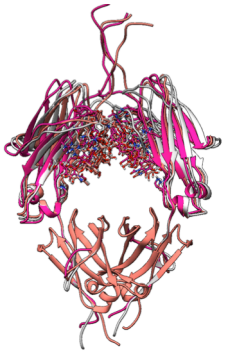
Fc plasticity studies by Small Angle X-ray scattering (SAXS)

Soumya G. Remesh[1], Anthony Armstrong[2], Jinquan Luo[2], Andrew Mahan[2], Michal Hammel[1]
[1]Molecular Biophysics and Integrated Biology, Lawrence Berkeley National Laboratory, Berkeley, CA - 94720
[2] Janssen 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 Fcγ receptors (FcγR) 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 FcγRs, 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.



Ensemble model of Fc1 based on SAXS represents accurate dynamic structure in solution.

1. IgF Fc Receptors. *Annu. Rev. Cell. Dev. Biol.* **19**, 275-290 (2001).
2. Fc Receptors and their interactions with Immunoglobulins. *Annu. Rev. Cell. Dev. Biol.* **12**, 181-220 (1996).
3. Structural basis for Fc gammaRIIa recognition of human IgG and formation of inflammatory signaling complexes. *J Immunol* **187**, 3208-3217 (2011).
4. The structure of a human type III Fcgamma receptor in complex with Fc. *J Biol Chem* **276**, 16469-16477 (2001).
5. Structure and flexibility within proteins as identified through small angle X-ray scattering. *General Physiology and Biophysics* **28**, 174-189 (2009).
6. Immunoglobulin G1 Fc domain motions: implications for Fc engineering. *J Mol Biol* **426**, 1799-1811 (2014).

Support: This work was supported by Jansen R&D, LLC.