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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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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