s1.m1.o1a **In Vitro Protein Ligation and its Application in Structural Analysis of Lipidated Proteins**. Alexey Rak, Olena Pylypenko, Thomas Durek, Anja Watzke, Susanna Kushnir, Lucas Brunsveld, Herbert Waldmann, Roger S. Goody, Kirill Alexandrov, *Max-Planck-Institute for Molecular Physiology, Department of Physical Biochemistry, Dortmund, Germany. E-mail: kirill.alexandrov@mpi-dortmund.mpg.de*

Keywords: Protein Synthesis; Prenylation; GTPases

Rab/Ypt GTPases represent a family of key regulators of membrane traffic in eukaryotic cells. Association of Rab proteins with their targeted membranes is facilitated by posttranslational modifications with isoprenoid lipids. GDP dissociation inhibitor (GDI) is a general and essential regulator of Rab recycling. Although knowledge of the structure of the Rab:GDI complex is central for understanding vesicular transport, progress in its determination has been hampered by the lack of methods for engineering post-translationally-modified proteins. Here we have used a combination of total-chemical synthesis, protein engineering and intein mediated *in vitro* protein ligation to generate preparative amounts of prenylated Ypt1:GDI complex. The structure of the complex was solved to 1.5 Å resolution and provides a mechanistic explanation for the ability of GDI to selectively interact with GDP bound Rab proteins and to inhibit the release of nucleotide. Unexpectedly, we found that the isoprenoid binding site of GDI is formed by the hydrophobic core its domain II. Moreover, the presented structure demonstrates that the I92P mutation of α-GDI, which causes mental retardation in humans, perturbs the fixation of the Ypt/Rab C-terminus on domain I of GDI.

s1.m1.o1b **A New Protein Expression Platform Based on a Eukaryotic Parasite *Leishmaina Tarentolae***. Breitling Reinhard, Kushnir Susanne, Callewaert Nico, Pietrucha Regina, Contreras Roland, Beverley Steven M. and Kirill Alexandrov. *Max-Planck-Institute for Molecular Physiology, Department of Physical Biochemistry, Dortmund, Germany. E-mail: kirill.alexandrov@mpi-dortmund.mpg.de*

Keywords: Protein Expression; Eukaryotic Expression Systems; Protien Glycosylation

The fundamental problem in the production of recombinant proteins in eukaryotic expression systems is rooted in the biology of the organisms chosen as expression hosts and has not been adequately recognized. All available eukaryotic protein expression systems are based on free-living organisms and utilize the endogenous RNA polymerase II for transcription. Due to constant changes in their environment, free-living eucaryotes are forced to control their gene expression very tightly, primarily at the transcriptional level. High-level over-expression of heterologous proteins affects the physiology of the host leading to down-regulation of protein expression via activation of transcriptional control mechanisms. The complexity of the transcription control machinery precludes engineering eucaryotes, which reproducibly produce large amounts of recombinant polypeptides. In order to exploit the potential practical benefits associated with *Trypanosomatidae* organisms essentially lacking transcriptional control we have developed a new protein expression system based on a protozoan parasite of lizards *Leishmania tarentolae*. To achieve strong transcription, the genes of interest were integrated into the small subunit ribosomal RNA gene. Expression levels obtained were up to 30 mg of recombinant protein per liter of suspension culture and increased linearly with the number of integrated gene copies. To assess the system's potential for production of post- translationally modified proteins, we have expressed human erythropoietin in *L. tarentolae*. The recombinant protein isolated from the culture supernatants was biologically active, natively processed at the N-terminus, and N-glycosylated. The N-glycosylation was exceptionally homogenous, with a mammalian-type biantennary oligosaccharide and the $Man_3GlcNAc_2$ core structure accounting for >90% of the glycans present.