2457-Pos Board B594

Rigidity of Poly-L-Glutamic Acid: Influence of Secondary and Supramolecular Structures

Stefania Perticaroli[1,2], Jonathan D. Nickels[3], Alexei P. Sokolov[1,2].
[1]Chemistry, University of Tennessee, Knoxville, TN, USA, [2]Chemical and Materials Sciences Division; Joint Institute for Neutron Sciences., Oak Ridge National Laboratory, Oak Ridge, TN, USA, [3]Joint Institute for Neutron Sciences, Oak Ridge National Laboratory, Oak Ridge, TN, USA.

Defining precisely the mechanical properties of bio-macromolecular systems on a nanometer length scale is crucial for the development of more efficient drug delivery systems and scaffolds-based tissue engineering. We characterized the structure, topology, and rigidity properties of poly-L-glutamic acid (PGA), prepared with different molecular weights and secondary structures, using the same approach that we recently applied to proteins[1-3]. We employed various techniques, including FT-IR, SEM, light scattering, neutron diffraction, and neutron scattering spectroscopy. Our results show that on the length scale of a few nanometers, rigidity of PGA powders is determined by hydrogen bonding interactions in presence of neutral species, and by electrostatic interactions when the polypeptide is negatively charged. On ~ a hundred nanometer length scale, the rigidity of these materials is modified by long range intermolecular interactions that are introduced in the supramolecular structures.

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2458-Pos Board B595

Bio-Lithography: A Novel Process for Modification and Patterning of Supported Lipid Bilayers using Lipopolysaccharide, a Biological Amphiphile

Peter G. Adams[1], Kirstie L. Swingle[1,2], Walter F. Paxton[3], John J. Nogan[3], Loreen Lamoureux[4], Millicent A. Firestone[1], Harshini Mukundan[5,6], Gabriel A. Montaño[1].
[1]Center for Integrated Nanotechnologies, Los Alamos National Laboratory, Los Alamos, NM, USA, [2]Department of Biology, University of New Mexico, Albuquerque, NM, USA, [3]Center for Integrated Nanotechnologies, Sandia National Laboratories, Albuquerque, NM, USA, [4]Center for Biomedical Engineering, University of New Mexico, Albuquerque, NM, USA, [5]Physical Chemistry and Applied Spectroscopy, Los Alamos National Laboratory, Los Alamos, NM, USA, [6]New Mexico Consortium, Los Alamos, NM, USA.

Biological cell membranes are highly organized, multi-component systems that carry out complex energy and information processing. Supported lipid bilayers (SLBs) have shown great promise as model membranes but generating biologically-relevant complexity is challenging using existing membrane patterning techniques. Here we demonstrate an unconventional method that allows the controlled formation and *in situ* modification of complex membrane arrangements. Lipopolysaccharide (LPS) is a membrane-inserting amphiphile and an important human toxin. We recently reported that LPS can insert into and destabilize fluid-phase SLBs, leading to the formation of voids [Adams et al. (2014) *Biophys J.* 106, 2395]. We noted that the fluidity and continuity of the remainder of the SLB was maintained. In the current study, we exploit this effect of LPS to generate SLBs containing voids that can be backfilled to introduce desired functional components. This 'biochemical-assisted lithography' procedure was used to generate hierarchically organized membrane domains and microscale 2-D array patterns of domains. These domains could be formed using proteins, gel-phase lipids or even synthetic polymers. Alternatively, the voids could be healed by introducing new fluid-phase lipids. Significantly, this technique can be used to repeatedly modify membranes allowing iterative control over membrane composition. This approach expands our toolkit for functional membrane design, with potential applications for enhanced materials templating, biosensing and mimicking biological processes. Furthermore, we present fundamental insights into the interaction of lipids and other amphiphiles, demonstrating that line tension and alkyl chain packing effects can direct the organisation of a membrane-inserted amphiphile into geometric patterns.

2459-Pos Board B596

The Bacterial Spore as an Energy-Rich Adaptive Material

Michael DeLay[1], Xi Chen[1], Jonathan Dworkin[1], Adam Driks[2], Ozgur Sahin[1].
[1]Columbia University, New York City, NY, USA, [2]Loyola University, New York City, NY, USA.

Stimuli responsive materials are in high demand for myriad applications within the fields of biomedicine, adaptive-architecture, robotics, and alternative energy. The non-pathogenic bacterial spores of Bacillus subtilis have the ability to swell as much as 12% in response to humidity gradients. Within the spore unique biomaterials drive this dynamic response, resulting in energy densities greater than 10 MJ*m−3, more than a hundred times the potential of existing synthetics. We hypothesize that the spore's cortex, a protective layer surrounding the genetic core, is largely but not solely responsible for this peculiar water-responsive behavior. To evaluate the spore's constituent biomaterials we developed AFM techniques that assay individual spores during a thermodynamic cycle of varied applied force and relative humidity. This method resulted in energy density and strain profiles for multiple spore variants and structural mutants. Electron microscopy (FIB SEM) was then used to ascribe radial dimensions to biomaterial layers within the spore. Quantifying precise substructural contributions to the spore's hygrodynamics will allow for in vitro optimization of composite spore biomaterials and may even facilitate artificial synthesis in the future. The bacterial spore's inherent ability to form monolayers and superior performance in highly efficient energy conversion and actuation qualifies it as an intriguing material worthy of broad development.

2460-Pos Board B597

Stress-Induced Lamellar Order in Spider Silk Fibers

Eduardo R. Cruz-Chu[1], Patil Sandeep[1], Imke Greving[2], Martin Mueller[2], Frauke Graeter[1].
[1]MBM, HITS, Heidelberg, Germany, [2]Centre for Materials and Coastal Research, Helmholtz Zentrum Geesthacht, Geesthacht, Germany.

Spider dragline silk, solely made from protein, outperforms synthetic fibers in terms of toughness and extensibility, which is thought to originate from its refined nano-scale hierarchical structure. The key for understanding the outstanding mechanical performance of dragline silk lies in a detailed description of the underlying molecular structure and dynamics under load conditions. Here, we aim at determining the ingredients for silk's outstanding toughness using multi-scale computational simulations. First, we present large-scale molecular-dynamics simulations of the structure and dynamics of silk protein. We introduce the most comprehensive, to date, spider silk models comprising of hundred 500-residue chains of two spider proteins, namely MaSp1 and MaSp2, using a collapsing-annealing protocol [1]. Our systems are composed of crystalline beta-sheet segments completely embedded in and connected with an amorphous phase. On this basis, we developed a three-dimensional continuum model of dragline silk for finite element analysis. Such model takes into account the plasticity of the beta-sheets, the rate-dependent dynamics of the amorphous phase, and the viscous friction between them [2]. Tensile properties, velocity-dependent effects and hysteresis are in good agreement with experimental data. Intriguingly, the simulations revealed that under load conditions, crystals rearrange into lamellar bands. We could confirm this trend by small-angle neutron scattering of silk fibers under stress, with simulations and experiments showing quantitatively the same shift and intensity in the signal from the periodically forming bands. The increased order results in a more homogenous stress distribution and higher fiber toughness. Our combined atomistic and fiber-level simulations and experiments suggest that the test stress-induced order is a common feature in materials combining crystalline and amorphous phases on the nano-scale.

[1] Cruz-Chu, E. R et al. Faraday Discussions (2009) 143, 47-62
[2] Sandeep Patil et al. PLOS One (2014) 9, e104832

2461-Pos Board B598

Live Cell Interactions with Biocompatible Ultra-Short Carbon Nanotube Porins

Jia Geng[1,2], Whitney Stannard[1], Arthur Escalada[3], Kyunghoon Kim[1,4], Michael P. Thelen[1], Vadim A. Frolov[3,5], Aleksandr Noy[1,2].
[1]Lawrence Livermore National Laboratory, Livermore, CA, USA, [2]University of California, Merced, Merced, CA, USA, [3]University of the Basque Country, Leioa, Spain, [4]University of California, Berkeley, Berkeley, CA, USA, [5]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain.

Various techniques, such as peptide chemistry, DNA origami, or nanotubes cutting, have been tried to develop synthetic analogues of biological membrane channels for transmembrane transport of ions and molecules, but challenges still remain to achieve the desired affinity, transport properties and biocompatibility for delivery applications. Here we report the development of CNT porins, an ultra-short biocompatible carbon nanotube with the dimension of 5-15 nm in length and 1.5 nm in diameters and with structure and function resembling membrane channel. We explored concentration-dependent interaction of the CNT porins with CHO cells using microscopy and OmniLog phenotype microarray system. Interestingly, the CNT porins didn't inhibit the cell viability at low concentration over a 72 hr co-incubation period. Single channel recording experiments also showed well-defined channels formation on the cell membrane. Those results indicated those artificial transmembrane channels could be established as a promising biomimetic platform for developing cell interfaces, studying transport in biological channels, and creating stochastic sensors. Their inherent robustness towards biological and chemical challenges