CONSTANT SYSTEMS
CELL DISRUPTION MADE EASY

MANCHESTER 1824
The University of Manchester

Application Note

Supporting Structural Analysis of Membrane Ion Channels Through Controlled Cell Disruption with Constant Systems

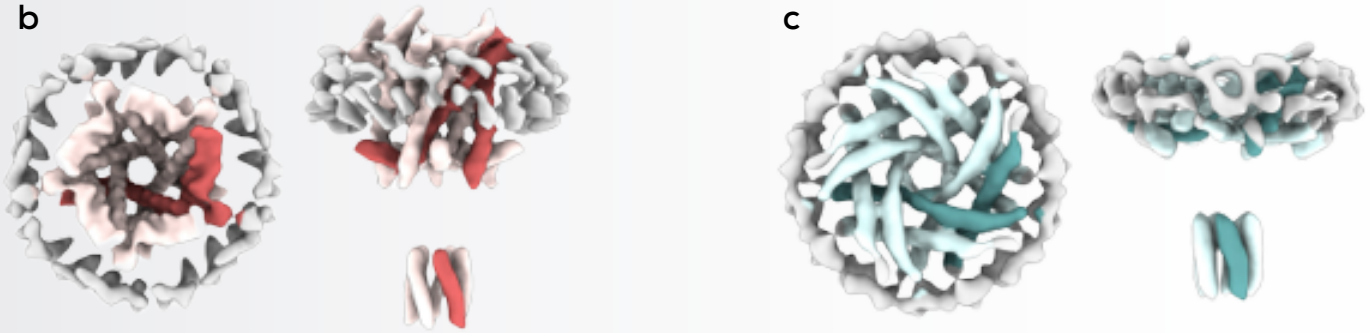
In membrane biophysics understanding how bacterial ion channel mechanosensors and essential proteins complexes behave allows us to target these gated rights of way with novel antimicrobial therapies.

The mechanosensitive channel of large conductance (MscL) is a tension gated pore-forming protein from E. coli (EcMscL) and is the most widely studied system to date. However, it has not been possible to determine the 3-dimensional structure of this challenging membrane protein until the arrival of novel technology in Dr Christos Pliotas' laboratory in Manchester.
The Pliotas Lab has solved two distinct conformational states of this channel protein, namely 'closed' and 'open-like' in lipid bilayers by cryoEM. PELDOR and EPR spectroscopy together with cryoEM among other state-of-the-art technologies allowed the Pliotas laboratory to see high-resolution images of MscL- never seen before and is now described in detail in Dr Pliotas' 2026 publication:

Structural basis of the gating mechanism of the large-conductance mechanosensitive channel from Escherichia coli

Staying with conformational states, such findings would only be possible by extracting these membrane-bound proteins from E. coli while maintaining their functional and structural integrity throughout the cell lysis, purification, and reconstitution processes, with the required yield achieved at each stage.

For membrane proteins such as EcMscL, cell disruption is a critical upstream step because it directly influences membrane recovery, oligomer stability and final sample quality. Structural workflows depend not only on releasing protein efficiently, but on preserving it in a form suitable for downstream analysis.



Figures b and c:
Top-and side-view density maps from cryoEM of the closed and expanded-state EcMscL structures in MSP1D1 DSPC nanodiscs, indicating the MSP-belt protein.

MSP 1D1: Membrane Scaffold Protein 1D1, a genetically engineered protein used to create nanodiscs

Constant Systems high-pressure cell disruption was particularly well aligned with these requirements by delivering controlled and reproducible cell breakage, performed at low temperatures (i.e. 4°C) (membrane proteins such as MscL are susceptible to heat damage) while maintaining high recovery of membrane material for purification and structural analysis.

This contributed value in three key areas:

1. **Preservation of native protein assemblies** – EcMscL exists as an oligomeric membrane complex (pentamer) and maintaining this organisation without dissociating to single subunits or aggregating due to reduced stability, is essential for structural studies.

2. **Recovery of intact membrane fractions** – efficient membrane isolation supports subsequent detergent extraction, purification and nanodisc reconstitution.

3. **Batch-to-batch consistency** – reproducible disruption conditions are important when generating material for cryo-EM and orthogonal analytical methods.

Alternative high-pressure technologies such as conventional homogenizers or interaction-chamber systems are highly effective for general cell disruption and emulsification applications. However, these systems are typically optimised around high shear processing and particle size reduction. For delicate membrane protein workflows with controlled temperatures, the priority shifts from maximum disruption efficiency to controlled recovery of intact membrane assemblies and structural-grade material.
For advanced membrane protein structural biology, success depends on preserving quality rather than simply maximising yield.

Constant Systems provides the controlled disruption environment needed to recover EcMscL in a state suitable for high-resolution structural analysis.

Testimonial - Christos Pliotas

"The CF-1 cell disruptor has been key to our research within the Pliotas laboratory at the University of Manchester. This system allows for rapid and consistent cell lysis of our bacterial, yeast, and human samples, ensuring prep-to-prep reproducibility of diverse membrane protein preparations for structural and functional characterisation. The CF-1 is very easy to use, which makes training new members of staff straightforward. Additionally, the ability to easily clean and maintain the system using the clean-in-place protocols set out during installation allows for quick transitions between different protein purification samples."

Further, the CF-1's ability to cool samples during the cell disruption process means that we are able to maintain the correct conformation of our membrane proteins for high resolution structural and EPR spectroscopy studies. Overall, we believe that the CF-1 has been instrumental in progressing our research as highlighted by a number of publications from the laboratory where the CF-1 played a key role in purification.

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