3425-Pos
Probing the Molecular Structure of Polymer DNA Nanoparticles Via Fluids-DFT
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Polymer-DNA nanoparticles (polyplexes), can be highly effective and safe vehicles for delivering therapeutic genes into cells, but despite great promise they have yet to emerge as a consistently effective tool. Though many attempts have been made to improve transfection efficiency by altering polymer chemistry, as of yet no hard and fast rules have emerged to guide the engineering design process. Meanwhile, little attention has been paid to the molecular architecture of the particles themselves. Through x-ray scattering measurements, we have recently observed that nanoparticles have highly complex, three-dimensional structure. Further, these structures are highly sensitive to the same changes in polymer chemistry that dramatically affect transfection efficiency. We propose that high-resolution, three-dimensional structure of a polymer-DNA nanoparticle is a determining characteristic of its ability to deliver DNA to a cell and, when fully understood, should be useful in guiding successful nanoparticle design. We have developed and applied computational Fluids-Density Functional Theory (Fluids-DFT) that allows us to predict and understand large-scale organization of polymers and DNA in polyplexes.

3426-Pos
Fixation of Self-Assembled DNA Nanostructures by Simultaneous Multicenter Click Chemistry
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Fast and specific self-assembly, together with steric rigidity by base stacking, makes DNA uniquely efficient for building supramolecular nanostructures. However, their non-covalent nature makes subsystems of DNA insufficiently robust for use as building blocks for large systems. We have developed a fixation technology and taken the first step towards the modular build-up of complex larger networks. This is demonstrated for a six membered DNA hexagon, each edge one turn of a double helix, which is covalently cross-linked using click chemistry, creating a robust module that can be readily adapted for building larger systems. This hexagonal module constitutes the smallest practical assembly unit of DNA, a system truly fit for molecular nanotechnology.

3427-Pos
Sensing Biomolecules with Ultra-Thin Film Organic Field Effect Transistors
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Organic field effect transistors (OFET), where ordered conjugated molecules act as charge transport material, are low-dimensional devices. Charge carriers are transported within the first few mono-layers in contact with the gate dielectric. The structural/morphological and electronic control of the first few monolayers of the organic semiconductor (e.g. pentacene) allows us to fabricate ultra-thin film transistors with the accumulation layer directly exposed to the outer environment. We developed OFETs as label-free biological transducers and sensors of biological systems. Unconventional patterning techniques and microfluidics have been adapted to proteins and nucleic acids to dose the molecules on the OFET channel with a high control of the concentration. Patterning is useful to impart conformations and architectures which are otherwise not accessible spontaneously by the biological systems.
Operations an ultra-thin film OFETs under water will be shown, together with the response of OFET parameters to different biomolecules (linear DNA, beta-amyloid 1-40 peptide) upon a systematic change of concentration, secondary structure, aggregation state. The sensitivity issues will be addressed. These results show a quantitative approach for the detection of biological molecules in vitro and monitoring their slow dynamics. Finally, first experiments using OFETs transducers of cell signaling at the molecular level will be presented in the presence of diffentiated murine stem cells NE4C forming neural networks. References: [1] P. Stoliar, E. Bystrenova, M. Facchini, P. Annibale, M.-J. Spijkman, S. Setayesh, D. de Leeuw, and F. Biscarini, ''DNA adsorption measured with ultra-thin film organic field effect transistors'' Biosens. Bioelectronics 29, 2935-2938 (2009).
[2] A. Shehu et al. submitted 2009. [3] E. Bystrenova, M. Jelitai, I. Tonazzini, A. Lazar, M. Huth, P. Stoliar, C. Dionigi, M. G. Cacace, B. Nickel, E. Madarasz, and F. Biscarini ''Neural networks grown on organic semiconductors'', Adv. Funct. Mater. 18, 1751-1756 (2008).

3428-Pos
The E.coli Chromosome is an Internally-Organized, Springy, Helical Ellipsoid, the Shape and Dynamics of Which, Through the Cell Cycle, are Determined by the Mechanical Constraints Associated with Replication-Driven Extrusion of DNA/chromatin into a Confined Space
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We have used 3-dimensional imaging of *E. coli* to further our analysis of chromosome dynamics. Using fluorescently labeled histone-like protein HU we can visualize the shape of the *E. coli* nucleoid in living cells in 3D as a function of time in the cell cycle and monitor the positions of specific chromosomal loci or protein complexes (also fluorescently labeled) with respect to this evolving shape. We find that the *E. coli* nucleoid in newborn (G1) cells is a football-shaped ''feather boa'', denser in the middle and highly compressible at its edges, that is twisted into an asymmetric left-handed helical shape. As replication progresses, one sister is immediately reincorporated back into the ''mother'' nucleoid while the other sister emerges into the ''new pole'' of the cell where it also acquires shape. Replication origins, replisome complexes, replication forks and sister loci exhibit specific behaviors that are defined by nucleoid shape, with activity occurring in the periphery and/or the low-density ''spaces'' created by the shape. These and other findings imply that nucleoid shape and chromosome dynamics are primarily governed by internal mechanical forces rather than via external determinants and that the nucleoid as a whole is stiff but ''springy''. Springiness may arise from repulsive interactions between plectonemic supercoiled loops that, after emerging from the replisome, form a series of radial arrays. Given that this stiff structure is confined within a cylindrical cell, energy-minimization may promote twisting of the ellipsoid into a helix, with left-handed bias conferred by the right-handed bias of the component negatively supercoiled plectonemes. Tests of this hypothesis are underway.

3429-Pos
Structure and Dynamics of the Bacterial Chromosome in *E. Coli* Monitored by Gfp-Fis
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The bacterial cell's ability to control the topology of the 1.5 mm-long DNA in the confined environment of the cell is quite remarkable. Despite a great number of studies on bacteria, and especially *E coli*, our understanding of the spatio-temporal organization of bacterial chromosomes is minimal, partly because their dynamics have been difficult to observe directly. Using fluorescent-protein techniques we can visualize bacterial chromosome conformation during cell growth and division through fluorescent microscopy. We have developed a bacterial strain containing fluorescent gfp-fusion versions of a chromosome folding protein, Fis, under inducible control. Bacterial chromosomes have been studied in cells and removed from cells, in order to establish their spatial organization and mechanical properties, and to study how those properties are changed by varied external conditions. Space-time studies of the nucleoid in live *E coli* cells shows how domain structure and overall conformation of chromosomes vary during rapid and slow growth, and it also shows a relation between chromosome segregation and cell division under these different growth conditions. In order to study the bacterial chromosome outside of the cell, we have developed methods for isolation of single bacterial chromosomes and our further objective will be to directly examine nucleoid mechanical properties as a function of protein levels using micromanipulation methods.

3430-Pos
Strong Intra-Nucleoid Interactions Organize the E. Coli Chromosome into a Nucleoid Filament
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The stochastic nature of chromosome organization was investigated by fluorescently labeling genetic loci in live E. coli cells. Measurements of the locus distributions reveal that the E. coli chromosome is precisely organized into a nucleoid filament. Loci in the body of the nucleoid show a precision of positioning within the cell of better than 10% the cell length. The precision of inter-locus positioning of genomically proximal loci was greater than just 4% of the cell