**Poster Sessions**

**MS36.P09**


**Structural analysis of Toll-like receptor 2-activating lipoprotein**

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IlpA, a surface protein of the human pathogen *Vibrio vulniﬁcus*, is the first lipoprotein to be characterized in *Vibrio* spp. as a major immunostimulant. Previously, it was characterized that IlpA was subject to lipidation at its N-terminal cysteine residue. The resulting IlpA then activates Toll-like receptor 2 in human cells, and induces overproduction of proinﬂammatory cytokines closely associated with septic shock in infected individuals. To identify structural features of IlpA, we determined the crystal structure of IlpA at 2.6 Å resolution. Speciﬁcally, IlpA consists of two homologous domains, each with α/β topology, similar to the structure of substrate-binding protein which is a component of ATP-binding cassette transporter. In fact, binding of α-methionine was observed in the pocket between the two domains, suggesting that IlpA is an α-methionine-binding protein. The structural features of IlpA in this study, along with the immunological properties of IlpA identiﬁed previously and other substrate-binding proteins, suggest that substrate-binding lipoproteins of ATP-binding cassette transporter present at the bacterial cell surface could serve as pathogen-associated molecular patterns to Toll-like receptor 2, causing host immune responses against infection.

The structure of IlpA-2

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**Keywords:** lipoprotein, structure, substrate-binding

**MS36.P10**


**Structure of the catalytic domain of *H. pylori* cholesterol-α-glucosyltransferase**

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α-Glucosyl cholesterol and its derivatives are the major cell wall components of *Helicobacter pylori*, also playing an important role in immune evasion and survival. *H. pylori* makes α-glycosyl cholesterol by glucosylating cholesterol extracted from the plasma membranes of human gastric mucosa cells, using the enzyme cholesterol-α-glucosyltransferase. Here we present the crystal structure of the catalytic domain of cholesterol-α-glycosyltransferase from *H. pylori* at 1.50 Å resolution, providing a platform for discovering speciﬁc inhibitors of *H. pylori* cholesterol-α-glucosyltransferase that could be developed as novel antibiotics. This work was funded by Korea Ministry of Education, Science, and Technology, National Research Foundation of Korea; Basic Science Outstanding Scholars Program, World-Class University Program, and Innovative Drug Research Center for Metabolic and Inflammatory Disease; Korea Ministry of Health, Welfare & Family Affairs (Korea Healthcare Technology R&D Project, Grant no. A092006).

**Keywords:** cholesterol-α-glucosyltransferase, HP0421, helicobacter pylori

**MS36.P11**


**Structure of the Streptococcus pyogenes β-NAD+ glycohydrolase-inhibitor complex**

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Streptococcus pyogenes (group A streptococcus; GAS) secretes several extracellular proteins which contribute to pathogenesis. Among them, β-NAD+ glycohydrolase (SPN) is an important virulence factor. The mechanism for pathogenesis is intracellular β-NAD+ depletion within the host cell due to the β-NAD+ hydrolytic activity of SPN. SPN is also toxic to the bacterium itself; therefore, GAS encodes the ifs gene, whose product (IFS) is an endogenous inhibitor of the NAD+ glycohydrolase. In order to understand the inhibition mechanism of SPN by IFS, we have determined the crystal structure of the SPN-IFS complex at 1.8 Å resolution. SPN is an atypical member of the ADP-ribosyltransferase superfamily, lacking a canonical binding site for the protein substrate. The SPN-IFS complex is stabilized by numerous hydrogen bonds and electrostatic interactions. In the complex structure, IFS covers the active site of SPN and blocks the binding of β-NAD+.

**Keywords:** β-NAD+ glycohydrolase, IFS, streptococcus pyogenes

**MS36.P12**


**Crystal structures of Eis proteins from *M. tuberculosis* and *M. smegmatis***

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Crystal structures of Eis proteins from *M. tuberculosis* and *M. smegmatis* were determined to 2.6 Å and 2.2 Å resolution, respectively. The structures of β-NAD+ glycohydrolase (β-NAD+ GHase) and β-NAD+ ADP-ribosyltransferase (β-NAD+ ARTase) from *M. tuberculosis* were solved at 2.6 Å and 2.2 Å resolution, respectively. The structures of β-NAD+ GHase and β-NAD+ ARTase from *M. smegmatis* were solved at 2.0 Å and 2.0 Å resolution, respectively. The structures of β-NAD+ GHase and β-NAD+ ARTase were solved at 2.0 Å and 2.0 Å resolution, respectively.
Enhanced intracellular survival protein (Eis), a secreted protein encoded by the Rv2416c gene of *M. tuberculosis*, was shown to enhance intracellular survival of *M. smegmatis* in macrophages. It modulates the host immune responses by suppressing macrophage autophagy, inflammation, and cell death through the inhibition of reactive oxygen species (ROS) generation. Its GCN5-related N-acetyltransferase (GNAT) domain at the N-terminus was found to be essential for the regulation of ROS generation and proinflammatory responses. Eis is also capable of acetylating kanamycin to confer resistance. To provide insights into its role in pathogenesis by *M. tuberculosis*, we have determined the crystal structures of *M. tuberculosis* Eis in both the ligand-free and ligand-bound states. It is comprised three domains. Domain 1 possesses the GNAT fold, as predicted by sequence analysis. Unexpectedly, domain 2 is also folded into the GNAT structure, while domain 3 structurally resembles the sterol carrier protein-2 domains with a hydrophobic cavity. In addition, we have also determined the crystal structure of Eis protein from *M. smegmatis*, as a complex with CoA, which is bound to domain 1 only.

**Keywords:** enhanced intracellular survival, immune response, *mycobacterium tuberculosis*  

**MS36.P13**


Crystal structure of the TNF-α inducing protein (Tipα) from *Helicobacter pylori*: DNA docking study  

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*Helicobacter pylori* infection is one of the highest risk factors for gastroduodenal diseases including gastric cancer. TNF-α is one of the essential cytokines for tumor promotion and thus a *H. pylori* protein which induces TNF-α is believed to play a significant role in gastric cancer development in humans. The HPS0596 gene product of *H. pylori* strain 26695 was identified as the TNF-α inducing protein (Tipα). Tipα is secreted from *H. pylori* as dimers and enters the gastric cells. It was shown to have a DNA binding activity. Here we have determined the crystal structure of Tipα from *H. pylori*. Its monomer consists of two structural domains (“mixed domain” and “helical domain”). Tipα exists as a dimer in the crystal and the dimeric structure represents a novel scaffold for DNA binding. The DNA-combined structures obtained from Haddo 2.1, high ambiguity driver docking, suggest possible binding mode and its biological role.


**Keywords:** tipα, *helicobacter pylori*, gastric cancer  

**MS36.P14**


LipL32, a virulence factor from pathogenic *Leptospira*,  

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Leptospirosis caused by *Leptospira* is one widespread zoonotic disease. The major target of *Leptospira* in kidney is the renal proximal tubular cells. *Leptospira* outer membrane proteins would lead to tubulointerstitial nephritis and acute renal malfunction. LipL32 is a virulence factor and the major lipoprotein of outer membrane proteins from pathogenic *Leptospira*. The crystal structure of LipL32 was determined by multiwavelength anomalous dispersion at 2.3 Å. LipL32 contains a novel polyD sequence with a cluster of seven aspartate residues, which form an acidic surface patch for Ca$^{2+}$ binding. The calcium binding to LipL32 was determined by ITC. A significant conformational change was induced when Ca$^{2+}$ bound to LipL32. LipL32 can recognize extracellular matrix components and adheres to the host cell to evade an immune response. The binding of fibronectin to LipL32 was observed by Stains-all circular dichroism and ELISA experiments. The interaction between fibronectin F30 and LipL32 is associated with Ca$^{2+}$ binding. The Ca$^{2+}$ binding to LipL32 might be important for extracellular matrix interaction with the host cell in *Leptospira*.


**Keywords:** Ca$^{2+}$ binding protein, fibronectin, leptospirosis  

**MS36.P15**


Crystal structure of Ia-Actin complex with novel ligand  

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ADP-ribosylation is one of the important enzyme modification after the protein translation. ADP-ribosylating toxin (ADPRT) adds ADP-ribosyl group of NAD to target and lead to disorganization of the cell. It is thought that some pathogenic bacteria use the ADPRT to infect into the host cell. ADPRT can be classified into four groups as the target difference. Actin specific ADPRT, such as iota toxin from *C. perfringens* ADP-ribosylates Arg-177 of α-Actin, inhibits actin polymerization and induces cell rounding. It finally causes diarrhea against human and domestic animals. Up to now, many actin ADPRT’s structures are available including Ia (catalytic subunit of iota toxin) by us [1], however, there was no information how toxin binds to actin and how proceeds the ADP-ribosylation reaction. Recently, we reported the first crystal structure of Ia-Actin complex with β-TAD, which is nonhydrolyzable NAD analog, as its ligand [2]. It provided the information of the interaction between ADPRT and actin.

Here we report a new complex structure of Ia, α-Actin and ADP instead of β-TAD. The crystal showed maximum X-ray diffraction to 2.6 Å resolution, space group = P212121, and unit cell parameters, a = b = γ = 90°, a = 56.8, b = 126.5, c = 138.1. We solved the structure with Molecular Replacement using Ia-Actin-β-TAD complex and refined with R factor = 25.8%. Currently, we are doing the refinement. Under the same crystallization condition, we found no crystals of complex of Ia-α-Actin without β-TAD or ADP. Together with these fact and complex structure analysis, we conclude the next binding scheme; Upon the binding of small ligand, such as β-TAD or ADP, it induces the small conformational change of Ia and it triggers further binding with actin.

**Keywords:** tipα, *helicobacter pylori*, gastric cancer