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The behavior of detergents around membrane proteins is more complex than supposed, as revealed by a new method of quantitation

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rembrane proteins (MPs) represent more than 60% of pharmaceutical targets for which different approaches require M 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. They are amphipathic molecules which compete with lipid and disrupt 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. A key information when MPs are extracted and maintained in a soluble detergent-MP complex is to know the true concentration of detergent present in the medium, largely dependent on the membrane protein of interest and directly influencing the level of aggregation, topology, crystal growth and stability. Unless using radiolabelled compounds, there is no method to get this information routinely, quickly and with any detergent. A method providing such information potentially for any detergent, quickly and with a high degree of accuracy have been set up. 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. The method was validated withfoscholine 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. 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 fromprokaryotic efflux systems) showing detergent:MP ratio ranging from 130 to 700mol/mol depending of the MP and the detergent, could be measured. Finally, 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 were detected.

Biography

Pierre Falson got his PhD at the LYON University. He is a CNRS (National Centre for Scientific Research) Research Director, enzymologist and membrane proteins biochemist, co-leading the Drug resistance mechanism and modulation team in the BMSSI CNRS-UCBL1 Research Unit. PFhas published 54 publications, patented 6 inventions and licensed2 to CALIXAR, a startup which he co-founded. He was awarded in 1991 by the Maurice Nicloux prize from the French Society of Biochemistry and Molecular Biology, in 2010 and 2011 by the "National competition of innovative start-ups" and by the Innovation and Transfer Technology prize from theCNRS.

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