MS05 O1

New Approaches in Structure based kinase drug discovery Aude Echalier[a], Anais Merckx [b], Alison Hole[a], Jane Endicott[a], Martin Noble[a], [a]*Department of Biochemistry, University of Oxford. Laboratory of Molecular Biophysics, Oxford, United Kingdom.* [b]*Institut Cochin, Université Paris Descartes, CNRS, Paris, France.*
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Cellular events in all living organisms are driven by the phosphorylation of key substrates. The phosphorylation status of proteins is controlled by a complex network of phosphatases and protein kinases (PKs). Many of the 518 Human PKs have been reported to be de-regulated in Human diseases, such as cancers and neurodegenerative diseases. Consequently PKs are very actively studied as potential targets for treatment of a number of diseases. Extensive effort is devoted to the discovery and optimization of selective and potent PK antagonists. At the moment ~ 60 PK inhibitors are undergoing clinical trials against conditions such as cancers and inflammation. The use of structural information to guide inhibitor design has long been recognized as an important key to success[1]. This was particularly well exemplified in the design of follow-up compounds to the anti-CMC drug Gleevec.
Cell proliferation is driven by the activity of cell cycle cyclin-dependent kinases. Several complex layers of regulation keep cell cycle CDKs under control. Deregulation of cell cycle CDKs leads to abnormal proliferation of cells often seen in cancers. One of the cell cycle regulatory mechanisms is the activatory phosphorylation of cell cycle CDKs by the CDK-activating kinase, CAK. CAK activity results from the presence of another CDK, CDK7. Inhibiting CAK activity by targeting CDK7 could potentially downregulate cell cycle progression. Because CDK7 is not readily amenable to structure guided inhibitor design, mutated CDK2 was used as a surrogate of CDK7. Central to our surrogacy approach has been the validation of the obtained surrogate protein by inhibitor fingerprinting. Progress towards the development of a method to quickly evaluate surrogates could be of general interest in the PK field as some PKs are not easily amenable to structure guided inhibitor design.
With their central role in every aspects of cell regulation, PKs can also be targeted in bacterial and parasitic therapies. *Plasmodium falciparum* (*Pf*), the apicomplexan responsible for malaria, has a kinome about 10 times smaller than Human. Sequence analysis of these *Pf*PKs showed that some of them are orphan and do not have homologues in the Human kinome and therefore could potentially be valid drug targets for the treatment of malaria[2]. The crystal structure of one of these atypical *Pf*PKs, *Pf*PK7 was determined and two classes of potent inhibitors were identified[3]. Analysis of the *Pf*PK7-inhibitor structures gives valuable insights into the development of more potent inhibitors, highlighting in particular a novel additional pocket to be exploited in the ATP-binding site.
These two new approaches in structure guided inhibitor design currently developed in Oxford will be presented and discussed.

[1] Noble, M.E., Endicott, J.A., Johnson, L.N. *Science*. 2004, 303, 1800; [2] Doerig, C., Billker, O., Pratt, D., Endicott, J.A. *Biochim. Biophys. Acta.* 2005, 58, 1253; [3] Merckx, A., Echalier, A., Langford, K., Sicard, A., Langsley, G., Joore, J., Doerig, C., Noble, M.E., Endicott, J.A. Submitted.

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Crystal structure of the X domain of human coronavirus NL63. Yvonne Piotrowski[a], Lia van der Hoek[b], Ralf Moll[a], Jeroen R. Mesters[a] and Rolf Hilgenfeld[a], [a] *Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, University of Lübeck.* [b] *Department of Human Retrovirology, Academic Medical Center, University of Amsterdam.*
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Coronaviruses are enveloped plus-strand RNA-viruses with a genome size of ~ 27-31 kb. Their genome encodes a large number of non-structural protein domains, which are required for genomic and subgenomic RNA synthesis (replication and transcription) and several structural proteins that are necessary for the assembly of new virus particles. The non-structural protein 3 is a multifunctional protein with several domains that mediate various enzymatic activities. It consists of an acidic domain, followed by the papain-like proteinase 1 ($PL1^{pro}$), the X domain, the $PL2^{pro}$, and a hydrophobic domain with a putative zinc-finger called Y domain. We determined the crystal structure of the X domain of human coronavirus NL63 at 1.8 Å resolution. This structure reveals a macro-domain-like fold. Macrodomains are found in several types of ssRNA viruses but also in bacteria, archea and eukaryotes [1]. This suggests them to be involved in an important and ubiquitous cellular process. As shown for other proteins with a macrodomain fold, ADP-ribose 1”-phosphatase activity could also be detected for the HCoV-229E and the SARS-CoV Nsp3 X domain as well as binding of poly(ADP-ribose) (PAR) [2,3]. A very weak enzymatic activity and non-conservation of putative catalytic residues among Nsp3 homologues suggest that the primary function of the X domain is in fact not that of an ADP-ribose-1”-monophosphatase. We will present new results on the HCoV-NL63 X domain against the backdrop of the functional [1-3] and structural [3,4] data available for these enigmatic domains.

[1] Kumaran D., Eswaramoorthy S., Studier F.W. and Swaminathan S., *Protein Sci.*, 2005, 14, 719. [2] Putics Á., Filipowicz W., Hall J., Gorbalenya A.E. and Ziebuhr J., *J. Virol.*, 2005, 79, 12721. [3] Egloff M.-P., Malet H., Putics Á., Heinonen M., Dutartre H., Frangeul A., Gruez A., Campanacci V., Cambillau C., Ziebuhr J., Ahola T. and Canard B., *J. Virol.*, 2006, 80, 8493. [4] Saikatendu K.S., Joseph J.S., Subramanian V., Clayton T., Griffith M., Moy K., Velasquez J., Neuman B.W., Buchmeier M.J., Stevens R.C. and Kuhn P., *Structure*, 2005, 13, 1665.

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Determination of Absolute Stereochemistry for Regulatory Submission Suzanne M Harte, Christopher Frampton , *Pharmorphix, Cambridge UK.*
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