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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Keywords: protein kinases, structure guided inhibitor design, *Plasmodium falciparum*

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.

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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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Keywords: coronaviruses, non-structural proteins, X-domain

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.

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MS20 O3

Determination of Absolute Stereochemistry for Regulatory Submission Suzanne M Harte, Christopher Frampton , *Pharmorphix, Cambridge UK.*
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Keywords: Absolute stereochemistry, **single-crystal X-ray diffraction, regulatory submission.**

The FDA requires that the absolute stereochemistry of any chiral drugs be determined for regulatory submission. However, chiral compounds may often prove to be the most desirable drug candidate. Thus reliable methods for establishing the absolute stereochemistry of these candidates are essential within the pharmaceutical industry. It has been known for many years that the absolute stereochemistry of structures can be determined by a single crystal X-ray diffraction anomalous dispersion experiment [1]. For structures containing atoms heavier than sulphur this process is fairly straightforward and can be carried out on a standard laboratory Mo Kα diffractometer. However, for structures consisting only of light atoms the process can prove more complex. In the work presented the use of single crystal x-ray diffraction for establishing the absolute structure of both light and heavy atoms will be discussed. This will include the use of the well-known Flack parameter [2] and a new method created by Hooft, Straver and Spek for determining the absolute structure of chiral compounds based on the Bijovet pair intensity differences and accessible via the program Platon [3].

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MS21 O4

Crystallochemical, vibrational and optic Studies of the silver doped $NaY(PO_3)_4$ structure. M. El Masloumi[a], (b), V. Jubéra[a], S. Pechev[a], J. P. Chaminade[a], J. J. Videau[a], M. Mesnaoui[b], M. Maazaz[b], B. Moine[c].
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Keywords : Crystal structure ; Phosphate ; Silver luminescence ; decay time

Since ten years, the study of the optical properties of monovalent silver phosphates was undertaken by the LCSM of Marrakech (Morocco) in collaboration with the ICMCB (France), these investigations related to the well crystallize materials as vitreous [2]. It made it possible to identify three centers of luminescence covering the whole of the visible one.
The aim of this work is, on the one hand, to widen the range of the compositions crystallized in order to facilitate a deepening of the comprehension of the mechanisms of luminescence of the various types of association of the ions Ag^+ while optimizing the output of photoluminescence and on the other hand, to associate the silver ions rare earth in new phosphates (crystals and glasses), in seen to examine their mutual influences (creation of new centers, transfer of energy between centers, interaction between silver clusters and ions rare earth, etc.) and to understand the mechanisms of them.
It is in this context that the structure $NaY(PO_3)_4$ in single-crystal form, could be established by diffraction of X-rays (**$P2_1/n$**).This phase will enrich the family by the metaphosphate of general formula $NaLn(PO_3)_4$ (**Ln = La**[3]**, Nd, Gd, Er**).A comparative study of the influence of rare earths on the structure could be carried out and interpreted.
In parallel, the study of luminescence carried out on crystals of composition $Na_{0.9}Ag_{0.1}Y(PO_3)_4$ showed two types of emission. Measurements of declines of luminescence make it possible to advance mechanisms of fluorescence of the silver

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A boron based antifungal agent in complex with its target protein domain

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Aminoacyl-transfer RNA (tRNA) synthetases, which catalyze the attachment of the correct amino acid to its corresponding tRNA during translation of the genetic code, are proven antimicrobial drug targets.
Recently, we showed that the broad-spectrum antifungal 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (AN2690), which is in development for the treatment of onychomycosis and inhibits the fungal leucyl-tRNA synthetase (LeuRS), also binds to bacterial LeuRS by formation of a stable LeuRS-AN2690 adduct in the editing site of the enzyme. Adduct formation is mediated through the boron atom of AN2690 and the 2'- and 3'-oxygen atoms of tRNA's3'-terminal adenosine. The trapping of enzyme-bound tRNA in the LeuRS editing site prevents catalytic turnover, thus inhibiting synthesis of leucyl-tRNA and consequentially blocking protein synthesis [1].
We now present x-ray structures of a fungal LeuRS editing domain, the real drug target, alone and in complex with a similar adduct. Together with our low resolution x-ray structure of the human LeuRS editing domain (3.1 Å) and homology modeling results, these data open the way for rational drug improvement.

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