



Interactions between Neurofilaments and L-DOPA

Kelsey Gorter

Biochemistry

Allan Hancock College

UCSB Materials Research Lab

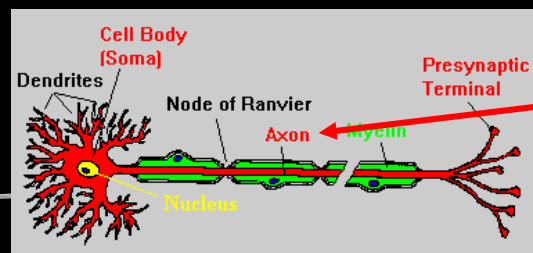
Jayna Jones, Mentor

Cyrus Safinya, Faculty Advisor



Motivation

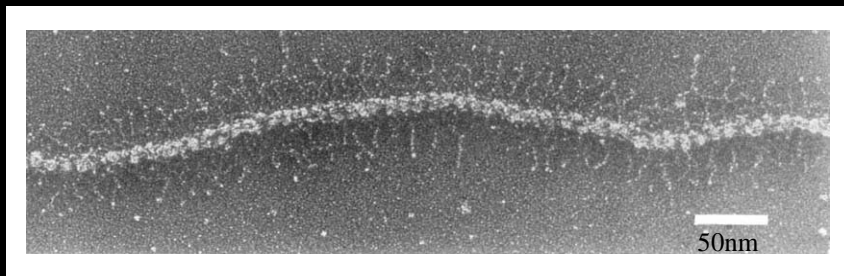
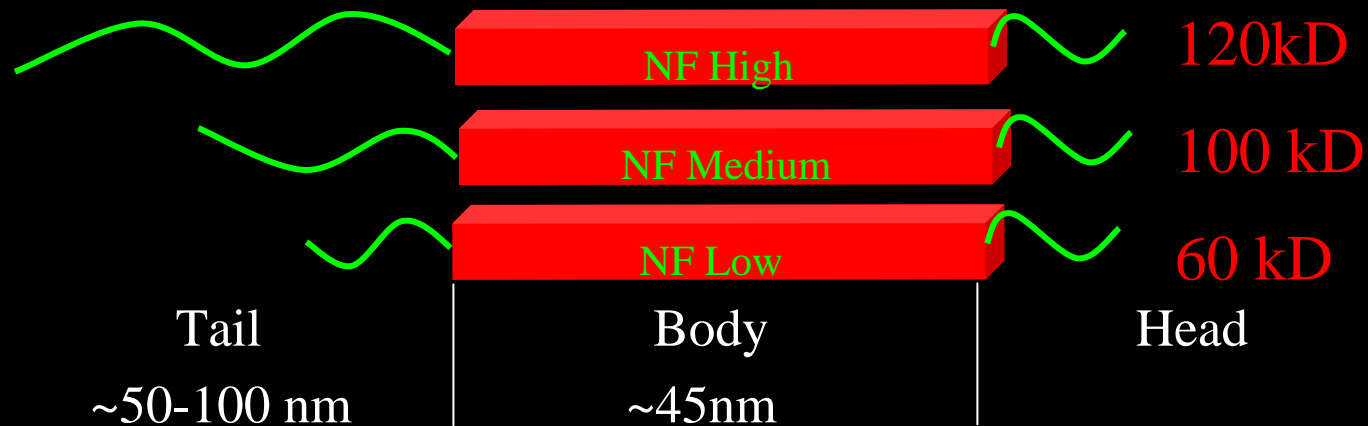
- ☞ Neurofilaments are structural proteins specific to neurons.
- ☞ Neurofilament non-specific aggregation is a hallmark several neurodegenerative diseases.
- ☞ Oxidative stress may cause Lewy body formation in Parkinson's patients.
- ☞ L-dopa is a treatment for Parkinson's disease that was previously shown to oxidize and crosslink NFs* .
- ☞ **Goal: To understand the mechanism by which L-dopa crosslinks NFs.**



Location of NFs

*Montine, T, Journal of Neuropathology and Experimental Neurology. 1995; 54(3): 311-319.

Neurofilament Assembly



Electron micrograph of a NF reassembled *in vitro**

NFs are made up of three subunit proteins which assemble into a rod about 10 nm in diameter with radiating sidearms.

*Fuchs et al., Science, 279, 514 (2000)

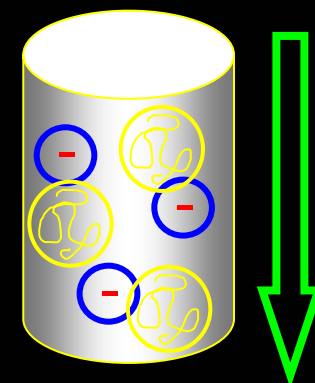
Isolating NFs from Bovine Spinal Cord



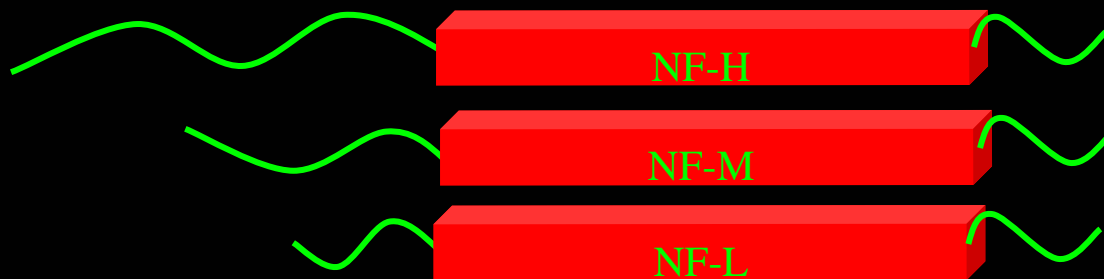
1. Homogenize spinal cord in a blender



2. Centrifuge to remove cell waste

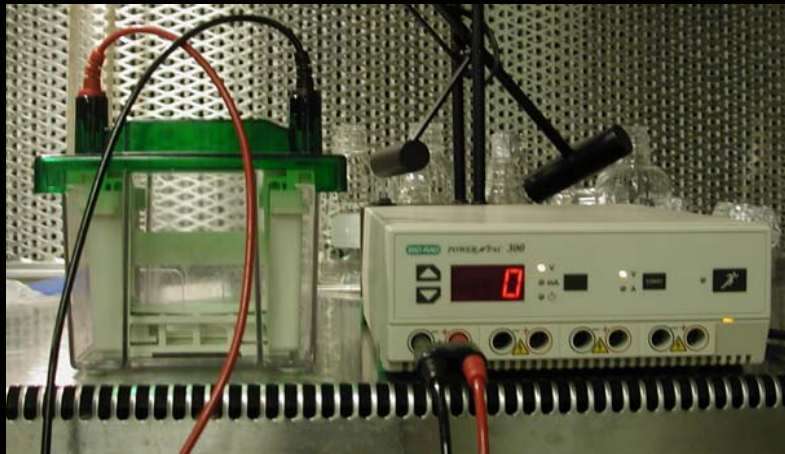


3. Use ion exchange chromatography to remove impurities

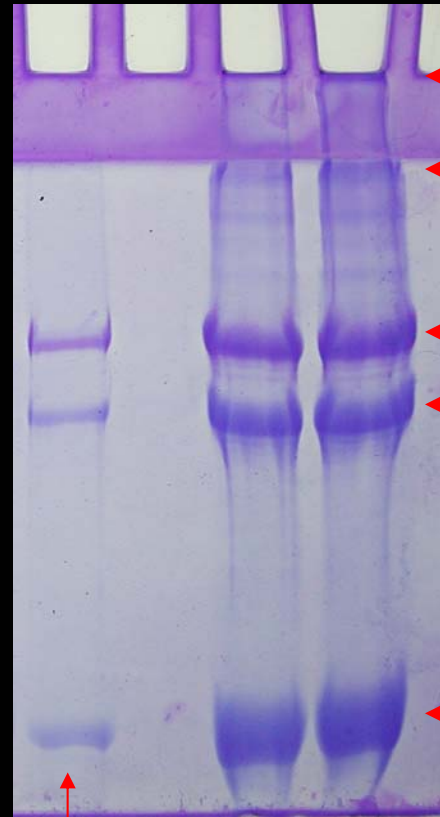


4. Results in pure NF subunits

Determining sample purity and extent of crosslinking using gel electrophoresis (SDS-PAGE)



SDS-PAGE separates molecules based on their molecular weight.



High MW bands indicate NF crosslinking

NF-H
NF-M

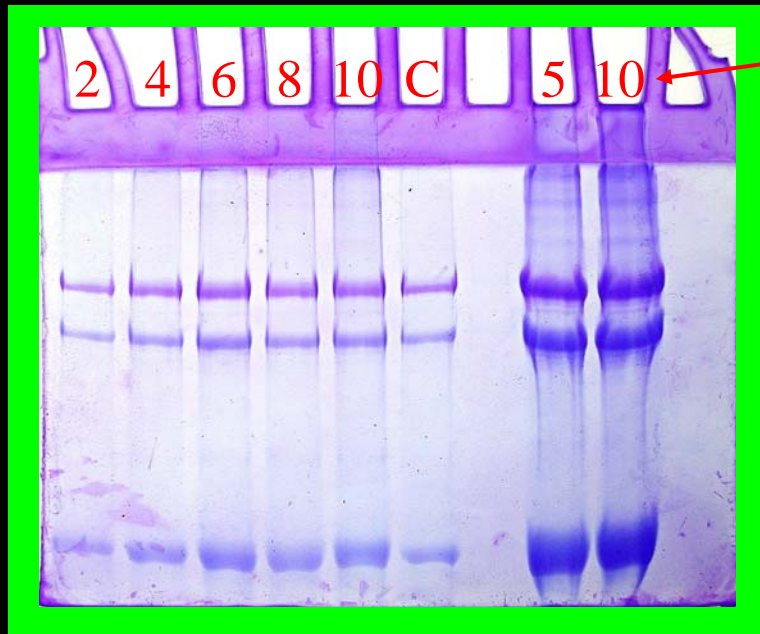
Characteristic NF banding pattern

NF-L

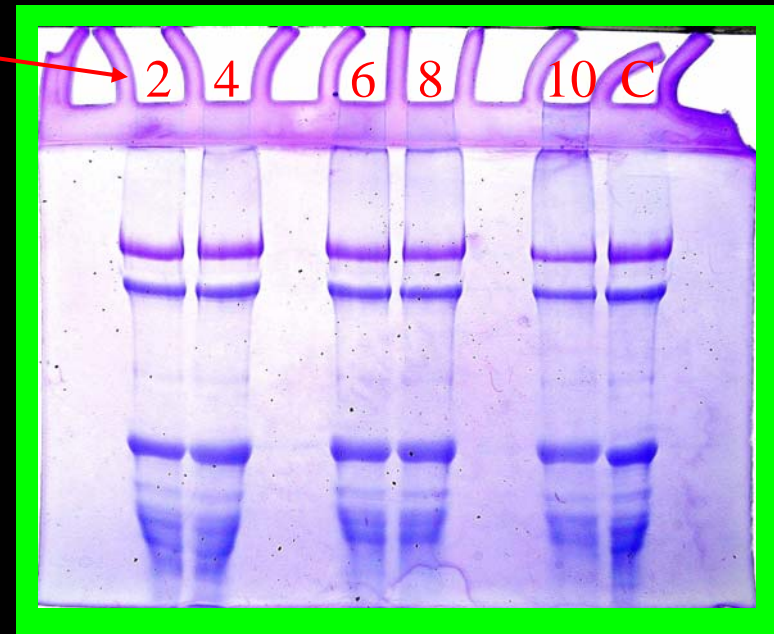
Control

Crosslinking Optimization

Purified NF with increasing concentrations of L-dopa



Crude NF with increasing concentration of L-dopa



L-dopa
(mM)

1.5 mg/ml

>50 mg/ml

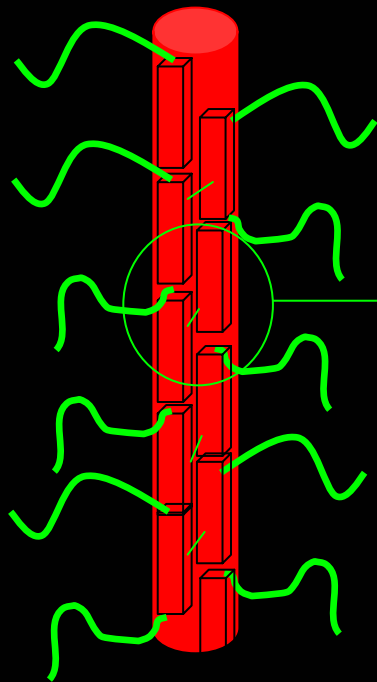
1.5 mg/ml

Mechanism of Crosslinking

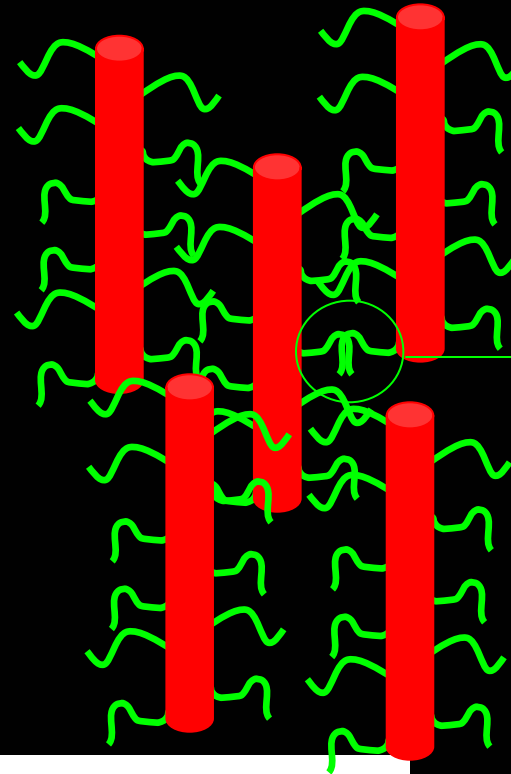
Intra-filament Crosslinking

Vs.

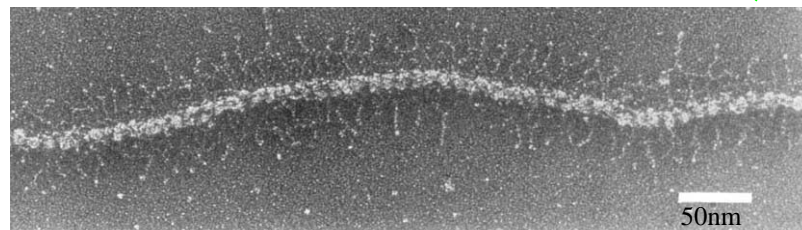
Inter-filament Crosslinking



Is the crosslinking within the filaments?



Or between adjacent filaments?

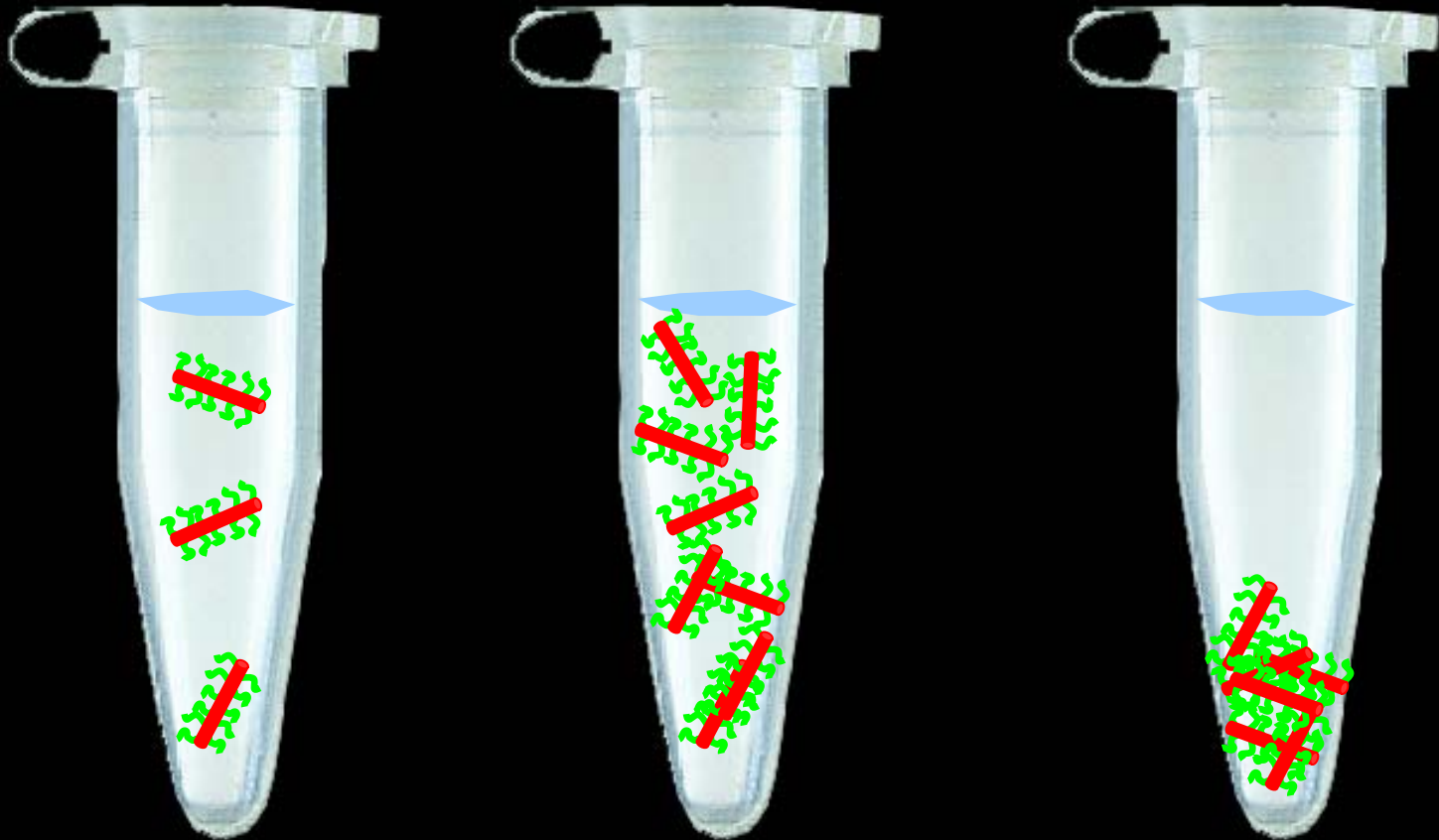


Amino acids	Oxidation products
Cysteine	Disulfides, cysteic acid
Methionine	Methionine sulfoxide, methionine sulfone
Tryptophan	2-, 4-, 5-, 6-, and 7-Hydroxytryptophan, nitrotryptophan, kynurenine, 3-hydroxykynurinine, formylkynurinine
Phenylalanine	2,3-Dihydroxyphenylalanine, 2-, 3-, and 4-hydroxyphenylalanine
Tyrosine	3,4-Dihydroxyphenylalanine, tyrosine-tyrosine cross-linkages, Tyr-O-Tyr, cross-linked nitrotyrosine
Histidine	2-Oxohistidine, asparagine, aspartic acid
Arginine	Glutamic semialdehyde
Lysine*	α -Aminoadipic semialdehyde
Proline*	2-Pyrrolidone, 4- and 5-hydroxyproline pyroglutamic acid, glutamic semialdehyde
Threonine	2-Amino-3-ketobutyric acid
Glutamyl	Oxalic acid, pyruvic acid

*Found in repeat units along NF sidearms.

Sample Preparation

✓ Concentration dependence of crosslinking implies inter-filament crosslinking



Conclusions & Future Research

- ☛ L-dopa concentrations of 6mM and higher yield detectable crosslinking.
- ☛ Samples with longer incubation times display more crosslinking.
- ☛ Remaining internship: determining the mechanism by which NF crosslinking occurs.

Acknowledgements

INSET Program

Jayna Jones, Mentor

Cyrus Safinya, Principal Investigator

Allan Hancock College

Allan Hancock College MESA Program

UCSB

NIH GM-59288,

NSF DMR- 0203755, & CTS-0404444



SDS-PAGE

