Docking and molecular dynamics simulations of the Fyn-SH3 domain with free and phospholipid bilayer-associated 18.5-kDa myelin basic protein (MBP) – Insights into a non-canonical and fuzzy interaction

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RUNNING TITLE: *Docking/MD of MBP with Fyn-SH3 in aqueous and membrane environments.*

KEYWORDS: amphipathic α-helix, poly-proline type II (PPII), Fyn-SH3, intrinsically-disordered protein (IDP); myelin basic protein (MBP); molecular dynamics (MD) simulations; molecular recognition fragment (MoRF); GROMACS;

HIGHLIGHTS:

- Myelin basic protein (18.5-kDa MBP) has 3 membrane-associated amphipathic α-helices
- Proline-rich region (P93-P98) participates in MBP/Fyn-SH3 interaction over 50-ns MD
- Energetically-favorable MBP/Fyn-SH3 interaction in aqueous and membrane contexts
- Docking complemented by MD led to a more comprehensive interaction model

ABSTRACT

The molecular details of the association between the human Fyn-SH3 domain, and the fragment of 18.5-kDa myelin basic protein (MBP) spanning residues S38–S107 (denoted as xα2-peptide, murine sequence numbering), were studied *in silico* via docking and molecular dynamics over 50-ns trajectories. The results show that interaction between the two proteins is energetically favorable and heavily-dependent on the MBP proline-rich region (P93-P98) in both aqueous and membrane environments. In aqueous conditions, the xα2-peptide/Fyn-SH3 complex adopts a “sandwich”-like structure. In the membrane context, the xα2-peptide interacts with the Fyn-SH3 domain via the proline-rich region and the β-sheets of Fyn-SH3, with the latter wrapping around the proline-rich region in a form of a clip. These results provide a more-detailed glimpse into the context-dependent interaction dynamics and importance of the β-sheets in Fyn-SH3 and proline-rich region of MBP.

ABBREVIATIONS

Aze, azetidine-2-carboxylic acid; CAPRI, Critical Assessment of PRediction of Interactions; CD, circular dichroism; DMPC, dimyristoylphosphatidylcholine; DMPS, dimyristoylphosphatidylserine; DPC, dodecylphosphocholine; EP, electrostatic potential; EPR, electron paramagnetic resonance; ESP, electrostatic potential; GrOMACS, GROningen Machine for Chemical Simulations; I-TASSER, Iterative Threading ASSEmbly Refinement; ITC, isothermal titration calorimetry; MBP, myelin basic protein (specifically classic 18.5-kDa splice isoform); MD, molecular dynamics; MM-PBSA, molecular mechanics (MM) with Poisson–Boltzmann (PB) and surface area (SA) solvation; MoRF, Molecular Recognition Fragment; MPI, message-passing interface; MS, multiple sclerosis; NMR, nuclear magnetic resonance; PDB, protein data bank; PME, particle-mesh Ewald; PPII, poly-proline type II; PTM, post-translational modification; RMSF, root mean-squared fluctuation; SASA, solvent-accessible surface area; SH3, Src homology 3; VMD, Visual Molecular Dynamics;

INTRODUCTION

Myelination of the central nervous system by oligodendrocytes is a complex process involving a highly-coordinated network of protein-protein, protein-membrane, and cell-cell interactions (*e.g.*, [1,2,3,4,5,6,7,8,9,10,11]). The classic isoforms of myelin basic protein (MBP) are essential to the formation of the myelin sheath, and the predominant 18.5-kDa isoform in the adult human brain is generally considered to be a marker of compact myelin, maintaining the cytoplasmic leaflets of the oligodendrocyte membrane closely to each other to form the major dense line observed in electron micrographs of thinly-sectioned internodal myelin [12,13,14,15,16]. This isoform of MBP (henceforth the one considered here) is highly positively-charged, and interacts with myelin membranes predominantly by three amphipathic α-helical segments that represent molecular recognition fragments (α-MoRFs), and that we have previously denoted the α1-, α2-, and α3-helices [17,18,14]. Although the topology of MBP sandwiched between membranes is still unknown, we consider that a single protein molecule can interact with the opposing leaflets [19,20,21,22,23]. Then the two-dimensional protein network forms a molecular sieve [24,25,26,27]. The nature of the interactions of 18.5-kDa MBP with myelin membranes is as for other peripheral membrane proteins, including electrostatic, hydrophobic, and (perhaps to a small extent) a role for N-terminal acyl modifications [28,29]. It should also be noted that the 18.5-kDa MBP isoform sequesters phosphoinositides strongly [30,31,32,33,34], and should be acknowledged as a prominent member of this category of membrane proteins [35,36].

Yet the adhesive maintenance of compact myelin multilayers is only one function of MBP. This classic protein family proffers a diverse interactome modulated by extensive post-translational modifications – it is a hub in various structural and signaling networks that arise in different microdomains of myelin at different stages of differentiation [16]. Many previous

studies suggest that MBP participates in Fyn-mediated signaling pathways by a direct but non-canonical association with the Fyn-SH3-domain (SH3 – Src homology 3) [37,38,39]. These protein-MBP associations take place dynamically in the membrane ruffles during membrane process extension, as suggested by fluorescence co-localization in model oligodendroglial cells, and by co-immunoprecipitation from primary oligodendrocyte cell cultures (**Figure 1**) [40,41]. Using complementary approaches such as isothermal titration calorimetry (ITC), solution spectroscopy (CD – circular dichroism, NMR – nuclear magnetic resonance), and fluorescence imaging of transfected model oligodendrocyte cell cultures, we have shown that the interactions of MBP with Fyn-SH3 also involve other segments beyond the primary ligand [37,39]. This is an important new example of non-canonical SH3-domain associations, and of a new "fuzzy" complex formed by intrinsically-disordered proteins [42,43].

In all mammalian species, the central amphipathic α2-helix of MBP partly overlaps a subsequent proline-rich TPRTPPPS (murine T92-S99) segment that comprises a minimal SH3-ligand (XP-x-XP) and that we have termed a "molecular switch" [44,45,46,15]. In our previous molecular dynamics (MD) simulations of models of the central segments of MBP comprising the α2-helix and SH3-ligand, the former has been membrane-associated and the latter has been left exposed to solvent [47,45,38,46]. Since canonical binding targets of SH3-domains form left-handed poly-proline type II (PPII) conformations, we chose to model this region of the protein in this way in these first MD simulations. Solution NMR spectroscopy suggested that the PPII conformation appeared to depend on there being a membrane environment, potentially enhanced by the presence of the fully-formed adjacent α2-helix which was membrane-associated. Intriguingly, our most recent solution NMR spectroscopy of the interaction of an extended MBP-derived peptide comprising the central α2-helix and the SH3-ligand, with the SH3-domain of

Fyn, did not demonstrate the significant adoption of an ordered PPII conformation [39]! This is not to say that the membrane-associated protein might not form PPII in this region – it just appears that association with the SH3-domain alone does not necessarily induce this particular disorder-to-order transition. Moreover, an upstream region of the canonical SH3-ligand was required for binding, a complexity being revealed for other proteins [48,49,50].

The interplay between α-helix stability and length, its association with the lipid bilayer (depth and angle of penetration), nature and position of phosphate modifications, and membrane composition, and the precise conformation of the proline-rich segment (PPII or not) all remain to be elucidated at the molecular level. It is important to do so, because Fyn kinase regulates a number of signal transduction pathways in the CNS and plays an important role in neuronal and oligodendrocyte differentiation, plasticity, and survival (*e.g.*, [51,52]). It has been suggested that missteps in these signaling networks during myelin development may result in structurally weakened segments of myelin from which damage can readily propagate [53,54,47,55,56,57,22,58,59].

Here, in order to gain further molecular insight into the interaction of membrane-associated MBP with the SH3-domain of Fyn, we have built the three-component system in its entirety *in silico*. Previously, we have only performed *in silico* docking of MBP-derived peptides with models of Fyn-SH3 in an aqueous milieu [60,61], or MD simulations of segments of MBP in association with either dodecylphosphocholine (DPC) micelles, or with dimyristoylphosphatidylcholine (DMPC) bilayers [38,46,45]. Solution NMR spectroscopy of MBP-derived peptides has also only been performed with either DPC micelles alone, or with Fyn-SH3 alone [38,46,39]. The size and complexity of the membrane-bound MBP with Fyn-SH3 makes it exceedingly difficult to study experimentally by any established form of solution spectroscopy

(particularly NMR), and *in silico* modelling approaches are valuable to guide future investigations.

METHODS

Modelling the xα2-peptide of 18.5-kDa MBP and Fyn-SH3

In this study, we simulate the extended xα2-peptide (S38-S107, 70 amino acids):

S38-I39-G40-RFFS-G45-DRGA-P50-KRGS-G55-KDSH-T60-RTTH-Y65-GSLP-Q70-KSQH-G75-RTQD-E80-NPVV-H85-FFKN-I90-VTPR-T95-PPPS-Q100-GKGRG105-L106-S107

which was first described in reference [39]. The residues are numbered with respect to murine 18.5-kDa MBP which is 168 residues in length (A1-R168, **Figure 2A**) [14,15,44,16]. This extended peptide comprises the second amphipathic α-helical MBP segment (V83-I90) shown to be involved in membrane-association, as described in detail later.

The 3D structures of both full-length MBP and even of the shorter xα2-peptide have yet to be elucidated, and hence, a model of the xα2-peptide was generated using the I-TASSER online server for "Iterative Threading ASSEmbly Refinement":

(http://zhanglab.ccmb.med.umich.edu/I-TASSER). The I-TASSER package builds molecular models using a protein threading approach in which a submitted amino acid sequence is aligned with template structures of similar folds from the protein data bank whereas unaligned regions are built by *ab initio* modeling [62,63]. The top template used by I-TASSER was the solution NMR structure of the shorter α2-peptide (S72-S107, 36 amino acids) in association with dodecylphosphocholine (DPC) micelles [18,38] (PDB ID code 2LUG).

The docking target was represented by the X-ray crystallographic structure (PDB ID code 1SHF) of the SH3-domain of human Fyn tyrosine kinase (UniProtKB: P06241), comprising 59

residues and obtained at 1.9-Å resolution (**Figure 2B**) [64]. Two scenarios of xα2-peptide-Fyn-SH3 interaction environments were considered: (Scenario-1) xα2-peptide and Fyn-SH3 in water; (Scenario-2) xα2-peptide and Fyn-SH3 on a dimyristoylphosphatidylcholine (DMPC) phospholipid membrane and water.

Docking of xα2-peptide and Fyn-SH3 using the ClusPro2.0 web-server

Empirical data were used to guide the docking between the xα2-peptide and the Fyn-SH3 model, specifically the interacting residues on MBP identified previously by solution NMR spectroscopy [38,37,39]. A total of 14 xα2-peptide interacting residues comprising T62-L68 and T92-P98 of the full-length 18.5-kDa murine MBP (**Figure 2A**) (*i.e.*, attraction residues) were used as *a priori* knowledge for the ClusPro2.0 program [65,66]. The ClusPro2.0 program uses rigid-body PIPER-based algorithm that scores a set of possible docked conformations according to surface complementarity and clustering properties, and has performed well in the CAPRI (Critical Assessment of PRediction of Interactions) docking challenge [67]. The advantage over other docking algorithms is in the availability of many fine-tuning options, including those for specification of attraction and repulsion residues (*i.e.*, prior knowledge). As recommended by the ClusPro2.0 authors, we used "balanced" energy function coefficients and the largest cluster of 324 members (*i.e.*, ligand positions) to select the final, central conformer, docked xα2-peptide/Fyn-SH3 complex model shown in **Figure 3**. All other docking parameters were set at default values including the energy scoring function with the "balanced" set of weight coefficients further described in [].

The docked system required local energy minimization in order to resolve clashes and high energy tensions. System assembly steps were done partly using the PyMOL visualization

package (Schrödinger). System assembly and all MD runs were done with the GROMACS 5.0.4 software package [68,69] using the Gromos96 ffG53a6 force-field [70]. The choice of the force-field was dictated by DMPC membrane parameterization done in the Gromos96 force-field [71]. In addition, this united-atom Gromos96 force-field was chosen due to its superior performance characteristics especially in protein simulations [72]. In particular, Gromos96 force-fields have been shown to be the best to capture secondary structure elements such as β-hairpins. Molecular dynamics production runs were performed using the Compute Canada/SharcNet facilities (https://www.sharcnet.ca). For visualization and analysis of structural files and trajectories, the GROMACS utilities and the Visual Molecular Dynamics (VMD) program [73] were utilized. In post-run MD analyses, particular attention was given to the MBP proline-rich region (P93-P98) representing the experimentally-determined interaction site with Fyn-SH3.

Binding free energy measurements

The MM-PBSA method (Molecular mechanics Poisson-Boltzmann surface area, implemented as a "*g_mmpbsa*" GROMACS tool) was used to measure the free binding energy of the xα2-peptide/Fyn-SH3 complex in solution, using the trajectory snapshots of complexes as input [74]. In its current implementation, the *g_mmpbsa* tool does not calculate the entropic contribution to binding. The free binding energy (ΔG_{bind}) comprises van der Waals, electrostatic, polar solvation, and solvent-accessible surface area (SASA) contributions.

Scenario-1: Water-only MD simulation

In the first scenario (water-only simulation), the docked complex was positioned in the center of a 10x10x10 nm^3 simulated box, and solvated with spc216 water molecules via the

“*genbox*“ tool. To neutralize the system, one CL- ion was added. The resulting system was energy-minimized using the default parameters summarized in the “minim.mdp” file (found in the online Supporting Information). The key energy-minimization parameters included the steepest descent minimization algorithm, and maximum force (F_{max}) tolerance for all atoms of the system set at 1000.0 kJ/mol/nm (the “*emtol*“ parameter).

Next the whole system was equilibrated through the NVT and NPT steps. The NVT step allowed the whole system to equilibrate for 100 ps at a constant volume and a temperature of 37°C, whereas the NPT step lasted for 1000 ps, also at 37°C, and a constant pressure of 1 bar. During the NVT and NPT steps, the movement of the complex was restrained by virtue of the “-*DPOSRES*” parameter of the mdp configuration file (see the online Supporting Information).

The equilibration steps were followed by a 50-ns MD production run which used the leap-frog integrator at 0.002-ps integrator time-steps. The thermal coupling groups were “Protein” and “Water and Ions”, the particle cut-off scheme was Verlet, and the temperature was set at 37°C. (For more detailed MD parameters, please refer to the mdrun_50ns.mdp file provided online in the Supporting Information.)

Scenario-2: DMPC membrane MD simulation

In the second scenario (DMPC membrane environment), the dimensions of the box were 9x9x12 nm^3. The docked complex was placed on top of one of the leaflets of the DMPC membrane such that the xα2-peptide α2-helix was partially embedded in the bilayer, and the proline-rich segment was accessible for Fyn-SH3 binding as illustrated in **Figure 4**, and as previously modelled in MD simulations of smaller MBP-derived peptides [[46,45]]. The positioning of the docked Fyn-xα2-peptide system was done using the PyMol (version 1.7.5) program,

particularly with the "*rotate*" and "*translate*" commands. The xα2-peptide was positioned at approximately 45° with respect to the DMPC phospholipid bilayer. To preserve the docked system positions, the same displacements/transpositions were echoed in Fyn-SH3 to preserve relative positioning of the xα2-peptide/ Fyn-SH3 selected model.

The main steps, including energy minimization and equilibration, were similar to the water-only system. The boxes of docked xα2-peptide/Fyn-SH3 were also resized to 9x9x12 nm^3 to accommodate the DMPC membrane box. Next, the DMPC-only 9x9x12 nm^3 box was solvated with spc216 water molecules (via "*genbox*"). The topology files were generated separately for each of the components of the docked xα2-peptide/Fyn-SH3 complex (via "*gmx editconf*"). The solvated DMPC box in water was next merged with the positioned xα2-peptide/Fyn-SH3 complex (again via the "*genbox*" tool). Water molecules occurring within the lipid bilayers were removed via the custom-made "*waterRemover*" tool available in the online Supporting Information. Similar to the water-only simulation, the assembled system passed through energy minimization, and NPT and NVT steps, using the same configuration settings defined by the *.mdp configuration files (see the online Supporting Information). The production MD runs were also for 50 ns, as for Scenario-1.

Availability

Trajectories, structures, MD parameters, and assembly scripts are deposited at the Sage Synapse repository (Synapse: syn5303692).

RESULTS and DISCUSSION

Rationale for model systems built in this study – the biological background

The mRNA for membrane-associated 18.5-kDa MBP is trafficked on microtubules to the cell periphery where it is translated [75,76]. It can be predicted that the protein associates with a single membrane leaflet shortly after synthesis (*cf*., [77]), and that surface-associated MBP is the species that would first sequester phosphoinositides and interact with Fyn and cytoskeletal proteins in extending membrane processes and ruffles [22]. The activation of Fyn kinase results in the localized translation of the MBP mRNA [78,79], and this phenomenon may be modulated by the direct interaction of 18.5-kDa MBP with the SH3-domain of Fyn (see **Figure 1**).

Rationale for model systems built in this study – the biophysical background

Our emergent *in silico* docking study of the 70 amino-acid long MBP-peptide with the Fyn-SH3 structure included the 13-residue T92-R104 segment comprising the minimal SH3-ligand (T92-P93-R94-T95-P86) [60,61]. In this, our first comprehensive study of this interaction, we modelled the region comprising the XP-x-XP consensus SH3-ligand as a left-handed poly-proline type II (PPII) helix, and performed *in silico* rigid-body docking experiments with the crystallographic structure of Fyn-SH3 [61]. The results showed the presence of interactions such as salt bridges, CH-π and cation-π. For example, this last interaction takes place between positively-charged lysyl and arginyl residues of the MBP-peptide and the aromatic residues of Fyn-SH3. Although the MBP-peptide phosphorylation caused significant changes in its structure, no loss of binding to SH3-ligands (*e.g.*, MBP) was observed [61]. At the time, this assumption of a PPII conformation in MBP was justified because of its propensity [80,81], and because we had observed transient PPII structuring in both free and dodecylphosphocholine-associated 18.5-kDa MBP (full-length protein) via collection of circular dichroism spectra at variable temperatures

[61,15]. These latter experiments, though, could not pinpoint where in the 168-residue protein the PPII conformation was formed.

Although these *in silico* simulations were insightful and interpretable, they could not be realized experimentally. A synthetic peptide produced by AnaSpec Inc. (Fremont, CA), which spanned amino acids F86-G103 (murine 18.5-kDa sequence numbering) and comprised the canonical SH3-ligand comfortably, appeared not to bind SH3-domains at all, as ascertained by ITC [37,39]. Indeed, we could only observe clear heats of interaction in ITC experiments by using full-length 18.5-kDa MBP charge variants, or by extended MBP-derived peptides encompassing residues S72-S107 or S38-S107, the so-called α2- or xα2-peptides, respectively (discussed further below). Moreover, even though NMR spectroscopic and mutagenesis studies indicated that the expected SH3-ligand on MBP was indeed the primary SH3-target [38,37], the ITC data with larger MBP-peptide fragments and with the whole protein had shapes indicating additional interaction sites (*cf*., [82]), which were confirmed subsequently to be upstream and to comprise MBP residues (T62–L68) [39]. These latter experiments involved solution NMR spectroscopy, co-transfection of an immortalised oligodendroglial cell line, and polyacrylamide gel electrophoresis of glutaraldehyde cross-linked complexes.

All things considered, to be biologically relevant, our simulation model required membrane-associated MBP comprising the minimal SH3-ligand, the overlapping α2-helix that lay on the surface and partially penetrated the lipid bilayer for presenting the SH3-ligand to the cytoplasm (**Figures 1, 4**), and the region upstream of these α-molecular recognition fragments (αMoRFs) represented by the three α-helical segments (α1, α2 and α3) defined in the next section [22]. We do not yet have a model for full-length 18.5-kDa MBP on a membrane surface, or

between two membranes, and chose here to model just the xα2-peptide for reasons articulated next.

Progression and nomenclature of recombinant and *in silico* MBP-derived peptide models

The murine 18-5-kDa MBP isoform consists of 168 amino acids (A1-R168), and it is this numbering that we have used consistently here and previously. A hydrophobic moment analysis of this sequence has highlighted the three amphipathic α-helices involved in membrane association: the α1-helix (T33-D46), the α2-helix (V83-T92), and the α3-helix (Y142-L154) [[14]]. This simple prediction has matched experimental analyses by electron paramagnetic resonance (EPR) and NMR spectroscopy (*e.g.*, [[17,83,19,20,84]]). For experimental purposes, we first constructed 3 recombinant forms encompassing these α-helices: the α1-peptide (A22-K56, 35 amino acids), the α2-peptide (S72-S107, 36 amino acids), and the α3-peptide (S133-S159, 27 amino acids) [[18,38,85]]. A solution NMR model of the α2-peptide on a DPC micelle has been determined and is available in the Protein Data Bank (RCSB ID code rcsb102847; PDB ID code 2LUG) [[38]]. Because of the unusual interaction of MBP with Fyn-SH3, we have also constructed a fourth recombinant variant called the extended xα2-peptide: residues S38-S107 (70 amino acids) [[39]].

Due to the limitations of computational resources available to us, as well as the lack of global conformational topology of the protein, previous MD studies of MBP-derived peptide models on membrane models have necessarily comprised smaller protein segments. These computational models have been referred to as the mdα1-peptide (murine 18.5-kDa residues R29-G48), and the mdα2-peptide (murine 18.5-kDa residues E80-G103), to distinguish them from recombinant forms used experimentally [[45,86,38]]. Molecular dynamics simulations of the

α2-peptide have been performed on DMPC bilayers to complement experimental studies and to assess the effects of site-specific threonyl phosphorylation at residues T92 and/or T95 [[46]]. Here, we present our largest MBP-peptide model yet simulated, that of the extended xα2-peptide (S38-S107, 70 amino acids). The starting conformation was modeled using I-TASSER, which utilized the solution NMR structure of the shorter α2-peptide (S72-S107, 36 amino acids) on a DPC micelle [[38]] (PDB ID code 2LUG) as the main structural template.

Detailed analysis of Scenario-1 (water-only MD)

In this environment, the xα2-peptide/Fyn-SH3 complex was highly mobile and loose, with an overall strong interaction being evident. The average RMSD value calculated for all time snapshots with respect to the time-zero structure for the region encompassing the α2-helix and PP-II (E80-Q100) was rather high (2.108 Angstrom). No dissociation event was observed along the 50-ns trajectory. The interacting residues of the xα2-peptide/Fyn-SH3 complex are shown in **Table 1**. The key three prolines of the SH3-associating segment (P96, P97, and P98) predominantly remained in contact with Fyn-SH3. The estimated energy of binding (ΔG_{bind}), estimated via the MM-PBSA model without the entropic term (-TΔS) [[74]], showed a strong propensity towards xα2-peptide/Fyn-SH3 association throughout the entire simulation supported by a negative slope of -40.67 obtained from a linear fitting of ΔG_{bind} *versus* time (**Table 1**). The MM-PBSA model showed that electrostatic forces are the major contributing component to binding [[48,49]], a trend that persisted over the entire simulation course. The non-covalent α2-peptide/Fyn-SH3 interactions were mostly dominated by H-bonds and van der Waals contacts, followed by salt-bridges. The largest number of salt-bridges (a total of 6) was observed at 50 ns.

The R94/N100, P93/Y93 and R94/N99 pairs were amongst the most frequent examples of the stated non-covalent interactions. (see Table 1 and online supplement).

The MBP α2-helix unraveled rapidly at the 10-15-ns time-point, perhaps because of the influence of the large disordered segment (S35-P82) next to the N-side of the α2-helix (see **Figure 5** at the 15-ns time-point). Specifically, this large N-terminal end segment before the α-helical region in the residue range S38-P82 (xα2-peptide numbering, see **Figure 2A**) seems to be pushed by the Fyn-SH3, and drastically changes its position with respect to all five Fyn-SH3 β-sheets (compare, for example, time-points 0 and 5 ns of **Figure 5**). Unfolding of the α2-helix in aqueous environment is not unusual [87,88], since water is known to disrupt hydrogen bonding between α-helical residues, causing the α-helix-coil transition discussed in [89]. Analysis of H-bonding patterns in the α-helical region of the xα2-peptide (V83-T92) showed a significant change. Out of eight initial inter-residual H-bonds one remains between residues N81 and V83 at 50 ns. The Fyn-SH3 structure is stable, in comparison.

At 10 ns, the Fyn-SH3 domain engulfs the xα2-peptide, adopting a dome-like configuration that is maintained throughout the whole 50-ns trajectory. This event is also supported by a larger number of interacting Fyn-SH3 residues and a significant increase in favorable binding energy from -1673 to -3108 kJ/mol (**Table 1**). The overall xα2-peptide/Fyn-SH3 complex fluctuations include adoption of the “loosest” state (in terms of overall spherical diameter) at 15 ns with the segment spanning residues (S38-P82) sticking out. The “loose” state at 15 ns is slowly transitioned to the most compact state observed at the 50-ns end-point. Specifically, there is a compact xα2-peptide/Fyn-SH3 complex structure at the 50-ns end-point with both polypeptide chains being on top of each other and five Fyn-SH3 β-sheets facing the

xα2-peptide denatured α-helical region (**Figure 5**). The denaturation of the α-helical region (V83-T92) is complete at 15 ns.

The behavior of the N- and C-termini of both polypeptide chains is similar, with a propensity for intra-molecular (*i.e.*, within the chain) pairing. Compared to a shorter α2-peptide (S72-S107), the xα2-peptide has a longer N-terminus positioned next to the α2-helix (V83-T92) providing an extra level of stability to the region via non-covalent interactions. The extended N-terminal of the xα2-peptide remains mainly in the extended conformation throughout the entire simulation. At 20 ns, both C-termini are at their closest proximity and move further apart only slightly during the subsequent 30 ns of simulation. The proline-rich region (P93-P98), defined in **Figure 2A**, formed a loop, shown in yellow in **Figure 5**, and was highly dynamic. The proline-rich region interacted with the loop residues found between the β1- and β2-sheets (L90-G106) throughout the entire trajectory (**Table 1**). Starting from 10 ns, this region also interacted with the β3- and β4- -sheet of the Fyn-SH3(W119-L125 and T130-P134, respectively). These β-sheets were in anti-parallel configuration (**Figure 5** at 10-50 ns). The β-sheet rich structure of Fyn-SH3 peptide was maintained throughout the simulation (*i.e.*, the total number of five β-sheets remained constant) with slight fluctuations in their lengths and positions.

Detailed analysis of Scenario-2 (DMPC membrane MD)

The MD simulation in the context of the DMPC membrane was quite different from that in the water-only Scenario-1. First of all, the membrane-associated α2-helix of the xα2-peptide was able to preserve its integrity throughout the 50-ns MD run (**Figure 6**). The strong torsion of the α-helix was observed at the 2nd α-helical turn spanning the F86-K88 residue range (**Figure 2**). Whereas the amphipathic α2-helical segment comprises residues V83-T92, and SDSL/EPR of

this immunodominant epitope showed an α-helical projection pattern [83,90], a solid-state NMR study provided more detailed molecular information on the secondary structure, and indicated that the core α-helical architecture was formed by residues V83-K88, *i.e.*, the C-terminus unraveled slightly [20]. Similarly, the various MD simulations of mdα2- and α2-peptides on the DMPC bilayer showed more transient membrane-association of the C-terminal end of the α2-helix, especially after threonyl phosphorylation [45,46]. One explanation is that the strong interaction of the xα2-peptide with Fyn-SH3 has the effect of pulling this α2-helix off the membrane. It has been experimentally shown that the interaction of membrane-bound 18.5-kDa MBP with Ca^{2+}-calmodulin disrupts the association of the α3-helix with the membrane [91], so this explanation is reasonable.

Overall, the xα2-peptide/Fyn-SH3 complex motions were more restricted compared to Scenario-1 (water-only simulation). Here in Scenario-2 (DMPC membrane), the five β-sheets of Fyn-SH3 remained stable (*i.e.*, no differences in length or position), with the Fyn-SH3 domain adopting a less-round hair-clip structure, still with the proline-rich region (P93-P98) as the center of the interaction (**Table 2**). The three key residues P96, P97, and P98 of the xα2-peptide SH3-ligand constantly interacted with Fyn-SH3 as supported by our interacting analysis results (**Table 2**, **Figure 6**). Specifically, the Fyn-SH3 β3- and β4- sheets (**Figure 2B**) and the loop between the β1- and β2-sheets (L90-G106) directly interacted with the P93-P98 region of the xα2-peptide (**Figure 2A**), of which the latter had already been ascertained experimentally [38,37,39]. No dissociation event was observed throughout the whole duration of the MD run.

The association energy measured via the MM-PBSA model remained negative, indicating an energy-favorable propensity towards xα2-peptide/Fyn-SH3 complex formation additionally supported by a negative slope of -1.382 of a linear fitting of ΔG_{bind} *versus* time (**Table 2**). The

association trend under the DMPC membrane Scenario-2 was less pronounced compared to the water-only Scenario-1, based on the slope comparisons (*i.e.*, a 29-fold decrease). Similar to Scenario-1, the predominant binding force of the complex over the entire course of simulation was electrostatic. The non-bonding interactions profile of the xα2-peptide/Fyn-SH3 complex was rather dynamic throughout the simulations but was again mostly dominated by H-bonding, followed by van der Waals interactions, followed by salt-bridges. In contrast to Scenario-1, the salt-bridges were more persistent throughout the simulation, reaching a maximum of 18 at 20 ns. The T95/S135, T95/N136 and R94/Y137 pairs exemplify each interaction type, respectively. (The online Supporting Information provides a complete set of interaction profiles for reference.)

Similar to Scenario-1, the N- and C-termini of the xα2-peptide and Fyn-SH3 showed a positive trend towards the intra-molecular (within), but not inter-molecular (between) associations. In terms of the membrane penetration, the xα2-peptide position did not change significantly with exception of the α2-helix. The region right after the 2nd α-helical turn (K88-T92) changed its position from ~45° included to a completely vertical position (**Figure 7**, 15-50 ns). This re-arrangement of the α2-helix most likely has to do with the Fyn-SH3 interaction and mobility of the proline-rich region.

The protein complex-membrane interface and hydrophobicity analyses showed that the majority of the non-covalent interactions were contributed by the xα2-peptide via van der Waals contacts and H-bonds with the lipid head groups. Examples of the xα2-peptide residues participating in H-bonding included S38, I39, H59, R61, F86, N89, and R94, amongst others. The non-bonding interactions were uniformly distributed over the interaction surface of the xα2-peptide. Hydrophobic analysis identified the xα2-peptide as less hydrophobic compared to Fyn-SH3, with total charges of +12 and -6, and pI values of 11.89 and 3.98, respectively. The most

hydrophobic regions of the xα2-peptide are located in the α-helical segment (V83-V91, **Figure 2A**), and those of the Fyn-SH3 segment in its N- and C-termini (V84-L90 and V138-V141, **Figure 2B**). Despite the rather weak mean hydrophobicity of the N-terminus of the xα2-peptide, ranging from 1.1 to 0.18, (calculated using the 5-residue sliding window), the xα2-peptide N-terminus rested embedded in the hydrophobic lipid environment over the course of the entire MD run, potentially aided by the large number of the H-bonds and vdW contacts with the lipid head groups and hydrophobic tails.

The xα2-peptide has a longer N-terminus (S38-K71) compared to α2-peptide (S72-S107). Similar to Scenario 1, analysis of the trajectory snapshots indicate that the N-terminus segment is also in a close proximity to the xα2-peptide helical region (V83-T92). Presence of large number of non-covalent interactions between residues of the S38-K71 and V83-T92 segment suggests possible stabilization effects.

Comparison of aqueous and membrane-associated MD scenarios

The trajectories under both considered scenarios differed significantly, showing the importance of the simulation context and accountability of all possible factors in an MD study. In the case of Scenario-1, the xα2-peptide/Fyn-SH3 complex was less compact with partial loss of the α-helix as compared to Scenario-2. Remarkably, in both scenarios the interaction with the proline-rich region was consistent independent of the environment context. It should be noted that the stated binding free energies in **Tables 1 and 2** should be interpreted as relative for purpose of comparing of binding strengths between scenarios and between trajectory snapshots. The MM-PBSA model has many caveats and requires a careful tuning and parameter selection in order to provide accurate absolute ΔG_{bind} predictions, as discussed in [[92]]. Consistent with our previous experimental evidence, MBP, represented by the xα2-peptide, is the ligand for Fyn-SH3 [[60,61,38,37,39]] supported by the stable complex formation throughout the 50-ns simulations.

Compared to the water-only Scenario-1, the number of interacting residues in Scenario-2 decreased, along with the 2.3-fold decrease in absolute value of the ΔG_{bind} value (compare -3453 and -1545 kJ/mol at 50 ns, **Table 2**). This result indicates the involvement of the membrane context in xα2-peptide/Fyn-SH3 interaction, possibly through modulation of SH3-domain accessibility and local electrostatic environment.

Importantly, these *in silico* results obtained here have confirmed previous experimental evidence that the interaction between MBP and Fyn-SH3 does not exclusively occur at the canonical SH3-ligand site (murine residues T92-S99, **Figure 2A**), but also critically involve upstream [39,48,49,50] and downstream [82] regions, as seen in **Table 2**. It is now clearer why a synthetic MBP-derived peptide (murine F86-G103) does not bind to SH3-domains [37]. A more detailed analysis revealed that, for example, L68, T77 P98, and Q100 participate in H-bonding with Fyn-SH3 residues. Overall, our current *in silico* and prior experimental investigations suggest a multi-site interaction model for MBP and SH3-domain interactions [37,39].

CONCLUDING REMARKS

We have presented a hybrid docking-MD analysis of the interacting residues of the essential myelin protein MBP, and the Fyn-SH3 domain, in aqueous solution and in the physiologically more relevant context of a membrane environment. Particularly, we illustrate *in silico* the molecular details of the interaction with Fyn-SH3 of the proline-rich MBP region (P93-P98) encompassed by the modelled xα2-peptide, that are consistent with prior experimental, spectroscopic evidence. Under aqueous conditions, Fyn-SH3 engulfs the xα2-peptide, adopting a dome-like structure. The xα2-peptide and Fyn-SH3 interaction in a membrane context is more ordered and restrictive (less entropy). Interestingly based on the interaction energy

measurements, the xα2-peptide/Fyn-SH3 complex formation is 2.3-fold weaker in the DMPC membrane context as compared to an aqueous environment, as measured by ΔG_{bind} values based on the MM-PBSA model without the entropic term. The proline-rich P93-P98 region of MBP is the primary interaction bridge between both proteins in both the water-only and membrane contexts, and involves the β3- and β4-sheets of Fyn-SH3 (W119-L125, T130-P134), and the loop between the β1- and β2-sheets (L90-G106).

Moreover, we have developed a virtual system for evaluating the effects of modifications to a critical central region of MBP, including phosphorylation [46,45], of proline mis-incorporation or isomerization [56,47,93,94,95], and their cross-talk [16,96,97], in the context of diseases such as multiple sclerosis [53,54,98,99]. Within the 8-residue -T92-P93-R94-T95-P96-P97-P98-S99- segment alone we have $2^8 = 256$ combinations of modifications – phosphorylation of Thr or Ser, deimination of Arg, and *cis*/*trans* isomerization of each Pro. We are currently extending the membrane model to include cationic lipids as found in myelin and to simulate more physiologically-relevant models [100,86], with the intent of exploring such combinatorial problems further by MD simulations, to define and guide subsequent experimental investigations.

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Appendix A. Supporting Information

Trajectories, structures, MD parameters, and assembly scripts are deposited at the Sage Synapse repository (Synapse: syn5303692).

FIGURE CAPTIONS

Figure 1. A schematic illustration of MBP/Fyn-SH3 interaction steps from expression until joining of the myelin membranes. **(A)** Delivery of the MBP-mRNA to the membrane periphery and its translation; **(B)** interaction of MBP with Fyn-SH3 kinase with potential activation of cytoskeletal proteins causing creation of membrane protrusions; **(C)** joining of two membranes via MBP acting as a “molecular Velcro” initiating myelin compaction.

(The colored version of this figure can be seen in the online version of this article.)

Figure 2. The amino acid sequence and secondary structural map of **(A)** 18.5-kDa murine myelin basic protein (MBP), and **(B)** the human Fyn-SH3 domain. **(A)** The classic MBP is intrinsically-disordered in aqueous solution but has three distinct α-helical regions that form transiently and are stabilized by membrane association, representing molecular recognition fragments (MoRFS). The sequence enclosed by the red rectangle represents the 70-residues long xα2-peptide encompassing residues S38–S107. The simulated peptide contains an amphipathic α-helix (denoted the α2-helix because it is the second of three such MoRFs), a proline-rich region (P93-P98) that is the primary SH3-ligand, as predicted and confirmed by NMR spectroscopy and mutagenesis, and which has been modeled here as a left-handed poly-proline II (PPII) structure. This central region of 18.5-kDa MBP is highly conserved evolutionarily and comprises also two mitogen-activated protein kinase sites at residues T92 and T95. **(B)** The human Fyn-SH3 sequence part of the crystal structure (PDB ID code 1SHF) is highlighted by the red rectangle. The β-sheets are shown by arrows and are numbered according to N→C directionality. The sequence shown here represents only part of the full human Fyn-SH3 domain sequence (UniProt: P06241).

(The colored version of this figure can be seen in the online version of this article.)

Figure 3: The selected pose of the ClusPro 2.0 docked α2-peptide/Fyn-SH3 complex. The xα2-peptide of 18.5-kDa murine MBP is shown in red, and the Fyn-SH3 domain is shown in blue. The proline-rich region (P93-P98) of MBP containing the SH3-ligand (XP-x-XP) is shown in yellow. Docking was done via the ClusPro2.0 web-server with constraints consisting of experimentally determined interacting residues (see Methods). The selected pose interacting residues numbers at ≤ 4.0 Å distance of the xα2-peptide encompassing 18.5-kDa MBP residues S38–S107 are G66-L68, K71-S72, Q78, K88-P98, and Q100 (see **Figure 2A** and **Table 1**).

(The colored version of this figure can be seen in the online version of this article.)

Figure 4. Initial positioning of the xα2-peptide/Fyn-SH3 complex with respect to the DMPC lipid bilayer, shown in green, with water molecules shown in blue. The xα2-peptide was tilted with respect to the membrane surface in order to embed its α2-helix partially, whilst maintaining solvent accessibility of the proline-rich segment shown in yellow. Approximately 60 out of 70 residues of the xα2-peptide (i.e. 85.7%) were embedded into DMPC lipid bilayer spanning R47-P50 and P93-P98 regions.

(The colored version of this figure can be seen in the online version of this article.)

Figure 5. Selected MD simulation snap-shots of the docked xα2-peptide/Fyn-SH3 complex in the water-only environment (Scenario-1), throughout the 50-ns MD simulation. The PPII segment is shown in yellow, xα2-peptide in red, and Fyn-SH3 in blue. The N- and C-termini are indicated by the corresponding letters. The S38-P82 xα2-peptide segment is highlighted by curly brackets.

(The colored version of this figure can be seen in the online version of this article.)

Figure 6. Selected snap-shots of the docked xα2-peptide/Fyn-SH3 complex in the DMPC lipid bilayer environment (Scenario-2), throughout the 50-ns MD simulation. The lipid bilayer is omitted for clarity; the PPII segment is shown in yellow, the remainder of the xα2-peptide in red, and the Fyn-SH3 domain in blue. The N- and C-termini are highlighted further by orange and green colors, respectively. Water is not shown for simplicity.

(The colored version of this figure can be seen in the online version of this article.)

Figure 7. Selected snap-shots of the docked xα2-peptide/Fyn-SH3 complex in the DMPC lipid bilayer environment (Scenario-2), throughout the 50-ns MD simulation. The DMPC lipids are shown here in green; the color scheme for the proteins is the same as in **Figure 6**, and water is not shown for simplicity.

(The colored version of this figure can be seen in the online version of this article.)

TABLES

Table 1 – The xα2-peptide/ Fyn-SH3 interaction profiles and binding energies of the water-only MD Scenario-1.

Time (ns)	xα2-peptide (MBP)*	Fyn-SH3*	ΔG_{bind} (kJ/mol)
0	66-68, 71, 72, 78, 88-98, 100	91, 93, 96, 119, 130, 134, 135, 136	-1597
5	41, 63-68, 71-78, 88-100	91-94, 99, 100, 132, 134, 136	-1693
10	41, 63-66, 68, 71-78, 88-100	91-94, 99, 100, 117-119, 132, 134, 136	-1673
15	41, 68, 70-79, 88-89, 91-102, 104, 106	91, 93, 94, 96, 99-101, 118, 135-137, 141, 142	-3108
20	40, 41, 68-73, 77-79, 85, 88-91, 93-95, 97-101, 104-106	91-94, 100, 101, 118, 136, 140-142	-3176
25	38-41, 66, 68-79, 85, 88-101, 104-106	91-94, 96, 100, 101, 118, 135, 136, 138, 140-142	-3488
30	38-41, 67-79, 85, 88-90, 93-101, 103-106	93-96, 100, 101, 104, 117, 118, 135, 136, 138, 140-142	-3553
35	38-41, 65, 68-79, 85, 88-101, 104, 106-107	91-94, 96, 100, 101, 117, 118, 135, 136, 140-142	-3608
40	39-41, 64, 67-79, 88-101, 104, 106	91, 92 94, 96, 100, 103, 117, 118, 136, 138, 140-142	-3518
45	41, 64, 68-74, 77-79, 88-101, 104, 106	91, 93, 94, 99, 100, 103, 104, 117, 118, 138, 140-142	-3238
50	41, 47, 64-74, 77-79, 88, 89, 93-101, 103-106	91-94, 96, 99, 101, 117, 118, 136, 138, 140-142	-3453

*Any residue pair from xα2-peptide and Fyn-SH3 that has any atom within 4.0 Å is considered interacting. Residues numbers follow the numbering scheme defined in **Figure 2**.

Table 2 – The xα2-peptide/ Fyn-SH3 interaction profiles and binding energies of the DMPC membrane MD Scenario-2.

Time (ns)	xα2-peptide (MBP)*	Fyn-SH3*	ΔG_{bind} (kJ/mol)
0	42, 64-68, 71, 72, 75, 77, 92-100, 107	91, 93, 117, 118, 135-137	-1609
5	41, 42, 64-68, 70, 71, 77, 91-98, 100, 107	93, 118, 132, 134-136	-1458
10	47, 65-68, 70-72, 77, 92-98, 100, 107	116-119, 120, 136, 140	-1487
15	47, 65-68, 70-72, 77, 93-98, 100, 107	113, 115, 116, 118, 135-137, 140	-1553
20	41, 42, 47, 63, 65-68, 70-72, 77, 93-98, 100, 107	115-118, 120, 135-137, 140	-1660
25	41, 42, 47, 62-68, 70-72, 77, 93-98, 107	91, 93, 135-137, 139, 140	-1756
30	41, 42, 47, 63-68, 70-72, 77, 93-96, 98, 100, 107	91, 93, 116, 118, 120, 135, 136, 140	-1565
35	41, 42, 44, 47, 63-68, 70-72, 77, 93-99, 100, 107	90, 91, 94, 105, 118, 120, 135-136, 139	-1638
40	41, 42, 47, 63-71, 94-96, 98, 100, 107	91, 94, 116, 118, 120, 135, 136,	-1607
45	42, 47, 63-72, 77-78, 93-96, 100, 107	90, 91, 93, 94, 118, 120, 135, 136	-1620
50	41, 42, 47, 63, 65, 66, 67, 68, 70-72, 77, 78, 93-96, 98, 100, 107	91, 93, 116, 117, 118, 120, 135-137, 139	-1545

*Any residue pair from xα2-peptide and Fyn-SH3 that has any atom within 4.0 Å is considered interacting. Residues numbers follow the numbering scheme defined in **Figure 2**.

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