Fractionation-Free Quantification of Nanoparticle-Protein Interactions by Synchrotron SAXS

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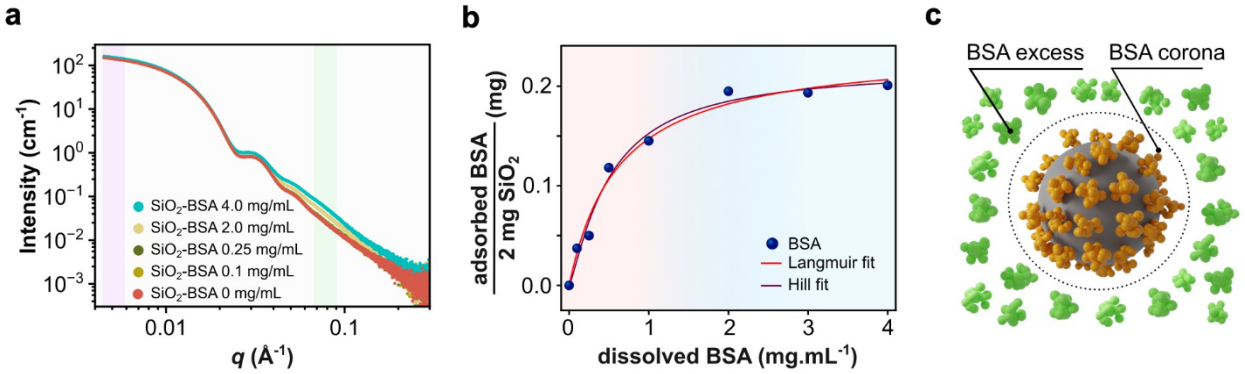
The protein corona (PC) at the nanoparticle (NP) biointerface governs biological identity and fate, yet its quantitative characterization remains limited by separation steps that perturb equilibrium and introduce artifacts [1,2]. Herein, we present a fractionation-free approach based on synchrotron small-angle X-ray scattering (SAXS) to directly quantify protein adsorption under native, *in situ* conditions. Experiments were performed at a high-throughput synchrotron beamline using an automated setup, ensuring sample integrity and statistical robustness (<0.3% intensity variation). The method exploits the proportionality between scattering intensity ($d\Sigma/d\Omega$) and mass concentration to decouple the contributions of free and adsorbed proteins, directly extracting the adsorbed mass in both single-protein (bovine serum albumin, BSA) and complex proteomic (human serum) systems in 30 nm silica NPs. Distinct SAXS signatures were observed, with corona formation dominating the low- q regime and free protein contributing primarily at high- q , as validated by analytical simulations. For SiO_2 -BSA systems, adsorption isotherms were well described by Langmuir and Hill models, yielding a maximum protein adsorption capacity of ~ 0.20 mg/mL, in agreement with theoretical predictions (0.22 and 0.23 mg/mL). Synchrotron radiation circular dichroism (SRCD) confirmed preservation of protein secondary structure upon adsorption. Zwitterionic-functionalized NPs suppressed low- q intensity changes, validating the sensitivity of the method to PC formation. Extension to human serum revealed markedly different behavior, with higher adsorption (~0.96 mg/mL) and isotherms best described by the Hill model, indicating negative cooperativity and competitive, multilayer adsorption. The absence of a clear saturation plateau and increased low- q slopes further support a heterogeneous, multilayer PC [3,4]. Hence, this work establishes synchrotron SAXS as a quantitative, non-invasive tool for probing PC formation without fractionation, preserving the corona's unique fingerprint and true physicochemical nature.

Keywords:
Small-Angle X-ray Scattering, protein corona quantification, fractionation-free, synchrotron SAXS



a) SAXS profiles of SiO2 and SiO2-BSA mixtures. b) BSA adsorption isotherm (Langmuir/Hill). c) Schematic of BSA corona.

References:

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