Automated Native Mass Spectrometry Screening of Membrane Proteins for Structural Biology Applications

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In the last five years a sharp increase in membrane protein structures have been determined, driven largely by the adoption of cryo-EM, which is well positioned to determine previously unobtainable integral membrane protein structures. As the automation and achievable resolution of cryo-electron microscopes is rapidly improving, the critical step is now in preparing good grids, which require stable and homogeneous sample. Typical characterization methods like SEC can be hard to interpret due to the heterogeneity introduced by the membrane mimetics required for stabilization of the membrane protein.

Here we present a fully automated native mass spectrometry-based workflow, designed for non-experts, to screen membrane proteins for stability, homogeneity, and composition.

This workflow was tested on several membrane protein classes to validate the performance. The workflow can be used in an automated fashion for up to 96 samples, ranging from applications like drug screening to buffer optimization. By switching or omitting detergents, the same workflow can be used to either provide intact analysis of membrane proteins without additional sample preparation or harsh denaturing conditions and even analysis of host cell proteins that are co-expressed or purified with the target protein.