

New approaches in biostructural EPR : from dynamics to organization of large biomolecular systems

Bruno GUIGLIARELLI

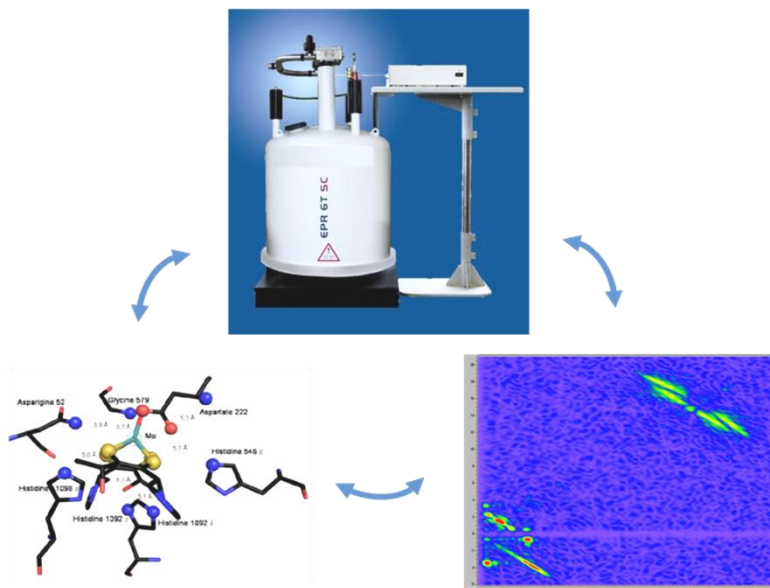
Bioénergétique et Ingénierie des Protéines

BIP – UMR 7281

CNRS and Aix-Marseille University

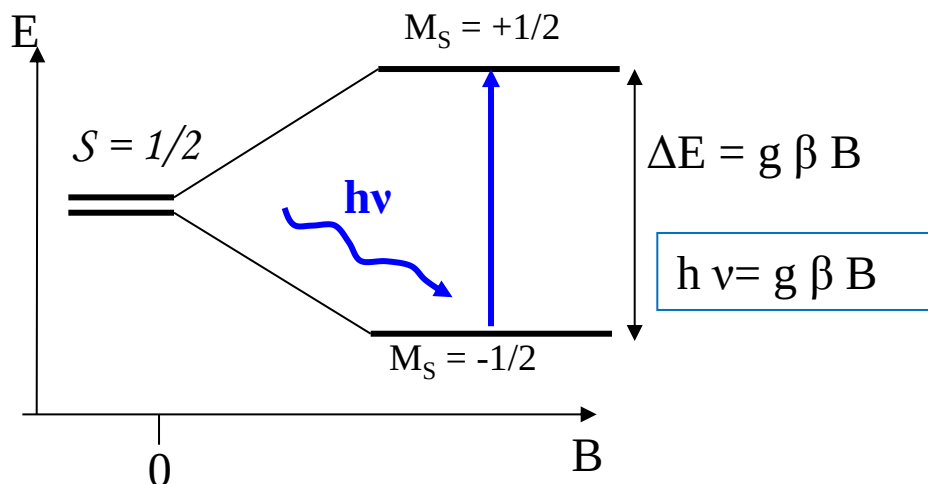
Institut de Microbiologie de la Méditerranée

MARSEILLE - FRANCE



Electron Paramagnetic Resonance (EPR):

A spectroscopy specific of **single electron systems** ($S = 1/2$)

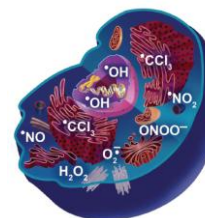
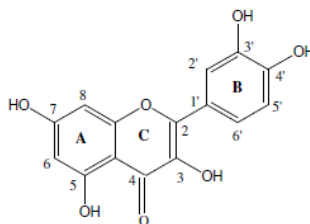
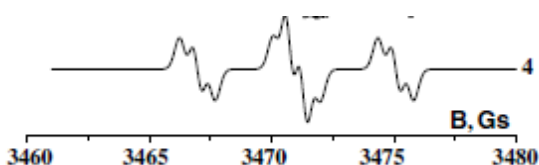


- Common EPR spectrometers : $B = 0.3$ T, Microwave Frequency = 9 GHz (X-band)

- Radicals : Oxidative stress (ROS, RNOS)

Reaction intermediates

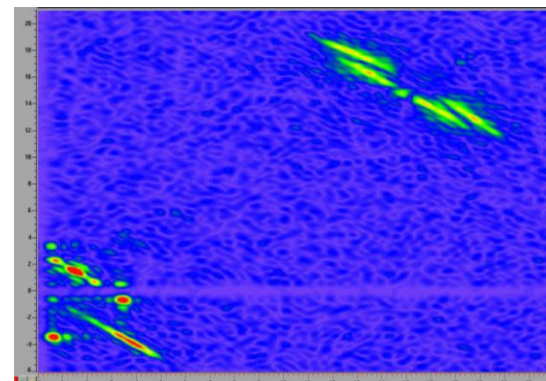
Semiquinones, FAD, FMN, Gly , Cys , Tyr , Trp , ..



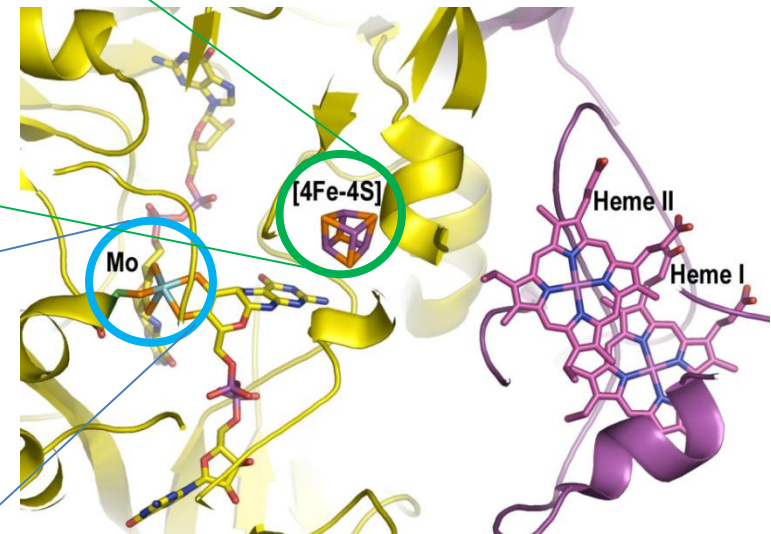
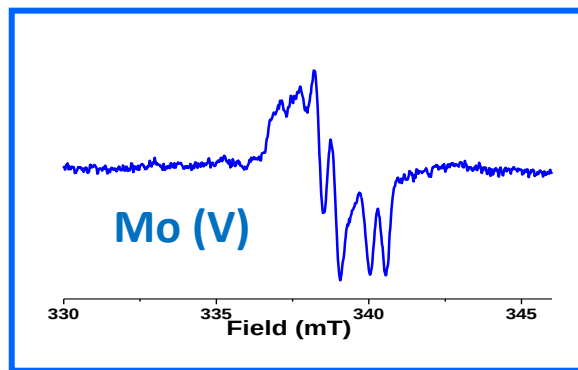
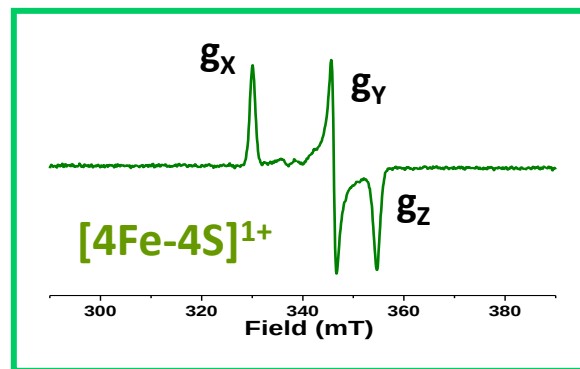
Hyperfine coupling : coupling of single electron with close magnetic nuclei (^1H , ^{14}N , ^{13}C , ^{17}O , ^{33}S ,...)

Pulsed EPR : ENDOR, ESEEM, HYSCORE

High resolution structural data



- Metal centers: hemes, Fe-S, Cu, Ni, Co, Mo, W, V,...
- Anisotropy of magnetic properties : g_x , g_y , g_z
- Common concepts in EPR and Solid state NMR
- EPR :
 - A selective view of magnetic centers and of their environment (magnetic nuclei)
 - No limit in size or physical state (solution, membrane, cells, crystals)

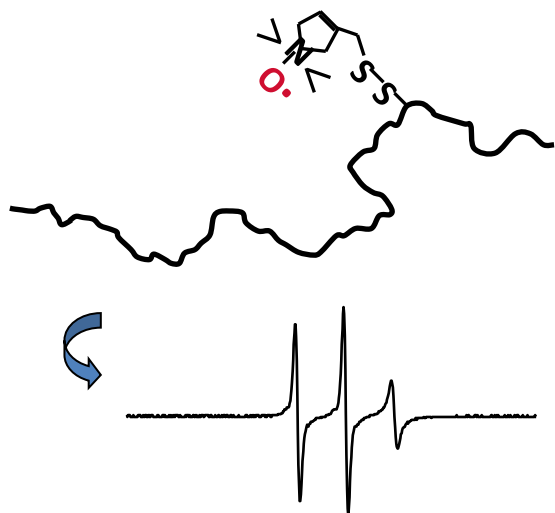
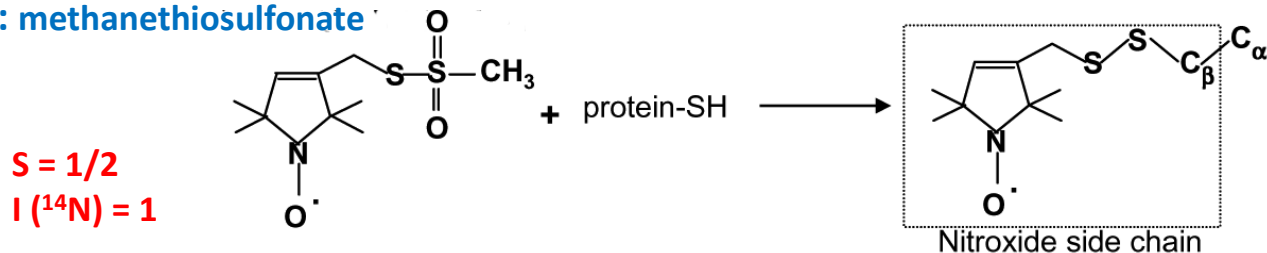


R. Sphaeroides periplasmic Nitrate reductase - NapAB

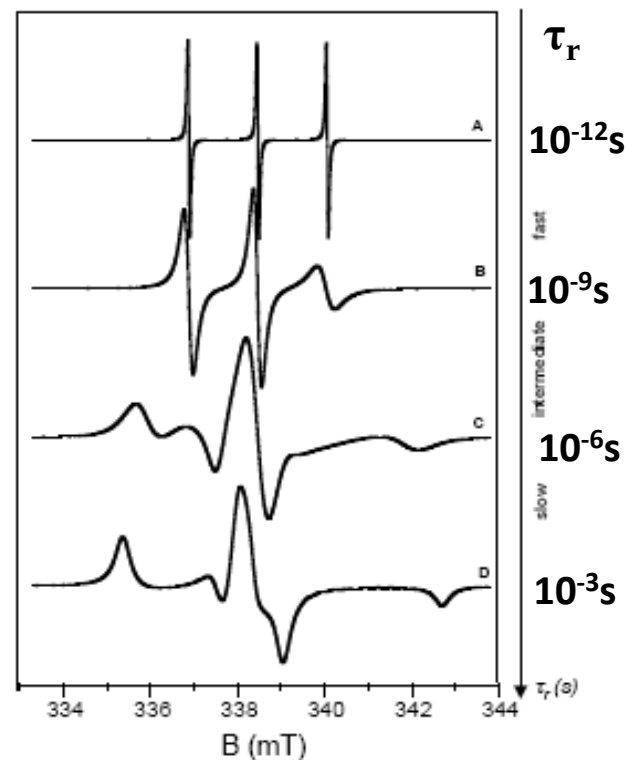
Arnoux *et al.*, Nat. Struct. Biol. 2003

SDSL = grafting of a stable nitroxide radical on a Cysteine residue

MTSL : methanethiosulfonate



- High sensitivity to radical mobility
- Local structural changes :
 - * Interactions between partners
 - * Structural transitions
- Low steric hindrance
- No size limitation
- Room T study, small amounts (100 pmol)



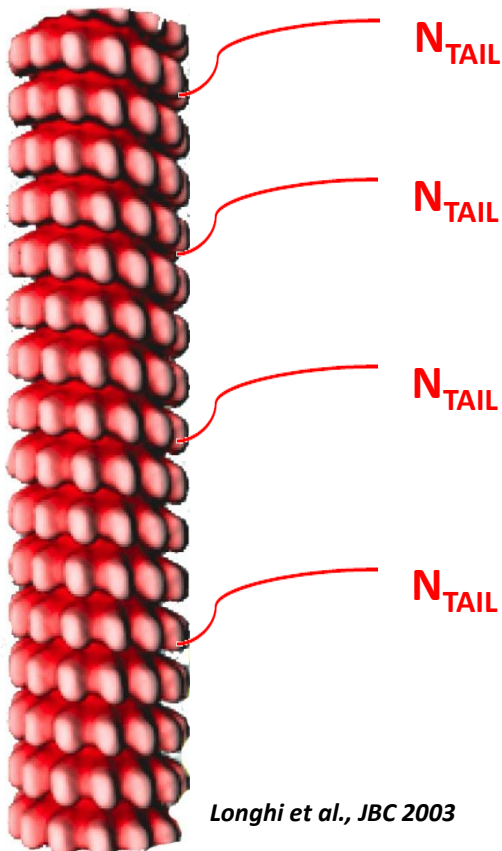
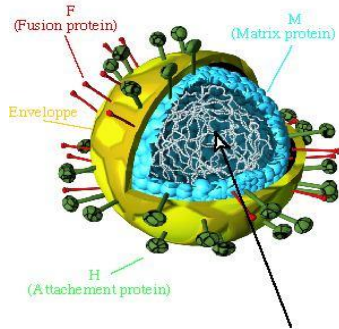
Intrinsically Disordered Proteins : No stable secondary structure
Hardly accessible to structural techniques

N = Nucleoprotein of the Measles virus involved in the replicative complex

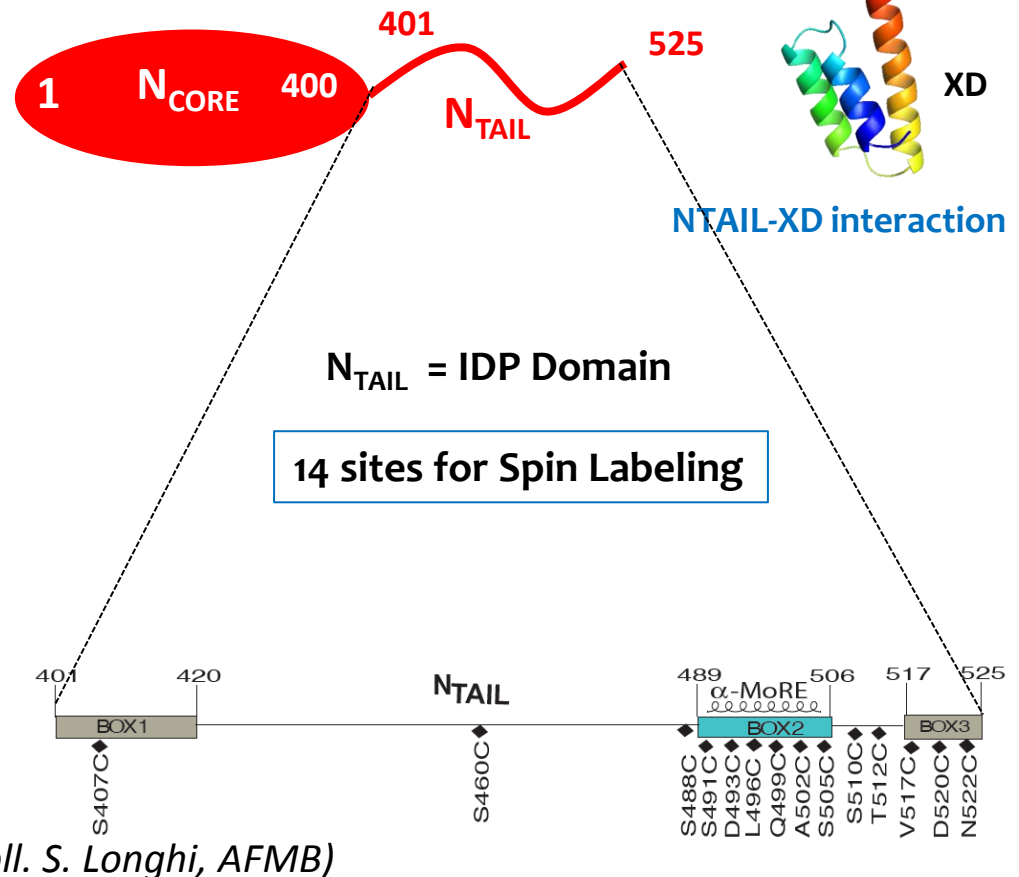
N_{TAIL} is intrinsically disordered at the surface of the nucleocapside

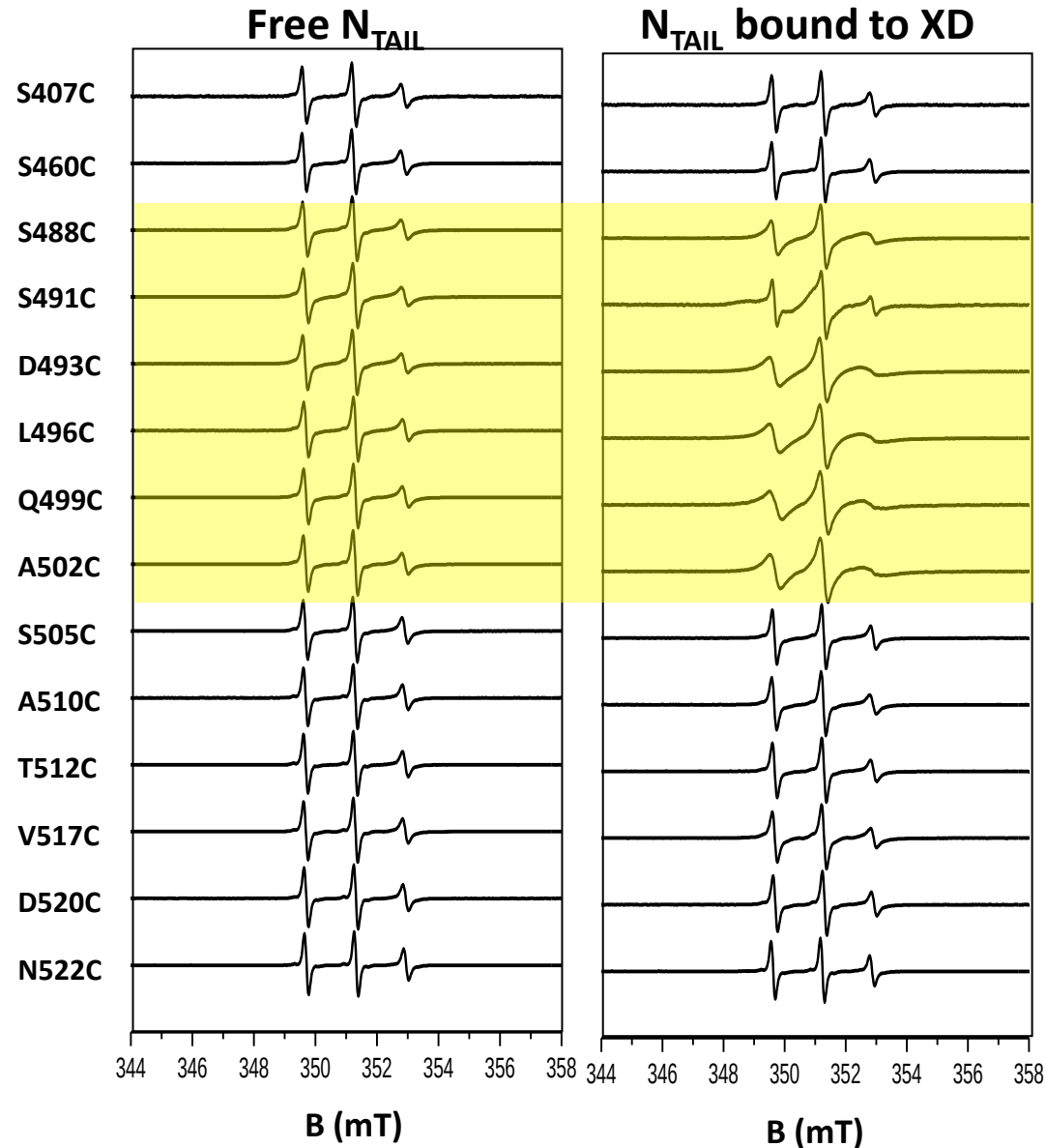
Interaction Nucleoprotein – Polymerase (XD)

Map the induced folding of N_{TAIL} in interaction with XD

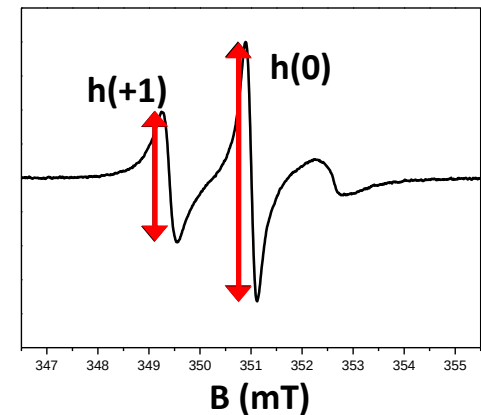


Longhi et al., JBC 2003

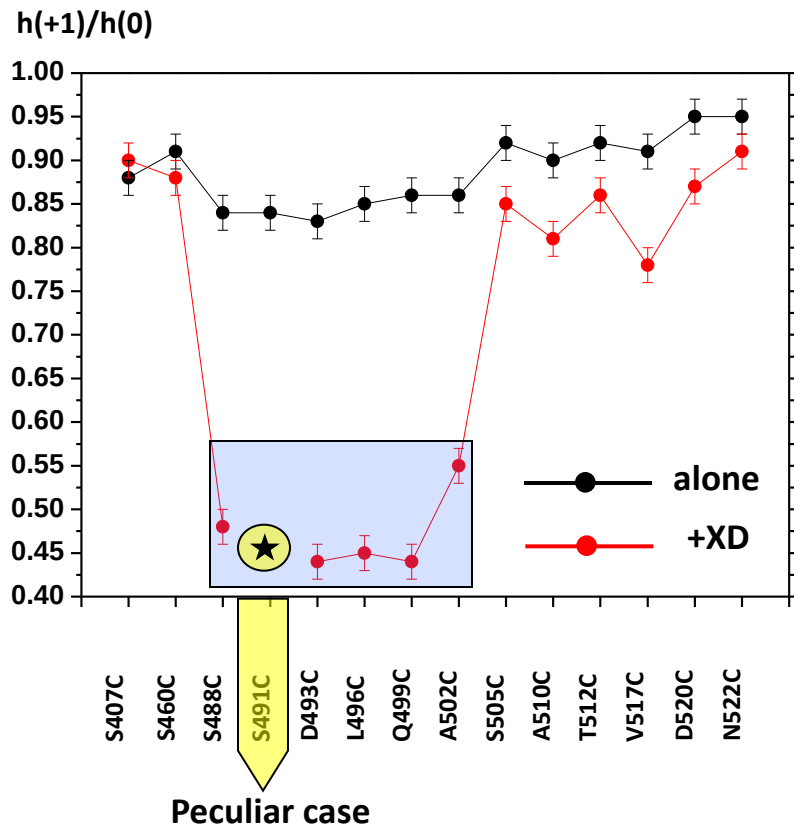
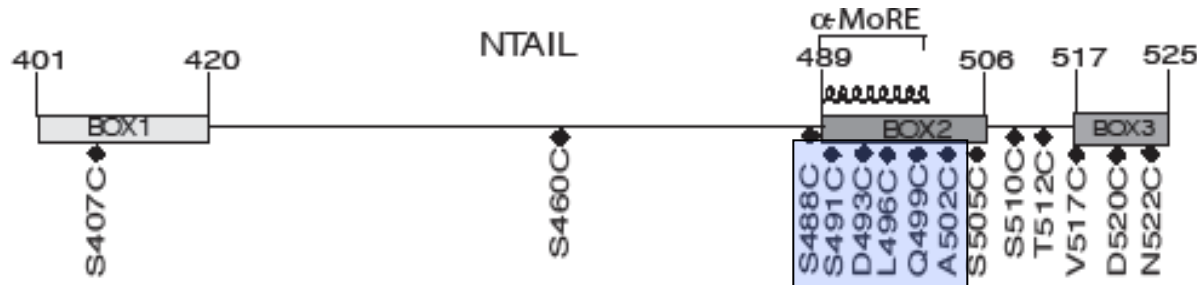




Semi-quantitative parameter



$h(+1)/h(0)$ good indicator
to report mobility
changes of the label

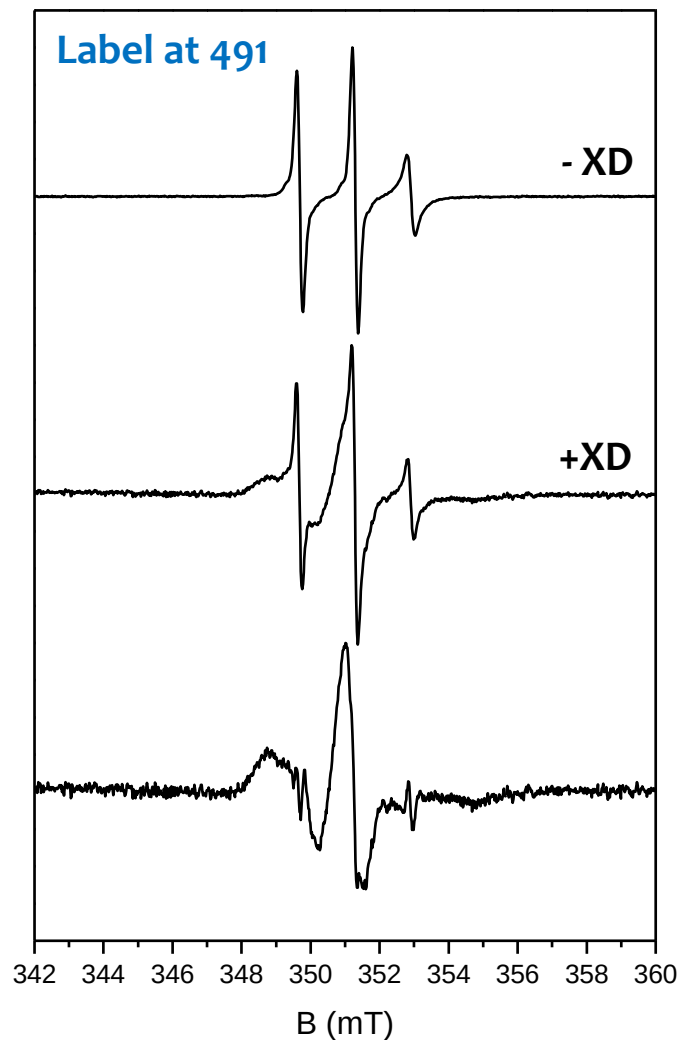


Strong decrease of the label mobility

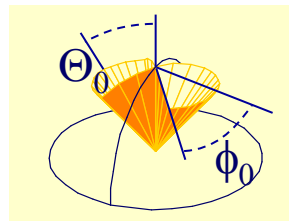
α -helical folding

Belle et al., J. Pept. Sci. 2010

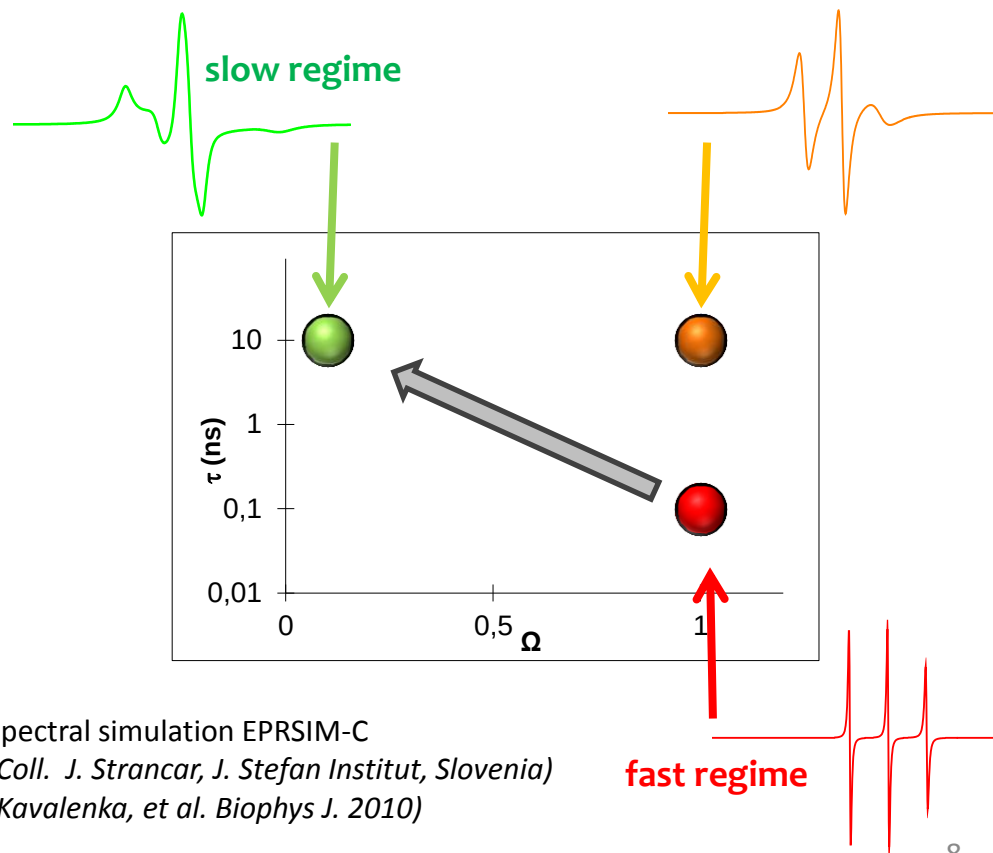
Composite spectrum
Broad component (75%)



Simulation of EPR spectra

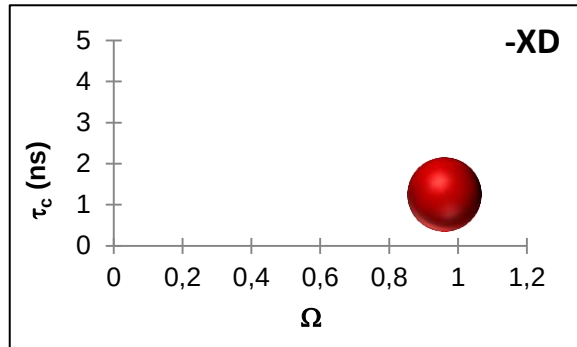


Rotation correlation time: τ (ns)
Free rotational space $\Omega = \theta_0 \phi_0 / (\pi/2)^2$
 $0 < \Omega < 1$
no space full space

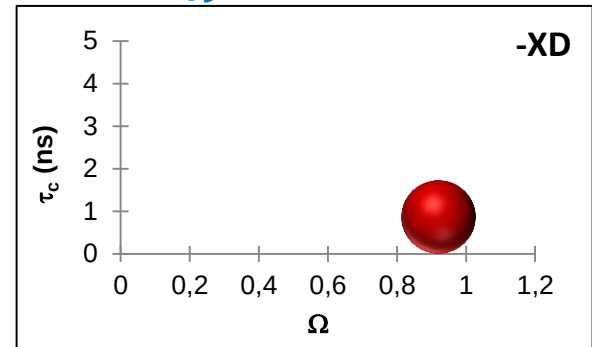


Spectral simulation EPRSIM-C
(Coll. J. Strancar, J. Stefan Institut, Slovenia)
(Kavalenka, et al. Biophys J. 2010)

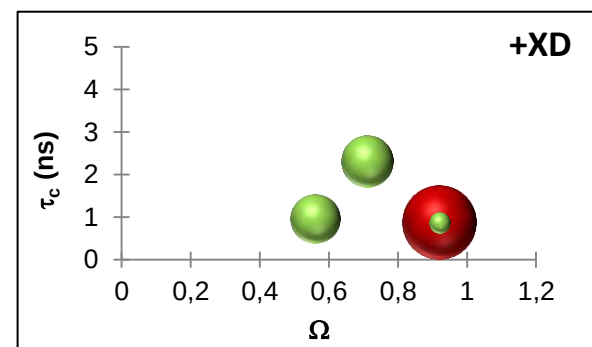
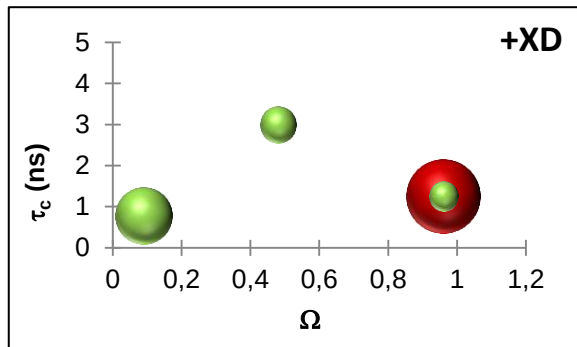
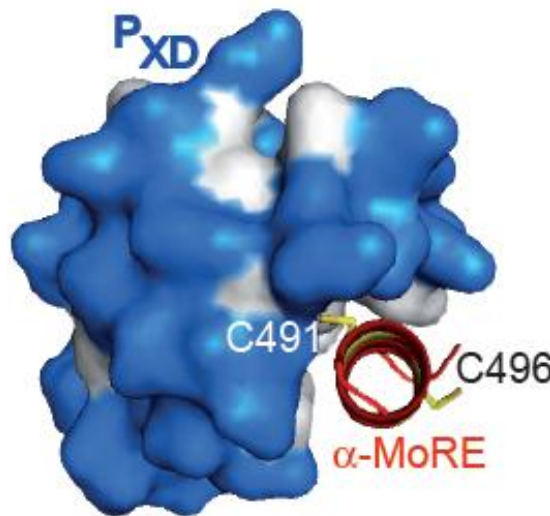
Label at 491



Label at 496

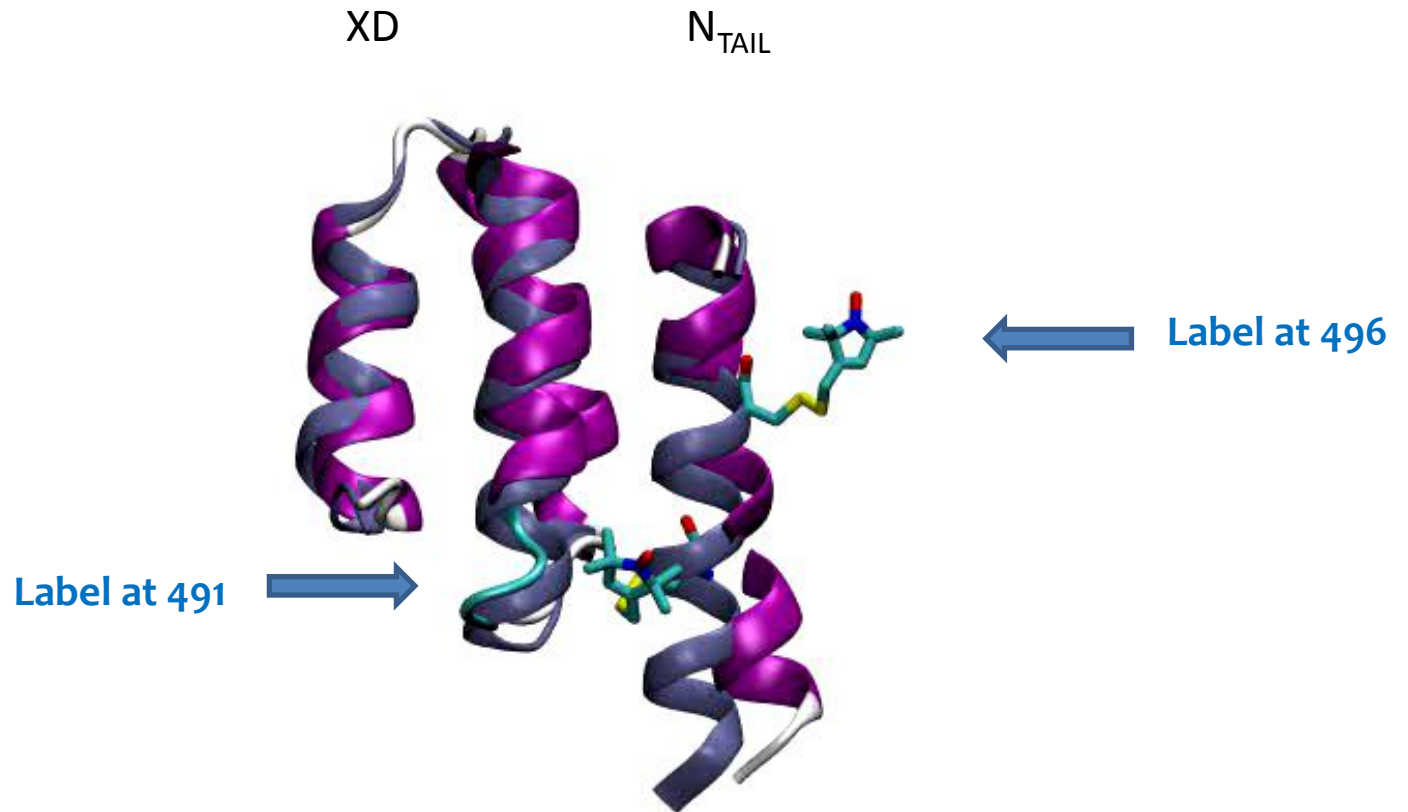


3D structure of a chimera construct (XD+18 aa NTAIL)
(Kingston et al, PNAS, 2004)



Validation of the structural model of the chimera

Martinho et al, JBSD, 2012



MD calculation (20 ns)

In collaboration

J. Golebiowski, LCMBA, Nice

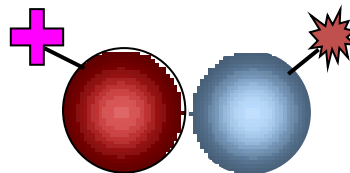
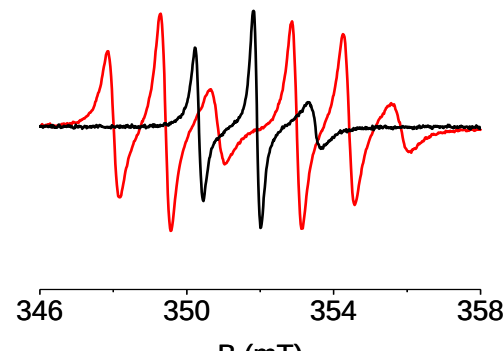
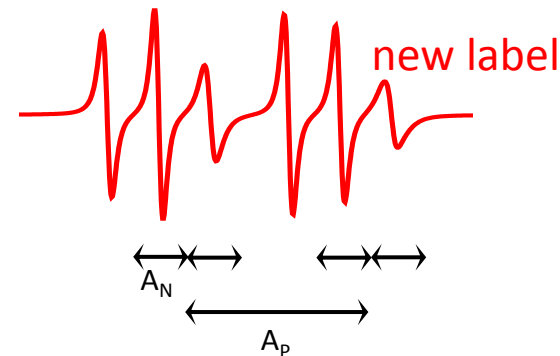
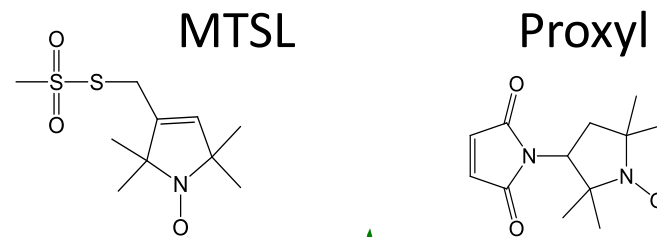
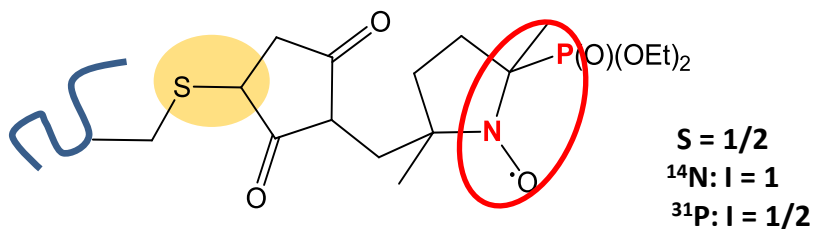
SDSL-EPR is a method of choice for studying:

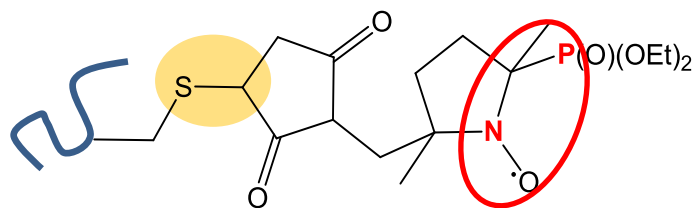
- Protein-protein interactions
- Conformation changes
- Structural and dynamic properties of IDP's
- Disorder to order transitions

But all commercial nitroxide Spin Labels

- give the same EPR signature
- need Cys to be grafted

Requirement of different EPR signatures :
Development of new spin labels : Phosphorylated Proxyl

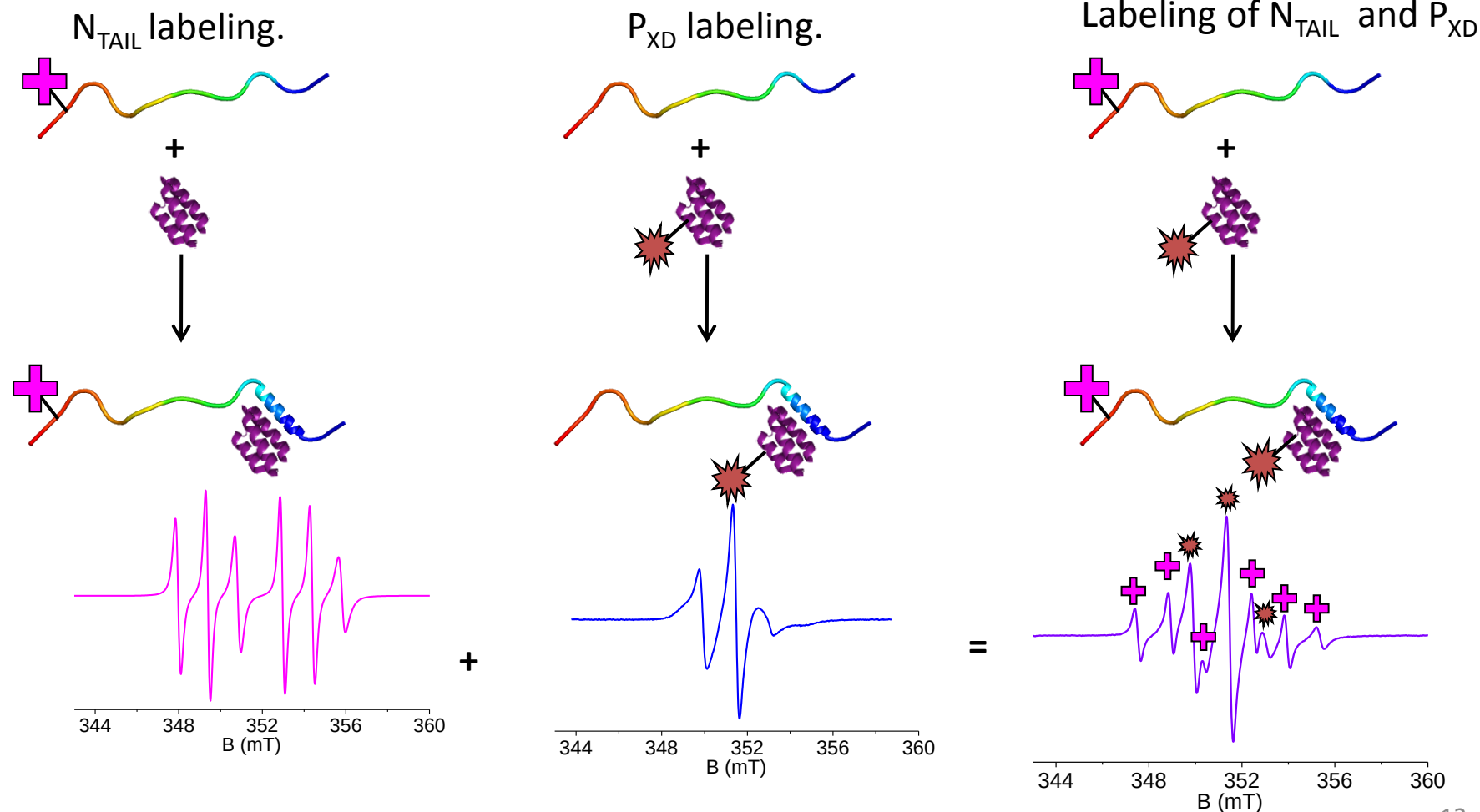




To probe structural changes of different regions of the complex simultaneously !

(Lebreton et al., PCCP, in press 2014)

(Coll. P. Tordo, S. Marque, ICR)



All available Spin labels need Cys for grafting

Strong limitation when Cys have functional role : S-S bridge, metal binding,...

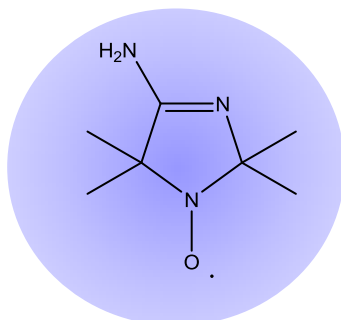


Development of new spin labels to be grafted on non-Cysteine residues

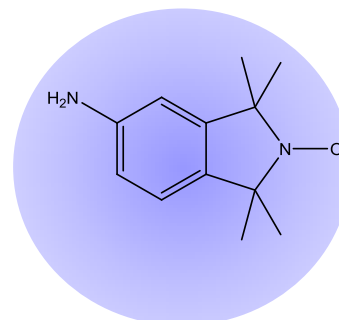
Targeting Tyr and Trp

(Coll. P. Tordo, S. Marque, ICR)

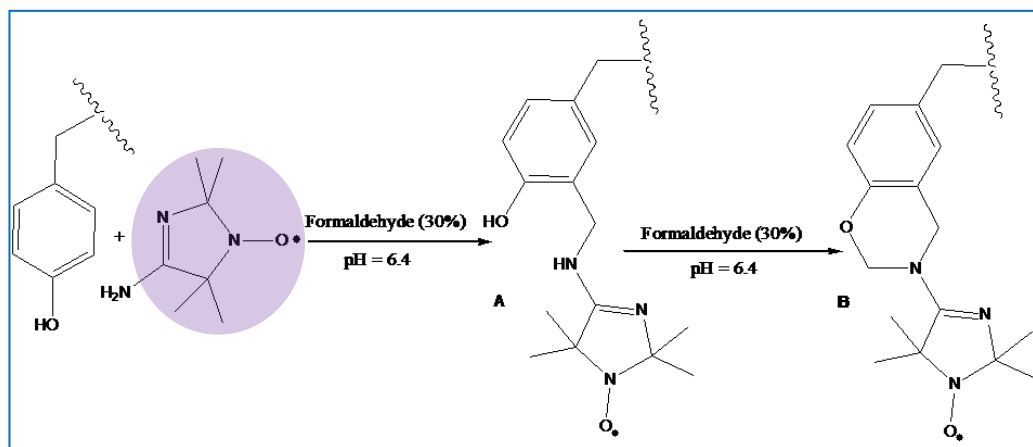
AGENCE NATIONALE DE LA RECHERCHE
ANR

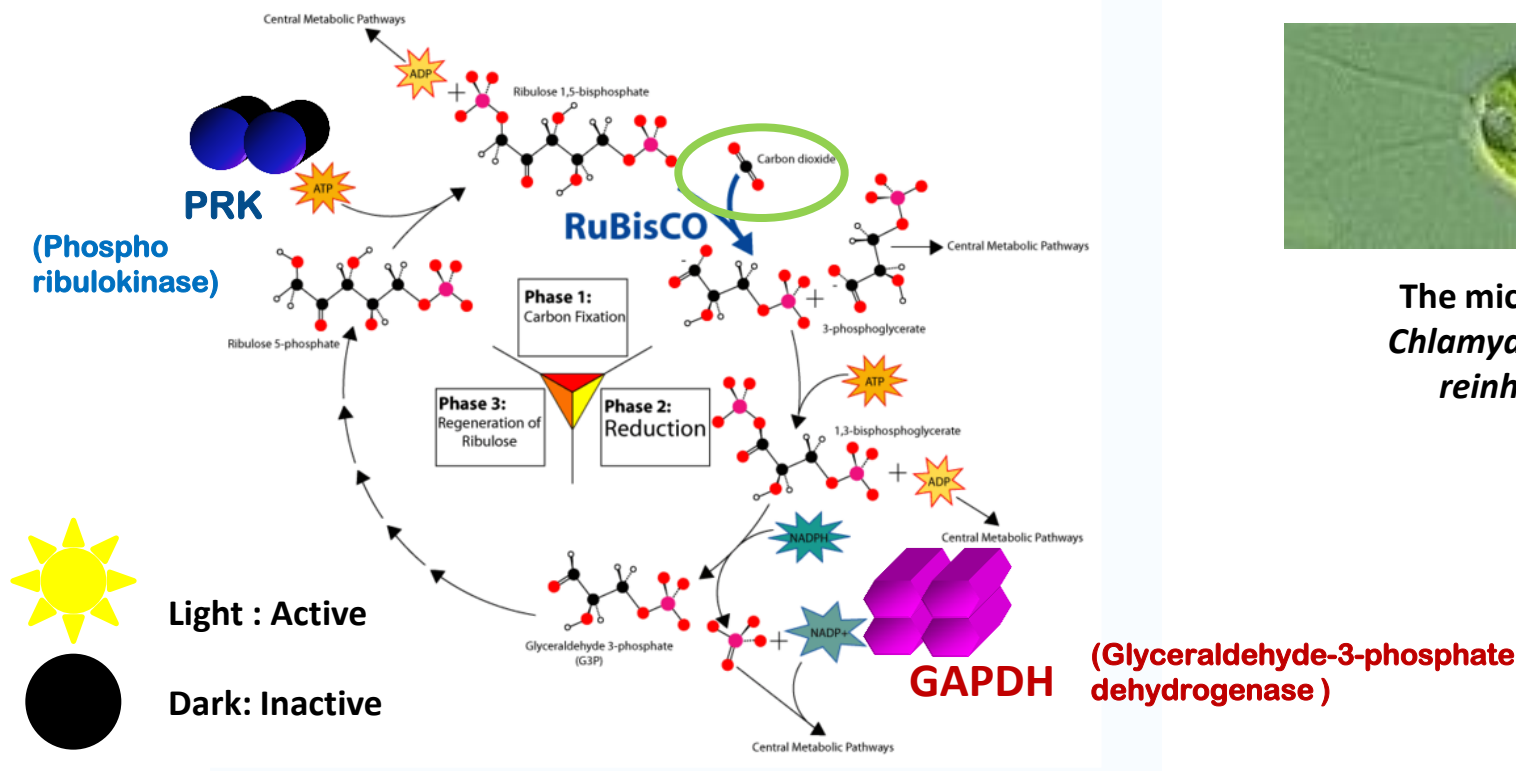


Lorenzi et al, 2011, Angew. Chem. Int. Ed.



Mileo et al, 2013, Bioconj. Chem.



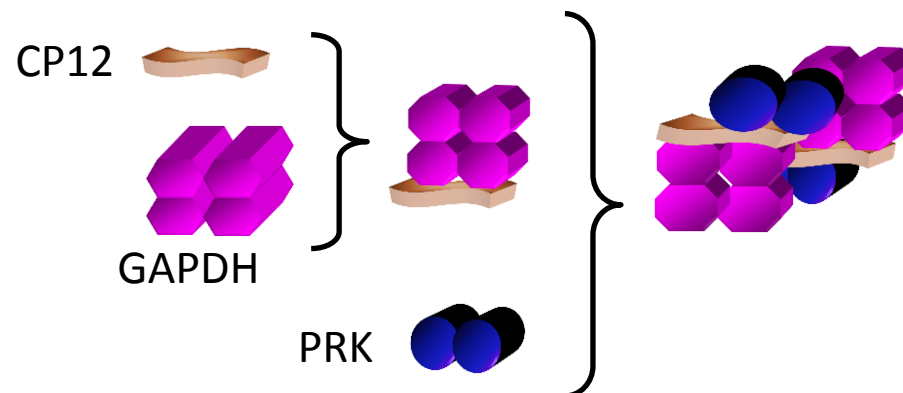


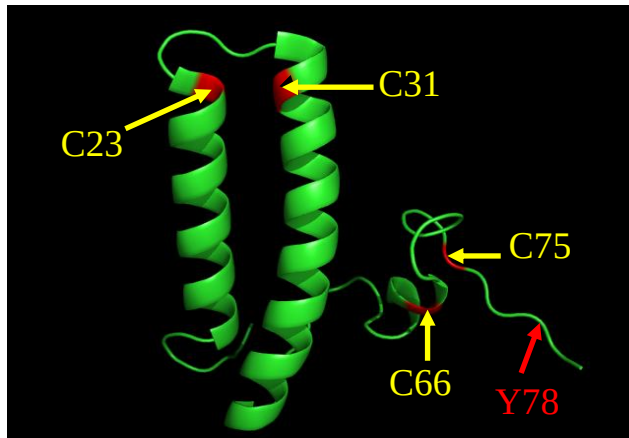
The microalgae
Chlamydomonas reinhardtii

Regulation of the Calvin cycle by CP12 :

Supramolecular complex CP12/GAPDH/PRK

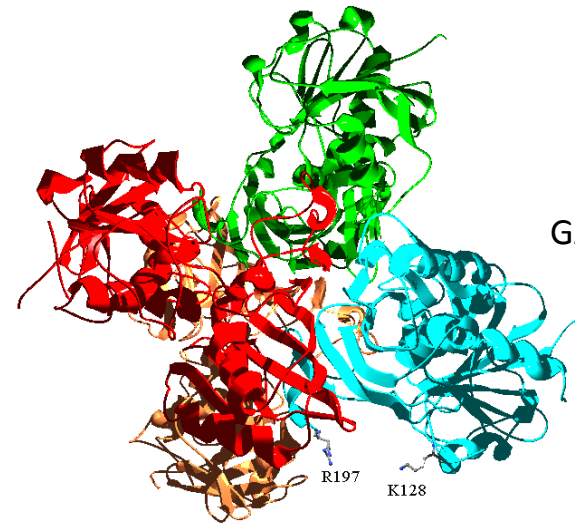
↔ Efficiency of the CO₂ assimilation





Structural model of the oxidized CP12
(Gardebien et al., 2006)

Regulation

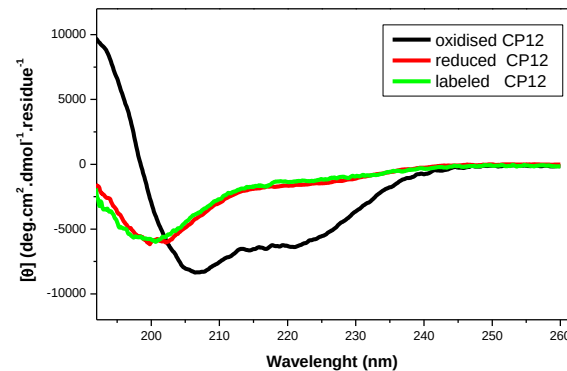


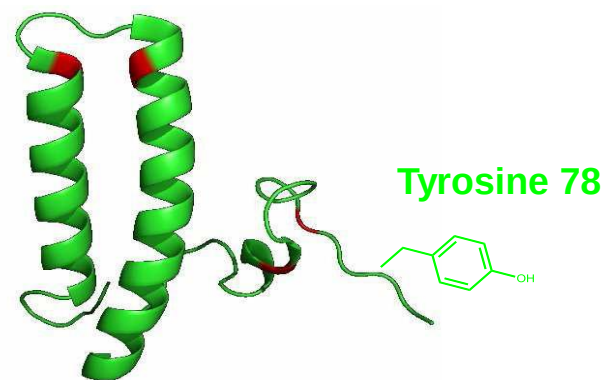
GAPDH (tetramer)

CP12 :

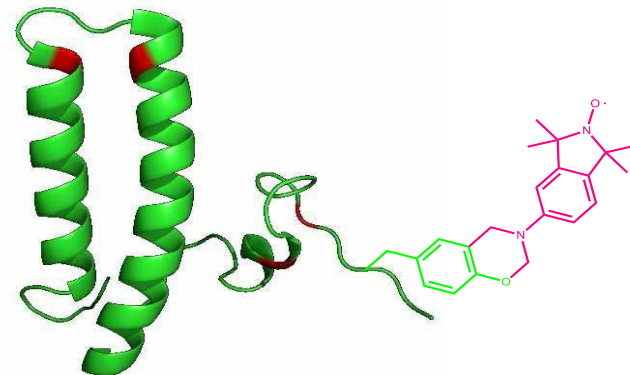
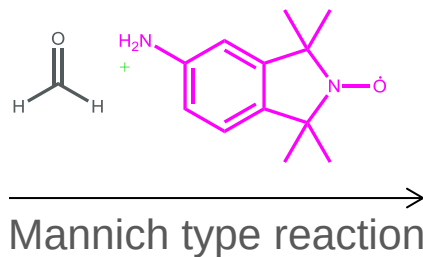
- Four cysteine residues : C23, C31, C66, C75
- Oxidized state : - 2 disulfide bridges
 - C23-C31 and C66-C75
 - Inhibition of GAPDH
 - IDP like protein (C-term)
- Reduced state : - breaking of disulfide bridges
 - fully disordered structure

**Study of CP12-GAPDH
regulation through SDSL-EPR
required non-Cys spin labels**

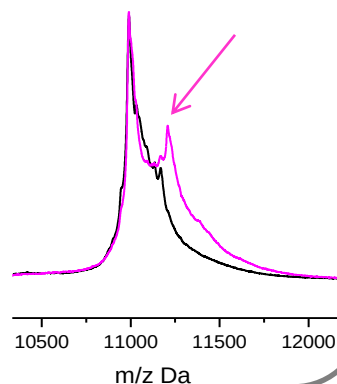




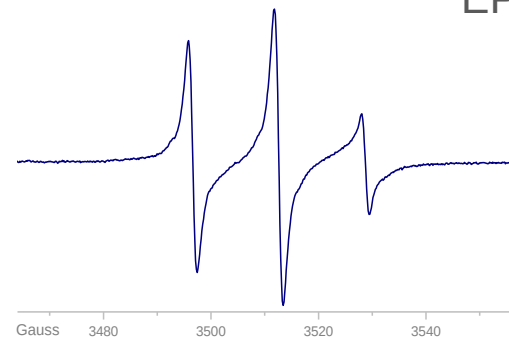
CP12 (Structural model)



Mass Spec.
(MALDI-ToF)



EPR

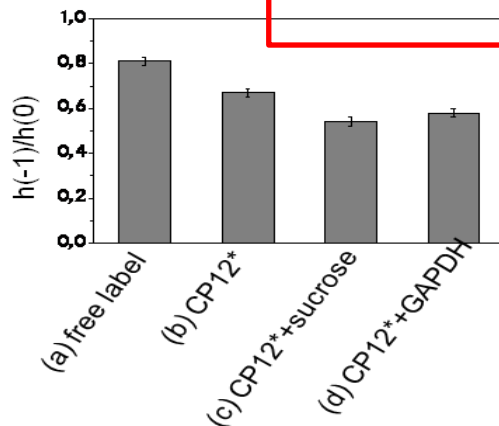
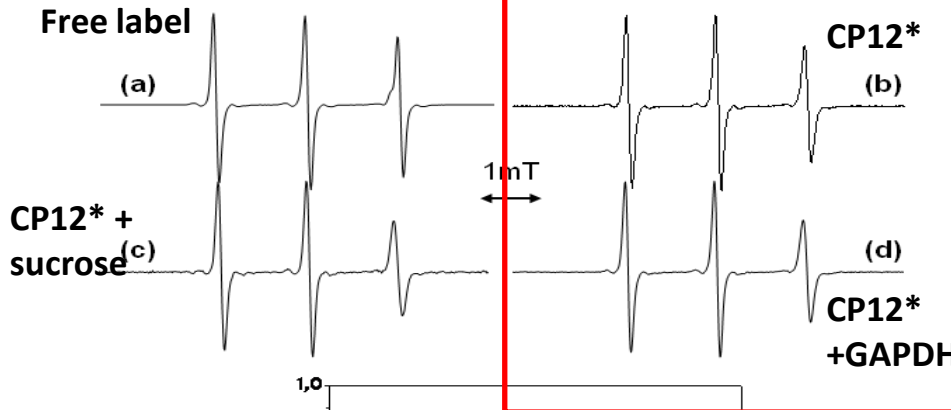
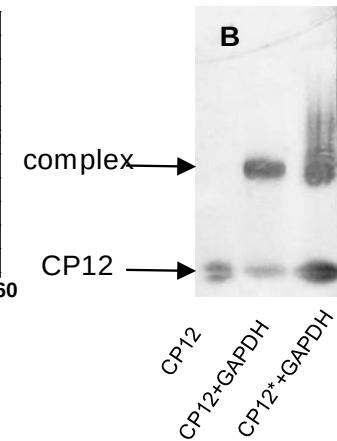
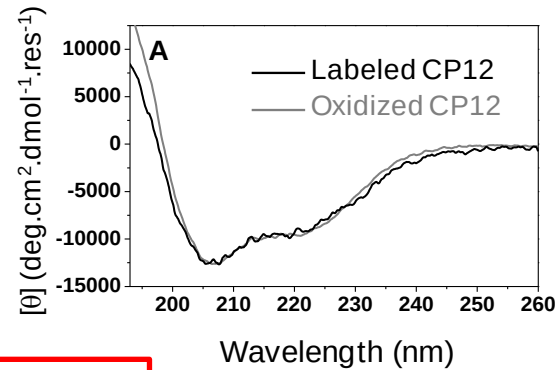


First spin-labeling of a Tyr in a protein

Lorenzi et al., Angew. Chem. Int. Ed. 2011
Mileo et al., Bioconj. Chem. 2013

Spin labeling of **Tyr78** in CP12 :

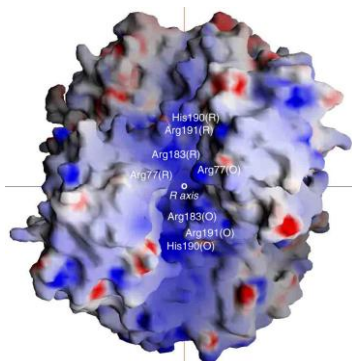
- Same structural properties of CP12
- Formation of a complex with GAPDH



- Yield of labeling : 70 %
- High mobility of the label on CP12* and the CP12*-GAPDH complex
- High flexibility of **CP12 C-term** in the complex
(Lorenzi et al., *Angew. Chem. Int. Ed.* 2011)

First spin-labeling of a Tyr in a protein

Evidence for Fuzzy complex by SDSL- EPR



GAPDH : a large charged crevice

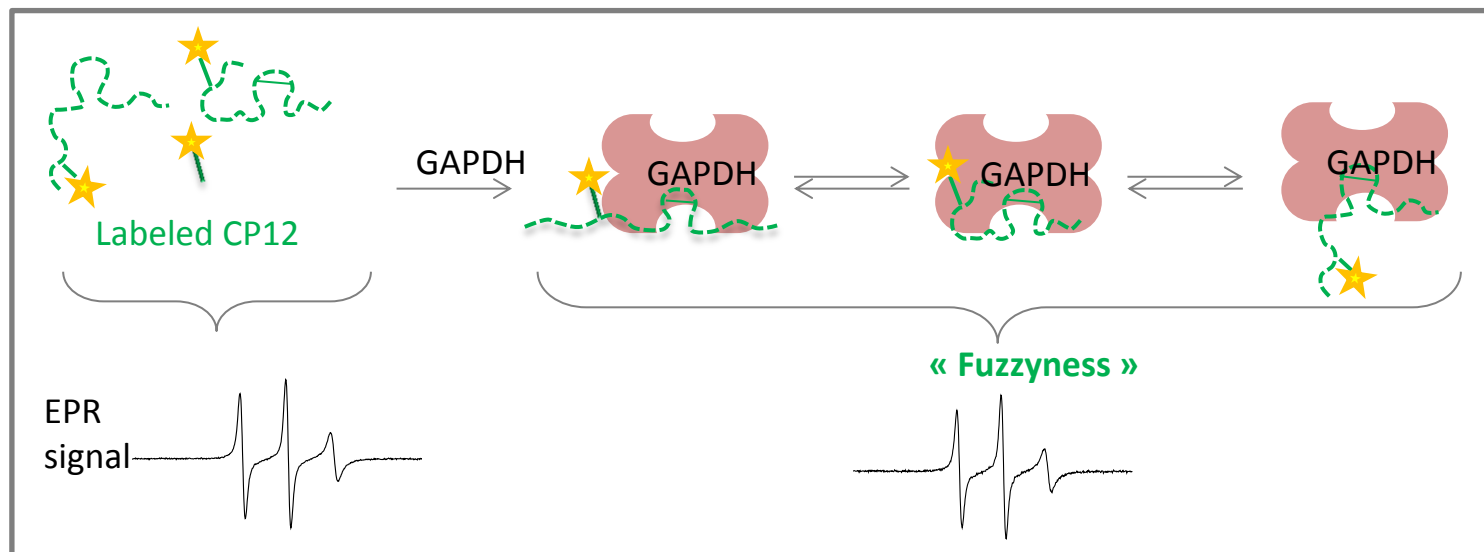
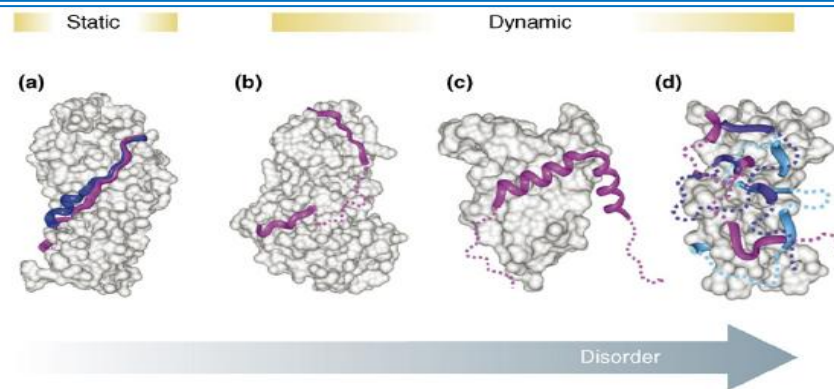
Dynamic disorder of CP12 in the GAPDH-CP12 complex
A highly dynamic “Fuzzy” complex
(Mileo et al., Mol. BioSyst. 2013)

Opinion

TRENDS in Biochemical Sciences Vol.33 No.1

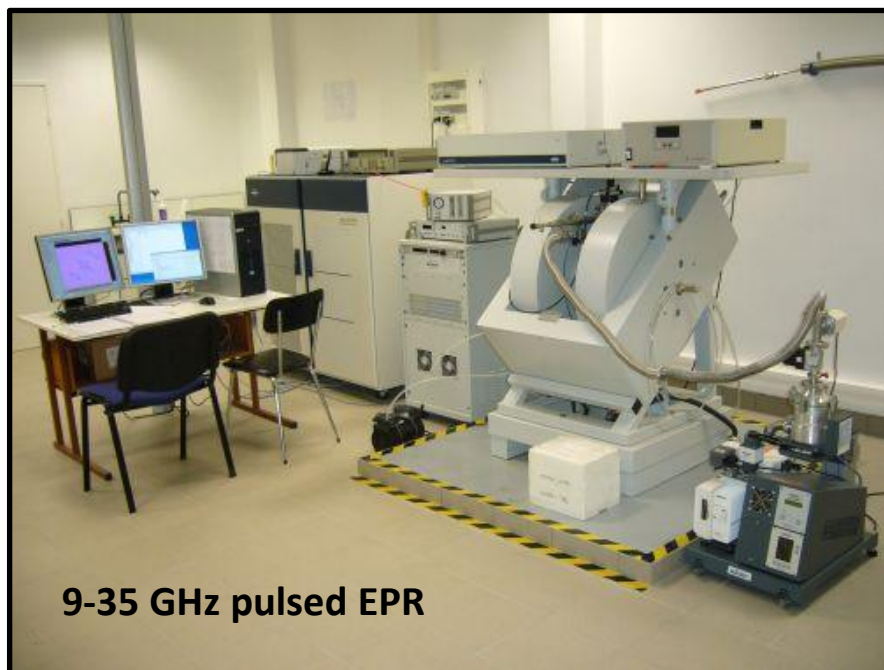
Fuzzy complexes: polymorphism and structural disorder in protein–protein interactions

Peter Tompa and Monika Fuxreiter



Multiple spin-labeling and pulsed EPR

Distance measurements by DEER (Double Electron-Electron Resonance)

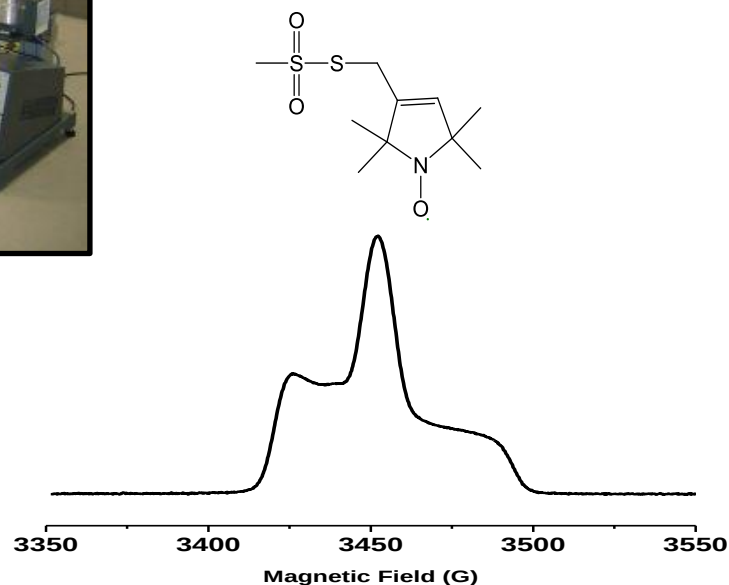


9-35 GHz pulsed EPR

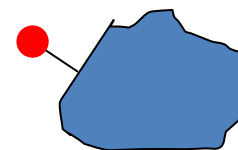
$$\pi/2 = 4 \text{ ns}$$



Echo Field Sweep



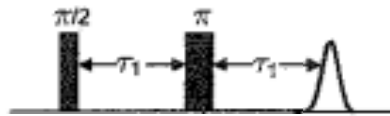
Nitroxide absorption EPR spectrum (70 K)



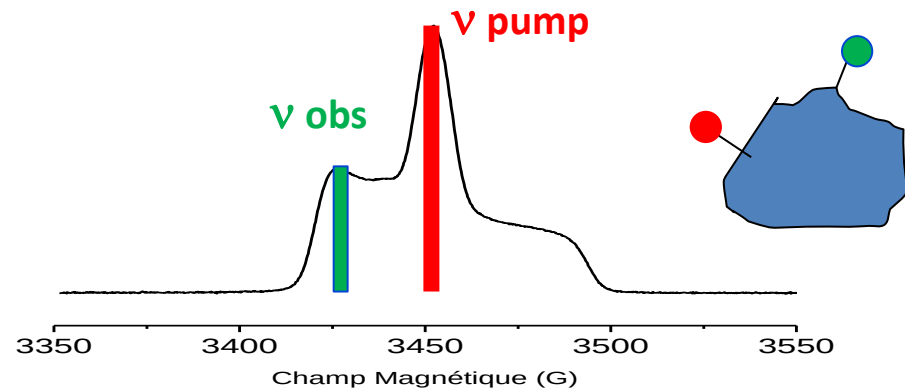
Multiple spin-labeling and pulsed EPR

Distance measurements by DEER (Double Electron-Electron Resonance)

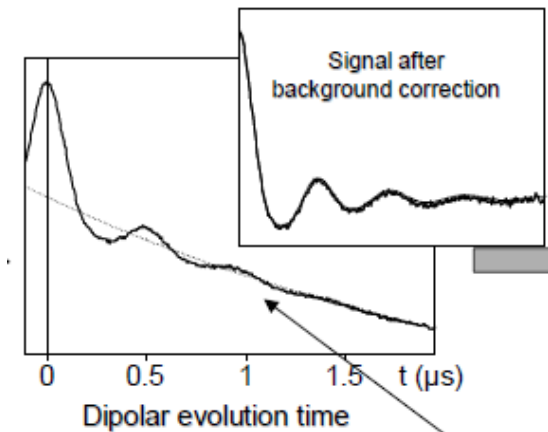
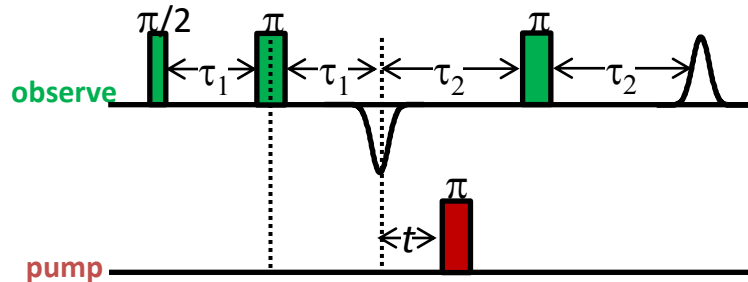
$$\Pi/2 = 4 \text{ ns}$$



Echo Field Sweep

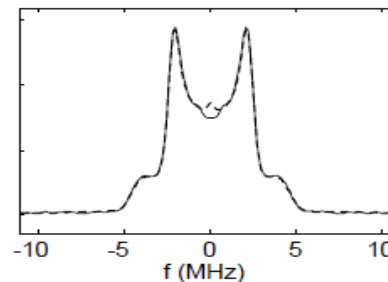


DEER Experiment



$$r(\text{nm}) = \sqrt[3]{\frac{52.04}{\nu_{\perp}(\text{MHz})}}$$

2.0 – 8.0 nm



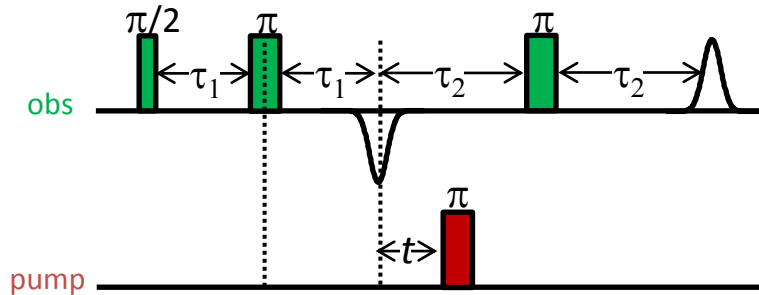
$$V_{\text{DEER}}(t) = \cos(\omega_{ee}t)$$

$$\omega_{ee} = \frac{\mu_0 g_A g_B \beta_e^2}{4\pi\hbar} \frac{1}{r_{AB}^3} (3\cos^2\theta_{AB} - 1)$$

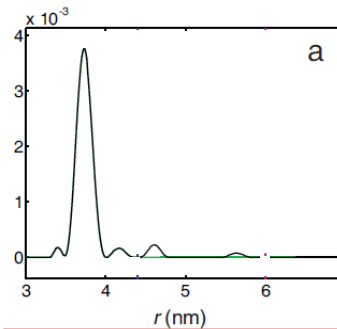
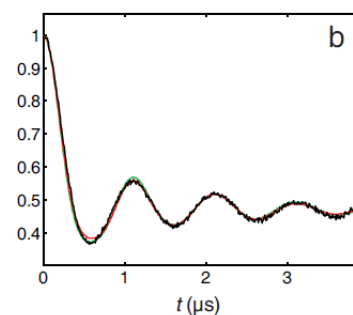
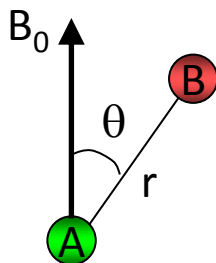
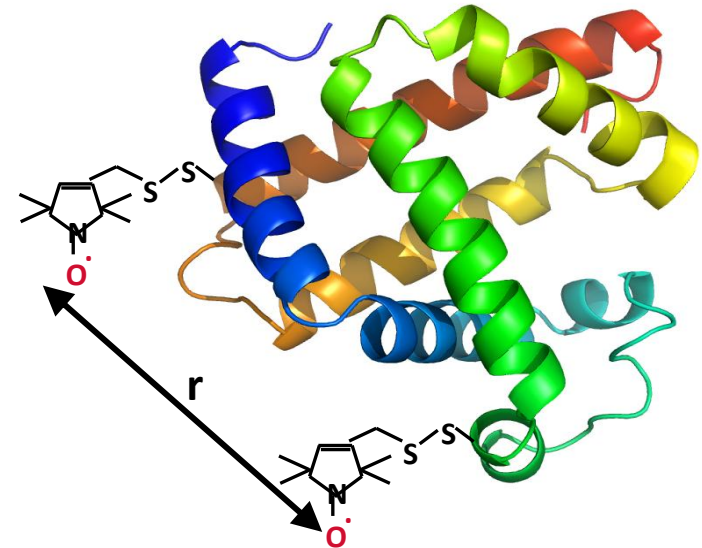
Multiple spin-labeling and pulsed EPR

Distance measurements by DEER (Double Electron-Electron Resonance)

Two labels $\longleftrightarrow$ Inter-label distance and distance distribution
Range [2 – 8 nm]



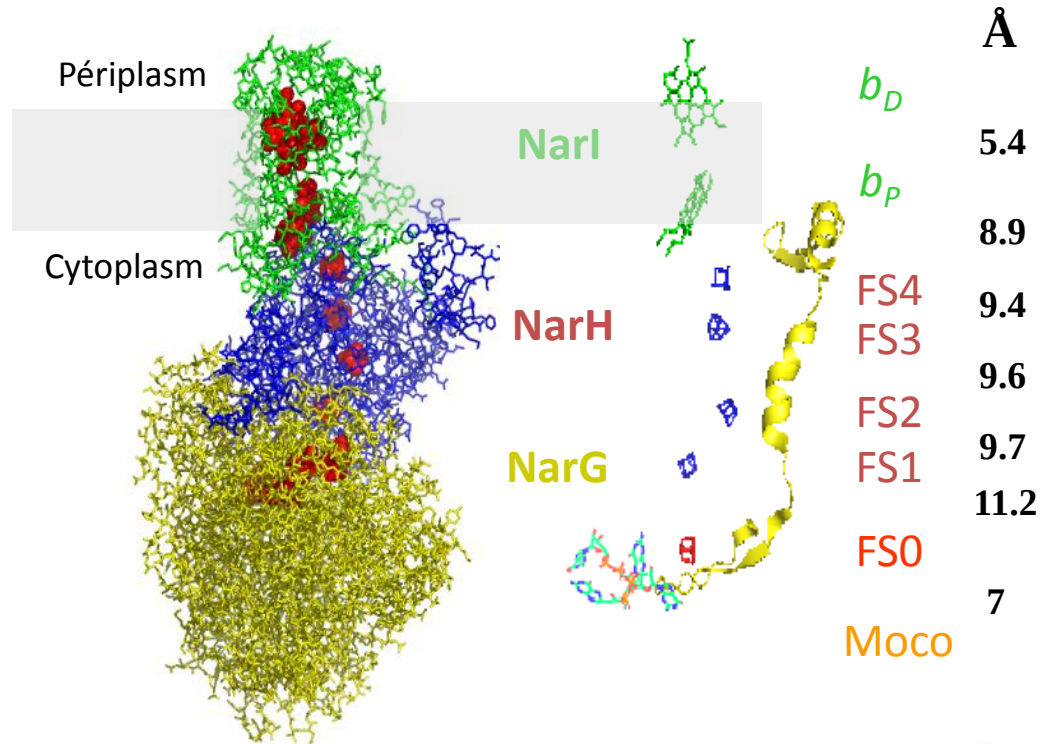
Frozen solution (60K), 2nmol, 3-4 h



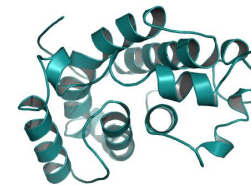
Dipolar interaction: $1/r^3$

Distance distribution $\longrightarrow$ Conformation ensemble

E. coli Respiratory Nitrate reductase complex NarGHI (MW =220 kDa)



Biogenesis of the Mo-cofactor subunit is a dynamic process involving a specific chaperone **NarJ**

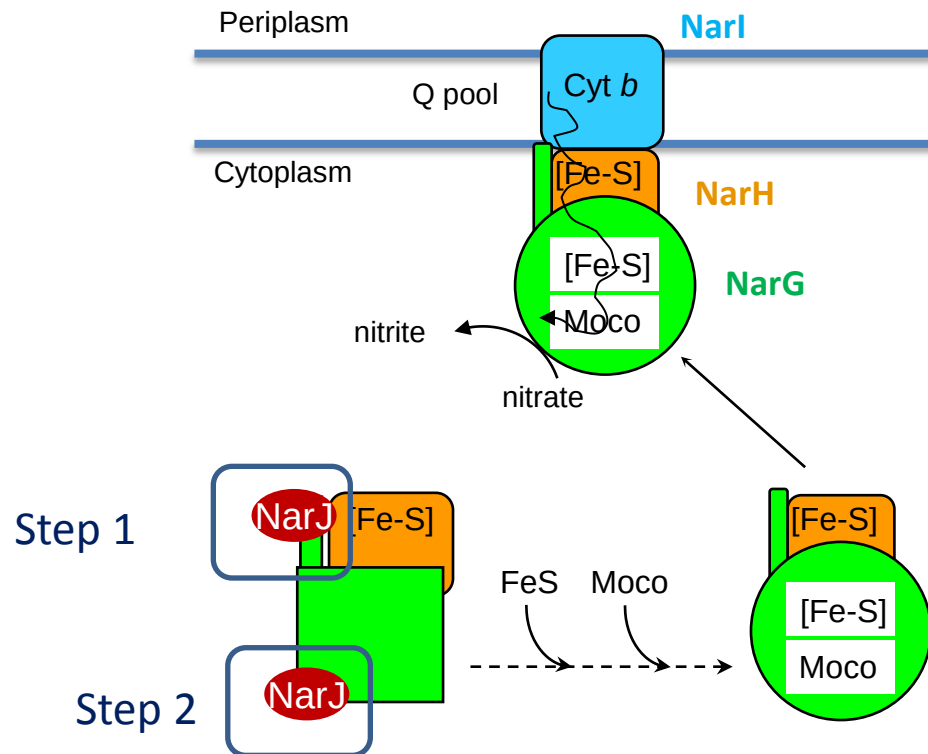


Deciphering the NarJ-NarGH interaction process by SDSL-EPR

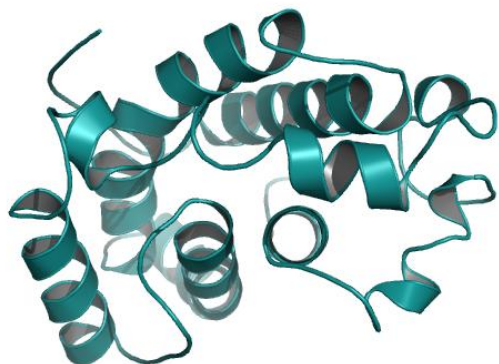
(Coll. A. Magalon, LCB)

NarJ : a specific chaperone of NarGHcomplex a multifunctional protein

- ✓ Association to the N-ter of NarG prevent premature membrane anchoring
- ✓ Insertion of metal centers (FeS et Moco)



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



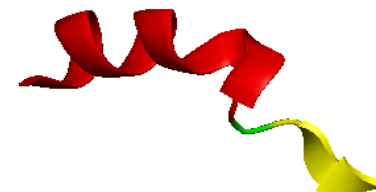
NarJ : No 3D structure - 3D model (homology modeling)

50 C-ter (over 236) aa missing due to lack of similarity

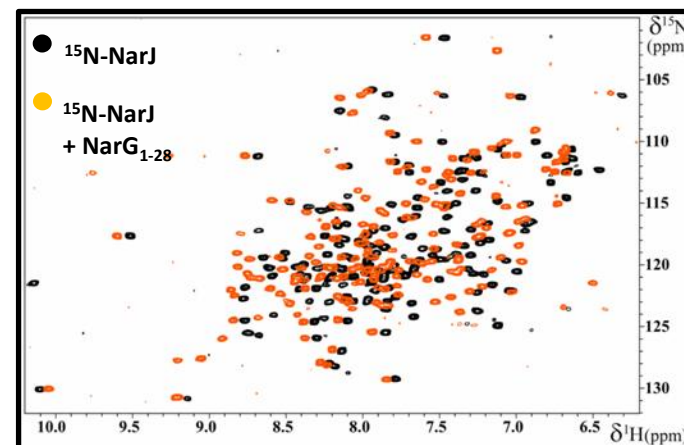
NMR studies of the NarJ/peptide association

No attribution (missing peaks)

Global shift of the peaks +/- partner



Interaction site : N-ter of NarG
= NarG(1-15)



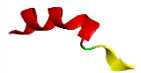
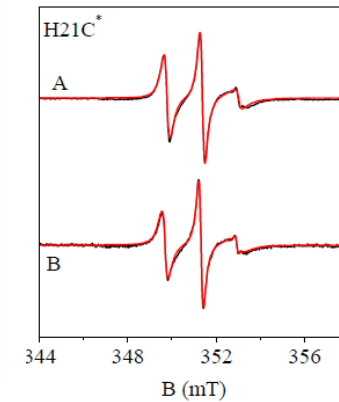
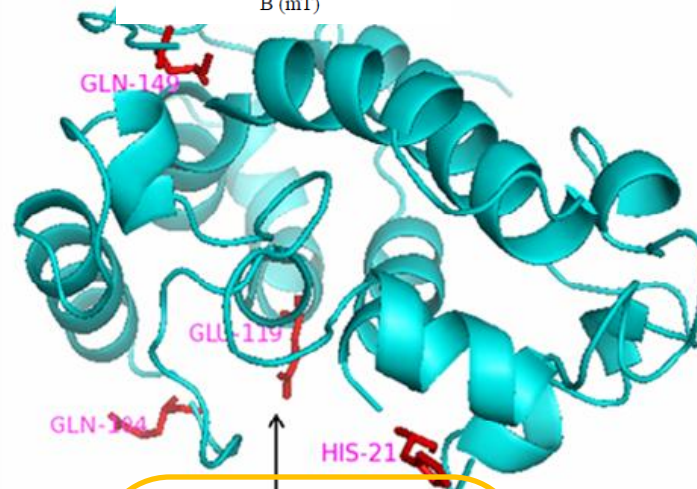
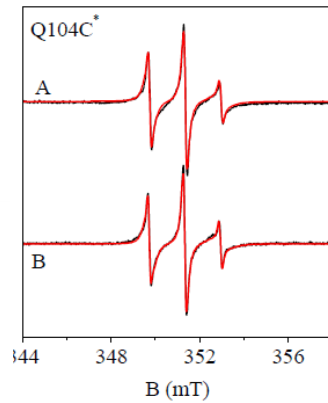
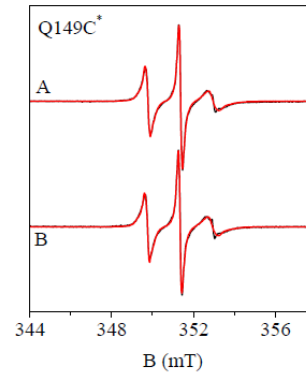
➔ Suggests global conformational change of NarJ

➔ Use of spin labels / EPR

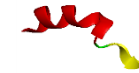
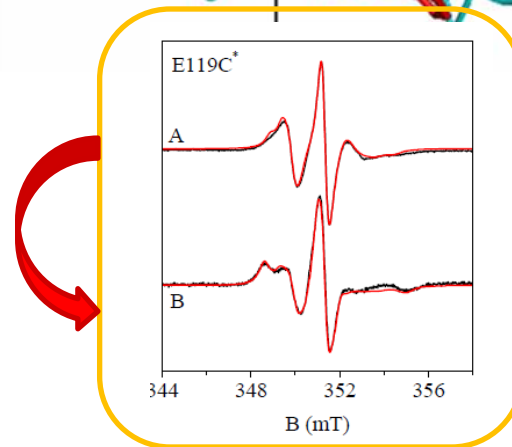
Interaction site ? Recognition Mechanism ?

(Zakian S. et al, 2010, *FEBS journal*)

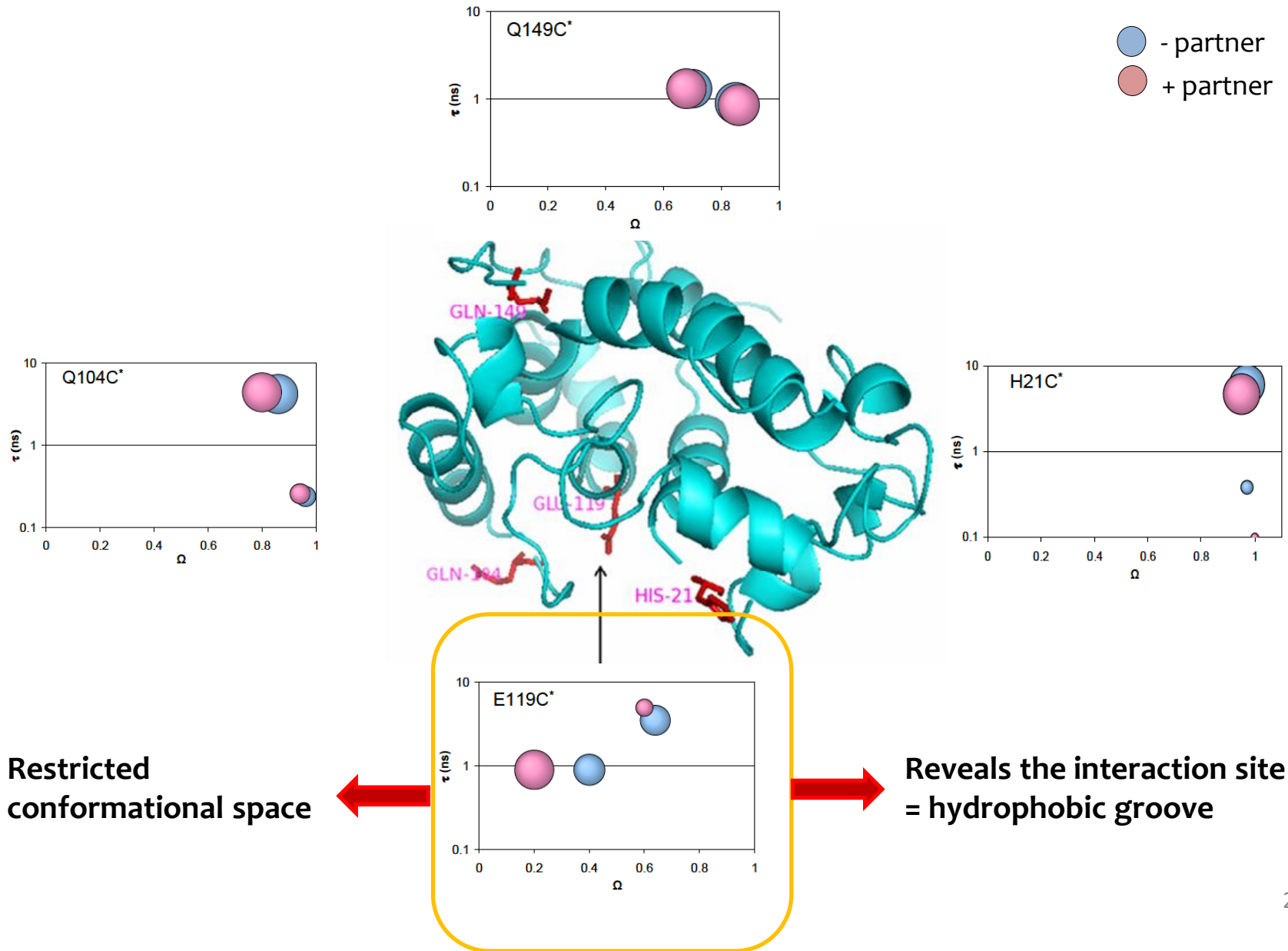
Black : experimental
Red : simulated



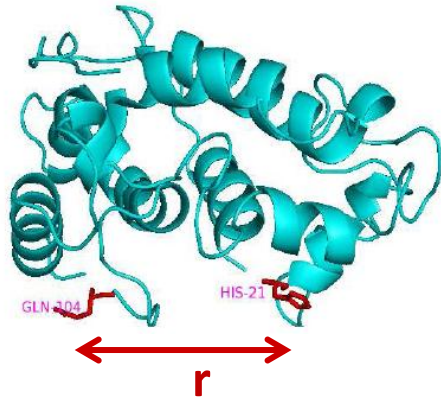
Strong spectral variations



Conformation change of NarJ upon binding

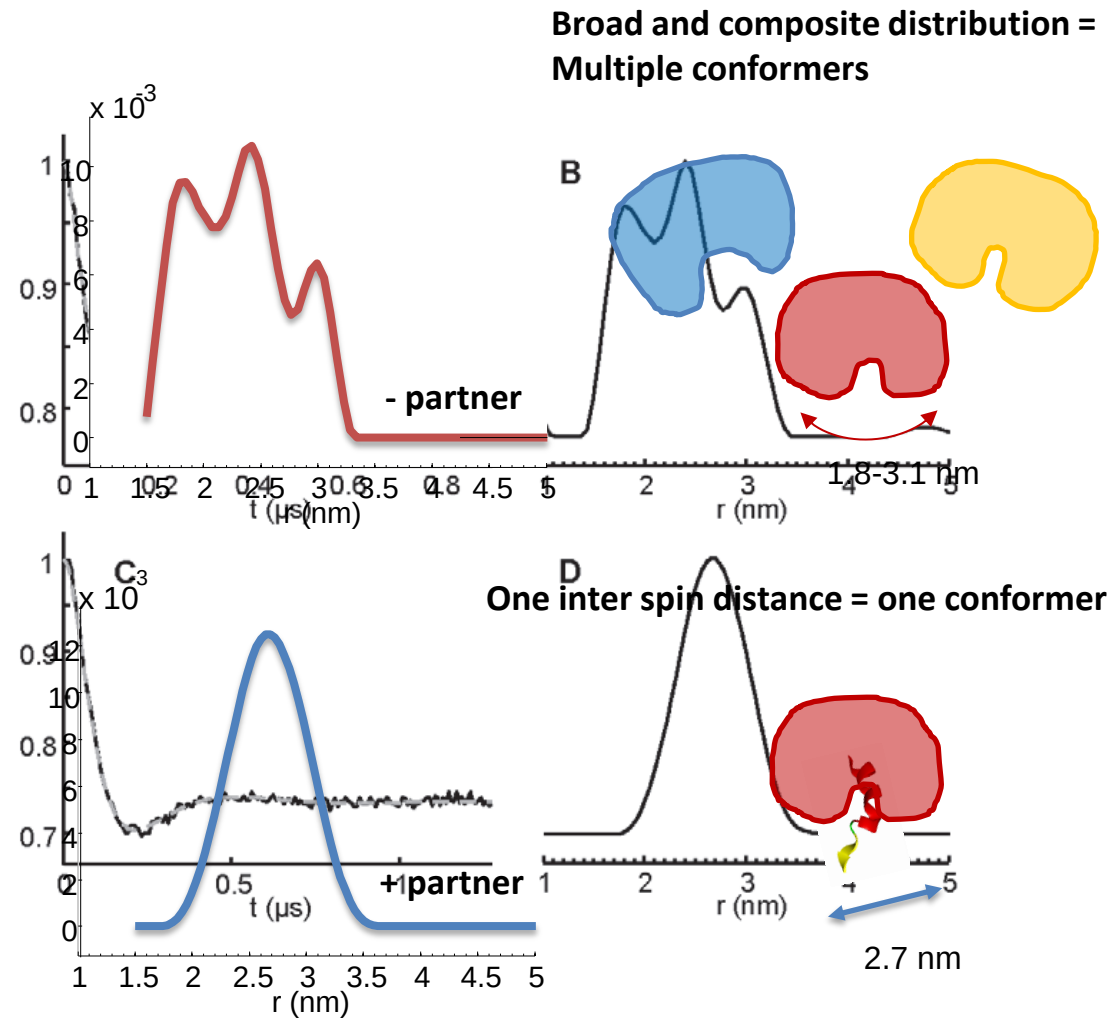


Double labeling of NarJ Q104-H21 and DEER study



No partner

With partner
NarG(1-15)

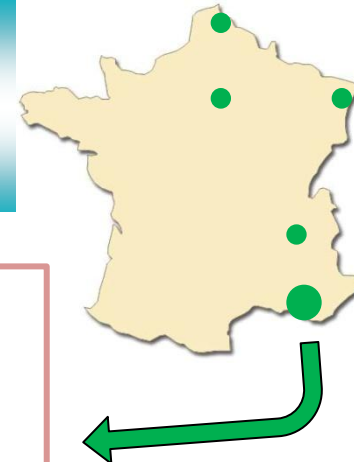


Demonstrate the structural flexibility of NarJ
Gain of rigidity of NarJ upon peptide binding

TGE-RPE - National EPR Network

Aix-Marseille Multidisciplinary EPR Center

BIP (INSB), ICR (INC), IM2NP (INP)



Multifrequency EPR (4-35 GHz)

BIP- UPR 7281 – CNRS, AMU

EPR for Life Sciences

ELEXSYS II (X, S, Q)

ELEXSYS 500 (X, S, Q)

ENDOR CW (X)

Cryogeny 1.5 – 300 K

MCD (8 T)

Pulsed EPR : ELEXSYS 580 (X, Q) :

ESEEM, HYSCORE,

ELDOR, DEER

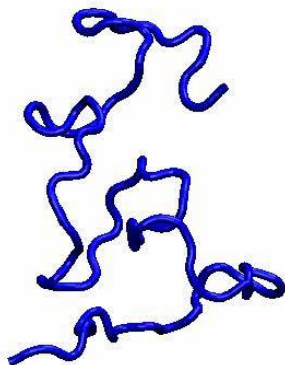


**Magnetic
Circular
Dichroism**



**Pulsed EPR
(9-35 GHz)**

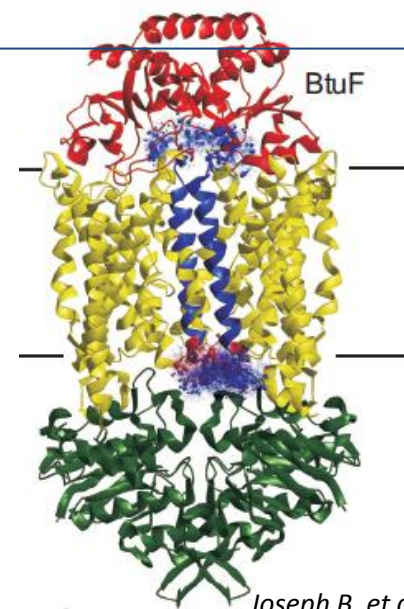
- No limit in size and complexity of biomolecules
 - Room T , Physiological conditions : in solution, membrane
 - Weak amounts : 100 pmol
 - New spin labels available (signatures, grafting sites)
 - DEER : Distances 2-8 nm (gap between Electron Microscopy, X-ray crystallography)
- Distances distribution, conformation ensemble

**Disordered Proteins**

Induced folding,
Dynamics

**Globular proteins**

Conformation transitions
Interaction with partners

**Membrane complexes**

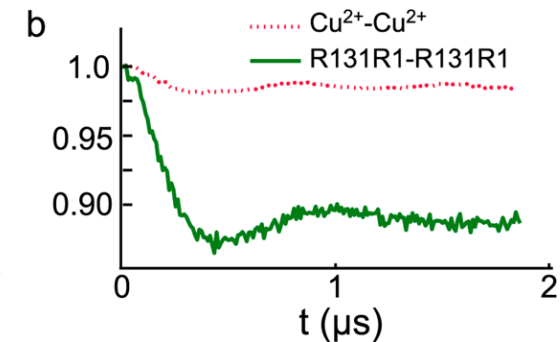
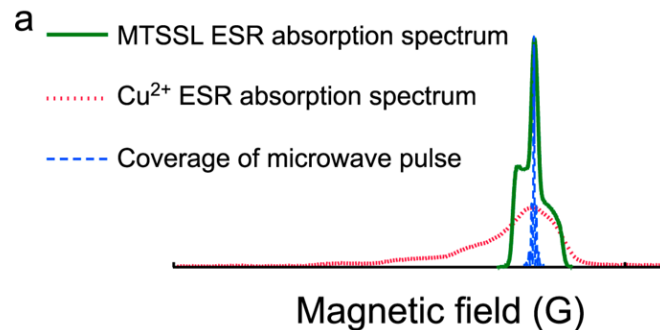
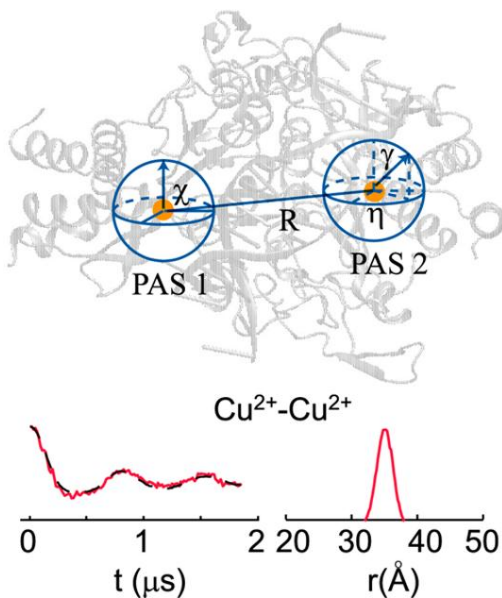
Topology and Organization
Long range mechanisms
Translocation

+ Anisotropy of Metal EPR signals → Orientation selection study

Distance and orientation of centers

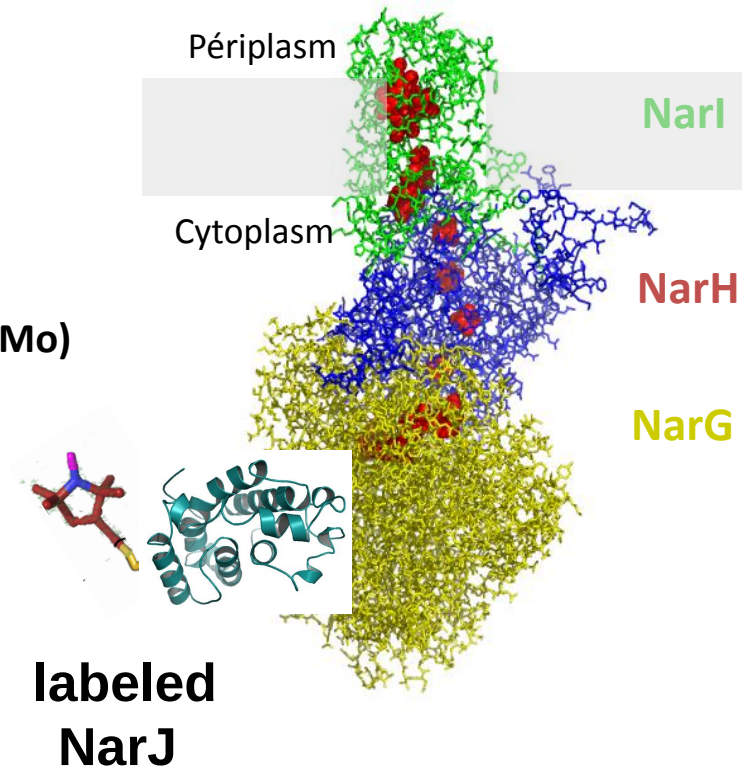
- Low sensitivity (broad signal)
- Fast relaxation properties (shorten distance range)

Cu^{2+} - Cu^{2+} DEER in *EcoR1*-DNA complex (Yang, PNAS, 2012)

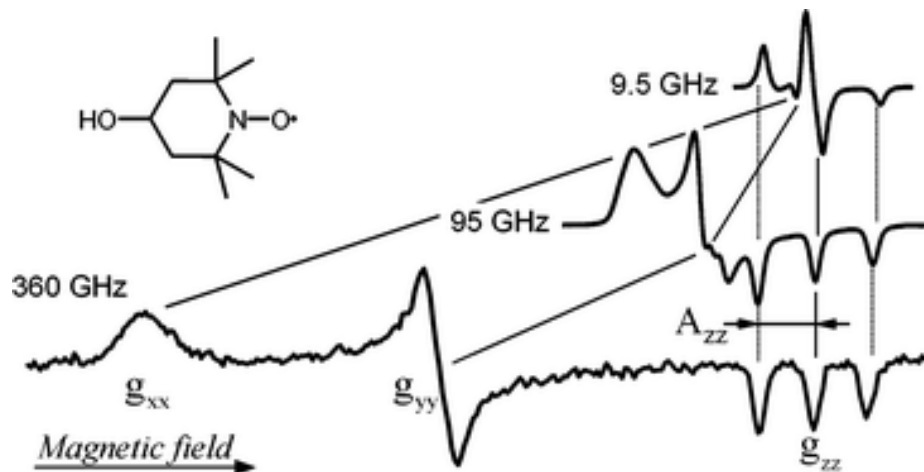


NarJ-NarGH interaction process by SDSL-EPR
(Coll. A. Magalon, LCB)

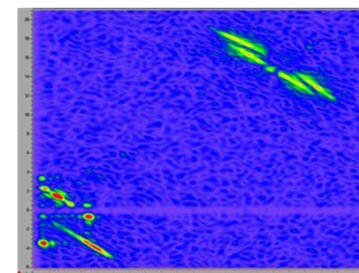
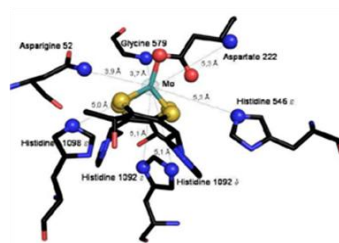
DEER NarJ-RNO^o/Metal cofactor (heme, FeS, Mo)



- + High resolution
- + Anisotropy of EPR signals
→ Orientation selection study
- Distance and orientation of centers**
- + High sensitivity



W-Band EPR (95GHz) project in BIP Marseille
- Venue of A. Ivancich group at BIP



BIP- CNRS & AMU



Valérie BELLE

Elisabetta MILEO

Marlène MARTINHO

Guillaume GERBAUD

Nolwenn LEBRETON

Bénédicte BURLAT

Frédéric BIASO

Stéphane GRIMALDI

Eric PILET

Emilien ETIENNE

Julien JACQUES

Julia RENDON



Sonia LONGHI



Axel MAGALON



Brigitte GONTERO

ICR

Paul TORDO

Sylvain MARQUE



Janez STRANCAR

Ljubljana

Région



Provence-Alpes-Côte d'Azur



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BIP- CNRS & AMU



**Valérie BELLE
Elisabetta MILEO
Marlène MARTINHO
Guillaume GERBAUD
Nolwenn LEBRETON**

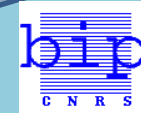
**Bénédicte BURLAT
Frédéric BIASO
Stéphane GRIMALDI
Eric PILET
Emilien ETIENNE
Julien JACQUES
Julia RENDON**



Sonia LONGHI



Axel MAGALON



Brigitte GONTERO

ICR

**Paul TORDO
Sylvain MARQUE**



**Janez STRANCAR
Ljubljana**

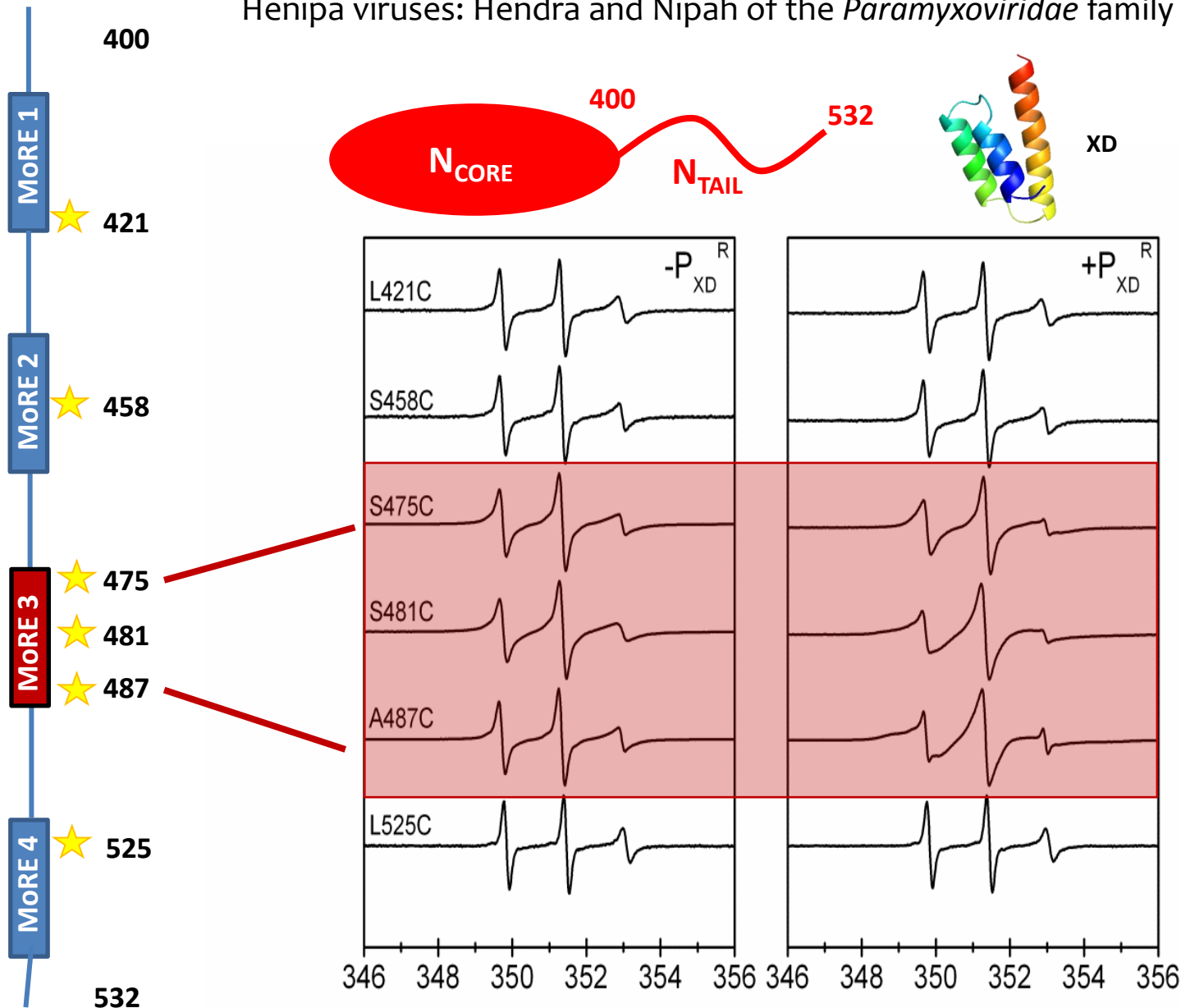
Région



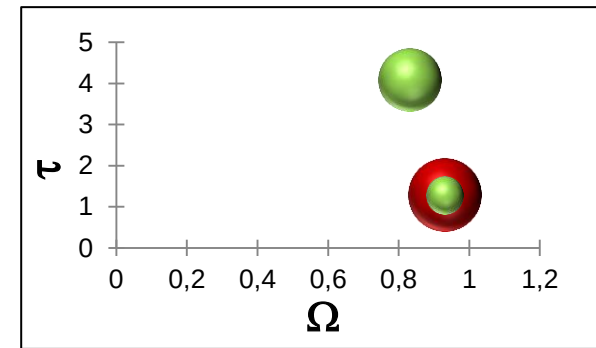
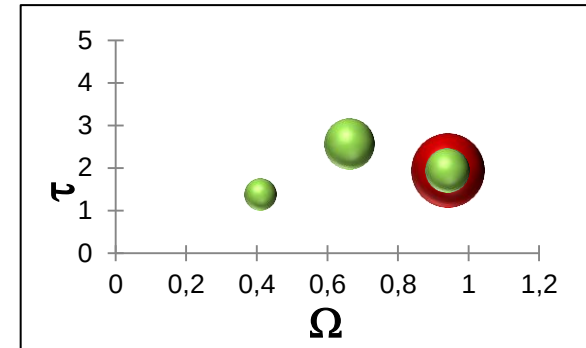
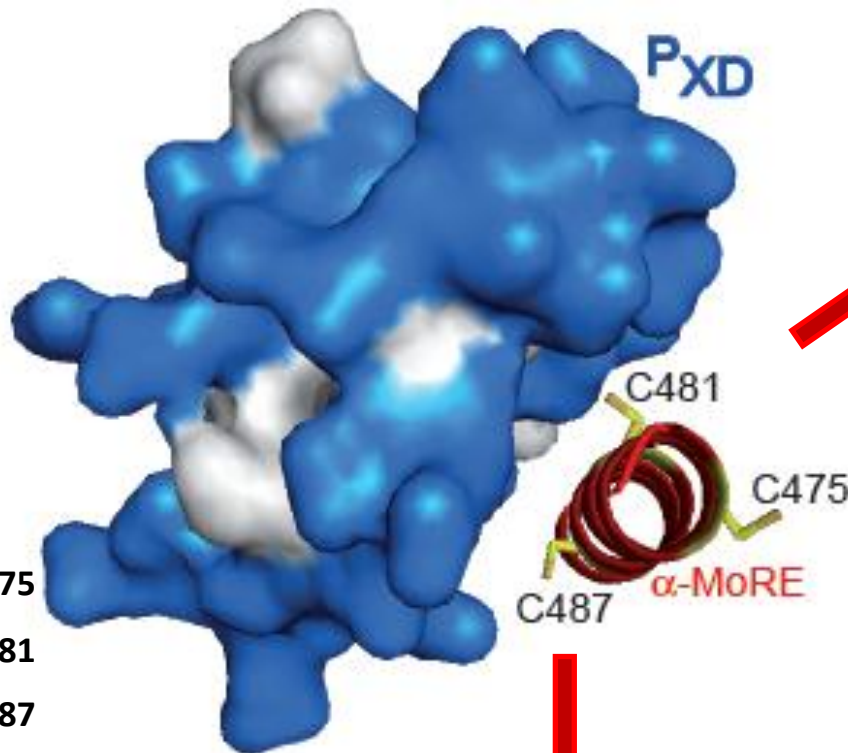
Provence-Alpes-Côte d'Azur



Henipa viruses: Hendra and Nipah of the *Paramyxoviridae* family



Habchi et al, JBC, 2011



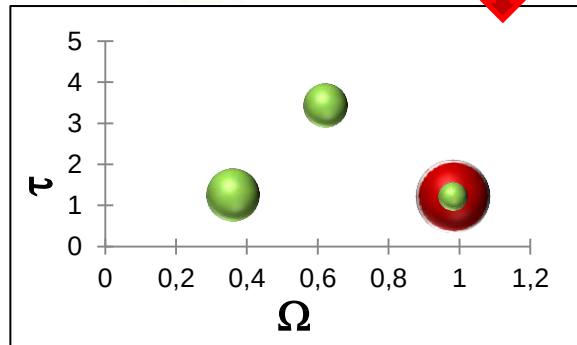
Induced α -helix in MoRE 3

481 and 487 in tertiary contact with P_{XD}

475 solvent-exposed

Validation of the structural model of the complex

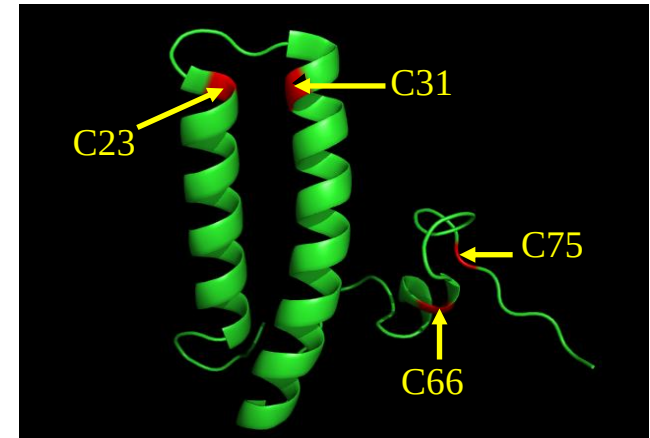
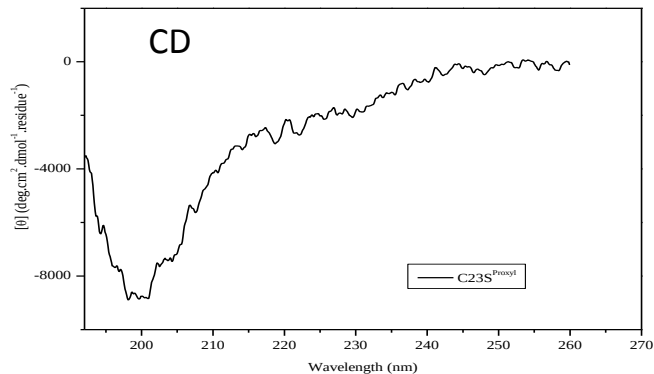
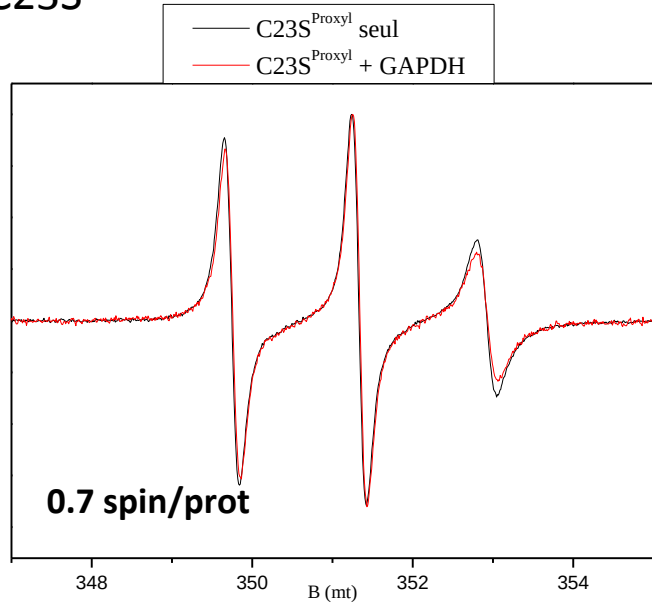
Martinho et al, JBSD, 2012



Mutants of CP12 labeled with proxyl

CP12 mutants C23S and C31S labeled with proxyl

C23S^{Proxyl}



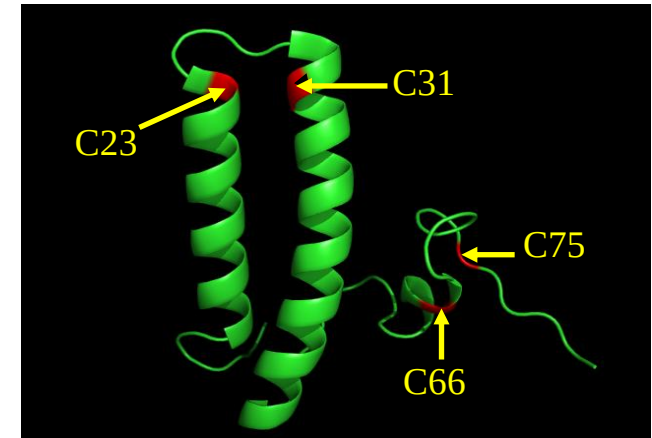
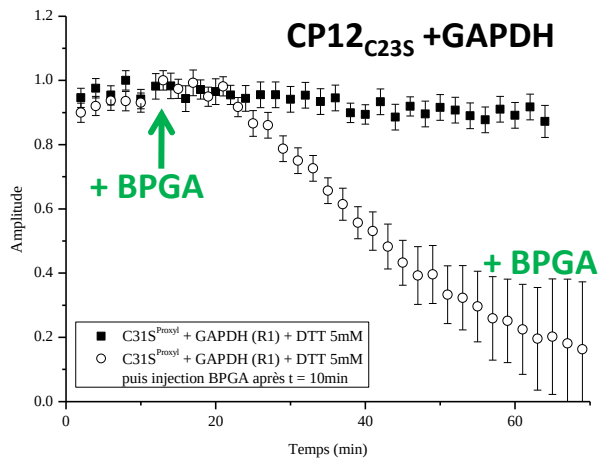
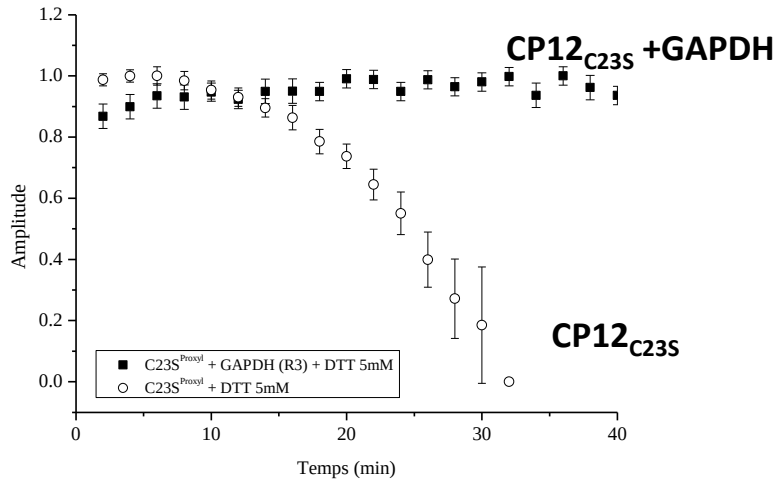
- MS : proxyl label on C31
- CD: Fully disordered protein
- Complex formation with GAPDH and inhibition
- No release of the proxyl spin-label
- **High mobility of the mutated CP12 in the CP12-GAPDH complex**

Similar results with C31S^{Proxyl}

Mutants of CP12 labeled with proxyl

CP12 mutants C23S and C31S labeled with proxyl

C23S^{Proxyl} : reduction of RNO[•] by DTT



- GAPDH protects the spin label in the complex
- Addition of BPGA remove this protection

Marquage de la région C-ter de NarJ

1

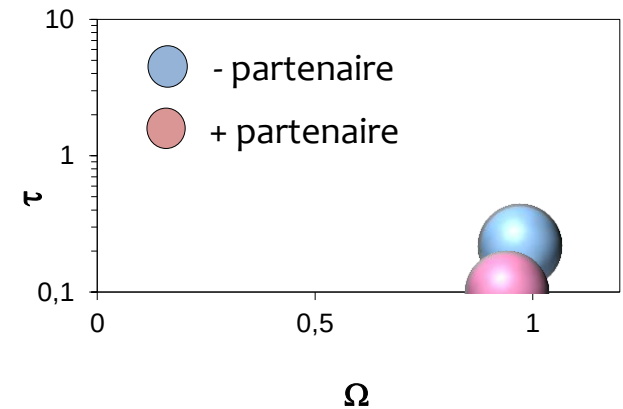
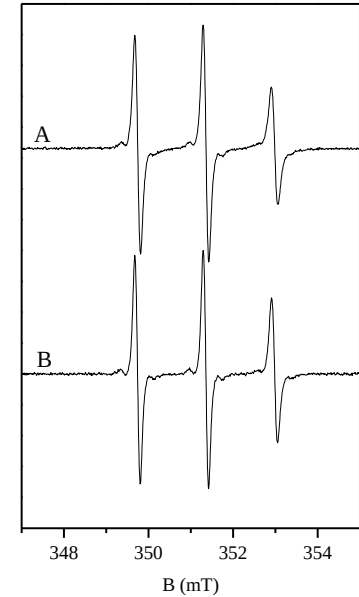
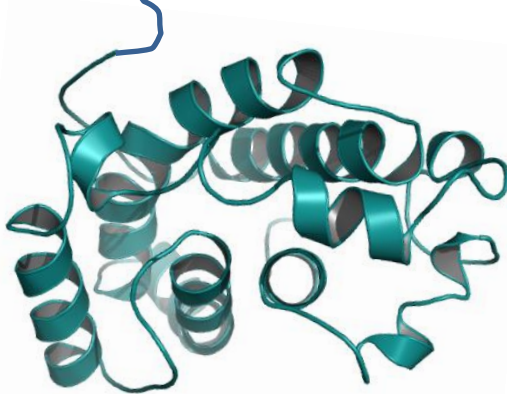
186

236



Cystéine naturelle en position 207

C207



➡ Révèle la région C-ter (à proximité de aa 207) de NarJ désordonnée
Reste désordonnée en présence du partenaire

Mise en évidence de différents états conformationnels

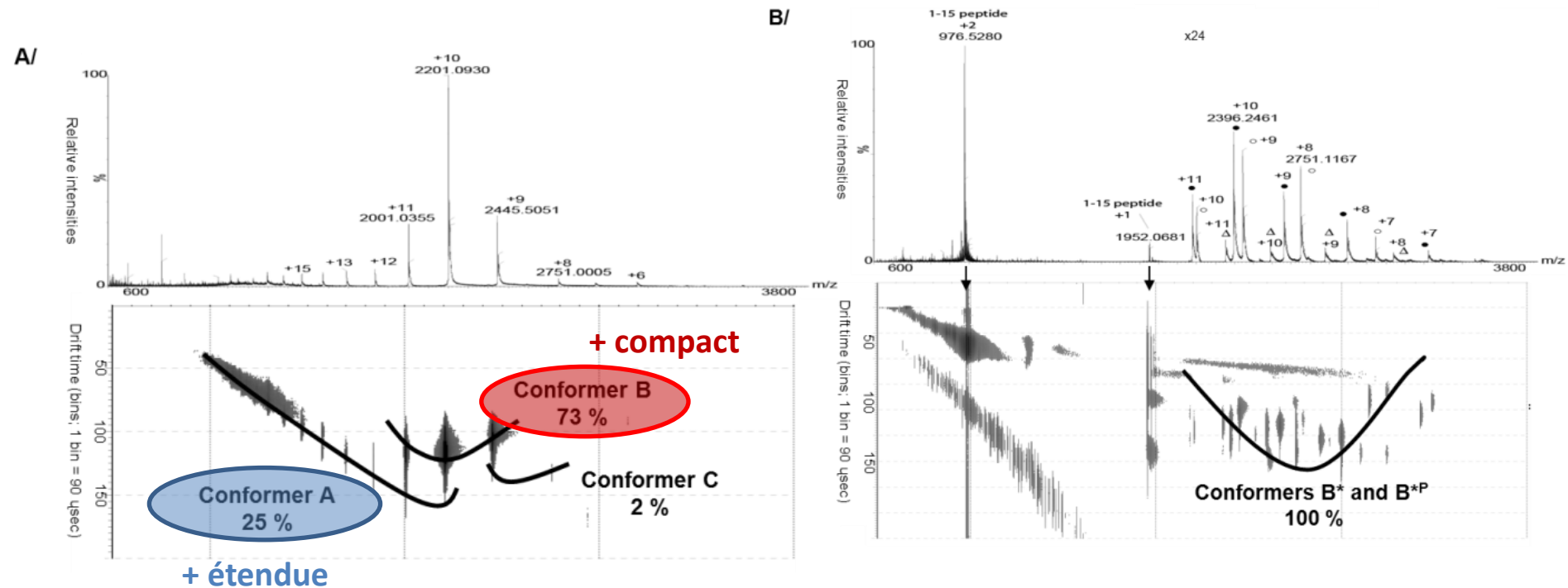
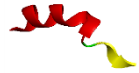
ESI MS et ESI-TWIM-MS (Mobilité Ionique)

ESI en condition non dénaturante (préserve le complexe)

IMS : à m/z cst temps de vol différent selon la section efficace de collision

sans partenaire

avec partenaire



- ✓ ensemble conformationnel qui évolue en 1 proche du conformère B = B*
- ✓ En accord et complémentaire des données RPE