3695-Pos Board B556
Repeated Nucleotide Detection with Duplex Interrupted Nanopore Sequencing in MspA
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Nanopore sequencing uses an ion current through a pore to identify nucleotides of single stranded DNA also driven through the pore. We previously demonstrated a technique where multiple double stranded sections are inserted in single stranded DNA. These double stranded sections halt the progression of the DNA, and provide enough time to use the current to identify the nucleotides held in the pore's constriction. When the force on the DNA dissociates a given duplex, the DNA moves to the next duplex section allowing a sequential read of the DNA. This sequencing method, termed duplex interrupt (DI) sequencing, does not easily detect repeated-nucleotides as they have the same current signature. Here we present a method to produce a distinct current level when the DNA progresses allowing repeat nucleotides to be determined.

3696-Pos Board B557
Electrostatic Trapping of DNA during Translocation through an Electrically Tunable Nanopore in a P-N Semiconductor Membrane
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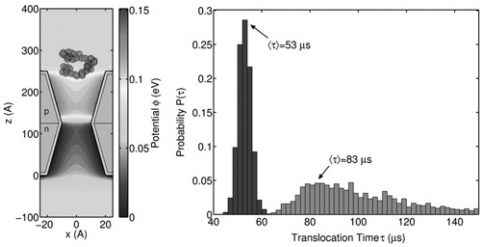
Discriminating between individual bases of a polynucleotide with the use a nanopore-based biosensing device is a promising idea, but one that has not yet been realized due to significant roadblocks. One such issue is the speed of translocation, as it has proven difficult to slow translocation enough to achieve a meaningful signal to noise ratio. We propose to mitigate this problem with the use of a nanopore-equipped semiconductor membrane containing a p-n junction. With the junction, it should be possible to create an electric field across the length of the pore that can momentarily trap DNA base pairs as they move down an electrophoretic gradient. We have created a computational method for modeling single-stranded DNA molecules as they undergo translocation through such a system. The results of our Brownian dynamics simulations indicate how effective this type of nanopore is at increasing translocation times. Furthermore, these simulations provide a better understanding of how to optimize such a nanopore for improved usefulness in a biosensing device.



3697-Pos Board B558
Characterization of Membrane Lipids and Protein Lateral Diffusion in Patterned Surfaces
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The construction of functionalized surfaces for uses in nanotechnology and biotechnology has opened a new area in materials science. Advances in microfabrication and nanofabrication processes are also opening new opportunities to investigate complex questions in Cell Biology in a way not previously possible. In particular, supported lipid bilayers (SLBs) are popular models of membranes with potential bio-technological applications. Investigation of lipid lateral mobility in biological membranes and their artificial models provides information on membrane dynamics and structure.
Fluorescence correlation spectroscopy (FCS) is a powerful technique to study the lateral organization of membranes. It measures fluorescence intensity fluctuations in the single molecule regime and allows the determination of diffusion coefficients.
In this work we present our progress in the use of the technique of Micro Contact Printing and results obtained on the modification of the topology of supported membranes and the ability of the membrane to bind ligands of interest, in particular, streptavidin and a biotinilated Antibody.
Finally, the lateral diffusion coefficients of the different components in each patterned surface were determined by z-scan FCS method.
Keywords: supported lipid bilayer, spectroscopy, topology, lateral diffusion
This work was supported by Agencia Foncyt.

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[2] Ana J. García-Sáez, Dolores C. Carrer, and Petra Schwille, V. Weissig (ed.), Liposomes: Methods and Protocols, Volume 2: Biological Membrane Models, Methods in Molecular Biology, vol. 606, © Humana Press
3 cm

3698-Pos Board B559
Towards Nanopore Sequencing: Comparing Experimental Results with Computational Modeling of DNA Interactions with α-Hemolysin
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The ability to discriminate current modulations produced by various nucleotides while single stranded DNA is being electrophoretically driven through a biological nanopore offers a simple and inexpensive technique for DNA sequencing. However, to realize the potential of nanopore sequencing, the molecular mechanism of DNA movement through the pore and the interactions of nucleotides with various pore residues have to be well characterized. Here, we applied computational approaches through atomistic Molecular Dynamics (MD) and Grand-Canonical Monte-Carlo/Brownian Dynamics simulations to investigate DNA translocation and interactions with the biological nanopore α-Hemolysin (αHL) and compared results with data obtained from experiments. Equilibrium and non-equilibrium (accounting for an electric field acting on a system) MD simulations of all-atom models of homopolymeric DNA (polydA or polydC) translocating through both wild-type and mutant αHL pores provided results that qualitatively match the contrast in ionic current blockade produced between the bases in experimental studies. Additionally, atomistic Free Energy Simulations with the ''swarm-of-trajectories'' method provides the first computational insight into the potential energy landscape governing DNA conformational dynamics within the pore, which agree with experiments indicating an asymmetric periodic potential. Finally, a truncated model of the αHL pore, in which the extramembrane vestibule is removed, has been generated and compared to the full protein. This simple model will allow longer simulation times, providing richer information on the dynamics of DNA translocation as well as a more accurate evaluation of ion conductance. The combined theory/experimental analysis of targeted modifications of different sensing regions within the pore allows the assessment of different factors (e.g. steric, electrostatic and van-der-Waals contributions to binding) governing specificity of pore-nucleotide interactions. The development of such in silico models will enable prediction of residues for site-directed mutagenesis yielding ideal pore properties for sequencing.

3699-Pos Board B560
Poration of Lipid Bilayers by Shock-Induced Nanobubble Collapse
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Sonoporation is a promising noninvasive drug- and gene-delivery approach, in which cavitation bubbles generated by ultrasound are used to enhance the cell membrane permeability. We have performed multimillion-atom molecular dynamics (MD) simulations to study the impact of shock waves on nanobubbles in the vicinity of a dipalmitoylphosphatidylcholine (DPPC) bilayer. The MD simulations reveal that the nanojet impact generates shear flow of water on bilayer leaflets and pressure gradients across them, which transiently enhance the bilayer permeability by creating nanopores and water molecules translocation across the bilayer. Effects of nanobubble size and temperature on the porosity of lipid bilayers will be discussed.

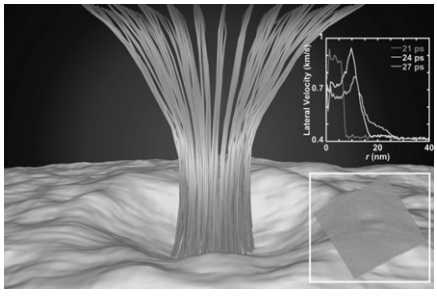

Fig.: Nanojet (blue, green and red are velocity streamlines) impact deforming the lipid membrane (yellow). The nanopore created in the membrane (blue) is shown in the bottom inset. The top inset shows the time evolution of lateral flow on the lipid membrane surface.