Phosphorylation: a smart biological switch for electrostatic interactions in neurons

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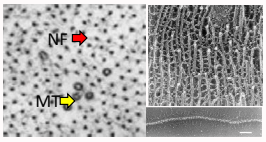
What is phosphorylation?

One of the processes by which cells modify proteins after their expression. Phosphorylation is the addition of a phosphate group to a protein. The phosphate charge is $\sim 2\ e^{-1}$.



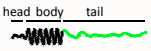
Fuchs & Cleveland, Science. **279**, 514 (1998)
Hirokawa, Science **279**, 519-526 (1998)
N. Hirokawa, Trends in Cell Biology **6**, 135-141 (1996)

Electrostatic interactions in neuron structure

Neurofilament proteins are the main constituents of axon cell, and provide it with mechanical and structural support. The proteins organize in a well- oriented, regularly spaced filamentous network which sets the axonal diameter. The network properties are governed by intricate electrostatic and steric interactions between their C-terminal highly charged tails.

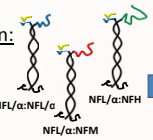
Self-Assembly of filaments

4 subunit proteins

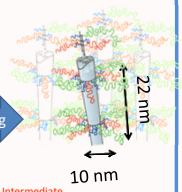


Coil-coiled interaction:

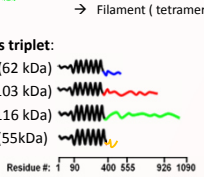
- Dimers
- Tetramers (2 dimers)
- Filament (tetramers)

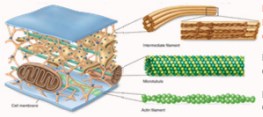


Concentrating



- **Neurofilaments triplet**:
  - NF-Low (62 kDa)
  - NF-Medium (103 kDa)
  - NF-High (116 kDa)
- **a-Internexin** (55kDa)

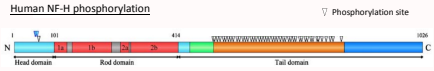




Bio 101: Phosphorylation and neurodegenerative diseases

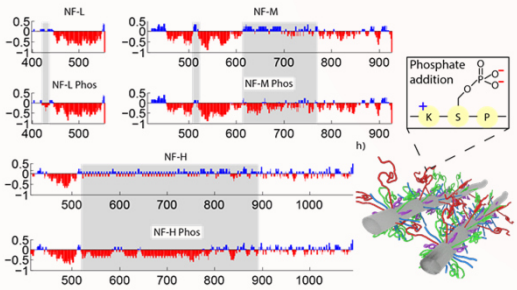
Sidearm phosphorylation increases the negative net charge density of NF-M and NF-H, which results with axonal caliber expansion: a prerequisite of normal axonal conduction and transport.

Aberrant neurofilament protein phosphorylation is a pathological hallmark of many human neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases.



Inter-filament tail interaction is modulated by phosphorylation

The sidearms mediate both repulsive and attractive interactions between neurofilaments in the condensed hydrogel. Phosphorylation significantly increases the net negative charge density of NF-M and NF-H, thus altering the electrostatic interactions between the filaments.



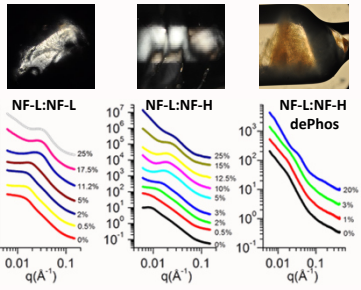
Protein	Number of AA		# in-vivo Phospho sites	Tail charge (e)
	Head+ body	Tail		
NF-L	397	158	1-3	-39.56
NF-L Phos				-45.30
NF-M	412	514	~26	-47.39
NF-M Phos				-99.04
NF-H (mouse)	411	679	~50	-7.06
NF-H Phos				-98.88
a-internexin	409	90	1	-2.97
a-internexin Phos				-4.89

Phosphorylation modulates the inter-filament distance and promotes structural orientation

Measurement of force and inter-filament distance with induced osmotic pressure shows differences in force response and structure.

Cross- polarizing microscopy shows nematic (oriented) domains in NF-L and in phosphorylated NF-L:NF-H networks compared with an isotropic scattering of dephosphorylated NF-L:NF-H networks.

Small angle X ray scattering profiles of dephosphorylated NF-L:NF-H networks agree with these findings: the correlation peaks suggest a lesser translational order.



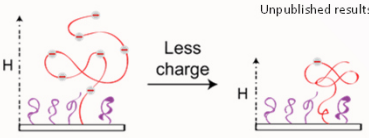
Synchrotron experiments performed at

SOLEIL SYNCHROTRON · diamond · DESY · MAX IV LABORATORY

What do we "naively" expect?

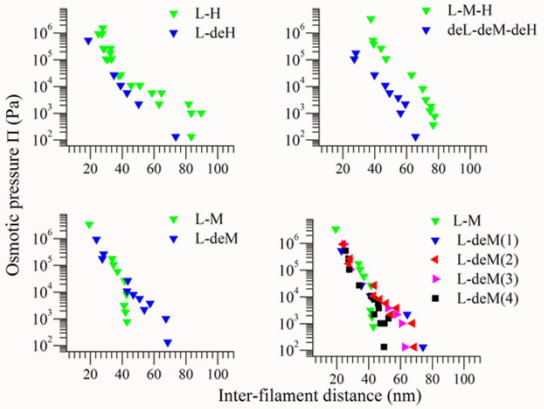
Unpublished results

Reducing the net charge of charged polymers in solution usually results with a decrease in polymer size. Similarly, removal of the phosphate negative charges is expected to reduce the brush height and inter-filament distance.



Results

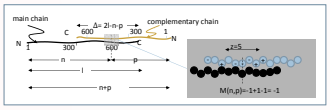
NF-H dephosphorylation reduces the inter-filament distance, as expected of charged polymers.
However, the phosphorylated NF-L:NF-M network is more condensed than the dephosphorylated NF-L:NF-M network, indicates that the negative phosphates on the NF-M tail are involved in attractive interactions.

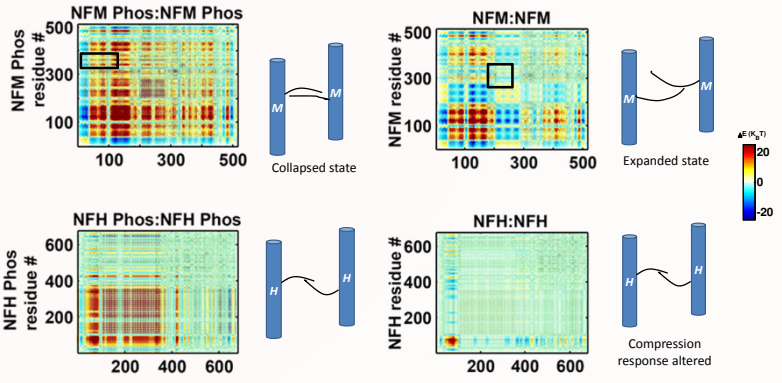


Handshake Analysis Matrices

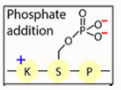
To reveal the potential attractive interactions cross-linking the negatively charged tail together; and to relate the tail charge distribution to our results, we employ a sequence-based electrostatic model.
Complementary amino-acid sequences that contribute to opposite chain interactions are represented by the matrix element:

$$\Delta E(n,p) = k_e \sum_{i=-w/2}^{w/2} \sum_{j=-m}^{m} \frac{eZ_1(n+i)eZ_2(p-i-j)}{|\vec{r}_1(n+i)-\vec{r}_2(p-i-j)|}$$

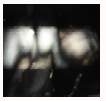
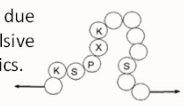
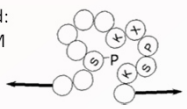




Neurofilament tails as "smart" polymers



How do polymers react to net charge increase?

	Conventional charged polymers	"Smart" charge distribution
Orientation	No: charge obstructs orientation 	Yes: NF-L:NF-H 
Tail size	Increased due to repulsive electrostatics. 	Decreased: NF-L:NF-M 

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