

# Advanced Applications of NMR:

## Samples, Dynamics and Examples

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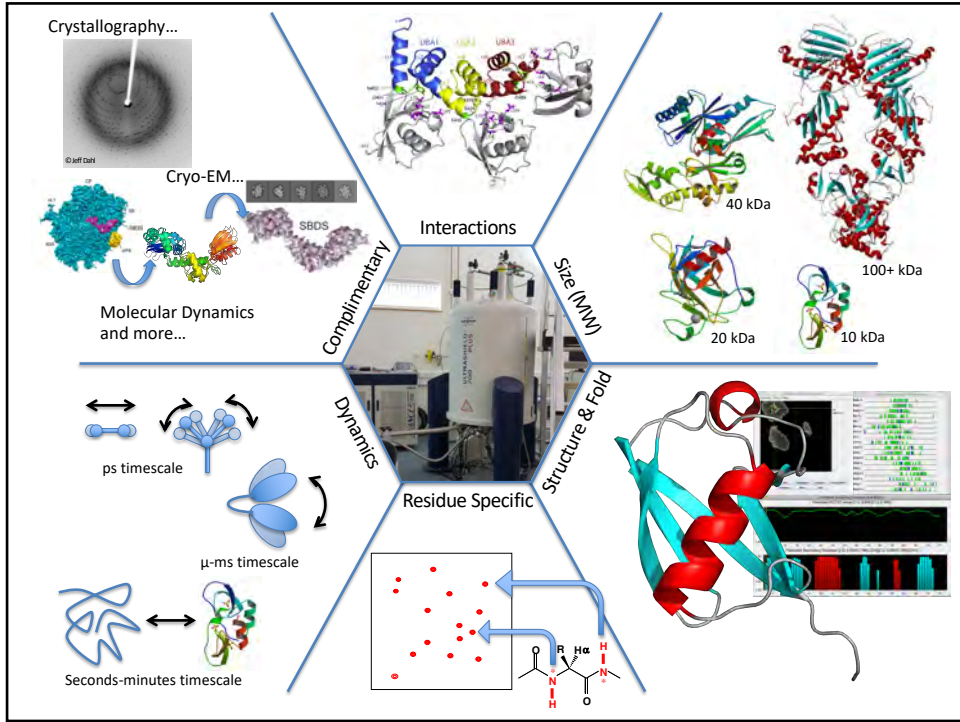


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### Where to find us!



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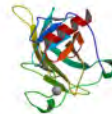


- Samples
- Dynamics
- Examples
  - Ubiquitin: phosphorylation and novel conformer
  - LITAF: modeling patient mutations in a membrane protein

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## Molecular weight range

			
10 kDa	20 kDa	40 kDa	>100-1000 kDa
Full 3D Structure, Dynamics	Assignments, 3D fold recognition, internal dynamics, interactions, functional studies		Individual residues, complex interactions, docking, IDP, mobile linker motion
2D/3D	2D/3D	nD TROSY	Methyl -TROSY
<sup>1</sup> H	<sup>1</sup> H, <sup>15</sup> N, <sup>13</sup> C	<sup>1</sup> H, <sup>15</sup> N, <sup>13</sup> C, <sup>2</sup> H	Selective / segmental labeling (Intein)
<b>“Complete Picture”</b>		<b>“Site Specific”</b>	

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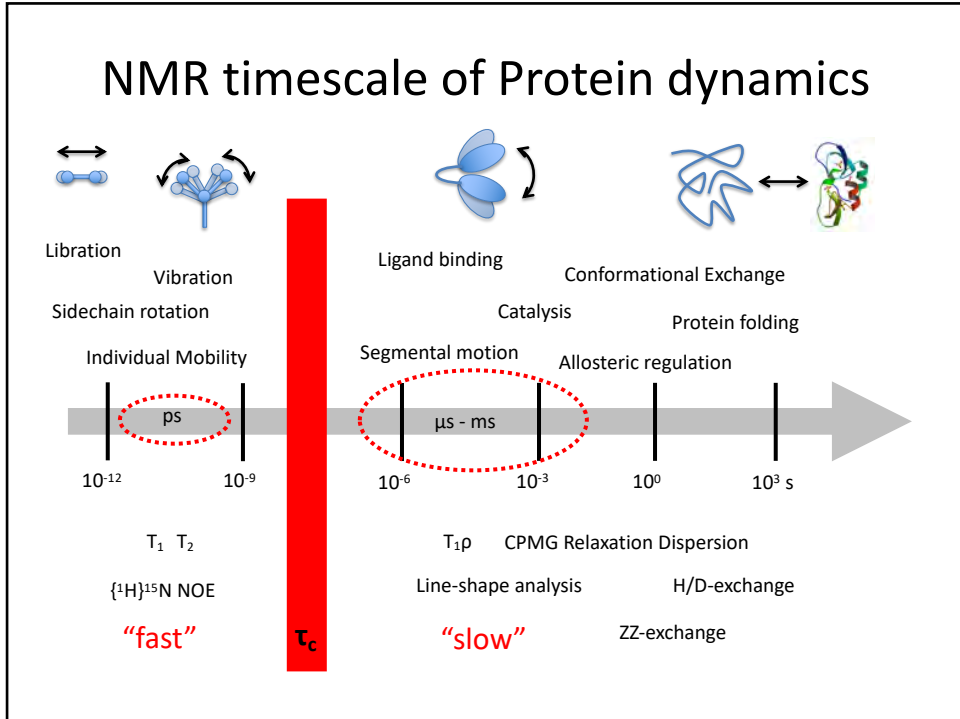
## Sample preparation

- Minimal Media
  - $^{15}\text{N}$  ammonium chloride
  - and/or  $^{13}\text{C}$  glucose ( $^2\text{H}$ & $^{13}\text{C}$  glucose)
  - $\text{D}_2\text{O}$
- Sample conditions
  - 550  $\mu\text{L}$  of 100-500  $\mu\text{M}$  in aqueous buffer
  - mid-range pH
  - 5 % by volume deuterated solvent ( $\text{D}_2\text{O}$ )

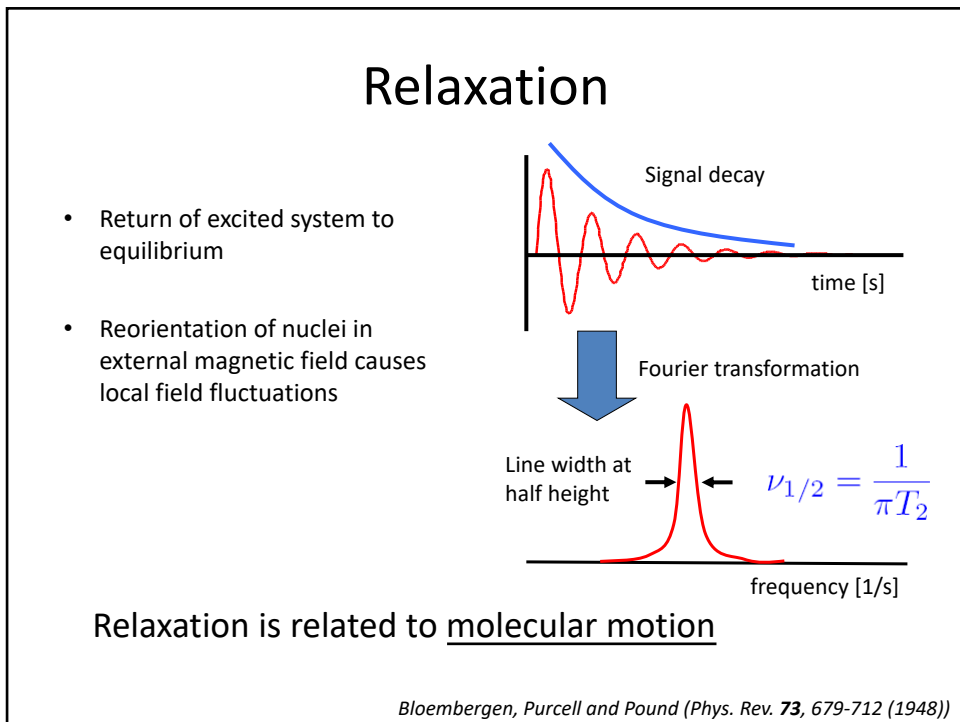
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## Inter-conversion between states

Inter-conversion on intermediate  $\mu\text{s}$ -ms timescale results in line broadening : **apparent  $R_2$  increase**

Observed for:

- Slow versus fast folding of proteins
- 2-state binding equilibrium
- Cis-trans proline isomerisation
- Segmental motions in proteins
- Monomer-oligomer equilibria in solution

rate constants from line shape

$$R_{ex} = p_1 p_2 \delta \nu^2 \tau_{ex}$$

$p_1$  and  $p_2$  populations of states A,B  
 $\tau_{ex} = p_1/k_{-1} = p_2/k_1$  and  $\delta\omega = |\omega_1 - \omega_2|$

A,  $\nu_1$       B,  $\nu_2$

Slow exchange  
 $K_{ex} \ll \delta\nu$

coalescence  
 $K_{ex} \sim \delta\nu$

fast exchange  
 $K_{ex} \gg \delta\nu$

Conformational Exchange

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## Cross-relaxation: $\{^1\text{H}\}^{15}\text{N}$ NOE

$\{^1\text{H}\}^{15}\text{N}$  NOE is the ratio between steady-state and equilibrium magnetisation :

$$^1\text{H}-^{15}\text{NNOE} = 1 + \frac{\sigma_{\text{HN}} \gamma_{\text{H}}}{R_{1\text{N}} \gamma_{\text{N}}}$$

Dipole-dipole interactions cause cross-relaxation :

$$\sigma_{\text{HN}} = \frac{d_{\text{oo}}}{4} \{-J(\omega_{\text{H}} - \omega_{\text{N}}) + 6J(\omega_{\text{H}} + \omega_{\text{N}})\} \text{ (OH)}$$

**Rigid**

**Flexible**

Probes fast picosecond mobility

Ho A, Wagstaff JL, et al. *BMC Biology*. (2016) 14 p109

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# Auto-relaxation of a <sup>15</sup>N nucleus

- Longitudinal relaxation rate  $R_1=1/T_1$

Spin-Lattice

$$R_1^{DD} = \frac{d_{oo}}{4} \{J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)\}$$



$$R_1^{CSA} = cJ(\omega_N)$$



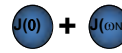
- Transverse relaxation rate  $R_2=1/T_2$

Spin-Spin

**DD**  $R_2^{DD} = \frac{d_{oo}}{8} \{4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)\}$



**CSA**  $R_2^{CSA} = \frac{c}{6} \{4J(0) + 3J(\omega_N)\}$

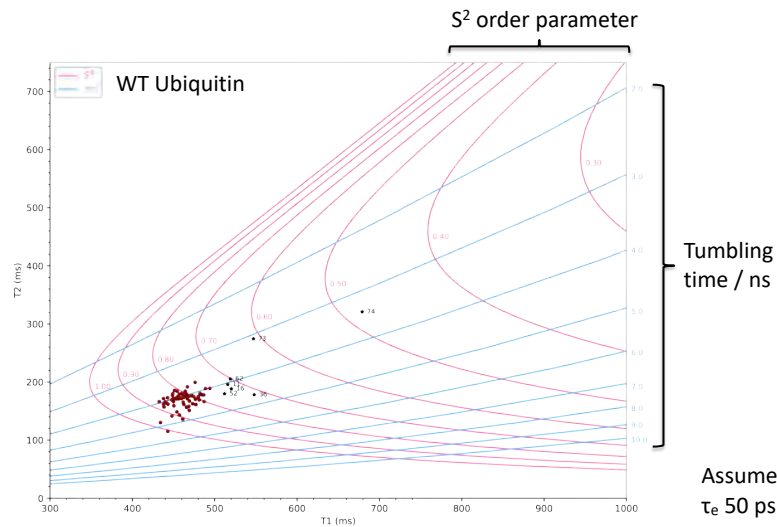


+ Exchange

with  $d_{oo} = \frac{\hbar^2 \gamma_H^2 \gamma_N^2}{r_{HN}^6}$  and  $c = (\sigma_{\parallel} - \sigma_{\perp})^2 \frac{\gamma_N^2 B_0^2}{3}$   
(Field dependent term!)

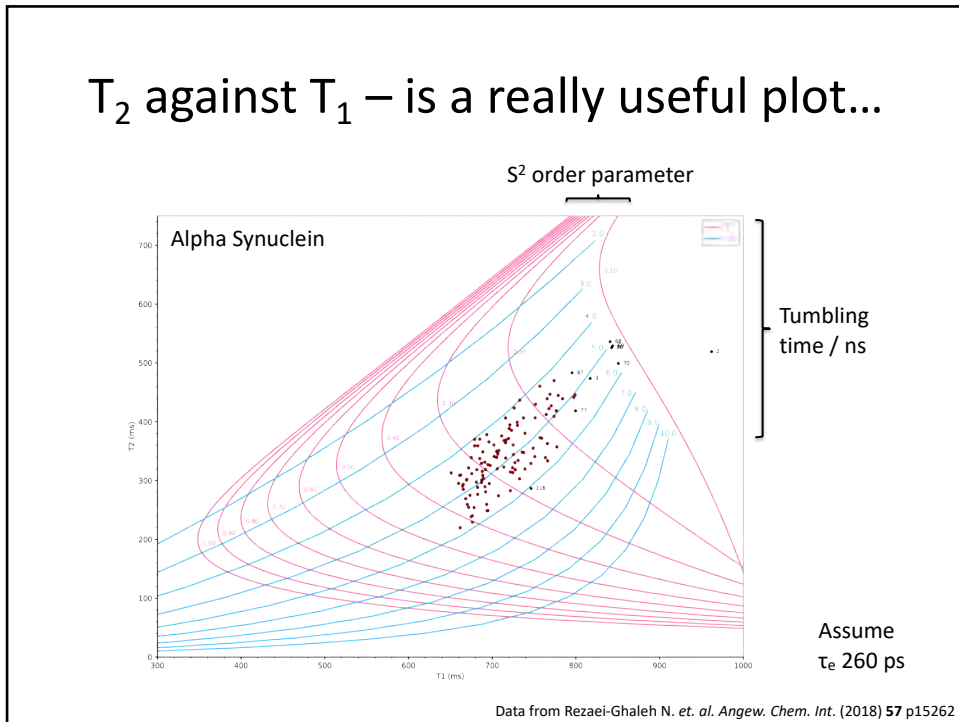
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# T<sub>2</sub> against T<sub>1</sub> – is a really useful plot...



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## $T_2$ against $T_1$ – is a really useful plot...



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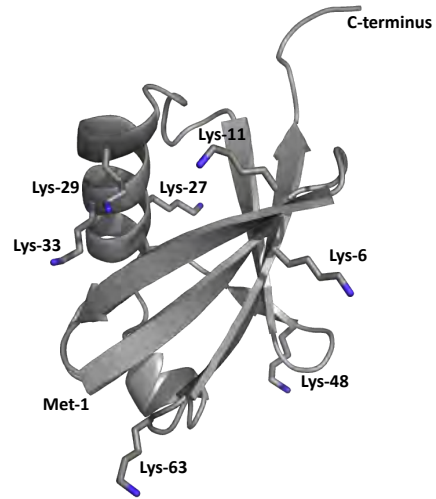
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# Ubiquitination

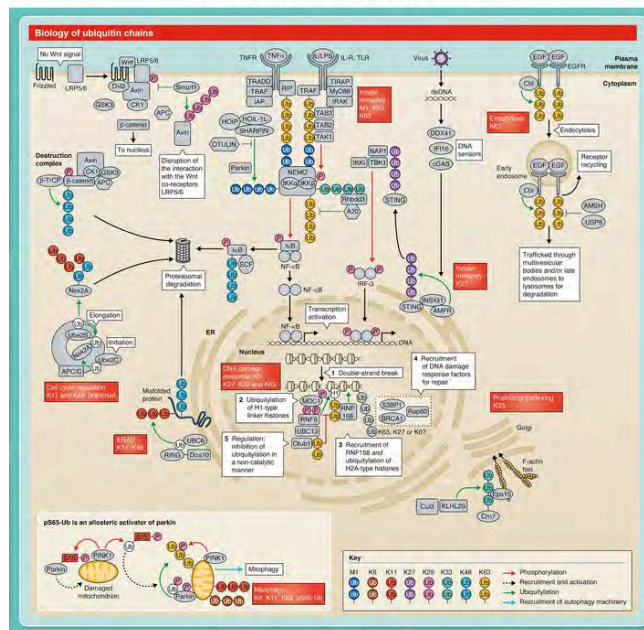
- Highly conserved
- Chain permutations
- Multiple functions



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## Ubiquitin Chain Diversity at a Glance

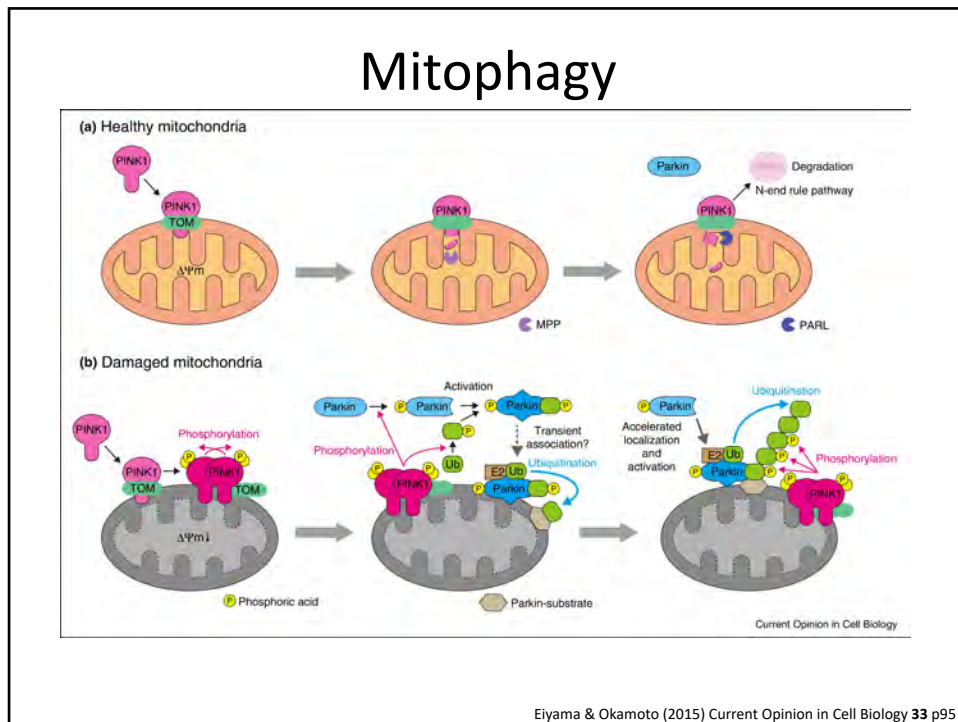
Masato Akutsu, Ivan Dikic and Anja Bremm



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Masato Akutsu et al. (2016) J Cell Sci 129 p875-880

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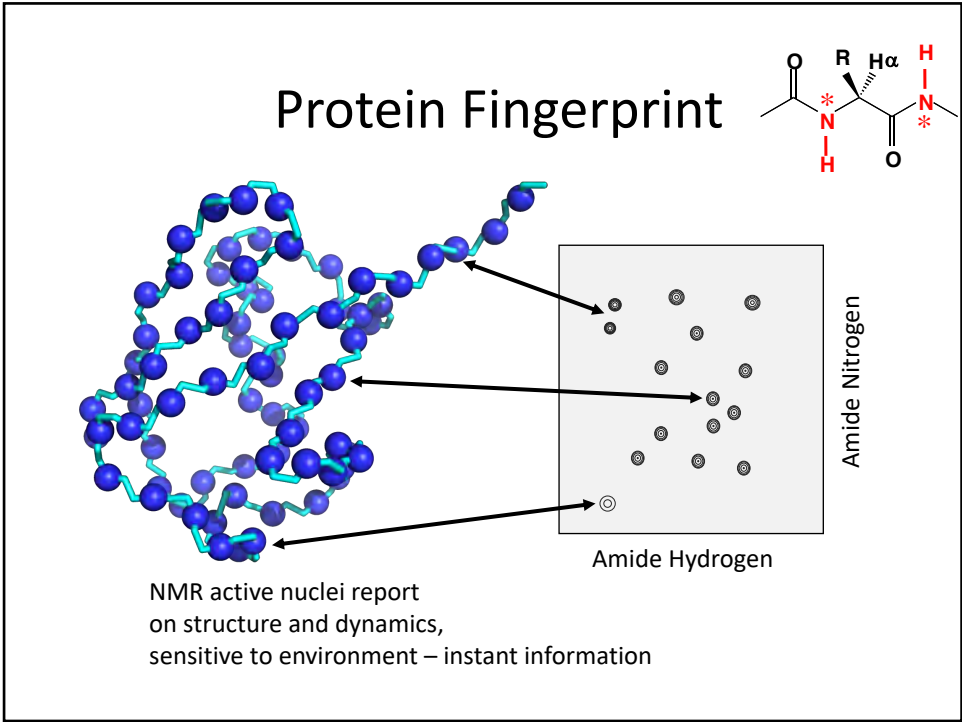


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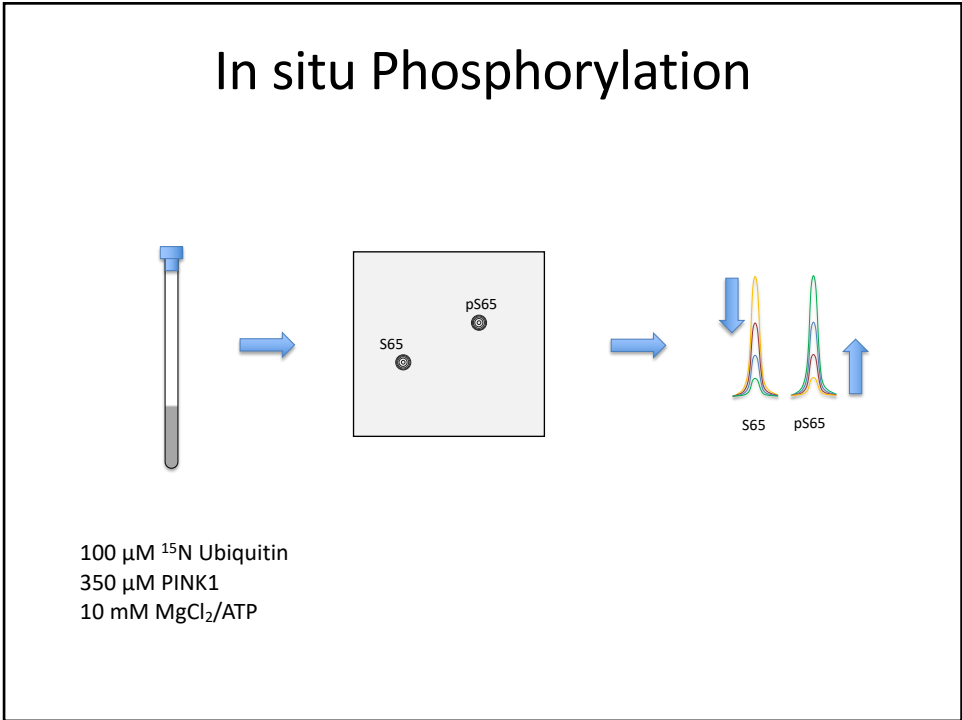
## Phosphorylation of Ubiquitin by PINK1

- Mutations in the Ubiquitin E3 ligase Parkin lead to propensity to develop autosomal juvenile Parkinsonism
- Parkin is activated by the Kinase PINK1
- Parkin has a UBL, and this is a target for PINK1 phosphorylation
- Phosphorylated ubiquitin can also activate Parkin
- PINK1 phosphorylates ubiquitin at S65 – what are the consequences ?

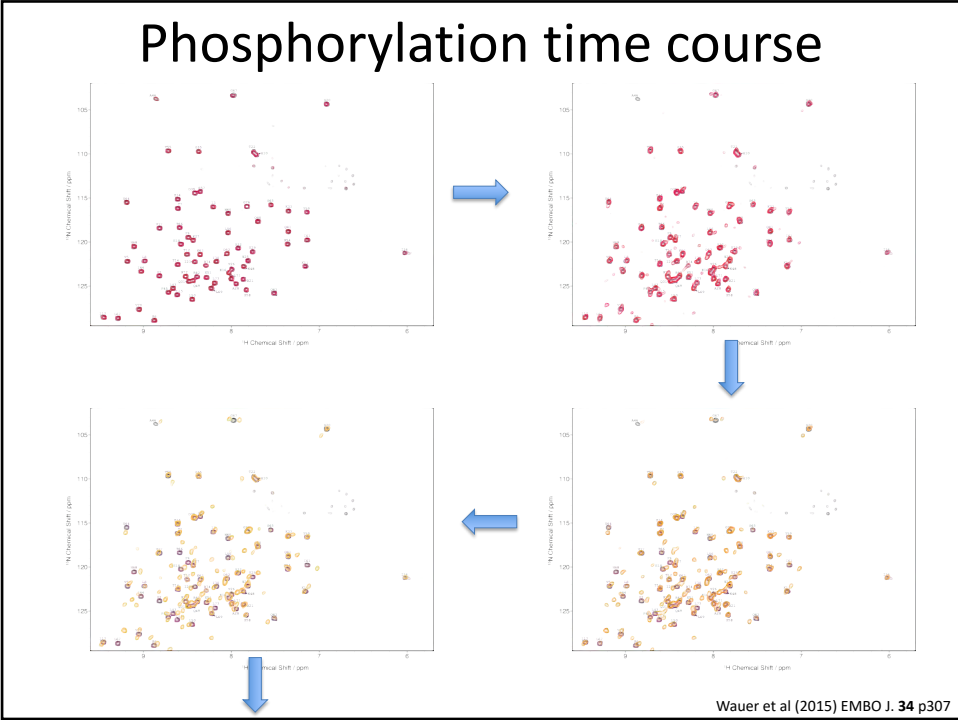
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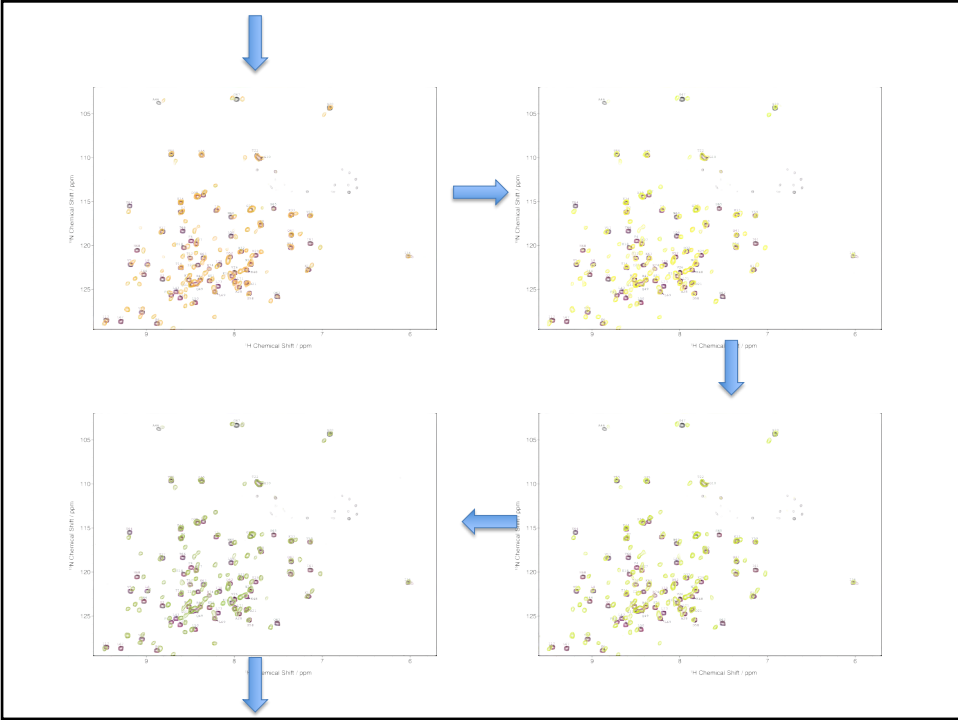
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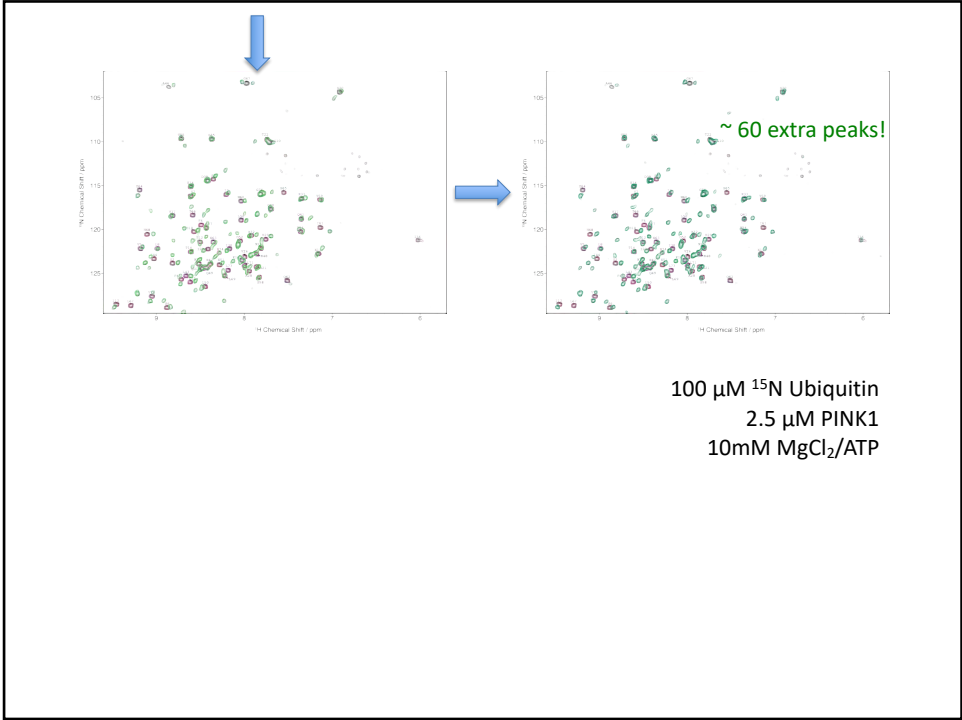
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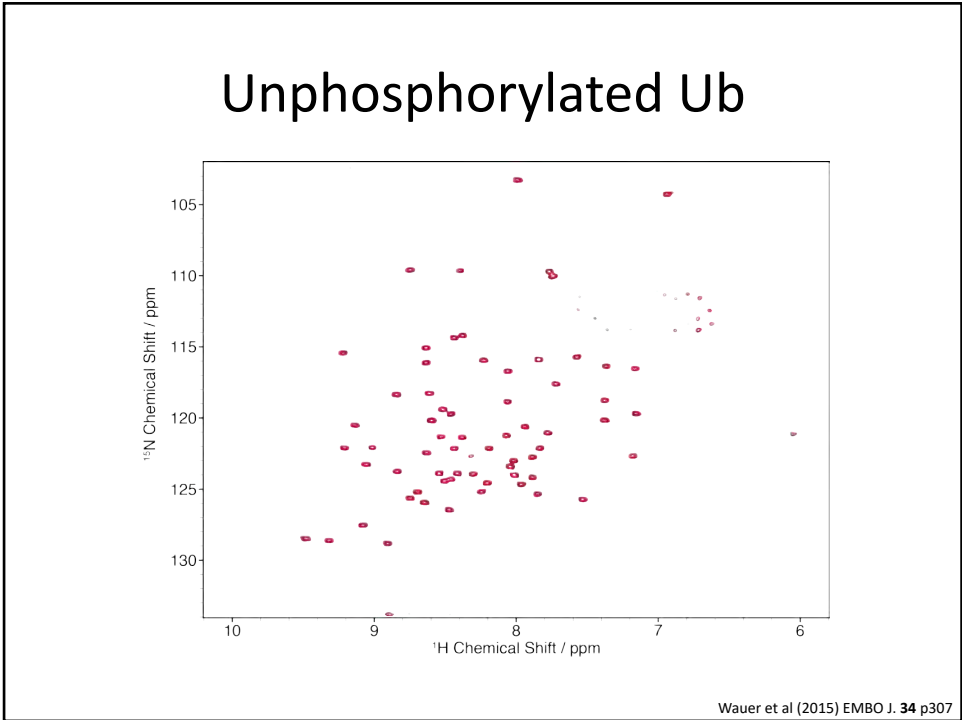
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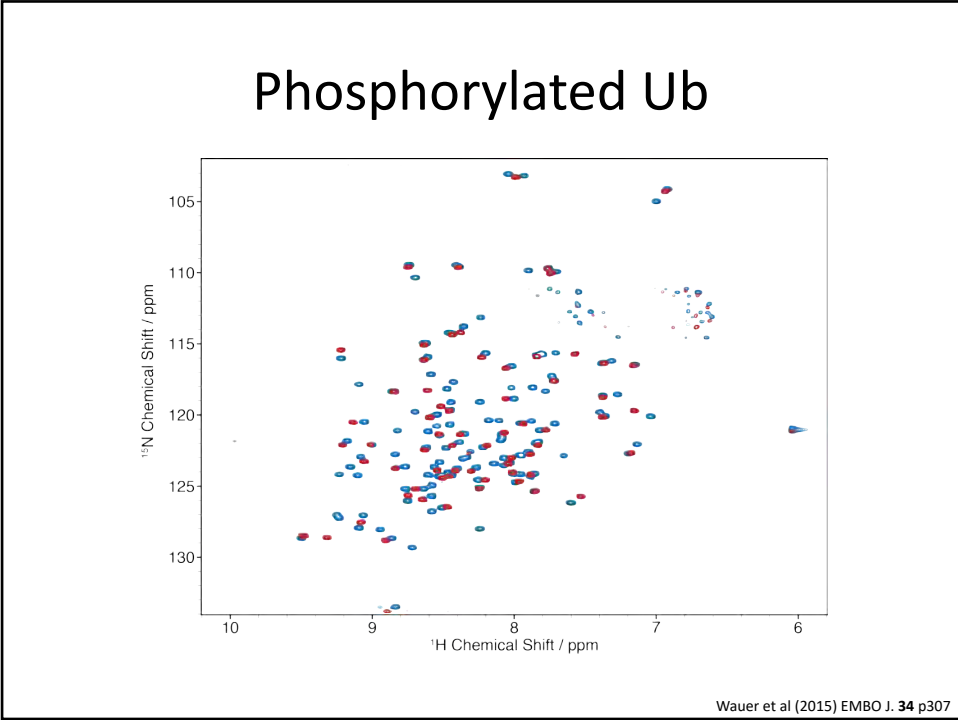
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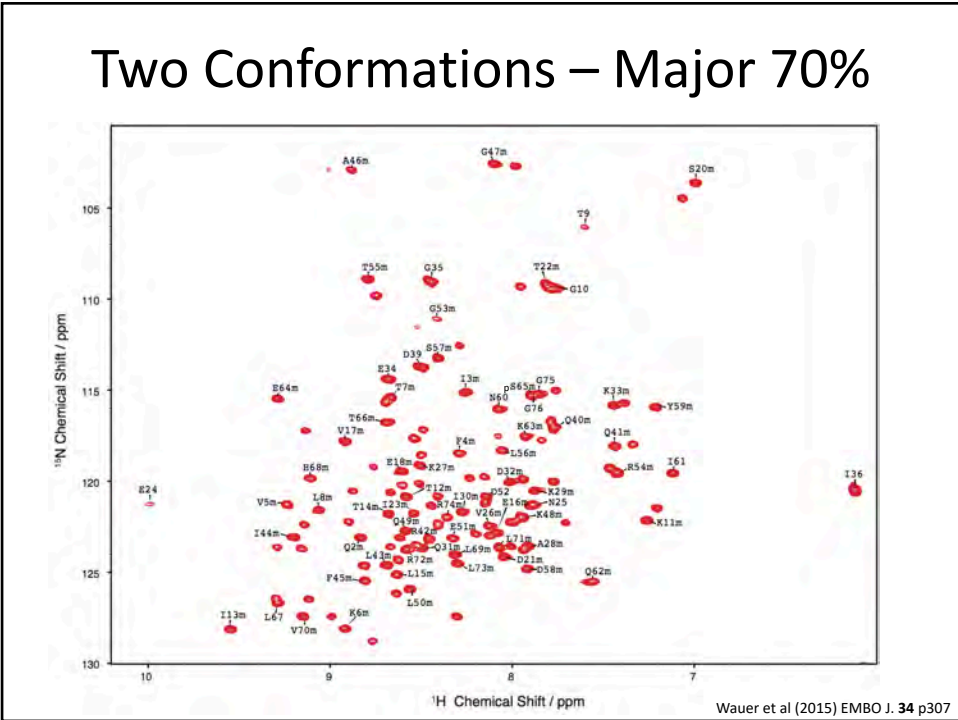
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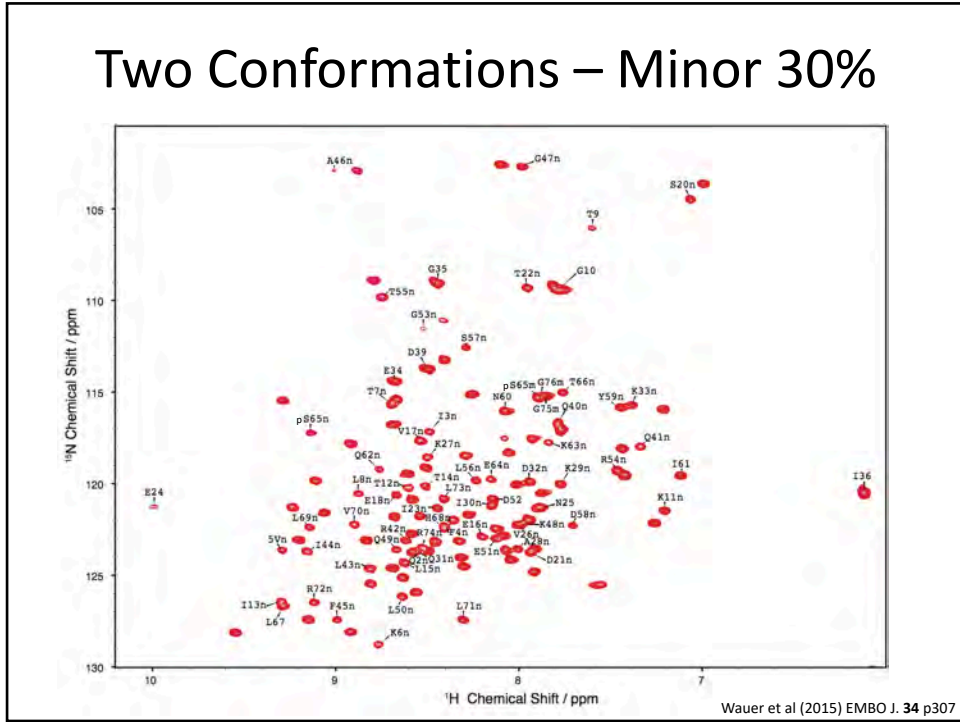


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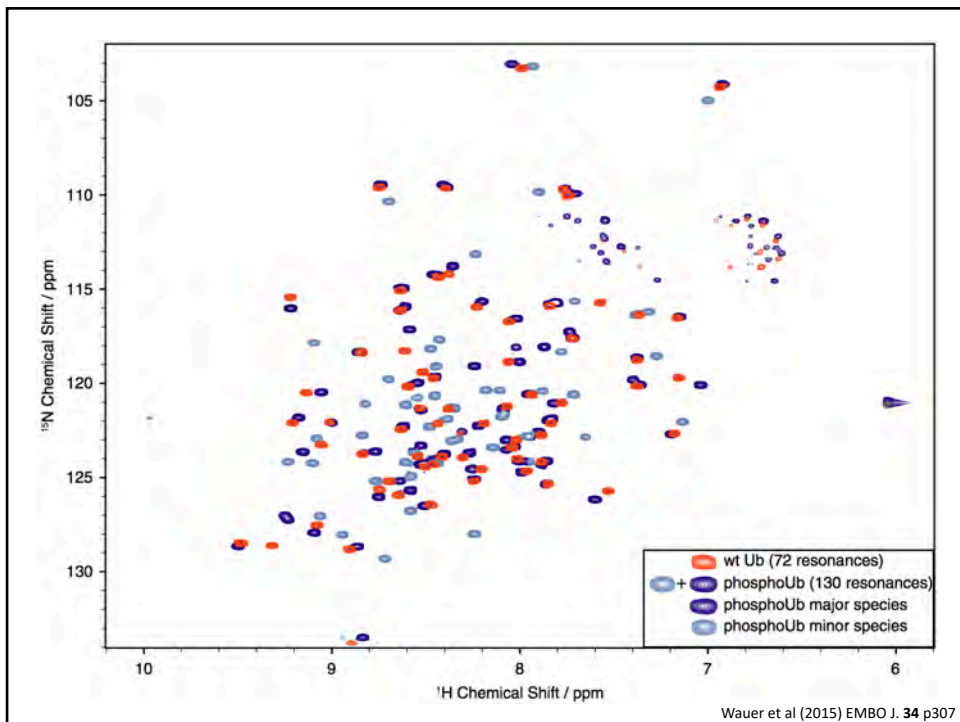


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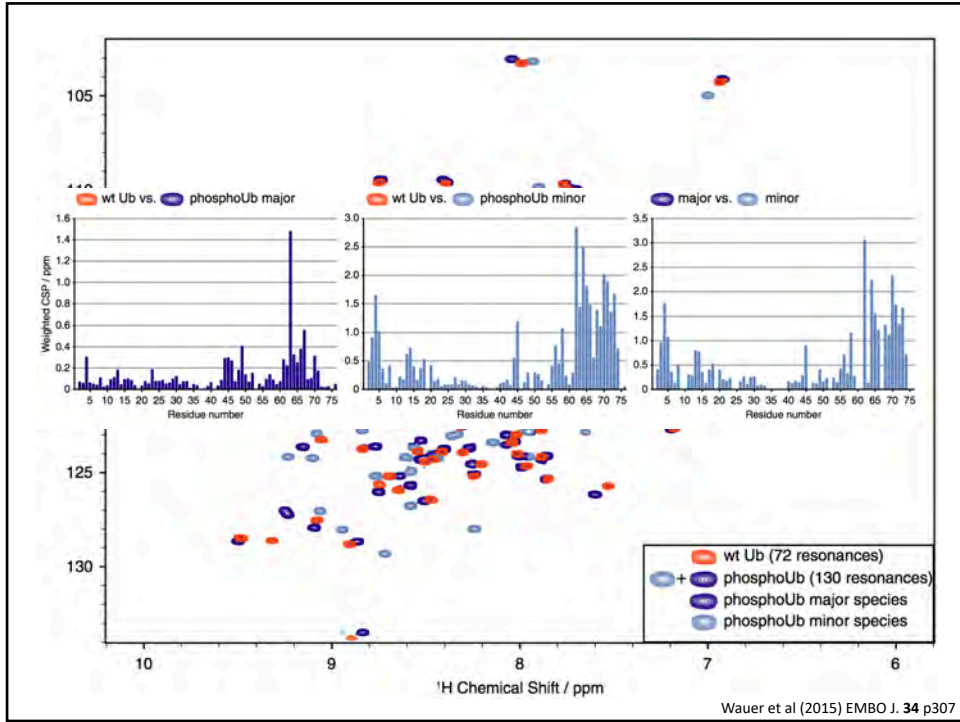
## Two Conformations – Minor 30%



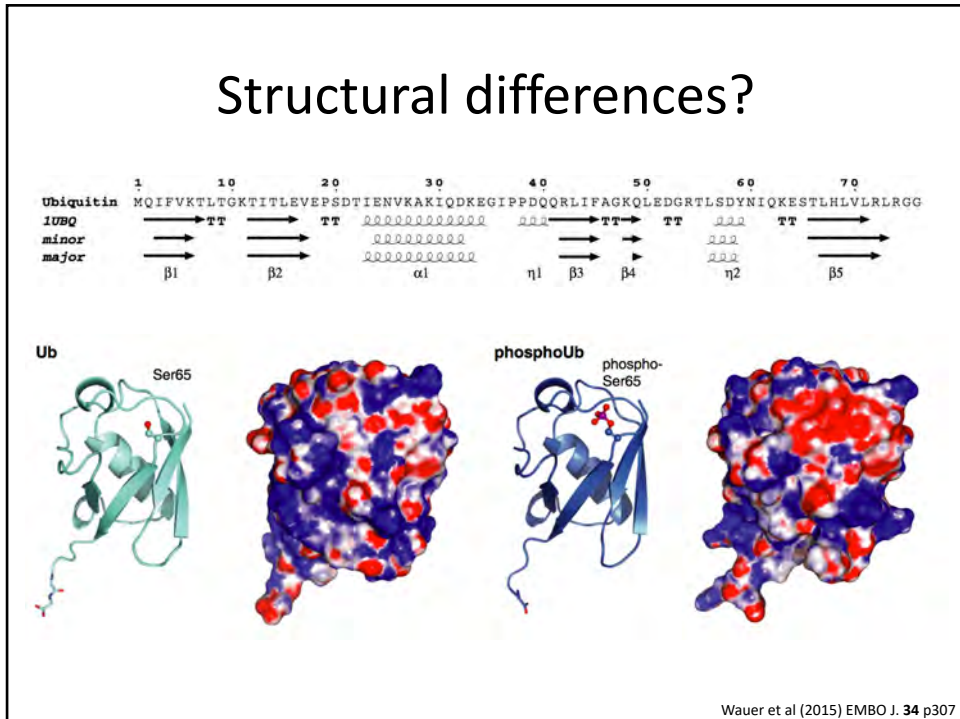
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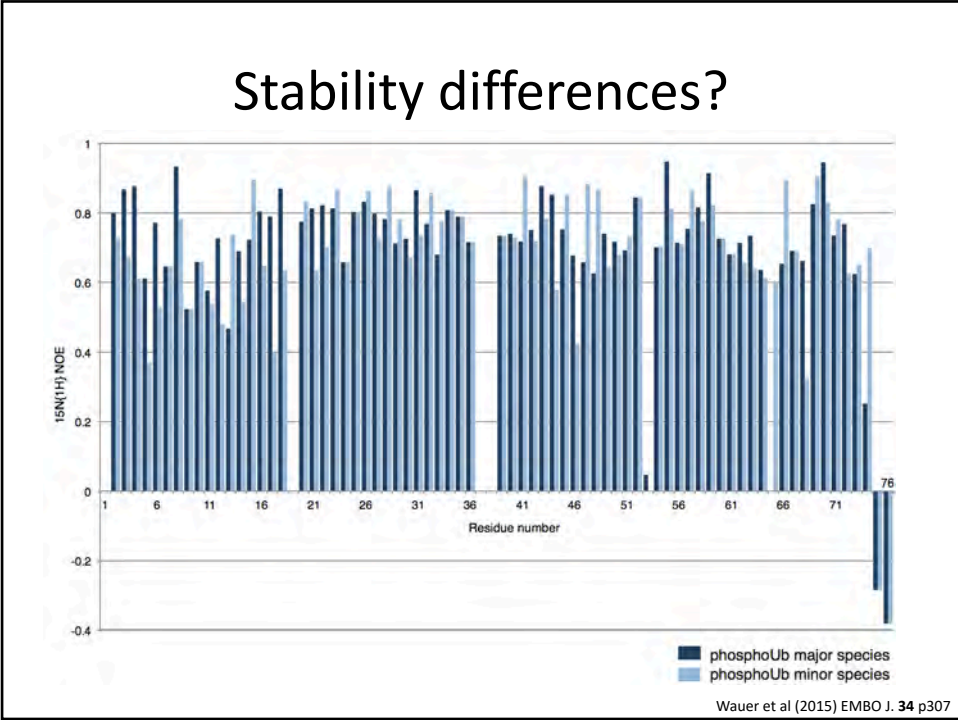


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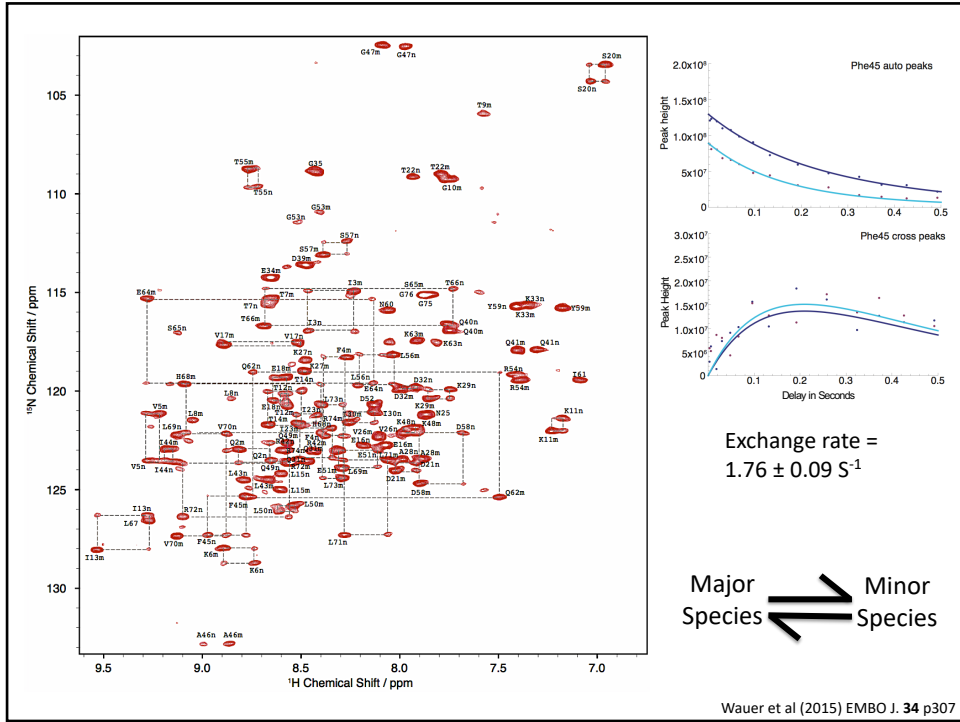
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### What could be happening?

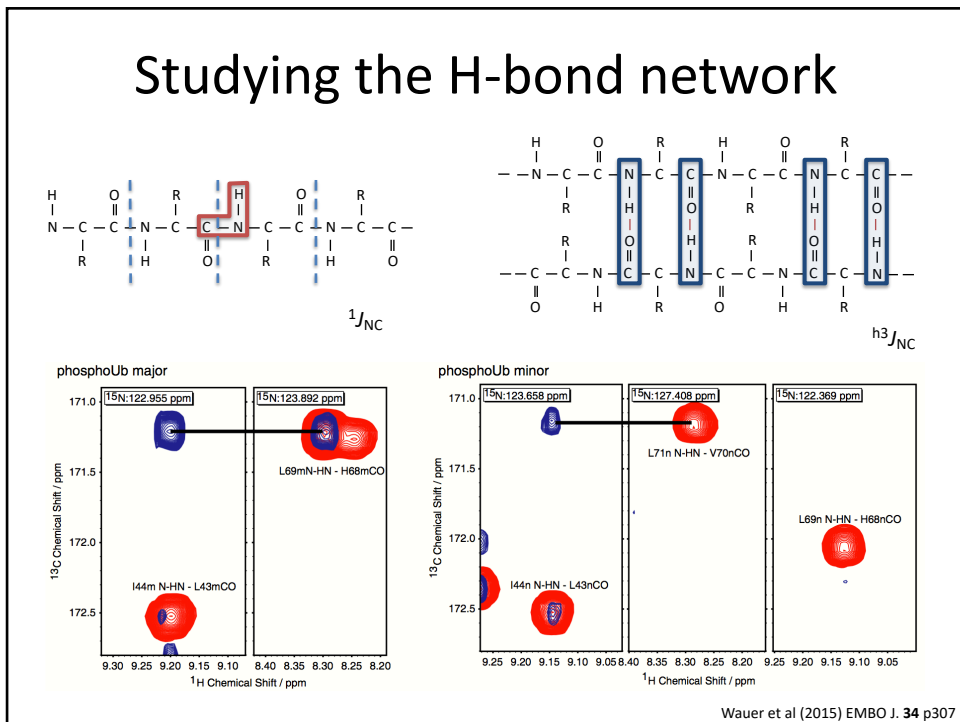
- Two Conformations

Major Species  $\rightleftharpoons$  Minor Species

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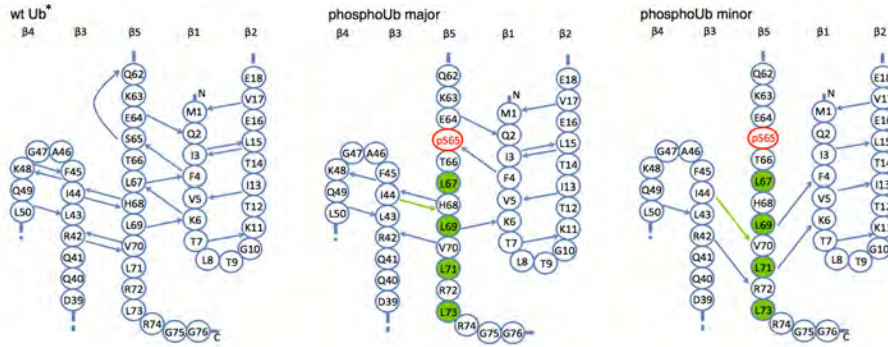


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# Long Range HNCO experiment

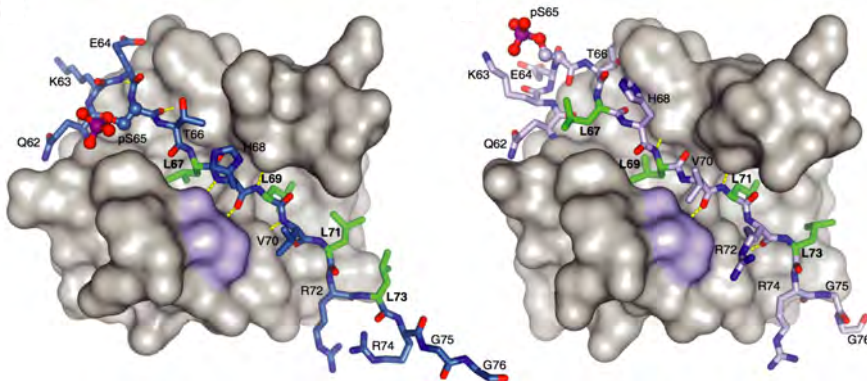


\*Ref: Cordier et al. *Nat Protoc* (2008) 3 p235

Wauer et al (2015) *EMBO J.* 34 p307

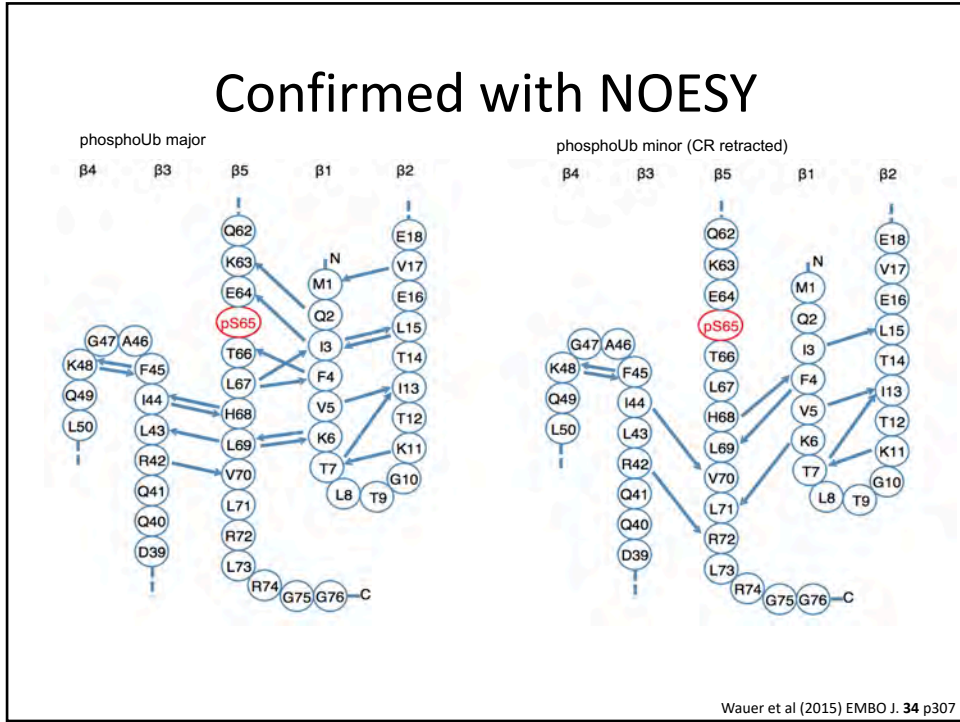
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# Long Range HNCO experiment

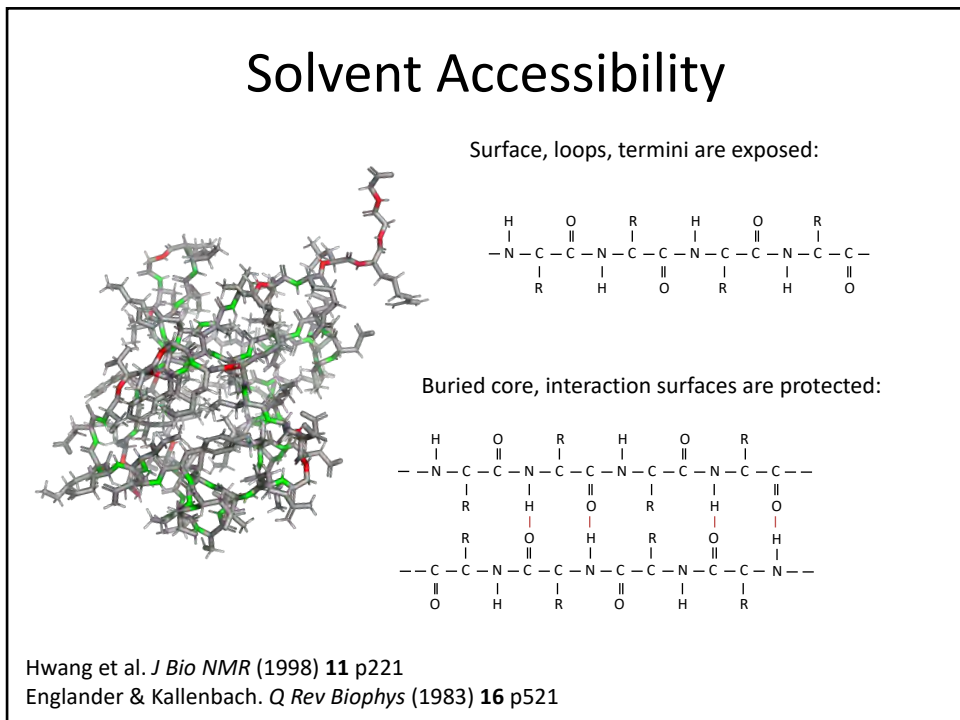


Wauer et al (2015) *EMBO J.* 34 p307

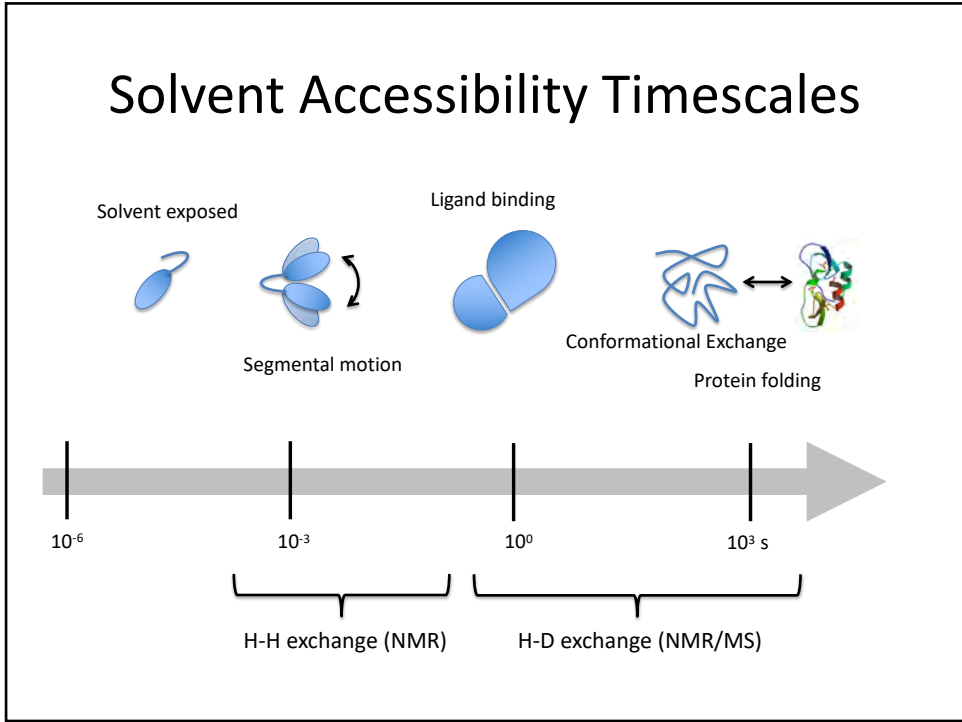
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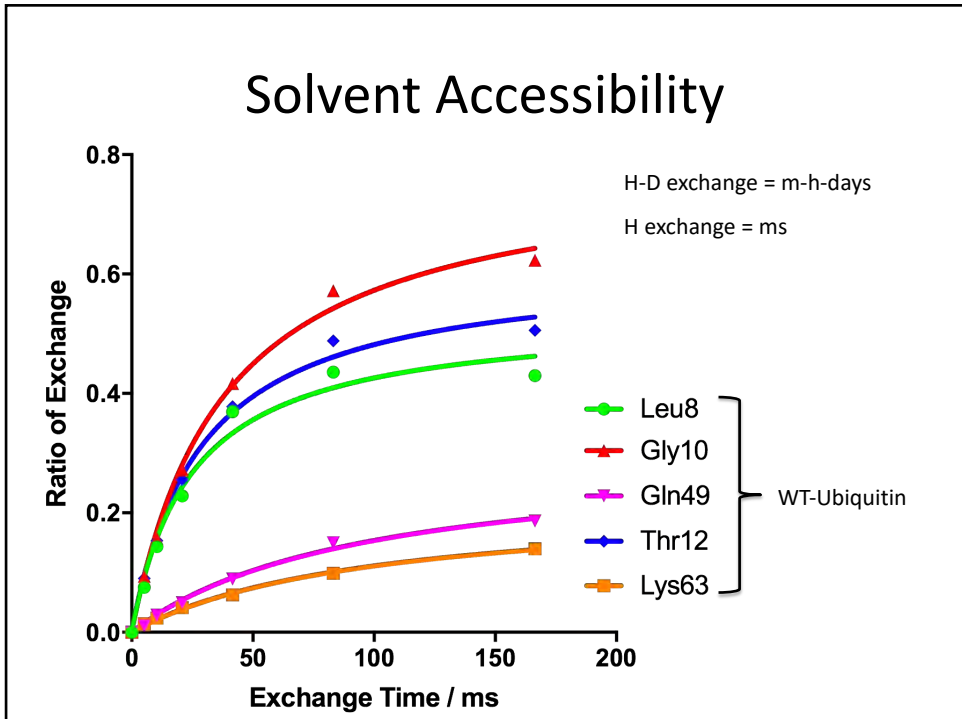
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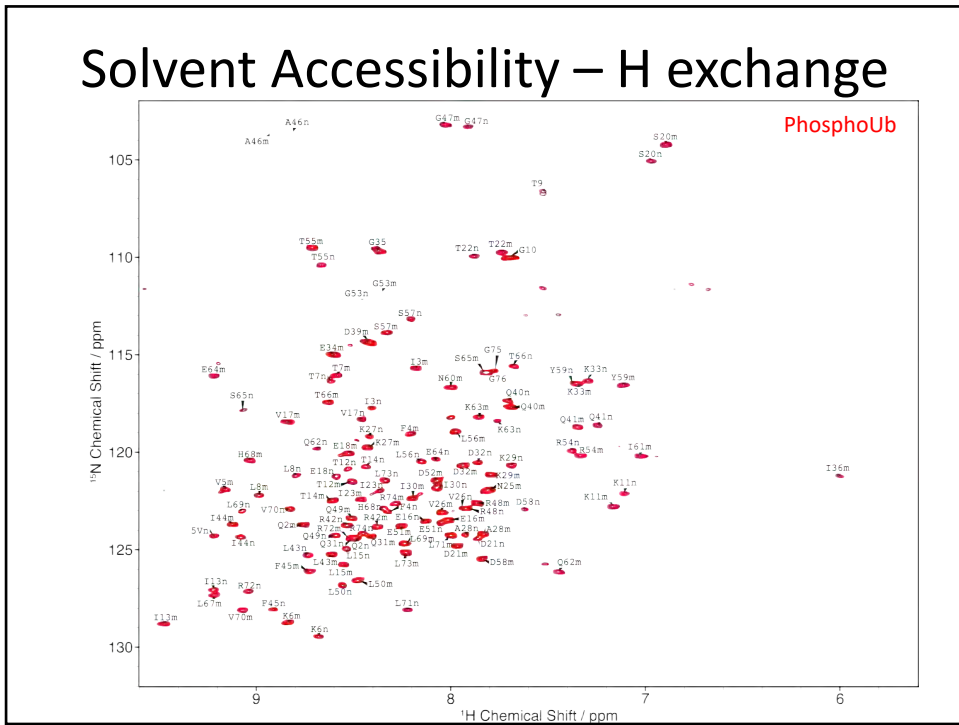


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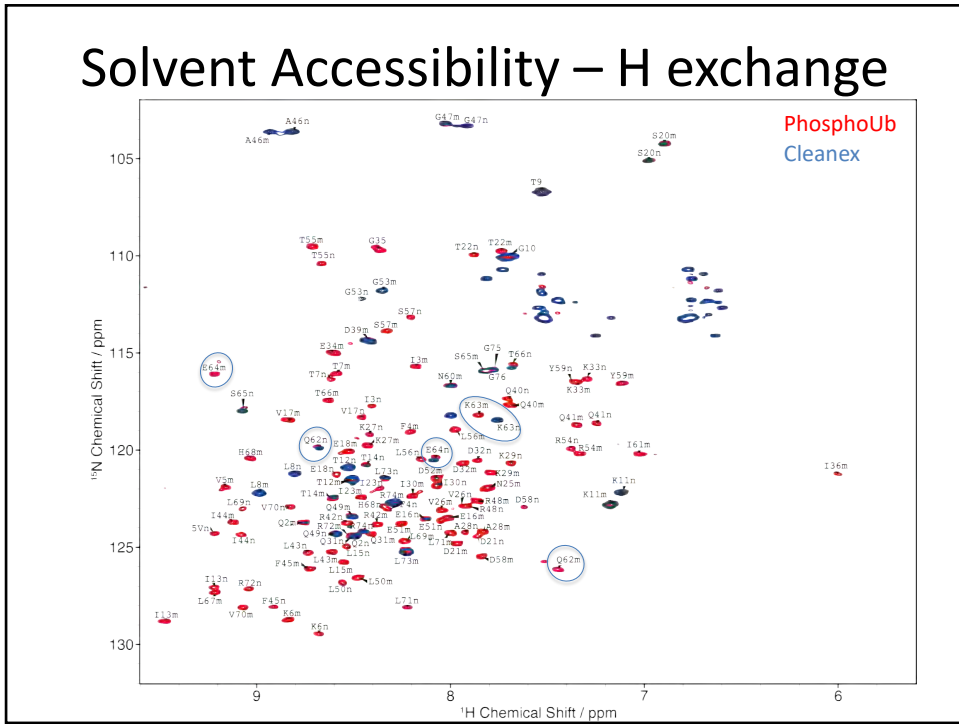
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# Solvent Accessibility – H exchange

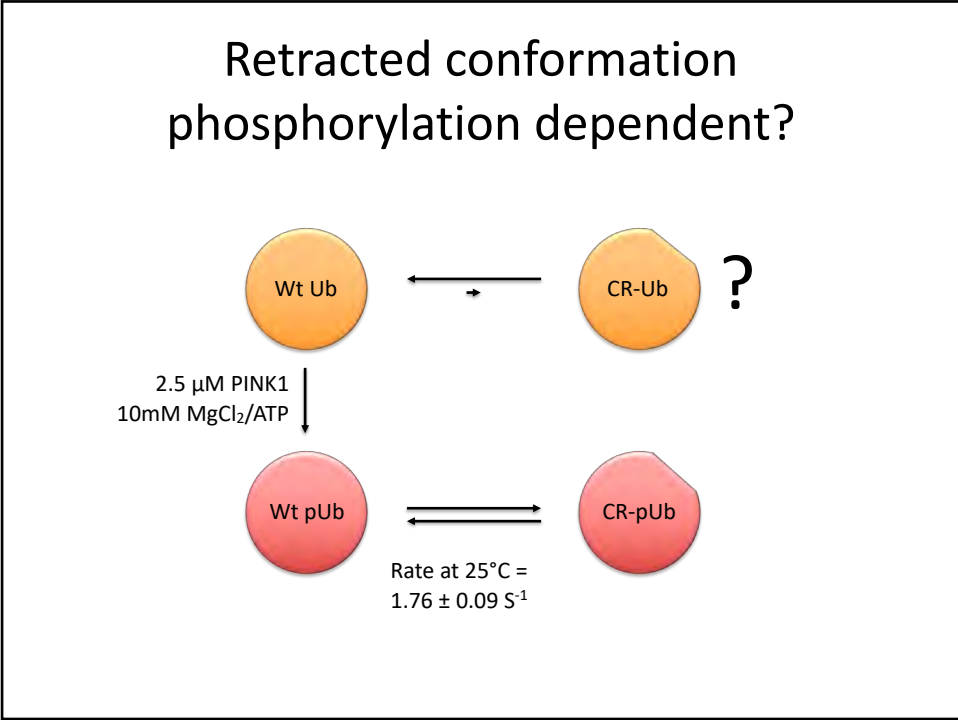


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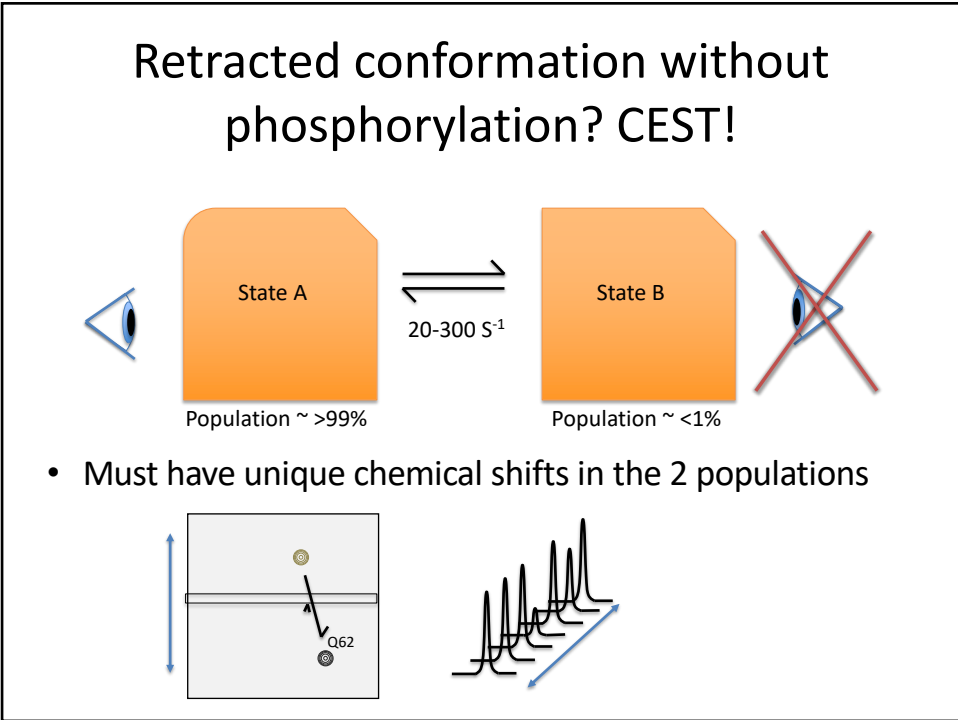
# Solvent Accessibility – H exchange



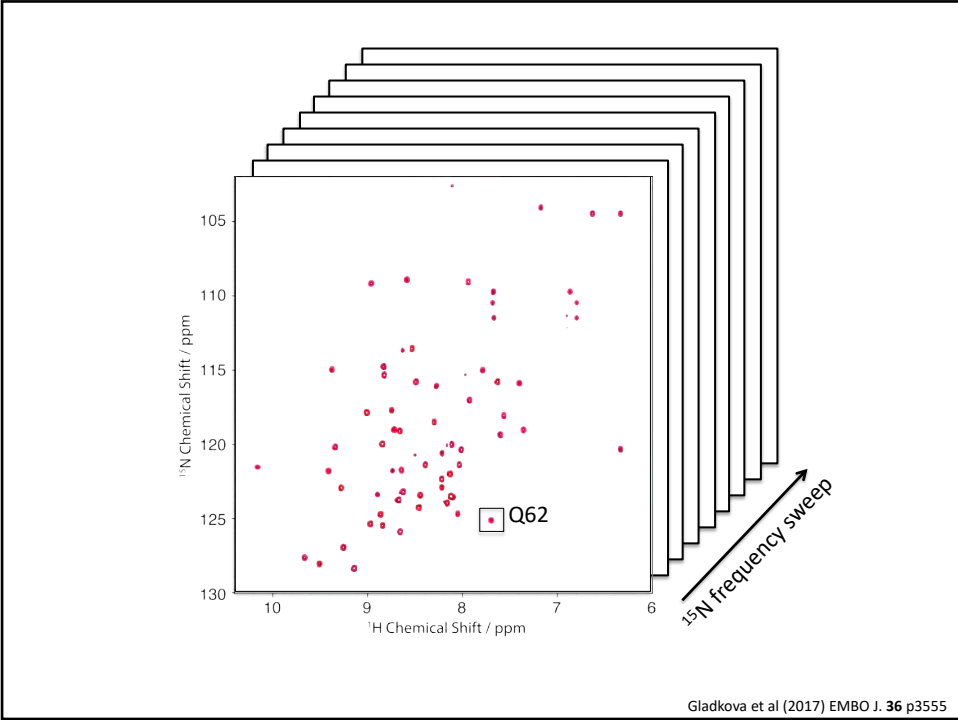
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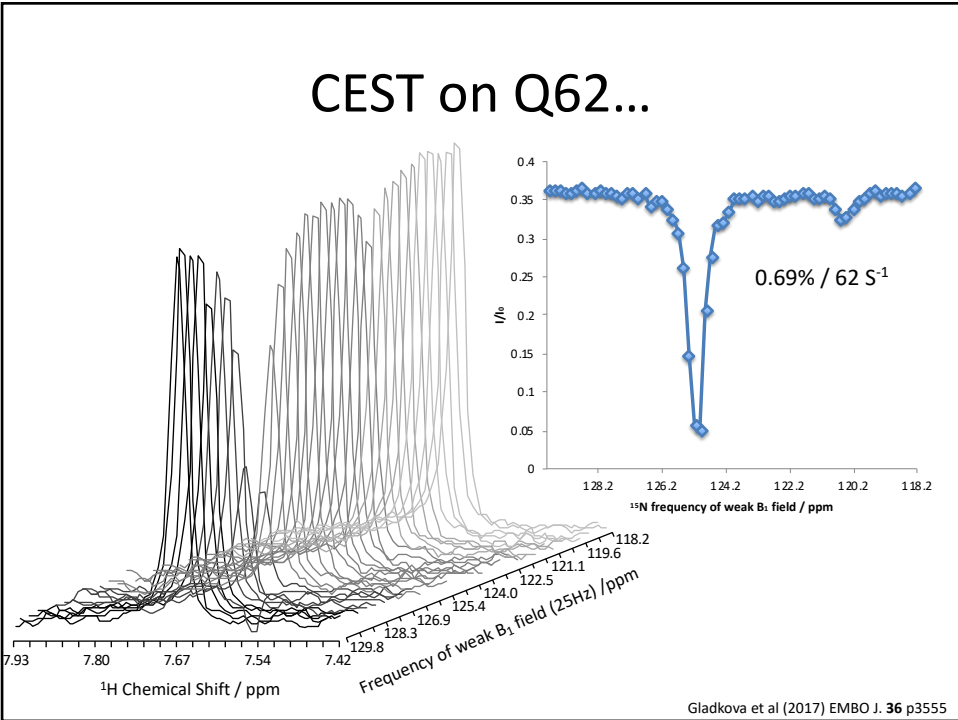
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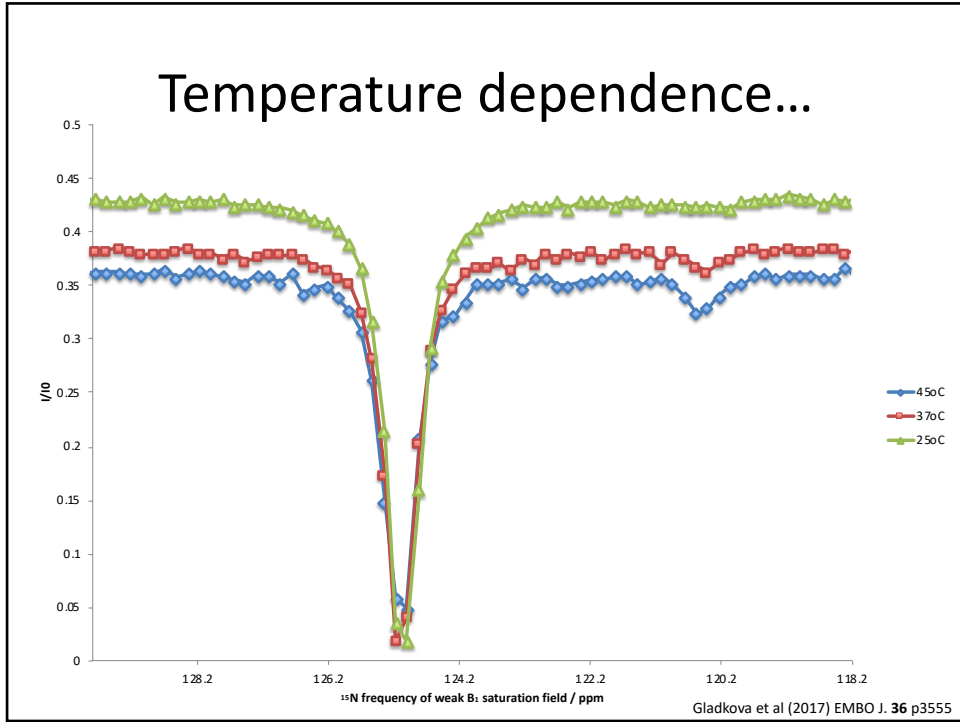


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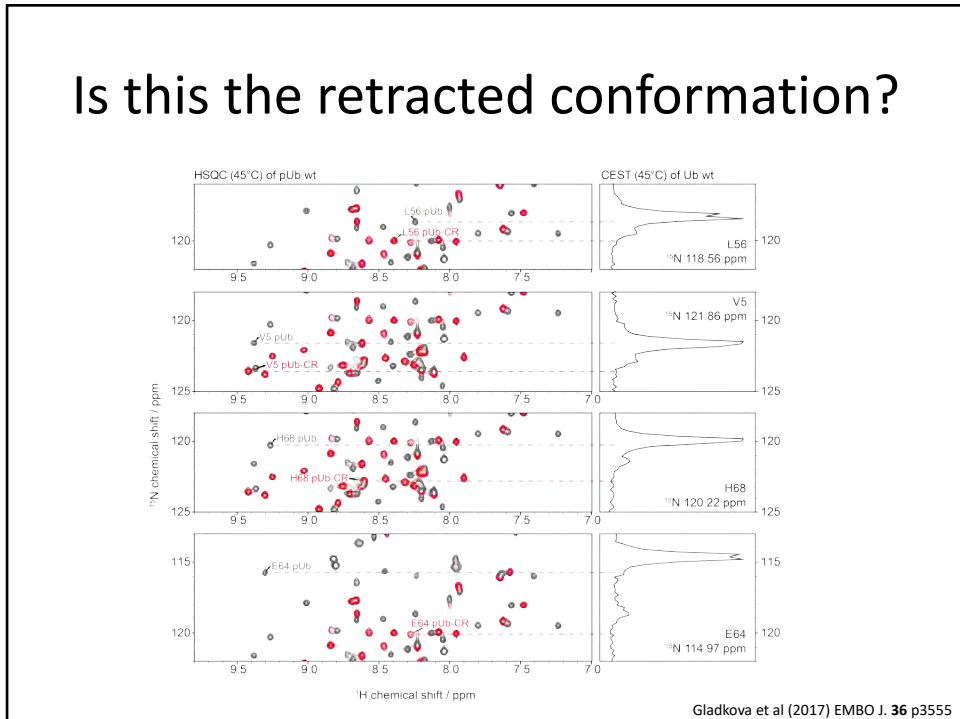


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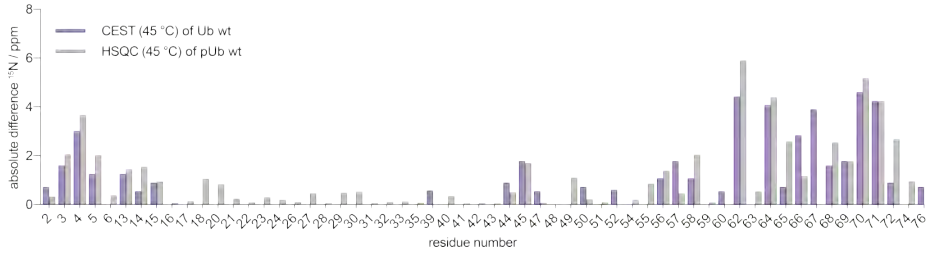


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# Is this the retracted conformation?

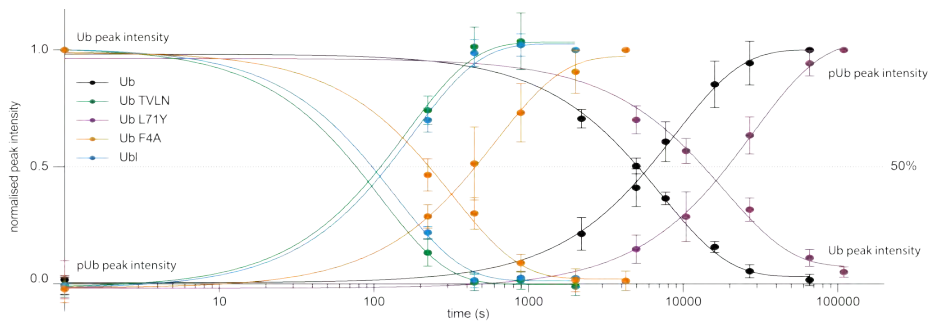


- Mutants TVLN (retracted) /L71Y (WT lock)
- Phosphorylation time courses

Gladkova et al (2017) EMBO J. 36 p3555

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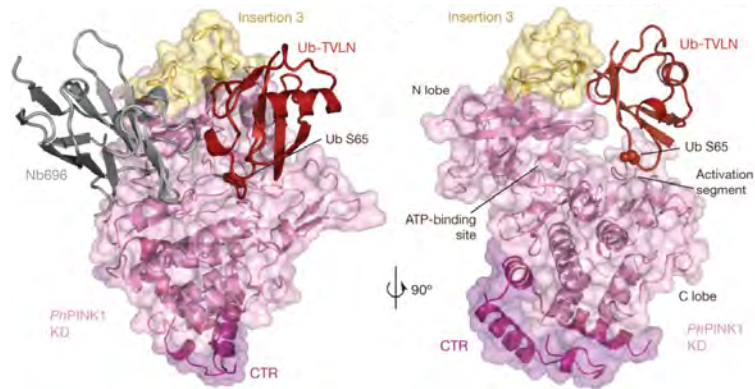
# Phosphorylation of mutants...



Gladkova et al (2017) EMBO J. 36 p3555

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## Implications...



Schubert et al (2017) Nature. 552 p51

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## Summary

- In-situ phosphorylation
- Exchange between two forms characterized
- Not observable by crystallography
- Invisible conformation detectable and quantifiable
- Retracted conformation required for PINK1 phosphorylation

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## Charcot-Marie-Tooth disease (CMT) and LITAF

- Most common inherited neuromuscular disorder (1:2500)
- Muscle weakness, loss of sensation: effects feet, lower legs, hands and forearms



Jean-Martin Charcot  
1825-1893



Pierre Marie  
1853-1940



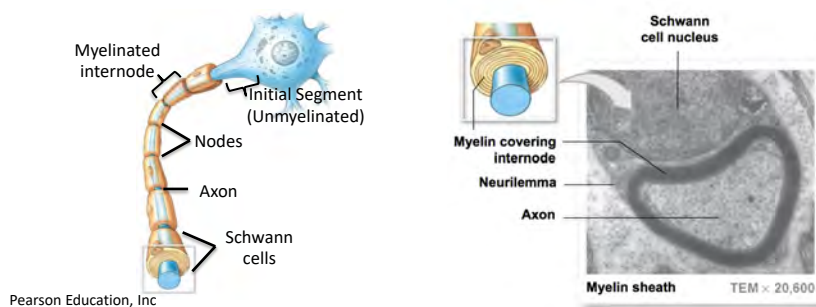
Howard Henry Tooth  
1856-1925



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## CMT: axons and myelin sheath

- Various genes associated with CMT, divided into subtypes
- Autosomal dominant & recessive
- Damage to Axon and Myelin sheath

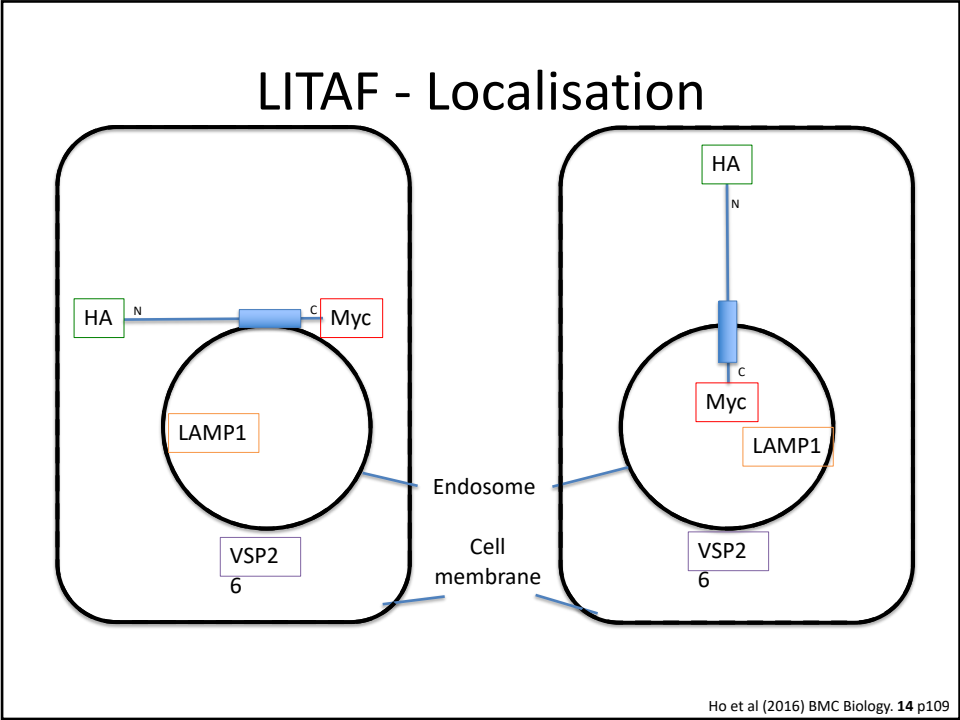


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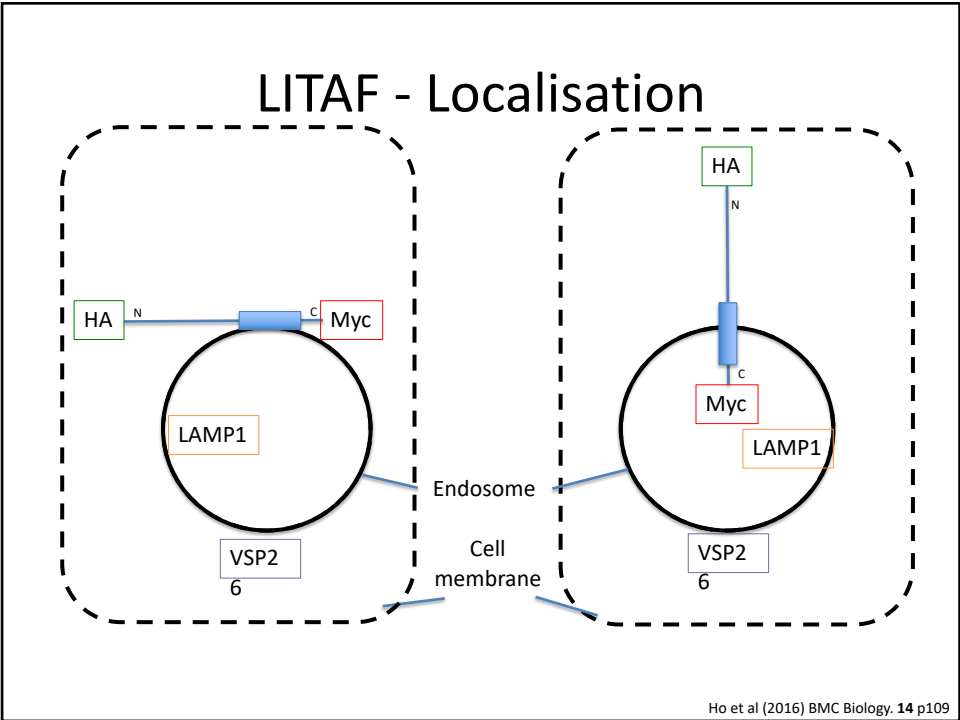
## CMT Type 1C: LITAF

- Lipopolysaccharide-induced tumour necrosis factor- $\alpha$  factor / small integral membrane protein of lysosome/late endosome (SIMPLE)
- 17kDa – possibly higher order complex
- Endosome associated
- N-terminal proline rich unstructured domain
- C-terminal “LITAF” domain containing hydrophobic helix
- Topology remained controversial in literature

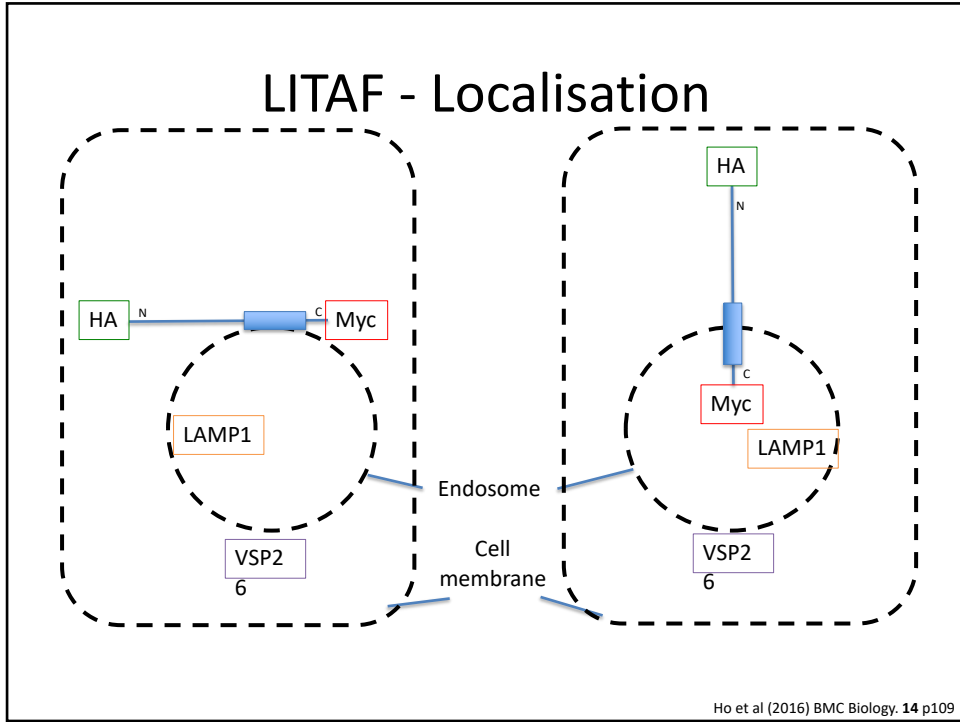
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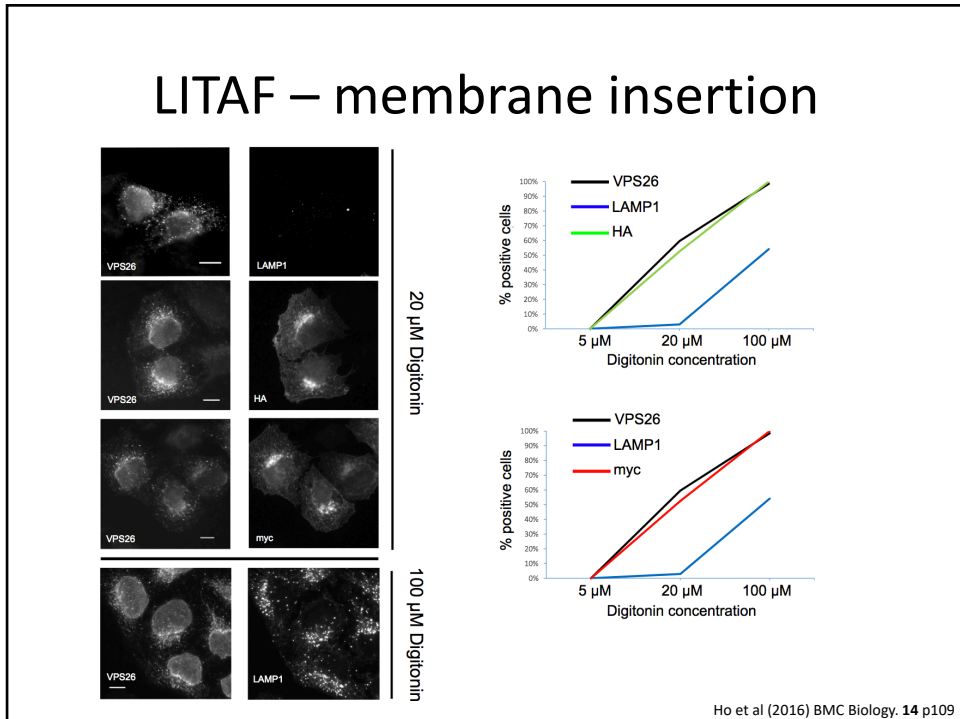
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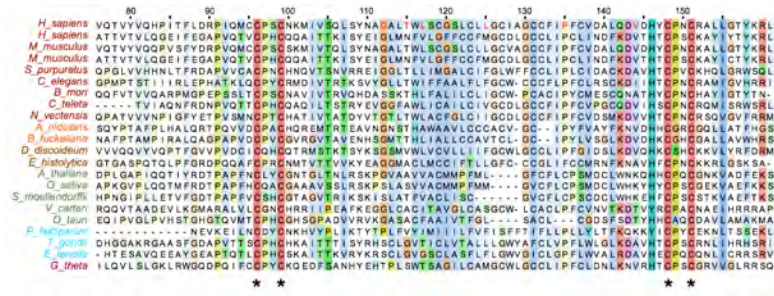


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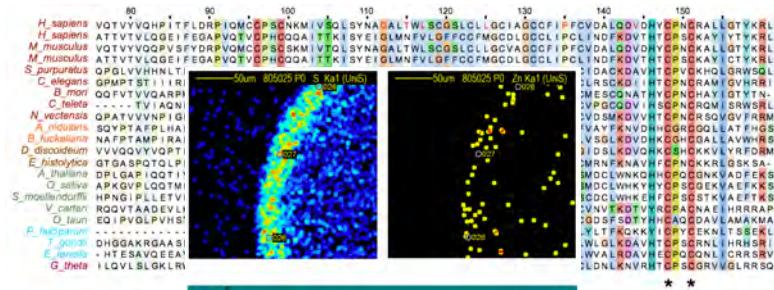
# LITAF - metal binding - microPIXE



Ho et al (2016) BMC Biology, 14 p109

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# LITAF - metal binding - microPIXE

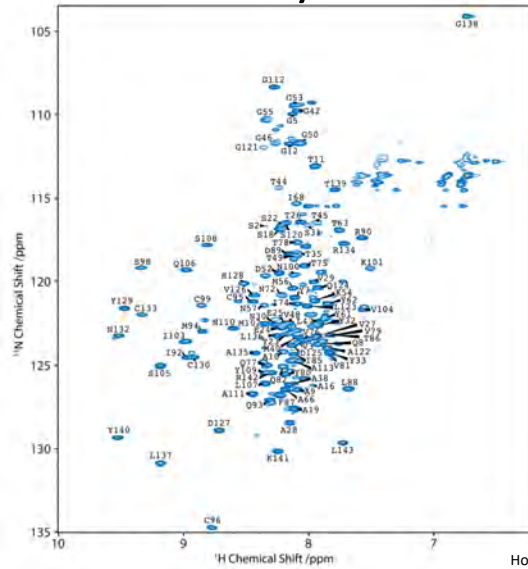


Ho et al (2016) BMC Biology, 14 p109

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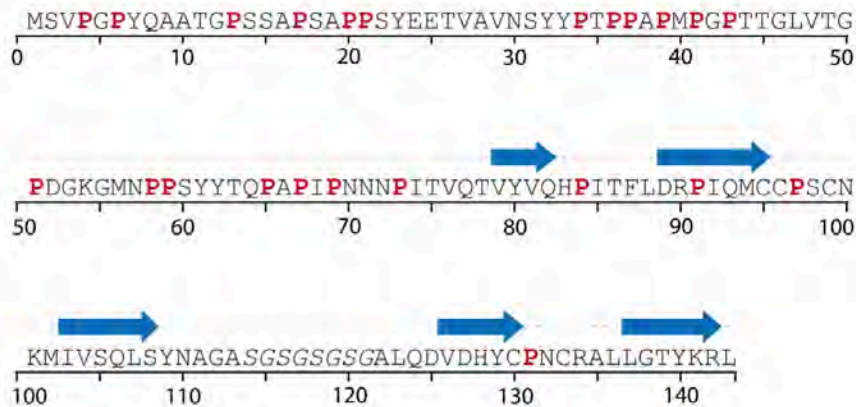
## Assignment and Secondary Structure Analysis



Ho et al (2016) BMC Biology. 14 p109

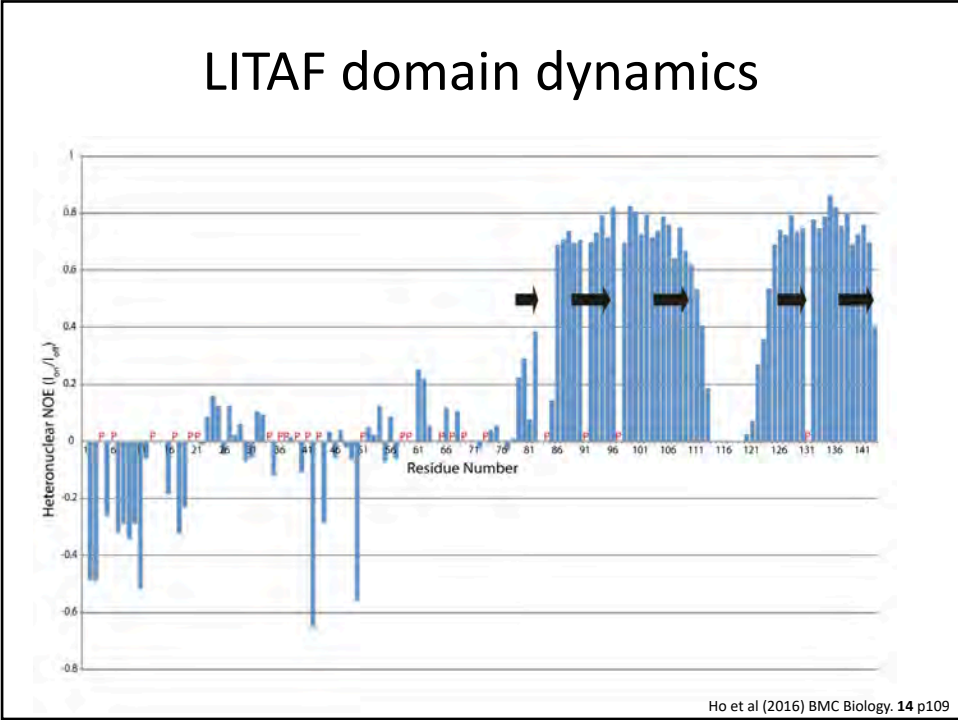
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## Assignment and Secondary Structure Analysis

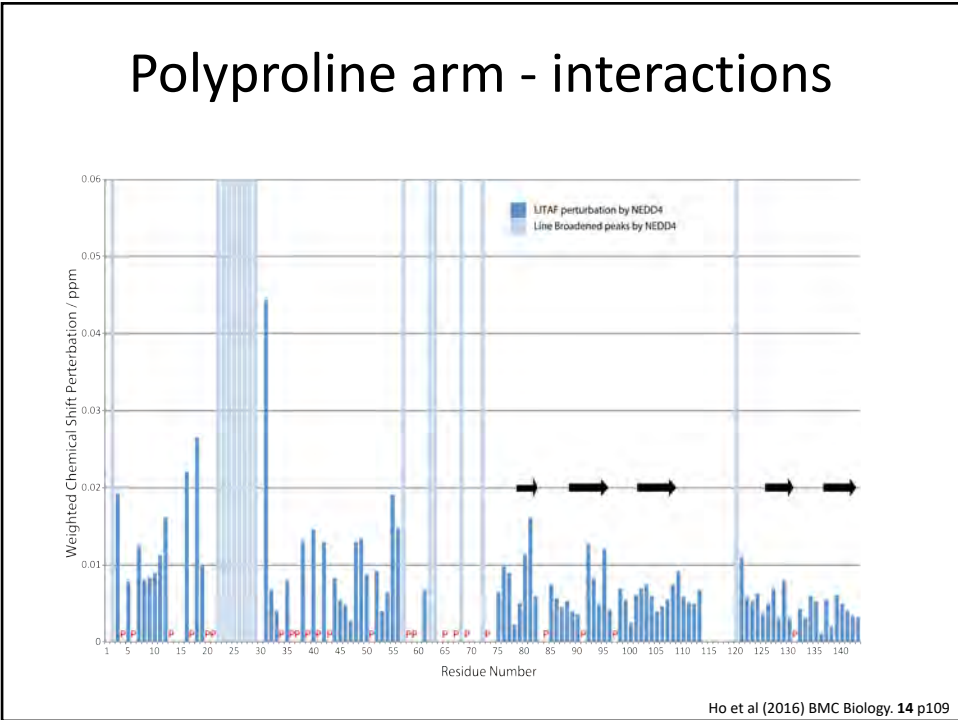


Ho et al (2016) BMC Biology. 14 p109

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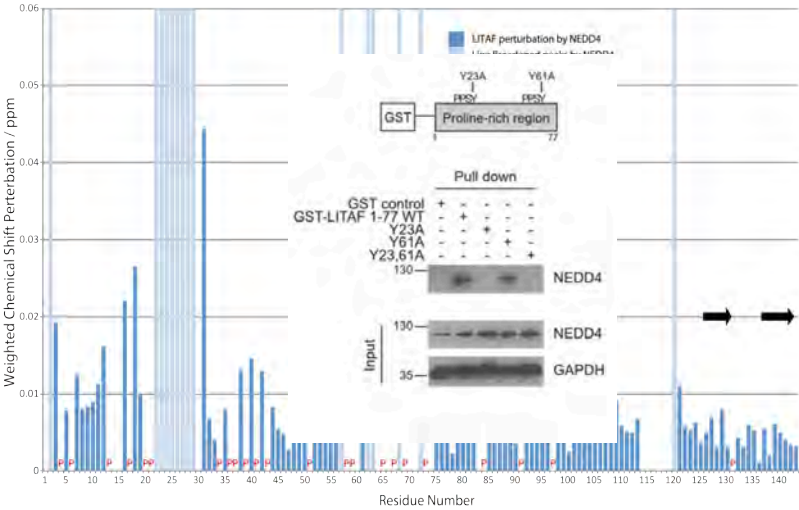


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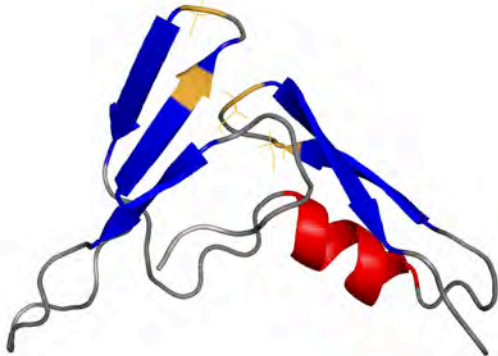
# Polyproline arm - interactions



Ho et al (2016) BMC Biology, 14 p109

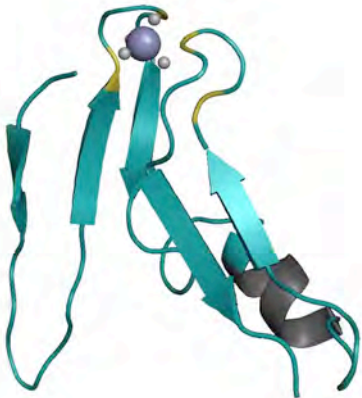
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# Model Building: $\Delta$ 114-139



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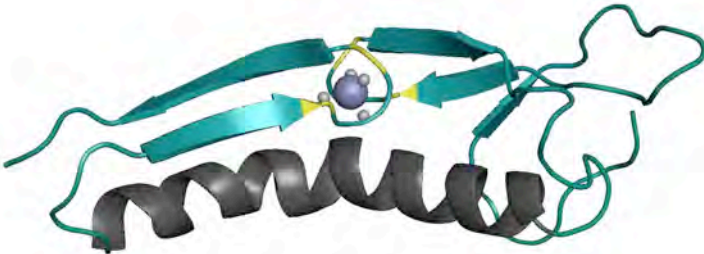
### Model building: $\Delta 114-139$



Ho et al (2016) BMC Biology. 14 p109

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### Wild-type structure modeling

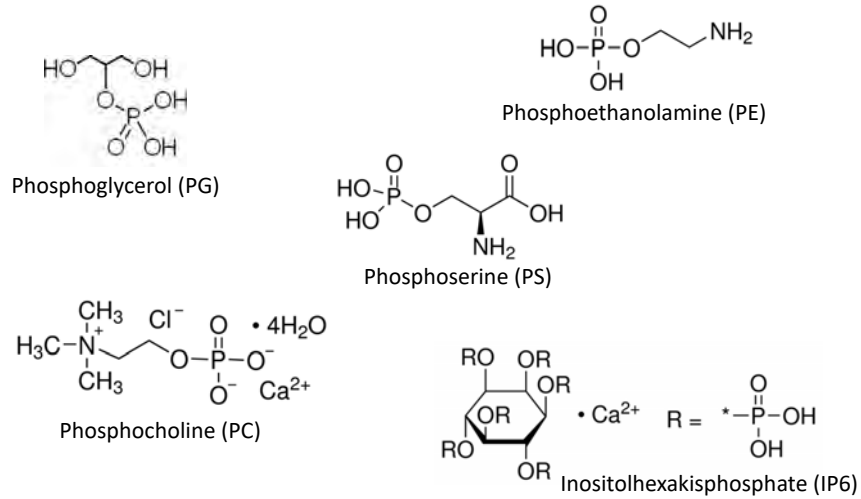


Ho et al (2016) BMC Biology. 14 p109

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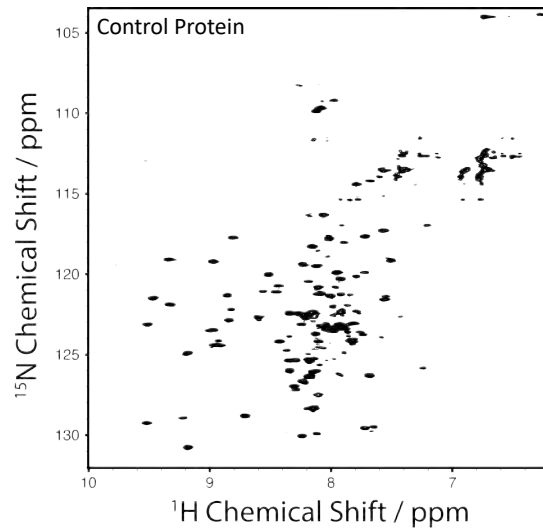
## Membrane mimetics...

- Soluble protein; soluble head group



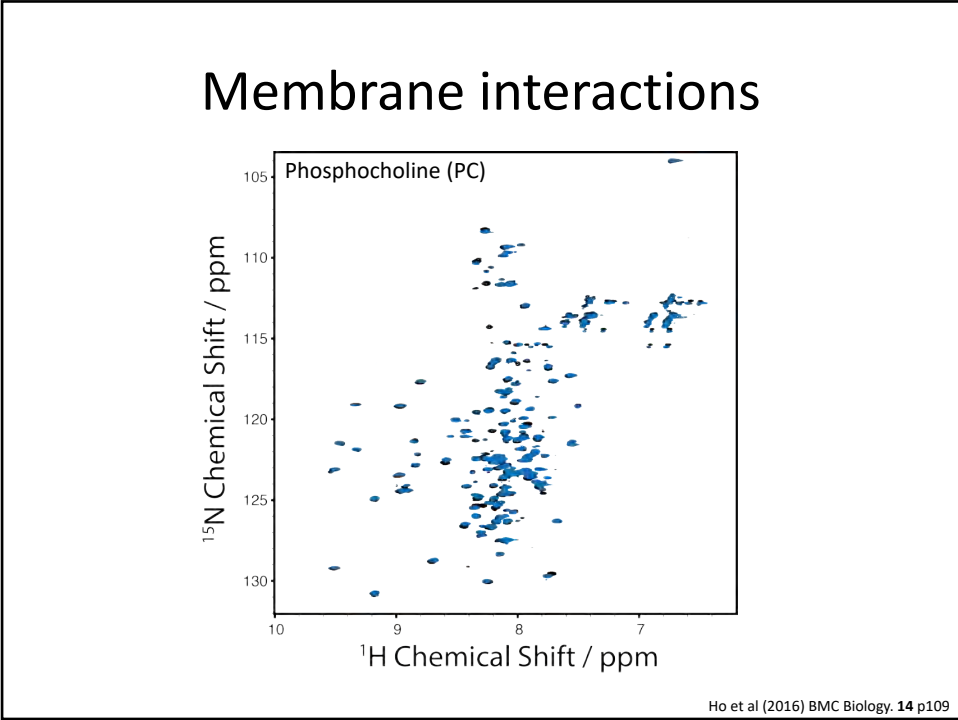
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## Membrane interactions

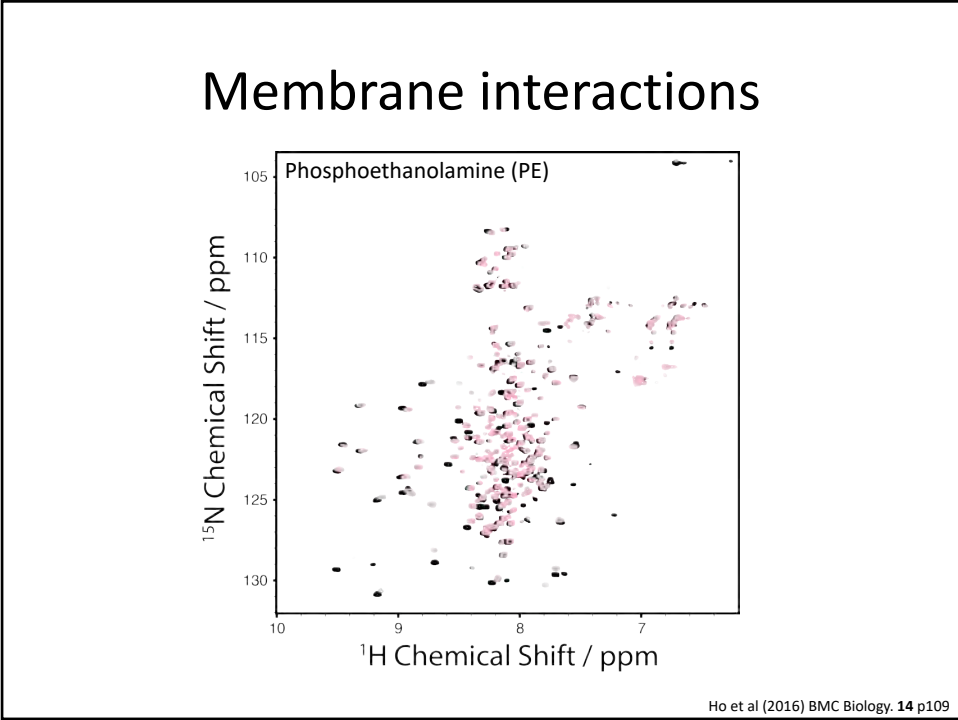


Ho et al (2016) BMC Biology, 14 p109

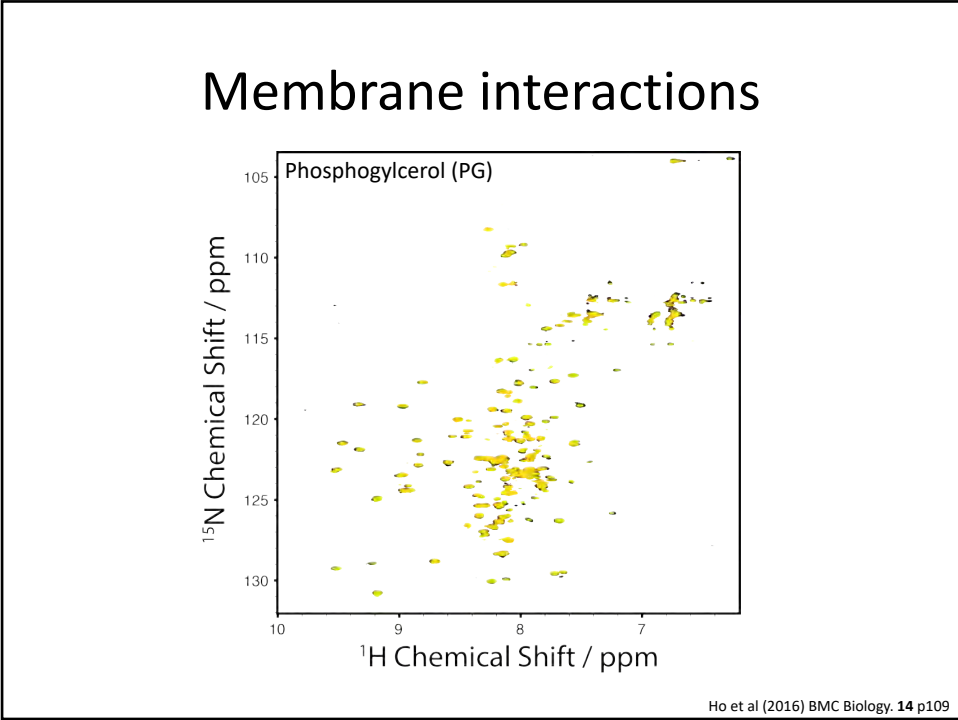
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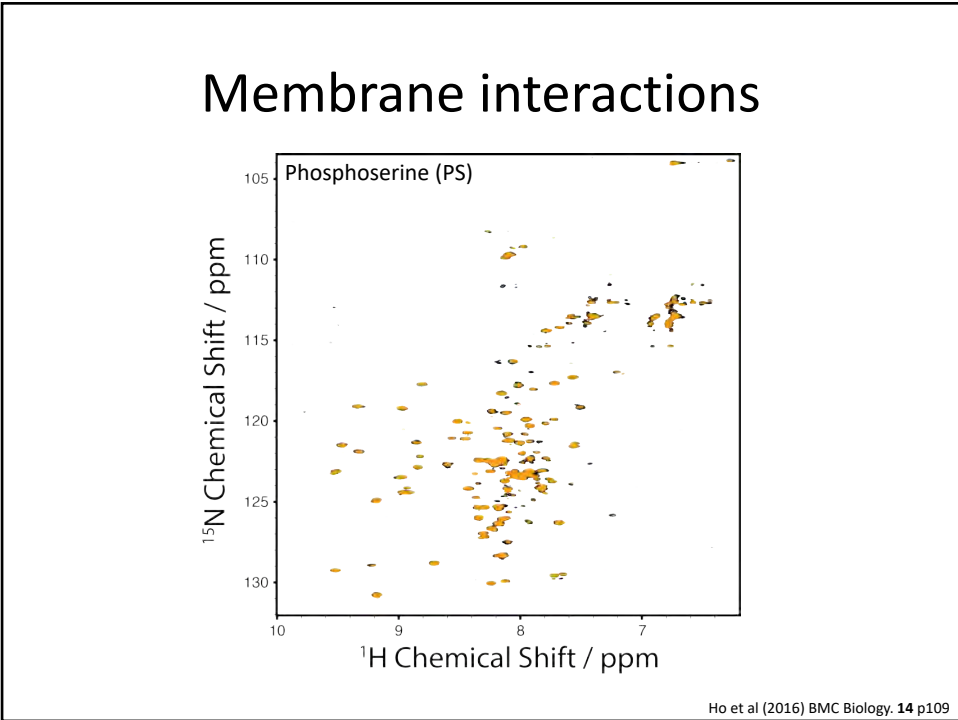
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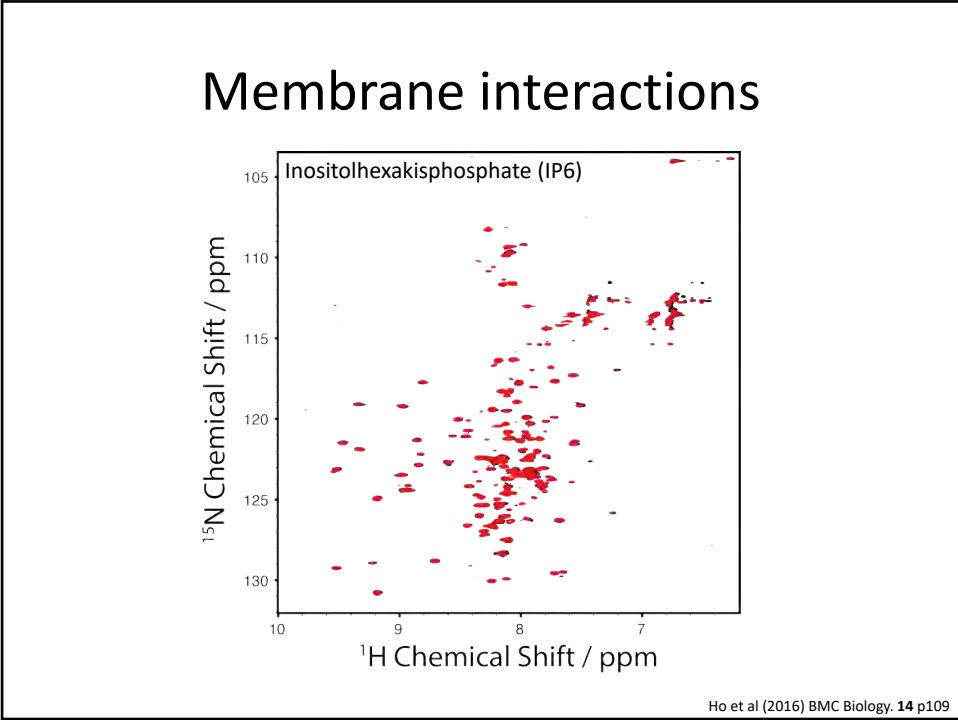
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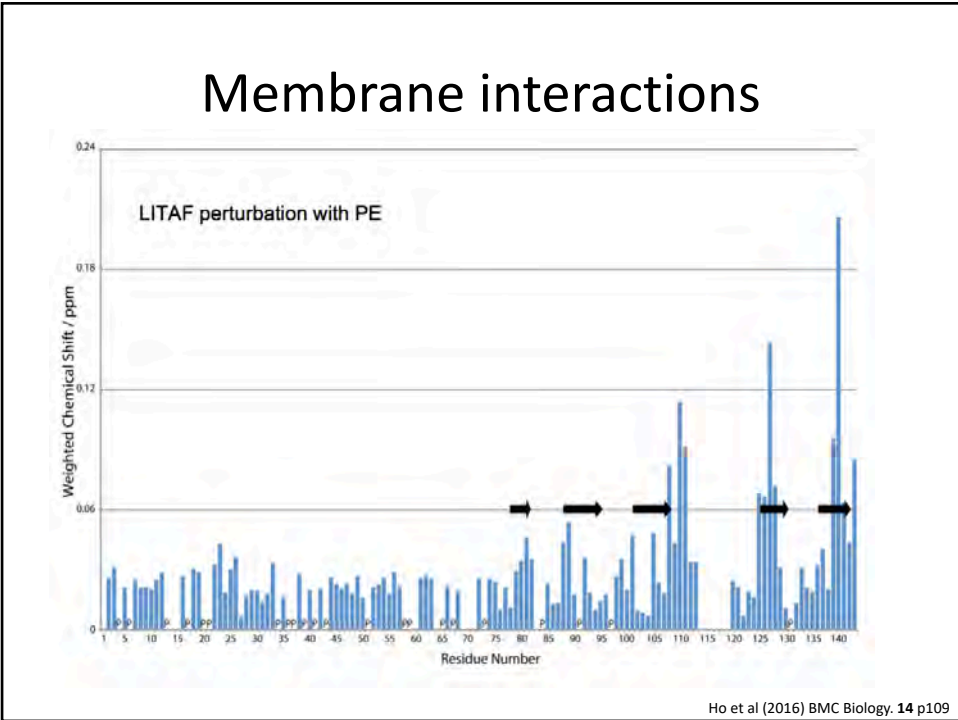
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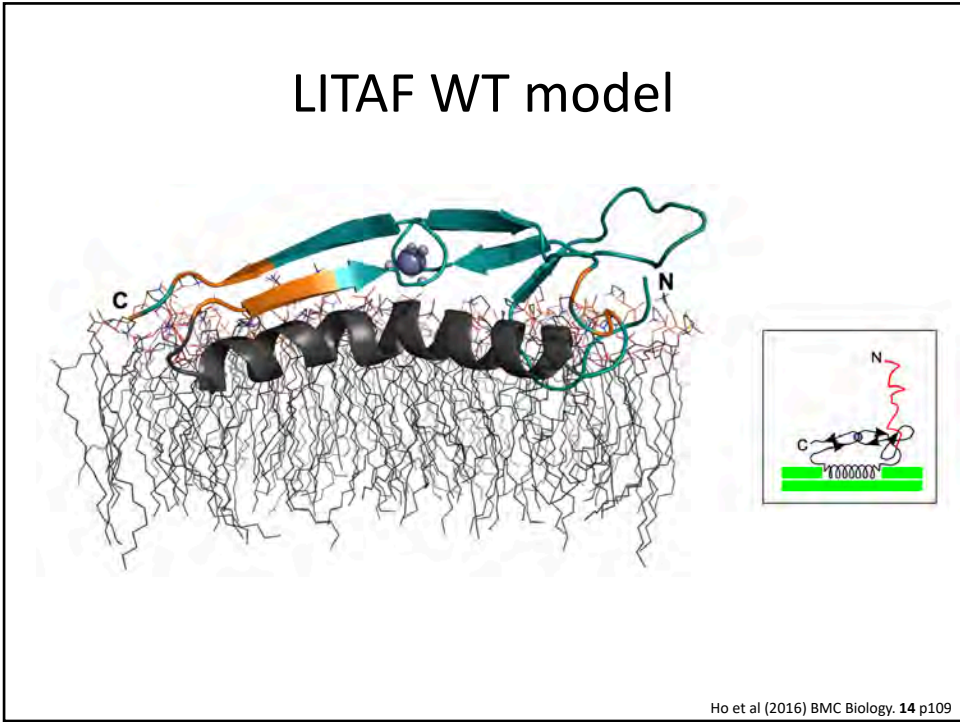


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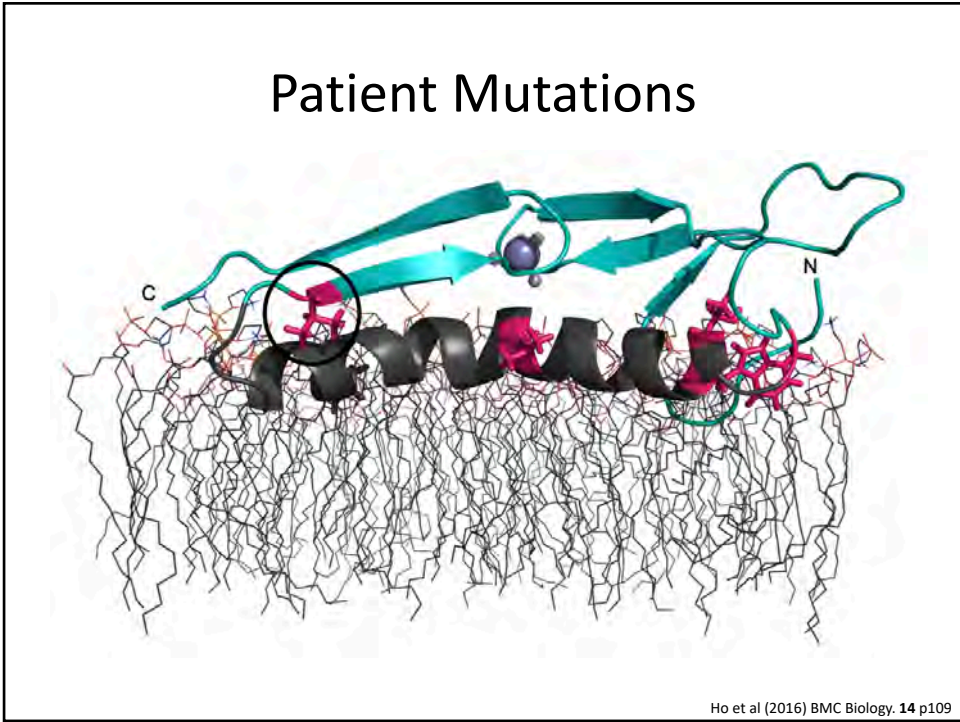


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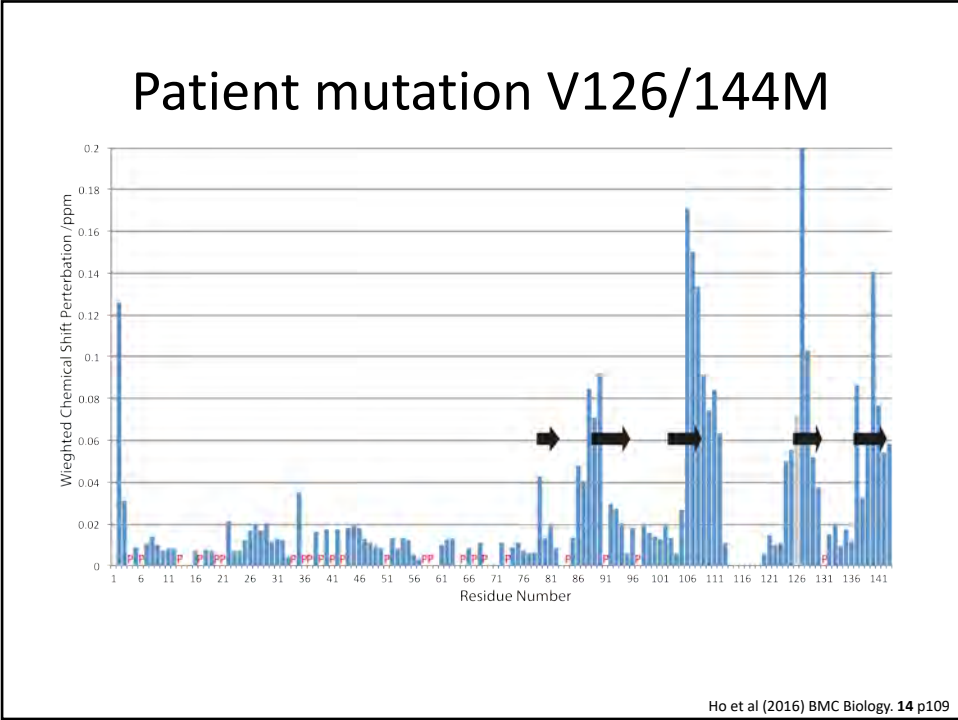




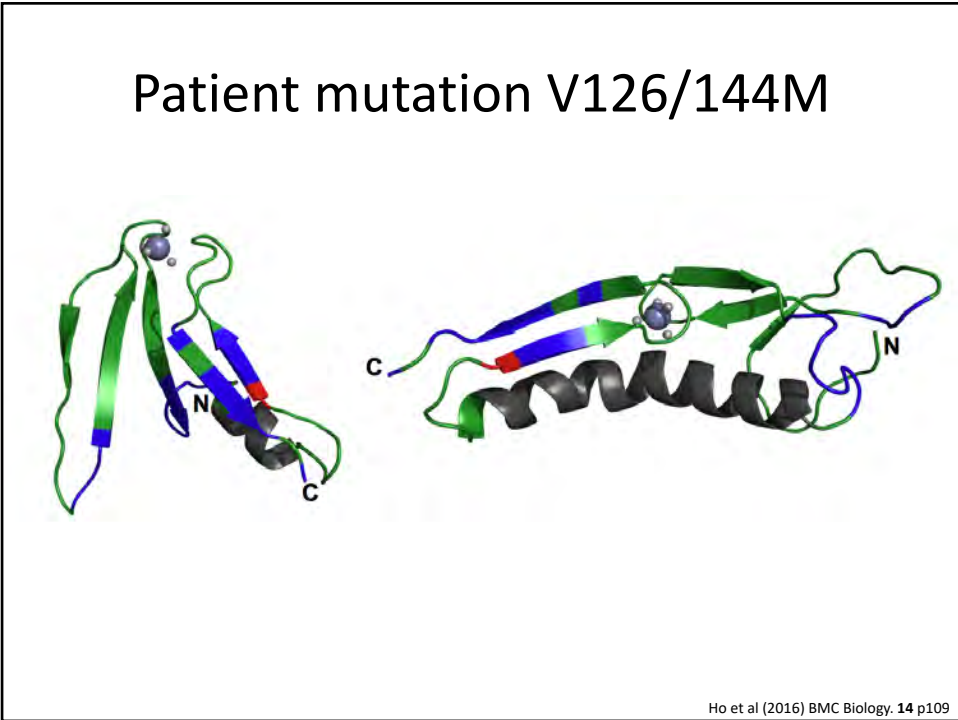
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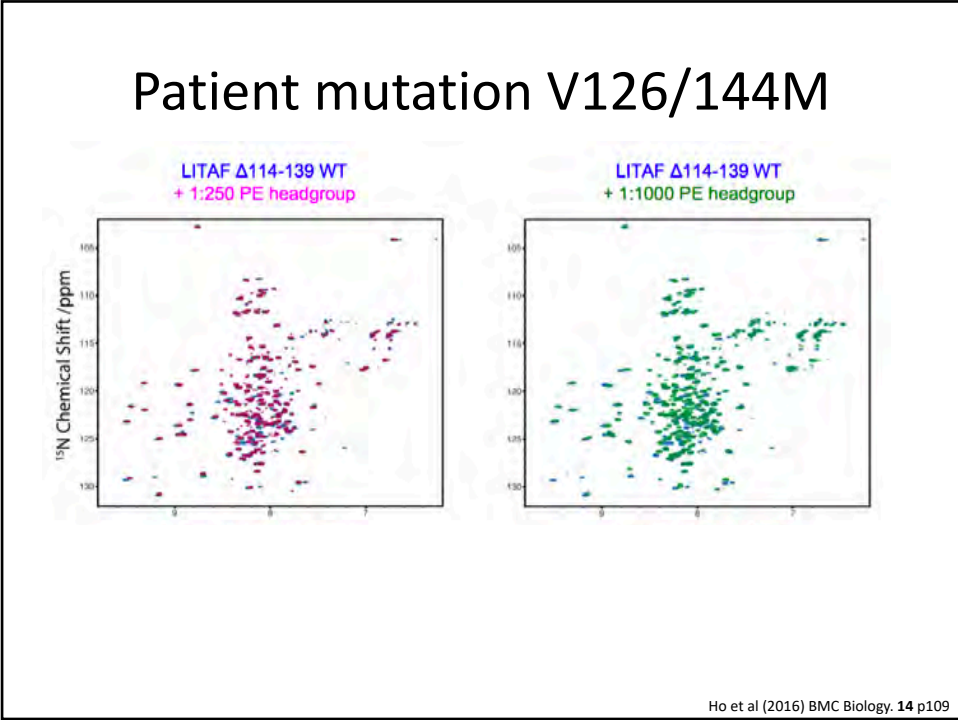
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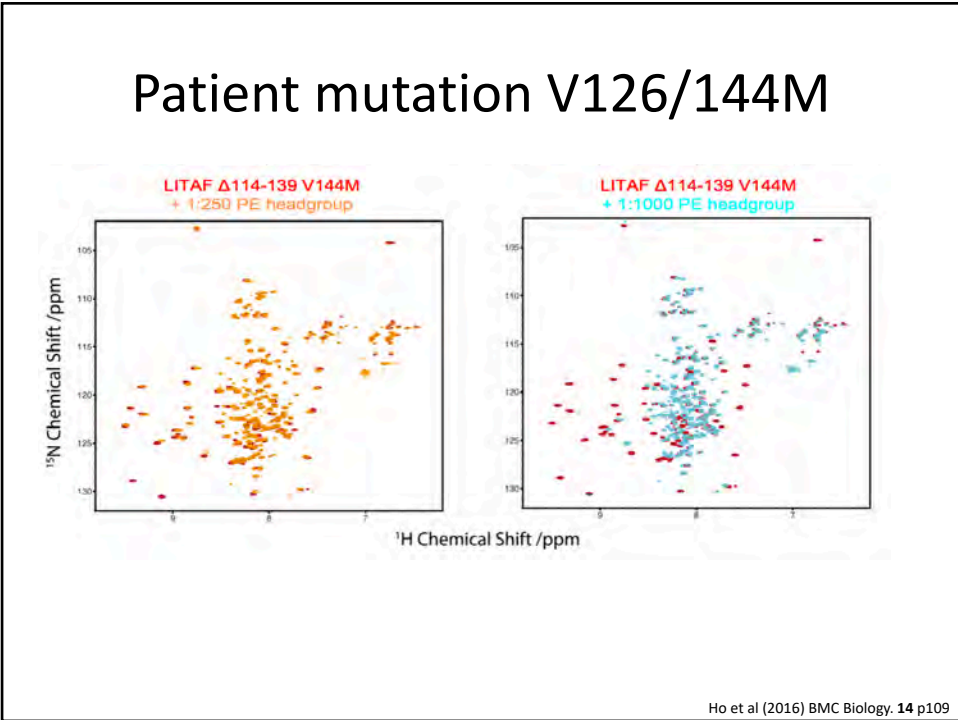
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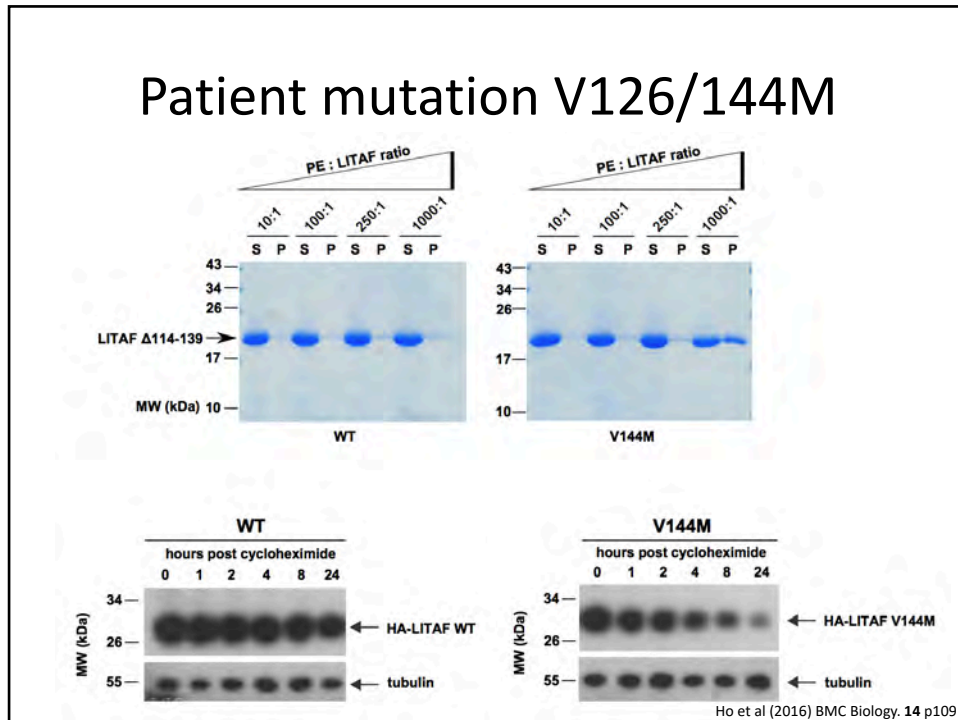
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