Cryo-EM structure of ion channel pannexin 1 using GeneArt and Salipro DirectMX platforms

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Abstract

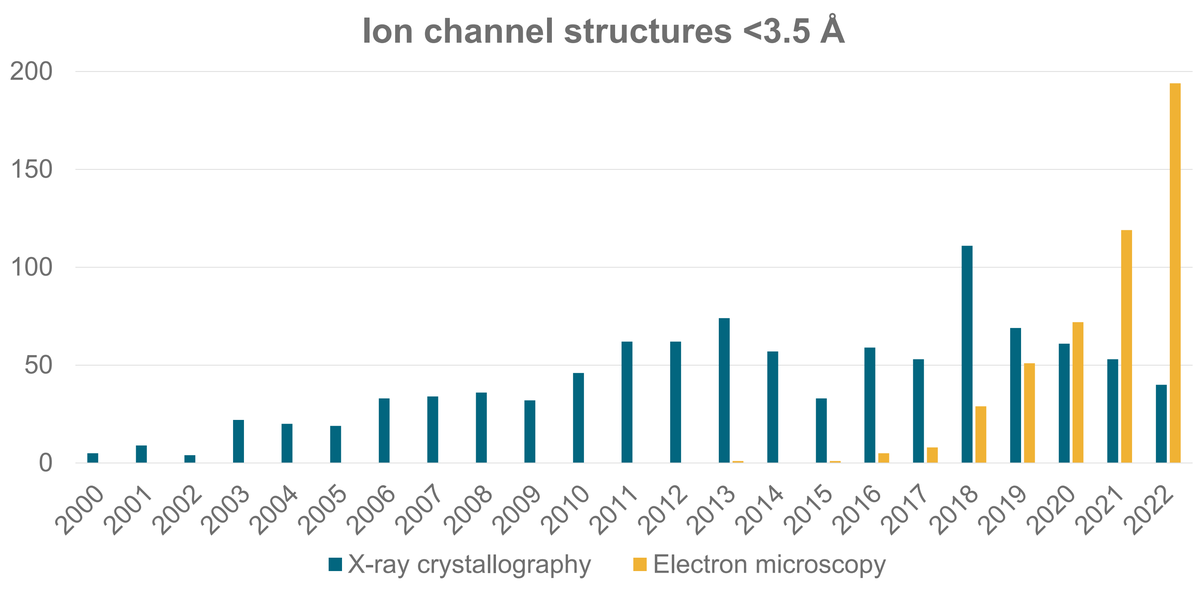
Membrane proteins are the largest group of therapeutic targets in a variety of disease areas, and yet, they remain particularly difficult to investigate[1]. DirectMX is a one-step approach for the incorporation of membrane proteins into lipid Salipro nanoparticles[2]. Here, using the pannexin1 channel as a case study, we demonstrate the applicability of this method for structure-function analysis using surface plasmon resonance (SPR) and cryo-electron microscopy (cryo-EM).

Introduction

Cryo-EM won the 2017 Nobel Prize in Chemistry and has since proven to be game-changing for the structure determination of membrane proteins, including ion channels (Figure 1)[2,3]. Cryo-EM doesn't depend on crystallisation and enables structure determination of challenging proteins in their native state, which are two significant advantages over existing approaches.

Salipro nano-membrane system was developed as a universal platform to stabilize membrane proteins in a native-like lipid environment using a scaffold of saposin proteins[4,5]. Here, we present a case study using a streamlined version of the DirectMX protocol working directly from crude cell pellets to purify the ATP release channel pannexin1 (PANX1) and investigate its structural and ligand-binding properties[6].

Figure 1. Ion channel structures of better than 3.5 Å resolution in the Protein Database (source: PDB).



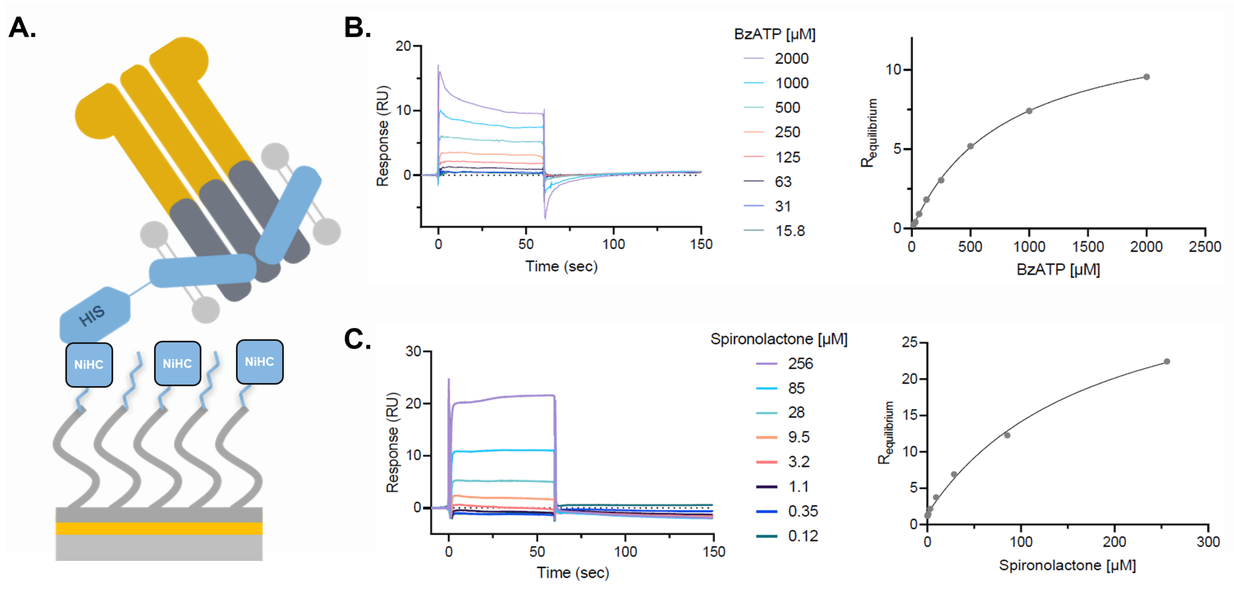
PANX1 binds to known inhibitors as measured by SPR

Mouse PANX1 (mPANX1) containing a cleavable C-terminal GFP and Twin-Strep-tag® was transiently expressed in Expi293 cells and purified as described in the published protocol[6]. Thermal stability of freeze-thawed Salipro-mPANX1 particles was assessed using analytic SEC, showing that protein particles remained stable and homogenous at 22 °C for up to 16 h.

Untagged mPANX1 embedded in a His-tagged Salipro nanoparticle was immobilized (Figure 2A) and challenged with benzoyl-ATP (bzATP), spironolactone, and carbenoxolone, representing known inhibitors with previously reported IC50 inhibition of PANX1 voltage stimulation values (Figure 2B-C).

Binding constants were determined for bzATP (KD 720 µM ± 133 µM) and spironolactone (KD 160 µM ± 10 µM). Binding to carbenoxolone was also confirmed, although the binding constant could not be determined most likely because higher carbenoxolone concentrations could not be explored due to limitations in solubility.

Figure 2. Salipro-mPANX1 functional analysis by SPR. (A) Schematic of His-Salipro-mPANX1 nanoparticles immobilisation to a sensor with Ni2+ ions (NiHC) binding to the His-tag. Sensogram plots and equilibrium of binding determined for bzATP (B) and spironolactone (C) compounds. All SPR data is representative of n=3.

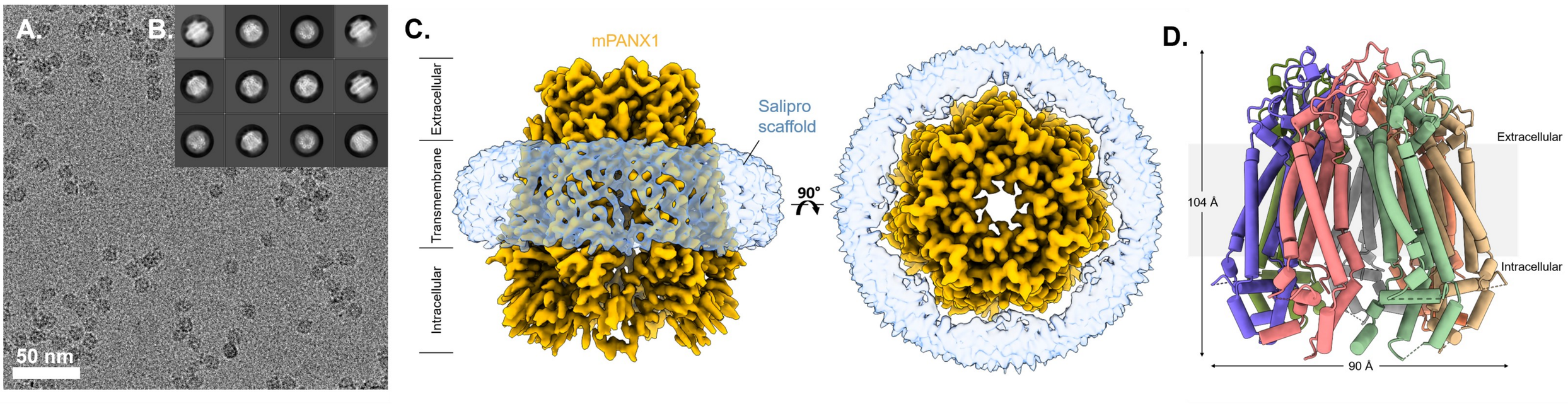


Cryo-EM reconstruction of Salipro-mPANX1 at 3.1 Å

Cryo-EM grids were prepared using Salipro-mPANX1 nanoparticles and a standard Vitrobot plunge-freezing protocol. A 300 kV Thermo Scientific™ Krios G4™ Cryo-TEM equipped with Selectris X Imaging Filter and Falcon 4™ Direct Electron Detector camera operated in Electron-Event representation mode were used for cryo-EM screening and data collection.

Cryo-EM micrographs revealed that frozen-hydrated mPANX1 particles were monodisperse with 2D class averages displaying secondary and tertiary structures, as well as heptameric organisation (Figure 3A-B). Using a total of 269,000 particles from approx. 8000 micrographs, the 3D structure was reconstructed to 3.1 Å resolution (Figure 3C). Extracellular and transmembrane regions of the Salipro-mPANX1 are well-resolved in the structure with clear side-chain density visible. In contrast, much of the intracellular regions were not well resolved in the cryo-EM map prohibiting complete atomic modelling (Figure 3D).

Figure 3. Cryo-EM single-particle analysis of Salipro-mPANX1. Representative micrograph (A) and 2D class averages (B) for the Salipro-mPANX1 cryo-EM dataset. (C) Cryo-EM reconstruction of Salipro-mPANX1 at 3.1 Å. The ion channel (orange) and Salipro lipid disk (blue) is shown as side view (left) and top view (right). (D) The structural model of mPANX1 with each subunit colored.



Conclusions

The cryo-EM structure of mouse PANX1 was determined (3.1 Å), representing the first published structure of a membrane protein extracted by Salipro nanoparticles directly from crude cell pellets.

An *in vitro* small molecular ligand-binding assay was developed for mPANX1, enabling screening of compounds for this drug target via SPR.

Future directions

Overall, the direct cell extraction presented here enables purification of stable and functional wildtype membrane proteins without the need for laborious and time-consuming detergent screenings, protein engineering or screening of applicable alternative scaffolding setups. We believe that the Salipro direct cell extraction may accelerate future membrane protein research, as well as enable the development of new therapeutic ligands in a time-efficient and streamlined manner.

Acknowledgments

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References

1. Overington JP, *et al*. How many drug targets are there? Nat Rev Drug Discov. 2006;5:993-996.
2. Cheng Y. Membrane protein structural biology in the era of single particle cryo-EM. Curr Opin Struct Biol. 2018;52:58-63.
3. Callaway E. Revolutionary cryo-EM is taking over structural biology. Nature. 2020;578(7794):201.
4. Frauenfeld J, *et al*. A saposin-lipoprotein nanoparticle system for membrane proteins. Nat Methods. 2016;13(4):345-351.
5. Lloris-Garcerá P, *et al.* DirectMX-one-step reconstitution of membrane proteins from crude cell membranes into salipro nanoparticles. Front Bioeng Biotechnol. 2020;8:215.
6. Drulyte I, *et al*. Direct cell extraction of membrane proteins for structure-function analysis. Sci Rep. 2023;13:1420.

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