Cryo-EM Snapshots of Nanodisc-Embedded Native Eukaryotic Membrane Proteins

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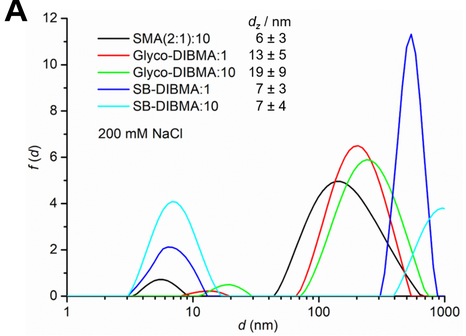
HALOmem
membrane protein structure & dynamics

Abstract

New membrane complex purification technologies in combination with cryo-electron microscopy (cryo-EM) recently allowed the exploration of near-native membrane protein complex architectures. Polymer nanodiscs in particular provide the basis to study overexpressed membrane proteins at high resolution while retaining protein–protein and protein–lipid interactions. However, how the majority of endogenous membrane proteins are organized remains elusive, mainly due to the inherent complexities that a hydrophobic environment poses to biochemical preparations. In this work, we combined biochemical enrichment protocols for native membrane complexes together with amphiphilic polymers to increase the quality of recovered endogenous membrane complexes. The derived protein-encapsulated nanodiscs were identified by mass spectrometry and imaged with cryo-EM. This set of technologies is applied to *Chaetomium thermophilum*, a thermophilic fungus, that confers additional advantages for protein structure determination due to the increased thermal stability of its biomolecular assemblies. Our results show a highly efficient recovery of protein-encapsulating nanodiscs, amenable to structural and biophysical characterization with a multitude of methods. Initial mass spectrometry results reveal ~1300 proteins while multiple 2D class averages from cryo-EM data show prominent nanodisc-embedded structural signatures. This combined methodological approach to isolate multiple endogenous membrane protein complexes provides unprecedented opportunities for a deeper understanding of the membrane proteome of a eukaryote.

Preliminary Studies

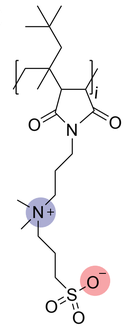
A



B

Lipid headgroup	%
Heart-PC	28
Heart-PE	40
Liver-PI	5
Heart-CL	27

C

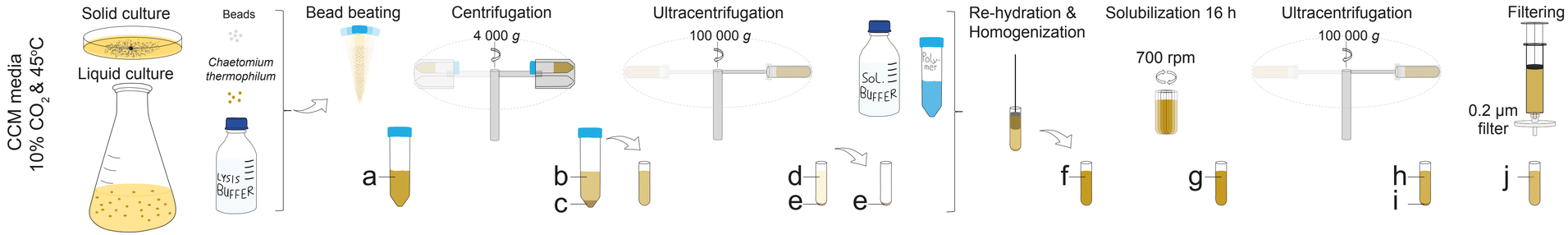


SB-DIBMA

Solubilization of inner mitochondrial membrane lipid mixture

(A) Intensity-weighted particle size distribution of solubilization experiments on vesicles mimicking inner mitochondrial membranes with different polymer derivatives.
(B) Lipid composition that was used, to mimick the inner mitochondrial membrane mixture, in the preliminary solubilization experiments.
(C) Chemical structure of the zwitterionic DIBMA-derivative, SB-DIBMA.

Workflow



Experimental workflow from culture to nanodiscs. The Lysed model organism (a) from which membranes are being isolated. Centrifugation of lysed cells to separate the cell extract (b) from any cell wall debris and unlysed cells (c). Ultracentrifugation is performed to isolate the membranes (e) from the soluble cell extract (d). Isolated membranes are rehydrated in solubilization buffer and supplemented with the chosen polymer concentration and, subsequently, homogenized (f).

The homogenized solution (f) is then incubated at 37°C and 700 rpm for 16 h (g). Incubated solution is subjected to ultracentrifugation to separate the soluble nanodiscs (h) from the unsolubilized material (i). Furthermore, nanodiscs are filtered through a 220 µm filter (j).

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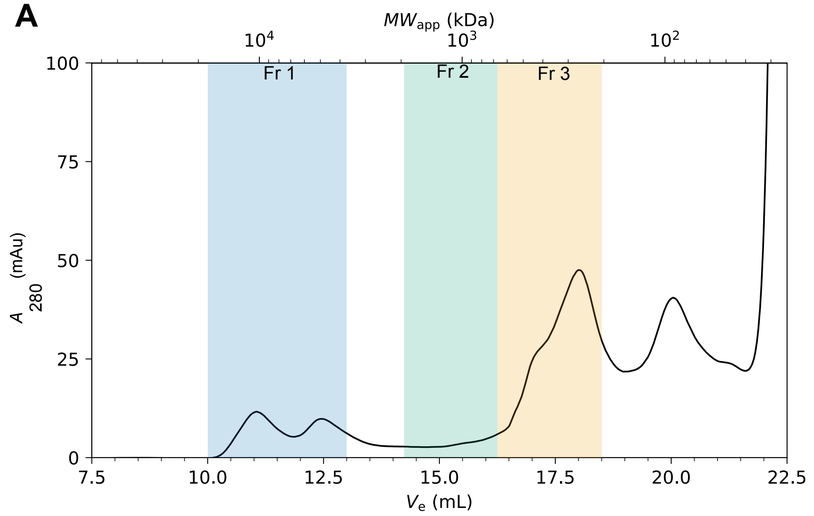
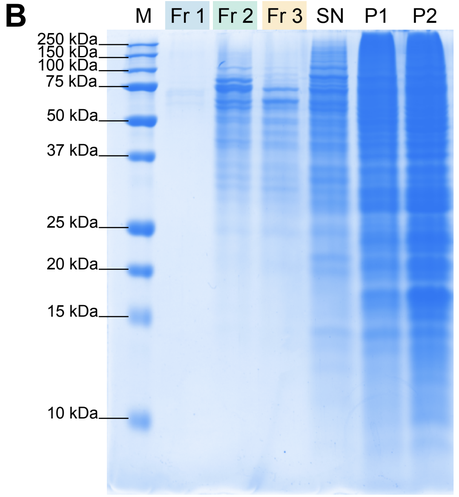
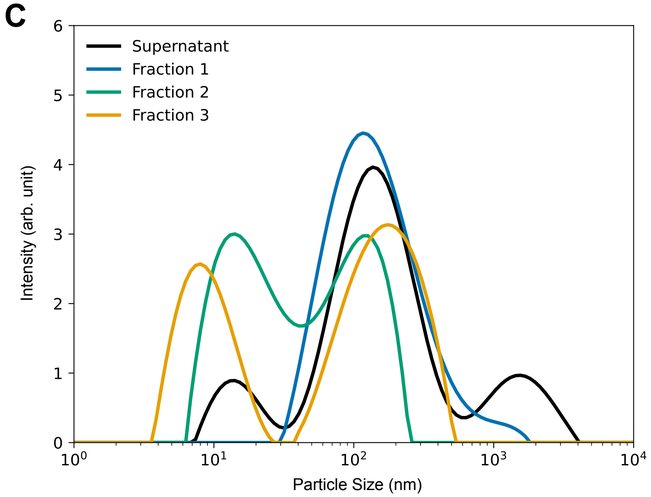
Biochemical Characterization and Cryo-EM of Nanodisc-Embedded Proteins

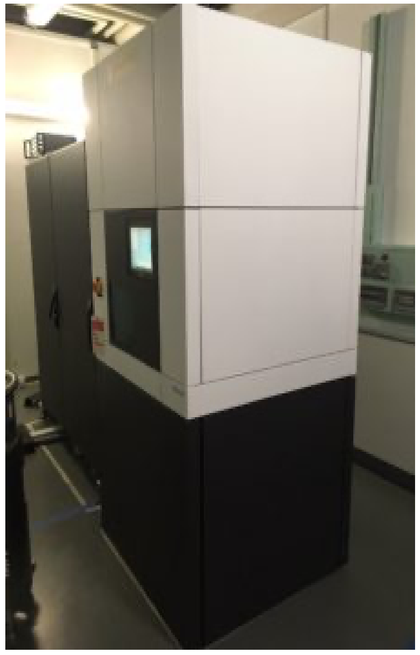


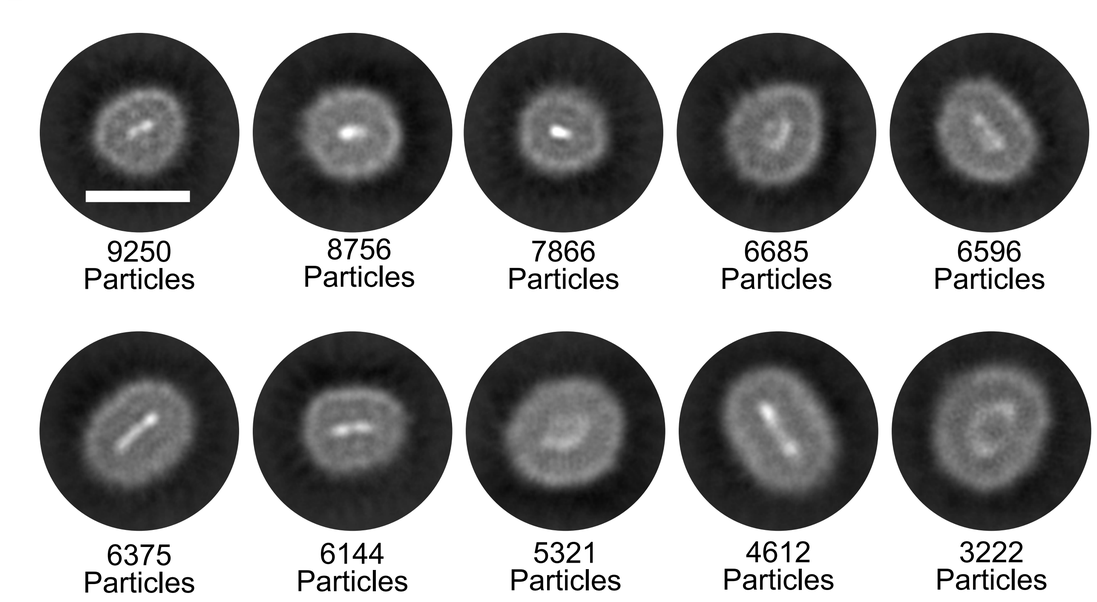


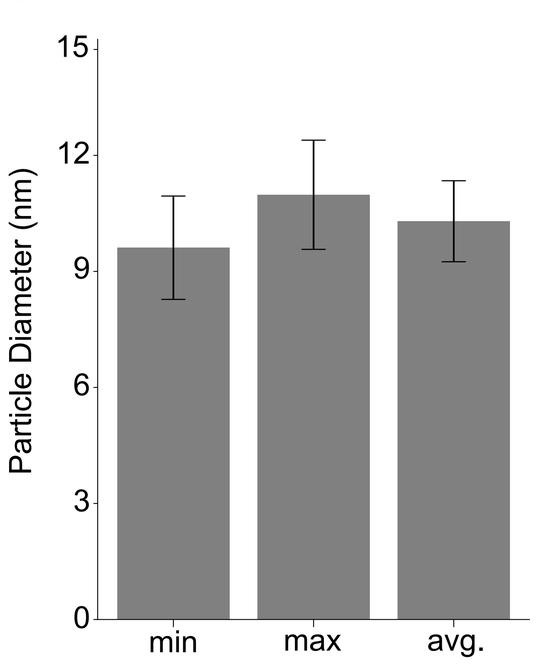


Biochemical characterization of Nanodiscs.

(A) Size-Exclusion Chromatogram of 250 mg/mL *C. thermophilum* membranes with 1.25 mg/mL SB-DIBMA. Colored columns indicate utilized elution volumes that were used to pool the samples.
(B) SDS-PAGE of the pooled fractions (Fr1-3), the supernatant (SN/j) that was injected into the SEC, the pellet (P1/e) that was initially used for the solubilization and the pelllet after the solubilization (P2/j).
(C) Intensity-weighted particle size distribution of the supernatant and the pooled fractions utilizing **DLS**.







2D classes and size distribution of native nanodiscs from *C. thermophilum* membranes in cryo-EM using our in-house Glacios microscope.

(A) Our in-house Glacios microscope
(B) Representative results from selected 2D classes. Scale bar = 10 nm
(C) Weighted size distributions of the minimum, maximum as well as the average size diameters observed in the 2D classes of nanodiscs.

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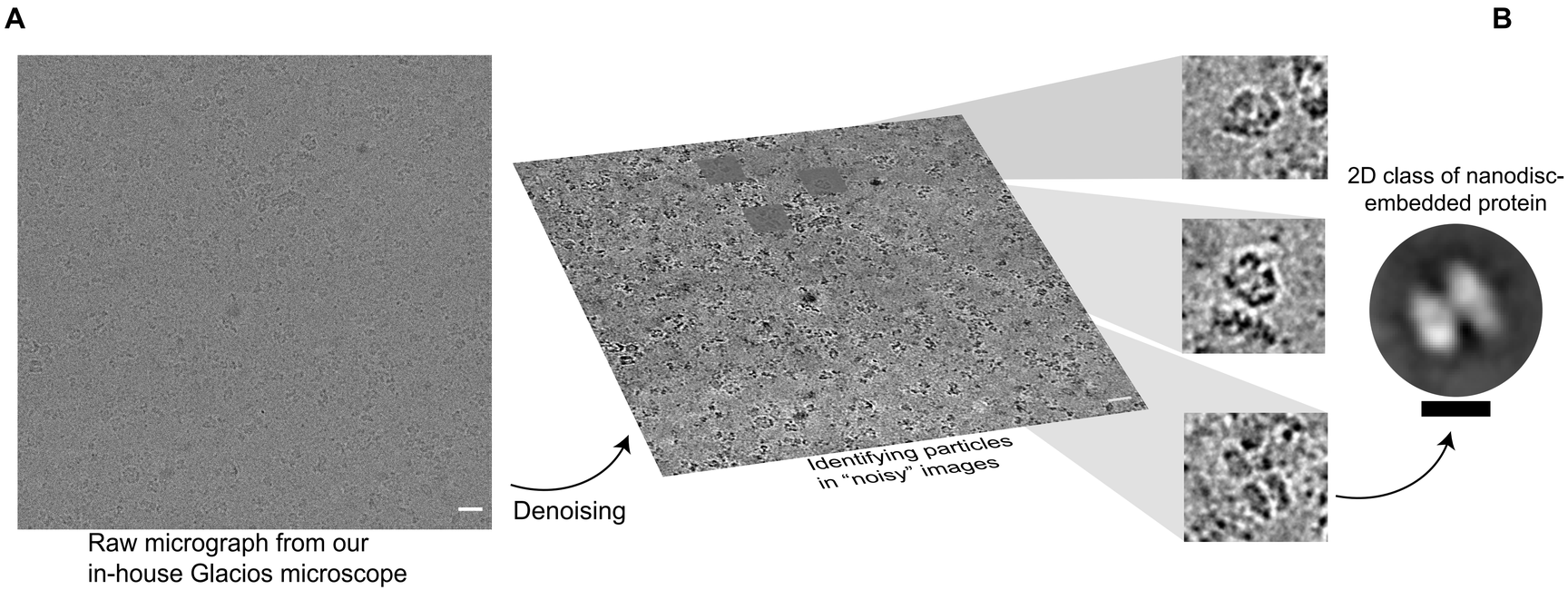
Cryo-EM and Mass Spectrometry of Nanodisc-Embedded Proteins



Cryo-EM and Mass Spectrometry of Nanodisc-Embedded Proteins.

(A) Screening data processing worklow. Cryo-EM of fraction 2 allows, after denoising, to detect numerous particles, including possible nanodisc-embedded proteins. 2D classification of particles, contained in denoised image data, reveals nanodisc-embedded proteins. Micrograph scale bar = 20 nm, 2D class scale bar = 10 nm

(B) Mass spectrometry data of fraction 2. Distribution of relative abundance of soluble (light grey) and membrane (blue) proteins. Dotted lines indicate the 1st and 3rd quartile, (25%/75%) and the dashed line the 2nd quartile (median). Membrane proteins above the median were BLASTed against the yeast proteome and annotaed according the top hits.

Conclusion

- SB-DIBMA is able to form nanodiscs at low lipid to polymer ratios previously unseen, here $R_{L/P}$=0.005.
- Nanodiscs formed from *C thermophilum* membranes are slightly larger, compared to nanodiscs formed from model membranes, having a previously uncharacterized plasticity.
- The developed method will have unprecedented effects on cryo-EM characterization of endogenous membrane complexes.

Outlook

- Analyzing single fractions rather than pooled ones will increase possibilities for higher resolution structural analysis.
- Reconstruction and identification of endogenous membrane complexes in nanodiscs will provide shape information of the endogenous membrane complexome.
- Complement structural and mass spectrometry data with classical biochemical assays to verify the activity with direct insights intro future biotechnologial potential.

Reference

[1] Janson, Kevin *et al.* "Solubilization of artificial mitochondrial membranes by amphiphilic copolymers of different charge." Biochimica et Biophysica Acta (BBA) - Biomembranes, Volume 1863, Issue 12, (2021); 183725

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