Isolating interfacial scattering in emulsions: A SAXS-based bulk subtraction approach for pro-tein-stabilized emulsion systems

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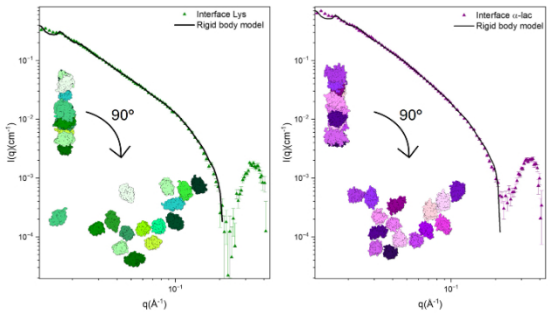
Interfaces are central to the behavior of colloidal systems, yet their structural characterization remains difficult due to their small and weak scattering signals compared to the bulk of the solution. Emulsions -prototypical soft matter systems- scattering illustrate this challenge well: although interfacial phenomena largely determine macroscopic properties, the interface itself is easily masked by bulk scattering.
In this study, we introduce an advanced small-angle X-ray scattering (SAXS) approach designed to isolate interfacial scattering in model oil-in-water emulsions stabilized by proteins. By combining centrifugation-induced phase separation with a theoretical reconstruction and subtraction of the bulk contribution, we extract the interfacial signal and analyze it through rigid-body modeling of model proteins. Our findings indicate a planar arrangement of protein monomers at the interface, with α-lactalbumin forming a denser interfacial layer than lysozyme—likely reflecting differences in molecular flexibility and destabilization of the protein at the interface. This methodology overcomes key challenges in conventional SAXS analyses, such as buffer mismatch and ambiguity between free and bound particles and provides a general framework for studying interfaces in complex fluids, biological assemblies, and functional materials.

Keywords:
Emulsions, Food interfaces, SAXS, Rigid body modeling



Absolute scale data results after bulk subtraction and corresponding fit for model proteins in a planar structure used in the study, Lysozyme and alpha-lactalbumin.