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

length. The measured dependence of inter-locus positioning on genomic distance singles out intra-nucleoid interactions as the mechanism responsible for chromosome organization, from which we infer the existence of an as-yet uncharacterized higher-order DNA organization in prokaryotic cells. We demonstrate that both the stochastic and average structure of the nucleoid is captured by a fluctuating elastic filament model. This organization is shown to be dependent on a number of structural genes. The quantitative analysis of the deletion phenotypes of these genes, in the framework of the nucleoid model, reveals new insights into the mechanisms by which these genes effect cellular-scale organization.

3431-Pos

I-Switch: A DNA Nanomachine that Maps Spatial and Temporal pH Changes Inside Living Cells

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DNA nanomachines are synthetic assemblies that switch between defined molecular conformations upon stimulation by external triggers. Previously, the performance of DNA devices has been limited to *in vitro* applications. Here we report the construction of a DNA nanomachine called the I-switch, which is triggered by protons and functions as a pH sensor based on fluorescence resonance energy transfer (FRET) inside living cells. It is an efficient reporter of pH from pH 5.5 to 6.8, with a high dynamic range between pH 5.8 and 7. To demonstrate its ability to function inside living cells we use the I-switch to map spatial and temporal pH changes associated with endosome maturation. The performance of our DNA nanodevices inside living systems illustrates the potential of DNA scaffolds responsive to more complex triggers in sensing, diagnostics and targeted therapies in living systems.

3432-Pos

Chromosomal Loci Move Subdiffusively Through a Viscoelastic Cytoplasm

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Tracking of fluorescently labeled chromosomal loci in live bacterial cells reveals a robust scaling of the mean square displacement (MSD) as $\tau^{0.39}$. We use Brownian dynamics simulations to show that this anomalous behavior cannot be fully accounted for by the classic Rouse or reptation models for polymer dynamics. Instead, the motion seems to arise from the interaction of the Rouse modes of the DNA polymer with the viscoelastic environment of the cytoplasm. To demonstrate these physical effects, we present a general analytical derivation of the subdiffusive scaling for a monomer in a polymer within a viscoelastic medium. The time-averaged and ensemble-averaged MSD of chromosomal loci exhibit ergodicity, and the velocity autocorrelation function is negative at short time lags. These observations are most consistent with fractional Brownian motion and rule out a continuous time random walk model.

3433-Pos

The Epigenetic Code and Algorithms: Complementary Biomolecular Imprint Interaction of Functional Non-Coding ncRNA, A "Rosetta Stone" for Hermeneutics of Genome Episcription

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OBJECTIVE: The genome orchestrates genetic [Mendelian] and epigenetic [non-Mendelian] information of same genotype into different organized or mess-chaotic [tumor] phenotype variations. By genetic complementary and triplet base codes, only ~2% total DNA transcription output [RNA] are translated into proteins. In entangled epigenetic cancer-angiogenesis-tolerance remodeling of cells activated by extrinsic [environmental] factors, functions of residual ~98% ncRNA transcripts were investigated what governs genome episcription [greek:"overscript"]: Chance or necessity for structural codes and algorithms? ***METHODS:*** Ann.N.Y.Acad.Sci.**1022**:163-184,2004; **1137**:316-342,2008. ***RESULTS:*** Nascently formed functional redox- and metalloregulated small hairpin ncRNA [<200n] as bioaptamers in RNP complexes were isolated and sequenced. Some are genomic DNA derivatives which are not base-complementary to protein-coding gene sequences. They may address defined conserved homologous helix-nucleating domains shared in epigenetic regulator proteins entangled in tolerated growth, vascularization, metabolic syndromes, cancer, epigenetic and genetic information indexing of the epigenome. At variance to usual interpretation in genetic complementary and triplet base codes, ncRNA are not "non-coding", if read in another "language alphabeth" [complementary biomolecular imprint interaction]. They code algorithmic [necessity] rather than stochastic [chance] and heuristic [trial/error] regulatory processes. ***CONCLUSIONS:*** The results suggest the epigenetic code consists of different associated intrinsic and extrinsic interactive complementary biomolecular imprints and factors: **[1]** Non-Mendelian nucleic acid 3D-episcripts in helix-nucleating complementary interaction with **[2]:** Conserved Mendelian genotype-originated homologous domains in epigenetic regulator protein and nucleic acid matrices, comprising variant, mutational, infectious [viral] and heritable disease implications. **[3]** Extrinsic and intrinsic factors upon which formation of [1] and interaction with [2] depends [e.g. redox-and metalloregulation]. Thus, the epigenetic code comprises more diversity, complexity and plasticity repertoires than genetic codes. It implies Darwin-Mendel's genetic principles in synergism with some environmental [Lamarck's] influences for epigenetic [phenotype] imprinting and inheritance.

3434-Pos

Epigenetics of Self-Organization of Biomolecules

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The Genetic Code table does not reveal the hidden pattern in the structure of the table. The only observed structural pattern reported is *assymmetry*, [1]. **C. H. Waddington** pointed out that "*Selection does not impinge directly on **genotypes**, but on **phenotypes**.*", [2]. In the case of the Genetic Code, the codons form the genotypes and amino acids phenotypes. The pattern of synthesis of the different groups in amino acids is observed in terms of Fibonacci sequence, 1 1 2 3 5 8. The asymmetric form of the Genetic Code table, the Genetic Tableau, has a pattern formed by the six right-hand Fibonacci *spirals*, 1 1 2. A similar pattern for the six Fibonacci *spirals* 1 1 2 3 5 8 is observed by matching the elements of the DO_{GU} group of *polyhedra* classifying proteins, [3]. This precision in pattern formation is ***intrinsic*** in the Fibonacci sequence, ($F_n = F_{n-2} + F_{n-1}$). Unlike the *cardinal* numbers, x_n , n =1,2,3,.., or *complex* numbers $z_n = x_n + iy_n$***Fibonacci numbers*** are *hexagonal*; therefore, six rotationally symmetric positions on the surface (2D) are possible. This creates *congruence* (*mod* 6). In addition, two consecutive ones (F_{n-2} and F_{n-1}) represented by two hexagons are rotated from each other by $\pi/6$. This *rotation* can be $+\pi/6$ or $-\pi/6$, creating the *right-handedness* or *left-handedness* of 2D (surface) tessellation by *spirals*. The size of the edges of each hexagon corresponding to a Fibonacci number is equal to F_n. For example, $F_6 = 8$. Covering surface (2D) can be *symmetric* or *asymmetric*. The 3D tessellation is accomplished by the formation of a *helix*. The expanding surface forms a *hyperboloid* obeying the golden ratio (F_n/F_{n-1}). This is the **Waddington-Thom***Epigenetic Landscape* [3]. [1] *Biophys J.* February **(1969)**; A-254 [2] *Annals NYAS* 231 (**1974**)32-42. [3] *Biophys J.* January (**2009**); (3038-Pos).

3435-Pos

Biophysical Features of Non-Coding Genome Sequences

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Non coding sequences of DNA represent most of the genome in eukaryotes. Such sequences are complex and probably include features which are essential for chromosome structure, such as chromosome condensation, axis formation, homologous chromosome pairing in meiosis, etc. It is unlikely that coding regions (exons) play a significant role in chromosome structure, since they have evolved to optimize protein synthesis. We are trying to determine by computational methods the presence of frequent sequences, which might play a role in chromosome structure. Such frequent sequences should also be considered as potential targets for drug binding. We have analyzed the occurrence of short words (2-4 nucleotides), as well as the distribution of frequent longer words (9-14 nucleotides) and of microsatellites (long repeats of short words). We have found that some sequences, such as $(AT)_n$ and $(AG)_n$, have a distribution in most eukaryotic genomes which suggests a structural role. Analysis of longer words shows the presence of many frequent sequences which contain clusters of purines/pyrimidines such as GGAA, TTT, CCC, etc. We have studied in more detail the genome of *Caenorhabditis elegans*: we have found words with a similar sequence which punctuate the whole genome and provide structural marks. Surprisingly very few structural data are available on such frequent sequences, as obtained by biophysical methods (x-ray crystallography, NMR): further work is required. Their eventual influence on nucleosome structure should also be established. As an example we present the structure of some $(AT)_n$ sequences which are polymorphic and frequently present Hoogsteen instead of the standard Watson-Crick base pairing. Interaction with pentamidine, for example, presents novel features when compared with highly studied sequences such as d(CGCGAATTCGCG). It is worth noting that the latter sequence is not frequent in most genomes.