



Lecture 3

Applications of EPR spectroscopy to the study of protein dynamics

Dr. Marlène Martinho

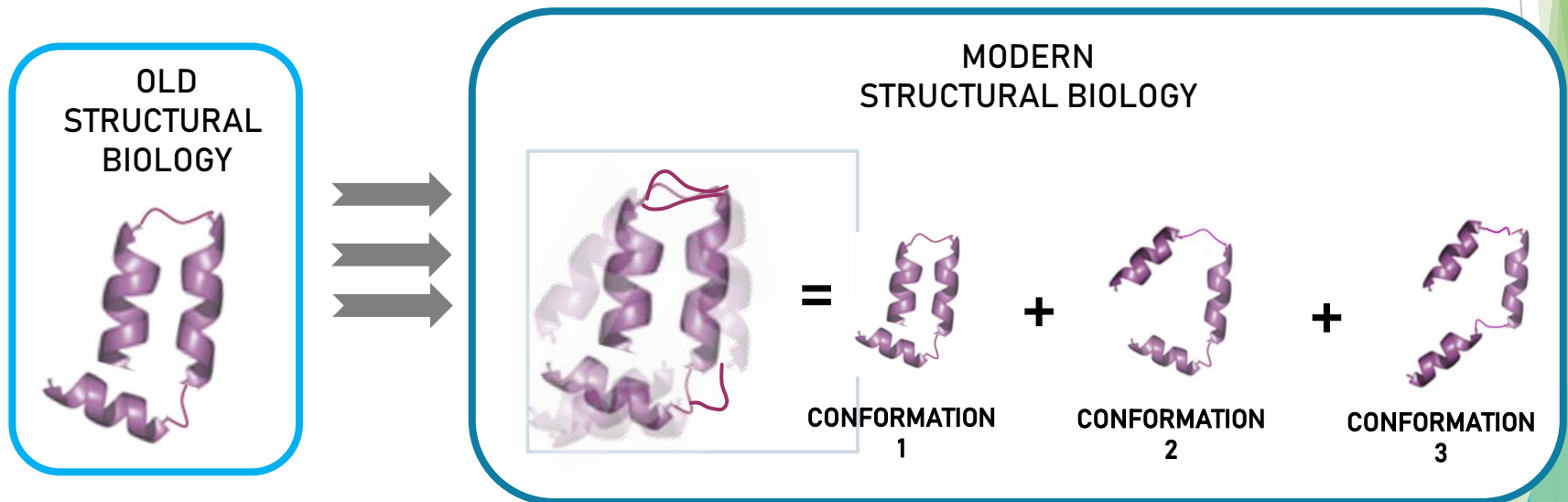
Laboratoire de Bioénergétique et Ingénierie des Protéines, BIP, Marseille, France

<https://bip.cnrs.fr/groups/bip07/>
mmartinho@imm.cnrs.fr

INTRODUCTION

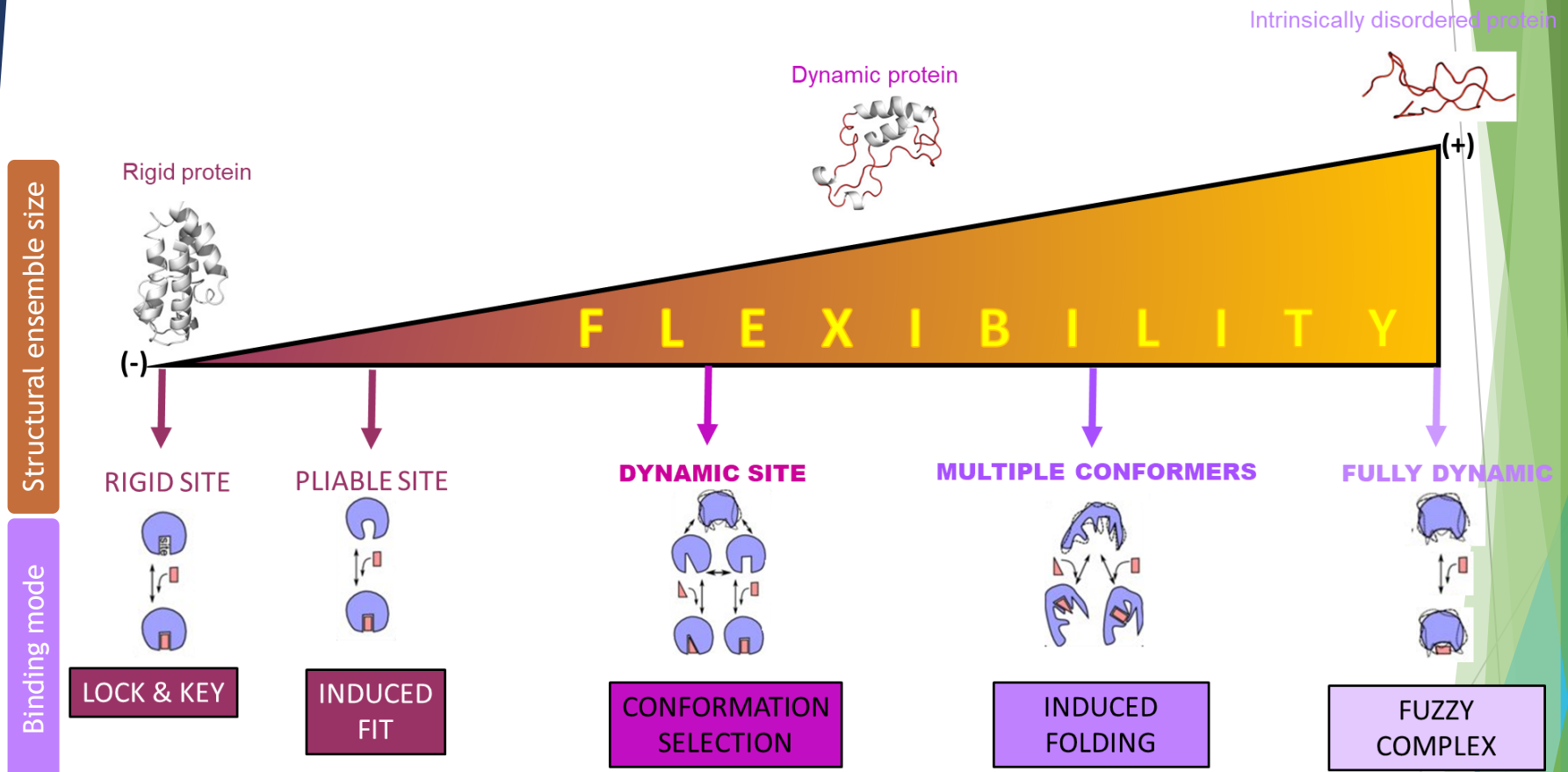
OLD : 1 SEQUENCE = 1 STRUCTURE = 1 CONFORMATION

MODERN : STRUCTURE = ENSAMBLE OF CONFORMATIONS



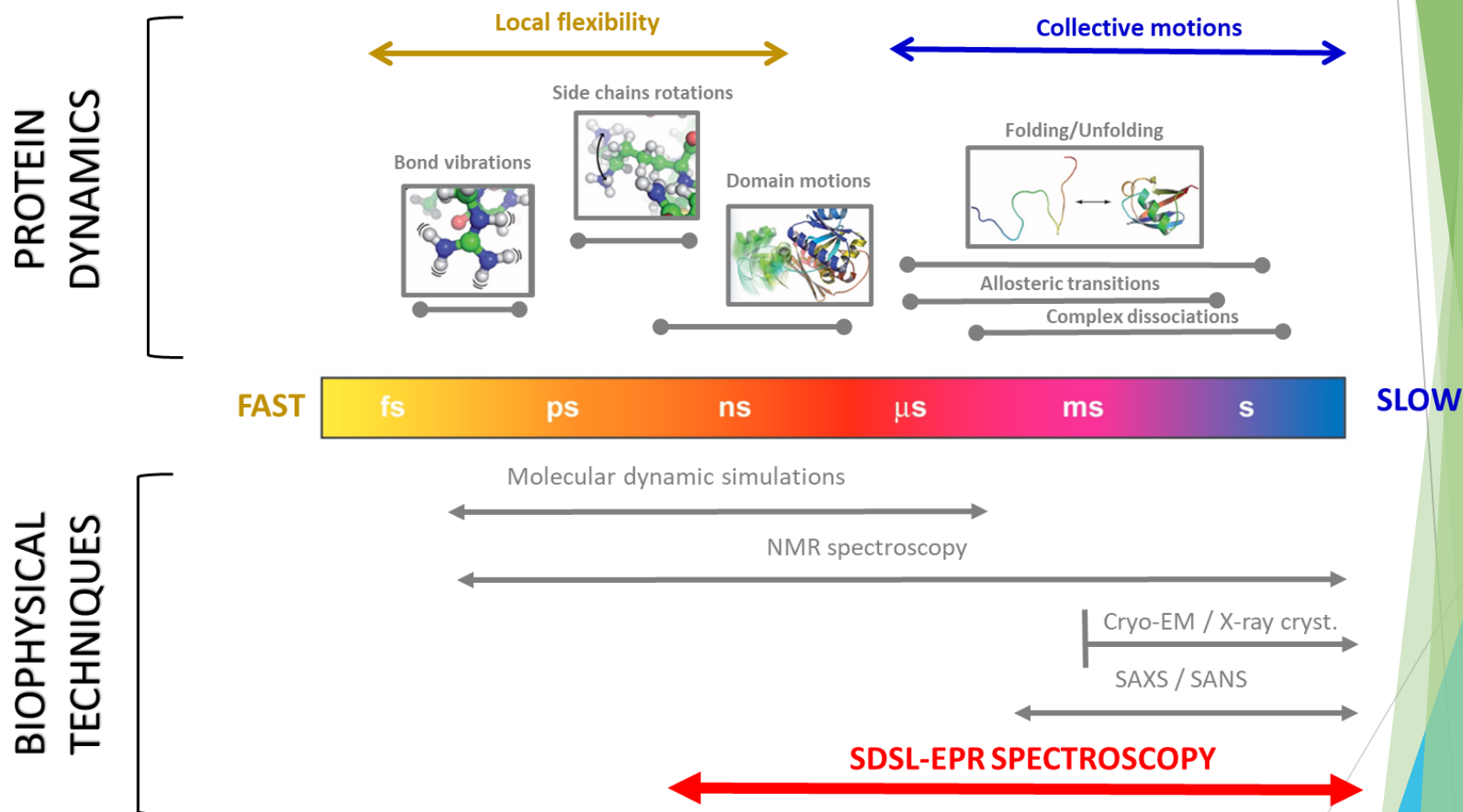
INTRODUCTION

INVESTIGATIONS ON PROTEIN DYNAMICS ARE FUNDAMENTAL TO DECIPHER THE FUNCTIONS OF A DYNAMIC PROTEINS



INTRODUCTION

Different approaches can be exploited to probe protein dynamics



Slide adapted from Alessio Bonucci

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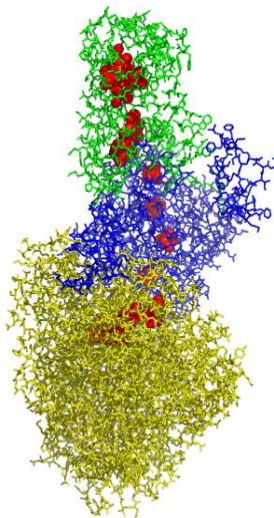
Paramagnetic species in biological systems

**EPR = Electron Paramagnetic Resonance
(or ESR = Electron Spin Resonance)**



**Paramagnetic
species**

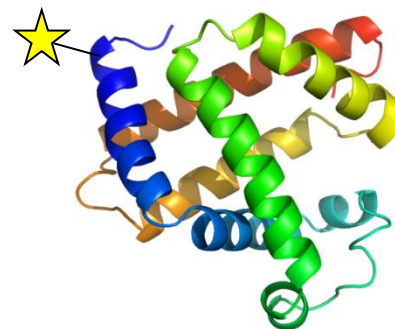
***Intrinsic paramagnetic
centers***



Metal centers or clusters: Fe, Cu, Ni, Mo ...
Radicals: Semiquinone, Tyr $^{\bullet}$, S $^{\bullet}$, ...

Catalytic mechanisms, ET mechanisms, ...

***Extrinsic paramagnetic
label***

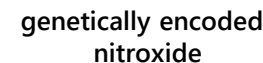
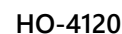
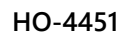
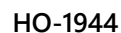
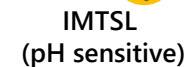



Site-Directed Spin Labeling (SDSL)

Structural transitions in proteins,
protein-protein interactions, ...

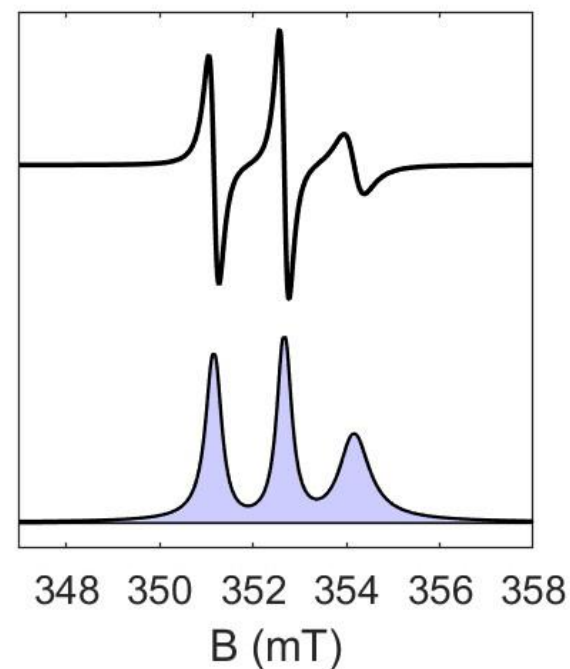
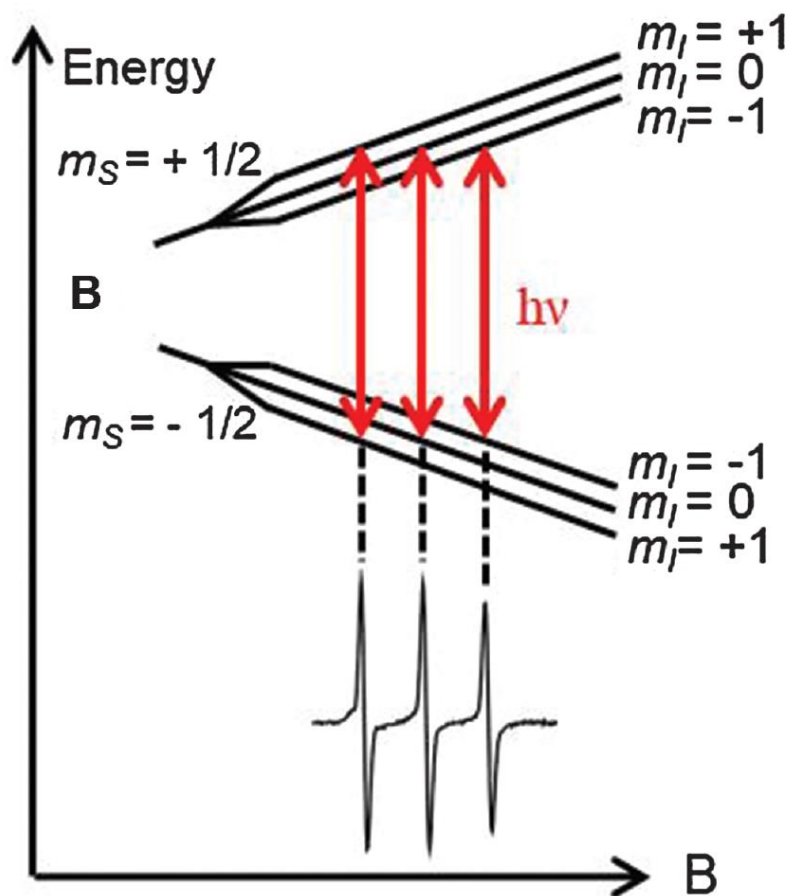
5

Spin labels for biomolecules



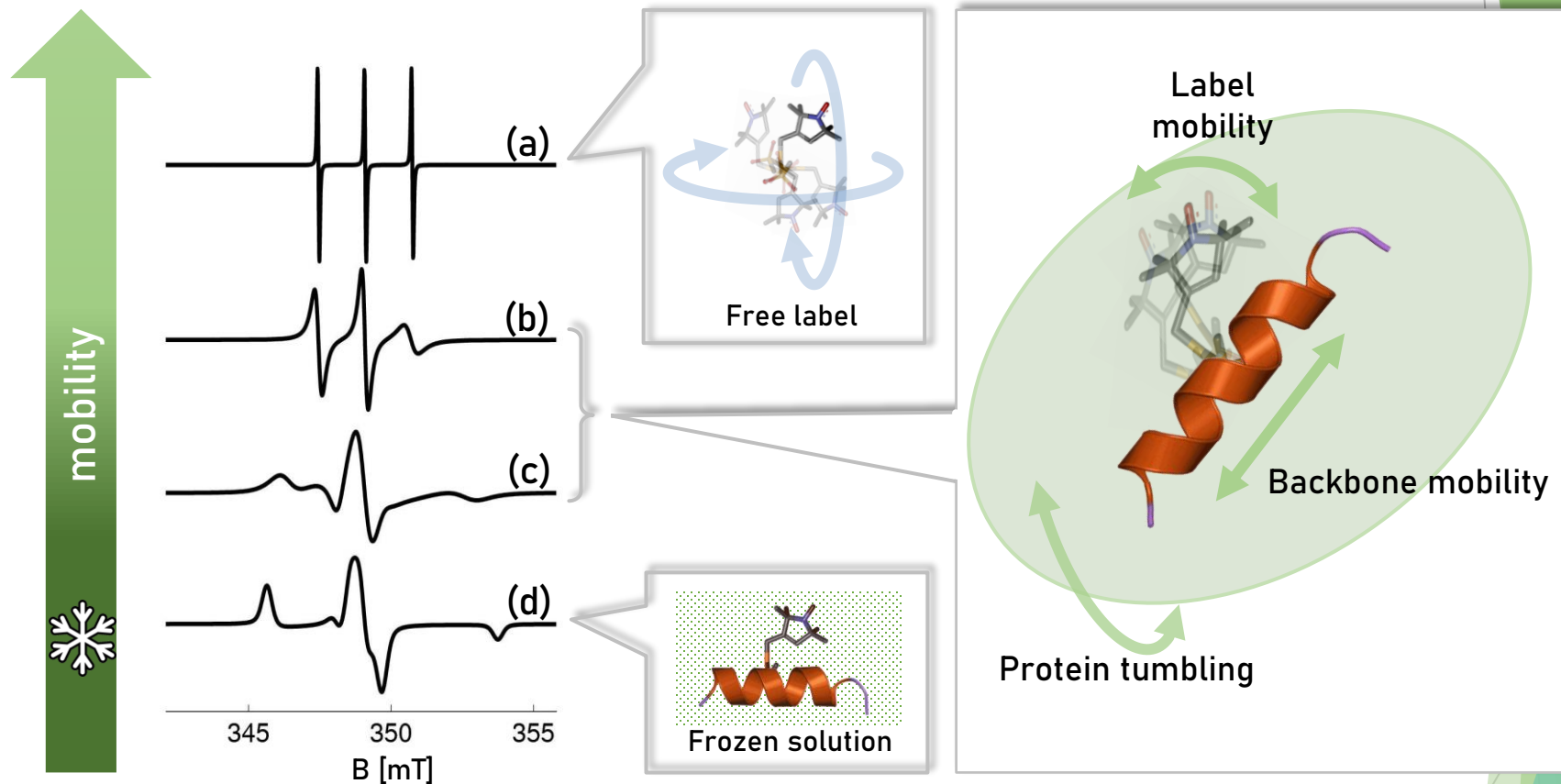
Le Breton, N. *et al. Front. Mol. Biosc.* **2015** ; M. Martinho *et al., Book of the RSC, Electron Paramagnetic Resonance* **2018** vol. 26 ; Lorenzi, M. *et al. Angew. Chem. Int. Ed.* **2011** ; Mileo, E. *et al. Bioconjugate Chem.* **2013** ; Fleissner, M.R. *et al. PNAS* **2009** ; Kalai, T. *et al. Tetrahedron Lett.* **2011** ; Kucher, S. *et al. JMR* **2017** ; Schmidt *et al. JACS* **2014** ; Paletta, *et al. Org. Lett.* **2012** ; Fleissner, M.R. *et al. PNAS* **2011** ; Smirnov, *et al. JACS* **2004** 

EPR spectroscopy of nitroxide spin labels



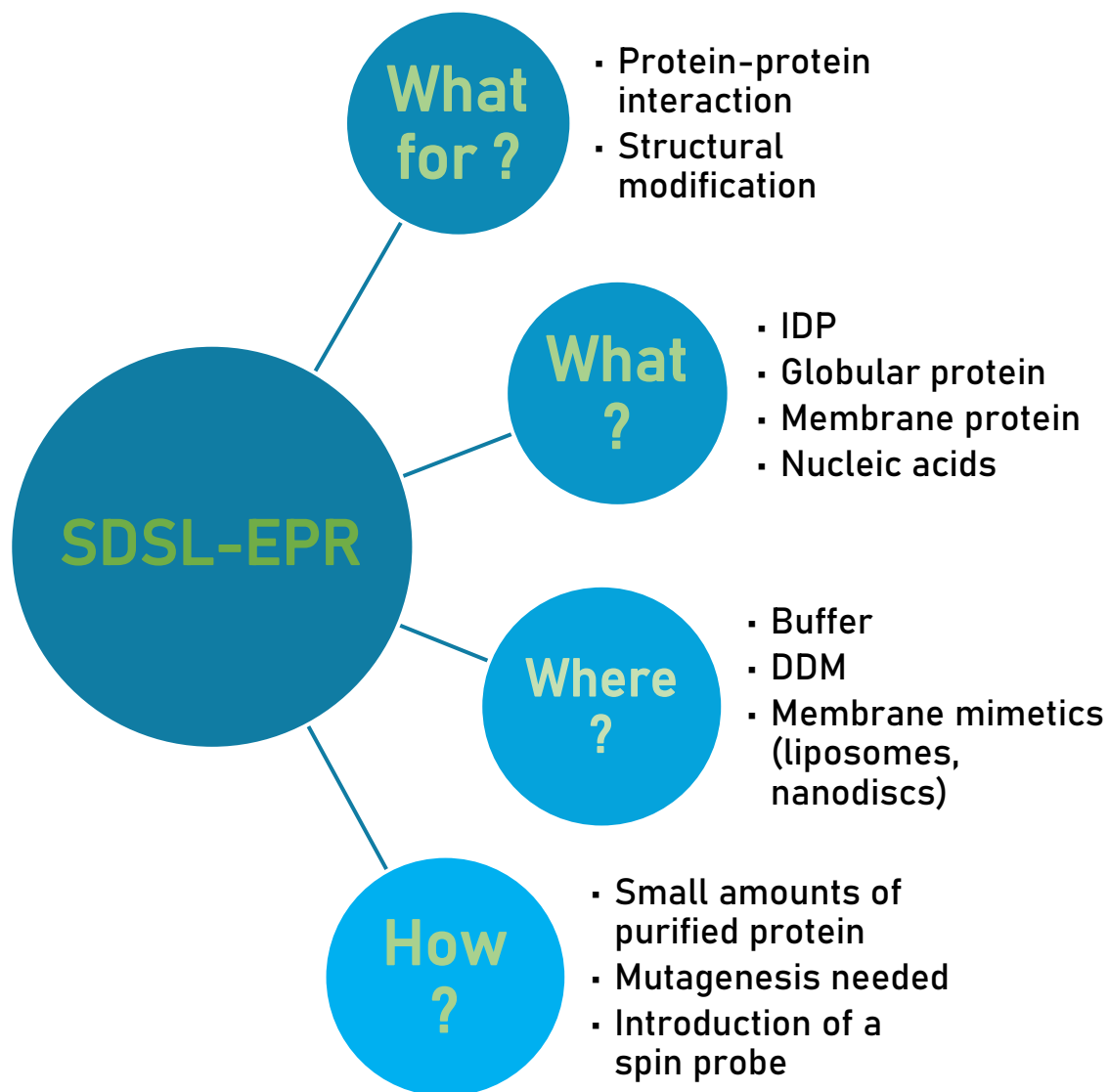
M. Martinho *et al.*, *Book of the RSC, Electron Paramagnetic Resonance vol. 26* 2018

EPR spectroscopy of nitroxide spin labels



Fournier, E, *et al. Actualité Chimique* 2019 443,13-16

SDSL-EPR : A powerful technique



The pioneer work

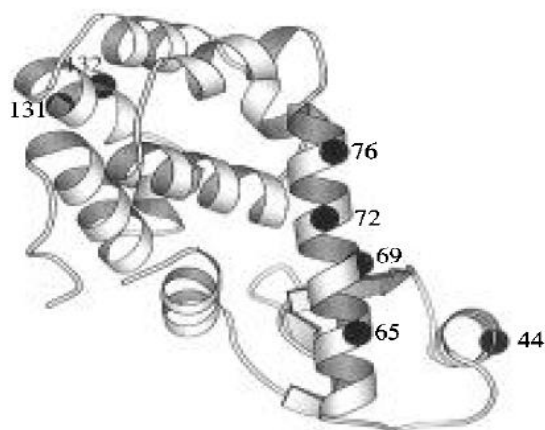
7692

Biochemistry **1996**, 35, 7692–7704

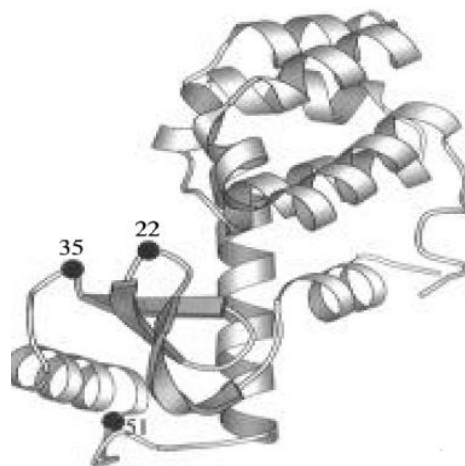
Motion of Spin-Labeled Side Chains in T4 Lysozyme. Correlation with Protein Structure and Dynamics[†]

Hassane S. Mchaourab,[‡] Michael A. Lietzow,[‡] Kalman Hideg,[§] and Wayne L. Hubbell^{*,‡}

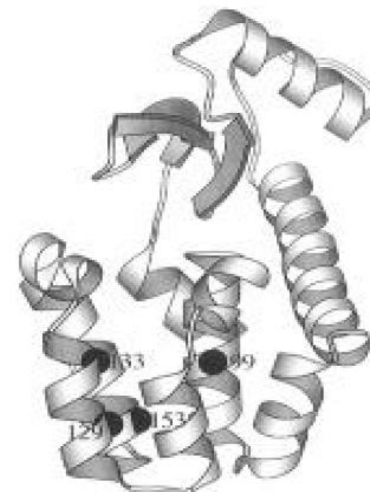
Jules Stein Eye Institute and Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095-7008, and Central Research Laboratory, Chemistry, University of Pecs, P.O. Box 99, H-7643 Pecs, Hungary



helices



loops



buried sites

Mobility



Structure

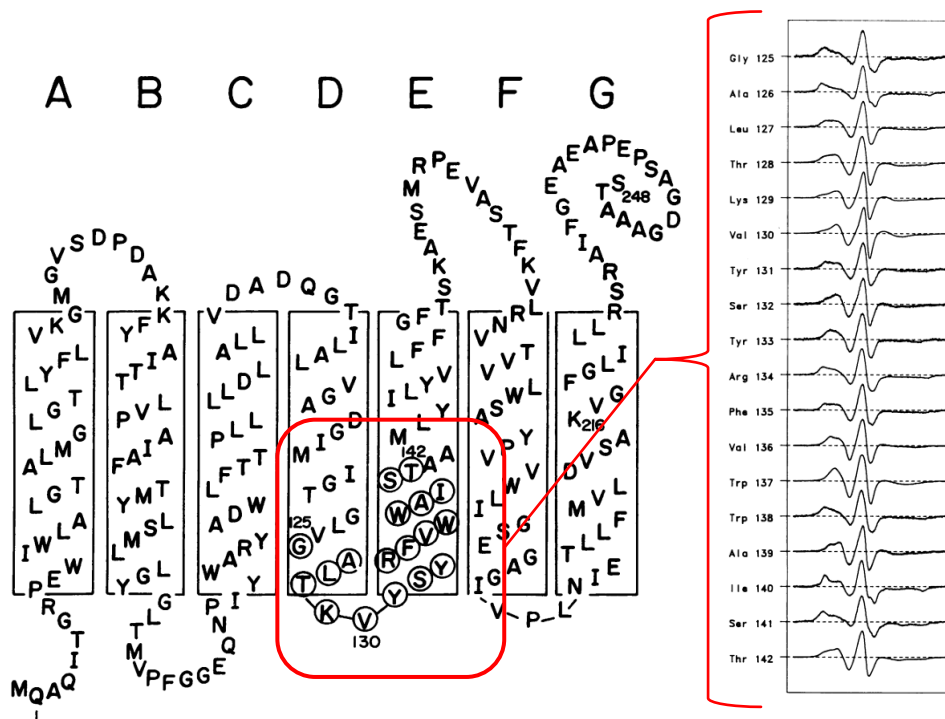
The pioneer work for membrane proteins

Structural Studies on Transmembrane Proteins. 2. Spin Labeling of Bacteriorhodopsin Mutants at Unique Cysteines[†]

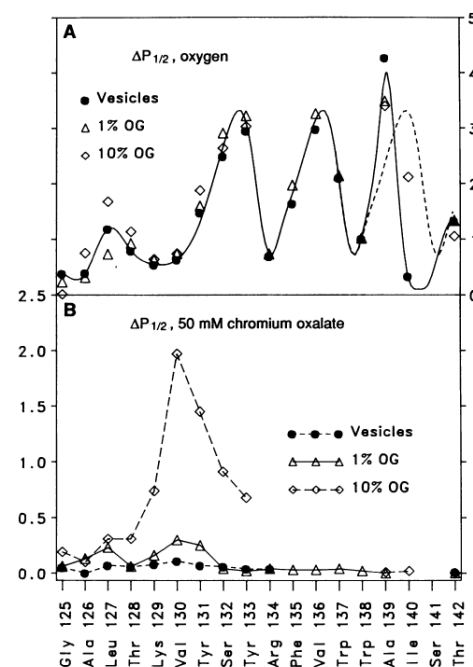
Christian Altenbach,[†] Sabine L. Flitsch,^{§,||} H. Gobind Khorana,[§] and Wayne L. Hubbell^{*,‡}

Jules Stein Eye Institute and Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, California 90024-1771, and Departments of Biology and Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139

Received February 9, 1989; Revised Manuscript Received June 1, 1989



EPR spectra of spin-labeled bacteriorhodopsin mutants reconstituted in soybean lipid vesicles



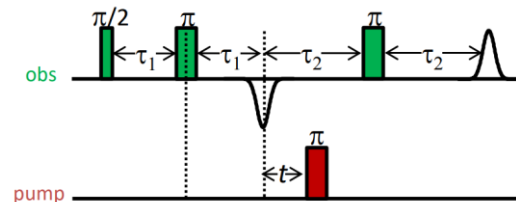
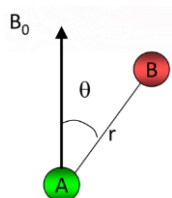
The accessibility parameter $\Delta P_{1/2}$ for oxygen (A) or chromium oxalate (B) versus position of the spin label in the bacteriorhodopsin sequence.

Altenbach, C. *et al. Biochem.* **1989** *28*, 7806-7812 ; Altenbach, C. *et al. Science* **1990** *248*, 1088-1092

DEER : distance measurements

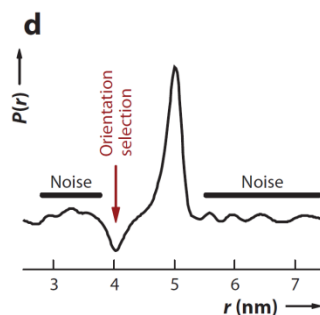
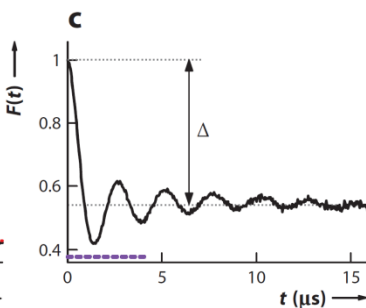
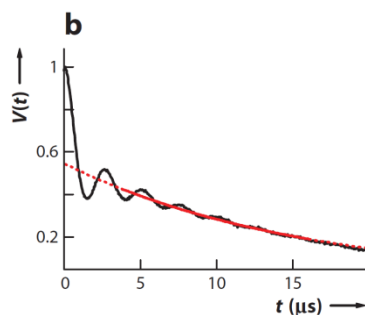
Spin A = observed spin

Spin B = flipped by the « pump » pulse



The flip of the spin B alters the local magnetic field of the population of spin A

Causes a modulation in the echo amplitude as the pump pulse is incremented (ω_{dd})



$$\omega_{dd} = \frac{1}{r^3} \beta^2 g_A g_B \frac{\mu_0}{4\pi \hbar (3\cos^2\theta - 1)}$$

nitroxide

Metal centers in proteins (Fe, Cu, Mg...)

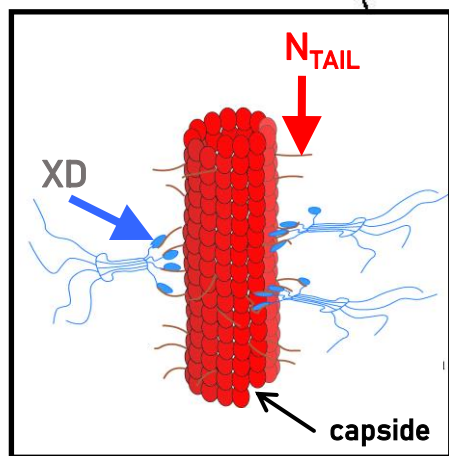
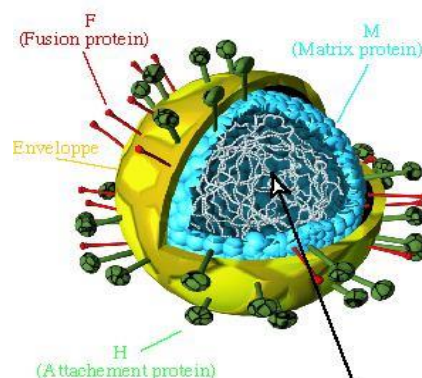
Exogenous spin labels (Gd³⁺, Cu²⁺, trityl ...)

M. Pannier *et al.*, *J. Magn. Reson.* **2000** ; M. Martinho *et al.*, *Book of the RSC, Electron Paramagnetic Resonance vol. 26* **2018** ; Jeschke. G. *et al.*, *Ann. Rev. Phys. Chem.* **20012** ; Fournier, E. *et al.* *Chemistry : A European J.* **2019**

Application 1 : Induced folding in nucleoprotein virus

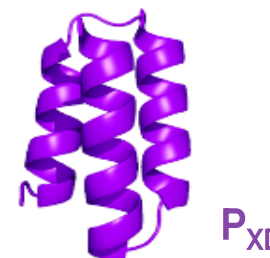
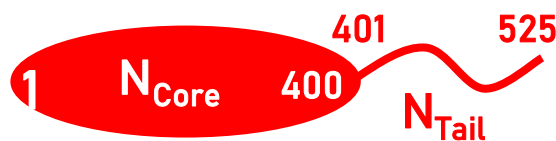
Main cause of childhood mortality in developing countries (164 000 deaths, OMS 2008).
To date, no antiviral treatment exists.

→ Understanding the mechanism of action of proteins involved in the replicative complex



★ Viral genome encapsulated by the nucleoprotein (N)

N : two regions



N_{CORE}

Structured
region

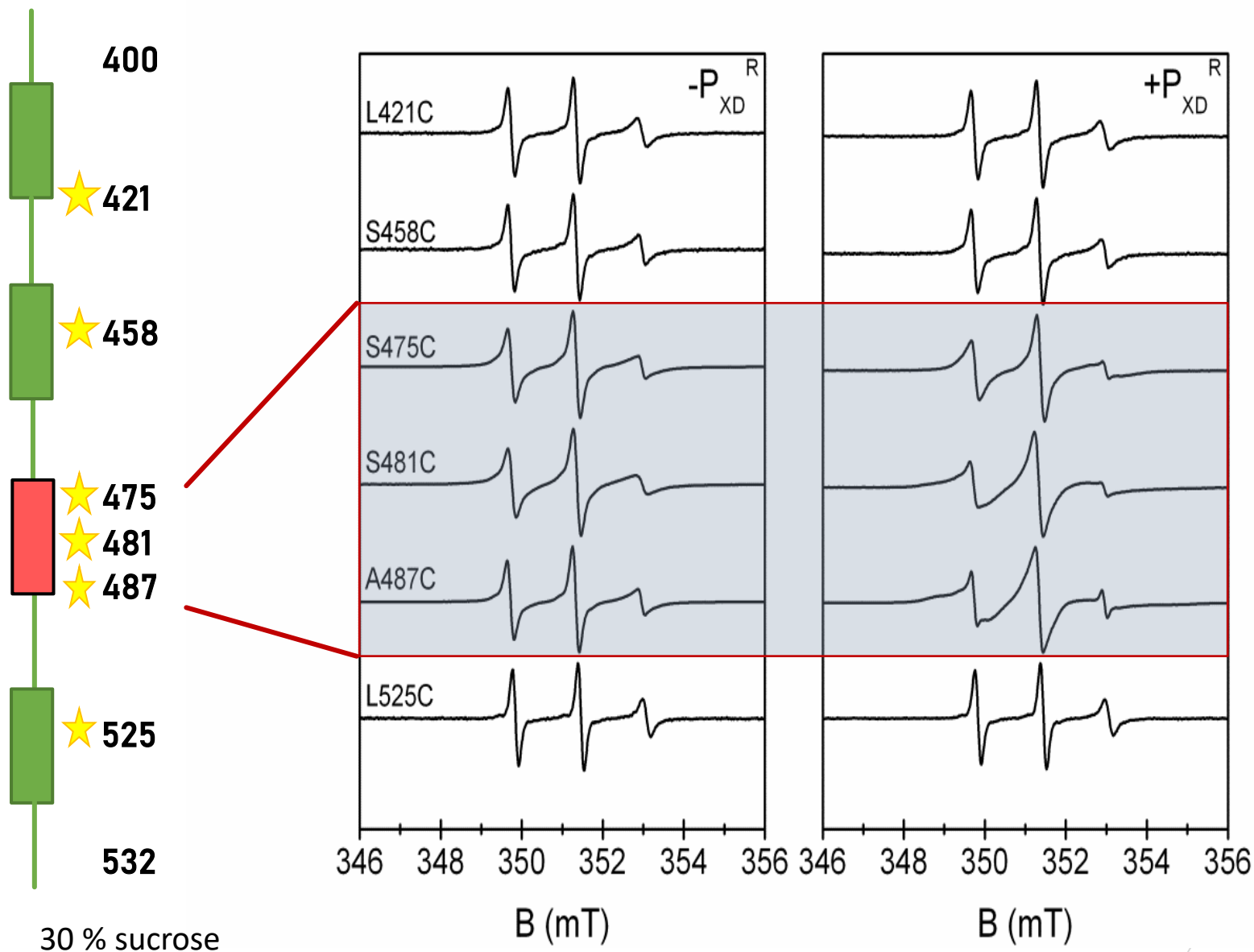
N_{TAIL}

Intrinsically disordered

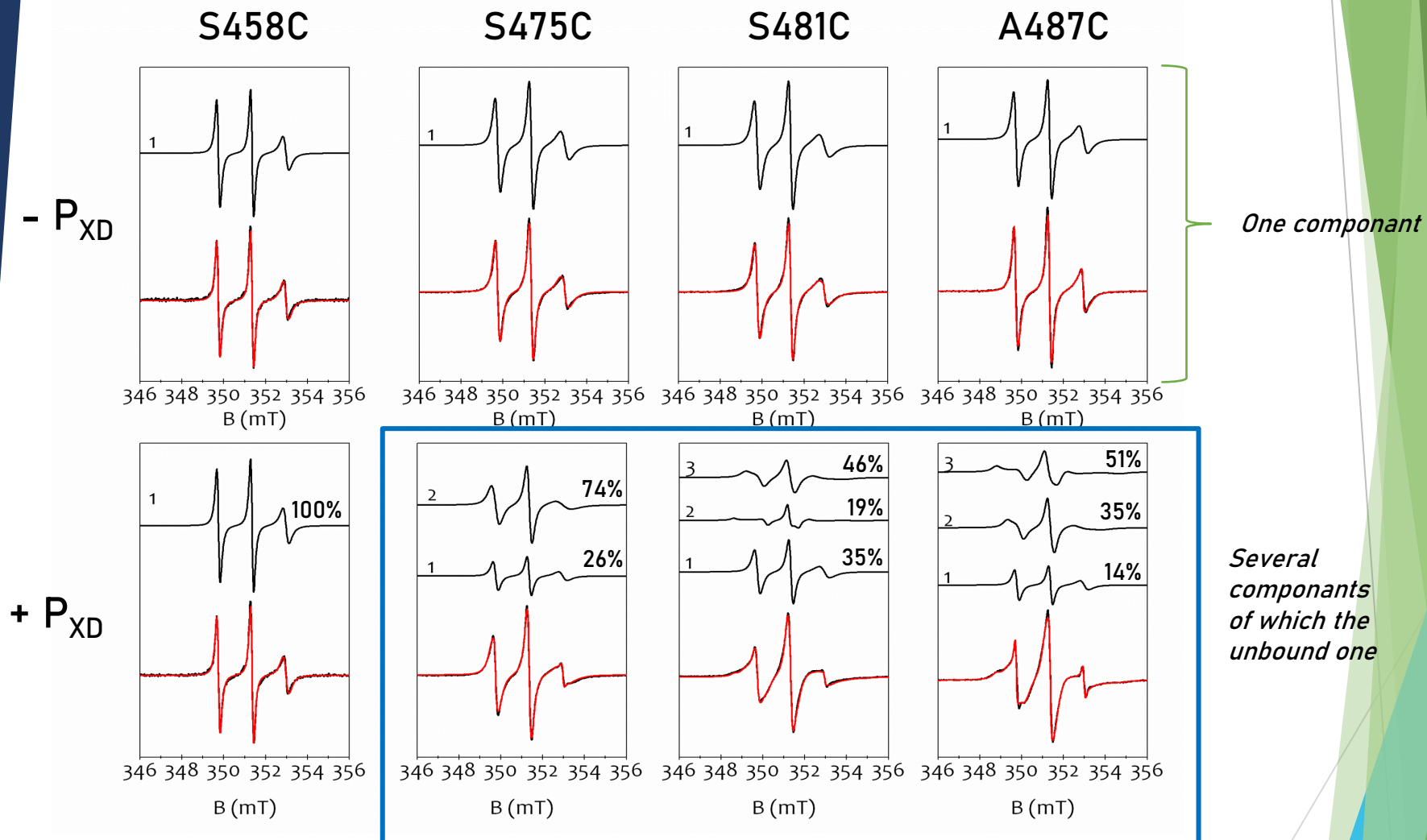
Induced folding when
interacting with P (P_{XD})

Bourhis *et al. Virology* 2006

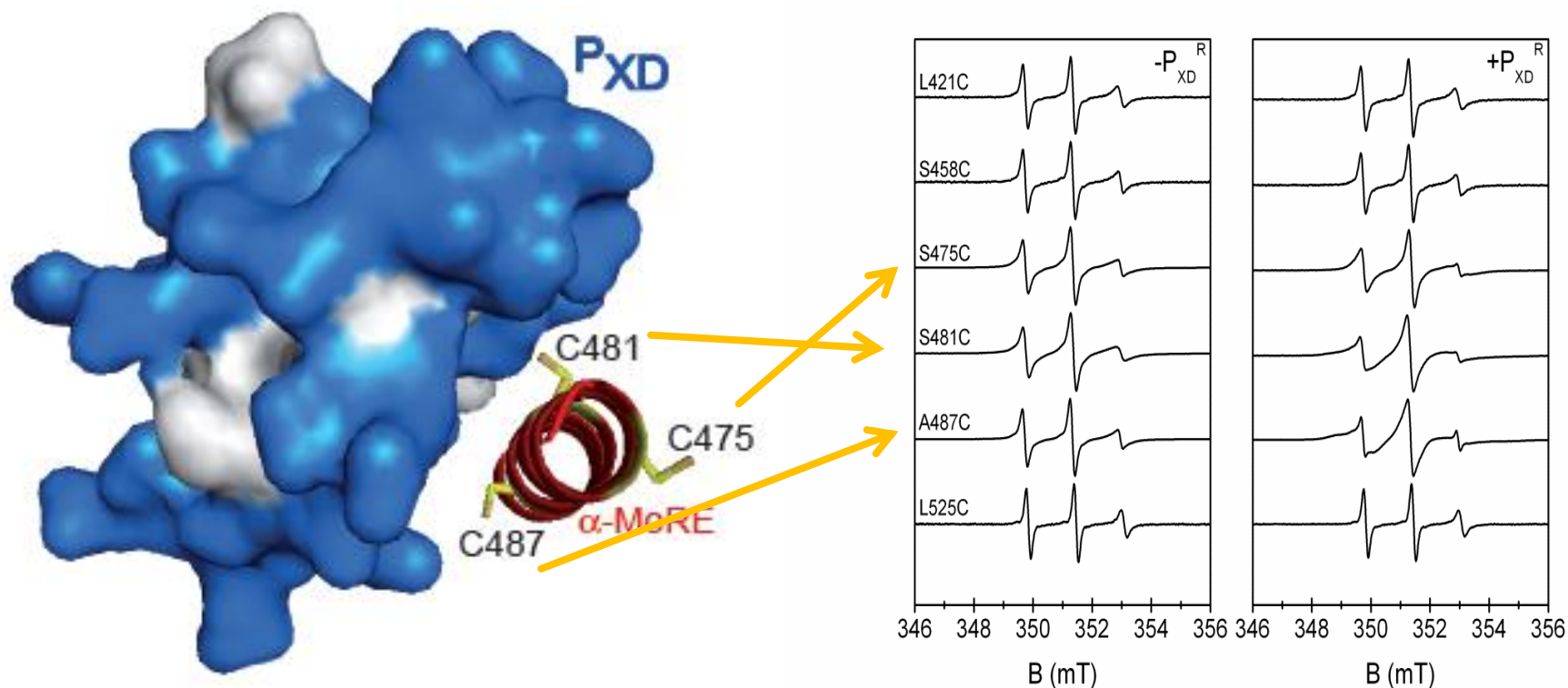
Application 1 : Induced folding in nucleoprotein virus



Application 1 : Induced folding in nucleoprotein virus



Application 1 : Induced folding in nucleoprotein virus



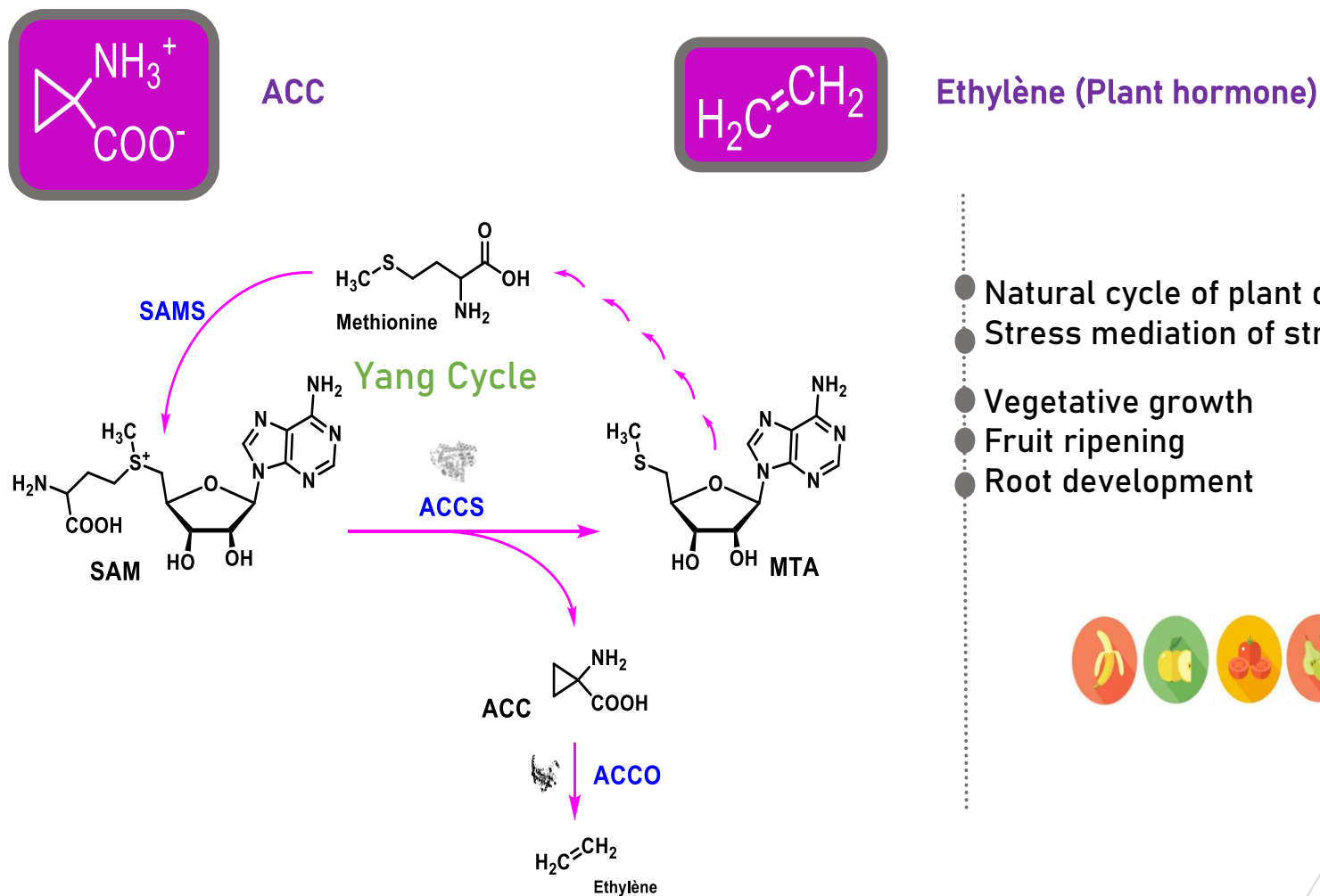
Validation of structural model of Hev N_{TAIL} - P_{XD} complex

Habchi *et al*, JBC 2011

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Application 2 : dynamics of ACCO

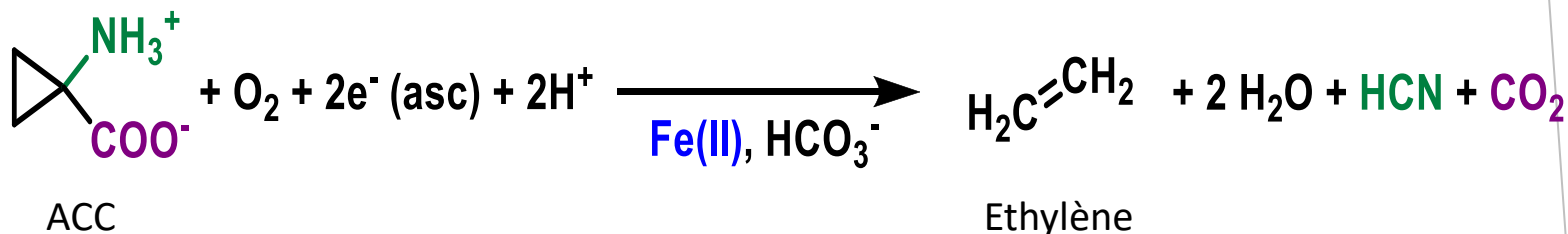
Acide 1-AminoCyclopropane 1-Carboxylique Oxydase (ACCO)



D.P. Murr *et al.*, *Plant Physiology*. **1975** ; A. Bleecker *et al.*, *Annu. Rev. Cell. Dev. Biol.* **2000**

Application 2 : dynamics of ACCO

Acide 1-AminoCyclopropane 1-Carboxylique Oxydase (ACCO)



Substrate
Electron donors

Not understood role BUT
essential for activity
 $\text{CO}_2 / \text{HCO}_3^- / \text{CO}_3^{2-}$

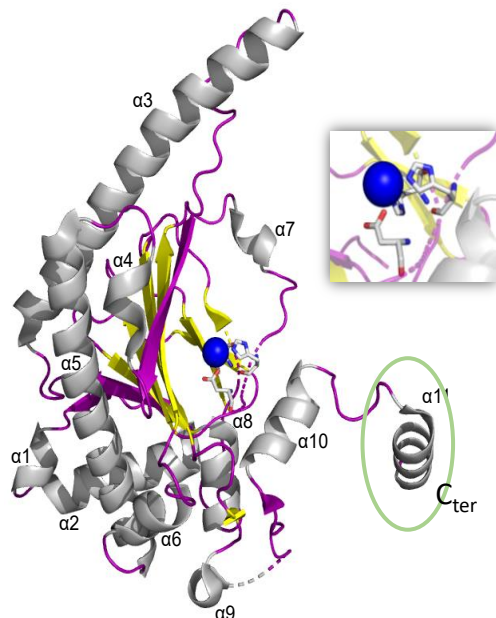
Metabolized in
plants

Application 2 : dynamics of ACCO

1

Crystallographic Structure from *Petunia hybrida*

Z. Zhang *et al.*, *Chem. Biol.* 2004



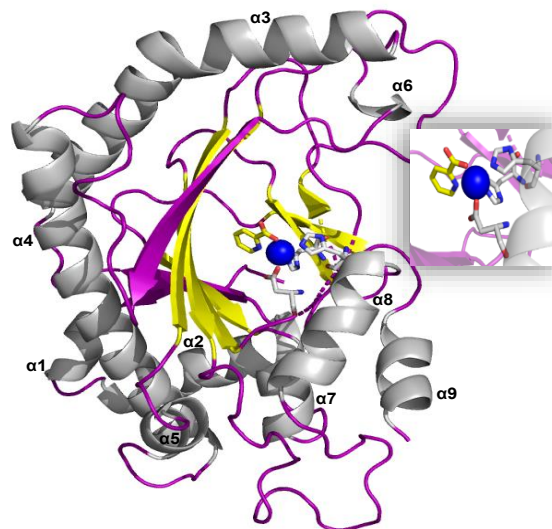
- 2 formes : apo and holo : ACCO-Fe(II)
- Tetramere : C_{term} extremity from each monomere interacts with C_{term} extremity from 2nd monomere C_{term}

Not active conformation

2

Crystallographic Structure from *Arabidopsis thaliana*

X. Sun *et al.*, *Nat. Commun.* 2017



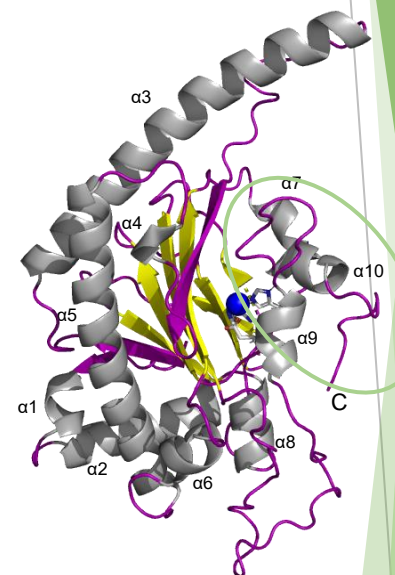
- 2 forms : ACCO-Zn(II) with inhibitors
- 17 truncated residues in C_{term} part

Inhibited form

3

Structural models from *Malus domestica* and *Lycopersicum esculentum*

Z. Zhang *et al.*, *Chem. Biol.* 2004 ; L. Brisson *et al.*, *JBIC.* 2012



- Other conformation of C_{term} part

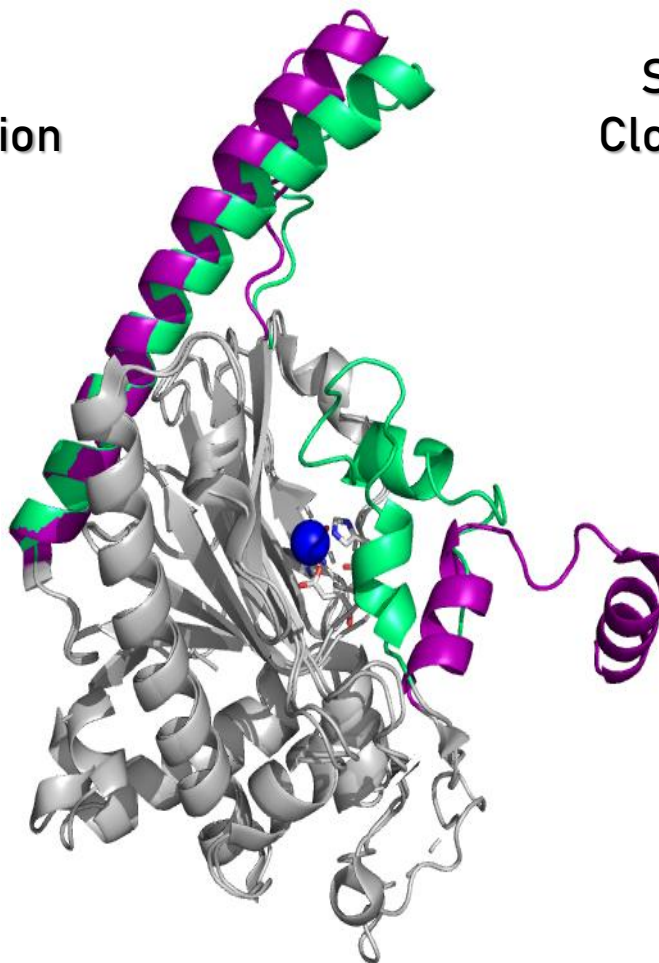
Proposed active conformation

Application 2 : dynamics of ACCO

1 RX Structure
Open conformation

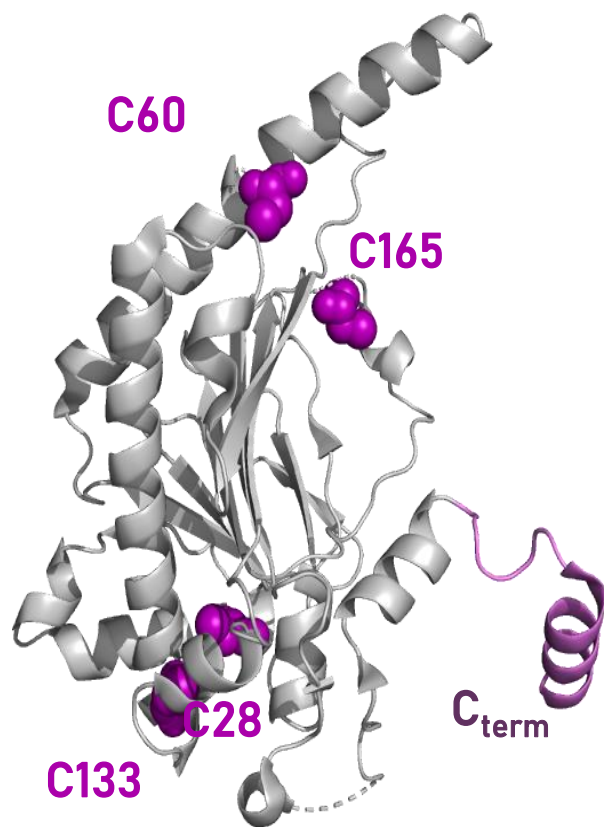
Structural Model
Closed conformation

3



Flexibility of the C_{term} region of *tomato* ACCO ??

Application 2 : dynamics of ACCO



Tomato ACCO



LOCAL ENVIRONNEMENT

1 cysteine on the body OR 1 cysteine on the C_{term} part

NITROXYDE-NITROXYDE DISTANCE

1 cysteine on the body AND 1 cysteine on the C_{term} part

METAL-NITROXYDE DISTANCE

1 cysteine on the C_{term} part AND 1 metal center

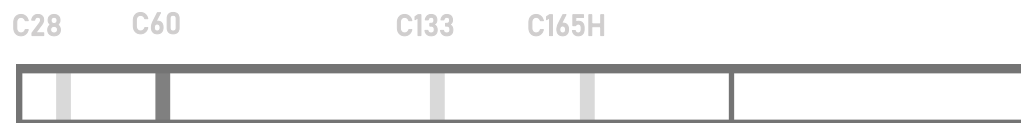
Application 2 : dynamics of ACCO

Grey cysteines : non accessible to the label

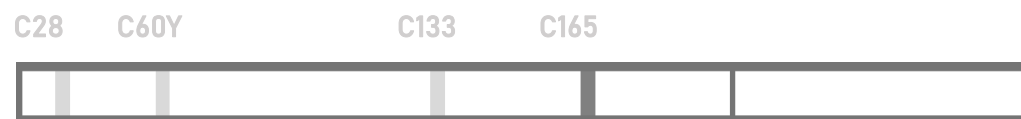
Active ?
Before / After labeling



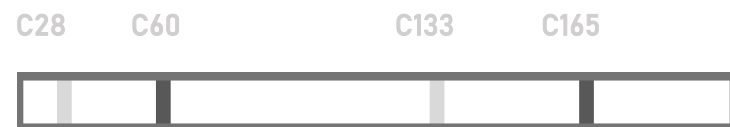
✓ X



✓ X



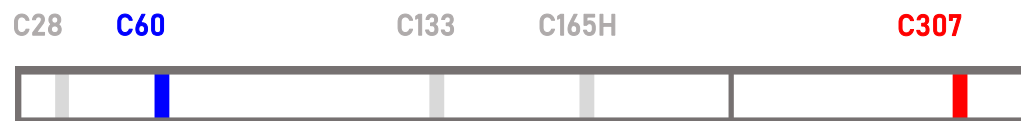
✓ ✓



X X



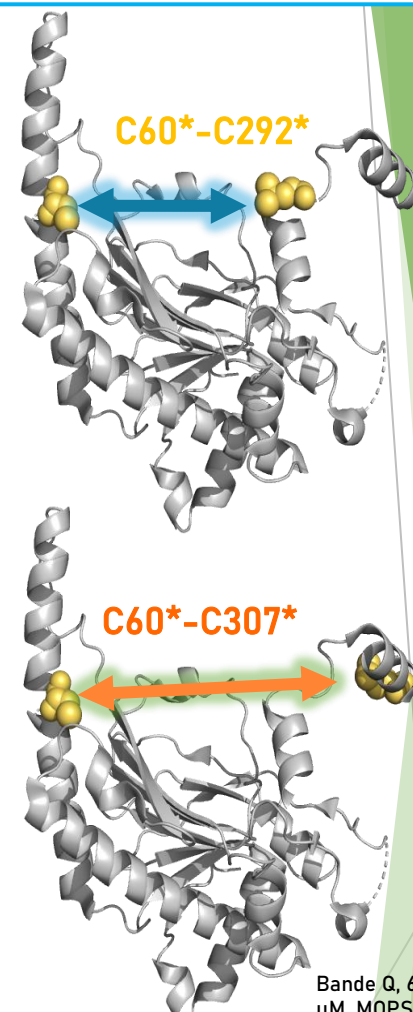
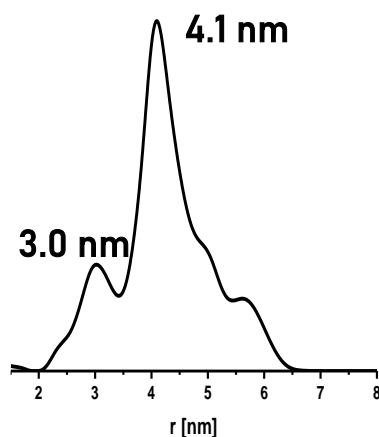
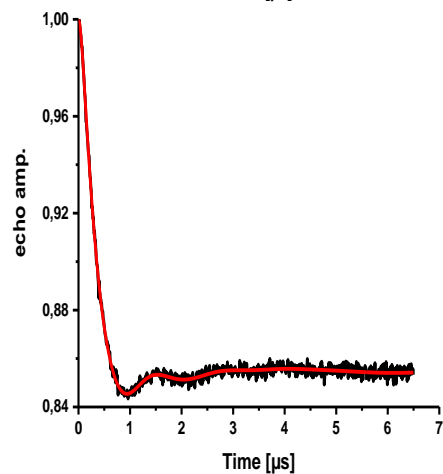
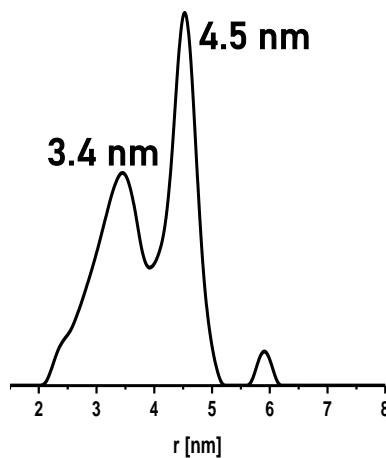
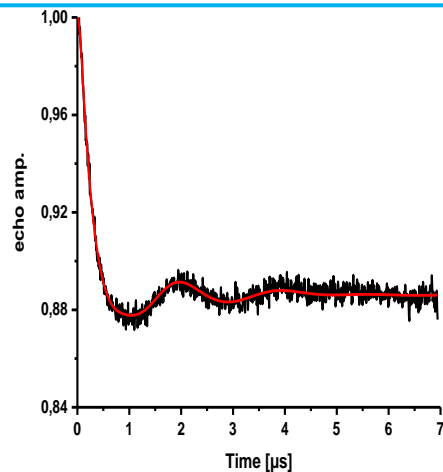
✓ ✓



✓ ✓



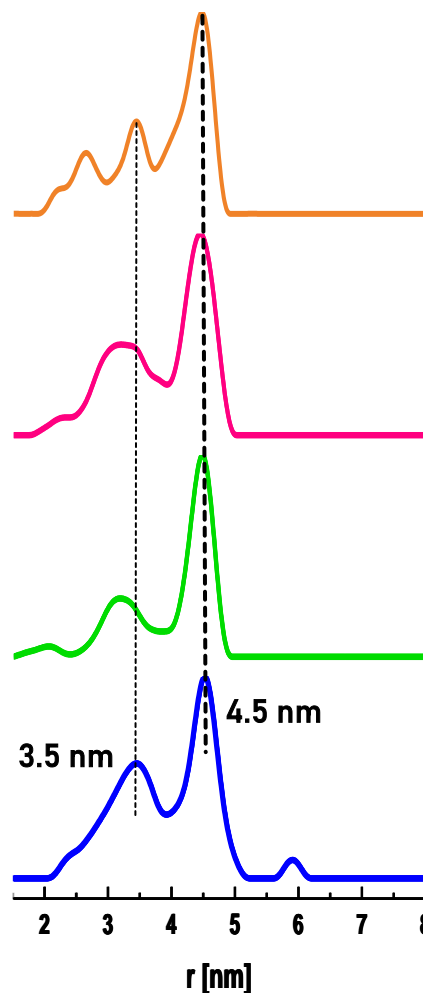
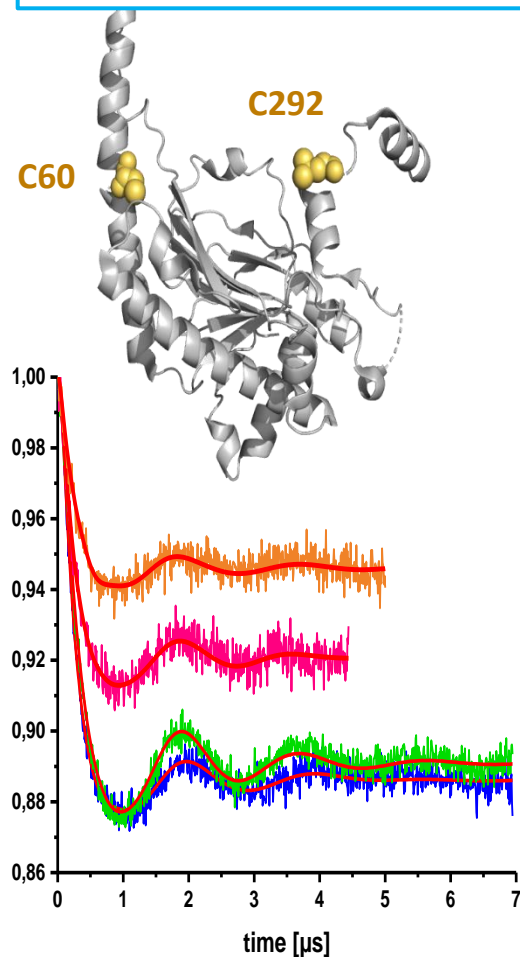
Application 2 : dynamics of ACCO



Bande Q, 60 K, [ACCO]=100 μ M, MOPS 50 mM pH7.5
 D_2O , 30 % glycéról d-8

Large distance distributions \rightarrow flexible region

Application 2 : dynamics of ACCO



- **Fe(II)-ACCO*+ ACC + HCO₃⁻ + Asc + O₂**
+ 2 min O₂
- **Fe(II)-ACCO*+ ACC + HCO₃⁻ + Asc**
+ 100 μM Asc
- **Fe(II)-ACCO*+ ACC + HCO₃⁻**
+ 5 mM ACC
25 mM Bic
- **Fe(II)-ACCO***
90 μM Fe(II)

Large distance distributions : Flexible C_{term}
Same results for position C307

Fournier, E. *et al. Chemistry : A European J.* 2019

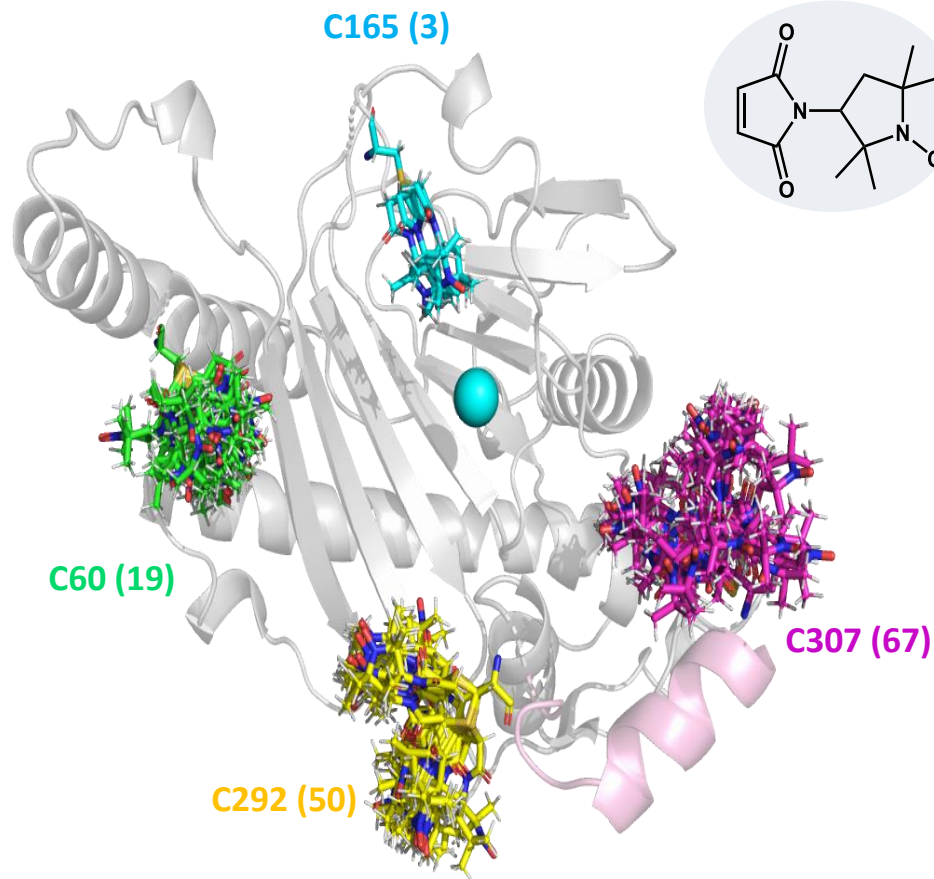
Bande Q, 60 K, [ACCO]=100 μM, MOPS 50 mM pH7.5
D₂O, 30 % glycérol d-8

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Application 2 : dynamics of ACCO

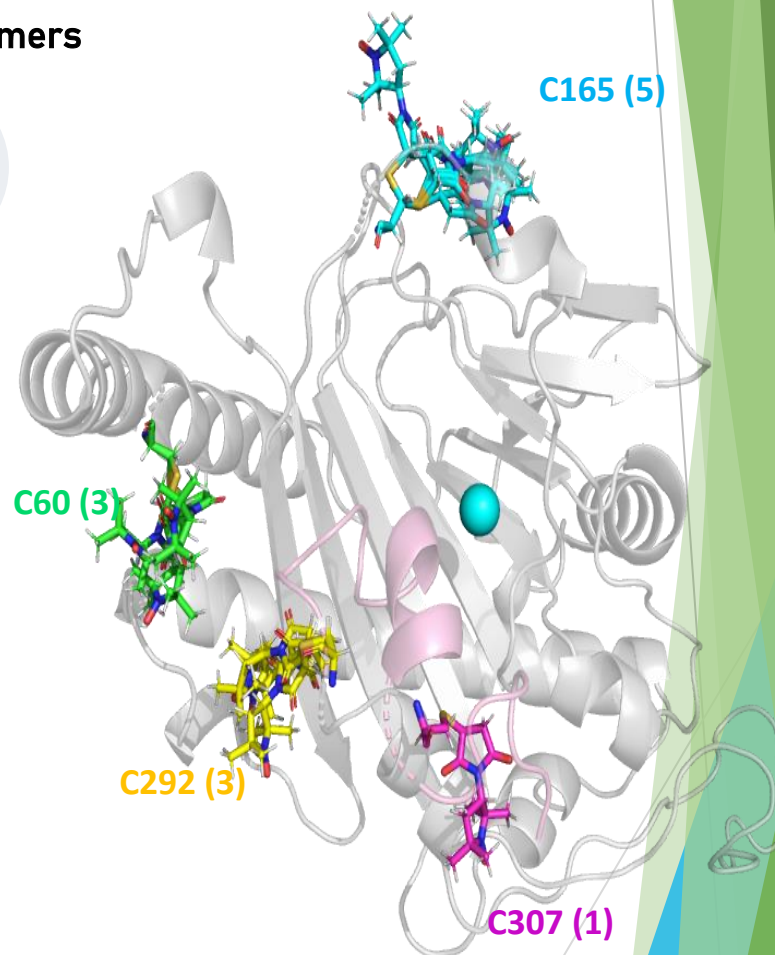
Open conformation

Crystallographic
structure



Closed conformation

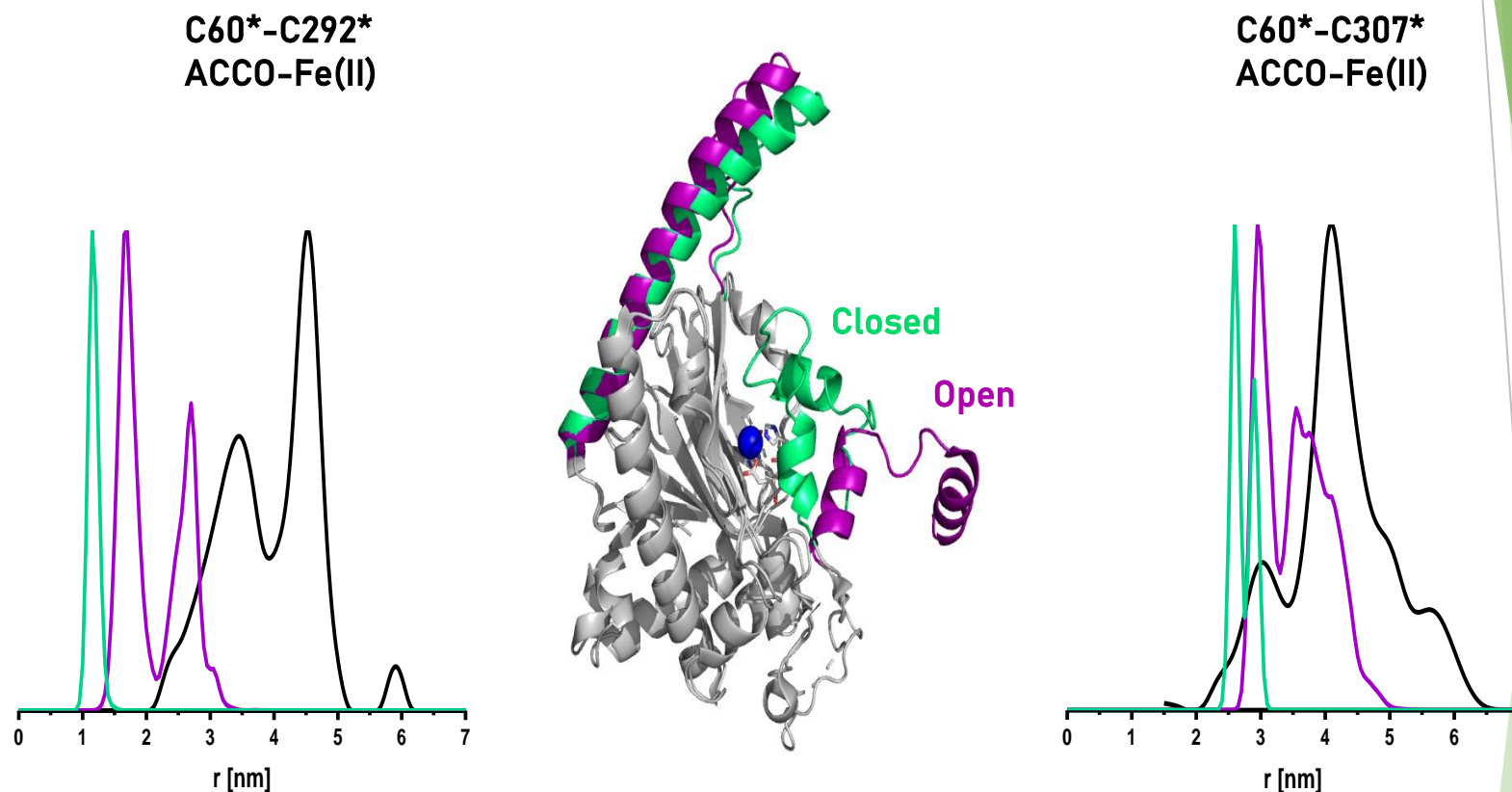
Structural model



Fournier, E. *et al. Chemistry : A European J.* 2019

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Application 2 : dynamics of ACCO



Crystallographic/model ACCO structures
do not correspond to ACCO populations in solution

Fournier, E. *et al. Chemistry : A European J.* 2019

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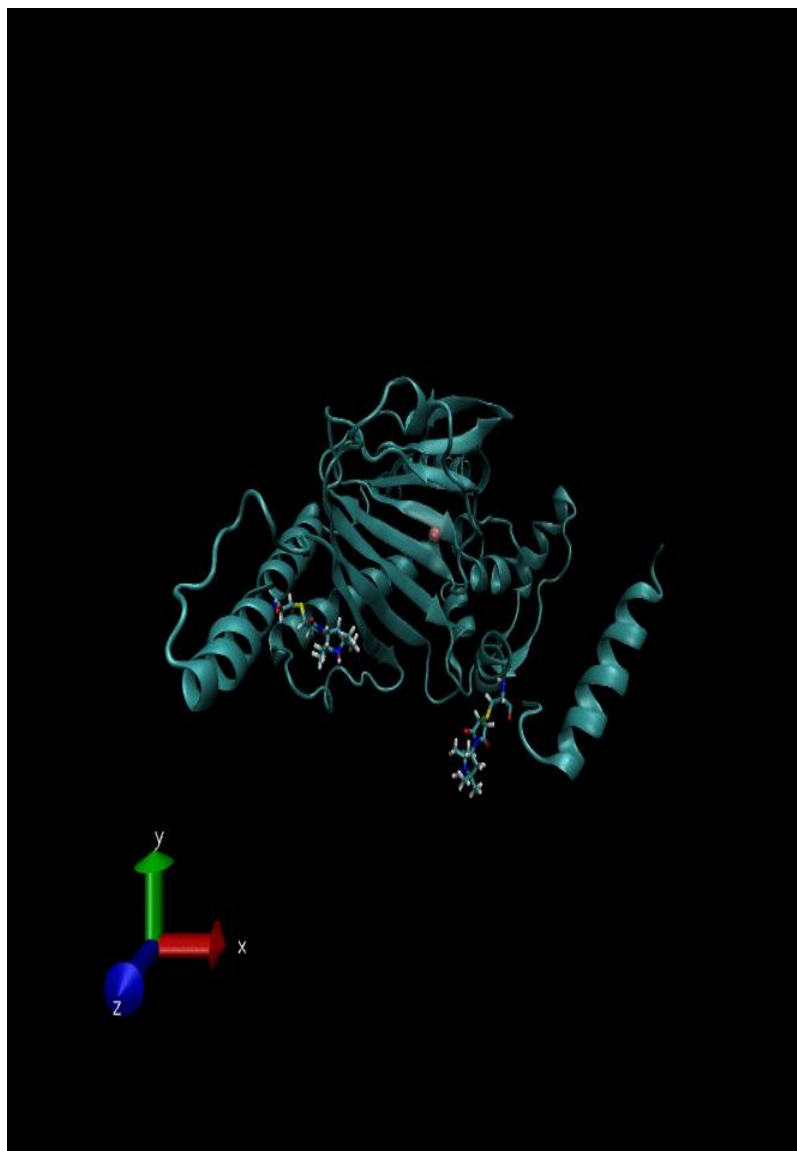
Application 2 : dynamics of ACCO



The University of Manchester

Sam P. de Visser
Nick Fowler

C60*-C292*
ACCO-Fe(II)



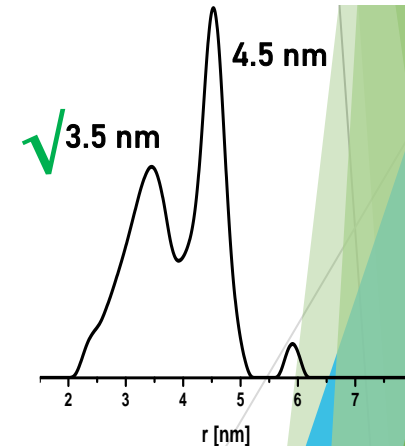
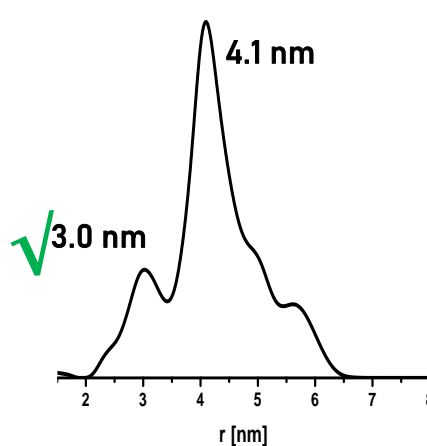
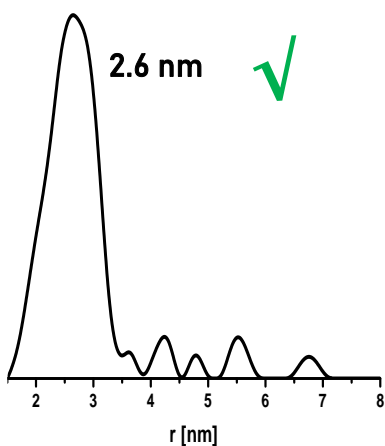
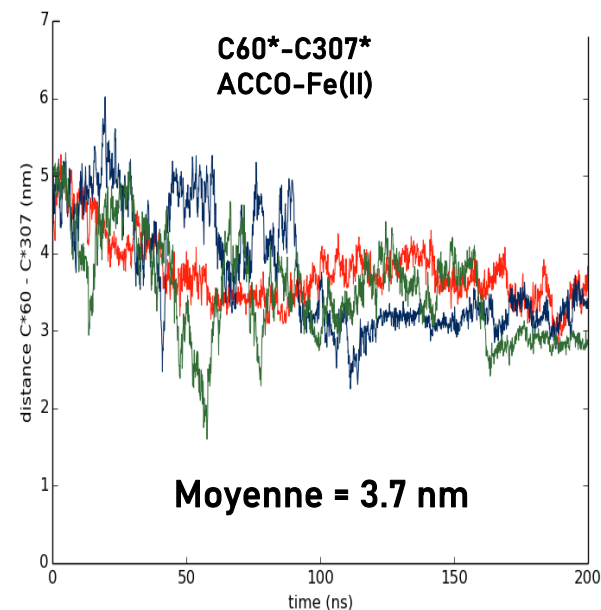
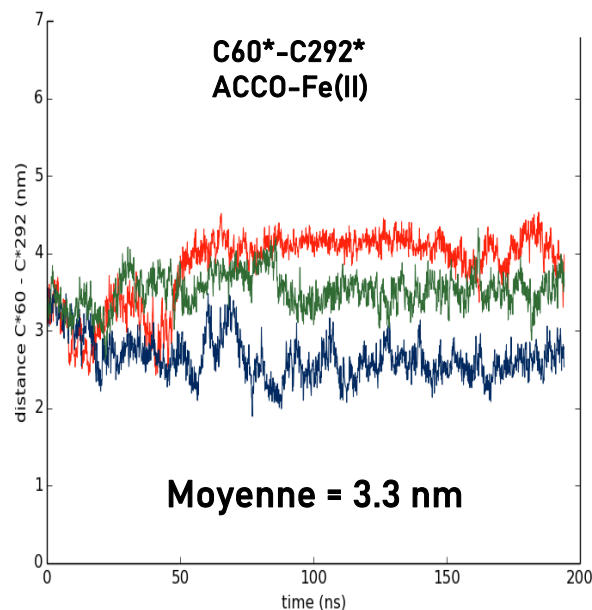
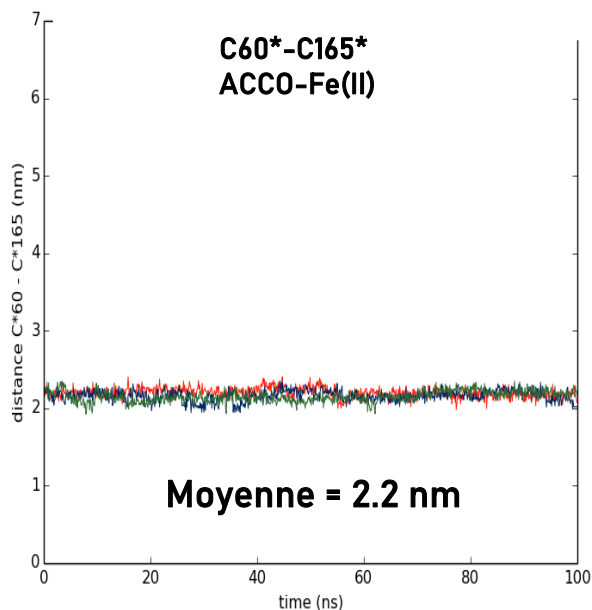
Fournier, E. *et al. Chemistry : A European J.* 2019

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

Application 2 : dynamics of ACCO



Fournier, E. *et al. Chemistry : A European J.* 2019

Flexible C_{term} region consistent with experimental results

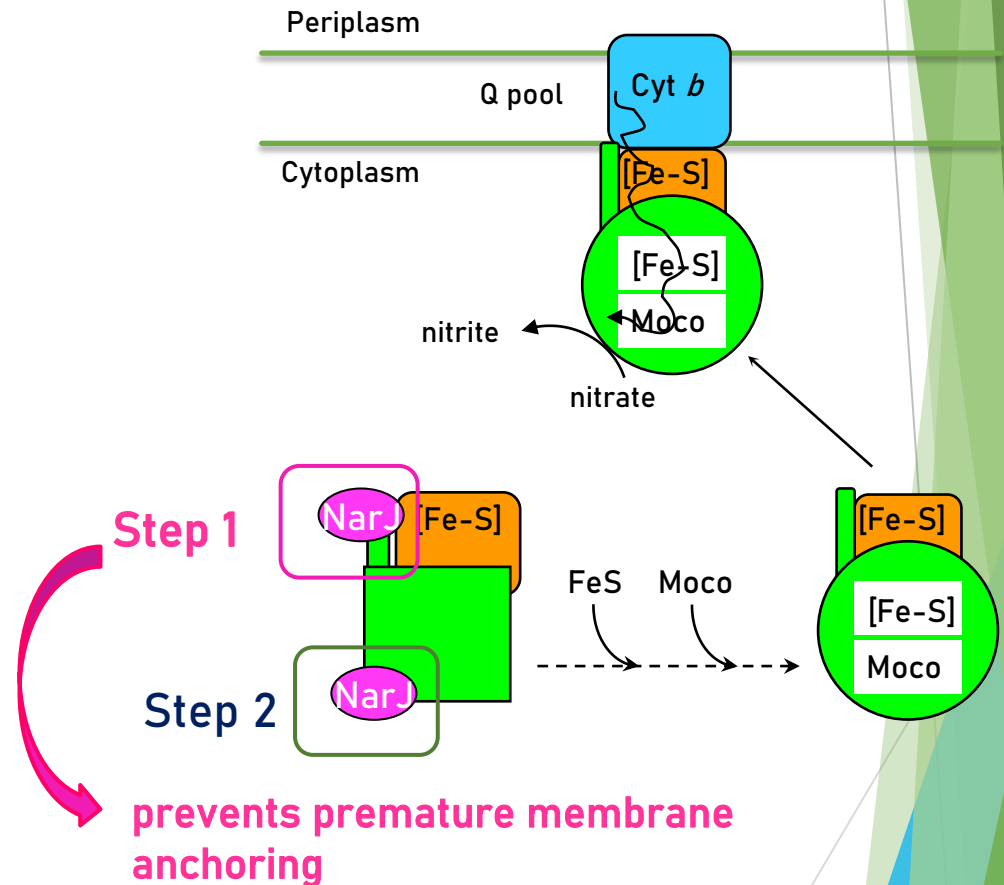
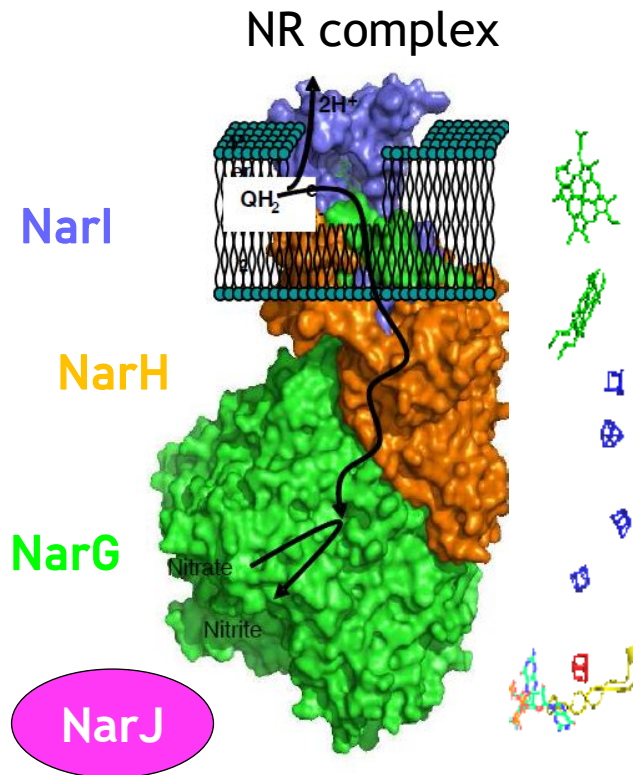


This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

Molecular-Scale Biophysics
Research Infrastructure

Application 3 : chaperone protein NarJ : looking for an interaction site

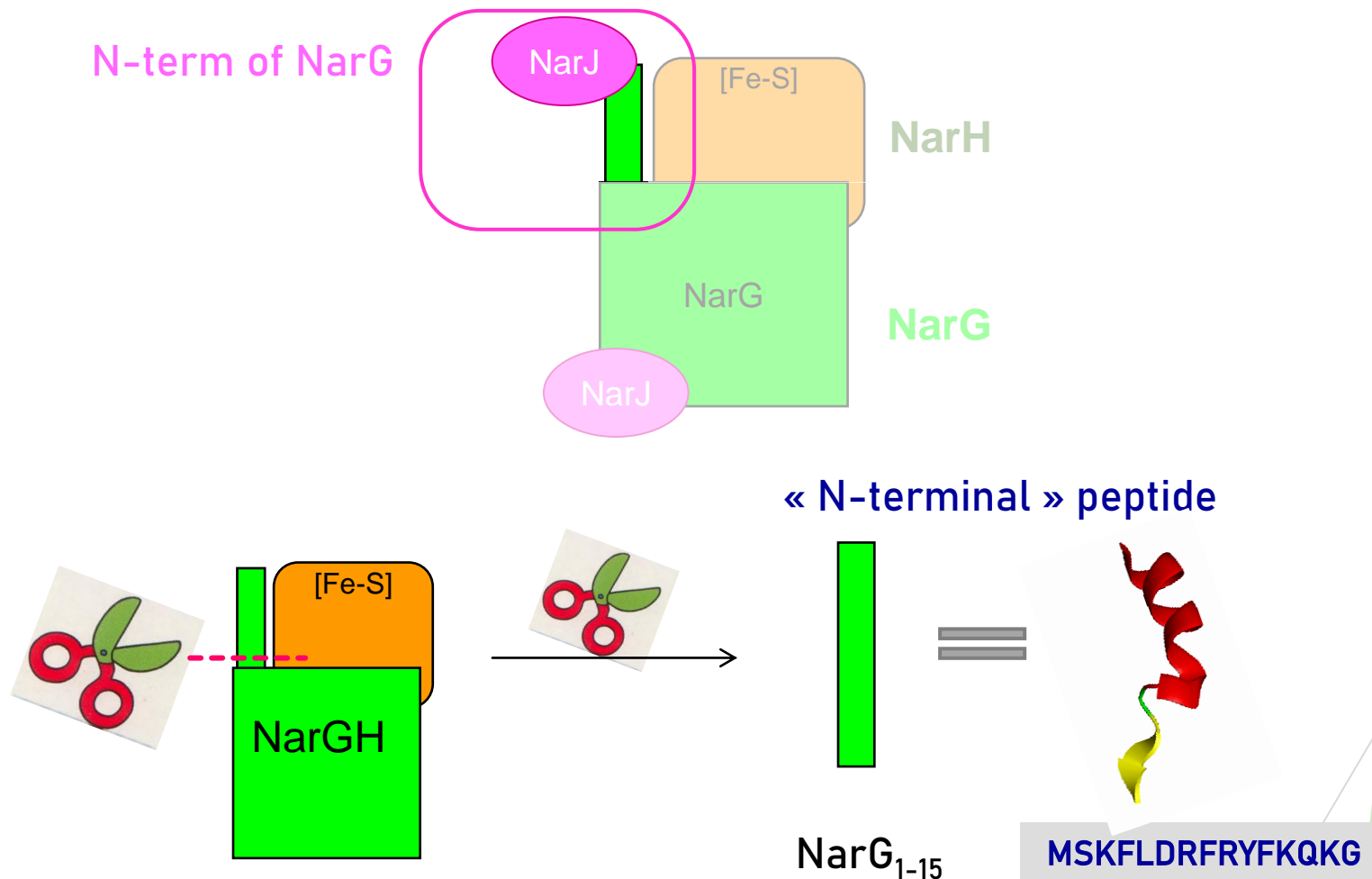
NarJ is involved in the **biogenesis** of the respiratory nitrate reductase (NR) complex *in E. coli*



Vergnes A. *et al*, 2006, *J. Biol. Chem.*
Lanciano P. *et al*, 2007, *J. Biol. Chem.*

Application 3 : chaperone protein NarJ : looking for an interaction site

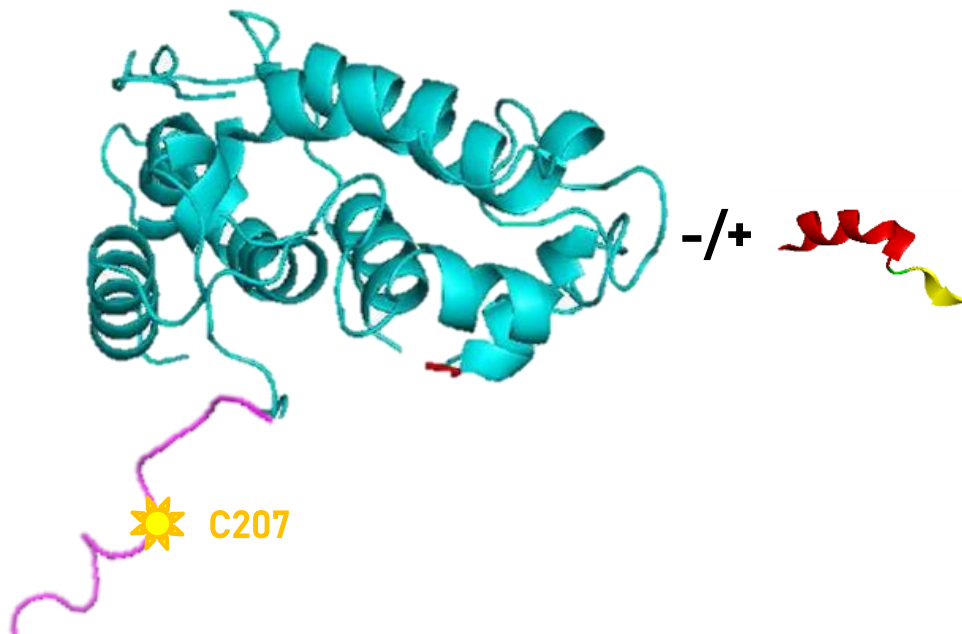
initial step : the recognition between NarJ and NR



Application 3 : NarJ wt : 1 Cys at 207

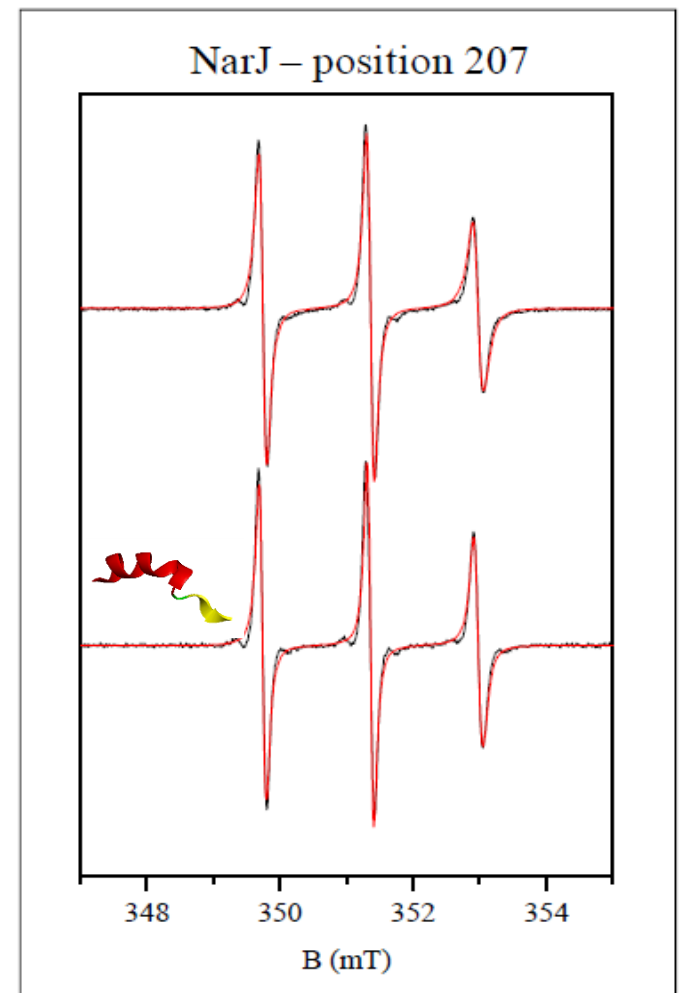


Truncated NarJ: model by homology modeling



1/ the C-ter of NarJ (close to aa 207) is highly mobile

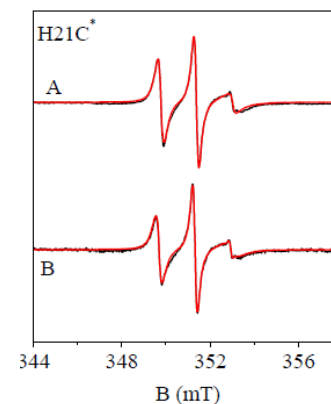
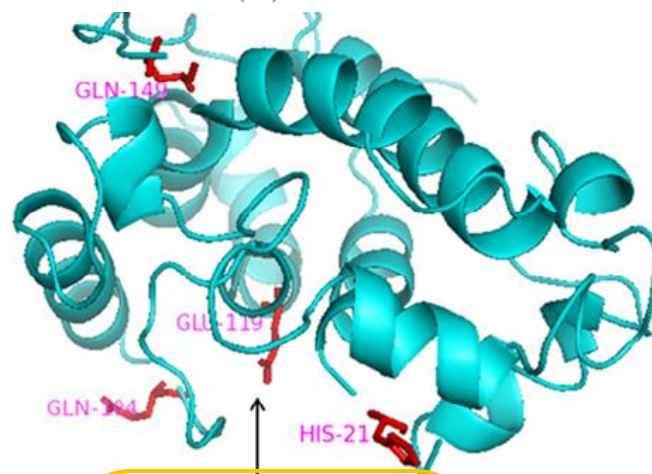
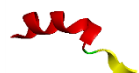
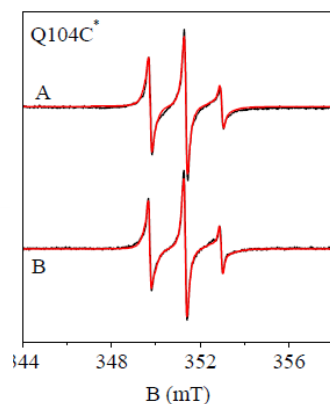
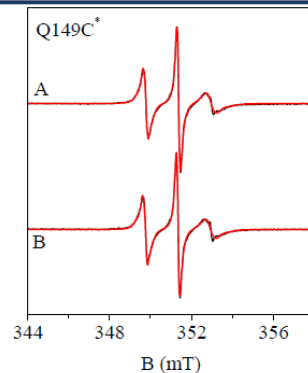
2/ Remains disordered in the presence of the peptide partner



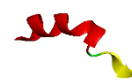
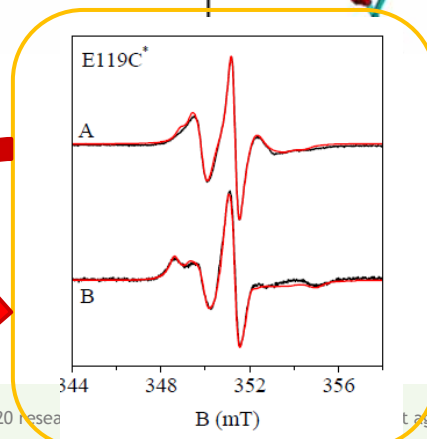
Application 3 : truncated NarJ

Black : experimental

Red : simulated

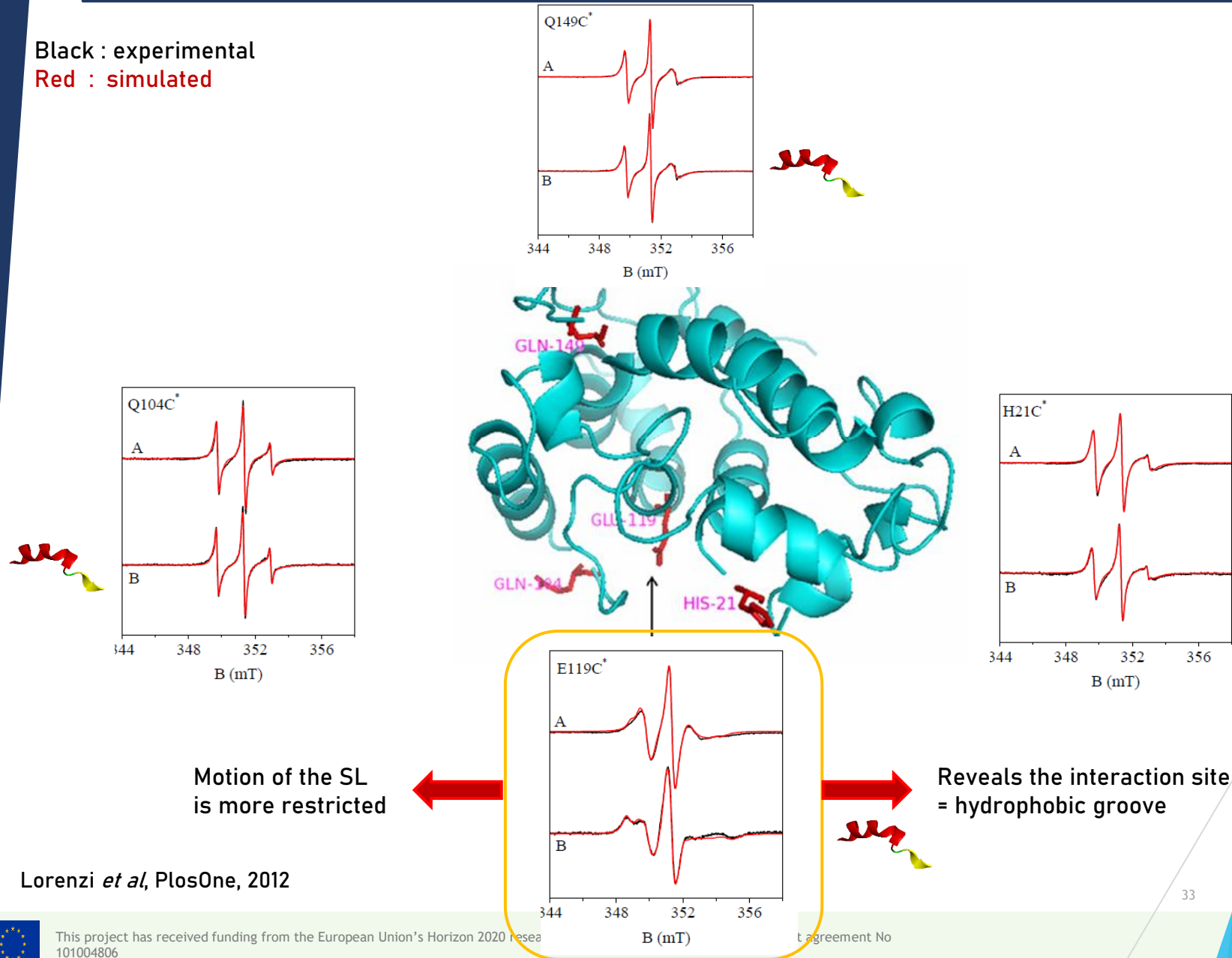


Important spectral
variation in
presence of peptide



Application 3 : truncated NarJ

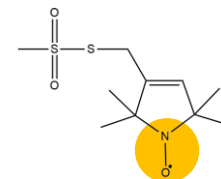
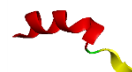
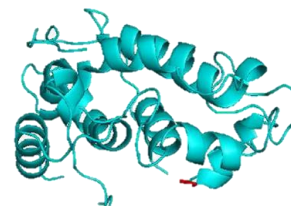
Black : experimental
Red : simulated



Lorenzi *et al*, PlosOne, 2012

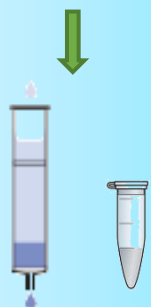
Application 3 : In the practicals

- ✓ Truncated NarJ
- ✓ 2 purified samples : NarJ C207 and NarJ E119C
- ✓ Labeling each sample with MTSL
- ✓ Interaction with the partner



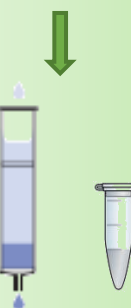
step 1 : reduction of Cys variant

*Cys variant + DTT (excess)
RT or ice, ~30 min*



step 2: labeling with the label

*Addition of MTSL (10 eq.)
RT or ice, dark ~ 45 min*



EPR and OD_{280nm}
of each fraction

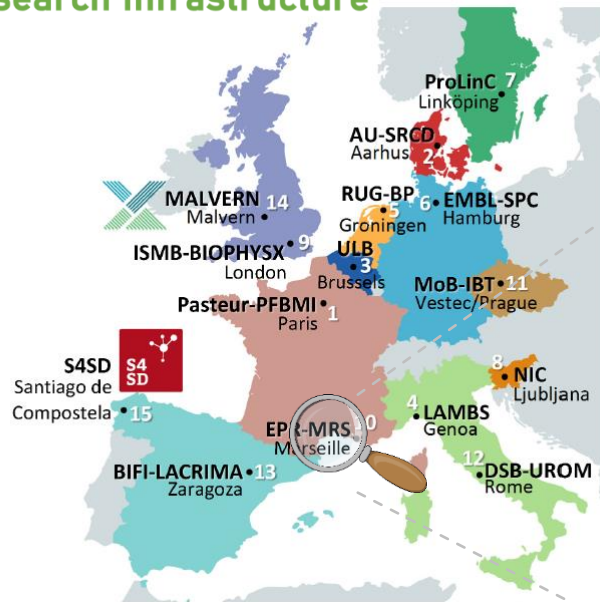
Concentration of the
protein
OD_{280nm} and EPR



M. Martinho *et al.*, *Book of the RSC, Electron Paramagnetic Resonance vol. 26* 2018

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