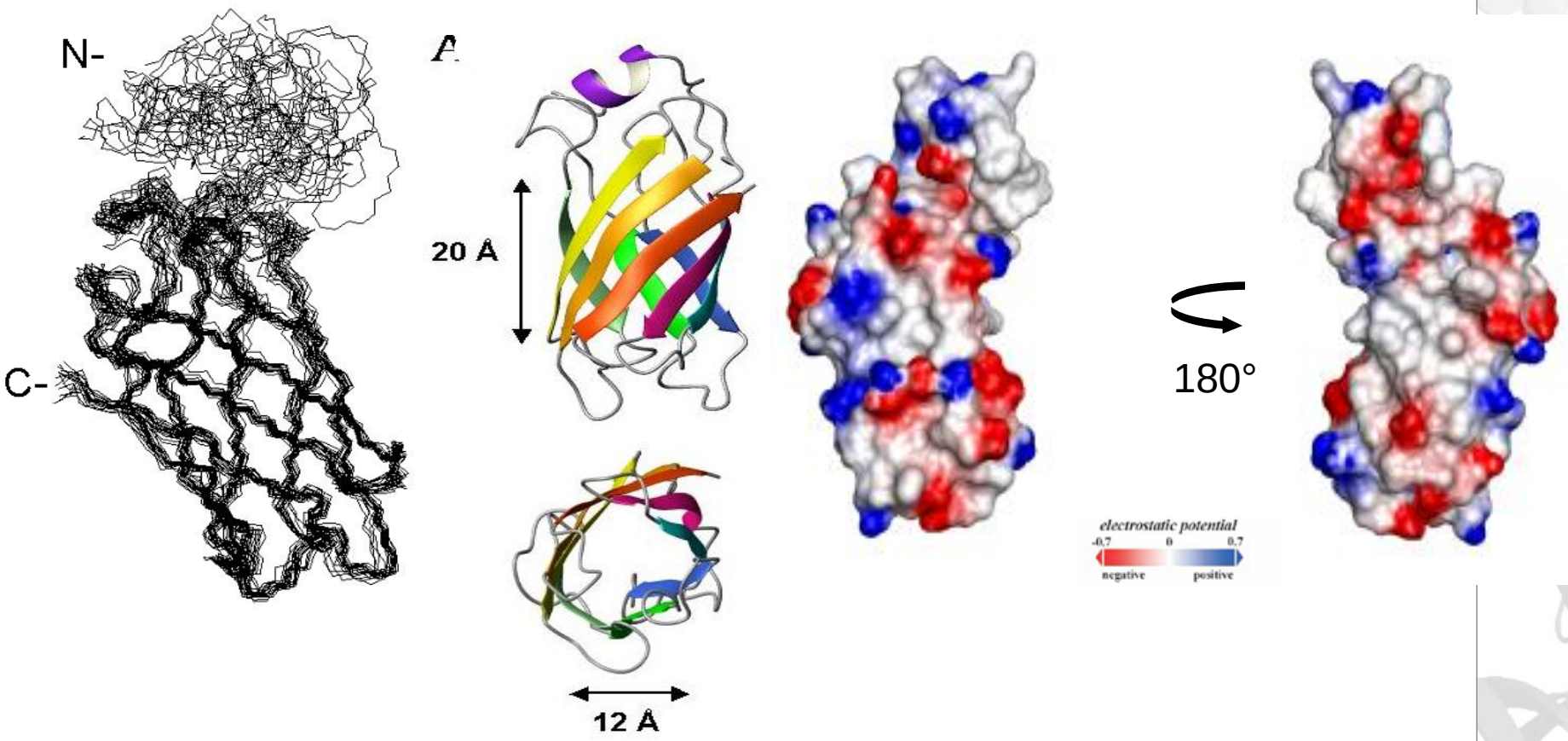
1-2 years

Antigen selection and validation

Rational design of target epitopes

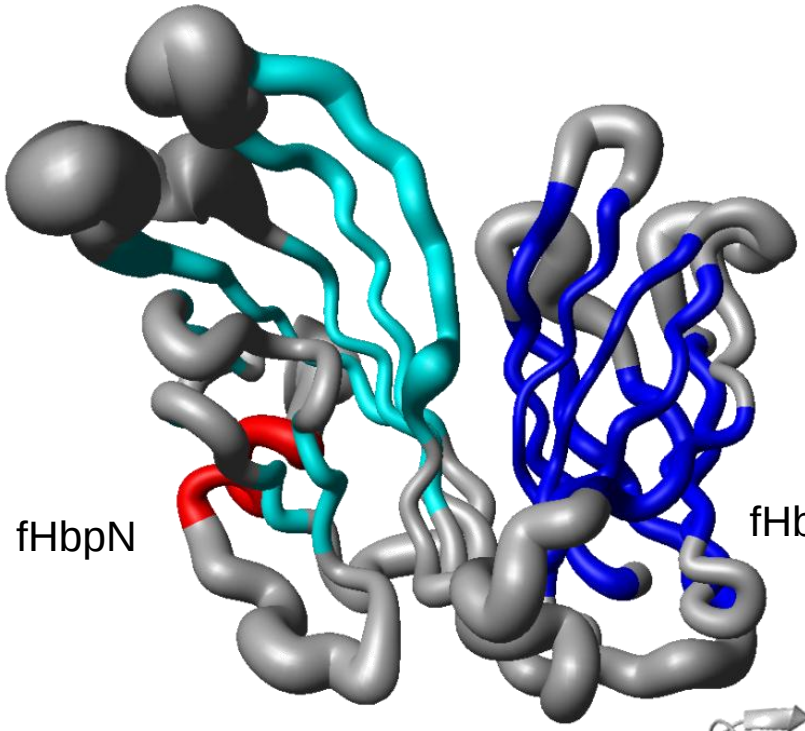
vaccine

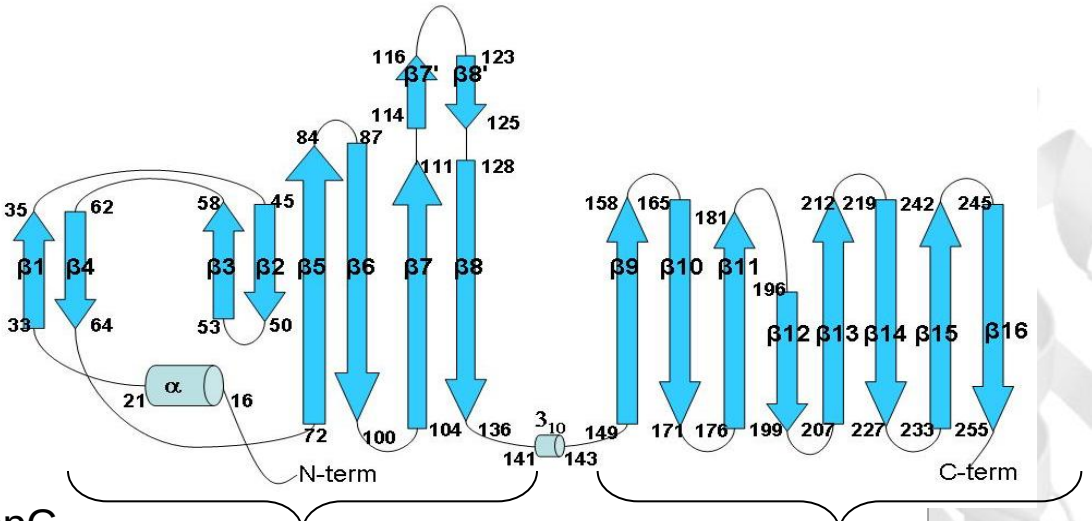
SOLUTION STRUCTURE OF THE IMMUNODOMINANT DOMAIN (C DOMAIN) OF ANTIGEN fHbp (Variant 1) OF *MenB*



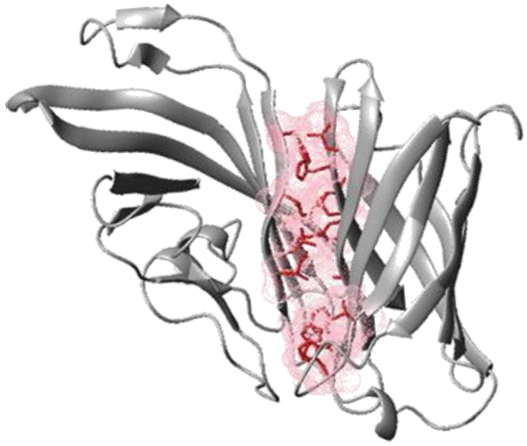
Cantini F, Savino S, Scarselli M, Masignani V, Pizza M, Romagnoli G, Swennen E, Veggi D, Banci L, Rappuoli R., *J Biol Chem*. 2006 , 281, 7220-7.

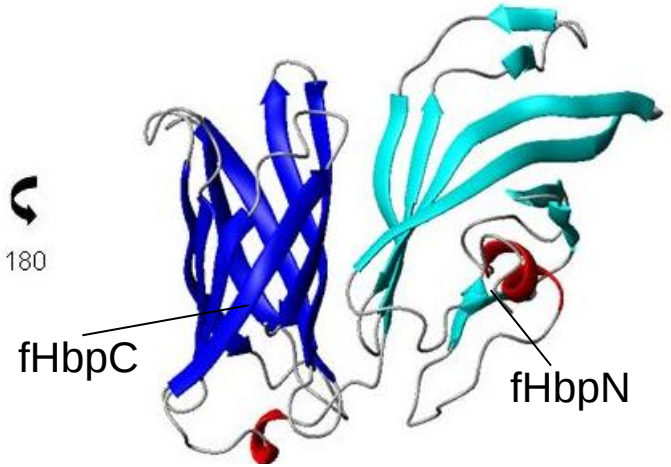
Structure of the full length fHbp (variant 1)





Hydrophobic interactions between the two domains stabilize the overall fold



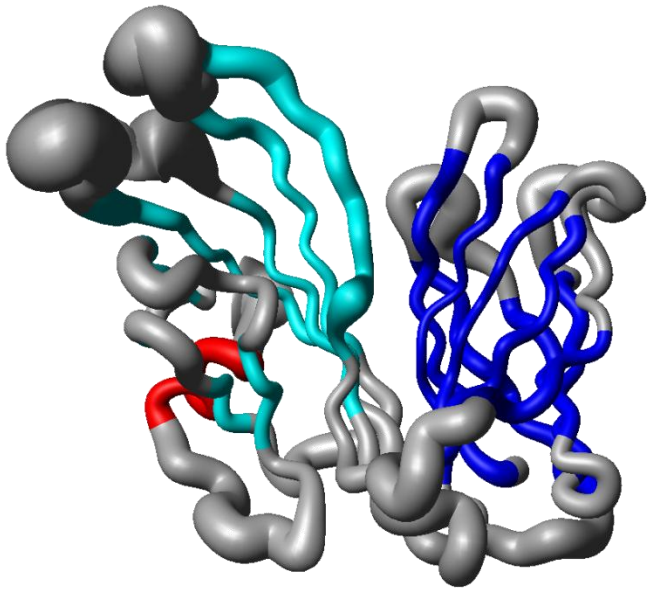


Cantini, F., Veggi, D., Dragonetti, S., Savino, S., Scarselli, M., Romagnoli, G., Pizza, M., Banci, L., and Rappuoli, R. (2009) ***J. Biol. Chem. 284*****, 9022-9026.**

Beyond the structure

Structural vaccinology

STRATEGY



STUDY THE INTERACTIONS

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

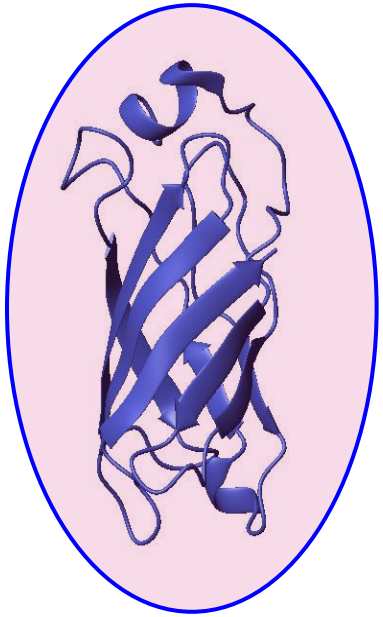
TO CREATE

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

The atomic resolution of the structures of potential antigens allowed us the rational design of target epitopes to be used as vaccine candidates.

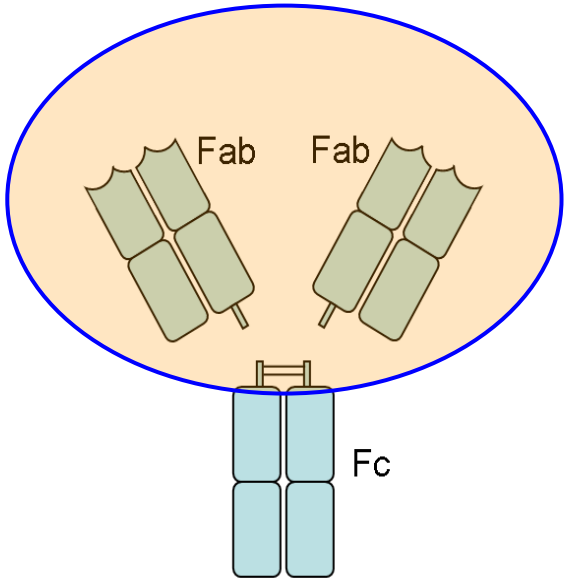
fHbpC & Antibodies Interaction

fHbpc
(18kDa)



+

fAb portion of mAb502
(50kDa)

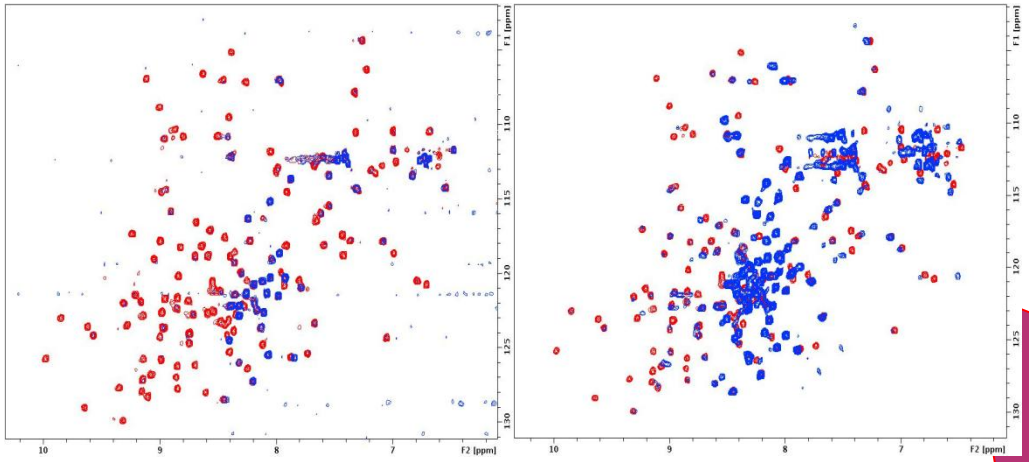


The knowledge of the 3D structure of fHpb allowed us to map the regions involved in antigen-antibody interactions, i.e between factor H binding protein and the bactericidal antibodies.

Scarselli M, Cantini F, Santini L, Veggi D, Dragonetti S, Donati C, Savino S, Giuliani MM, Comanducci M, Di Marcello F, Romagnoli G, Pizza M, Banci L, Rappuoli R. *J Mol Biol*. 2009 386, 97-108.

Interaction between fHbpC and a fAb portion of the antibody mAb502 (as studied by NMR)

- 900 MHz Spectrometer (298K)



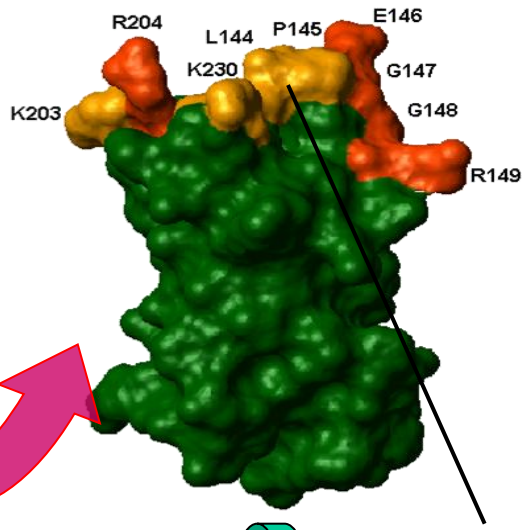
^{1}H-^{15}N HSQC-TROSY of fHbpC alone

^{1}H-^{15}N HSQC-TROSY of fHbpC:mAb502 mixture

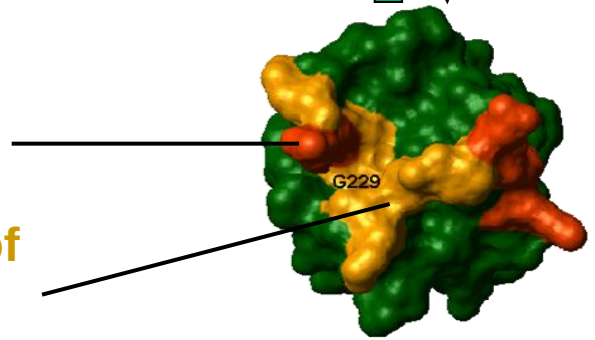
^{1}H-^{15}N HSQC-TROSY of fHbpC alone

^{1}H-^{15}N HSQC-TROSY-CRINEPT of fHbpC:mAb502 mixture

Chemical shift mapping



Residues showing Chemical Shift variation



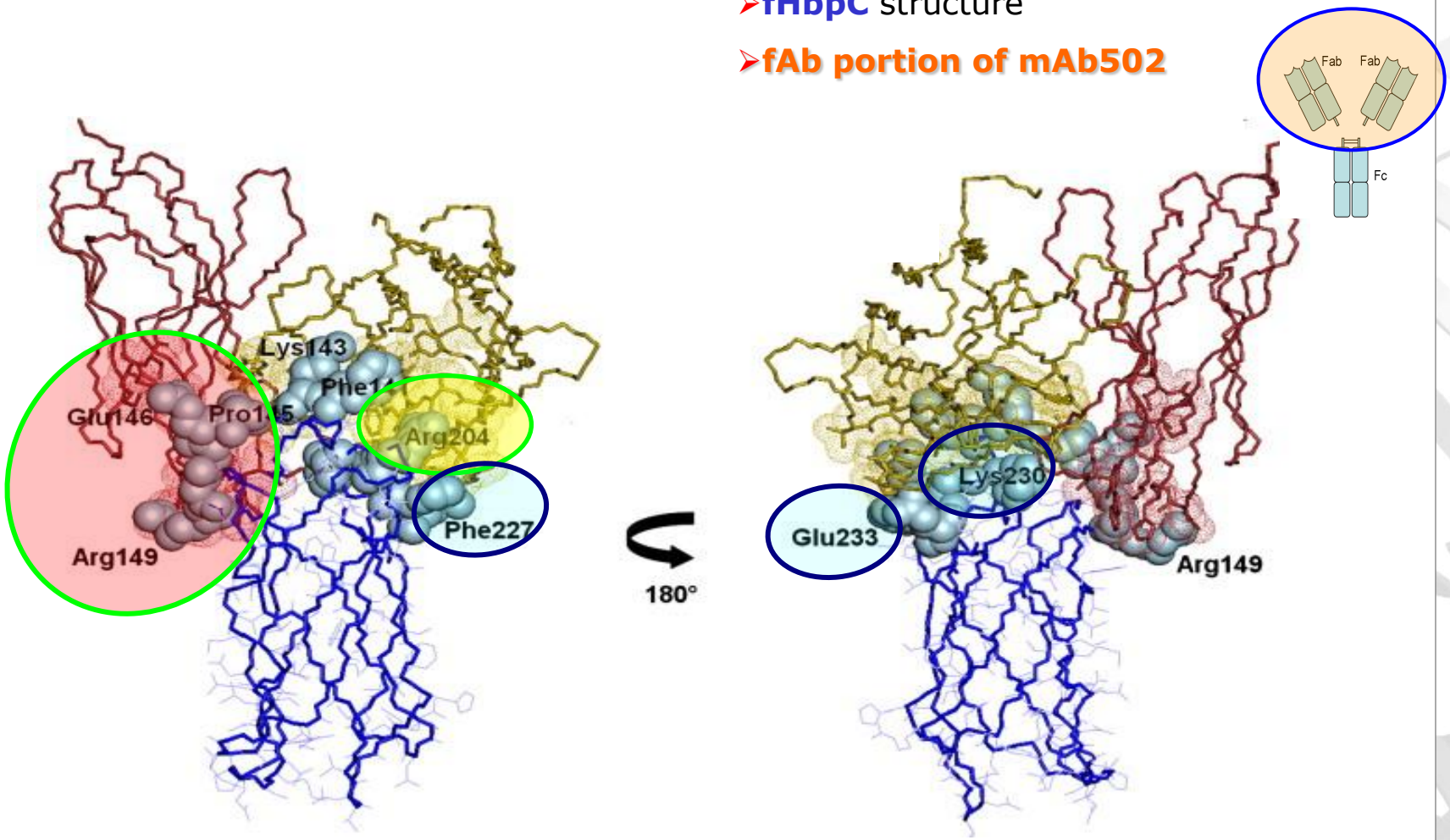
Residues predicted by immunological data and confirmed by NMR

NMR data suggested the involvement of other amino acids

The data show that mAb502 recognizes a conformational epitope within a well-defined area of the immunodominant C-terminal domain of fHbp.

Model of the complex between fHbpC & fAb portion of mAb502

- **fHbpC** structure
- **fAb portion of mAb502**

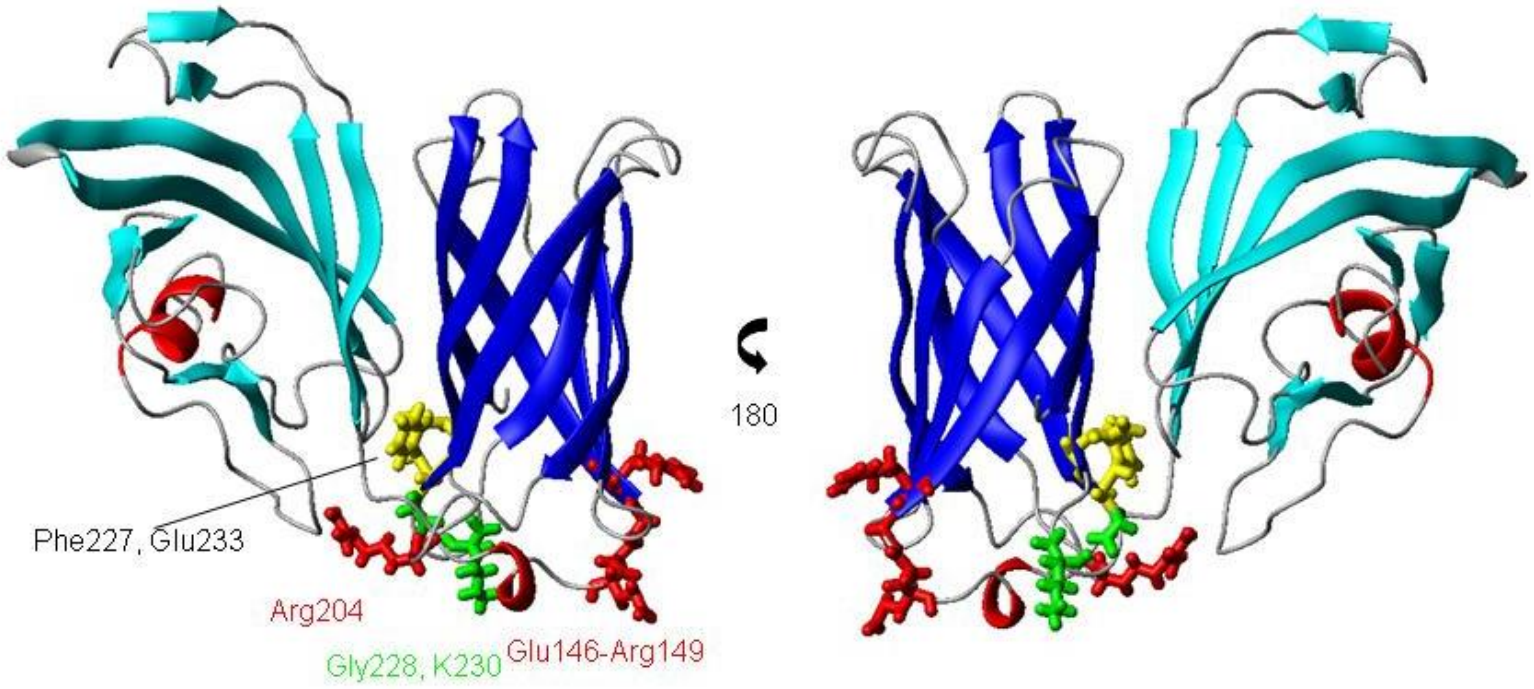


These results, obtained through NMR data and docking calculations, represented the first step of an experimental strategy in which vaccine candidates can be designed to contain broad repertoires of natural protective epitopes identified by molecular mapping.

…based on the structure of full length fHbp

Residues of **fHbpC** involved in binding to mAb502 mapped onto the

full length protein structure

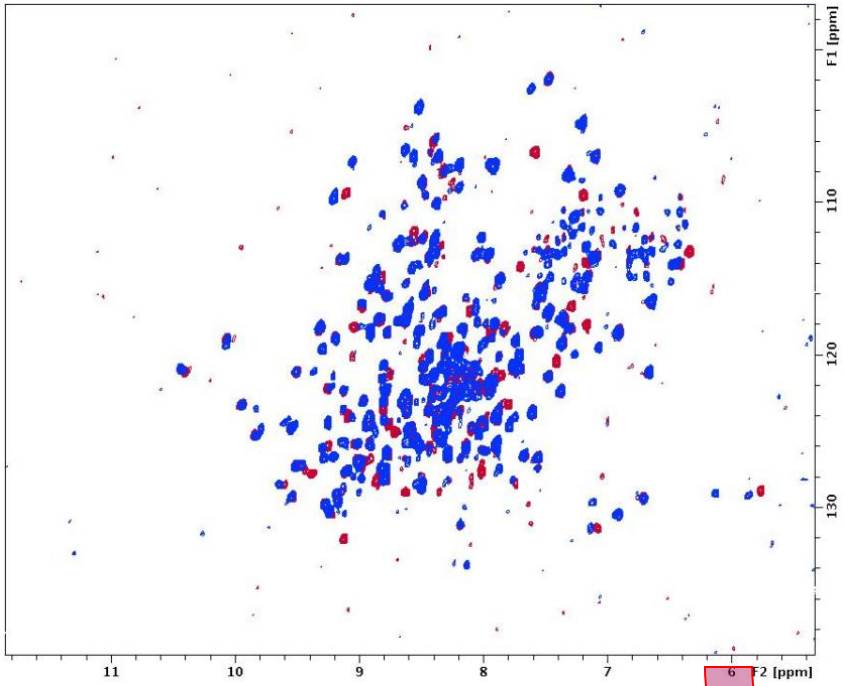


1. They are still solvent accessibles!

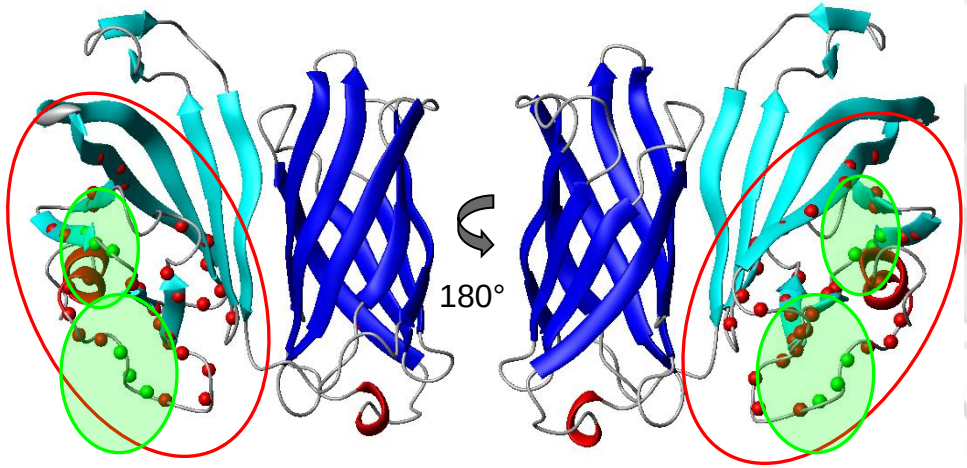
2. fHbp**C** contains the major part of the epitope

Interaction between fHbp and the fAb portion of mAb504

^{15}N ^{2}H fHbp and unlaballed **mAb**



Mapping onto the 3D structure



The N-terminal domain also is involved in the interaction

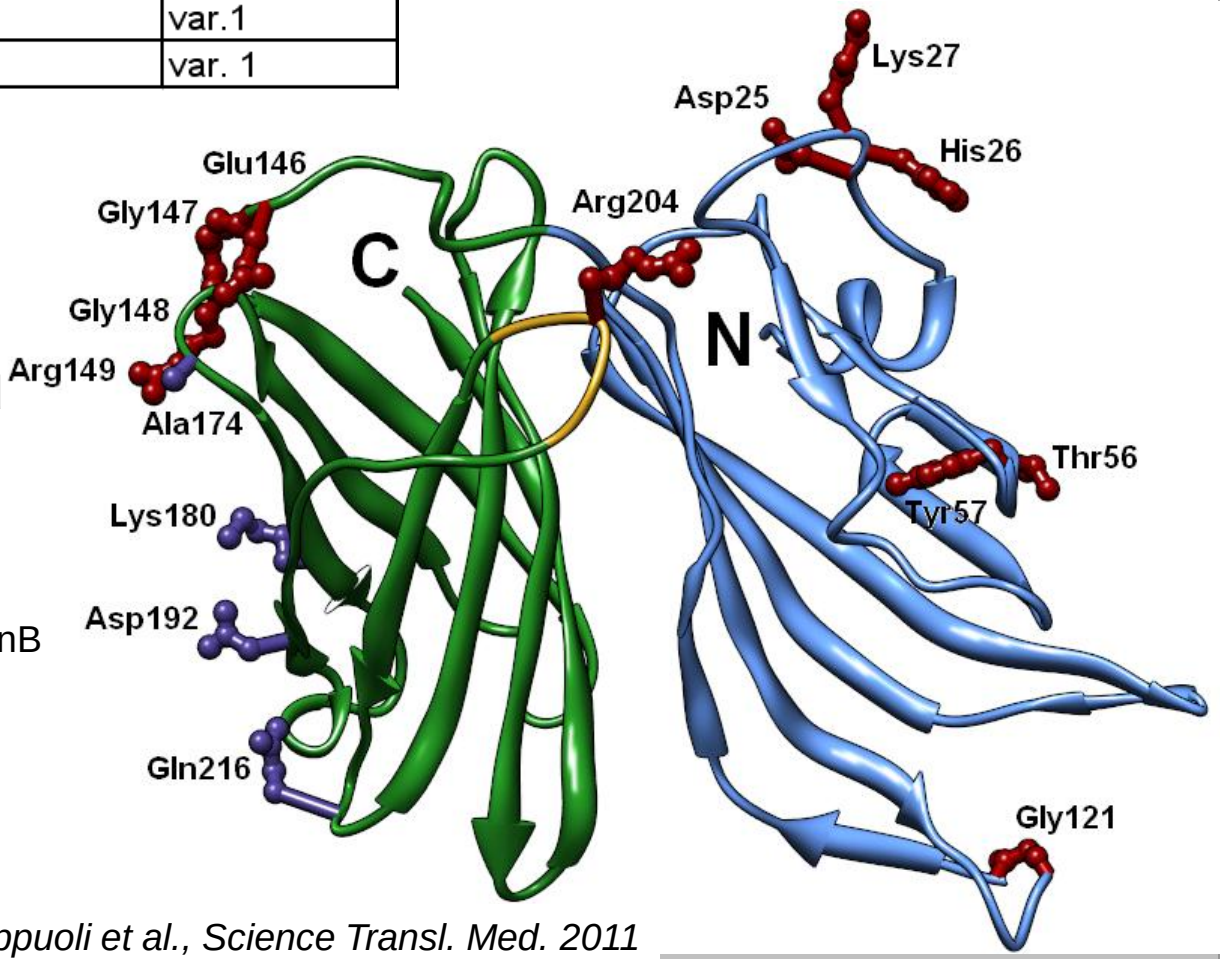
^{15}N ^{2}H HSQC TROSY fHbp

^{15}N ^{2}H HSQC TROSY-CRINEPT fHbp-mAb complex

Rational design of a meningococcal antigen inducing broad protective immunity

Residue	fHBP variant
R204	var.1
G121, T56, Y57	var.1
S216	var. 2
K180, E192, A174	var. 2 and 3
E146 - R149	var.1
D25 - K27	var. 1

Distribution of the essential amino acids recognized by mAbs elicited by variant 1 (red) and variants 2 and 3 (purple)



3D structure of fHbp from MenB strain MC58 (variant 1)

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

Rational design and structure of a meningococcal antigen inducing broad protective immunity

- **From the analysis of the 3D structure, it is clear that amino acids contributing to the immunogenicity of variant 1 or variants 2 and 3 are located in nonoverlapping regions.**

- **This important observation suggested that the immunodominant regions of the three variant groups are distinct and that an immunogenic epitope featuring variant 2 or 3 residues could be grafted onto the variant 1 backbone.**

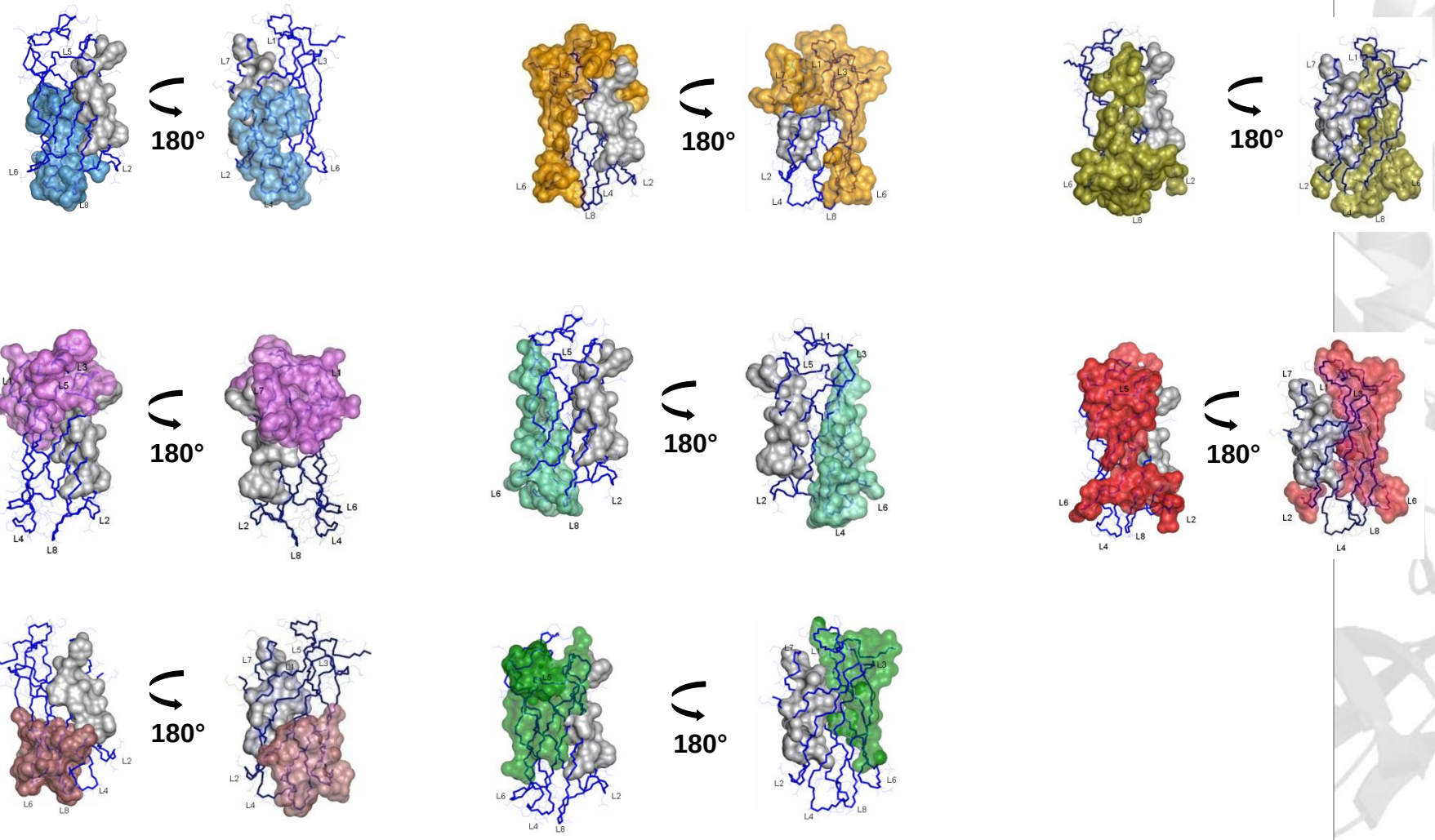
- **Portions of the antigen surface potentially recognizable by an antibody were engineered as a whole , instead of making single amino acid mutations**

Amino acid substitutions were introduced only for residues whose side chains were well exposed to the solvent, leaving the internal core of the protein unaltered.

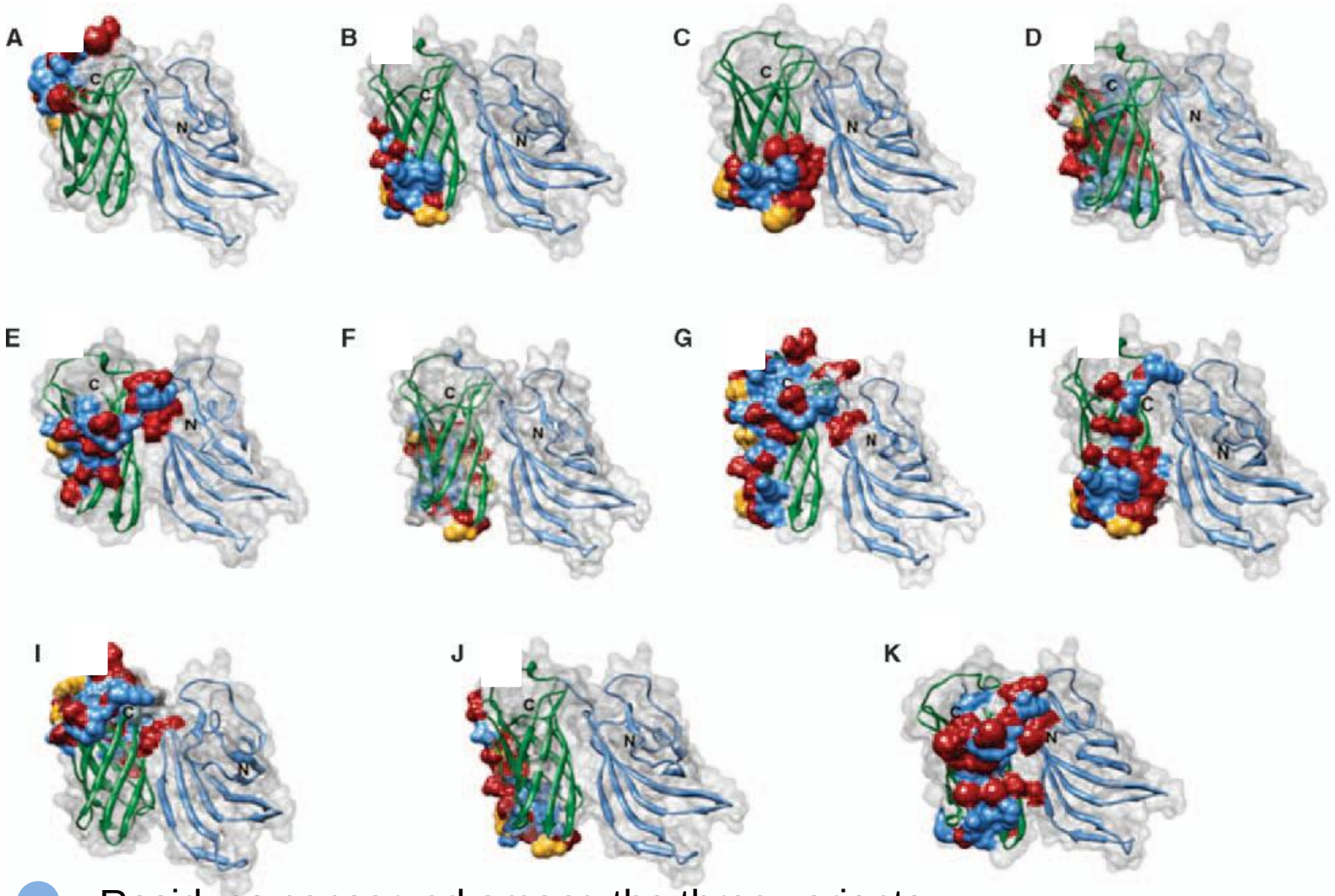
Scarselli, Banci, Cantini, Rappuoli et al., Science Transl. Med. 2011

Some of the selected 11 regions of the protein

The C-terminal domain of fHbp was divided into 11 partially overlapping surface areas large enough to hold at least one conformational epitope



Mutations on fHbp (variant 1) surface



Residues conserved among the three variants

Residues conserved between variants 2 and 3

Variable residues

54 different mutants were designed and produced

- **Of the 54 mutants tested, 18 gave more than a 10-fold reduction in bactericidal activity of the mouse sera against the MC58 strain (variant 1) and were therefore discarded.**

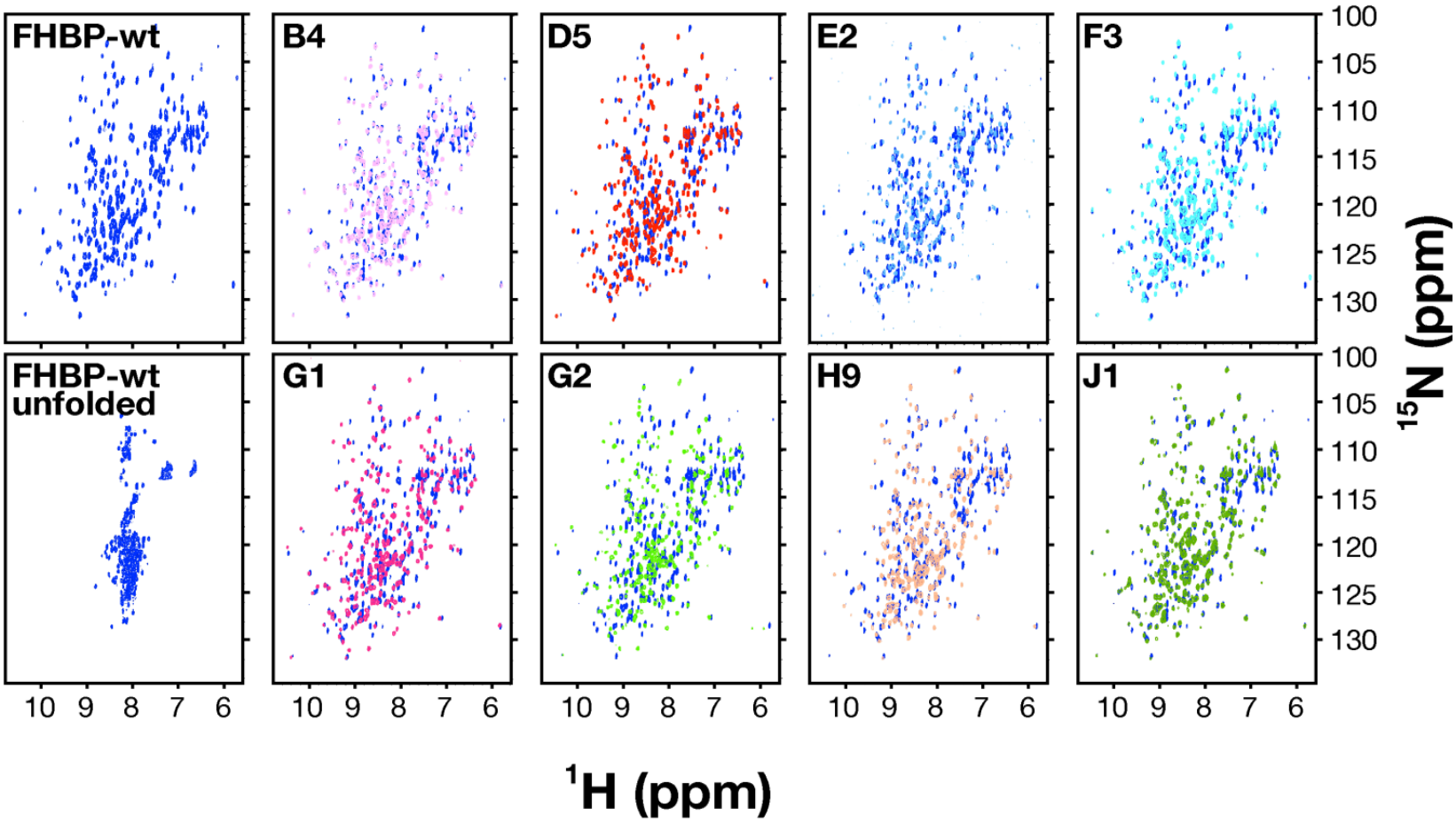
- **Among the remaining 36 molecules, 15 showed at least a 10-fold increase in bactericidal activity against the prototypic strain of variant 2 or were also positive against variant 3.**

- **This group of 15 proteins carried mutations within 6 of the 11 different engineered areas. We selected a representative set of them .**

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

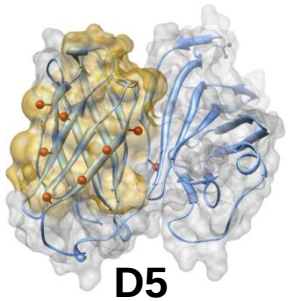
Structural characterization of fHbp mutants

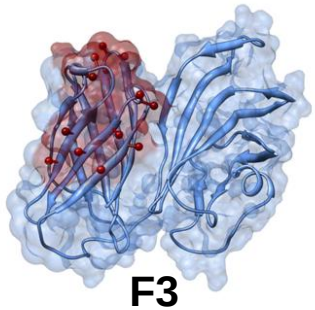
- Structural properties
  - All have the same profile, in terms of secondary structure content (CD) and of overall folding (NMR)

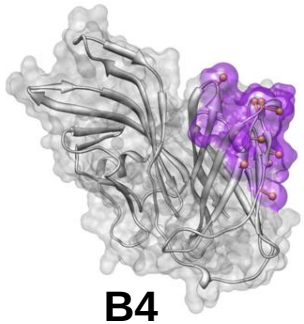


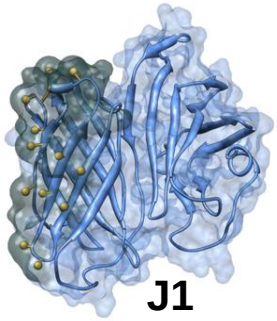
Scarselli, Banci, Cantini, Rappuoli et al., Science Transl. Med. 2011

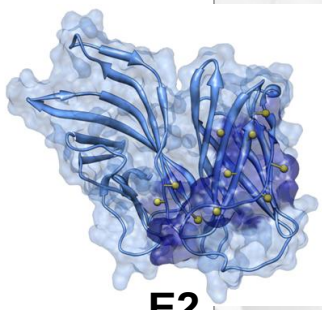
Bactericidal titers in mice elicited by the selected fHbp mutants


D5

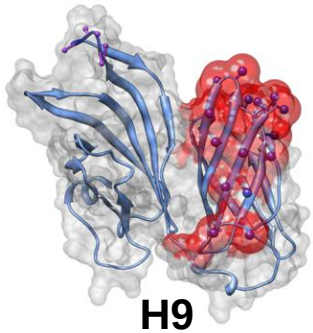

F3

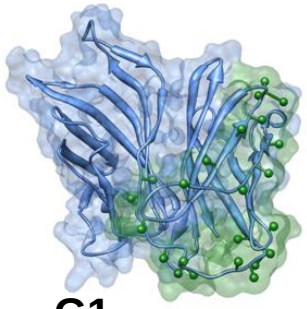

B4


J1


E2

	MC58 1.1	UK185 1.10	M4030 1.12	NZ98/254 1.14	961-5945 2.1	M3153 2.4	C11 2.7	M2552 2.10	M1239 3.1
fHBP var.1.1 wt	4096	2048	1024	128	16	256	<16	<16	<16
fHBP var.2.1 wt	16	16	<16	<16	32768	>8192	1024	2048	512
D5	2048	64	2048	<16	2048	512	<16	<16	<16
F3	8192	256	8192	1024	2048	1024	<16	<16	<16
B4	32768	256	8192	1024	2048	1024	16	512	<16
J1	16384	4096	>8192	256	256	2048	<16	<16	<16
E2	>65536	256	2048	4096	1024	128	<16	<16	<16
G2	16384	≥8192	4096	512	1024	>8192	32	64	128
H9	4096	512	4096	256	2048	2048	128	128	128
G1	8192	512	≥8192	1024	8192	8192	1024	1024	2048


H9

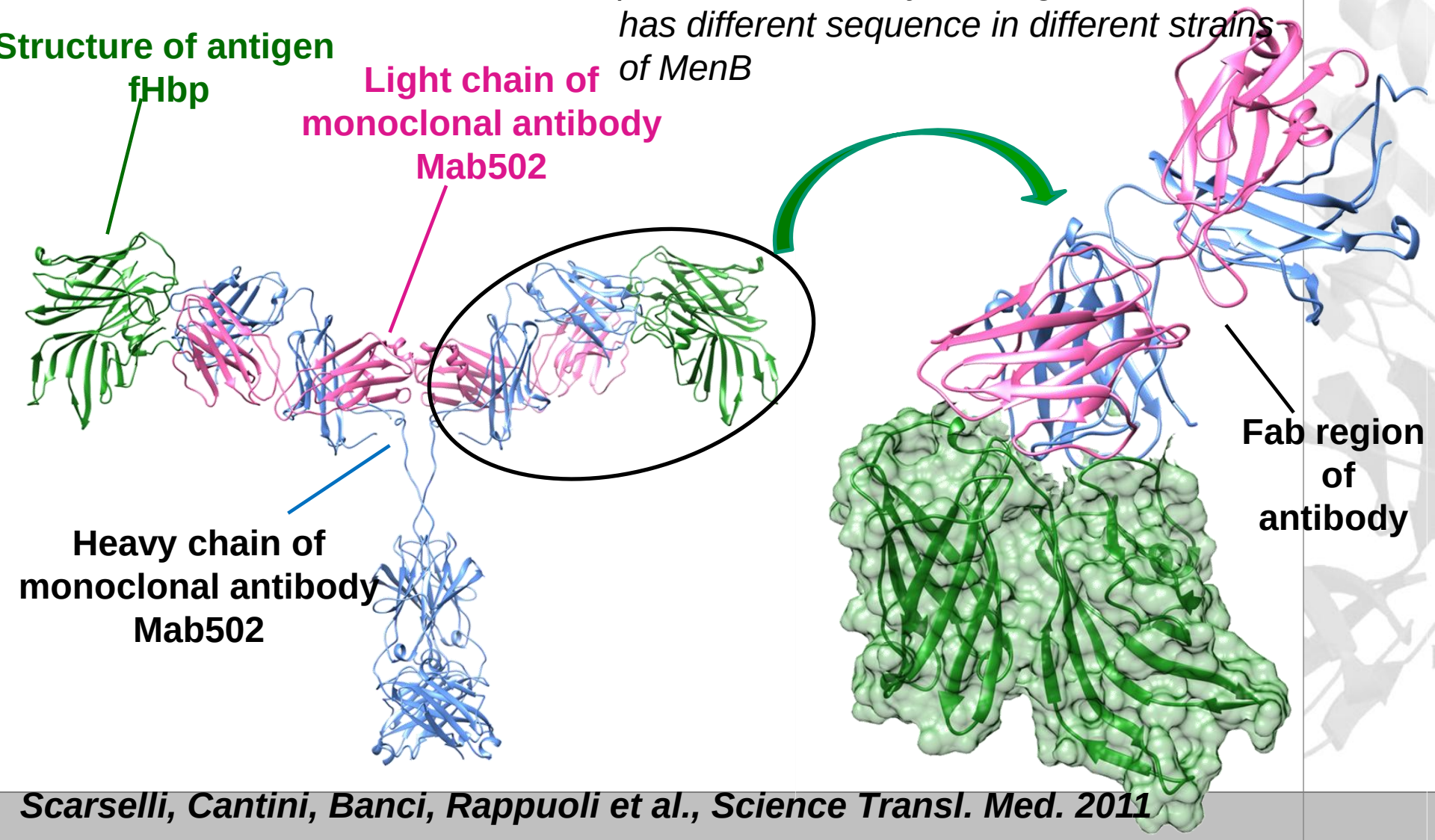

G1

Bactericidal activities of mouse serum (given as serum dilution level that resulted in a > 50% reduction of colony surviving)

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

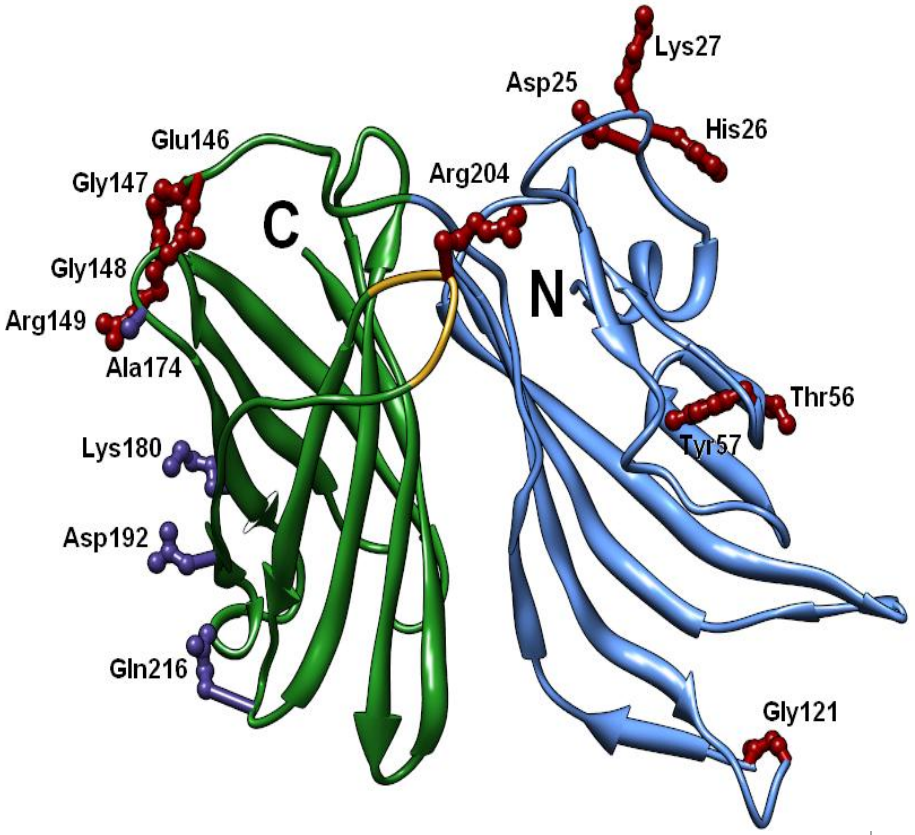
Complex of a monoclonal antibody with a Meningococcus B antigen (Factor H binding protein)

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



*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***

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

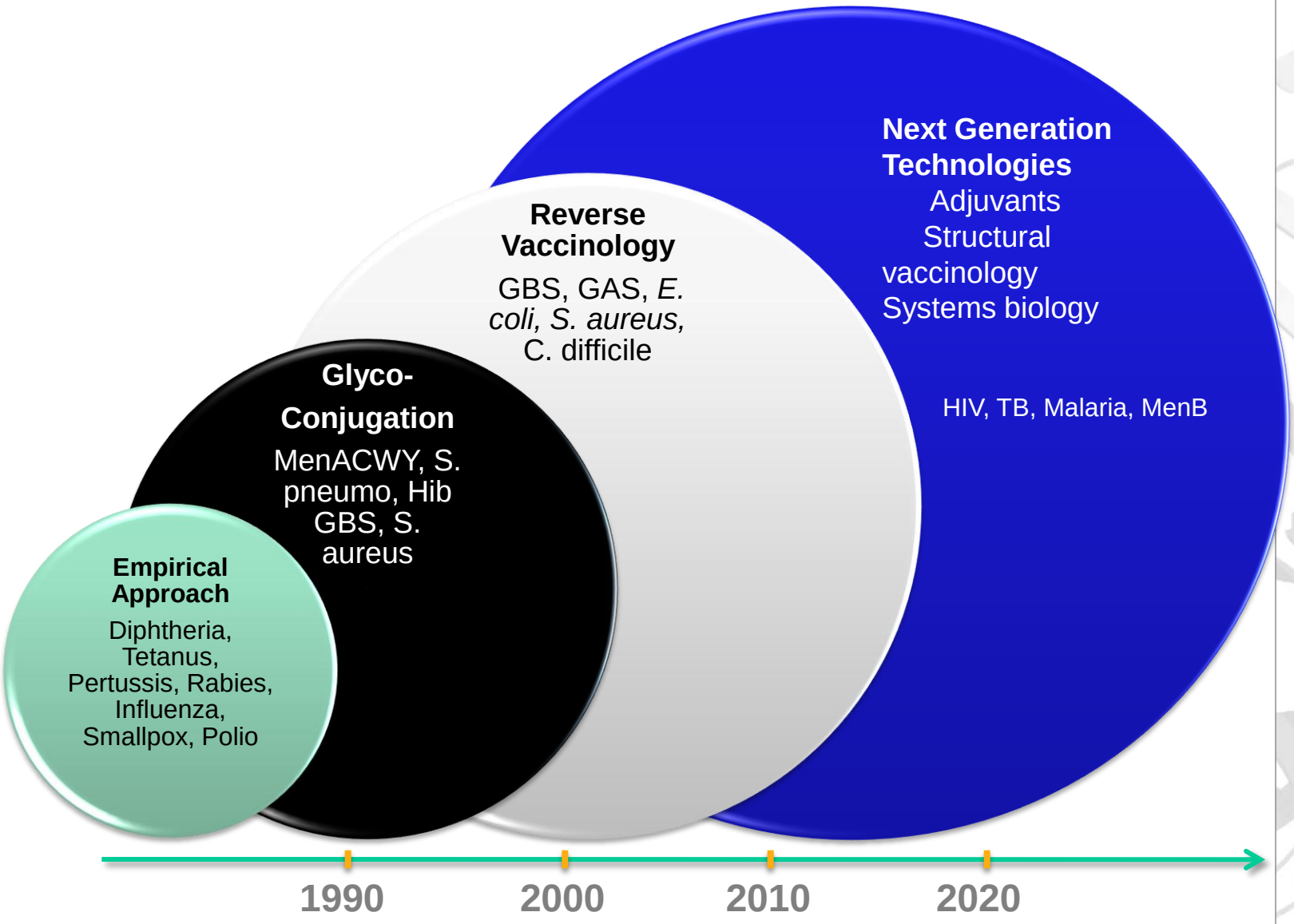
Conclusions

- Protective var 2/3-specific epitopes were succesfully introduced onto the var 1 fHbp molecule by rational engineering
- The knowledge of the 3D structure of the antigen is a necessary prerequisite to design the epitope grafting
- The new molecule shows a broad protective immunity
- The same approach can be in principle applied to all cases where variability hampers the use of otherwise effective immunogens

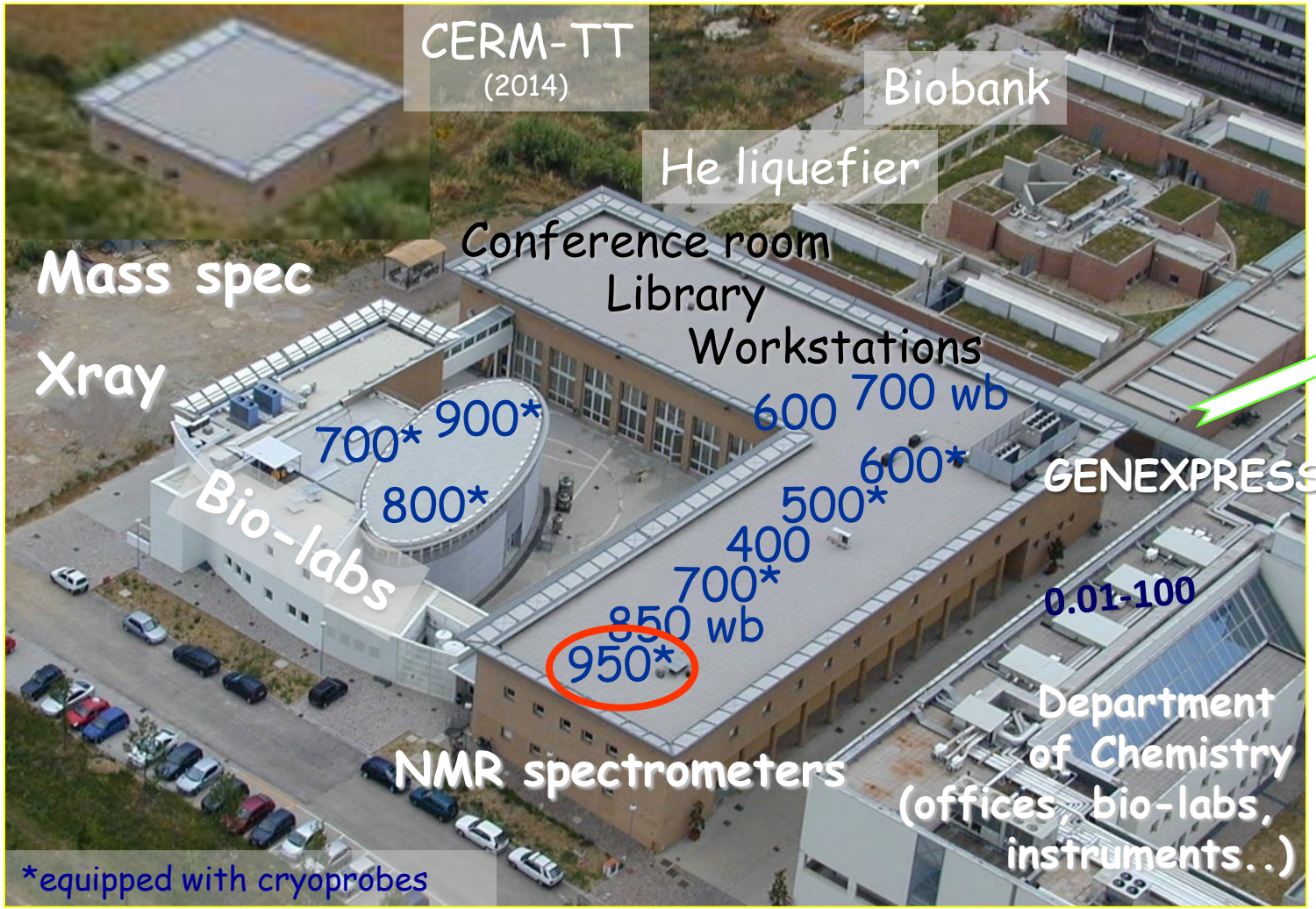
- The past: drugs identified by chance discovery.

- The future: understand how diseases and infection are controlled at the molecular level and target specific entities based on this knowledge.

Technology development to expand the number addressable diseases



CERM – University of Florence
An European facility for Biological NMR



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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 !!