

sufficient for resolving the different DNA bases and paving the way for DNA sequencing in the MHz regime.

2099-Pos Board B829

Electrochemical Detection of Acetylcholine using Enzyme Functionalized Nanoparticles

Jacqueline D. Keighron, Michael E. Kurczyk, Joakim Wigström, Ann-Sofie Cans.

Department of Chemical and Biological Engineering, Chalmers University of Technology, 41296 Gothenburg, Sweden.

Acetylcholine is a highly important non-electroactive neurotransmitter in the mammalian central nervous system. Its function is linked to memory and sleep, and it regulates, in part, mood and action via its connection to dopamine. A fast, sensitive method to detect the release of acetylcholine at the surface of a single cell is needed to gather data about the kinetics of exocytosis events in these systems.

To this end, carbon fiber electrodes have been modified with electrodeposited gold nanoparticles to increase the effective electrode surface area and provide a high curvature surface for enzyme attachment. Acetylcholinesterase and choline oxidase were then deposited onto the gold surfaces to catalyze acetylcholine to hydrogen peroxide for electrochemical detection. The functionalized electrodes have been characterized to determine the K_M and V_{max} of the enzymes as well as the total enzyme coverage and gold nanoparticle surface area in order to optimize retained enzyme activity. This optimized design has proven capable of detecting release events from an artificial exocytotic system on a sub-second time scale.

2100-Pos Board B830

A Single-Step Digital Nucleic Acid Amplification Platform by Digital Plasma Separation on a Chip

Erh-Chia Yeh¹, Chi-Cheng Fu Yeh¹, Lucy Hu¹, Meng-Yao Huang², Luke P. Lee¹.

¹Bioengineering, UC Berkeley, Berkeley, CA, USA, ²Electrophysics, National Chiao Tung University, Hsinchu, Taiwan.

Quantitative nucleic acid detection is the future for precision molecular medicine. However, multiple sample preparation steps and thermal heat cycles of PCR are necessary for nucleic acid (NA) analysis. We developed a single-step isothermal digital NA amplification microfluidic platform by a technique termed digital plasma separation. We invented an innovative biophysical microcliff design that allows the removal of blood cells based on inertia; it can skim plasma into 224 wells (100nl/well) in one-step (>99% separation efficiency for 6µm particles). By separating the blood cells that obstruct optical detection, the microcliff design enabled quantitative DNA detection via an isothermal DNA amplification technique called Recombinase Polymerase Amplification (RPA) in 30 minutes directly from human blood samples. We were able to perform endpoint quantitative digital RPA with a dynamic range of 10-1000 copies/µl via automatic compartmentalization of the samples. The precise control of fluid flow on chip is accomplished by passive degas-driven flow actuation, which does not require external tubing or pumps, thus making the chip portable. A large amount of sample (~100µl of blood samples) can be processed. We envision this single-step digital blood plasma separation platform will benefit low cost quantitative molecular diagnostics in both developed and developing countries.

2101-Pos Board B831

Single Molecule Detection of Insulin Autoantibodies in Type 1 Diabetes

Juliane Beyer¹, Ralf Paul², Ezio Bonifacio², Stefan Diez^{1,3}.

¹B CUBE - Center for Molecular Bioengineering, TU Dresden, Dresden, Germany, ²CRTD - Center for Regenerative Therapies Dresden, Dresden, Germany, ³Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Type 1 diabetes (T1D) is characterized as a chronic autoimmune disease caused by a selective inflammatory destruction of the insulin producing beta cells in the pancreatic islets of Langerhans. Closely associated to T1D are insulin autoantibodies (IAAs), representing early markers of the disease. Therefore, their reliable detection is needed i) to predict the onset of T1D, ii) to implement successful regenerative therapies and iii) to prevent loss of the beta cell mass. For this purpose, we developed a novel optical assay for the detection of IAAs using single molecule detection. Our quantitative approach specifically detects IAAs in the low pM range using quantum dots and total internal reflection microscopy (TIRF). This method overcomes the limitations associated with current clinical diagnostic approaches where IAAs are detected using radiolabelled antigens in a time-consuming, hazardous and expensive manner. Thus, we believe that in the future our assay could be used as a point of care measurement for T1D, readily usable in the health care sector combining the prognostic and diagnostic measurements of autoantibodies in T1D.

2102-Pos Board B832

Nanomaterials-Enhanced Electrochemical Biosensor for Detection of Cancer Biomarkers

Bing Jin, Hongju Mao.

Shanghai Institute of Microsystem and Information Technology, Chinese Academy of Science, Shanghai, China.

Electrochemical detection strategies employing nanotechnologies which include a variety of new materials and fabrication processes offer new opportunities for highly sensitive detection of cancer biomarkers. This work reports an electrochemical biosensor based on a graphene (GR) platform, combined with magnetic beads (MBs) and enzyme-labeled antibody-gold nanoparticle bioconjugates. MBs coated with capture antibodies (Ab1) were attached to isolated GR sheets by an external magnetic field, to avoid reducing the conductivity of graphene. Sensitivity was also enhanced by modifying the gold nanoparticles (AuNPs) with horseradish peroxidase (HRP) and the detection antibody (Ab2), to form the conjugate Ab2-AuNPs-HRP. Electron transport between the electrode and analyte target was accelerated by the multi-nanomaterial, and the limit of detection (LOD) for carcinoembryonic antigen (CEA) reached 5ng/mL, which meets clinical requirements. The multi-nanomaterial electrode GR/MBs-Ab1/Antigen/Ab2-AuNPs-HRP can be used to detect biomolecules such as CEA. The EC biosensor is sensitive and specific, and has potential in the detection of disease markers.

Keywords: electrochemical, graphene, magnetic beads, gold nanoparticles, horseradish peroxidase.

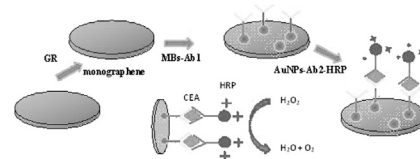


Figure 1. Preparation of the multi-nanomaterial EC biosensor and CEA detection procedure.

2103-Pos Board B833

Electrical Pumping of Potassium Ions Against an External Concentration Gradient in a Biological Ion Channel

Maria Queralt-Martin¹, Elena Garcia-Gimenez¹, Vicente M. Aguilera¹, Patricio Ramirez², Salvador Mafe³, Antonio Alcaraz¹.

¹Laboratory of Molecular Biophysics, Department of Physics, Universitat Jaume I, Castellón de la Plana, Spain, ²Departament de Física Aplicada, Universitat Politècnica de València, València, Spain, ³Departament de Física de la Terra i Termodinàmica, Universitat de València, Burjassot, Spain.

We show experimentally and theoretically that significant currents can be obtained with a biological ion channel, the OmpF porin of *Escherichia coli*, using zero-average potentials as driving forces. The channel rectifying properties can be used to pump potassium ions against an external concentration gradient under asymmetric pH conditions. The results are discussed in terms of the ionic selectivity and rectification ratio of the channel. The physical concepts involved may be applied to separation processes with synthetic nanopores and to bioelectrical phenomena.

2104-Pos Board B834

Rapid Detection of Protein Aggregation and Inhibition by Dual Functions of Gold Nanoplasmonic Particles: Catalytic Activator and Optical Reporter

Inhee Choi, Elizabeth Lee, Minsun Song, Luke P. Lee.

UC Berkeley, Berkeley, CA, USA.

Neurodegenerative diseases share common molecular mechanisms in disease pathogenesis: protein misfolding and abnormal aggregation. Current detection methods of protein aggregations require time-consuming and labor-intensive steps, which delay the process of drug discovery and understanding the mechanism of protein aggregation. Here, we present a rapid detection method for studying protein aggregation and inhibition under a variety of perturbation conditions by dual functions of gold nanoplasmonic particles (GNPs): catalytic activator and optical reporter. As a proof-of-concept demonstration, we employed the acidic perturbation permitting rapid cooperative assemblies of GNPs and amyloid-β (Aβ) peptides (implicated in Alzheimer's disease) via their surface charge modulation. Because of roles of GNPs as effective nucleation sites for fast-catalyzing protein aggregation and colorimetric optical reporters for tracking protein aggregation, we accomplished the fast aggregation assay in less than 1 min by the naked eyes. Under the given acidic perturbation condition, we characterized the concentration-dependent colorimetric responses for aggregation at physiologically relevant Aβ concentration levels without any instrumentation. We also demonstrated the GNP/acidic condition-based rapid inhibition assay of Aβ aggregation by using well-known binding reagents such as antibody and serum albumin. Consistent results were also obtained from the tests with superoxide dismutase protein implicated in Lou Gehrig's disease. The proposed methodology can be a powerful