resembles the fusion of secretory vesicles. The size of the barrel-stave pore is fixed because it is framed with the peptide assembly, but the toroidal pore shrinks during dehydration because of loss of water around the pore. This condition is indeed the case of a melittin/MLM pore, of which the diameter decreased to 0.7 nm. Neutron scattering showed the melittin pores in the fully hydrated lipid bilayer to have a diameter of 4.4 nm. This work used beamlines **BL13A1** and **BL23A1** in at the TLS.

The use of SAXS and membrane diffraction provides unique ways to observe membrane structures directly at a molecular level. Many other peptides and drugs interact with a membrane. These techniques will improve our understanding of the membrane structure and dynamics.

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Protein-Protein Interaction

*This report features the work of Gennaro De Libero and his co-workers published in Nat. Immunol. **14**, 908 (2013) and of Jayaraman Sivaraman and his co-workers published in Nat. Commun. **4**, 2546 (2013).*

Most proteins do not act alone; they interact with each other to promote various activities in cells. Such interactions can typically be investigated structurally with protein X-ray crystallography. This year, two studies are selected to illustrate the use of synchrotron radiation in investigating such protein-protein interactions.

Antigens that trigger an immune response of T-cells must be presented by other cells, such as macrophages; this process distinguishes invaders from the self. Antigens are generally presented by major histocompatibility complex (MHC) or CD1 molecule. Gennaro De Libero led a group of scientists in Switzerland and Singapore to find and to study the antigen-presenting molecule (APM) for the human T-cell antigen receptor (TCR) containing a variable region 9 in the γ-chain and a variable region 2 in the δ-chain (Vγ9Vδ2), which senses phosphorylated prenyl metabolites.[1] The antigen-presenting molecule for Vγ9Vδ2 is neither MHC nor CD1, and was unclear before their work, but the evidence indicates the existence of an APM dedicated for phosphorylated antigens, such as isopentenyl-pyrophosphate (IPP) or (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP).

These scientists first developed a method to avoid a complication caused by the autopresentation phenomenon of human T-cells, and pinpointed the gene encoding the APM on chromosome 6. Using micro-array to analyze the expression profile of cell lines that efficiently present IPP and HMBPP, they narrowed the regions to 81 genes. After some analy-

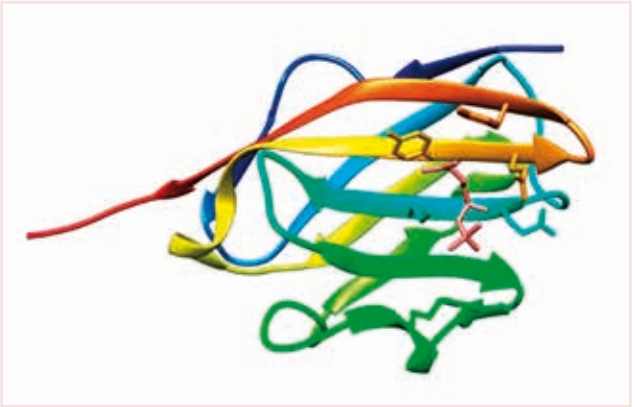

Fig. 1: Structure of BTN3A1 in complex with IPP (color in salmon). Residues interacting with IPP have sidechains presented in sticks.

sis, genes belonging to the butyrophilin (BTN) family became the candidates for APM, which had previously been linked to immunoregulation. All BTN proteins have two extracellular domains that resemble the immunoglobulin variable (V) and constant (C) domains; they have also a membrane-spanning region. After some experiments, they finally concluded that BTN3A1 is the APM for Vγ9Vδ2 TCR, and the immunoglobulin V-like domain of BTN3A1 forms a complex with the phosphorylated antigen and stimulates the γδ T-cells.

Besides the biochemical work on BTN3A1 with antigen IPP and HMBPP, they crystallized BTN3A1 in a complex with IPP and HMBPP to understand their interactions, and used protein X-ray crystallography to solve their structures, at beamline **BL13B1**. The solved structure of BTN3A1 V domain is indeed similar to the immunoglobulin V domain. The BTN3A1 V domain adopts a compact β-sandwich topology with an intersheet disulfide bond (Cys25-Cys99), characteristic of many immunoglobulin V domains. The IPP molecule is bound in a shallow groove in which a diphosphate moiety forms strong electrostatic interactions and an isopentenyl chain forms van der Waals interactions with surrounding residues. HMBPP binds similarly but with small and notable differences. They found also that IPP enhances the binding of BTN3A1 to Vγ9Vδ2 TCR. The finding of BTN3A1 to be an APM additional to MHC and CD1 establishes a new paradigm in the field of human immunological recognition, and has far-reaching implications for clinical immunotherapy.

Another example of a protein-protein interaction is the GrlR–GrlA complex. GrlA is a positive regulator of the LEE1 promoter, whereas GrlR inhibits GrlA activity. The LEE1 promotor is part of the *locus of enterocyte effacement (LEE)*, which contains most genes for the type-III secretion systems (T3SS) found in pathogens including enterohaemorrhagic *Escherichia coli* (EHEC) and enteropathogenic *E. coli* (EPEC). These pathogens cause many severe conditions, such as diarrhea. The LEE1 promotor is under a tight control by multiple factors, including Ler and H-NS; its activation triggers the entire virulent system of EHEC and EPEC.

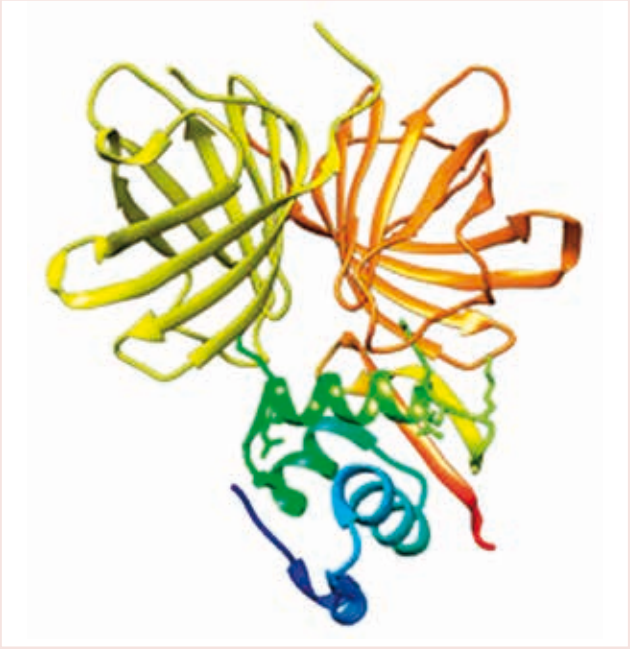

Fig. 2: Structures of GlrR-GlrAΔ. GrlR are colored in yellow and orange. GlrAΔ is in rainbow and the interacting residues (R53, R54, R64, R65 and R66) is presented in stickes.

At beamline **BL13B1**, Jayaraman Sivaraman led a group of scientists from Singapore, Israel, Spain, Canada and China to study the structure of GlrR in complex with part of GlrA (residues 1-106, termed GrlAΔ), to understand the regulatory mechanism of GlrA and GlrR on the *LEE* locus.[2] Despite that crystals of the full length of GlrA in complex with GlrR cannot be obtained, the structure GlrR-GlrAΔ shows that GlrR is a β-barrel protein similar to previously solved structures, and GlrAΔ comprises a HTH motif and an anti-parallel β-sheet at the carboxy terminus. One dimer of GlrR binds to one GlrAΔ; this stoichiometric ratio 2:1 is confirmed by analytical ultracentrifugation. The structural homologues of GlrAΔ found with a DALI search, even though of small sequence similarity, are also transcriptional regulators containing the HTH motif, indicating similar functions in these homologues. A careful investigation of residues between GlrR and GlrAΔ indicates that both the HTH motif and the C-terminal region are important for the GlrR-GlrAΔ interaction. Previous work indicated that GlrA binds with DNA through this HTH motif.

GlrR thus competes with DNA for the HTH motif of GlrA. These scientists used gel shift to show that GlrR can compete with DNA from GlrA at a concentration above 0.3 μM, but DNA cannot displace GlrR from a GlrR-GlrA complex. They showed also that the GlrR-GlrA complex regulates the ehx promoter positively and the fhhDC promoter negatively. Such a differential regulation allows pathogens to control precisely the gene expression involving its pathogenesis.

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Drug Discovery with Protein X-ray Crystallography

*This report features the work of Ya-Hui Chi, Hsing-Pang Hsieh, Su-Ying Wu and their co-workers published in Proc. Natl. Acad. Sci. USA **110**, E1779 (2013); of Su-Ying Wu, Hsing-Pang Hsieh and their co-workers published in J. Med. Chem. **56**, 3889 (2013); and of Pür Nordlund, E. Andreas Larsson and their co-workers published in J. Med. Chem. **56**, 4497 (2013).*

The objective of the huge investment in human genome projects and the subsequent structural genomics is to improve the quality of life. A direct result of this investment is the production of new and superior drugs to combat human diseases. Structurally based drug design is thus a main focus of beamlines for protein X-ray crystallography. Taiwan's government has supported our protein X-ray crystallography beamlines through National Research Program for Genomic Medicine in 2002-2010 and National Core Facility Program for Biotechnology since 2011. This year, three publications on the design of improved drugs are selected for this report.

One way to find suitable drug targets is to inspect proteins that are expressed abnormally in cells associated with certain diseases. Aurora kinases are the targets for several cancers. A few compounds for aurora kinases are currently in clinical trials. Aurora kinases are involved in chromosomal activities, especially during cell division and the separation of duplicated DNA. A group of scientists led by Ya-Hui Chi, Hsing-Pang Hsieh and Su-Ying Wu in National Health Research Institutes, Taiwan, characterized the inhibitors of aurora kinases, IBPR001 and IBPR002, and found efficacy better than MLN8237 and VX-680, the first-generation small molecules, to inhibit

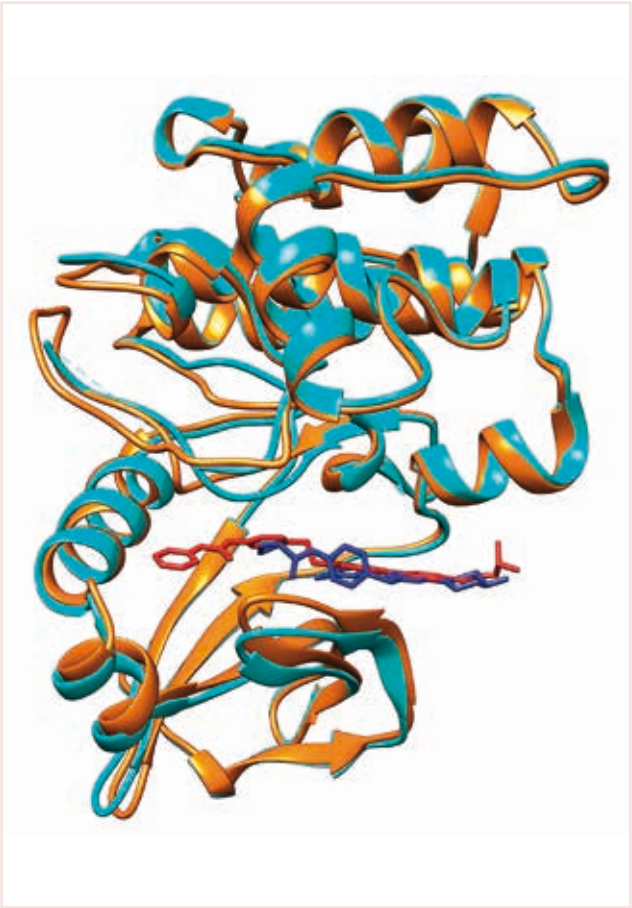

Fig. 1: Compare structures of Aurora A/IBPR001 and Aurora A/VW-680. Superimpose Aurora A/IBPR001 (Aurora A in orange and IBPR001 in red) and Aurora A/VX-680 (Aurora A in cyan and VX-680 in blue). IBPR001 clearly extends further into back pocket of Aurora A, thus, the binding affinity is increased.