Extensive substrate recognition by streptococcal antibody-degrading enzymes

Abigail S. L. Sudol[1], John Butler[1], Dylan P. Ivory[1], Ivo Tews[1] and Max Crispin[1]

[1]University of Southampton, Highfield Campus (Building 85), University Road, SO17 1BJ:

as6c23@soton.ac.uk

The bacterium *Streptococcus pyogenes* employs several enzymes in its evasion of the human immune system. Such enzymes include IgG-degrading enzyme of *S. pyogenes* (IdeS) [1], a protease which deactivates IgG antibodies by cleaving within the lower hinge region, thereby separating the antigen-binding $F(ab')_2$ regions from the immune-engaging Fc region. Additionally, the bacterium utilises two endoglycosidases, EndoS and EndoS2 [2], which inactivate IgG by removing *N*-linked glycosylation from the antibody Fc region. These enzymes are highly specific for IgG, which has led to their use in a wide range of clinical and biotechnological settings. IdeS has been clinically approved as a pre-treatment for hypersensitised patients undergoing kidney transplantation [3], while EndoS and EndoS2 have additional applications in antibody glycan remodelling [4]. We sought to understand the molecular basis of IgG recognition by these enzymes using X-ray crystallography; however, crystallisation of antibody-protein complexes is often hindered by the inherent ability of the IgG Fc region to self-crystallise. Here, we present a panel of IgG1 Fc variants engineered to disfavour preferential Fc crystallisation, and have subsequently used this strategy to crystallise IdeS, EndoS and EndoS2 in complex with IgG1 Fc, all of which display extensive recognition towards their Fc substrate [5, 6]. Understanding the molecular basis of antibody recognition by bacterial enzymes will facilitate the development of next-generation enzymes for a range of clinical and biotechnological applications.

[1] von Pawel-Rammingen, U., Johansson, B. P. & Björck, L. (2001) *EMBO J.* **20**, 3046–3055

[2] Sjögren, J., Cosgrave, E. F. J., Allhorn, M., Nordgren, M., Björk, S., Olsson, F., Fredriksson, S. & Collin, M. (2015) *Glycobiology.* **25**(10), 1053– 1063

[3] Huang, E., Maldonado, A. Q., Kjellman, C. & Jordan, S. C. (2022) *Am J Transplant.* **22**, 691–697

[4] Tong, X., Li, T., Li, C. & Wang, L. X. (2018) *Biochemistry.* **57**, 5239–5246

[5] Sudol, A. S. L., Butler, J., Ivory, D. P., Tews, I. & Crispin, M. (2022) *Nat Commun.* **13**(1), 7801

[6] Sudol A. S. L., Crispin, M. & Tews, I. (2024) *J Biol Chem.* **1**, 107245

We are grateful to the beamline scientists on I03 at Diamond Light Source (DLS) and ID30A-3 at the European Synchrotron Radiation Facility (ESRF), and to Chris Holes for his support with the macromolecular crystallisation facility at the University of Southampton. This work was supported by DLS, ESRF (MX2373) and the School of Biological Sciences, University of Southampton.