Agréger ou pas : une question d'oubli

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Overview

A protein, a polymer like another?

- Protein folding or folded proteins

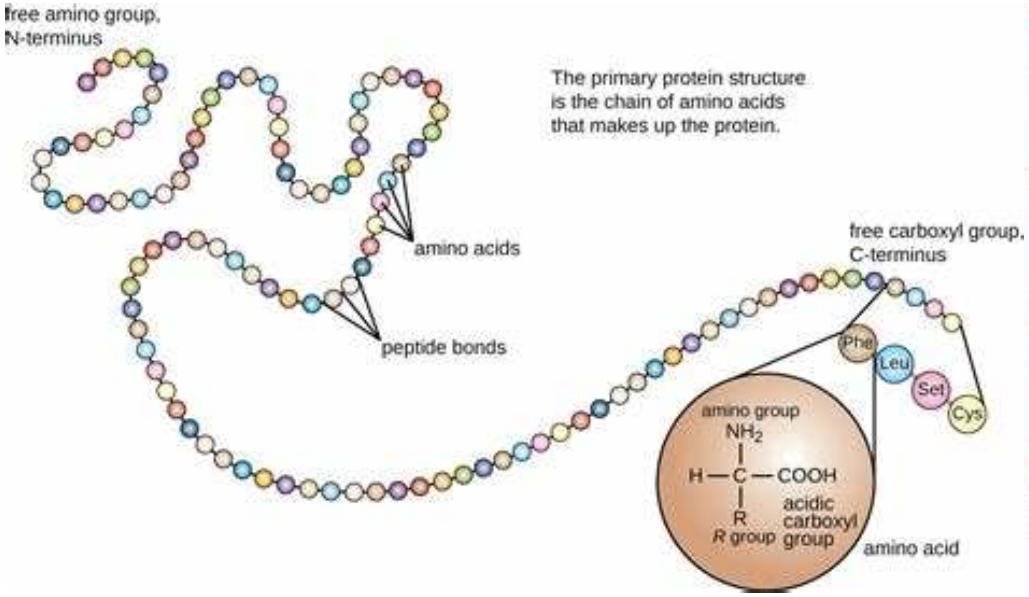
- IDPs

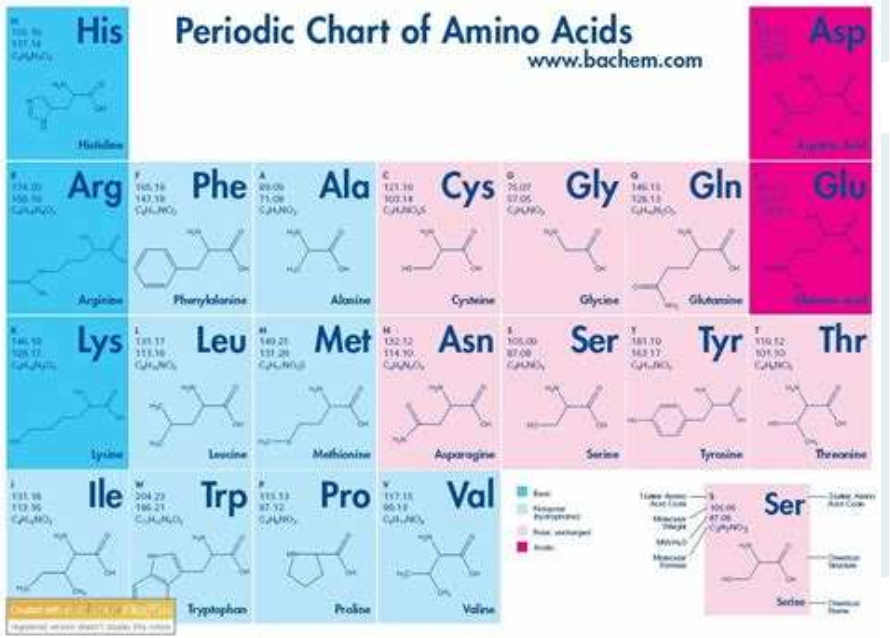
NMR as a tool to study IDPs

Tau protein : function and (dys)function

Homogeneous solution, amyloid form and in between

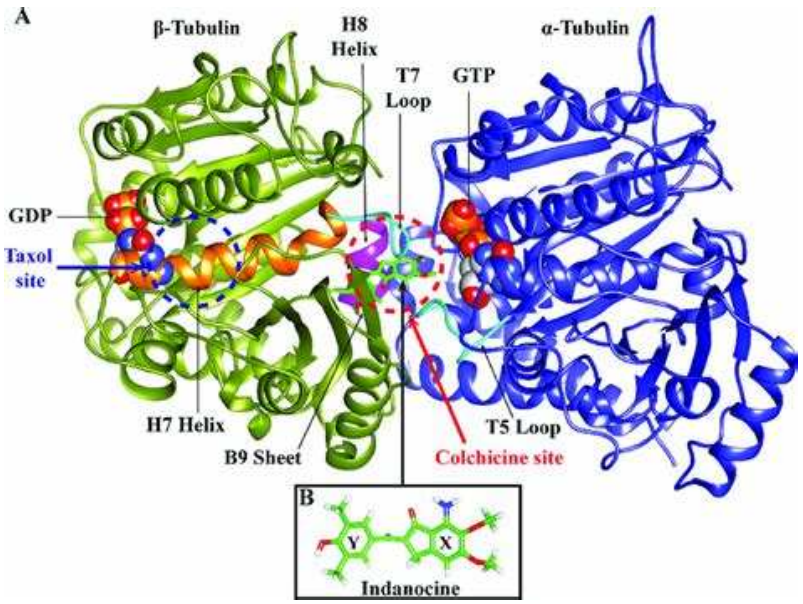
A protein, a polymer like another?





A protein, a polymer like another?

Protein folding or folded proteins : Structure-Function paradigm



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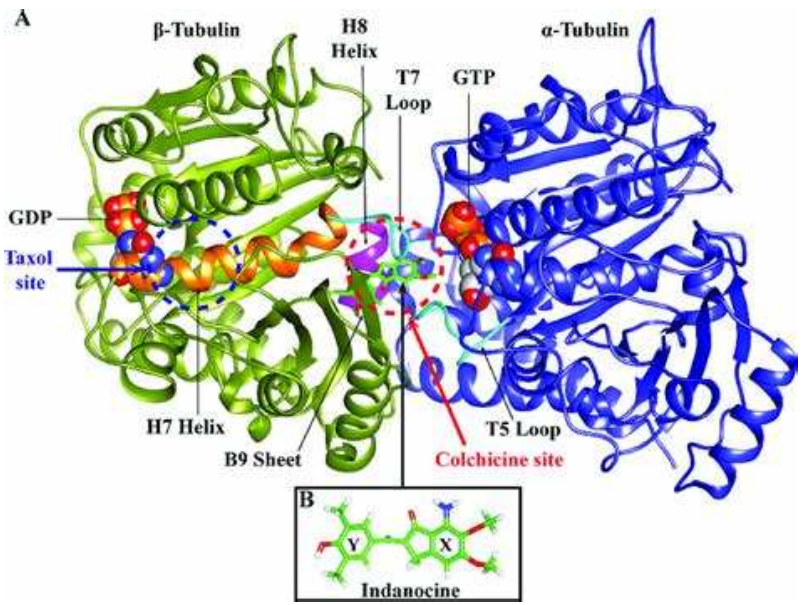

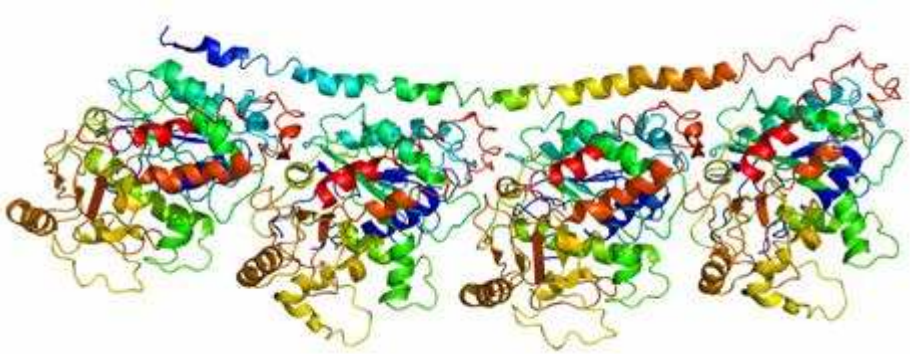

T2R complex : 2 tubulin dimers sequestered by stathmin helix

M. Knossow & B Gigant, Gif-sur-Yvette

A protein, a polymer like another?

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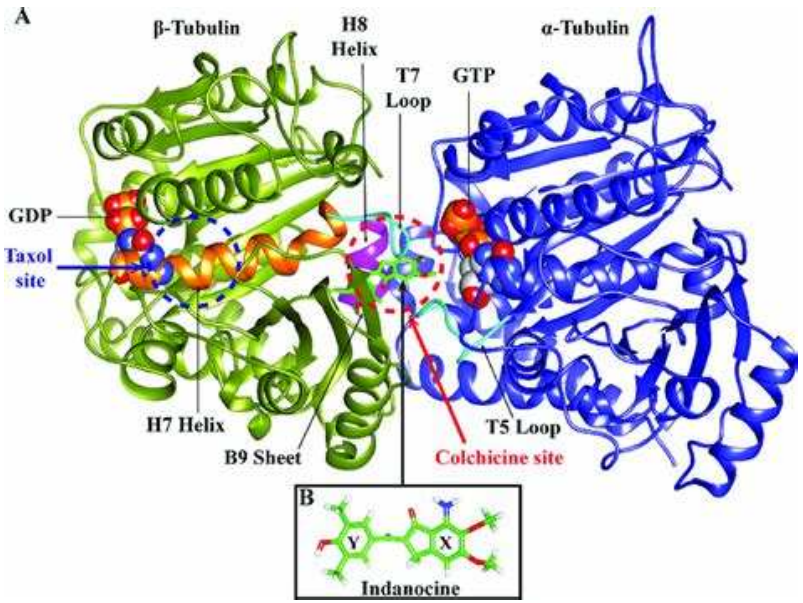

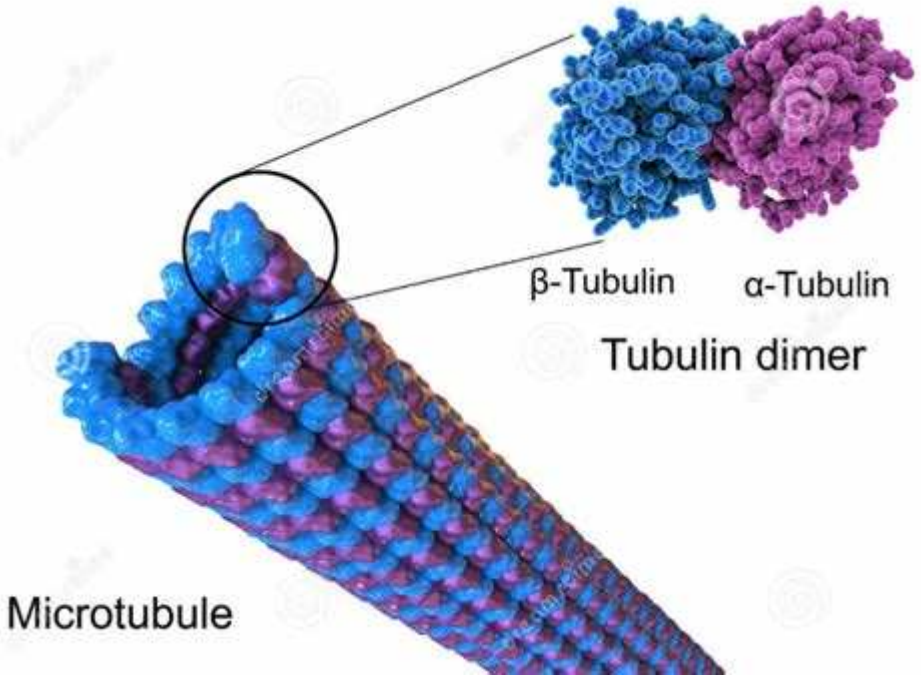

A protein, a polymer like another?

Intrinsically disordered proteins

Tau = Tubulin Associated Unit (M Kirschner, 1975)

MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT
PTEDGSEEPG SETSDAKSTP TAEDVTAPLV DEGAPGKQAA AQPHTEIPEG
TTAEEAGIGD TPSLEDEAAG HVTQARMVSK SKDGTGSDDK KAKGADGKTK
IATPRGAAPP GQKGQANATR IPAKTPPAPK TPPSSGEPPK SGDRSGYSSP
GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK SRLQTAPVPM
PDLKNVKSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGSKDNIKHV
PGGGSVQIVY KPVDLSKVTS KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV
QSKIGSLDNI THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS
GDTSPRHLSN VSSTGSIDMV DSPQLATLAD EVSASLAKQG L

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GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK SRLQTAPVPM
PDLKNVKSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGSKDNIKHV
PGGGSVQIVY KPVDLSKVTS KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV
QSKIGSLDNI THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS
GDTSPRHLSN VSSTGSIDMV DSPQLATLAD EVSASLAKQG L
```

Amino Acid composition

	#	%
Gly	49	11.4
Ser	45	10.2
Lys	44	10.0
Pro	43	9.8
Ala	34	7.7

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5 aa = 50% !!!

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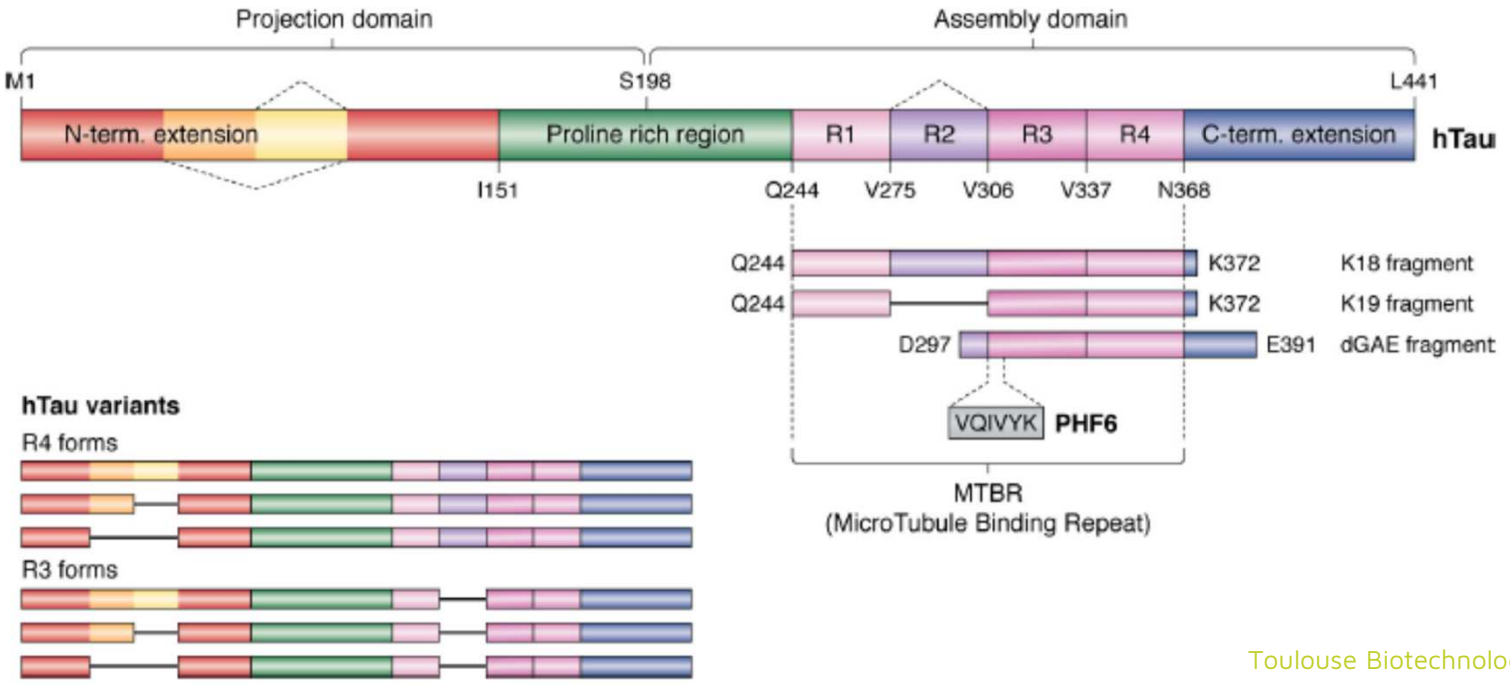

A protein, a polymer like another?

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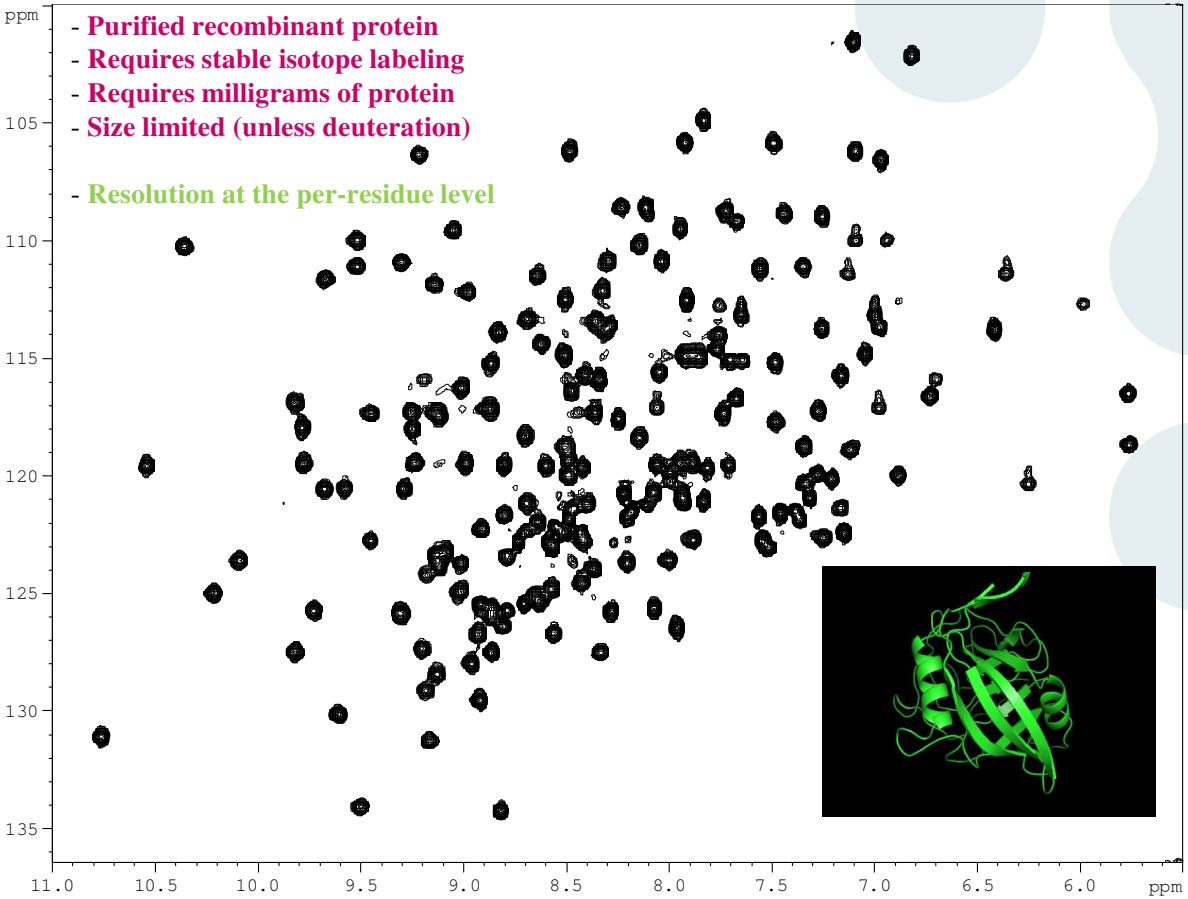
GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK SRLQTAPVPM
PDLKNVKSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGS
PGGGSVQIVY KPVDLSKVTS KCGSLGNIHH KPGGGQVEVK SEK
QSKIGSLDNI THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIV
GDTSPRHLSN VSSTGSIDMV DSPQLATLAD EVSASLAKQG L

Although dispersed, noncooperative and unstructured binding interactions may seem unusual, they may be very common in cell biology. To date, our most detailed structural information has been obtained from x-ray crystallography, which has concentrated on easily crystallizable and generally rigid structures. Thus, the protein and DNA, protein and other protein, or protein and ligand interactions studied so far are probably of structures with only a few stable conformations. Several important interactions in biology may not conform to such models.

NMR, a tool to study IDPs

Workhorse of biomolecular NMR spectroscopy= HSQC spectrum



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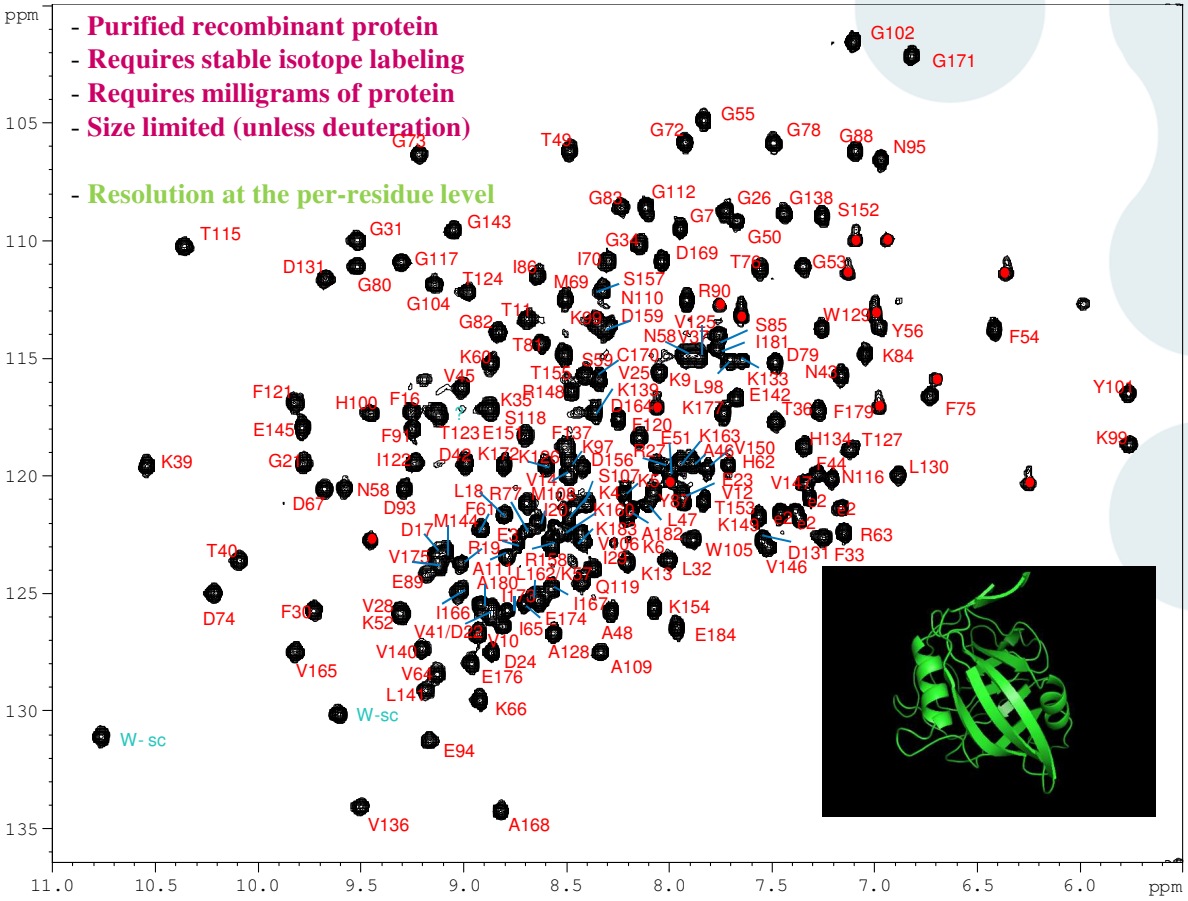

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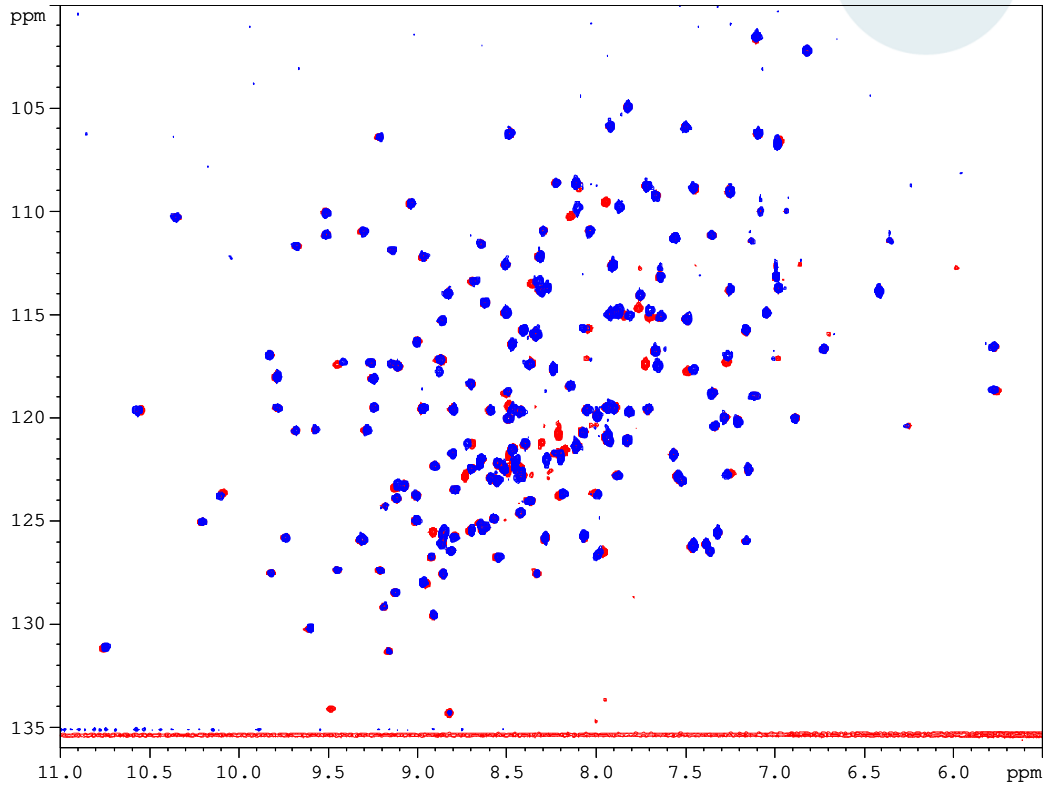

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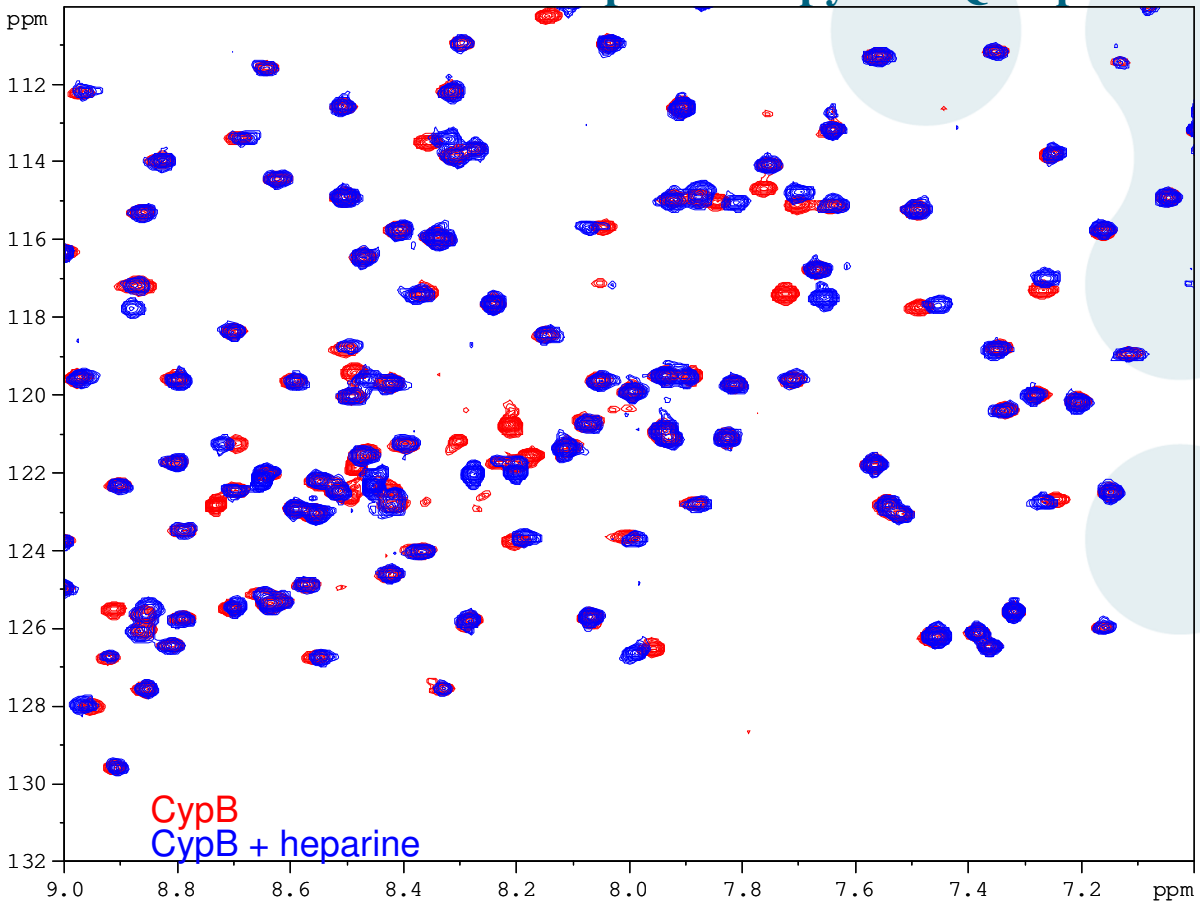

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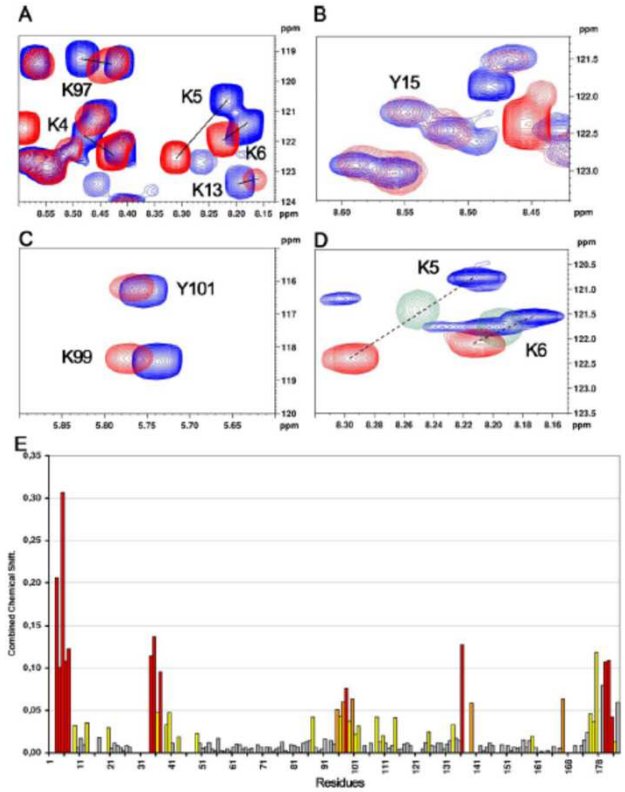

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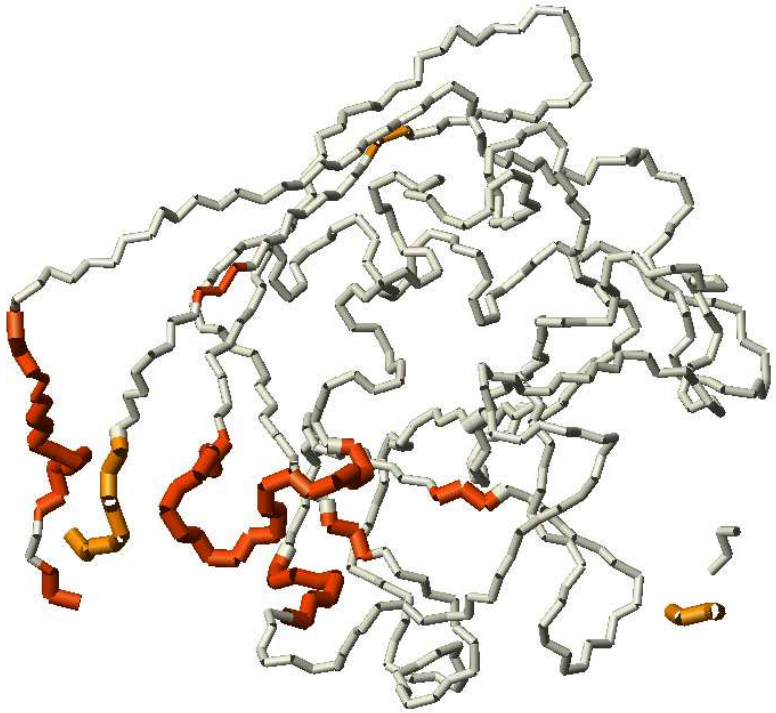
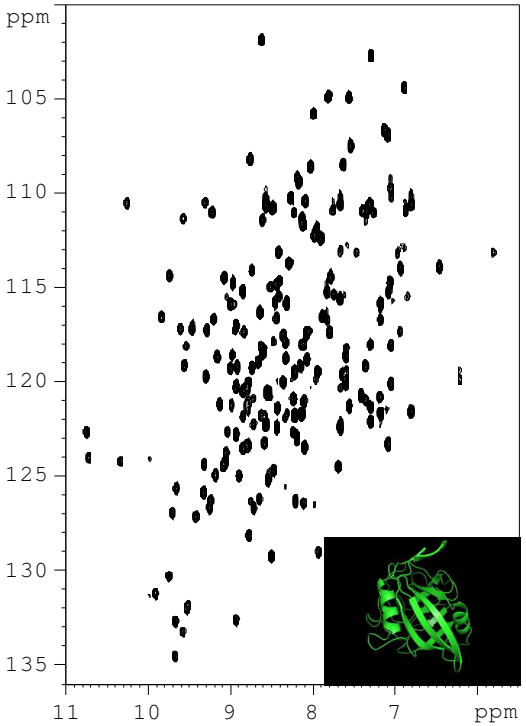
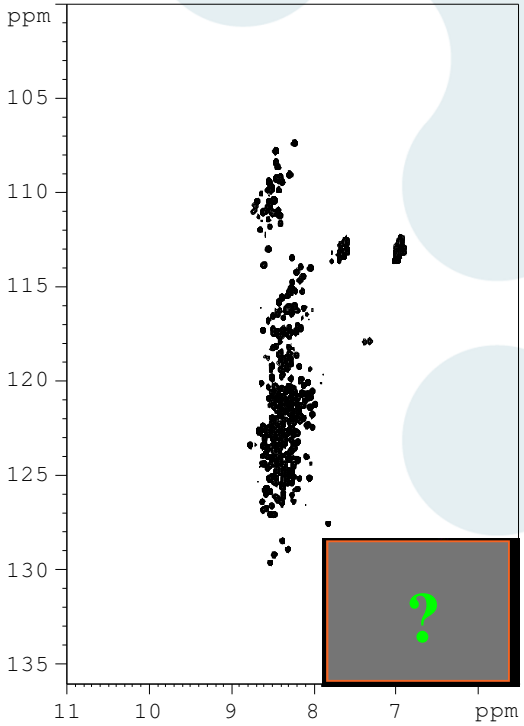


NMR, a tool to study IDPs

Lack of tertiary fold of Tau leads to a « random coil » spectrum





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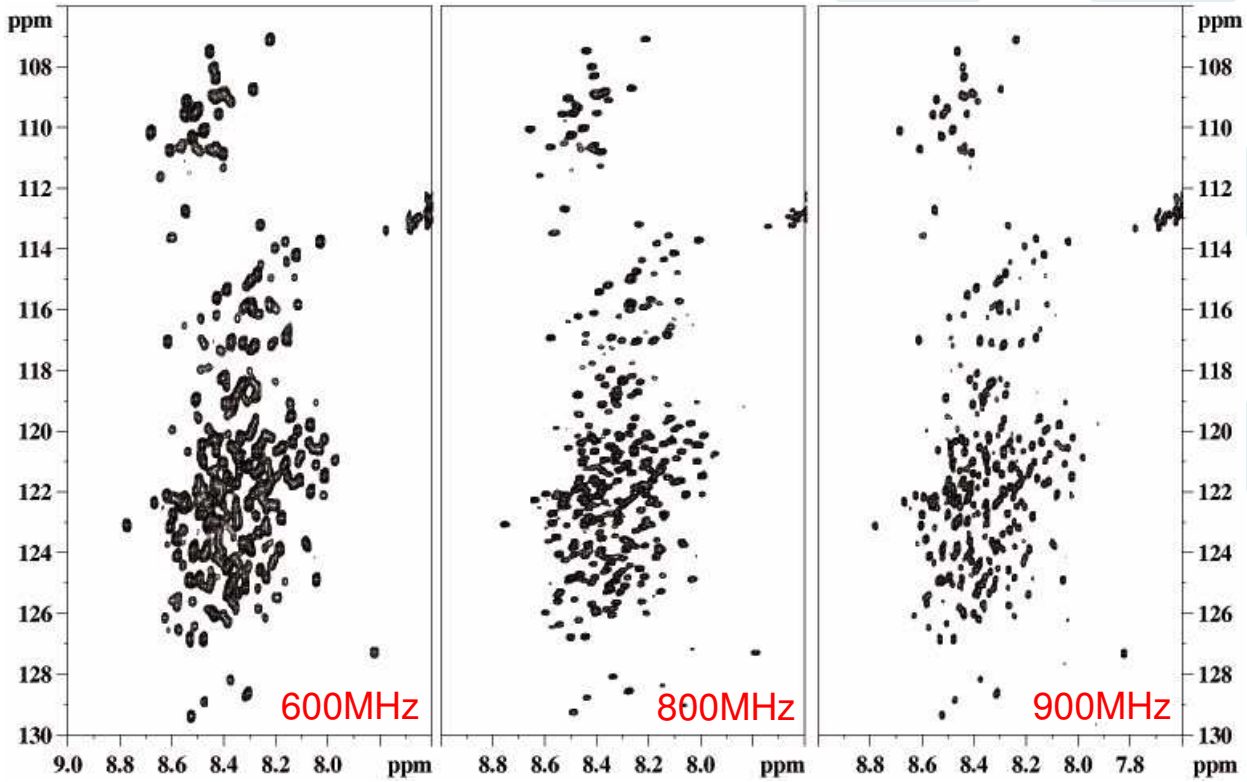

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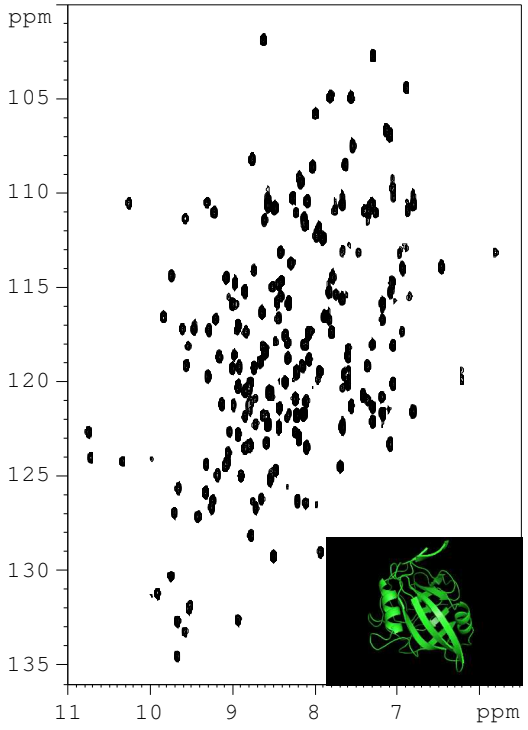
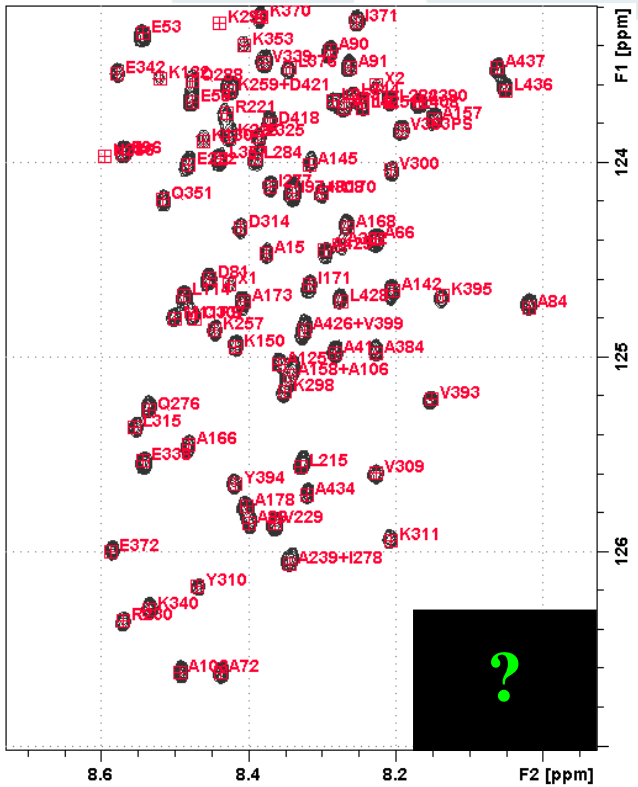
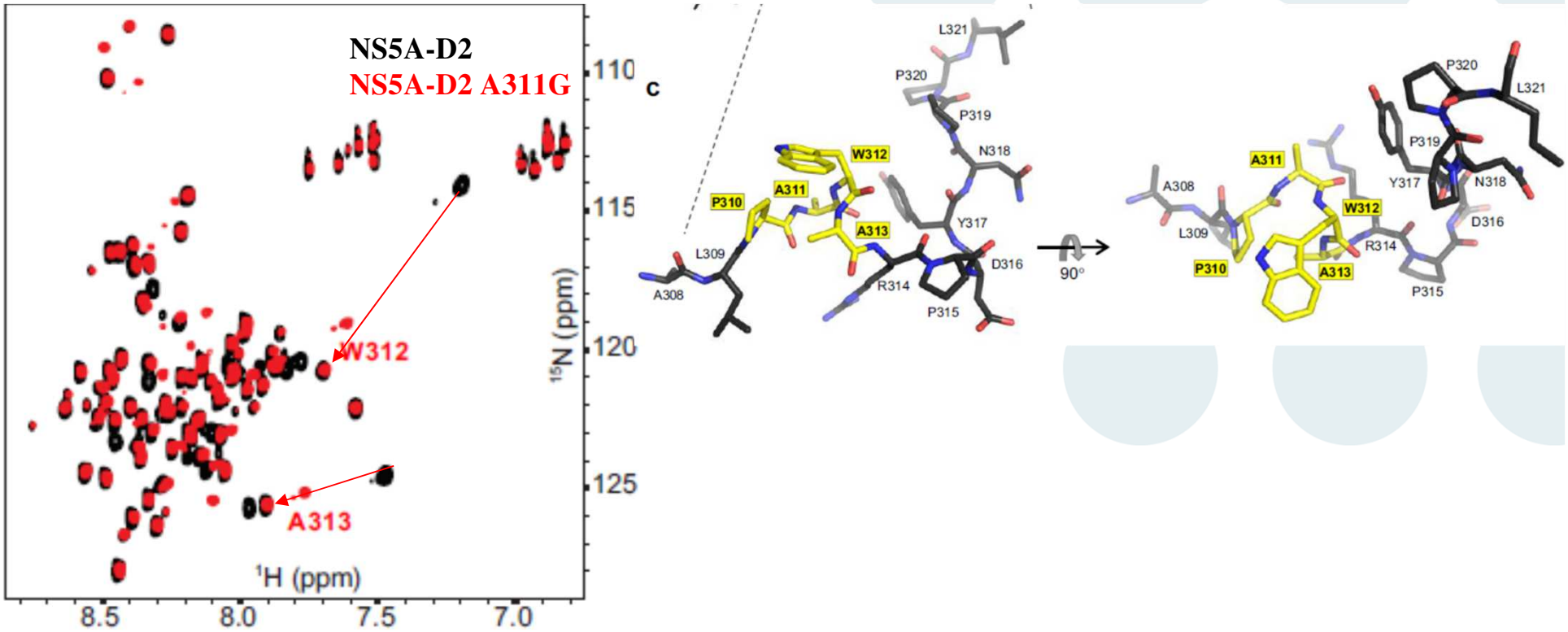




G Lippens *et al.* (2004)

NMR, a tool to study IDPs

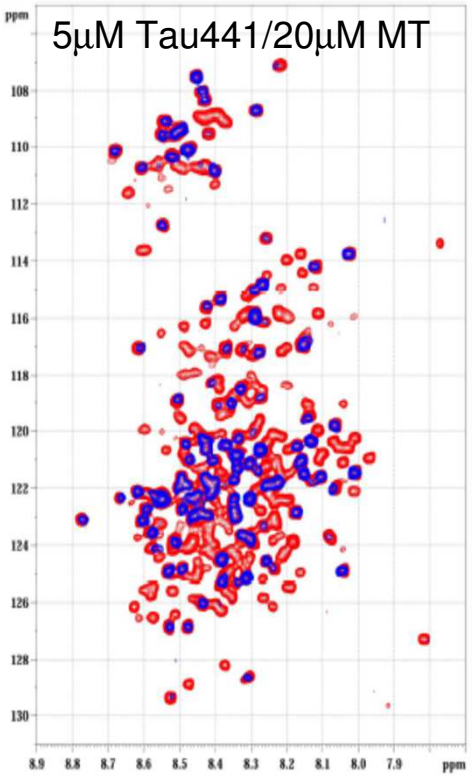
NMR detects tiny but vital structural features

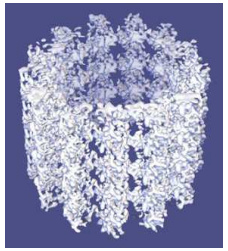


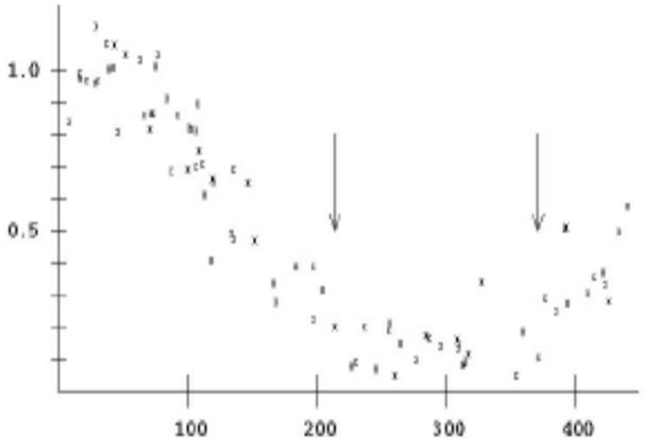
Dujardin & Hanoulle (2019)

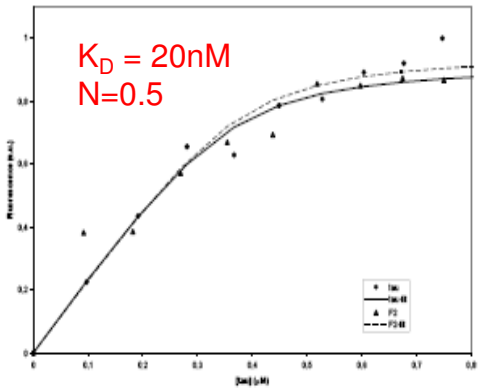
Tau protein : function

Interaction Tau:taxol stabilized MTs







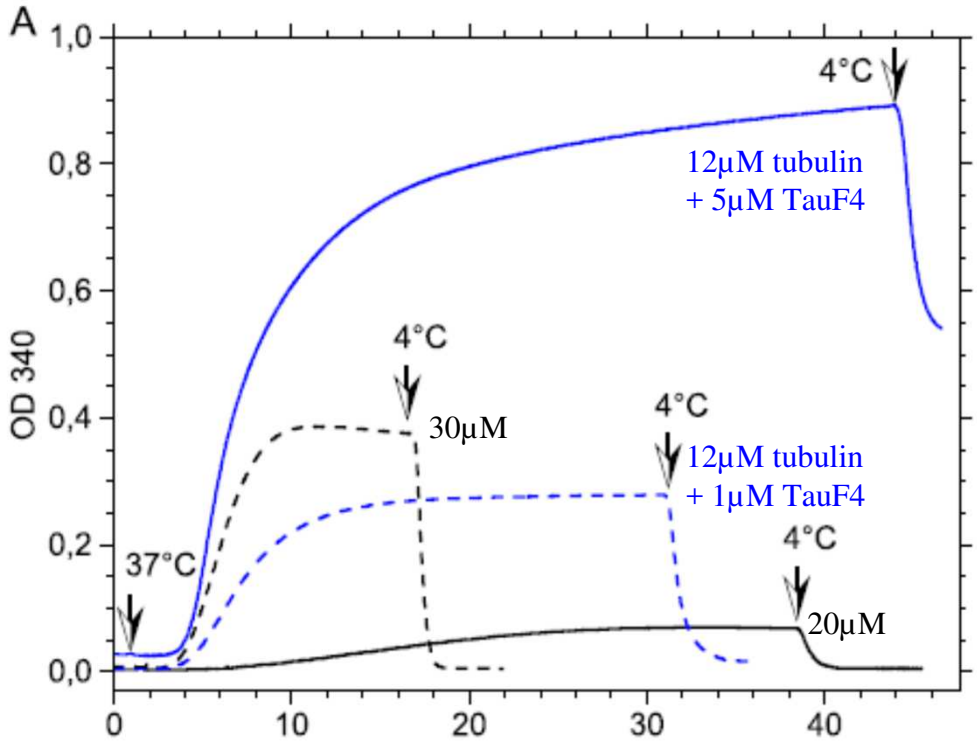


FRET between acrylodan-Tau and MTs

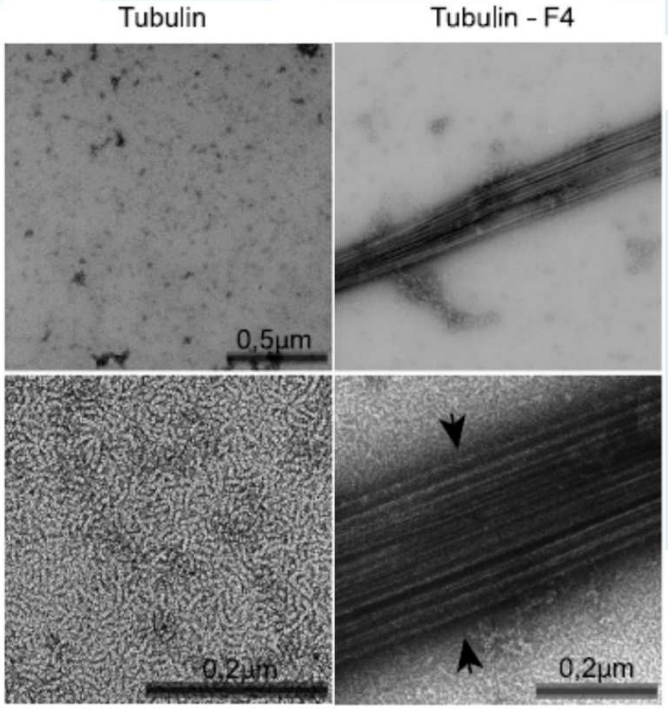
Sillen & Lippens 2006

Tau protein : function

Tubulin polymerization assay



Fauquant & Knossow (2011)

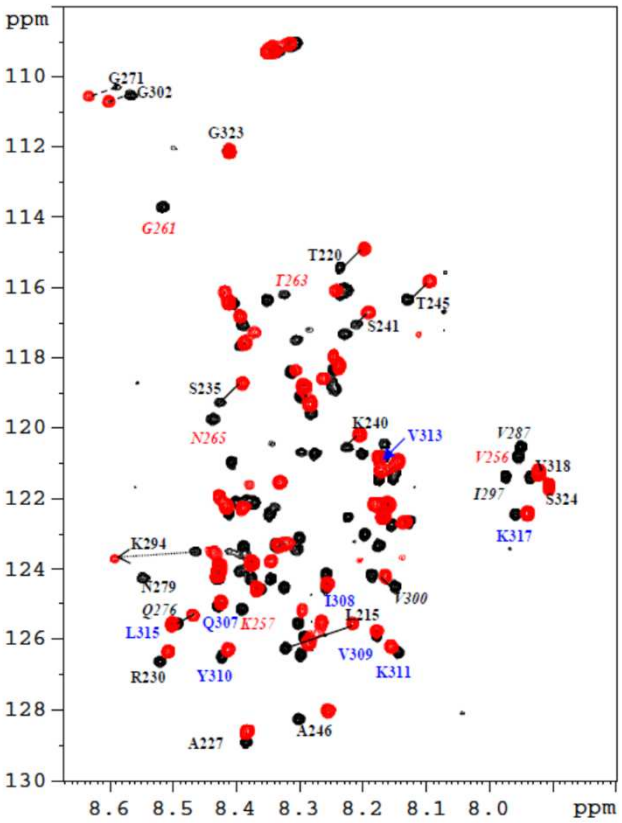


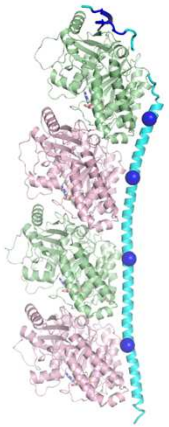
13µM tubulin

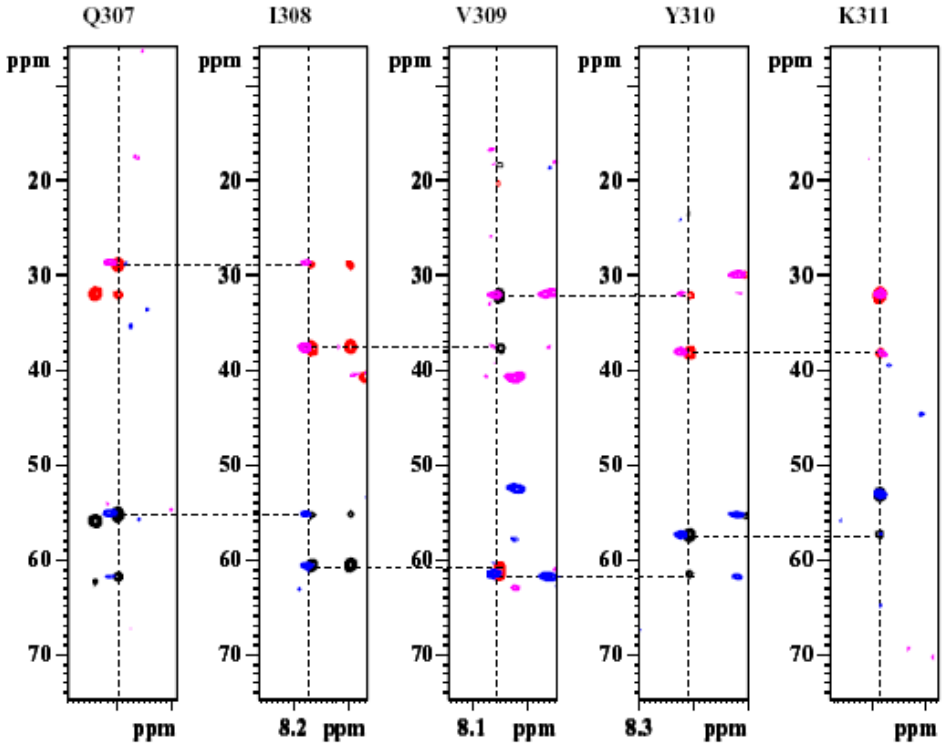
13µM tubulin
+ 5µM TauF4

Tau protein : function

TauF4 and T_2R by NMR





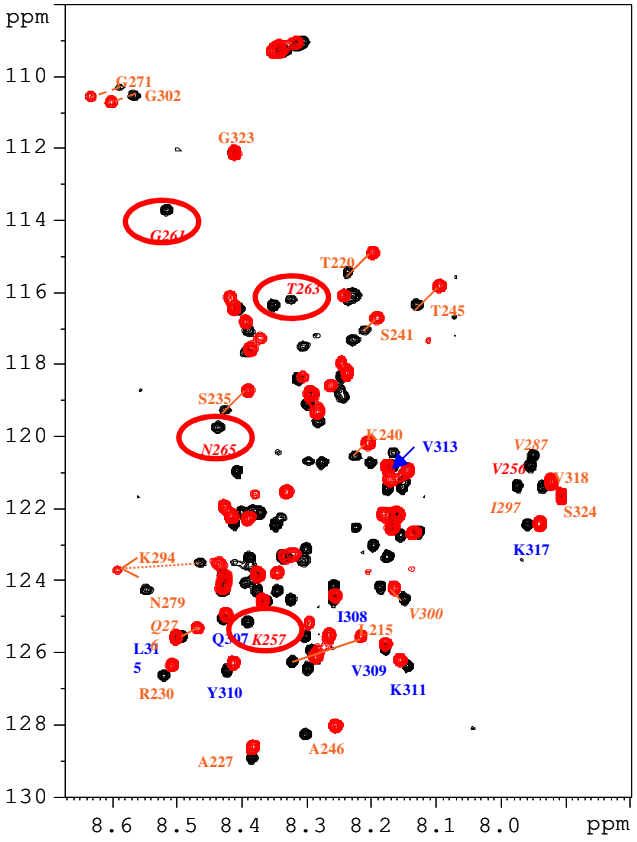


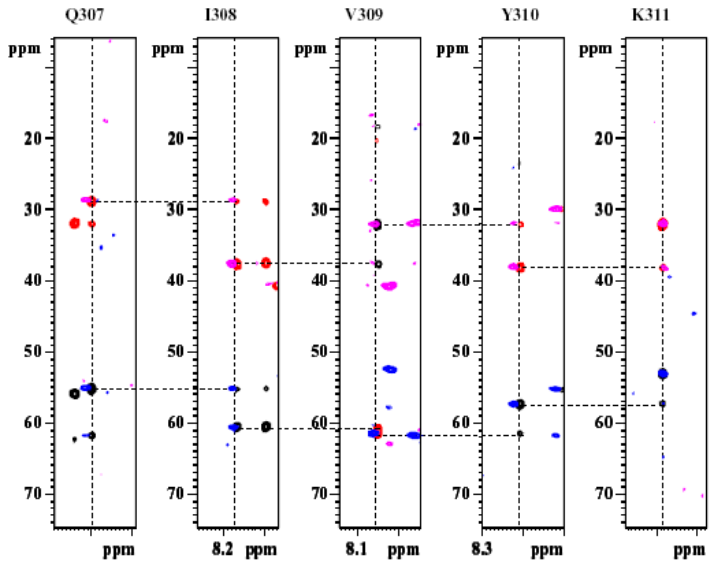
Important mobility at the tubulin surface

No defined structure on T_2R

Tau protein : function

TauF4 and T_2R by NMR





VKSKIGS$_{262}$TEN peptide in R1 invisible

Ser262 : MARK kinase site that interferes with Tau's MT assembly capacity

Gigant et al., JACS 2014

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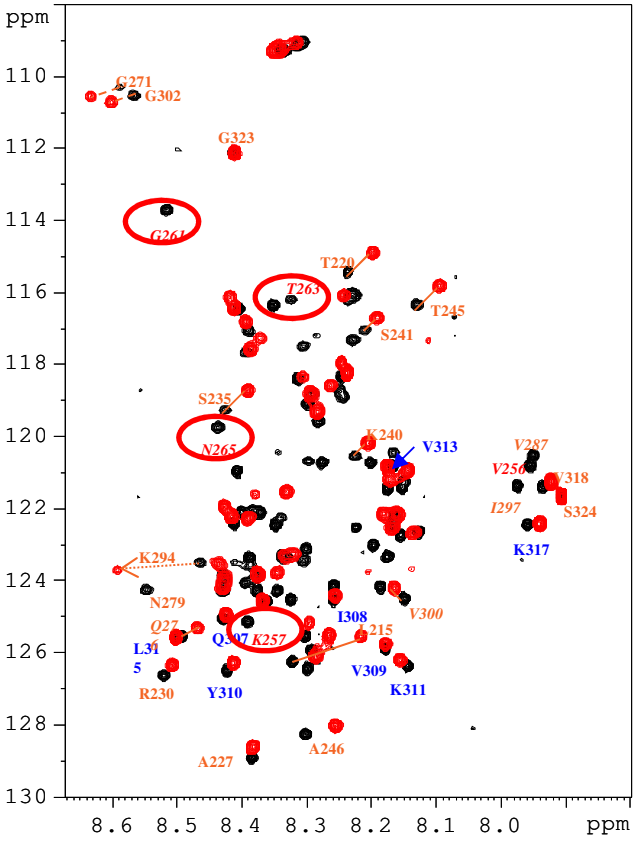

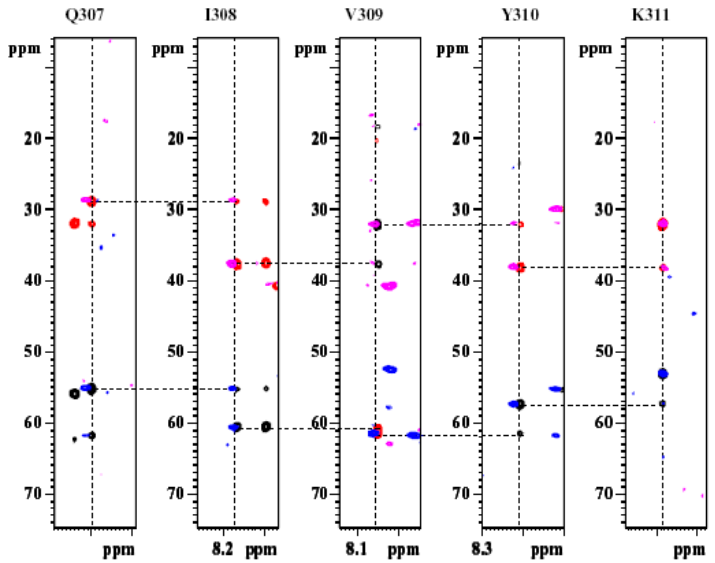

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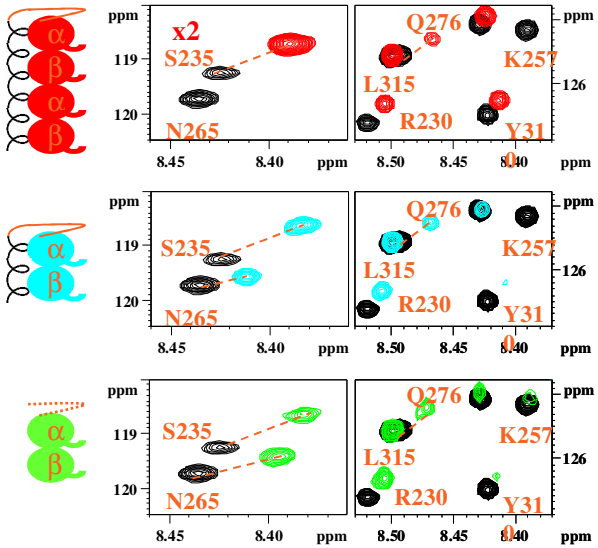


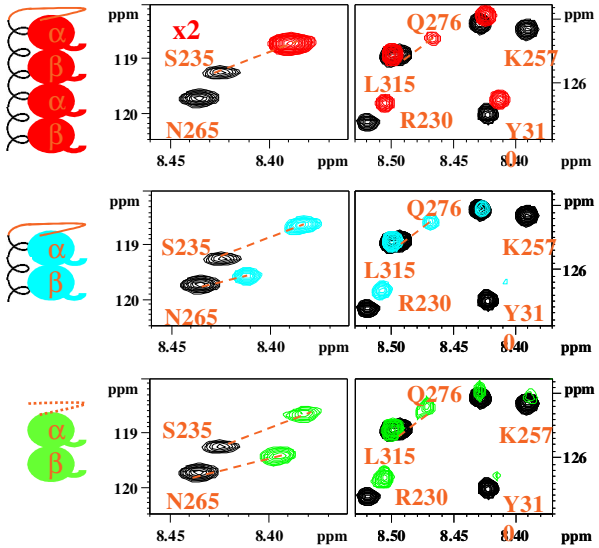
V_{256}KSKIGSTENLKHQPGGG$_{273}$ peptide becomes visible in constructs with a single tubulin heterodimer

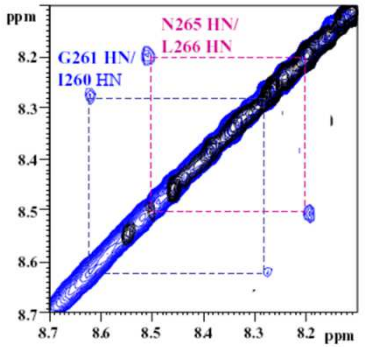
PHF6 loses its intensity in the same constructs

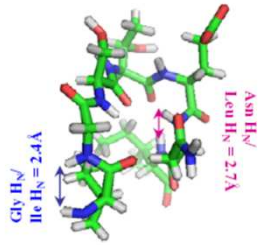
Gigant et al., JACS 2014

Tau protein : function

TauF4 and T_2R by NMR







Citrate synthase
(PDB code 3MSU)

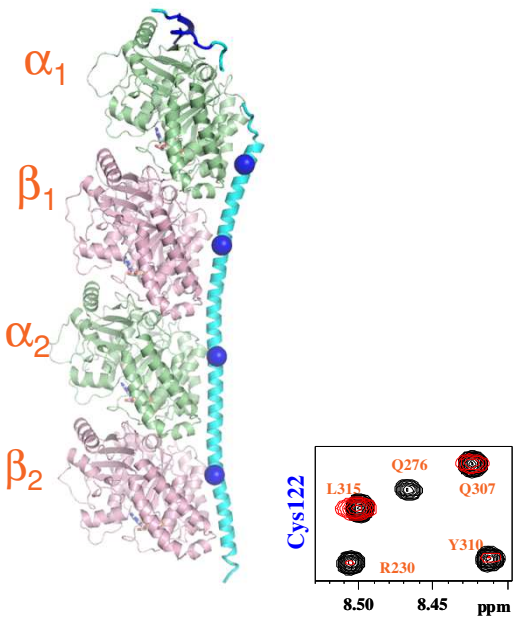
$V_{256}KSKIGSTENLKHQPGGG_{273}$ peptide : T_2R 40 : 1

Ser262 : MARK kinase site that interferes with Tau's MT assembly capacity

Gigant et al., JACS 2014

Tau protein : function

TauF4 and T_2R by NMR



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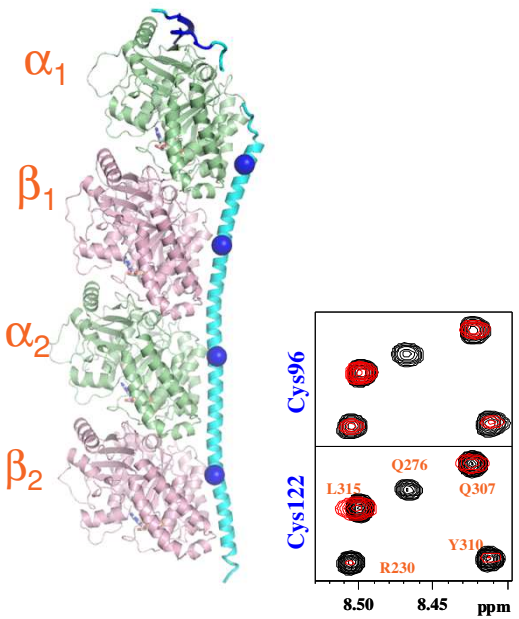

Gigant et al., JACS 2014

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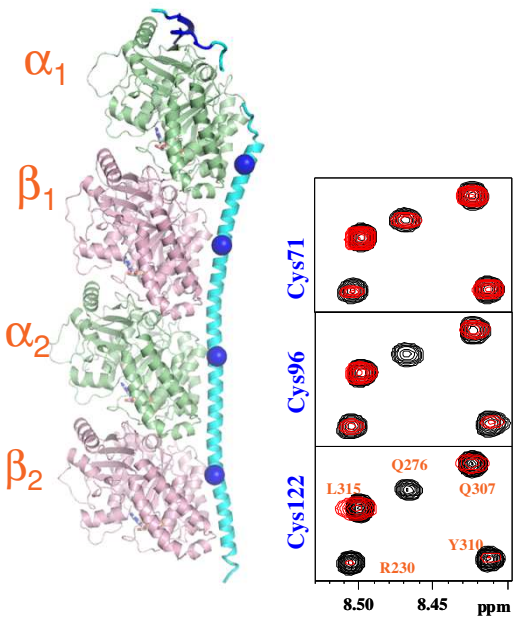

Gigant et al., JACS 2014

Tau protein : function

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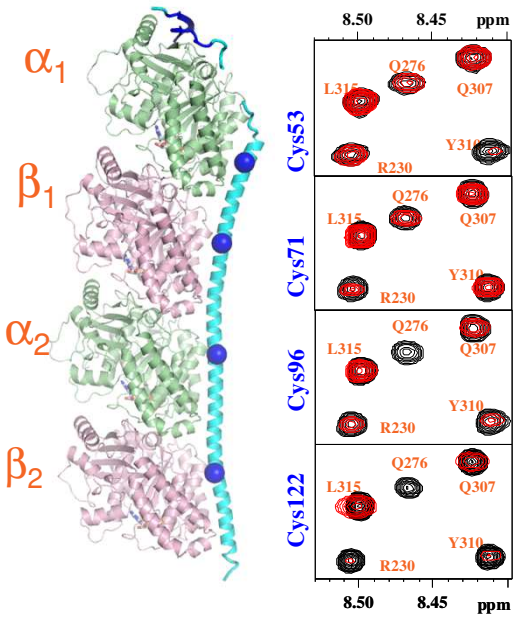

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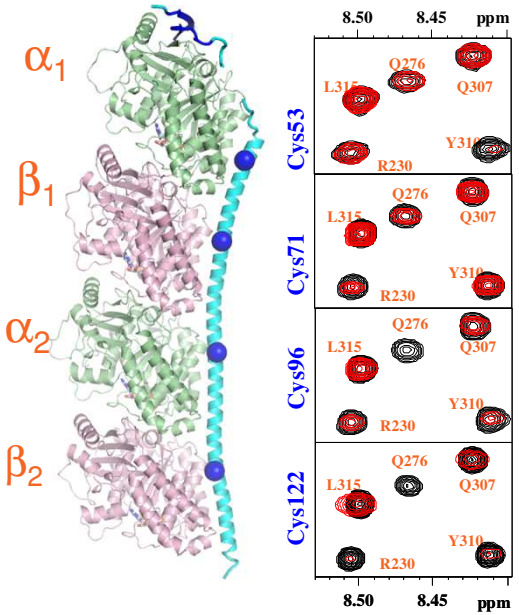

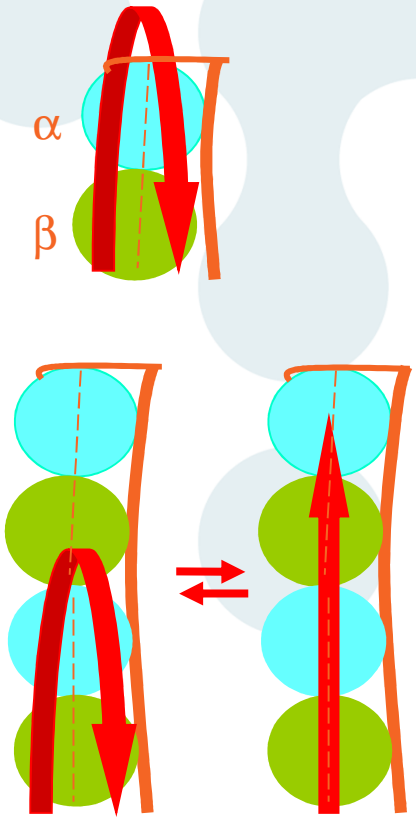

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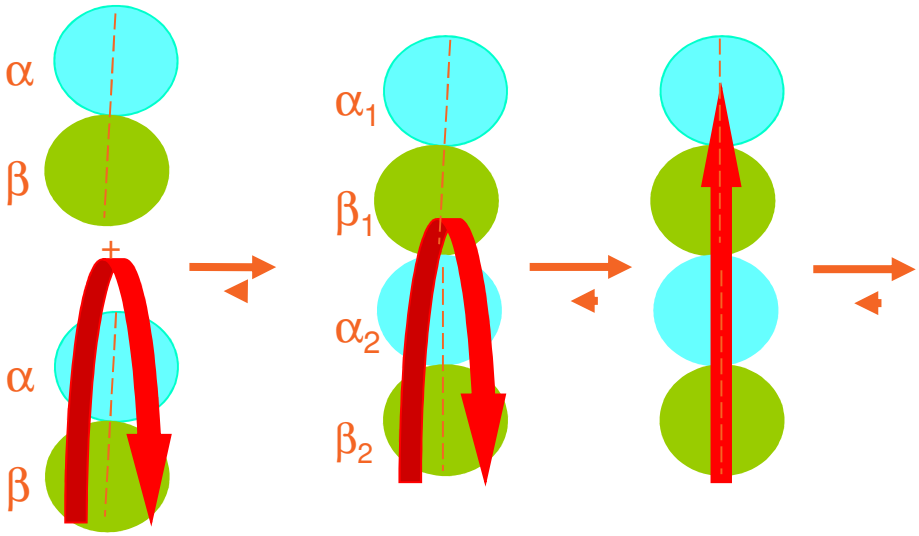
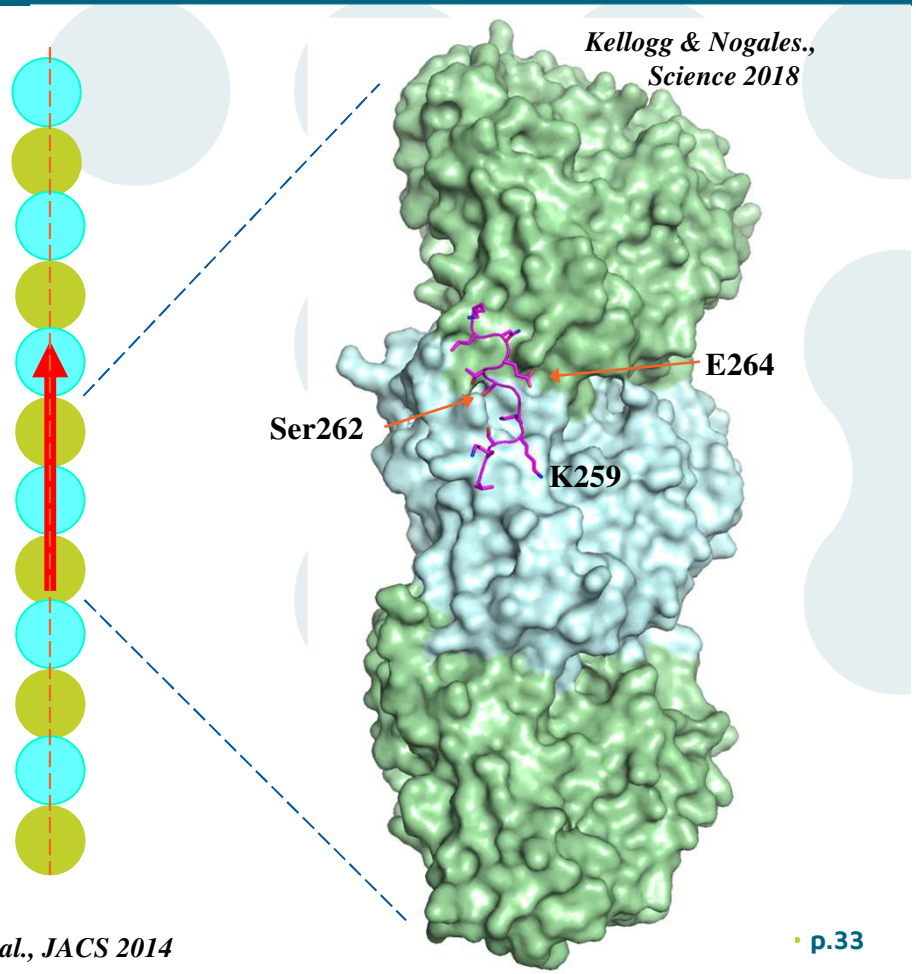


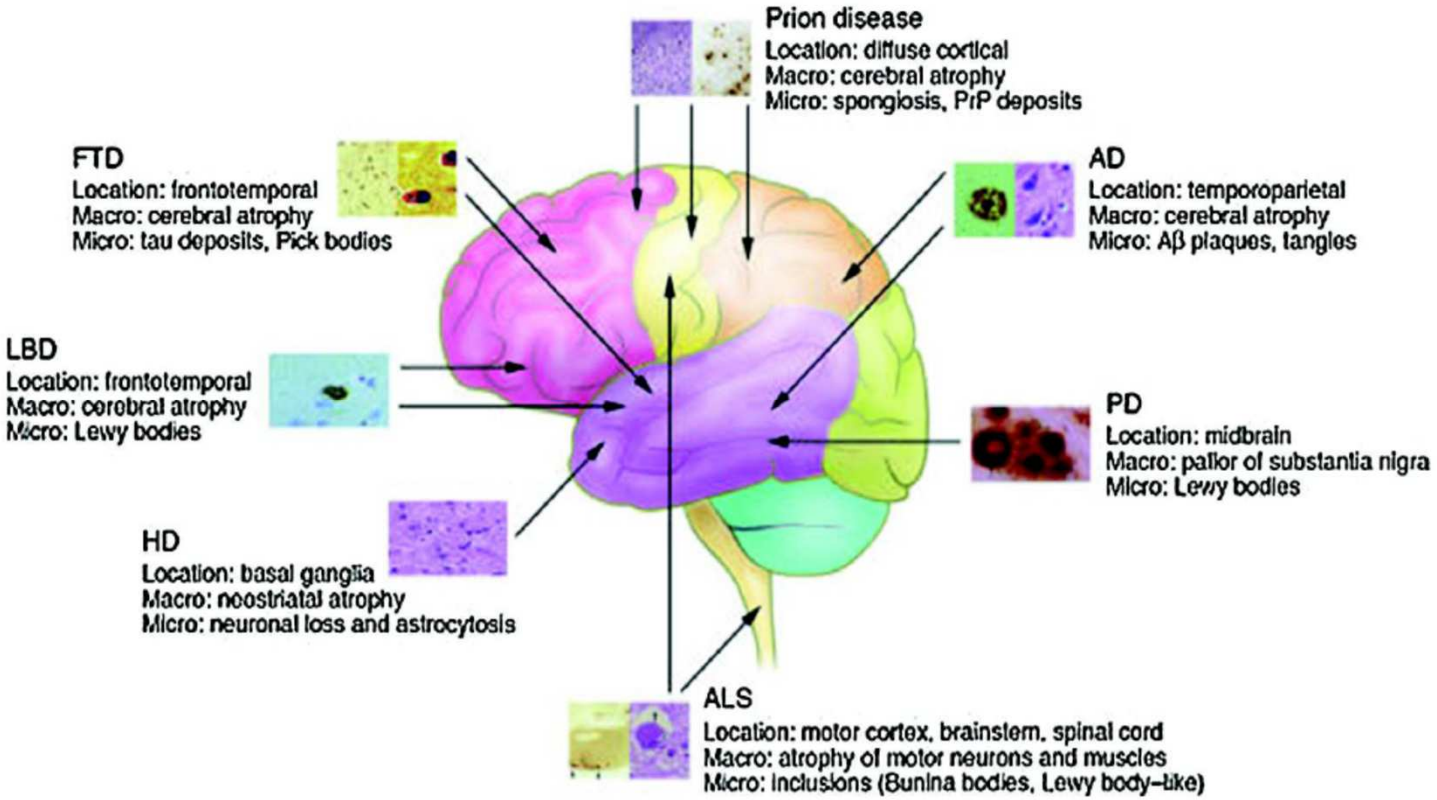
If $pSer_{262}$: not possible anymore



Gigant et al., JACS 2014

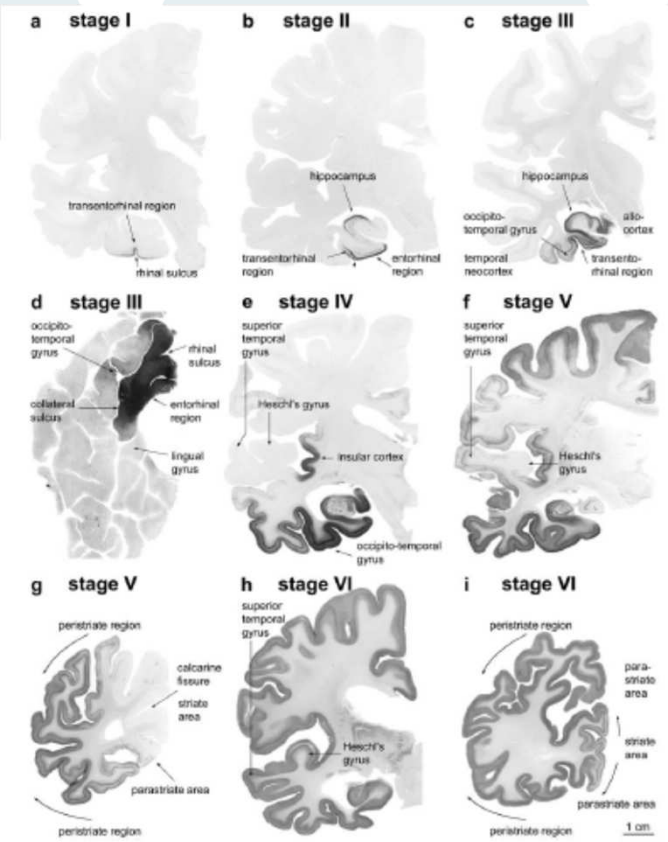
Tau protein : dysfunction

Amyloid diseases



Tau protein : dysfunction

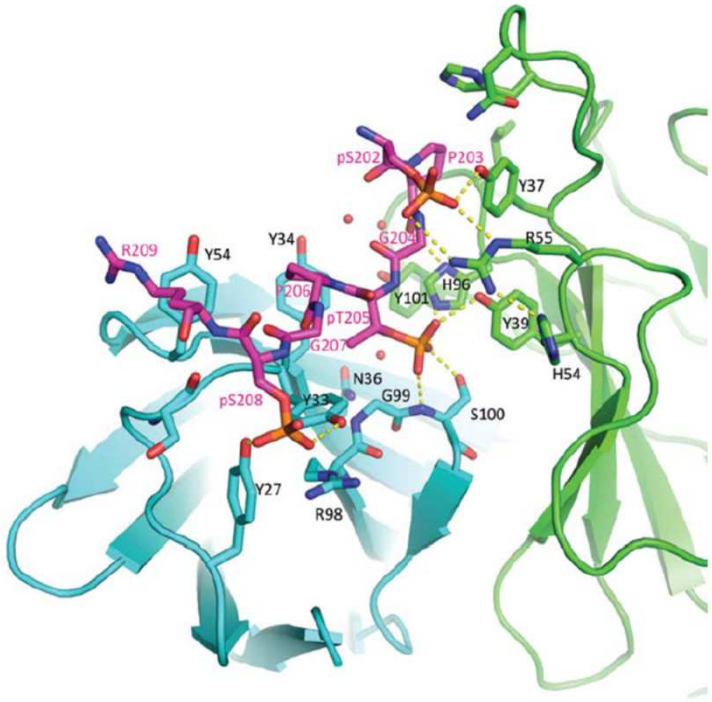
Braak immunodetection of Alzheimer's disease



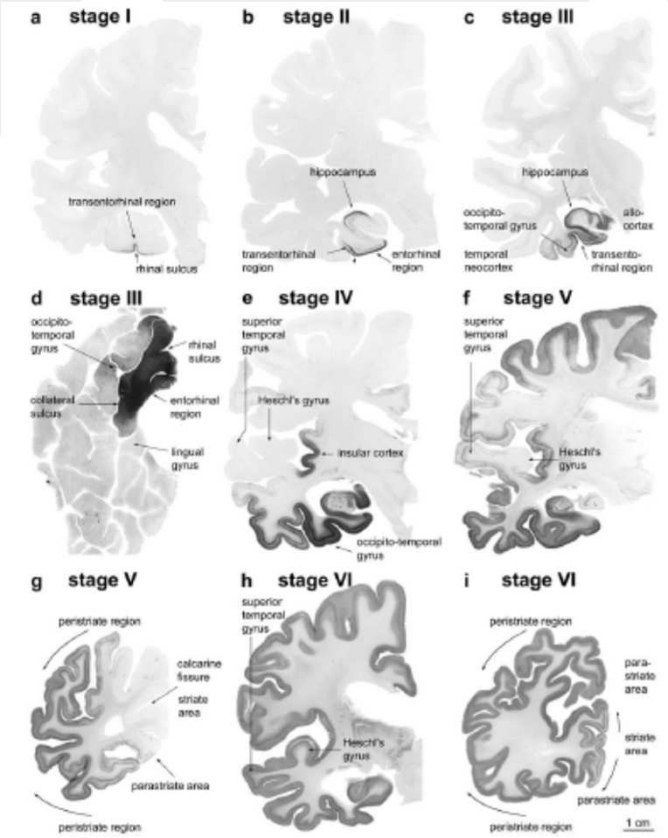
Braak et al., Acta Neuropath 2006

Tau protein : dysfunction

Braak immunodetection of Alzheimer's disease



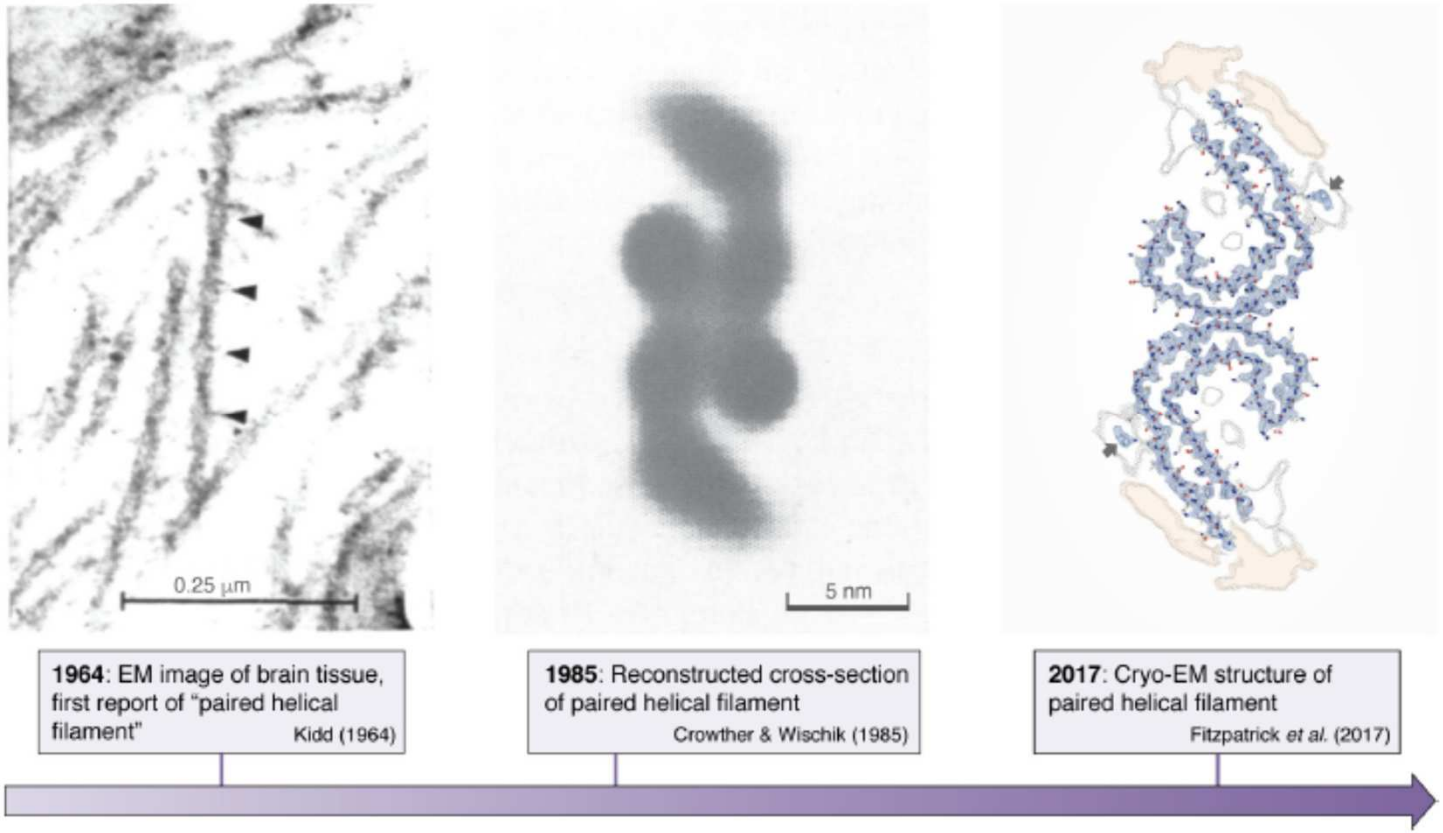
Malia et al., Proteins 2016



Braak et al., Acta Neuropath 2006

Tau protein : dysfunction

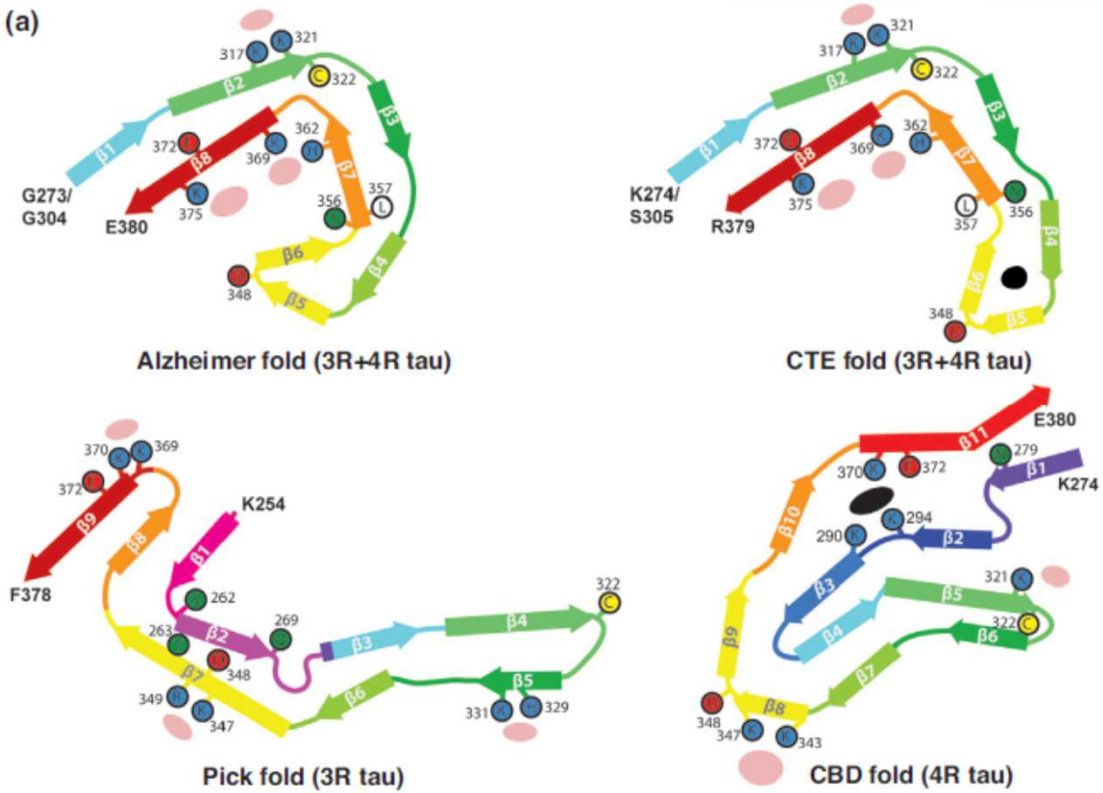
Resolution of Tau fibrils in Alzheimer's disease



Lippens & Gigant 2019

Tau protein : dysfunction

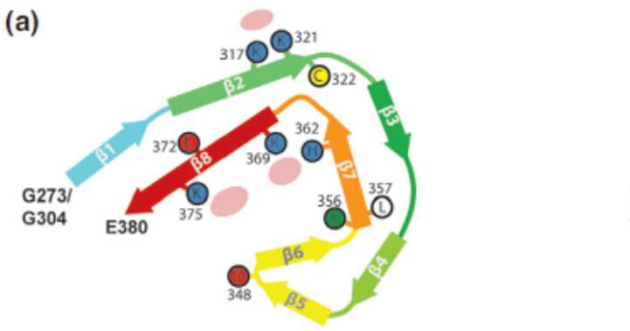
Resolution of Tau fibrils in taupathies



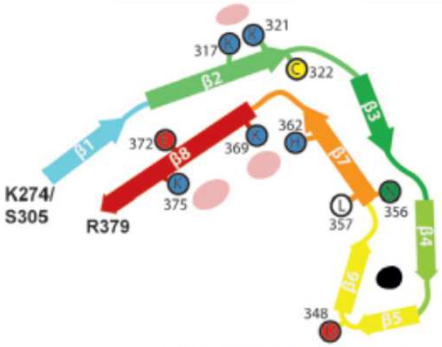
Scheres & Goedert 2020

Tau protein : dysfunction

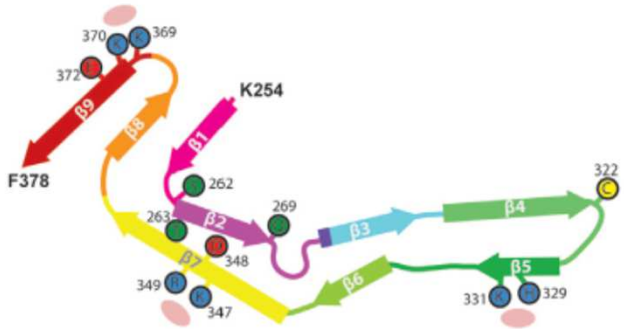
Resolution of Tau fibrils in taupathies



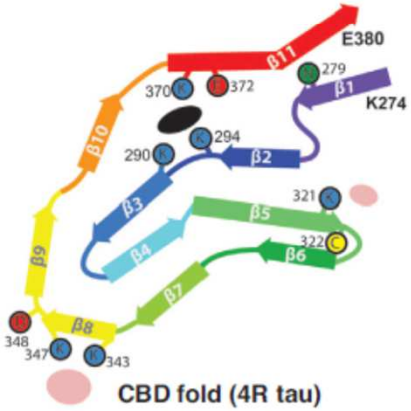
Alzheimer fold (3R+4R tau)



CTE fold (3R+4R tau)



Pick fold (3R tau)



CBD fold (4R tau)

Heparin induced synthetic fibers

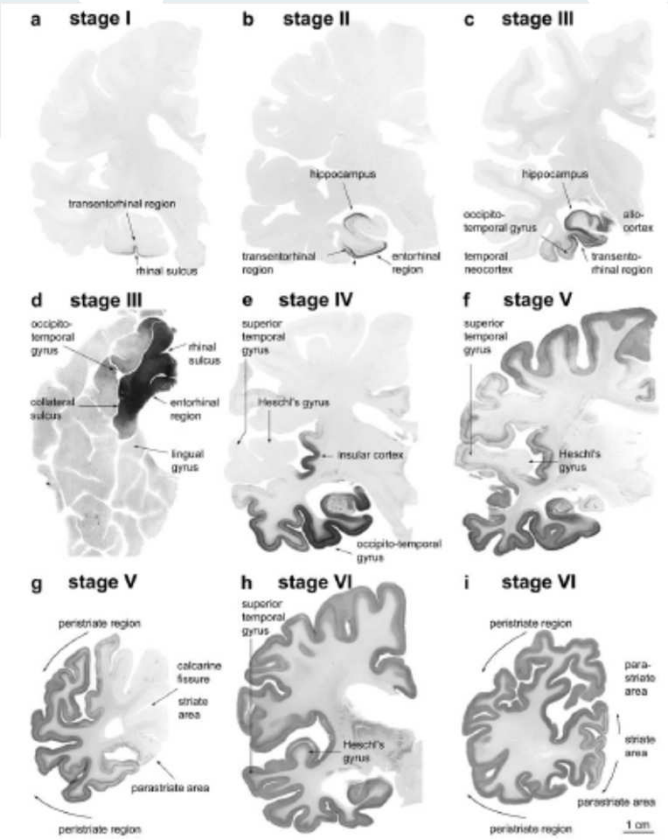
Scheres & Goedert 2020

Tau protein : dysfunction

Propagation

Crapper & De Boni **AD soluble fraction**

Paired helical filaments of the Alzheimer type in cultured neurones.
Nature 1978



Braak et al., Acta Neuropath 2006

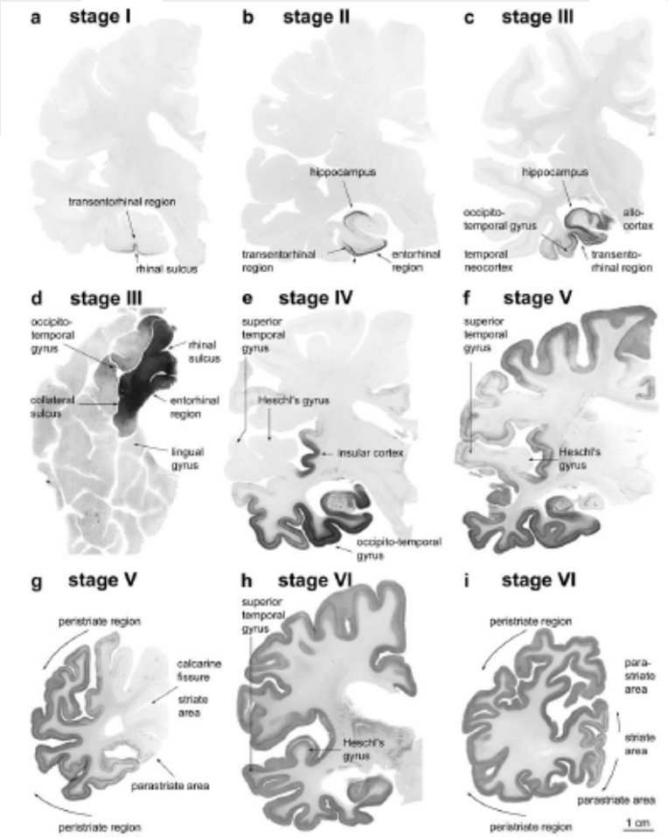
Tau protein : dysfunction

Propagation of Tau

Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW.
A protein factor essential for microtubule assembly.
Proc Natl Acad Sci U S A. 1975 **Tau**

Crapper & De Boni **AD soluble fraction**
Paired helical filaments of the Alzheimer type in cultured neurones.
Nature 1978

Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM.
Tau. A component of Alzheimer's disease paired helical filaments. J Biol Chem. 1986



Braak et al., Acta Neuropath 2006

Can we characterize the propagating species???

Crapper & De Boni Nature 1978	**AD soluble fraction**	***Paired helical filaments of the Alzheimer type in cultured neurones.***
Michel et al., JBC 2014	**Monomer**	***Extracellular Monomeric Tau Protein Is Sufficient to Initiate the Spread of Tau Protein Pathology***
Kim et al., Sci Reports 2014	**Dimer**	***Identification of disulfide crosslinked tau dimer responsible for tau propagation***
Mirbaha et al., JBC 2015	**Trimer**	***Tau Trimers Are the Minimal Propagation Unit Spontaneously Internalized to Seed Intracellular Aggregation***
Wu et al., JBC 2013	**LMW oligomers**	***Small Misfolded Tau Species Are Internalized via Bulk Endocytosis and Anterogradely and Retrogradely Transported in Neurons***
Takeda et al., Nat Comm 2015	**HMW oligomers**	***Neuronal uptake and propagation of a rare phosphorylated high-molecular-weight tau derived from Alzheimer's disease brain***

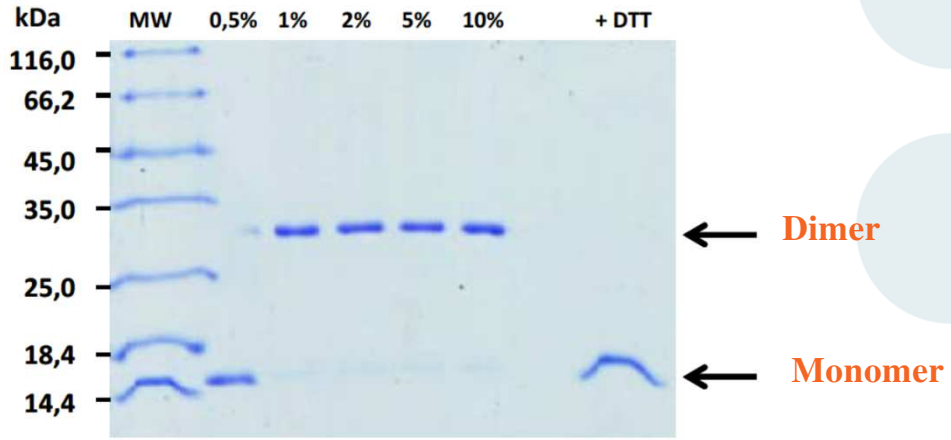
Tau protein : dysfunction

Propagation of Tau

Kim et al., **Dimer**
Sci Reports 2014

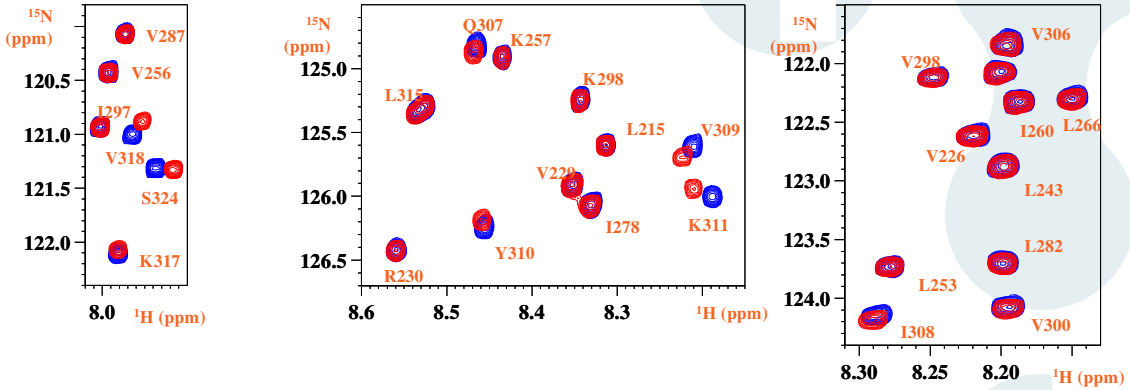
Identification of disulfide crosslinked tau dimer responsible for tau propagation

TauF4 C291S



Tau protein : dysfunction

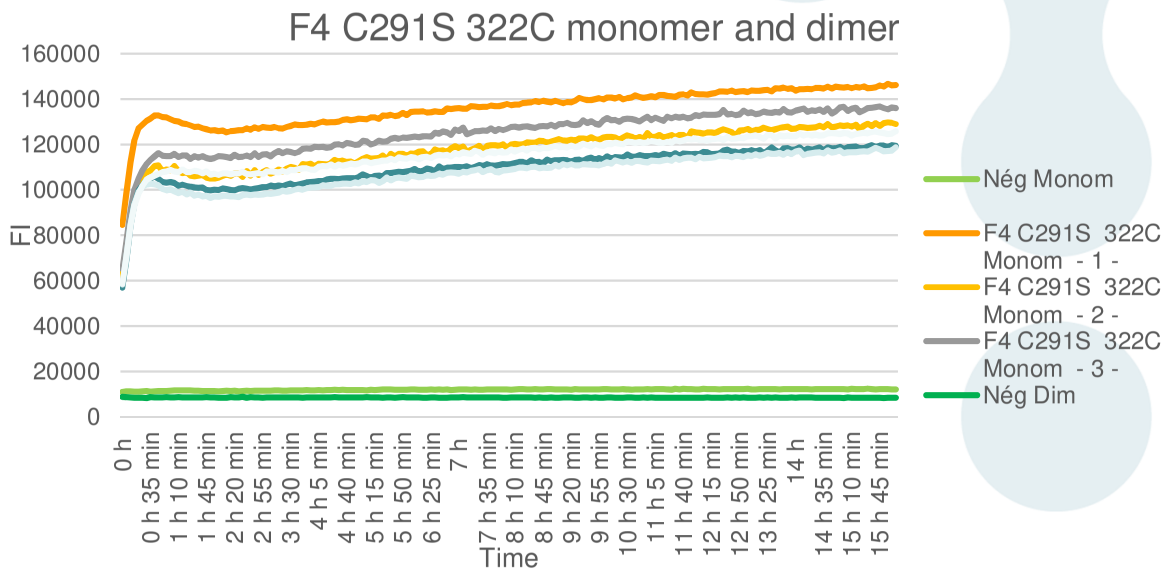
Propagation of Tau



TauF4 C291S dimer without or with DTT

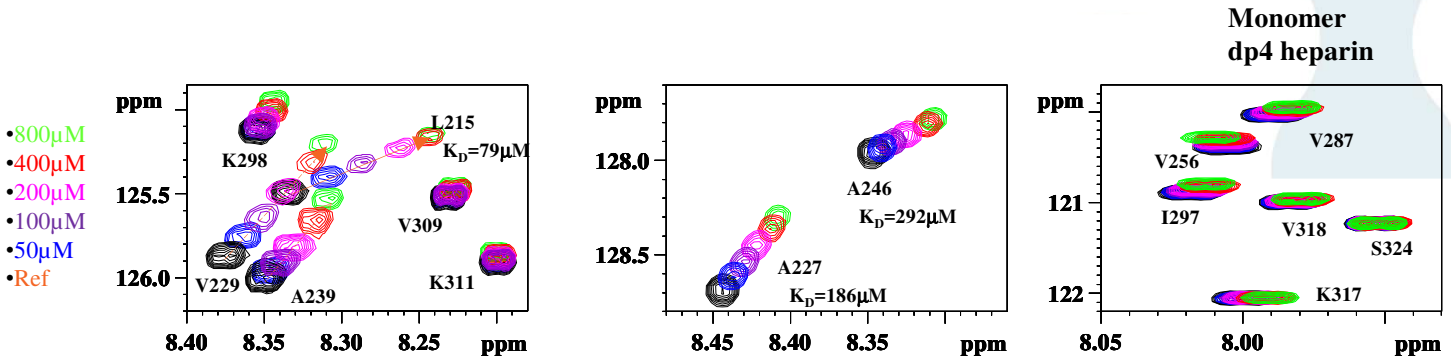
Tau protein : dysfunction

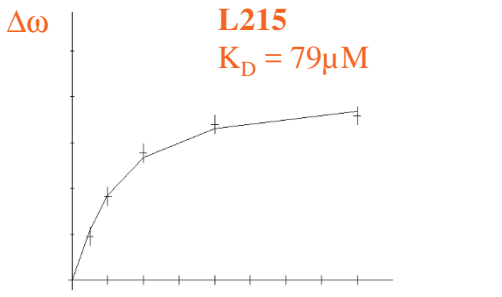
Propagation of Tau

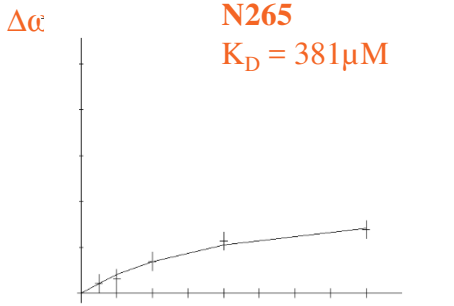


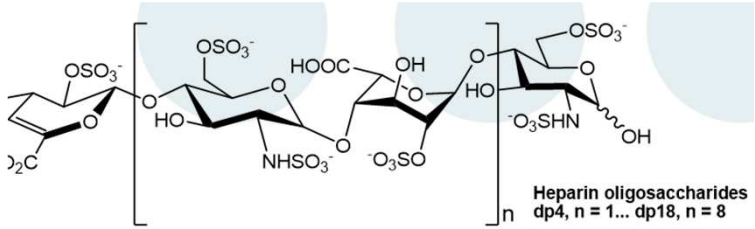
Tau protein : dysfunction

Propagation of Tau





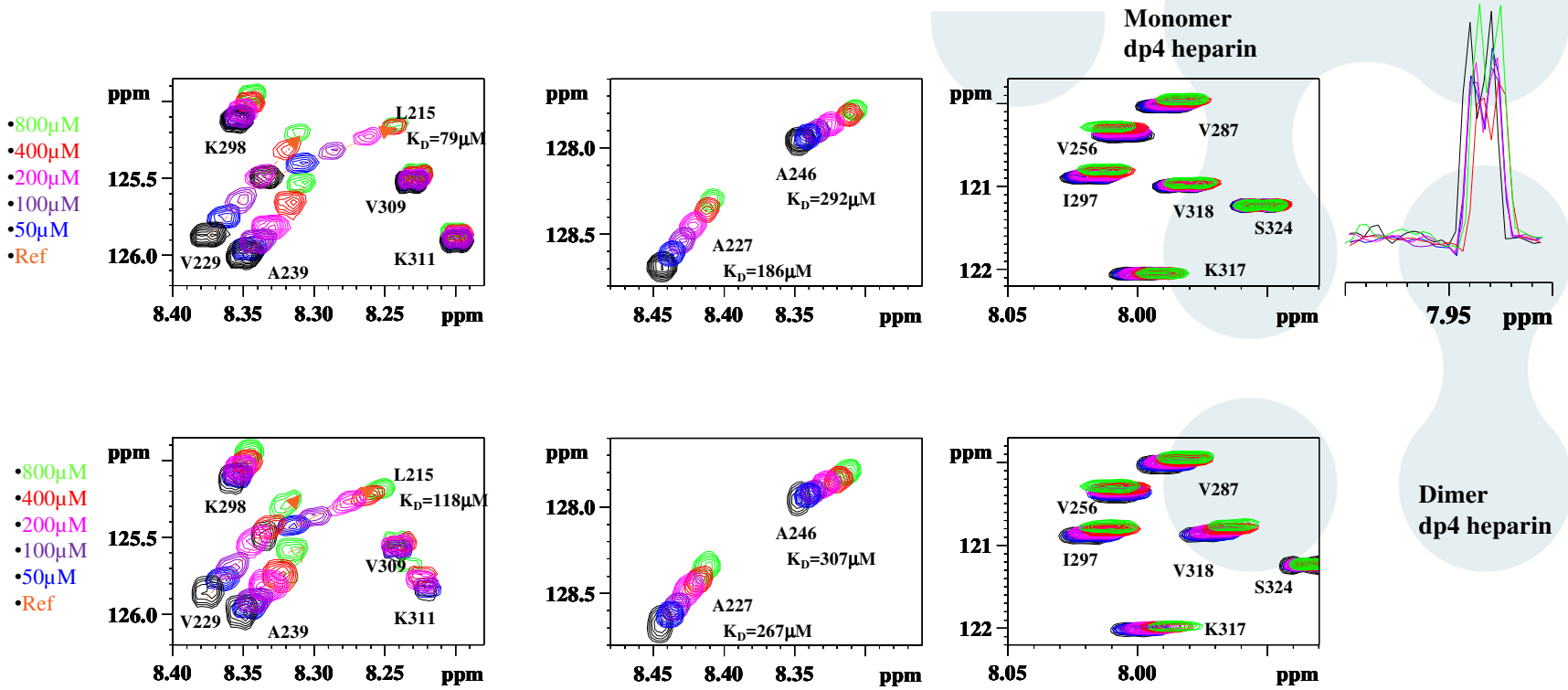




n=4

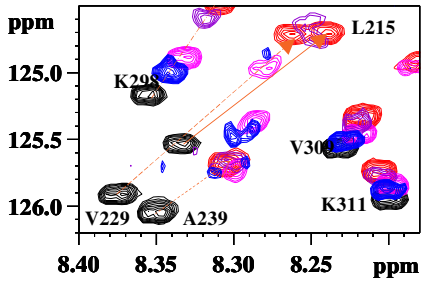
Tau protein : dysfunction

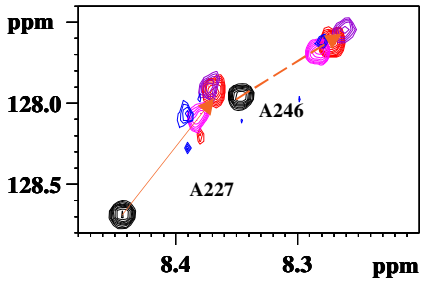
Propagation of Tau

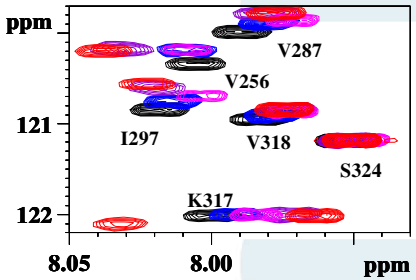


Tau protein : dysfunction

Propagation of Tau

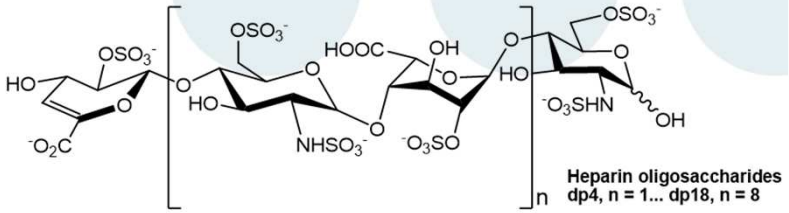






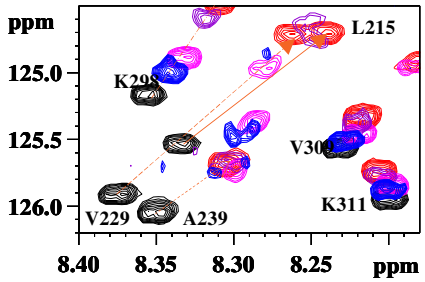
Monomer
dp16 heparin

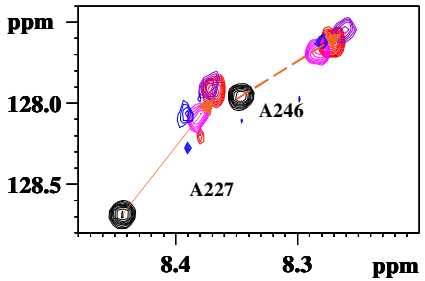
Larger HS bind tighter - slow exchange

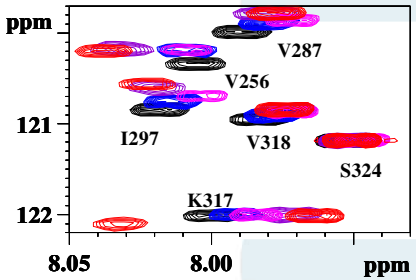


Tau protein : dysfunction

Propagation of Tau







Monomer
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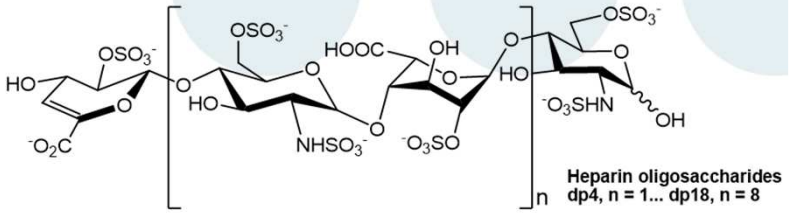
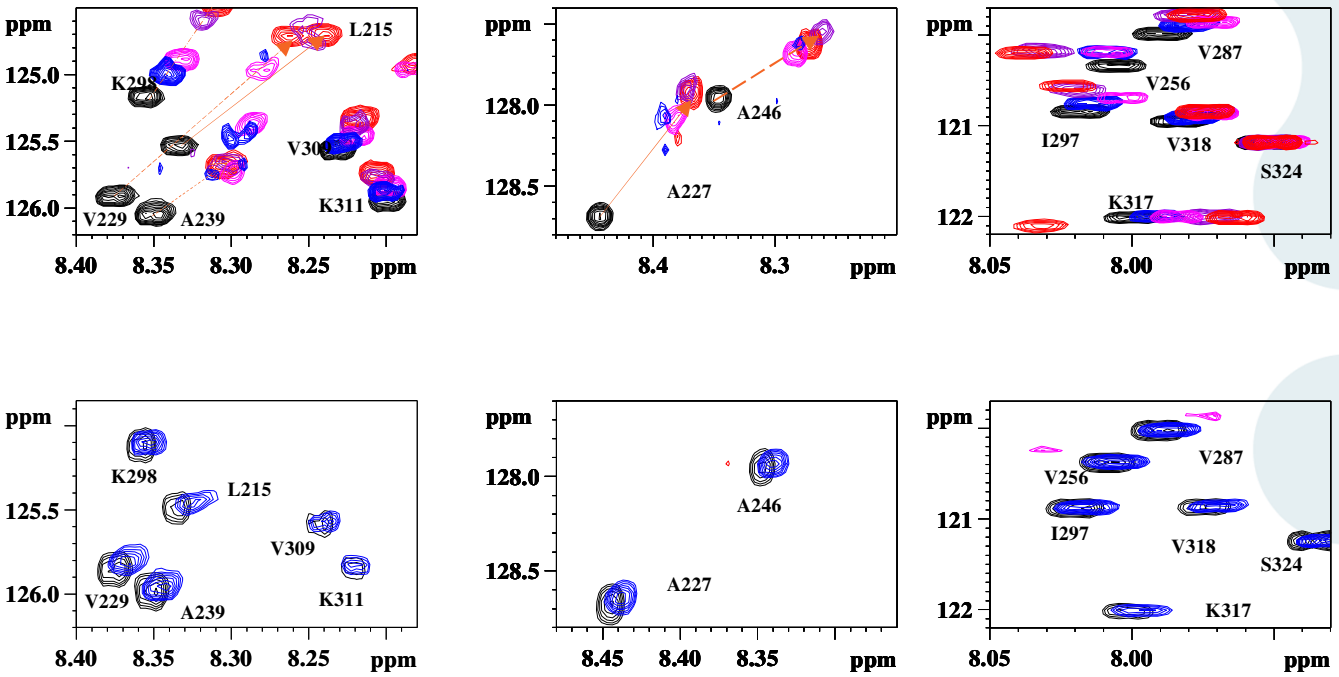


Tau protein : dysfunction

Propagation of Tau



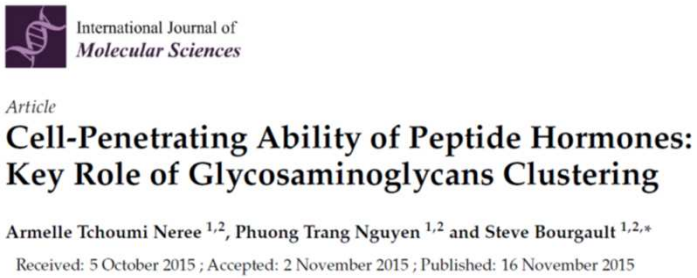
Monomer
dp16 heparin

Dimer
dp16 heparin

At the same absolute concentration of TauF4, addition of >25µM of dp16 leads to aggregation of the TauF4 dimers but not monomers.

Tau protein : dysfunction

Propagation of Tau



International Journal of *Molecular Sciences*

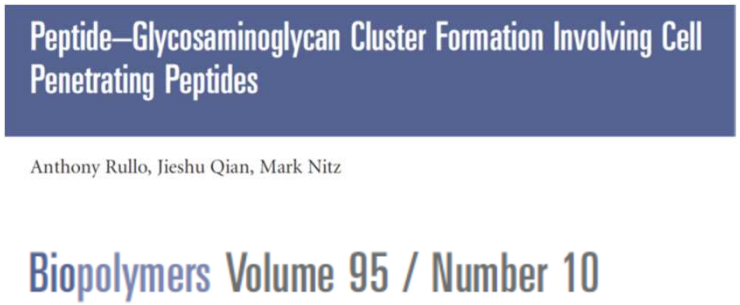
Article

Cell-Penetrating Ability of Peptide Hormones: Key Role of Glycosaminoglycans Clustering

Armelle Tchoumi Neree [1,2], Phuong Trang Nguyen [1,2] and Steve Bourgault [1,2,*]

Received: 5 October 2015 ; Accepted: 2 November 2015 ; Published: 16 November 2015

This study demonstrates that the uptake efficiency of a given cationic CPP does not necessarily correlate with its affinity to sulfated GAGs and that its ability to cluster GAGs should be considered for the identification of novel peptidic sequences with potent cellular penetrating properties.



Peptide–Glycosaminoglycan Cluster Formation Involving Cell Penetrating Peptides

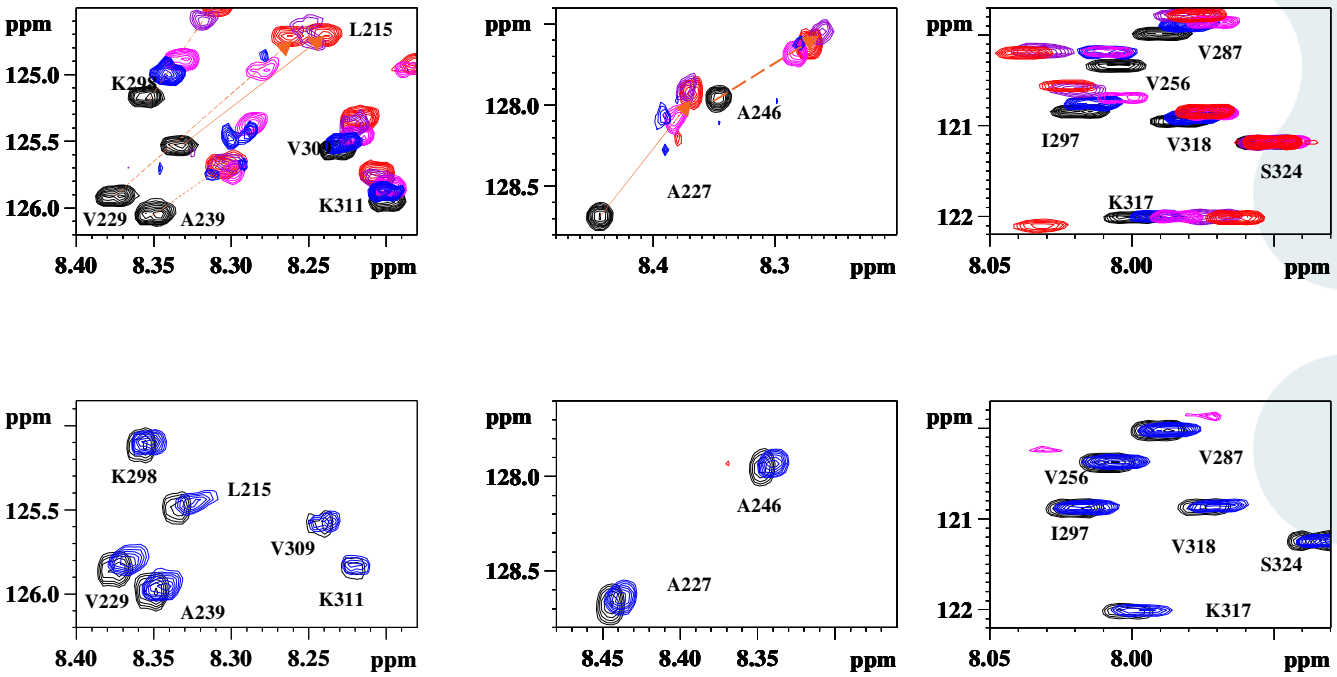
Anthony Rullo, Jieshu Qian, Mark Nitz

Biopolymers Volume 95 / Number 10

We have demonstrated that peptides with similar binding affinity for heparin can differ significantly in their ability to cluster with heparin. (…) The differences in cluster stability observed between these two peptides provide a possible explanation for their differing cell uptake routes.

Tau protein : dysfunction

Propagation of Tau



Monomer
dp16 heparin

Dimer
dp16 heparin

At the same absolute concentration of TauF4, addition of >25µM of dp16 leads to aggregation ??? of the TauF4 dimers but not monomers.

Tau protein : dysfunction

Liquid liquid phase separation

Koazervation.

(Entmischung in kolloiden Systemen.)

Vorläufige Mitteilung. (Eingegangen am 4. Oktober 1929.)

Von H. G. Bungenberg de Jong (Leiden) und H. R. Kruyt (Utrecht).

LLPS of Tau

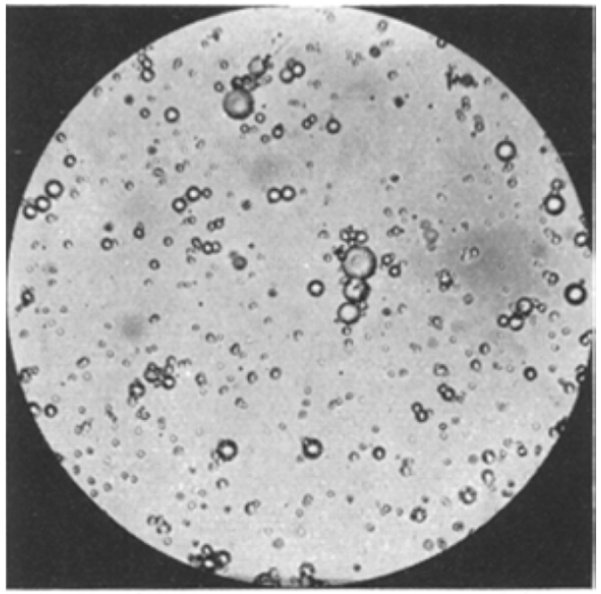


Fig. 3
Gelatine + Tannin. 162× vergr

LLPS of Tau

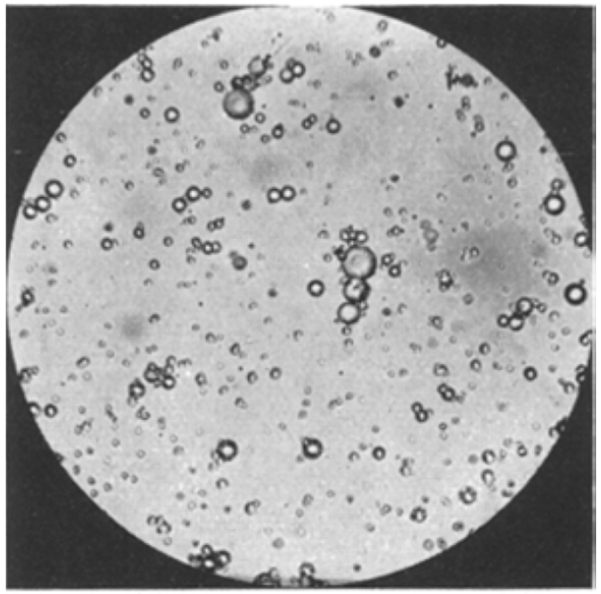


Fig. 3
Gelatine + Tannin. 162× vergr

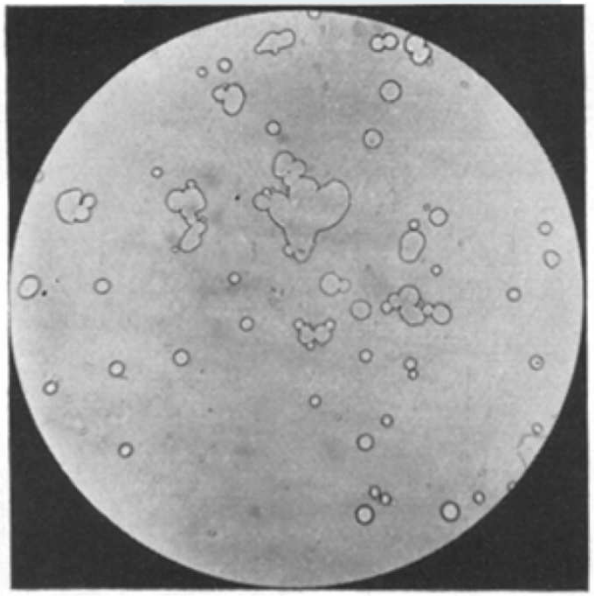
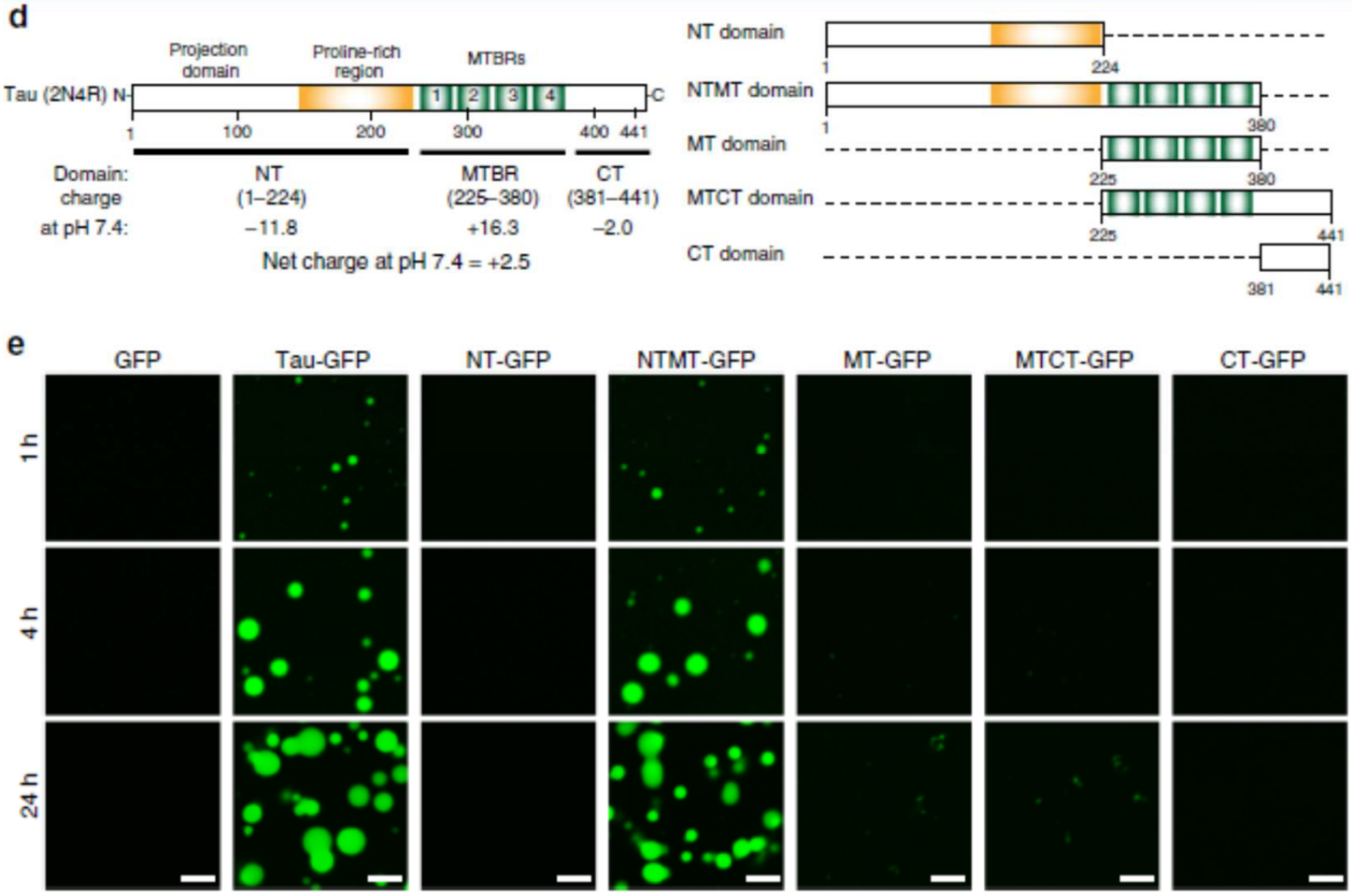


Fig. 8
Serumalbumin + arab. Gumm. 72× vergr.

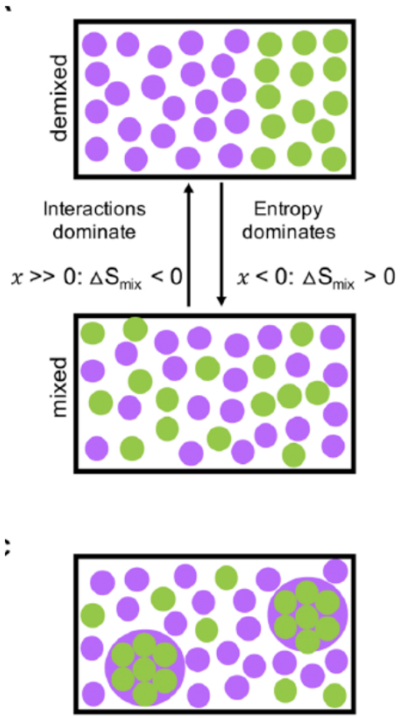
LLPS of Tau



2µM Tau – 10% PEG8000

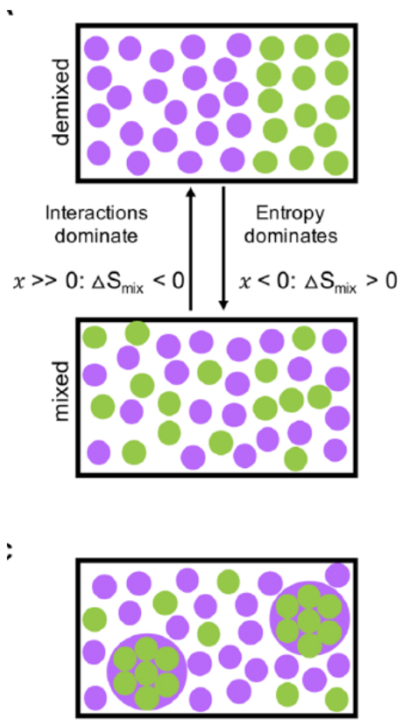
Kanaan *et al.* Nat Comm 2020

LLPS of Tau



Ukmar-Godec *et al.* Sem Cell Biol 2020

LLPS of Tau



Liquid–liquid phase separation of tau protein: The crucial role of electrostatic interactions

Boyko et al. *J. Biol. Chem.* (2019) 294(29) 11054–11059

Liquid-liquid phase separation of tau driven by hydrophobic interaction facilitates fibrillization of tau

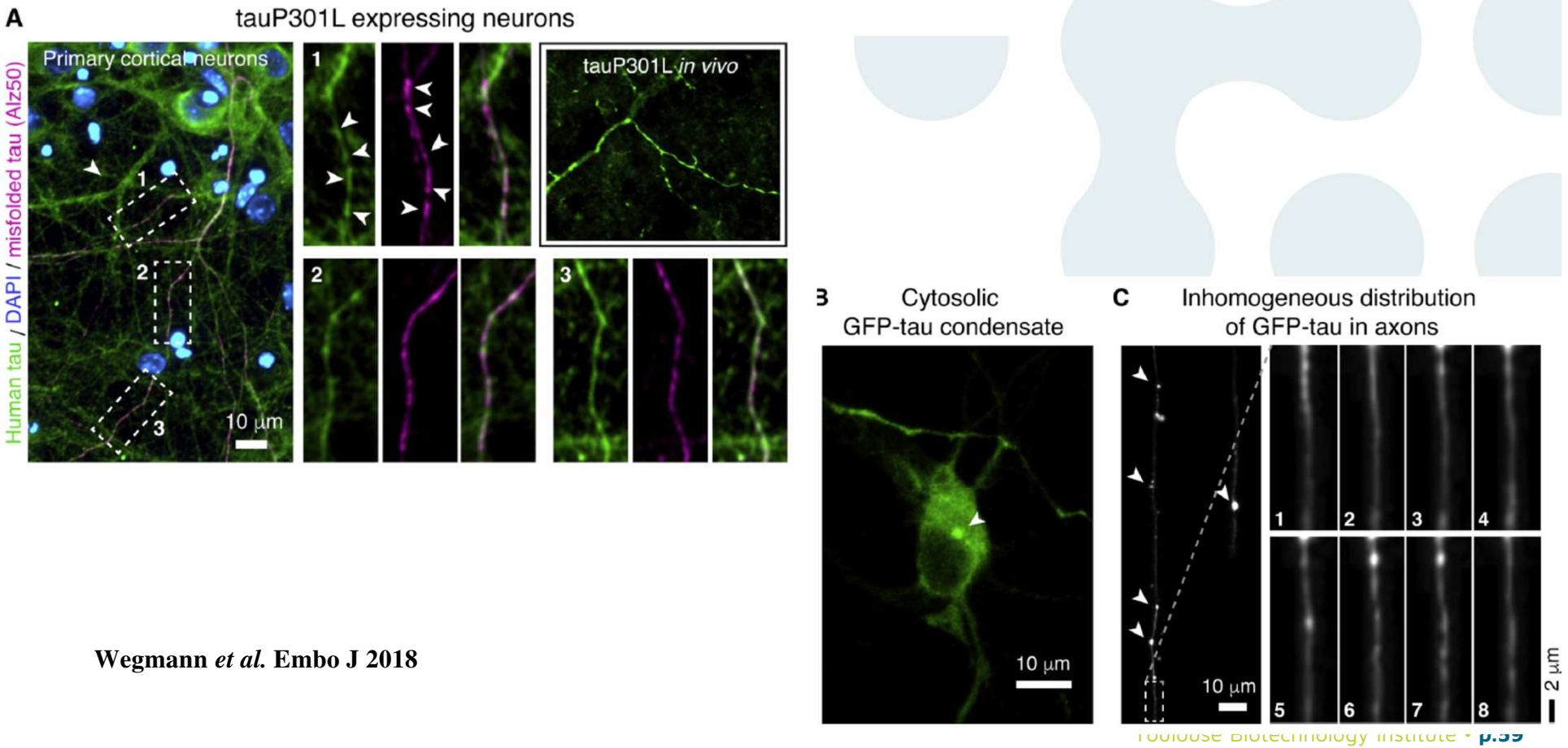
Lin et al., https://doi.org/10.1101/2020.08.05.237966 doi: bioRxiv preprint

The proline-rich domain promotes Tau liquid–liquid phase separation in cells

Zhang et al., J. Cell Biol. 2020 Vol. 219 No. 11 e202006054

Narrow equilibrium window for complex coacervation of tau and RNA under cellular conditions Lin *et al.* eLife 2019;8:e42571

LLPS of Tau



Wegmann *et al.* Embo J 2018

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