NEUROFILAMENT STRUCTURE AND UNFOLDING FORCES OF SIDEARMS

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Intro - Biological context

Flexibility and NF network elasticity

Charge, condensation, and charge reversal

Structure and unfolding of NF sidearms
Flexibility, Self-Interaction, and Counterion-Induced Collapse of Neurofilaments

Structure of a neuronal cell

cell body

dendrites

axon

processes or neurites

terminal arbor

Spinal cord neurons on glass surface grow only on underlying glial cells
Neurons on soft gels (200 Pa) grow directly on laminin-covered gel surface

Electron micrograph of NFs and MTs in axon
Hirokawa et al
Neurofilaments are intermediate filaments, which are found only in neurons.

Neurofilaments are made up of three proteins. They are named NFL, NFM, and NFH after their apparent molecular weight in SDS-PAGE.
Three types of NF subunits.
All are anionic and phosphorylated
Filament diameter is 10-12 nm, sidearm can be >50 nm
surface charge is between 1.4 and 2.8 charges / nm² depending on phosphorylation

\[ \lambda_p = 500 \text{nm} \]

Non-linear elasticity of NF networks

Strain-hardening at larger strains than for actin
Cytoskeletal polymer gels display strain-hardening not seen in synthetic gels.

Neuronal intermediate filament networks are also strain hardening but at larger strains than F-actin.
What is the diameter of a neurofilament?

How much of the axon volume do NFs occupy?

Neurofilaments have small protrusions called sidearms.
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Sidearms come from NFM and NFH proteins.

- NFH
- NFM
- NFL

Measurements from electron microscopy:
- 20 - 90 nm length
- 22 nm periodicity
- 4 - 6 nm thickness
Phosphorylation effect on sidearms and neurofilaments.

<table>
<thead>
<tr>
<th>Phosphorylated</th>
<th>Dephosphorylated</th>
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<tbody>
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<td>~ 92 nm</td>
<td>~ 61 nm</td>
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NF sidearms appear to limit spacing of NFs
NF sidearms have unusually high electrostatic charge and unknown folded structure.

Can a charged polypeptide have folded domains without secondary structure?
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Charge distribution along the sidearm from the AA sequence for both phosphorylated and dephosphorylated sidearms.

Net charge: -1
Phosphorylated:
Net charge: -113

Net charge: -1
Dephospho
Phospho

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Circular dichroism for phosphorylated and dephosphorylated sidearms.

Atomic Force Microscopy of sidearms.
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Force versus displacement curves depend on the type of biopolymer.

Typical saw-tooth patterns for sidearms:

- **no ATP**: 50 pN
- **ATP**: 50 pN
- **Dephosphorylated**: 25 pN
Number of peaks (domains) of sidearms. Sidearms have 4 domains,

\[ N_{pk} \]

The graph shows the distribution of peaks with different states:
- Green bars: no ATP
- Blue bars: ATP
- Red bars: Dephosphorylated

Total unfolding length \( L_u = L - L_f \approx 124 \text{ nm} \)

Contour length \( L \approx 224 \text{ nm} \)

Folded length \( L_f \approx 100 \text{ nm} \)
CONCLUSIONS

• AFM predicts the existence of four domains in the sidearm. The number of domains is not modified by ATP or dephosphorylation.

• The domain size remains constant at 20 nm.

• The force required to unfold dephosphorylated sidearms is almost half of the force required to unfold phosphorylated sidearms.

• Dynamic light scattering shows that the size of sidearms is not modified by ATP. Dephosphorylation, on the other hand, decreases the length of the sidearm.

• Circular dichroism measurements may indicate differences in the secondary structure of phosphorylated and dephosphorylated sidearms.
NFs are aggregated by physiologic concentrations of multivalent counterions

Bundle formation depends on NF surface charge

At low counterion concentration, NF self-interactions lead to a variety of looped structures

High counterion concentrations leads to redissolution of bundles and charge reversal

Surface charge of NFs affects bundling by lysine hexamers
Bundling of NFs by spermine depends on NF phosphorylation

DLS of Side-arms
On the effect of spermine and ATP
Neutron scattering

Formation and redissolution of NF bundles by spermine

[spemine]

0

0.1 mM

10 mM

0.1 mg/ml RhoB-labeled NFs
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15 nM NFs in RB 0.8 M sucrose exhibit a couple of characteristic structures. Because fluorescence imaging is much easier than AFM, one can characterize multiple typical structures of highly diluted NFs...
Lassos

Proposed evolving of lassos via the formation of pre-lassos:

Small and large rings

Rods and snakes

'double lasso'

Regarding branches it is not directly obvious how the two NFs are attached and why the attachment reveals a defined angle: zooming…
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Effect of simply adding sidearms to NF? Do sidearms interact with sidearms?

Bundling? Branching, cross-linking? Intrafilamentous shape changes?
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"Real-time" capturing of 'branching'

2 min
Rho-NFs (negatively charged) do accumulate at the plus pole

Spermine treated rho-NFs do accumulate at the minus pole

Binding of NFs to oppositely-charged beads

Structures depend on diameter of bead.

NF wrap around larger (>μm) beads but are decorated by small (20 nm) beads
Probing surface charge of NF with polystrene microspheres of different charges

Amino modified beads 0.2 µm (positively charged)

One can see NF-structures on 0.1 µm amino beads
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20 nm amino modified beads

Carboxylated beads (coated polystyrene beads with a hydrophilic polymer containing multiple carboxylic acids)
Another example of snuggling (small) mitos to NF:

Actin + Arp2/3
NFs can make extensive side-to-side attachments, mediated by sidearms.

**AFM**

**EM**

Net charge: -1
s = 56
ksp = 42

Phosphorylated:

Net charge: -113

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**Net charge:**

-56

**Phosphorylation:**

$k_{sp} = 42$
Primary structure and electrostatic charge of human NFH

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mmsfggadallgapafaphgssgshyalarkggagtrisaqsssgfhsdsortrsvvsaspsrfgagaastr
dstltspegecmvavatsrsekeqlaqlndrfagyidkvrqalehnrsolegeaalrqqagrsangely
evrevremsgavrligargqrllqehhlediahvqr1lddeearqreeaearalafqaeaeavrdldqkk
aqalqecgylrrhheeqgegllqiqgsaqaqmqatrdalkcdvtsairatreairaglghavqstqlqeeewf
rvrdrileaakvntdamrscqeeiteyrqrlqartelealkstkdslrqrseledrhqadiasqeqaaiqql
aelrntkwemaaqrlreyqdlvnkmaldeiaayrkilegeecrig
```

| No net charge | 56 phospho sites | 40 KSP |

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