Assessing Membrane Protein Structural Dynamics within Lipid Nanodiscs

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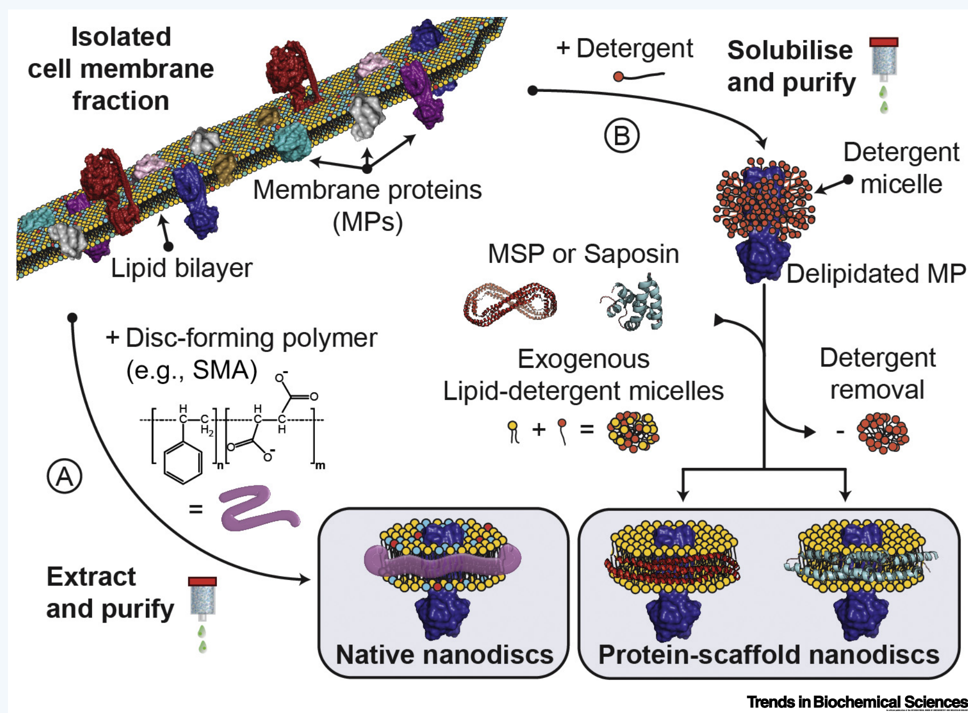
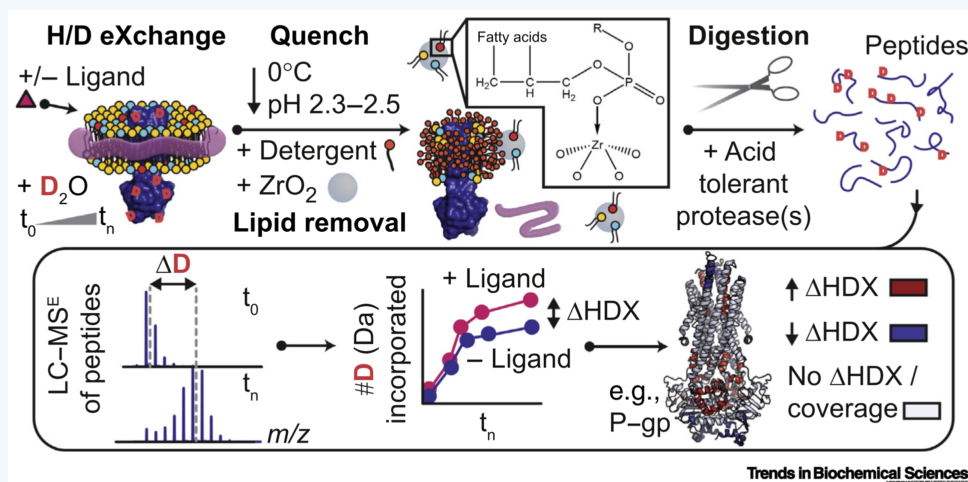


Membrane protein structure, dynamics, and function have an intimate relationship with the complex and varied cellular lipid membranes they reside in. Lipid nanodisc technologies provide a membrane protein with a soluble lipid bilayer environment – either through direct extraction within their native lipid surround (Figure 1A) or though reconstitution with isolated lipids (Figure 1B) – amenable to a variety of *in vitro* investigations.



Protein structural dynamics have increasingly been recognised as essential for cellular protein functional behaviour. Hydrogen/deuterium exchange mass spectrometry (HDXMS; Figure 2) bridges the gap between protein structure and function by directly examining structural dynamics, which are the structural fluctuations biological molecules undergo under equilibrium conditions; these fundamental conformational rearrangements remain poorly understood and are undetectable by current high-resolution techniques.

ADVANTAGES:

Native nanodiscs enable membrane proteins to be studied within native lipid compositions.

Protein-scaffold nanodiscs – formed by either membrane-scaffold protein (MSP) or Saposin protein – enable the lipid environment to be determined by exogenous lipid reconstitution.

HDXMS typically delivers peptide-level resolution information on membrane protein–protein interactions, ligand/drug binding, and structural dynamics.

HDXMS is a sensitive, nonperturbing labelling technique which can be performed on complex samples and does not require prohibitively large sample concentrations or crystalline or vitrified samples.

CHALLENGES:

During HDXMS workflows, it is essential for the sample to be quenched (to acidic pH 2.3–2.5), rapidly digested, and analysed at 0°C for the minimisation of H/D back exchange and, therefore, structural information loss.

Lipids lead to overlapping MS spectra and poorer chromatography resolution, as well as protease inhibition and instrument fouling during HDXMS workflows, which means that lipids must be rapidly removed before analysis.

HDXMS workflows on lipid nanodiscs are currently low-to-moderate throughput and, thus, require further improvement in automation technologies.

Lipid nanodiscs lack many cellular membrane properties such as the presence of other cellular proteins, protein crowding, membrane curvature, ion gradients, the native membrane phase, and membrane protein topology.

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