Supramolecular Structure and Assembly of Neurofilaments

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CALIFORNIA NANOSYSTEMS INSTITUTE

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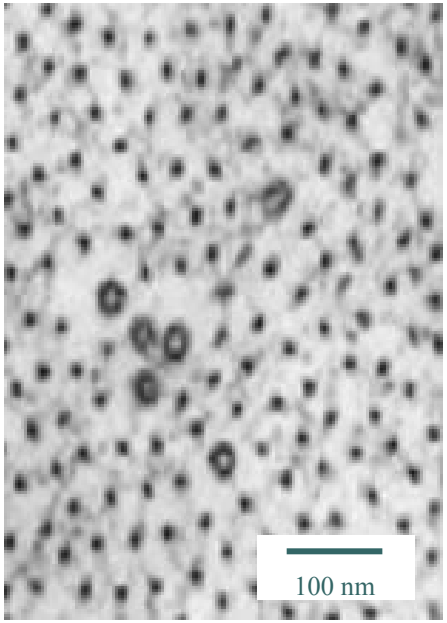
Introduction

- Neurofilaments (NFs) are cytoskeletal proteins located in the axon of neurons.
- NF aggregation is a hallmark of several neurological disorders such as Parkinson's disease and ALS.
- We study the structure and assembly of purified NFs *in vitro.*

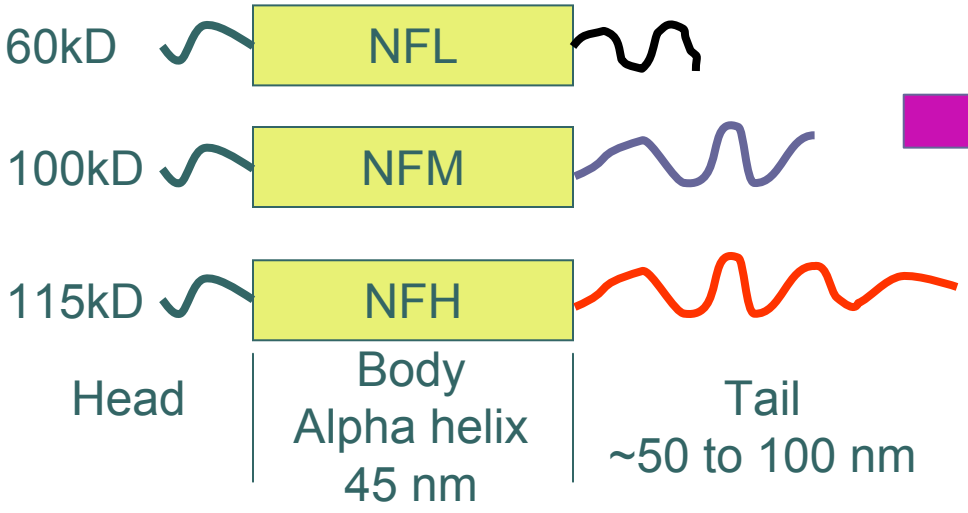
Goal:

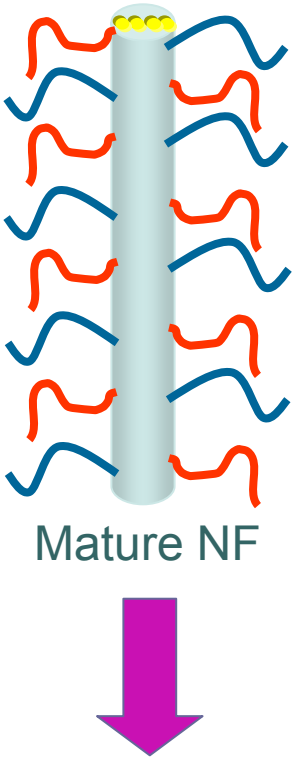
Find the saturation ratio of NFs assembled from NF-L and NF-H.



Mol. Bio.Cell, 3rd ed, 73-74 (1995).

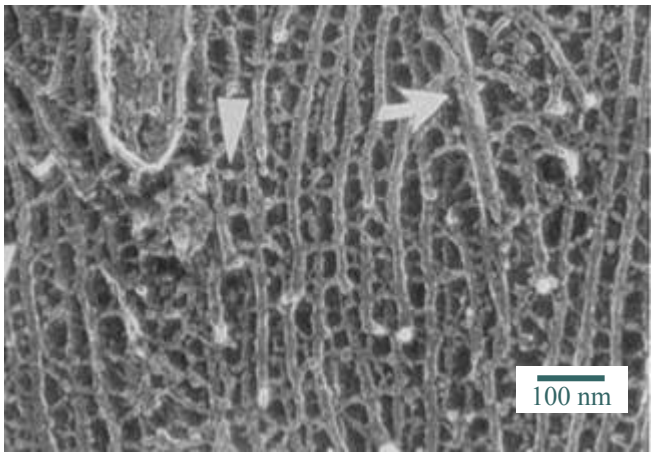
NF Assembly





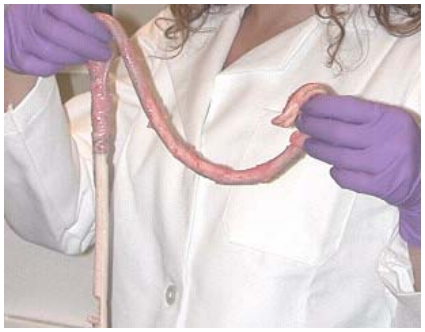
- *In vivo*, mature NF have a characteristic stoichiometric ratio of 7:3:2 (NFL:NFM:NFH)
- Other ratios occur in developing, regenerating, and diseased neurons
- We will look specifically at the ratio of NFs assembled from NF-L and NF-H subunits

Bundled Neurofilaments *in vivo*



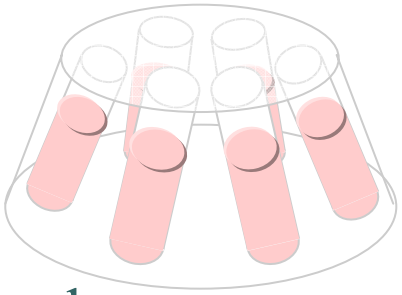
*N. Hirokawa et al, J. Cell Biol. 98, 1523 (1984).

NF Purification from Bovine Spinal Cord



1. Homogenize spinal cord in blender

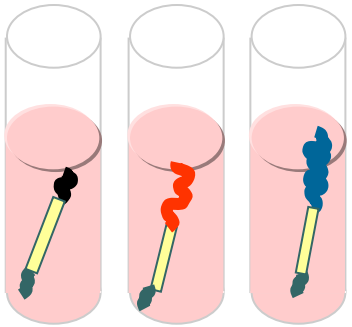
2. Centrifuge to get rid of cell waste



3. Incubate supernatant in glycerol and pellet neurofilaments

4. Clarify by centrifuging through sucrose gradient

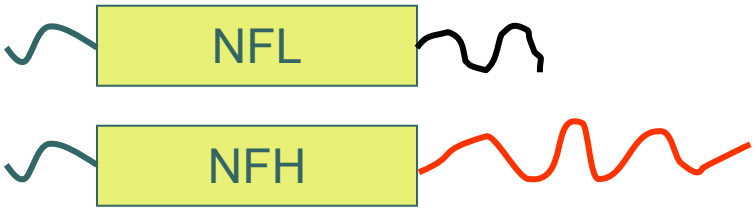
5. Remove remaining impurities with ion exchange column

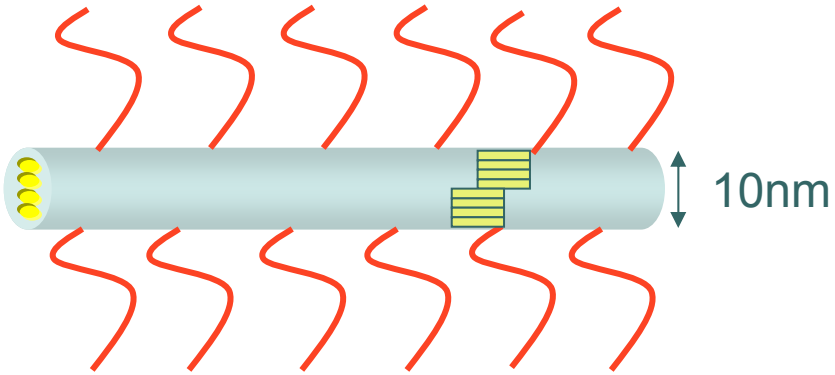


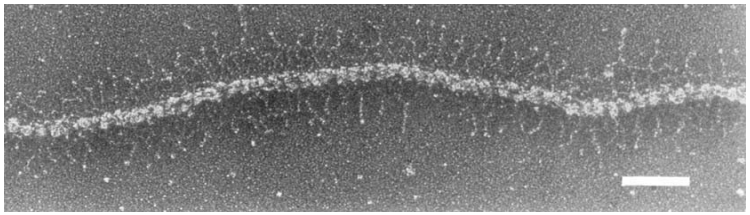
Result: Pure NF subunits

Delacourte et al, Biochem. J., 191, 543-546 (1980) and Scott et al, J. Bio. Chem., 260, 10736-10747 (1985).

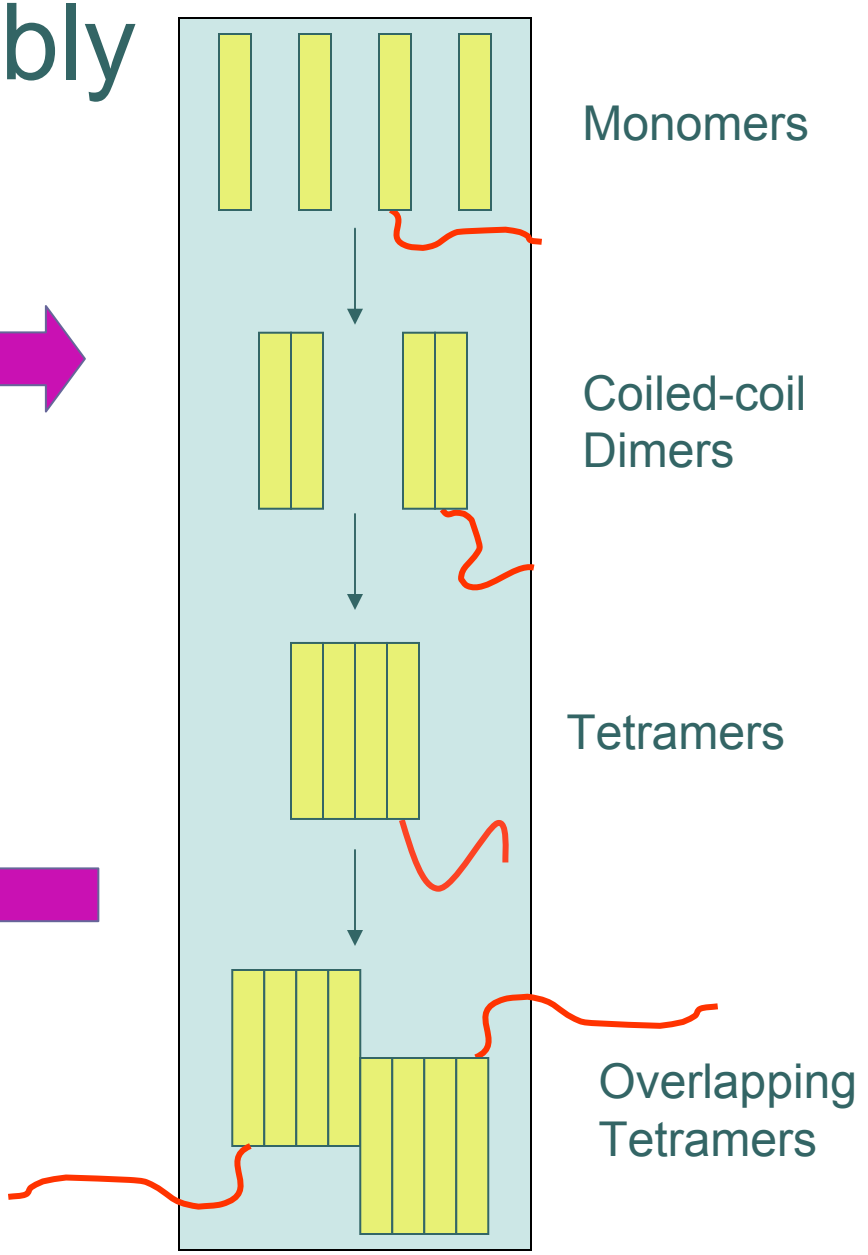
NF-L H Assembly





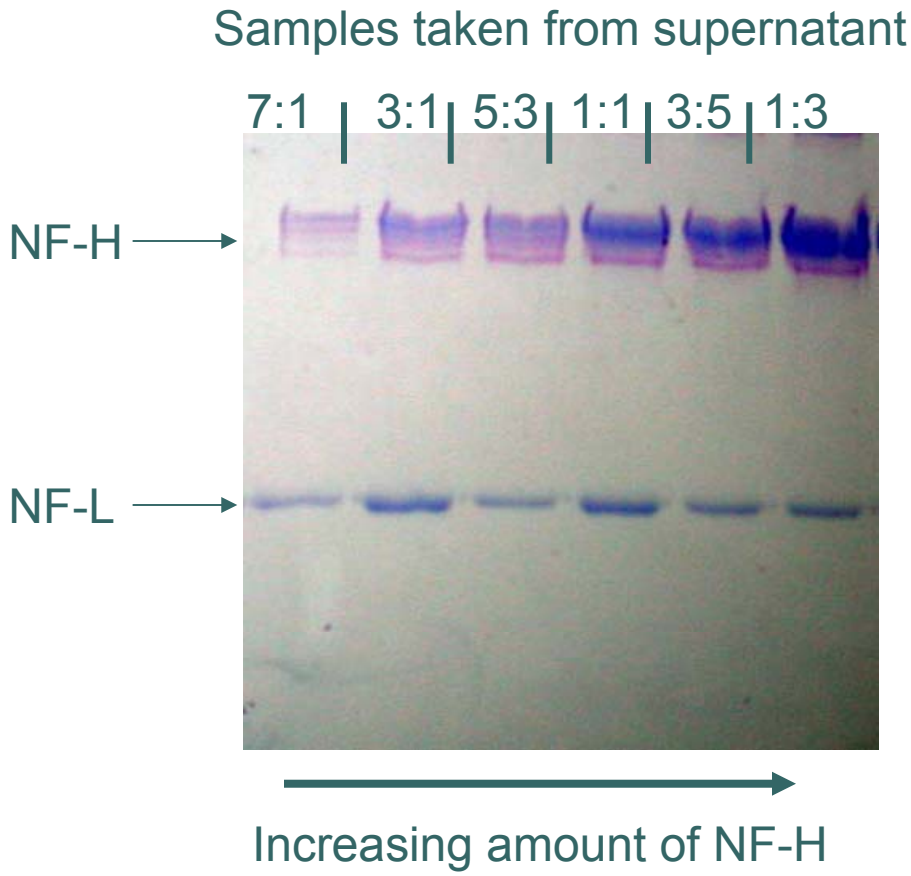


* Fuchs et al., Science, 279, 514 (2000)

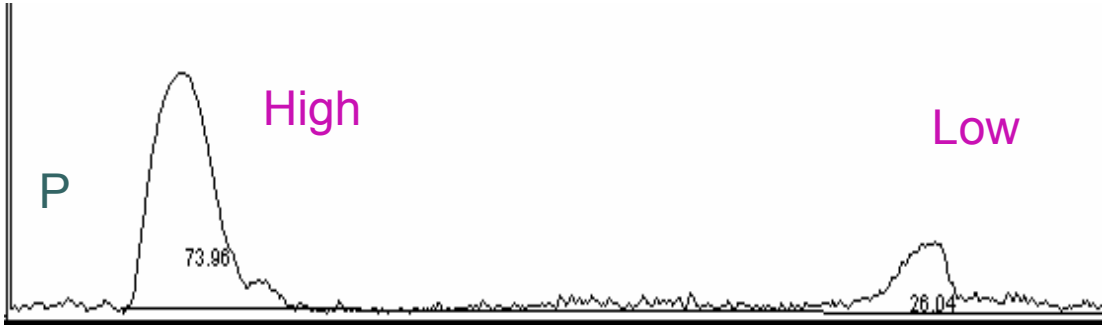


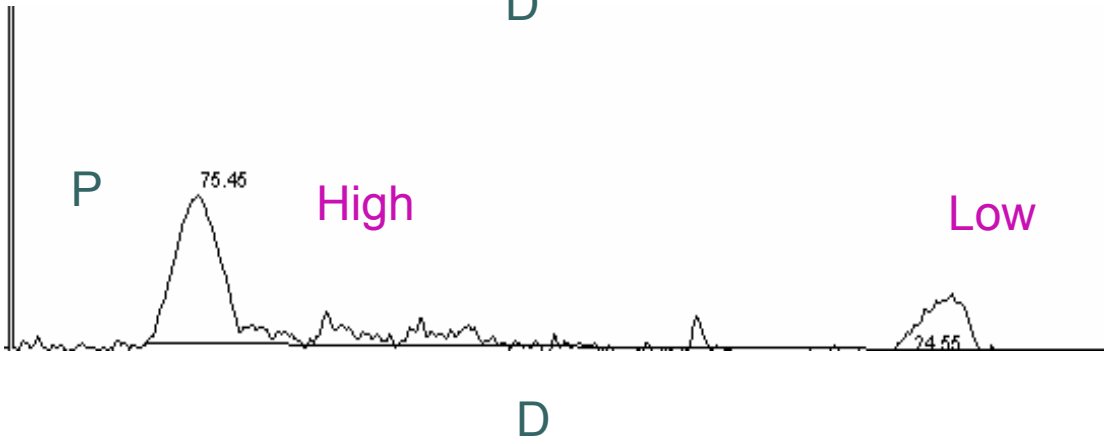
Sample Preparation

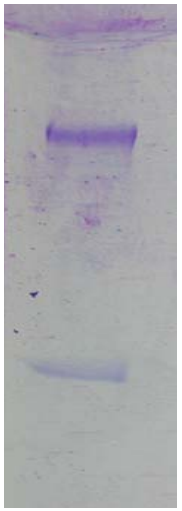
- Add increasing amount of NF-H to NF-L
- Centrifuge samples to form a pellet containing a network of NFs
- Using gel electrophoresis, determine the ratio of NF-L:NF-H that formed the NF network versus what NFs remained in the supernatant



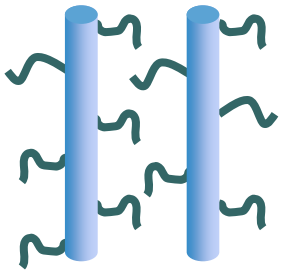
Gel Analysis



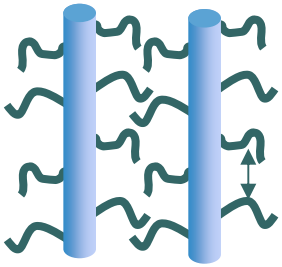




NF-L:NF-H (17:3)



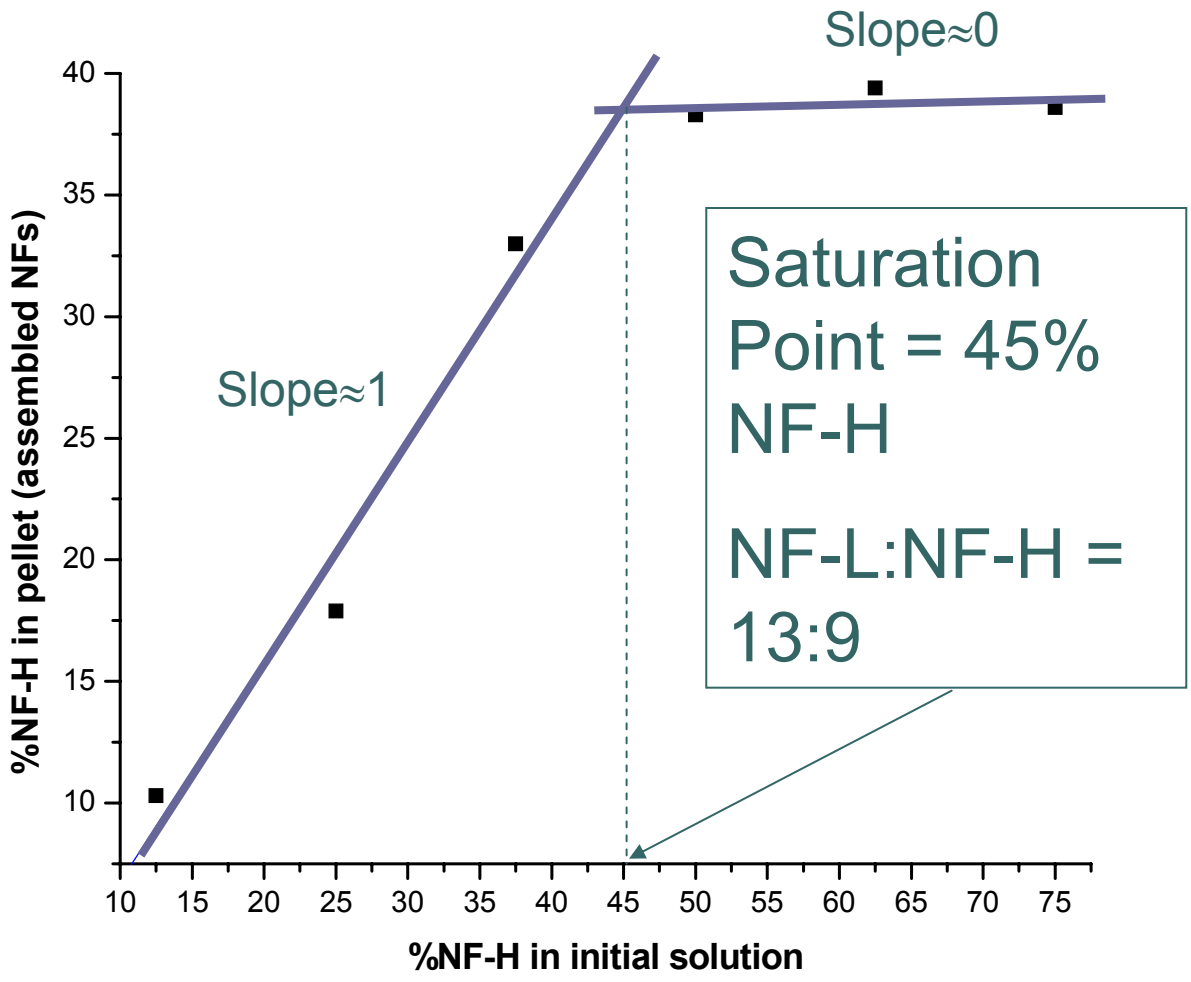
NF-L:NF-H (3:1)



As higher concentrations of NF-H are added, more side arms are also added causing side arm interaction and assembling

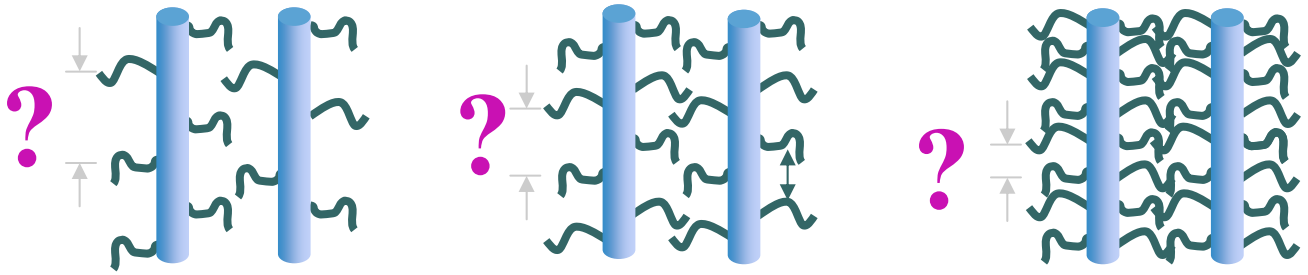
P: pixels
D: distance down the gel

Saturation Point



Future Research

- Reproduce the value for the NF-LH saturation point.
- Use the saturation point ratio to calculate the distance between the sidearms on the NF core.



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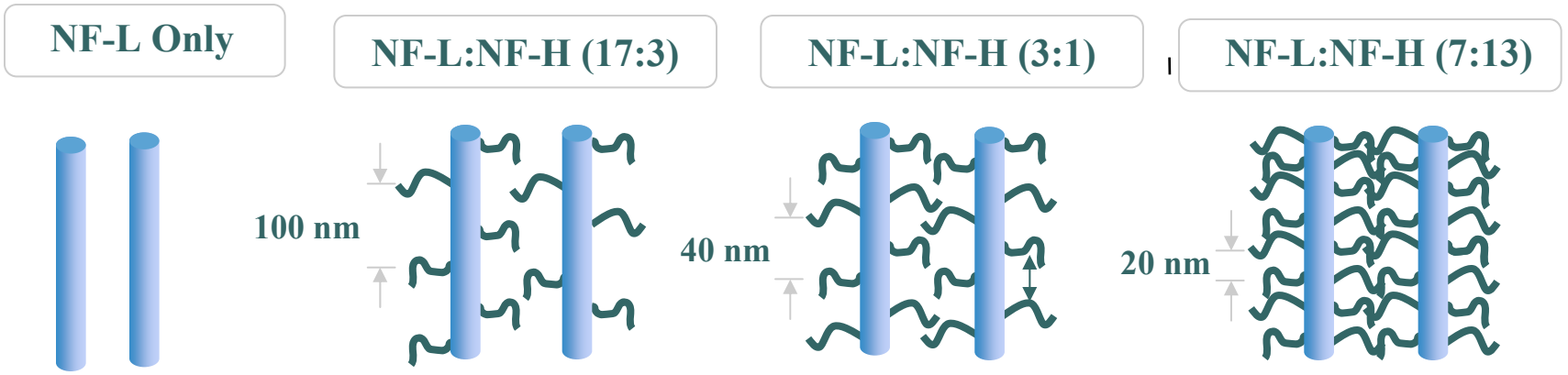
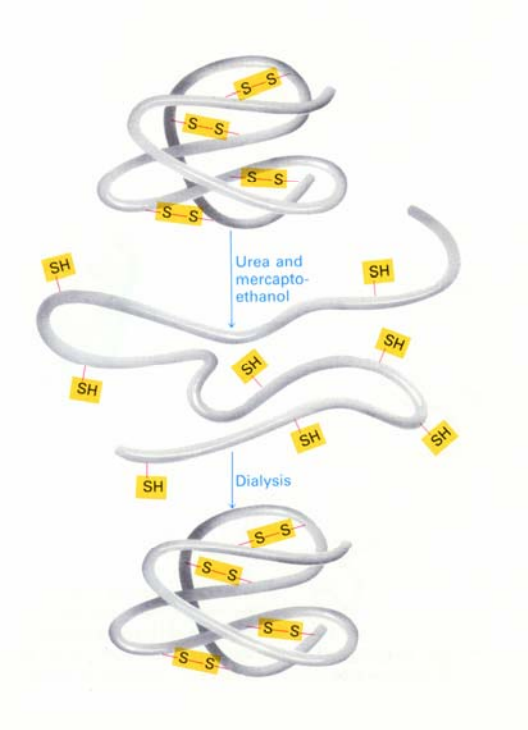
Saturation Point of Assembly for Neurofilaments



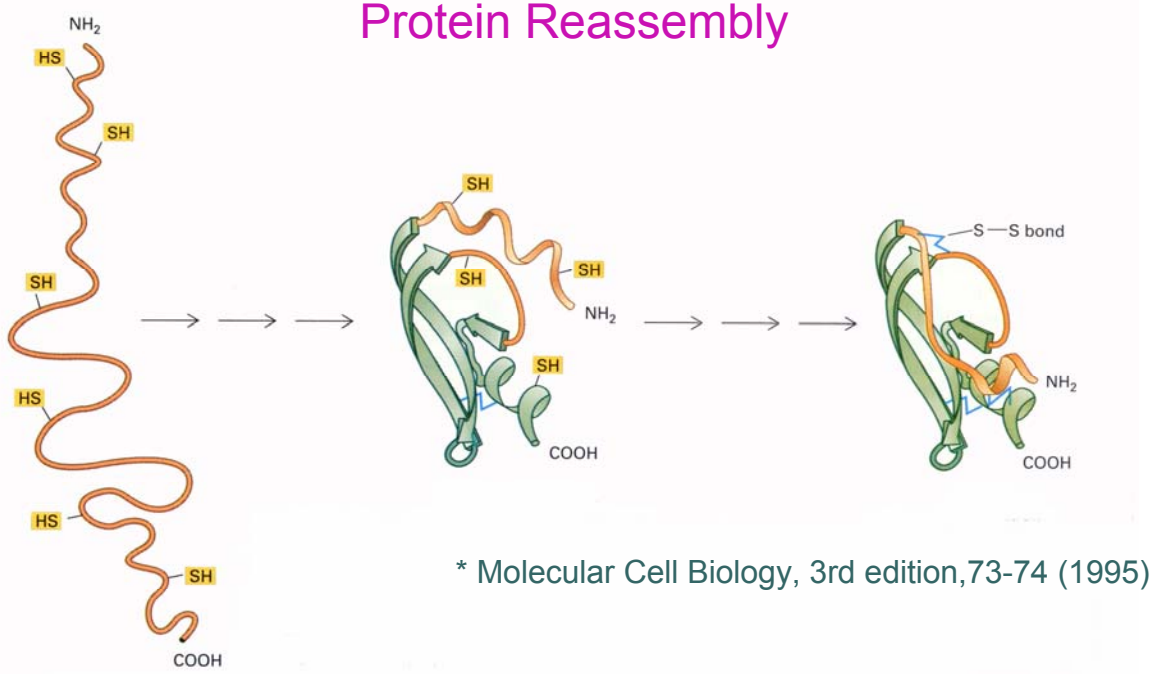
- Saturation point is the limiting factor in how many NF's can become coiled around one another at any given time
- NF-L has no side arms. NF-M and NF-H have interacting side arms.
- Theory: NF-H and M are attracted to each other causing a cross linkage.

- Ion Exchange Column separates sub-units
- Polyacrylamide gel to confirm fractions
- Dialysis with MES buffer to reassemble proteins



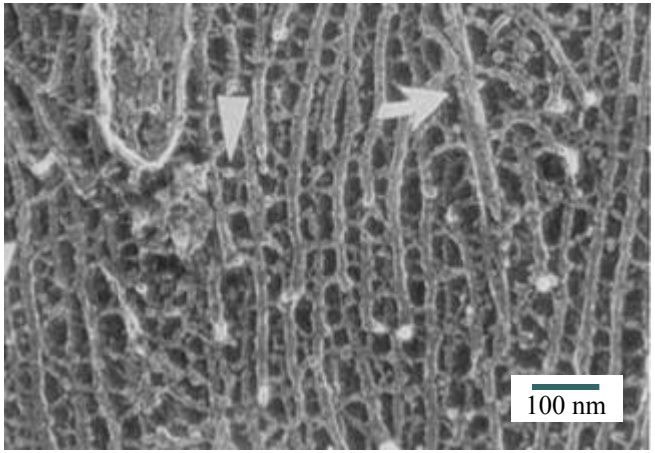
Urea buffer denatures NF and separates into sub-units

Protein Reassembly



* Molecular Cell Biology, 3rd edition,73-74 (1995)

MES Buffer assembles sub-units back together. Sub-units refold to form mature NF.



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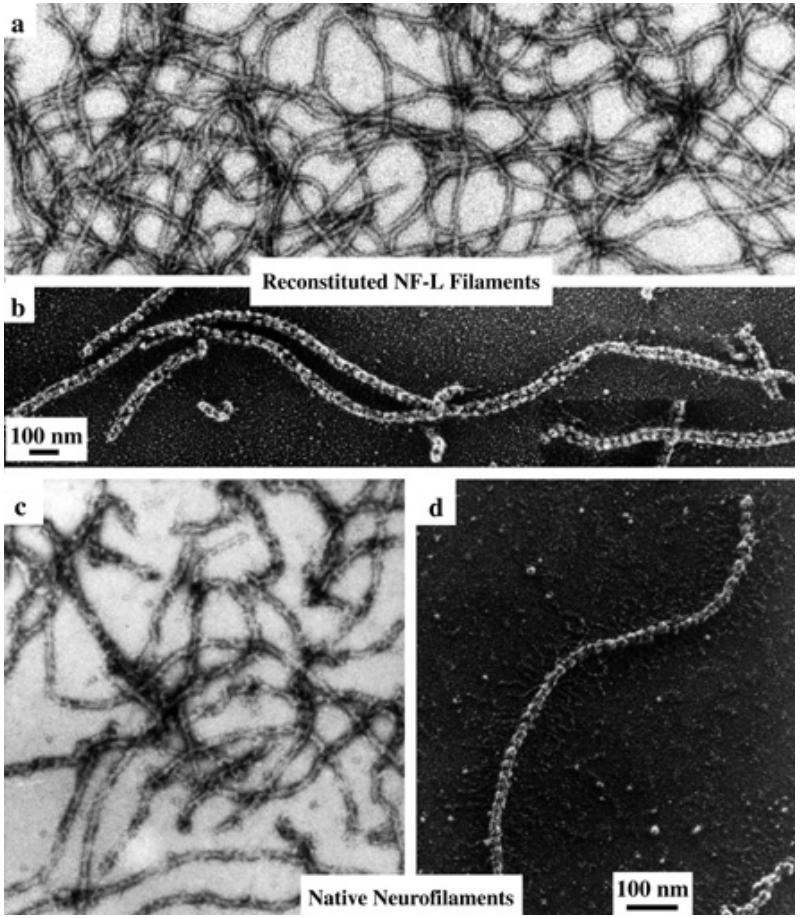
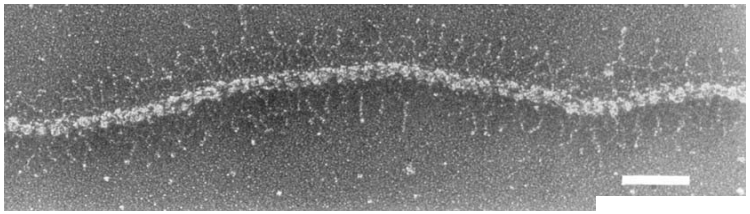
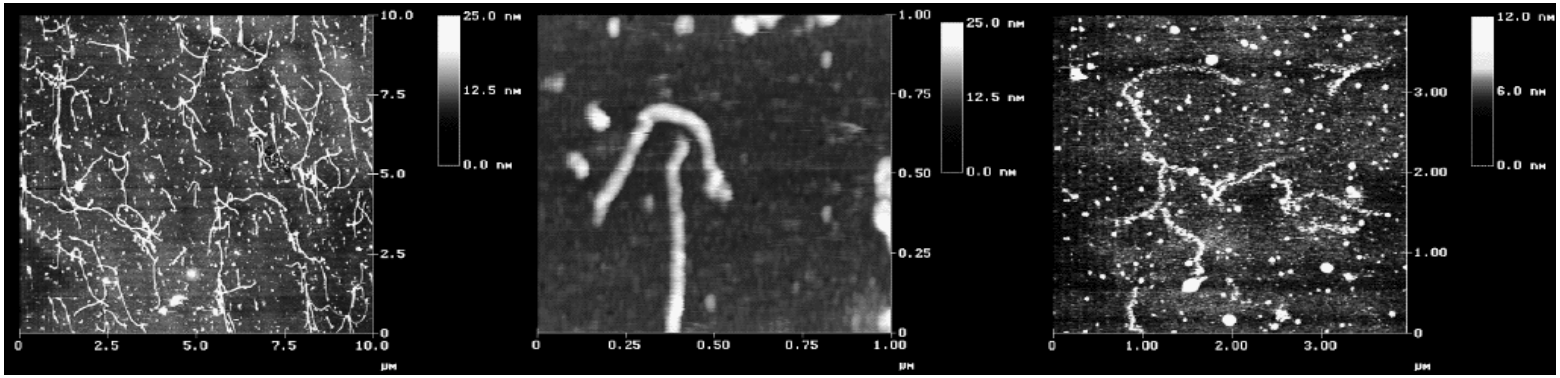


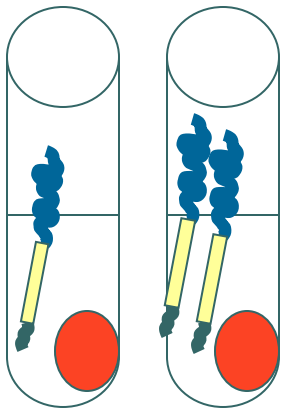


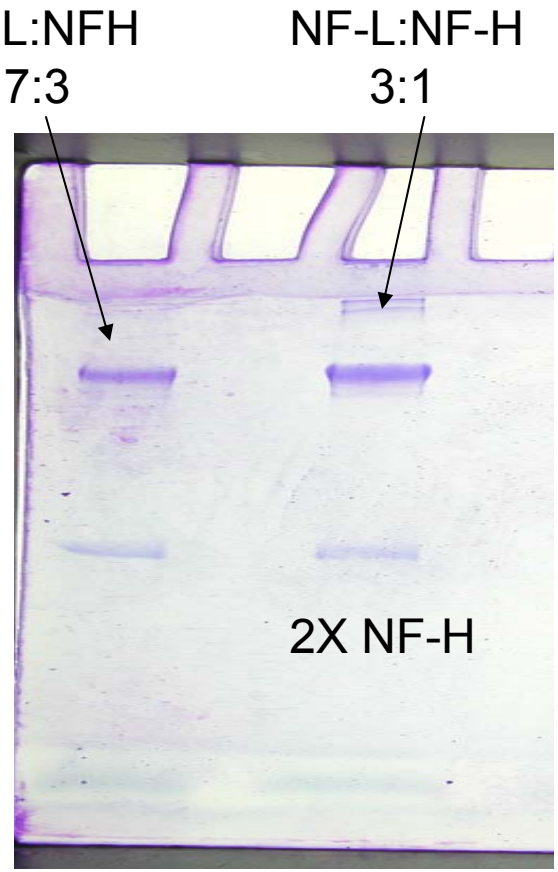
*Courtesy of Biozentrum

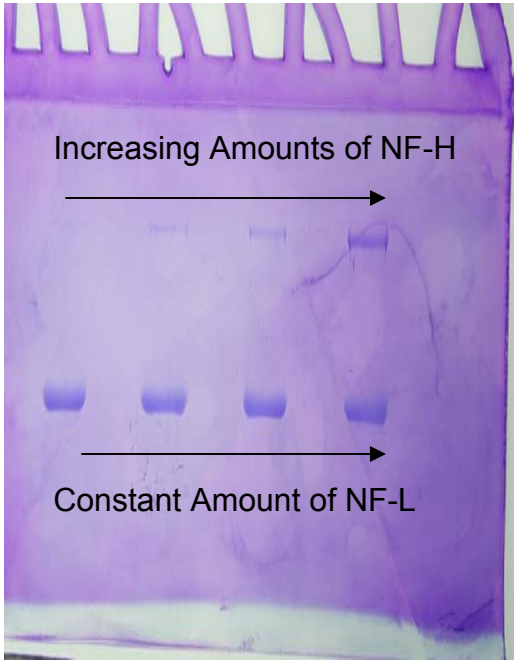


*Courtesy of Simone Karrasch

Saturation Point







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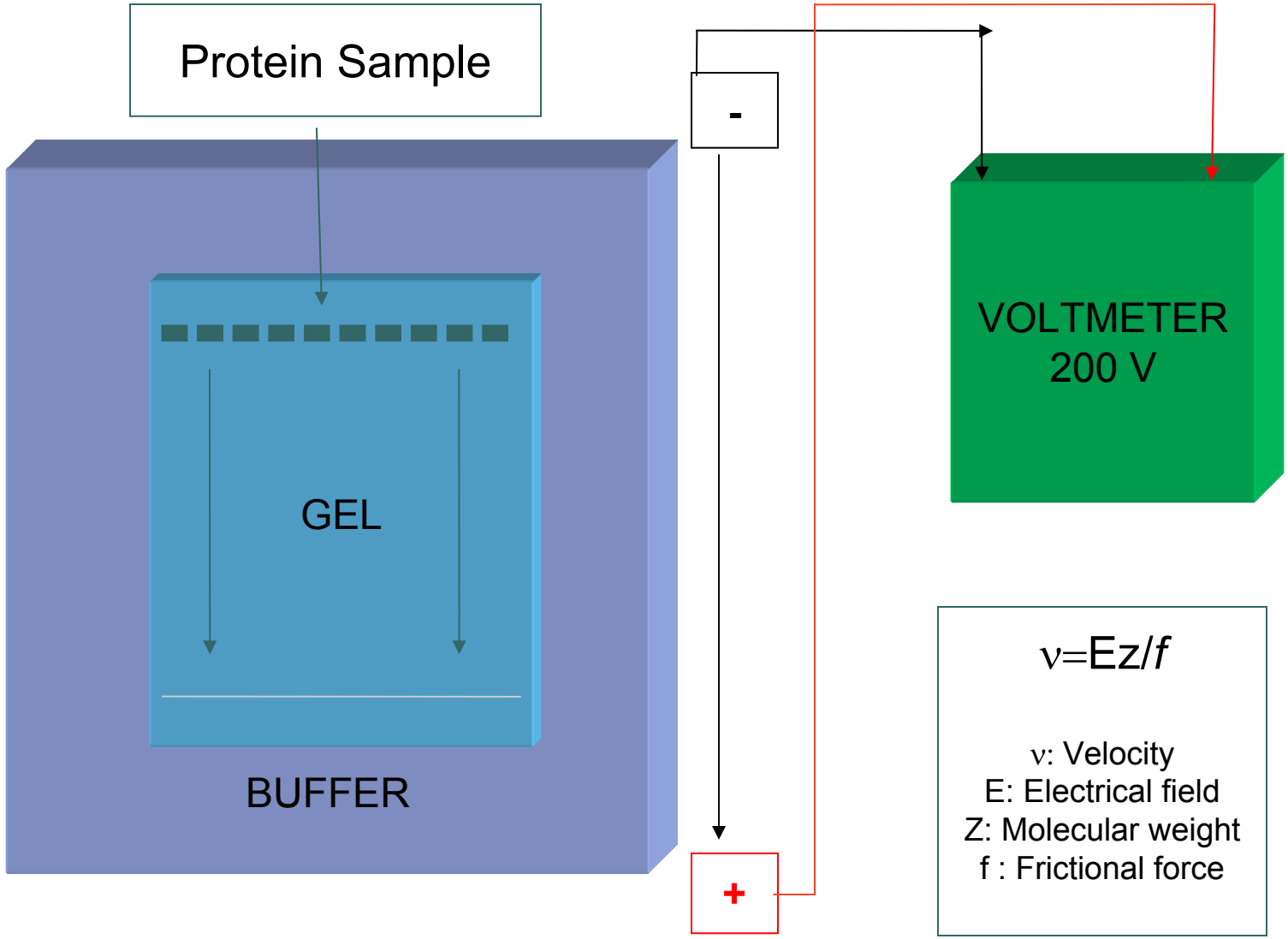
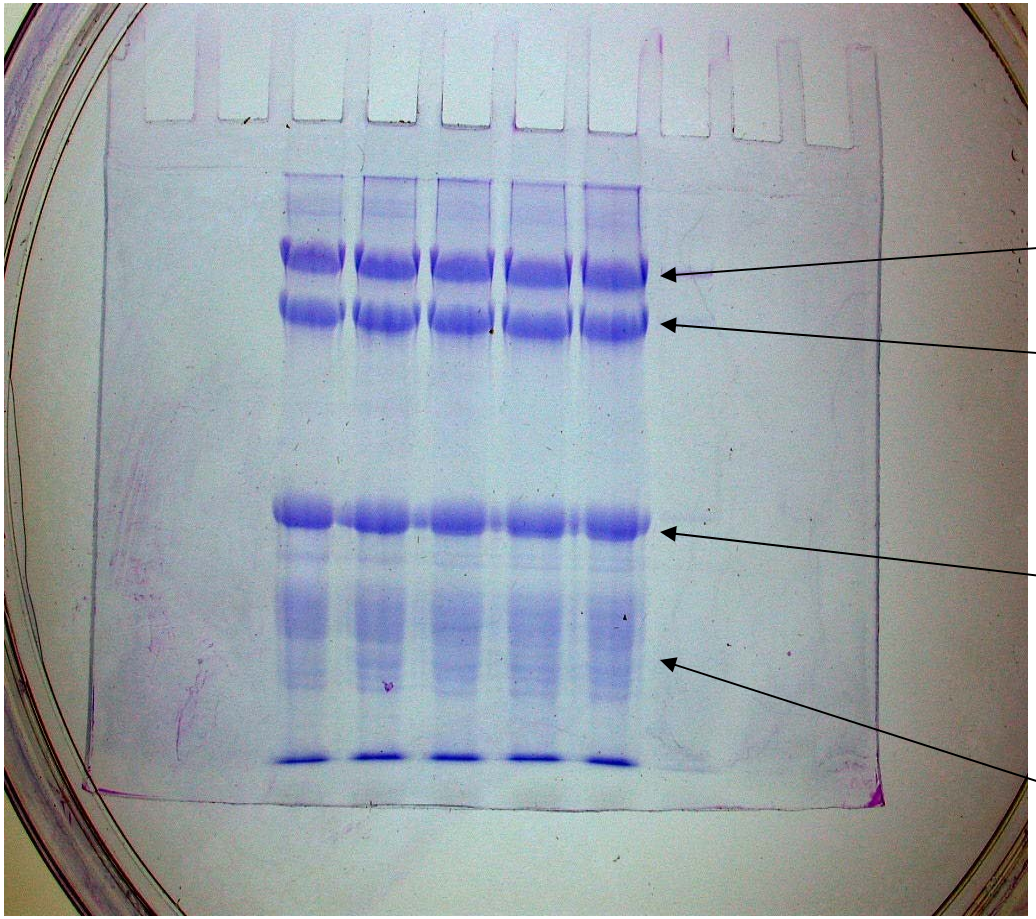
2004


Protein Sample
-
VOLTMETER
200 V
GEL
BUFFER
+
ν=Ez/f
ν: Velocity
E: Electrical field
Z: Molecular weight
f : Frictional force

Results



Jones,Jayna 2003