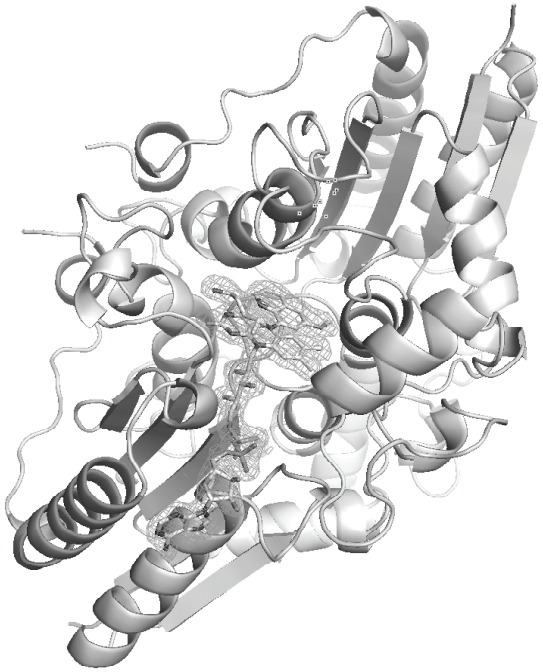
FA1-MS03-P01

Primaquine and Chloroquine as Inhibitors for Human Quinone Reductase 2 Enzyme. Majed M. AbuKhader. *Faculty of Pharmacy, Philadelphia University, PO Box: 1, 19392 Amman, Jordan.*
E-mail: majed.abukhader@gmail.com

Quinoline-based drugs such as primaquine and chloroquine are pupolar antimalaria drugs. In addition to their action on malaria parasite, they show a variation in their inhibition property of quinone reductase 2 enzyme (NQO2) which is a dominant enzyme in erythrocyte [1, 2]. The inhibiton of this particular enzyme in erythrocyte suggests the accumulation of superoxide radicals which kill malaria parasite. Through structural studies at the atomic level using x-ray crystallography, it was possible to explain the inhibition properties for primaquine and chloroquine towards NQO2. Primaquine and chloroquine were co-crystallized with NQO2 and the crystal structures of these complexes were resolved at 1.65Å and 1.83Å respectively. These structures illustrated that primaquine is bound tightly in a compact manner with NQO2. This is due to the presence of polar as well as hydrophobic interactions with amino acids of NQO2 active site. In case of chloroquine, the complex structure suggests a very weak binding with only hydrophobic interaction. The results explain the reason behind the strong and weak inhibition ability for primaquine and chloroquine towards NQO2 respectively. The information presented in this work could be further exploited in drug design studies to develop new possible antimalaria agents.



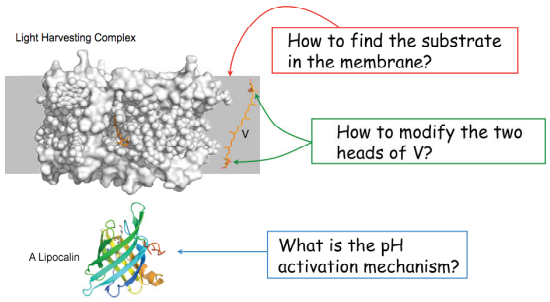
[1] Graves P.R., Kwiek J. J., Fadden P., Ray R., Hardeman K., Coley A. M., Foley M., Haystead T. A., *Mol. Pharmacol.*, **2002**, 62(6), 1364-1372. [2] Kwiek J.J., Haystead T. A., Rudolph J., *Biochemistry*, **2004**, 43, 4538-4547.

Keywords: quinonr reductase2; primaquine; chloroquine; antimalaria drugs

FA1-MS03-P02

A Structural Basis for the pH-Dependant Xanthophyll Cycle. Pascal Arnoux[a], Tomas Morosinotto[b], Giorgia Saga[b,c], Roberto Bassi[c], David Pignol[a]. [a]*Laboratoire de Bioénergétique Cellulaire, Saint-Paul-lez-Durance, 13108, France.* [b]*Università degli studi di Padova, Padova, Italy.* [c]*Università degli studi di Verona, Verona, Italy.*
E-mail: pascal.arnoux@cea.fr

Plants adjust their photosynthetic activity to changing light conditions. A central regulation is the xanthophyll cycle in which the carotenoid violaxanthin (V) is converted into zeaxanthin (Z) in strong light, with the dissipation of the excess absorbed energy as heat and the scavenging of reactive oxygen species. Violaxanthin de-epoxidase (VDE), the enzyme responsible for Z synthesis, is activated when the thylakoid lumen becomes acidified as a result of high photosynthetic activity: at neutral pH VDE is a soluble and inactive enzyme whereas it attaches to the membrane with a marked cooperativity at acidic pH. VDE also uses ascorbate as a co-substrate with a pH-dependent K_m that may reflect a preference for ascorbic acid.
We determined the structures of the central lipocalin domain of VDE (VDE_{cd}) at acidic and neutral pH. At neutral pH, VDE_{cd} is monomeric with its active site occluded in a lipocalin barrel. Upon acidification, the barrel opens up and the enzyme appears as a dimer. The channel linking the two active sites of the dimer can harbour the entire carotenoid substrate and thus may permit the parallel de-epoxidation of the two violaxanthin β-ionone rings, making VDE an elegant example of the adaptation of an asymmetric enzyme to its symmetric substrate.



Keywords: photosynthetic proteins; membrane protein; regulation and reaction mechanisms of enzymes

FA1-MS03-P03

The Structure of DapD from *Mycobacterium Tuberculosis.* Linda Schuldt[a], Simone Weyand[a], Georgia Kefala[a], Manfred S. Weiss[a]. [a]*EMBL Hamburg Outstation, c/o DESY, Notkestraße 85, D-22603 Hamburg, Germany.*
E-mail: lschuldt@embl-hamburg.de

Tuberculosis (TB) is a bacterial infectious disease predominantly caused by the pathogenic bacterium

Mycobacterium tuberculosis (*Mtb*). The lysine biosynthetic pathway, consisting of nine enzymatic reactions, is known to be essential for the survival of the *Mtb* [1]. Five out of nine enzymes have been structurally described by our group till now. Here we present the structure of DapD (tetrahydrodipicolinate N-succinyltransferase; Rv1201c; EC 2.3.1.117) from the *Mtb*-strain H37Rv [2], which catalyzes the fifth reaction step of the lysine biosynthetic pathway: the conversion of cyclic tetrahydrodipicolinate (THDP) to acyclic *N*-succinyl-2-amino-6-oxopimelate by the use of succinyl-CoA.
Mtb-DapD was cloned, expressed recombinantly in *E. coli* and purified to homogeneity. It was crystallized as cubic crystals, which diffracted X-rays to about 2 Å resolution [3]. The structure was solved by MAD Phasing. *Mtb*-DapD assembles as the biological active homotrimer, with each individual monomer being composed of three distinct domains, containing a left handed, three stranded *β*-helix domain. A complex of *Mtb*-DapD with its cofactor succinyl-CoA was generated to elucidate protein:cofactor interactions. Activity experiments revealed that enzymatic activity of *Mtb*-DapD is metal dependant, although the metal binding sites are about 14 to 24 Å away from the active site.

[1] Hutton *et al.* (**2007**). *Mini Rev. Med. Chem.* 3, 115-127. [2] Cole *et al.* (**1998**). *Nature* 393, 537-544. [3] Schuldt *et al*. (**2008**), *Acta Cryst* F64**,** 863-866.

Keywords: biological crystallography; enzymatic protein; MAD

FA1-MS03-P04

Structural and Mechanistic Properties of Grape Leucoanthocyanidin Reductase. Chloé Maugé[a], Thierry Granier[a], Jean Chaudière[a], Mahmoud Gargouri[a], Béatrice Langlois d'Estaintot[a], Claude Manigand et Bernard Gallois[a]. [a]*CBMN, UMR CNRS 5248, Bât B8, Avenue des Facultés, Université Bordeaux 1, 33405 Talence Cédex. France.*
E-mail: c.mauge@cbmn.u-bordeaux.fr

Flavan-3-ols (such as catechin and epicatechin) and their polymeric condensation products, the proanthocyanidins or condensed tannins, constitute the most common group of flavonoids consumed in the diet. In plants, they play a role in defense against herbivores. When ingested, they act as antioxidants with beneficial effects for human health and also contribute to the organoleptic properties of fruits and other plant products such as fruit juices and wine. It was only recently that their biosynthetic pathway was elucidated. Two NADPH-dependent enzymes, anthocyanidin reductase (ANR) and leucoanthocyanidin reductase (LAR), catalyze the formation of flavan-3-ols of 2,3-cis and 2,3-trans stereochemistry respectively. Both enzymes belong to the SDR (short chain dehydrogenase/reductase) family.
We recently succeeded to overexpress the LAR1 gene from *Vitis vinifera*, to purify the protein and to stabilize it in solution. The enzyme activity was evidenced by RP-HPLC and LC-MS experiments and the stereochemistry of the reaction product characterized by chiral chromatography. Crystals corresponding to different complexation states were obtained. The 3D structures of the apoenzyme, holoenzyme (LAR – NADPH) and ternary abortive complex (LAR – NADPH – catechin) were determined at a resolution of 2.72, 1.75 and 2.28 Å respectively, providing one of the rare examples in the SDR family for which three different states could be described. They suggest that the reaction mechanism involves the formation of a quinone methide intermediate prior to reduction. The formation of this intermediate is assumed from a hydrogen-bonding network that should favour the deprotonation of the substrate 7-hydroxyl and the extrusion of hydroxide from the C4 position, as recently described for basil eugenol synthase [1].

[1] Louie G.V., Baiga T.J., Bowman M.E., Koeduka T., Taylor J.H., Spassova S.M., Pichersky E., Noel J.P., *Plos One*, **2007**,2(10), e993.

Keywords: flavonoids; enzyme structure function; short chain dehydrogenase reductases

FA1-MS03-P05

Crystallization and X-ray Analysis of MKK4 Kinase Domain. Kensaku Hamada[a], Takayoshi Kinoshita[b], Takashi Matsumoto[a], Yuki Nakamura[a], Yasuyuki Kirii[c], Koichi Yokota[c], Toshiji Tada[c]. [a]*PharmAxess, Inc., Ibaraki, Osaka, 567-0085, Japan.* [b]*Department of Biological Science, Graduate School of Science, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan.* [c]*Carna Biosciences, Inc., Kobe 650-0047 Japan.*
E-mail: k-hamada@pharmaxess.com

MKK4 is a widely expressed dual-specificity mitogen-activated protein kinase kinase, and plays a central role in the stress-activated protein kinase signaling pathway. In response to pro-inflammatory cytokines or various environment stress such as UV radiation, heat and osmotic shock, activated MKK4 can phosphorylate either the c-Jun NH2-terminal kinase (JNK) or p38 mitogen-activated protein kinases (MAPK). MKK7 also phosphorylates and activates JNK. However, MKK4 and MKK7 indicate different substrate specificities. MKK4 phosphorylate JNK preferentially on Tyr within a Thr-Pro-Tyr motif, whereas MKK7 phosphorylate JNK preferentially on Thr.
There are questions how MKK4 recognizes two MAP-kinases and how it phosphorylate JNK preferentially on Tyr residue. In order to address these questions, we need to determine the structures of the apo MKK4 and MKK4-substrate peptide complex. As the first step of this approach, we have crystallized the MKK4 kinase domain. Crystals of MKK4 with AMP-PNP diffracted to 2.8 Å resolution and belonged to space group $P2_12_12_1$. X-ray structure determination is in progress.

Keywords: kinase structure; molecular replacement; X-ray structure