FA1-MS13-P01

Crystal Structure of the TGEV Papain-like Protease 1 (PLpro1). Justyna Aleksandra Wojdyla[a], Eric J.Snijder[b], Alexander E. Gorbalenya[b], Paul Albert Tucker[a]. [a]*EMBL Hamburg Outstation, c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany.* [b]*Molecular Virology Lab, Department of Medical Microbiology, Center of Infectious Diseases, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands.*
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The *Coronaviridea* family consists of enveloped, single stranded, positive sense RNA viruses. Two thirds of the viral genome encodes two replicon polyproteins, which are autocatalytically processed by cysteine proteases, a chymotrypsin-like protease (3CLpro) and papain-like proteases (PLpro1 and PLpro2). The only papain-like domain structure that has been published belongs to the SARS virus [1]. In this poster we present results obtained from our work on the PLpro1 domain of the Transmissible Gastroenteritis Virus (TGEV). The protein was purified to homogeneity and crystallization trials took place in EMBL-Hamburg HT Crystallization facility. Single crystals, belonging to the space group $P4_12_12$, were obtained. The structure of the TGEV PLpro1 has been determined to 2.4 Å resolution by single wavelength anomalous diffraction (SAD) phasing. The functional characterization of the TGEV PLpro1 domain is in progress.

[1]Ratia, K., Saikatendu, K. S., Santarsiero, B. D., Barretto, N., Baker, S. C., Stevens, R. C., Mesecar, A. D., **2006**, *Proc. Natl. Acad. Sci USA*, 103(15), p.5717-22

Keywords: viral structure and function; proteases; single anomalous diffraction

FA1-MS13-P02

Structure of the C-terminal Domain of NSP4 from Feline Infection Peritonitis Virus. Ioannis Manolaridis[a], Justyna Aleksandra Wojdyla[a], Santosh Panjikar[a], Eric J. Snijder[b], Alexander E. Gorbalenya[b], Bruno Coutard[c], Paul Albert Tucker[a]. [a]*EMBL Hamburg Outstation, c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany.* [b]*Molecular Virology Lab, Department of Medical Microbiology, Center of Infectious Diseases, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands.* [c]*Laboratoire Architecture et Fonction des Macromolécules Biologiques, UMR 6098, AFMB-CNRS-ESIL, Case 925, 163 Avenue de Luminy, 13288 Marseille, France.*
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Feline infectious peritonitis virus (FIPV) belongs to the coronavirus family of positive-stranded RNA viruses. The crystal structure of the C-terminal domain of non-structural protein 4 (NSP4ct) from FIPV has been determined to 2.8 Å resolution. Native and SeMet-substituted proteins were crystallised under similar conditions, resulting in tetragonal crystals belonging to space group $P4_3$. The phase problem was solved initially by single isomorphous replacement with anomalous scattering (SIRAS) followed by molecular replacement (MR), using a SIRAS-derived composite model for MR. This is the first crystal structure of any part of the NSP4 protein reported for a coronavirus. It consists of a single domain with a predominantly α helical content. Sequence alignment of NSP4ct with homologous proteins from all other sub-groups of the *Coronaviridae* demonstrates high amino acid conservation, suggesting a similar fold and, therefore, function.

Keywords: viral proteins; domain structure; SIRAS

FA1-MS13-P03

What is So Sweet About Neutron Crystallography? Susana Teixeira[a,b,c], Matthew Blakeley[b], Ricardo Leal[b,c,d], Edward Mitchell[d], Trevor Forsyth[a,b,c]. [a]*EPSAM & ISTM, Keele University, Keele, Staffordshire ST5 5 BG, UK.* [b]*EMBL-ILL Deuteration Laboratory, PSB, 6 rue Jules Horowitz, 38042 Grenoble cedex 9, France.* [c]*Institut Laue Langevin, 6 rue Jules Horowitz, 38042 Grenoble cedex 9, France,* [d]*ESRF, 6 rue Jules Horowitz, BP 220, 38043 Grenoble, France.*
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The molecular mechanisms underlying the perception of taste and, in particular, the structural-functional relationship that makes thaumatin 1600 times sweeter than sucrose (weight basis [1]) is poorly understood. Thaumatin shows structural similarity to non sweet proteins [2], but shares no common structural motifs with other sweet proteins [3]. The surface charge distribution and protonation states of the thaumatin residues are likely to play a role in the interaction with the sweet receptor [4-5]. These are structural characteristics where neutrons cannot be matched by any other technique, due to their sensitivity to hydrogen and/or deuterium nuclei even at moderate resolution.
A neutron crystallographic study on thaumatin, a protein currently commercialised as a natural sweetener, is described. Neutron data were collected to a resolution of 2Å on the LADI-III diffractometer at the Institut Laue Langevin (ILL) [6]. X-ray data were collected at an in-house X-ray source. A joint neutron/X-ray refinement of the structure is ongoing and the results will be presented.

[1] Wel, H. van der & Loeve, K. *Eur. J. Biochem.* **1972**, 31, 221. [2] Tattersall, D. B., van Heeswijck, R. & Høj, P. B. *Plant Physiol.* **1997**, 114, 759. [3] Somoza, J. R., Jiang, F., Tong, L., Kang, C. H., Cho, J. M. & Kim, S.-H. *J. Mol. Biol.* **1993**, 234, 390. [4] Kaneko, R. & Kitabatake, N. *Biosci. Biotechnol. Biochem.* **2001**, 65, 409. [5] Perri, F., Romitelli, F., Rufini, F., Secundo, F., Stasio, E. D., Giardina, B. & Vitali, A. *Protein J.* **2008**, 27, 13. [6] Teixeira, S., Blakeley, M., Leal, R., Mitchell, E., Forsyth, T., *Acta Cryst.* **2008**, F64, 378.

Keywords: neutron; X-ray; crystallography