

NEUROFILAMENT STRUCTURE AND UNFOLDING FORCES
OF SIDEARMS

Oliver Wagner, H. Aranda-Espinoza, P. Carl,
D. Discher, P. Janmey

Institute for Medicine and Engineering,
University of Pennsylvania

Jean-Francois Leterrier

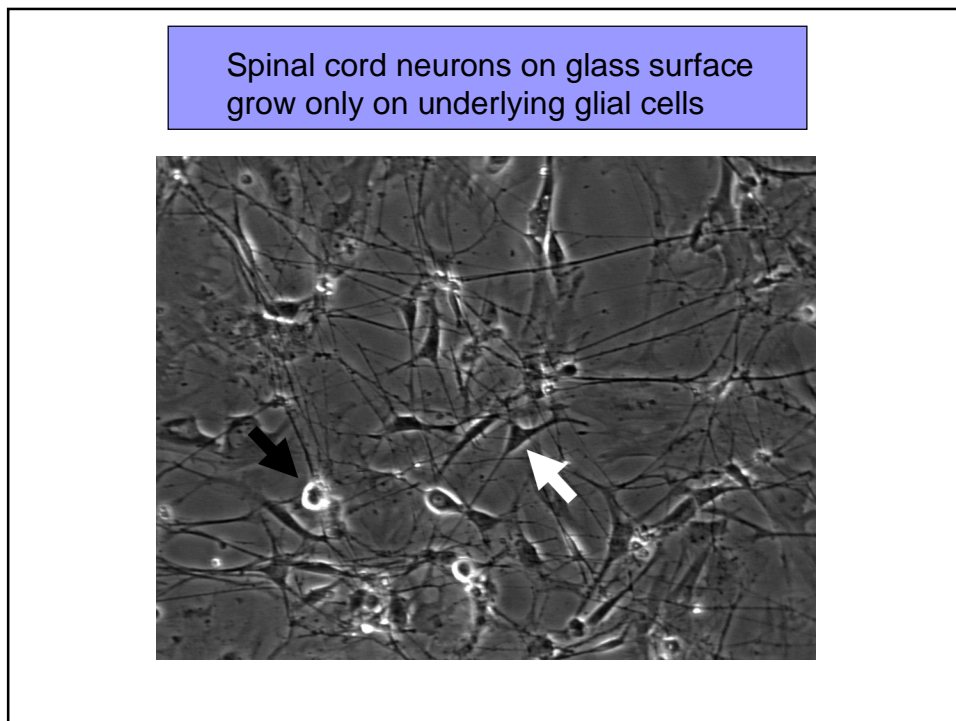
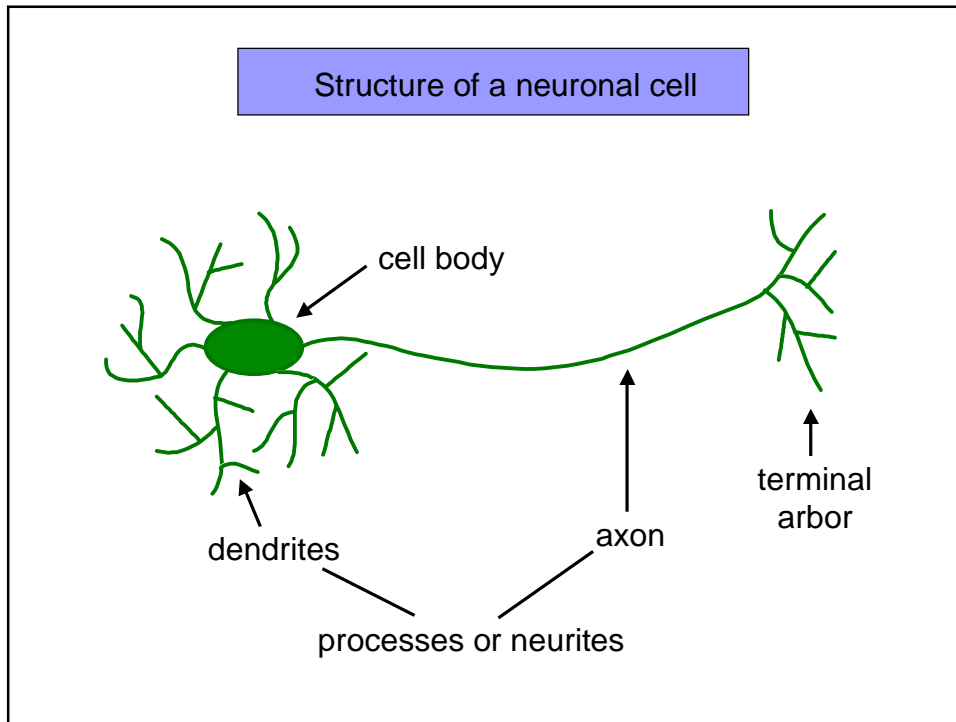
Universite de Poitiers, France.

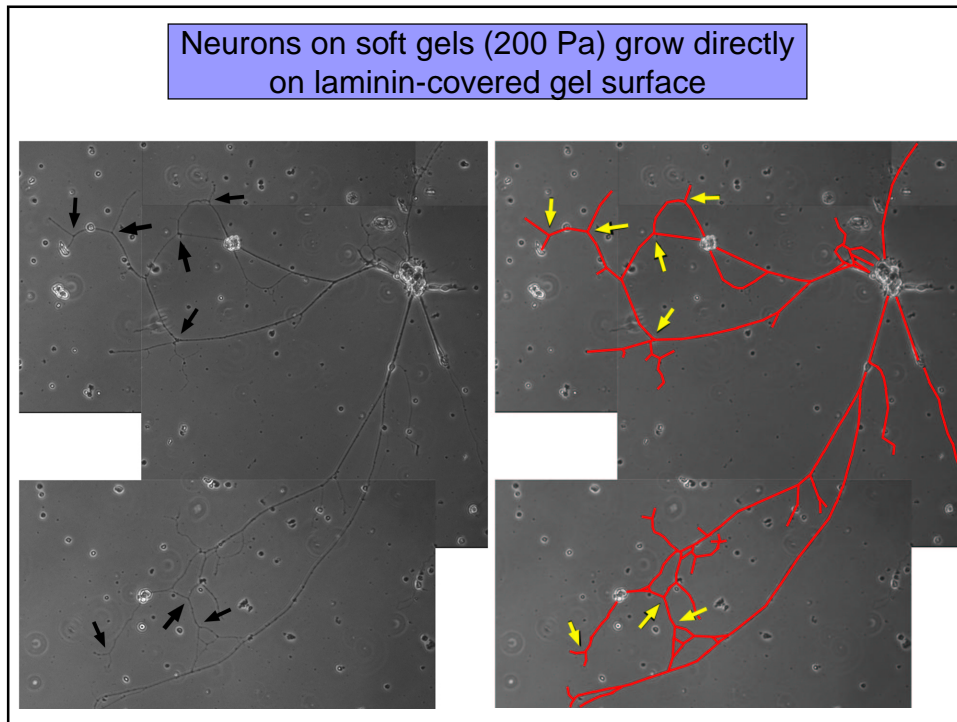
Intro - Biological context

Flexibility and NF network elasticity

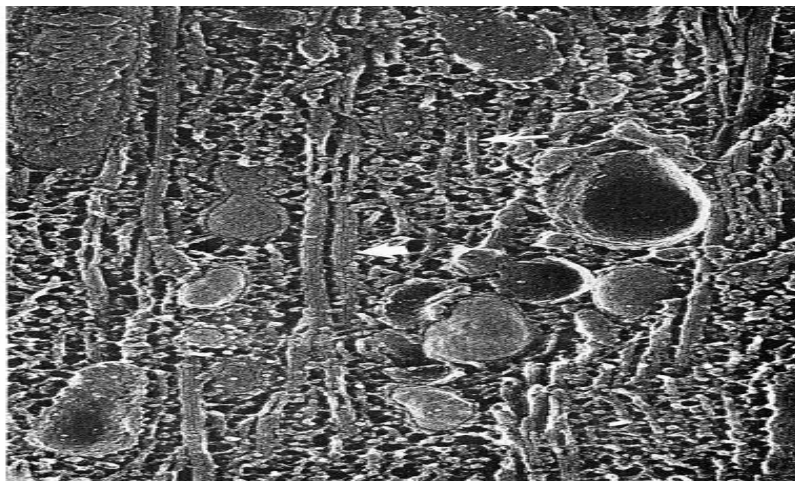
Charge, condensation, and charge reversal

Structure and unfolding of NF sidearms



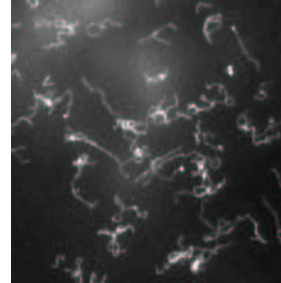
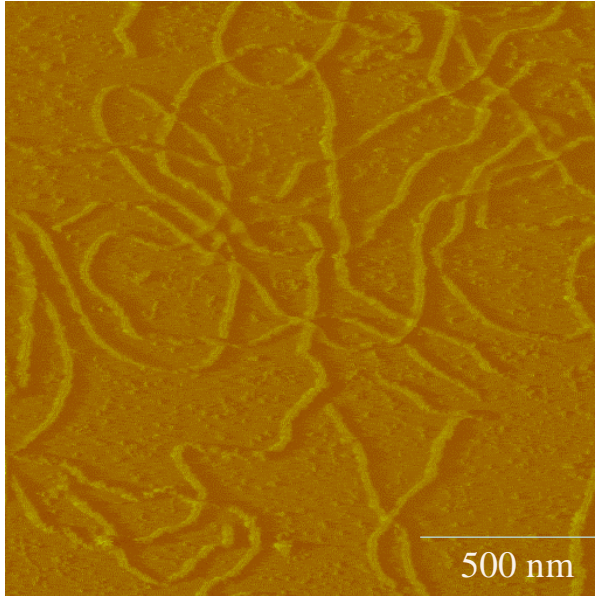


Electron micrograph of NFs and MTs in axon
Hirokawa et al

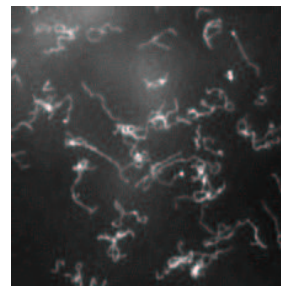
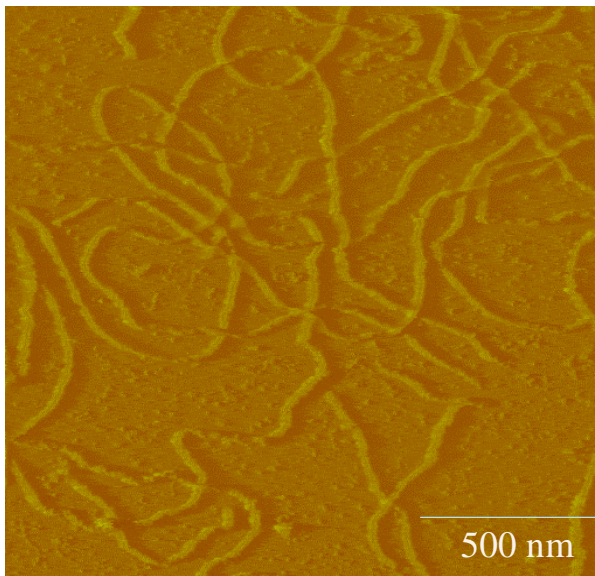


Flexibility, Self-Interaction, and Counterion-Induced Collapse of Neurofilaments

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.




NFH 

NFM 

NFL 

Neurofilament subunit domain organization

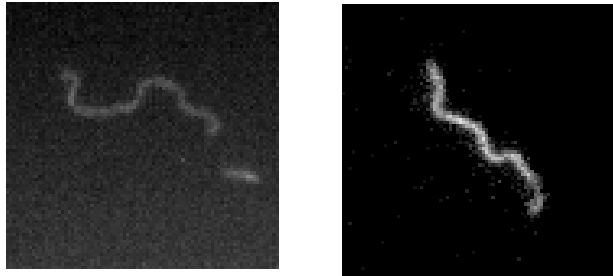
Helical rod domain



Amino terminus Carboxyl terminus

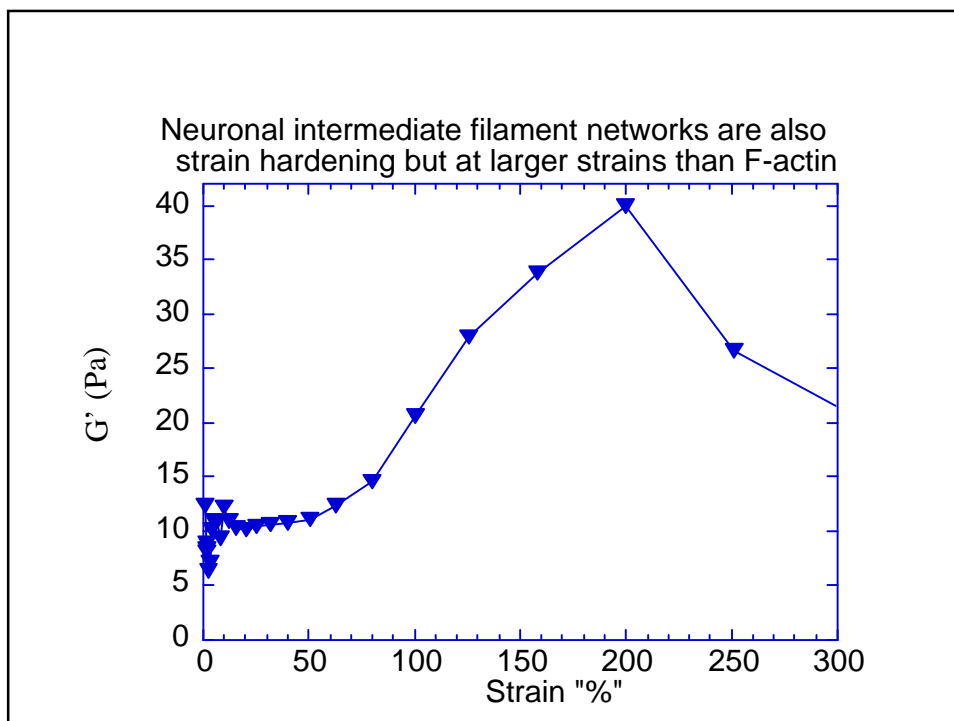
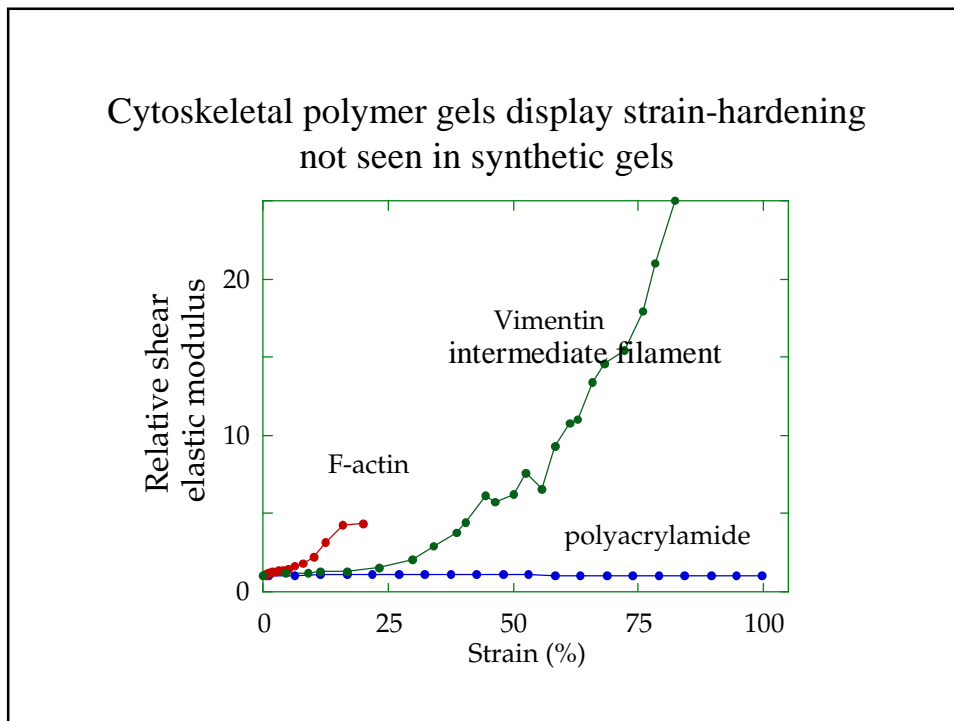
Three types of NF subunits.
All are anionic and phosphorylated
Filament diameter is 10-12 nm, sidearm can be >50 nm
surface charge is between 1.4 and 2.8 charges / nm² depending on phosphorylation

$\lambda_p = 500\text{nm}$



Non-linear elasticity of NF networks

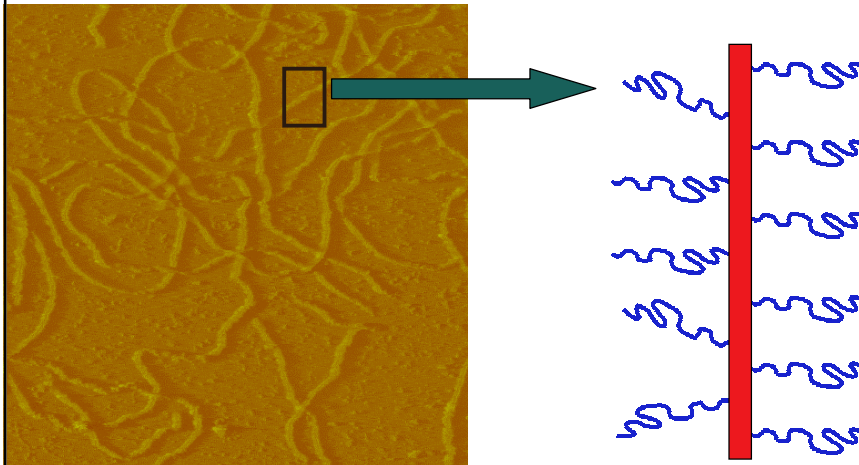
Strain-hardening at larger strains than for actin

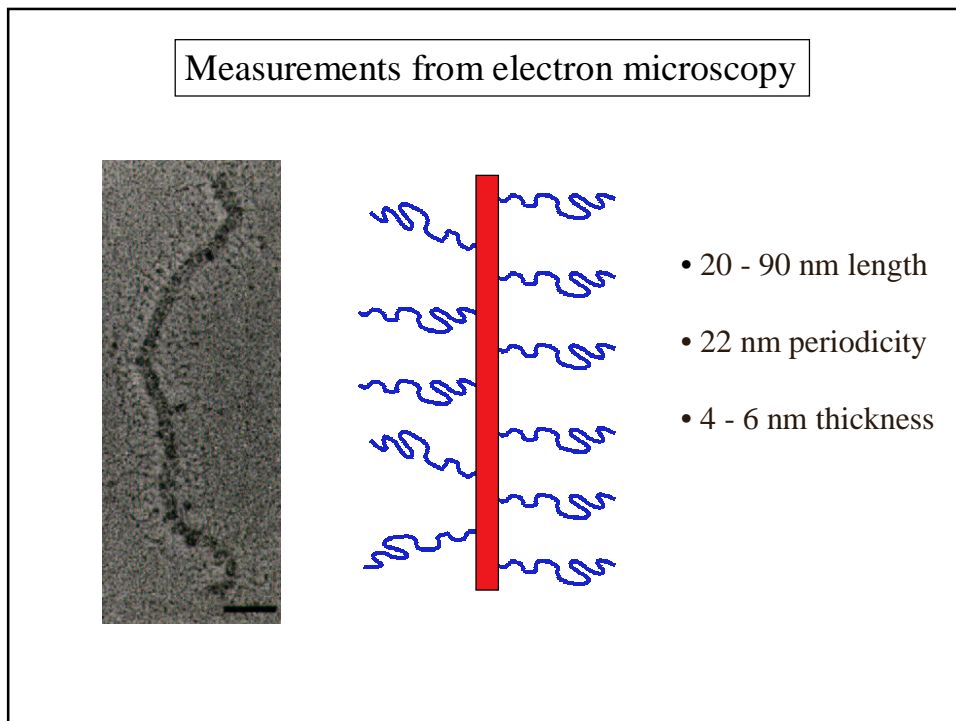
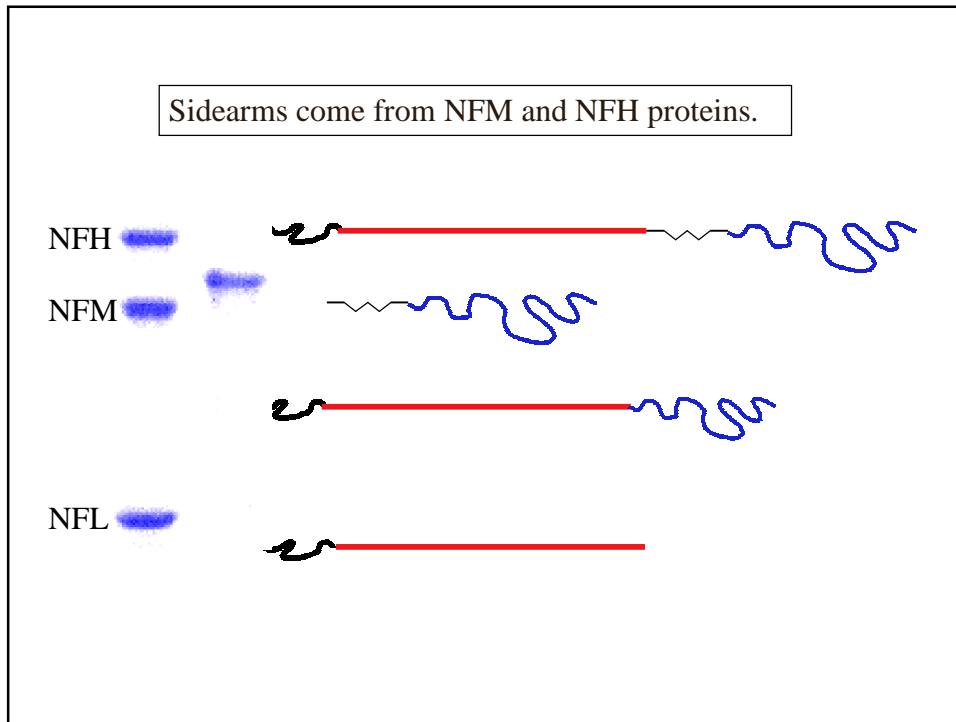


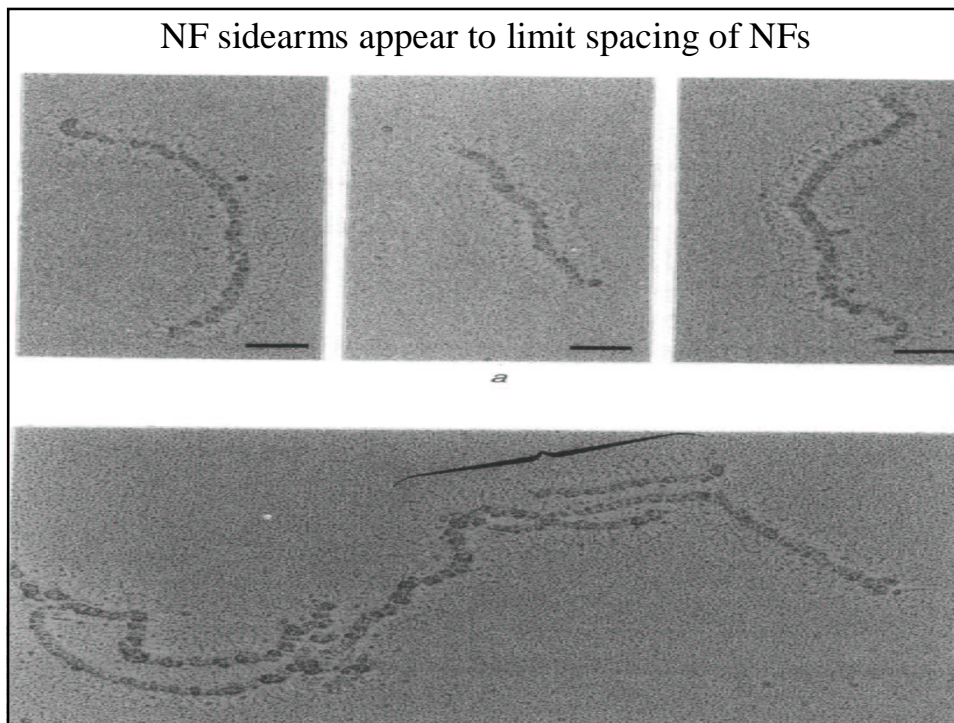
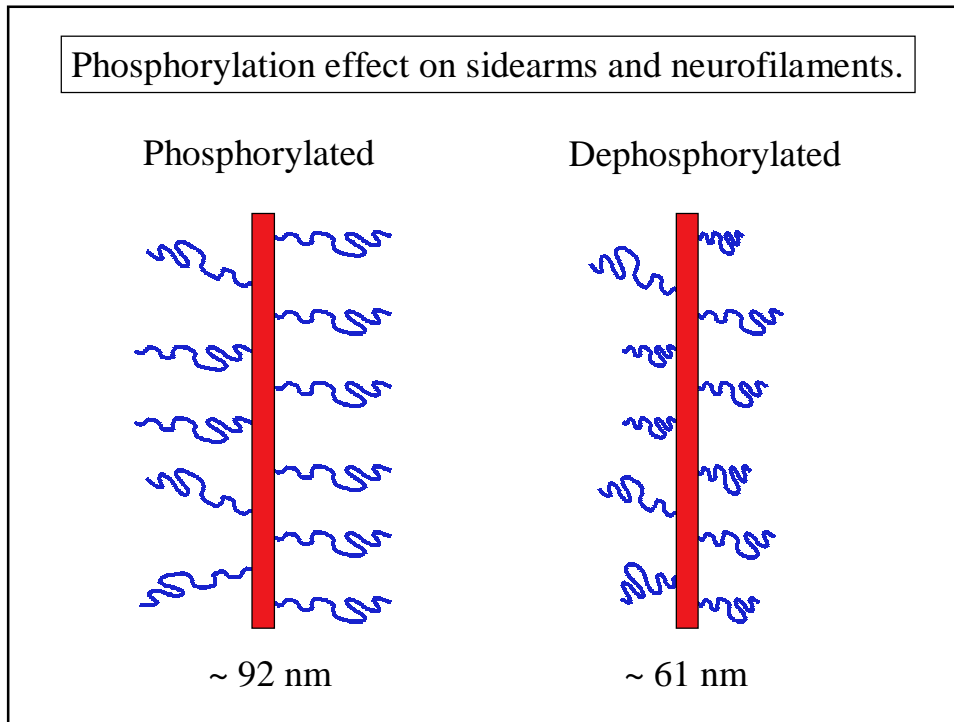
What is the diameter of a neurofilament?

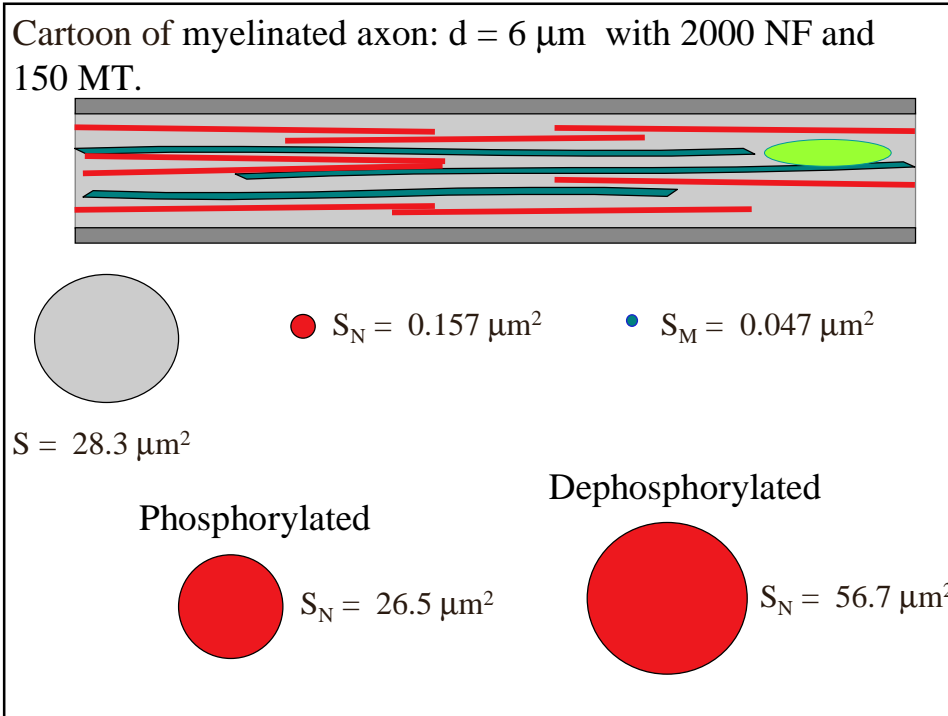
How much of the axon volume do NFs occupy?

Neurofilaments have small protrusions called sidearms.





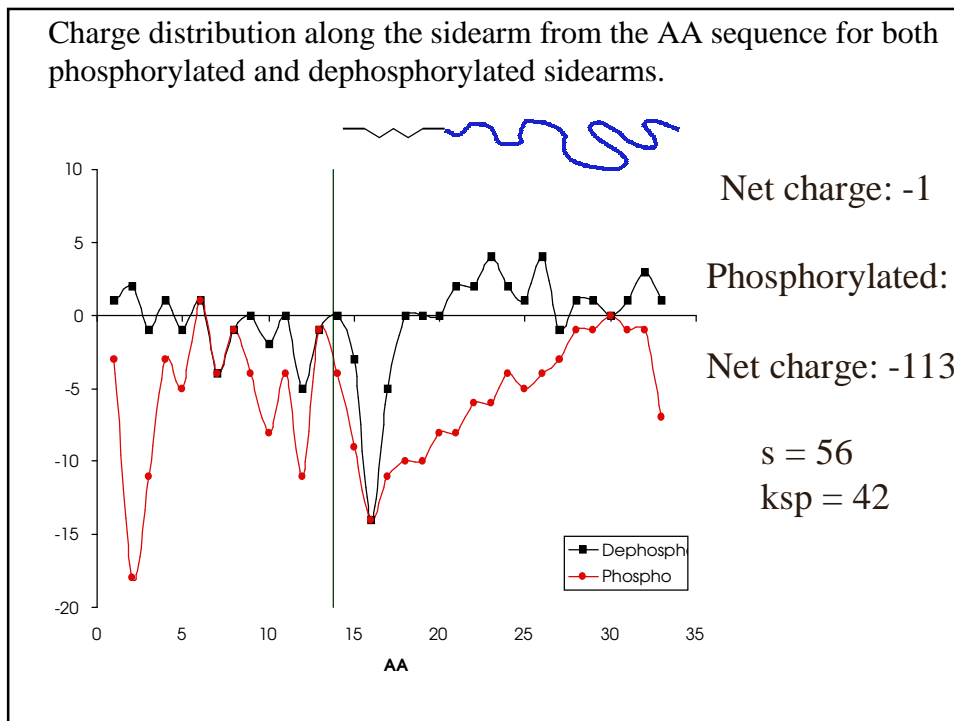
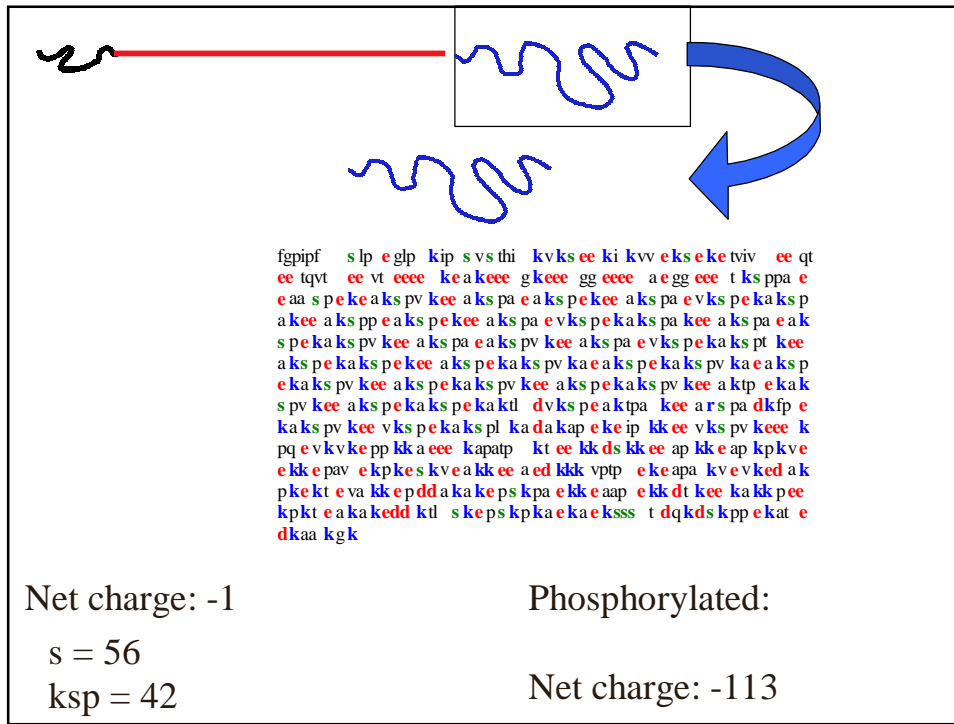




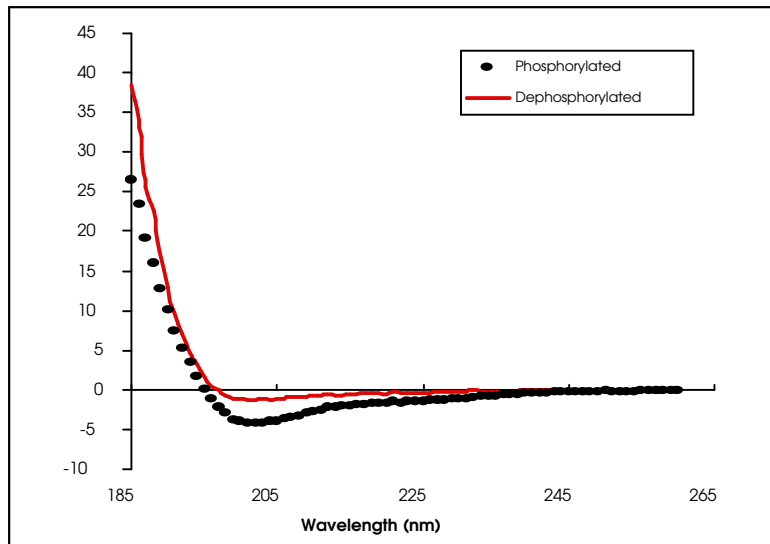
NF sidearms have unusually high electrostatic charge and unknown folded structure.

Can a charged polypeptide have folded domains without secondary structure?

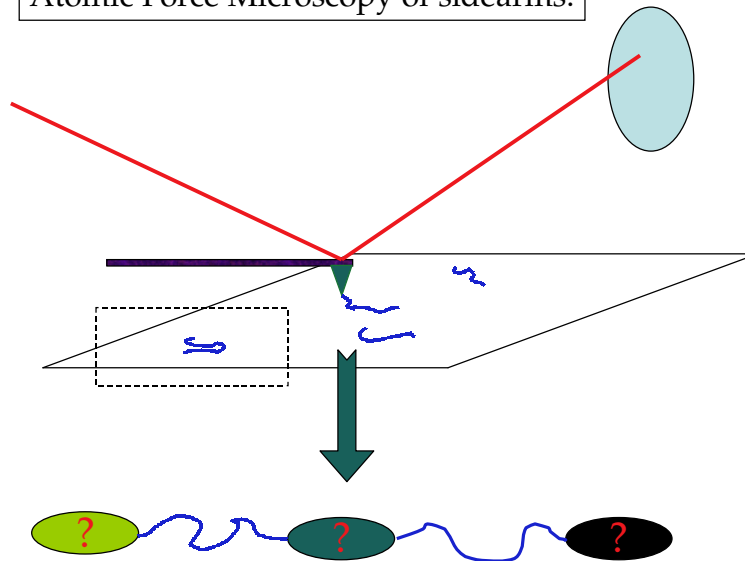
Flexibility, Self-Interaction, and Counterion-Induced Collapse of Neurofilaments

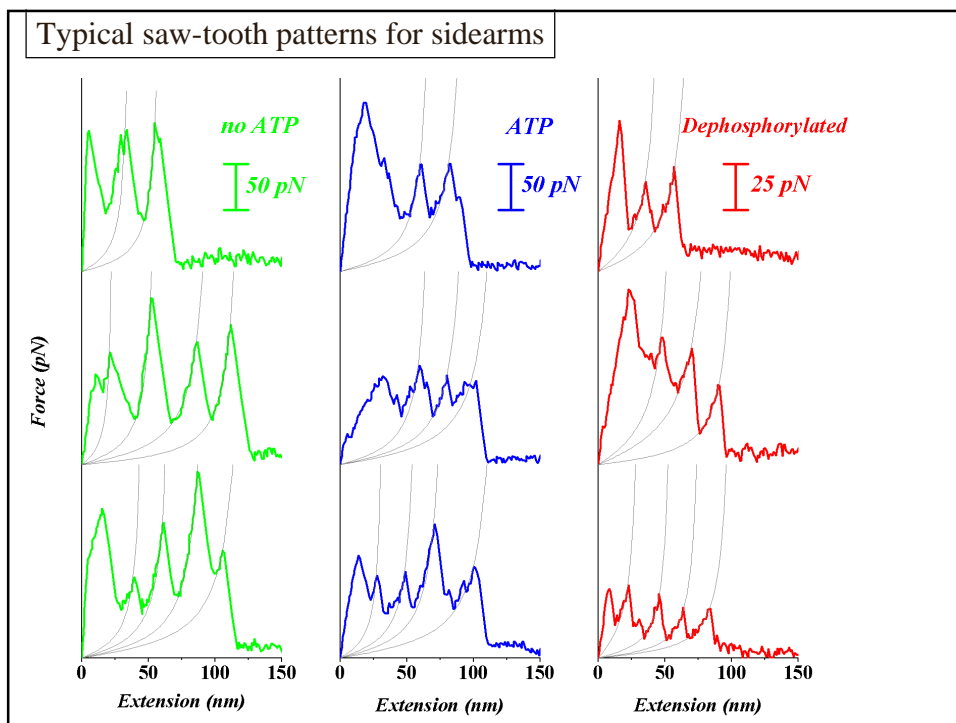
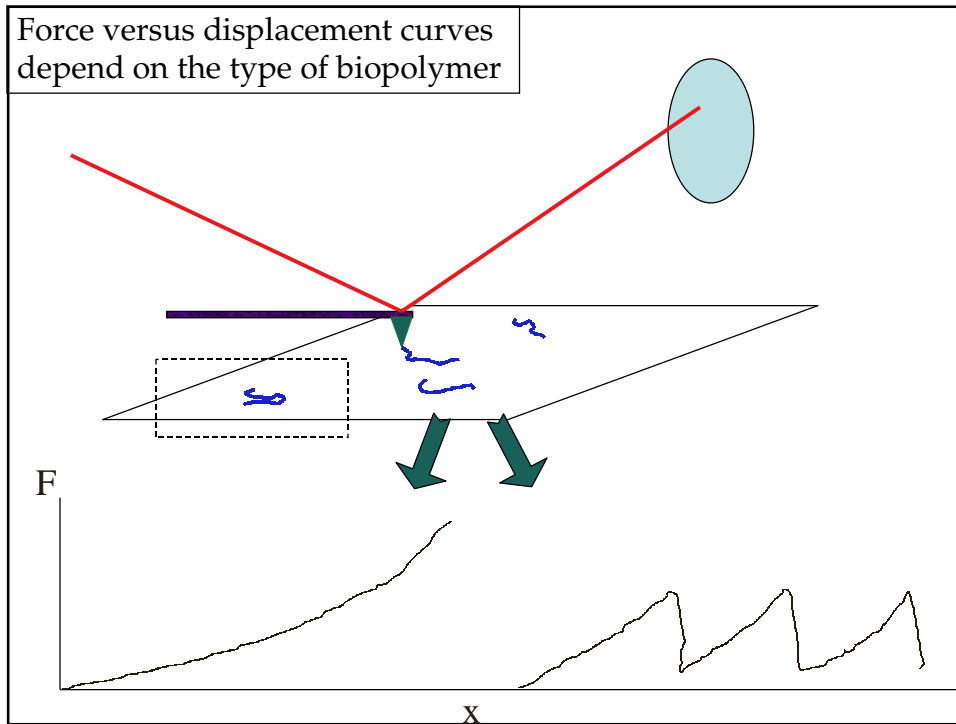


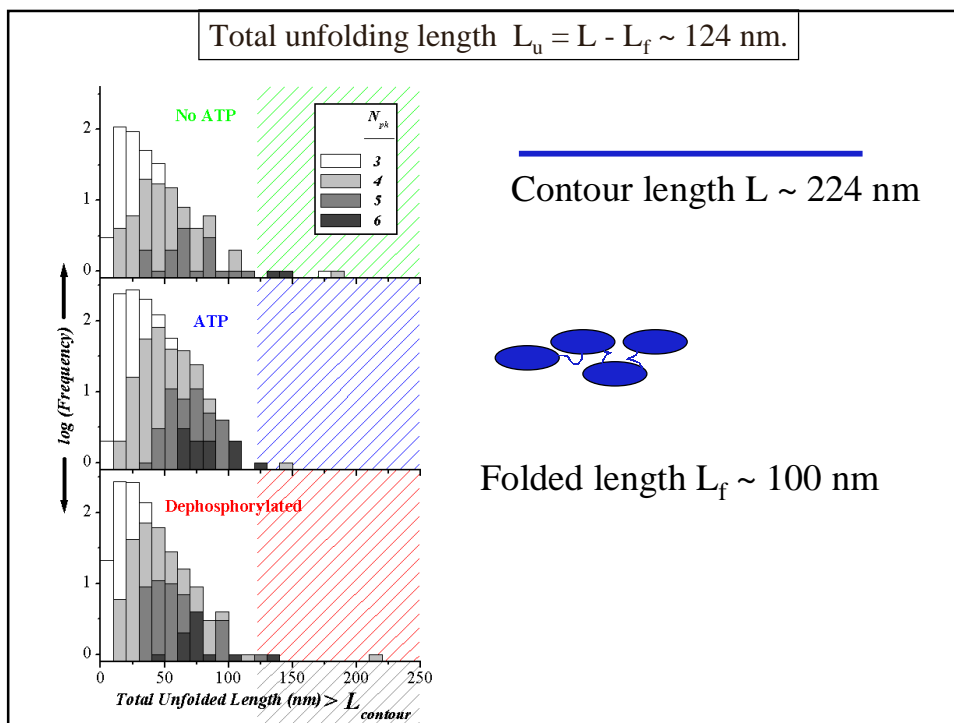
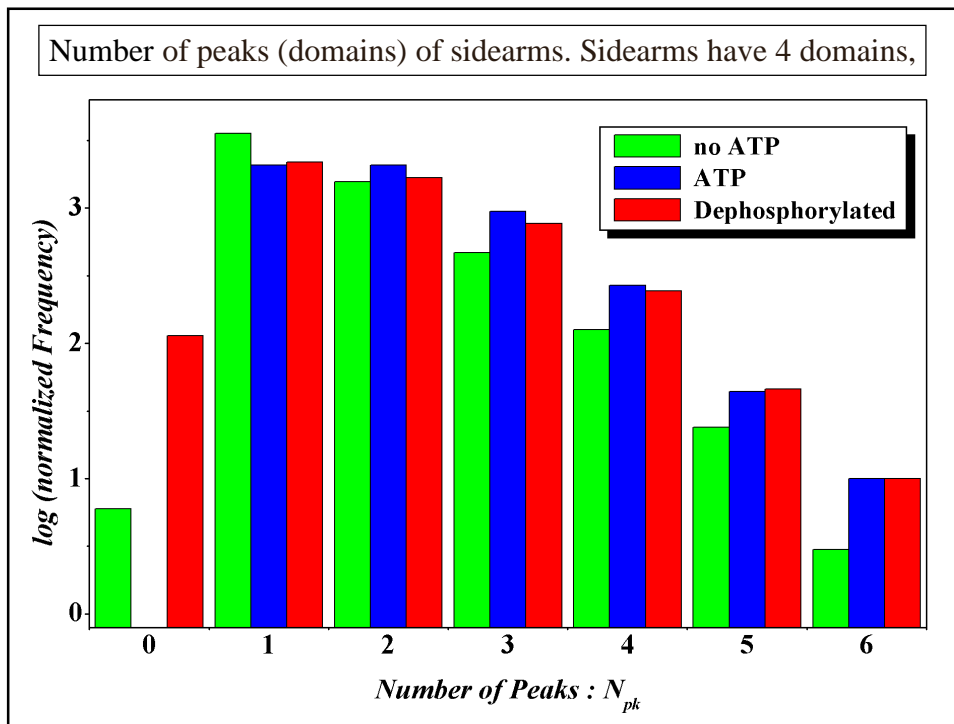
Circular dichroism for phosphorylated and dephosphorylated sidearms.

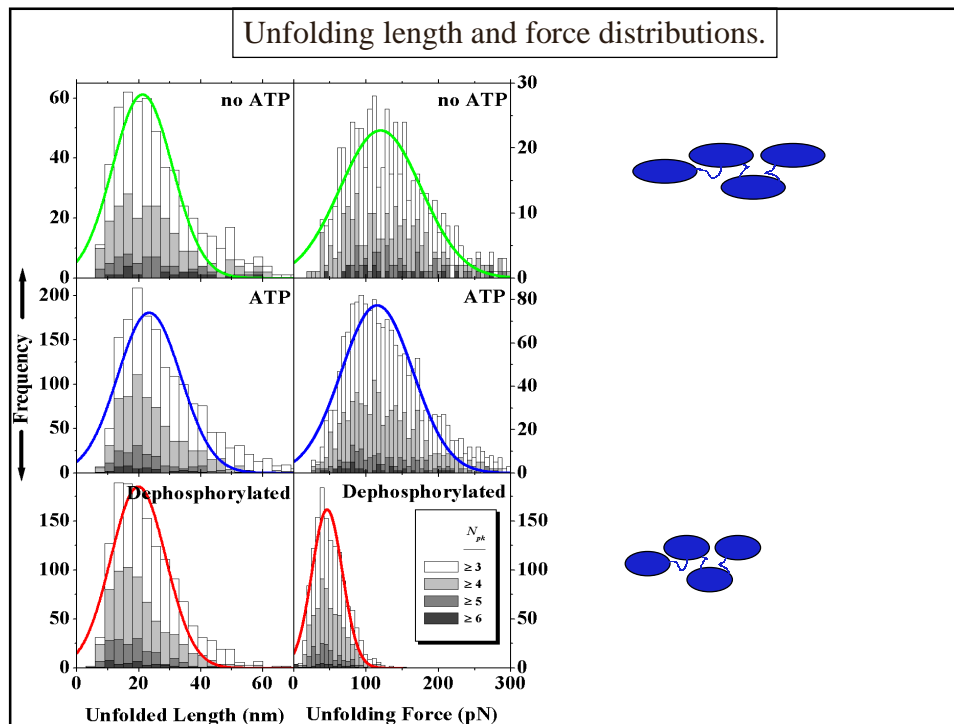


Atomic Force Microscopy of sidearms.









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.

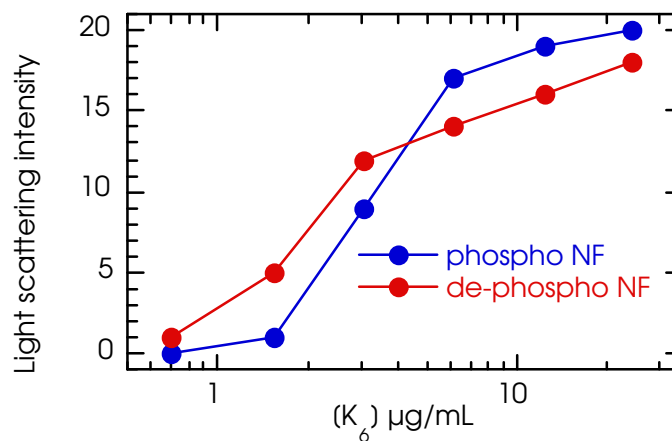
NFs are aggregated by physiologic concentrations of multivalent counterions

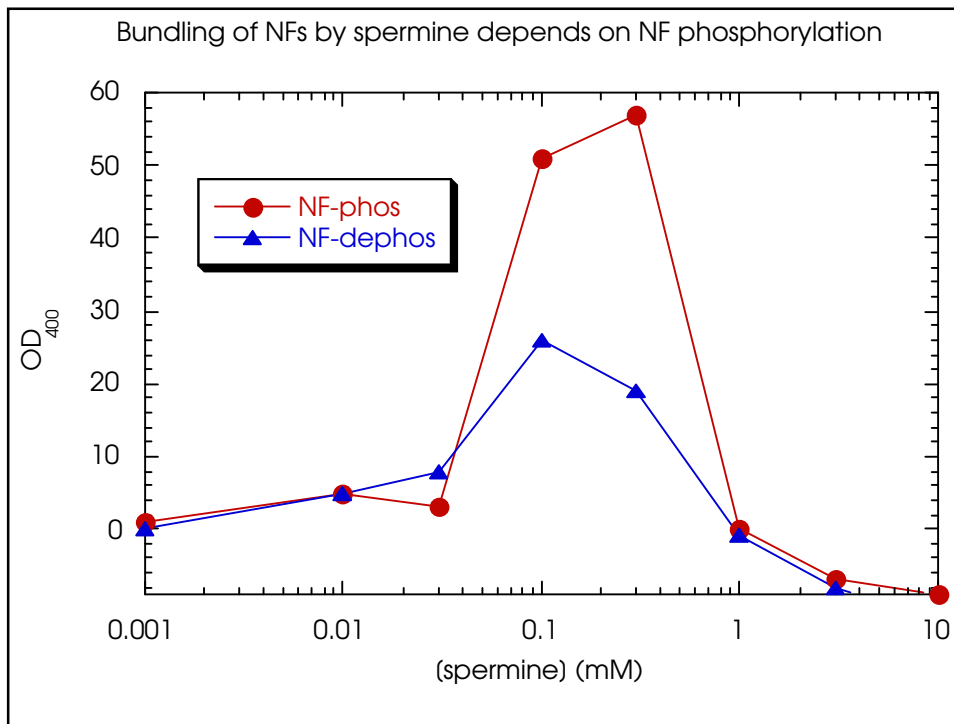
Bundle formation depends on NF surface charge

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

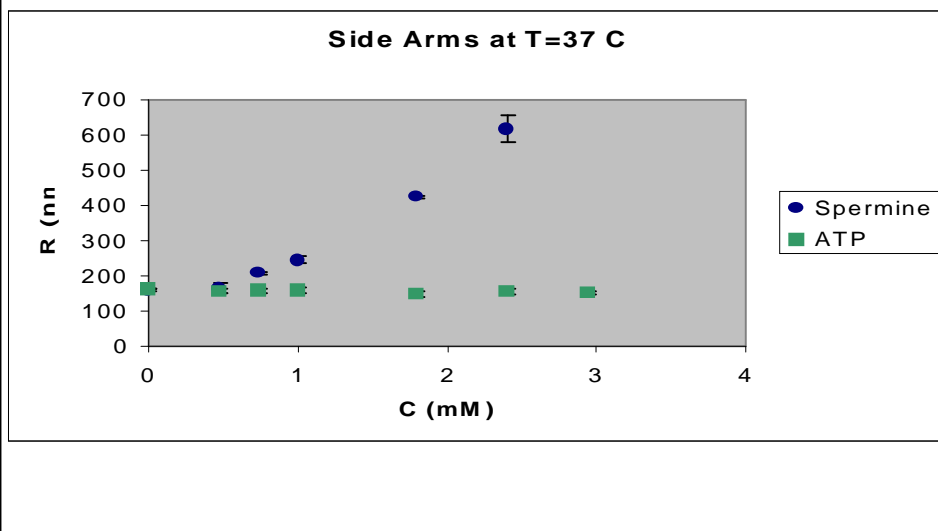
Surface charge of NFs affects bundling by lysine hexamers



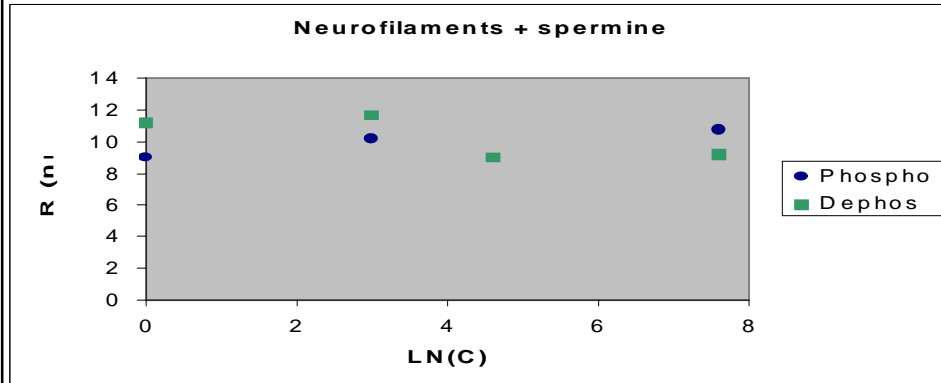


DLS of Side-arms

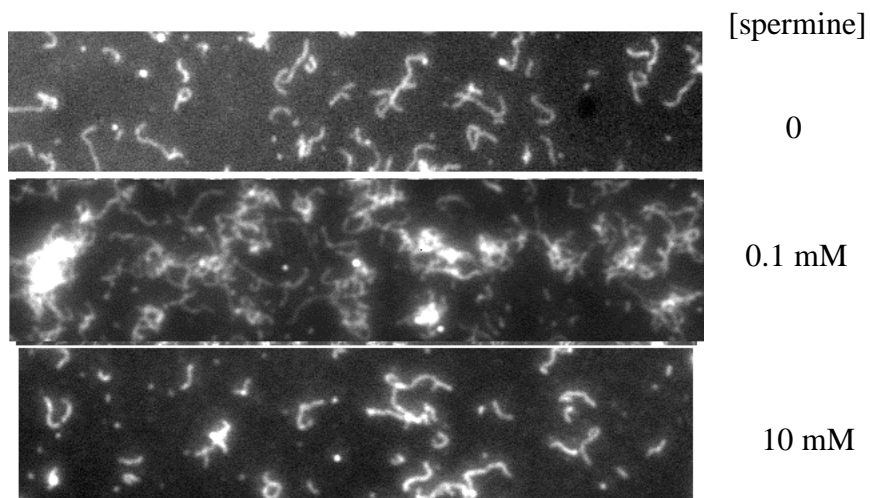
On the effect of spermine and ATP



Neutron scattering

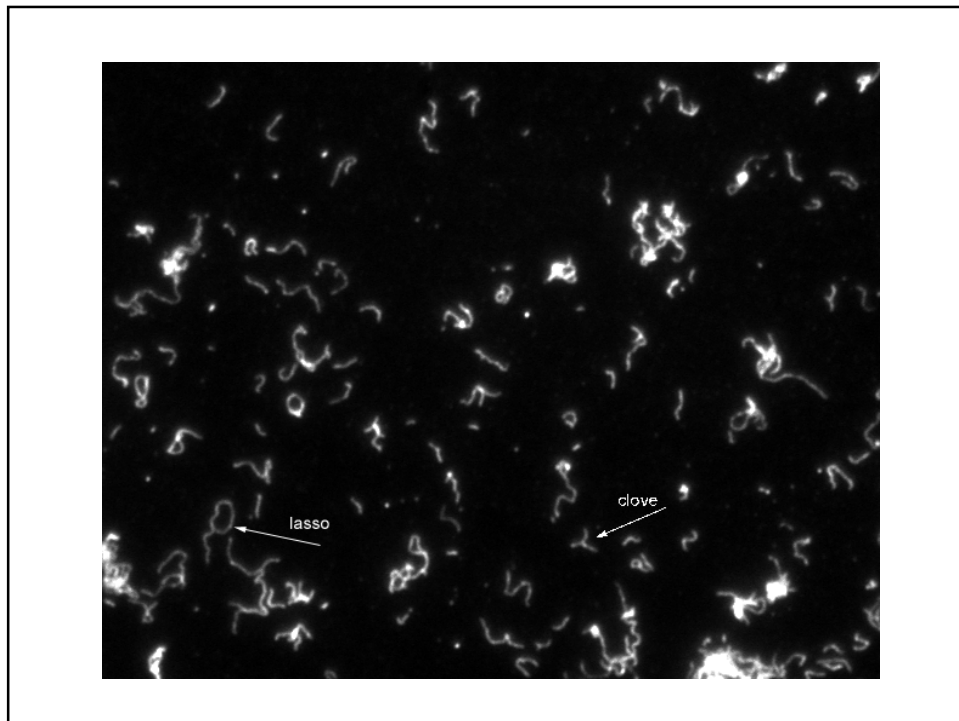
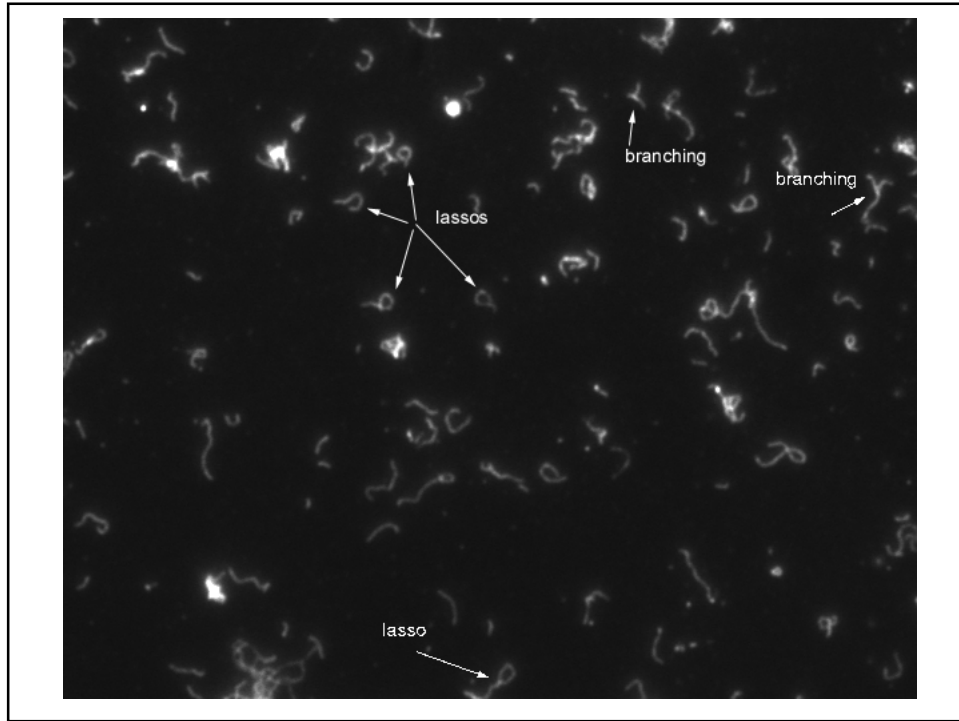


Formation and redissolution of NF bundles by spermine

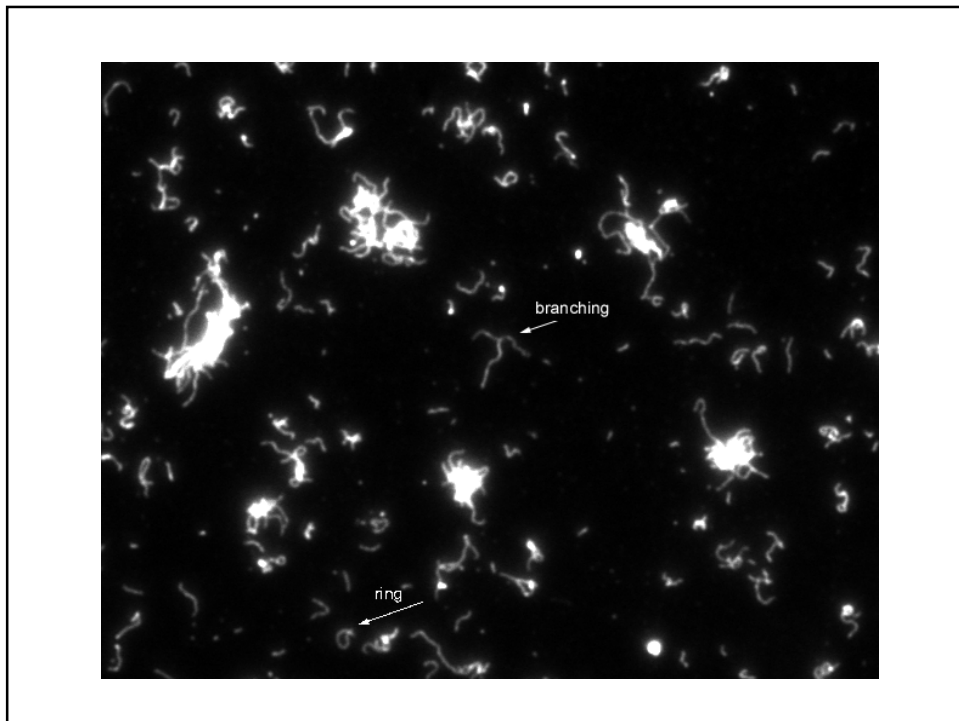
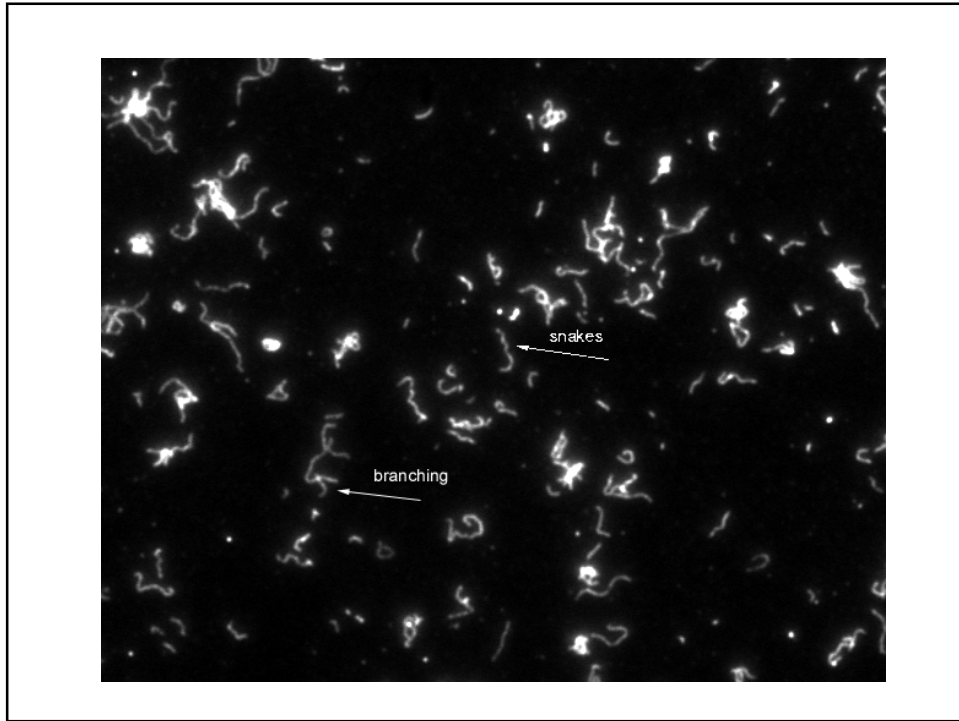


0.1 mg/ml RhoB-labeled NFs

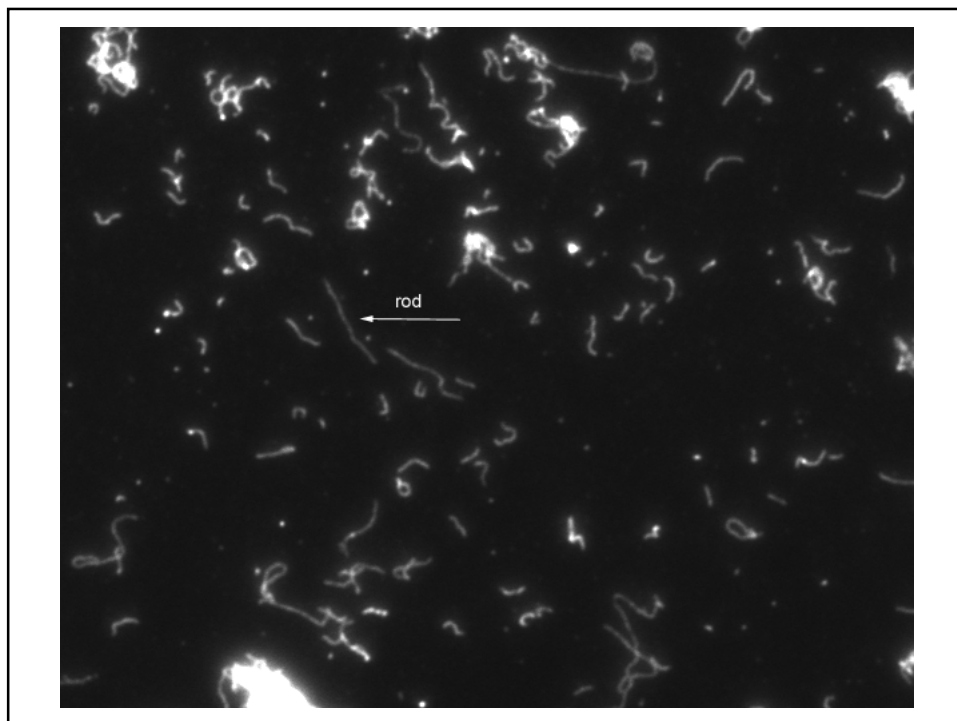
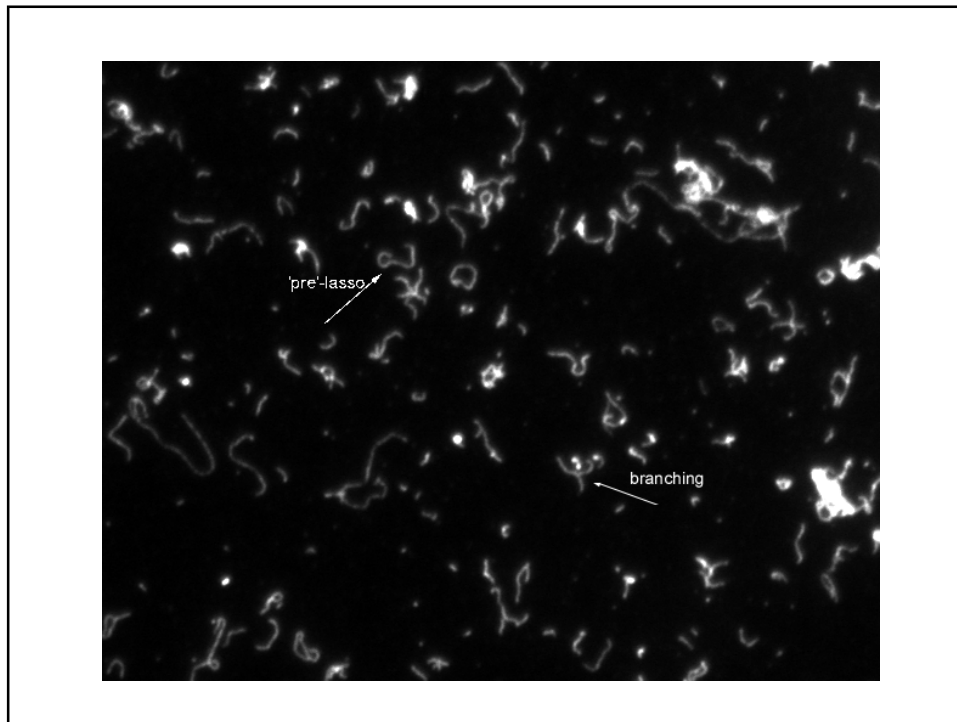
Flexibility, Self-Interaction, and Counterion-Induced Collapse of Neurofilaments



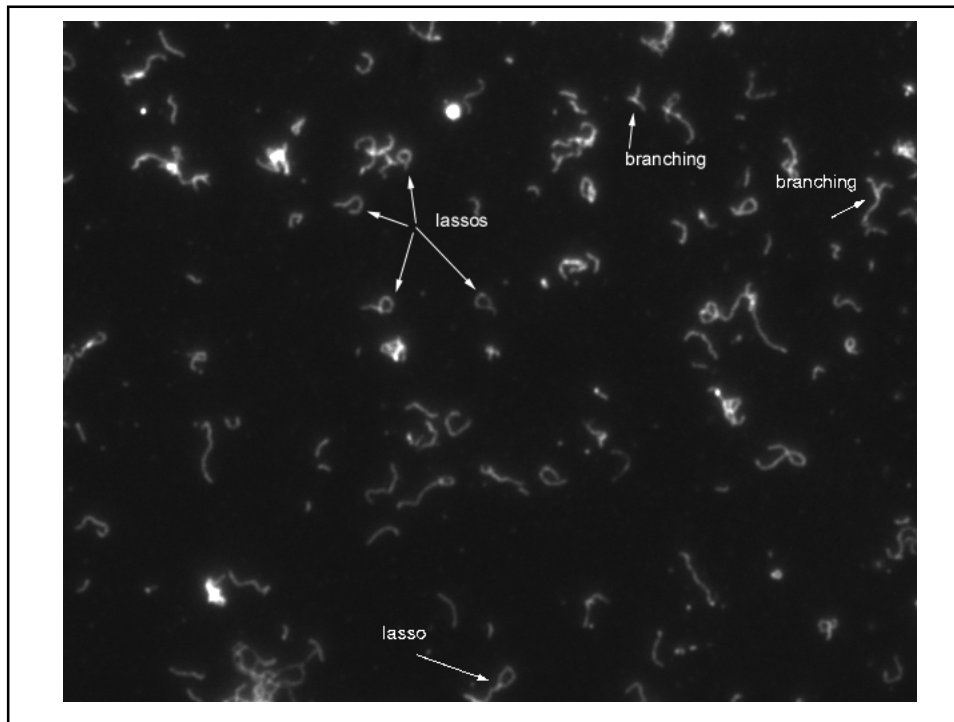
Flexibility, Self-Interaction, and Counterion-Induced Collapse of Neurofilaments



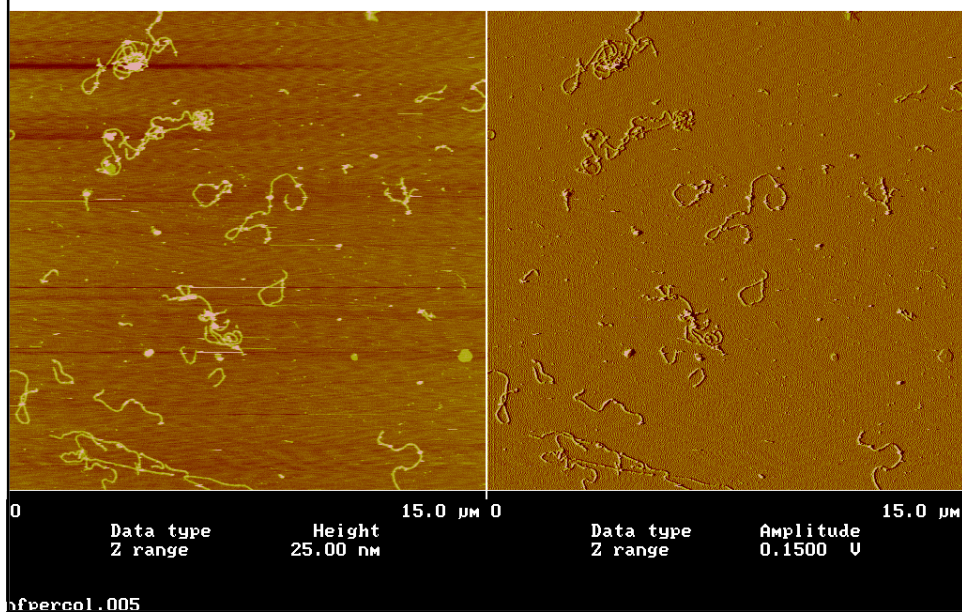
Flexibility, Self-Interaction, and Counterion-Induced Collapse of Neurofilaments



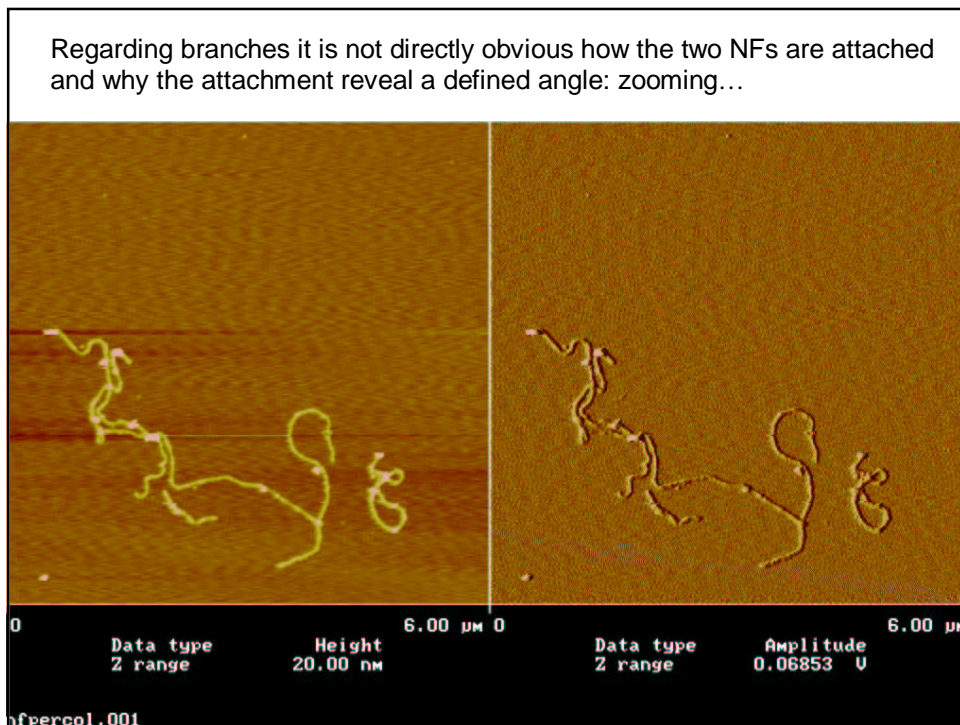
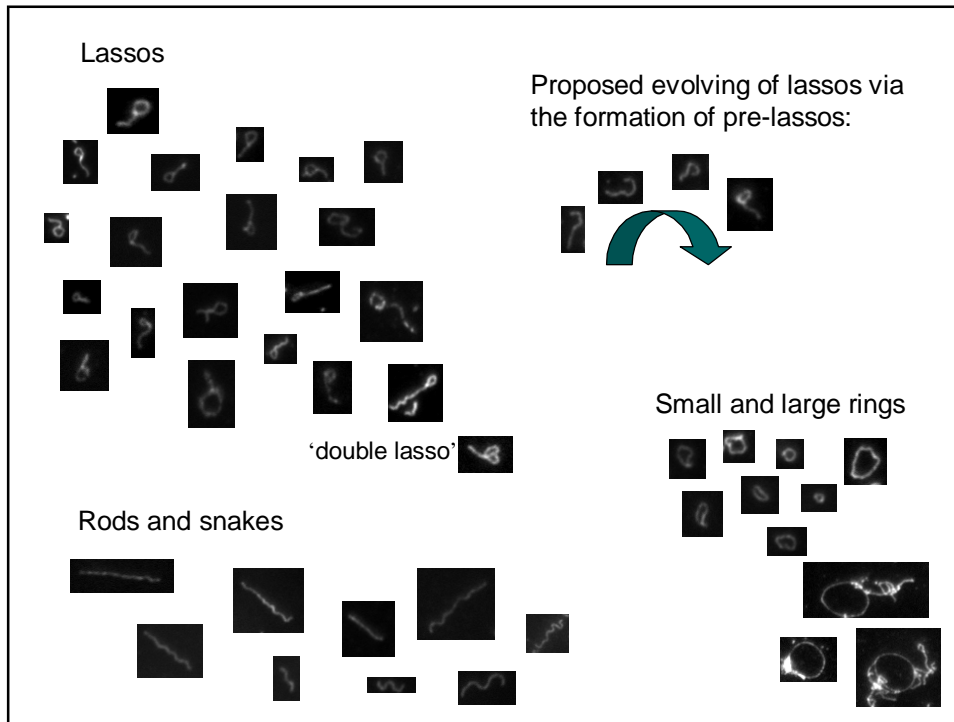
Flexibility, Self-Interaction, and Counterion-Induced Collapse of Neurofilaments

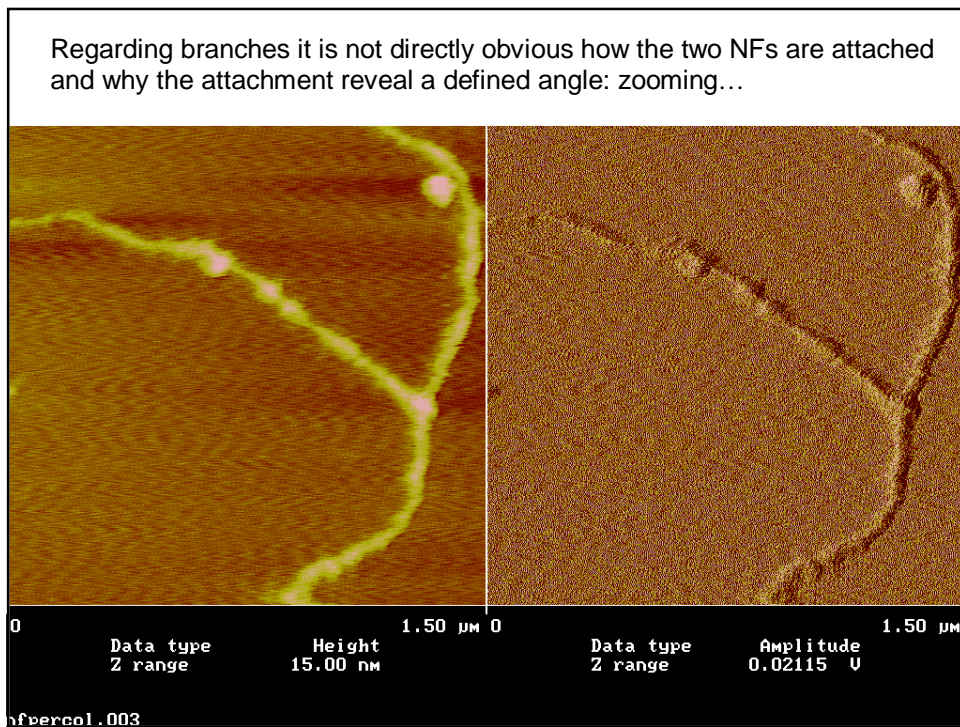
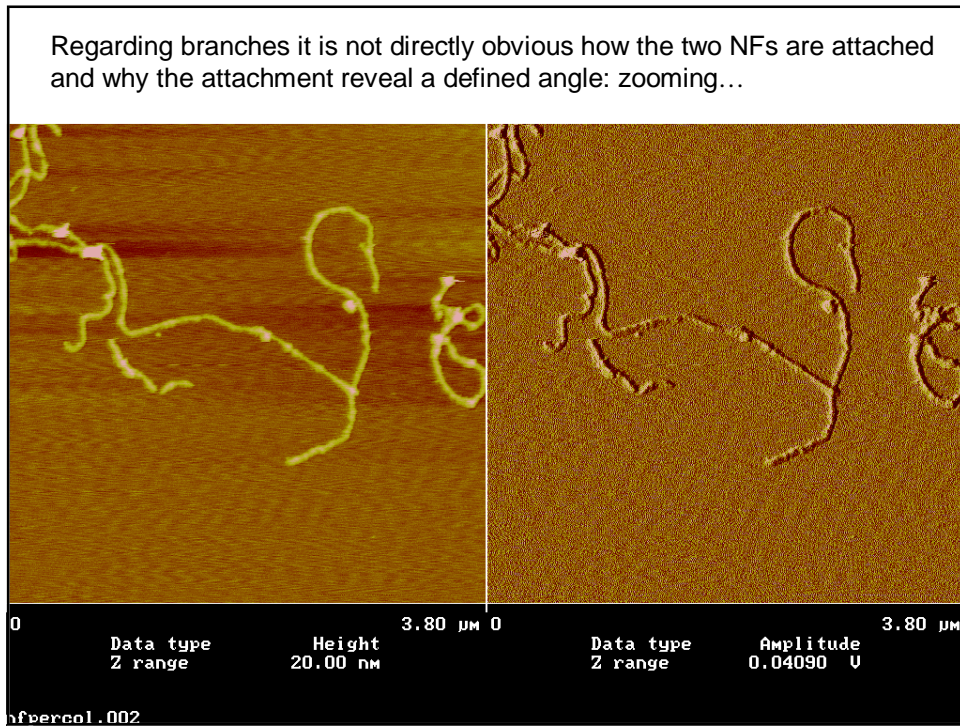


15 nM NFs in RB 0.8 M sucrose exhibit a couple of characteristic structures. Because fluorescence imaging is much easier than AFM, one can characterize multiple typical structures of highly diluted NFs...

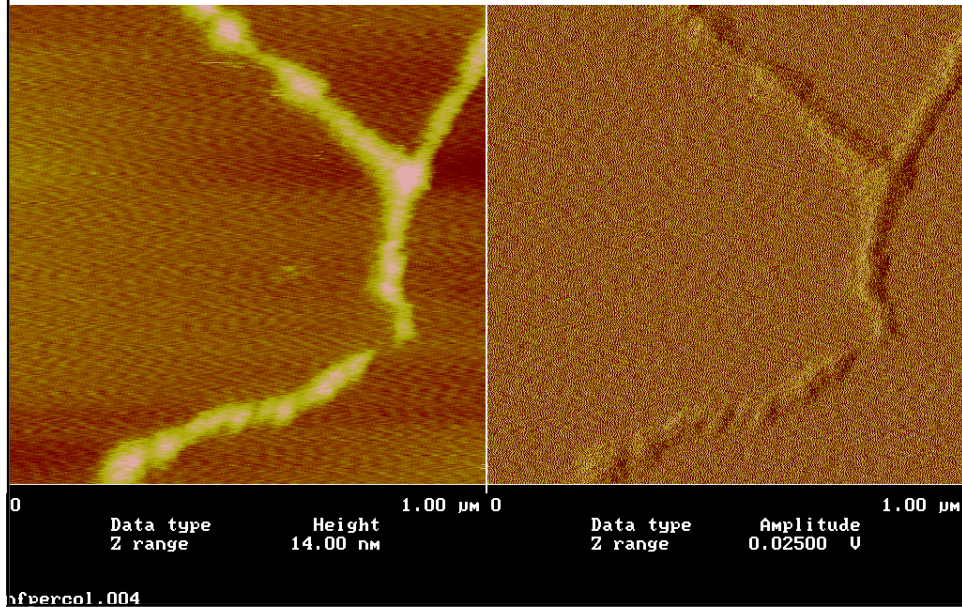


Flexibility, Self-Interaction, and Counterion-Induced Collapse of Neurofilaments

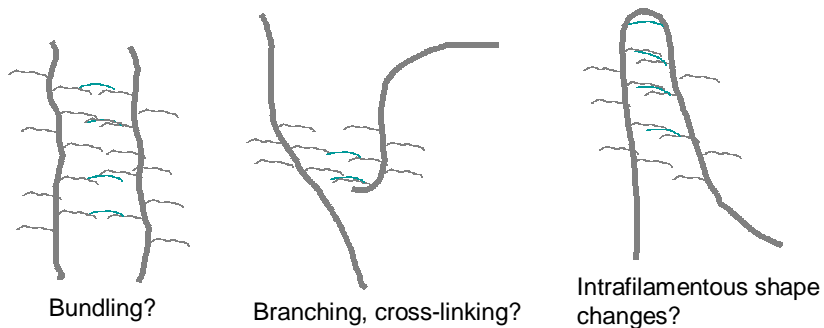




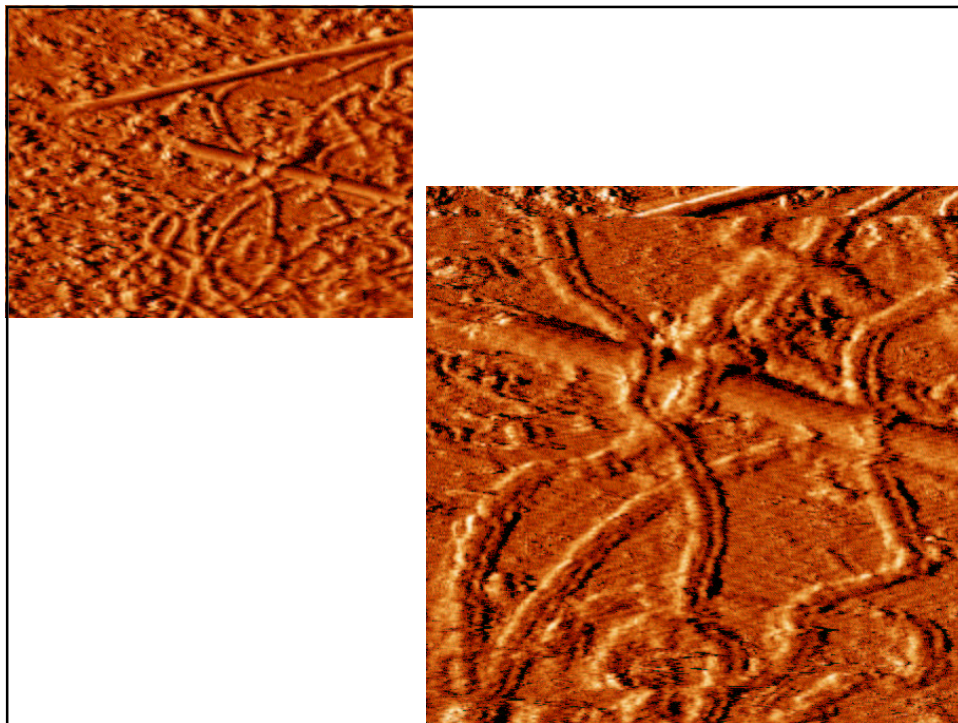
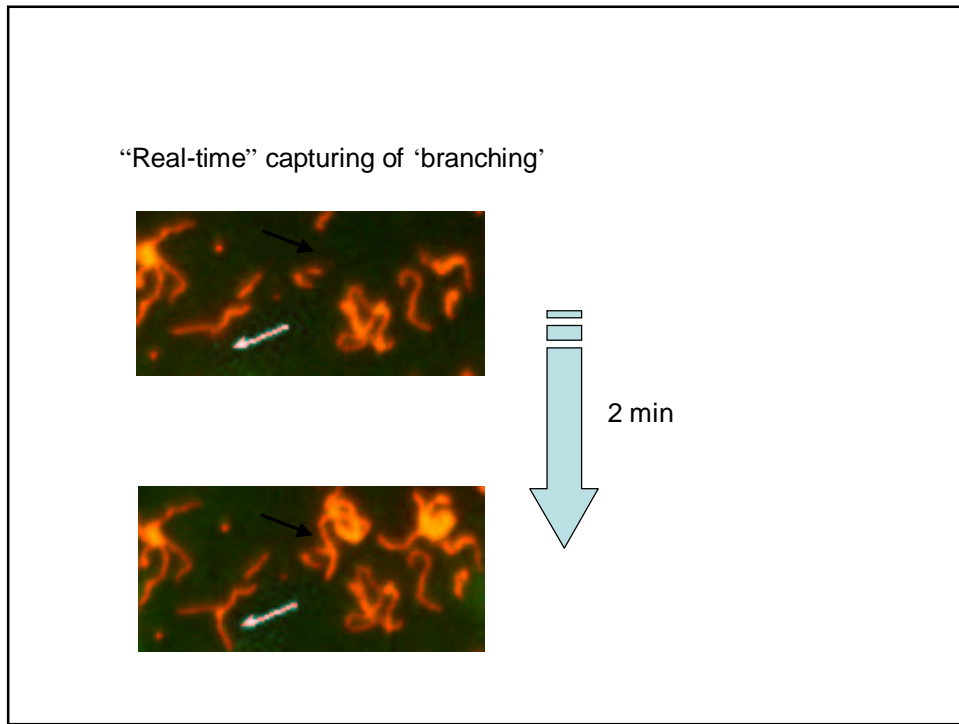
Regarding branches it is not directly obvious how the two NFs are attached and why the attachment reveal a defined angle: zooming...



Effect of simply adding sidearms to NF?
Do sidearms interact with sidearms?

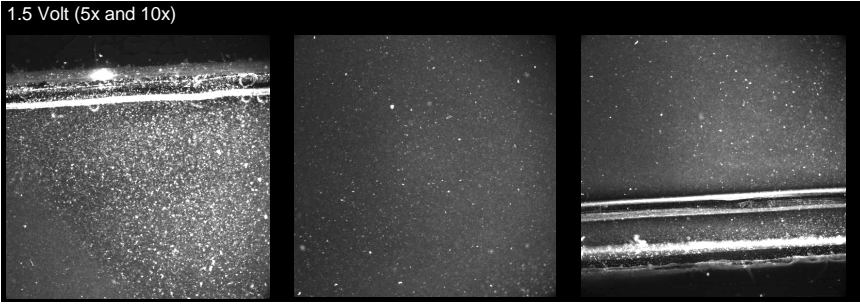


Flexibility, Self-Interaction, and Counterion-Induced Collapse of Neurofilaments



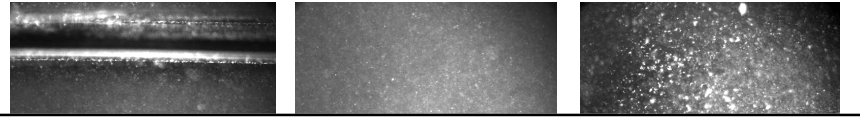
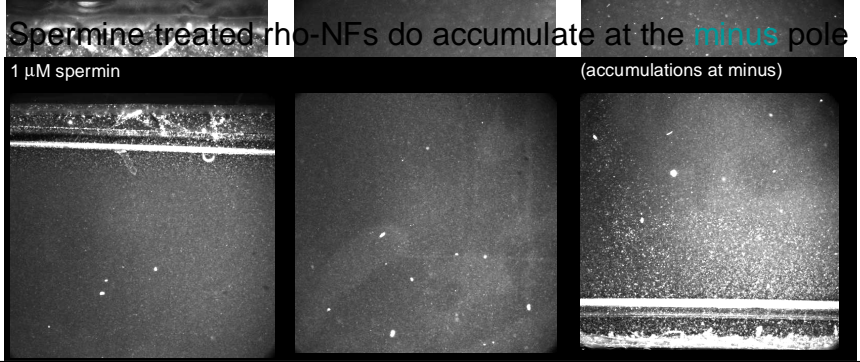
Rho-NFs (negatively charged) do accumulate at the **plus** pole

1.5 Volt (5x and 10x)



Spermine treated rho-NFs do accumulate at the **minus** pole

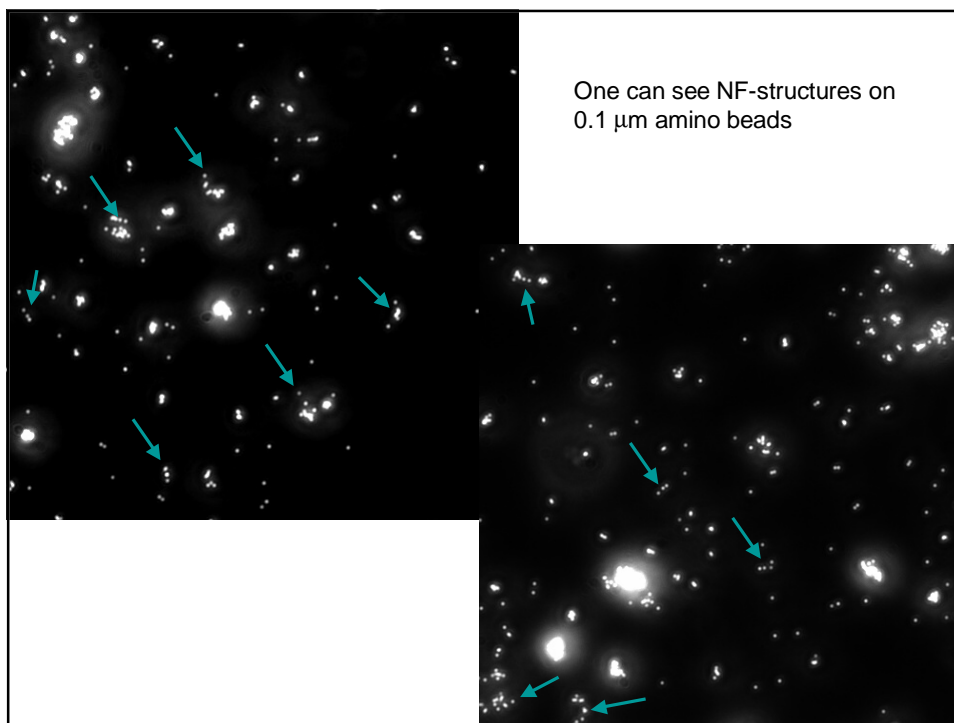
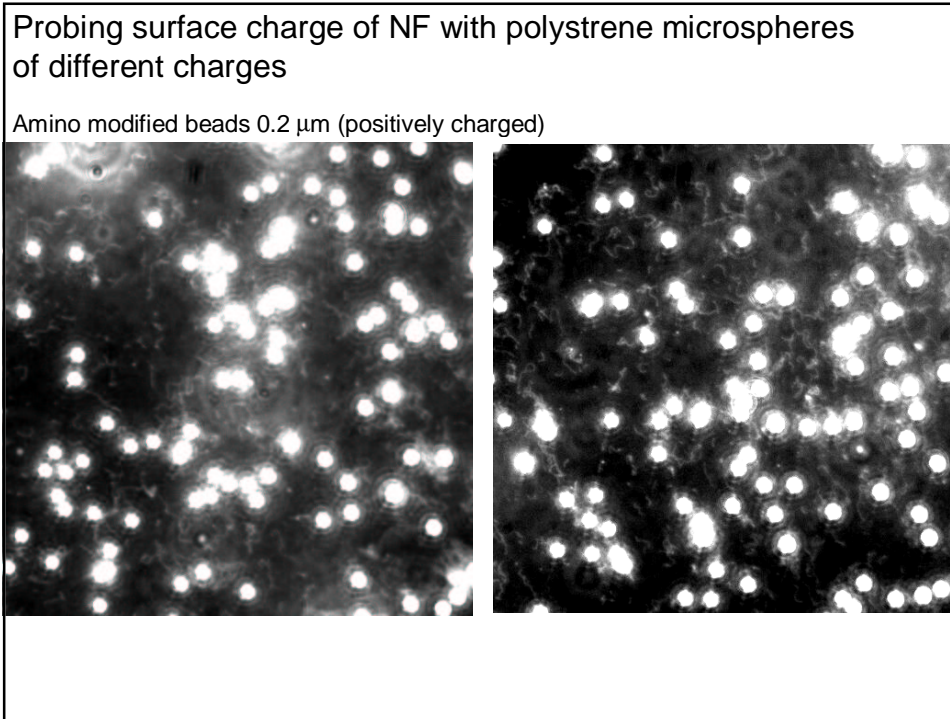
1 μ M spermin (accumulations at minus)



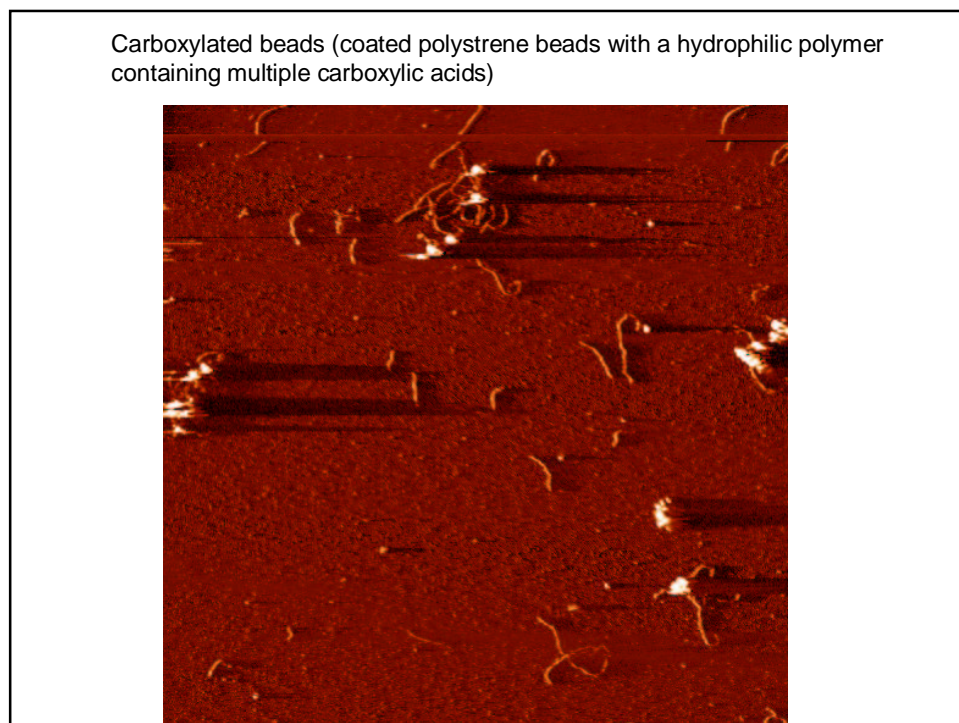
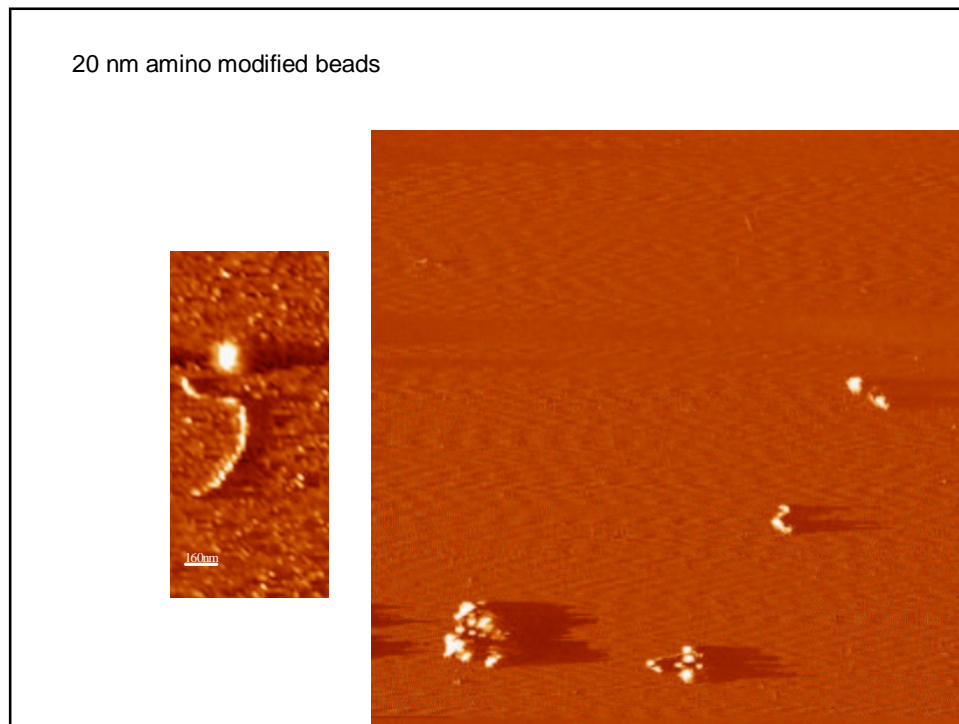
Binding of NFs to oppositely-charged beads

Structures depend on diameter of bead.

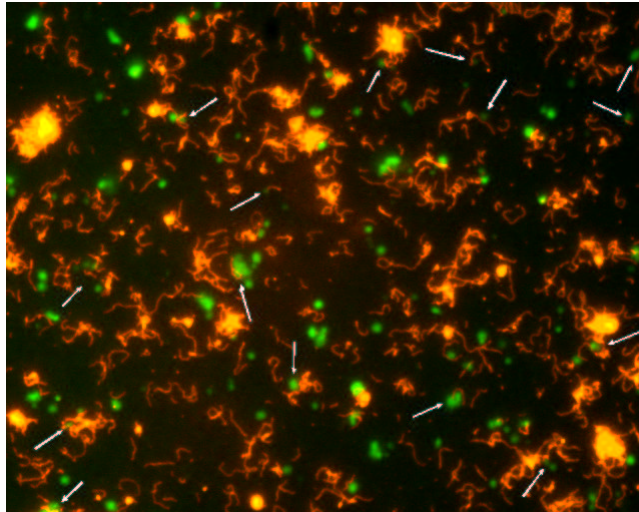
NF wrap around larger ($>\mu\text{m}$) beads but are decorated by small (20 nm) beads



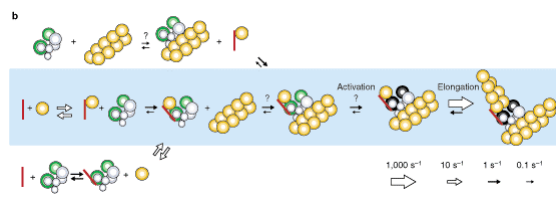
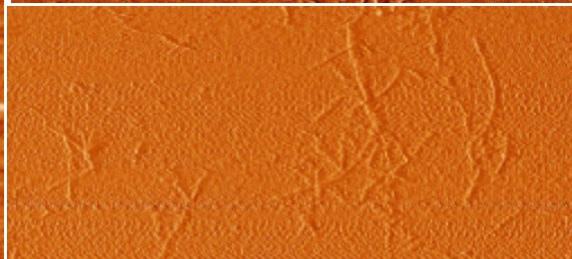
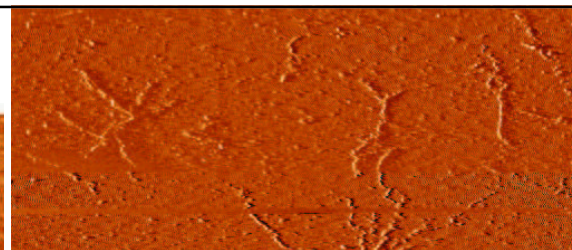
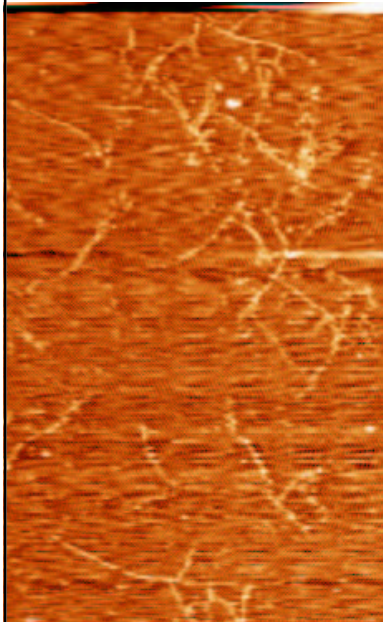
Flexibility, Self-Interaction, and Counterion-Induced Collapse of Neurofilaments



Another example of snuggling (small) mitos to NF:



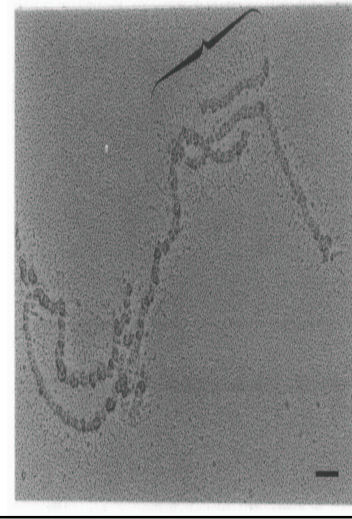
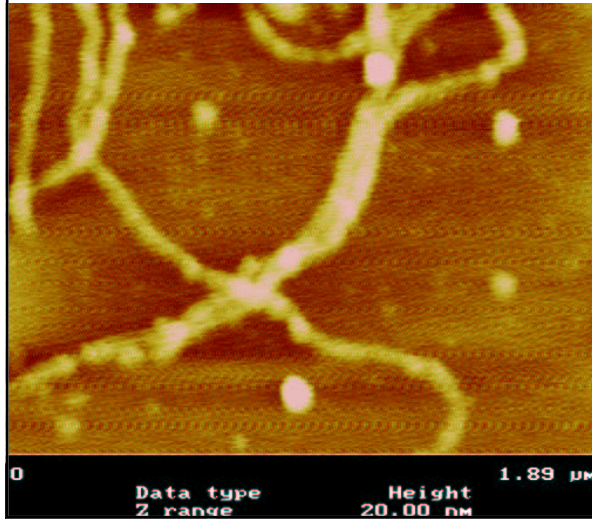
Actin + Arp2/3



NFs can make extensive side-to-side attachments, mediated by sidearms

AFM

EM



fgpipf slp eglp kip svs thi kvks ee ki kvv eks eke tiv ee qt
 ee tqvt ee vt eeee ke akeee gkeee gg eeee a e gg eee t ks ppa e
 e aa s pe ke aks pv kee a ks pa e aks pe kee a ks pa ev ks pe ka ks p
 a kee a ks pp e a ks pe kee a ks pa ev ks pe ka ks pa kee a ks pa e a k
 s pe ka ks pv kee a ks pa e aks pv kee a ks pa ev ks pe ka ks pt kee
 aks pe ka ks pe kee a ks pe ka ks pv ka e aks pe ka ks pv ka e aks p
 e ka ks pv kee a ks pe ka ks pv kee a ks pe ka ks pv kee a ktp e ka k
 s pv kee a ks pe ka ks pe ka ktl dvks pe a ktpa kee a rs pa dkfp e
 ka ks pv kee v ks pe ka ks pl ka da kap e ke ip kk ee v ks pv keee k
 pq ev kv ke pp kk a eee kapatp kt ee kk ds kk ee ap kk e ap kp kve
 e kke pav e kp ke s kve a kke e a ed kkk vptp e ke apa kv ev ked a k
 p ke kt e va kk e p dd a ka ke ps kpa e kke aap e kk dt kee ka kk pee
 kp kt e a ka ked d ktl s ke ps kp ka e ka e ksss t dqk ds kpp e kat e
 dkaa kgk

Net charge: -1
 s = 56
 ksp = 42

Phosphorylated:
 Net charge: -113

