# Nanoscale myelin ultrastructural dynamics from out-of-equilibrium functional state to degraded state

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Ultrastructural fluctuations at nanoscale are fundamental to assess properties and functionalities of advanced out-of-equilibrium materials. We have taken myelin as a model of supramolecular assembly in out-of-equilibrium living matter. In this physical condition, structural fluctuations at nano- and meso-scales are needed to understand the physics behind its biological functionality. Myelin sheath is a simple stable multilamellar structure (Figure 1) of high relevance and impact in biomedicine. Although it is known that myelin has a quasi-crystalline ultrastructure [1], there is no information on its fluctuations at nanoscale in different states due to limitations of the available standard techniques. To overcome these limitations, we have used scanning micro X-ray diffraction, which is a unique non-invasive probe of both reciprocal and real space to visualize statistical fluctuations of myelin order of the sciatic nerve of Xenopus laevis. The results show that the ultrastructure period of the myelin is stabilized by large anticorrelated fluctuations at nanoscale, between hydrophobic and hydrophilic layers. The ratio between the total thickness of hydrophilic and hydrophobic layers defines the conformational parameter, which describes the different states of myelin. Our key result is that myelin in its out-of-equilibrium functional state fluctuates point-to-point between different conformations showing a correlated disorder described by a Levy distribution [2,3]. As the system approaches the thermodynamic equilibrium in an aged state, the disorder loses its correlation degree and the structural fluctuation distribution changes to Gaussian (Figure 2). In a denatured state at low pH, it changes to a completely disordered stage [2].
Moreover, there is no information on the relationship between this correlated disorder and the dynamics of the intrinsically disordered Myelin Basic Protein (MBP) [4] which is expected to influence the membrane structure and overall functionality. Then, we have investigated the role of this protein structural dynamics in the myelin ultrastructure fluctuations in and out-of-equilibrium conditions, by using synchrotron Scanning micro X Ray Diffraction and Small Angle X ray Scattering. We have induced the crossover from out-of-equilibrium functional state to in-equilibrium degeneration changing the pH far away from physiological condition. While the observed compression of the cytosolic layer thickness probes the unfolding of the P2 protein and of the cytoplasmic P0 domain ($P0_{cyt}$), the intrinsic large MBP fluctuations preserve the cytosol structure also in the degraded state. Thus, the transition of myelin ultrastructure from correlated to uncorrelated disordered state, is significantly affected by the unfolding of the P2 and P0 proteins, which in this latter state do not act in synergistic manner with MBP to determine the membrane functionality (Figure 3) [5]. Our results aim to clarify the degradation mechanism in biological systems by associating these states with ultrastructural dynamic fluctuations at nanoscale.

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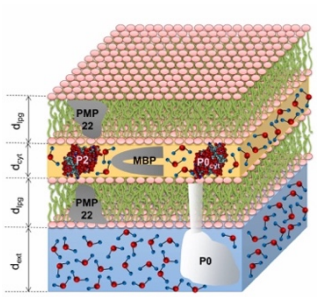


Figure 1. Myelin structure and proteins.

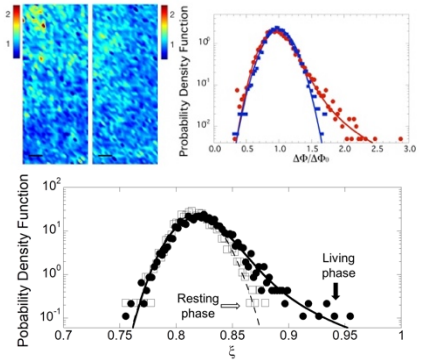


Figure 2. levy distribution on living state and gaussian after aging. Up) angle orientation of the axons; Down) conformational parameter.

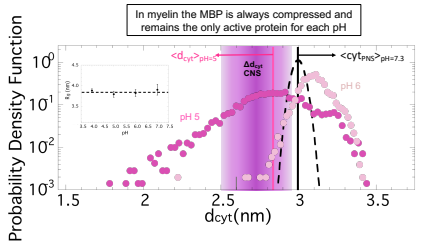


Figure 3. PDF of $d_{cyt}$ , at pH 5, in comparison with samples at pH 7.3 and pH 6 in semi-log plot. Insert) Constant MBP radius of gyration as a function of the pH. MBP is always compressed and the only active protein at each pH.