STOICHIOMETRIC CONSTRAINTS AS A ROUTE TO MORE RELIABLE SAXS ANALYSIS OF MEMBRANE PROTEINS IN DETERGENT

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Membrane proteins are central to signal transduction, transport and bioenergetics, yet their quaternary structure in solution is often difficult to establish by high-resolution methods alone. Crystallography and cryo-EM provide indispensable structural detail, but they do not always report the oligomeric organization that is most relevant in detergent or membrane-mimetic environments. Small-angle X-ray scattering is therefore an important complementary approach, since it probes membrane-protein assemblies directly in solution and can be highly informative with respect to their oligomeric state and overall architecture. This point is illustrated by the cyanobacterial rhodopsin ASR: although it forms dimers in the crystal, SAXS and NMR studies indicated predominantly trimeric species, showing that the crystal structure alone did not fully capture the relevant oligomeric state [1]. For oligomeric microbial rhodopsins, SAXS-type solution analysis is therefore not merely supportive but can address a structurally important question that may remain unresolved by a high-resolution structure alone.

At the same time, SAXS analysis of detergent-solubilized membrane proteins is intrinsically complicated by two factors: the detergent corona surrounding the hydrophobic part of the protein and, in many cases, oligomeric polydispersity. Our previous study showed that both effects can strongly bias data interpretation and may even produce misleading low-resolution models if not treated explicitly [2]. Hybrid approaches such as MEMPROT substantially improve this situation by describing the detergent corona with pseudo-atoms representing hydrophobic and hydrophilic regions and fitting the resulting protein-detergent models against the SAXS curve [3,4]. However, more recent work also makes clear that such SAXS-guided modeling remains intrinsically non-unique: several detergent-corona geometries may fit the same data nearly equally well, and physically implausible solutions may survive if model selection is guided only by fit quality. In practice, this means that even an incorrect oligomeric hypothesis can sometimes be artificially compensated by an alternative detergent-belt geometry.

We suggest that this ambiguity can be reduced by introducing stoichiometric constraints, namely the detergent-to-protein ratio in the protein-detergent complex. Such information may be obtained by orthogonal biophysical methods, for example from UV and differential refractive index measurements or from infrared spectroscopy. These approaches are useful, but their accuracy depends on external parameters such as extinction coefficients, dn/dc values and the quantitative calibration of the spectroscopic method. An attractive complementary route is SANS contrast variation, which provides access to the relative volume fractions of the protein and detergent components in scattering terms by comparing the contrast-matching point of the whole complex with those of the individual components. In this way, the detergent-to-protein ratio can be estimated in a form directly relevant to scattering-based modeling and then used as an a priori restriction in SAXS analysis, or later in joint SAXS/SANS refinement.

We are currently testing this concept on DDM-solubilized microbial rhodopsins. In particular, SANS contrast-variation experiments have been performed for KR2, a sodium-pumping rhodopsin whose functional form is pentameric and whose oligomerization is directly important for activity [5]. The ongoing analysis addresses whether stoichiometric information extracted from SANS can help to reduce the effective parameter space of detergent-corona models and thereby improve the uniqueness and physical plausibility of SAXS-based structural interpretation of protein-detergent complexes.

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