MX2447 interim report

The **Southampton, Bristol, Cardiff, Exeter, KCL, UCL Royal Free Block Allocation Group (BAG)** reports four papers, six SAXS datasets deposited in the SASBDB, and six structures deposited in the PDB in the past year. We meanwhile have successfully used ID29 serial during commissioning / first user operation (MX 2438) and propose to integrate the activity into the bag by joining Exeter. The BAG submits three highlight reports and one ESRF general news (https://www.esrf.fr/home/news/general/content-news/general/antibody-rigidity-regulates-immune-activity.html).

Beamline performance

The bag thanks for support at beam lines. Particular thanks to the BM07 beamline that provided excellent support on preparation for X-Fel beamtimes at SACLA and PAL. Again, the *ic*OS and online spectroscopy has provided essential information. Also, with many thanks for the excellent support at BM29 which has been delivering essential data on structure dynamics (see ESRF general news).

PDB codes (beamline) published by the bag:

6TKF (ID23-1), 6TKB (ID30B), 8A3I (ID30B), 8A3K (ID30B), 8A47 (ID30A-3), 8A48 (ID30A-3).

SADBDB depositions published by the bag:

SASDLG4, SASDLH4, SASDLF4, SASDLL4, SASDLJ4, SASDLK4 (BM29)

Publications by the bag:

- Yu X, Orr CM, Chan HTC, James S, Penfold CA, Kim J, Inzhelevskaya T, Mockridge CI, Cox KL, Essex JW, Tews I, Glennie MJ, Cragg MS. Reducing affinity as a strategy to boost immunomodulatory antibody agonism. Nature. 2023 Feb;614(7948):539-547. doi: 10.1038/s41586-022-05673-2. Epub 2023 Feb 1.
  This paper uses the structure 6FAX (ID23-1) reported earlier.
- Naudin EA, Albanese KI, Smith AJ, Mylemans B, Baker EG, Weiner OD, Andrews DM, Tigue N, Savery NJ, Woolfson DN. From peptides to proteins: coiled-coil tetramers to single-chain 4-helix bundles. Chem Sci. 2022 Sep 20;13(38):11330-11340. doi: 10.1039/d2sc04479j. eCollection 2022 Oct 5.
  This paper published structures 8A3I (ID30B) and 8A3K (ID30B).
- Sudol ASL, Butler J, Ivory DP, Tews I, Crispin M. Extensive substrate recognition by the streptococcal antibody-degrading enzymes IdeS and EndoS. Nat Commun. 2022 Dec 17;13(1):7801. doi: 10.1038/s41467-022-35340-z.
  This paper published structures 8A47 (ID30A-3), 8A48 (ID30A-3).
- Orr CM, Fisher H, Yu X, Chan CH, Gao Y, Duriez PJ, Booth SG, Elliott I, Inzhelevskaya T, Mockridge I, Penfold CA, Wagner A, Glennie MJ, White AL, Essex JW, Pearson AR, Cragg MS, Tews I. Hinge disulfides in human IgG2 CD40 antibodies modulate receptor signaling by regulation of conformation and flexibility. Sci Immunol. 2022 Jul 15;7(73):eabm3723. doi: 10.1126/sciimmunol.abm3723. Epub 2022 Jul 8.

This paper published structures 6TKF (ID23-1), 6TKB (ID30B) and the SASBDB entries SASDLG4, SASDLH4, SASDLF4, SASDLL4, SASDLJ4, SASDLK4 (BM29).

Note on sending samples:

We continue to have problems with shipping form the UK to the EU, however the situation has somewhat improved from the last round. As we have learnt that hard way to avoid terms like “serum free”, “affinity purified” and “free of microorganisms” we did not encounter customs problems, but still have had severe delays in receiving samples. Since we had earlier lost samples in one dewar (that had warmed up) we took the decision at the last trip to split expensive samples over two shipments (at our own cost). Again, thank you for the help we have received from ESRF stores, the beamline scientists, and the User office to resolve various shipping problems!

Note on travel:

SAP Concur works reasonably well with travel planning and tickets, though seems somewhat inflexible for some air combinations (time, airports), which meant that we booked some tickets ourselves, thank you for re-imbursing these. If one wanted to travel by train then the booking system would not allow this

Extensive substrate recognition by the streptococcal antibody-degrading enzymes IdeS and EndoS

Abigail S.L. Sudol, John Butler, Dylan P. Ivory, Ivo Tews & Max Crispin

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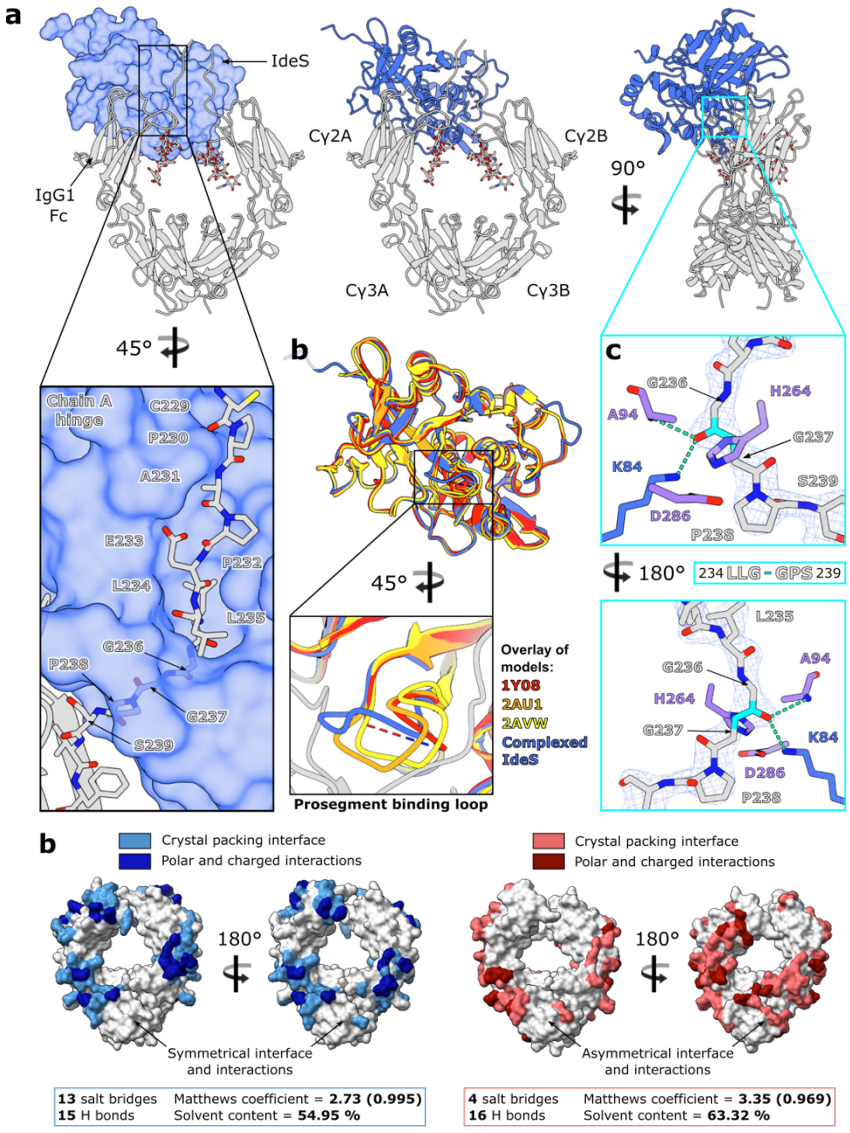
Enzymatic cleavage of IgG antibodies is a common strategy used by pathogenic bacteria to ablate immune effector function. The *Streptococcus pyogenes* bacterium secretes the protease IdeS and the glycosidase EndoS, which specifically catalyse cleavage and deglycosylation of human IgG, respectively. IdeS has received clinical approval for kidney transplantation in hypersensitised individuals, while EndoS has found application in engineering antibody glycosylation. The work reveals the molecular basis of antibody recognition by bacterial enzymes, providing a template for the development of next-generation enzymes.



(a) The IdeS protease displays extensive Fc recognition and encases the antibody hinge. Understanding the molecular basis of this interaction is critical for expanding clinical and biotechnological use, as deactivation of serum IgG can strengthen the potency of therapeutic antibodies. The structural information may thus help in the development of anti-streptococcal biologics resistant to enzyme-mediated degradation.

(b) Key to success in this study was Fc-engineering, where variation of the protein led to loss of the classic interaction in crystal contacts. The "less crystallisable" Fc variant was then used to obtain the crystal complexes.

Published in Nature Communications 13 (2022): 7801.
PDB depositions 2: 8A47, 8A48.
Crystallographic data were collected at ID30A-3.

From peptides to proteins: coiled-coil tetramers to single-chain 4-helix bundles

Elise A. Naudin[1], Katherine I. Albanese[1,2], Abigail J. Smith[3], Bram Mylemans[1,2], Emily G. Baker[1,3], Orion D. Weiner[4], David M. Andrews[5], Natalie Tigue[6], Nigel J. Savery[3,7] & Derek N. Woolfson[1,2,3,7]

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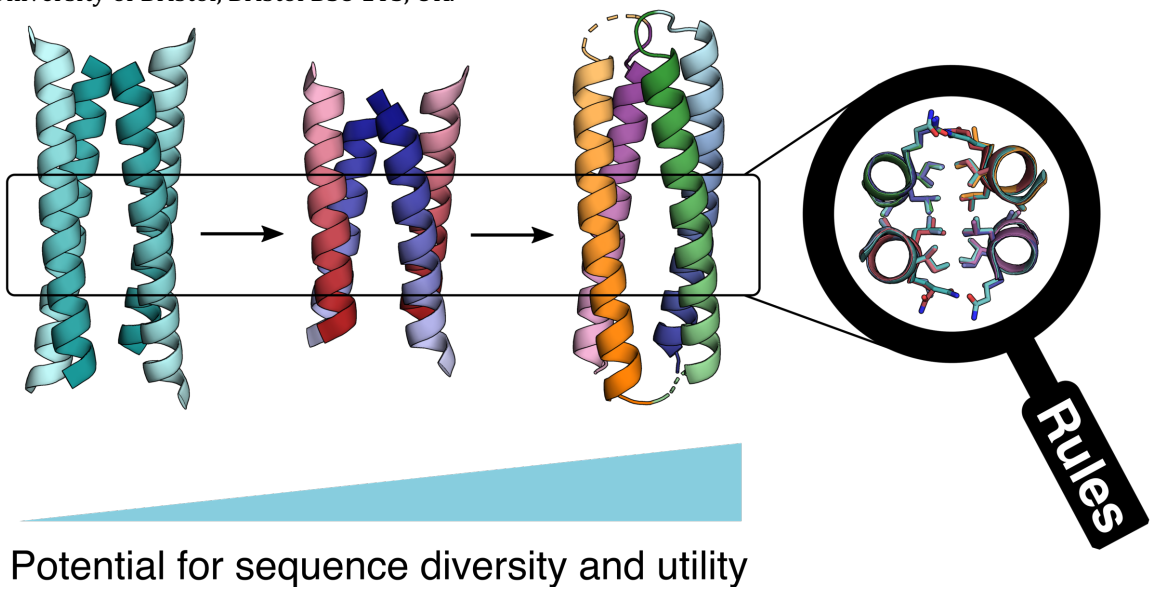
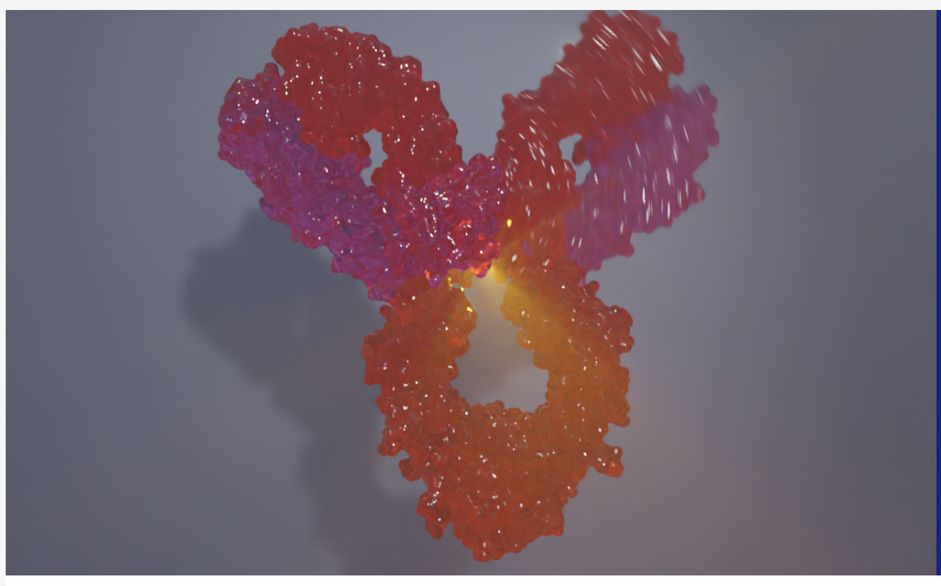


The design of completely synthetic proteins from first principles –de novo protein design– is challenging. This is because, despite recent advances in computational protein-structure prediction and design, we do not understand fully the sequence-to-structure relationships for protein folding, assembly, and stabilization. Antiparallel 4-helix bundles are amongst the most studied scaffolds for de novo protein design. We set out to re-examine this target, and to determine clear sequence-to-structure relationships, or design rules, for the structure. Our aim was to determine a common and robust sequence background for designing multiple de novo 4-helix bundles. In turn, this could be used in chemical and synthetic biology to direct protein-protein interactions and as scaffolds for functional protein design.

Our approach starts by analyzing known antiparallel 4-helix coiled-coil structures to deduce design rules. In terms of the heptad repeat, abcdefg –i.e., the sequence signature of many helical bundles– the key features that we identify are: a = Leu, d = Ile, e = Ala, g = Gln, and the use of complementary charged residues at b and c. Next, we implement these rules in the rational design of synthetic peptides to form antiparallel homo- and heterotetramers. Finally, we use the sequence of the homotetramer to derive in one step a single-chain 4-helix-bundle protein for recombinant production in *E. coli*. All of the assembled designs are confirmed in aqueous solution using biophysical methods, and ultimately by determining high-resolution X-ray crystal structures. Our route from peptides to proteins provides an understanding of the role of each residue in each design.

Published in Chem. Sci. 13 (2022), 11330–11340.
PDB depositions 2: 8A3I, 8A3K.
Crystallographic data were collected at ID30B.

Antibody rigidity regulates immune activity



13-07-2022

Scientists at the University of Southampton have gained unprecedented new insight into the key properties of an antibody needed to stimulate immune activity to fight off cancer, using the ESRF's structural biology beamlines, among others.

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The interdisciplinary study, published in Science Immunology, revealed how changing the flexibility of the antibody could stimulate a stronger immune response. The findings have enabled the team to design antibodies to activate important receptors on immune cells to "fire them up" and deliver more powerful anti-cancer effects. The researchers believe their findings could pave the way to improve antibody drugs that target cancer, as well as automimmune diseases.

In the study, the team investigated antibody drugs targeting the receptor CD40 for cancer treatment. Clinical development has been hampered by a lack of understanding of how to stimulate the receptors to the right level. The problem being that if antibodies are too active they can become toxic. Previous research by the same team had shown that a specific type of antibody called IgG2 is uniquely suited as a template for pharmaceutical intervention, since it is more active than other antibody types. However, the reason why it is more active had not been determined. What was known, however, is that the structure between the antibody arms, the so called hinges, changes over time.

This latest research harnesses this property of the hinge and explains how it works: the researchers call this process "disulfide-switching". In their study, the team analysed the effect of modifying the hinge and used a combination of biological activity assays, structural biology, and computational chemistry to study how disulfide switching alters antibody structure and activity.

Ivo Tews, Associate Professor in Structural Biology at the University of Southampton, explains the methodology: "Our approach was to analyse the structure of the antibody in atomic detail, using X-ray crystallography at the ESRF and Diamond Light Source. While the resulting picture is very accurate, the information on how they move their 'arms' is missing".

In order to overcome this obstacle, the team joined forces with Mark Tully, scientist at the ESRF, and used small-angle X-ray scattering at beamline BM29. "Using this technique we could show, in solution, the variation of conformational stability of the various hinge region mutants", he says. Through this detailed study of the hinge, the team revealed that more compact, rigid antibodies are more active than their flexible counterparts.

Professor Mark Cragg, of the Centre for Cancer Immunology at the University of Southampton, explains the repercussions of the findings: "This study has given us new information about how to engineer antibodies to deliver a better immune response. We propose that more rigid antibodies enable the receptors to be bound closer together on the cell surface, promoting receptor clustering and stronger signalling for activity. This means by modifying the hinge we can now generate more or less active antibodies in a more predictable way."

"The results could provide a highly controlled and tractable means of developing antibodies for clinical use in future immunostimulatory antibody drugs", concludes Tews.

REFERENCE:

Orr, C. M. et al, Science Immunology, 8 July 2022. DOI: 10.1126/sciimmunol.abm3723. www.science.org/doi/10.1126/sciimmunol.abm3723

Top image: Flexibility of the monoclonal antibody F(ab) arms is conferred by the hinge region disulphide structure. Credits: C. Orr