

Fc plasticity studies by Small Angle X-ray scattering (SAXS)

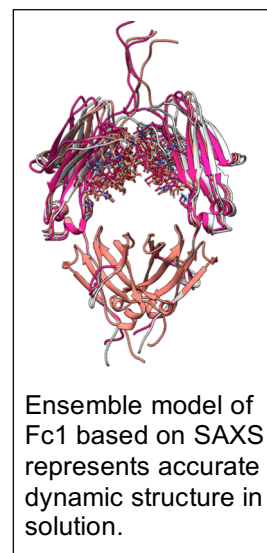
Soumya G. Remesh¹, Anthony Armstrong², Jinquan Luo², Andrew Mahan², Michal Hammel¹

¹Molecular Biophysics and Integrated Biology, Lawrence Berkeley National Laboratory, Berkeley, CA - 94720

²Janssen BioTherapeutics, Janssen R&D, LLC., 1400 McKean Road, Spring House, PA - 19477

sgremesh@lbl.gov.

The fragment crystallizable (Fc) region of immunoglobulin G (IgG) serves a variety of biological functions including binding to specific Fc γ receptors (Fc γ R) to trigger immunological events like, antibody-dependent cell-mediated cytotoxicity (ADCC) and opsonization (1, 2). Here we study how the positional variation of Fc-CH2 domains may affect the selectivity of different Fcs for distinct Fc γ Rs, which is specific for immune activation (3, 4). We developed SAXS based modeling approach to elucidate conformational plasticity of Fcs variants (IgG1-Fc1, IgG2-Fc2, IgG4-Fc4 and an IgG1-FcYTE mutant). Although, the differences in radii of gyration (Rg) as determined by SAXS were negligible, the SAXS indicator for flexibility (Kratky plot) showed that Fc2 is the most and Fc1 the least flexible molecule. Next, we developed a modeling tool using relatively inexpensive molecular dynamics (MD) simulations in combination with the SAXS measurements to validate the conformational plasticity of Fcs (5). By analyzing selected conformers in the ensemble we show that Fc flexibility derives primarily from the tilt and twist motion of CH2 domain, which closely correlates with the theoretical prediction (6). Intriguingly, SAXS modeling also correlated the extent of deglycosylation within some degree of confidence compared to our mass spectroscopy analysis. To our knowledge the results presented here are the first known experimental visualization of the plasticity of Fcs in solution and their differences between various Fc isotypes.



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