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Expression, purification and crystallization of LMW-PBP 4 and 5 from *Haemophilus influenzae*

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The gene encoding penicillin binding protein 4 and 5 of *Haemophilus influenzae* were cloned into the high-expression plasmid pET28 and overexpressed in Escherichia coli BL21 (DE3) star / pLysS respectively. Each proteins were purified more than 95 % and initial crystals were obtained by sitting-drop vapour-diffusion method at 293 K in a lot of conditions. After optimization of each conditions, the single crystals were grown at 293 K, 1 week for data collection. The HiPBP4 crystals belonged to space group $P2_1$ with unit-cell parameters a = 64.55 Å, b = 92.59 Å, c = 104.88 Å and β = 107.75°. and the HiPBP5 crystals belonged to space group $P2_12_12_1$ with unit-cell parameters a = 41.13 Å, b = 53.01 Å, c = 201.79 Å. These crystals complete data set were collected at Photon factory.

Keywords: penicillin binding protein, Haemophilus influenzae, PBP4 PBP5

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The proteome of M. tuberculosis in 3D: Towards structure based drug discovery

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The availability of the molecular structures of the proteome from M. tuberculosis serves as an essential tool to advance the understanding of the biological processes during the different stages of its life cycle within the human host. During the last five years, the molecular structures of about 200 unique targets from M. tuberculosis have been determined, comprising about 5% of its entire proteome. The majority of them have been provided by structural genomics consortia from around the world. As an example, we present the approach and some of the key achievements of the recent X-MTB consortium based in Germany (1). The targets have been selected based on comparative analyses for up or down-regulation specific gene or protein expression patterns (2). More than 100 targets have been expressed and purified, and present count of structures is 40. In parallel, purified targets were provided for compound library screening, either using assay-based tools or NMR spectroscopy. We summarize and discuss some recent highlights of potential drug targets of M. tuberculosis involved in lipid metabolism, amino acid biosynthesis and unknown function. The achievements are providing a solid framework to support coordinated international approaches for future structure-based drug discovery programs at the interface of industrial enterprises and academic research. One of the objectives will be to focus on target complexes, in addition to single targets that dominate the present depository of structures from the M. tuberculosis proteome.

References:
(1) Holton et al. (2006). Curr Protein Pept Sci. 8, 365-75.
(2) Rachman et al. (2006) Microbes Infect 8, 747-57.

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Keywords: structural genomics, drug targets, infectious diseases

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Inhibition of human pancreatic alpha-amylase by montbretin A: A new drug for diabetes and obesity?

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The World Health Organization estimates that the number of deaths related to diabetes will increase by 50% over the next ten years due largely to an alarming increase in obesity. Patients with diabetes suffer from reduced quality of life as well as an increased risk of serious complications including hyperlipidemia, cardiovascular disease, hypertension, stroke, kidney failure and nerve damage. Human pancreatic alpha-amylase (HPA) provides a unique opportunity for the development of potential therapeutic agents for the treatment of diabetes and obesity. HPA plays a vital role in the breakdown of starch in the diet, and its activity has been correlated to postprandial blood glucose levels, the control of which is essential for maintaining quality of life for diabetic patients. Specific and high affinity inhibitors of HPA, however, have been elusive, and currently available therapies that target this enzyme cause many deleterious side effects due to their activity on a wide range of glycosidases. To find new inhibitors of HPA that exhibit both high selectivity and specificity, we have screened over 80,000 pure chemicals and crude biological extracts, and have found several previously unknown HPA inhibitors. One of the most promising is montbretin A, a glycosylated acyl flavonol that acts as a competitive HPA inhibitor with a Ki of 8.1 nM. The crystallographic characterization of this inhibitor has been undertaken on two fronts: co-crystallization of the enzyme with montbretin A and crystal soaking experiments using fragments of the montbretin molecule, including myricetin and ethyl caffeate which are also HPA inhibitors demonstrating competitive and non-competitive inhibition, respectively. Supported by the Canadian Institutes of Health Research.

Keywords: drug design, glycosyl hydrolase, molecular

screening

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Structure of the catalytic subunit of human protein kinase CK2alpha prime with a potent inhibitor

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Casein kinase ll (CK2) is a serine/threonine kinase and widely distributed in various tissues. CK2 predominantly exists in the form of heterotetramer composed of two catalytic subunits (CK2alpha or CK2alpha prime) and two regulatory subunits (CK2beta). Recently, the CK2alpha inhibition has been revealed to prevent the progression of glomerulonephritis. On the other hand, inhibition of CK2alpha prime in testis affect the spermatogenesis. In order to develop of novel CK2 inhibitor for nephritis, we determined the first structure of CK2alpha prime complexed with a potent inhibitor and compared with the structure of CK2alpha. The crystal structure of a C-terminal deletion mutant of human Ck2alpha prime was solved and refined to 3.2 Å resolution. Two isozymes, CK2alpha and CK2alpha prime, reveal the high similarity of the overall structure. The largest structural difference between CK2alpha prime and human Ck2alpha occurs at the loop connecting the strands beta 4 and beta 5. The corresponding region belongs on one hand to the CK2alpha/CK2beta interface in the holoenzyme and on the other hand to the catalytic core, which is structurally highly conserved among the eukaryotic protein kinases. This observation is consistent with the growing evidence that CK2alpha prime and CK2alpha may possess the difference of the relation in vivo with CK2beta and the substrate recognition.

Keywords: protein kinase CK2, structure-based drug design, X-ray crystallography

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Therepeutic antibodies target a locally misfolded region of tumour-specific EGFR

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Epidermal Growth Factor Receptor (EGFR) is a significant target for cancer therapy. In most normal tissues EGFR is expressed at low levels and is inactive but it stimulates growth in many human tumours. EGFR inhibitors have been developed as therapeutic agents but success has been limited by interference from the presence of EGFR on normal tissues. All current therapeutic antibodies bind both active and inactive EGFR. We have characterized antibodies mAb806 and mAb175 with the curious property of binding wild type EGFR on tumour cells but not wtEGFR expressed on normal cells. Fab structures with the EGFR epitope show that the epitope adopts a conformation similar to that in wild type receptor. However, binding is prohibited by significant steric clashes of the Fab with the CR1 domain in both observed conformations of the receptor. From the EGFR structure it appeared that breaking a disulfide bond just before the epitope should allow the CR1 domain to open up sufficiently for antibody binding. Mutant EGFRC271A/C283A binds mAb806 and mAb175 with significantly greater affinity than wtEGFR. While mAb806 fails to inhibit the in vitro growth of cells expressing wtEGFR, mAb806 completely inhibits ligand-associated stimulation of cells expressing EGFRC271A/C283A. Our results provide the first view of how an antibody can recognise a cryptic epitope in a cell surface receptor. The mechanisms of binding of antibodies mAb806 and mAb175 requires a form of the EGFR where the epitope is preferentially exposed during receptor activation. Detection of this locally misfolded form of EGFR associated with tumour cells suggests that it might be possible to produce therapeutics which target local misfolding when other cell surface proteins are overexpressed or activated on tumour cells.

Keywords: therapeutic antibody, cryptic epitope, cancer

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Crystal structures of SERCA in complex with inhibitors with potential as prostate cancer drugs

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Currently no treatment significantly prolongs the survival of men suffering from androgen insensitive prostate cancer. Treatment is complicated by a slow proliferation rate making the cells insensitive to standard chemotherapeutics. The cytotoxin thapsigargin (Tg) inhibits the membrane protein sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), thus obstructing the Ca^{2+} homeostasis and eventually invoking apoptosis. Conjugation of Tg with a specific peptide carrier produces an inert prodrug, which selectively is unmasked by the prostate-specific antigen (PSA) protease at prostate tissue sites, including prostate cancer cells. The structure of SERCA with the Boc-12-aminododecanoyl derivative of Tg was determined at 3.3 Å and revealed a highly unusual binding mode where the 12-aminododecanoyl group penetrates the transmembrane region of SERCA, reaching the surface on the opposite side of the protein, corresponding to one of the Ca^{2+} binding sites (Site II). Furthermore structures of complexes of SERCA with other inhibitors possessing a guaianolide nucleus esterfied with a long un-branched acyl goup have been obtained: i) nortrilobolide (Nor), ii) two additional thapsigargin-based compound (Hzl2308 and Hzl130407), and iii) a synthetic derivative of thapsigargin, in which the guaianolide nucleus has been hydrogenated (Tg2), have been determined at 2.65 Å, 2.65 Å, 2.85 Å and 3.1 Å resolution, respectively. The structures can now be compared to SERCA in the same functional state, but in the absence of inhibitors (Olesen et al. 2007), and give valuable new knowledge on the further development of Tg-derivatives and other SERCA-inhibitors as resources for drug design, both in cancer and