View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

1035-Pos Board B821

A DNA Methylation Sensitive Nanopore Engineered from the phi29 Portal Protein GP10

David Wendell[1], Murali Venkatesan[2], Rashid Bashir[2].
[1]University of Cincinnati, Cincinnati, OH, USA, [2]University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Here we report the electrophysiology of an engineered nanopore mediated by the capsid portal protein GP10 and several mutants in a freestanding lipid bilayer. The measured conductance is on par with some of the largest biological nanopores, like those of the mechanosensitive channels and porins found in many prokaryotes. Conductance appears to be restricted by both the variable region and the c-terminal crown, areas known to interact with the viral DNA but unresolved in the crystal structure. We have used the c-terminal interaction as a basis for distinguishing dsDNA methylation state by engineering a methylated DNA binding domain onto the crown of GP10. The engineered pore has the ability to electrically distinguish methylated and hydroxymethylated DNA from the unmodified form. We envision this sensor as a future tool for detecting the alterations in DNA methylation state commonly associated with carcinogenesis.

1036-Pos Board B822

Metal Organic Materials as Biomimetic Heme Catalysts

Randy W. Larsen, Carissa M. Vetromile, Lukasz Wojtas, Christy Young.
University of South Florida, Tampa, FL, USA.

The catalytic diversity of heme proteins is an ongoing target for biomimetic chemistry and a wide array of systems have been developed to capture the salient catalytic features of heme proteins with limited success. Heme proteins perform a plethora of chemical reactions utilizing a single iron porphyrin active site embedded within an evolutionarily designed protein pocket. Here the first class of metal-organic framework materials (MOFs) that mimic heme enzymes in terms of both structure and reactivity will be presented. This class of materials is based upon a prototypal MOF, HKUST-1, into which catalytically active metalloporphyrins are selectively encapsulated ''ship-in-a-bottle'' fashion within one of the three nanoscale cages that exist in HKUST-1. These new materials display catalytic activity towards peroxide degradation similar to the peroxidase activity of metallporphyrins in solution as well as heme proteins. In addition, encapsulation of photo-active porphyrins into HKUST-1 like frameworks provides an opportunity for the development of novel porphyrin based MOF materials capable of both photocatalytic chemistry and optically based sensor technologies.

1037-Pos Board B823

Effect of Nanoparticles in Top Consumers

Karin Mattsson.
Biochemistry, Lund University, Lund, Sweden.

The use of nanoparticles in products, like cosmetics and food, increase even if the knowledge of their effect on the organic metabolism is limited. How the nano-sized particles effect the metabolism is not known.
It is known that when a nanoparticle enters a biological fluid the nanoparticle is surrounded by biomolecules such as proteins that will bind to the nanoparticle. These interactions progress over time and other biomolecules will bind to the particle. The proteins and the nanoparticles together create a corona and this corona will affect how the particles are transported in the fluid.
We characterise how commercially manufactured polystyrene nanoparticles affect the metabolism in fish. Labelled nanoparticles are fed to the fish through an aquatic food chain from algae through zooplankton to fish. The behaviour of the fish is studied and compared with a control group. The organs and the blood are studied and we investigate which proteins that bind to the nanoparticles.

1038-Pos Board B824

Manipulating the Size of Hen Lysozyme Nanoparticles Created by Controlled Self-Assembly

Vijay K. Ravi, Nividh Chandra, Tulsi Swain, **Rajaram Swaminathan**.
Indian Institute of Technology Guwahati, Guwahati, India.

Natural protein-based nanostructures like viral capsids, ferritin present attractive platforms for vaccine development, catalysis, nanomaterial synthesis, drug/gene delivery owing to their biocompatability and rich functional chemistry. These container-like protein cage architectures possess multiple interfaces (interior, exterior and between subunits) for imparting functionality. However, assembling such structures artificially, with defined molecular dimensions has proved challenging. Current approaches do not permit spatial tuning, yielding bulky nanostructures (>50 nm). Here we show that controlling the monomer concentration of hen egg-white lysozyme (HEWL) in micro-nanomolar range during its spontaneous aggregation at pH 12.2 under room temperature (298 K) tunes the average hydrodynamic radii of lysozyme nanostructures between 2 and 15 nm and their rotational correlation times (measured using nanosecond time-resolved fluorescence anisotropy decay) between 4.3 and 26 ns. As lysozyme self-associates by an isodesmic mechanism at this pH, limiting the monomer concentration halts the growth of larger aggregates, making the size of the aggregate directly dependent on HEWL monomer concentration. These nanoparticles are stabilized by intermolecular disulphide bonds after 120 hours, which is shown to preserve their nano-architecture after transfer to neutral pH. Differently sized HEWL nanoparticles, thus produced, are shown to possess interiors with a size-dependent gradation in polarity and molecular packing, suitable for optimising non-covalent binding of desired cargo.

1039-Pos Board B825

Applying Nanostructures to Biology: An Understanding of the Nano-Bio Interface through Transmission Electron Microscopy

Lindsey Hanson, Chong Xie, Ziliang Lin, Yi Cui, Bianxiao Cui.
Stanford University, Stanford, CA, USA.

In recent years there have been many efforts to take advantage of the unique properties of nanoscale structures for the study of biological systems. In order to both fully exploit these properties and properly interpret the applications, we must develop a better understanding of the interactions between the inorganic nanostructures and the living cell. We have successfully used transmission electron microscopy to characterize the interface between vertical nanopillars and several mammalian cell types, most notably neurons. The high spatial resolution of the technique allows us to observe the morphology of subcellular structures, such as the nucleus or the neuronal axon, as well as the membrane topography around the nanopillars. We have seen that the structure of the surface has marked effects on cell attachment, and that the presence of the nanopillars yields a tight seal between the cell membrane and the surface. As a result, the nanopillar platform provides a wide array of opportunities for study, including electrical recording, localized observation of cell signaling, and delivery of molecules into the cell.

1040-Pos Board B826

The Inchworm: Construction of a Biomolecular Motor with a Power Stroke

Martina Balaz[1], Cassandra Nimen[1], Mariusz Graczyk[1], Gerhard A. Blab[2,3], Paul M.G. Curmi[4,5], Roberta Davies[4], Nancy R. Forde[6], Derek N. Woolfson[7,8], Heiner Linke[1,3].
[1]The Nanometer Structure Consortium (nmC@LU), Lund University, Lund, Sweden, [2]Molecular Biophysics, Universiteit Utrecht, Utrecht, Netherlands, [3]Materials Science Institute, University of Oregon, Eugene, OR, USA, [4]School of Physics, University of New South Wales, Sydney, Australia, [5]Centre for Applied Medical Research, St. Vincent's Hospital, Sydney, Australia, [6]Department of Physics, Simon Fraser University, Burnaby, BC, Canada, [7]School of Chemistry, University of Bristol, Bristol, United Kingdom, [8]School of Biochemistry, University of Bristol, Bristol, United Kingdom.

Essentially all approaches to artificial molecular motors rely on diffusional stepping, i.e. the free energy input that powers the motor is used to rectify thermal motion. However, many models for biological motors, e.g. myosins or kinesins, include directed motion due to a ''power stroke''. In this project we are employing a bottom-up approach to developing a relatively simple experimental model system, the ''Inchworm'', with the intention to create the first artificial motor with a power stroke. Specifically, the Inchworm consists of a stretch of DNA confined inside a nanochannel, with ligand-gated repressor proteins immobilized on the nanochannel walls. By cyclically stretching and contracting the Inchworm DNA, via changes in buffer ionic strength, and simultaneously externally controlling the DNA repressor proteins' binding and unbinding states, the motor is designed to achieve processive and unidirectional motion along the nanochannel. Here we report the status of this project: we have constructed the Inchworm DNA, expressed the ligand-gated repressor proteins and tested their specificity. In addition, we have developed a protocol for immobilization of the repressor proteins to the nanochannel walls and produced nanochannels (100-200 nm) in quartz, which can be interfaced with preexisting micro-fluidic systems.

Acknowledgment: This work was supported by MONAD, HFSP, the Swedish Research Council and nmC@LU.