Intrinsic dynamical fluctuations of PNS myelin ultrastructure

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Abstract

The ultrastructure fluctuations and complex dynamics of the multi-layered membrane structure of myelin are fundamental for understanding and control its formation process and its degeneration and repair in neurological diseases such as multiple sclerosis (MS). Myelin is considered a liquid-crystal but there are no information on its multiscale fluctuations from nanoscale and mesoscale due to limitations of the available standard techniques. To overcome this limitation in this work we have used Scanning micro X-ray Diffraction (SµXRD), which is a unique non-invasive probe of both k-space and real space allowing to visualize statistical fluctuations of myelin order with high spatial resolution in real space. We have used this method to examine the myelin sheath in the sciatic nerve of Xenopus laevis. We have found first that the myelin ultrastructure period is stabilized by large anti-correlated fluctuations at nanoscale, between hydrophobic and hydrophilic layers. Our key result is that the correlated disorder of myelin spatial ultrastructural fluctuations follows a Levy distribution in the functional state, which is lost with early stage degeneration. Our results open could open new venues for understanding formation and degradation in biological ultrastructure by associating these states with dynamical structural fluctuations at nanoscale.

Keywords: micro X Ray Diffraction, Myelin, Correlated disorder

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**Introduction**

The compact myelin sheath is an elaborated multi-layered membrane that surrounds selected axons in the central and peripheral nervous systems (CNS, PNS) of vertebrates. It is fundamental for normal nervous system function. Its main role is to decrease the capacitance of the membrane; it covers the axons, so that the speed of propagation of action potentials is increased via saltatory conduction, facilitating nerve signal transmission. Myelin, respect to the other biological membranes, have a dry mass very lipid-rich, containing 75-80% of lipid and only 25-20% of proteins. The major lipids in PNS myelin are cholesterol, phospholipids and galactolipids, which are important for membrane structure and assembly. The major PNS compact myelin proteins are myelin basic protein, MBP, an unstructured protein, (present at only 5-18%), the major structural protein of PNS myelin P0 glycoprotein, the peripheral myelin P2 and the peripheral myelin protein-22 (PMP-22). These proteins interact closely with the membrane.

Recently, there is increasing interest on the dynamics arising from these interactions, for a better understanding neurodegenerative diseases, such as multiple sclerosis and nerve injuries. Most of the studies have been focused on the dynamics of the atomic structure of individual proteins and on understanding their structure and function. Other studies have been focused on the influence of the proteins on the membrane dynamics, identifying the protein that control the fluctuations of the membrane layers thickness. Also the myelin biogenesis has been studied, showing the dynamic development of the sheath and of his chemical components and his metabolities. There have been other studies on the dynamics of the diseases that incur on myelin and on the dynamics of re-myelination and repair of the myelin sheath after a trauma. In addition, we mention the change of the internal dynamics as response to the variation of the myelin humidity. In this framework, there is interest and need to investigate the functional heterogeneity and the intrinsic structural fluctuations of myelin for a better understanding of myelination and demyelination processes and to discover the dynamic of supramolecular structure at nanoscale.

Myelin ultrastructure has been studied using a wide variety of approaches, the most used are X-ray diffraction (XRD), electron microscopy (EM) and neutron diffraction. The myelin ultrastructure can be seen as a multilamellar lattice with the repeating of a structural unity constituted by the stacking of the four following membranes: i) cytoplasmatic (cyt), ii) lipidic (lipid polar group, lpg), iii) extracellular (ext) and iv) another lipidic (lpg).
This structural unit appears to be very stable because of intrinsic limitations of used experimental methods. Indeed, conventional XRD is limited since it provides insight only into the periodic structure of myelin probing the k-space (or reciprocal space) with no spatial resolution. On the other hand, electron microscopy approach (e.g. TEM) is a local, highly spatially resolved probe, but it is an invasive probe, which suffers from sample fixation and dehydration artefacts.

Therefore, we need a non-invasive probe with high spatial resolution to map functional spatial structure fluctuations. At this aim, we used Scanning micro X-ray Diffraction (µXRD), which probes with high resolution both the k-space (reciprocal space) and real space inhomogeneity of complex materials; in a second step we applied tools of statistical physics to the diffraction collected data to unveil the “statistical distributions of the fluctuating structural order”. This approach has been recently used to carry out a mapping of biological tissues with high spatial resolution, such as bone\textsuperscript{38}, cells\textsuperscript{39} and myelin\textsuperscript{18,40}.

In this work, we report the statistical fluctuations of myelin order structure of sciatic nerve extracted from Xenopus laevis. We have measured the spatial fluctuations of the four membranes thickness at thousands of discrete locations in freshly extracted and aged Xenopus sciatic nerves using a short time scale for data collection, obtained by using bright synchrotron radiation focused on 1\(\mu\)m\(^2\) spot and the use of precise micro translation stages. Thus, we applied statistical physics tools to probe the structural statistical fluctuations of myelin nanoscale order. Our results indicate that a) the quasi-crystalline periodicity of the myelin lattice in the functional state is due to intrinsic anti-correlated fluctuations between the lipidic hydrophobic layers and the cytoplasmatic and external hydrophilic layers; b) the probability distribution of the fluctuations between the hydrophobic and the hydrophilic layers shows a fat tail probability distribution in the functional, fresh nerve. In the early stage of degeneration due to nerve aging, despite the constant thickness of the structural unit, both the spatial fluctuations of membranes thickness and their spatial correlations decrease.

**Results and discussion**

The experimental apparatus for µXRD is shown schematically in Figure 1A. The scheme of the multilamellar ultrastructure of myelin is shown in Figure 1B. It is made of the stacking of i) cytoplasmatic (cyt), ii) lipidic (lpg), iii) extracellular (ext) and iv) another lipidic (lpg\textsuperscript{31,32,33}) layers. The individual thickness of each layer in 1 \(\mu\)m\(^2\) area, named \(\lambda_c\), \(d_{cyt}\), \(d_{lpg}\), \(d_{ext}\), have been extracted.
from electron density profiles computed by Fourier analysis of the diffraction patterns, as described in details in Materials and Methods (Figure 1).

We have built the maps of the thickness of structural unit, $\lambda$, and of each layer of myelin, $d_{cyt}$, $d_{lipg}$, $d_{ext}$ in 400x125 $\mu$m$^2$ central Regions of Interest (ROI) selected along the vertical axis of the nerve shown in Figure 2A, 2C, 2E and 2G, respectively. The Probability Density Function, PDF, of these thicknesses are shown in Figures Figure 2B, 2D, 2F and 2H respectively. From these probability distributions we have extracted the mean values, $\mu$, and the standard deviations, $\sigma$, for each thickness; in this way we have calculated the spatial fluctuations of each layer defined by the relative standard deviation, RSD=$\sigma/\mu$, on the whole ROI. Let us consider the period (Fig. 2A, 2B). The average myelin periodicity of the nerve, $\lambda$, is found to be 17.350 $\pm$ 0.024 nm, in agreement with previous works$^{18,32}$. Its spatial fluctuation, $\sigma(\lambda)/<\lambda>$, results to be about the 0.14%, quite smaller than the fluctuations for the other individual substrates, having RSD between 1.1-1.7% as reported in Table 1. Here we meet the “paradox of myelin sheath” quasi-crystallinity due to the tinier spatial fluctuation of period in comparison with the larger sublayers’ fluctuations.

To shade light on this paradox, we have studied the correlations between the spatial fluctuations $\sigma(d_{lipg})/<d_{lipg}>$, $\sigma(d_{cyt})/<d_{cyt}>$ and $\sigma(d_{ext})/<d_{ext}>$. At this aim, we have first calculated the Pearson’s correlation coefficients, $c_{ij}$, between maps of cytosolic apposition (Fig. 2C), extracellular apposition (Fig. 2G), lipidic membrane (Fig. 2E) and the results are shown in Table 2. We observe that the spatial fluctuations of $d_{cyt}$ are smaller than those of $d_{ext}$ but waving together in the same direction, being $c_{cyt-ext}$ positive; on the other hand fluctuations of $d_{lipg}$ go in the opposite direction since $c_{lipg-ext}$ and $c_{cyt-lipg}$ are negative. This means that the lipid membrane fluctuations are strongly anti-correlated with both cytosolic and extracellular layers fluctuations. It is natural to associate this anti-correlation with the anti-correlated dynamics of the myelin proteins shown in Fig. 1B$^{3,10,11,12}$.

These fluctuations and their anti-correlation can be measured and visualized by introducing the appropriate conformational parameter, $\xi$, characterizing the state of myelin through the ratio between hydrophilic and hydrophobic layers:

$$\xi=(d_{ext}+d_{cyt})/2d_{lipg}.$$  

A typical map of $\xi$ measured on a central 400x125 $\mu$m$^2$ ROI is shown in Figure 2I. The red spots represent areas where hydrophilic layers are larger, while in the blue spots they become smaller and
the thickness of hydrophobic layer increase. The PDF of $\xi$ shows a skewed line shape modelled by using Levy stable distributions\textsuperscript{41} (see Figure 2L). The Levy stable distribution provide a general statistical description of complex signals deviating from normal behaviour and in recent years have found increasing interest in several applications in diverse fields\textsuperscript{42-46}. This class of probability distributions are generally represented by a characteristic function defined by four parameters: stability index $\alpha$, skewness parameter $\beta$, scale parameter $\gamma$ taking into account the width of the distribution, and location parameter $\delta$ with varying ranges of $0<\alpha\leq2$, $-1\leq\beta\leq1$, $\gamma>0$ and $\delta$ real. We stress the fact that the closed form expressions of density and distribution functions of Levy stable distributions are not available except for few particular cases such as the well-known Gaussian distribution where the stability parameter $\alpha$ is 2. Here we have used basic functions in the numerical evaluation of these parameters and goodness of data fitting as described by Liang and Chen\textsuperscript{47}. The Levy fitting curve, indicated by the continuous line in Figure 4B, gives a stability index of 1.78 ($<2$), a location of 0.8242, a skewness of 1 and a scale parameter $\gamma$ of 0.0138. The fact that the conformational parameter, $\xi$, is always less than 0.9 grabs that myelin has a high lipid content\textsuperscript{4}. The ultrastructure dynamic and the myelin quasi-crystallinity paradox can be seen in a compact and clear way in Figure 2M where the dispersion of the layers, $\Delta d_{\text{ext}}$, $\Delta d_{\text{lpg}}$, $\Delta d_{\text{cyt}}$ and the period, $\lambda$, are plotted as a function of $\xi$. We observe that the almost total independence of the period from $\xi$ is due to the positive correlations between the fluctuations of hydrophilic layers, which increase with increasing $\xi$, and their anti-correlation with hydrophobic lpg fluctuations, which decrease with the increase of $\xi$. This clearly shows that hydrophilic fluctuations compensate the hydrophobic fluctuations to keep the period almost constant giving the apparent crystalline character to the myelin ultrastructure.

In order to check that the described structure fluctuations are an intrinsic feature of the functional state of myelin, we have measured an aged state of the unfresh myelin of a sciatic nerve left 18 hours in a Ringer solution after the dissection. From a biochemical point of view, we expect a loss of protein dynamics\textsuperscript{3,10,11,12}, owing to the decreasing ATP content with time. In fact ATP provide the energy to the myelin, controlling its functionality and degeneration\textsuperscript{48,49}. The spatial distribution of the ultrastructure of the unfresh myelin is shown in Figure 3. Typical maps of the $\lambda$, $d_{\text{cyt}}$, $d_{\text{lpg}}$, $d_{\text{ext}}$ thicknesses in a central (400x125) jm$^2$ ROI of the nerve are shown in panels 3A, 3C, 3E and 3G, respectively, and the PDF of these thicknesses in panels 3B, 3D, 3F and 3H. The dashed lines represent the PDF of the thicknesses in the functional fresh nerve, for comparison. The average myelin period, $\lambda = 17.350 \pm 0.021$ nm, results to be the same as in the fresh state. This indicates that the myelin is not yet in any degenerate state where the period is
expected to change significantly\textsuperscript{18,32}. On the other hand, we observe that both the spatial fluctuations and the mean value of each individual layer change in comparison with the fresh functional state. In particular, the spatial fluctuations are found to decrease significantly in the \textit{unfresh} nerve, as we can see by comparing the RSD values in \textbf{Table 1a} and \textbf{Table 1b}. In particular, we observe that the lipidic hydrophobic thickness, $d_{\text{lip}}$, reaches smaller values down to about 4.6 nm in the fresh functional state in comparison with the unfresh state. At the same time the hydrophilic extracellular layer reaches larger values up to 5.1 nm in the fresh nerve while it does not exceeds 5 nm in the \textit{unfresh} state. The decreasing thickness fluctuations in the unfresh nerve are accompanied by the reduction of the spatial correlations, as indicated by comparing the correlation coefficients between cytosolic, extracellular and lipidic membrane apposition in \textbf{Table 1b} and \textbf{Table 2b}.

Thus, although the maintained stability of the period in the aged unfresh nerve we find \textit{i)} decreasing fluctuations and \textit{ii)} decreasing spatial correlations between fluctuations. This means that the aged system acquires rigidity and order, losing correlated disorder. This is quite intriguing since it tells us that in the living system the functionality is associated to the correlated disorder while the early degeneration shows the tendency towards a \textit{frozen-like} state with mayor rigidity and order. These results can be clearly summarized in \textbf{Figure 3I} where we can compare the dispersions of the layers, $\Delta d_{\text{ext}}$, $\Delta d_{\text{lip}}$, $\Delta d_{\text{cyt}}$, and the period, $\lambda$, as a function of $\xi$, in the fresh and unfresh samples, to observe the changes with the aging of the sample. We find a marked decrease of fluctuations on all layers in the unfresh sample.

Our main result can be shown by comparing the PDF of $\xi$ maps, in the unfresh and fresh samples, in Figure 6. Typical map of $\xi$ in the unfresh nerve is shown in \textbf{Figure 4A}. The probability distribution of the conformational parameter, $\xi$, become Gaussian in the unfresh nerve, losing the fat tail modelled by Levy flight in the fresh sample, as shown in \textbf{Figure 4B}. Indeed, the stability parameter here becomes equal to 2. Further, there is a decrease of both the location parameter $\delta = 0.807$ and of the scale parameter $\gamma = 0.0130$ compared to the fresh sample, which identifies the narrowing of hydrophilic and hydrophobic layers.
Conclusion

In summary, we demonstrate the feasibility of applying SµXRD for non-invasive imaging, to provide unique information on the correlated disorder in biological systems\textsuperscript{18}. This technique allowed us to map the nanometric distribution of the myelin structural unity as well as the cytoplasmatic, lipidic and extracellular subcomponents in the sciatic nerve of frog Xenopus leavis. From our measurements and from statistical analysis of distributions of these quantities we got the following main evidences: i) the quasi-crystalline periodicity of the myelin lattice is due to intrinsic anti-correlated fluctuations between hydrophobic and hydrophilic layers, given by anti-correlated dynamic of the proteins of the layers, ii) the spatial variations of the thickness of hydrophilic lipid extracellular and cytoplasmatic layers is compared with the hydrophobic extracellular layer by introducing a conformational parameter showing a long-tailed distribution that follows a Levy stable behaviour in the functional myelin state.

The fat tail with a power law distribution of myelin structural fluctuations of the hydrophilic to hydrophobic ratio in the fresh state, freezing in the un-fresh aging myelin state is a key result of this work. The statistical fluctuations of the myelin supramolecular structure open new venues in this field; in fact supramolecular assembly is a robust rapid and spontaneous process that although it occurs widely in nature it is poorly understood since it takes place in multiple scales and involve very weak intermolecular interactions. Today it is of high relevance in health care, engineering [50] and photosynthetic processes [51]. It is of high relevance for polymers where partially crystalline and amorphous nanoscale regions coexist. The interests in modelling autonomous supramolecular assembly of materials is a vast challenging area in nanotechnology [52]. Therefore, wide varieties of supramolecular structures are possible depending on the nature of weak forces in biological liquid crystals like cholesterol and myelin ranging from micro- to nano- structures.

It is interesting to remark that particular power law distributions of the supramolecular structure fluctuations has been predicted theoretically to promote quantum coherence at high temperature [53, 54]. Using the same SµXDR experimental method the power law fat tail distribution has been found for the structural fluctuations in lamellar materials, which show the emergence of quantum coherence at high temperature. This result has been related with complex non Euclidean geometry that has been proposed to be a key factor promoting quantum coherence in living matter expected to be triggered by a critical percolation point [55-57].
Finally, this work offers new perspectives for the study of emergence of neuro degenerative process. In fact, the present paradigm associates the onset of the neurodegenerative process to the transition from the ordered quasi-crystalline ultrastructure to a disordered structural phase. On the contrary, this work proposes a new paradigm where the early stage of degenerative process can be detected by a deviation of structural fluctuations away from the physiological fluctuations. More specifically, both the decreasing of structural fluctuations at nanoscale and the losing of their spatial correlations constitute a measure of the degeneration degree. Therefore, this new paradigm opens the way to further investigations into the ultrastructure dynamics, by studying time evolution of spatial fluctuations under external factors such as diseases and drug response.
Materials and Methods

Sample preparation
Two adult female frogs (Xenopus laevis; 12 cm length, 180-200 g weight, Xenopus express, France) were housed and euthanized at the Grenoble Institute of Neurosciences with kind cooperation of Dr Andre Popov. The local committee of Grenoble Institute of Neurosciences approved the animal experimental protocol. The frogs were individually transferred in water to a separate room for euthanasia that was carried out using a terminal dose of tricaine (MS222) by immersion, terminal anaesthesia was confirmed by the absence of reflexes. Death was ensured by decapitation. Two sciatic nerves were ligated with sterile silk sutures and extracted from both thighs of freshly sacrificed Xenopus frog at approximately the same proximal-distal level through a careful dissection of the thigh. After dissection, the sciatic nerves were equilibrated in culture medium at pH 7.3 for at least 3 hours at room temperature. The culture medium was a normal Ringer’s solution, containing 115 mM NaCl, 2.9 mM KCl, 1.8 mM CaCl2, 5 mM HEPES (4-2-hydroxyethyl-1-piperazinyl-ethanesulfonylic). Following equilibration, one of the freshly extracted nerves was immediately placed in a thin-walled quartz capillary, sealed with wax and mounted on the sample holder for the SμXRD imaging measurements. The other sciatic nerve of second frog, after dissection was left in Petri dish and equilibrated in culture medium at pH 7.3 for 18 hours at room temperature. Following equilibration in the same described conditions, the nerve was prepared for a further SμXRD imaging session in the same day.

Experimental and data analysis
The experimental methods were carried out in “accordance” with the approved guidelines. The Scanning micro X ray Diffraction measurements of myelin of frog’s sciatic nerve were performed on the ID13 beamline of the European Synchrotron Radiation Facility, ESRF, France. A scheme of the experimental setup is shown in Figure 1A. The source of the synchrotron radiation beam is a 18 mm period in vacuum undulator. The beam is first monochromatized by a liquid nitrogen cooled Si-111 double monochromator (DMC) and then is focused by a Kirkpatrick-Baez (KB) mirror system. This optics produces an energy X-ray beam of \( \lambda = 12.6 \text{ KeV} \) on a 1x1 \( \mu \text{m}^2 \) spot. The sample holder hosts the capillary-mounted nerve with the horizontal (y) and vertical (z) translation stages with 0.1 \( \mu \text{m} \) repeatability. The sample was scanned by using a step size of 5 \( \mu \text{m} \) in both y and z direction. A Fast Readout Low Noise (FReLoN) camera (1024x1024 pixels of 100x100\( \mu \text{m}^2 \)) is placed at a distance of 565.0 mm from the sample to collect the 2-D diffraction pattern in transmission. Diffraction images were calibrated using silver behenate powder (AgC_{22}H_{44}O_{2}), which has a
fundamental spacing of $d_{001}=58.38\text{Å}$. We choose an exposure time of 300 ms for minimizing the radiation damage and for didn’t sacrifice sensitivity at the same time$^{18}$. The crossed bundle are of approximately 50 myelinated axons. Therefore, the diffraction frames are an average of these axons. Considering the scale of our problem, this is an acceptable average.

We measured different regions of interest (ROIs) in the central part of the nerves around their axis to minimize the capillary geometry effect on the X-ray absorption. A typical 2-D diffraction pattern with the expected arc-rings corresponding to the Bragg diffraction orders $h = 2, 3, 4, 5$ is shown in Figure 1C. The 2-D diffraction patterns have been radially integrated to provide 1-D intensity profiles, $I(s)$, vs. transfer moment $s=2\sin(\theta)/\lambda$, after background subtraction and normalization with respect to the impinging beam (Fig. 1C). The 1-D radial profile shows the four characteristic peaks of myelin modelled with a Lorentian line shape from which we get the peak amplitude, $A(h)$, and full width at half height, $w(h)$, used for the Fourier’s analysis yielding electron density for each pixel according to:

$$|F(h)| = \sqrt{hA(h)w(h)} = \sqrt{hI(h)}.$$

for each reflection of order $h$. These structure factors, $|F(h)|$, were employed in a Fourier analysis to extract the Electron Density Distribution (EDD) of myelin:

$$\rho = \frac{F(0)}{d} + \left(\frac{2}{d}\right) \sum_{h_{\text{min}}}^{h_{\text{max}}} \pm F(h/d) \cos(2\pi rh/d),$$

where the phases were taken from literature$^{30,31,32}$:

$$F(h) = |F(h)|e^{i\phi_h} = \pm |F(h)|,$$

since the nerve is a centrosymmetric structure, so we consider just the real terms of Fourier series. The EDD obtained from the diffraction patterns measured at different sample positions in Fig. 1C, are shown in Figure 1D. From the differences between two adjacent maxima in the EDD profile the widths of the inter-membrane spaces at the cytoplasmic ($d_{\text{cyt}}$) and extracellular ($d_{\text{ext}}$) appositions and the thickness of the lipid bilayer ($d_{\text{lip}}$) were obtained. From these we got the period of the structural unit$^{18,30,33}$:

$$\lambda = 2d_{\text{lip}} + d_{\text{ext}} + d_{\text{cyt}}$$
The extrapolation of EDD at each pixel of the ROI, was performed using a customized in-house developed code written in MatLab (Mathworks, Natick, MA).
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Figures and figure captions

**Figure 1:**  
A) Main components of the SµXRD apparatus. A typical 2D single diffraction frame, collected with the FReLon camera, shows 4 concentric arcs. B) Pictorial view of the protein depleted membrane layers made of polar lipid groups, lpg, with thickness $d_{lpg}$, intercalated by two hydrophilic layers: the Schwann cell cytoplasm, cyt, and the extracellular apposition, ext, with thickness $d_{cyt}$ and $d_{ext}$, respectively. The specific myelin sheath protein PMP22, P0, P2 and MBP are schematized. C) X-ray diffraction patterns measured in the fresh sample, at different sample spots. The intensity is plotted against the reciprocal distance (nm$^{-1}$) and the Bragg reflection of order 2, 3, 4, 5 are indicated. D) Electron density distribution computed from the diffraction patterns shown in A). The curve presents troughs and maxima, with a period $\lambda = 2d_{lpg} + d_{ext} + d_{cyt}$, where $d_{lpg}$, $d_{ext}$ and $d_{cyt}$ refer to the thickness of lipid lpg, extracellular ext, cytoplasmatic cyt zones respectively.
**Figure 2:** Maps (400x125 μm²) of λ, d_{cyt} d_{lpg} and d_{ext} in A), C), E), and G) respectively. PDFs of these thickness maps are shown in B), D), F), H). We notice the larger fluctuations of d_{cyt}, d_{ext} and d_{lpg} given by σ(d_{cyt}), σ(d_{ext}) and σ(d_{lpg}) respectively, with respect to σ(λ) fluctuations of the period λ (see Table 1a).

I) Map of the conformational parameter, ξ, in a selected central zone of the nerve (400x125 μm²). L) Probability density function of ξ in semi-log plot. Here we notice the fat tail of that PDF fitted with Levy distribution. M) Scatter plot of absolute dispersion λ, Δd_{ext}=d_{ext}^0, Δd_{lpg}=d_{lpg}^0, Δd_{cyt}=d_{cyt}^0 as a function of ξ, where d_{ext}^0=d_{cyt}^0=2.2 nm and d_{lpg}^0=3.7 nm. The period, λ, is reported enclosed between two dotted lines at λ=17.4 nm and λ=17.1 nm. This plot solves the paradox of the period stability. We note the almost total independence of the period from ξ due to the anti-correlated fluctuations between the hydrophilic layers (cyt and ext) and the hydrophobic one (lpg).
Figure 3: Maps (400x125 µm²) of λ, d_{cyt}, d_{lpg}, and d_{ext} in A), C), E), and G) respectively, measured in the unfresh sample. PDFs of these thickness maps are shown in B), D), F) and H). Despite the unchanged period, in comparison with the fresh nerve, we observed reduced fluctuations of d_{cyt}, d_{ext} and d_{lpg} (see RSD values in Table 1b). These narrower fluctuations are associated with the loss of correlated disorder, as can be seen by the decreasing of the Pearson’s correlation coefficient for the unfresh nerve in Table 2b. I) Scatter plot of the period λ and the dispersions Δd_{ext}, Δd_{lpg}, Δd_{cyt} (defined in Fig. 4) as a function of ζ. The period fluctuations are enclosed between two dotted lines at λ=17.4 nm and λ=17.1 nm. The grey full circles representing the fluctuations found in the fresh nerve are reported for comparison.
Figure 4: A) Map of the conformational parameter, $\xi$, in a selected central zone of the unfresh nerve (400x125 $\mu m^2$). B) Probability density function of $\xi$ in the unfresh sample (open circles) in semi-log plot. The Levy PDF curve found in the fresh sample (dashed line) is also reported, for comparison. We notice the loosing of the fat tail in the distribution assuming a Gaussian profile in the unfresh sample.
Tables:

|        | µ(nm) | σ(nm) | RSD (%) |
|--------|-------|-------|---------|
| d_{cyt} | 2.999 | 0.036 | 1.20    |
| d_{ext} | 4.860 | 0.082 | 1.68    |
| d_{lpg} | 4.746 | 0.055 | 1.15    |
| λ      | 17.350| 0.024 | 0.14    |

Table 1a: Mean values µ, standard deviations, σ, and Relative Standard Deviation RSD = σ/µ of the thickness d_{cyt}, d_{ext}, d_{lpg} and λ in the fresh sample.

|        | µ(nm) | σ(nm) | RSD (%) |
|--------|-------|-------|---------|
| d_{cyt} | 2.957 | 0.028 | 0.95    |
| d_{ext} | 4.797 | 0.049 | 1.03    |
| d_{lpg} | 4.798 | 0.034 | 0.71    |
| period | 17.350| 0.021 | 0.12    |

Table 1b: Mean values µ, standard deviations, σ, and Relative Standard Deviation RSD = σ/µ of the thickness d_{cyt}, d_{ext}, d_{lpg} and λ in the unfresh sample.

| c_{i-j} | cyt   | ext   | lpg   |
|---------|-------|-------|-------|
| cyt     | 1     | 0.4452| -0.6406|
| ext     | 1     | 1     | -0.9474|
| lpg     |       | 1     | 1     |

Table 2a: Correlation coefficients c_{i-j} between maps of the spatial fluctuations between the different layers i, j = cyt, ext and lpg in the fresh sample. Negatively correlated coefficients are indicated by grey cells. These correlations are well explained by Figure 4C.

| c_{i-j} | cyt   | ext   | lpg   |
|---------|-------|-------|-------|
| cyt     | 1     | 0.3426| -0.5293|
| ext     | 1     | 1     | -0.8290|
| lpg     |       | 1     | 1     |

Table 2b: Correlation coefficients c_{i-j} between maps of the spatial fluctuations between the different layers i, j = cyt, ext and lpg in the unfresh sample. Negatively correlated coefficients are indicated by grey cells. These correlations are well explained by Figure 5I.
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Author contributions
All authors contributed equally to the work. M.B. provided the XRD station at ESRF; N.P. and T.A.H. prepared the samples; M.D.G. G.C., A.B. and A.R. performed the data analysis; G.C., M.D.G., A.B., together wrote the paper.

Additional information
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