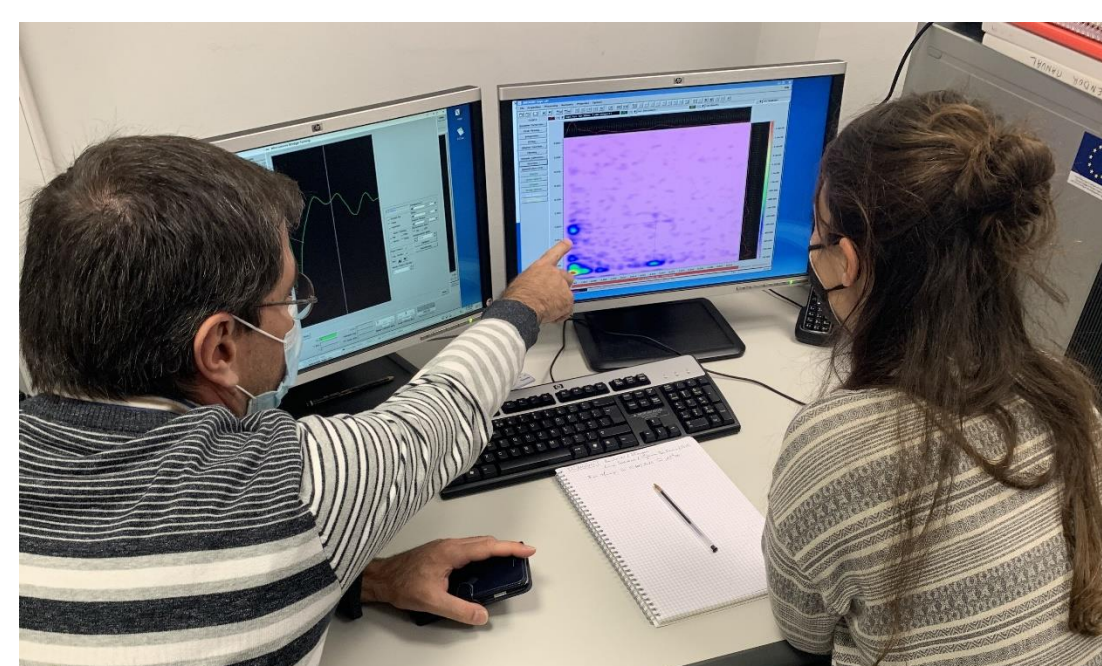
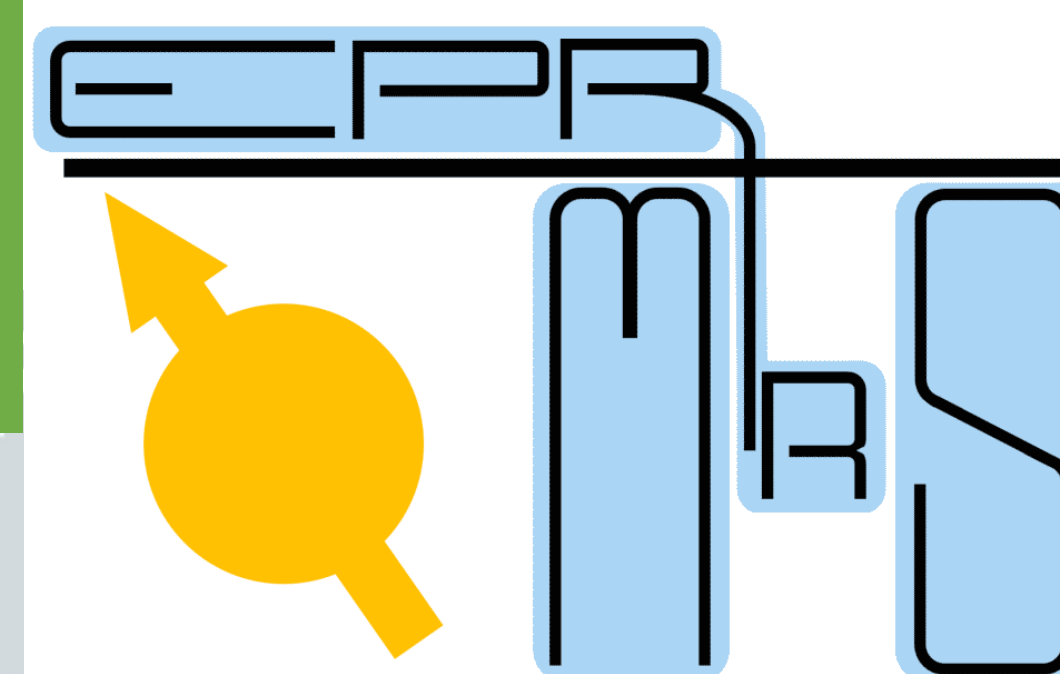


EPR-MRS, Marseille, FR

Electron Paramagnetic Resonance facility



Valérie BELLE
Pierre DORLET
Guillaume GERBAUD
Emilien ETIENNE
Bruno GUIGLIARELLI

Probe paramagnetic centers and their near vicinity

Paramagnetic centers = metals or free radicals
(electronic spin $S \neq 0$)

- 1) Endogeneous electronic spin (ex redox enzymes)
 - determine the metal content
 - provide the magnetic characterization of these centers
 - reveal magnetic nucleus in the vicinity
- 2) Exogeneous electronic spin
 - => insert paramagnetic labels!
 - study macromolecule dynamics
 - detect structural transitions, folding events, ...
 - measure inter-label distances

EPR-MRS specificities

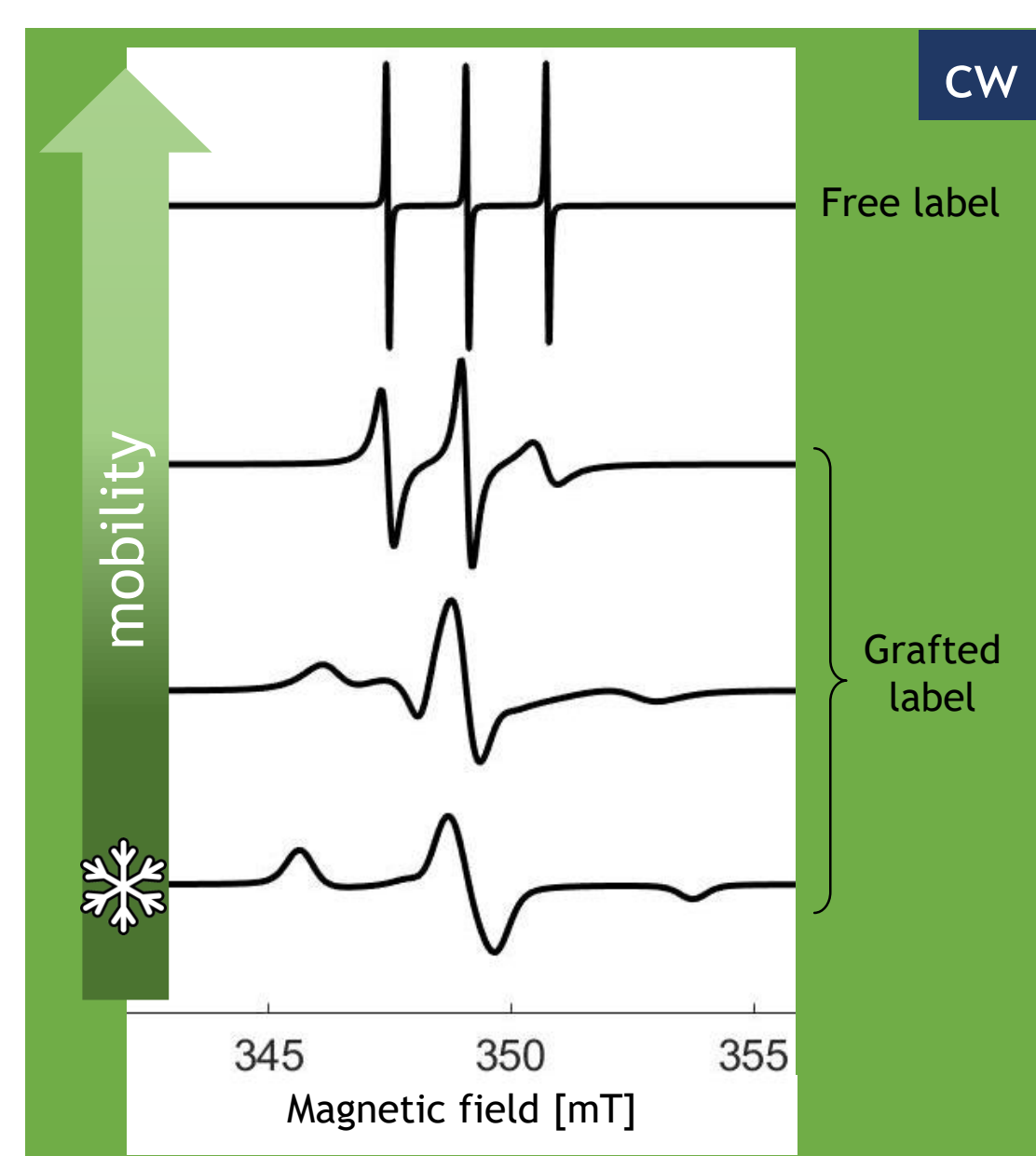
- outstanding multifrequency spectrometers (S, X, Q, W bands),
- continuous wave (cw) and pulsed mode,
- 2 facility managers and 9 researchers,
- the most important French EPR facility dedicated to life sciences.



Methodology

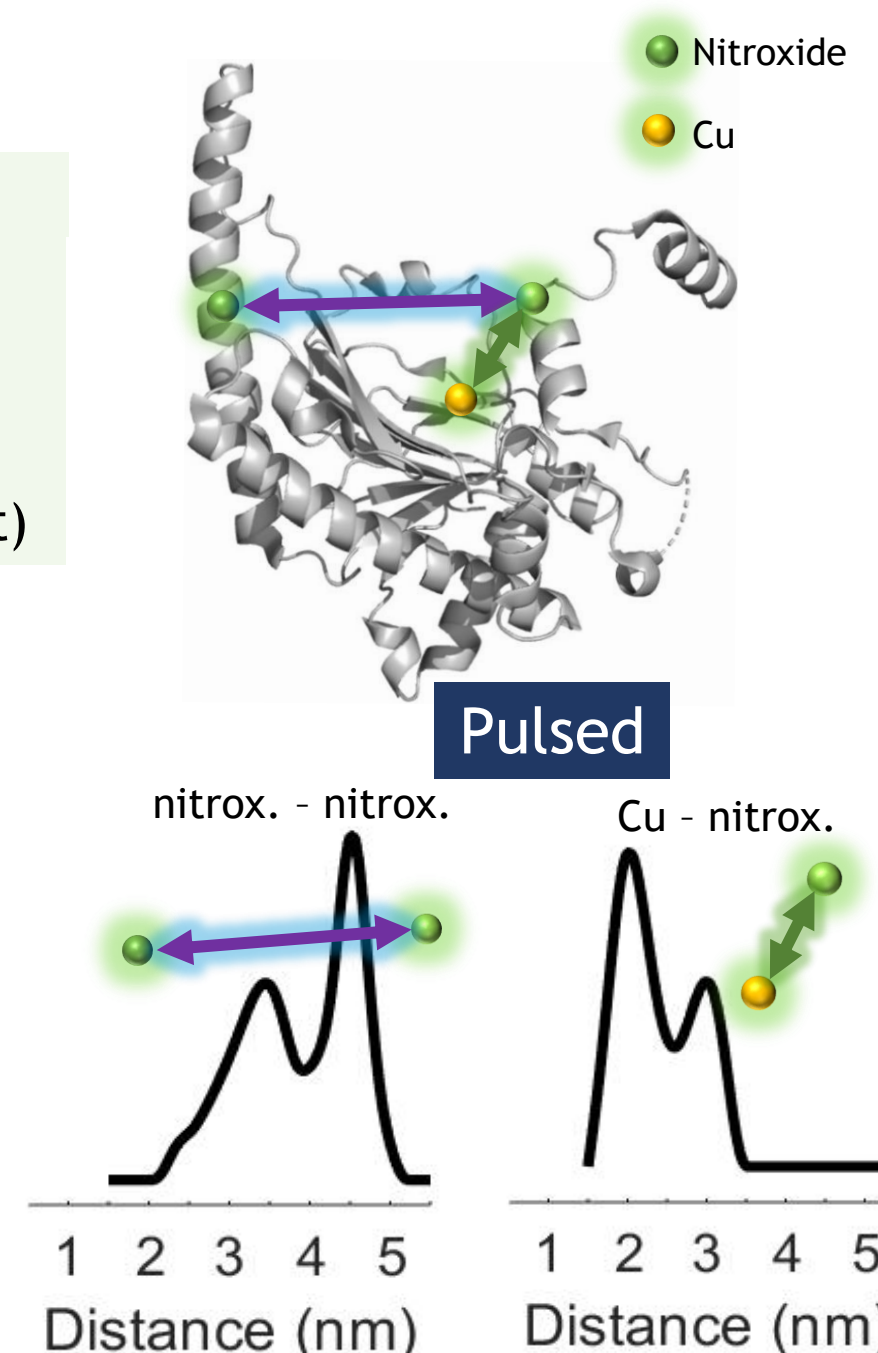
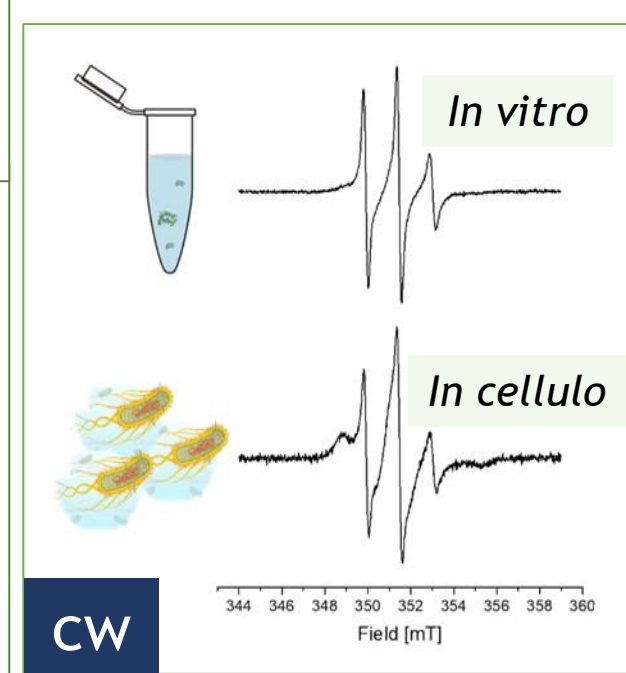
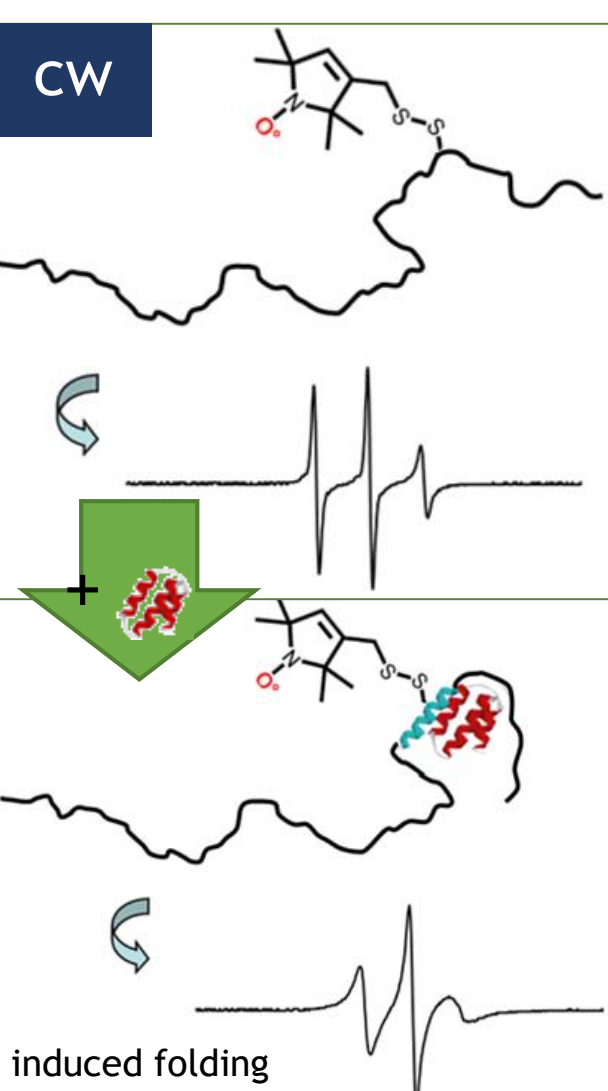
No electronic spin inside!

Site directed Spin Labelling coupled with EPR



Grafted spin label (usually nitroxide on Cys):

- Mono-labelling
=> cw EPR
- Bi-labelling
=> pulsed EPR (distance measurement)



Sample requirements

- For Spin Labelling, 50-100 nmoles are required.
cw-EPR: 10-50 μ M in 50 μ L (RT - ~min)
pulsed EPR 60-100 μ M in 15 μ L (50K - hours)
- For metalloproteins
cw and pulsed EPR: typical concentration 50-100 μ M in 100-150 μ L (4K to RT)

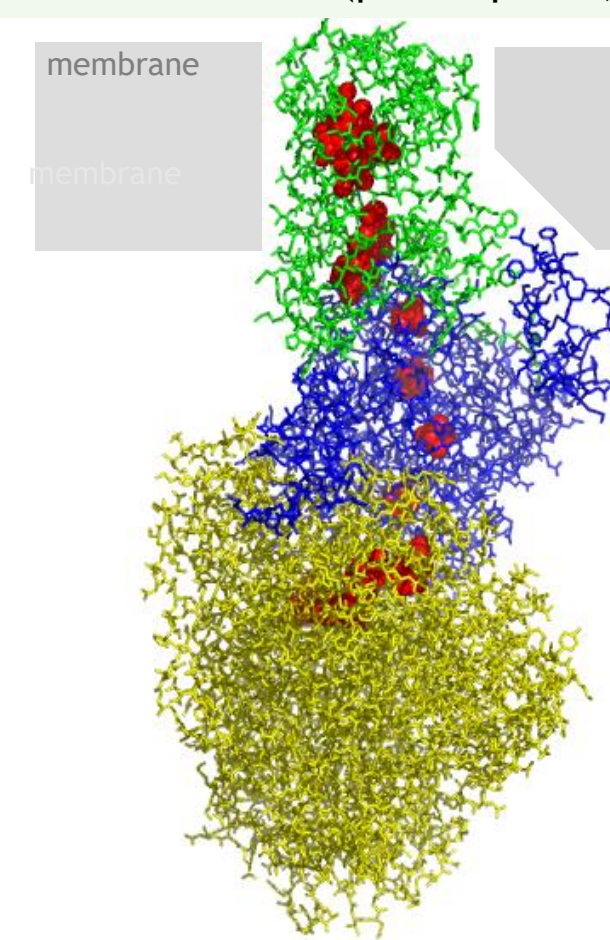


- Mapping molecular interactions between physiological partners
- Study of structural transitions and folding / unfolding events
- In-cell biostructural EPR

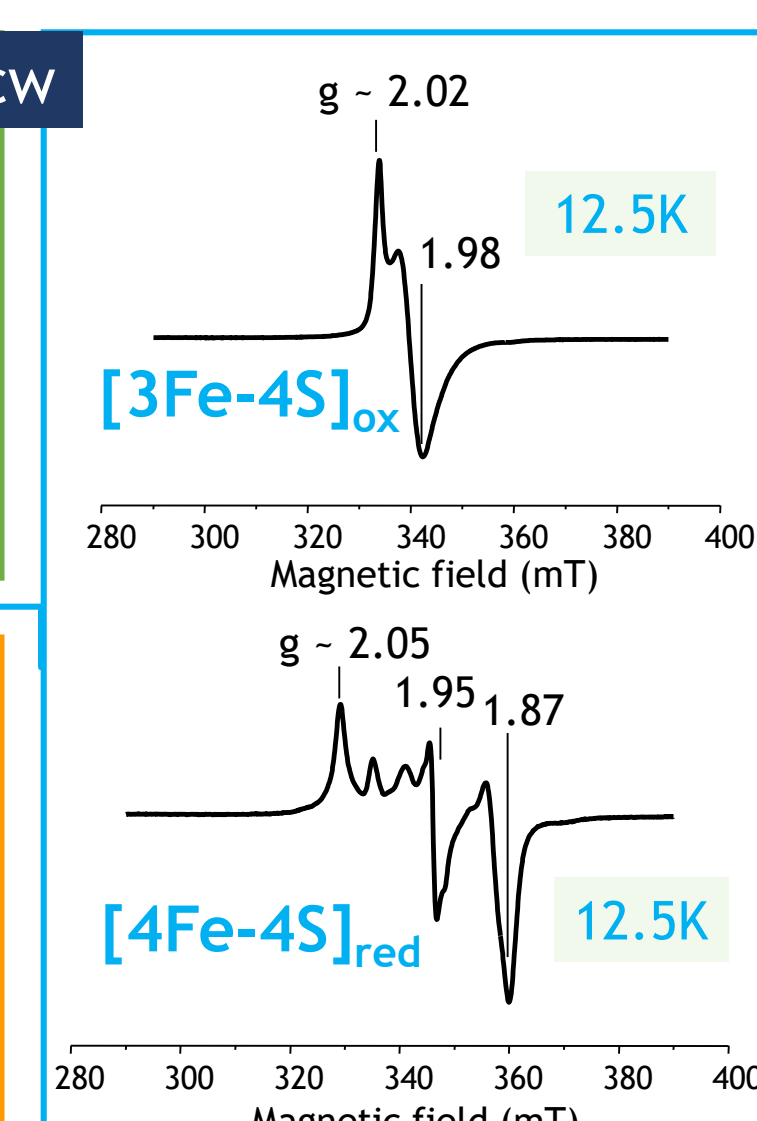
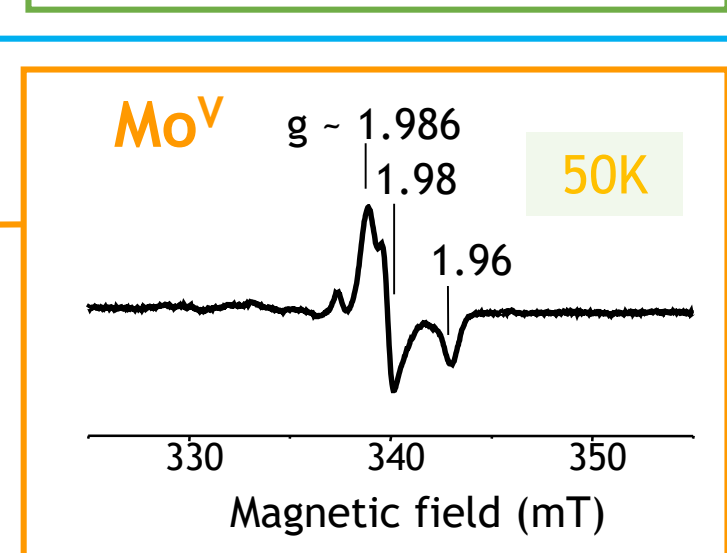
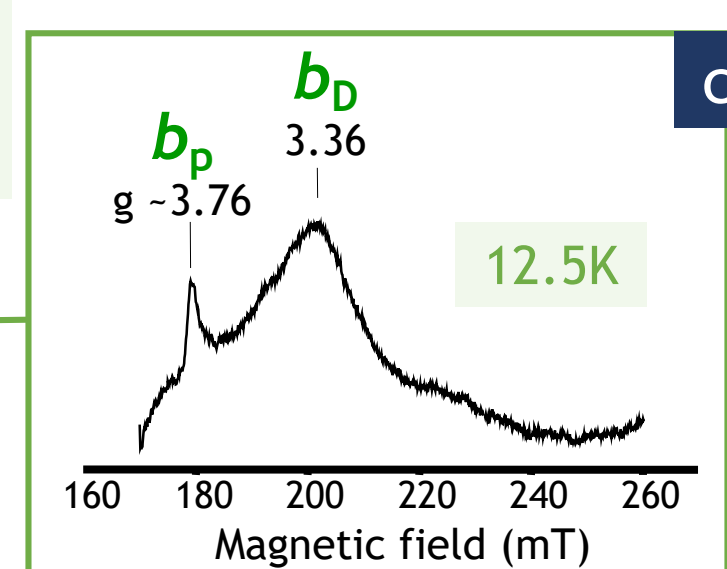
Electronic spin inside!

Determination of metal content / Analysis of metal cofactors / Detection of free radicals

- Specific spectral signature
- Magnetic centers selected by experimental conditions (μ wave power, temperature, redox state)

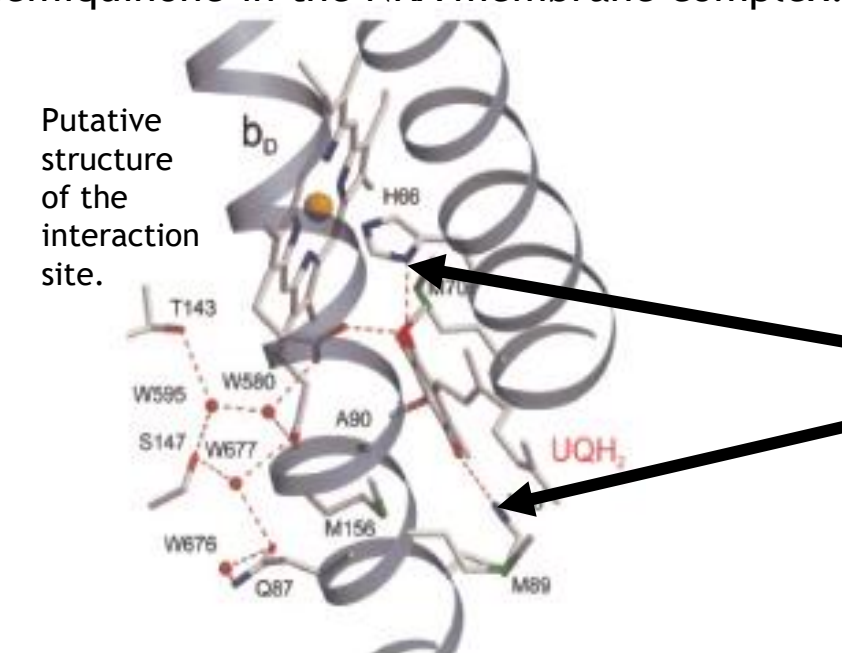


Heme b_D
Heme b_P
FS4
FS3
FS2
FS1
FS0
Moco

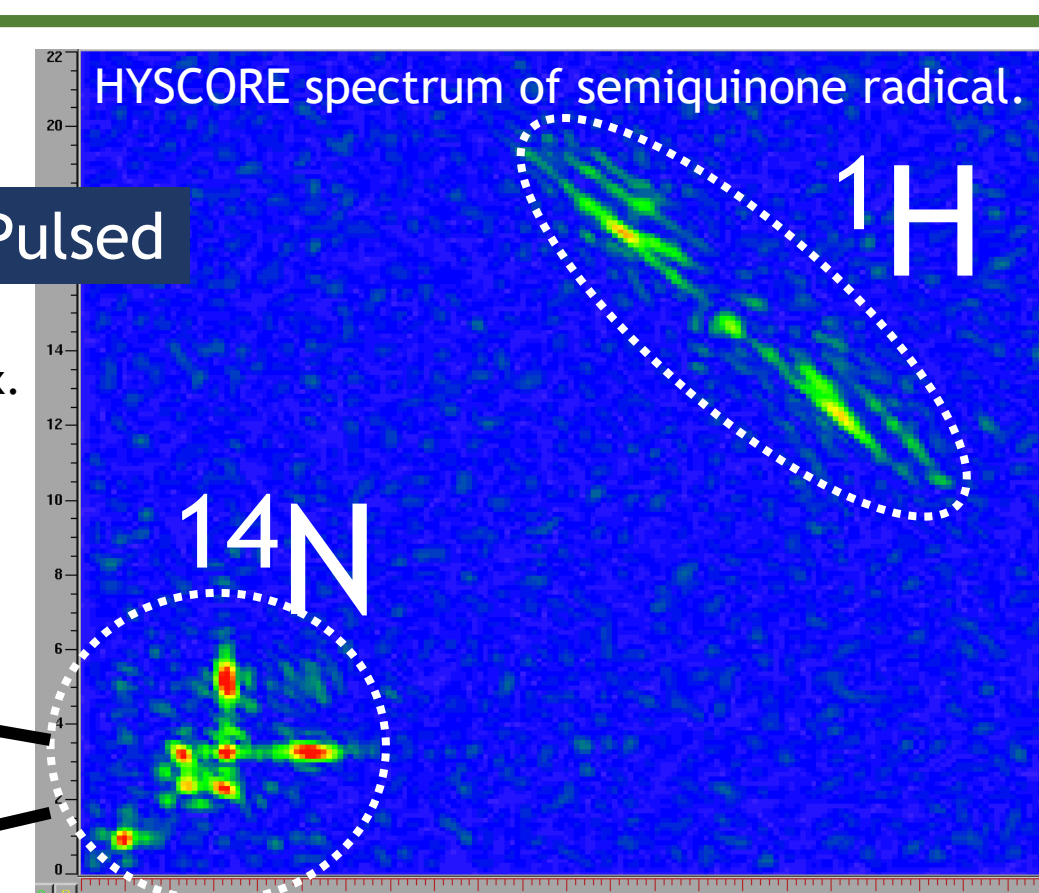


Atomic resolution

Detection of nitrogen atoms stabilizing semiquinone in the NRA membrane complex.



Pulsed



- Microbial bioenergetic metabolism
- Molecular sensitivity to stress factors (oxidative, thermal, chemical stress)
- Enzymatic engineering

Some recent publications :

1. Pierro, et al., Biomolecules 2020
2. Torricella et al., Biochim. Biophys. Acta 2021
3. Karthikeyan et al., Angew. Chem. Int. Ed., 2018
4. Bonucci et al., ChemBioChem, 2020
5. Fournier, et al., Chemistry - A European Journal 2019
6. Fournier, et al., Actualité Chimique, 2019
7. Rendon et al., Inorg. Chem. 2017
8. Seif Eddine et al., Chem. Phys. Chem. 2017
9. Arias-Cartin R. et al., J. Biol. Chem. 2022
10. Koebke et al., Angew. Chem. Int. Ed., 2021
11. Al Attar et al., ACS Catalysis, 2021