Supporting Information for

Structural Transition in Myelin Membrane as Initiator of Multiple Sclerosis

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Supporting Information S1: Materials and Methods.

S1.1. Sample preparation. The lipids used in this study represent those that are abundant (above 2 mol %) in native and modified composition as reported before.¹ Phosphatidylserine (porcine brain PS⁻), sphingomyelin (porcine brain SM), phosphatidylcholine (porcine brain PC), phosphatidylethanolamine (porcine brain PE), and cholesterol (ovine wool), all of purity >99%, were purchased from Avanti Polar Lipids, and were used without further purification. The lipids and cholesterol, at desired mole ratios, were mixed in chloroform, which was evaporated under reduced pressure (8 mbar) in a Buchi rotary evaporator vacuum system (Flawil, Switzerland). The resulting lipid film was resuspended in an 10 mM ammonium bicarbonate solution, lyophilized, and subsequently hydrated in the reassembly buffer (10 mM MOPS, 150 mM NaCl, and 2 mM CaCl₂). Bovine MBP (Sigma) powder was dissolved in the same assembly buffer; its concentration determined by measuring its absorption at 280 nm (NanoDrop 2000 spectrophotometer, Thermo Scientific). The MBP solution (20 mg/ml) was added to 50 µl of the MLV stock solution (20 mg/ml) to the desired MBP wt%. In addition to 18.5 kDa isomer, the purified MBP contains small fraction of other native isomers.^{2,3}

S1.2. Small-angle X-ray scattering measurements. Lipid-protein solution was well mixed, and 30 µl transferred to 1.5 mm quartz capillaries (Hilgenberg-GMBH), and centrifuged at 8000 rpm for 90 minutes. That resulted in a phase-separated MLV pellet at the bottom of the capillary and excess buffer on top. The capillaries were flame-sealed and glued to prevent volatile evaporation. Preliminary small-angle X-ray scattering (SAXS) experiments were carried out in home-built apparatus with a Cu ($\lambda = 1.54 \text{ \AA}$) tube source (Xenocs), Pilatus 300K detector (Dectris), about 1 meter sample-to-detector distance, and a scatterless slit set-up.⁴ Synchrotron measurements at room temperature were performed at EMBL P12 (1.24 Å), Diamond light source I22 (1.24 Å) and Max-Lab I911 SAXS (0.91 Å) beam lines. The 2D diffraction data were radially integrated, using data reduction software (SAXSi) developed in our lab. Background scattering intensities, collected from the buffer above the pellet, were measured and subtracted from the pellet's scattering curves. The full scattering intensity curves (Figure SI-1) were analyzed using the *X+* software.⁵ Form-factor analysis used a stack of infinite flat uniform slabs membrane with a box electron

densities model as shown in Figure SI-1 inset. Structure-factor analysis applied Gaussian line-shape correlation peaks (Figure SI-1).

Figure SI-1. SAXS analysis for (A) native lipid composition, (B) native with 20 wt% MBP, (C) modified lipid composition, (D) modified with 20 wt% MBP. SAXS measurement (dotted black line) is fitted (red solid line) with a symmetric layer slabs form-factor model (dashed green line) and Gaussian structure-factor, using X+ software.⁵ Inset shows the electron density profiles of the lipid head and tail groups, and the water layer. We used identical form-factor for all data analysis.

S1.3. Cryo-TEM imaging *in vitro*. Cryogenic transmission electron microscopy (Cryo-TEM) was used as a direct technique to image the nanostructures of selected lipid compositions. Cryo-TEM samples were prepared using a Controlled Environment Vitrification System (CEVS) as described by Bellare *et al.*^{6,7}, at 25°C and humidity at saturation, to prevent evaporation from the specimen. Sample (3-4 μ l) drops of solution were placed on a carbon-coated perforated polymer film, supported on a 200-mesh TEM grid. Prior to specimen preparation the grids were plasma-etched in a PELCO EasiGlow glow-discharger (Ted Pella Inc., Redding, CA) to increase their hydrophilicity. Most of the liquid was then blotted away to obtain a thin film,

and the specimens were vitrified by quickly plunging into liquid ethane at its freezing point. The cryo-specimens were kept in liquid nitrogen before transfer to TEM. Specimens were examined in an FEI Tecnai T12 G² TEM operating at 120 kV. To enhance contrast, images were recorded at a defocus of $\sim 1\ \mu\text{m}$, with a typical electron exposure beam^{7,8} of less than $20\ \text{e}^-/\text{\AA}^2$. The coexisting H_{II} phase was found to be more pronounced within thicker lipid layers, and after condensation with centrifugation. Such behavior is typical for hexagonal phases, as previously documented.⁹

S1.4. TEM imaging *in vivo*. Spinal cord white matter pathology was characterized using transmission electron microscopy as in.¹⁰ Briefly, spinal cords of mice inflicted either by relapsing remitting proteolipid protein (PLP) induced EAE or by chronic oligodendrocyte glycoprotein (MOG) induced EAE, as well as of naïve control mice, were dissected and postfixed. In total 2 of normal, and 7 PLP and MOG induced spinal cords were characterized. Cervical cross-sections were embedded in 7% agarose and sectioned ($200\ \mu\text{m}$) by vibratome. Tissues were incubated in 1% osmium tetroxide in 0.1 M cacodylate, stained with 1% uranyl acetate in water, dehydrated in graded ethanol solutions, and embedded in Epon 812 (Electron Microscopy Science).

Ultrathin sections ($70\text{--}90\ \text{nm}$) cut by ultramicrotome were imaged 120 kV at SPIRIT Transmission Electron Microscope (FEI, Eindhoven, Netherlands), equipped with an Eagle CCD camera, using the TIA software (FEI). The electron microscopy studies were conducted at the Irving and Cherna Moskowitz Center for Nano and Bio-Nano Imaging at the Weizmann Institute of Science.

Supporting Information S2: Lipid composition.

In order to determine which of the lipid modifications induce the H_{II} phase, we gradually changed each lipid mole fraction, while keeping the remaining lipids with a constant relative mole fraction, as is seen in the original lipid composition. For example, SAXS data was collected for samples ranging from modified lipid compositions with $[\text{PC}] = 20.1\ \text{mol}\%$ up to $[\text{PC}] = 25.9\ \text{mol}\%$ (as in the native lipid composition), while the remaining lipid composition ratios were fixed (Figure SI-2A). Above $[\text{PC}] \approx 24\ \text{mol}\%$, and in the absence of MBP, the H_{II} phase diminishes. Similar experiments were conducted with native lipid compositions, changing the PC content (Figure SI-2B). Here, modifying a single component does not induce H_{II} phase

formation; in such instances, a single lamellar phase, with a nearly identical interlamellar spacing, is present.

Figure SI-2. SAXS profiles for PC changes. (A) Increasing PC mol% while keeping other components constant from modified back to a native lipid composition, results in a decrease in the hexagonal phase, until it vanishes completely. (B) Decreasing the PC mol% while keeping other components constant from native to modified lipid composition causes no changes in the lamellar phase.

Another large composition variation was seen in the SM content. Here, even 1 mol% variation in SM content completely abolishes the H_{II} phase in the modified lipid composition (Figure SI-3A). Further decreasing SM content results in two lamellar phases with 121 $\text{\AA}$ and 110 $\text{\AA}$ interlamellar spacings, respectively (Figure SI-3B). Similar behavior was observed with native lipid compositions and with other lipid variations, although they represent only minor alterations in composition (data not shown).

Figure SI-3. SAXS profiles for SM changes. (A) Decreasing SM mol% while keeping other components constant from a native to a modified lipid composition results in two lamellar phases. (B) Increasing SM by 1 mol% while keeping other components constant from a modified back to a native lipid composition results in a diminished hexagonal phase.

Supporting Information S3: TEM analysis: TEM membrane roughness analysis.

The membrane roughness was evaluated by calculating the local curvature of the membranes contour. Membrane contours were manually traced using custom MATLAB code (Figures SI-4A, B). Traced contours were fitted to a 3rd order piece-wise polynomial (*i.e.*, a smoothing spline) with breaks every 200 Å (2,000 Å) for *in vitro* (*in vivo*) samples. The fitted contours along with their 1st and 2nd derivatives were sampled at equal distance of 10 Å along the contour for following computation of curvature statistics. Local curvature ($1/R$, where R is the local radius of the membrane) was computed as $y''x' - y'x''$ from the sampled derivatives. Final statistics in Figure 4 and Figure SI-4C are accumulated from 34, 195, 29, 116 traced contours for *in vitro* native and modified models and *in vivo* normal and diseased samples respectively, gathered from multiple images.

Statistical characterizations of the local curvature from TEM images clearly indicate a much wider distribution of curvatures in specimens taken from EAE induced mice,

as is the case for the modified lipid composition *in vitro* model (Figures 4C, F and Figure SI-4C). Native lipid composition and specimens taken from normal mice exhibit a higher mean and sharper distribution of curvature as expected.

Figure SI-4. TEM structural analysis for *in vitro* lipid composition models. (A) Tracing membrane contours on cryo-TEM images of modified lipid composition and (B) native lipid composition (each color represents different traced membrane). (C) Local curvature frequency of native lipid composition with and without 20 wt% MBP, and modified lipid composition with 20 wt% MBP. In all cases the mean curvature lies in the range $3.89\text{--}5.14 \times 10^{-4} \text{ \AA}^{-1}$ and the STD $7.71\text{--}8.73 \times 10^{-4} \text{ \AA}^{-1}$. Statistics are accumulated from 34, 36, and 66 traced contours for lipid compositions of native with 20 wt% MBP, native, and modified with 20 wt% MBP respectively, gathered from multiple images. For native lipid composition, *in vitro* measurements show similar low roughness (STD) regardless of MBP inclusion. Scale bars = 50 nm (A) and 100 nm (B).

Supporting Information S4: References.

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