pids composed of ceramide are recognized by CD1-restricted T and NKT cells and that a non-peptide antigen is recognized by γδT cells. The mechanisms of host immune responses against these SPLs remain unknown. The immune response against bacterial lipid components is considered to play important roles in microbial infections. We demonstrated that these bacterial SPLs activated murine bone marrow macrophages (BMMs) via Toll-like receptor (TLR) 4 but not TLR2, although they slightly activated CD1d-restricted NKT and γδT cells. Interestingly, this TLR 4-recognition pathway of bacterial SPLs involves the fatty acid composition of ceramide in addition to the sugar moiety. A non-hydroxy fatty acid composed of ceramide was necessary to activate murine BMMs. The bacterial survival was significantly higher in TLR4-/- mice than in TLR2-/- and wild-type mice. The results indicate that activation of the TLR4-dependent pathway of BMMs by SPLs induced an innate immune response and contributed to bacterial clearance. In this study, we clarified the immune recognition of bacterial SPLs, identified structure/function relationships in this recognition, and assess the impact of SPLs on bacterial burdens.

Keywords: Sphingobacterium, sphingolipid, Toll-like receptor 4.

SUN-397
In vivo studies of aquaporin 0 interaction with calmodulin using bimolecular fluorescence complementation

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Protein-protein interactions (PPIs) are important in close to all vital processes in the cell. Ranging from stable protein complexes to transient interactions the studies of PPIs is not only critical in the understanding of protein functions but also difficult to monitor. Bimolecular fluorescence complementation (BiFC) allows PPIs to be studied by analyzing fluorescence as a result of an interaction. Non fluorescent fragments of YFP is fused to the protein targets and upon assembly into a complex fluorescence is emitted.

We show that the BiFC method in *S. cerevisiae* can be used to confirm the tetramerization of human aquaporin0 (hAQP0) which was used as a positive control for the assay. Also, no tetramerization is observed between hAQP0 and hAQP2, acting as a negative control.

The method was then applied to the the well-known interaction between aquaporin 0 and calmodulin (CaM). Whereas previous studies have an in vitro approach, we can show the complex formation of hAQP0-CaM in vivo. By truncating the C-terminal of hAQP0 just before the putative CaM interaction site the fluorescence is drastically decreased as expected when the interaction site is removed.

S. cerevisiae is an excellent system for PPI studies and in the case of hAQP0-CaM it has been shown to also work for interactions between a membrane protein and a soluble protein. This particular complex can now be studied more easily compared to the mammalian cell systems used previously and the consequence of minor changes in either protein can be quantitated and related to differences in the affinity of the complex.

Keywords: bimolecular fluorescence complementation, membrane proteins, protein-protein interactions.

SUN-398
Intramembrane helix–helix interactions of receptor tirosine kinases: structural biology and implications for signaling and human pathologies

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Cell membrane is important part of living cell. Receptor tyrosine kinases play a key role in biological processes occurring within the membrane. However detailed mechanism of their functioning has not been completely understood yet as there are no structures of full-length receptor tyrosine kinases. Fibroblast growth factor receptor 3 (FGFR3) transduces biochemical signals via lateral dimerization in the plasma membrane, and plays an important role in human development and disease. The most frequent pathogenic mutations G380R and A391E in the transmembrane (TM) region of FGFR3 are associated both with cancer and with disorders in skeletal development. Relatively small size of complexes of TM fragments of FGFR3 (with membrane adjacent regions, tmFGFR3) with detergents or lipids allows one to study their detailed spatial structure using three-dimensional heteronuclear high-resolution NMR spectroscopy. An effective expression system and purification procedure for preparative-scale production of tmFGFR3 in norma and with point mutations for structural and functional studies were developed. The purified peptides were reconstituted in lipid/detergent DPC/SDS (9/1) micelles and characterized using dynamic light scattering, CD and NMR spectroscopy. In the solved NMR structure, the two transmembrane helices pack into a symmetric left-handed dimer, with intermolecular stacking interactions occurring in the dimer central region. Some pathogenic mutations fall within the helix-helix interface, whereas others are located within a putative alternative interface. This implies that although the observed dimer structure is important for FGFR3 signaling, the mechanism of FGFR3-mediated transduction across the membrane is complex. We propose an FGFR3 signaling mechanism that is based on the solved structure, available structures of isolated soluble FGFR domains, and published biochemical and biophysical data.

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Keywords: membrane protein receptor, NMR Spectroscopy, structural biology.

SUN-399
Isolation of mycobacterial mutants that disrupted the phospholipid synthetase gene, and their properties

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In pathogenic bacteria, cell envelopes are essential in evading various attacks on the host defense system. The membranes of the gram-positive bacteria are overlaid cell walls consisting of a thick layer of peptidoglycans. Phospholipids are one of the indispensable components for formation of bacterial membranes. The synthesis of phospholipids is thus important in mycobacteria.