This strand passage mechanism allows type II topoisomerases to unlink, unknot, and relax supercoiled DNA to below equilibrium levels[1]. The mechanisms underlying this non-equilibrium topology simplification remain speculative. Several theoretical models have been proposed but experimental data has not been able to distinguish among them. One model developed by Yan and Marko[2], postulates that the strand passage mechanism of type II topoisomerases is governed by a kinetic proofreading process. In practice, this model suggests that type II topoisomerases require two collisions between the T- and G-segments prior to strand passage. The first collision of a T-segment with the enzyme-bound G-segment transiently activates the enzyme and the second collision of the T-segment with the activated enzyme results in strand passage. The model predicts that the strand passage probability scales as the square of the collision rate, which has not been tested. We directly tested this prediction of the kinetic proofreading model using a single-crossing DNA unlinking assay. We measured the rate that topoisomerase IV, a bacterial type II topoisomerase, unlinked DNA as a function of the strand collision probability obtained from Monte Carlo simulations of the DNA crossings. The unlinking rate was linearly related to the collision probability, which is inconsistent with the kinetic proofreading model.

1.Rybenkov, V. V., Ullsperger, C., Vologodskii, A. V. & Cozzarelli, N. R. Simplification of DNA topology below equilibrium values by type II topoisomerases. *Science* **277**, 690-693 (1997).
2.Yan, J., Magnasco, M. O. & Marko, J. F. A kinetic proofreading mechanism for disentanglement of DNA by topoisomerases. *Nature* **401**, 932-935, doi:10.1038/44872 (1999).

2481-Pos Board B251

An Enthalpic "On/Off Switch" Linking DNA Binding and Nucleotide Incorporation Activity in Pol I DNA Polymerases

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Primer-template DNA (pt-DNA) binding by DNA polymerases is the first step of the polymerization cycle. We have previously characterized the thermodynamics of pt-DNA binding with respect to temperature for the Klenow and Klentaq ''large fragment domains'' of DNA polymerase I, from *Thermus aquaticus* and *Escherichia coli*, respectively. DNA binding affinities for both polymerases are quite tight across wide temperature ranges, 5-70 °C for Klentaq and 5-37 °C for Klenow. Both polymerases show significant heat capacity changes upon binding, yielding curved ΔG versus temperature dependences. This results in large changes in ΔH and $T\Delta S$ with temperature, including a sign change from positive to negative for both enthalpy and entropy as temperature increases. For both polymerases, DNA binding is enthalpy-driven near their respective physiological temperatures.

Herein, nucleotide incorporation activity was measured with respect to temperature to examine how the thermodynamics of initial pt-DNA binding relates to the enzymatic activities of Klentaq and Klenow. It is found that both polymerases are enzymatically inactive until the temperature reaches the point where the enthalpy of binding becomes negative (favorable), despite the fact that they both bind DNA quite well at lower temperatures. The data suggest that, for both polymerases, a negative free energy of binding alone is insufficient to drive catalysis, and that a negative enthalpy of initial binding (ΔH) is required for nucleotide incorporation activity. This work is supported by the National Science Foundation.

Membrane Active Peptides II

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The Clustering of Anionic Lipids by Highly Cationic Cell Penetrating Peptides, as with Antimicrobial Peptides, can Contribute to their Antimicrobial Activity

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It has been reported that there is some overlap of biological activities between cell penetrating peptides (CPPs) and antimicrobial peptides (AMPs). We have studied a group of 9 AMPs, a fusion peptide and 7 CPPs, with regard to their conformational state in the presence and absence of a lipid mixture mimicking the cytoplasmic composition of Gram negative bacteria, their ability to cluster anionic lipids and their bacteriostatic effect on several different species of bacteria. Generally those peptides with the highest number of charges per residue were more effective in clustering anionic lipids. Among the peptides studied, six AMPs and five CPPs were found to have anionic lipid clustering activity. Remarkably, these peptides also had bacteriostatic activity against several species of bacteria, particularly against *E. coli*, a Gram negative bacteria sensitive to lipid clustering agents. In contrast, those AMPs and CPPs that did not cluster anionic lipids were not toxic to *E. coli*. As shown for several types of AMPs previously, we suggest that anionic lipid clustering may contribute to the mechanism of antibacterial action of the more highly cationic CPPs as well. Furthermore, it is a mechanism that should be further considered to explain the entry of these agents into mammalian cells or their escape from intracellular endosomes.

2483-Pos Board B253

Effects of Tryptophan Content and Backbone Spacing on Uptake Efficiency of Cell-Penetrating Peptides

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Cell-penetrating peptides (CPPs) have recently gained considerable interest due to their ability to cross cellular membranes and deliver macromolecular cargo. It is of great importance for the pharmaceutical industry to find molecular tools that can assist drug delivery across the cell plasma membrane and CPPs have emerged as promising candidates for this purpose. Despite being efficient vectors for intracellular delivery, the mechanisms behind CPP uptake into cells are not yet fully understood. Endocytosis has been recognized as a major pathway, however direct translocation pathways also exist and several routes may even act in parallel depending on peptide sequence and concentration.

Our goal is to better understand the mechanistic details of how CPPs enter cells. Previous studies suggest that arginines and tryptophans contribute to cell internalization efficiency. In order to investigate the effect of tryptophan content and backbone spacing on peptide uptake, we have designed a series of peptides all containing eight arginines and 1-4 tryptophan residues with different positions and spacing. Uptake efficiency, cellular distribution and toxicity of these peptides in live mammalian cells were explored using flow cytometry and confocal microscopy. In addition, peptide induced leakage and binding affinity to liposomes were investigated.

Our data show that the uptake efficiency and intracellular distribution varies between peptides of different tryptophan content and backbone spacing. Highest uptake was observed for the peptide with four tryptophans scattered in the amino acid sequence. The peptides were found to have binding constants of the same magnitude, indicating that the differences in uptake are caused by peptide structure rather than variations in binding affinity. All peptides showed relatively low levels of cytotoxicity and liposome leakage, thus making them interesting for future therapeutic applications.

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Direct Penetration of Cell-Penetrating Peptides Across Lipid Bilayers

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The short, charged, amphipathic and sometimes structured cell-penetrating peptides (CPPs) have been shown promising carriers for delivering protein, RNA, DNA, and even nanoparticles into cells. The mechanistic understanding of how CPPs cross the cell membrane and what biophysical property is critical for efficient penetration is nevertheless largely unknown, mostly due to the lack of proper experimental tools to directly assess the penetration process.

In order to gain insights in the universal rules of penetration mechanism of CPPs, we selected six peptides from three different classes of proposed mechanisms: (1) Tat and poly-arginine as structurally ''disordered'', cationic peptides that most likely enter the cell via endocytosis; (2) penetratin and pVEC as beta-stranded amphipathic peptides shown capable of directly translocate through the membrane; and (3) TP10 and modeled amphipathic peptide (MAP) as alpha-helical amphipathic peptides also being able to penetrate directly. We have developed new techniques based on atomic force microscopy (AFM) to measure energetic and dynamic properties as CPPs go through the bilayer. CPPs were attached via thiol-gold linkage to Au-coated AFM probes and brought into contact with lipid bilayers. The displacement-time trajectory of CPPs inside the bilayers was directly measured during bilayer breakthroughs under different force loading rates. Biophysical characteristics, such as potential energy barrier of bilayer failure, dynamics of penetration pathways, and bilayer structural rearrangement, were derived and compared among the six peptides, which will lead to new classification of CPPs sharing distinguishable biophysical traits.