Structural analysis of antibody complexes by inverse contrast-matching small-angle neutron scattering combined with size exclusion chromatography (SEC-iCM-SANS)

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Small-angle neutron scattering (SANS) has been effectively utilized for structural analysis of biomacromolecular complex in solution because each component can be distinguished by using contrast matching method with selectively deuterated molecules. In particular, inverse contrast matching (iCM) method is quite useful because it can suppress incoherent scattering from hydrogen of solvent water by measuring nearly 75% deuterated biomolecules in 100% heavy water. Meanwhile, SEC-SAS, a combination of inline size-exclusion chromatography (SEC) and small-angle scattering measurements (SAS), has recently been developed to address the problem that undesirable contamination of aggregates and dissociated fragments prevent the precise analysis of target molecules. Currently SEC-SAS has become a popular option for small-angle X-ray scattering (SAXS), but not widely available yet for SANS. In this study [1], we applied the SEC-SAS technique to the iCM-SANS measurements (SEC-iCM-SANS) of antibody interaction systems: Immunoglobulin G (IgG) or its Fc fragment and 75% deuterated Fc-binding proteins (Fig. 1). As a result, we could confirm that bound species were successfully fractionated by SEC excluding aggregates and unbound molecules and immediately subsequent iCM-SANS measurements provided the scattering profiles of the target complexes alone, in which hydrogenated components in the complexes were selectively observable.

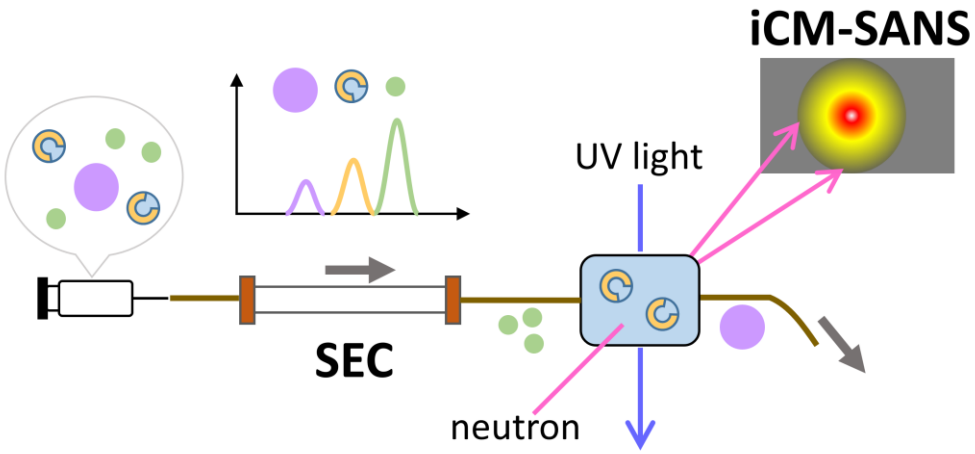

Figure 1. Schematic illustration of SEC-iCM-SANS measurement.

[1] Sato, N., Yogo, R., *et al.* (2021). *J. Biochem.*, mvab012.

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