Bovine Neurofilament Interactions

Research Experience for Teachers (RET) at University of California, Santa Barbara

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Mentor: PhD Candidate Joanna Deek

Undergraduate Assistant: Bernice McLaurin

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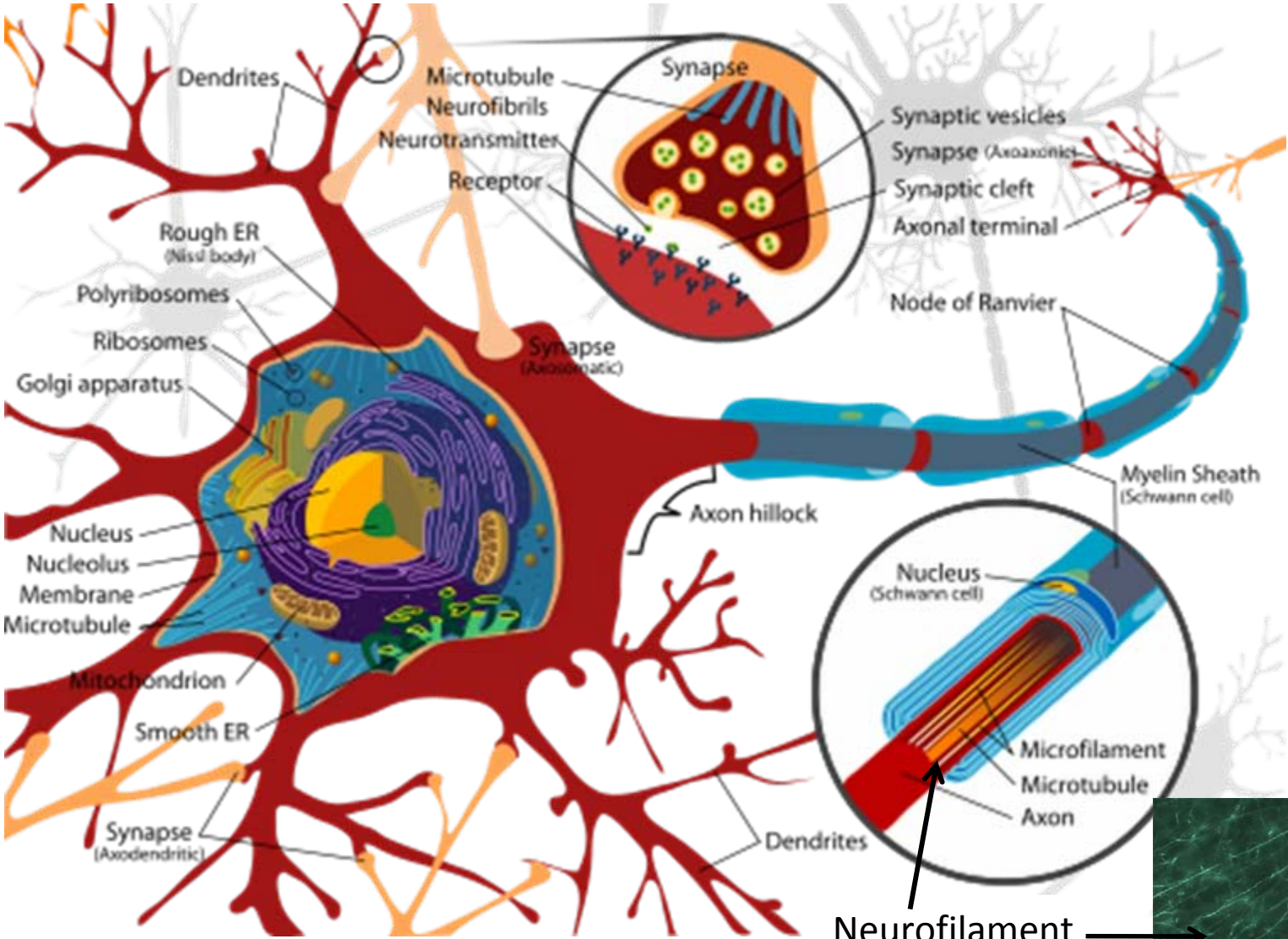
Introduction

- Neurofilaments, which make-up part of the cytoskeleton of neurons found in the spinal cord, may play a role in neurodegenerative diseases like amyotrophic lateral sclerosis (ALS), Parkinson and Alzheimer disease.

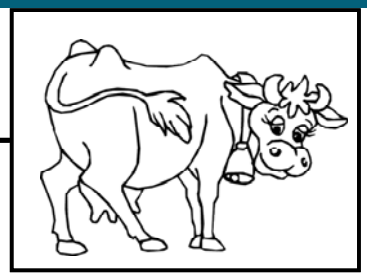
Neuron

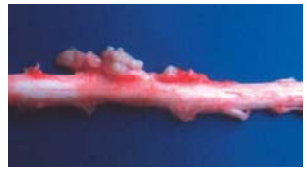

Dendrites
Microtubule
Neurofibrils
Neurotransmitter
Receptor
Synapse
Synaptic vesicles
Synapse (Axoaxonic)
Synaptic cleft
Axonal terminal
Rough ER
(Nissl body)
Polyribosomes
Ribosomes
Golgi apparatus
Synapse
(Axosomatic)
Node of Ranvier
Myelin Sheath
(Schwann cell)
Axon hillock
Nucleus
Nucleolus
Membrane
Microtubule
Mitochondrion
Smooth ER
Synapse
(Axodendritic)
Dendrites
Nucleus
(Schwann cell)
Microfilament
Microtubule
Axon
Neurofilament

Project Goals:

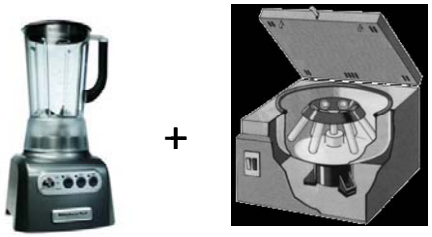
- Overall Goal: To understand the interactions between neurofilaments.
- Project Goal: To understand the specific effects of salts on neurofilament interactions.

Experimental Methods

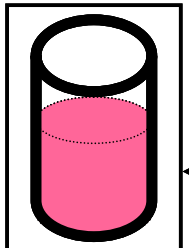




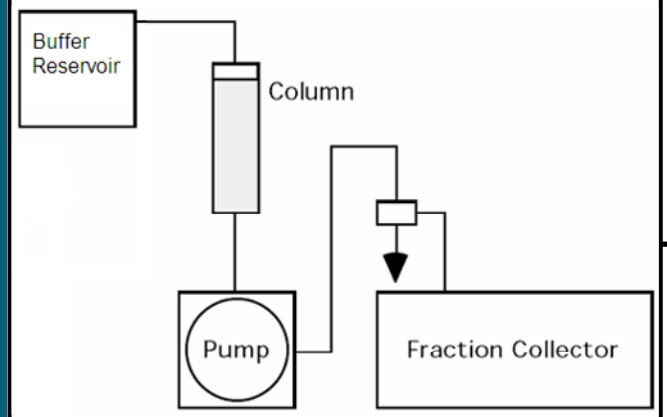
Bovine Spinal Cord



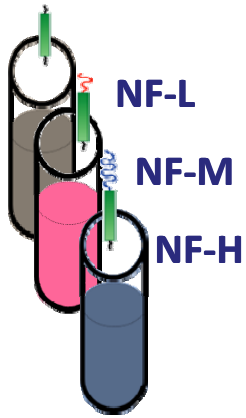
Homogenization and Centrifugation

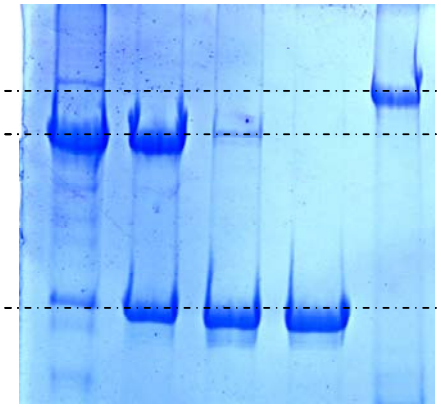


Crude Protein



Anion Exchange Chromatography

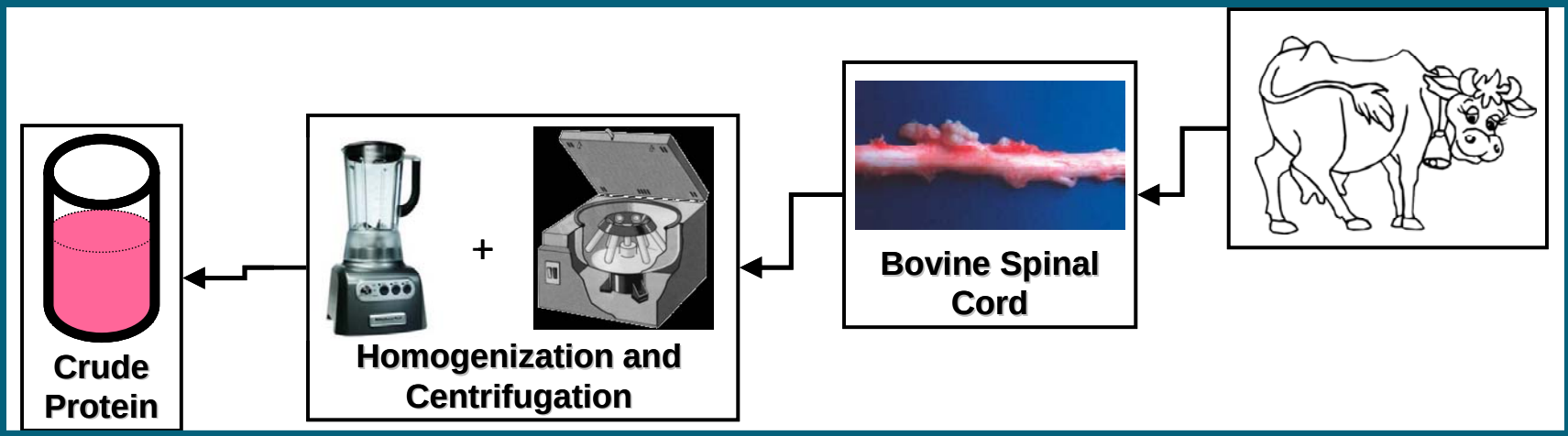




Separated subunits: NF-L, NF-M, and NF-H

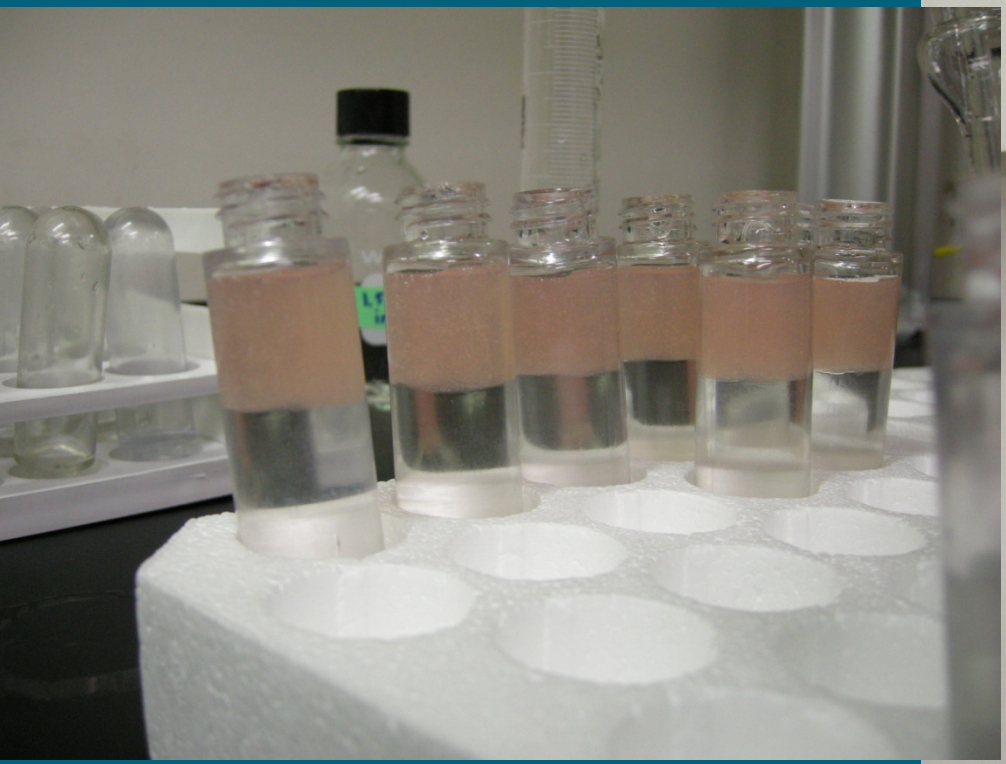
Purification Yield ~ 12 mg of total NF protein

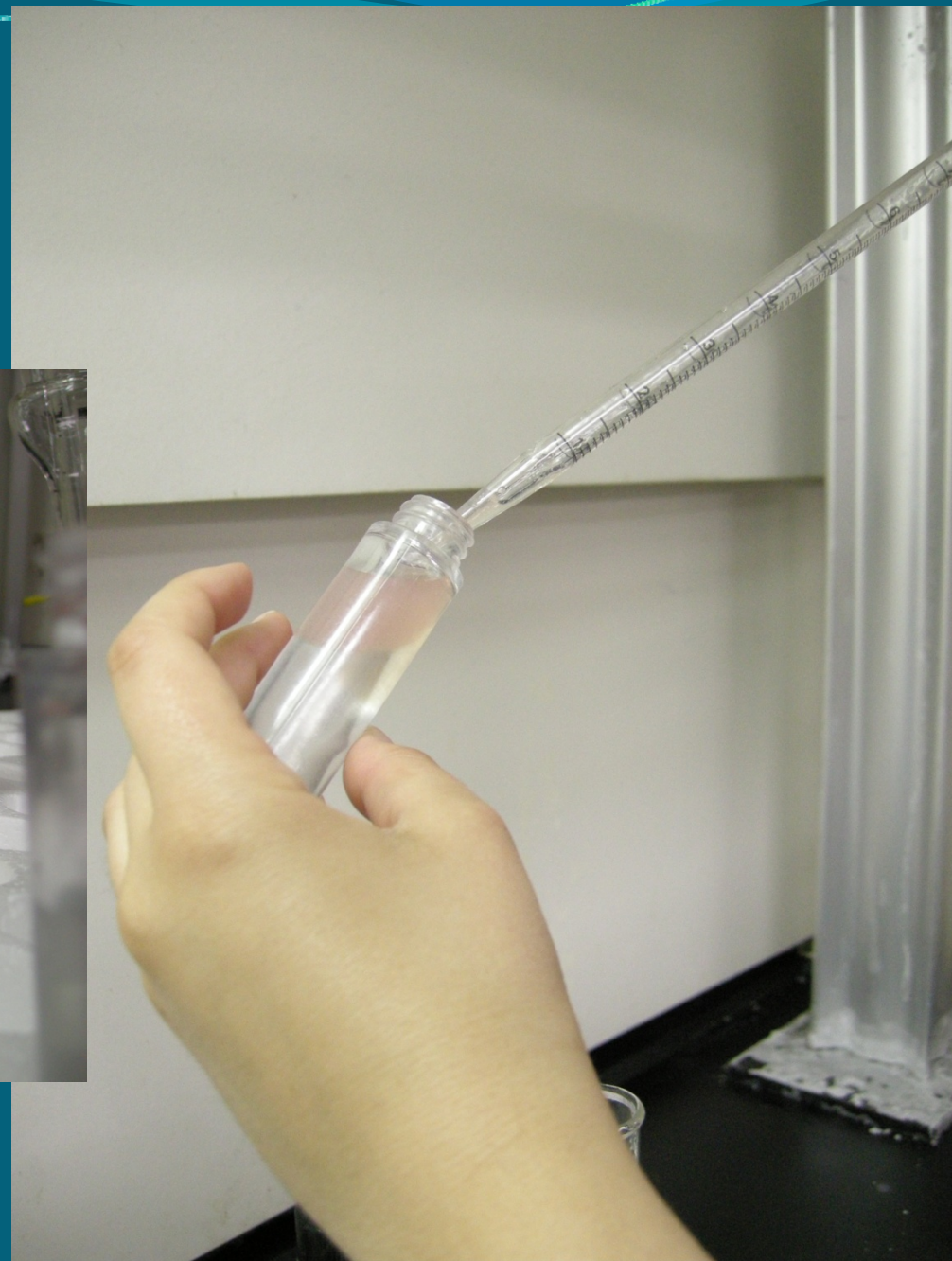
Experimental Methods



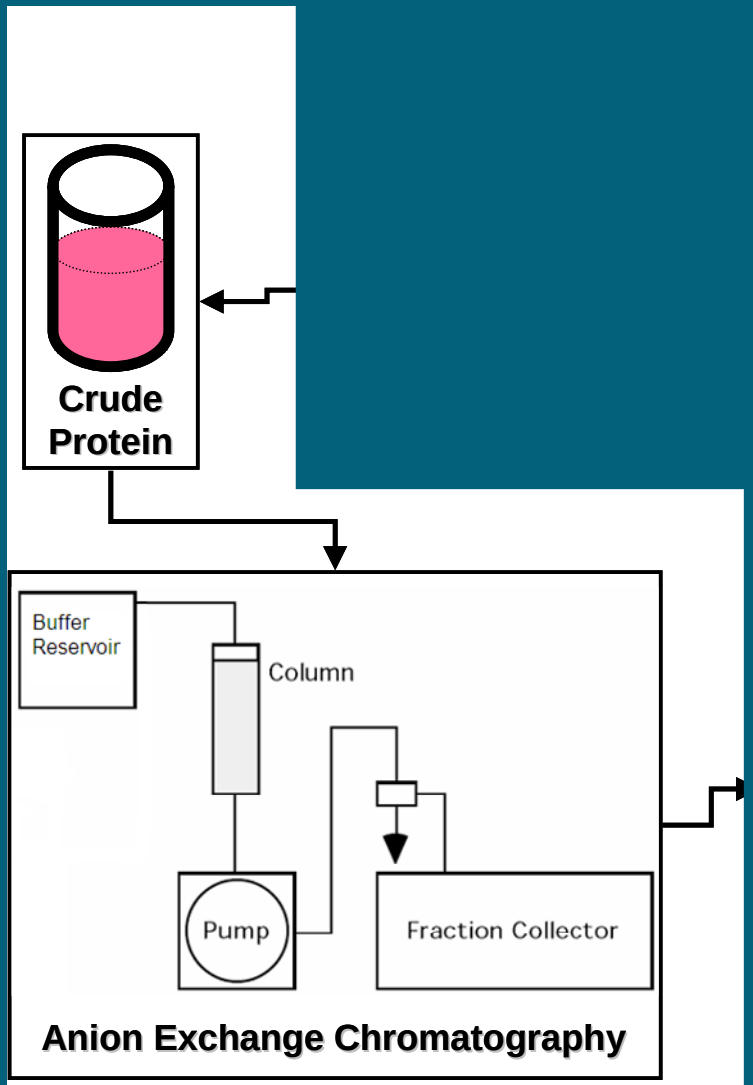
Bovine Spinal Cord Extraction

Neurofilament Purification





Experimental Methods



Purification Yield ~ 12 mg of total NF protein

Anion exchange chromatography using various buffers.

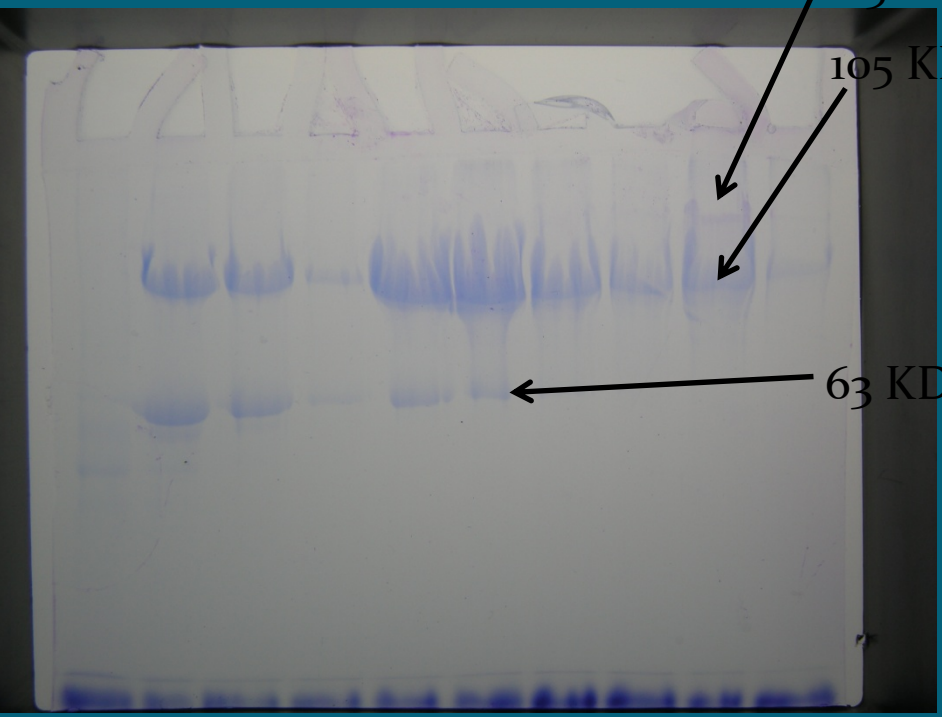
- Collect different subunit neurofilaments in a fraction collector.

Collecting the Three Types of Subunit Neurofilaments

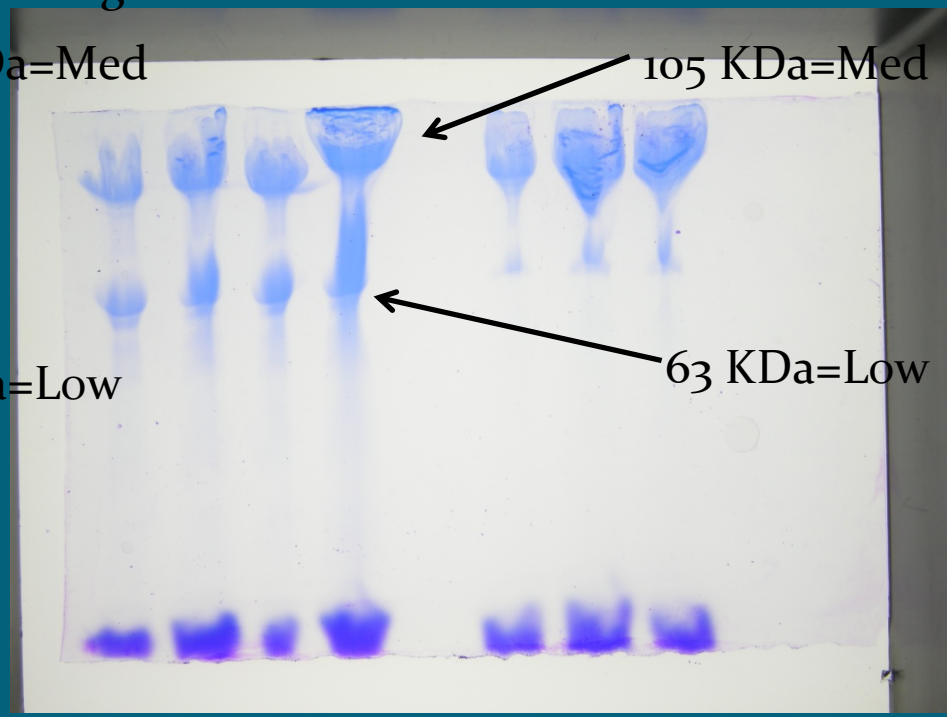
1. Low
2. Medium
3. High

- Confirmation of neurofilaments: Bradford test and acrylamide gel electrophoresis.

Acrylamide Gel Electrophoresis Neurofilament Protein Data



High-Med-Low

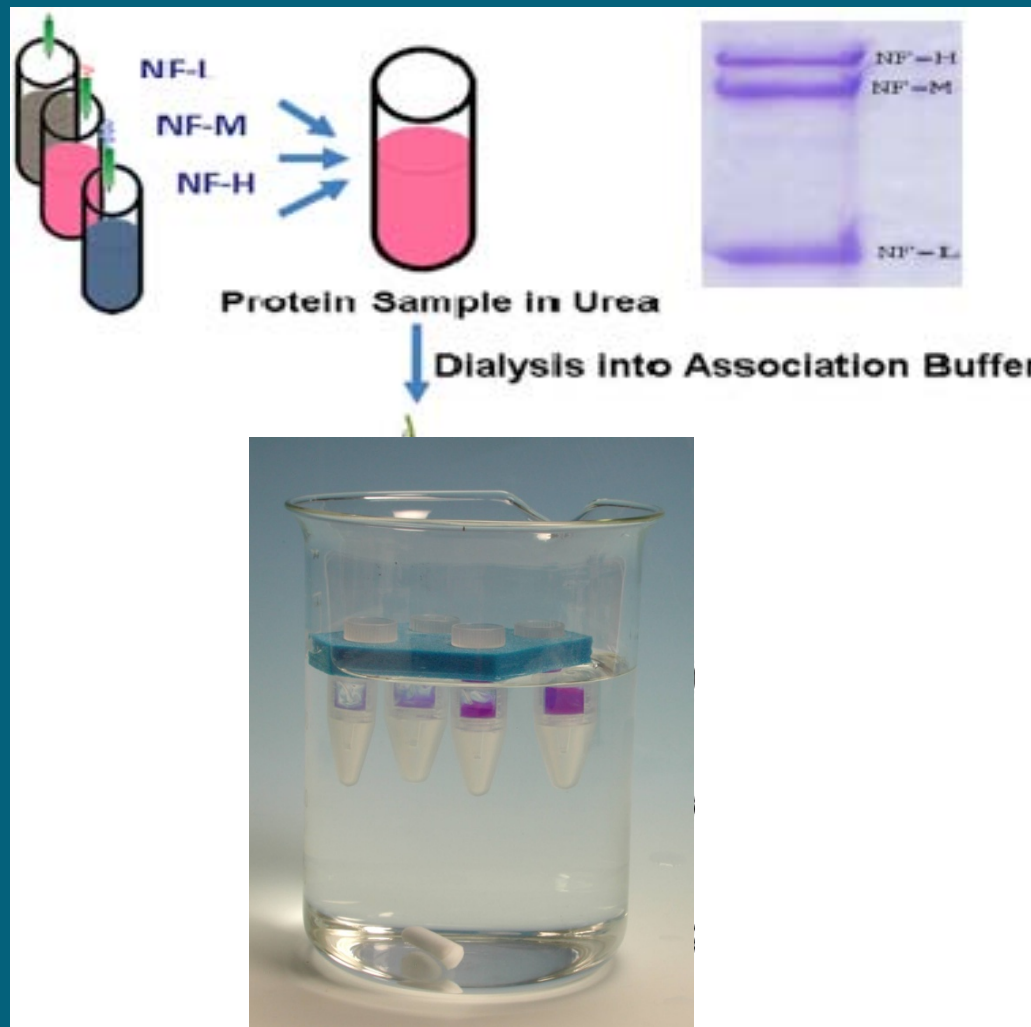


Med-Low

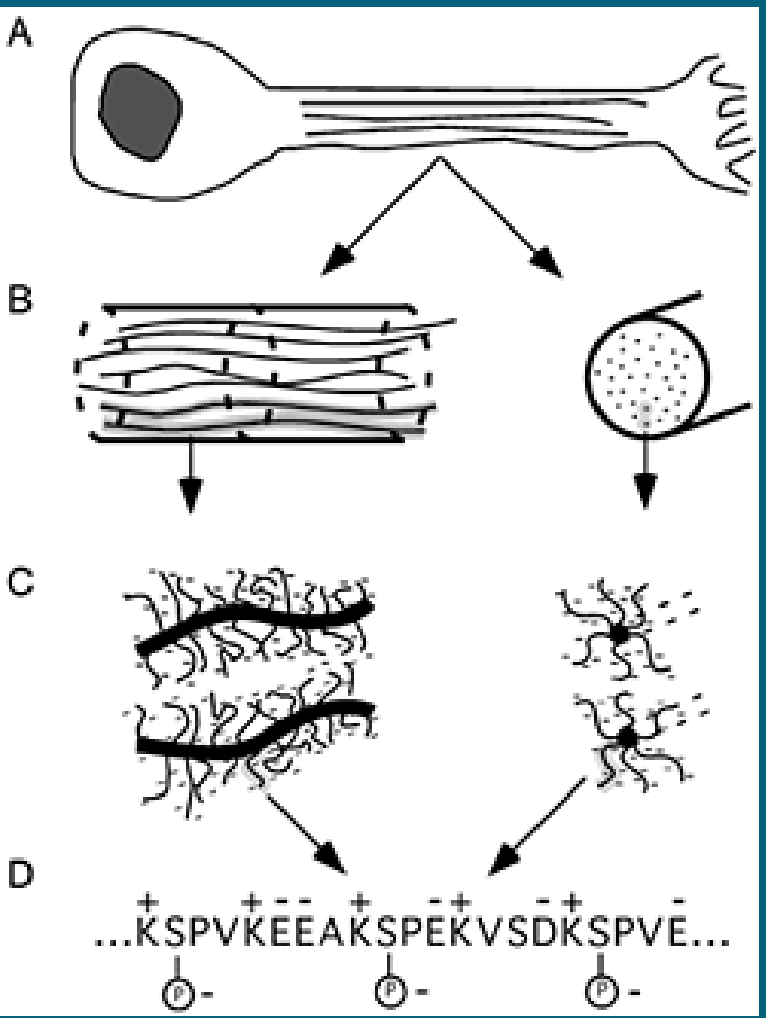
KDa = 1g/mol

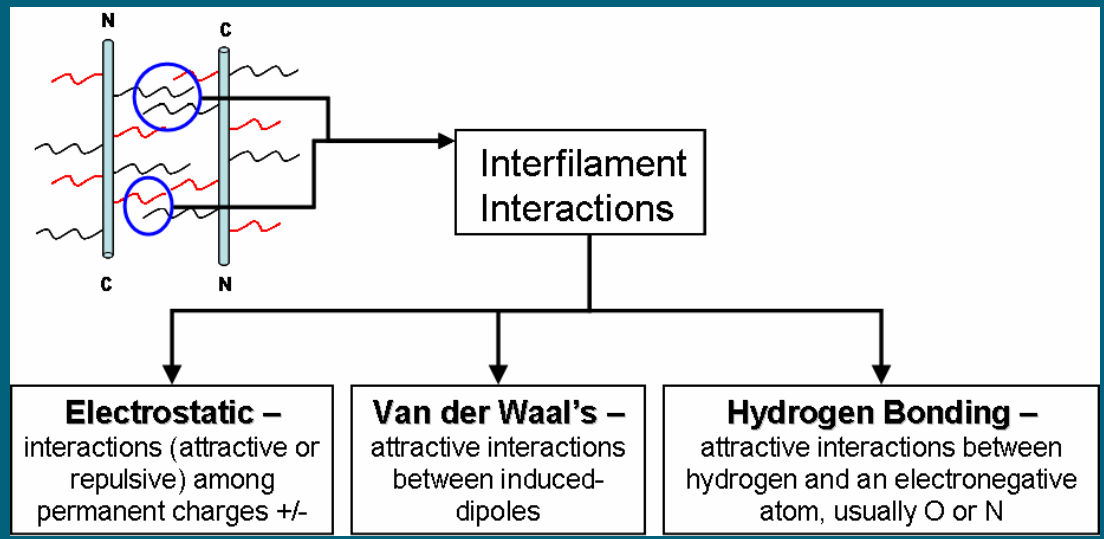
Experimental Methods Continued

- Reassemble neurofilaments into desired subunit ratios using dialysis.



Understanding neurofilament interactions





- If neurofilaments have electrostatic interactions, then salts will effect the neurofilament interactions.

Neurofilaments in different subunit ratios are treated with different concentrations of salts.

- Potassium chloride
- Magnesium chloride
- Spermidine hydrochloride
- Spermine hydrochloride

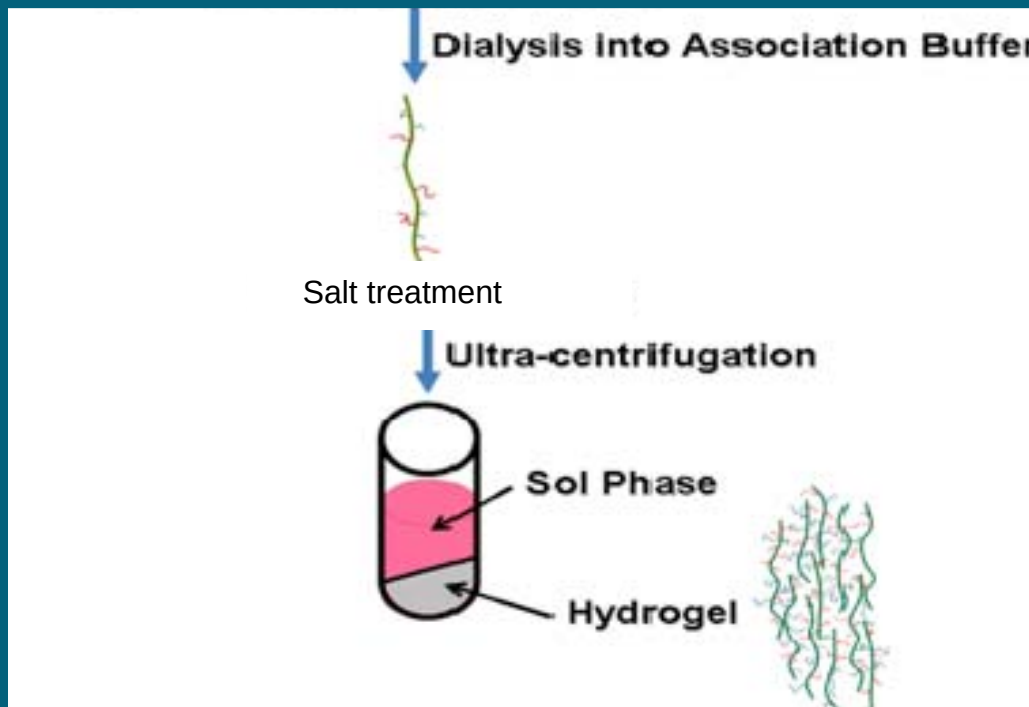
Treated neurofilaments are made into hydrogels and are put into capillaries for analysis.

Sol Phase:

- Tested for neurofilament using a Bradford protein assay

Hydrogel:

- Analysis of Neurofilaments



Bradford Protein Assay

- We use a spectrophotometer to determine the light absorbance of our samples, which will determine our protein concentration in our experiment.

Experimental Samples

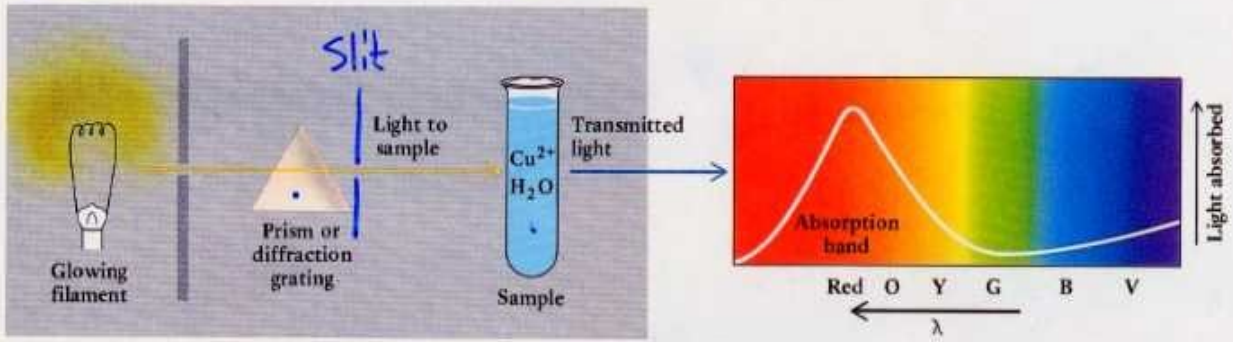
No Protein

Lots of Protein

UV-Visible Spectrophotometer

Figure 25.30 Spectrophotometer and spectrum of Cu^{2+}

Light Source Wavelength Selector Sample Cell



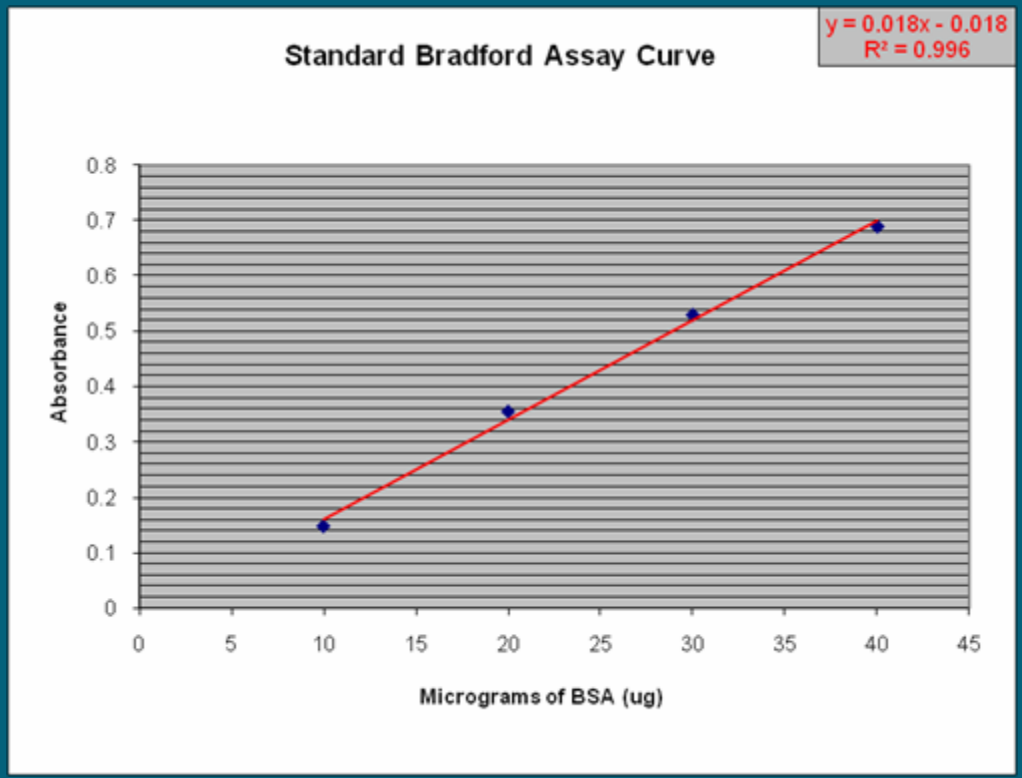
SPECTROPHOTOMETER

700 600 500 400 nm

Wavelength

Bradford Data

[[y (absorb.) + 0.0185]/0.018]/5ml = x (protein conc.)



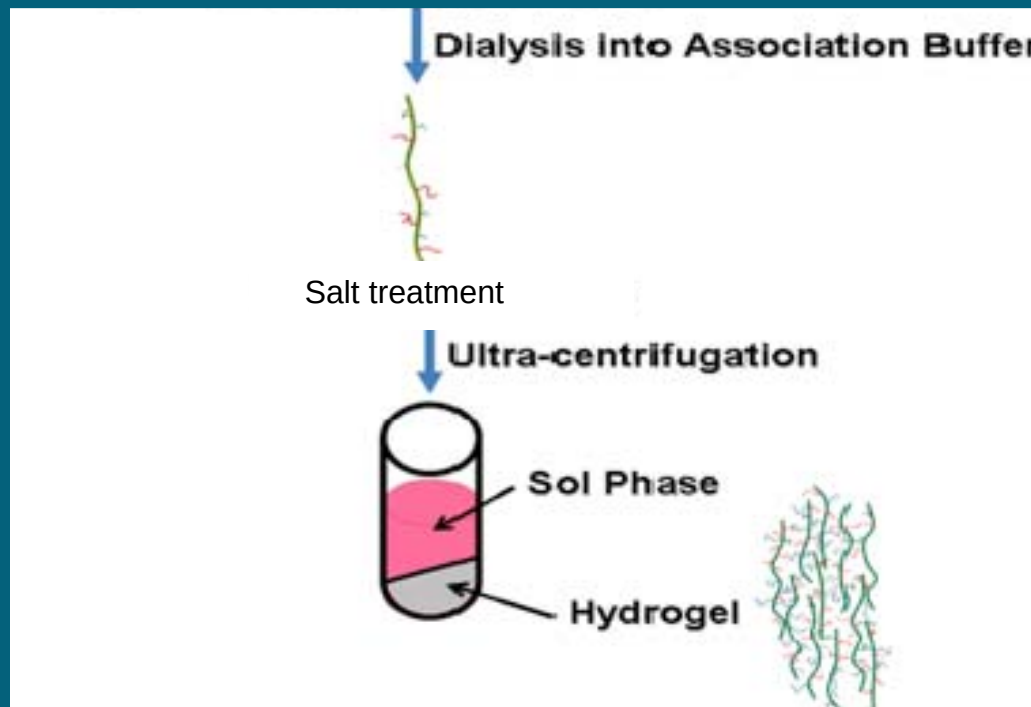
Bovine serum albumin (BSA) = Known protein standard

Sample Number	Absorbance 1 (Recorded Data)	Absorbance 1 (Data with no negative Data)	Volume of Sample Used (uL)	Final Sample Concentration (mg/mL)
1	0.016	0.016	5	0.38
2	0.028	0.028	5	0.52
3	0.019	0.019	5	0.42
4	0.005	0.005	5	0.26
5	0.006	0.006	5	0.27
6	0.009	0.009	5	0.31
7	0.002	0.002	5	0.23
8	0.008	0.008	5	0.29
9	0.002	0.002	5	0.23
10	0.005	0.005	5	0.26
11	0.002	0.002	5	0.23
12	-0.004	0	5	0.21
13	0.001	0.001	5	0.22
14	0.004	0.004	5	0.25
15	0.017	0.017	5	0.39
16	0.001	0.001	5	0.22
17	0	0	5	0.21
18	-0.002	0	5	0.21
19	0	0	5	0.21
20	-0.003	0	5	0.21
21	0.001	0.001	5	0.22
22	0.003	0.003	5	0.24
23	0.003	0.003	5	0.24
24	0.003	0.003	5	0.24
25	0	0	5	0.21
26	0.001	0.001	5	0.22
27	0.034	0.034	5	0.58
28	0.001	0.001	5	0.22
29	0.001	0.001	5	0.22
30	-0.27	0	5	0.21

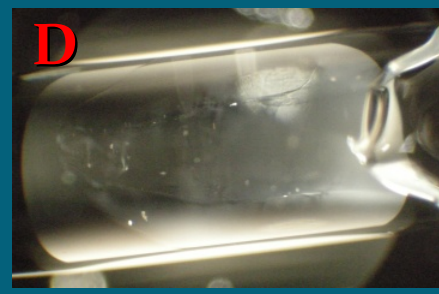
Treated neurofilaments are made into hydrogels and are put into capillaries for analysis.

Hydrogel Analysis:

- Light Microscopy
- Transmission Electron Microscopy (TEM)
- Small angle X-ray Scattering

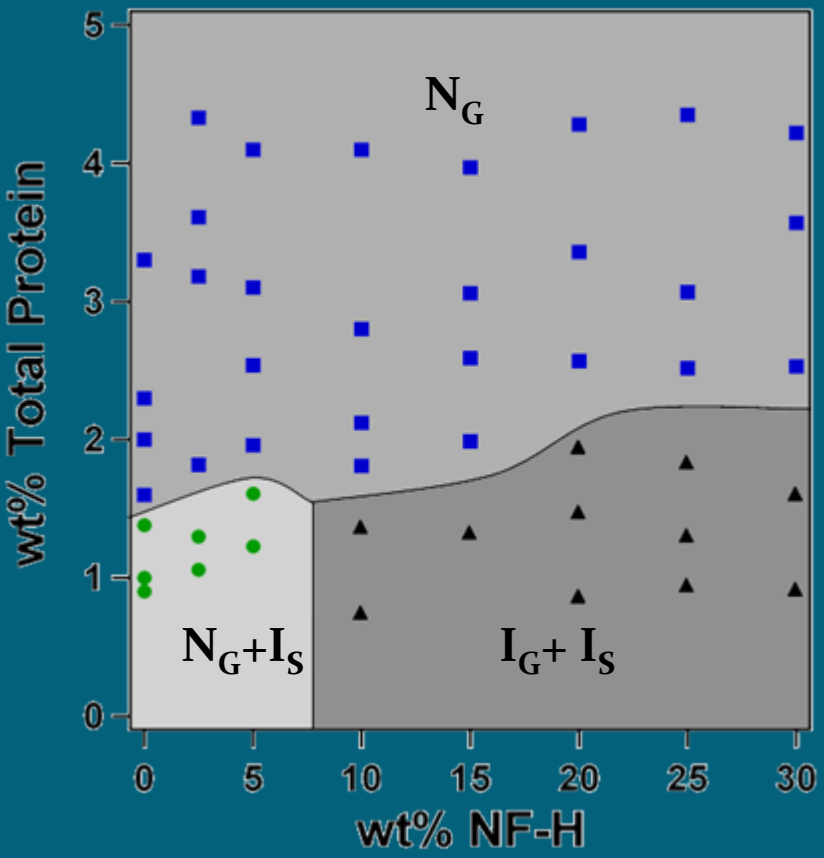


Light Microscopy Data



- Images A and C have birefringent properties because they have rotated light and are nematic gels. Nematic gels contain alligned neurofilaments.
- Images B and D have not rotated polarized light and are isotropic gels. Isotropic gels have neurofilaments that are not ordered.

Light Microscopy Phase Diagram Data

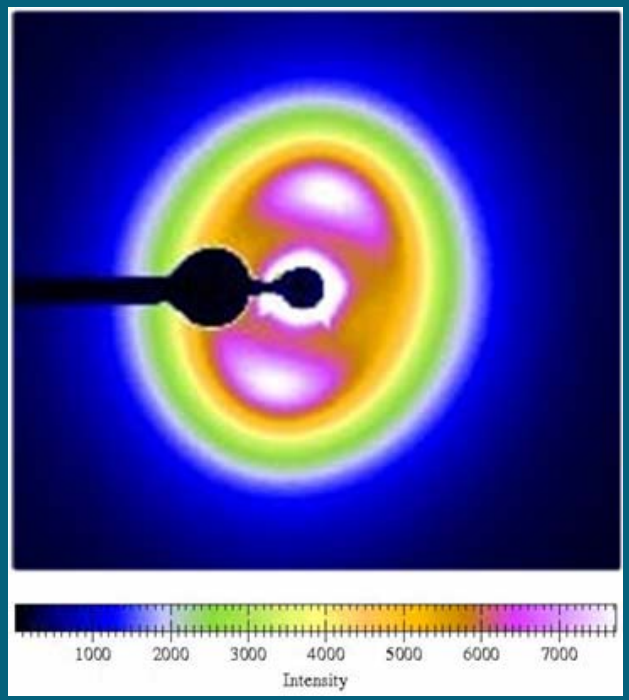


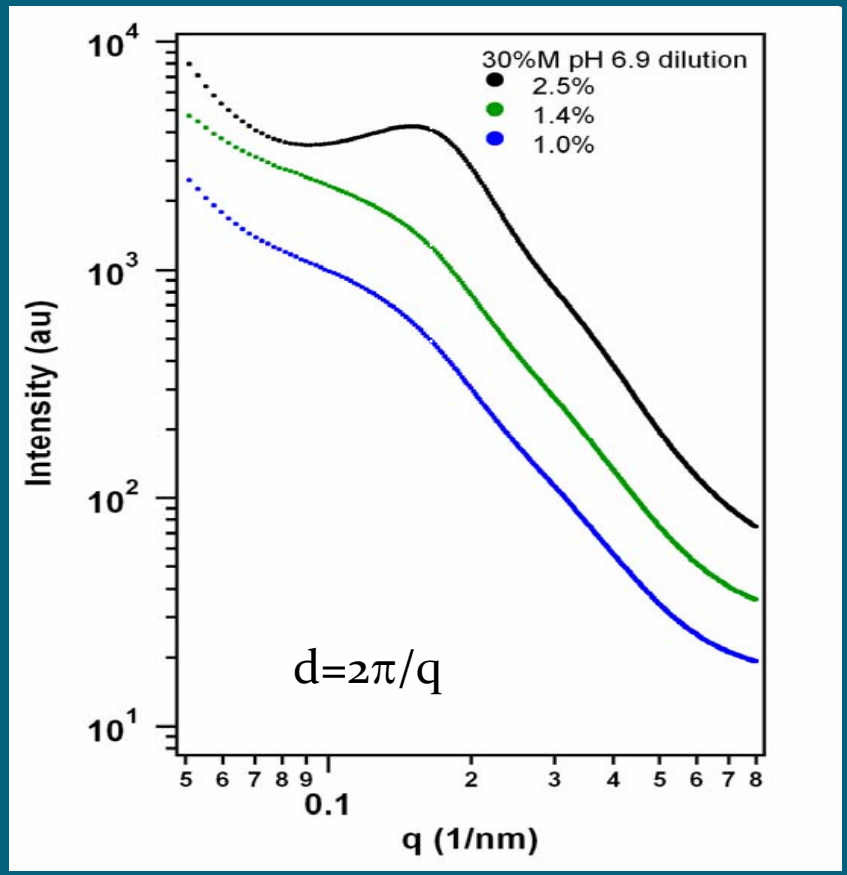
N_G = Nematic gel

I_S = Isotropic sol/gel

Phase diagram is constructed to understand phase behavior.

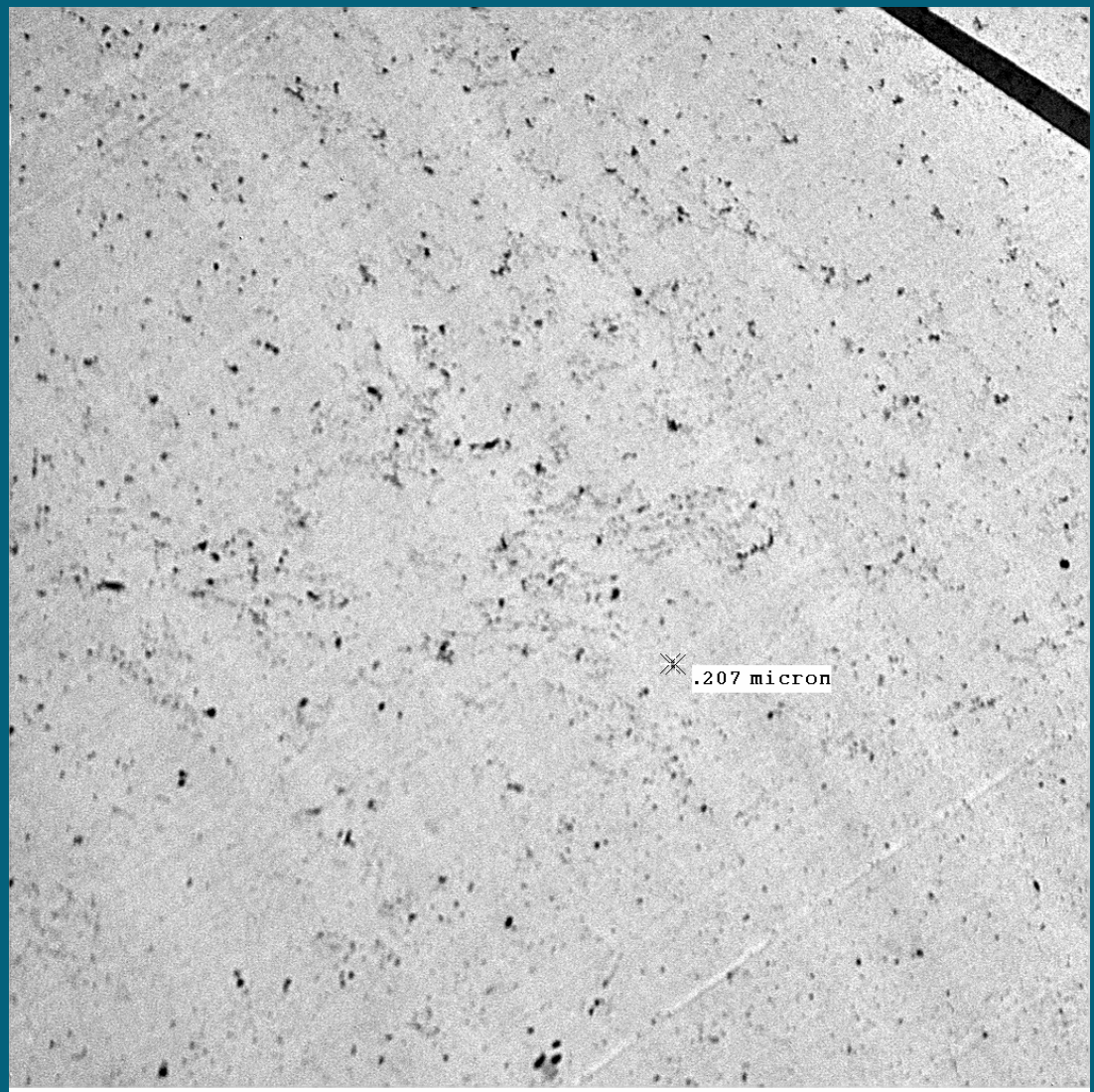
Small Angle X-ray Scattering



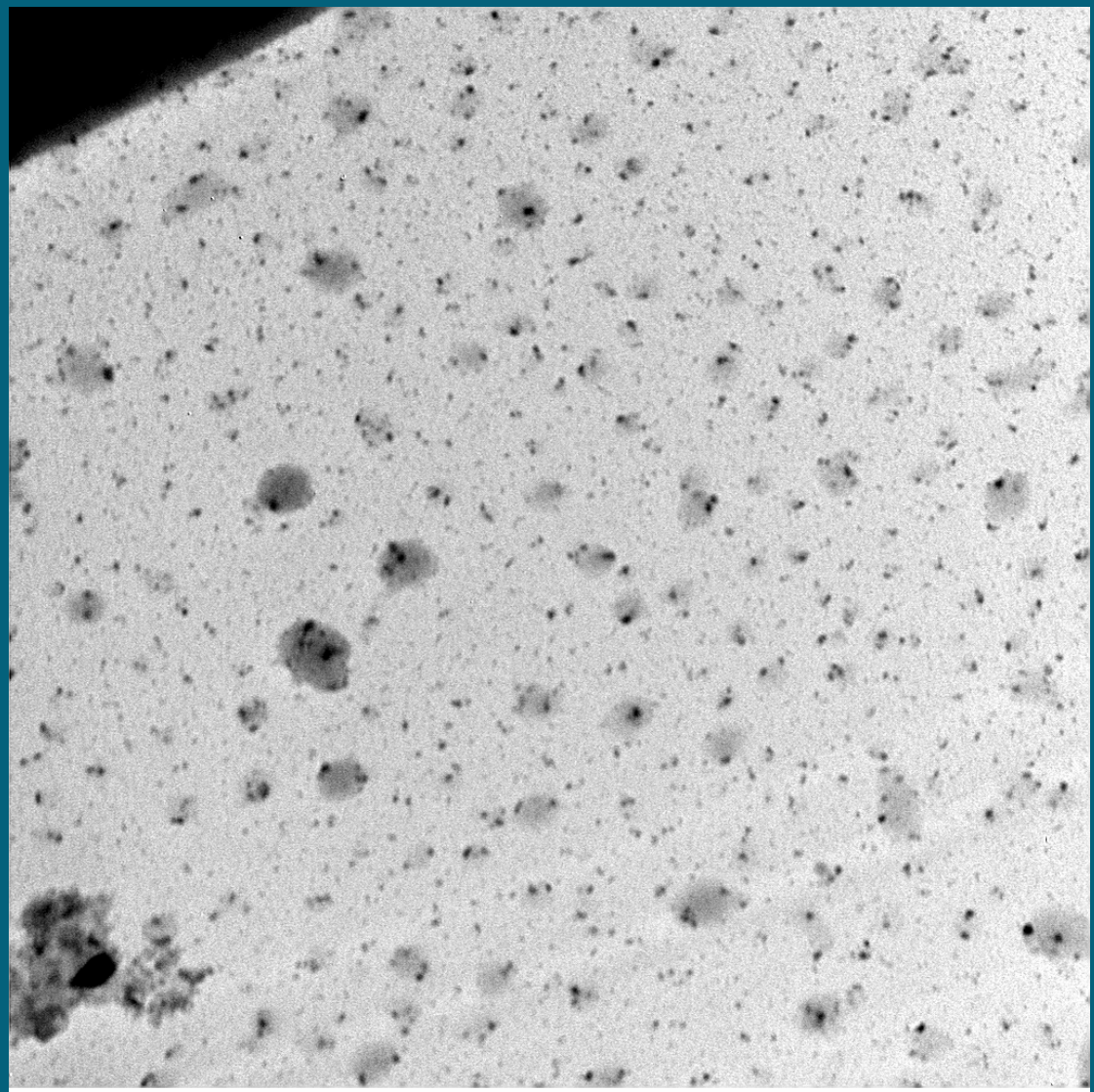


d-value is the average distance between neurofilaments for a salt treated sample.

TEM Data


.207 micron
I12.tif
I12
Print Mag: 4570x @ 7.5 in
16:03 07/08/09
10 microns
HV=80kV
Direct Mag: 3000x

TEM Data


I21.tif
I21
Print Mag: 30100x @ 7.5 in
11:17 07/09/09
500 nm
HV=80kV
Direct Mag: 20000x

General Conclusions

- High neurofilaments have more repulsive forces because they are less ordered based on light microscopy and small angle X-ray scattering data.
- Medium neurofilaments have more attractive forces.

Personal Conclusions & Thanks

- Science and research is fun!

Thank You:

- Mentor PhD Candidate: Joanna Deek
- Undergraduate Assistant: Bernice McLaurin
- Faculty Supervisor & Group: Professor Cyrus Safinya & Safinya Group
- Dr. Martina Michenfelder & RET Staff