Characterizing the interaction between Tau and microtubules using Site-Directed Spin labeling EPR spectroscopy

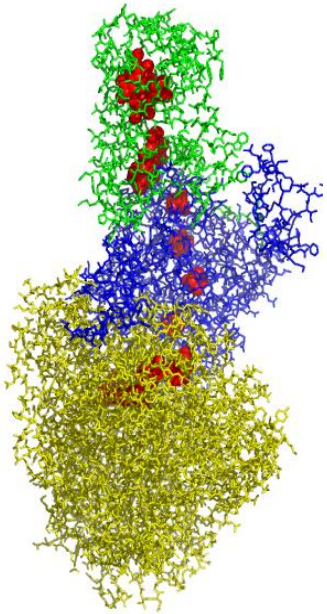
DR. MARLÈNE MARTINHO

LABORATOIRE DE BIOÉNERGÉTIQUE ET INGÉNIERIE DES PROTÉINES, BIP MARSEILLE, FRANCE

EPR and Site-Directed Spin Labeling (SDSL)

EPR is a technique for studying systems with unpaired electrons

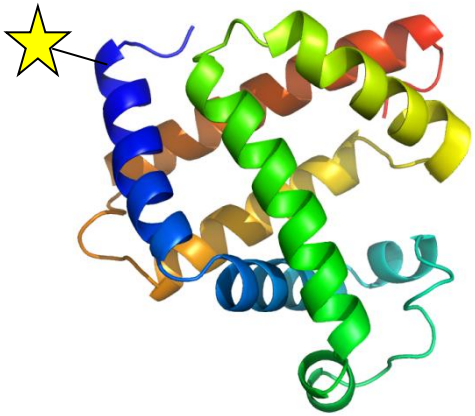
Intrinsic paramagnetic probe



Centers or metal clusters: Fe, Cu, Ni, Mo ...
Radicals: Semiquinone, Tyr °, S °, ...

Catalytic mechanisms
Electron transfer mechanisms

Extrinsic paramagnetic label



Site-Directed Spin Labeling (SDSL)
Structural transitions in proteins

SDSL-EPR : A powerful technique

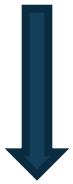
Structural transitions in proteins

Structural transitions involved in many biological processes

Conformational changes
Protein-protein interactions

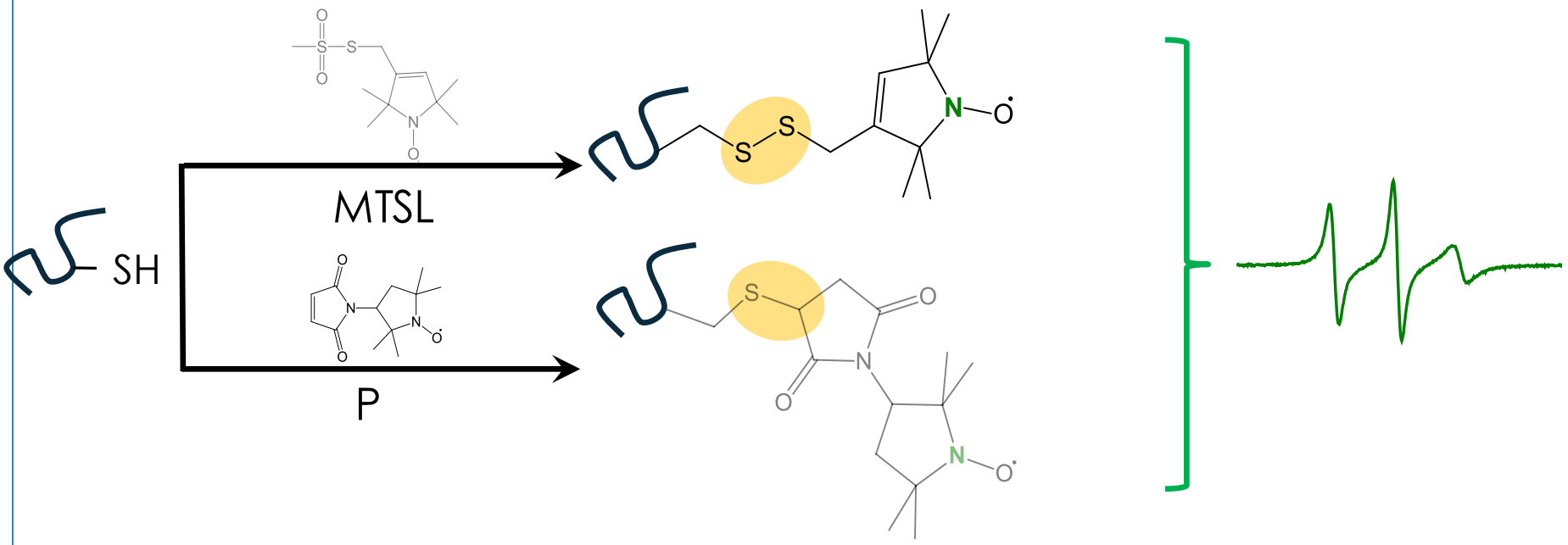
Various biological systems

From disordered to globular proteins

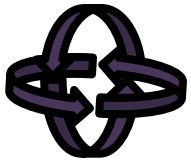


Site-Directed Spin Labeling combined with EPR spectroscopy

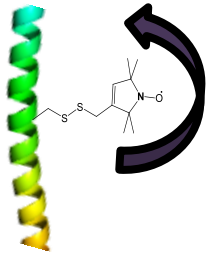
Site-Directed Spin Labeling approach

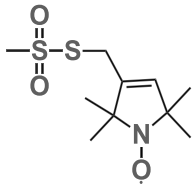


SDSL and CW EPR

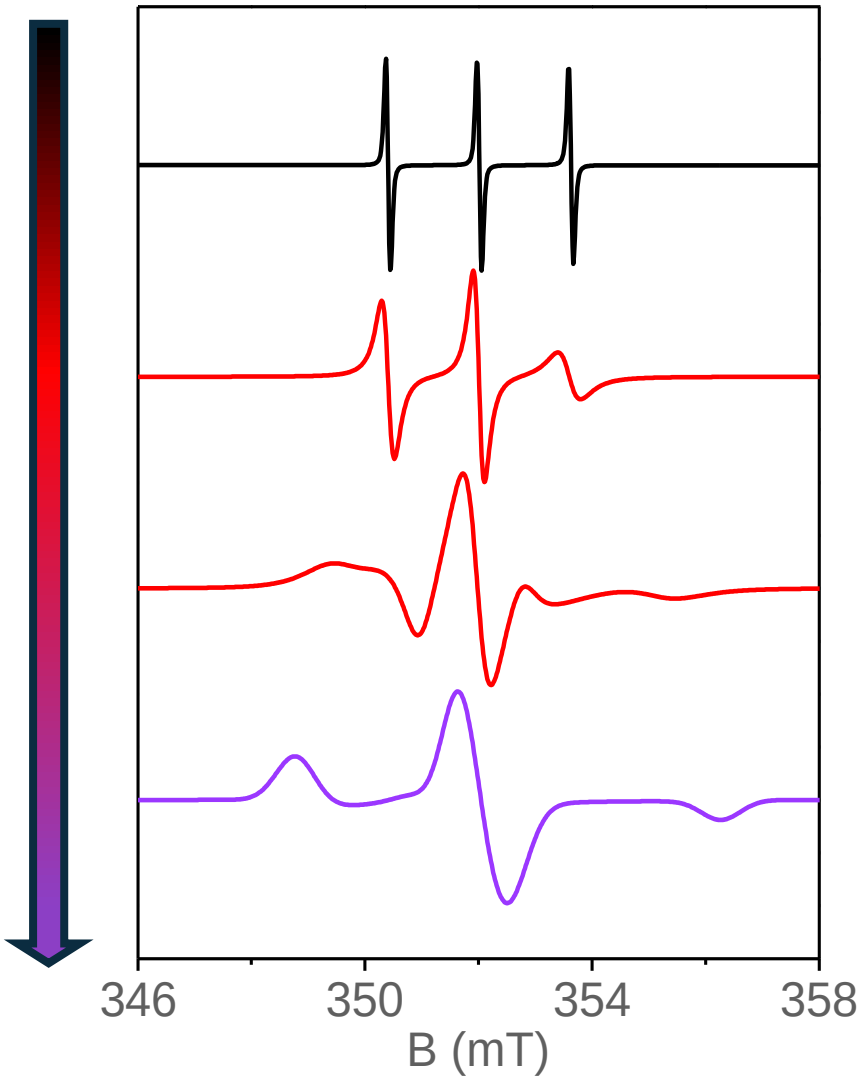


Free label
in solution

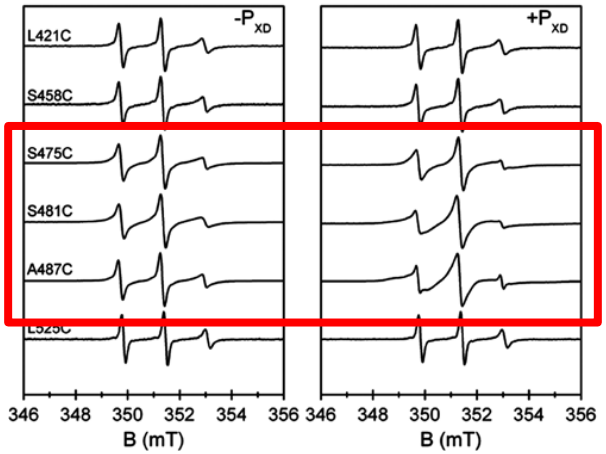




Immobilized
label



Mapping protein-protein
interaction region



S481C N_{TAIL} + P_{XD}

63 rotamers

A487C N_{TAIL} + P_{XD}

53 rotamers

Martinho, M., et al. *J. Biomol. Struct. Dynam.* **2013**

SDSL – several approches

Spin labeling

SDSL – several approches

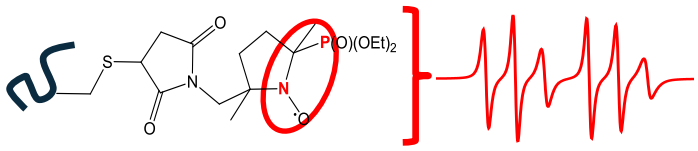
Martinho, M., et al. *J. Biomol. Struct. Dynam.* **2013**

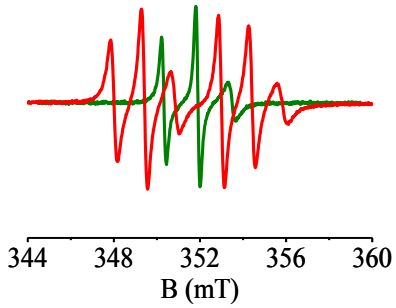
E. Etienne* N. Le Breton, M. Martinho, E. Mileo and V. Belle* *Magn. Res. Chem.* **2017**

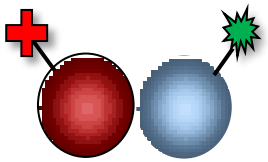
SDSL – several approches

Spin labeling → **on cysteine**

Spin labeling → **new spectral signature**







MTSL or P

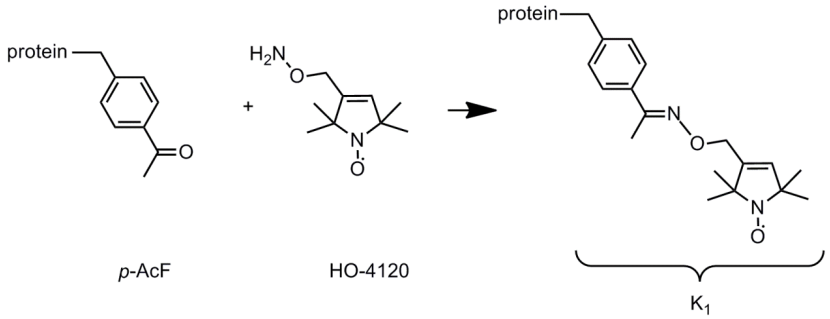
PP

Le Breton, N.; Martinho, M.*; Kabytaev, K.; Topin, J.; Mileo, E.; Blocquel, D.; Habchi, J.; Longhi, S.; Rockenbauer, A.; Golebiowski, J.; Guigliarelli, G.; Marque, S. R. A.; Belle, V.* *PCCP* ***2014***

SDSL – several approches

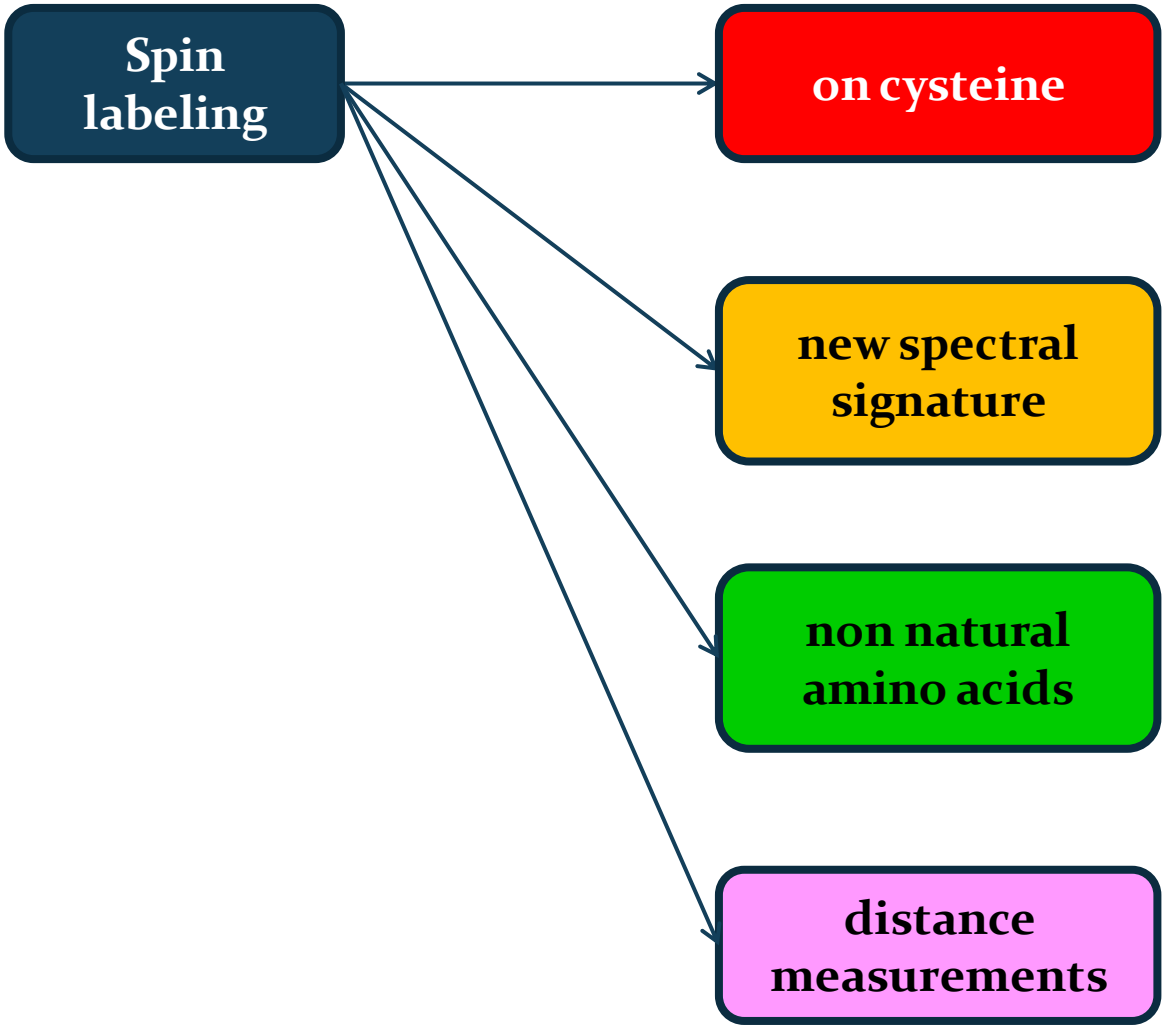
Spin labeling

- **on cysteine**
- **new spectral signature**
- **non natural amino acids**



Fleissner, M.R. *et al. PNAS* **2009** ; Kalai, T. *et al. Tetrahedron Lett.* **2011** ; Kucher, S. *et al. JMR* **2017**

SDSL – several approches



Le Breton, N.; Adrianaivomananjaona, T.; Gerbaud, G.; Etienne, E.; Bisetto, E.; Dautant, A.; Guigliarelli, B.; Haraux, F.; Martinho, M.; Belle, V. *BBA* **2016**

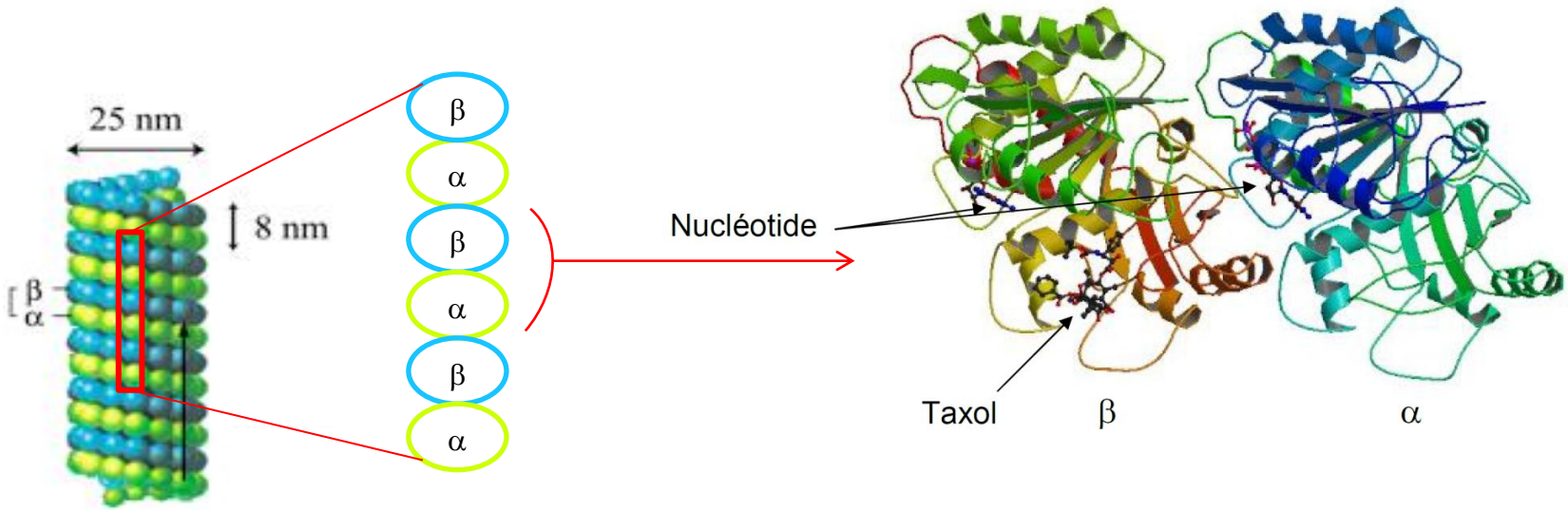
Tau protein : an IDP case

Interaction between Tau and MTs

Tau protein : an IDP case

Microtubules (MTs)

- cytoskeleton component, involved in a variety of cellular processes : cell division …
- tubular polymer composed of protofilaments of αβ-tubulin heterodimers
- dynamic structure regulated by ***MTs Associated Protein (MAP)***



Tubulin structure, 3.5Å resolution.
PDB : 1JFF (Lowe *et al.* J. Mol. Biol. **2001**)

Tau/MTs interaction

Tau is a MAP found in nerve cells

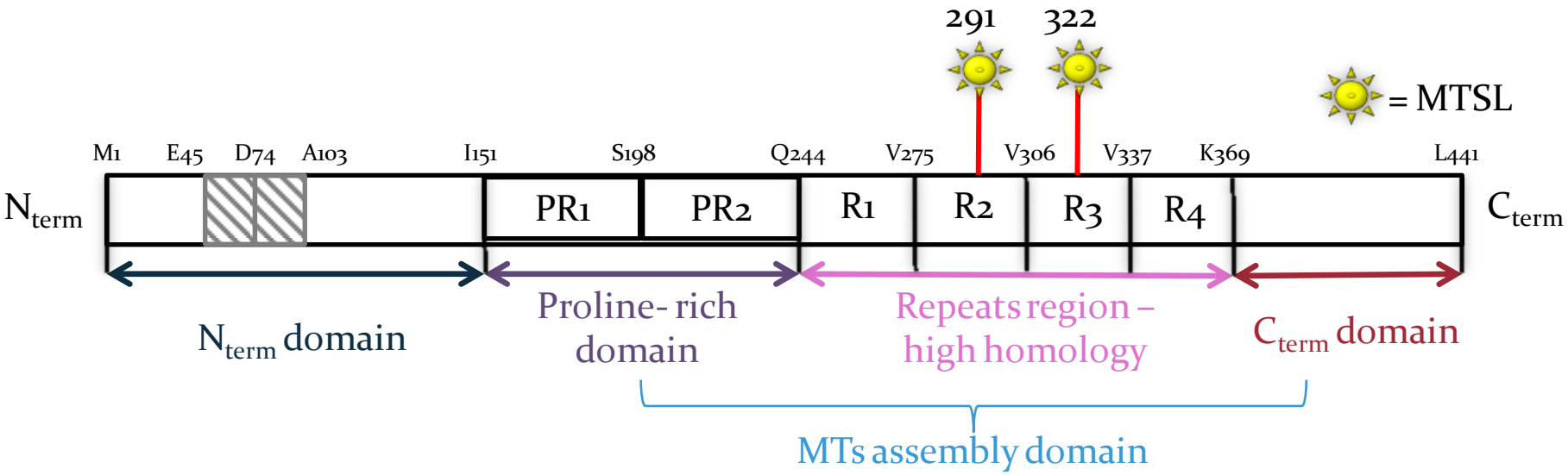
Function

- Stabilize MTs and promote their assembly
- In numerous neurodegenerative diseases called Tauopathies (Alzheimer disease)

Structure

- Very long Intrinsically Disordered Protein (IDP)
- Imperfect Repeats Region
- Tau remains extended and dynamics upon binding
- Where / how does Tau bind to MTs ?

Objective:
to study Tau/MTs interaction



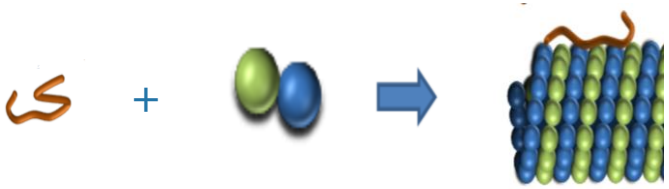
Sillen, A. *et al. Biochemistry* **2007**

Tau/MTs interaction

Experimental conditions

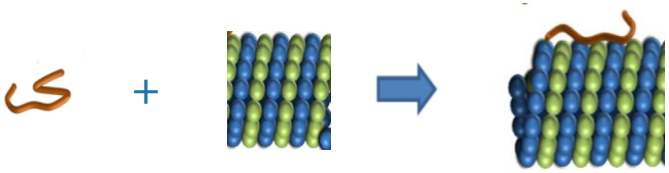
Tau induced MTs

- Physiological conditions
- Tau has a role of inducing MTs polymerization and stabilization



Taxol- stabilized MTs

- Non physiological conditions
- Tau has a role of stabilization of MTs

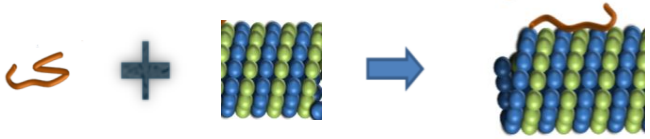


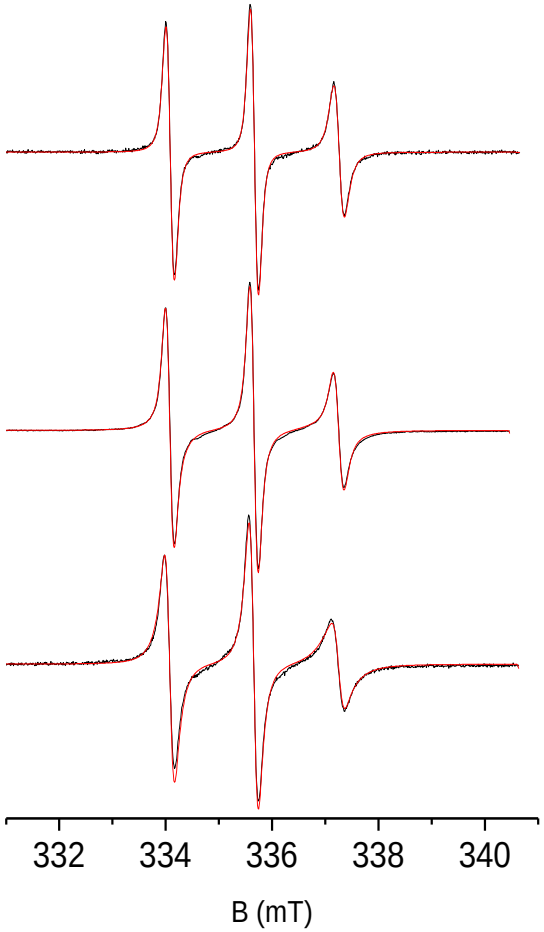
Tau/MTs interaction : with Proxyl

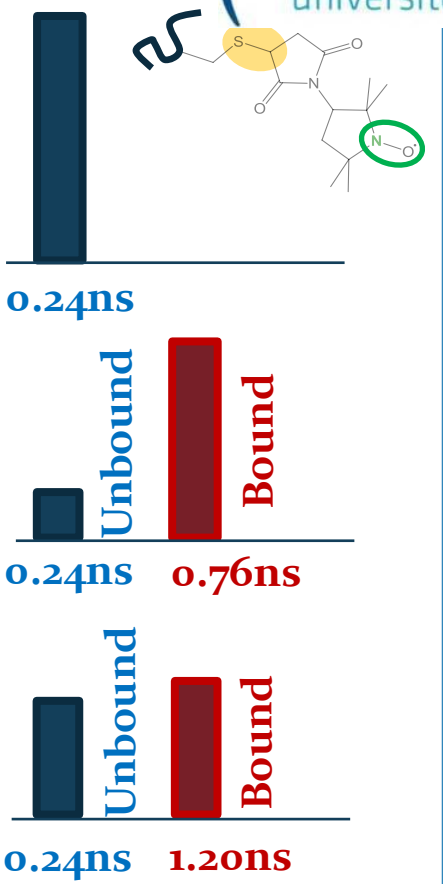
Tau^{Proxyl} alone

Tau^{Proxyl}-induced MTs

Taxol-stabilized MTs





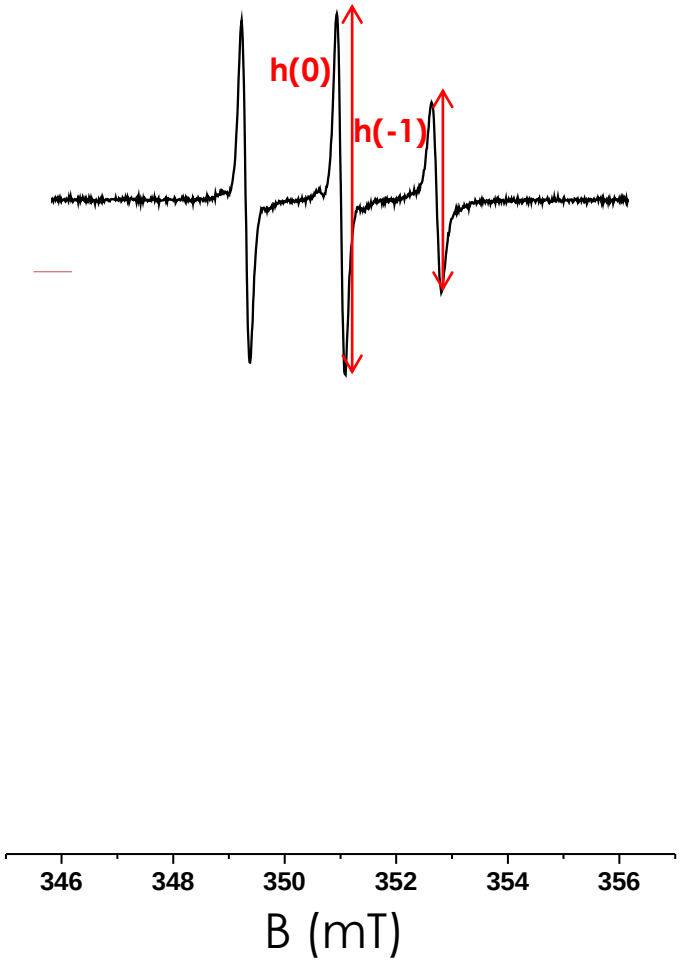


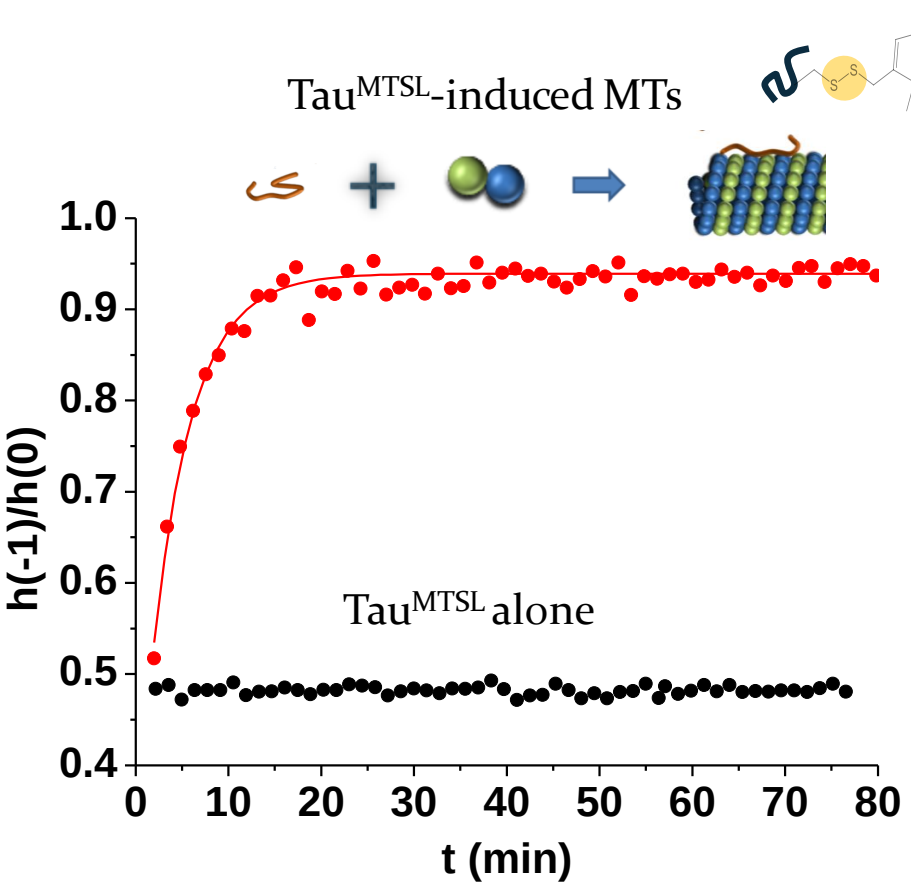
Analyses SimLabel

- Labels at C291 and C322 not discernible
- **Bound form:** different t_c between 2 cases = different environment / rapid regime

Tau remains highly flexible: Tau/MTs = fuzzy complex

Tau/MTs interaction : with MSTL

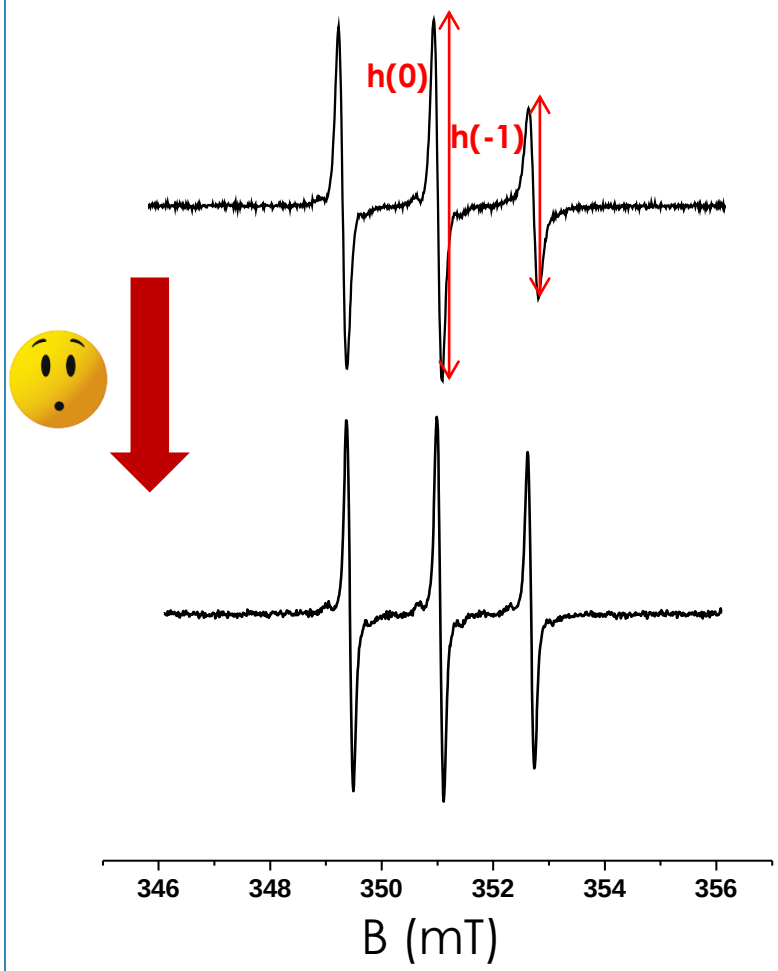


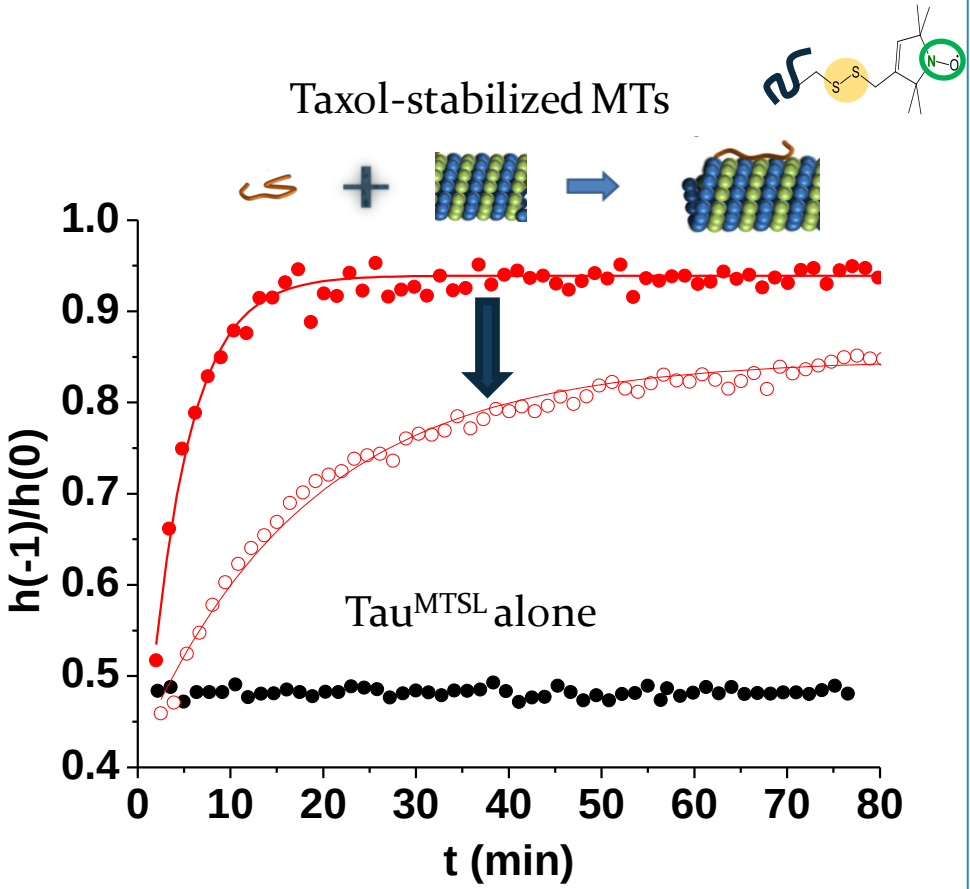


Unexpected release of the label upon interaction !!

Martinho. M. *et al. In preparation*

Tau/MTs interaction



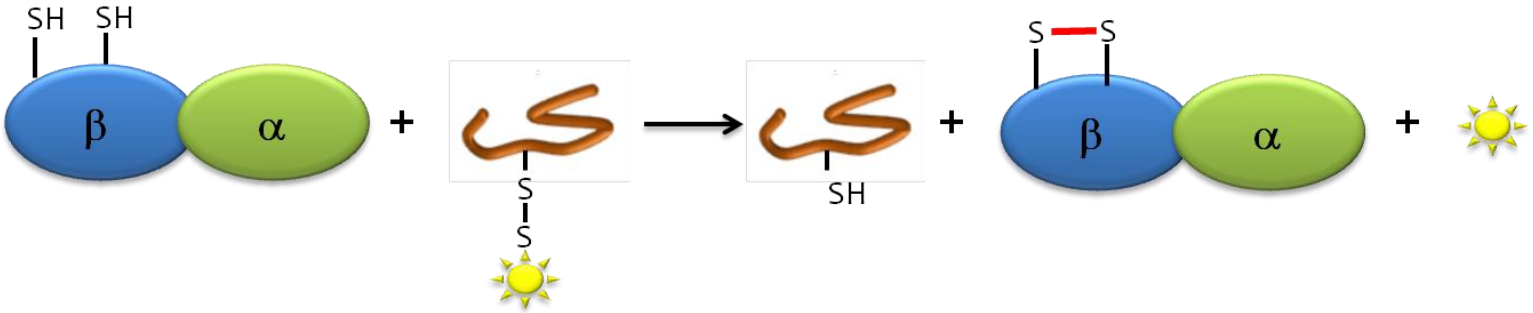


Slower kinetics when MTs are preformed

Tau is able to perform a thiol / disulfide exchange with MTs

Martinho. M. *et al. In preparation*

Proposed mechanism of S-S transfer between Tau and MTs



Which Cys as binding sites ?

Remember the slower kinetics for preformed MTs compared to induced MTs

Looking for structural differences between the 2 cases

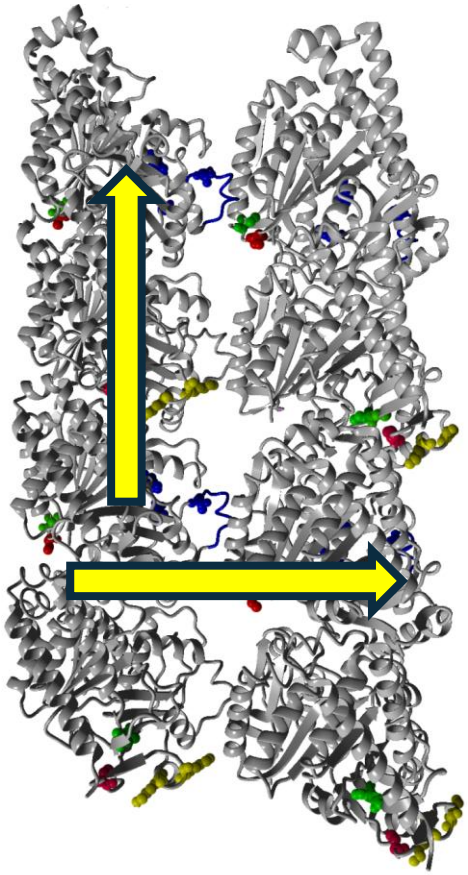
Martinho. M. *et al. In preparation*

Comparison of two 3D structures of MTs

PBDsum: Analyses of interactions between monomers of tubulin: longitudinal / transversal

Without Taxol (pdb 3j6f)

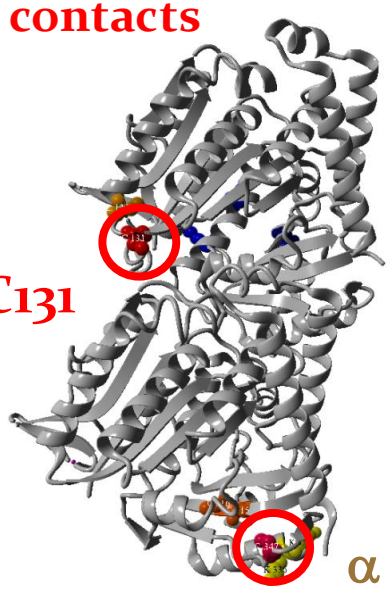
No Cys involved



(Alushin, Cell 2014)

With Taxol (pdb 3j6g)

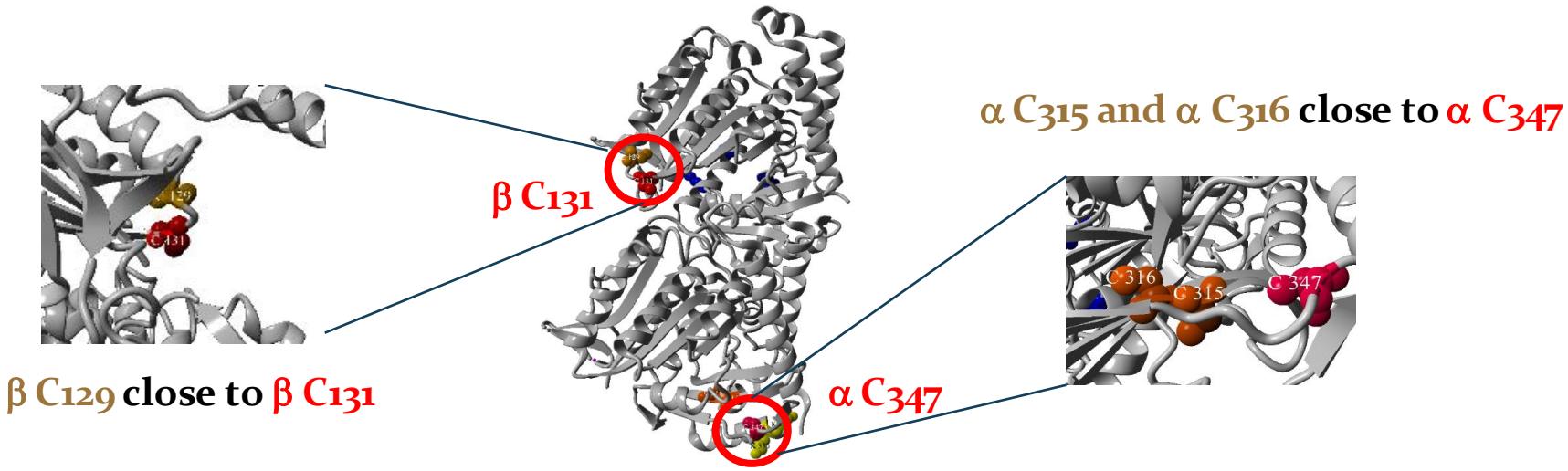
2 Cys in longitudinal contacts



- a C347 and b C131 less accessible due to these contacts
- can explain the slower kinetics in the case of Taxol stabilized MTs

Proposed mechanism of S-S transfer between Tau and MTs

Need of Cys in tubulin and in close vicinity to be able to form a S-S bridge

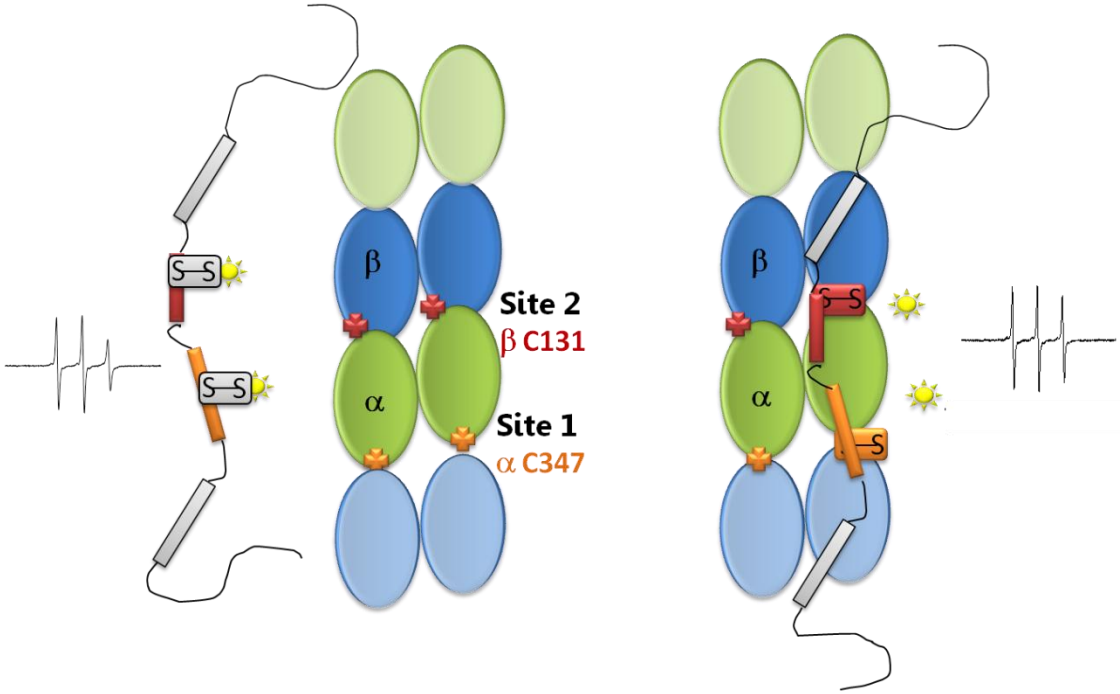


α-C347 and β-C131 are good candidates for Tau binding sites on MTs

Martinho. M. *et al. In preparation*

Tau/MTs interaction

- Ability of Tau to exchange a disulfide bridge with MTs
- Unusual use of SDSL-EPR spectroscopy approach
- Localization of 2 binding sites



Martinho. M. *et al. In preparation*

Acknowledgements

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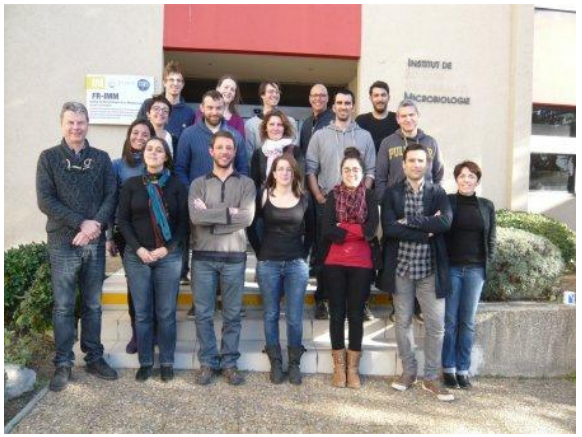
- Prof. Valérie Belle, Aix-Marseille Univ, UMR 7281, BIP
- Dr. Emilien Etienne, Aix-Marseille Univ, UMR 7281, BIP



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- Dr. Isabelle Landrieu, Lille Univ, UMR 8576, CNRS, UGSF, Lille

Poster 19

Thank you for your attention

ARBRE MOBIEU
BETWEEN ATOM AND CELL