Simulation of Neurofilament Proteins: Structure of Unstructured Proteins

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Intrinsically Disordered Proteins

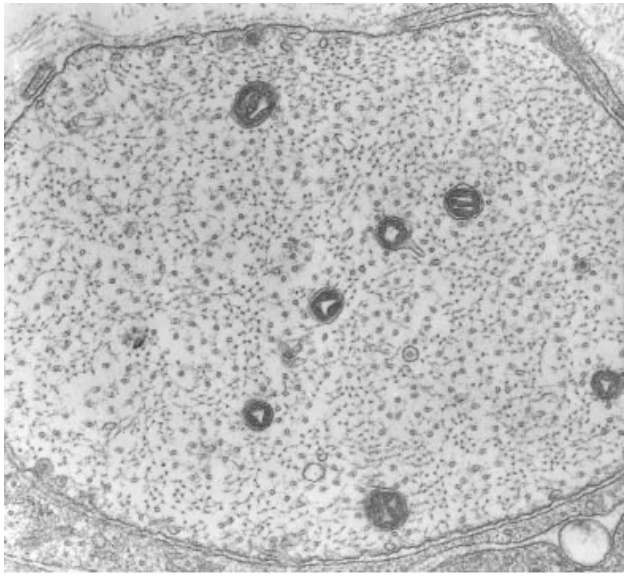
- Not all proteins (fully) fold

  i.e. the polymer is not in a collapsed state

  – tend to have low hydrophobicity and high net charge
  – many bind to other biomolecule and then become structured
- Two broad classes
  – disordered on whole length of protein
  – long section of residues disordered
- Biologically functional
  – many bind to DNA
  – kinases
- Why don't proteases eat them all up?
  – bound or hidden
- Intrinsically unstructured proteins are a significant fraction of eukaryotic genome (~ 30%)
- Anthony L. Fink, Current Opinion in Structural Biology, **15**, 35 (2005)

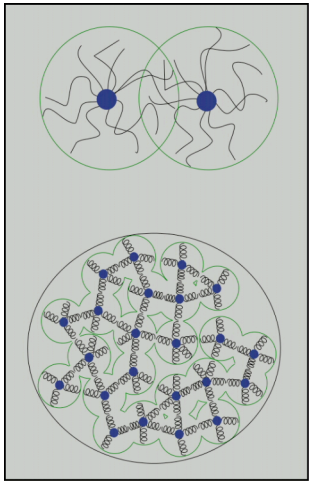
Axons and Polymer Brushes: Neurofilaments and MAPs

Neurofilaments and microtubules (MT) run axially along axons. The neurofilaments (NF) have branches which are part of the NF proteins. Microtubule associated proteins (MAPs) bind to MTs and extend as a polymer brush. The idea of Hoh & Brown is that this system of polymer brushes provides structural integrity of the axons, without a rigid chemical crosslinked structure.

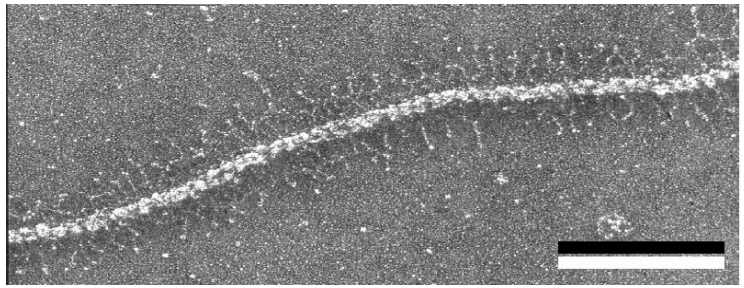


EM Cross Section of an Axon

brush vs. network

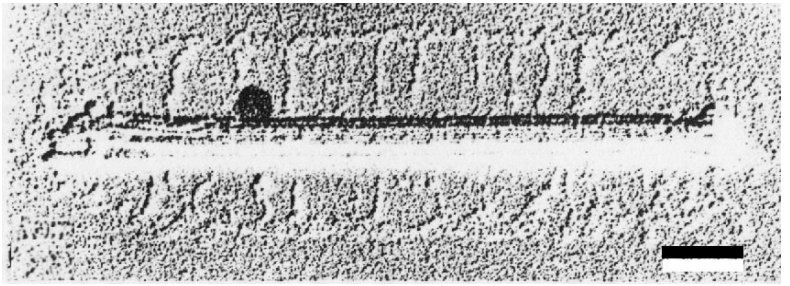


Neurofilament with “Side-Arms”



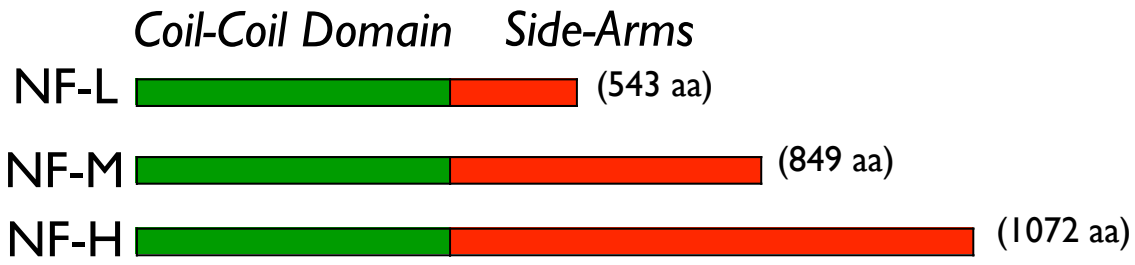
Aebi

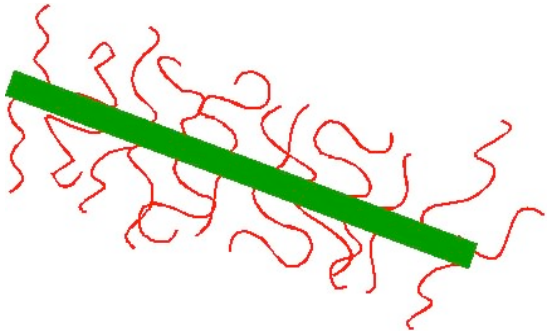
Microtubule with Microtubule Associated Protein “Projection Domains”



(Voter & Eriksson, J. Ultrastruct. Res., 80:374)

Neurofilament Proteins





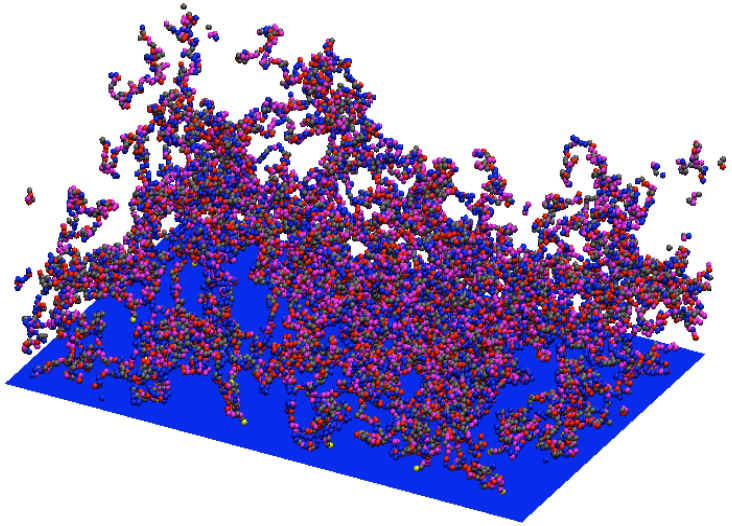
- Oligomerize through coil-coil domain
- Heteropolymers in vivo
- Side-Arms
  - Many charged amino acids
  - NF-M and NF-H phosphorylated

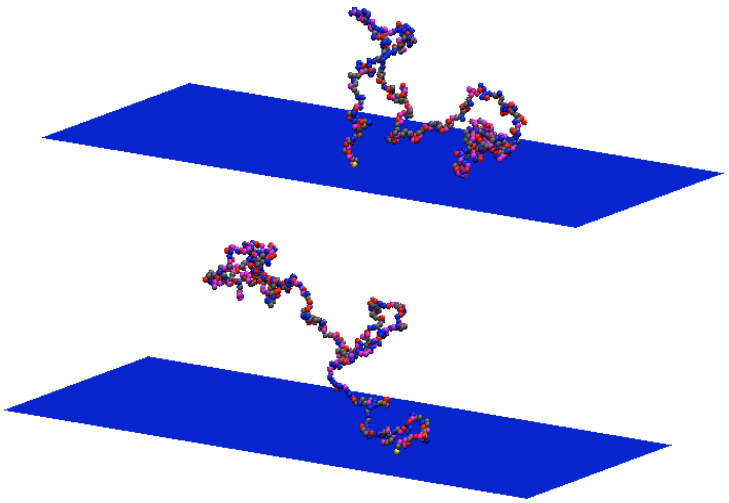
NF-M tail sequence:

ITISSKIQKTKVEAPKLKVQHKFVEEIIEETKVEDEKSEMEEALTAITEELAASMKEEKK
EAAEEKEEEPEAEEEEVAAKKSPVKATAPEVKEEEGEKEEEEGQEEEEEEEDEGAKSD
QAEEGGSEKEGSSEKEEGEQEEGETEAEAEGEEAEAKEEKKVEEKSEEVATKEELVAD
AKVEKPEKAKSPVPKSPVEEKGKSPVPKSPVEEKGKSPVPKSPVEEKGKSPVPKSPVEE
KGKSPVSKSPVEEKAKSPVPKSPVEEAKSKAEVGKGEQKEEEEKEVKEAPKEEKVEKK
EEKPKDVPEKKKAESPVKEEAVAEVVTITKSVKVHLEKETKEEGKPLQQEKEKEKAG
GEGGSEEEGSDKGAKGSRKEDIAVNGEVEGKEEVEQETKEKGSGREEEKGVVTNGLD
LSPADEKKGGDKSEEKVVVTKTVEKITSEGGDGATKYITKSVTVTQKVEEHEETFEEK
LVSTKKVEKVTSHAIVKEVTQSD

Bead-Spring Polyampholyte

- Basic system
  - 4 bead types: +, —, hydrophobic (poor solvent), hydrophilic (good solvent)
  - M = 36, 100 grafted chains of N monomers
  - triangular lattice: spacing = 7.2 nm or area/chain = 45.6 nm^2 (from experiments)
  - 100 mM salt
  - NF: L (N=87), M (487), H (613)

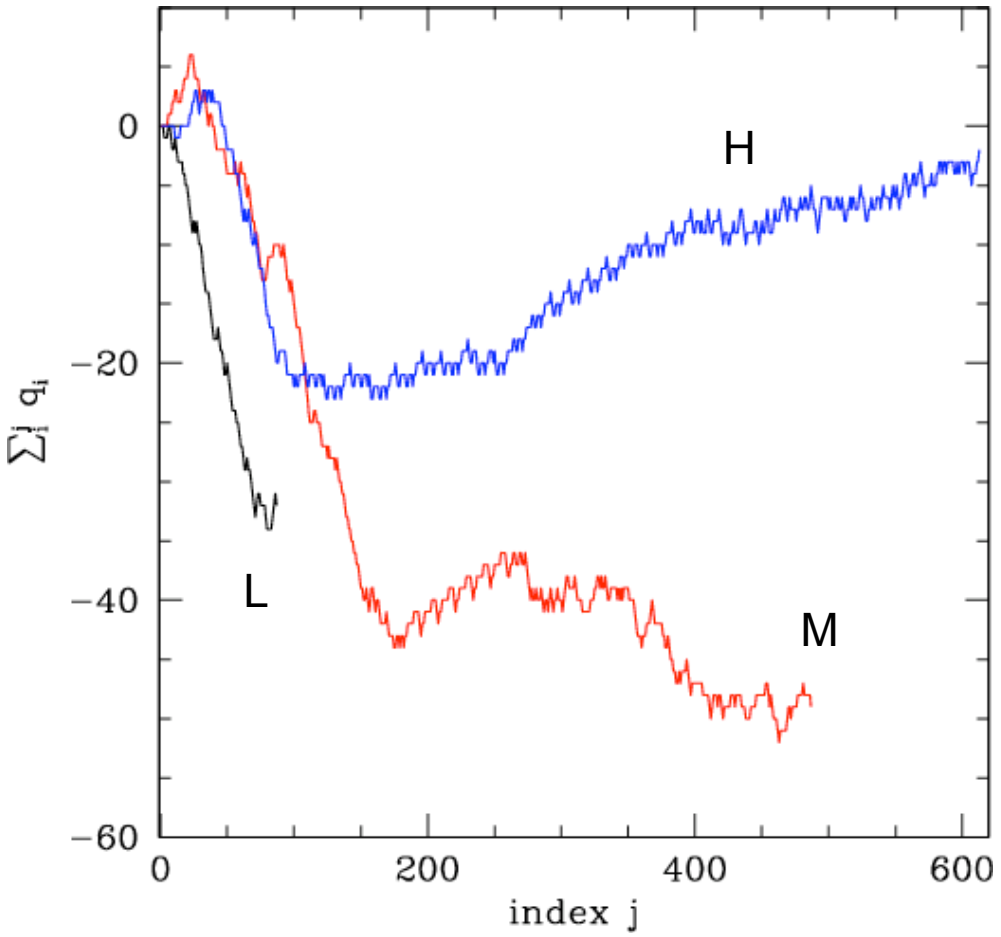




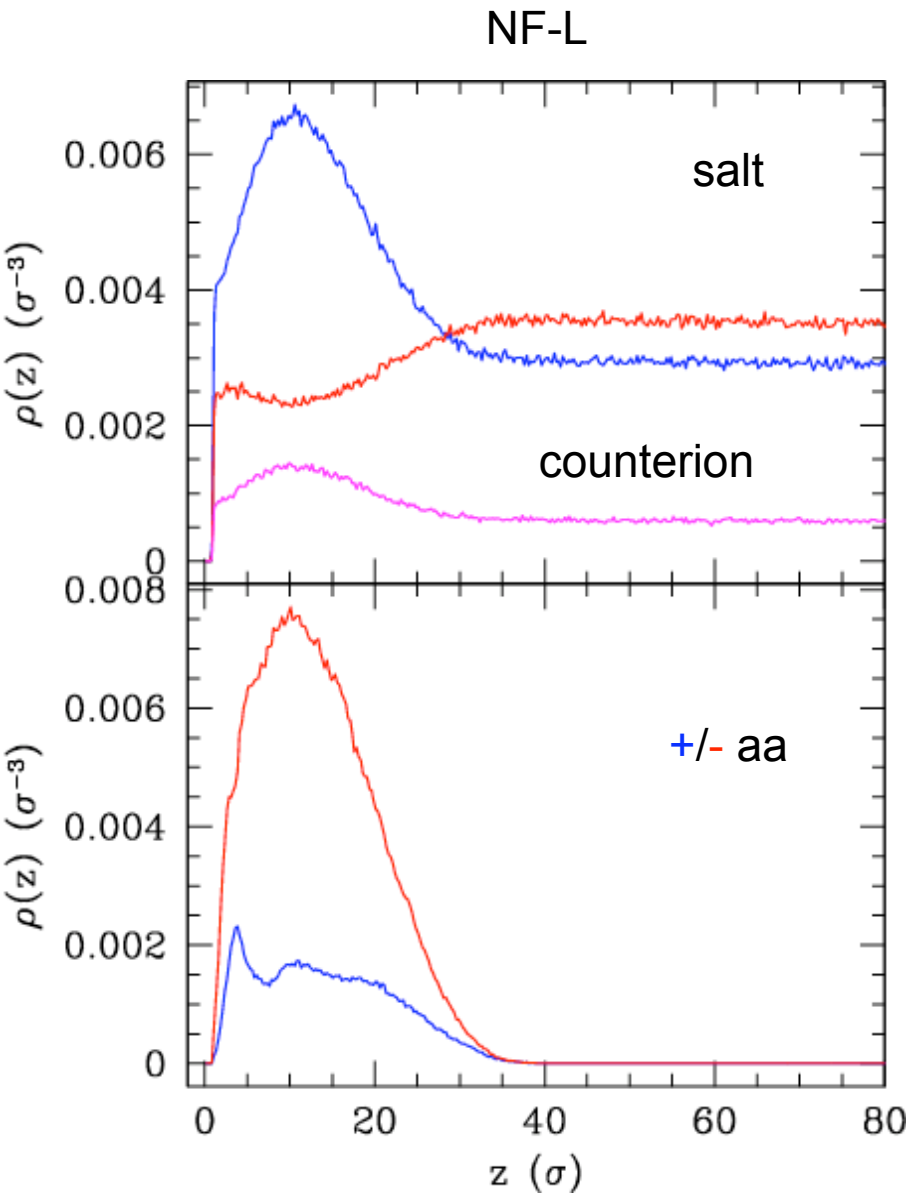
NF-M

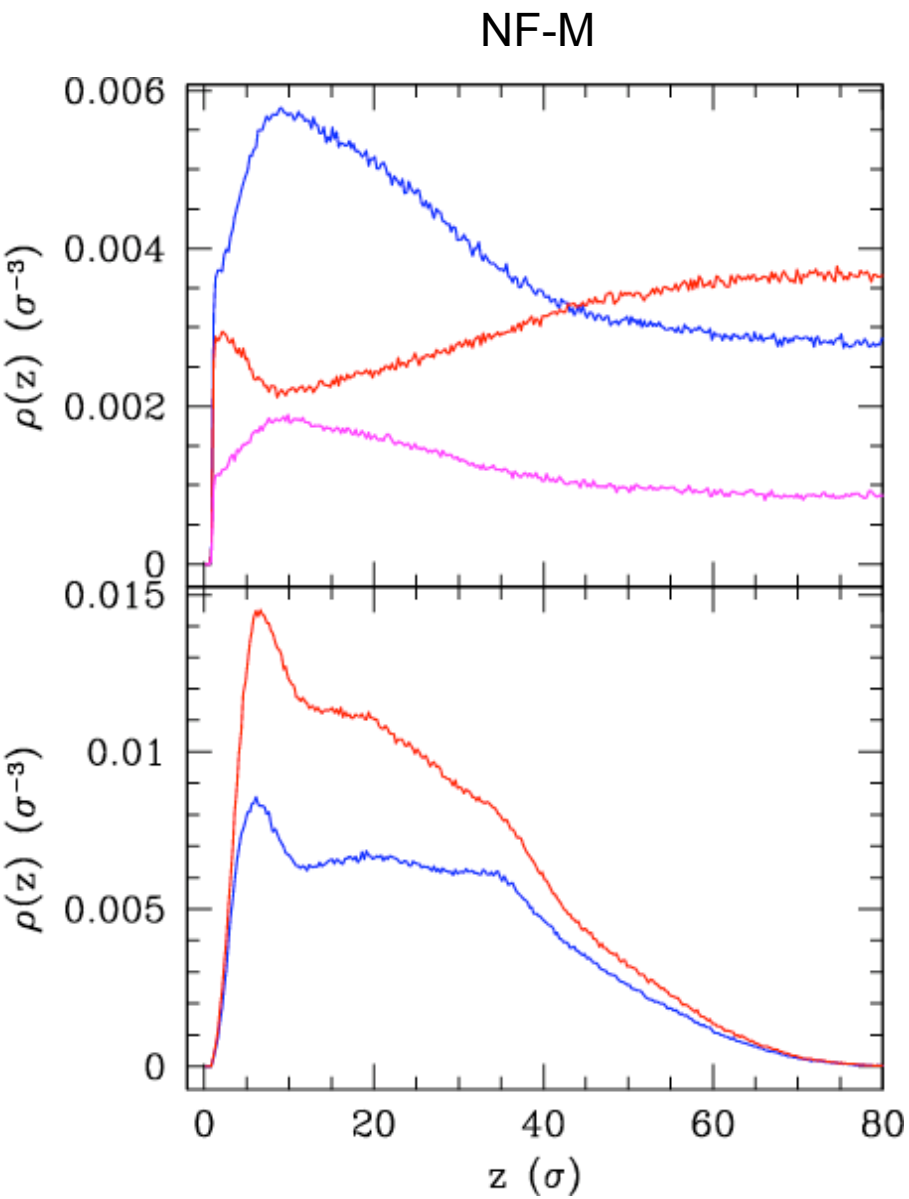
Charge as a function of sequence

Plots of the total charge as a function of position along the sequence of the NF tails shows the similarities and differences among the 3 types. All 3 types have an initial region that is negatively charged. For the short NF-L, that is all there is. For NF-M, the chain is increasingly negatively charge until the 180th aa and then the sequence is predominantly neutral. For NF-H the amount of initial negative charge is smaller than the other two and the latter half of the sequence is positive almost yielding a neutral chain. In cells, NF-H is highly phosphorylated making the chain net negative.



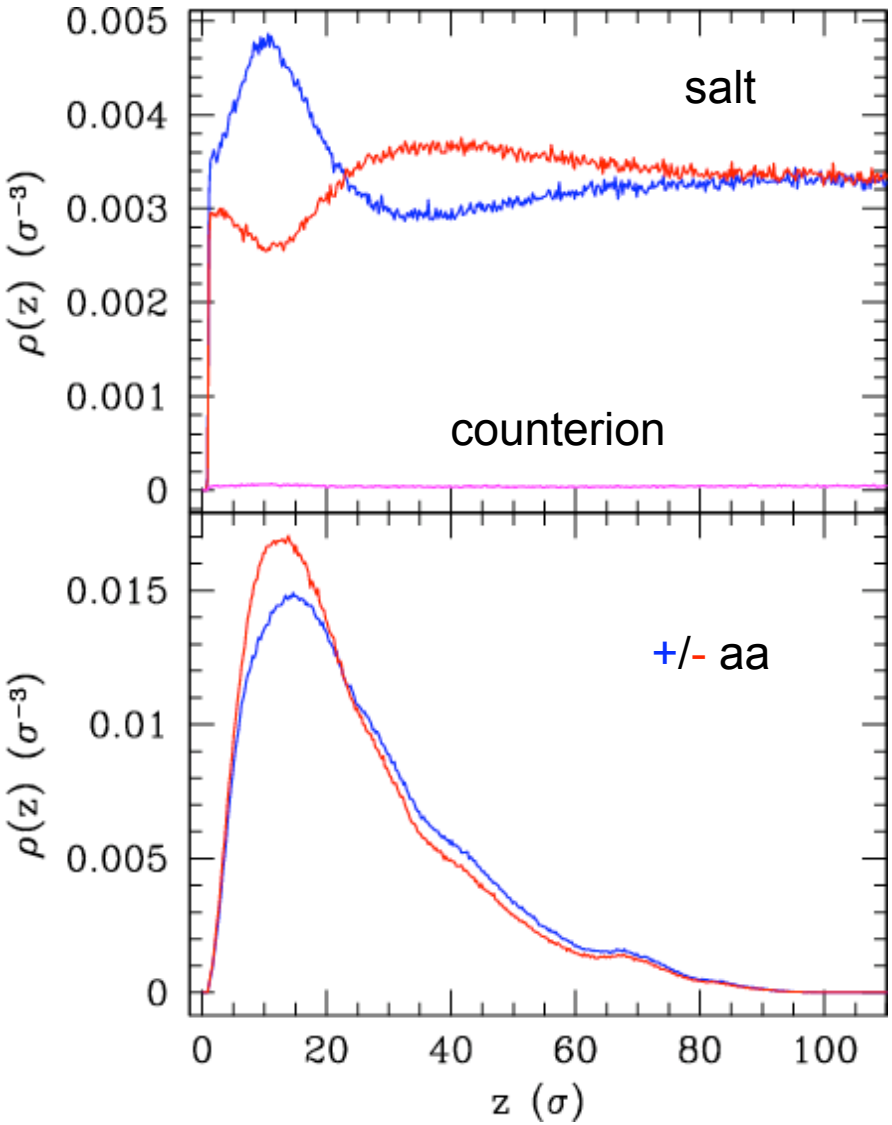
Density Profiles

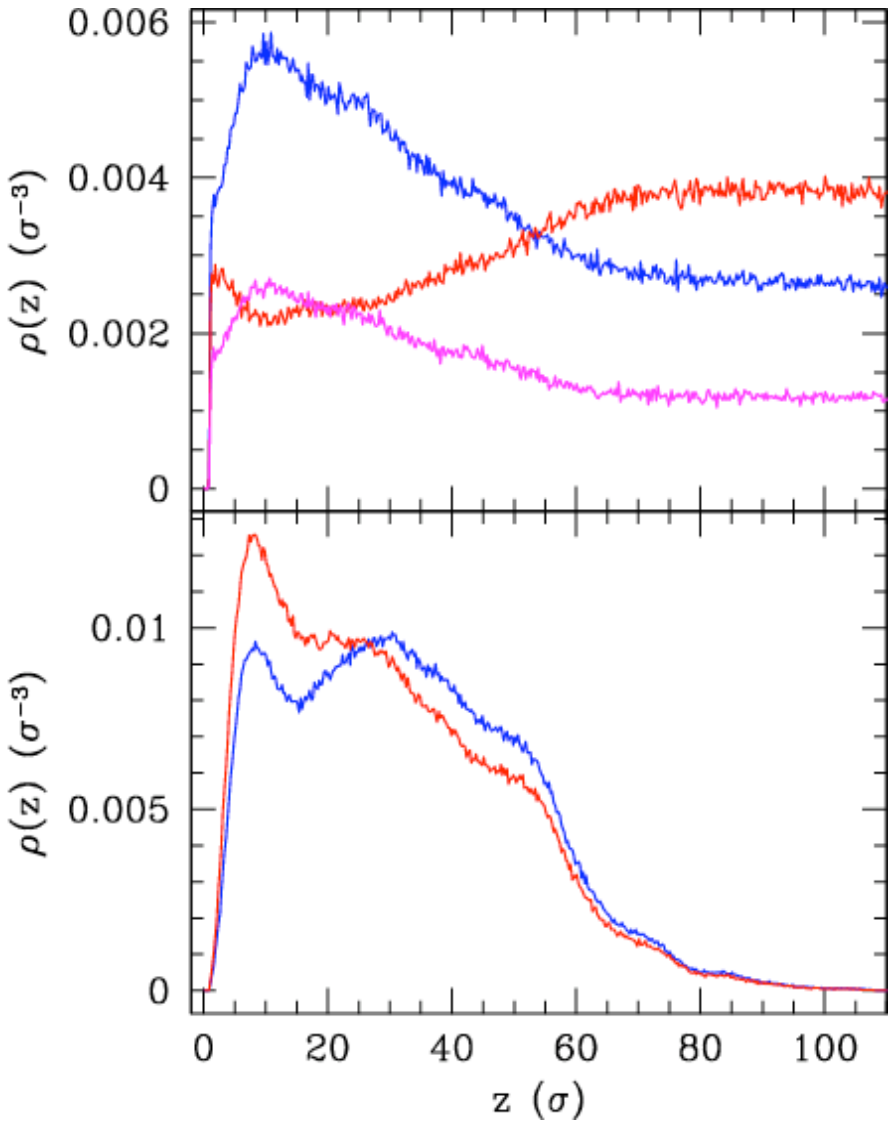




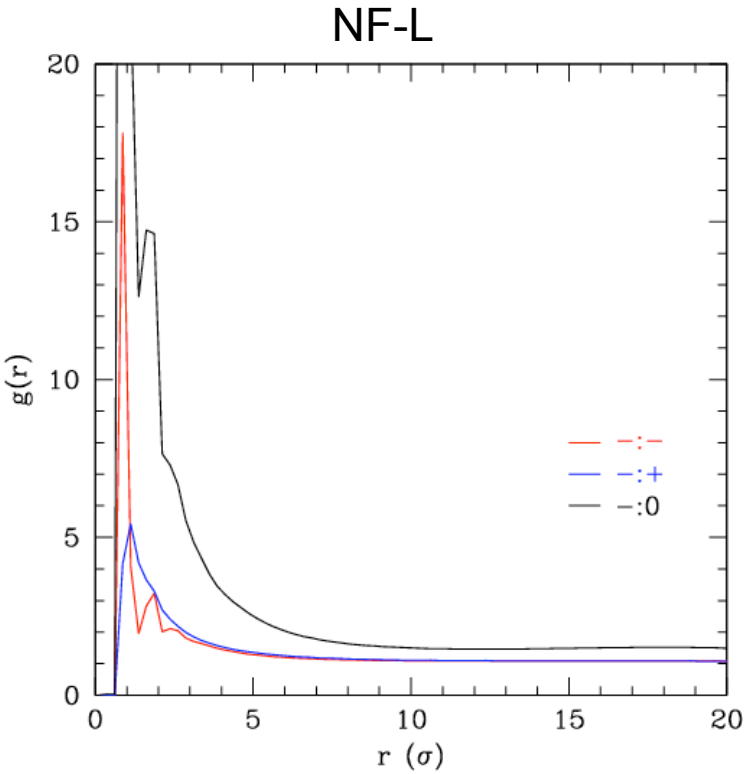
Density Profiles

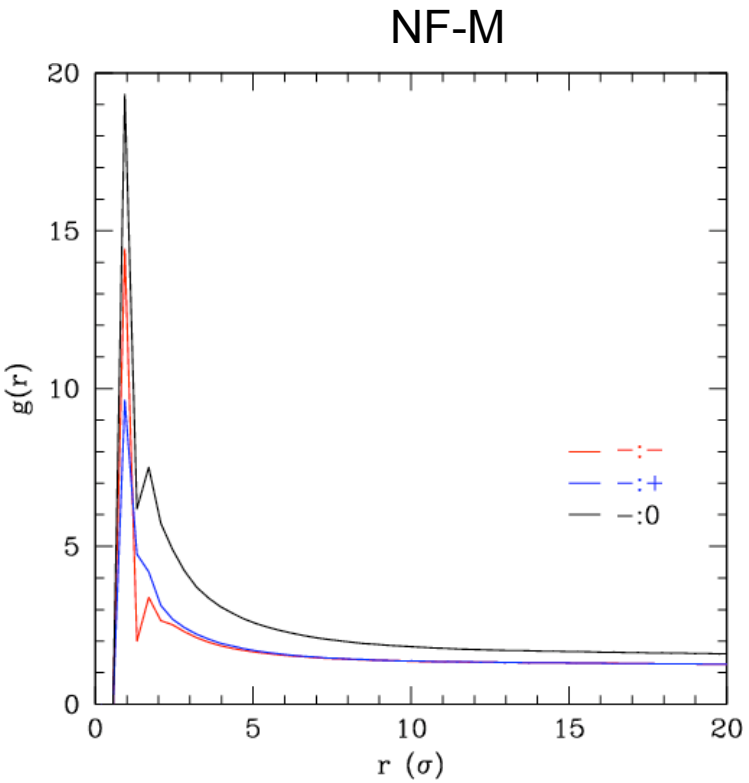
NF-H (Q=-2) (not equilibrated) NF-H+phos Q=-70





Radial Distribution Functions





The rdfs were calculated based on the charge of the bead. Since NF-L and NF-M are net negatively charged, we plot the rdfs for negative beads with respect to negative, positive and neutral beads. All the g(r) decay on a scale smaller than the spacing between chains in the brush (19 σ). The large peaks in the —:— g(r) represent the large number of repeats in the amino acid sequence. However, the function has decayed to 1 by 5 s, implying that there is plenty of salt nearby screening the interactions.

Final Comments

Comparison with SCFT calculations of Zhulina *et al*. Biophys. J 2007.

We are seeing more features in the density profile than the SCFT calculations give. Our simulations are for 100 mM salt concentration and the SCFT calculations have been done for 10-1000 mM. The SCFT calculations have been done for the natural mixture of L, M and H. Direct comparison must really wait for further simulations. In any case it will be interesting to see what the result is for mixtures. One of the insights in the Zhulina work is that the different NF types tend to occupy volumes at different distances from the substrate.

To Do:

- natural mixture of L, M, H
- grafted to a line (cylinder) and examine effect of geometry
- force between two grafted surfaces as a function of separation