DETERGENTS AS TOOLS FOR MANIPULATING MEMBRANE PROTEINS ARE MORE COMPLEX THAN SIMPLE EXTRACTING COMPOUNDS

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Membrane proteins (MPs) represent more than 60% of pharmaceutical targets for which different approaches require to maintain them in aqueous solution in a native state, e.g. crystallography, ligand screening, antibody production, immunization and other applications. Such solution state is essentially obtained by using detergents, amphipathic molecules able to compete with lipids for disrupting biological membranes in which MPs are embedded. Detergents replace lipids around hydrophobic patches of MPs, thereby keeping them in solution and preventing their aggregation during the process from extraction to purification.

If detergents are efficient competitors of lipids, they have not the same efficacy for stabilizing membrane proteins once extracted. To solve this problem we are developing a new concept of anionic detergents for improving such stabilization. These compounds are unique in being able to interact with membrane proteins not only through hydrophobic interactions but also by generating a network of salt bridges with the bulk of positively-charged residues located at the cytosol-membrane interface of membrane proteins, rather abundant in that region.

Detergents behave in different states in aqueous solution, as single molecules or either forming micelles, pure or mixed with membrane proteins when present. The true concentration of detergent, free or associated to the membrane protein of interest is therefore hard to know, especially along processes such as membrane protein purification that involves multiple steps of dilution/concentration. Such information is however critical as it influences the level of membrane protein aggregation, topology, crystal growth and stability .... Unless using radiolabelled compounds, there is no method to get this information routinely, quickly and with any detergent. We have set up a method providing such information potentially for any detergent, quickly and with a high degree of accuracy. The method based on the determination by MALDI MS of the ratio of deuterated/protonated detergents or that of structurally close molecules when a deuterated version is not available. We validated the method with foscholine 12 (FC12), dodecylmaltoside (DDM), octylglucoside (OG), Maltose Neopentyl Glycol (MNG), Calix[4]arene-based detergents (C4Cn), CHAPS and cholate, by measuring their concentrations in different extraction conditions / purification, concentration by ultrafiltration, dialysis and gel filtration of various membrane proteins. We have measured the amount of detergent associated with a variety of membrane proteins with different topologies, membrane spanning domains, functions and oligomerization states (ABC transporters, GPCR, ADP/ATP exchanger, proteins from prokaryotic efflux systems) revealing a detergent:MP ratio ranging from 130 to 700 mol/mol, depending of the MP and the detergent. Finally, we detected an extra amount of detergent released after ultrafiltration followed by gel filtration revealing that MPs are not simply embedded in a detergent micelle but rather sequester twice more detergent to protect their hydrophobic area through a gradient from tight to weak interactions. Such new state has yet to be characterized: a challenge for computer simulations?