Interaction forces and adhesion of supported myelin lipid bilayers modulated by myelin basic protein

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Force–distance measurements between supported lipid bilayers mimicking the cytoplasmic surface of myelin at various surface coverages of myelin basic protein (MBP) indicate that maximum adhesion and minimum cytoplasmic spacing occur when each negative lipid in the membrane can bind to a positive arginine or lysine group on MBP. At the optimal lipid/protein ratio, additional attractive forces are provided by hydrophobic, van der Waals, and weak dipolar interactions between zwitterionic groups on the lipids and MBP. When MBP is depleted, the adhesion decreases and the cytoplasmic space swells; when MBP is in excess, the bilayers swell even more. Excess MBP forms a weak gel between the surfaces, which collapses on compression. The organization and proper functioning of myelin can be understood in terms of physical noncovalent forces that are optimized at a particular combination of both the amounts of and ratio between the charged lipids and MBP. Thus loss of adhesion, possibly contributing to demyelination, can be brought about by either an excess or deficit of MBP or anionic lipids.

biomembrane adhesion | lipid–protein interactions | multiple sclerosis | myelin membrane structure | experimental allergic encephalomyelitis

The myelin sheath is a multilamellar membrane surrounding the axons of neurons in both the central nervous system (CNS) and peripheral nervous system (PNS) (1) as shown in Fig. 1 A and B. The myelin sheath consists of repeating units of double bilayers separated by 3- to 4-nm-thick aqueous layers that alternate between the cytoplasmic and extracellular faces of cell membranes (2) (Fig. 1C). Dehydrated myelin is unusual in that it is composed of 75–80% lipid and 20–25% protein by weight, compared with ≈50% of most other cell membranes (3) (Fig. 1 C and D). Multiple lipids make up the myelin sheath (Table 1), and each sheath, with its own distinct physical properties, contributes to the structure, adhesive stability, and possibly the pathogenesis of the myelin membrane. The asymmetric distribution of lipid composition on the cytoplasmic and extracellular faces likely also plays an important role (4). Myelin basic protein (MBP) constitutes 20–30% of total protein by weight and is located only between the 2 cytoplasmic faces, where it acts as an intermembrane adhesion protein.

The myelin sheath acts as an electrical insulator, forming a capacitor surrounding the axon, which allows for faster and more efficient conduction of nerve impulses than unmyelinated nerves (5). According to cable theory, the time to transmit a signal over a distance \( x \) is \( \tau = \frac{1}{2} R C_{\text{myelin}} \), where \( R \) is the resistance per unit length, and \( C_{\text{myelin}} \) is the capacitance between the axon and its surroundings, which is given by (5)

\[
C_{\text{myelin}} = \frac{2 \pi e_{\text{myelin}}}{\log(R_0/R_1)} \quad \text{per unit length,}
\]

where \( R_0 \) and \( R_1 \) are the outer and inner radii (Fig. 1B), \( e_{\text{myelin}} \) is the permittivity of free space, and the mean dielectric constant of the myelin sheath is

\[
e_{\text{myelin}} = \frac{2 e_{\text{HC}} D_B + e_P D_P + e_W(D_1 + D_0)}/D_{\text{Tot}}.
\]

where \( D_{\text{Tot}} = [2D_B + D_1 + D_P + D_0] \) (Fig. 1C). Low capacitance necessitates a low value of \( e_{\text{myelin}} \) which is promoted by the much lower dielectric constants of the lipid chains (\( e_{\text{HC}} \approx 2 \)) and proteins (\( e_P = 2.5–4.0 \)) (6) compared with that of water (\( e_w \approx 80 \) for bulk water and \( e_w \approx 40 \) for “interfacial” or “partially trapped” water in thin films) (7). Increasing the thickness of the myelin sheath increases \( R_0 R_1 \), and tighter membrane binding within the sheath decreases the water gaps, both of which decrease \( C_{\text{myelin}} \). For squid axon, a measured value of \( e_{\text{myelin}} \approx 8.5 \) has been reported (8). Inserting the following measured or estimated values into Eq. 2: \( e_P = 2.5–4.0 \) (6), \( D_B \approx 4.5 \) nm, \( D_0 \approx 3 \) nm, \( D_1 = D_P \approx 1 \) nm (assuming 50% of the cytoplasmic gap is protein) (1), and \( e_w \approx 40 \) (7), we obtain \( e_{\text{myelin}} \approx 13 \), which is close to the measured value.

Myelin dysfunctions vary from deterioration of signal transduction to demyelinating diseases such as multiple sclerosis (MS) (3). MS is characterized by the appearance of lesions, reflecting loss of membrane adhesion, swelling across the water gaps, vacuolization, vesiculation, and eventual disintegration of the

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myelin structure (9). It has been shown in previous work that in experimental allergic encephalomyelitis (EAE) in the cerebroside sulfate (CS) (10) model, the myelin membrane is a composite bilayer consisting of a nonhydrophobic lipid membrane and an inner protein bilayer. This structure is believed to be responsible for the formation of myelin (3) and is the key to understanding the formation of myelin membranes (9).

Table 1. Lipid compositions of healthy and diseased bilayers calculated as mole % of lipid in the extracellular (EXT) and cytoplasmic (CYT) faces of myelin membranes

<table>
<thead>
<tr>
<th>Lipid type (i)</th>
<th>Healthy (EXT:CYT)</th>
<th>EAE (EXT:CYT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (CHOL)</td>
<td>22.4:10.6</td>
<td>25.8:12.1</td>
</tr>
<tr>
<td>Phosphatidylserine (PS)</td>
<td>6.7:2.4</td>
<td>4.7:2.4</td>
</tr>
<tr>
<td>Hydroxylated cerebrosides (HCER)</td>
<td>13.9:0</td>
<td>14.7:0</td>
</tr>
<tr>
<td>Nonhydroxylated cerebrosides (NCER)</td>
<td>2.3:0</td>
<td>2.4:0</td>
</tr>
<tr>
<td>Cerebroside sulfate (CER)</td>
<td>6.4:0</td>
<td>3.8:0</td>
</tr>
<tr>
<td>Sphingomyelin (SM)</td>
<td>2.8:2.1</td>
<td>0.9:0.7</td>
</tr>
<tr>
<td>Phosphatidycholine (PC)</td>
<td>12.1:8.7</td>
<td>8.9:6.5</td>
</tr>
<tr>
<td>Phosphatidylethanolamine (PE)</td>
<td>6.0:9.7</td>
<td>6.5:10.6</td>
</tr>
<tr>
<td>Total mole %, X_i and X_j</td>
<td>66.5:33.5</td>
<td>67.8:32.2</td>
</tr>
</tbody>
</table>

\( X_i^{\text{EXT}} \) is the total mole fraction (percent of lipid of type i, so that \( \Sigma X_i^{\text{EXT}} = 100\% \)), \( X_i^{\text{CYT}} \), and \( X_j^{\text{EXT}} \) and \( X_j^{\text{CYT}} \) are the total mole fractions of lipid of type i in the extracellular and cytoplasmic lipid face, so that \( X_i^{\text{EXT}} = X_i^{\text{EXT}} + X_i^{\text{CYT}} \), and \( X_j^{\text{EXT}} = X_j^{\text{EXT}} + X_j^{\text{CYT}} = 100\% \). \( X_i^{\text{CYT}} \) are the mole fractions of lipid of type i in the cytoplasmic face, only, so that \( \Sigma X_i^{\text{CYT}} = 100\% \). The \( \nu \) and \( \chi \) values are related by \( \nu = 100(1 - \chi) \). We may note that the total mole fraction of lipids in the extracellular lipid domain \( \Sigma X_i^{\text{EXT}} \) is typically twice that for the cytoplasmic lipids \( \Sigma X_i^{\text{CYT}} \) (see bottom row). The “missing” volume is likely taken up by the MBP in the cytoplasmic spaces. Myelin membranes are from marmoset white matter (10).

*These compositions were used in the experiments.

**Results**

**SFA Force–Distance Measurements.** Fig. 2 shows the long- (4) and short- (B) range forces measured between EAE cytoplasmic bilayers in the absence and presence of various amounts of MBP. Fig. 2A is a semilog plot showing the strong repulsive force on approach, and Fig. 2B is a linear plot showing the weak (negative) adhesion forces on separation as a function of bulk MBP concentration.

To relate the measured adhesive forces to the surface coverage rather than the bulk solution concentration of MBP, we have separately measured the surface coverage at each concentration. The same fringes-of-equal-chromatic-order (FECO) optical technique (18) used to measure the distance between mica surfaces can be used to determine the refractive index \( n(D) \) of the aqueous space between the bilayers, which can then be used to estimate the surface coverage of MBP. Because in a first approximation, \( n^2(D) \) is a linear function of the volume fraction \( \phi_i \) of each component i, \( n(D) \) is given by (19)

\[
n^2(D) = \sum \phi_i n_i^2 = \phi_{\text{MBP}} n_{\text{MBP}}^2 + \phi_{\text{MBP}} n_{\text{MBP}}^2 \phi_{\text{MBP}} \phi_{\text{MBP}} \phi_{\text{MBP}},
\]

where \( \phi_i = 1 \) and \( \phi_{\text{MBP}} = 0, \phi_{\text{MBP}} = 0.3 \), and \( \phi_{\text{MBP}} = 0.4 \), respectively. In the absence of MBP, \( \phi_{\text{MBP}} = 0, \) Eq. 3 reduces to \( n^2(D) = n_{\text{w}}^2 + \phi_{\text{w}} n_{\text{w}}^2 \).

In the presence of MBP, \( \phi_{\text{w}} = D_{\text{w}}/D_{\text{b}} \), and we have:

\[
n^2(D) = n_{\text{w}}^2 + \frac{2D_{\text{b}}}{D} (n_{\text{b}}^2 - n_{\text{w}}^2),
\]

where \( D = 2D_{\text{b}} + D_{\text{w}} \) is the total mica–mica gap thickness and \( n_{\text{w}} = 1.333 \). Thus, by plotting \( n^2(D) - n_{\text{w}}^2 \) as a function of \( n_{\text{b}}^2 - n_{\text{w}}^2 \), we can determine \( 2D_{\text{b}} \) and \( D_{\text{b}} \). This analysis gives \( 2D_{\text{b}} = 7.4 \) nm by using \( n_{\text{b}} = 1.47 \).

\( D \)

①Negative (adhesion) forces cannot be displayed on log plots.

②Most of the MBP injected into the solution ends up on the negatively charged surfaces; i.e., there is an equilibrium distribution of MBP molecules between those molecules that are the bulk and those molecules adsorbed on the surfaces, which is strongly biased in the direction of the surfaces. From the concentration giving rise to monolayer coverage, the equilibrium affinity or association constant is estimated to be \( 10^{-8} \) M, which is typical of antibody-antigen and ligand–receptor bonds.
In the presence of MBP ($\phi_{\text{MBP}} > 0$):

$$n^2(D) = (1 - \phi_B - \phi_{\text{MBP}})n_m^2 + \phi_Bn_B^2 + \phi_{\text{MBP}}n_{\text{MBP}}^2,$$

i.e.,

$$\phi_{\text{MBP}} = \frac{n^2(D) - \phi_Bn_B^2 - (1 - \phi_B)n_m^2}{n_{\text{MBP}}^2 - n_m^2}. \quad [5]$$

Assuming that the bilayer thickness, $D_B$, and hence $\phi_B$, do not change on addition of MBP and during compression, $\phi_{\text{MBP}}$ can be readily obtained by using calculations of $n^2(D)$ and also assuming that the refractive index of the protein is known. Here, we assume $n_{\text{MBP}} \approx 1.55$ (20, 21). Finally, the surface coverage of MBP trapped between the surfaces, $\Gamma_{\text{MBP}}$, can be estimated from $\phi_{\text{MBP}}$ as (18)

$$\Gamma_{\text{MBP}} = \frac{1}{2} \rho_{\text{MBP}} D \phi_{\text{MBP}}. \quad [6]$$

in terms of the known density $\rho_{\text{MBP}}$ (~1.38 g/cm$^3$) (22).

Fig. 3 shows the measured surface coverage of MBP ($\Gamma_{\text{MBP}}$) between the 2 surfaces at the different bulk MBP concentrations tested. On correlating the data of Figs. 2 and 3, we find that as the bulk MBP concentration increases, the adhesion initially increases, then decreases. Simultaneously, the equilibrium spacing initially decreases, then increases. Significantly, maximum adhesion occurs when the equilibrium separation is the least (green and turquoise circles in Fig. 2), i.e., when the membranes are in their most tightly packed configuration at a separation of ~12 nm. At MBP concentrations >0.041 µg/mL, where the surface coverage exceeds some critical “saturation” value, the adhesion disappears, and the magnitude and range of the repulsion increases precipitously.

At MBP concentrations below the saturation value ($C_{\text{MBP}} < 0.041$ µg/mL), quantitative analysis of the repulsive forces on
approach (Fig. 1A) shows the forces to be because of the electrostatic "double-layer" repulsion between the charged surfaces: The forces are exponentially repulsive with a decay length that is close to the theoretically expected Debye length of 0.8 nm in 0.15 M NaNO$_3$ solution (straight line in Fig. 2A). Thus, in these near-physiological conditions, the forces are well-described by the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory (23) of attractive van der Waals and repulsive electrostatic "double-layer" forces. However, in the presence of MBP, the depths of the adhesive wells are much deeper than can be accounted for by van der Waals forces acting alone, indicating that the adhesion is also because of electrostatic bridging forces [discrete ionic bonds between the negatively charged PS and lipids and the positively charged lysine or arginine amino acid (AA) groups in MBP] as illustrated in Fig. 4B and ref. 15. It is also noticeable that the presence of MBP brings the 2 membranes closer together than in the absence of MBP, even as MBP gets sandwiched between the 2 bilayers. At higher MBP concentrations, i.e., \( \geq 0.047 \) µg/ml, there is excess MBP and, presumably, excess positive charge at the interface, which causes the bilayers to repel each other electrostatically and the water gap to swell (analogous to demyelination in vivo). But partial swelling also occurs in the absence of MBP, where \( D_1 + D_P \) increases by \( \approx 2 \) nm (Fig. 2B).

Figs. 4 and 5 give further details about the effects of the number of approach–separation cycles (successive compressions and decompressions), contact time, hysteretic effects in the forces, and the likely conformations of the MBP molecules between the bilayers as a function of the coverage. We note, as has been observed in many previous force measurements between biological samples (24), that the approach is more repulsive than the separation. This effect is probably due to the initially rougher and less-correlated surfaces, which become smoothed out, where attractive species diffuse toward each other on contact to make the separation more attractive (or less repulsive). The short-range forces measured on separation are therefore likely to be closer to the "equilibrium" adhesion forces (14).

At low MBP coverage, when \( C_{\text{MBP}} = 0.031 \) µg/ml (Fig. 4A), there was little hysteresis in the force vs. distance curves. Fig. 4A further indicates that the adhesion force can be enhanced by repeated approach–separation cycles and also by increasing the equilibrium contact time between the 2 surfaces. This enhancement shows that the lipids and proteins are laterally mobile and "adaptable" and can diffuse and rearrange to find their optimum configuration (14, 25). It should be noted that the adhesive force in run 3 shows a noticeable deviation from the linear relationship shown by the straight line in Fig. 4A, supporting the idea that increasing the contact (equilibration) time allows the binding sites on the mobile lipid and protein molecules to find each other (24).

Another related observation is that the final contact and jump-out distances move farther in by repeated cycling. This decreasing trend in \( D_1 \) suggests that the hydrophobic segments of MBP molecules penetrate into the lipid bilayers as shown schematically in Fig. 4B, resulting in a thinning of the membranes. Because change occurs at constant lipid and protein coverage, it suggests a deeper penetration of the MBP into the bilayers, i.e., the thinning is more of a smoothing of the membranes. Nevertheless, this process is accompanied by more water being forced out from the cytoplasmic gap, which further lowers the dielectric constant of myelin, which decreases the capacitance of the myelin sheath.
The forces are very different above the critical saturation concentration of MBP. Fig. 5 shows the very large hysteresis observed at \(C_{\text{MBP}} = 0.156 \, \mu g/mL\), well above the saturation concentration of 0.041 \(\mu g/mL\). The corresponding pressure–distance (or \(P-V\) plot) between 2 planar surfaces has a shape that reveals a first-order phase transition of the MBP layer between the surfaces, suggesting that it forms a weak and/or flexible gel when present in excess (26, 27).

Analysis and Discussion of the Results. In the experiments, the mean area per lipid molecule is \(a = 0.4 \, nm^2\), measured during LB depositions. This value appears to be lower than that expected for double-chain lipids and is attributed to the high amount of cholesterol present in myelin (see Table 1). The fraction of negatively charged lipids is \(f \approx 0.074\), which corresponds to a negative surface-charge density of 0.4/0.074 = 5.4 nm\(^2\) per unit charge \(\varepsilon\) . The estimate gives the excess number of positive charges (arginine and lysine AAs) per 18.5 kDa MBP molecule to be \(\approx 20\) (15). Thus, for full-charge neutralization each protein molecule should cover an area of 20 \(\times 5.4 = 108\) nm\(^2\), which corresponds to a surface coverage of \(f_{\text{MBP}} \approx (18,500)(6.02 \times 10^{21} \times 108)/g \, nm^2 = 0.28 \, mg/m^2\) per surface. This value, when compared with the measured coverage of \(f_{\text{MBP}} \approx 2 \, mg/m^2\) obtained at maximum adsorption (Fig. 3), suggests that other nonelectrostatic interactions also contribute to the adsorption.

Although the repulsive forces appear to follow the continuum theory of double-layer forces, the attractive forces do not because at the measured jump-out distances the charges on the lipid head groups and the charges on MBP must be very close to each other, if not actually in contact, so that continuum or mean field theories can no longer be used. Therefore, regarding the maximum membrane–membrane adhesion force (as mediated by MBP), assuming that at maximum adhesion every negative lipid is bound to a lysine or arginine group of MBP and that the adhesion force \(F/R\) is given by \(-2\pi \varepsilon \alpha /a\) (14, 24), where again \(f \approx 0.074\) is the fraction of negatively charged lipids, \(a = 0.4 \, nm^2\) is the mean area per lipid molecule, \(\alpha = 1\) is the fraction of positive charges on MBP that are bound to the membranes, and \(\varepsilon = 2kT\) is the Coulomb energy for an ionic bond in water (24), then the maximum \(F/R\) is calculated to be \(\approx 9\) mN/m. This value is lower than the maximum adhesion force of \(\approx 19\) mN/m measured at \(C_{\text{MBP}} = 0.031 \, \mu g/mL\) (Fig. 3), suggesting that other interactions such as van der Waals, hydrophobic (15, 28), hydrogen-bonding interactions (29), and weak dipolar interactions (28) also contribute to the attractive forces, which is also consistent with the structural-thickness and refractive-index changes (adsorbed MBP) observed. We note that hydrophobic- and hydrogen-bonding interactions have been shown to be important in the interactions of P0 glycoprotein in CNS myelin (29).

Finally, the MBP undergoes conformational changes when it adsorbs to the lipid bilayer (25). In the presence of excess MBP, a phase transition is observed across highly swollen bilayers, suggesting the formation of a weak, dilute, but extended gel-like structure of MBP rather than a simple electrostatic repulsion (26, 27).

We conclude that MBP maintains the structure and stability of the cytoplasmic region of the myelin sheath by acting as an electrostatic “glue” holding the negatively charged bilayers together through its positively charged amino acids and that hydrophobic and other attractive (adhesive) interactions are also involved. Maximum adhesion coincides with minimum water-gap thickness of the cytoplasmic space, which further implies minimum dielectric constant (or capacitance) of the myelin and, therefore, maximum transmission of nerve signals.

To achieve optimum electrostatic conditions in the amounts and charge ratio (or balance) of the positive MBP and negative lipids, both must be optimized with maximum adhesion occurring at maximum densities, where each negative charge on a lipid serum head group is bound to a positive arginine or lysine group on MBP. Hydrophobic and other interactions also appear to be involved both in the adsorption and intermembrane adhesion, which are currently being investigated. Any compositional changes that lead to demyelination must also include changes to the solution, such as pH and ionic charges or addition of solutes, if these altered solutions lead to changes in the surface-charge density and/or dielectric constant that in turn affect the electrostatic and hydrophobic interactions. We do not identify the primary causes that lead to these compositional changes, only noting that these changes result in demyelination.

Removed from optimum conditions, the bilayers repel and the water gap swells, which may correspond to the onset of demyelination. This swelling can be attributed to the charge neutralization, followed by charge overcompensation, which is also common in the flocculation of colloidal particles by polyelectrolytes (30). Excess negative charge causes electrostatic swelling, but excess positive charge, i.e., excess MBP, causes swelling through the formation of a weak gel of MBP (26), possibly with some lipid (11, 26, 31) that forces the bilayers apart. This gel exhibits a phase transition on being compressed, which allows it to be collapsed when subjected to a compressive pressure of \(\approx 1\) MPa (10 atm). When the swelling pressures are not too great (e.g., 0.047 and 0.063 \(\mu g/mL\) in Fig. 2), the bilayers can be pressed together again to almost the same separation, within 0–2 nm, as the separation for adhesive, nonswelling systems, even from distances as large as 80 nm (Fig. 2).

These results indicate that swelling can have different causes: Excess or deficient charges on the lipid and/or MBP. For example, arginine-deficient MBP has been found to be present in some MS cases (32). To counteract the swelling, drainage of the water gap by using nontoxic hydrophilic polymers like polyethylene glycol (PEG) (33) has been successfully used to treat spinal-cord injuries in animals (34). PEG induces a high osmotic pressure (attractive depletion force) that forces the bilayers together again, perhaps collapsing the gel layer produced by unbound MBP. It also appears that there needs to be an as-yet-unknown mechanism to regulate production of MBP to match the existing surface charge in the cytoplasmic space. The insight into cytoplasmic myelin bilayer interactions and swelling presented here suggests guidelines for assessing and possibly arresting or reversing myelin swelling.

Materials and Methods

Our previous work (10) identified the changes in overall lipid composition between normal (control) and EAE myelin in the white matter of the common marmoset. Previous force measurements (14) by using the mean total lipid composition of normal and EAE myelin were made in the absence and presence of an undetermined quantity of MBP. However, as shown in refs. 2 and 4, the cytoplasmic and extracellular faces of the myelin bilayer have very different compositions. We used the distributions of the lipids determined by Inouye and Kirschner (2, 4) to calculate the composition of the cytoplasmic and extracellular monolayers of the control and EAE membranes (Table 1), given the overall lipid compositions of normal and EAE myelin measured in our previous work (10). The asymmetry in lipid composition between the extracellular and cytoplasmic monolayers was believed to be the same for both control and EAE (4, 15).

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 stored in chloroform until used. The major

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(3)This estimate assumes that each protein is adsorbed fully extended on each surface as in Fig 2A. If the MBP bends back when bridging 2 surfaces as in Fig. 3A, this does not change the calculated coverage per surface.

(4)Repulsion is also enhanced by polyunsaturated lipids through their increased repulsive undulation forces. Interestingly, EAE lipids have a greater degree of polyunsaturation than in healthy bilayers, which may also play a role in promoting demyelination (10).
chain lengths of the 3 major phospholipids (PC, PE, and PS) are 16:0, 18:0, 18:1, and 20:4. Dipalmitoylphosphatidylethanolamine (DPPE), sodium nitrate, calcium nitrate, and morpholinopropanesulfonic acid (Mops) sodium salt were purchased from Sigma-Aldrich. MBP was isolated from bovine brain white matter as described (35). The MBP used was unfraccionated, heterogeneous, bovine MBP. An SFA 2000 was used for the force measurements (23, 24). The cross- 
cylinder geometry of the surfaces (each cylinder of radius R) is locally equiva-
ient to J (1966) in Dr. Max A. Ratan’s Theory with a flat surface or 2 spheres of 

radius R. A simple geometric transformation, called the Derjaguin approxi-
mation (23), converts the force–distance curve $F(D)$ measured between the 
2 curved surfaces to the energy–distance curve, $E(D) = (F(D)/2 \pi r$, or pressure– 

distance curve, $P(D) = -dE(D)/dD$, between 2 flat (planar) surfaces (23), corre-

csponding to the interaction geometry of myelin membranes.

The lipid bilayers were deposited on the mica surfaces by using conven-
tional Langmuir–Blodgett deposition (36). The composition used was the 
cytosolic side of EA membranes, because MBP is found exclusively on the 
cytosolic side of the membrane. Monolayers were spread from solvent onto a 

ph7.2 Mops buffer (150-mM sodium nitrate/10 mM Mops sodium salt/2 mM calcium nitrate) and compressed to the desired surface pressure after solvent 

evaporation. As the first layer, DPPE was deposited onto the mica substrates at a surface pressure of 35 mN/m from the solid phase. The second monolayer of 

23.0 mass % (37.4 mole %) cholesterol, 9.6 mass % (7.4 mole %) PS, 2.6 mass % (2.2 mole %) SM, 24.3 mass % (20.1 mole %) PC, and 39.0 mass % (32.9 mole %) PE was made in a 11:5:4 (vol/vol) solution of hexane/chloroform/ 

ethanol. After the second “myelin” monolayer deposition at 30 mN/m onto the 

first DPPE monolayer, one of the bilayer-covered surfaces was transferred into the SFA chamber in buffer solution. The other surface was incubated with 
different amounts of MBP in buffer solution for 30 min, then rinsed twice with

pure buffer solution to remove MBP molecules from the bulk and those loosely 

adsorbed to the surface before transferring into the SFA chamber, producing an “asymmetric” bilayer system consisting of a pure lipid bilayer facing an 

MBP-covered bilayer.

The forces $F$ as a function of distance D were measured between the 2 
curved mica-supported bilayers in solutions of varying MBP concentrations as 

previously described (14). The optical technique used in the measurements 
gave the surface radius $R$ (2 cm), surface separation D (to $-0.1$ nm), and independently the refractive indices of the various components (water, bi-

layer, protein) between the surfaces and hence an estimate of the in situ 

amounts of these components during an interaction.

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