~3 orders of magnitude weaker K_a compared to conventional CDB nanopores, is thought to be inextricably linked with the Ohmic nature, extremely stable open-pore current during analyte translocation (hours), minimum analyte sticking with self-correction of current signal (if analyte sticking causes current deviation) and higher analyte responsiveness. The transport phenomena through these chemically modified nanopores are successfully tested by translocating 1 kb dsDNA and the human serum transferrin (hSTf) protein, with the highlight being the yield of an excess of 200 thousand DNA translocation events. Moreover, a comprehensive noise analysis done for the low frequency regime of this new class of SSNs revealed interesting characteristics which are distinct from the conventionally fabricated SSNs.

1750-Pos

Smooth Voltage-Driven Translocation of Full-Length Proteins Through Nanopores

Luning Yu[1], Xinqi Kang[2], Mohammad Amin Alibakhshi[1], Fanjun Li[3], Bezhad Mehrafrooz[4], Aleksei Aksimentiev[4], Min Chen[3], Meni Wanunu[1].
[1]Physics, Northeastern University, Boston, MA, USA, [2]Bioengineering, Northeastern University, Boston, MA, USA, [3]Dept Chemistry, University of Massachusetts-Amherst, Amherst, MA, USA, [4]Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Biological nanopores have become useful nanosensors that can read single DNA and RNA molecules with high resolution. The most common way to use these nanosensors is to insert a protein channel (e.g. α-hemolysin) into a phospholipid bilayer and apply voltage to drive ion current through the pore. When a DNA molecule traverses the pore, electromotive force on the DNA molecule drives it through the pore in a single-file manner. Compared to DNA, proteins have more complex secondary structures and non-uniform backbone charge, which makes single-file translocation of proteins through the pore difficult to achieve. Here we use a polymer-based membrane and a custom wedge-on-pillar aperture to allow high voltage and chemical denaturing of proteins for nanopore analysis. In comparison with a bilayer lipid membrane, the polymer membrane has superior mechanical and chemical stability under these conditions, which allows full-length protein translocation through alpha-hemolysin. By tagging full-length proteins (range from 200 - 800 aa) with a charged tail (10 aa), we demonstrate the efficient translocation of these proteins through the hemolysin pore, and further, we demonstrate that mean translocation times are linearly related to the protein length, irrespective of its native charge. Further, translocation times are slow enough to allow sufficient sampling (~0.01 ms/aa). Simulations that probe the mechanism of such smooth translocations of these weakly-charged proteins reveal the role of denaturant-induced electroosmosis on the translocation process, which is key to successful translocations. When combined with a higher-resolution nanopore, we anticipate the ability to fingerprint unmodified protein molecules based on single molecule signals.

1751-Pos

Develpoment and Characterization of Novel Probes for Photoacoustic Microscopy

Francesco Garzella[1,2], Barbara Storti[3], Ranieri Bizzarri[3], Aba Losi[2], Wolfgang Gärtner[4], Stefania Abbruzzetti[2], Paolo Bianchini[1], Cristiano Viappiani[2], Alberto Diaspro[1,5].
[1]Nanoscopy, Istituto Italiano Tecnologia, Genoa, Italy, [2]Department of Mathematical, Physical and Computer Sciences, University of Parma, Parma, Italy, [3]Scuola Normale Superiore and NANO-CNR, Pisa, Italy, [4]Institute for Analytical Chemistry, Lipsia, Germany, [5]DIFILAB, Department of Physics, University of Genoa, Genoa, Italy.

The photoacoustic effect (PA) is a physical phenomenon based on the emission of sound waves following light absorption in material samples. The photon absorption and subsequent non-radiative depletion of the chromophores rapidly rises the temperature within the sample, increasing the pressure and inducing a thermoelastic expansion, leading to the emission of a pressure wave called photoacoustic wave. The reduced scattering of acoustic waves enables high-resolution, deeply penetrating imaging in biological tissues. The difference among the absorption coefficients of tissue components or suitable transgene labels in the sample gives the base of contrast in PA imaging but the few number of probes showing high PA efficiency reduces the applications to living systems and processes at the cellular and subcellular levels. The development of reversibly switchable fluorescent proteins (rsFPs) has revolutionized the life science imaging contributing to optical nanoscopy as agents able to improve contrast-to-noise ratio and spatial resolution. The competiveness between light and heating emission in rsFPs optimized for fluorescence imaging considerably reduces the acoustic emission efficiency making them not suitable for PA microscopy. The aim of this project is the development of a novel approach in photoacoustic microscopy inspired by the toolbox of knowledge we have for fluorescence imaging. The key point lies in the probes, belonging to two different families of photochromic proteins, which will be characterized and applied in the novel photoacoustic microscope: GAF3, and two new mutants of GFPs obtained adding a fluorescence-decreasing mutation to wildQ and wildQT proteins.

1752-Pos

Bent DNA Bows as Sensing Amplifiers for Detecting DNA-Interacting Salts and Molecules

Yong Wang[1], Jack Freeland[1], Lihua Zhang[2], Shih-Ting Wang[2], Mazon Ruiz[1].
[1]Physics Department, University of Arkansas, Fayetteville, AR, USA, [2]Center for Functional Nanomaterials, Brookhaven National Laboratory, Upton, NY, USA.

Due to the central role of DNA, its interactions with inorganic salts and small organic molecules are important. For example, such interactions play important roles in various fundamental cellular processes in living systems and are involved in many DNA-damage related diseases. Strategies to improve the sensitivity of the existing techniques for studying DNA interactions with other molecules is always appreciated in situations where the interactions are too weak. Here we report our development and demonstration of bent DNA bows for amplifying, sensing, and detecting the interactions of >10 inorganic salts and small organic molecules with DNA. This method is based on perturbing energy landscapes using mechanical energy stored in the bent DNA bows. With the bent DNA bows, these interactions were easily visualized and quantified in gel electrophoresis, which were difficult to measure without bending. In addition, the strength of the interactions of DNA with the various salts/molecules were quantified using the modified Hill equation. This work highlights the amplification effects of the bending elastic energy stored in the DNA bows and the potential use of the DNA bows for quantitatively measuring DNA interactions with small molecules as simple economic methods; it may also pave the way for exploiting the bent DNA bows for other applications such as screening DNA-interacting molecules and drugs.

1753-Pos

iGlow: Real-Time Fluorescence Reporting, Intra-Vesicular Activity, and Helix-8 Independence in a CPGFP-Tagged Mechanosensitive GPCR

Alper D. Ozkan[1], Tina Gettas[1], Audrey Sogata[2], Wynn Phaychanpheng[2], Jerome J. Lacroix[1].
[1]Western University of Health Sciences, Pomona, CA, USA, [2]Chino Hills High School, Pomona, CA, USA.

GPR68 is a class A G-protein coupled receptor activated by protons and mechanical stimuli. Here, we engineered a novel fluorescent reporter of GPR68 signaling by inserting a circularly permuted GFP (cpGFP) into the third intracellular loop of the receptor, borrowing design principles from the dopamine sensor dLight1.2. This reporter, which we call "*iGlow*" (indicator of GPR68 activation by flow and low pH), elicits robust and transient fluorescent responses when stimulated with extracellular protons, shear stress, or the synthetic agonist ogerin. iGlow activation is not abolished by treatment with chemical modulators of GPCR signaling, cytochalasin D, or GsMTx4, an inhibitor of stretch-activated ion channels. Like GPR68, iGlow is not solely localized at the plasma membrane, but also populates intracellular compartments. Remarkably, intracellular iGlow activates in response to shear stress, suggesting that the internalized pool of endogenous GPR68 may also sense and respond to shear flow. Furthermore, iGlow activation is not abrogated by the deletion of helix 8, a conserved motif proposed to mediate mechanosensation in other class-A GPCRs. As ligand- and force-sensing properties are interdependent in GPR68 but decoupled in other mechanosensitive GPCRs, it is plausible that GPR68 and related proton-sensing GPCRs (GPR4, GPR65 and GPR132) use a 'non-canonical', helix-8 independent pathway to detect mechanical forces.

1754-Pos

Black Dots: Microcontact Printed Reference-Free Traction Force Microscopy

Kevin M. Beussman[1], Molly Y. Mollica[2], Sangyoon J. Han[3], Ashley Emery[1], Wendy E. Thomas[2], Nathan J. Sniadecki[1,2].
[1]Department of Mechanical Engineering, University of Washington, Seattle, WA, USA, [2]Department of Bioengineering, University of Washington, Seattle, WA, USA, [3]Biomedical Engineering, Michigan Technological University, Houghton, MI, USA.

Cells generate mechanical force to proliferate, locomote, and perform other cellular functions. Measuring cell forces is vital for understanding their health and behavior, but it has been challenging to find techniques that are compatible with live and fixed samples and do not adversely affect cell function. To