Characterizing the pH and Salt Dependence of Neurofilament Grafting Densities

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Introduction:

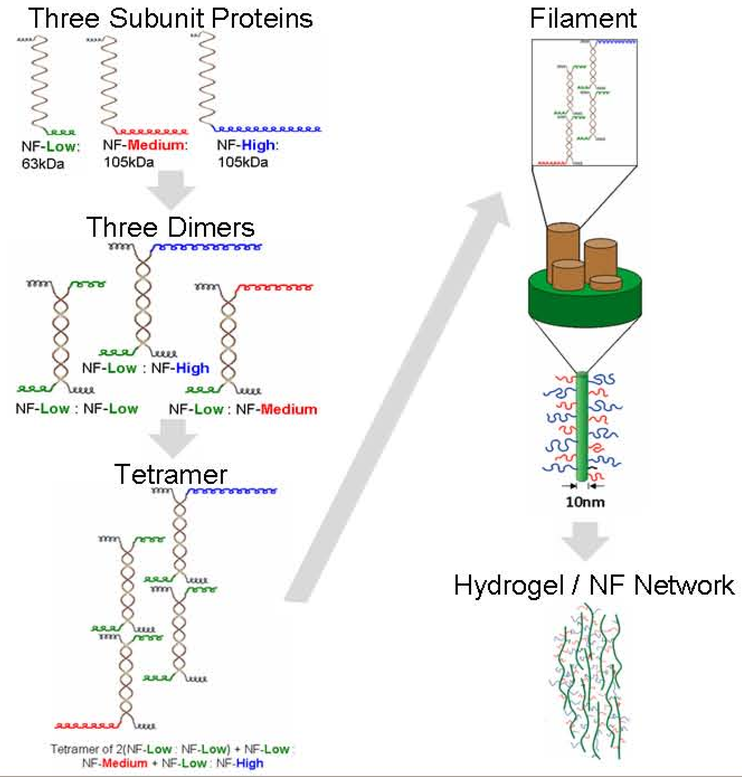
Neurofilaments

- A class of intermediate filaments
- 10nm-wide protein rods composed of three subunits: low, medium and high (NF-L, NF-M, NF-H)
- Once assembled into filaments, the C-terminus tails of the subunits radiate outward, interacting with the sidearms of adjacent filaments to form extensive neurofilament (NF) networks within the cell
- Stress-buffering members of the axoplasm
- Implicated in maintenance of neuronal cell structure, as support structures (scaffolds) for transport that occurs on microtubules, and in dendritic aborization

Motivation

- Implication of NF's in such neurodegenerative diseases as Amyotrophic lateral sclerosis, Parkinson disease and Alzheimer disease

Subunit Proteins to Functional Hydrogel



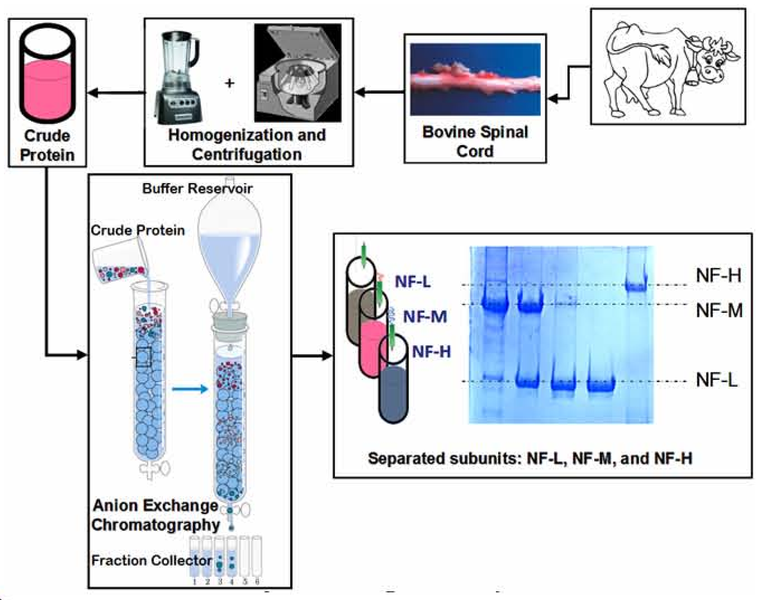
Grafting Density

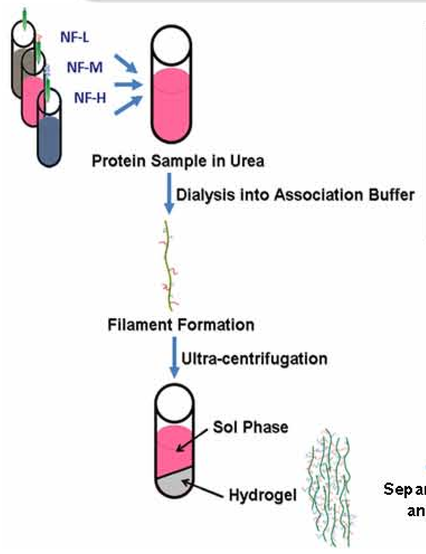
- NF-M and NF-H can only dimerize with NF-L
- The ratio of assembled NF-M and /or NF-H to NF-L in the filament

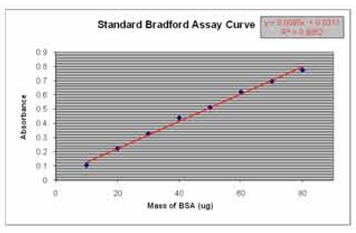
Objective

- To quantify the percentage of NF-M added to the protein solution that has grafted to NF-L at the given assembly buffer salt concentrations and pH's, and to compare this percentage to that obtained for the standard assembly buffer conditions
  - Assembly conditions tested include salt concentrations of approximately 40mM, 90mM, 150mM, 240mM and 500mM at pH's of 6 and 6.8
  - Initial grafting densities of approximately 12%, 20%, 27%, 34%, 36%, 40%, 46%, 51% and 57% for each buffer condition
  - Total of 99 samples tested

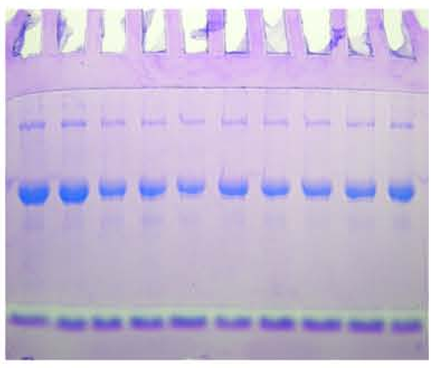
Methodology:







Left: the Bradford Assay compares a measured absorbance to a known protein concentration. Measured absorbance of sol phase quantifies subunit presence using the resultant curve.

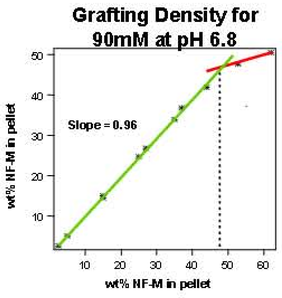


Above: 15% polyacrylamide gel run on 10% NF-M. Relative intensities for each band in a lane are measured with ImageJ, giving %NF-M for each sample; the stock solution represents initial conditions for all subsequent samples.

Results:

Previous Work

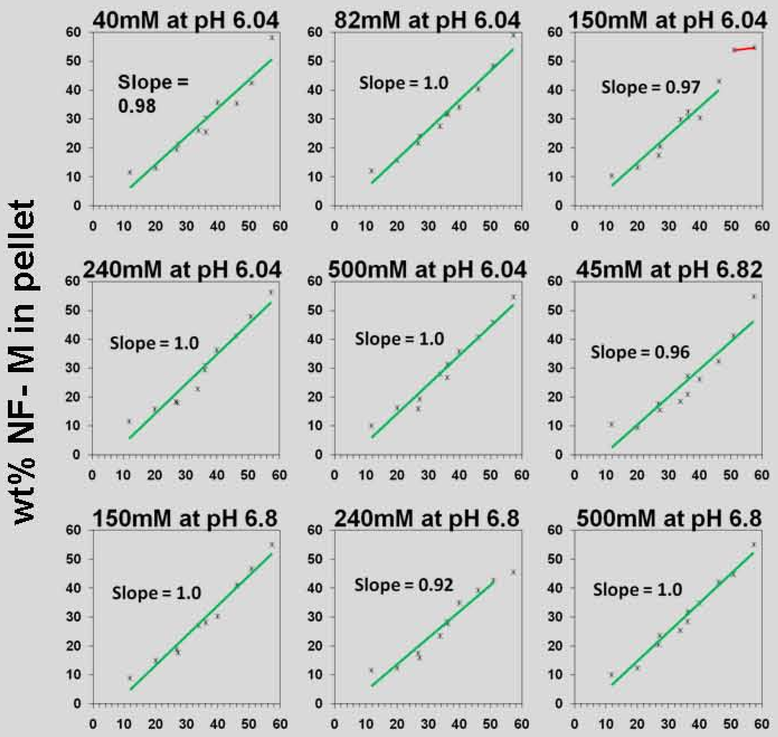
Following the characterization of grafting densities for NF networks at standard conditions, there was a need to determine the pH and salt dependence of grafting density, if any. Quantifying any such effect was the focus of this study.



Current Work

An ideal network grafting density (wt% NF-M in pellet) is equivalent to the subunit's initial density prior to assembly (wt% NF-M initial), giving a slope of one when plotted against each other. Because subunits may only form dimers containing at least one NF-L (the second subunit may be NF-L, NF-M or NF-H), a saturation point of 50% by unit, 62.5% by weight gives the maximum possible composition of NF-M. Samples were tested up to 57 weight% NF-M; consequently, only two buffer conditions (150mM at pH 6.04 and 240mM at pH 6.8) displayed clear saturation points within the range tested.

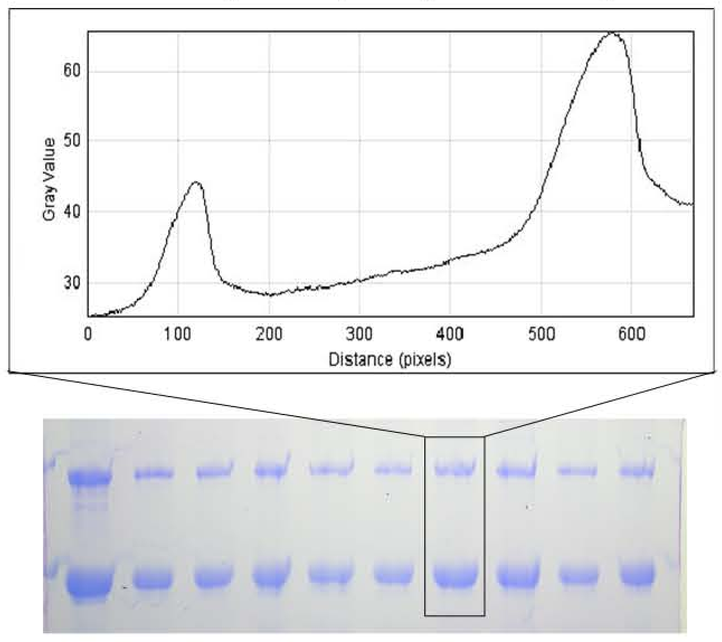
Grafting Densities



As illustrated above, the grafting densities of samples at all assembly buffers are consistently good, with slopes at or near one. The consistency indicates a lack of clear correlation between changes in salt concentration or pH and changes in grafting density. The results suggest that NF networks are highly stable and largely unaffected by fluctuations of neuronal cell conditions.

Polyacrylamide Gel Analysis

Lane 7 Intensity Histogram (35% 45mM pH 6.82)



Above: Intensity of each band in eleven acrylamide gels was analyzed using a histogram created in ImageJ (pictured here with the lane it represents). The comparative area of each peak—the first of which represents NF-M, the second NF-L—correlates directly to the wt% of the corresponding subunit.

Acknowledgement:

Funding provided by DOE DE-FG-02-06ER46314, NIH GM-59288, NSF DMR-0503347, NSF DMR-0803103, NSF MRSEC Program Award No. DMR05-20415