of strains of this malaria parasite resistant to conventional drug therapy has stimulated the search for antimalarials with novel modes of action. Fosmidomycin [3-(*N*-formyl-*N*-hydroxyamino) propylphosphonic acid] has proved to be efficient in the treatment of uncomplicated *P. falciparum* malaria in recent clinical trials conducted in Gabon and Thailand. It has a novel mode of action through the inhibition of 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), an enzyme of the non-mevalonate pathway of isoprenoid biosynthesis, which is absent in humans. However, the structural detail of the DXR inhibition by fosmidomycin in *P. falciparum* is unknown.

Here, we report the crystal structures of *P. falciparum* DXR (PfDXR) in its inhibitor-free ternary complex, fosmidomycin-bound quaternary complex, and FR900098 (*N*-acetyl derivative of fosmidomycin)-bound quaternary complex at 1.86, 1.9, and 2.15 Å resolution, respectively [1], [2]. The disordered loop region in the inhibitor-free form is well defined in the inhibitor complexes. Fosmidomycin and FR900098 bind to PfDXR in a similar manner, in which each inhibitor is buried in the active site and is shielded from the solvent environment. The methyl group of FR900098, which is not found in fosmidomycin, is accommodated in the active site by the induced-fit movement of the side chain of Trp296 belonging to the flexible loop, which is disordered in the inhibitor-free form. These flexible features demonstrate that the active site of PfDXR has the ability to accommodate an additional functional group of fosmidomycin derivatives. The quaternary complex structures revealed that a *cis* arrangement of the oxygen atoms of the hydroxamate group of the bound inhibitor is essential for tight binding of the inhibitor to the active site metal. We expect present structures to be useful guides for the design of more effective antimalarial compounds.

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**Keywords: antimalarial, disease, drug,**

## MS16.P48

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**Structural Investigation of Pim1 Kinase**

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The incidence and death rate of prostate cancer, the most common type of malignancy diagnosed in men in developed countries, is rapidly increasing. Unfortunately, the low response rate to current treatment regimes makes treating prostate cancer extremely difficult. The expression of Pim1 kinase, a serine/threonine kinase, is reported to be upregulated in the prostate. Overexpression has been correlated with unsuccessful clinical outcome in prostate cancer patients. Pim1 is also overexpressed in other human leukemia including acute myeloid leukemia and hematopoietic malignancies.

Pim1 plays a role in cell survival/apoptosis, differentiation, and proliferation. It adopts a constitutively active conformation so is presumed to be linked to cell proliferation and development of tumorogenesis. Specific regions of Pim1 have been identified as essential for activity. The conserved Lys67-Asp186 salt bridge is responsible for constitutive activity and the formation of the Lys67, Asp186, Glu89, triad is crucial. Mutation of Lys67 results in inactivation.

Pim1 binds to its natural substrate, ATP, through a single hydrogen-bond interaction with Glu121, in the hinge region (formed by Leu120, Glu121, Arg122, Pro123, Glu124, Pro125, Val126 and Gln127). The glycine rich P-loop comprises residues Leu44, Gly45, Ser46, Gly47, Gly48, Phe49, Gly50, Ser51 and Val52. The P-loop is flexible and may play a role in specificity and selectivity, it may affect the affinity of inhibitors via induced fit binding modes. Available structures of Pim1 inhibitor complexes show, generally, this region adopts a fairly conserved conformation. The binding of AMP-PNP, however, causes the P-loop to be significantly displaced. It has been reported that this displacement destabilizes the protein, resulting in an unfavorable conformation.

Currently, therapies that target Pim1, mostly employ ATP competitive inhibitors. A consequence of targeting the ATP binding site of Pim1 is that these compounds also tend to interact, non-specifically, with the ATP binding site of many other kinases. This lack of specificity results in a significant reduction in efficacy of the compound and thus its potential in clinical use. Unspecific binding is also often responsible for the emergence of many unwanted side effects.

To date, no small compounds targeting Pim1 have successfully progressed through to clinical use because of lack of specificity. Thus, the design of drugs that can specifically inhibit Pim1 activity is the focus of our research. Recently, we employed insilico-screening techniques to identify potential inhibitors. The crystal structures of these protein-inhibitor complexes were subsequently solved. Interestingly, these compounds cause the P-loop to adopt a completely novel conformation upon binding. Unlike the conformational change caused by AMP-PNP interactions, this loop position seems to be stabilized by additional interaction with the protein. These new inhibitors have relatively high affinity, as well as specificity, and have been shown to be effective *in vitro* and *in vivo*. We believe that the interactions these compounds make with the protein, and the consequent conformational repositioning of the P-loop, may be responsible for the high potency and specificity achieved for this series of compounds. Investigations into their potential as drug candidates are currently under investigation.

**Keywords: Kinase, Rational Drug Design, Disease**

## MS16.P49

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**Crystal structure of Cbl-b TKB domain in complex with Cblin (Cbl-b inhibitor)**

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Cbl-b is RING-type ubiquitin ligase established as a negative regulator of receptor tyrosine kinase signaling in variety of cells. In previous studies, we showed that the Cbl-b-mediated ubiquitination and degradation of IRS-1 (insulin receptor substrate 1), a key intermediate of skeletal muscle growth, contribute to muscle atrophy during unloading [1]. Furthermore, we found that a pentapeptide, named "Cblin (Cbl-b inhibitor)", mimetic of tyrosine 608-phosphorylated IRS-1 inhibited Cbl-b-mediated IRS-1 ubiquitination and degradation under in vitro and in vivo experimental conditions [1]. Cbl-b contains N-terminal TKB domain (tyrosine kinase binding domain) which mediates direct binding to phosphorylated tyrosine residues of substrates. We predicted that Cblin directly bound to TKB domain of Cbl-b resulting in inhibition of the interaction between Cbl-b and IRS-1. However, the direct binding of Cblin to Cbl-b was not investigated. Thus, in order to evaluate the mechanism of inhibition of IRS-1 ubiquitination by Cblin, we determined the crystal structure of the Cbl-b TKB domain in complex with Cblin at resolution of 2.8 Å.

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.

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**Keyword: complex, inhibitor, interaction**

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**Crystal structure of the leishmania major MIX protein**

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*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

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**Identification of two imidazole binding sites in VAP-1**

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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**

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

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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