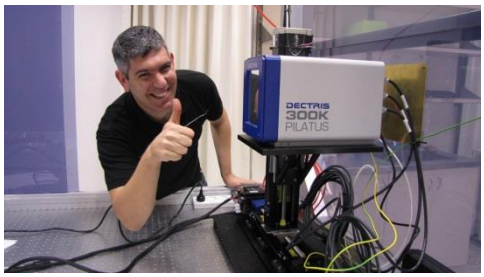
"Self-Assembly, Structures and Interactions of Cell Specific Cytoskeleton"

EU FP7- Career Integration Grant, Dr. Roy Beck, Tel-Aviv University

Our research is motivated by the desire to find a quantitative description for biological systems and interactions using the analysis toolkit of physicists. Using the CIG grant, we studied the complex self-assembly nature of neurofilaments, the cytoskeletal proteins of the nervous system, and in particular the forces maintained by their intrinsically disordered domains.

The concept that a given amino-acid sequence of a protein will not form a stable 3D folded structure but still have biological functionality has been developed only in the last ~15 years. The discovery rate and characterization of such intrinsically disordered proteins have been increasing continually, becoming one of the fastest growing areas of proteomics. It is now estimated that 40-50% of eukaryotic proteins contain large intrinsically disordered domains, involved in a wide range of cellular functions including transcription, translation, signaling and regulation of protein assembly. Structural flexibility and plasticity originating from the lack of an ordered structure suggest a major functional advantage for these proteins, enabling them to interact with a broad range of binding partners.

Our expertise lies in extracting inter and intra-molecular interactions from the self-assembled structures they form under different conditions using small angle X-ray scattering (SAXS) technique. Our methodology is to map structural changes as a function of subunit compositions and/or environmental conditions (*e.g.* temperature, osmotic pressure, salinity, *p*H etc.) into highly informative "phase diagrams". Such diagrams are not common in biology but are an instrumental tool in physics since they can be supported by theoretical modeling and calculation as well as affording a way to sort out current conflicting paradigms.



Dr. Roy Beck at his SAXS laboratory in TAU

The cytoskeleton is the construction scaffold of the cell, which supports its shape and provides its elasticity. There are three major cytoskeleton proteins groups – microfilaments, microtubules and intermediate filaments (IF). While the first two are common among all cell types, IF proteins are cell-type specific and therefore are a natural candidate to meet cell-type specific mechano-elastic requirements. In neurons, different IF proteins are expressed at different developmental stages and it is speculated that this sequential expression is related to altered mechano-elastic needs of a neuron during its development.

From a physical point of view, IFs in general and neuronal IF in particular, are interacting self-assembled polymers with bottlebrush geometry. This geometry is composed of flexible backbone and radiating long intrinsically disordered tail domains. Therefore, the interactions between filaments lead

eventually to the viscoelastic properties of their dense hydrogel networks. It is therefore vital to unravel these interactions in order to properly characterize their functionality in the cytoskeleton.

Using synchrotron SAXS as well as electron, light and atomic force microscopy, we investigated the structural and mechanical properties of neuronal IF hydrogel networks. Most importantly, we showed that the properties of composite filament networks are the result of synergistic interactions between the short and long proteins. In contrast to previous predictions, we demonstrate that the short proteins have a key role in determining the neuronal IF hydrogel network properties and inter-filament spacing. Our study explored filaments comprising different subunit compositions to unravel the individual physical and structural roles of neurofilament proteins in the hydrogel network.

PhD student, Micha Kornreich, at synchrotron SAXS beamline

Moreover, our findings suggested a structural explanation for the differential expression pattern of neuronal IF proteins during embryonic development. We found that composite filaments of α-Inx and NF-M form an expanded network with ~80 nm inter-filament spacing. These proteins are expressed during early developmental stages. In contrast, the network generated from NF-L and NF-M composite filaments is condensed, exhibiting a reduced inter-filament spacing of ~40 nm. The expression level of these proteins increases postnatally. We show that a "mature" combination of any three or all four proteins results in expanded networks. These findings explain the functional role of α-Inx which was only recently found to be the fourth neuronal IF subunit protein. It thus emphasizes the importance to address α-Inx expression levels in future studies of neuronal development .

From a physical point of view, the C-terminal tails can be studied as polymer brushes: arrays of macromolecules end-attached to a substrate. However, neuronal IF protein tails are polyampholyte brushes, *i.e*, containing both negative and positive charges. Such brushes display a richer behavior than their neutral counterparts which we discuss in detail in our analysis. Our experimental findings are explained by an ionic-bridge model taking into account the specific charge heterogeneity of each subunit protein. This model captures the essence of the interactions leading to the observed non-trivial hydrogel properties, where short neutral brushes induce network expansion.