FA1-MS07-O1

Structural Studies on Bacterial Type IIA Topoisomerases-Targets for Quinolone and Coumarin Antibiotics. Ben Bax[a], Martin Hibbs[a], Emma Jones[a], Andrew Theobald[a], Andrew Fosberry[a], Claus Spitzfaden[a], Anthony Shillings[a], Alexandre Wohlkonig[a], Kristin K. Koretke[b], Jianzhong Huang[b], Neil Pearson[b], Michael N. Gwynn[b] . [a]*GlaxoSmithKline, Harlow, Essex, UK.* [b] *GlaxoSmithKline, Pennsylvania, USA.*
E-mail: benjamin.d.bax@gsk.com

Bacterial type IIA topoisomerase inhibitors continue to be developed as clinical antibacterial agents. While quinolones have been succesfully used in the clinic for many years, there is increasing resistance to marketed quinolone antibiotics and and an urgent need for new antibiotics. Bacterial type IIA topoisomerases (DNA gyrase and topoisomerase IV) are flexible molecular machines that regulate DNA topology by producing a double stranded break in one DNA segment and passing another double stranded segment through this break. Quinolone antibiotics target the central DNA cleavage gate of the enzyme, while coumarins bind to the amino-terminal ATPase domain of the GyrB subunit. The structure-based design of novel chemotypes as inhibitors of the ATPase domain has been facilitated by crystal structures of many complexes. More recently progress has been made with crystallographic studies of constructs containing the catalytic core of the enzyme, containing both the C-terminal region of GyrB and the N-terminal region of GyrA. These contructs are capable of cleaving DNA in the presence of quinolone antibiotics.

Keywords: antibiotic; topoisomerase; protein structure

FA1-MS07-O2

Discovery and Optimization of Inibitors of Hepatitis C Virus: A Structure-Based Approach. Stefania Di Marco. *IRBM P. Angeletti, Via Pontina Km 30.600, 00040 Pomezia-Rome, Italy.*
E-mail: stefania_dimarco@merck.com

Hepatitis C virus (HCV) is a small positive-strand RNA virus responsible for a considerable proportion of acute and chronic hepatitis in humans. Worldwide, more than 170 million people are infected by HCV. The size of HCV epidemic and the limited efficacy of the current therapy, which is based on the use of alpha interferon, have driven intense research efforts toward the development of novel antiviral drugs targeting essential HCV enzymes. Although all HCV enzymes are, in theory, equally appropriate for therapeutic intervention, the NS3-4A serine protease and the NS5B RNA-dependent RNA polymerase have emerged as the most popular targets. A number of active-site inhibitors of the NS3 protease as well as nucleoside and non-nucleoside inhibitors of the NS5B polymerase are being developed. For the NS3 protease, structural information has guided the optimization of active-site inhibitors. For the NS5B polymerase, crystallography has revealed several binding sites for non-nucleoside inhibitors and has underlined the importance of taking into account the dynamic protein surface to find small molecules to bind. An overview of our structural work with both NS3 protease and NS5B polymerase will be presented.

Keywords: HCV; NS3 protease; NS5B polymerase

FA1-MS07-O3

Complexes of Tubulin with Inhibitors that Bind to the Colchicine Domain. Marcel Knossow[a], Audrey Dorléans[a], Raimond Ravelli[b], Benoît Gigant[a]. [a]*L.E.B.S., CNRS, Gif-sur-Yvette, France.* [b]*Grenoble Outstation, EMBL, Grenoble, France.*
E-mail: knossow@lebs.cnrs-gif.fr

Microtubules are hollow cylindrical assemblies of alpha-beta tubulin heterodimers (tubulin). They participate in numerous processes such as cell division, where they form the mitotic spindle, or intra-cellular trafficking where they constitute the roads along which microtubule-based motors move. To fulfil their wide range of functions, microtubules alternate phases of assembly and disassembly in a process known as dynamic instability [1]. The assembly-disassembly cycle is accompanied by a structural cycle in which the tubulin structure undergoes changes. Their most prominent feature is a transition from a straight microtubular structure [2], in which tubulin subunits are related by a translation, to a curved structure of soluble tubulin, in which an additional rotation is needed to superimpose these subunits [3]. Information on curved tubulin has come from the structure of a protofilament-like complex of two tubulins with the stathmin-like domain of the RB3 protein. The overall curvature of the complex is due to reorientations of neighbouring tubulin subunits with respect to each other both within a heterodimer and at the inter-heterodimer interface. These orientation changes accommodate variations at the inter-subunit contact surfaces due to rearrangements of tubulin domains with respect to the structure in straight protofilaments [4, 5]. The microtubule cycle is disturbed by small molecule compounds, a class of which targets the colchicine binding site and prevents microtubule assembly. It is presently not known whether compounds in this broad class, with very different chemical structures, prevent microtubule assembly by the same mechanism. To address this issue, we have determined the structures of tubulin complexed with a set of colchicine-site ligands. We show that colchicine-site ligands interfere with several of the movements of tubulin subunits structural elements that occur upon its transition from curved to straight. We also determined the structure of tubulin unliganded at the colchicine site. In the absence of ligand, a loop of the polypeptide chain flips into the site. This prevents a helix of the beta subunit from occupying its location in straight protofilaments and destabilizes the assembly of tubulin subunits that characterizes microtubules. When a colchicine site ligand binds to tubulin, this interference gets frozen. Our results also suggest that in the absence of such ligands this interference contributes to microtubule dynamic instability by participating in the resistance to straightening

that opposes microtubule assembly.

[1] Mitchison, T. & M. Kirschner, *Nature*, **1984**. 312: p. 237-42. [2] Nogales, E., et al., *Cell*, **1999**. 96: p. 79-88. [3] Gigant, B., et al., *Cell*, **2000**. 102: p. 809-16. [4] Gigant, B., et al., *Nature*, *2005*. 435: p. 519-22. [5] Ravelli, R.B., et al., *Nature*, **2004**. 428: p. 198-202.

Keywords: cancer drug design; cytoskeleton; inhibitor binding

FA1-MS07-O4

Snapshot of Translesion Synthesis of a Cisplatin 1,3-GTG Intrastrand Cross-link. Sabine Schneider[a], Thomas Reißner[a], Thomas Carell[a]. [a]*Department of Pharmacy and Chemistry, Ludwig Maximilians University, Munich, Germany.*
E-mail:sabine.schneider@cup.uni-muenchen.de

First discovered in the 60s, cisplatin (*cis*-diaminedichloridoplatinum(II)) is still one of the most widely used anticancer chemotherapeutic agents. ts cytotoxic properties rely on its ability to form stable and persisting DNA adducts, resulting in cell cycle arrest and apoptosis (reviewed in [1]). The major formed DNA adduct of cisplatin is the 1,2-intrastrand cross-link of adjacent purines In addition 1,3-intrastrand cross-links of nonadjacent guanines as well as interstrand cross-links were also found [2, 3]. Its second generation analogue carboplatin (cis-diamine-[1,1-cyclobutanedicarboxylato]platinum(II)) is less toxic and has fewer side-effects than cisplatin, and was introduced into the clinics in the mid 1980s. Interestingly, carboplatin and cisplatin only differ in their leaving groups and form comparable DNA adducts, but show a different distribution of these adducts, with the 1,3-intrastrand cross-links of nonadjacent guanines, Pt-GTG, as the major adduct of carboplatin [4]
Nevertheless, tumours can commonly be intrinsically resistant or acquire resistance during the course of therapy against these drugs. The major cellular mechanism contributing to clinical cisplatin resistance include: decreased cellular uptake and increased efflux of the drug, increased cytoplasmic detoxification, inhibition of apoptosis, repair and increased bypass of the DNA adducts (reviewed in [1]). The mechanism of DNA lesion tolerance, known as translesion synthesis (TLS) is a universal, often error-prone response to arrested replication forks. TLS involves a multitude of specialized DNA polymerases, often belonging to the Y-family of polymerases, characterized by their ability to replicate across damaged sites and their low fidelity when replicating undamaged DNA. This can either be error-prone, resulting in mutations or accurate in respect to the damaged site (reviewed in [5])
DNA polymerase η is a key player in TLS and responsible for the suppression of UV induced mutations [6]. Its abundance also has been correlated to the susceptibility of tumor cells to treatment with cisplatin [7-9].
Here we investigated the ability of Pol η to replicate template DNA containing a platinum 1,3-dGTG intrastrand cross-link, the major DNA adduct of the anticancer drug carboplatin. In contrast to the 1,2-dGG cisplatin adduct [10], Pol η is not able to bypass this lesion. The X-ray crystal structure of Pol η in complex with template DNA containing the 1,3-dGTG adduct reveals the molecular basis of this lesion to prevent TLS by Pol η.

[1] H. Zorbas, B. K. Keppler, *Chembiochem*, **2005**, 6, 1157. [2] E. R. Jamieson, S. J. Lippard, *Chem Rev*, **1999**, 99, 2467. [3] A. E. Egger et al., *Inorg Chem*, **2008**, 47, 10626. [4] A. M. Fichtinger-Schepman, H. C. van Dijk-Knijnenburg, S. D. van der Velde-Visser, F. Berends, R. A. Baan, *Carcinogenesis*, **1995**, 16, 2447. [5] S. D. McCulloch, T. A. Kunkel, *Cell Res*, **2008**, 18, 148. [6] R. E. Johnson, L. Haracska, S. Prakash, L. Prakash, *Mol Cell Biol*, **2001**, 21, 3558. [7] R. E. Johnson, C. M. Kondratick, S. Prakash, L. Prakash, *Science*, **1999**, 285, 263. [8] C. Masutani et al., *Nature*, **1999**, 399, 700. [9] E. Bassett et al., *Cancer Res*, **2004**, 64, 6469. [10] A. Alt et al., *Science*, **2007**, 318, 967.

Keywords: DNA replication; DNA damage; DNA-drug interactions

FA1-MS07-O5

Improvement of Drug Virtual Screen by GA/GP: Docking Studies on Tubulin Inhibitors as Anticancer Agents. Po-Tsang Huang[a], Chi-Hwa Wang[a], Shiao-Chun Wang[a], Chin-Tzong Pang[a], Kuo-Long Lou[a]. [a]*Institutes of Biochemistry and Molecular Biology, College of Medicine, National Taiwan University, Taiwan.*
E-mail: d91442010@ntu.edu.tw

Conventional procedures for drug design have been very expensive and time-consuming. Due to the enormous progress in information technology during recent years, it is expected to shorten the required research time spent in the early stage of development through computer calculation. CADD (Computer Aided Drug Design) is one of the most powerful concepts applied to satisfy such demand. Upon docking simulations, it is allowed to find out the binding sites and orientations between target proteins and drug molecules in several days. This is not only to save the time and the cost used in drug development, but also for us able to understand the structural implications used for further design. However, it is still currently difficult to formularize efficient software to carry out the docking simulations as a standard procedure leading to definite results with high accuracy. Therefore, we are in attempt to propose a new category of programming, for which the standard effectiveness for docking procedure can be anticipated in the near future. To initiate such computer simulations, many factors have to be taken into consideration. The first is to decide which algorithms should be applied to perform the job. GA (Genetic Algorithms) and GP (Genetic Programming) seem to be excellent candidates to solve this problem. The next concern is the determination of scoring function, which is appropriate for either GA or GP to generate their scores. As being the best commercially available scoring function with high accuracy and flexibility, X-score is used to satisfy this purpose. Our study has been thus concentrated in the search of binding site(s) between protein and the drug molecule through docking simulations by applying the aforementioned special algorithms and