

## 5. Susan Lea

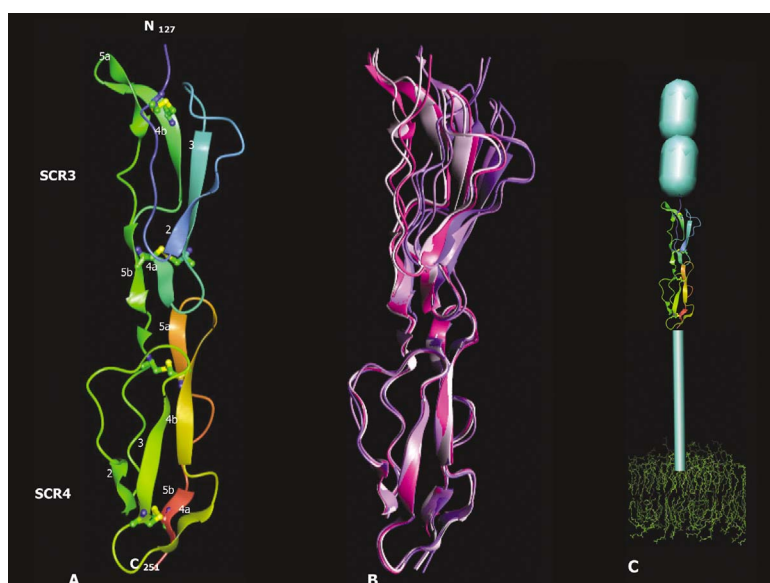
### Towards a structural understanding of pathogenesis.

Knowledge of the way in which an invading pathogen interacts with its host at a molecular level is an essential aid to understanding the nature and extent of disease caused. My group aims to use a variety of techniques to probe the interactions that characterise different disease processes. Central to this approach is the use of X-ray crystallography to determine the structures of individual host- or pathogen components, with a view in the longer term to examining the atomic structure of important host- pathogen complexes. The major targets are protein based but we are also involved in projects where folded RNAs provide the structural target. To aid understanding of biochemical and structural data we use a variety of other biophysical techniques (including surface plasmon resonance) to further characterise the biological systems under study. The main systems studied during 2000-2001 are summarised below.

#### 5.1 Decay Accelerating Factor (CD55)

Jason Billington, Petra Lukacik with the groups of Dr. D. Evans (University of Glasgow) and Dr. R. Smith (AdProTech Ltd)

Decay Accelerating Factor (DAF, CD55) is a membrane associated regulator of complement activation that contains four ~60 amino acid long consensus sequences termed complement control protein repeats (CCPs) or short consensus repeats (SCRs). The four SCRs comprise the functional portion of the protein and are linked to the plasma membrane via a heavily glycosylated serine/threonine rich linker and a glycosylphosphatidylinositol (GPI) anchor. CD55 is a multi-functional molecule accelerating the decay of both the classical and alternative pathway C3 convertases, binding CD97 a member of the EGF-TM7 family whose expression is rapidly upregulated on T and B cells following activation, and acting as the receptor for a variety of viral and bacterial pathogens. Domain swapping studies for a variety of CD55 interactions have shown that multiple domains are essential for biological function and the precise arrangement of any one SCR domain with respect to the others is therefore crucial to a full understanding of the biology.



**Figure 1** All figures drawn using AESOP (M.E.M. Noble, unpublished program).

(A) Secondary structure and location of disulphide bonds in CD5534. Strands are labeled according to the convention. Note there is no strand 1 as the hydrogen-bonding pattern of these residues does not meet the strict criteria for definition of a b-strand. (B) Variation in orientation between SCR domains 3 & 4 in the five independent copies of CD5534 found in the different crystal forms. (C) Model for topology of CD55 in the membrane based on our structure of CD5534

Using data collected from ESRF and Daresbury we have solved the structure (at 1.7Å) of the lower two SCR domains of CD55 in several crystal forms using MIRAS (Figure 1 - Williams et al in the press). This structure suggests that pathogen binding of CD55 has arisen via convergent evolution, but a better understanding of the natural, complement regulating, functions of the molecule requires structural information about the other SCR domains. We have collected various data from P1 crystals of the all four extracellular SCR domains including native, potentially Au and Pt derivatised crystals and SeMet crystals. The crystals are fairly poorly ordered and it has required much optimisation of crystal growth and freezing conditions to collect data to a moderate resolution. None of the data collected have allowed phasing of the structure to date.

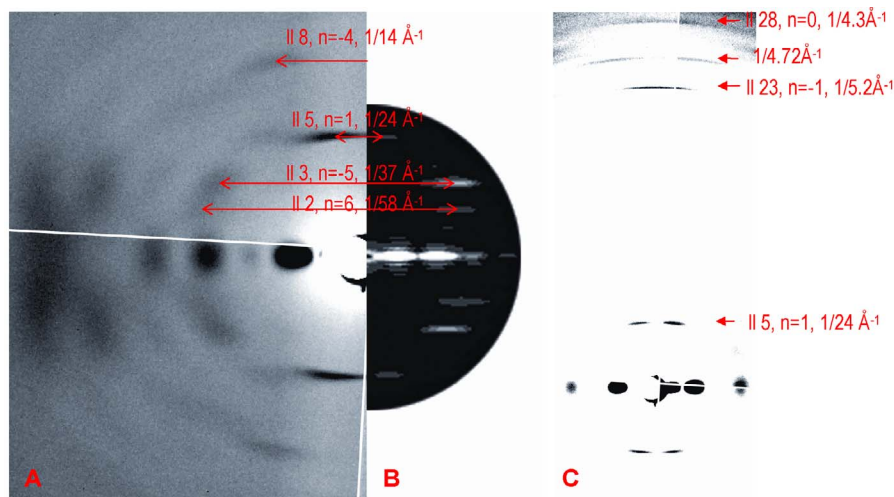
## References

1. Williams, P., Chaudhry, Y., Goodfellow, I., Billington, J., Spiller, B., Evans, D. & Lea, S. (2003) Mapping CD55 function: the structure of two pathogen-binding domains at 1.7Å. *J.Biol.Chem.* (in the press)
2. Lea, S. (2002) Interactions of CD55 with non-C ligands. *Biochem. Soc. Trans* (in the press)
3. Lea, S., Powell, R., and Evans, D. (1999). Crystallization and preliminary X-ray diffraction analysis of a biologically active fragment of CD55, *Acta-Crystallogr-D-Biol-Crystallogr* 55, 1198-200.
4. Lea, S., Powell, R. M., McKee, T., Evans, D. J., Brown, D., Stuart, D. I., and van der Merwe, P. A. (1998). Determination of the affinity and kinetic constants for the interaction between the human virus echovirus 11 and its cellular receptor, CD55, *J-Biol-Chem* 273, 30443-7.

## 5.2 *Shigella flexnerii* Type Three Secretion System

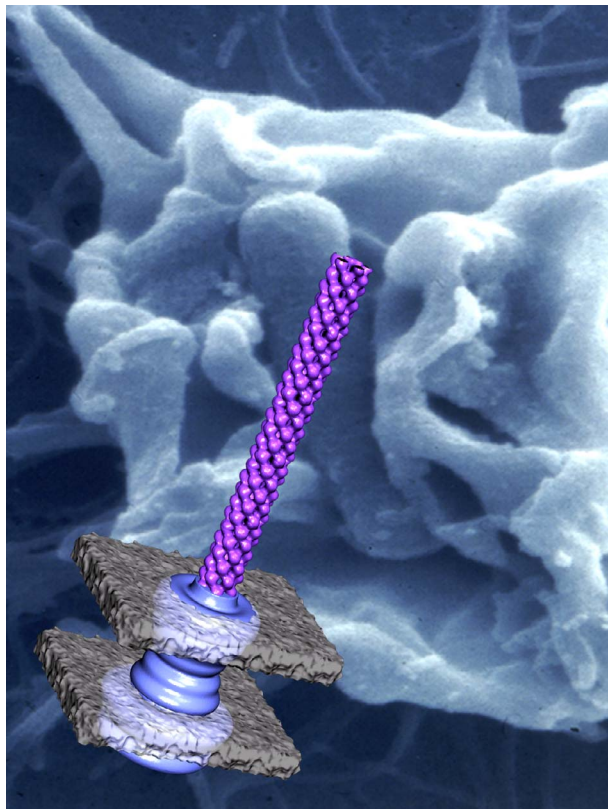
### Frank Cordes with the group of Dr. A. Blocker (Sir William Dunn School of Pathology)

Gram-negative bacteria commonly interact with animal and plant hosts using type III secretion systems (TTSSs) for translocation of proteins into eukaryotic cells during infection. Ten of the twenty-five TTSS encoding-genes are homologous to components of the bacterial flagellar basal body which the TTSS needle complex morphologically resembles. This indicates a common ancestry although no TTSS sequence homologues for the genes encoding the flagellum are found. We are studying a variety of proteins involved in this system ranging from structural components of the secretion system (using X-ray fibre diffraction and EM reconstruction) to the ATPase that powers the system.



**Figure 2** (A) Half of the central region of X-ray fibre diffraction pattern from aligned needles taken at station 14.2 of the SRS, Daresbury. (B) Half of the averaged power spectrum generated from 5402 partially overlapping segments extracted from images of 108 negatively stained needles. For both images the peaks corresponding to specific layer lines are indicated with the layer line spacings and indices (determined as in text) shown. (C) XRFD pattern as shown in (A), but displaying the high-resolution layer lines.

We have used X-ray fibre diffraction (Figure 2) to determine the helical arrangement of the needle component of the TTSS (~5.6 subunits per turn, 24Å helical pitch) and negative stain EM reconstruction to yield a structure at ~16Å resolution (Figure 3). This structure has confirmed the analogies to the flagellar system and allows us to speculate about the structural basis of activation of secretion. Further studies of the needle will include attempts



**Figure 3** Composite image giving a model for the structure of the needle complex in the bacterial membranes using the needle reconstruction in combination with the earlier reconstruction of the basal body of the needle complex (Blocker et al 2001) overlaid on an image of a HeLa cell engulfing a single bacterium.

to purified needles in different activation states and X-ray crystallographic studies of the needle component and other isolated proteins from the TTSS complex.

## Reference

1. Cordes, F., Komoriya, K., Larquet, E., Yang, S., Egelman, E., Blocker, A. & Lea, S. Helical Structure of the Needle of the Type III Secretion System of *Shigella Flexneri* (manuscript submitted)

## 5.3 Bacterial Adhesins

**Jason Billington & David Pettigrew**

Bacterial adhesins are important virulence factors that allow colonisation of the human urogenital tract by *Escherichia coli*. The observation that many *E. coli* would haemagglutinate human erythrocytes led eventually to the realisation that a large number of these adhesins recognised and bound to CD55. These so-called Dr haemagglutinins include the fimbrial Dr, X and diarrhoea-associated F1845 adhesins and the afimbrial Afa adhesins. This family of molecules share a common genetic organisation and have similar nucleotide sequences but detailed analysis of the interaction determinants shows that they are dependent on different portions of CD55 for recognition and binding. To date we have no further information about the structures of these molecules or a more detailed characterisation of the interaction but recent work has begun to demonstrate a role for pathogen-CD55 interactions that goes beyond simply providing a convenient hook for the invading pathogen to catch at.

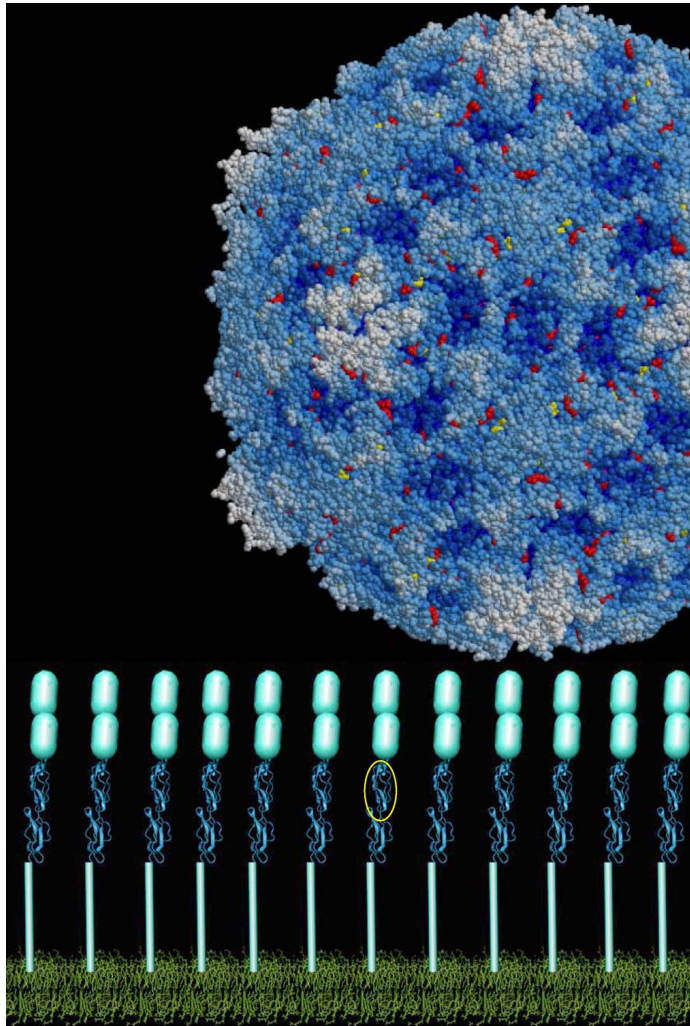
We have expression systems for members of all of these classes of bacterial adhesins and crystallisation trials of the proteins in isolation and in complex with CD55 are underway.

## 5.4 Echovirus 11

David Pettigrew with the group of Dr. D. Brown (University of Cambridge)

Picornaviruses and their receptors have been much studied by the crystallographic community. We have determined the structure of a clinical isolate (Figure 4) of one of these viruses that is known to use CD55 as the receptor for cellular infection. We now wish to extend these studies to attempt to determine the structure of the virus in complex with truncated forms of the receptor.

To date all data have been collected from crystals mounted at RT for reasons of disease security – we hope to develop contained freezing protocols to allow collection of complete data sets from single crystals so speeding data collection. Complexes will be generated by soaking or co-crystallisation of virus with receptor.



**Figurer 4** The Illustration depicts (at the same scale) a single particle of Echovirus 11 (Stuart *et al*, J.Virol. 2002) approaching a cell membrane in which multiple copies of our current model for CD55 are embedded. The domain to which more than 80% of the binding energy for the virus-receptor interaction can be attributed (Lea *et al*. J.Biol.Chem. 1995) is highlighted.

### Reference

1. Stuart, A., McKee, T., Williams, P., Stuart, D., Brown, T. & Lea, S. (2002) The structure of a DAF binding clinical isolate of echovirus 11 at 2.9Å resolution and mapping of variant virus sequences suggest location of a DAF binding site on the virion. J. Virol 76:7694-7704

## 5.5 EGF-TM7 family proteins

**Saskia Neudek, Rachel Abbott & Petra Lukacik in collaboration with the groups of Dr. P. Handford (Department of Biochemistry) and Dr. S. Gordon (Sir William Dunn School of Pathology)**

The EGF-TM7 family is a group of cell-surface molecules characterised by a unique chimaeric structure in which tandem EGF (Epidermal Growth Factor-like) repeats are coupled to a G-protein coupled receptor moiety via a mucin-like stalk. They are implicated in a range of biological function but are of particular interest to us due to the identification of one of these proteins (CD97) as a T-cell ligand for CD55. To date we have grown crystals of a natural variant of CD97 termed EMR2.

We have collected an optimised SAD data set for a Gd derivative of EMR2 to 2.5Å – these data allow positioning of the Gd sites but due to the low solvent content of the crystals (35%) do not allow complete structure solution.

We have Ba, Sr and Ca crystal forms (although the symmetry varies) and are currently producing SeMet labelled protein – a combination of data from these crystals should allow phasing of this structure. In the future we hope to study other isoforms of this molecule and a complex between CD97 and CD55

### Reference

1. Hsi-Hsien, L., Stacey, M., Saxby, C., Knott, V., Chaudhry, Y., Evans, D., Gordon, S., McKnight, A., Handford, P. & Lea, S. (2001) Molecular dissection of the CD55-CD97 complex provides insights into EGF-like domain mediated cell-cell interaction, *J-Biol. Chem.* 276:24160-24169

## 5.6 Complement System Components

**Jason Billington & Petra Lukacik in collaboration with the groups of Dr. P. Morgan (University of Wales College of Medicine) and Dr. R. Sim (MRC Immunochemistry Unit)**

The complement system is a highly evolved system of proteins that together constitute a major element of host defences, functioning in both innate and adaptive immunity. Activation of complement by bacterial or other pathogens proceeds through enzymatic amplification steps (which are tightly regulated by specific proteins) to generate protein fragments and complexes that mediate acute inflammatory reactions, clearance of foreign cells and killing of invading pathogenic organisms. Conditions that result in misguided, excessive or uncontrolled activation of complement lead to human disease. We have six complement proteins in crystallisation trials at present.

## 5.7 RNA Structure

**Antu Dey in collaboration with the groups of Dr. W. James (Sir William Dunn School of Pathology) and Prof. A. Lever (University of Cambridge)**

Folded RNAs are important for the life cycles of many viral pathogens and have provided us with long-term crystallographic challenges. Our work at present is focussed on RNAs derived from HIV (from which we have small crystals) and synthetic RNA aptamers designed to block binding of HIV gp120 to CD4. This work is currently at the stage of design of suitable crystallographic targets and determination of the affinities and kinetics that characterise aptamer-gp120 binding.