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Cryo-EM: spinning the micelles away

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Structural characterization of integral membrane proteins (MPs) demands that the samples be pure, monodisperse, and stable. Detergents are required to extract MPs from the lipid bilayer in which they reside and to stabilize them for downstream biophysical analyses. Some of the best MP-stabilizing detergents pose problems for cryo-EM studies, but Hauer et al. (2015) now offer a solution called GraDeR.

Lauryl maltose-neopentyl glycol (LMNG) is one of a new class of detergents that solubilizes and stabilizes a wide range of membrane proteins (MPs) much better than traditional detergents (Chae et al., 2010). Methods of solubilizing MPs are of great interest for single-particle electron cryo-microscopy (cryo-EM), which is rapidly yielding MP structures at resolutions rivaling those of X-ray crystallography (Cheng, 2015). LMNG can be problematic for cryo-EM studies however because of its extremely low critical micelle concentration (CMC). The CMC is, roughly speaking, the free monomer concentration as buffered by micelles in a detergent solution. Dodecylmaltoside (DDM), the mainstay "low-CMC" detergent has a CMC around 120–180 micromolar, whereas LMNG has CMC estimated to be about 11 *nanomolar* (Chung et al., 2012). Although low-CMC detergents pose technical challenges for cryo-EM studies, Hauer et al. (2015) now report a very successful gradient-centrifugation approach for preparing MP complexes with LMNG.

In single-particle cryo-EM specimens, the presence of excess detergent micelles creates a severe interfering background in the micrographs. Indeed, micelles of DDM and LMNG are so large that they are often indistinguishable from protein particles (see Fig. S1 of Hauer et al. (2015) for an example). The presence of detergent also affects the surface tension of the thin buffer film that is vitrified in cryo-EM sample preparation. The removal of excess detergent is therefore essential but notoriously difficult in the low-CMC case. Removal of detergent monomers by dialysis is intolerably slow and with size-exclusion chromatography (SEC) the micelles often run at the same velocity as a protein peak of similar molecular weight. Even adsorption to polystyrene beads, usually the standard means for removing low-CMC detergents, may require considerable time and effort to determine empirically the appropriate amount to use.

In cryo-EM one successful approach has been to use amphiphilic polymers (amphipols; Popot et al., 2011) instead of detergents. These bind tightly to protein, and the excess can be removed completely by SEC. Hauer et al. now show that with their straightforward "GraDeR" approach, LMNG-solubilized complexes can similarly be made with negligible excess detergent and micellar background. GraDeR merely involves centrifuging the sample

in the presence of two gradients of inverse directionality: that of glycerol or other crowding agent increasing downward and that of the low-CMC detergent decreasing downward. The authors demonstrate that GraDeR is suitable for three diverse specimens from across the evolutionary spectrum: a prokaryotic V-type ATPase, a lower-eukaryotic gap junction, and a mammalian F_oF_1 ATP synthase. The good cryo-EM results are indicative of the technique's potentially widespread utility.

Beyond cryoEM, GraDeR could conceivably be exploited in X-ray crystallography. In the process of concentrating MP samples for crystallization—to concentrations as high as 60 mg/ml for lipid cubic phase (Caffrey, 2015)—the micelles of low-CMC detergents are often inadvertently concentrated as well. Inordinately high detergent concentrations may hinder crystallization, and GraDeR could be adapted to lower the concentration.

Another potential use for GraDeR in MP biochemistry is in the reconstitution of proteins into lipid vesicles, bilayers, or nanodiscs. Insufficient removal of excess detergent from MP reconstitution samples, especially those of secondary transporters into liposomes, is particularly problematic. This is because the detergent micelles or monomers can easily destabilize the bilayer to dissipate the electrochemical gradient, rendering an uptake or efflux assay useless or, at a minimum, drastically skewing the results. GraDeR offers an alternative means of removing extra detergent.

In conclusion, this paper introduces the innovative but surprisingly simple GraDeR. Although the authors of this paper focus exclusively on its use in cryoEM, we feel that this technique could eventually be expanded to other methods employed to study MP structure and function.

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