The impact of phosphorylation modulation in the binding of NLS peptides to importin-alpha

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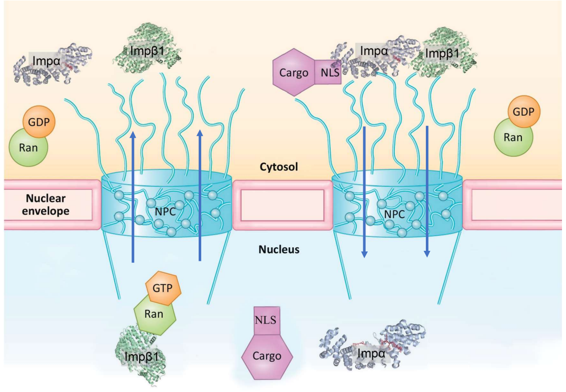
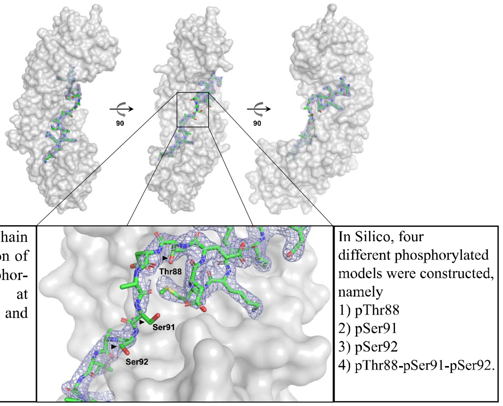
The import of cargos into the nucleus initiates by the interaction between nuclear localization signal (NLS) and nuclear import receptors in the cytoplasm.



The process of cargo proteins passing the nuclear envelope through Nuclear Pore Complex (NPC) is facilitated by members of the importin superfamily

Importin alpha, TDP-43 nuclear localization signal (NLS)



NLS mainchain and the position of putative phosphorylation sites at Thr88, Ser91 and Ser92.

In Silico, four different phosphorylated models were constructed, namely
1) pThr88
2) pSer91
3) pSer92
4) pThr88-pSer91-pSer92.

Markov State Model (MSM) analysis from MD simulation

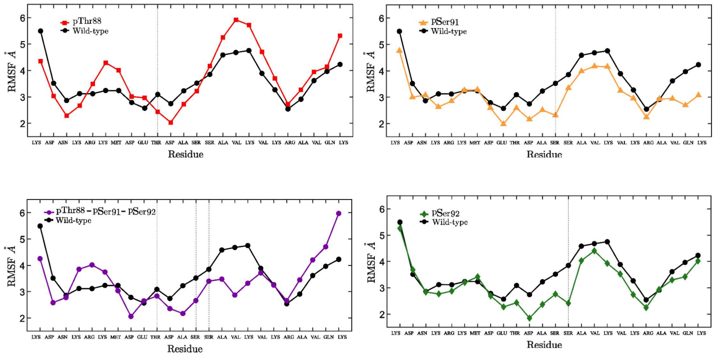
Kinetic maps generated by Markov state model to analyze the conformational dynamics of **TDP-43 NLS**

A four-state MSM using the PCCA++ algorithm and the Mean First-Passage Time (MFPT) were measured between different states of each phosphorylated system

Time-lagged independent component analysis (TICA) Features
1) Radius of gyration, 2) root-mean-square distance.
3) Distances between Cα atoms on the N- and C-terminus residues
4) Distances between Cα atoms on the N-terminus and Ala90 residues
5) Distances between Cα atoms on the C-terminus and Ala90 residues

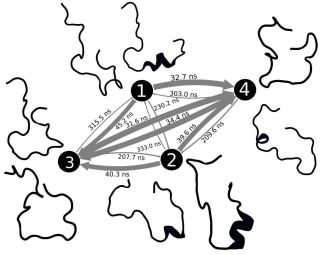
Molecular Dynamics Simulation detail		Simulated Systems				
		Wild-Type	pThr88	pSer91	pSer92	pThr88-pSer91-pSer92
Importin-α:TDP-43 NLS	Simulation Time	**100 ns**	**100 ns**	**100 ns**	**100 ns**	**100 ns**
	Simulation Size (Atoms)	**65,800**	**65,778**	**65,769**	**65,787**	**65,782**
TDP-43 NLS	Simulation Time	**1 μs × 6**	**1 μs × 6**	**1 μs × 6**	**1 μs × 6**	**1 μs × 6**
	Simulation Size (Atoms)	**19,614**	**19,618**	**19,615**	**19,603**	**19,603**

The NLS's Dynamics at the P2 and P2' binding sites significantly modulated by phosphorylation



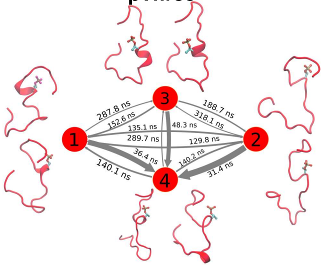
Kinetic maps of phosphorylated NLS via MSM analysis

Wild-Type

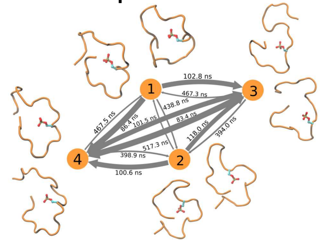


Arrows show Mean First-Passage Time (MFPT) between different morkov states. The lowest MFPT belongs to the wild-type system (< 333.0 ns), suggesting NLS has the highest probability of transitioning to the conformation that is favored for binding to importin α. The longest MFPT belongs to hyperphosphorylated TDP-43 NLS (< 1862.0 ns) and indicates that the spontaneous transition between different states is relative to other phosphorylated NLS peptides is less probable.

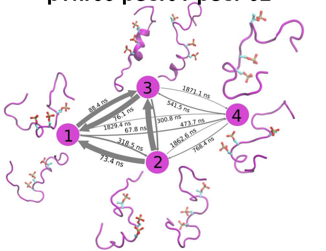
pThr88



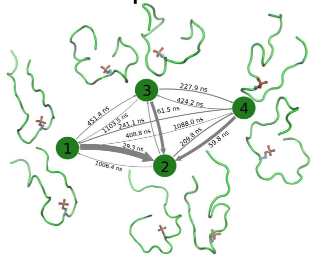
pSer91



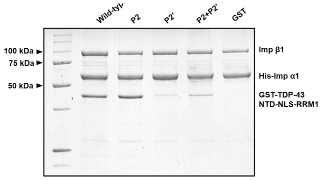
pThr88-pSer91-pSer-92

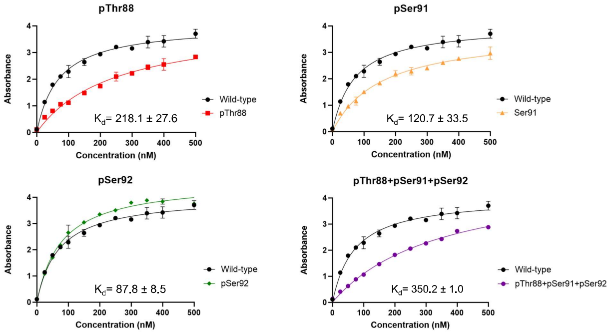


pSer92

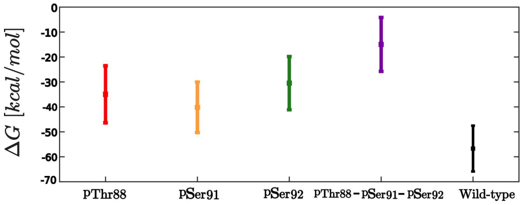


ELISA-based microtiter binding assay shows phosphorylation significantly perturbs NLS binding





In silico free energy of binding analysis shows poly-phosphorylation and wild-type have the highest and lowest biding energy, respectively



Conclusion

Phosphorylation of the TDP-43 NLS significantly perturbs Importin-α binding affinity, thereby regulating nuclear import. The binding energies of each construct with Importin-α, corroborate the dramatic reduction in affinity for 88pThr, 91pSer, 92pSer, and poly-phosphorylated constructs compared to wild-type NLS.

References

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