MS11-P2 **Crystallization and Structure Determination of A *C. Sativus* Cysteine Protease** Sadaf Iqbal,[ab] Ahmed Akrem,[b] Friedrich Buck,[c] Markus Perbandt,[b] Sankaran Banumathi,[d] M. Iqbal Choudhary,[a] and Christian Betzel.[b]
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A papain-like cysteine protease from bulbs of *crocus sativus* belonging to the C1 protease family was identified, purified, crystallized and the structure was solved and refined to 1.3 Å resolution. Proteins of *crocus sativus* are already identified having pharmaceutical potential. Then crystals of *crocus sativus* cysteine protease (CSCP) belong to the H3 space group with cell dimensions of a = b = 97.97 Å and c = 48.78 Å. The synchrotron radiation at DESY, Hamburg, Germany and at the Advanced Light Source (ALS) Berkeley, CA, USA was used to collect high resolution data and to solve the three-dimensional structure of CSCP. The final model was refined to R_{factor} of 0.14 and R_{free} of 0.19. The high resolution electron density at 1.3 Å resolution allowed to identify the entire sequence, so far only partially known. The final model contained 1596 protein atoms and 192 solvent atoms. The coordinates of the structure has been deposited at the protein data bank (PDB) with ID: 3U8E. The structure of CSCP shows homologies with plant as well as mammalian papain-like cysteine proteases. The enzyme is active and stable over the wide range of pH and temperature and thus can be applied for food, pharmaceutical and biotechnical applications. Details will be presented.

Keywords: *crocus sativus* cysteine protease; 1.3 Å resolution; structural comparison and interpretation

MS11-P3 **Structural determinants of substrate specificity in a nitrilase superfamily amidase** Serah Kimani,[ab] Trevor Sewell,[bc] [a]*Dept of Molecular and Cell Biology, University of Cape Town, South Africa,* [b]*Division of Medical Biochemistry, University of Cape Town, South Africa,* [c]*Electron Microscope Unit, University of Cape Town, South Africa*
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Nitrilase superfamily amidases catalyze the conversion of amides to their corresponding acids and ammonia. They perform essential metabolic roles *in vivo* and are useful in the manufacture of fine chemicals, pharmaceuticals and plastics *in vitro*. The amidase from *Nesterenkonia* species (NitN) has a preference for short aliphatic amides (SAAs) despite having an active site pocket that is considerably larger than these substrates. While its natural substrates are still unknown, rational design approaches to improve the industrial capability of NitN are hindered by a lack of understanding of the mechanism of substrate binding and catalytic conversion. This study was aimed at understanding the basis for NitN specificity on SAAs as well as identifying more potent substrates for its industrial application. The catalytic Cys (C165) and Lys (K131) residues of NitN were mutated to Ala and Gln respectively and both the wild-type (WT) and the mutant enzymes co-crystallized with a range of SAAs with the aim of visualizing bound substrate molecules in the active site pocket. The co-crystal structures of the C165A mutant with propionamide (PMD) and butyramide (BMD) had two substrate molecules bound back-to-back in the active site pocket, which suggested a possible mechanism for enhancing catalytic efficiency and also indicated that NitN would be capable of binding larger substrates. Despite its ability to bind two SAA molecules, the NitN pocket was unable to adequately restrain these substrates as the crystal structure of the WT enzyme in the presence of acrylamide (ACR) showed that ACR was bound in multiple orientations resulting in multiple covalent adducts. The crystal structure of the K131Q mutant had an unexpected adipamide (ADM) thioester intermediate at C165, which provided hints of NitN being capable of hydrolyzing ADM or related compounds. An activity assay with the WT NitN confirmed that ADM is indeed a 'better' substrate than the known SAAs, which shows a great potential in using NitN as a biocatalyst for mass production of adipic acid, one of the precursors of nylon:6.6.

Keywords: substrate preference; amide binding; amidase