Elucidating the role of phosphoserines in Tau protein by combined computational and synthetic methods

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Introduction

- The cause of Alzheimer's disease (AD) is still unknown but is correlated with alteration of neuron proteins like Tau, which functions by the assembly and stabilization of microtubules, which helps normal neuronal functions. In AD, Tau loses that capacity and does not bind microtubules, due to posttranslational modifications such as hyper-phosphorylation at serine residues, resulting in misfolding.[1]

- In some bacteria, enzymatic β-elimination of phosphoserines by the amine of a nearby Lys sidechain is reported (**Figure 1**).[2] This leads to a Dha intermediate which is then capable of undergoing crosslinking reactions with Lys, His and Cys residues, as well as glutathione.

- Is it possible: ➢ to apply this type of mechanism to Tau protein in human brain?
  ➢ that a proximal Lys amine sidechain could lead to Dha formation, cross-linking, and subsequent misfolding of Tau protein?

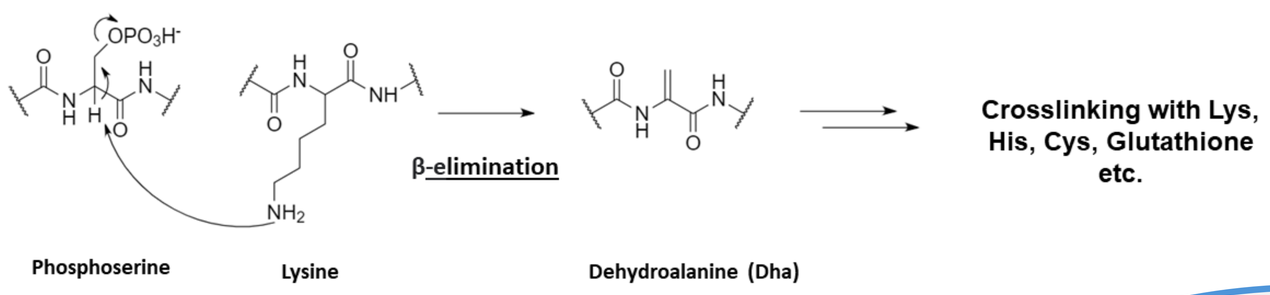
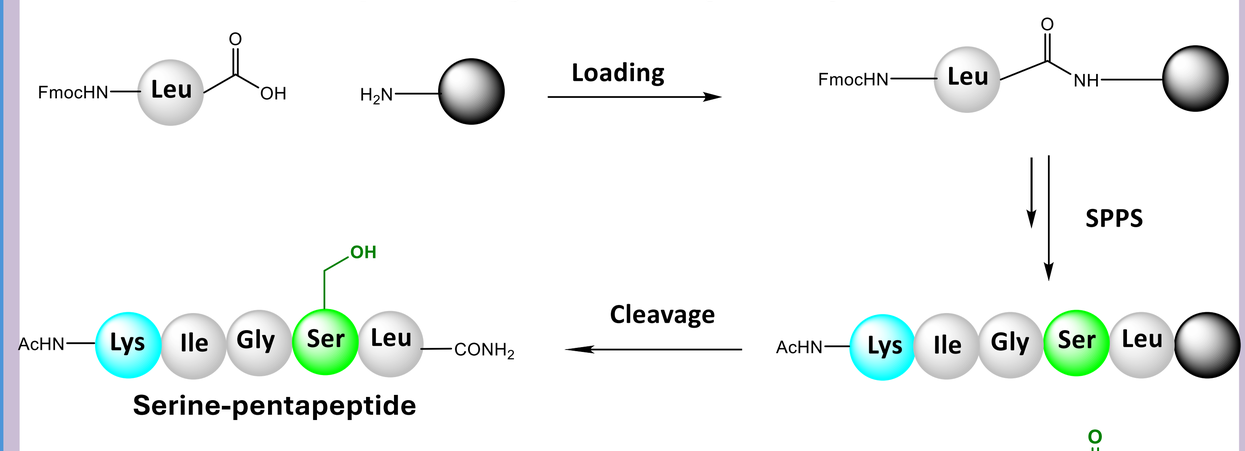


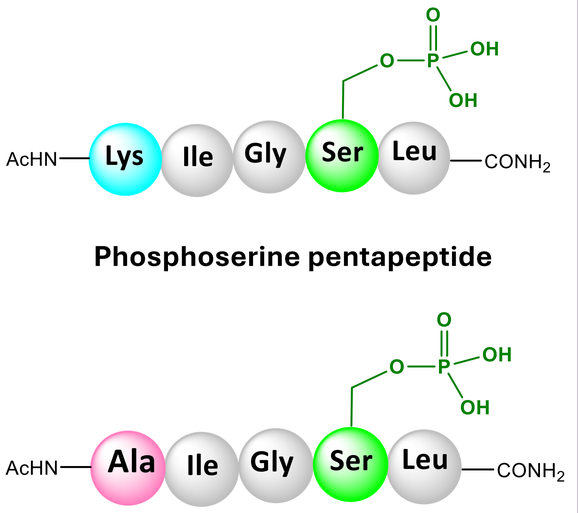
Figure 1. Mechanism of β-elimination of phosphoserine by Lys, with Dha formation

Solid–Phase Peptide Synthesis (SPPS)



Similar method used to synthesise phosphoserine and Ala-containing pentapeptide. This one is used to check if β-elimination occurred without Lys side chain.

Scheme 1. SPPS of Serine, Phosphoserine and Ala - pentapeptides.



Molecular Dynamics (MD) Simulations

A library of Tau (PDB entries) was prepared and the distance between Ser and Lys was measured with a Python script **(Fig. 2**).

All-atom MD simulations were performed using *NAMD 2.14* on (i) pentapeptide (Lys^{353}-Leu^{357}) of Tau and on (ii) the same fragment with $pSer^{356}$ **(Figure 3)**

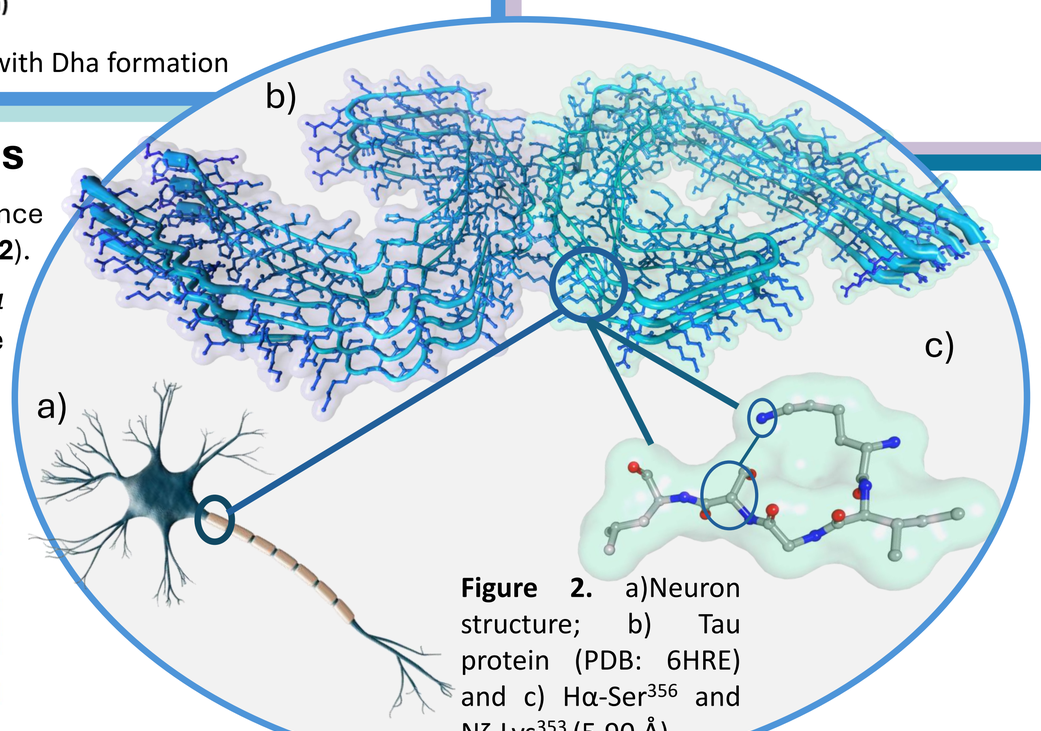


Figure 2. a)Neuron structure; b) Tau protein (PDB: 6HRE) and c) Hα-Ser^{356} and Nζ-Lys^{353} (5.90 Å)

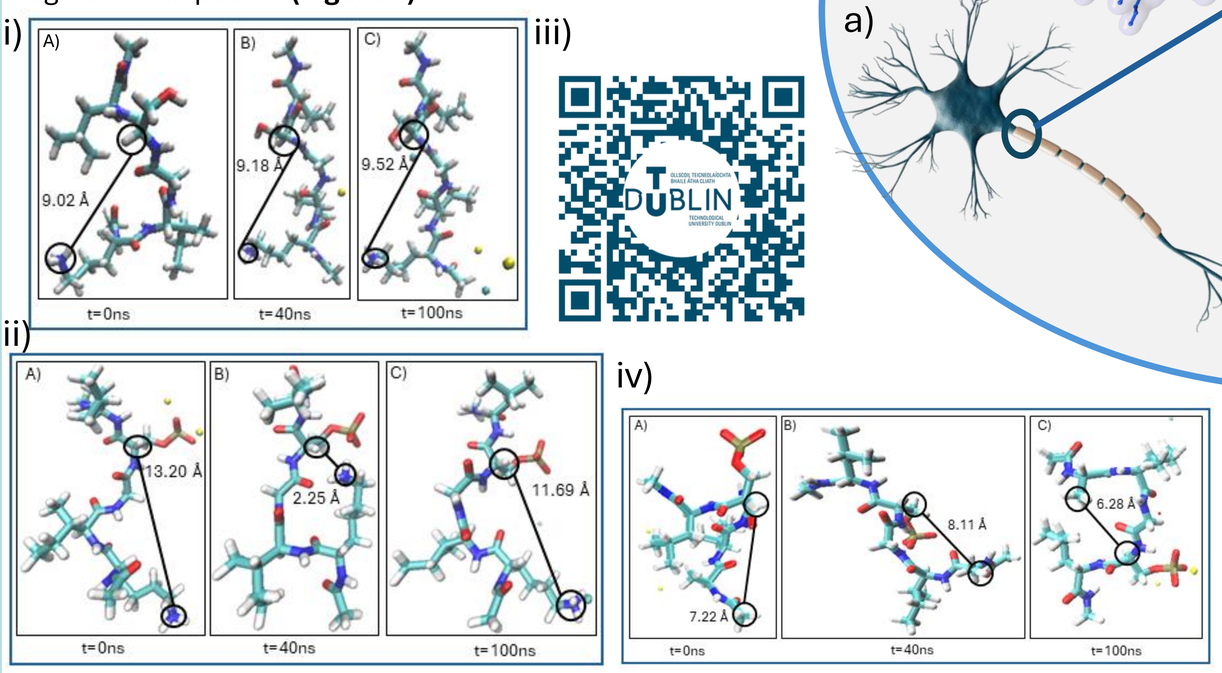
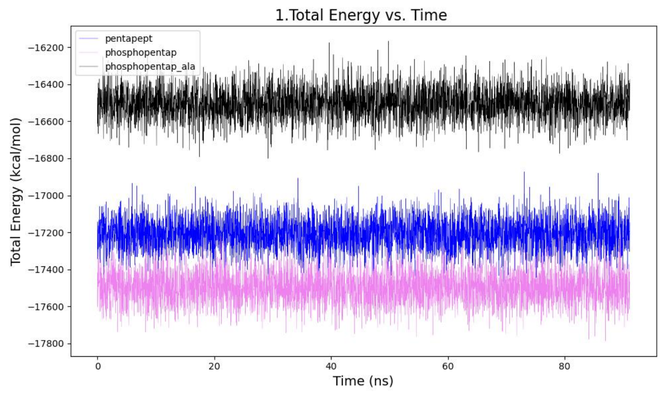
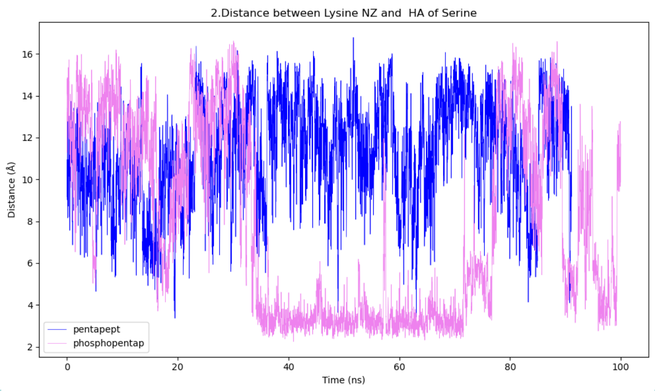


Figure 3. Snapshots of **i**) non-phosphorylated chain versus;**ii**) phosphorylated chain $pSer^{356}$ and $Lys^{353}$and; **iii)** phosphorylated chain $pSer^{356}$ and Ala^{357} are highlighted; **iv)** QR code of phophopentapeptide simulation movie



MD simulation	% of Occupancy
Pentapeptide	0.11
Phosphopentaptide	35.92

Table 1. % of Occupancy of interaction with a cut off < 4 Å between Nζ-Lys^{353} of and Hα-Ser^{356} of the first MD simulation and Hα-$pSer^{356}$ for the second.

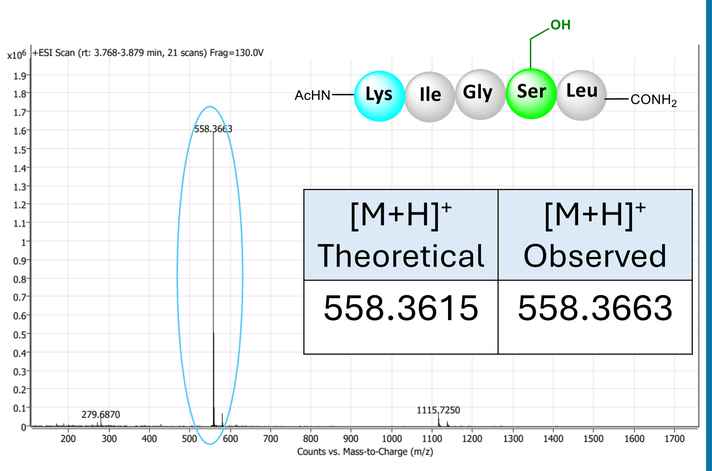


1. Total Energy is a measure of how the system is stable after heating at 310K and equilibrating after 5ns

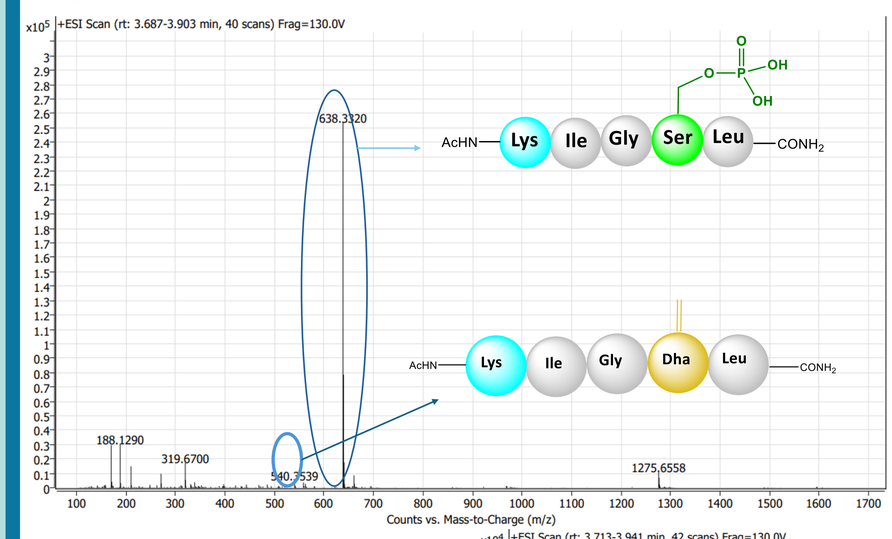
2. Average distance between Hα-Ser^{356} and Nζ-Lys^{353} and Hα-$pSer^{356}$ and Nζ-Lys^{353}

(blue) Pentapeptide
(pink) Phosphopentapeptide
(black) Phosphopentapeptide with K353A

LC-MS Analysis of synthesised fragments



[M+H]+ Theoretical	[M+H]+ Observed
558.3615	558.3663



[M+H]+ Theoretical	[M+H]+ Observed
638.3278	638.3320

[M+H]+ Theoretical	[M+H]+ Observed
540.3509	540.3539

[M+H]+ Theoretical	[M+H]+ Observed
581.2700	581.2756

- *Column Poroshell 120 RP18, 3.0x 50mm, 2,7; eluent H_2O/ACN (90% : 10%-100%) +Formic acid, flow rate 0.400 ml/min, UV-VIS detection 208nm.*

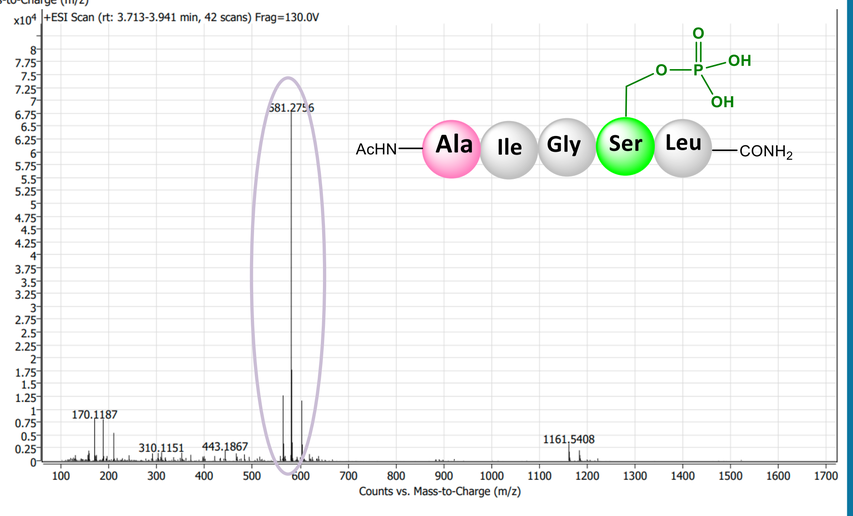


Figure 4: Serine pentapeptide fragment (top*);* Phosphoserine and Dha-containing peptide fragment (middle); Phosphoserine with alanine instead of lysine (end).

Conclusions Structural analysis, and computational studies identified a phosphoserine-containing Tau protein pentapeptide fragment, that was synthesised by SPPS. LC-MS analysis shows the desired phosphopeptide fragment, along with a minor amount of the Dha-containing peptide being detected. Future work will study the aqueous stability of the phosphopeptide fragments at varied pH values and computational studies will focus on the Dha peptide, and pH.

1. Šimi´c, G; et al.: Tau Protein Hyperphosphorylation and Aggregation in Alzheimer's Disease and Other Tauopathies, and Possible Neuroprotective Strategies. *Biomolecules*,**2016**, 6;6(1):6
2. Goto, Y; et al.; Mechanistic Studies of Ser/Thr Dehydration Catalyzed by a Member of the LanL Lanthionine Synthetase Family, *Biochemistry,* **2011**, 50, 891-898

Acknowledgement :
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