Bilayers at the ILL

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Interdomain flexibility within NADPH oxidase revealed by SANS using LMNG stealth-carrier

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Membrane protein solubilization implies the use of amphipathic detergents that form protective belts around hydrophobic patches to maintain the protein's structural integrity outside of the lipid environment. Consequently, the resulting SANS signal includes both protein and detergent belt signatures leading to a global outer shell larger than the envelope expected for the protein alone; masking the detergent signal represents an important issue. Moreover, specific detergent characteristics can disrupt the proper folding of the membrane protein, greatly impacting the structural parameters of the SANS envelope and thus necessitating significant effort in the preparation of an optimized sample for SANS. A strategy to overcome these specific issues was tested using the LMNG detergent, recently characterized as a strongly stabilizing detergent. We studied the membrane protein SpNox, a prokaryotic homolog of NADPH oxidase originally identified in phagocytic cells where it produces Reactive Oxygen Species. Detergent screening confirmed the improved thermostability of SpNox and limited aggregation when solubilized in LMNG. Through contrast variation experiments, we were able cancel the LMNG contribution to the SANS signal, and we determined the percentage of D20 corresponding to the buffer matching point. Then, to improve protein contrast, we produced deuterated SpNOX and subsequently solubilized and purified it in LMNG. Finally, since SANS studies are very sensitive to the presence of aggregates or oligomers in the sample, the development of a new in-situ size exclusion chromatography (SEC) system on a SANS instrument enabled data collection on line from an aggregation-free homogeneous sample. This strategy led to the determination of a low-resolution envelope of SpNox confirming the monomeric state of the protein in solution. Parallel to the structural study, a homology model of SpNox was developed; the model was successfully docked in the SANS envelope. The general strategy may be applied to many other membrane proteins in the future.

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