

6. Martin Noble

Adhesive interactions, the cell cycle, and NAT enzymes

Work in this group addresses structure-function relationships of medically important proteins from three different areas: adhesive cellular interactions, the eukaryotic cell cycle, and the enzyme arylamine N-acetyltransferase (NAT). We study these proteins by both experimental and theoretical approaches. Experimentally, proteins are subject to biochemical and biophysical characterisation, as well as structural analysis by X-ray crystallography and nuclear magnetic resonance. Development of theoretical methods has centred on developing tools for molecular analysis. These include programs to study protein surfaces in terms of their chemical character as indicated by sequence conservation, electrostatic potential, or other potentials calculated by the program GRID. We have also been exploring the use of this approach to identify probable sites of protein-protein interaction.

Adhesion and signalling from adhesive complexes

The cell membrane is a location of bidirectional signalling. External events may activate signalling networks within the cell, while internal signals may be transduced through modulation of the properties of cell-surface proteins that mediate cellular adhesion to the extracellular matrix or to other cells. We are studying two prototypical signalling systems that involve both outside-in and inside-out signalling. These are CD44, a transmembrane protein found on the surface of many cell-types in mammals, where it acts as the major receptor for the glycosaminoglycan hyaluronan (HA), and focal adhesions, the cellular substructure that forms where integrins bind to proteinaceous components of the extracellular matrix. To date we have explored the structure of the HA binding domain of CD44, as well as various aspects of the structure and function of different parts of the focal adhesion kinase (FAK) molecule.

CD44

(With Dr. D. Jackson, IMM, Dr. A.J. Day, MRC Immunochemistry)

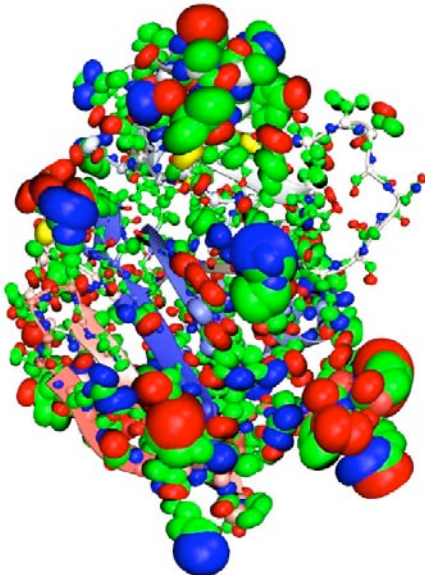


Figure 1: The 1.25 Å structure of mouse CD44, drawn in ribbon trace, with anisotropic thermal ellipsoids at atomic positions.

Adhesive interactions involving CD44, the cell surface receptor for hyaluronan, underlie fundamental processes such as limb morphogenesis, wound healing, leukocyte migration and tumor metastasis. Critical to these events, the regulation of CD44's hyaluronan-binding activity is known to be effected by changes in N-glycosylation, switching the receptor "on" under appropriate circumstances. How glycosylation influences CD44 function has until now been unclear. Like many hyaluronan-binding proteins found in extracellular matrix, CD44 contains a conserved lectin-like domain termed the Link module. However,

CD44 is unique in that regions of the extracellular domain additional to the Link module are required for receptor function, and evidence suggests these "extensions" are involved in regulation. We have shown using X-ray crystallography and NMR spectroscopy that sequences flanking the Link module form a supplementary structural lobe that extends the main hyaluronan-binding surface. Moreover, the location of key N-glycosylation sites revealed for the first time how such glycans might regulate CD44 function. This year we have pursued crystallographic studies with mouse CD44 with a view to producing crystals that lend themselves to binding studies.

The Focal adhesion targetting domain

Maria Hoellerer and Sonja Lorentz (With Dr. S. Arold, CNRS Montpellier, Prof. I.Campbell, Biochemistry)

FAK is localised to focal adhesions via its C-terminal Focal Adhesion Targetting (FAT) domain. FAT performs this function by binding to paxillin and talin, both of which are in turn associated with the cytoplasmic tails of integrins. FAT is also required for binding of FAK substrates: a phospho-tyrosine motif within FAT binds to the Src homology (SH) 2 domain of the adapter protein Grb2. A proline-rich sequence immediately upstream of the FAT domain binds to the SH3 domains of p130 CAS, Graf, and the p85 subunit of phosphatidylinositol-3 kinase.

This year we have established that the FAT domain binds to long-forms of all 5 LD motifs of paxillin, indicating a highly relaxed specificity, the implications of which we are exploring by further binding studies.

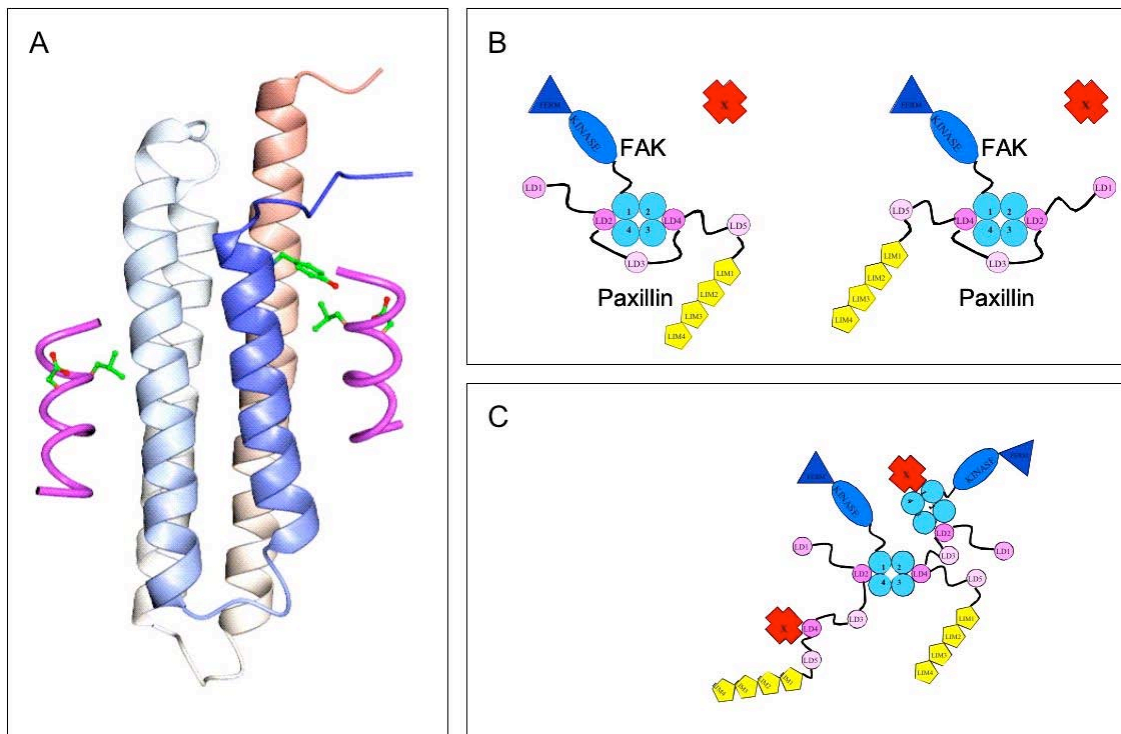


Figure 2: A) Binding sites for paxillin LD peptides on FAK, and resulting models for association in B) bimolecular and C) multimolecular complexes.

Cell cycle

Dr. Paul Barrett

We have collaborated with other groups in the LMB on various aspects of cell-cycle regulation over recent years, the results of which are discussed elsewhere in this report (see reports from Prof. Louise Johnson, Dr. Jim McDonnell, and Prof. Jane Endicott).

This group has focussed this year on characterising the dynamic properties of CDK molecules. To this end, we have developed a web-based server that evaluates the essential dynamics of submitted proteins (http://s12-ap550.biop.ox.ac.uk:8078/dynamite_html/dynamite_splash.html).

This analysis, together with others based on molecular dynamics, have revealed a surprisingly dynamic structure for CDK2/cyclin A, and demonstrated an intrinsic preference for this complex to undergo a characteristic set of motions.

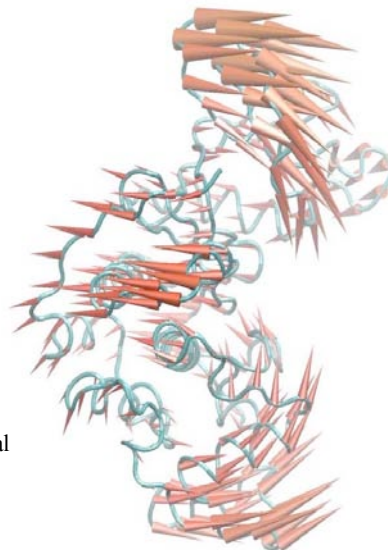


Figure 3: First Eigenvector of structural variability of cyclin-complexed CDK2.

NAT enzymes

Simon Holton, James Sandy (Pharmacology) (With Prof. E. Sim, Pharmacology)

This year we have extended our structure function studies of the arylamine N-acetyltransferase family of enzymes by developing a structure activity relationship for *M. smegmatis* NAT with reference to the known structure for the complex of NAT with isoniazid. This has further offered insights into “handles” within the active site that might be exploited in inhibitor design.

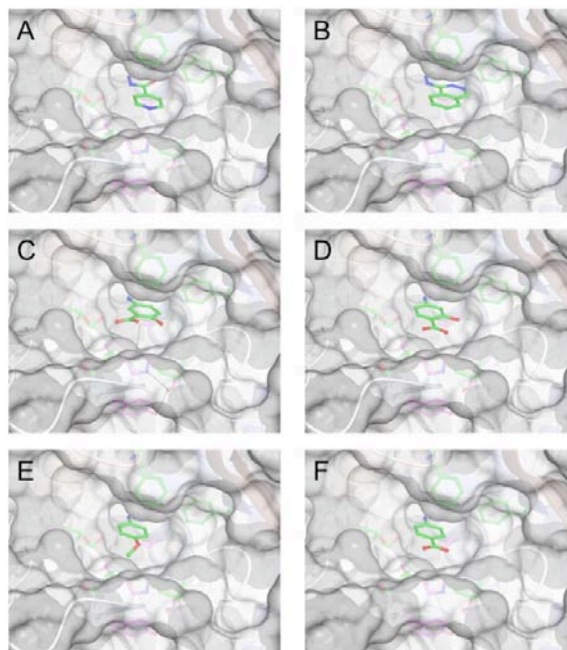


Figure 4: A) Observed and B-F) putative substrate complexes of *M. smegmatis* NAT.

Methods

Visualisation/analysis

Jan Gruber, Alexander Zawaira

This year we have developed and implemented algorithms for the analytical calculation of protein surfaces, and for the evaluation of electrostatic potential through solving the Poisson Boltzmann equation by finite difference methods. These algorithms have been implemented in the programs AESOP, ccp4mg, and coot. We have also designed a novel algorithm for assigning amino-acid conservation from analysis of aligned sequences that promises to reveal important functional surfaces.

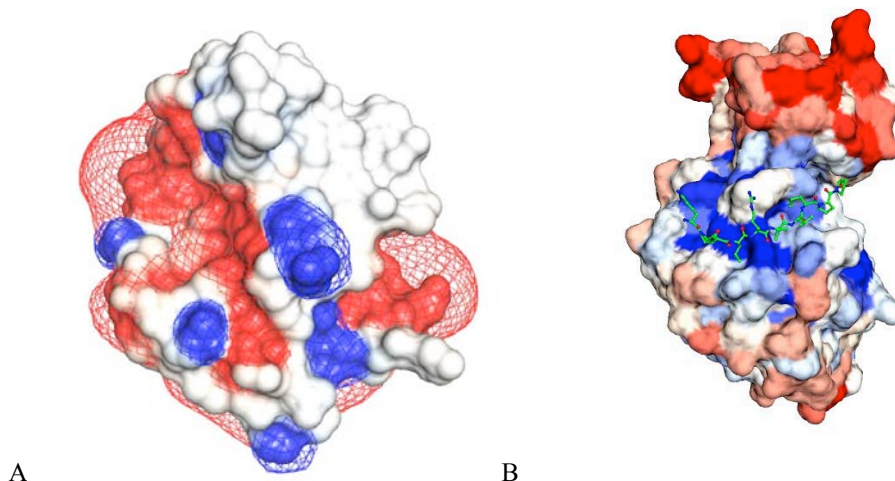


Figure 4: Analytical tools: Surfaces of A) cd44A with associated electrostatic potential map, and B) p38 kinase coloured by sequence conservation, and revealing the site of substrate recruitment.

References

1. Pratt DJ, Endicott JA, Noble ME: The role of structure in kinase-targeted inhibitor design. *Curr Opin Drug Discov Devel* 2004, 7:428-436.
2. Hardcastle IR, Arris CE, Bentley J, Boyle FT, Chen Y, Curtin NJ, Endicott JA, Gibson AE, Golding BT, Griffin RJ, et al.: N2-substituted O6-cyclohexylmethylguanine derivatives: potent inhibitors of cyclin-dependent kinases 1 and 2. *J Med Chem* 2004, 47:3710-3722.
3. Noble ME, Endicott JA, Johnson LN: Protein kinase inhibitors: insights into drug design from structure. *Science* 2004, 303:1800-1805.
4. Holton S, Merckx A, Burgess D, Doerig C, Noble M, Endicott J: Structures of *P. falciparum* PfPK5 test the CDK regulation paradigm and suggest mechanisms of small molecule inhibition. *Structure (Camb)* 2003, 11:1329-1337.
5. Hoellerer MK, Noble ME, Labesse G, Campbell ID, Werner JM, Arold ST: Molecular recognition of paxillin LD motifs by the focal adhesion targeting domain. *Structure (Camb)* 2003, 11:1207-1217.
6. Sayle KL, Bentley J, Boyle FT, Calvert AH, Cheng Y, Curtin NJ, Endicott JA, Golding BT, Hardcastle IR, Jewsbury P, et al.: Structure-based design of 2-arylamino-4-cyclohexylmethyl-5-nitroso-6-aminopyrimidine inhibitors of cyclin-dependent kinases 1 and 2. *Bioorg Med Chem Lett* 2003, 13:3079-3082.
7. Sim E, Pinter K, Mushtaq A, Upton A, Sandy J, Bhakta S, Noble M: Arylamine N-acetyltransferases: a pharmacogenomic approach to drug metabolism and endogenous function. *Biochem Soc Trans* 2003, 31:615-619.
8. Mettey Y, Gompel M, Thomas V, Garnier M, Leost M, Ceballos-Picot I, Noble M, Endicott J, Vierfond JM, Meijer L: Aloisines, a new family of CDK/GSK-3 inhibitors. SAR study, crystal structure in complex with CDK2, enzyme selectivity, and cellular effects. *J Med Chem* 2003, 46:222-236.
9. Kawamura A, Sandy J, Upton A, Noble M, Sim E: Structural investigation of mutant *Mycobacterium smegmatis* arylamine N-acetyltransferase: a model for a naturally occurring functional polymorphism in *Mycobacterium tuberculosis* arylamine N-acetyltransferase. *Protein Expr Purif* 2003, 27:75-84.