The Doyle group is working on glutamate receptors (NMDAR) interactions, playing a central role in cognitive processes and long-term memory. Loss of NMDAR activity in synapses is associated with neurological diseases such as Alzheimer's. The proteins PSD-95 and SAP-102 contain multiple protein-protein interaction domains and localise to the glutamate receptors, of which structures of the PDZ domains were determined (PDZ stands for post synaptic density protein PSD95, Drosophila disc large tumor suppressor Dlg1, and zonula occludens-1 protein zo-1). ONgoing work studies the selectivity of SAP-102 and PSD-95 PDZ domains to the NMDA receptors NR2A and NR2B by co-crystal structures.

The Tews group study proteins of the human immune system and (anti)-microbial targets. The work in immunology focuses on study of structures of complexes between monoclonal antibodies (Fab fragments) and the receptors CD32 and CD40. Studies are undertaken with the vision of improving the use of mAb in cancer or in therapy of autoimmune diseases. The work on CD40, delivering a key complex between Fab and CD40, has recently been published in Cancer Cell. The CD32 project includes a structure determination, SAXS data, and molecular modelling and is in revision with Biophys J. Work on Ox40 has been reviewed in Molecular Immunology. Work on lipid antigen presentation by the CD1 receptor is continued and was published in PNAS. In the microbiology field, we continue to explore mechanisms of biofilm formation and dispersal and have determined bacterial phosphodiesterases and PAS signalling domains, and published some of the data in Scientific Reports. The study of nutrient stress in marine microbiology has lead to determination of ABC transporter substrate binding domains (manuscript in preparation) and housekeeping enzymes induced under nutrient stress, such as the fructose-bisphosphate aldolases.

The Roach group have two areas of interest for structure determination, members of the radical SAM superfamily and antibacterial targets. The group have perfomed structure determinations of proteins in the complex maturation system that leads to the activation of [FeFe]-hydrogenase, namely structure and interactions between the three maturases HydG, HydE and HydF. A recent success was in the study of antibacterial targets, where polyphosphate kinases have been studied and been published in PNAS.

The Werner group pursues an integrative biology approach to antigen processing and cell signalling, studying the MHC I antigen presentation and peptide selection by endoplasmic reticulum aminopeptidase I (ERAP1). MHC I is studied for plasticity in the selection process of antigen peptides and its implications for immune system modulation. ERAP1 is studied for allotype variation that is associated with several autoimmune diseases, rendering the enzyme a potential drug target. The multi-technique structural biology approaches to define molecular mechanisms of disease phenotypes. The functional implications of protein dynamics are
a particular focus of the group. This proposal integrates SAXS and NMR data.

The Littlechild / Isupov groups study thermophilic hydrolase enzymes including novel esterases, lactonases and epoxide hydrolases, all of which have potential applications for industrial biotechnology. The Harmer group has determined structures of enzymes involved in the synthesis of the unusual sugar dihydohydroxystreptose in the *Coxiella burnetii* O-antigen. The group have also studied gene regulation of Vitamin B6 biosynthesis, the biosynthesis of the *Burkholderia pseudomallei* capsular polysaccharide, and determined the structure of the PdxR protein.

The Hough group are investigating the structure and function of copper nitrite reductase (CuNiR) where we aim to understand the mechanisms of Cu incorporation and substrate approach, binding and product escape. Multiple site-directed mutants of CuNiR are studied to (i) measure anomalous data at wavelengths of 1.2 and 1.33 Å allowing the use Cu (rather than Zn) loading at the catalytic centre; (ii) characterise the substrate-binding mode; (iii) assess substrate specificity by soaking small molecules into the enzyme active site (e.g. formate, malonate, acetate). The use of online UV-vis microspectrophotometry is required for assessment of the type 1 Cu redox state in the crystals before and after data collection. Binding of ligands to two heme peroxidases is also examined: the multifunctional dehaloperoxidase and a dye-type peroxidase A. The aim is to determine high-resolution structures of azole and alcohol substrate complexes with correlated single crystal UV-vis or Raman spectroscopy.

The Coker group address viral targets and continue to work on neurodegenerative diseases. Jon Cooper submits a highlight report on pullulan-hydrolysing enzymes, more commonly known as debranching enzymes for starch and other polysaccharides.

The Kolstoe group is leaving the bag.

PDBs

- 6FAX
- 5LL0, 5LLB and 5LLF

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Structure and function of the type III pullulan hydrolase from *Thermococcus kodakarensis*

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Pullulan-hydrolysing enzymes, more commonly known as debranching enzymes for starch and other polysaccharides, are of great interest and have been widely used in the starch saccharification industry. Type III pullulan hydrolase from *Thermococcus kodakarensis* (TK-PUL) possesses both pullulanase and *α*-amylase activities. Until now, only two enzymes in this class, which are capable of hydrolysing both *α*-1,4- and *α*-1,6-glycosidic bonds in pullulan to produce a mixture of maltose, panose and maltotriose, have been described. TK-PUL shows highest activity in the temperature range of 95 - 100 °C and has a pH optimum in the range of 3.5 - 4.2. Its unique ability to hydrolyse maltotriose into maltose and glucose has not been reported in other homologous enzymes. The crystal structure of TK-PUL has been determined at a resolution of 2.8 Å using synchrotron radiation and represents the first analysis of a type III pullulan hydrolyse (Fig. 1). The structure reveals that the last part of the N-terminal domain and the C-terminal domain are significantly different from homologous structures. In addition, the loop regions at the active site end of the central catalytic domain are quite different with important implications for substrate specificity. The enzyme has a well-defined calcium binding site and possesses a rare vicinal disulphide bridge. The thermostability of TK-PUL and its homologues may be attributable to several factors including the increased content of salt bridges, helical segments, Pro, Arg and Tyr residues and the decreased content of Ser.

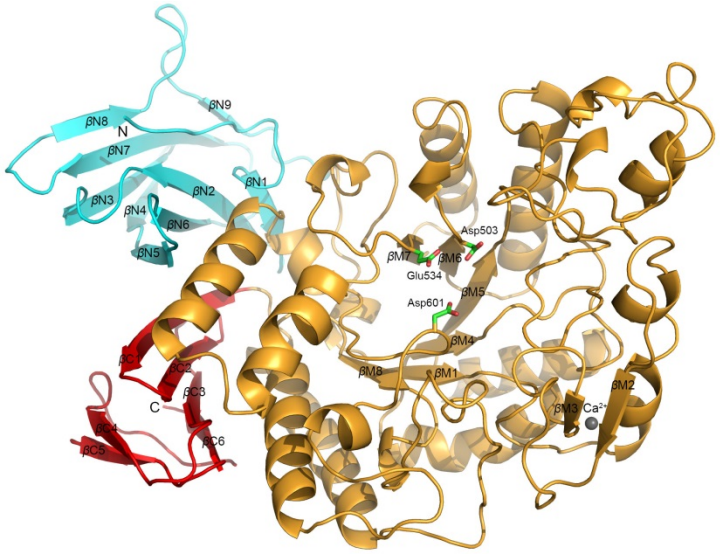

Fig. 1. The crystal structure of TK-PUL. The N-terminal, central and C-terminal domains are coloured as cyan, orange and red, respectively. The three residues forming the catalytic triad are shown in ball-and-stick representation and the calcium ion is shown as grey sphere. The calcium-binding subdomain is visible on the lower right hand side of the central domain and the kernel-like subdomain appears on the upper-right.

Complex interplay between epitope specificity and isotype dictates the biological activity of anti-human CD40 antibodies

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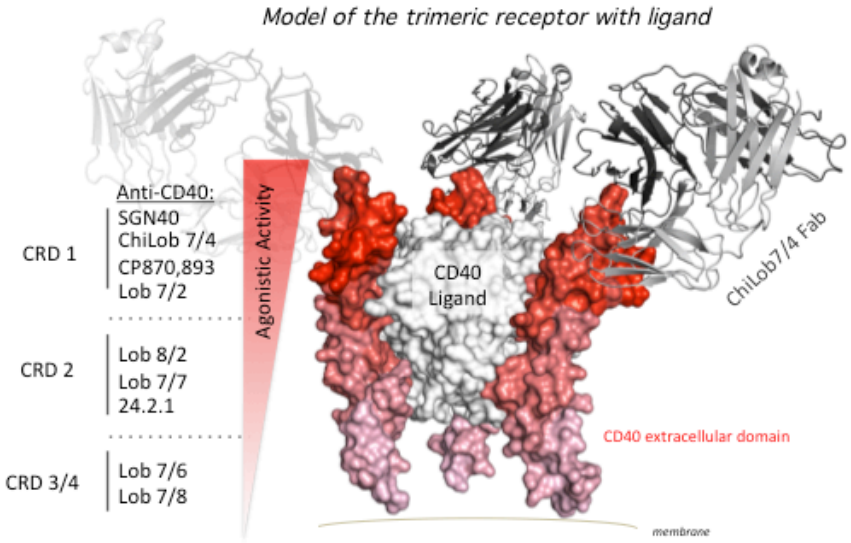
Monoclonal antibodies (mAb) that regulate immune responses are providing potent therapeutics for treating cancer and autoimmune disease. One target is CD40, a Tumor Necrosis Factor Receptor (TNFR) superfamily member that is expressed on antigen presenting cells involved in regulating adaptive immunity. mAb targeting this receptor show diverse activities, from strong agonism to powerful antagonism, however the rules determining activity are unclear. Using a comprehensive panel of anti-hCD40 mAb and a range of *in vitro* and *in vivo* approaches we demonstrate that agonistic activity is largely dependent on mAb epitope, which dictates their level of activity.



How do we suggest epitope recognition modulates activity? Both epitope specificity and isotype have separately been shown to regulate anti-hCD40 mAb function. Mouse IgG1 constant regions can promote CD40 mAb agonism through interaction with FcgRIIB on adjacent cells. The mAb tested here were expressed with the same m1 backbone, but a range of activities were observed. Those mAb engaging epitopes in CRD1 (ChiLob 7/4, SGN40, CP870,893 and Lob 7/2) were agonistic, whereas those that bound CRD2-4 were not. We propose dependence on membrane-distal CRD1 binding reflects the requirement for the mAb Fc to engage FcgRII on adjacent cells, while steric constraints may prevent optimal FcgR engagement for mAb that bind epitopes closer to the membrane.

Cancer Cell, in press. PDB 6FAX.