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Molecular Basis of D-Bifunctional Protein Deficiency. Maija Malin[a], Laura Pietikäinen[a], Kalervo Hiltunen[a], Tuomo Glumoff[a]. [a]*Department of Biochemistry and Biocenter Oulu, University of Oulu, Oulu, Finland.*
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Structure-function studies and clinical data were used to establish a genotype-phenotype correlation for D-bifunctional protein deficiency, a metabolic syndrome resulting from nonfunctional or residually active multifunctional enzyme type 2 (MFE-2) of peroxisomal fatty acyl β-oxidation in humans [1]. A milder form of the disease associated with expanded life time is apparent in patients carrying certain types of mutations.
We are testing mutant MFE-2 variants for their stability and reversal of the stability/folding defect. Methods include urea and guanidinium chloride denaturation monitored with tryptophan fluorescence, thermal stability measured with CD spectroscopy, and chemical or pharmacological chaperone screening. Enzyme activities are determined, and results correlated with the known crystal structure and properties of the wild-type protein. Mutant proteins are also subject to crystallization trials.
Mutant proteins under study are T15A, N158D, E232K, R248C, W249G, which based on the available structural information are expected to be rather folding or stability defective than inactivated through substrate binding or catalytic site effects. All of these variants have been expressed as recombinant proteins and purified. Preliminary results on stability have been obtained. Latest results on stability and structural studies will be presented.

[1] S. Ferdinandusse, M.S. Ylianttila, J. Gloerich, M.K. Koski, W. Oostheim, H.R. Waterham, J.K. Hiltunen, R.J.A. Wanders & T. Glumoff. *Am. J. Hum. Genet*. 78, **2006**, 112-124.

Keywords: beta-oxidation; MFE-2; deficiency

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Domains of Coronaviral Nsp3: Important Players in the Molecular Battle Between Virus and Host Cell. Rolf Hilgenfeld[a,b], Yuri Kusov[a], Christian L. Schmidt[a], Yvonne Piotrowski[a], Jinzhi Tan[a]. [a]*Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, University of Lübeck, Germany.* [b]*Laboratory for Structural Biology of Infection and Inflammation, c/o DESY, Hamburg, Germany.*
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Of the 15 – 16 non-structural proteins of coronaviruses, Nsp3 is by far the largest. Its polypeptide chain of ≈1920 residues is organized into at least seven domains: an acidic domain (Ac), the X-domain, the SARS-unique domain (SUD, only present in the SARS virus), one or two papain-like protease domains, a transmembrane domain, and the Y domain. Determination of the three-dimensional structures of these domains by X-ray crystallography or NMR spectroscopy has helped derive ideas concerning their functions [1]. This presentation will focus on the coronaviral X-domains and on the SUD. Having a macrodomain fold, the X-domain has been proposed to have an ADP-ribose-1"-phosphate phosphatase activity [2] or to bind poly(ADP-ribose) [3]. We have shown that ADP-ribose binding is not a conserved feature of all coronaviral X-domains. For example, in Infectious Bronchitis Virus, the binding site for ADP-ribose is blocked by a mutation, suggesting that this module may also have other functions [4]. For the X-domain of Human Coronavirus NL63, we have demonstrated that it hydrolyzes NAD^+ [5]. Reduction of NAD^+ levels in the host cell may reduce poly(ADP-ribosylation), an apopotosis signal in infected cells. Our crystal structure of the SUD revealed that it contains two further copies of the macrodomain, bringing the number of these modules in the SARS coronavirus to three [6]. SUD does neither bind NAD^+ nor ADP-ribose, but G-quadruplexes [7], unusual nucleic-acid structures formed by consecutive guanosine nucleotides, where four strands of nucleic acid are forming a superhelix. G-quadruplexes occur in the 3'-nontranslated regions of mRNAs coding for host cell proteins involved in apoptosis or signal transduction. This suggests an involvement of SUD in suppressing host-cell apoptosis (e.g, through inhibiting translation of *bbc3* mRNA) and/or expression of NF**κ**B and of the antiviral type-I interferons (through TAB3 or MAPK). Some of these functional assignments are being tested using our newly established SARS-CoV replicon system. The latest results will be reported.

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Structural Bases for the Selection of a Public TCR Against the HCMV NLV Epitope. Jean-Baptiste Reiser[a], Stéphanie Gras[a], Xavier Saulquin[b], Emilie Debeaupuis[b], Klara Echasserieau[b], Adrien Kissenpfennig[c], François Legoux[b], Anne Chouquet[a], Madalen Le Gorrec[a], Paul Machillot[a], Bérangère Neveu[b], Nicole Thielens[a], Bernard Malissen[c], Marc Bonneville[b], Dominique Housset[a]. [a]*Institut de Biologie Structurale Jean-Pierre Ebel, UMR 5075 (CEA, CNRS, UJF, PSB), 41 rue Jules Horowitz, F-38027 Grenoble, France.* [b]*INSERM, U892, Institut de Biologie, 9 quai Moncousu, F-44035 Nantes, France.* [c]*Centre d'Immunologie de Marseille-Luminy,*