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Myelin biogenesis

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Document Version

Publisher's PDF, also known as Version of record

Publication date:
2014

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Ozgen, H. (2014). *Myelin biogenesis: Dynamics of MBP, PLP and galactolipids*. [Thesis fully internal (DIV), University of Groningen]. [S.n.].

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Chapter 2

Oligodendroglial Membrane Dynamics in Relation to Myelin Biogenesis

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Manuscript In Preparation

Abstract

In the central nervous system, oligodendrocytes synthesize a specialized membrane, the myelin membrane, which enwraps the axons in a multilamellar fashion to provide fast action potential conduction and to ensure axonal integrity. When compared to other membranes, the composition of myelin membranes is unique with its relatively high lipid to protein ratio. However, their biogenesis is quite complex and requires a tight regulation of sequential events, which are deregulated in demyelinating diseases such as multiple sclerosis (MS). To devise strategies for remedying such defects, it is crucial to understand molecular mechanisms that underlie myelin assembly and dynamics, including the ability of specific lipids to organize proteins and/or mediate protein-protein interactions in healthy versus diseased myelin membranes. The tight regulation of myelin membrane formation has been widely investigated with classical biochemical and cell biological techniques, both *in vitro* and *in vivo*. However, our knowledge about myelin membrane dynamics, such as membrane fluidity in conjunction with the movement/diffusion of proteins and lipids in the membrane, the specificity and the role of distinct lipid-protein and protein-protein interactions, is limited. Here, we provide an overview of recent findings about myelin structure in terms of myelin lipids, proteins and membrane microdomains. To give insights into myelin membrane dynamics, we will particularly highlight the application of model membranes and advanced biophysical techniques, *i.e.*, approaches which clearly provide an added value to insight obtained by classical biochemical techniques.

1. Introduction

In the central nervous system (CNS), processes protrude from oligodendrocytes (OLGs) at the end of which sheet-like extensions are formed, the myelin membranes, which ensheath axons in a multilamellar fashion to provide proper saltatory nerve conduction [1]. Myelin membranes are unique in that 70% of their dry weight consists of lipids, in particular cholesterol and galactolipids, such as galactosylceramide (GalC) and sulfatide (table 1, [1,2]). Myelin also contains a specific repertoire of myelin proteins, among which proteolipid protein (PLP) and myelin basic protein (MBP) are the most abundant ones [1–3]. The interactions between lipids and proteins are pivotal for myelin formation and maintenance, regulating protein transport to and the molecular organization within the myelin sheath [2,4,5]. Thus, biochemical and biophysical properties of the lipids actively control (myelin) protein sorting,

while (myelin) proteins, in turn, are able to organize lipids, thereby creating regions of specialized molecular packing (e.g. lipid rafts) and dynamics, relevant to their functioning [6–8]. Indeed, the “lipid raft” concept is also very important for myelin research given that such rafts play a role in orchestrating signaling platforms that regulate OLG behavior, and sorting of several myelin proteins [2,4,6,9,10]. All these vital cellular functions are precisely regulated in space and time, and any alteration in the complex organization of oligodendroglial (OLG)-myelin membranes leads to severe neurological disorders such as multiple sclerosis (MS), acute-disseminated encephalomyelitis, and Pelizaeus-Merzbacher disease (PMD) [11,12]. Therefore, insight into the spatio-temporal architecture of oligodendrocytes (OLGs) and their myelin membranes is crucial to improve our understanding of the (re)myelination machinery, and hence the potential in developing (novel) therapeutic strategies.

So far, the majority of studies in myelin membrane research have been performed with living cells. However, model membranes such as large unilamellar (LUVs) or giant unilamellar vesicles (GUVs) are quite promising tools to investigate the lipid organization and domain assembly, as well as lipid-lipid and lipid-protein interactions [3,13–16]. Additionally, next to conventional biochemical and cell biological techniques, (single-molecule) biophysical optical microscopy offers a set of highly sophisticated tools for gaining detailed molecular insight into the dynamic organization of lipids and proteins, which, most importantly, can be accomplished in a non-invasive manner. These approaches include photon counting histogram (PCH) analyses [17], F techniques [e.g. fluorescence recovery after photobleaching (FRAP) [18], fluorescence correlation spectroscopy (FCS) [19], advanced FCS techniques (e.g. scanning [20], dual focus [21], z-scan [22], spot variation FCS [23]), fluorescence cross-correlation spectroscopy (FCCS) [24], and Förster resonance energy transfer (FRET) [25,26]), image correlation techniques (e.g. raster image correlation spectroscopy (RICS)[27,28], and number and brightness analyses (N&B) [29]]).

Here, we will summarize and discuss myelin assembly and maintenance in terms of the interaction of its structural elements, i.e., lipids, enriched in the myelin membrane and myelin-specific proteins. Additionally, we will highlight the versatility of biophysical technology in OLG-myelin research, and the impact of such approaches on improving our understanding of how lipids and proteins regulate the tight spatio-temporal organization of the myelin membrane and, thereby, its physiological function.

2. Myelin biogenesis and structure: involvement of a set of specialized proteins and lipids

The multilayered myelin membrane displays a distinct and complex architecture, i.e., during enwrapping, the outer leaflets of the myelin membranes appose each other, thereby creating the intraperiod line, while the condensed cytoplasmic surface constitutes the major dense line (Fig. 1A and B, [1,5]), as readily visualized by electron microscopy (EM). The myelinated segments of the axons, the so-called internodes, are interchanged with myelin-devoid areas, named the ‘nodes of Ranvier’, where sodium channels are localized that generate a membrane potential that drives the action potential along the axon in a saltatory manner. Besides axonal enwrapping, myelin compaction takes place, which thus gives rise to areas of compact and non-compact myelin [1,5]. Recent findings suggest that the compaction of the myelin sheath starts from the outer tongue (leading edge of the sheath) and gradually shifts towards the inner tongue [30]. Within the internodes different degrees of compaction can be discerned. Although mainly compact within the internode, regions at their edges, known as ‘paranodes’, consist of non-compact myelin (Fig. 1A). Interestingly, the molecular composition of compact and non-compact myelin differs; i.e., the major myelin proteins PLP and MBP together with the glycosphingolipid GalC reside in compact myelin, whereas other myelin proteins, such as NF155, together with the glycosphingolipid sulfatide localize in non-compact myelin (paranodes) [2,31]. The proper compartmentalization of the myelin sheath is crucial for its function, because any alterations in myelin structure might cause more or less severe demyelinating diseases.



Figure 1: Myelin Structure.

A. Schematic model that shows the enwrapment of axons by myelin and the localization of major myelin proteins. The major myelin protein PLP is represented in red and MBP is represented in green. B. Detailed schematic model of myelin membrane organization and the localization of the major myelin lipids GalC, sulfatide, cholesterol and myelin proteins PLP and MBP within the myelin membrane. Note that extracellular lipids GalC and sulfatide face each other in enwrapped myelin. C. The synthesis scheme of sulfatide and GalC. Note that GalC is synthesized from ceramide by CGT (ceramide glucosyltransferase); sulfatide is synthesized from GalC by CST (cerebroside sulfotransferase).

For myelin biosynthesis to occur, progenitors cells of the myelin producing OLGs first have to mature to myelin-competent cells along a well-defined differentiation timeline [1,32]. In this process, OLGs synthesize myelin specific proteins and lipids in a sequential and time-dependent manner. Specifically, the major myelin galactolipids GalC and sulfatide are produced prior to the expression of PLP and MBP. Therefore, the timing of the myelination machinery is crucial. In vivo, the complete synthesis of the myelin sheath over one axon occurs over a time interval of approximately 5 hours in zebra fish [33], but 24 hours in rodents [4]. Thus, over a relatively short period of time myelin biogenesis may require vast amounts of building blocks to be produced within an OLGs for sheet assembly. Major issues to be resolved in this regard are the identification and functioning of key players that orchestrate these events in a strictly temporal and spatial manner. Throughout myelin biogenesis and its maintenance, OLGs sort and transport different cargos from cell body, via primary processes to growing myelin sheath. In order to understand these events it has to be taken into account that like epithelial and neuronal cells, OLGs are polarized cells, implying that polarized sorting and transport is likely instrumental in myelin biogenesis [2,34]. Indeed, previous studies from our laboratory revealed the existence of such pathways in OLGs, the myelin sheet being targeted by a basolateral-like trafficking mechanism [2,35], whereas transport of newly synthesized plasma membrane proteins reach this membrane via an apical-like mechanism. Consistently, the t-SNAREs syntaxin 3 and 4, which are distributed in a polarized fashion, localize in OLGs to the plasma membrane and myelin membrane, respectively [2]. Similarly, the apical membrane protein marker hemagglutinin (HA) of influenza virus, as reported for its transport in epithelial cells, is delivered to the apical-like plasma membrane in OLGs, while the VSV G protein, a basolateral marker in epithelial cells, is delivery to the myelin sheet [35]. More specifically, in more recent work evidence supports a transcytotic transport mechanism for PLP, indicating transport of de novo synthesized PLP from the endoplasmic reticulum (ER) to the myelin membrane via the OLG plasma membrane (chapter 3). From the latter membrane, newly arrived PLP appears to be internalized via a clathrin independent but cholesterol dependent pathway, and it

has been proposed that endosomal compartments may subsequently serve as site of storage, prior to a neuronal signal-triggered delivery of the protein to the myelin membrane, as inferred from data obtained in Oli-Neu cells [36]. By contrast, the peripheral myelin membrane protein MBP is translated 'on site', following transport of MBP mRNA containing granules towards the myelin sheet [3]. During the process of myelin wrapping around the axon, both PLP protein and MBP mRNA are continuously trafficking towards the myelin membrane via cytoplasmic channels near the noncompacted edges of the newly assembling myelin membrane [37]. Furthermore, stimulation of myelin synthesis leads to an opening or closing of these cytoplasmic channels, which further emphasizes the dynamic structure of the myelin sheet [30].

Although considerable progress has been made in recent years in clarifying aspects of the underlying mechanism in myelin biogenesis, further improvement will require detailed insight into the fundamental role of myelin lipids and proteins in myelin assembly. We will therefore first focus on current knowledge of the involvement of the major myelin glycosphingolipids, i.e., the galactolipids GalC and sulfatide.

3. Major myelin lipids galactosylceramide and sulfatide and their role in the myelin membrane

The lipid pool of myelin consists of phospholipids, cholesterol and glycosphingolipids (GSL) [2,10], and does not contain unique, i.e., myelin specific lipids. Nevertheless, the GSL galactosylceramide (GalC) and its sulfated derivative, sulfatide (Fig. 1C) can be considered as 'typical' myelin lipids because of their relative high abundance, representing approx. 23 % and 4 %, respectively, of the total lipid pool. These lipids, often referred to as galactolipids, are highly ordered lipids with long saturated and monosaturated fatty acid chains, containing 22-26 carbon atoms [38]. Cholesterol is another abundant (approx. 28 % of the total lipid pool) and important structural lipid element of the myelin membrane (table 1, [39,40]), not in the least because of its ability to engage with the galactolipids in the formation of specific membrane microdomains, as will be discussed below.

Although galactolipids are important key players in safeguarding integrity and long term maintenance of myelin membranes, their presence does not seem to be essential for myelin biogenesis and assembly [41–45]. Based upon in vitro studies, it has been proposed that particularly GalC is important in OLG maturation [46], whereas sulfatide plays a role in OLG differentiation [47,48]. However, more specific insight has been obtained in studies in which enzymes of galactolipid biosynthesis were downregulated, as in ceramide galactosyltransferase (CGT) knock-out mice, which are deficient in GalC, and consequently in sulfatide, for which GalC serves as

a precursor [45]. Even though myelin ultrastructure abnormalities are observed in this model, the biosynthesis of seemingly compacted myelin membranes does occur. Nevertheless, abnormal nodal and paranodal structures, a decrease in myelin stability over time and, importantly, disrupted axo-glia interactions are observed [43,45]. This pathological phenotype of CGT null mice can be rescued by OLG-specific re-expression of CGT, which strongly supports the notion that the abnormalities observed in CGT null mice were indeed caused by GalC and/or sulfatide deficiency in OLGs [49]. Insight into a specific role of sulfatide could be obtained when the possibility was provided to create an animal model in which cerebroside sulfotransferase (CST), which is responsible for sulfatide synthesis from GalC, could be knocked-out [44]. Although the clinical phenotype was less severe, CST null mouse also revealed unstable myelin with age, disrupted paranodal compartments, axonal swellings and disruption in sodium channel clustering [10,43]. Accordingly, these data evidently indicate that both GalC and sulfatide play distinct roles in OLG differentiation, myelin maintenance and overall stability and proper functioning of the myelin membrane.

Table 1: Major Myelin Lipids

Lipid	% Total Dry Weight of Myelin			Comments	Reference	Notes
	Human	Bovine	Rat			
Cholesterol:	27.7	28.1	27.3	-Rate limiting for CNS myelination -Insulator function of the myelin	Saher et al.,2005 Salzer et al.,2003	
Galactolipids:						
Galactosylceramide(GalC)	22.7	24	23.3	-Role in OLG maturation -Role in proper CNS node and paranode formation* -Role in electrophysiological properties of myelin* -Stabilization and maintenance of axoglial adhesion at the paranode* -Maintenance of compact myelin* -Role in myelin integrity and stability*	Dyer and Bejamins, 1988,1990 Dupree et al, 1998a,b, 1999 Coetzee et al.,1996; Bosio et al.,1996	mainly C(24:1)
Sulfatide	3.8	3.6	7.1	-Negative regulator of OLG differentiation -Role in sodium channels clustering -Role in paranode formation -Role in NF155 organization	Bansal et al., 1999, Ishibashi et al.,2002, Honke et al., 2002 Schafer et al., 2004	mainly C(24:1)

* The mentioned comment are obtained from CGT knock-out mouse studies, therefore these findings are also relevant for the function of sulfatide.

4. Major myelin proteins PLP and MBP and their role in the myelin membrane

Unlike its lipids, myelin expresses a unique set of proteins including proteolipid protein (PLP), myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), myelin associated glycoprotein (MAG), 2', 3'-Cyclic-nucleotide 3'-phosphodiesterase (CNP) and neurofascin 155 (NF155), of which PLP and MBP are the most abundant ones (Fig.1A, [1,2]). Their participation in myelin biogenesis is a carefully regulated and timed process [1]. The integral membrane protein PLP with

four membrane spanning domains consists of two different isoforms, i.e., PLP and DM20 [50]. PLP is synthesized at the endoplasmic reticulum (ER), passed through the Golgi apparatus, and then transported to the myelin membrane via a vesicular transport pathway [51]. The protein primarily plays a role in stabilizing the intraperiod line by bringing together the extracellular leaflets of the myelin membrane (Fig. 1B), although PLP also participates in many other cellular processes, such as cholesterol transport and migration [52–55]. Nevertheless, as noted above and as demonstrated in knock-out animal models, for actual formation of the myelin sheet, PLP is not essential [56]. Interestingly though, as yet it cannot be excluded that another transmembrane protein with four membrane spans, M6B, can compensate for the lack of functional PLP in PLP knock-out animals [55], particularly since in case of a double knock-out of both PLP and M6B, a severe hypomyelination was observed. PLP also appears to play a role in regulating cholesterol levels in myelin membranes, which makes its presence very important in governing assembly of cholesterol-containing membrane microdomains [55].

Different postnatal isoforms of the peripheral protein MBP are expressed in a species-dependent manner, i.e., four isoforms in rats, six in mice and four in humans. The MBP variants are produced from a single eleven exons containing gene complex (in mice), called Golli (gene in the OLG lineage) [3,57]. The classical MBP isoforms are derived from alternative splicing of a single MBP mRNA, which includes seven most downstream exons of the Golli gene complex. Among these isoforms, the ones containing exon-II, i.e., 17 and 21.5 kDa, are localized within the nucleus, whereas the others, 14 and 18.5 kDa, localize in compact myelin [3,58,59]. MBP is the only known structural myelin protein required for myelin membrane formation because MBP plays a role in myelin membrane compaction by bringing the cytoplasmic leaflets together [60,61]. Besides, (exon-II minus) MBP is a multifunctional protein that has many different roles in signaling, cytoskeleton (actin, tubulin) polymerization and stability, and calcium-calmodulin binding [62–65]. On the other hand, (exon-II minus) MBP serves as a molecular sieve for the integration of proteins with a large cytoplasmic domain to the myelin membrane in a mechanism where MBP co-clusters in a condensed network, which triggers a phase transition in the myelin membrane and subsequently determines the exclusion or inclusion of other proteins into the myelin membrane [59,66]. Because of its positive charge, MBP can interact with anionic phospholipids (e.g. PS, PI) in the inner leaflet of the myelin membrane [3]. Interestingly, MBP dynamics is also affected by changes of the dynamics of extracellular leaflet galactolipids [67], suggesting that ‘indirect’ interactions may occur between the galactolipids and MBP, facing the cytoplasmic surface of the membrane. Therefore, by considering its actin binding properties to the myelin membrane

[62,68], MBP might be a key player in transmitting galactolipid-derived signals .

5. Membrane microdomains in oligodendroglial-myelin

5.1. Role of GalC and sulfatide

For proper myelin functioning, the presence or absence of galactolipids is not the only key factor, but also their biochemical structure. Myelin membranes are mainly composed of galactolipids with very long saturated fatty acyl chains (C22-24) [69] and these galactolipid species, together with cholesterol and membrane proteins, are known to assemble into important and specialized membrane microdomains, so-called lipid rafts [70], operationally defined as detergent-insoluble or detergent-resistant membranes (for detailed reviews the reader is referred to [6,7,9]). Possibly, very long fatty acyl chain galactolipids are crucial for proper myelin maintenance and stability because they exert their function via these membrane microdomains. In this respect, it has been well established that membrane domain formation of the galactolipids is dependent on the chain length, their hydroxylation and saturation levels [71,72]. Thus, hydroxylated or unsaturated lipids reveal a strongly diminished membrane microdomain forming capacity. For instance, the acyl chain length of sulfatide is developmentally regulated; i.e., prior to the onset of myelin formation (day 10 in rats), stearic acid (C18:0) is the main hydrocarbon chain present in sulfatide, whereas between day 10-32, sulfatide (C24:1) is upregulated [73]. Not only chain length but also hydroxylation of the fatty acyl chains of sulfatide is developmentally regulated; i.e., the extent of hydroxylation of sulfatides decreases with age [73]. Therefore, it is also important to take into account the nature of the fatty acid chain and its state of hydroxylation when investigating the specific role of galactolipids. Indeed, in an animal CerS2 knock-out model, CerS2 being responsible for the synthesis of sphingolipids with very long (C22-24) fatty acid chains, a marked decrease in GalC and sulfatide levels was observed, while the phenotype showed unstable, and noncompacted myelin, with abnormalities in the inner lamellae [74]. Moreover, myelin lipid extracts obtained from CerS2 deficient mice, in contrast to such extracts from control animals, do not give rise to formation of membrane domains, when reconstituted in a model membrane system. [69]. Finally, with regard to hydroxylation, the hydroxylation levels of sulfatide are remarkably increased in MS patients. Since a decrease in hydroxylation promotes the formation of membrane microdomains in model membranes [72], these data suggests that such membrane microdomains in MS patients may be relatively decreased which may have severe consequences for the proper assembly, organization and functioning (e.g. signaling) of the myelin

membrane.

To appreciate the complexity of the myelin structure and organization, it is crucial to understand the ability of galactolipids to induce functional membrane microdomains and their role in membrane compaction. Such knowledge will require further insight as to how extracellular leaflet lipids transmit signals from to the intracellular environment. For example, it has been proposed that galactolipids, given their relatively long fatty acid chains, might interfere with the (lipid) organization in the inner leaflet as a result of acyl chain interdigitation, thereby ‘transmitting’ the signal. Consistent with this proposal, studies performed in model membranes indeed revealed that ceramide with C24:0 chains forms interdigitated gel phases [75]. Alternatively, it is also possible that galactolipid-mediated signal transmission proceeds via integral myelin membrane-specific proteins such as PLP, MAG or MOG. Consistent with such a possibility are observations that clustering of GalC in OLGs, occurring upon interaction of the cells with GalC-sulfatide liposomes, also causes clustering of the membrane spanning proteins PLP and MOG [76]. However, as yet, there is no direct evidence of a direct interaction between galactolipids and these integral membrane proteins, although such interactions might well be revealed by applying appropriate biophysical techniques or model membranes, as will be discussed in detail in section 5.

5.2. Myelin proteins and their membrane microdomain association

Membrane microdomains are composed of lipids and specific membrane proteins [9,70,77]. Hence, the partitioning behavior of integral or peripheral membrane proteins might have a pivotal effect on cellular activities dominated by lipid rafts, acting for example as signaling platforms. Several major and minor myelin membrane proteins are known to reside in membrane microdomains. Operationally, these domains are often defined by their different detergent solubility behavior. For example, in myelin sheet directed transport, following de novo biosynthesis, the major myelin protein PLP initially displays triton-X100 (TX-100) insolubility, and acquires 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) insolubility at later steps in the transport pathway [chapter 3,2,77,78]. Remarkably, as a peripheral protein, MBP also displays different detergent solubility behavior, as observed at different stages of OLG differentiation [80] and especially phosphorylated forms of MBP become CHAPS insoluble in the myelin membrane [81]. Proteins such as NF155, MAL, and MOG display TX-100 insolubility or acquire TX-100 solubility by crosslinking (MOG); MAG, however, is Lubrol WX insoluble [6,82]. The different detergent solubility properties might reflect the dynamic partitioning of the proteins in different

membrane micro-domains in OLGs and myelin.

It is still poorly understood whether myelin lipids represent the driving force in the lateral organization of myelin proteins or vice versa. In this regard, in differentiating OLGs, myelin galactolipids are expressed prior to myelin proteins [32]. Consistently, also our recent findings, derived from work in which conventional raft isolation procedures with detergent extraction were combined with optical biophysical techniques, revealed that sulfatide determines PLP's partitioning into CHAPS resistant membrane microdomains whereas for MBP, the presence of GalC rather than sulfatide was pertinent for the protein's partitioning in detergent-insoluble microdomains (chapter 3 and 4). In further support, several knock-out animal studies also suggest the importance of the membrane lipid composition in organizing the lateral distribution of myelin proteins into distinct membrane microdomains within the myelin sheath [78,83]. Thus, in the CGT knock-out animal model the absence of galactolipids altered PLP's association with CHAPS-insoluble membrane microdomains, i.e., under knock-out conditions PLP is largely CHAPS-soluble [78]. Similarly, inhibition of sphingolipid synthesis in primary OLGs disrupts the CHAPS insolubility of PLP and MBP [78,83]. However, in these studies it was also reported that in shiverer mice, where MBP is absent, a large fraction of PLP appears CHAPS-soluble, which could suggest that next to the presence of membrane galactolipids, the protein composition of the myelin membrane is also important for the lateral segregation of proteins [83]. However, in shiverer mice the galactolipid content decreases as well [61], implying that the membrane microdomain association of PLP might also be a consequence of an altered galactolipid content, which will require additional experimental work.

6. Biophysical tools to investigate oligodendroglial-myelin membranes

6.1. Model systems to study myelin lipid and proteins

6.1.1. Cell Systems and model membranes

A need for further detailed mechanistic studies into the fundamental role of GalC and sulfatide in myelin biogenesis and maintenance is apparent from observations reported above in knock-out animal models. A convenient model for such studies appears to be OLG rat progenitor cell lines such as OLN-93 cells, which express neither GalC nor sulfatide [84]. However, in this OLG-derived cell line, GalC or GalC and sulfatide can be readily and selectively expressed by cellular transfection with appropriate constructs of CGT, giving rise to the exclusive expression of GalC, and CGT/

CST, which results in production of both GalC and sulfatide (Baron et al., 2014, chapter 4, [79]). However, an obvious limitation of this cell system is that the effect of sulfatide cannot be investigated as such, given that GalC serves as its precursor. Although the addition of extracellular sulfatide to the parental OLN-93 cells is an option, it is questionable whether exogenously inserted sulfatide will reside in the correct membrane domain. Alternatively, Oli-neu cells, an immature OLG cell line from mice is also widely used in OLG-myelin research. However, these cells have the 'disadvantage' that they are not suitable for myelin lipid-dependent research, since they do express GalC and sulfatide, which is promoted upon differentiation by cAMP [85].

An attractive option to study specific myelin lipid-related questions is the application of simple membrane systems such as large unilamellar vesicles (LUVs), which may provide detailed insight into lipid-protein interactions between myelin lipids and protein [16], as will be discussed in next section. An obvious choice would also be the use of model membranes such as Giant Unilamellar Vesicles (GUVs) in which liquid ordered (Lo) and disordered (Ld) phases can coexist in a lipid composition dependent manner [86]. In contrast to the relatively small LUVs, GUVs display diameters that may vary between 10-100 μm which makes them most convenient for visual inspection by optical microscopy. GUVs can be prepared from synthetic or natural lipids, allowing a large variation in fatty acid chain length, hydroxylation levels, nature of the lipid head groups, and overall lipid composition. [87,88]. For example, GUVs reconstituted with GalC, sulfatide and glucosylceramide at similar ratios as present in myelin, revealed that depending on its concentration, glucosylceramide containing GUVs are fairly unstable (our unpublished observations), which might explain why in the absence of GalC and sulfatide, the upregulation of glucosylceramide synthesis cannot compensate for these galactolipids [89]. GUVs can also be prepared from natural lipid extracts [90,91] or isolated native cell membranes [92]. In this manner, the lateral segregation of lipids extracted from different animal models, displaying different degrees in myelin perturbation, have been investigated in GUVs. Thus, in GUVs prepared from myelin extracts obtained from MBP-deficient shiverer mice and *Cers2* deficient mice, no membrane microdomain formation could be detected [69]. Nevertheless, domain formation in GUVs can be readily visualized using a fluorescent marker, which specifically attaches to the lipid of interest. For example, domain formation in GUVs, reconstituted with neuronal lipid extracts, was visualized with fluorescently-tagged cholera toxin, which specifically binds to GM1, and with monoclonal antibody R24, which specifically binds to GD3, to study the heterogeneity of the lipid rafts [93]. It is also possible to visualize phase separation behavior by using fluorescent lipid probes such as DIC, DIO, DiD, which prefer partitioning in membrane domains, depending on their fluidity [94]. However,

variability in results when applying these probes in detecting the liquid ordered or disordered phases have been reported, implying that care should be taken when interpreting the data. For example, DiIC18 showed preferential partitioning into the gel phases in GUVs, prepared with POPC/sphingomyelin, whereas the probe partitioned into the fluid phase in GUVs prepared with POPC/DPPE [94]. Moreover, although fluorescently labeled lipids can be directly used in the preparation of GUVs, one should however be cautious by using those probes, as various factors, including chemical structure of the fluorescent probe, the positioning of the fluorophore in the lipid analogue, the lipid composition of the bilayer, the chemical structure of the fatty acyl chain of the lipid analogue, and some thermodynamic properties such as the temperature, might change dramatically the domain formation behavior as such (reviewed in [95]).

The specific preference of myelin proteins for microdomains composed of distinct lipids can also be visualized in reconstituted model membrane systems (GUVs) by optical microscopy. Particularly as a myelin membrane model, it is relevant of being able to reconstitute GUVs selectively with either sulfatide or GalC. An important step of such procedures is of course the proper integration of the protein of interest in the reconstituted membrane. There are in fact several options to incorporate proteins into these model membranes [14,96–98] and the preparation procedure depends on the nature of the protein.

6.1.2. *Mimicking myelin membranes*

To study the function of myelin membrane proteins or lipids can be troublesome with simple cell line systems or model membranes, because myelin membranes are the only membrane platform of which the extracellular leaflets of the same membrane are opposing each other, thereby giving rise to a multilamellar complex structure (Fig. 1B). Therefore, by using in vitro model systems consisting of a combined system of cells and apposing model membranes, the major dense line or intraperiod line can thus be mimicked, allowing a better understanding of the function of the major myelin proteins MBP and PLP, and myelin galactolipids.

Upon myelination, the sugar moieties of the myelin galactolipids, which exclusively localize at the extracellular leaflet in myelin, are facing each other. This fact prompted by Boggs et al. to propose that myelin membranes might have a ‘glycosynapse’, where carbohydrate groups from opposing membranes are interacting [67,76,99]. Indeed, liposomes and nanoparticles, consisting of either GalC or both GalC and sulfatide, when incubated with primary OLGs, mimicking the intraperiod line (IPL) as described above, are capable of inducing (i) a redistribution/clustering of GalC at

the extracellular leaflet, (ii) a reorganization of MBP at the cytoplasmic side of the membrane, and (iii) a disruption of the actin cytoskeleton and tubulin network in OLGs [67,100]. These fascinating observations highlight a potential role of galactolipids in creating signaling platforms, thereby regulating myelin compaction by perturbing MBP-membrane interaction and affecting cytoskeletal dynamics. In addition, galactolipids might act as their own receptors, receiving and/or transmitting signals via the glycosynapse in a density dependent manner [67]. Indeed, in this context, it has been recently demonstrated that the density and confluency of the coat of negatively charged oligosaccharides (i.e., the glycocalyx, in particular provided by the galactolipids in OLGs), may cause repulsion of the opposing membranes, and that this density-dependent electrostatic repulsion between opposing membranes is effectively downregulated upon differentiation of the OLG, i.e., conditions at which the myelin membrane is generated [101]. Furthermore, in the same study, the formation of the IPL was also mimicked by addition of liposomes or IPL membranes from PLP knock-out mice. However, not unexpectedly, a proper interaction between the liposomes and the membranes of mature OLGs [101] was perturbed, supporting the role of PLP in the stabilization of IPL and adhesion of extracellular leaflets of myelin to each other. Taken together, a partial loss of the glycolcalyx might create an appropriate molecular and spatial environment for engagement of the galactolipids, especially GalC as it localizes in compact part of internodes, in (transient) interactions between opposing membranes in a PLP-dependent manner, thereby promoting myelin development and compaction.

Recently, it has been proposed that MBP is a major molecular factor in maintaining the high lipid to protein ratio in myelin by a mechanism in which it acts as a molecular sieve and diffusion barrier [59]. Next to the primary cell system, a biomimetic system was applied to study the physical barrier properties of MBP in which the apposition of the cytoplasmic part of the myelin membrane (major dense line) was mimicked by using supported bilayers (SPLs), consisting of cytoplasmic leaflet lipids of myelin, and GUVs prepared with PS and PC. In this system, the distribution of a positively charged membrane anchored GFP was monitored in the presence or absence of MBP. In the absence of MBP, membrane-anchored GFP, sandwiched between SPLs and GUVs, revealed a homogenous distribution whereas addition of MBP to this system caused a reallocation of GUVs to distinct areas on the SPLs, from which GFP was partially excluded. When MBP was sandwiched between SPLs and GUVs prior to addition of membrane-anchored GFP, a total exclusion of GFP from the spread areas to which GUVs were attached, was observed. These data support the notion that MBP can also exert its physical barrier function in in vitro systems, a conclusion that is most conveniently reached employing a biomimetic system that allows freedom in

the addition of relevant compounds. The sequential inclusion of MBP thus supports the protein's ability to exclude homogeneously distributed proteins from compacted sheaths .

6.2. *Measuring oligodendroglial -myelin membrane dynamics*

In general, the molecular organization of the lipids within the membrane creates a special environment for the correct distribution of the protein and presumably, lipids and proteins may be considered as an 'interactive team', which determines the general structure and the assembly of the membranes. In the previous sections, we summarized findings indicative of the existence of lipid/protein cross-talk in OLGs and myelin membranes, largely inferred from biochemical and cell biological approaches. This cross-talk, as reflected by microdomains, or "rafts", is also proposed to be very important for specific cellular functions because of their highly dynamic properties, i.e., they can be transiently formed upon external stimulation, which then might stimulate internal signaling, as occurs via the 'glycosynapse' [99,102]. However, when and how 'rafts' affect the dynamic properties of a membrane is still poorly understood. Therefore, a better understanding of these dynamics within the OLG plasma membrane and myelin might shed light on crucial mechanisms, playing a role in the proper organization of the myelination machinery. Within this context, the application of advanced methodologies such as a combined approach of cell biology and biophysics might be most rewarding in improving our understanding (recent achievements are summarized in table 2).

6.2.1. *Measuring membrane order (fluidity)*

Membrane ordering/fluidity is determined by the presence or absence of membrane microdomains, and regulates membrane dynamics and rigidity and thereby the functioning of the membrane. Evidence is appearing that myelin membrane order/fluidity is largely dependent on two major factors; firstly, the molecular composition of the myelin membrane; i.e., the abundant presence of long chain microdomain forming galactolipids, and the presence of the peripheral membrane protein MBP. For instance, GUVs reconstituted with membrane extracts from *Cers2*-deficient mice devoid of long fatty acid galactolipids, or from shiverer mouse, devoid of MBP, revealed a decrease of membrane order compared to that of wild type animals [69]. In a similar way, the membrane fluidity increases (i) following fumonisin B1-mediated inhibition of sphingolipid synthesis in primary OLGs and (ii) when the measurement are performed in OLGs derived from shiverer mice [69,83].

Secondly, myelin membrane fluidity is under tight control of neuron-derived soluble signals. The incubation of primary OLGs and Oli-Neu cells with conditioned neuronal medium displayed increased membrane condensation [83,103].

Table 2: Optical Microscopical Biophysical Techniques Applied in OLG-myelin Field in a Chronological Order

References	Biophysical techniques	Major Findings
Gielen et al., 2005	FCS	-Diffusion coefficient of MOG-eGFP and Bodipy FL-C5 sphingomyelin
Fitzner et al., 2006	C-Laurdan FRAP STED FRET	In co-culture systems (neuron-derived signals): -Increased membrane order in primary OLGs by neuron-derived soluble factors -Increased membrane condensation in the presence of MBP -Formation of highly dynamic GalC clusters in OLGs -Increased number of large GalC clusters in the presence of neurons -Self-interaction of GalC within the large clusters
Kippert et al., 2007	FRET C-Laurdan	-Decrease in Rho activity in Oli-neu cells in the presence of neuronal-conditioned medium -Increased membrane condensation in the presence of conditioned neuronal medium or RhoGTPase inactivation
Gielen et al., 2008	RICS, FRAP	-Diffusion coefficient of MOG-eGFP in OLN-93 cells
Nawaz et al., 2009	FRET	-Interaction of 14-kDa MBP with PIP2 (sensed by CFP-PH-PLC δ 1) in Oli-neu cells
Gielen et al., 2009	RICS, FRAP	-Diffusion coefficient of Dil-C18 in primary OLGs
Yurlova et al., 2011	C-Laurdan FCS FRAP	-Higher lipid order in GUVs prepared from myelin compared to Oli-neu cells -Higher lipid order in GUVs prepared from wild type animal compared to shiverer mouse ¹ or CerS2-deficient mouse ² -Higher lipid order in primary OLGs compared to FB1 ³ -treated OLGs -Slower diffusion of DiD in GUVs prepared from wild type animal compared shiverer ¹ mouse or CerS2 deficient mouse -Slower diffusion of Cell MaskOrange ⁴ in control OLGs compared to FB1 ³ treated OLGs
Aggarwal et al., 2011	FRAP	-Detection of highly mobile MAG in OLGs isolated from shiverer mouse compared to control to show the diffusion barrier function of 14-kDa MBP -Higher mobility of truncated Tmem10 (without cytosolic domain) compared to full length Tmem10
Ozgen et al., 2013	FRAP	-Dynamic of role of 21.5-kDa MBP-RFP in OLN-93 cell proliferation
Aggarwal et al., 2013	FRAP FRET	-Decreased 14-kDa MBP diffusion due to its oligomerization -Self association of 14-kDa MBP
Ozgen et al., 2014	s-FCS Z-scan FCS, Z-scan RICS	-Decreased mobility of PLP -eGFP in the presence of sulfatide (rather than GalC) on poly-L-lysine and laminin-2 in OLN-93 cells -Increased mobility of PLP -eGFP even in the presence of sulfatide on fibronectin in OLN-93 cells -Increased mobility of 18.5-MBP-eGFP in the presence of only GalC in OLN-93 cells

¹MBP expression deficient mouse; ²Mouse model unable to synthesize long acid fatty acyl chain lipids; ³inhibitor of sphingolipid synthesis ; ⁴Membrane dye

To determine the state of membrane ordering, fluorescent probes which specifically partition into liquid ordered or disordered phases are often used to investigate such domain formation in model membranes. However, the choice of probe in terms of obtaining unambiguous results, can be rather challenging. For that reason, the application of different types of environmentally sensitive membrane dyes, such as di-4-ANEPPDHQ, Laurdan, PY3304, PY3174, and PY3184, is recommended [94,104]. Particularly the use of Laurdan has greatly contributed to clarifying important biophysical issues in myelin membrane dynamics [105,106]. The fluorescent probe Laurdan is sensitive to membrane phase transitions and membrane fluidity. The emission spectrum of membrane inserted Laurdan undergoes a blue shift from 500 to 430 nm, when the membrane domain in which the probe partitions undergoes a phase change from a liquid-disordered to liquid-ordered state. By collecting, therefore, the fluorescent signal from two channels, it is possible to compose a generalized polarization (GP) image which provides the

possibility to calculate the membrane order [94,107]. The application of C-Laurdan, which has a greater membrane environment-dependent sensitivity and a diminished susceptibility towards photobleaching, further boosted its versatile use [106]. A particular advantage of this dye is that it homogeneously distributes in the lateral plane of the membrane, while differences in membrane order are simply inferred from changes in its emission spectrum. Another advantage is that, (C-)Laurdan is suitable for use in both model and cell membranes. Taken together, further applications of (C-)Laurdan can rule out other factors, i.e., soluble signals such as pro-inflammatory cytokines present in MS lesions or different ECM proteins, affecting the OLG-myelin membrane order and therefore dynamics. Moreover, the application of (C)Laurdan to model membranes reconstituted from MS patient material or myelin lipids with modifications such as chain length, and hydroxylation (see above), can clarify to the extent to which membrane order is affected or changed in diseases such as MS.

6.2.2. Measuring membrane dynamics

Even though membrane probes, such as (C-)Laurdan, provided insight into the general OLG-myelin membrane dynamics, such tools cannot extract dynamic information, originating from a specific lipid or protein, i.e., its lateral mobility. Therefore, for more detailed information on the molecular dynamics, more advanced optical microscopic techniques, which can be applied to living cells as well as model membranes, combined with sophisticated analysis methods, are required. One of such techniques relies on fluorescence recovery after photobleaching (FRAP) which is widely used to investigate molecular dynamics in biological systems [58,66,108] as well as in myelin field (recent findings are summarized in table 2). FRAP is a technique which is able to quantify dynamic information such as the environment-dependent diffusion coefficient as well as the mobile fraction, providing information on the interaction of the molecule of interest with the adjacent molecular environment, by applying a high laser power to bleach a region of interest (ROI), and recording the rate of recovery (Fig. 2A). However, strictly speaking, FRAP cannot be considered as a non-invasive technique because of the usage of a high laser power to bleach ROI. In addition, FRAP cannot provide single molecule specificity, as is possible for other biophysical techniques such as fluorescence correlation spectroscopy (FCS). FCS is a powerful non-invasive biophysical technique, allowing determination of dynamical properties by applying very low laser light on a single point on the cell and recording the fluorescence fluctuations created by the diffusion of the fluorescently labeled molecules in and out the area of interest, which allows the calculation of diffusion coefficients/lateral mobility (Fig. 2B,[19,86,108,109,]). Given the complexity of

the plasma membrane of OLGs and the myelin membrane, the application of more advanced forms of FCS should be considered, as they might be more informative and effective (as reviewed in [110], Fig. 2B). Examples include scanning FCS which provides the possibility to measure diffusion of the molecules simultaneously at different points [111], and z-scan FCS, which provides the possibility to measure diffusion in different z planes, such as near the upper or bottom plasma membrane [22].

Even though FCS has a high temporal resolution, it lacks spatial resolution, which may hamper determination of the membrane dynamics in cell types with complex morphology, like OLGs. Accordingly, image correlation techniques have been developed to extract dynamic information from live cell systems; however, these techniques were not ideal because of their poor temporal resolution. A more recent technique, named raster image correlation spectroscopy (RICS) combined the temporal and spatial resolution in a confocal setup to make it more convenient for live cell systems (Fig. 2C, [27,28,111]). RICS can be applied with a conventional scanning confocal setup and makes it possible to eliminate the immobile fractions from the obtained image or provides a possibility to select more precise spots within the ROI. However, it appears very challenging to apply RICS to very heterogeneously distributed proteins such as PLP, which localizes to a variety of transport vesicles, plasma and myelin membrane and, presumably, other intracellular (endosomal) membranes.

There are emerging evidences, obtained by techniques as mentioned in the previous paragraph, that the myelin lipids as such act as key players in regulating the lateral mobility behavior of myelin proteins, and thereby OLG-myelin membrane dynamics. By using an FCS approach, Gielen et al. showed that the lateral mobility of BODIPY-labeled sphingomyelin was changed in parental OLN-93 plasma membranes upon cholesterol depletion, a procedure that destroys membrane microdomains, showing diffusion of BODIPY-labeled sphingomyelin changes in a membrane microdomain dependent manner [113]. Additionally, we recently applied circular scan FCS to determine the dynamics of PLP-eGFP, and z-scan FCS and RICS to determine the dynamics of MBP-eGFP in relation to the myelin lipids GalC and sulfatide. The mobility of PLP decreased in the presence of sulfatide, which presumably reflects PLP-eGFP's association with CHAPS-insoluble membrane microdomains at those conditions, as determined by classical biochemical means (detergent extraction). Additionally, the same study revealed that MBP dynamics was mainly governed by the presence of GalC, thus suggesting a signal transmission between exoplasmically expressed GalC and MBP, which localizes at the cytoplasmic surface (chapter 4, table 2). Similarly, the lipid mobility measured by FCS in GUVs, reconstituted from myelin membranes of *Cers2*-deficient mice, devoid of long fatty acid chain galactolipids

and where there is no domain formation, was significantly faster than the rates determined in reconstituted membranes from healthy animals [69]. Hence, these findings provided new insight as to how the membrane microdomain forming nature of myelin galactolipids might regulate the lateral mobility of myelin specific lipids and proteins. In line with these findings, a combination of biochemical and biophysical studies suggested that presence of the ECM protein fibronectin, a pathological condition which inhibits myelin membrane formation and impairs remyelination in MS lesions, might alter membrane microdomain organization of primary OLG membranes by shifting sulfatide out of membrane microdomains [79]. This alteration might then affect the membrane microdomain association and the lateral mobility of the major myelin protein PLP (chapter 3). These studies further suggested that the perturbation of the equilibrium of membrane microdomains in OLGs under diseased conditions may result in alterations in lateral mobility of myelin specific proteins. However, obviously further investigations are needed to improve our understanding concerning the link between membrane microdomain association of a protein/ lipid and its lateral mobility , which will be further discussed below.

6.2.3. Measuring membrane dynamics in relation to membrane microdomains

The mobility of microdomain associated fluorescently labeled molecules, i.e., lipid, was studied extensively in model membrane systems by biophysical techniques such as FCS [114,115]. For example, by FCS, it has been shown that in GUVs, the raft marker GM1 diffuses relatively slowly, whereas the non-raft marker dialkylcarbocyanine dye diffuses considerably faster [114]. These kind of studies support the conclusion that the dynamic behavior of a lipid or protein is closely related to its microdomain association; i.e, microdomain associated lipids diffuse slower in the membrane. However, unlike model membrane systems, where domain formation can be easily visualized, in biological membranes it is impossible to directly visualize membrane microdomains. Accordingly, a study performed by Kenworthy et al showed by FRAP analysis that the lateral mobility of raft associated proteins is not necessarily slower than that of non-raft proteins [116]. In that respect, different approaches have been proposed in order to obtain a more direct clue on the dynamics of membrane proteins or lipids in relation to their membrane microdomain association. Marguet et al, proposed for the first time the spot variation FCS (sv-FCS, [23]) based on the idea of the diffusion law, which was reviewed in detail in [8]. Briefly, in this method, the focal volume size where molecules pass through as a result of their diffusion properties, is changed and the transient time that molecules spend in each focal volume is measured. Subsequently, the plotted transient time versus

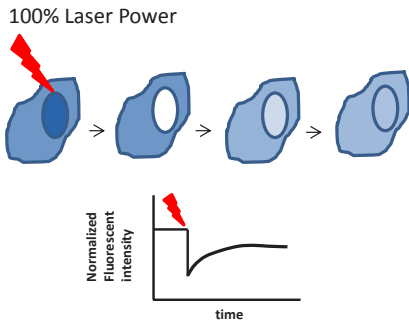
focal volume (focal spot area) gives three different diffusion patterns; free diffusion, hindered diffusion by either the cytoskeleton and/or a membrane microdomain (Fig. 2B). In that respect, sv-FCS can be very informative for myelin research because it provides direct information about whether the lipids and proteins diffuse in a membrane microdomain or in a cytoskeleton-dependent manner. Alternative to spot variation FCS, an approach based on the principle of z-scan FCS makes it possible to determine the different diffusion types extracted by sv-FCS in a commercial confocal setup [22]. In the z-scan, the diffusion time is plotted versus the ratio between the particle number at each z plane and the initial particle number. The first comparative study of diffusion behavior of DiD in SPV and OLN-93 cells by z-scan FCS, suggested that DiD diffuses freely in SPV while in OLN-93 cells the probe displays hindered diffusion by rafts [22]. Therefore, an in-depth investigation about the type of diffusion behavior of myelin proteins or lipids, especially in primary OLGs, can provide further insight into the various diffusion types, such as hindered diffusion by microdomain association or cytoskeleton, which can be altered under pathological conditions.

6.3. Lipid-protein and protein-protein interactions in oligodendroglial-myelin membranes

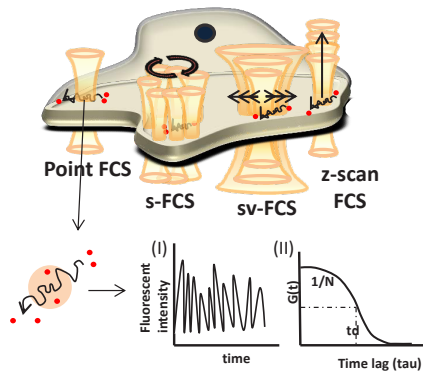
As evidenced by both biophysical and biochemical studies, the influence of myelin lipids on myelin proteins seems very important. However, the above mentioned studies do not provide direct information about the interactions between myelin lipids and proteins. To study such interactions, previous *in vitro* studies performed with artificial membranes such as LUVs with purified myelin proteins (especially MBP) provided detailed insight into myelin protein- inner leaflet lipid interaction [3,68]. For instance, these studies revealed that MBP/ inner leaflet lipid ratios in myelin membranes are quite crucial for MBPs binding to the negatively charged cytoplasmic leaflet lipids such as PI and PS; i.e., in the case of MBP/lipid ratios, lower than the one in compact myelin, MBP's ability to adhere lipid bilayers decreases dramatically [117–119]. Accordingly, since the concentration of the acidic phospholipid PS is dramatically increased in OLGs of MS patients. the MBP/ lipid ratio is relatively decreased, which may thus lead to an impairment of MBP's ability to mediate the adhesion of cytoplasmic leaflets [120]. Additionally, studies performed with LUVs suggested that post-translational modifications of MBP such as deamination, which is dramatically increased in MS patients, might also change MBPs adhesion to the membrane [3,118]. Moreover, a potential interaction of MBP with PIP(2), a minor component of the cytoplasmic leaflet of myelin, was first suggested with LUVs studies [121]. However these kind of approaches only provides detailed information at the

model membrane level. At the cellular level, these interactions can be much more complex, especially by considering the presence of the extracellular leaflet lipids. In this sense, optical biophysical techniques such as FRET or FCCS (Fig. 2D), where the measurements are done in live intact cells, can be very effective tools to investigate these interactions. The FRET technique relies on the principle of energy transfer between two chromophores, which depends on the distance between the molecules; i.e., if the distance between two molecules decreases the efficiency of energy transfer increases (for details see [122], Fig. 2D). For example, by means of FRET evidence was obtained showing that MBP and PIP2 interact at the plasma membrane of Oli-Neu cells, providing the possibility to investigate the interactions of molecules within special compartments of the cells [123]. However in some cases, FRET cannot detect these interactions due to the distance limit. In those cases FCCS might serve as a more informative tool because FCCS determines whether two molecules are co-diffusing within the same confocal volume, independent of the distance [24,109]. In that regard, it can be interesting to explore co-movement of major myelin proteins with (fluorescently labeled) lipids under different conditions; i.e., ‘glycosynapse’.

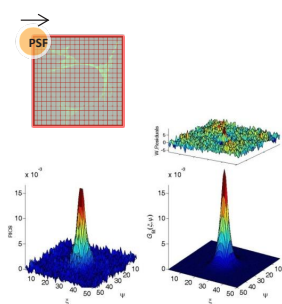
A FRAP



B FCS Applications



C RICS



D FRET and FCCS

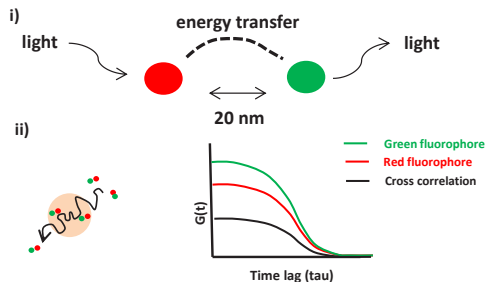


Figure 2: Biophysical Applications:

A. FRAP application in a living cell (for more details see the text). The laser beam depicted in red reflects 100% laser power. The corresponding graph shows the fluorescence recovery after bleaching. B. FCS applications in a living cell (for more details see the text). The laser beam is depicted in orange and the diffusing molecules in red. Fluorescently labeled molecules diffusing through the detection volume give rise to fluorescence fluctuations in time (I) which can be converted to the autocorrelation curve to determine the half decay. By fitting the autocorrelation curve with mathematical models, particle number, diffusion time/coefficient can be calculated (II). C. Schematic representation of RICS. Temporal information can be extracted from raster scan images as these images are recorded pixel by pixel (for details see [27,28]). A representative autocorrelation curve, the weighted residuals and corresponding 2D1C fit model is shown from a z-scan RICS measurement for 18.5kDa MBP-eGFP. D. I) The red fluorophore which is excited by laser light transfer its energy to the green fluorophore which emits light. For this energy transfer the distance between two fluorophores should be 20 nm. II) The red and green fluorophore diffuse together through the confocal volume (see B) which reveals cross correlation depicted with black cross-correlation curve in the corresponding graph.

Alternatively, FRET can also be used to investigate the ‘self-interactions’ of the proteins. For instance, the self-association of MBP, and hence its oligomerization, has been revealed by a FRET study [66]. Moreover, a different approach for investigating the ‘self-interactions’ of proteins may also rely on specific techniques such as number and brightness analysis (N&B) [29] or photon counting histogram (PCH) [17], which determines the degree of oligomerization. Such applications can provide further options to investigate the potential oligomerization of PLP, which has been proposed to be regulated by sulfatide (chapter 3).

Especially by considering the polarized composition of OLGs, these techniques can provide opportunities to specifically investigate ‘local’ lipid-protein and protein-protein interactions as well as protein oligomerization in the different compartments of OLGs, as provided by the unique opportunity of visualization by optical microscopy in living, intact cells.

7. Conclusion and Outlook

OLGs are exposed to many changes in their internal and external environment from early to late development. Until the myelin sheath is synthesized and all the myelin related molecules reach their proper destination, OLG-myelin membranes undergo a non-stop reorganization. Hence, OLGs should display a very dynamic structure in order to regulate this series of events. Our knowledge about myelin biogenesis and OLG-myelin membrane dynamics is gradually improving. However, there are still plenty of open questions. Why does myelin have this unique lipid composition, and more specifically, why are membrane microdomain forming lipids highly enriched in myelin? To what extent do membrane microdomains composed of galactolipids

determine myelin membrane dynamics? In order to answer all these questions, a detailed understanding of membrane dynamics of OLGs and myelin is needed, as these dynamic properties might shed light on the vital internal mechanisms.

Here, we have summarized the role of myelin proteins and lipids in myelin biogenesis, introduced different models to study myelin biogenesis related questions and illustrated how we can make use of biophysical techniques (table 2). For instance, optical microscopic applications, next to conventional biochemical techniques, already served as very informative tools to investigate the sophisticated biology of OLGs and myelin membranes. In addition to that, optical biophysical techniques will bring us one step further and provide new insight into the dynamics of living OLGs in a non-invasive way.

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