PORTLAND PRESS

Review Article

Durable vesicles for reconstitution of membrane proteins in biotechnology

Paul A. Beales[1], Sanobar Khan[1], Stephen P. Muench[2] and Lars J.C. Jeuken[2]

[1]School of Chemistry and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, U.K. and [2]School of Biomedical Sciences and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, U.K.

Correspondence: Paul A. Beales (p.a.beales@leeds.ac.uk)

OPEN ACCESS

The application of membrane proteins in biotechnology requires robust, durable reconstitution systems that enhance their stability and support their functionality in a range of working environments. Vesicular architectures are highly desirable to provide the compartmentalisation to utilise the functional transmembrane transport and signalling properties of membrane proteins. Proteoliposomes provide a native-like membrane environment to support membrane protein function, but can lack the required chemical and physical stability. Amphiphilic block copolymers can also self-assemble into polymersomes: tough vesicles with improved stability compared with liposomes. This review discusses the reconstitution of membrane proteins into polymersomes and the more recent development of hybrid vesicles, which blend the robust nature of block copolymers with the biofunctionality of lipids. These novel synthetic vesicles hold great promise for enabling membrane proteins within biotechnologies by supporting their enhanced *in vitro* performance and could also contribute to fundamental biochemical and biophysical research by improving the stability of membrane proteins that are challenging to work with.

Introduction

The significant challenge of maintaining membrane proteins in their native state, preserving their structure and function *in vitro*, calls for experimental tools that facilitate their study and handling, which are being continually developed [1,2]. These efforts are driven by their abundance, comprising approximately one-third of the proteome, and their importance in biological function, as pharmaceutical targets [3] and their future potential within emerging nano- and bio-technologies [4,5].

Integral membrane proteins (IMPs) span the lipid bilayers that form functional barriers at the interface of cellular and subcellular compartments. They perform diverse roles such as adhesion, material transport, signal transduction and catalysis. Besides their well-established importance in drug-screening programmes, IMPs are of interest as biofunctional components within technologies, including sensors, nanoreactors, protocells and nanomedical drug delivery systems [6–8]. The construction of such novel artificial biological devices comprises the field of Synthetic Biology [9,10], which is a major growth area in current fundamental and applied research.

The biggest hurdle to overcome for manipulation of IMPs *ex vivo* is their inherent instability in water. In their native state, a large proportion of the exterior surface of IMPs is hydrophobic to enable their stable insertion into a biomembrane. These non-polar surface residues must be shielded from direct contact with water, as is achieved by the liquid crystalline amphiphilic solvation within a lipid bilayer. A wide variety of self-assembling and macromolecular materials have been applied to IMP handling; these soft materials combine both liquid-like and solid-like properties, known as mesophases, and can mimic important aspects of their native environment [11]. The most commonly used IMP-stabilising systems are detergent micelles, but other materials have been developed for enhanced stability, more native-like environments or practical simplicity: these include amphiphilic polymers called amphipols [12], and disc-shaped lipid-containing structures such as bicelles [13], lipoprotein-mimetic

nanodiscs [14], saposin lipoprotein nanoparticles [15] and styrene maleic acid lipid particles (SMALPs) [16]. Structurally complex bicontinuous cubic phases of surfactants are also used in crystallisation studies [17]. These soft, self-organised systems have facilitated important advances in our understanding of the structure and function of membrane proteins.

Despite the variety and success of the aforementioned materials, an important feature of natural organisation is lost: compartmentalisation [18,19]. Many IMPs transport ions or molecules from one distinct aqueous compartment to another or are driven by the release of energy from transmembrane potential differences. Moreover, the loss of a 'closed' system is problematic for structural studies where the detergent-extracted IMP may not exist in its native state due to the lack of a membrane potential. Compartments can be achieved by reconstitution into liposomes with the added benefit of the native-like lipid bilayer structure. More complex droplet interface bilayer systems can also provide compartmentalised architectures [20,21]. Liposome reconstitution protocols have widely been reported [22], yet novel materials and methods are still continually being developed [23,24].

While liposomes solve the challenges of compartments and native-like solvation, their major drawback is the lack of long-term stability of these systems [25,26]. This is particularly problematic for biotechnological applications, where considerable stability and durability are necessities. Alternative soft matter systems are known to form tougher vesicles than liposomes and so have become candidate constructs for IMP stabilisation: polymersomes.

Polymersomes

Polymersomes are composed of amphiphilic polymers that spontaneously self-assemble in water to form vesicles, analogous to the formation of liposomes from their constituent lipids [27]. While there are many commonalities between liposomes and polymersomes, there are also many important fundamental differences. The advantages of polymer membranes over their lipid counterparts are their broader parameter space of physical and chemical properties due to the variety of polymer chemistries that can be applied and their broad range of possible molecular masses. Amphiphilic block copolymers that are known to self-assemble into vesicles can have several architectures, the most common being diblock AB copolymers (A = hydrophophilic polymer, B = hydrophobic polymer), or triblock ABA or ABC copolymers, where A and C are chemically distinct hydrophilic blocks (Figure 1). The relative block lengths required for vesicle assembly can be correlated with packing parameter models for amphiphile self-assembly [28], for example worm-like micelles commonly assemble if the hydrophilic to hydrophobic block volume ratios are slightly too large for vesicles to be preferred [29].

Unlike lipid membranes, block copolymer membranes do not form a distinct bilayer structure, i.e. two molecular monolayers aligned in apposition to one another. Instead the hydrophobic polymer blocks interdigitate and penetrate at random within the hydrophobic core of the membrane in an entangled polymer melt [32]. Polymersome membranes have been reported ranging in thickness from that of a lipid membrane (3–5 nm) up to 40 nm [33]. Polymersomes generally have a membrane permeability that is lower than those of lipid membranes due to the inverse dependence of permeability on membrane thickness [34]. The membrane fluidity of viscous polymer membranes is also typically at least an order of magnitude less than for lipid bilayers, and the mechanical properties of tough, robust polymer membranes can range over at least three orders of magnitude [35].

Common hydrophobic blocks include polybutadiene (PBd), polystyrene (PS) and polydimethylsiloxane (PDMS), while common hydrophilic blocks are poly(ethylene oxide) (PEO), poly(acrylic acid) (PAA) and poly (2-methyl oxazoline) (PMOXA). Despite their vastly different chemistry, structure, mechanics and dynamics, polymersomes have proved successful reconstitution systems for some IMPs. Key to this success are the flexibility of the polymer chains and the hydrophobic thickness of the membrane: flexible, linear hydrophobic polymers allow conformational adaption to the preferred hydrophobic thickness of the protein (Figure 1) and, while membrane thickness may in some cases be less critical, membranes closer to the natural hydrophobic thickness of a biomembrane can be preferable. While not IMPs *per se*, gramicidin and ionomycin have been shown to be able to create ion selective pores in polymersome membranes with thicknesses up to approximately 12 nm, but no greater [36,37]. Successful reconstitution of IMPs into polymer membranes >10 nm in thickness, resulting in a large hydrophobic mismatch with the protein, has also been readily achieved [30].

Several highly stable membrane proteins have been successfully reconstituted into polymer vesicles, with outer membrane protein F (OmpF), bacteriorhodopsin (BR) and AquaporinZ (AqpZ) being common examples. OmpF, for example, is known to be able to withstand harsh conditions, such as high temperatures, proteases and denaturing detergents [38,39]. A broader range of examples of membrane proteins reconstituted into
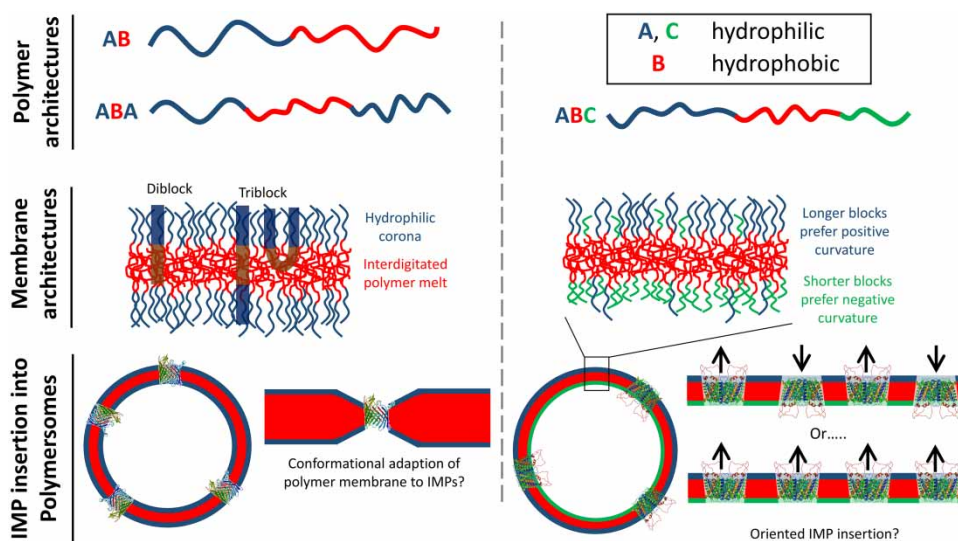
PORTLAND
PRESS

Figure 1. Polymersomes.

Polymer membranes are formed from amphiphilic block copolymers that often have AB, ABA or ABC polymer architectures. These membranes are interdigitated with a viscous polymer melt at its core and a hydrophilic corona of polymers in an extended brush-like conformation. Triblock copolymers may be a mixture of transmembrane and hairpins, which have both their hydrophilic blocks displayed at the same membrane surface. AB and ABA architectures and resulting polymerosome structures are shown on the left-hand side. Asymmetric ABC polymers (right-hand side) can give rise to asymmetric membrane chemistries if the hydrophilic block lengths are different: longer polymers prefer the positive curvature at the exterior of the vesicle and vice versa. Membrane proteins can be inserted into these polymer membranes even if the polymer membrane is much thicker than the hydrophobic thickness of the IMP, suggesting conformational adaption of the polymers to the protein, shown for OmpF (PDB ID: 2OMF) on the left-hand side [30]. Asymmetric ABC membranes may also help drive preferential orientation of the IMPs within the membrane, shown for Aqp0 (PDB ID: 2B6P) on the right-hand side [31].

polymersomes is given in Table 1. This list is not intended to be exhaustive; a more comprehensive record can be found in Table 1 of reference [40].

Reconstitution methods into polymersomes bear similarities to those used for proteoliposomes. For example, polymersomes can be formed from lipid-detergent micelles [41], temporary destabilisation of preformed polymersomes detergents [42] and spontaneous insertion of the protein into the membrane [43]. Detergent removal can be achieved by the use of BioBeads or dialysis. Removing detergent by diluting them to below the critical micelle concentration (CMC) and harvesting the polymersomes by centrifugation may not be possible as, unlike liposomes, many polymersomes are less dense than water and so cannot be spun down into a pellet. Direct incorporation of membrane proteins into polymersomes from cell-free synthesis has also been reported [44]. However, differences in properties between lipid and polymer systems mean that, for a given IMP, successful proteoliposome reconstitution protocols are not necessarily directly transferable to polymersome systems. Unlike lipid–detergent interactions [11,45], detergent–copolymer interactions are currently not as well understood, making rational modification of IMP–polymersome reconstitution protocols a major challenge.

Several studies show the potential of polymersomes as platforms for a wider range of advanced IMP systems. Different IMPs have been functionally reconstituted into the same polymersome, most elegantly demonstrated by the coupling of BR and $F_0 - F_1$ ATP synthase (F-ATPase) for light-generated ATP synthesis [46,47]. Reconstitution of more challenging IMPs into polymersomes may also be possible through careful optimisation of polymer structure, chemistry and reconstitution protocols. For example, complex I of the electron transport chain has been functionally reconstituted into PMOXA–PDMS–PMOXA triblock copolymer vesicles [42].

Polymer membranes might also offer some functional advantages over traditional proteoliposomes. Oriented reconstitution of an Aquaporin0 (Aqp0) channel has been achieved using asymmetric ABC PEO–PDMS–PMOXA triblock membranes (Figure 1), opening the possibility, for example, of directional substrate transport into/out of vesicles containing multiple reconstituted IMPs [31]. While natural biomembranes have an

Table 1 Examples of IMPs reconstituted into polymersomes
<div align="right">Part 1 of 2</div>

Protein	Polymer(s)	Notes	Ref.
OmpF	PBd_{12}-PEO_8	• Magnetic fields can be used to drive OmpF crystallisation.	[58]
	$PMOXA_6$-$PDMS_{44}$-$PMOXA_6$	• OmpF gated with a pH-responsive cap.	[59]
BR/ F-ATPase	$PEtOz_{11}$-$PDMS_{76}$-$PEtOz_{11}$	• Co-reconstitution of BR and F-ATPase allows coupling of membrane protein function: light-driven ATP synthesis.	[46,47]
		• Polymer membranes support pH gradients sufficient to create a proton-motive force to drive secondary IMP functions.	
		• Choice of reconstitution method flipped the preferred orientation of BR in vesicles allowing selection of vectorial proton transport into or out of the vesicle.	
PR	$P4MVP_x$-PS_y-$P4MVP_x$ $(x,y) = (21.26), (21.38),$ or (29.42)	• Reconstitution into highly stable glassy membranes.	[43]
		• Electrostatically driven protein reconstitution.	
		• Membrane acts as an allosteric regulator of PR function.	
AqpZ	$PMOXA_{15}$-$PDMS_{110}$-$PMOXA_{15}$	• Polymer membranes alone are impermeable to water.	[60]
		• AqpZ allows water to cross the membrane but not larger solutes.	
Aqp0	$PMOXA_{20}$-$PDMS_{75}$-$PMOXA_{20}$ PEO_{25}-$PDMS_{40}$-$PMOXA_{110}$ PEO_{67}-$PDMS_{40}$-$PMOXA_{45}$	• Investigates oriented insertion into symmetric ABA and asymmetric ABC membranes.	[31]
		• Relative sizes of PEO and PMOXA hydrophilic blocks determine polymer orientation in the vesicle membrane: the larger block generates positive curvature and forms the outer membrane surface.	
		• Preferential protein orientation only observed for ABC polymer vesicles.	
	PBd_{10}-PEO_{12} PBd_{22}-PEO_{14} $PMOXA_{20}$-$PDMS_{42}$-$PMOXA_{20}$ $PMOXA_{12}$-$PDMS_{55}$-$PMOXA_{12}$	• High densities of Aqp0 can be functionally reconstituted into polymersomes.	[61]
		• Effects on vesicle morphology observed for high protein concentrations.	
FhuA	PIB_{18}-PEO_{136}-PIB_{18}	• The protein is re-engineered to increase the hydrophobic β-barrel length by 1 nm to allow for more favourable solvation interactions with the membrane.	[51]
KcsA	$PMOXA_x$-$PDMS_y$-$PMOXA_x$ $(x,y) = (6.34), (7.49),$ or (12.63)	• Flexibility of PDMS block allows insertion into membranes with large hydrophobic mismatch.	[30]
		• Even with a large hydrophobic mismatch, the fluidity of the PDMS chains means that the protein diffusion constant is only one order of magnitude slower than in a lipid bilayer.	
		• No evidence for functional incorporation of KcsA in these membranes.	
Integrin $α_vβ_3$	PBd_x-PEO_y $(x,y) = (22.14), (17.6),$ or (12.9)	• *In vitro* (cell free) membrane-assisted protein synthesis.	[44]
		• Integrin incorporation efficiency is not found to be dependent on the polymer block length.	
Complex I	$PMOXA_x$-$PDMS_y$-$PMOXA_x$ 8 polymers	• Transmembrane electron transfer from NADH to an encapsulated quinone.	[42]

<div align="right">Continued</div>

Table 1 Examples of IMPs reconstituted into polymersomes

Protein	Polymer(s)	Notes	Ref.
	$9 \leq x \leq 65$ $23 \leq y \leq 165$	• Increasing membrane thickness increases the activity of complex I. • Increasing hydrophilic polymer length at fixed hydrophobic thickness decreases complex I activity. • Specific inhibition by 10 µM piericidin A reduces activity by >90%.	
LamB	$PMOXA_{11}$-$PDMS_{73}$-$PMOXA_{11}$	• LamB acts as a specific receptor for λ phage to trigger DNA loading into the polymersome lumen.	[62]
TsX	$PMOXA_{20}$-$PDMS_{54}$-$PMOXA_{20}$	• 200 nm polymersomes with the nucleoside-specific porin TsX. • Encapsulation of thymidine phosphorylase for enzyme-replacement therapy for mitochondrial neurogastrointestinal encephalomyopathy. • Nanoreactors are functional in serum at 37°C, show low cytotoxicity and do not stimulate a significant inflammatory response.	[63]
Claudin-2	PBd_{21}-PEO_{12}	• Cell-free protein expression and directed insertion into polymersomes. • Protein in polymersomes confirmed by specific antibody binding (SPR).	[64]
GPCR (5-$HT_{1A}R$)	PBd_{12}-PEO_9	• Functional reconstitution into giant polymer vesicles. • Oriented protein insertion with ∼90% of GPCR in its native orientation. • GPCR activity retained after lyophilisation and rehydration of vesicles.	[50]

asymmetric lipid distribution, asymmetric lipid bilayer vesicles are much more challenging to fabricate for oriented membrane protein reconstitution [48,49]. Furthermore, while conventional thinking says that block copolymers for IMP reconstitution should be in a fluid state, above their glass transition temperature, proteorhodopsin (PR) has been functionally reconstituted into highly stable glassy block copolymer membranes with PS hydrophobic blocks [43]. Proteopolymersomes have also shown stability under lyophilisation: following rehydration, PBd–PEO vesicles are restored without a significant loss of IMP function, in this case a G-protein-coupled receptor [50].

Polymersomes are not always favourable environments for IMP reconstitution and modifications to the protein or membrane environment may be required to achieve the desired function. Evolution has optimised membrane proteins for function within lipid membrane matrices; however, protein engineering might be used to adapt a membrane protein for more favourable insertion into synthetic polymer membranes. The ferric hydroxamate protein uptake component A (FhuA) has been engineered to increase its hydrophobic surface by 1 nm, reducing hydrophobic mismatch and lowering the insertion penalty into thicker polymer membranes [51]. However, hydrophobic mismatch is not the only challenge; many membrane proteins require specific interactions with lipids for native function [52,53]: for example, recent reports of specific lipid regulation of the TrpV1 ion channel [54]. Even if specific lipid interactions are not required, the delicate balance of forces experienced by an IMP in a biomembrane may be important for maintaining its native structure and/or function, i.e. establishing a lateral pressure profile in the membrane that mimics the natural biomembrane [55–57]. These issues motivate the modification of polymersome properties to enhance their biofunctionality. This has been approached by blending block copolymers and phospholipids to create hybrid vesicles with the goal of combining the best features of these two materials: the chemical versatility and robustness of polymersomes with the biocompatibility and biofunctionality of liposomes.

Hybrid vesicles

Given the disparity in properties between lipid and block copolymer membranes, in particular differences in membrane thickness and structure, it may seem surprising that blends of lipids and block copolymers can form mixed, hybrid vesicles (Figure 2). Therefore, the study of the mixing behaviour between lipids and polymers in hybrid vesicles has been at the fore of their material characterisation.

Several block copolymer classes have been investigated in vesicle blends with phospholipids; examples include PBd-PEO [65–67], PDMS-PMOXA [68], PIB-PEO [69], PChA-PNIPAAm [70] and PDMS-PEO [71,72]. These mixtures can form homogeneous, well-mixed membranes or phase separate into lipid-rich and polymer-rich domains dependent on several factors, including lipid polymorphism and phase transitions, hydrophobic mismatch, cross-linking between lipids or polymers and specific mixing interactions between the individual components. While well-mixed vesicles [65] may give optimal, modified local membrane environments for protein reconstitution, lipid-rich domains within a bulk polymer matrix may also be desirable for tuning optimal activity and durability. In particular, nanodomains of lipids have been reported in PDMS-PEO/POPC hybrid vesicles [73], where the PDMS hydrophobic block is less compatible with the lipid tail groups than a PBd polymer block based upon their respective solubility parameters [74]. Stable, nanoscale lipid-rich islands within a sea of mechanically robust polymer-rich membrane might be particularly attractive IMP-functionalised constructs for combining native-like lipid solvation with the enhanced structural stability of polymersomes.
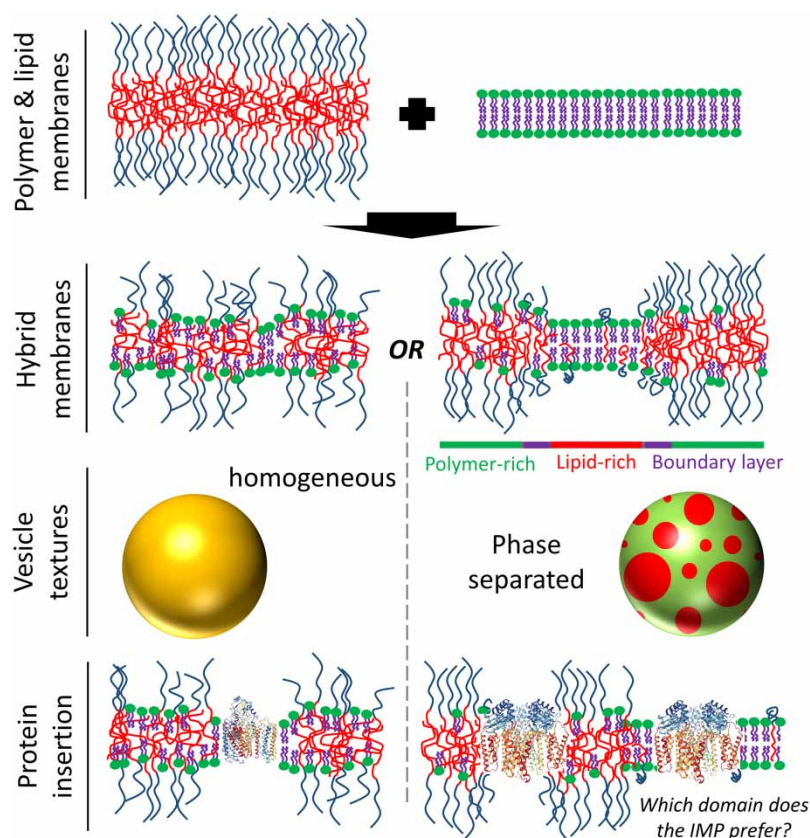

Figure 2. Hybrid vesicles.

Hybrid vesicles combine lipids and block copolymers into blended membranes. These hybrid membranes can either be well mixed, giving homogeneous properties across the surface of the vesicle (left-hand side), or phase separated into lipid-rich and polymer-rich domains, which give rise to textured vesicle morphologies with coexisting domains of different structures and properties (right-hand side). Membrane proteins can be inserted into these hybrid membranes, either into homogeneous membranes (left-hand side, showing cyt bo$_3$; PDB ID: 1FFT) or phase-separated membranes, where the preferred location of the IMP in the membrane is dependent on the relative properties of these two coexisting phases (right-hand side, showing MloK1; PDB ID: 4CHW).

Hybrid vesicles tune the dynamical and mechanical properties of vesicle membranes between those of pure lipids and pure polymers [66,72,75]. Lipid-specific stimuli can also be used to address the hybrid membranes, particularly when phase-separated into lipid-rich domains, modifying structure and permeability [67]. We point the reader to more comprehensive reviews of the physicochemical properties of hybrid vesicles for further details on this topic [74,76].

The study of membrane proteins in hybrid lipid–polymer systems is still in its infancy with only one report currently studying vesicle architectures. A summary of current reports is presented in Table 2. Phase separation and mixing phenomena are important properties in hybrid materials, as we discuss above. This begs the question as to where a membrane protein would prefer to reside in textured, phase-separated hybrid membranes. The partitioning of OmpF in lipid–polymer Langmuir monolayers at the air–water interface have shown that the protein can prefer to reside in polymer-rich domains [77]. At first sight this is a surprising result as the motivation for using hybrid membranes is the preference of membrane proteins for native lipid environments. However, the lipid-rich domains (DPPC) in the present study are in a non-native gel phase: the ordering and tight packing of the lipids in this phase exclude impurities, e.g. OmpF, causing them to instead reside in the more flexible and fluid polymer-rich regions of the membrane.

Similarly, the observation that membrane proteins (MloK1) prefer polymer-rich membrane over solid-like gel phase lipid (DPPC, DPPE) was further demonstrated in planar, solid-supported membranes (Figure 2) [78]. Importantly, in separated PDMS-PMOXA/DOPC membranes, where DOPC-rich domains were observed, MloK1 preferentially partitions into the fluid lipid-rich phase. Furthermore, PDMS-PMOXA/POPE membranes form well-mixed membranes with uniform protein distribution. This reveals an important control parameter: the location of protein within a membrane can be manipulated between polymer-rich or lipid-rich domains or a uniform membrane distribution by judicious choice of the lipid.

Crucially, these two aforementioned studies do not investigate native structure or function of the membrane protein reconstituted within hybrid membranes. This shortfall has been addressed for OmpF in PI-PEO membranes (90:10 polymer:protein) [79]. Electrochemical analysis of the protein conductivity shows comparable values between lipid (DPhPC) and polymer-rich (PI-PEO/DPhPC) membranes. This result was independent of the length of polymer used. OmpF also exhibited native-like voltage-dependent channel closing. However, protein insertion into polymer-rich membranes was suppressed in hybrid membranes compared with pure lipids. The authors suggest that this may be due to excess residual chloroform in hybrid films that denature the protein. Notably, there is no comparison of protein properties within 100% polymer membranes that convincingly justifies the requirement of a more complex hybrid membrane; OmpF has been functionally reconstituted into polymersomes as earlier discussed.

The advantage of the hybrid system became evident for cytochrome bo_3 (cyt bo_3) reconstituted into PBd-PEO/POPC vesicles [80]. Here, the protein is not functional in the pure polymersome system. However, there is only minimal loss in protein activity for vesicles with up to 50% polymer content. Impressively, increased polymer content enhances the functional lifetime of the protein, with 50% polymer hybrids providing the best combination of high initial activity and durability of function. Intriguingly, purification of these 50% polymer proteo-hybrid vesicles by size exclusion chromatography to remove coexisting micelles suggests a further improvement in cyt bo_3 durability with a ∼20% drop in activity after one week followed by a very slow decline in function up to week 6 where >70% of its initial activity still remains. As a comparison, control proteoliposomes rapidly lost function and were inactive within four weeks. In future it will be of interest to conduct a more long-term study of the activity of these hybrid vesicles, beyond 6 weeks, to understand the full extent of their extraordinary stability.

Outlook

Stability and durability are important characteristics for membrane proteins to make a successful impact in biotechnologies. Where compartmentalisation via vesicular architectures is required, proteoliposomes are unlikely to meet these obligations. More robust block copolymer membrane systems will be important in this provision but, beyond the most stable and robust membrane proteins, the non-native polymersome environment will limit the inventory of viable protein components. Protein engineering to optimise the protein's structure for incorporation within the polymer membrane may yield some success, e.g. by matching their hydrophobic thickness, but optimisation of the membrane matrix for biofunctionality is likely to be a more straightforward and fruitful approach with broad applicability to a wide range of membrane proteins. Blending lipids and polymers within a membrane environment will allow native-like lipid solvation of the protein and facilitate specific

Table 2 Overview of membrane protein reconstitution into hybrid lipid–polymer systems

Protein	Polymer(s)	Lipid(s)	Membrane architecture	Notes	Ref.
OmpF	$PMOXA_{15}$-$PDMS_{110}$-$PMOXA_{15}$	DPPC	Langmuir monolayers	• Phase separation between lipid and polymer components creates textured films. • Protein preferentially partitions with fluid polymer domains, excluded from the non-native lipid gel phase domains. • Native structure or function of the reconstituted protein is not studied.	[77]
	PI_x-PEO_y (x,y) = (9.6), (16.10) or (30.26)	DPhPC	Planar membranes	• Three different polymer lengths studied at a 90:10 ratio with lipid. • Channel conductance comparable with that in pure lipid for hybrid membranes of all polymer lengths. • Native-like voltage-dependent channel closing is observed. • Hybrid membranes inhibit protein insertion compared with pure lipid. • Comparison of hybrids with the pure polymer system is not made.	[79]
MloK1	$PDMS_x$-$PMOXA_y$ (x,y) = (65.12), (37.9) or (16.9)	DPPC, DOPC, DPPE or POPE	Solid-supported planar membranes	• Three different length polymers are studied in conjunction with one of four different lipids. • Protein insertion into phase-separated lipid–polymer membranes shows that the protein partitions based on the fluidity of coexisting domains, disfavouring lipid gel phases and favouring fluid lipid domains. • Native structure or function of the reconstituted protein is not studied.	[78]
Cyt bo_3	PBd_{22}-PEO_{14}	POPC	Vesicles	• Compositions from 0 to 100% polymer content in 25% increments. • Only a small drop in protein activity is observed for up to 50% polymer; activity drops significantly above 50% polymer content. • The functional lifetime of the protein is significantly extended with increasing polymer content. • Evidence that purification of vesicles from coexisting micelles could further enhance the durability of function.	[80]

lipid–protein interactions that may be important for function and to tune the local fluidity and mechanics of the membrane environment that the protein experiences.

Investigation of hybrid vesicles for membrane protein reconstitution is still at an immature stage of development. Much work needs to be done to further optimise the hybrid membrane environment, which will be best achieved through a more detailed fundamental understanding of the coupling between the membrane composition and its bulk physicochemical properties. Furthermore, a much wider range of membrane proteins needs

to be investigated when reconstituted into hybrid vesicles to test the generality of this approach. This selection of proteins must extend beyond the most stable model IMPs into important IMPs that are more difficult to handle in order to fully challenge the abstraction of this principle. However, there is nothing unique about cyt bo_3 that would suggest that its enhanced durability in hybrid vesicles is a special case [80]. This enhanced stability may not only be critical to synthetic biology but could also become an important tool in handling membrane proteins in fundamental biochemical studies where protein stability has proved to be a major impediment. A more long-term appeal for these systems is not just to create a stable, functional environment, but to use techniques such as lipidomics to accurately reflect the lipids found within the native environment, as has been done with the SMALP platform [81]. Rapid recent advances in vesicle engineering are beginning to overcome the practical challenges in realising robust biotechnologies based on these hollow capsules. This augurs a bright future for emerging applications, encompassing nanoreactor, drug delivery and biosensor technologies, among others.

Abbreviations

Aqp0, Aquaporin0; AqpZ, AquaporinZ; BR, Bacteriorhodopsin; Cyt bo_3, Cytochrome bo_3 ubiquinol oxidase; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DPhPC, 1,2-diphytanoyl-sn-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine; F-ATPase, F_0F_1 ATP Synthase; FhuA, Ferric-hydroxamate uptake protein A; GPCR, G-protein-coupled receptor; IMP, Integral membrane protein; KcsA, Potassium crystallographically sited activation channel; LamB, Bacteriophage lambda receptor (maltoporin); MloK1, Cyclic nucleotide-modulated potassium channel 1; OmpF, Outer membrane protein F; P4MVP, Poly(4-vinyl-N-methylpyridine iodide); PBd, Polybutadiene; PChA, Poly (cholesteryl acrylate); PDMS, Poly(dimethyl siloxane); PEO, Poly(ethylene oxide); PEtOz, Poly(2-ethyl-2-oxazoline; PI, Poly(1,4 isoprene); PIB, Polyisobutylene; PMOXA, Poly(2-methyl oxazoline); PNIPAAm, Poly (N-isopropylacrylamide); POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; POPE, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine; PR, Proteorhodopsin; PS, Polystyrene; SPR, Surface Plasmon Resonance; TsX, Nucleoside-specific channel forming protein TsX

Funding

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1   Früh, V., Ijzerman, A.P. and Siegal, G. (2011) How to catch a membrane protein in action: a review of functional membrane protein immobilization strategies and their applications. *Chem. Rev.* **111**, 640–656 doi:10.1021/cr900088s
2   Shen, H.-H., Lithgow, T. and Martin, L. (2013) Reconstitution of membrane proteins into model membranes: seeking better ways to retain protein activities. *Int. J. Mol. Sci.* **14**, 1589–1607 doi:10.3390/ijms14011589
3   Arinaminpathy, Y., Khurana, E., Engelman, D.M. and Gerstein, M.B. (2009) Computational analysis of membrane proteins: the largest class of drug targets. *Drug Discov. Today* **14**, 1130–1135 doi:10.1016/j.drudis.2009.08.006
4   Bayley, H. and Jayasinghe, L. (2004) Functional engineered channels and pores (Review). *Mol. Membr. Biol.* **21**, 209–220 doi:10.1080/09687680410001716853
5   Kim, Y.-R., Jung, S., Ryu, H., Yoo, Y.-E., Kim, S.M. and Jeon, T.-J. (2012) Synthetic biomimetic membranes and their sensor applications. *Sensors* **12**, 9530–9550 doi:10.3390/s120709530
6   Tanner, P., Baumann, P., Enea, R., Onaca, O., Palivan, C. and Meier, W. (2011) Polymeric vesicles: from drug carriers to nanoreactors and artificial organelles. *Acc. Chem. Res.* **44**, 1039–1049 doi:10.1021/ar200036k
7   Molinaro, R., Corbo, C., Martinez, J.O., Taraballi, F., Evangelopoulos, M., Minardi, S. et al. (2016) Biomimetic proteolipid vesicles for targeting inflamed tissues. *Nat. Mater.* **15**, 1037–1046 doi:10.1038/nmat4644
8   Walde, P., Cosentino, K., Engel, H. and Stano, P. (2010) Giant vesicles: preparations and applications. *Chembiochem* **11**, 848–865 doi:10.1002/cbic.201000010
9   Channon, K., Bromley, E.H.C. and Woolfson, D.N. (2008) Synthetic biology through biomolecular design and engineering. *Curr. Opin. Struct. Biol.* **18**, 491–498 doi:10.1016/j.sbi.2008.06.006
10 Schwille, P. (2011) Bottom-up synthetic biology: engineering in a Tinkerer's world. *Science* **333**, 1252–1254 doi:10.1126/science.1211701
11 Seddon, A.M., Curnow, P. and Booth, P.J. (2004) Membrane proteins, lipids and detergents: not just a soap opera. *Biochim. Biophys. Acta Biomembranes* **1666**, 105–117 doi:10.1016/j.bbamem.2004.04.011

12 Popot, J.L., Althoff, T., Bagnard, D., Baneres, J.L., Bazzacco, P., Billon-Denis, E. et al. (2011) Amphipols from A to Z. In *Annual Review of Biophysics* (Rees, D.C., Dill, K.A. and Williamson, J.R., eds), vol. 40, pp. 379–408, Annual Reviews

13 Dürr, U.H.N., Gildenberg, M. and Ramamoorthy, A. (2012) The magic of bicelles lights up membrane protein structure. *Chem. Rev.* **112**, 6054–6074 doi:10.1021/cr300061w

14 Bayburt, T.H. and Sligar, S.G. (2010) Membrane protein assembly into Nanodiscs. *FEBS Lett.* **584**, 1721–1727 doi:10.1016/j.febslet.2009.10.024

15 Frauenfeld, J., Löving, R., Armache, J.-P., Sonnen, A.F.-P., Guettou, F., Moberg, P. et al. (2016) A saposin-lipoprotein nanoparticle system for membrane proteins. *Nat. Meth.* **13**, 345–351 doi:10.1038/nmeth.3801

16 Lee, S.C., Knowles, T.J., Postis, V.L.G., Jamshad, M., Parslow, R.A., Lin, Y.-p. et al. (2016) A method for detergent-free isolation of membrane proteins in their local lipid environment. *Nat. Protoc.* **11**, 1149–1162 doi:10.1038/nprot.2016.070

17 Landau, E.M. and Rosenbusch, J.P. (1996) Lipidic cubic phases: a novel concept for the crystallization of membrane proteins. *Proc. Natl. Acad. Sci. U. S.A.* **93**, 14532–14535 doi:10.1073/pnas.93.25.14532

18 Ichihashi, N. and Yomo, T. (2014) Positive roles of compartmentalization in internal reactions. *Curr. Opin. Chem. Biol.* **22**, 12–17 doi:10.1016/j.cbpa.2014.06.011

19 Go, Y.-M. and Jones, D.P. (2008) Redox compartmentalization in eukaryotic cells. *Biochim. Biophys. Acta Gen. Subjects* **1780**, 1273–1290 doi:10.1016/j.bbagen.2008.01.011

20 Maglia, G., Heron, A.J., Hwang, W.L., Holden, M.A., Mikhailova, E., Li, Q. et al. (2009) Droplet networks with incorporated protein diodes show collective properties. *Nat. Nanotechnol.* **4**, 437–440 doi:10.1038/nnano.2009.121

21 Booth, M.J., Schild, V.R., Graham, A.D., Olof, S.N. and Bayley, H. (2016) Light-activated communication in synthetic tissues. *Sci. Adv.* **2**, e1600056 doi:10.1126/sciadv.1600056

22 Rigaud, J.-L. and Lévy, D. (2003) Reconstitution of membrane proteins into liposomes. *Method Enzymol.* **372**, 65–86 doi:10.1016/S0076-6879(03)72004-7

23 Motta, I., Gohlke, A., Adrien, V., Li, F., Gardavot, H., Rothman, J.E. et al. (2015) Formation of giant unilamellar proteo-liposomes by osmotic shock. *Langmuir* **31**, 7091–7099 doi:10.1021/acs.langmuir.5b01173

24 Biner, O., Schick, T., Müller, Y. and von Ballmoos, C. (2016) Delivery of membrane proteins into small and giant unilamellar vesicles by charge-mediated fusion. *FEBS Lett.* **590**, 2051–2062 doi:10.1002/1873-3468.12233

25 Armengol, X. and Estelrich, J. (1995) Physical stability of different liposome compositions obtained by extrusion method. *J. Microencapsul.* **12**, 525–535 doi:10.3109/02652049509006783

26 Grit, M. and Crommelin, D.J.A. (1993) Chemical stability of liposomes: implications for their physical stability. *Chem. Phys. Lipids* **64**, 3–18 doi:10.1016/0009-3084(93)90053-6

27 Discher, B.M., Won, Y.-Y., Ege, D.S., Lee, J.C.-M., Bates, F.S., Discher, D.E. et al. (1999) Polymersomes: tough vesicles made from diblock copolymers. *Science* **284**, 1143–1146 doi:10.1126/science.284.5417.1143

28 Israelachvili, J.N., Mitchell, D.J. and Ninham, B.W. (1976) Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers. *J. Chem. Soc. Faraday Trans. 2 Mol. Chem. Phys.* **72**, 1525–1568 doi:10.1039/f29767201525

29 Kita-Tokarczyk, K., Grumelard, J., Haefele, T. and Meier, W. (2005) Block copolymer vesicles — using concepts from polymer chemistry to mimic biomembranes. *Polymer* **46**, 3540–3563 doi:10.1016/j.polymer.2005.02.083

30 Itel, F., Najer, A., Palivan, C.G. and Meier, W. (2015) Dynamics of membrane proteins within synthetic polymer membranes with large hydrophobic mismatch. *Nano Lett.* **15**, 3871–3878 doi:10.1021/acs.nanolett.5b00699

31 Stoenescu, R., Graff, A. and Meier, W. (2004) Asymmetric ABC-triblock copolymer membranes induce a directed insertion of membrane proteins. *Macromol. Biosci.* **4**, 930–935 doi:10.1002/mabi.200400065

32 Battaglia, G. and Ryan, A.J. (2005) Bilayers and interdigitation in block copolymer vesicles. *J. Am. Chem. Soc.* **127**, 8757–8764 doi:10.1021/ja050742y

33 Chen, Q., Schönherr, H. and Vancso, G.J. (2009) Mechanical properties of block copolymer vesicle membranes by atomic force microscopy. *Soft Matter* **5**, 4944–4950 doi:10.1039/b903110c

34 LoPresti, C., Lomas, H., Massignani, M., Smart, T. and Battaglia, G. (2009) Polymersomes: nature inspired nanometer sized compartments. *J. Mater. Chem.* **19**, 3576–3590 doi:10.1039/b818869f

35 Palivan, C.G., Goers, R., Najer, A., Zhang, X., Car, A. and Meier, W. (2016) Bioinspired polymer vesicles and membranes for biological and medical applications. *Chem. Soc. Rev.* **45**, 377–411 doi:10.1039/C5CS00569H

36 Lomora, M., Itel, F., Dinu, I.A. and Palivan, C.G. (2015) Selective ion-permeable membranes by insertion of biopores into polymersomes. *Phys. Chem. Chem. Phys.* **17**, 15538–15546 doi:10.1039/C4CP05879H

37 Lomora, M., Garni, M., Itel, F., Tanner, P., Spulber, M. and Palivan, C.G. (2015) Polymersomes with engineered ion selective permeability as stimuli-responsive nanocompartments with preserved architecture. *Biomaterials* **53**, 406–414 doi:10.1016/j.biomaterials.2015.02.080

38 Schirmer, T. and Rosenbusch, J.P. (1991) Prokaryotic and eukaryotic porins. *Curr. Opin. Struct. Biol.* **1**, 539–545 doi:10.1016/S0959-440X(05)80075-2

39 Phale, P.S., Philippsen, A., Kiefhaber, T., Koebnik, R., Phale, V.P., Schirmer, T. et al. (1998) Stability of trimeric OmpF Porin: the contributions of the latching loop L2. *Biochemistry* **37**, 15663–15670 doi:10.1021/bi981215c

40 Habel, J., Hansen, M., Kynde, S., Larsen, N., Midtgaard, S., Jensen, G. et al. (2015) Aquaporin-based biomimetic polymeric membranes: approaches and challenges. *Membranes* **5**, 307–351 doi:10.3390/membranes5030307

41 Marsden, H.R., Quer, C.B., Sanchez, E.Y., Gabrielli, L., Jiskoot, W. and Kros, A. (2010) Detergent-aided polymersome preparation. *Biomacromolecules* **11**, 833–838 doi:10.1021/bm1001763

42 Graff, A., Fraysse-Ailhas, C., Palivan, C.G., Grzelakowski, M., Friedrich, T., Vebert, C. et al. (2010) Amphiphilic copolymer membranes promote NADH: ubiquinone oxidoreductase activity: towards an electron-transfer nanodevice. *Macromol. Chem. Physics* **211**, 229–238 doi:10.1002/macp.200900517

43 Kuang, L., Fernandes, D.A., O'Halloran, M., Zheng, W., Jiang, Y., Ladizhansky, V. et al. (2014) 'Frozen' block copolymer nanomembranes with light-driven proton pumping performance. *ACS Nano* **8**, 537–545 doi:10.1021/nn4059852

44   Zaba, C., Ritz, S., Tan, C.-W.D., Zayni, S., Müller, M., Reuning, U. et al. (2015) Functional cell adhesion receptors (Integrins) in polymeric architectures. *ChemBioChem* **16**, 1740–1743 doi:10.1002/cbic.201500176

45   le Maire, M., Champeil, P. and Møller, J.V. (2000) Interaction of membrane proteins and lipids with solubilizing detergents. *Biochim. Biophys. Acta Biomembr.* **1508**, 86–111 doi:10.1016/S0304-4157(00)00010-1

46   Choi, H.-J. and Montemagno, C.D. (2005) Artificial organelle:ATP synthesis from cellular mimetic polymersomes. *Nano Lett.* **5**, 2538–2542 doi:10.1021/nl051896e

47   Choi, H.-J., Germain, J. and Montemagno, C.D. (2006) Effects of different reconstitution procedures on membrane protein activities in proteopolymersomes. *Nanotechnology* **17**, 1825–1830 doi:10.1088/0957-4484/17/8/003

48   Pautot, S., Frisken, B.J. and Weitz, D.A. (2003) Engineering asymmetric vesicles. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 10718–10721 doi:10.1073/pnas.1931005100

49   Kamiya, K., Kawano, R., Osaki, T., Akiyoshi, K. and Takeuchi, S. (2016) Cell-sized asymmetric lipid vesicles facilitate the investigation of asymmetric membranes. *Nat. Chem.* **8**, 881–889 doi:10.1038/nchem.2537

50   Gutierrez, M.G., Jalali-Yazdi, F., Peruzzi, J., Riche, C.T., Roberts, R.W. and Malmstadt, N. (2016) G protein-coupled receptors incorporated into rehydrated diblock copolymer vesicles retain functionality. *Small* **12**, 5256–5260 doi:10.1002/smll.201601540

51   Muhammad, N., Dworeck, T., Fioroni, M. and Schwaneberg, U. (2011) Engineering of the *E. coli* outer membrane protein FhuA to overcome the hydrophobic mismatch in thick polymeric membranes. *J. Nanobiotechnol.* **9**, 8 doi:10.1186/1477-3155-9-8

52   Hunte, C. (2005) Specific protein–lipid interactions in membrane proteins. *Biochem. Soc. Trans.* **33**, 938–942 doi:10.1042/BST0330938

53   Barrera, N.P., Zhou, M. and Robinson, C.V. (2013) The role of lipids in defining membrane protein interactions: insights from mass spectrometry. *Trends Cell Biol.* **23**, 1–8 doi:10.1016/j.tcb.2012.08.007

54   Gao, Y., Cao, E., Julius, D. and Cheng, Y. (2016) TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action. *Nature* **534**, 347–351 doi:10.1038/nature17964

55   Marsh, D. (2007) Lateral pressure profile, spontaneous curvature frustration, and the incorporation and conformation of proteins in membranes. *Biophys. J.* **93**, 3884–3899 doi:10.1529/biophysj.107.107938

56   Cantor, R.S. (1997) The lateral pressure profile in membranes: a physical mechanism of general anesthesia. *Biochemistry* **36**, 2339–2344 doi:10.1021/bi9627323

57   Marsh, D. (2008) Protein modulation of lipids, and vice-versa, in membranes. *Biochim. Biophys. Acta Biomembr.* **1778**, 1545–1575 doi:10.1016/j.bbamem.2008.01.015

58   Klara, S.S., Saboe, P.O., Sines, I.T., Babaei, M., Chiu, P.-L., DeZorzi, R. et al. (2016) Magnetically directed two-dimensional crystallization of OmpF membrane proteins in block copolymers. *J. Am. Chem. Soc.* **138**, 28–31 doi:10.1021/jacs.5b03320

59   Einfalt, T., Goers, R., Dinu, I.A., Najer, A., Spulber, M., Onaca-Fischer, O. et al. (2015) Stimuli-triggered activity of nanoreactors by biomimetic engineering polymer membranes. *Nano Lett.* **15**, 7596–7603 doi:10.1021/acs.nanolett.5b03386

60   Kumar, M., Grzelakowski, M., Zilles, J., Clark, M. and Meier, W. (2007) Highly permeable polymeric membranes based on the incorporation of the functional water channel protein Aquaporin Z. *Proc. Natl Acad. Sci. U.S.A.* **104**, 20719–20724 doi:10.1073/pnas.0708762104

61   Kumar, M., Habel, J.E.O., Shen, Y.-x., Meier, W.P. and Walz, T. (2012) High-density reconstitution of functional water channels into vesicular and planar block copolymer membranes. *J. Am. Chem. Soc.* **134**, 18631–18637 doi:10.1021/ja304721r

62   Graff, A., Sauer, M., Van Gelder, P. and Meier, W. (2002) Virus-assisted loading of polymer nanocontainer. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 5064–5068 doi:10.1073/pnas.062654499

63   De Vocht, C., Ranquin, A., Willaert, R., Van Ginderachter, J.A., Vanhaecke, T., Rogiers, V. et al. (2009) Assessment of stability, toxicity and immunogenicity of new polymeric nanoreactors for use in enzyme replacement therapy of MNGIE. *J. Control. Release* **137**, 246–254 doi:10.1016/j.jconrel.2009.03.020

64   Nallani, M., Andreasson-Ochsner, M., Tan, C.-W.D., Sinner, E.-K., Wisantoso, Y., Geifman-Shochat, S. et al. (2011) Proteopolymersomes: *in vitro* production of a membrane protein in polymersome membranes. *Biointerphases* **6**, 153–157 doi:10.1116/1.3644384

65   Lim, S.K., de Hoog, H.-P., Parikh, A.N., Nallani, M. and Liedberg, B. (2013) Hybrid, nanoscale phospholipid/block copolymer vesicles. *Polymers* **5**, 1102–1114 doi:10.3390/polym5031102

66   Nam, J., Beales, P.A. and Vanderlick, T.K. (2011) Giant phospholipid/block copolymer hybrid vesicles: mixing behavior and domain formation. *Langmuir* **27**, 1–6 doi:10.1021/la103428g

67   Nam, J., Vanderlick, T.K. and Beales, P.A. (2012) Formation and dissolution of phospholipid domains with varying textures in hybrid lipo-polymersomes. *Soft Matter* **8**, 7982–7988 doi:10.1039/c2sm25646k

68   Winzen, S., Bernhardt, M., Schaeffel, D., Koch, A., Kappl, M., Koynov, K. et al. (2013) Submicron hybrid vesicles consisting of polymer-lipid and polymer-cholesterol blends. *Soft Matter* **9**, 5883–5890 doi:10.1039/c3sm50733e

69   Schulz, M., Glatte, D., Meister, A., Scholtysek, P., Kerth, A., Blume, A. et al. (2011) Hybrid lipid/polymer giant unilamellar vesicles: effects of incorporated biocompatible PIB-PEO block copolymers on vesicle properties. *Soft Matter* **7**, 8100–8110 doi:10.1039/c1sm05725a

70   Panneerselvam, K., Lynge, M.E., Riber, C.F., Mena-Hernando, S., Smith, A.A.A., Goldie, K.N. et al. (2015) Phospholipid—polymer amphiphile hybrid assemblies and their interaction with macrophages. *Biomicrofluidics* **9**, 052610 doi:10.1063/1.4929405

71   Chemin, M., Brun, P.-M., Lecommandoux, S., Sandre, O. and Le Meins, J.-F. (2012) Hybrid polymer/lipid vesicles: fine control of the lipid and polymer distribution in the binary membrane. *Soft Matter* **8**, 2867–2874 doi:10.1039/c2sm07188f

72   Chen, D. and Santore, M.M. (2015) Hybrid copolymer-phospholipid vesicles: phase separation resembling mixed phospholipid lamellae, but with mechanical stability and control. *Soft Matter* **11**, 2617–2626 doi:10.1039/C4SM02502D

73   Dao, T.P.T., Fernandes, F., Er-Rafik, M., Salva, R., Schmutz, M., Brûlet, A. et al. (2015) Phase separation and nanodomain formation in hybrid polymer/lipid vesicles. *ACS Macro Lett.* **4**, 182–186 doi:10.1021/mz500748f

74   Le Meins, J.-F., Schatz, C., Lecommandoux, S. and Sandre, O. (2013) Hybrid polymer/lipid vesicles: state of the art and future perspectives. *Mater. Today* **16**, 397–402 doi:10.1016/j.mattod.2013.09.002

75   Cheng, Z., Elias, D.R., Kamat, N.P., Johnston, E.D., Poloukhtine, A., Popik, V. et al. (2011) Improved tumor targeting of polymer-based nanovesicles using polymer–lipid blends. *Bioconjug. Chem.* **22**, 2021–2029 doi:10.1021/bc200214g

76   Schulz, M. and Binder, W.H. (2015) Mixed hybrid lipid/polymer vesicles as a novel membrane platform. *Macromol. Rapid Commun.* **36**, 2031–2041 doi:10.1002/marc.201500344

77   Thoma, J., Belegrinou, S., Rossbach, P., Grzelakowski, M., Kita-Tokarczyk, K. and Meier, W. (2012) Membrane protein distribution in composite polymer–lipid thin films. *Chem. Commun.* **48**, 8811–8813 doi:10.1039/c2cc32851h

78   Kowal, J., Wu, D., Mikhalevich, V., Palivan, C.G. and Meier, W. (2015) Hybrid polymer–lipid films as platforms for directed membrane protein insertion. *Langmuir* **31**, 4868–4877 doi:10.1021/acs.langmuir.5b00388

79   Bieligmeyer, M., Artukovic, F., Nussberger, S., Hirth, T., Schiestel, T. and Müller, M. (2016) Reconstitution of the membrane protein OmpF into biomimetic block copolymer–phospholipid hybrid membranes. *Beilstein J. Nanotechnol.* **7**, 881–892 doi:10.3762/bjnano.7.80

80   Khan, S., Li, M., Muench, S.P., Jeuken, L.J.C. and Beales, P.A. (2016) Durable proteo-hybrid vesicles for the extended functional lifetime of membrane proteins in bionanotechnology. *Chem. Commun.* **52**, 11020–11023 doi:10.1039/C6CC04207D

81   Prabudiansyah, I., Kusters, I., Caforio, A. and Driessen, A.J.M. (2015) Characterization of the annular lipid shell of the Sec translocon. *Biochim. Biophys. Acta Biomembr.* **1848**, 2050–2056 doi:10.1016/j.bbamem.2015.06.024