composed of PDMS micro compartments, supported lipid bilayers and purified proteins we engineered a biomimetic minimal system in which the dynamic oscillations of Min proteins were reconstituted in vitro [1]. By systematically varying the shape of the micro compartments, this biomimetic system enables us to investigate how compartment geometry influences pattern formation of a self-organizing protein system in vitro. Furthermore this system provides a platform to study protein gradient formation of the Min system and Min protein regulated localization of downstream proteins in a well-defined environment.

[1] Zieske, K. and Schwille, P. (2013), Reconstitution of Pole-to-Pole Oscillations of Min Proteins in Microengineered Polydimethylsiloxane Compartments. Angew Chem Int Ed Engl. 52: 459-462.

948-Pos Board B703

Symmetry Breaking and Plasticity of Min Protein Oscillators in Living Bacteria Sculptured into Defined Geometries

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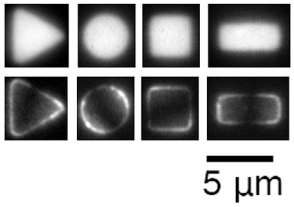
Cell shape is a pervasive feature which guides the orchestration of the subcellular organizations. By inoculating Escherichia coli cells with cell-wall-targeting agents in nanofabricated structures, we guide individual cells to adopt predefined shapes such as squares, circles, and triangles, with volumes ranging from 4 to 72 μm3. We use these sculptured bacteria to explore the shape-recognition mechanism and spatial plasticity of the MinCDE protein system, which is involved in positioning cell division machinery through MinD proteins that oscillate in time along the long axis of rod-shape E. coli. Surprisingly, Min proteins are able to sustain stable spatial oscillations in a broad spectrum of geometries, exhibiting remarkable plasticity. We observe multi-modal switches in dynamic Min patterns as the lateral dimensions increase from 2μm to 11μm in identical shapes. Our data reveal an intrinsic wavelength dictating the orientation of the Min oscillations, challenging various models that attribute the Min pattern formation to membrane curvature, binding-zone size, or long-axis recognition. Computational simulations show that this wavelength is a result of a MinD cytosolic transfer distance restricted by the rates of MinD corporative-binding and its sequestration by MinE.



949-Pos Board B704

Computational and Biomolecular NMR Guided Design of Peptide Therapeutics for Influenza A

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Peptidomimetic drugs are a rapidly growing field for treatment of disease. With the current screening-based approach yielding fewer and fewer drug candidates, peptide drugs appear to be the next frontier of disease treatment. Still, the issues of structural stability and half-life exist for peptides due to the vast number of proteases in the cell. We combine computational structure-guided design and solution NMR techniques including 19F NMR to rapidly design and screen peptide inhibitors for both protein:protein interactions (PPI) and protein:nucleotide interactions (PNI). Structural stability is greatly increased by utilizing D-amino acids at alpha helix termini, which also may enhance metabolic half-life. Both PPI and PNI interfering peptides demonstrate promising binding activity to target sites.

950-Pos Board B705

Utilizing a Reconfigured Hdl Particle to Target and Deliver Sirna to Mantle Cell Lymphoma Cells

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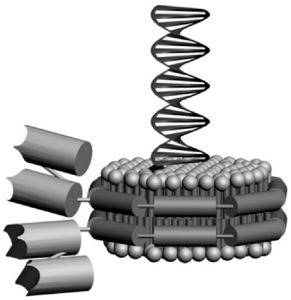
We have reconfigured the biological high-density lipoprotein (HDL) particle such that it has a high preference for binding and delivering siRNA to a subclass of B-cell lymphoma, mantle cell lymphoma (MCL) cells. We utilize a *chimeric* protein comprised of a CD20 specific single-chain variable fragment antibody fused to apolipoprotein A-1, in our HDL reconstitution (*chimeric*-rHDL). The amphipathic apoA-1 component of the *chimera* is proposed to circumscribe the edge of a discoidal lipid bilayer (see figure). The size and function of the *chimeric*-rHDL particle has been evaluated by biophysical and biochemical techniques. Fluorescence-activated cell sorting studies showed that the *chimeric*-rHDL exhibits much higher binding towards MCL cells compared to apoA-1-rHDL particles. Interestingly, immunoblot studies show that the protein cyclinD1, that is responsible for the G1→S transition in MCL cells, is efficiently knocked down when incubated with cholesterol-linked-siRNA (specific for cyclinD1) incorporated into *chimeric*-rHDL particles (see figure). The results suggest *chimeric* rHDL carrying siRNA tethered via covalent linkage to cholesterol, provides a means to deliver siRNA specifically to CD20 expressing cells.



951-Pos Board B706

Genetic Engineering of Membrane Lipid Composition in E. Coli

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Understanding the functional basis for cell membrane composition is a challenge because of the scarcity of available tools for modulating lipid composition in vivo. Here I introduce a synthetic biology approach for studying the effects of lipid composition in the model bacterium Escherichia coli. This approach involves knocking out lipid synthesis genes from the chromosome, thus freeing them from endogenous regulation pathways, and reintroducing them under the control of plasmid-based titratable expression promoters. Using this strategy, I have developed a set of strains in which several parameters of lipid composition - acyl unsaturation and cyclopropane content, cardiolipin levels - can be systematically modulated by varying the concentration of inducer in the culture. In the case of acyl unsaturation, this has allowed me to directly test the phenotypic effects of varying membrane fluidity, which is otherwise homeostatically maintained via transcriptional regulation of unsaturated fatty acid synthesis. These experiments suggest a novel role for membrane fluidity as a physical regulator of central metabolism.

952-Pos Board B707

Chloride Transport Across Planar Lipid Bilayers and Cell Membranes by Steriod-Based Synthetic Anion Transporters

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Cholapods are steroid-based synthetic anion transporters derived from cholic acid that bind anions with high affinity and promote their efflux from liposomes. To understand better cholapod-mediated anion transport, we studied the cholapods AS09, LJ09 and TL145 in planar lipid bilayers, single cells and polarised epithelia. When compared with AS09, LJ09 has higher anion affinity, whereas TL145 has greater lipid solubility. In planar lipid bilayers, cholapod-mediated currents relaxed to a steady-state after an initial peak when membrane voltage was stepped. Anion transport by cholapods was concentration-dependent with anion conductance decreasing with increasing anionic radius. Evaluation of the rate constants for anion transport by cholapods using the method of Läuger (Science 1972;178:24-30) suggested that movement of the anion-transporter complex across the membrane is the rate-limiting step of cholapod-mediated anion transport. To investigate cholapod-mediated anion transport in single cells, we used Fischer rat thyroid epithelial cells stably expressing the halide-sensitive yellow fluorescent protein (FRT-YFP) (gift from A Verkman, UCSF) to monitor anion influx. In the absence of cholapod, addition of iodide (10 mM) to FRT-YFP cells had little or no effect on fluorescence. However, in the presence of cholapod, YFP fluorescence decreased following iodide influx into FRT-YFP cells and when iodide was washed from the external solution fluorescence quenching reversed. Finally, to evaluate cholapod-mediated transport at the tissue level, we grew FRT-YFP cells as polarised epithelia and measured transepithelial Cl^- movement in Ussing chambers. Treatment of FRT-YFP epithelia with cholapods led to the development of transepithelial Cl^- current that was absent in untreated epithelia. Because TL145 was the most effective transporter in all assays, we conclude that lipid solubility is an important determinant of transmembrane