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further using of this novel system as an adequate tool to study real time changes of cellular junction dynamics.

2232-Plat
Point Mutation in the Hydrophobic Region Drives Selectivity and Activity of OP-145, a Derivative of Human Cathelicidin LL-37

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The antimicrobial peptide OP-145 (1) was selected as template for the development of a therapeutic agent for coatings of implants due to its potent activity against diverse microbial species and its success in the treatment of patients with chronic otitis. The focus of our study is based on biophysical characterization of the interaction of OP-145 with bacterial and mammalian membrane mimetics, e.g. liposomes composed of phosphatidylglycerol and lipoteichoic acid as well as phosphatidylcholine. Similar to earlier findings on human cathelicidin LL-37 (2), OP-145 interacted with all lipid systems, but exhibited different incorporation mechanisms into the lipid bilayer. Thermodynamic and structural studies revealed the formation of disk-like aggregates in mammalian mimics and induction of a quasi-interdigitated structure in bacterial mimics. In agreement with in vitro studies these results indicate limited ability to distinguish bacterial from eukaryotic membranes. In order to improve selectivity of OP-145 we designed a new peptide by substitution of a single hydrophobic by a cationic amino acid residue in the hydrophobic face of OP-145. Comparable to earlier data using this strategy (3), the resulting peptide 1236-04 showed enhanced membrane selectivity in model systems inducing complete leakage of PG vesicles at very low concentrations, but none for PC. Surprisingly, peptide 1236-04 exhibited rather poor antimicrobial activity towards S. aureus and selected Gram-negative bacteria in vitro, but no toxicity in human dermal fibroblast. Differences in the interaction with other cell wall components may explain the discrepancy of findings between the model systems and living bacterial cells.

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2233-Plat
Localized Permeabilization of E. Coli Membranes by the Antimicrobial Peptide Cecropin A

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Fluorescence microscopy enables detailed observation of the effects of the antimicrobial peptide Cecropin A on the outer membrane (OM) and cytoplasmic membrane (CM) of single E. coli cells with subsecond time resolution. Fluorescence from periplasmic GFP decays and cell growth halts when the OM is permeabilized. Fluorescence from the DNA stain Sytox Green rises when the CM is permeabilized and the stain enters the cytoplasm. The initial membrane disruptions are localized and stable. Septating cells are attacked earlier than non-septating cells, and curved membrane surfaces are attacked in preference to cylindrical surfaces. Below a threshold bulk Cecropin A concentration, permeabilization is not observed over 30 min. Above this threshold, we observe a lag time of several minutes between Cecropin A addition and OM permeabilization and 30 s between OM and CM permeabilization. The long lag times and the existence of a threshold concentration for permeabilization suggest a nucleation mechanism. However, the roughly linear dependence of mean lag time on bulk peptide concentration is not easily reconciled with a nucleation step involving simultaneous insertion of multiple peptides into the bilayer. Monte Carlo simulations suggest that within seconds, the OM permeability becomes comparable to that of a pore of 100 nm diameter or of numerous small pores distributed over a similarly large area. Studies with fluorescently labeled Cecropin A provide information about the kinetics of peptide binding to the OM, and subsequent entry of peptide molecules into the periplasm and cytoplasm. Single molecule imaging of fluorescently labeled Cecropin A allows measurement of trajectory lengths and diffusion constants of the peptide on the outer membrane surface.

2234-Plat
Development of Functional Artificial Ion Channels using Peptide Nanostructures

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Ion channel proteins are of fundamental importance in numerous biological processes and constitute a key target in pharmaceutical research. However, many aspects of their mechanism of action still remain poorly understood. In order to shed light on these complex membrane transport systems, we have developed a family of peptide nanostructures that create channels for ions by aligning crown ethers on top of each other when adopting an α-helical conformation. We will present different analogs of such artificial ion channels and how they were used to study ion translocation processes in membranes. Also, we will report on the engineering of their ion-selectivity and their incorporation into new biodetection systems.

2235-Plat
Switching the Antimicrobial Activity of Gramicidin S by Light

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Light-driven reversible conformational changes of proteins are of fundamental importance to nature. Here, we present the development of a photo-controllable antimicrobial peptide (AMP), based on a novel photo-switch in the backbone. AMPs are an essential part of innate immune system of living organisms and defend them against various bacteria, viruses, and fungi. They are promising therapeutic sources for future antibiotics as they permeabilize bacterial membranes, but they tend to suffer from cytotoxic side effects against eukaryotic cells. These problems, preventing clinical applications, could be eliminated by remotely switching the membranolytic activity ON and OFF. We have thus designed and synthesized a reversibly photo-isomerizable amino acid analogue based on a diarylethylene scaffold. The amino acid analogue was incorporated into the cyclic backbone of the antimicrobial peptide Gramicidin S. Several peptidomimetics were successfully synthesized, their photochromic features were recorded in the ON and OFF states, and the corresponding molecular conformations were analyzed by CD spectroscopy and MD simulations. Antimicrobial assays and hemolysis tests showed that the biological activity could be directly controlled by irradiation with visible and UV light. These results open the way to develop new strategies for treating bacterial infections or other localized pathologies, by activating the antibiotic with light only in those specific areas where needed. The use of such 'smart' antibiotics will largely eliminate the harmful side effects on the whole body and should also prevent the concomitant development of bacterial resistance.

Platform: Microtubules and Motors

2236-Plat
3.8 Angstrom Resolution Structure of Microtubule by Cryo-Em

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Microtubules (MTs) are hollow tubes assembled from α/ß tubulin heterodimers, which align in a polar head-to-tail manner. In living cells, the "plus-end" of MTs are highly dynamic and undergo rapid episodic switches between growth and shrinkage.

The intrinsic dynamic property of MTs is carefully regulated by a large family of Microtubule Associated Proteins (MAPs). Of particular importance is a set of MAPs called plus-end tracking proteins (+TIPs), which selectively localize to the MT growing ends and mediate interactions with various cellular structures such as kinetochores and cell cortex.

End-binding proteins (EBs) are the central hub of the +TIPs interaction networks. Previous studies (Surrey et al., 2011) showed that EBs and their yeast homologue Mal3 can recognize MTs at different nucleotide states, with high affinity for GTPγS MTs, which is believed to be a good mimic of the GTP-cap of MT.

With the start-of-the-art technology of K2 direct detector (Gatan Inc) and improved helical-reconstruction programs, we determined the cryo-EM structure of EB-decorated GTPγS microtubule to 3.8 Angstrom resolution, which allowed us to de novo build a complete atomic model of microtubule at high level of accuracy. This model is a big step towards our detailed understanding of the two key questions in microtubule biology, i.e. the molecular mechanism of MT dynamic instability and MT +TIPs' end tracking behavior.

2237-Plat
Tubulin Cofactors Form a Multi-Protein Platforms that Regulate the Soluble Tubulin Pool and Promote Microtubule Polymerization

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The Microtubule (MT) cytoskeleton mediates intracellular organization and trafficking, and is responsible for force generation during cell division and