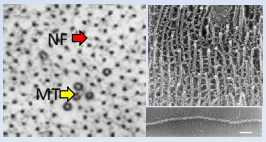
Neurofilaments: from disordered subunits to a well ordered complex

M. Kornreich*, E. Malka-Gibor*, A. Laser-Azogui, O. Doron, R. Beck
School of Physics and Astronomy, Center for Nanosciecne and Nanotechnology, Tel Aviv University, Israel
*michakor@post.tau.ac.il, *etimalka@post.tau.ac.il

What are Neurofilaments?

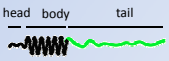
Neuronal cell-specific cytoskeleton intermediate filaments that have direct influence on axonal diameter and mechanical properties. Neurofilaments expression changes during cell development, growth and recovery from injury. The interaction between filaments is mainly driven by the intrinsically disordered C-terminal tail domains of the proteins.



Fuchs & Cleveland, Science. **279**, 514 (1998)
Hirokawa, Science **279**, 519-526 (1998)
N. Hirokawa, Trends in Cell Biology **6**, 135-141 (1996)

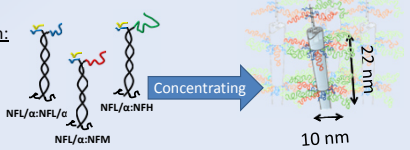
Self-Assembly of filaments

4 subunit proteins

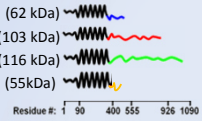


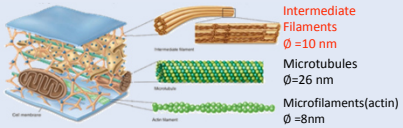
Coil-coiled interaction:

- → Dimers
- → Tetramers (2 dimers)
- → Filament (tetramers)



- **Neurofilaments triplet**:
  - NF-Low (62 kDa)
  - NF-Medium (103 kDa)
  - NF-High (116 kDa)
- **a-Internexin** (55kDa)

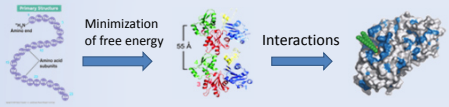




Bio 101: Structured Proteins

Sequence-to-structure-to-function paradigm

Sequence to Structure to Function



Mirsky, A. E. & Pauling, L. (1936), Anfinsen CB (1973)
Tony Futerman's Lab, Weizmann institute

Bio 101': Unstructured

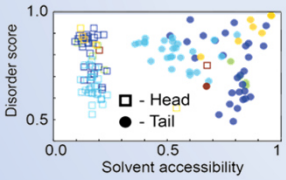
~50% lack unique 3D structure

3-D structure is not a prerequisite for function

Richfield, D. "Medical gallery of David Richfield 2014".
Dyson HJ and Wright PE, Nat. Rev. (2015)

Disorder in intermediate filament proteins

We employ bioinformatical analysis of 70 human intermediate filament proteins to reveal their common and differentiating properties.

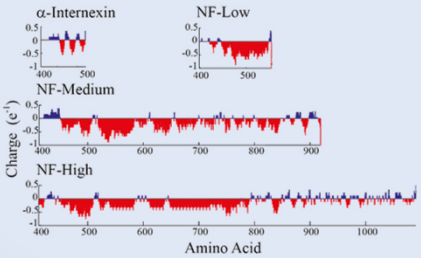


- Disorder is conserved
- Tails are solvent accessible
- Neuronal tails are unique in charge and length

Kornreich M. *et. al.*, FEBS Letters (2015)

Inter-filament interaction through polyampholytic Bottle-Brushes

The sidearms mediate both repulsive and attractive interactions between neurofilaments in condensed hydrogel.

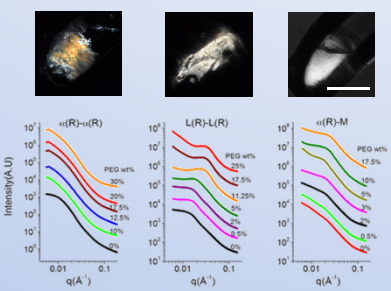


The effects of protein composition and tail charge on the hydrogel is revealed by small-angle X-ray and polarizing microscopy

Measurement of force and inter-filament distance with induced osmotic pressure shows differences in mechanical response and structure.

Cross- polarizing microscopy shows nematic domains in NF-L and co-polymer networks compared with a diffusive scattering observed in α- int networks, indicative of many micro- domains.

Small angle X ray scattering profiles of α-Inx agree with these findings: unlike NF-L and co-polymer networks, no translational order is observed for the filaments.



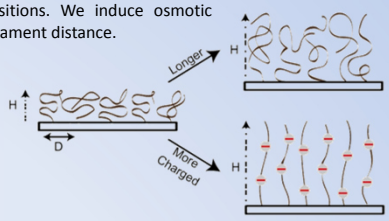
Kornreich M., Malka-Gibor E. *et. al.*, Soft Matter (2015)

Composition → Structure & Mechanics

In order to reveal the individual structural and mechanical roles of each tails, we self-assemble networks of various compositions. We induce osmotic pressure and measure the corresponding inter-filament distance.

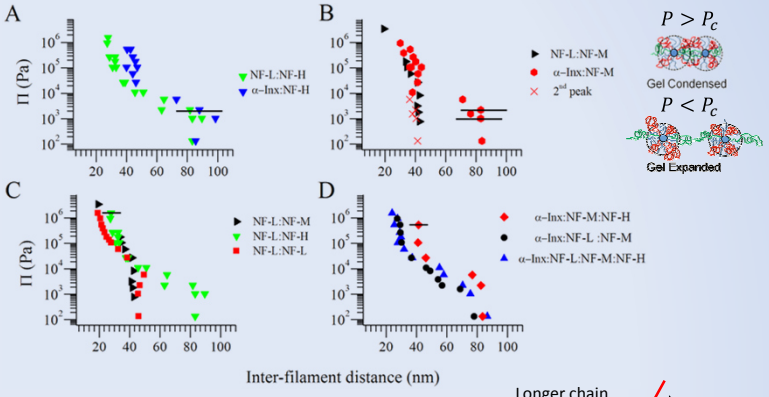
What do we "naively" expect?

Polymer brush theory predicts that longer, more charged tails, form a more expanded layer.



Results

We find a general mechanical response trend: an expanded conformation at low pressures and a collapsed, stiff, conformation at high pressures. The transition from gel-expanded to gel-condensed occurs at $\Pi \approx 10^4$[Pa], and is irreversible.

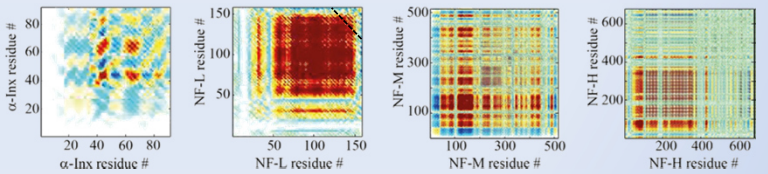


Longer chain
More charge
expansion

Handshake analysis matrices

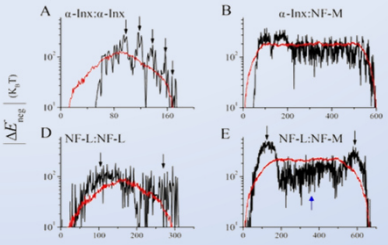
Complementary amino-acid sequences that contribute to opposite chain interactions are represented by a matrix element:

$$\Delta E(n,p) = k_e \sum_{i=-w/2}^{w/2} \sum_{j=-m}^{m} \frac{eZ_1(n+i)eZ_2(p-i-j)}{|\vec{r}_1(n+i)-\vec{r}_2(p-i-j)|}$$



Cumulative electrostatic attraction

We sum the negative energy along the constant $C = n_1 + n_2$. The results show significant deviations from random charge distributions.

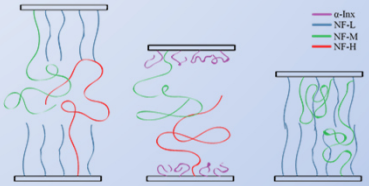


$c = n_1 + n_2$ ~ interfilament spacing

Permuted sequences (polymer)
Based on sequence (protein)

Botanics of neurofilaments

Our results and analysis suggest a novel brush phase, termed "truffle". The new phase is the result of electrostatic binding sites situated on the disordered, flexible, rails.



brush & Flower | collapsed brush & flower | brush & truffle

Kornreich M., Malka-Gibor E. *et. al.*, Soft Matter (2015)

Synchrotron experiments performed at diamond SOLEIL MAX IV

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Tel Aviv University Center for Nanoscience & Nanotechnology