NMR in Cellular Structural Biology: from Single Molecules to Pathways

Lucia Banci
Magnetic Resonance Center (CERM)
University of Florence
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 Vaccinology
  Rational vaccine design based on the structural knowledge of the antigen
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.
Integrating Atomic Resolution with the Cellular Context

Copper trafficking in human cells

No free copper ions in the cytoplasm

$E^\circ$ 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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Following SOD1 maturation steps in human cells

Combining in-cell NMR with X-ray fluorescence microscopy

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

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.


The mutations do not affect zinc binding in vitro

This unstructured species DOES NOT bind zinc.

It could be a precursor of SOD1 aggregates.

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.
Cox17 mitochondrial import

Reduced apoCox17 is unstructured

Cox 17 is transporting Cu to CcO

Cytosol

Mia40_{3S-S} + Cox17_{6SH} → apoCox17_{3SH-SH}

Mia40_{2S-S/2SH} + Cox17_{1S-S/4SH} → apoCox17_{2S-S}
Cox17 is unfolded in the cytoplasm detected in living cells

The protein folding state depends on the cellular compartment

The first step in Cox17 folding

The hydrophobic cleft of Mia40 is the interaction site for Cox17

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. O₂ or glutathione can now rapidly form the second disulphide bond.

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

Structure of Cox17-Mia40 intermediate

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

Steps in a mitochondrial pathway

**COX17**
CcO copper chaperone

**Mia40**
Mitochondrial intermembrane space Import and Assembly protein 40

**ALR**
Augmenter of Liver Regeneration

ALR regenerates the active import redox state of Mia40, i.e. with a disulfide bond in the CPC site.
Hydrophobic interactions between Mia40 and the N-terminus domain of ALR mediate efficient electron transfer from Mia40 to FAD in ALR.

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

Mia40

N-terminus domain of ALR is unstructured
Electron shuttling mechanism

ALR oxidized

Mia40$_{2S-S}$

Through $^{13}$C NMR on $^{13}$C Cys

ALR reduced

Mia40$_{3S-S}$

ALR then transfers electrons to Cyt c

**IMS protein import**

- **COX17**
  - CcO copper chaperone
  - $e^-$

- **Mia40**
  - Mitochondrial intermembrane space Import and Assembly protein 40
  - $e^-$

- **ALR**
  - Augmenter of Liver Regeneration
  - $e^-$

- **CytC**
  - Cytochrome c
  - $e^-$
$\text{Cu}_A$ assembly in the mitochondrion

$\text{Cu(I)}$-Cox17

Sco1

Sco2

$\text{Cu}_B$

CCO

Matrix

IMS

Cox2  $\text{H} - x_{34} - \text{C}x\text{ExC} - x_3 - \text{HxxxM}$
**Cox17 binds Cu(I) and transfers it to apo-Sco1/Sco2**

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.

**K_D** = 3.1/3.7 x 10^{-15} M

**K_D** = 1.7 x 10^{-14} M

Sco1 transfers Cu(I) to apo-CuA

KD = 3.1/3.7 x 10^{-15} M

KD = 0.7 x 10^{-15} M

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.

- **COX11**
  - CcO assembly protein ctaG
  - Synthesis of CcO
  - Cu(I) subunit 1 of CcO
  - Cu(I) subunit 2 of CcO
  - CcO copper chaperone

- **SCO**
  - Synthesis of CcO
  - Cu(I) subunit 1 of CcO
  - Cu(I) subunit 2 of CcO

- **Mia40**
  - Mitochondrial intermembrane space import and assembly protein 40
  - ALR Augmenter of Liver Regeneration
  - CytC cytochrome c

- **COX17**
  - CcO copper chaperone
  - Cu(I) subunit 1 of CcO
  - Cu(I) subunit 2 of CcO

- **Cu(I)**
  - Electron shuttling reactions
  - Copper transfer processes

- **CytC**
  - Cytochrome c Oxidase Complex IV
Copper trafficking in human cells

- **Cu(II)**
- **Cu(I)**

Redox state of copper ions in human cells.

1. **Nucleus**
   - Ctr
   - Regulators
     - MNK/WLN
     - SOD
     - Ceruloplasmin

2. **Golgi**
   - Amine Oxidase, Lysyl oxidase
   - CCS

3. **Mitochondria**
   - Cox17
   - Cox19
   - SCo1
   - SCo2
   - Cox23
   - SOD
   - CCS
   - Cox11
   - CCO
   - MT

No free copper ions in the cytoplasm.
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:

\[ K_D \]

- GSH $10^{-12}$
- Cox17 $10^{-14}$
- Hah1 $10^{-14}$
- CcS $10^{-15}$
- Sco1 $10^{-15}$
- MNK(1-6) $10^{-15}$
- CcO $10^{-16}$
- Sod1 $10^{-16}$

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.
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

past

Conventional vaccinology

Cultivate microorganism

5-15 years

Antigen selection and validation

vaccine

present

Reverse vaccinology

Genome-based approaches

Pan-genome approach

Determination of antigens structure

1-2 years

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

- 900 MHz Spectrometer (298K)

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

Residues predicted by immunological data and confirmed by NMR

NMR data suggested the involvement of other amino acids
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.
They are still solvent accessibles!

2. \textit{fHbpC} contains the major part of the epitope
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.

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*
CERM/CIRMMP - a core center of Instruct

11 NMR spectrometers + 1 Relaxometer,
The largest available magnetic field range (0.01 – 950 MHz)
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 !!