A Two Nanopore System for Controlling DNA Motion

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Two nanopores placed in sufficiently close proximity to capture single biomolecules can enable new studies of stretching and relaxation dynamics as well as sequence or structure information. To realize such a system, we have designed a hollow AFM-like chip geometry with an integrated nanopore that can be precisely positioned relative to a second nanopore fabricated in a flat membrane, using a combination of electronic and optical feedback. This approach has allowed us to probe single molecules of DNA interacting with both pores simultaneously and to progress towards exerting control on the DNA directly.

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Ion Transport Through Synthetic Nanopores Deposited in Porous Manganese Oxide Wires

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Synthetic nanopores emerged as an important tool to understand ionic and molecular transport at the nanoscale. Properties of ion current passing through nanopores can reveal geometric as well as electrochemical characteristics of the structures. Ionic selectivity, for example, is indicative of the presence of surface charges, while ion current rectification indicates broken electrochemical symmetry in the form of patterned surface charge or geometry. In this study, we utilized synthetic nanopores to perform conductivity experiments on manganese oxide, a porous material whose electrical state can be modulated. The measured ion current provided information on the effective size of the voids as well as the polarity of the surface charges. Membranes containing many pores, as well as single pores, were coated with a gold layer via sputter deposition, and then electrochemically deposited with manganese oxide wires. The gold layer extended inside the pores, in direct contact with the MnO2 wires, which permitted electrochemical modification of the wires. Measurements of ionic current through the wires were performed immediately after deposition, after an initial reduction with lithium, and after discharging the wires. These measurements revealed that each electrical state demonstrated different conductivities and provide strong evidence that the material has been successfully altered inside the nanopores. Experiments performed at a range of electrolyte concentration indicate the voids’ diameter of the porous MnO2 is dependent on the oxidation state of the material.

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Improved Protocol for the Hydrophobization of Glass Pipettes for Use in Patch-Clamp Experiments; Tera-Seals and Tenths of Fa Noise

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Patch-clamp experiments are used to study a variety of electrophysiological responses such as single-channel recordings and channel kinetics. Although an effective and extensively used technique, there exist several problems when using it, for example leakage through the lipid-glase interface or even through the thin glass wall. It is also important that the bilayer is stable and long lasting. In this study we present an improved experimental protocol which is intended to alleviate the above mentioned problems. Borosilicate glass pipettes of 1 μm inner diameter (I.D.) are coated via immersion in poly-dimethylsiloxane (PDMS) and curing agent to be then cured with ultraviolet (UV) light. PDMS is bound to the surface of the glass via a chemical reaction accelerated by the UV light, thus rendering the surface of the glass micropipette hydrophobic. This hydrophobic surface produces better membrane patch seals, with seal resistances in the order of Tera-Ohms (1012 Ohms) and noise level of < 100 fA. Normally micropipettes of 1 μm, would not yield lipid bilayer (LB) by the tip-dip method. The I.D. of the micropipettes are measured using the bubble number method and the results show that the I.D. of the micropipettes does not vary significantly when treated, hence no blocking occurs. The results indicate that the proposed treatment is very convenient for improving the patch-clamp technique by increasing seal resistance, and therefore stability and the lifet ime of the patch, as well as eliminating the possibility of ionic leakage through the thin glass wall of the tip and finally, reproducibility is increased due to the possibility of using micropipettes of 1 μm to obtain LB.

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Multiscale Diffusion Measurements in Biological Gels Using Photoactivatable Fluorescent Nanoparticles

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Diffusion through biological gels is crucial for effective nanoparticle drug delivery. In this work, we develop a method to measure diffusivity over a large range of length scales—from tens of nanometers to tens of microns—using photoactivatable nanoparticle probes. Nanoparticles composed of block copolymers of poly(lactic-co-glycolic acid) (PLGA) and polylethylene glycol (PEG) were synthesized and confirmed to possess dense PEG coatings that resist bioadhesion, and these particles were conjugated with caged rhodamine to make them photoactivatable. Using confocal microscopy, we activated a region of these particles with a brief, targeted exposure to UV light, uncaging the rhodamine and causing the particles to become fluorescent. We observed their collective diffusion over tens of minutes and tens of microns, thus obtaining a measure of their diffusivity. This technique is complementary to traditional multiple particle tracking (MPT); it extends the range over which particle motion can be directly observed. We confirmed the accuracy of this technique with reference to MPT measurements and the known diffusivity of particles in water. We applied the method to a model fibrin gel system, and found that both our method and MPT measurements show an immobile fraction of particles and mobile fraction that diffuses over long scales. Finally, we examined nanoparticle diffusion in sputum collected from cystic fibrosis patients, and we measured particle diffusion over distances relevant to drug delivery in the lungs. Coupled with traditional MPT, this technique enables multiscale characterization of particle mobility in complex biological fluids.

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Behavior Response of Caenorhabditis Elegans to Physical Complex Stimuli in a Controlled Microfluidic System

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Caenorhabditis elegans is a nematode species that has a great potential as a model organism because of its genetic homology with human. So various studies on human neural network in organism level have been studied with C.elegans. Furthermore, C.elegans live under the environment with complex combination of these stimuli but previous studies only have focused on the single stimuli conditions. Here, we developed a microfluidic system for analyzing the behavioral response of C.elegans to physical complex stimuli; temperature gradient and electric field. Also, we compared the responses of wild type (N2) and mutant worms (PR678, IK589, BC347, and CB78) with this device to show that the specific gene affects on the behavior of C. elegans. The developed device has a potential to be a tool for the behavior of C. elegans and to be applied for studying of human nerve system.

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Powered DNA Logic Gates

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Chemical computers process information at the molecular scale, facilitating the exploration of biological phenomena, and enabling bottom-up control over physical and biological materials. Synthetic DNA circuits are chemical computers noted for their scalability and ease of design. However, most existing DNA circuits cannot respond to changing input signals, because their reactants are consumed in the process of computing a single output product. Here we demonstrate a mechanism to continuously supply DNA circuits with fresh reactants while degrading old output products. This mechanism powers DNA based circuits to operate continuously in dynamic environments.

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A Systematic Investigation to Determine the Optimal Lipid Coating for Nanopore-Based Sensing Experiments

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Despite the importance of proteins, nanopore sensing has so far been mostly focused on single molecule DNA and RNA characterization. One factor that limited experiments with proteins were nonspecific interactions of proteins with the walls of synthetic nanopores. We showed recently, that nanopores with fluid coatings of phospholipid bilayers circumvented this problem. In

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