# Solution structure of multi-domain protein as studied by small-angle neutron scattering

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Many proteins are composed of multiple structural domains connected by flexible linkers, and their biological functions are governed not only by the structure of each individual domain but also by the relative arrangement and dynamic behavior of these domains in solution. Small-angle neutron scattering (SANS) is well suited to characterizing solution structures of protein.

Antibodies are a representative example of such multi-domain proteins. They recognize antigens through their Fab regions and subsequently exert effector functions, such as cytotoxic activity, through their Fc regions. We have previously shown that an allosteric coupling exists between the Fab and Fc regions. These findings suggest that the effect of antigen binding is transmitted through molecular pathways hidden within the flexible architecture of the antibody molecule, thereby inducing allosteric conformational changes at distant interaction sites for effector molecules and exposing new functional sites. Elucidating this mechanism at the atomic level would enable the design of biomolecular functions by exploiting the intrinsic flexibility of biomolecules. In this study, we characterized the solution structure of human immunoglobulin G (IgG) as a model multi-domain protein. In particular, we prepared single-arm IgG (sIgG; Fig. 1a), in which one Fab region of IgG was removed, and performed SANS measurements in 100% $D_2O$. The SANS experiments were carried out using SANS-U at ISSP, JRR-3. Measurements were performed at sample-to-detector distances of 4 m and 1 m to cover a $q$-range of 0.01 - 0.4 $Å^{-1}$.

Figure 1b shows the analytical ultracentrifugation (AUC) results of the sample used in the SANS measurements. Although a small amount of aggregates (weight fraction = 2.9%) was present, sIgG was confirmed to be the major component. Figure 1c shows the SANS profile obtained after removing the aggregate contribution using the AUC-SAS treatment. The radius of gyration, $R_g$, obtained from Guinier analysis (inset of Fig. 1c) was 45.7 ± 1.8 Å, which is smaller than that of full-length IgG, 50.0 ± 1.3 Å. By combining these data with molecular dynamics simulations, we clarify the solution structure in more detail.

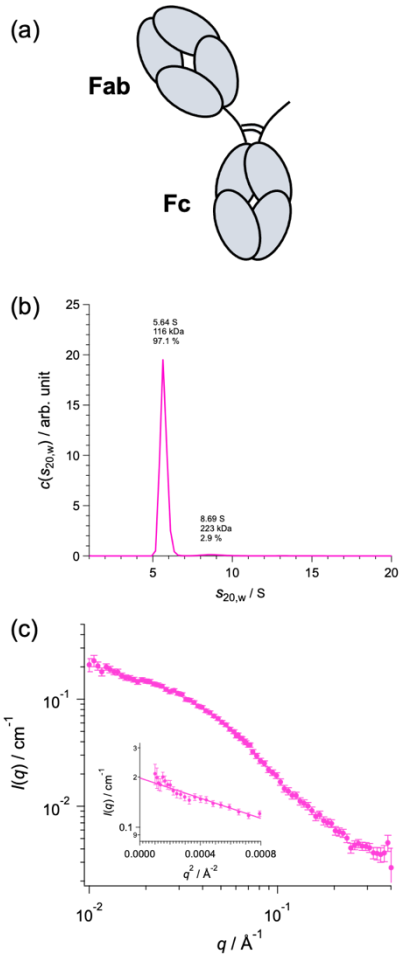


Figure 1. (a) Schematic illustration of single-arm IgG (sIgG). (b) Weight concentration distribution $c(s_{20,w})$ as a function of sedimentation coefficient $s_{20,w}$ obtained by AUC. (c) AUC-SAS treated SANS profile of sIgG. The inset shows its Guinier plot. The solid line represents the least-squares fit using the Guinier formula.