It is revealed that the phosphotyrosine residue of Cblin mediates the main contacts with positively charged pocket in Cbl-b TKB domain. Moreover, the detail binding mode between Cbl-b TKB domain and Cblin will be discussed.

[1] R. Nakao, K. Hirasaka, J. Goto, K. Ishidoh, C. Yamada, A. Ohno, Y. Okumura, I. Nonaka, K. Yasutomo, K. M. Baldwin, E. Kominami, A. Higashibata, K. Nagano, K. Tanaka, N. Yasui, E. M. Mills, S. Takeda, and T. Nikawa, *Molecular and cellular biology* **2009**, *29*, 4798-4811.

Keyword: complex, inhibitor, interaction

MS16.P50

Acta Cryst. (2011) A**67,** C305

Crystal structure of the leishmania major MIX protein

Michael A. Gorman,[a] Alex D. Uboldi,[b] Peter J. Walsh,[a] Kher Shing Tan,[a] Guido Hansen,[a] Trevor Huyton,[c] Hong Ji,[d] Joan Curtis,[b] Lukasz Kedzierski,[b] Anthony T. Papenfuss,[b] Con Dogovski,[e] Matthew A. Perugini,[e] Richard J. Simpson,[d] Emanuela Handman,[b] Michael W. Parker,[a,e] [a]*Biota Structural Biology Laboratory, St. Vincent's Institute Australia,* [b]*The Walter and Eliza Hall Institute of Medical Research, Australia,* [c]*The Australia Synchrotron,*[d]*Ludwig Institute for Cancer Research, Australia,* [e]*Bio21 Molecular Science and Biotechnology Institute, Australia,* E-mail: mgorman@svi.edu.au

Leishmania and *Trypanosoma* protozoan parasites of the order kinetoplastida are pathogenic to humans. These parasites shuttle between insect vectors and mammalian hosts where they cause disease. *Leishmania* species cause leishmaniasis, a spectrum of diseases that range in severity from skin lesions to serious disfigurement and fatal systemic infection. *Trypanosoma rhodesiense* and *Trypanosoma gambiense* are responsible for African sleeping sickness, while *Trypanosoma cruzi* causes Chagas disease in Central and South America. The WHO estimates that there are at least 2 million new cases of leishmaniasis each year. African sleeping sickness and Chagas disease, which are vastly underreported, each account for tens of thousands of cases per year.

There are currently no effective vaccines against these pathogens and existing drugs suffer from toxicity, variable efficacy and high costs. In addition, emerging drug resistance prompts the search for novel drugs, ideally directed against new targets. In our quest for such drug targets we have recently identified a mitochondrial membrane-anchored protein, designated MIX, which occurs exclusively in kinetoplastids. In *Leishmania,* MIX is expressed in all life-cycle stages including the amastigote stage present in the mammalian host. Deletion of one allele of *MIX* in *L. major* shows morphological and mitochondrial abnormalities, effects that are also seen in *T. brucei* epimastigotes in which MIX expression has been down-regulated by MIX gene-specific RNAi. Importantly, These parasites display reduced infectivity *in vitro* and reduced virulence *in vivo*.

We have determined the crystal structure of Leishmania major MIX (residues 45-195, referred to as MIX⊗45) using X-ray crystallography by the Multiple Isomorphous Replacement technique, to a resolution of 2.4 Å. MIX forms an all ⟨-helical fold comprising seven ⟨-helices that fold into a single domain. The distribution of helices is similar to a number of scaffold proteins, namely HEAT repeats, 14-3-3, and tetratricopeptide repeat (TPR) proteins, suggesting that MIX mediates protein–protein interactions. Accordingly, using co-purification and mass spectroscopy we were able to identify several proteins that may interact with MIX *in vivo*. Being parasite specific, MIX is a promising new drug target and, thus, the structure and potential interacting partners provide a basis for structure-guided drug discovery.

Keywords: parasite, protein, crystallography

MS16.P51

Acta Cryst. (2011) A**67,** C305

Identification of two imidazole binding sites in VAP-1

Eva Bligt, Vimal Parkash, Yvonne Nymalm-Rejström and Tiina Salminen, *Department of Biosciences, Biochemistry, Åbo Akademi University, Turku (Finland).* E-mail: ebligt@abo.fi

Amine oxidases oxidatively deaminate amines to the corresponding aldehydes in a reaction where hydrogen peroxide and ammonia are produced. The mammalian amine oxidase vascular adhesion protein-1 (VAP-1) is classified as a copper containing amine oxidase (CAO). VAP-1 is a 180 kDa membrane-bound glycoprotein, which has a 2,4,5-trihydroxyphenylalanine quinone (TPQ) as its cofactor. In addition to the enzymatic function, VAP-1 is an adhesion protein, which is primarily involved in leukocyte trafficking to sites of inflammation. It has also been shown to be involved in glucose metabolism, differentiation of adipose cells, potentiation of hypertension and the vascular degradation that often occurs in Alzheimer's disease.

We have solved two new structures of a soluble, proteolytically cleaved form of VAP-1, which was extracted from human plasma. The structures were refined to 2.6 Å and 2.95 Å respectively. In the structures we found imidazole molecules, which were derived from the crystallization buffer. In the 2.6 Å structure, an imidazole molecule is hydrogen bonded to the TPQ cofactor, which is in an inactive on-copper conformation. In the 2.95 Å structure, an imidazole molecule is covalently bound to the active off-copper conformation of TPQ. A second imidazole binding site was also identified in both structures, as an imidazole is bound to Tyr394 close to Thr212 in the substrate channels. The imidazoles block the entrance to the active site and act as inhibitors of the enzymatic activity.

As VAP-1 is involved in leukocyte trafficking to sites of inflammation, inhibition of the enzyme could be of great importance in chronic diseases like rheumatoid arthritis, asthma and psoriasis. Small molecular inhibitors that block leukocyte trafficking to sites of inflammation could be used as new anti-inflammatory drugs against these chronic diseases. The new VAP-1 structures give new insights in the drug design based on secondary amine inhibition. Furthermore, inhibitors bridging the two imidazole binding sites might acquire specificity towards VAP-1, since the second binding site is unique in VAP-1.

Keywords: amine oxidase, vascular adhesion protein-1, imidazole

MS16.P52

Acta Cryst. (2011) A**67,** C305-C306

Crystal Structure of a dimeric anti-HER2 human single domain antibody

Shu-ying Wang,[a] Toya Nath Baral,[b] Shi-yu Chao,[a] Shenghua Li,[b] Jamshid Tanha,[b] Mehdi Arbabi-Ghahroudi,[b] Jianbing Zhang,[b] [a]*Department of Microbiology and Immunology, National Cheng Kung University, Tainan, (Taiwan).* [b]*Institute of Biological Sciences, National Research Council , Ottawa, ON, (Canada).* E-mail: sswang23@mail.ncku.edu.tw

Human epidermal growth factor receptor-2 (HER2) is well described as a target for antibody in various tumor models [1]. Single-domain antibodies (sdAbs) derived from human VH are considered to be less soluble and prone to aggregate which makes it difficult to determine the crystal structures [2]. In this study, we isolated and characterized two anti-HER2 sdAbs, Gr3 and Gr6, from a synthetic human V_H phage display library. Size exclusion chromatography and surface plasmon resonance analyses demonstrated that Gr3 is a monomer, but that Gr6

P.MS.16