Pore Forming Toxins as useful Tools in Studies of Lipid Membrane Organization

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Pore forming toxins are one of the most ubiquitous groups of toxins found in Nature. They are able to efficiently perforate cell lipid membranes in a multi-step mechanism, where each step is represented by a distinct conformational state. They are usually produced and secreted as water-soluble proteins. After the binding onto a membrane they oligomerize on its surface and after a conformational change, that exposes hydrophobic regions, the insertion of a part of their polypeptide chain across the lipid membrane takes place. Due to these characteristics they represent useful models for studying protein-membrane interactions, unfolding on the membrane surface and protein-protein interactions within the membrane milieu. Pore forming toxins were also shown to be useful in biotechnological and medical applications. In recent years, they have been used in studying the membrane structure and fate of particular membrane lipids. In particular those that interact with lipid components of membrane lipid rafts, as is the case of lysenin and perfringolysin-O, have recently attracted considerable attention. In this work, we summarize current knowledge on two eukaryotic pore forming toxins: ostreolysin from the edible mushroom Pleurotus ostreatus and equinatoxin, an actinoporin from sea anemone Actinia equina. Both proteins might be potentially useful in studies of lipid rafts due to their specific interaction with these membrane micro-domains.

Key Words: Pore forming toxin, Lipid membrane, Lipid domain, Lipid raft, Detergent resistant membranes, Ostreolysin, Actinoporin, Equinatoxin

Pore Forming Toxins
Pore forming toxins (PFT) represent an important group of natural toxins. They are found in all kingdoms of life, where they serve in attack or defence strategies. They are heterogeneous in terms of size, molecular structure and the structural features of the final transmembrane pore. Members of PFT range from short peptides with lytic properties, (e.g. melittin from honey-bee venom), to large multidomain proteins. The most studied examples are found in the bacterial world, as many of these toxins are important virulence factors.

Despite their heterogeneity, the path that leads from a water-soluble monomer to the membrane-inserted aggregate is similar in most of the groups and proceeds in well defined steps (Gouaux 1997). PFT are usually produced and secreted as water-soluble proteins. In the next step they bind to the host lipid membrane, either unspecifically or by using specific membrane lipids or proteins as receptors. Further, few molecules oligomerize in the plane of the membrane and, finally, part of their polypeptide chain is translocated across the lipid bilayer to form the final conductive transmembrane pore. Pores are various in size and shape, but the common consequence of their formation is that the membrane becomes permeable for small solutes or larger molecules. Diffusional exchange of intra- and extracellular molecules through the pores results in an osmotic imbalance that usually leads to cell swelling, lysis and final cell death. According to the secondary structural element that builds the final transmembrane channel, PFT can be divided into two groups, α-helical and β-barrel toxins. In the α-helical group of PFT, pores are formed by bundles of pre-existing α-helices. The most studied examples are colicins from Escherichia coli (Lacey & Slaton 2001), the T-domain of diptheria toxin (Choe et al. 1992), Cry1 toxins from Bacillus thuringiensis (Li et al. 1991) and
actinoporins from sea anemones (Anderlhu & Maček 2002). In the β-barrel toxins, each monomer contributes two β-strands that are, together with strands from other monomers, assembled in a transmembrane β-barrel. β-barrel PFT form structurally stable oligomeric membrane-embedded structures that resist heat or detergent, and consequently, some high-resolution structures are available. In the contrast, pores of α-helical toxins are not structurally stable and little information on the functional pore state exists. The most studied examples of β-barrel toxins are α-hemolysin family of lysins from *Staphylococcus aureus* (Menestriña et al. 2001), cholesterol-dependant cytolysins from Gram positive bacteria (Tweiten et al. 2001), aerolysin from *Aeromona hydrophila* (Parker et al. 1996), and protective antigen of Anthrax toxin (Peta & Liddington 1996).


**Use of PFT in Cell Biology and Membrane Structure Studies**

Due to the above mentioned characteristics PFT are considered as useful tools for studying some of the fundamental processes in biochemistry and biology, such as protein-protein interactions in solution and within the membrane milieu, protein binding to membranes, and protein unfolding induced by interaction with the lipid bilayer. Some of the most studied PFT were also proposed as useful tools in biotechnology and in some biomedical applications. Illustrative applications of PFT include: controlled permeabilization of cell membranes (Russo et al. 1997, Erglu et al. 2000), use as biosensors for heavy metals (Walker et al. 1994, Braha et al. 1997, Kasiowicz et al. 1999, Gu et al. 1999), possible applications in DNA sequencing strategies (Howorka et al. 2001, Vercoutere et al. 2001), and employment as a part of immunotoxins directed against tumor or parasite cells (Avila et al. 1988, Avila et al. 1989, Pederzolli et al. 1995, Tejuca et al. 1999, Potrich et al. 2000). Recently, PFT have gained particular interest as potential tools to study lateral organisation of lipid membranes.

A nice example of how PFT can be used to study a particular membrane lipid, its membrane distribution and metabolism is lysenin, a 41 kDa protein derived from the coelomic fluid of earthworm *Eisenia fetida* (Shakor et al. 2003). It was shown by using various techniques that it specifically recognizes sphingomyelin (SM) and binds to sphingomyelin-containing membranes with a low dissociation constant ($K_D = 5.3 \times 10^4$ M) (Yamaji et al. 1998). This property has enabled few interesting experiments. In the study performed by Hanada et al. (1998), lysenin-resistant mammalian cell lines were selected and characterised. (Hanada et al. 1998). One of the resistant lines was specifically defective in SM but not in glycosphingolipid synthesis. It was shown that this cell line had normal activity of SM synthase but is defective in intracellular translocation of ceramide used for SM synthesis from ER to Golgi. This cell line was used to select revertants (Hanada et al. 2003). A cDNA was then retrieved that encoded a CERT protein of 68 kDa. CERT acts directly as a ceramide carrierr that shuttles between the two organelles. So the use of the PFT lysenin enabled selection of SM deficient mutants and gave important insights in biosynthesis of SM by discovering the important lipid courier. At the same time, lysenin, together with some other PFT described below, was further used in model lipid systems to characterise the heterogeneity and organisation of sphingomyelin-containing membranes (Ishitsuka et al. 2004 and 2005).

**Lipid Membrane Organization**

The fluid mosaic model of the structure of lipid membranes (Singer et al. 1972), in which proteins and lipids move freely within the membrane milieu has quite evolved in the last few years. According to the current view, particular lipids and proteins of the plasma membrane are confined to defined domains, in this way optimising their physiological function (Jacobson et al. 1995). The great heterogeneity in the lipid composition of the natural membranes can easily explain this feature. Saturated and unsaturated phospholipids, sphingolipids, gangliosides or cholesterol show particular physical properties, namely, defined gel-fluid transition temperature ($T_m$), different polar heads, or acyl-chain length. When mixing these lipids together, some of them having similar properties tend to interact together while some other tend to segregate, forming what we call different lipid domains (Vlijic and McConnell 2003). These domains are enriched in certain lipids and are responsible for the restriction of movements that many proteins show in the plane of the membrane (Simons & Ikonen 1997). In the last years the most studied lipid domains are those enriched in cholesterol (Chol) and SM, called lipid rafts, detergent resistant membranes (DRM), or detergent insoluble glycolipid-enriched fraction (DIG). Rafts can be isolated due to their resistance to solubilization by cold non-ionic detergents (Simons & Ikonen 1997). The preferential co-localization of SM with Chol and the low miscibility that both together have with the major constituent of the membrane, unsaturated
phosphatidylcholine (PC), is thought to be the basis for the raft formation (Rietveld and Simons 1998). Model membrane experiments have shown that in ternary mixtures of low T<sub>m</sub> PC, high T<sub>m</sub> SM and Chol, the latter interacts with the gel phase SM forming the so-called liquid-ordered phase (L<sub>o</sub>). This phase has a higher degree of chain order than the PC-rich fluid phase, which allows for the detergent resistance, but also a higher fluidity than the SM-rich gel phase (Ahmed et al. 1997). The L<sub>o</sub> phase is believed to be the physical state in which lipid rafts exist (Brown & London 1998, Brown & London 2000, London 2002). Rafts have been visualized in different model membranes by using fluorescent probes that are specifically partitioned or excluded from the L<sub>o</sub> phase (Brown 2001, Dietrich et al. 2001, Samsonov et al. 2001) or by taking advantage of the different height of L<sub>o</sub> and the surrounding fluid phases present (Rinia et al. 2001). The physical properties of the L<sub>o</sub> phase favour the presence of a variety of integral proteins, proteins acylated with saturated fatty acids, and of glycosylphosphatidylinositol (GPI)-anchored proteins (Schoeder et al. 1998, Foster et al. 2003). Many of these proteins are implicated in cellular signal transduction processes, so lipid rafts have also been implicated in their modulation (Foster et al. 2003). Lipid rafts also serve for attachment and entry of several cell pathogens, toxins and other ligands (Simons & Ebehalt 2002, Lafont et al. 2004).

In the last years, an increasing number of toxins that interact with the rafts during their toxic action have been reported (Fivaz et al. 2000). Examples include a Helicobacter pylori vacuolating toxin (Geisse et al. 2004), multivalent cholera toxin and related toxins (Wolf et al. 1998, Fujinaga et al. 2003), and tetanus toxin (Herreros et al. 2001). Among the toxins that interact with the rafts, or with components that are localized in rafts, we can also find various PFT. Aerolysin from Aeromonas hydrophila and Cry1A δ-endotoxin from Bacillus thuringiensis bind to GPI-anchored receptors accumulated in rafts (Abrami & Van der Goot 1999, Zhuang et al. 2002), lysozyme binds specifically to SM (Lange et al. 1997), osteolysin from the edible mushroom Pleurotus ostreatus binds preferentially to SM/Chol complexes (Sepčič et al. 2004), and perfingolysin O, a cholesterol-dependent cytolysin from Clostridium perfringens, binds selectively to cholesterol-rich rafts (Waheed et al. 2001). In most cases, the accumulation of the toxin monomers within lipid rafts facilitates the oligomerization, a prerequisite in order to create a transmembrane pore.

One shortcoming in the raft research is the lack of routinely applicable techniques to measure raft dynamics without perturbation by detergents. One promising strategy to overcome this problem is the use of fluorescently labelled raft-specific toxins in order to visualize the raft behaviour under the microscope. This strategy has already been applied to some toxins like the ganglioside binding cholera-toxin B subunit (Bacia et al. 2004), the cholesterol binding domain IV of perfingolysin O (Shimada et al. 2002), and lysozymin (Ishitsuka et al. 2005).

In the continuation, we will describe more in detail recent findings obtained with two other types of PFT that have been recently shown to have considerable potential in the study of lateral membrane domains, osteolysin and equinatoxin from the sea anemone Actinia equina.

Osteolysin

Osteolysin (Oly) is a cytolytic protein that has been isolated from young fruit bodies of the edible oyster mushroom Pleurotus ostreatus (Berne et al. 2002). The protein has a molecular weight of 15 kDa, and isoelectric point at pH 5.0. According to the analysis performed by Fourier-transformed infrared spectroscopy, it is composed of approximately 20% α-helix, 50% β-structure, and 30% random coil (Sepčič et al. 2003). The sequence of its 50 N-terminal amino acids has been determined and found to be homologous to Asp-hemolysin from the pathogenic mould Aspergillus fumigatus (Ebina et al. 1994), pleurotoxin A from Pleurotus ostreatus (Tomita et al. 2004), aegeroxin from the mushroom Agrocybe aegerita (Fernandez Espinhar & Labarere 1997, Berne et al. 2002), two Clostridium bifermantans hemolysin-like proteins (Barloy et al. 1998), a hypothetical protein PA0122 from Pseudomonas aeruginosa (Stover et al. 2000), and a putative protein from Neospora crassa (Galagan et al. 2003). All these proteins were assigned to a new aegeroxin protein family. Their exact biological role has not yet been elucidated. It was proposed that they might play a role in the initiation of fungal fruiting (Fernandez Espinhar and Labarere 1997, Berne et al. 2002, Vidic et al. 2005), virulence (Ebina et al. 1994), or bacterial sporulation (Barloy et al. 1998).

Osteolysin, in nanomolar concentration, lyse erythrocytes and other mammal cells (Sepčič et al. 2003, Sepčič et al. 2004). Hemolysis is induced by a colloid-osmotic mechanism, due to the formation of a pore with an estimated inner radius of ~ 2 nm. Systematic search for lipid acceptor(s) revealed that, in contrast to an erythrocyte total lipid extract, lipid dispersions of pure lipids (or combinations of the latter with egg PC) could not inhibit osteolysin-induced hemolysis. Only lysophospholipids and fatty acids, that have already been reported to inhibit a homologous Asp-hemolysin (Kudo et al. 2002), effectively inhibited it, but their
direct interaction with the protein could not be proven (Sepčić et al. 2003). Similarly, no lysis of calcine-loaded large unilamellar vesicles (LUV) composed of egg PC in combination with Chol, lysophospholipids, glycerophospholipids, or sphingolipids was observed. Separation of erythrocyte lipids and their reconstitution into lipid vesicles clearly showed that exclusively LUV composed of extracted neutral lipids (mainly Chol) and phospho- and sphingolipids (mainly SM) were effectively lysed by Oly. As these lipids are known to be the main constituents of lipid rafts, we became interested in further studying the interaction of Oly with these particular membrane domains. Studies on Chinese hamster ovary wild type CHO-K1 and CHO-215 cells with deficient Chol synthesis showed that Oly partitions into their DRMs. Moreover, its binding to these membrane fractions was proportional to their Chol content (Sepčić et al. 2004).

Studies on sonicated lipid vesicles with various lipid composition (figure 1) demonstrated that Oly effectively lyse vesicles composed of Chol/SM, which correlates with the abundance of Oly in their DRMs (Sepčić et al. 2003). The lytic activity on these Chol-containing vesicles was (i) unchanged if they were supplemented with 1% of ganglioside G_{1a}, and (ii) diminished if Chol was replaced by ergosterol or if (iii) SM was replaced with fully saturated PC. Addition of mono-unsaturated PC into the Chol/SM vesicles dramatically reduced the lytic activity, probably as a consequence of shifting the L_{α}/I_{α} ratio in favour of the liquid-disordered phase, as implied by phase diagrams for this particular lipid mixture (de Almeida et al. 2003). Direct binding of Oly, monitored by surface plasmon resonance technique (Sepčić et al. 2004), confirmed the preferential interaction of the protein with Chol/SM monolayers, while much lower or no binding at all was detected if SM was substituted by mono- or fully unsaturated PC, respectively.

Studies of interaction of Oly with sonicated Chol/SM vesicles (figure 2) showed that the detectable vesicle permeabilization was achieved only above 30 mole% Chol, coinciding with the transition from I_{α} to I_{β} phase (de Almeida et al. 2003), and was increasing with increasing Chol contents (up to 60 mole%). The estimation of the initial rate from the kinetic curves was used for determination of the coefficient n, an estimate of molecularity, with respect to Chol concentration. An unusual ever-increasing apparent Chol cooperativity was found, implying the interactions of Oly with specific Chol-containing complexes.

To date, all cytolytic proteins partitioning into lipid rafts were shown to interact with only one species of raft lipids. For example, lysozyme binds to SM (Yamaji et al. 1998), cholera toxin interacts with GM_{1} (Schengrund & Ringler 1989), and a large group of cholesterol-dependant bacterial cytolsins have high affinity for Chol (Giddings et al. 2003). Derivatives of a perfringolysin O, devoid of lytic activity and able to bind to pure and membrane-bound Chol, have been proposed as cytotoxicological probes for membrane Chol (Ohno-Iwashita et al. 1990, Shimada et al. 2002), and for lipid rafts (Ohno-Iwashita et al. 2004). In contrast,

**Figure 1:** Effect of membrane lipid composition on permeabilization activity of osteoselin. Percentage of release of encapsulated fluorescent dye calcine from sonicated lipid vesicles (20 µg/ml) with osteoselin (65 µg/ml) (Egg, ergosterol, DPPC, dipalmitoylphosphatidylcholine (fully saturated PC); POPC, palmitoyl-oleoylphosphatidylcholine (mono-unsaturated PC); DOPC, dioleoylphosphatidylcholine (fully unsaturated PC). Modified from Sepčić et al. 2004.

**Figure 2:** Effect of cholesterol to sphingomyelin ratio on permeabilization of sonicated lipid vesicles (20 µg/ml) with osteoselin (65 µg/ml). The graph shows the percentage of calcine release from osteoselin-treated vesicles composed of cholesterol and sphingomyelin in different molar ratios. Modified from Sepčić et al. 2004.
to these proteins, Oly specifically recognizes a combination of Chol with SM or other fully saturated glycerophosphatides, and cannot bind to pure lipids. Our combined results strongly suggest that Oly specifically binds to a Chol-rich phase (probably the \( L_0 \) phase) that is the main constituent of lipid rafts, and integrity of which is highly sensitive to unsaturated glycerophosphatides. Actually, besides direct interaction with cytolsins (as in the case of perfringolysin O), Chol was shown to modulate the activity of some bacterial toxins by changing the physical properties of lipid membranes, such as fluidity, lateral phase segregation or induction of non-bilayer structures (Palmer 2004). In this regard, it would be interesting to test the binding of Oly and its lytic activity on vesicles containing other sterols instead of Chol. It was already shown that in SM/ergosterol (1/1) vesicles both processes were reduced in comparison to SM/Chol (1/1) vesicles (Sepčić et al. 2004). Moreover, it was shown that vesicles reconstituted from \( P.\) ostreatus total lipids (containing ergosterol as a major sterol component) are totally resistant to Oly, while those composed from total erythrocyte lipids (containing mainly Chol) retain their sensitivity to the protein (Sepčić et al. 2003). It is well-known that different sterols have different abilities to promote formation of ordered lipid domains (Xu et al. 2001, Wang & London 2004, Halling & Slotte 2004), and we plan to investigate whether their domain-promoting activity will coincide with binding and lytic activity of Oly. Finally, mammal cells and artificial lipid vesicles will be treated with sublytic concentration of either fluorescently labelled Oly, or Oly bound to fluorescently labelled antibodies, and the distribution of the fluorescent signal at the membrane will be monitored by confocal microscopy.

**Equinatoxin II**

Equinatoxin II (EqII) is a member of the actinoporin family, comprising closely related cation-selective pores in lipid membranes (Anderluh & Mac'ek 2002). Bernheimer and Avigad (1976) showed for the first time that actinoporins are SM-specific toxins, i.e. they act preferentially upon membranes that contain sphingomyelin, and this has been shown for all newly discovered actinoporins. It is not clear whether this specificity is due to the recognition by the toxins of the sphingomyelin headgroup or whether is due to physical properties of sphingomyelin-containing membranes. In addition, it was shown that in some conditions cholesterol enhances the lytic activity of actinoporins (de los Rios et al. 1998, Barlić et al. 2004).

Equinatoxin-II (EqII) from *Actinia equina* and Sticholysin II (StII) from *Stichodactyla helianthus* are the most studied actinoporins, for which high resolution 3D structures have been recently solved (Athanasiadis et al. 2001, Hinds et al. 2002, Mancheho et al. 2003). Both toxins are composed of a tightly folded \( \beta \)-sandwich flanked on two sides by \( \alpha \)-helices. Pore formation by EqII has been shown to be a multi-step process (Hong et al. 2002). First, the toxin binds to the lipid bilayer. This step is governed by the aromatic amino acid cluster located on a broad loop at the tip of the \( \beta \)-sandwich and on the C-terminal \( \alpha \)-helix (Malovrh et al. 2000, Hong et al. 2002). Recent studies involving co-crystallization of StII together with phosphocholine have revealed the existence of a phosphocholine-binding pocket that probably participates in the initial binding to the membrane (Mancheho et al. 2003). In the next step, the amphipatic N-terminal helix translocates to the lipid-water interface (Hong et al. 2002, Malovrh et al. 2003, Gutiérrez-Aguirre et al. 2004), and finally, a transmembrane pore is formed by helices from three or most probably four monomers (Belmonte et al. 1993, Malovrh et al. 2003, Gutiérrez-Aguirre et al. 2004).

Recently, the preferential interaction of EqII with the boundary regions between different co-existing lipid domains has been described (Barlić et al. 2004). The effect of different lipid compositions on the pore-forming activity of EqII was tested by means of permeabilization of LUV and insertion into lipid monolayers. Compositions that don’t favour the formation of different lipid domains, i.e., pure PC membranes and pure SM membranes, are not susceptible to the toxin action, whereas, SM/PC mixtures, PC/Chol mixtures, and mostly PC/SM/Chol mixtures, which all promote the segregation of lipids in different phases, show high susceptibility to the membrane insertion and pore-forming activity.

At 25°C, in the binary mixture of PC and SM, fluid domains rich in PC coexist with gel-phase domains rich in SM (Untrach & Shipley 1977, de Almeida et al. 2003). Under these conditions, Barlić et al. found that the EqII pore-formation and its interaction with lipid monolayers were maximal when both components were present at equimolar proportion, while when PC or SM were predominant the toxin didn’t show almost any effect. The authors concluded that EqII might show a preferential interaction with the boundary regions between the two distinct phases. These boundaries are absent in the pure PC and SM membranes but are at maximum in the equimolar mixtures. Additionally, if SM/PC (1:1) LUV were warmed beyond the SM gel to fluid transition temperature (37°C) the activity of the toxin was reduced due to the increased miscibility between the two lipids and the consequent reduction of the boundary regions. After these studies a
reinterpretation of the SM role on the EqtII activity may be required. SM has been thought to be a low affinity receptor for actinoporins for many years (Bernheimer & Avigad 1976), but the results by Barlić et al. show that SM acts as an effector of the toxin action, more by inducing the formation of lipid domains than by acting as a receptor itself.

The mixture of SM/PC/Chol (50:15:35) was shown to be the most susceptible to the action of the EqtII. The permeabilization of LUV composed of this mixture was faster and reached a higher final extent in comparison with the SM/PC (1:1) mixture (Barlić et al. 2004). Interestingly, the dependence of the leakage percentage with the lipid to toxin (L/T) ratio was not so evident. At toxin concentrations where only 20% of leakage was observed in the SM/PC (1:1) LUV, percentages close to 60% were observed with the SM/PC/Chol (50:15:35) LUV. The presence of a new coexisting phase (Lc) improved the lytic process even at smaller toxin concentrations. In the case of the toxin insertion into lipid monolayers, the result was similar. The critical pressure is the initial surface pressure at which no more toxin can insert in a monolayer of defined lipid composition. This parameter correlates with the affinity that the toxin shows for the monolayer under assay. EqtII binding exerts a critical pressure of 36 mN/m, when interacting with SM/PC (1:1) monolayers. This value is above the described lateral pressure for a lipid bilayer of 30 mN/m (Demel et al. 1975), so the toxin easily inserts and permeabilizes LUV of SM/PC. In the case of the raft promoting mixture SM/PC/Chol (50:15:35), the critical pressure increases to 47 mN/m. This means that the toxin has a markedly higher affinity for this composition and explains the improved permeabilization of LUV of SM/PC/Chol (50:15:35).

As another evidence for the positive influence of the membrane irregularities, arising from the coexistence of different lipid phases, on the EqtII pore forming activity, Barlić et al. took advantage of the phospholipase-C (PLC) activity. The phosphodiesterase activity of PLC hydrolyzes PC and generates diacylglycerol (DAG). The localized accumulation of the product favours the creation of DAG-rich domains, which promote vesicle aggregation and fusion without leakage of encapsulated solutes (Nieva et al. 1989, Basáñez et al. 1996, Goji and Alonso 1999). The PC LUV are not susceptible to the permeabilization by EqtII, but when PLC was added to the toxin-LUV incubation mixture, leakage was observed. The DAG-rich lipid domains originated by the PLC activity made the PC LUV sensitive to the EqtII pore forming activity, once more demonstrating the preference of this toxin for membranes in which different lipid domains co-exist.

Figure 3: 3D structure of equinatoxin. The N-terminal helix is on the left, aromatic rich region is placed around broad loops on the bottom of the molecule and C-terminal helix.

As a final and direct proof of the preferential interaction of EqtII with the boundaries between different lipid domains, Barlić et al. showed epifluorescence images of monolayers composed of SM/PC/Chol (50:15:35) (Figure 4). A Texas-Red labelled variant of the toxin was allowed to insert into the monolayer until a stable surface pressure signal was reached, followed by the transfer of the monolayer to a coverslip and visualization under the microscope. The presence of the fluorescent NBD-PC in the monolayer allows distinguishing between L, and Lc phases as it is excluded from the ordered phases. EqtII appears to be localized preferentially within the boundaries between both phases (figure 4). This direct evidence explains all the previously reported tests.

A: NBD-PC
B: EqtII-TexasRed

Figure 4: Epifluorescence images of TexasRed labelled EqtII inserted in SM/PC/Chol/NBD-PC (49:15:35:1) monolayers. A and B panels correspond to images taken with the microscope filter fixed at the emission wavelength of NBD-PC and TexasRed, respectively. The bar represents 50 mm. Adapted from Barlić et al. 2004, with permission.
In conclusion, recent advances in cell membrane biology revealed intriguing details of the cell membrane lateral organization. In this respect, lipid-binding specificity of some PFT might be a useful complementary tool in tracking specific membrane lipid domains as already shown for perfringolysin O and its derivatives, and lysozymin. It appears that the diversity of plasmatic and intracellular membrane microdomains is much wider as ever expected, and novel, lipid domain-specific labels are prerequisite in their studies. Oly and EqTII are emergent interesting examples of PFT that interact with either specific raft lipids or with the contact regions between different lipid domains. These properties open the door for their future application in studies of structure and dynamics of cell membranes.

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