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Cryo-electron microscopy studies of actinoporin pores highlight the pore assembly mechanism and details of protein-membrane interactions

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ABSTRACT

Actinoporins are a protein family of α-pore-forming proteins (PFPs), initially isolated from sea anemones. These proteins are key components of the venom system used by sea anemones and some other cnidarians, the most ancient extant lineage of venomous animals, where they play a role in predation and predator deterrence through rapid membrane disruption. The scientific journey to understand these potent molecules began more than five decades ago with their initial isolation from crude anemone extracts. In recent years structural studies enabled the determination of multiple structures of monomeric actinoporins in solution and in complexes with ligands, elucidating their mechanism of membrane binding and pore formation, highlighting in the fragaceatoxin C (FraC) pore structure. Recently, two groups independently produced multiple pore structures of actinoporins and a close homologue, further elucidating the mechanism of pore formation and investigating the role of lipids in the pore structure, which is even more prominent than initially thought. Hereby, we highlight the achievements of the two studies and discuss some remaining open questions.

1. Introduction

Pore formation in cellular membranes is a common strategy in the attack or defense of organisms across all domains of life. Pore-forming proteins (PFPs) are typically secreted as soluble monomers that bind to target membranes, oligomerize at the plane of the membrane, and undergo conformational changes that enable insertion into the lipid bilayer and formation of transmembrane pores. PFPs appear in all domains of life and viruses and are involved in programmed cell death, immunity, or act as toxins that attack other cells or organisms (Pore-forming toxins; PFTs) (Bischofberger et al., 2009; Peraro and van der Goot, 2015). Based on the secondary structure element that builds the transmembrane channel part of the pore, PFPs are classified into α-helical and β-barrel PFPs (Gouaux, 1997).

Actinoporins are α-helical PFPs from sea anemones, with a molecular mass of approximately 20 kDa (Podobnik and Anderluh, 2017). They are venom components used in predation and digestion, as well as in defense (Anderluh and Maček, 2002). Actinoporins are one of the most studied families of PFPs with a rich tradition dating back to the early 70s. In 2025, two papers (Arranz et al., 2025; Šolinc et al., 2025) revealed fascinating details about the actinoporin pore-formation and structure. This paper aims to highlight the achievements of these works, briefly illustrating the five-decade-long body of work that paved their way and discuss some of the remaining open questions.

Actinoporins were isolated from crude anemone extracts in 1974, a protein that would later be named equinatoxin II (EqtII) from *Actinia equina* (Ferlan and Lebez, 1974) and sticholysin I and II (StnI, StnII) from *Stichodactyla helianthus* (Devlin, 1974) (Fig. 1). From these initial studies until now (early 2026), actinoporins research can be broken down into three periods with some overlap (Fig. 1). During initial biochemical characterization, soon after first isolations of actinoporins, it was discovered that these hemolytic toxins could be inhibited by incubation with sphingomyelin (Bernheimer and Avigad, 1976) and that the mechanism of action is likely membrane pore formation (Varanda and Finkelstein, 1980). In 1988, Kem proposed a name actinoporins, highlighting a group of pore forming toxins (PFTs) from sea anemones with some common functional properties (Kem, 1988).

Activities of the first two decades of actinoporins research on one of the model proteins, EqtII, are comprehensively summarized in a contemporary review by (Maček et al., 1994). Determination of

Abbreviations: ALPs, actinoporin-like proteins; cryoEM, cryogenic electron microscopy; EqtII, equinatoxin II; FraC, fragaceatoxin C; PFPs, pore-forming proteins; PFTs, pore-forming toxins; StnI, sticholysin I; StnII, sticholysin II.

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complete amino acid sequences (Belmonte et al., 1994; Morera et al., 1995; Simpson et al., 1990) and the use of recombinant technology (Anderluh et al., 1996) resulted in relatively easily obtainable large amounts of pure actinoporins, enabling the next period of functional and structural studies. These were initiated with the determination of the structure of monomeric EqtII (Athanasiadis et al., 2001) and electron microscopy images of membrane-bound oligomers of StnII (Mancheň et al., 2003). Actinoporins structure is conserved and consists of a central compact β-sandwich of ten β-strands flanked by two α-helices, one on each side (Fig. 2A). Known actinoporin structures in combination with advances in bioinformatics also led to the discovery of numerous proteins structurally similar to actinoporins. These actionoporin-like proteins (ALPs) are common in other cnidarians but can be found all over the tree of life (Birck et al., 2004; Gutiérrez-Aguirre et al., 2006; Kawashima et al., 2003; Ruparčič et al., 2025). It was soon established that the flexibility of the N-terminal amphipathic α-helix is crucial for pore formation (Hong et al., 2002; Kristan et al., 2004). In addition, it was discovered that lipid composition affects the formation of pores (Alvarez Valcarcel et al., 2001) and that membrane lipids likely form part of the pore wall (Anderluh et al., 2003). In 2008, a sphingomyelin molecule was confirmed to be a specific membrane lipid receptor by surface plasmon resonance and other biophysical approaches (Bakrač et al., 2008).

The next crucial paper was published in 2015 when the crystal structure of Fragaceatoxin C (FraC) pore, an EqtII homologue from *Actinia fragacea,* was solved by Tanaka et al. (2015). This work revealed that actinoporin pores are (at least mostly) octameric. The pore can be divided into two parts (Fig. 2B): i) the extracellular pore cap formed by circularly arranged β-sandwiches of protomers, which remain structurally almost unchanged compared to their conformation in monomeric form (Fig. 2A), and ii) a transmembrane bundle of amphipathic α-helices formed by the now extended N-terminal of protomers and arranged in an iris-like pattern. This work also revealed that lipids, likely sphingomyelin, remain tightly bound to the pore after its formation. Moreover, lipids (at the so-called lipid position L1) form an integral part of the pore by indirectly increasing the protomer-protomer interaction surface. Lipid at the same position is also exposed to the lumen of the pore, thus confirming prior hypotheses that actinoporins form protein-lipid pores (as discussed in Rojko et al., 2016).

This work was important also for utilization of FraC pore as it was extensively used in nanopore sensing experiments (Lucas et al., 2021; Restrepo-Pérez et al., 2019; Wloka et al., 2016). This modern analytical approach utilizes protein pores to detect and characterize individual molecules. The field of nanopore sensing underwent rapid development in the past decades, from proof-of-concept experiments with α-hemolysin in the 90s and early 2000s (Bayley and Cremer, 2001; Bezrukov et al., 1994; Kasianowicz et al., 1996) to mature and powerful analytical and commercial DNA and RNA sequencing technology, while also diversifying toward the detection of organic and inorganic molecules and protein analysis (Crnković et al., 2021). Particularly attractive for sensing applications were the funnel shape of the interior of the FraC pore and breathability of the α-helical region, which showed great potential. FraC pore was recently also used as a scaffold for nanoreactors (Robles-Martín et al., 2023). Beyond the scope of nanopore sensing, actinoporins have recently been used as scaffold proteins for pore-based nanoreactors, breaking down nano-sized polyethylene terephthalate (Robles-Martín et al., 2023) and as senotoxins, selectively targeting senescent cells (Moral-sanz et al., 2025).

In a recent decade, a cryogenic electron microscopy (cryoEM) quickly established itself as the preeminent approach to study membrane proteins, which are notoriously difficult to crystallize (Kühlbrandt, 2022). The first protein pore structure deposited to Protein Data Base solved by cryoEM was the low-resolution reconstruction of pleurotolysin in 2015 (Lukoyanova et al., 2015), followed by the reconstruction of anthrax toxin protective antigen pore at 2.9 Å resolution (Jiang et al., 2015) the same year, with lower resolution studies of protein pores dating even further back (Law et al., 2010; Tilley et al., 2005). Additional strength of cryoEM is the possibility of reconstructing multiple different structures from the same sample and thus ability to capture intermediate states. One such case of PFTs is aerolysin, where multiple prepore states as well as the final pore were resolved by cryoEM (Anton et al., 2025; Iacovache et al., 2016).

2. CryoEM structures of actinoporins pores reveal intricate interactions with lipids

In Šolinc et al. (2025), Fav, an actinoporin homologue form the coral *Orbicella faveolate* was utilized. Pores were prepared on membranes of various lipid compositions and structurally characterized with cryoEM. Based on the FraC pore structure (PDB-ID 4TSY), it was expected for Fav pore to be a protein-lipid complex, and indeed it proved to contain the high number of lipids associated with the pore. When pores were prepared on 1,2-dioleoyl-*sn*-glycero-3-phosphocholine:sphingomyelin membranes, like the FraC pore previously (Tanaka et al., 2015), six phospholipid molecules per protomer were observed, twice as much as in the case of the crystal structure. But when the membrane used for pore

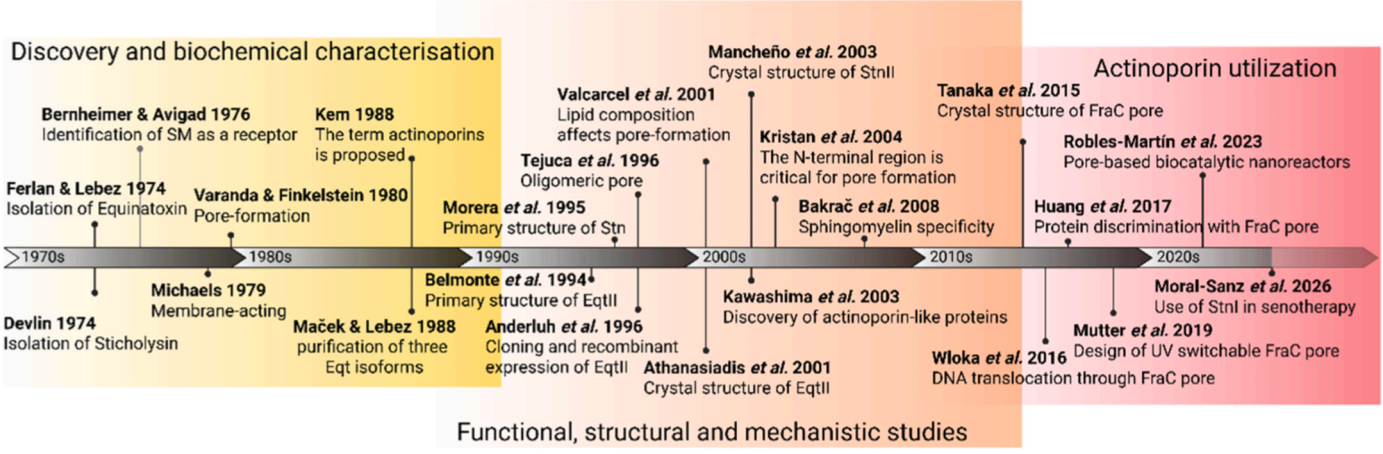

Fig. 1. The timeline of actinoporins research separated into three overlapping periods. Starting with the discovery and isolation from sea anemone extracts, closely followed by initial biochemical characterization (yellow rectangle). Primary structure data kicked off a period of biophysical, functional, mechanistic, and detailed 3D structural studies (orange rectangle), the information from which led to the period of actinoporin utilization in biotechnology (red rectangle). Selected publications with a short description of the contribution are highlighted. The list is not exhausted and we apologise for any inadvertent omission. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

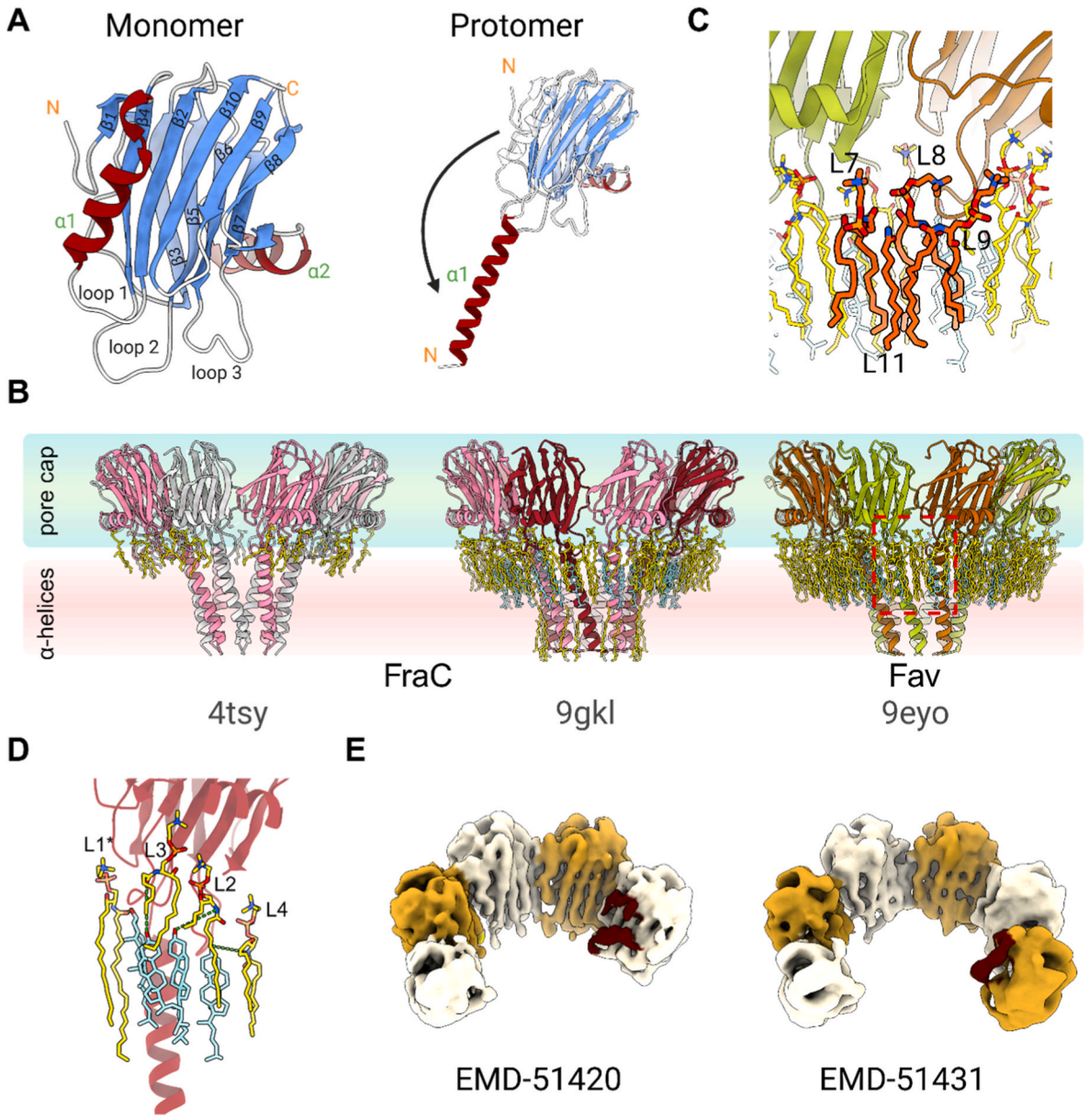

Fig. 2. Structures of actinoporins. (A) The structure of monomeric FraC (PDB-ID 3LIM) with labelled N and C-termini and secondary structural elements (left). Superposition of a single protomer of the FraC pore (PDB-ID 4TSY) and monomeric FraC (grey; right). The arrow indicates the extension of the N-terminal region to long α-helix during pore-formation. (B) The FraC pore (PDB-ID 4TSY) compared to FraC (PDB-ID 9GKL) and Fav pores (PDB-ID 9EYO). Phospholipids are colored in yellow and cholesterol in blue. (C) A closeup look at the bridging lipids (L7-L9, L11) present in the Fav pore structure. (D) A closeup view of sphingomyelins at positions L1-L4 paired with corresponding cholesterol molecules (PDB-ID 9GKL). The asterisk at L1 indicates that the lipid at this position is assigned to the neighbouring protomer. Green dashed lines represent potential hydrogen bonds prior to protein insertion. (E) CryoEM maps of StnII pore intermediates. Part of the map, corresponding to the N-terminal region still attached to the β-sandwich core, is shown in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

preparation also contained cholesterol, a known enhancer of actinoporins activity (Barlič et al., 2004; Palacios-Ortega et al., 2019), the number of phospholipids associated with the pore increased to eleven. Furthermore, additional four cholesterol molecules were observed forming a compact cholesterol nanodomain under the pore cap. What is especially fascinating is that all these lipids were resolved on the pore extracted from the membranes of large unilamellar lipid vesicles with detergent and all of the resolved lipids belong to the extracellular (upper) leaflet of the membrane. Analysis of the cryoEM-based structural models revealed that lipids have distinct structural and functional roles. Some lipids have a structural role (*structural lipids*), others are receptors anchoring the monomer and final pore to the membrane (*receptor lipids*), and some have limited contact with the protein but are mostly stabilized by interactions with other lipids (*bridging lipids*) (Fig. 2C). These findings were further supported with atomistic simulations that highlight a complex network of protein–lipid and lipid–lipid interactions around the cap of the pore. This work showed that lipids are much more than passive membrane components as they critically shape pore structure and stability (Šolinc et al., 2025).

Another interesting observation made possible by the higher resolution structures of FraC and StnII pores is that sphingomyelin at position L1 and cholesterol at position CH1 assigned to the neighboring protomer (as named in Šolinc et al., 2025) form a hydrogen bond (Arranz et al., 2025). A closer look at the entire cholesterol nanodomain and the lipids at positions L1-L4 (Fig. 2D), all of which were present in all the structures of Fav, FraC and StnII, show an arrangement where

each of the cholesterol molecules has a sphingomyelin partner, which indicate lipid-lipid interaction prior to the protein binding and pore-formation. This also indicates that actinoporins, similar to their distant homologue ostreolysin A from mushroom *Pleurotus ostreatus,* may discriminate between different conformations of sphingomyelin in presence of cholesterol (Endapally et al., 2019), and that it is not the general membrane properties effected by cholesterol that increase the pore-forming activity.

3. CryoEM structures of actinoporin pores reveal the details of the mechanism of pore assembly

Arranz *et el.* (2025) used a slightly different direction and builds on the FraC pore foundation by using cryoEM to visualize FraC and StnII pores formed on lipid membranes directly, using large unilamellar vesicles and lipid nanodiscs as model lipid systems. Both pores have architectures highly similar to Fav (and of course to the crystal structure of FraC) with fewer lipids compared to Fav, but capturing the pore in a more physiological context and at greater resolutions (Fig. 2B). Crucially, they resolved multiple membrane-bound oligomeric intermediates (Fig. 2E), providing direct structural evidence for a sequential, multistep assembly pathway from membrane-bound monomers to complete pores. This study proposes that protein oligomerization displaces Phe14, which triggers conformational changes of the N-terminal helix. This has been previously proposed by Tanaka et al., based on crystal structures of a dimer with bound phosphorylcholine groups (2015), but those dimers were formed in solution and not on the membrane.

The Arranz et al. (2025) study provides structural evidence for arc-shaped oligomeric intermediates formed by protomers with the disordered N-terminal helices and supports a sequential assembly pathway. However, it remains unclear what governs the fate of these intermediates (Fig. 3A). Specifically, it is still unknown whether arcs are: i) obligatory on-pathway precursors that inevitably close into full pores. And if this is the case, how the trapped lipids (Fig. 3A) are displaced during pore formation. ii) metastable, functionally relevant end states that can already, albeit transiently, permeabilize membranes as proposed before by Palacios-Ortega et al. (2020). Non-circular forms of membrane-perforating oligomers that can function as pores, sometimes referred to as arcs, are known to be formed by some other PFPs (Gilbert, 2016; Gilbert et al., 2014), such as members of the membrane attack complex/perforin (MAC/PF)-cholesterol depandant cytolisins (CDC) superfamily (Bhakdi et al., 1985; Czajkowsky et al., 2004; Gilbert et al., 2013; Morgan et al., 1994; Olofsson et al., 1993; Young et al., 1986) and gasdarmin D (Mulvihill et al., 2018) belong to the group of β-PFPs and form pores an order of magnitude larger than actinoporins formed by more subunits (Peraro and van der Goot, 2015). The arrangement of lipids forming part of such pores is thus likely very different compared to actinoporins. A rare example of α-PFPs that form arcs is BAX (Epand et al., 2002) from the BCL-2 family of proteins (García-Sáez, 2012). These arcs are, however, irregular in shape without consistent curvature (Peña-Blanco and García-Sáez, 2018; Zhang et al., 2025).

Both studies also show evidence of non-octameric stoichiometries of pores. When imaging pores on vesicles Šolinc et al. (2025) observed nonameric pores in addition to octameric while Arranz et al. (2025) show stable but unfavourable heptameric FraC pores and show a 2D class average of nonameric StnII ring with disordered N-terminal helices. Additionally, ALPs from molluscs, such as Mytiporin 1, form hexameric pores (Koritnik et al., 2022). FraC pores with different diameters were previously proposed and also utilized in nanopore sensing (Huang et al., 2019). The differences in conductance measured by using these pores were attributed to differences in diameter caused by multiple possible pore stoichiometries. While the differences in the structure of monomers between molluscan ALPs and actinoporins are likely the reason for differences in pore stoichiometry, the mechanism that dictates actinoporin pore stoichiometry remains to be discovered (Fig. 3B). A detailed understanding of this mechanism would allow for the production of homogeneous pore samples of desired diameters expanding the repertoire of analytes that could be addressed by nanopore sensing based on actinoporin pores.

4. Conclusions

Together, these studies mark a conceptual shift from viewing actinoporin pores as pre-defined protein assemblies to understanding them as protein-lipid supramolecular complexes whose structure depends on membrane lipids. Lipids have multiple roles in the formed pore, and the pore itself affects the dynamics of lipids in its surroundings. The structures of actinoporin pores show that on membranes, oligomerization is sequential and triggers the conformational changes crucial for pore-formation, and that the final oligomeric state can vary. All of this was made possible by using cryoEM, which allows for direct visualization of pores and oligomeric intermediates embedded in lipid bilayers.

Ethical statement

This is a review manuscript, no ethics issues are associated with the topics described in the manuscript.

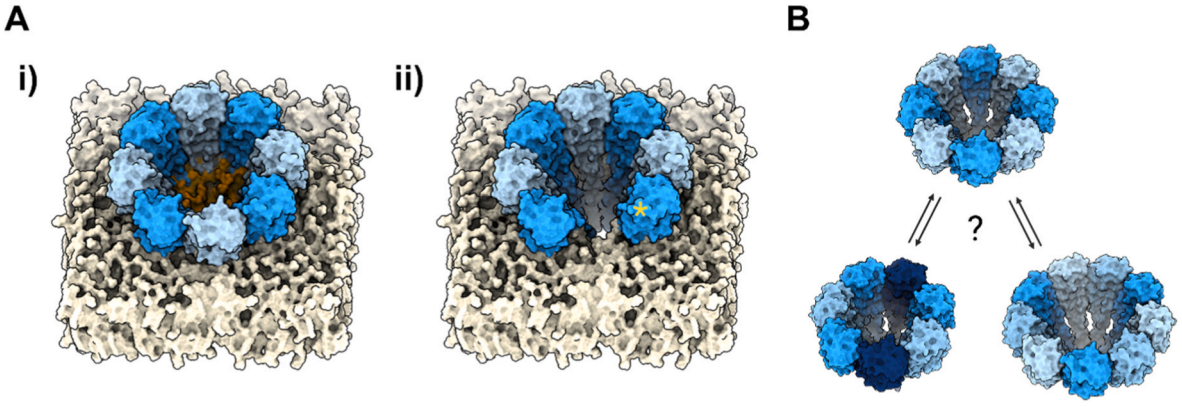

Fig. 3. Some outstanding questions in actinoporin research. (A) Two possible pre-final steps in pore formation. i) Completed membrane-bound oligomeric ring where the N-terminal helices of all protomers are detached from the β-sandwich core but don't yet form a transmembrane channel. Lipids 'trapped' inside the oligomer are shown in orange. ii) Near-complete pore with single protomer missing, where the N-terminal helices of all but the marked protomer (yellow star) are extended across the membrane. (B) The three observed pore stoichiometries of actinoporins and cnidarian ALPs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

CRediT authorship contribution statement

Gašper Šolinc: Writing – original draft, Writing – review & editing.
Gregor Anderluh: Writing – original draft, Writing – review & editing.

Declaration of competing interest

Authors have no competing interests to declare.

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Data availability

No data was used for the research described in the article.

References

Alvarez Valcarcel, C., Dalla Serra, M., Potrich, C., Bernhart, I., Tejuca, M., Martinez, D., Pazos, F., Lanio, M.E., Menestrina, G., 2001. Effects of lipid composition on membrane permeabilization by Sticholysin I and II, two cytolysins of the sea anemone Stichodactyla helianthus. Biophys. J. 80, 2761–2774. https://doi.org/10.1016/s0006-3495(01)76244-3.

Anderluh, G., Dalla Serra, M., Viero, G., Guella, G., Macek, P., Menestrina, G., 2003. Pore formation by equinatoxin II, a eukaryotic protein toxin, occurs by induction of nonlamellar lipid structures. J. Biol. Chem. 278, 45216–45223. https://doi.org/10.1074/jbc.M305916200.

Anderluh, G., Pungerčar, J., Štrukelj, B., Maček, P., Gubenšek, F., 1996. Cloning, sequencing, and expression of equinatoxin II. Biochem. Biophys. Res. Commun. 220, 437–442. https://doi.org/10.1006/bbrc.1996.0391.

Anderluh, G., Maček, P., 2002. Cytolytic peptide and protein toxins from sea anemones (Aanthozoa: Actiniaria). Toxicon 40, 111–124. https://doi.org/10.1016/S0041-0101(01)00191-X.

Anton, J.S., Iacovache, I., Bada Juarez, J.F., Abriata, L.A., Perrin, L.W., Cao, C., Marcaida, M.J., Zuber, B., Dal Peraro, M., 2025. Aerolysin nanopore structures revealed at high resolution in a lipid environment. J. Am. Chem. Soc. 147, 4984–4992. https://doi.org/10.1021/jacs.4c14288.

Arranz, R., Santiago, C., Masiulis, S., Torre, E.R., Gavilanes, J.G., Palomo, E.A., Pozo, Á. M., García, S., Martín, J., 2025. Elucidating the structure and assembly mechanism of actinoporin pores in complex membrane environments. https://doi.org/10.1126/sciadv.adv0683.

Athanasiadis, A., Anderluh, G., Maček, P., Turk, D., 2001. Crystal structure of the soluble form of equinatoxin II, a pore-forming toxin from the sea anemone Actinia equina. Structure 9, 341–346. https://doi.org/10.1016/S0969-2126(01)00592-5.

Bakrač, B., Gutiérrez-Aguirre, I., Podlesek, Z., Sonnen, A.F.P., Gilbert, R.J.C., Maček, P., Lakey, J.H., Anderluh, G., 2008. Molecular determinants of sphingomyelin specificity of a eukaryotic pore-forming toxin. J. Biol. Chem. 283, 18665–18677. https://doi.org/10.1074/jbc.M708747200.

Barlič, A., Gutiérrez-Aguirre, I., Caaveiro, J.M.M., Cruz, A., Ruiz-Argüello, M.B., Pérez-Gil, J., González-Mañas, J.M., 2004. Lipid phase coexistence favors membrane insertion of equinatoxin-II, a pore-forming toxin from Actinia equina. J. Biol. Chem. 279, 34209–34216. https://doi.org/10.1074/jbc.M313817200.

Bayley, H., Cremer, P.S., 2001. Stochastic sensors inspired by biology. Nature 413, 226–230. https://doi.org/10.1038/35093038.

Belmonte, G., Menestrina, G., Pederzolli, C., Kri, I., Guben, F., Turk, T., Maeek, P., 1994. Primary and secondary structure of a pore-forming toxin from the sea anemone, Actinia equina L., and its association with lipid vesicles. Biochim. Biophys. Acta Biomembr. 1192, 197–204.

Bernheimer, A.W., Avigad, L.S., 1976. Properties of a toxin from the sea anemone Stoichacis helianthus, including specific binding to sphingomyelin. Proc. Natl. Acad. Sci. USA 73, 467–471. https://doi.org/10.1073/pnas.73.2.467.

Bezrukov, S.M., Vodyanoy, I., Parsegian, V.A., 1994. Counting polymers moving through a single ion channel. Nature 370, 279–281. https://doi.org/10.1038/370279a0.

Bhakdi, S., Tranum-Jensen, J., Sziegoleit, A., 1985. Mechanism of membrane damage by streptolysin-O. Infect. Immun. 47, 52–60. https://doi.org/10.1128/iai.47.1.52-60.1985.

Birck, C., Damian, L., Marty-Detraves, C., Lougarre, A., Schulze-Briese, C., Koehl, P., Fournier, D., Paquereau, L., Samama, J.P., 2004. A new lectin family with structure similarity to actinoporins revealed by the crystal structure of Xerocomus chrysenteron lectin XCL. J. Mol. Biol. 344, 1409–1420. https://doi.org/10.1016/j.jmb.2004.10.007.

Bischofberger, M., Gonzalez, M.R., Goot, F.G. Van Der, 2009. Membrane injury by pore-forming proteins, 589–595. https://doi.org/10.1016/j.ceb.2009.04.003.

Crnković, A., Srnko, M., Anderluh, G., 2021. Biological nanopores: engineering on demand. Life 11, 1–30. https://doi.org/10.3390/life11010027.

Czajkowsky, D.M., Hotze, E.M., Shao, Z., Tweten, R.K., 2004. Vertical collapse of a cytolysin prepore moves its transmembrane β-hairpins to the membrane. EMBO J. 23, 3206–3215. https://doi.org/10.1038/sj.emboj.7600350.

Devlin, J.P., 1974. Isolation and partial purification of hemolytic toxin from sea anemone, Stoichactis helianthus. J. Pharm. Sci. 63, 1478–1480. https://doi.org/10.1002/jps.2600630936.

Endapally, S., Frias, D., Grzemska, M., Gay, A., Tomchick, D.R., Radhakrishnan, A., 2019. Molecular discrimination between two conformations of sphingomyelin in plasma membranes. Cell 176, 1040–1053.e17. https://doi.org/10.1016/j.cell.2018.12.042.

Epand, R.F., Martinou, J.-C., Montessuit, S., Epand, R.M., Yip, C.M., 2002. Direct evidence for membrane pore formation by the apoptotic protein Bax. Biochem. Biophys. Res. Commun. 298, 744–749. https://doi.org/10.1016/S0006-291X(02)02544-5.

Ferlan, I., Lebez, D., 1974. Equinatoxin, a lethal protein from Actinia equina—I purification and characterization. Toxicon 12, 57–58. https://doi.org/10.1016/0041-0101(74)90099-3.

García-Sáez, A.J., 2012. The secrets of the Bcl-2 family. Cell Death Differ. 19, 1733–1740. https://doi.org/10.1038/cdd.2012.105.

Gilbert, R.J.C., 2016. Protein-lipid interactions and non-lamellar lipidic structures in membrane pore formation and membrane fusion. Biochim. Biophys. Acta Biomembr. 1858, 487–499. https://doi.org/10.1016/j.bbamem.2015.11.026.

Gilbert, R.J.C., Mikelj, M., Dalla Serra, M., Froelich, C.J., Anderluh, G., 2013. Effects of MACPF/CDC proteins on lipid membranes. Cell. Mol. Life Sci. 70, 2083–2098. https://doi.org/10.1007/s00018-012-1153-8.

Gilbert, R.J.C., Serra, M.D., Froelich, C.J., Wallace, M.I., Anderluh, G., 2014. Membrane pore formation at protein-lipid interfaces. Trends Biochem. Sci. 39, 510–516. https://doi.org/10.1016/j.tibs.2014.09.002.

Gouaux, E., 1997. Channel-forming toxins: tales of transformation. Curr. Opin. Struct. Biol. 7, 566–573.

Gutiérrez-Aguirre, I., Trontelj, P., Maček, P., Lakey, J.H., Anderluh, G., 2006. Membrane binding of zebrafish actinoporin-like protein: AF domains, a novel superfamily of cell membrane binding domains. Biochem. J. 398, 381–392. https://doi.org/10.1042/BJ20060206.

Hong, Q., Gutiérrez-Aguirre, I., Barlič, A., Malovrh, P., Kristan, K., Podlesek, Z., Maček, P., Turk, D., González-Mañas, J.M., Lakey, J.H., Anderluh, G., 2002. Two-step membrane binding by equinatoxin II, a pore-forming toxin from the sea anemone, involves an exposed aromatic cluster and a flexible helix. J. Biol. Chem. 277, 41916–41924. https://doi.org/10.1074/jbc.M204625200.

Huang, G., Voet, A., Maglia, G., 2019. FraC nanopores with adjustable diameter identify the mass of opposite-charge peptides with 44 dalton resolution. Nat. Commun. 10, 835. https://doi.org/10.1038/s41467-019-08761-6.

Iacovache, I., De Carlo, S., Cirauqui, N., Dal Peraro, M., Van Der Goot, F.G., Zuber, B., 2016. Cryo-EM structure of aerolysin variants reveals a novel protein fold and the pore-formation process. Nat. Commun. 7, 1–8. https://doi.org/10.1038/ncomms12062.

Jiang, J., Pentelute, B.L., Collier, R.J., Zhou, Z.H., 2015. Atomic structure of anthrax protective antigen pore elucidates toxin translocation. Nature 521, 545–549. https://doi.org/10.1038/nature14247.

Kasianowicz, J.J., Brandin, E., Branton, D., Deamer, D.W., 1996. Characterization of individual polynucleotide molecules using a membrane channel. Proc. Natl. Acad. Sci. USA 93, 13770–13773. https://doi.org/10.1073/pnas.93.24.13770.

Kawashima, Y., Nagai, H., Ishida, M., Nagashima, Y., Shiomi, K., 2003. Primary structure of echotoxin 2, an actinoporin-like hemolytic toxin from the salivary gland of the marine gastropod Monoplex echo. Toxicon 42, 491–497. https://doi.org/10.1016/S0041-0101(03)00226-5.

Kem, W.R., 1988. 20 - Sea anemone toxins: structure and action. In: Hessinger, D.A., Lenhoff, H.M. (Eds.), The Biology of Nematocysts. Academic Press, pp. 375–405. https://doi.org/10.1016/B978-0-12-345320-4.50025-0.

Kühlbrandt, W., 2022. Forty years in cryoEM of membrane proteins. Microscopy 71, i30–i50. https://doi.org/10.1093/jmicro/dfab041.

Koritnik, N., Gerdol, M., Šolinc, G., Švigelj, T., Caserman, S., Merzel, F., Holden, E., Benesch, J.L.P., Trenti, F., Guella, G., Pallavicini, A., Modica, M.V., Podobnik, M., Anderluh, G., 2022. Expansion and neofunctionalization of actinoporin-like genes in Mediterranean mussel (Mytilus galloprovincialis). Genome Biol. Evol. 14. https://doi.org/10.1093/GBE/EVAC151.

Kristan, K., Podlesek, Z., Hojnik, V., Gutiérrez-Aguirre, I., Guncar, G., Turk, D., González-Mañas, J.M., Lakey, J.H., Macek, P., Anderluh, G., 2004. Pore formation by equinatoxin, a eukaryotic pore-forming toxin, requires a flexible N-terminal region and a stable beta-sandwich. J. Biol. Chem. 279, 46509–46517. https://doi.org/10.1074/jbc.M406193200.

Law, R.H.P., Lukoyanova, N., Voskoboinik, I., Caradoc-Davies, T.T., Baran, K., Dunstone, M.A., D'Angelo, M.E., Orlova, E.V., Coulibaly, F., Verschoor, S., Browne, K.A., Ciccone, A., Kuiper, M.J., Bird, P.I., Trapani, J.A., Saibil, H.R., Whisstock, J.C., 2010. The structural basis for membrane binding and pore formation by lymphocyte perforin. Nature 468, 447–451. https://doi.org/10.1038/nature09518.

Lucas, F.L.R., Sarthak, K., Lenting, E.M., Coltan, D., Van Der Heide, N.J., Versloot, R.C.A., Aksimentiev, A., Maglia, G., 2021. The manipulation of the internal hydrophobicity of FraC nanopores augments peptide capture and recognition. ACS Nano 15, 9600–9613. https://doi.org/10.1021/acsnano.0c09958.

Lukoyanova, N., Kondos, S.C., Farabella, I., Law, R.H.P., Reboul, C.F., Caradoc-Davies, T. T., Spicer, B.A., Kleifeld, O., Traore, D.A.K., Ekkel, S.M., Voskoboinik, I., Trapani, J. A., Hatfaludi, T., Oliver, K., Hotze, E.M., Tweten, R.K., Whisstock, J.C., Topf, M., Saibil, H.R., Dunstone, M.A., 2015. Conformational changes during pore formation by the perforin-related protein pleurotolysin. PLoS Biol. 13, 1–15. https://doi.org/10.1371/journal.pbio.1002049.

Maček, P., Belmonte, G., Pederzolli, C., Menestrina, G., 1994. Mechanism of action of equinatoxin II, a cytolysin from the sea anemone Actinia equina L. belonging to the family of actinoporins. Toxicology 87, 205–227. https://doi.org/10.1016/0300-483X(94)90252-6.

Mancheñ, J.M., Martín-Benito, J., Martínez-Ripoll, M., Gavilanes, J.G., Hermoso, J.A., 2003. Crystal and electron microscopy structures of sticholysin II actinoporin reveal insights into the mechanism of membrane pore formation porin (Kem, 1988) produced by the anemone Stichodac-tyla helianthus (order actiniaria), currently classified as a transmembrane solute transporter from the pore-form-ing equinatoxin family 1.C.38 (Saier, 2000). Actinoporins are PFPs displaying similar molecular masses (20 kDa). Structure 11, 1319–1328. https://doi.org/10.1016/j.str.2003.09.019.

Moral-sanz, J., Fernández-carrasco, I., Ramponi, V., Rivera-de-torre, E., Elsallabi, O., Martín-hernández, R., López-aceituno, J.L., Price, N.L., Sanchez, L., Cabo, R. De, Serrano, M., Ikonomopoulou, M.P., 2025. Senotoxins target senescence via lipid binding specificity , ion imbalance and lipidome remodeling. https://doi.org/10.1038/s43587-025-01030-w.

Morera, V., Gomez, J., Besada, V., Estrada, R., Pons, T., Alvarez, C., Tejuca, M., Padron, G., Lanio, M.E., Pazos, F., 1995. Primary structure analysis of the haemolytic polypeptide sticholysin isolated from a sea anemone. Biotecnol. Apl. 12. https://doi.org/10.1108/10650750910961929.

Morgan, P.J., Hyman, S.C., Byron, O., Andrew, P.W., Mitchell, T.J., Rowe, A.J., 1994. Modeling the bacterial protein toxin, pneumolysin, in its monomeric and oligomeric form. J. Biol. Chem. 269, 25315–25320. https://doi.org/10.1016/S0021-9258(18)47249-3.

Mulvihill, E., Sborgi, L., Mari, S.A., Pfreundschuh, M., Hiller, S., Müller, D.J., 2018. Mechanism of membrane pore formation by human gasdermin-D. EMBO J. 37. https://doi.org/10.15252/embj.201798321.

Šolinc, G., Srnko, M., Merzel, F., Crnković, A., Kozorog, M., Podobnik, M., Anderluh, G., 2025. Cryo-EM structures of a protein pore reveal a cluster of cholesterol molecules and diverse roles of membrane lipids. Nat. Commun. 16, 1–11. https://doi.org/10.1038/s41467-025-58334-z.

Olofsson, A., Hebert, H., Thelestam, M., 1993. The projection structure of perfringolysin O (Clostridium perfringens θ-toxin). FEBS Lett. 319, 125–127. https://doi.org/10.1016/0014-5793(93)80050-5.

Palacios-ortega, J., Rivera-de-torre, E., Gavilanes, J.G., Slotte, J.P., 2020. Evaluation of different approaches used to study membrane permeabilization by actinoporins on model lipid vesicles. BBA - Biomembr. 1862, 183311. https://doi.org/10.1016/j.bbamem.2020.183311.

Palacios-Ortega, J., García-Linares, S., Rivera-de-Torre, E., Gavilanes, J.G., Martínez-del-Pozo, Á., Slotte, J.P., 2019. Sticholysin, sphingomyelin, and cholesterol: a closer look at a tripartite interaction. Biophys. J. 116, 2253–2265. https://doi.org/10.1016/j.bpj.2019.05.010.

Peña-Blanco, A., García-Sáez, A.J., 2018. Bax, Bak and beyond — mitochondrial performance in apoptosis. FEBS J. 285, 416–431. https://doi.org/10.1111/febs.14186.

Peraro, M.D., van der Goot, F.G., 2015. Pore-forming toxins: ancient, but never really out of fashion. Nat. Rev. Microbiol. 77–92. https://doi.org/10.1038/nrmicro.2015.3 advance on.

Podobnik, M., Anderluh, G., 2017. Pore-forming toxins in Cnidaria. Semin. Cell Dev. Biol. 72, 133–141. https://doi.org/10.1016/j.semcdb.2017.07.026.

Restrepo-Pérez, L., Huang, G., Bohländer, P.R., Worp, N., Eelkema, R., Maglia, G., Joo, C., Dekker, C., 2019. Resolving chemical modifications to a single amino acid within a peptide using a biological nanopore. ACS Nano 13, 13668–13676. https://doi.org/10.1021/acsnano.9b05156.

Robles-Martín, A., Amigot-Sánchez, R., Fernandez-Lopez, L., Gonzalez-Alfonso, J.L., Roda, S., Alcolea-Rodriguez, V., Heras-Márquez, D., Almendral, D., Coscolín, C., Plou, F.J., Portela, R., Bañares, M.A., Martínez-del-Pozo, Á., García-Linares, S., Ferrer, M., Guallar, V., 2023. Sub-micro- and nano-sized polyethylene terephthalate deconstruction with engineered protein nanopores. Nat. Catal. 6, 1174–1185. https://doi.org/10.1038/s41929-023-01048-6.

Rojko, N., Dalla Serra, M., MačEk, P., Anderluh, G., 2016. Pore formation by actinoporins, cytolysins from sea anemones. Biochim. Biophys. Acta Biomembr. 1858, 446–456. https://doi.org/10.1016/j.bbamem.2015.09.007.

Ruparčič, M., Šolinc, G., Caserman, S., Galindo, J.C.G., Tenorio, M.J., Anderluh, G., 2025. The biological role of conoporins, actinoporin-like pore-forming toxins from cone snails. Toxins 17, 1–21. https://doi.org/10.3390/toxins17060291.

Simpson, R.J., Reid, G.E., Moritz, R.L., Morton, C., Norton, R.S., 1990. Complete amino acid sequence of tenebrosin-C, a cardiac stimulatory and haemolytic protein from the sea anemone Actinia tenebrosa. Eur. J. Biochem. 190, 319–328. https://doi.org/10.1111/j.1432-1033.1990.tb15579.x.

Tanaka, K., Caaveiro, J.M.M., Morante, K., González-Manãs, J.M., Tsumoto, K., 2015. Structural basis for self-assembly of a cytolytic pore lined by protein and lipid. Nat. Commun. https://doi.org/10.1038/ncomms7337.

Tilley, S.J., Orlova, E.V., Gilbert, R.J.C., Andrew, P.W., Saibil, H.R., 2005. Structural basis of pore formation by the bacterial toxin pneumolysin. Cell 121, 247–256. https://doi.org/10.1016/j.cell.2005.02.033.

Varanda, W., Finkelstein, A., 1980. Ion and nonelectrolyte permeability properties of channels formed in planar lipid bilayer membranes by the cytolytic toxin from the sea anemone, Stoichactis helianthus. J. Membr. Biol. 55, 203–211. https://doi.org/10.1007/BF01869461.

Wloka, C., Mutter, N.L., Soskine, M., Maglia, G., 2016. Alpha-helical fragaceatoxin C nanopore engineered for double-stranded and single-stranded nucleic acid analysis. Angew. Chem. Int. Ed. 55, 12494–12498. https://doi.org/10.1002/anie.201606742.

Young, J.D.-E., Hengartner, H., Podack, E.R., Cohn, Z.A., 1986. Purification and characterization of a cytolytic pore-forming protein from granules of cloned lymphocytes with natural killer activity. Cell 44, 849–859. https://doi.org/10.1016/0092-8674(86)90007-3.

Zhang, Y., Tian, L., Huang, G., Ge, X., Kong, F., Wang, P., Xu, Y., Shi, Y., 2025. Structural basis of BAX pore formation. Science 388 (80). https://doi.org/10.1126/science.adv4314 eadv4314.