ESRF FULL 2-YEAR BLOCK ALLOCATION GROUP REVIEW REPORT

BAG RESPONSIBLE: **NOBLE**
EXPERIMENT NO: **MX-452**
LAST REVIEW DATE: **March 04**

Shift usage since last Biennial Review:

Allocated	123	Used	123	Cancelled by Users	0	Cancelled by ESRF	0
Total Number of Visits		28	Total Number of Visitors	112			

BAG Principle Investigators (indicate by # those left since last review, * those new since last review.)

Principal Investigator	Institute
Prof. M.E.M. Noble	University of Oxford
Prof. J.A. Endicot	University of Oxford
Prof. L.N. Johnson	University of Oxford
Dr. Elspeth Garman	University of Oxford
Dr Susan M. Lea	University of Oxford
Dr Ed Lowe	University of Oxford
Dr Jasper van Thor	University of Oxford
Dr Rick Lewis	University of Newcastle
Dr Mark Banfield*	University of Newcastle
Dr Kim Watson	University of Reading

Total Number of PDB submissions from data from ESRF beam lines since last report	26
Total Number of Publications resulting from data from ESRF beam lines since last report	21

List below the five most important publications directly resulting from data recorded either wholly or partially on ESRF beamlines (you must indicate [1] ESRF data only; [2] data from more than one source):

1. F.S. Cordes *et al.*, (2005) A novel fold for the factor H-binding protein BbCRASP-1 of *Borrelia burgdorferi*. *Nat. Struct. & Molec. Biol.* **12**, 276-277[1].

2. J.W. Murray *et al.*, (2005) Structure of a nonheme globin in environmental stress signaling. *Proc. Natl. Acad. Sci. USA* **102**, 17320-5[1].

3. N. MacDonald *et al.*, (2005) Molecular basis for the recognition of phosphorylated and phosphoacetylated histone H3 by 14-3-3. *Molec. Cell* **20**, 199-211[1].

4. J.F. Trempe *et al.*, (2005) Mechanism of Lys48-linked polyubiquitin chain recognition by the Mud1 UBA domain. *EMBO J.* **24**, 3178-89[1].

5. R. Honda *et al.*, (2005) The structure of cyclin E1/CDK2: implications for CDK2 activation and CDK2-independent roles.. *EMBO J.*. **24**, 452-63[1].

Summary (250 words maximum) of the results obtained since last biennial review:

The structures solved by *ab initio* phasing and molecular replacement relate to how both eukaryotic and prokaryotic cells respond to their environment. They extend from extracellular receptors (CD44 in the group of MN), and membrane-associated proteins (BbCRASP-1 from *Borrelia burgdorferi*, Dr and Afa adhesins from pathogenic *E. coli* and MxiH, the *Shigella flexneri* type three secretion system needle subunit in the group of SL) to the intracellular kinases (*Plasmodium falciparum* PfPK7 from the group of JE and CDK7 and CDK2/cyclin E from the group of LJ) that interface regulatory networks with the metabolic and structural proteins that effect cellular responses (14-3-3/phosphohistoneH3 complex from the group of JE). Our structural studies have provided insight into the diverse mechanisms used by UBA domains to recognise ubiquitin (the Dsk2 UBA/Ubl complex and Mud1 UBA domain determined in the groups of LJ, JE and MN). We have also solved structures of mutants and complexes of the proteins that we are studying, both to illuminate their structure-function relationships, and for the purpose of developing protocols in inhibitor design (e.g. mutants of the arylamine N-acetyltransferases (MN) and of CDK/cyclin complexes (MN, JAE)). We have probed the mechanism of action of a stress-responsive phosphatase from *Bacillus subtilis* and determined the structure of a kinase-recruitment domain, homologous in function and structure to the regulatory domains of a number of eukaryotic phosphatases (RL). We have also recently determined the structure of N-RsbR, and deduced that RsbT binds to RsbU and RsbR through a common binding motif (RL).

Global Summary:

This should occupy **one A4 page at maximum**. As well as a more complete overview of the activities of the BAG during the period under review, this section should also contain comments on the overall usefulness of the scheme to your BAG. These may include suggestions at to how the scheme can be improved and should be made in a constructive manner.

This has been a particularly rich review period for new structures. Elaboration of structures for members of the CDK family notably the structures of CDK7, PfPK7, Tyr15-phosphorylated CDK2/cyclin A, CDK2/cyclin A/Cks1, CDK2/cyclin E, and of a CDK2/cyclin A/peptide substrate complex in the groups of *Jane Endicott, Louise Johnson* and *Martin Noble* continue to reveal novel aspects of the regulation of this essential protein family. Our determination of the structure of a 14-3-3/phosphoacetylhistone H3 peptide complex uncovered a distinct mode of 14-3-3 phosphopeptide binding and provided a structural understanding for the lack of affect of acetylation at Lys 9 and Lys14 on the interaction of 14-3-3s and Ser10-phosphorylated histone H3.

The ubiquitin (Ub)-mediated protein degradation pathway regulates many critical eukaryotic cellular functions. Results from the groups of *Jane Endicott* and *Louise Johnson* have revealed that different UBA domains can recognise Ub and polyUb in diverse ways. Whereas the Mud1 UBA domain binds diUb with a 1:1 stochiometry, the Dsk2 UBA domain forms a 1:1 UBA:monoUb complex. Our recent determination of the structure of the VWA domain from the *S. pombe* proteasomal subunit Pus 1 (Mts4/S5a) that binds to polyubiquitinylated substrates has now provided an opportunity to extend these studies to characterise the first steps in substrate recognition by the proteasome.

In the group of *Rick Lewis*, we have probed the mechanism of action of a stress-responsive phosphatase and determined the structure of a kinase-recruitment domain, homologous in function and structure to the regulatory domains of a number of eukaryotic phosphatases. We have also recently determined the structure of *B. subtilis* N-RsbR, which has a globin-like fold that we propose has evolved from an ancestral oxygen sensor, and deduced that RsbT binds to RsbU and RsbR through a common binding motif. In collaboration with electron microscopists, we will dock this domain into an electron microscopy-derived molecular envelope of the supramolecular signaling 'stressosomes in order to build an atomic model of how *B. subtilis* senses and responds to stress.

During the period under review *Susan Lea's* group has determined structures for a series of proteins from bacterial pathogens: BbCRASP-1 from *Borrelia burgdorferi*, Dr and Afa adhesins from pathogenic *E. coli* and recently, the structure of MxiH, the *Shigella flexneri* type three secretion system needle subunit. These proteins have demonstrated the wide range of protein architecture associated with bacterial virulence and give insights into specific protein functioning and hopes for future design of novel antibacterials.

As we continue to determine structures for novel drug targets, we have also progressed several established structure-lead inhibitor design studies, the targets of which include: (i) CDK7, CDK2/cyclin A and CDK2/cyclin A mutants as structural surrogates where we have targeted both the ATP binding site and the cyclin recrutiment site, (ii) the p53 binding domain of MDM2, and (iii) *P. falciparum* PfPK5 and PfPK7 where insights derived from studies on human CDKs may provide an opportunity to rapidly develop selective *P. falciparum* CDK inhibitors as probes to promote *falciparum* cell cycle research.

Hyaluronan (HA) is the major glycosaminoglycan component of the extracellular matrix. In a network that includes proteins such as TSG6, glycosaminoglycans dictate the viscous and mechanical properties of tissues in all multicellular organisms. In addition, interactions with HA, mediated by receptors such as CD44 are responsible for regulated adhesive processes that permit cells to survey and attach to their environment. In *Martin Noble's* group we have built upon our previously described structure of human CD44 to determine 1.3 Å resolution structures for mouse CD44 (mCD44), and for a mCD44:HA complex. We have also determined the first crystal structure of the HA-binding LINK domain of TSG6. We are continuing to explore HA-binding proteins and the complexes they form, including the CD44, TSG6, the proteoglycans aggrecan versican and adapter molecules such as cartilage LINK-protein. We are also targeting these interactions for inhibitor design. Within cells, the complexes that form after cell adhesion or receptor engagement play joint signaling and structural roles. Our research into components of such signalling complexes has lead in this period to the structure of the tandem SH2 domains of PLC-gamma.

The BAG scheme is critical to our success – the combination of certainty of regular access combined with flexibility to choose samples is key. The web-based forms are a tremendous improvement and the facility to submit sample sheets on-line from multiple users is warmly welcomed.

List <u>all publications</u> directly resulting from the use of data recorded on ESRF beamlines since last report (you must indicate [1]ESRF data only; [2] data from more than one source): (Please delete the examples)

1. P. Teriete *et al.*, (2004) Structure of the regulatory hyaluronan binding domain in the inflammatory leukocyte homing receptor CD44. *Molec. Cell.* **13**, 483-96[1].
2. S. Holton *et al.*, (2004) Structures of P. falciparum PfPK5 test the CDK regulation paradigm and suggest mechanisms of small molecule inhibition *Structure.* **11**, 1329-38[1].
3. K.L. Anderson *et al.*, (2004) An Atomic Resolution Model for Assembly, Architecture, and Function of the Dr Adhesins *Molecular Cell* **15**, 647-657[2].
4. J. White *et al.*, (2004) Biological activity, membrane-targeting modification and crystallization of soluble human decay accelerating factor expressed in *E.coli*. *Prot. Sci.* **13**, 2406-2415[2].
5. E. Blanc *et al.*, (2004) Refinement of severely incomplete structures with maximum likelihood in Buster-TNT. *Acta Cryst.* **D60**, 2210-2221[2].
6. R. Abbott *et al.*, (2004) Crystallisation and preliminary X-ray diffraction analysis of three EGF domains of EMR2, a 7-TM immune system molecule. *Acta Cryst.* **D 60**, 936-938[2].
7. F.S. Cordes *et al.*, (2004) Crystallisation and preliminary crystallographic analysis of BbCRASP-1, a complement regulator-acquiring surface protein of *Borrelia burgdorferi. Acta Cryst.* **D 60**, 929-932[1].
8. D. Pettigrew *et al.*, (2004) High resolution studies of the Afa/Dr adhesin DraE and its interaction with chloramphenicol *J. Biol. Chem.* **279**, 46851-46857[2].
9. O. Delumeau *et al.*, (2004) Functional and structural characterisation of RsbU, a stress signalling protein phosphatise 2C. *J. Biol. Chem.* **279**, 40927-40937[1].
10. G. Lolli *et al.*, (2004) The crystal structure of human CDK7 and its protein recognition properties.. *Structure* **12**, 2067-79[1].
11. I.R. Hardcastle *et al.*, (2004) N2-substituted O6-cyclohexylmethylguanine derivatives: potent inhibitors of cyclin-dependent kinases 1 and 2. *J. Med. Chem.* **15**, 3710-22[1].
12. J. Sandy *et al.*, (2005) Binding of the anti-tubercular drug isoniazid to the arylamine N-acetyltransferase protein from Mycobacterium smegmatis. *Prot. Sci.* **14**, 775-82[1].
13. F.S. Cordes *et al.*, (2005) A novel fold for the factor H-binding protein BbCRASP-1 of *Borrelia burgdorferi*. *Nat. Struct. & Molec. Biol.* **12**, 276-277[1].
14. J.W. Murray *et al.*, (2005) Structure of a nonheme globin in environmental stress signaling. *Proc. Natl. Acad. Sci. USA* **102**, 17320-5[1].
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16. N. MacDonald *et al.*, (2005) Molecular basis for the recognition of phosphorylated and phosphoacetylated histone h3 by 14-3-3. *Molec. Cell* **20**, 199-211[1].
17. J.F. Trempe *et al.*, (2005) Mechanism of Lys48-linked polyubiquitin chain recognition by the Mud1 UBA domain. *EMBO J.* **24**, 3178-89[1].
18. I.M. Westwood *et al.*, (2005) Expression, purification, characterization and structure of Pseudomonas aeruginosa arylamine N-acetyltransferase. *Biochem. J.* **385**, 605-12[1].
19. E.D. Lowe *et al.*, (2006) Structures of the Dsk2 UBL and UBA domains and their complex. *Acta Cryst.*. **D62**, 177-88[1].
20. J.E. Deane *et al.*, (2006) Expression, purification, crystallization and preliminary crystallographic analysis of MxiH, the subunit of the *Shigella flexneri* type III secretion system needle. *Acta. Crystallogr.* **F 62**, dio:10.1107/S174430910600555[1].
21. J.A. Endicott *et al.*, (2006) Molecular recognition of indigoids. (review) In "Indirubin, the red shade of indigo", Ed L. Meijer, Roscoff, in press.

Highlight reports.

This section should consist of a series of single page reports (a maximum of **three** per BAG) bringing scientific highlights of your overall BAG programme to the attention of the ESRF. Each report should include a colour figure. The reports themselves may be incorporated (with permission) into the ESRF Highlights Annual Booklet or ESRF Newsletters.

Molecular Basis of Evasion of the Innate Immune System By The Lyme Disease Spirochaete, *Borrelia burgdorferi*.

Cordes, F.S.[1], Kraiczy, P.[2], Roversi, P.[1], Simon, M.M.[3], Brade, V.[2], Jahraus, O.[4], Wallis, R.[5], Goodstadt, L.[6], Ponting, C.P.[6] Skerka, C.[7], Zipfel, P.F.[7], Wallich, R.[4], & Lea, S.M.[1]

[1]Laboratory of Molecular Biophysics & [5]Glycobiology Institute, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU. [2] Institute of Medical Microbiology, University Hospital of Frankfurt, Paul-Ehrlich-Str. 40, D-60596 Frankfurt, Germany [3]Max-Planck-Institute for Immunobiology, Stübeweg 51, D-79108 Freiburg, Germany [4]Infectious Immunology Group, Institute for Immunology, University of Heidelberg, Im Neuenheimer Feld 305, D-69120 Heidelberg, Germany [6] MRC Functional Genetics Unit, University of Oxford, Department of Human Anatomy and Genetics, South Parks Road, Oxford OX1 3QX, UK. [7] Leibniz Institute for Natural Products Research and Infection Biology : Hans Knoell Institute, Beutenbergstr. 11a, D-07745 Jena, Germany

Borrelia burgdorferi, a spirochaete transmitted to human hosts during feeding of infected *Ixodes* ticks, is the causative agent of Lyme disease, the most frequent vector-borne disease in Eurasia and North America. Sporadically Lyme disease develops into a chronic, multisystemic disorder. Serum-resistant *B. burgdorferi* strains bind complement factor H (FH) and FH-like protein 1 (FHL-1) on the spirochaete surface. This binding is dependent on the expression of proteins termed Complement-Regulator Acquiring Surface Proteins. We have determined the atomic structure of BbCRASP-1 (1), the key FHL-1/FH-binding protein of *B. burgdorferi*. Our analysis indicates that its protein topology apparently evolved to provide a high affinity interaction site for FH/FHL-1 at the centre of the dimeric protein (2). This work demonstrates that pathogens interact with complement regulators in ways that are distinct from the mechanisms used by the host and are thus obvious targets for drug design.

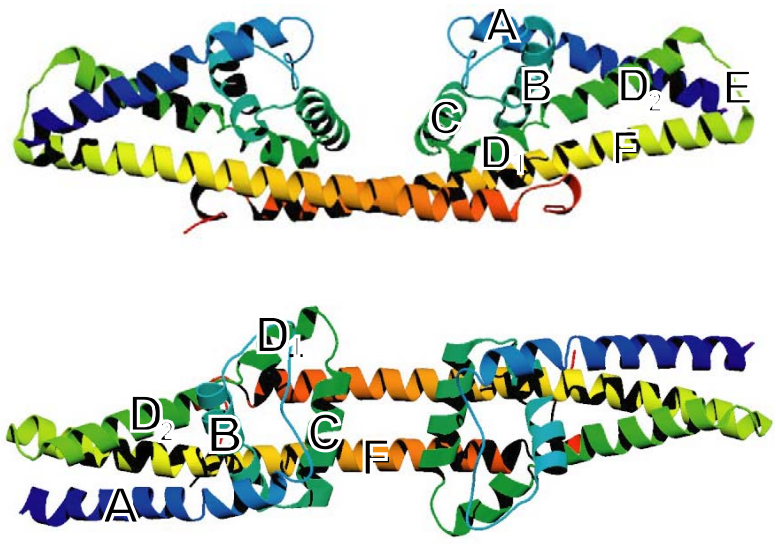


Figure: Structure of the BbCRASP-1 dimer. Cartoon representation of the BbCRASP-1 dimer coloured from blue at the N-terminus to red at the C-terminus. The existence of a dimmer had not been suspected prior to structure determination but the dimer has since been shown to be the functionally relevant form. Mutagenesis and functional analyses imply that the protein ligand, Factor H, binds in the cleft at the centre of the dimer.

1. F.S. Cordes *et al.*, (2005) A novel fold for the factor H-binding protein BbCRASP-1 of *Borrelia burgdorferi*. *Nat. Struct. & Molec. Biol.* **12**, 276-277.

2. F.S. Cordes *et al.*, (2006) Structure-function mapping of BbCRASP-1, the key complement factor H and FHL-1 binding protein of *Borrelia burgdorferi*. *Int J. Medical. Micro. doi:10.1016/j.ijmm.2006.01.011*

Structural views of Hyaluronan-binding proteins: an adhesion receptor-ligand complex and an HA-remodelling protein .

Suneale Banerji[1], Alan J. Wright[2], Vicky Higgman[2], Martin Noble[3], David J. Mahoney[2], Iain D. Campbell[4], Anthony J. Day [2,4], and David G. Jackson[1].

[1]1Medical Research Council Human Immunology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DS, United Kingdom, [2]Medical Research Council Immunochemistry Unit, [3]Laboratory for Molecular Biophysics and [4]Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU United Kingdom.

Hyaluronan (HA) is the major glycosaminoglycan component of the extracellular matrix. In a network that includes proteins such as TSG6, glycosaminoglycans dictate the viscous and mechanical properties of tissues in all multicellular organisms. In addition, interactions with HA, mediated by receptors such as CD44 are responsible for regulated adhesive processes that permit cells to survey and attach to their environment.

Within this reporting period we have built upon our previously described structure of human CD44 to determine 1.3 Å resolution structures for mouse CD44 (mCD44), and for a mCD44:HA complex. We have also determined the first crystal structure of the HA-binding LINK domain of TSG6.

Regulation of transient interactions between cells and the ubiquitous matrix glycosaminoglycan hyaluronan is critical to such fundamental processes as embryonic development and leukocyte homing. CD44, the primary cell surface receptor for hyaluronan binds ligand via a lectin-like fold termed the Link module, but only after appropriate functional activation. The structural basis for this activation and the molecular details of the CD44-hyaluronan interaction are however unknown. We have solved the first crystal structures of CD44 complexed with a hyaluronan octasaccharide that define two different conformational forms most probably corresponding to the “on” and “off” states of the receptor. Moreover, NMR analysis reveals that binding of HA can induce switching to the “on” state via a conformational change centred on a critical arginine (R45/R41 in mouse/human CD44) at the centre of the ligand-binding surface. These data reveal both the mechanism and allosteric regulation of a fundamentally important cell adhesion interaction.

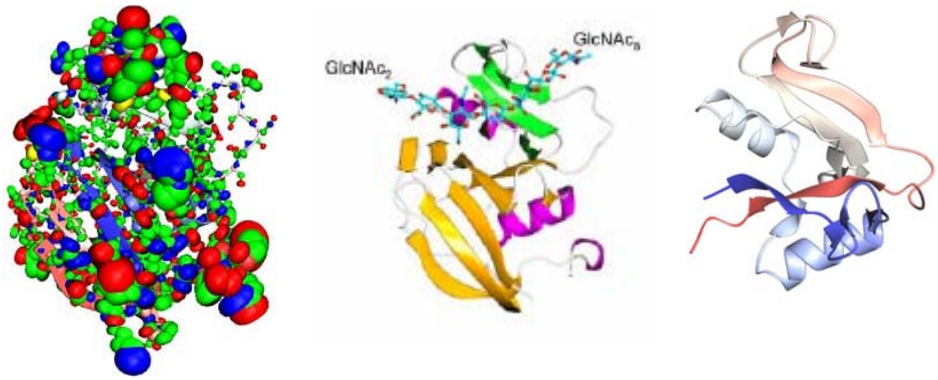


Figure: Structures involved in HA binding. A) The 1.3 Å resolution structure of the HA-binding domain of murine CD44. Ellipsoids indicate the magnitude and character of anisosotropic B-factors.

B) The complex between mCD44 HABD and an HA 8mer. A ribbon representation of CD44 is shown, with an atomic representation of the ligand. C) Crystallographic structure of the LINK domain of TSG6. Drawn in a similar orientation to panel B, the 1.8 Å resolution structure of TSG6 reveals a conserved fold, but a functionally significant conformational variability in the HA-contacting part of the structure.

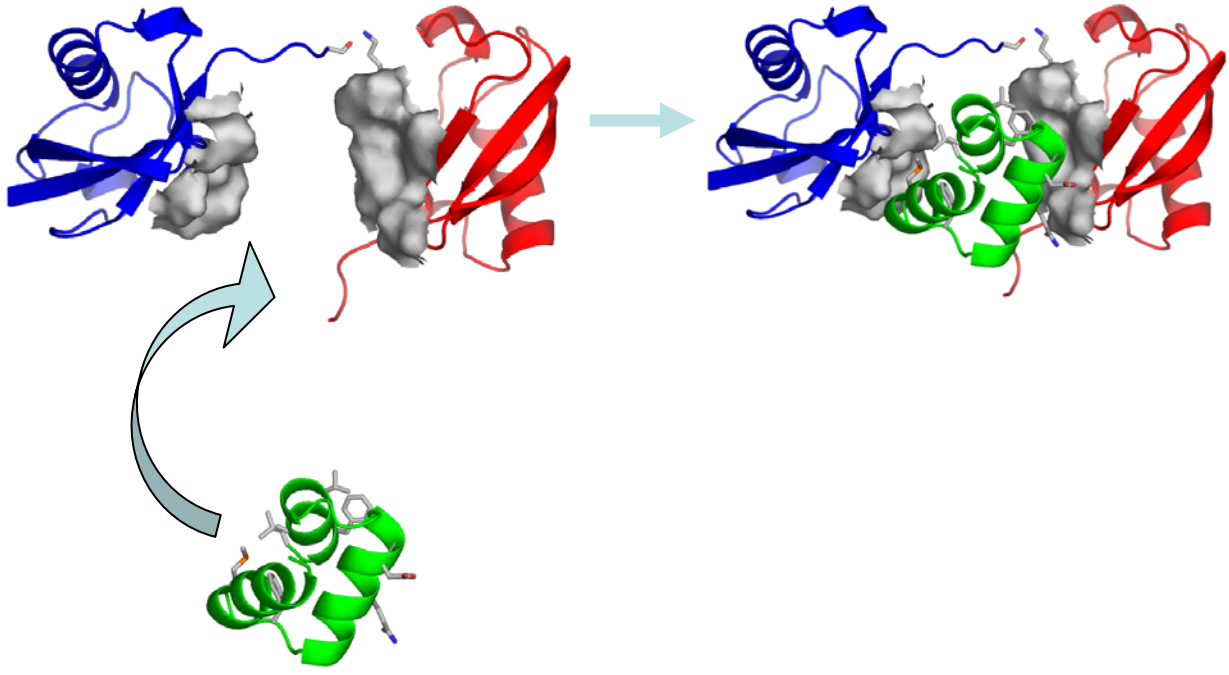
Mechanism of Lys48-linked polyubiquitin chain recognition by the Mud1 UBA domain

J-F. Trempe[1,3], N.R. Brown[1,3], E.D. Lowe[1,3], C. Gordon[2], I.D. Campbell[3], M.E.M. Noble[1,3] and J.A. Endicott.[1,3]

[1]Laboratory of Molecular Biophysics and [3]Department of Biochemistry, South Parks Road, Oxford and [2]MRC Human Genetics Unit, Western General Hopsital, Edinburgh.

The ubiquitin (Ub)-mediated protein degradation pathway regulates many critical eukaryotic cellular functions. Proteins targeted for degradation are tagged with a polyUb chain consisting of at least 4 monomers of ubiquitin (Ub_4), linked through K48 isopeptide bonds. The Ub-pathway associated (UBA) domain interacts with Ub and more specifically with K48- and K63-linked polyUb. Mud1 consists of a UBA domain and an associated aspartyl protease domain and was isolated as a suppressor of a temperature-sensitive mutant of the proteasome base subunit Mts4 (Rpn1 in *S. cerevisiae*), suggesting a role in protein targeting to the proteasome. We have determined the crystal structure of the Mud1 UBA domain and investigated its Ub-binding properties. The results have lead to a model in which the Mud1 UBA domain binds diubiquitin (Ub_2) through the same hydrophobic cluster on each Ub moiety using two hydrophobic pockets, one formed by the previously characterised MGF and dileucine motifs and the other by residues within helices 2 and 3 that comprise a novel Ub binding site (Figure 1). Only when Ub_2 is K48-linked are the two UBA binding sites of each Ub correctly disposed to bind to both Mud1 UBA Ub-binding sites. This model accounts for the observed stoichiometry, the difference in affinity between monoUb and Ub_2 and the observed linkage-type specificity.

Fig. 1.Molecular model for the interaction of Mud1 UBA with K48-linked Ub_2. The two hydrophobic clusters of Ub_2 (surfaces rendered in gray) are available for binding of a single UBA domain (green) via a primary and a secondary Ub-binding sites.



List of BAG participants (including Principle Investigators) during current reporting period

Researcher	Institute	Group[a]	Position
Susan Lea	Oxford	Lea	PI
Pietro Roversi	Oxford	Lea	Researcher
Steven Johnson	Oxford	Lea	Researcher
Janet Deane	Oxford	Lea	Researcher
Beverly Prosser	Oxford	Lea	Student
Rachel Abbott	Oxford	Lea	Student
David Pettigrew	Oxford	Lea	Student
Joyce Tay Zi	Oxford	Lea	Student
Rick J. Lewis	Newcastle	Lewis	PI
James W. Murray	Newcastle	Lewis	Researcher
Lorraine Hewitt	Newcastle	Lewis	Researcher
Mark J. Banfield	Newcastle	Banfield	PI
A.E.A. Openshaw	Newcastle	Banfield	Student
Martin Noble	Oxford	Noble	PI
Ed Lowe	Oxford	Noble	Researcher
Ewa Pilka	Oxford	Noble	Researcher
Sonja Lorenz	Oxford	Noble	Researcher
Eugene Valkov	Oxford	Noble	Student
Christiane Riedinger	Oxford	Endicott	Student
Karen Davies	Oxford	Johnson	Student
Jean-Francois Trempe	Oxford	Endicott	Student
Graziano Lolli	Oxford	Johnson	Student
Aude Echalier	Oxford	Noble	Researcher
Sarah Major	Oxford	Endicott	Student
Anais Merckx	Oxford	Endicott	Researcher
Julie Welburn	Oxford	Endicott	Student
Tim Johnson	Oxford	Endicott	Researcher
Enrique Rudino-Pinera	National Univ. of Mexico		Academic Visitor
Maria Hoellerer	Oxford	Noble	Researcher
Suneale Bannerji	Oxford	Noble	Researcher
James Sandy	Oxford	Noble	Researcher
Helen Fielder	Oxford	Noble	Student

[a]Give PI name where relevant

Beam line performance: No more than half an A4 page. Please comment on the beam line performance during your visits, together with any constructive suggestions about possible enhancements to the facilities.

Beamline operation remains of an extremely high standard. The presence of a consistent GUI across all beamlines has improved the ease and efficiency of data collection and the presence of on-axis microscopes and 3-click centering tools has greatly improved the visualisation and mounting of samples. The presence of a mini-diffractometer on all beamlines, equipped with the new beamstop/columnator assembly, has greatly improved the measurements we are able to record from weakly diffracting samples and aids in minimising the exposure time required for all samples, thereby reducing radiation damage. When problems have been encountered, such as the cooling system problems that occurred several times in 2005, they have been dealt with very rapidly and efficiently. Our only ongoing concern is with the sample changers fitted on the beamlines, which in our experience are quite unreliable. Specifically, of those times that the sample changer is functional upon arrival, we have always found that it breaks within a few hours, and we have formed the impression from beamline scientists that this is not an exceptional experience. The hours of success demonstrate the promise of this mode of data collection, but the inevitable failure makes scientists reluctant to commit to this approach. There are also some questions about transport of frozen samples in the supplied "pucks", although we have yet to try the new polystyrene insert that promises to secure them somewhat.