2883-Pos Automatic Modelling Of Olfactory Receptor 3D Structures And Classification Of Odor - Receptor Affinities

Stéphane Téletchéa[1], Antoine Marin[1], Jean-François Gibrat[1], Edith Pajot[2], Guénhaël Sanz[2]

[1] *INRA, MIG, Jouy-en-Josas, France*
[2] *INRA, NOPA, Jouy-en-Josas, France.*

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Olfaction is a complex process involving the recognition of odor molecules by olfactory receptors (ORs), the largest G protein coupled receptor (GPCR) category. They account for more than 1% of total mammalian genome. A receptor can recognize different odor molecules and one odor molecule can bind different receptors: these observed cross recognitions define the combinatorial coding.

To decipher its complexity, we focus on producing a generalized GPCR structure modelling tool and to link it to a scoring function measuring odor - receptor affinities. To date, no experimental three-dimensionnal (3D) structure of an olfactory receptor is available. In order to study precisely how the recognition is performed between the receptor and its ligand, it is thus necessary to build a model structure. The only known structure of a GPCR is the one of bovine rhodopsine that thus serves as a template for the modelling. Molecular modelling is based on the homology relationship existing between the query protein whose 3D model is needed and a protein template. A sequence alignement is carried out between the two sequences to find residues that occupy similar positions in the 3D structures and the template 3D is used to build the query protein model. The alignment accuracy is very important to obtain a reliable model. Unfortunately, for remote homologs, such as ORs and rhodopsine, the sequences are highly divergent and the alignment is error prone.

We will present the strategy we have developed in order to have a robust modelling approach, using a fold recognition method developed in our laboratory. This approach has been extensively tested using available experimental data. Detailed results of the model construction and its comparison to the available experimental data will be presented.

Chromatin & Nucleic Acids

2884-Pos Dynamics Of Individual Nucleosomes Analysed With Single Molecule Spectroscopy And Multiparameter Detection

Alexander Gansen[1], Florian Hauger[1], Katalin Toth[1], Alessandro Valeri[2], Suren Felakyan[2], Claus A.M. Seidel[2], Jörg Langowski[1]

[1] *German Cancer Research Center, Heidelberg, Germany*
[2] *University of Düsseldorf, Düsseldorf, Germany.*

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The structure and dynamics of nucleosomes affect the subsequent compaction into the higher order chromatin structure, which is an important regulatory mechanism of gene activity. We utilize a custom build confocal SMD system to investigate the dynamics within individual nucleosomes diffusing free in solution. Special focus is laid on the effect of histone acetylation and nucleosome remodelling. DNA fragments of the ribosomal 5S and the artificial 601 sequence from J. Widom are labelled with Alexa488 and Alexa594 and reconstituted onto histone octamers to form nucleosomes with 20 – 80 % average FRET, depending on the dye position. To ensure sample stability under SMD concentrations, an excess of inert protein and unlabeled nucleosomes was added to the solution.

In spFRET experiments heterogeneities were observed between the different nucleosome positioning sequences, which agree with PAGE gel analyses. Nucleosomes formed with the natural 5S sequence lead to broader distributions and larger dissociation compared to the highly affine 601 sequence. For both sequences acetylation of all histones caused a significant change in the linker DNA distribution of the nucleosome, but had only minor effect on DNA sites close to the dyad axis. Besides acetylation caused a destabilisation of the nucleosome which we monitored in a SMD based dissociation assay.

To further probe the intrinsic dynamics of the DNA we applied the method of Probability Distribution Analysis (PDA) recently developed in the Seidel lab. For 601 nucleosomes we found a salt-induced redistribution between at least two FRET conformations, pobbibly reflecting a symmetric and asymmetric nucleosome positioning. This approach is currently extended to investigate the activity of remodelling factors on the mononucleosome substrate.

2885-Pos AFM Imaging Of Chromatin Remodeling Complex Action : Mapping The Nucleosome Sliding

Fabien Montel, Hervé Menoni, Dimitri Angelov, Stefan Dimitrov, Martin Castelnovo, Cendrine Faivre-Moskalenko

Ecole Normale Superieure de Lyon, Lyon, France.

Board B188

The organization of DNA into nucleosome represents a physical barrier for the transcription factors binding to their target DNA sequences and interferes with several basic cellular processes. Histone modifications, ATP-remodeling machines and the incorporation of histone variants within chromatin are used by the cell to overcome the nucleosomal barrier and modulate DNA accessibility by the control of nucleosome dynamics. Here we use a single molecule technique (Atomic Force Microscopy, AFM) to visualize isolated nucleosomes, to quantify the influence of histone octamer composition (H2A-Bbd variant) on the equilibrium nucleosome conformation and to map nucleosome mobility induced by a remodeling complex (SWI/SNF, RSC).

AFM allows to determine simultaneously the DNA complexed length and nucleosome position distributions in various contexts [Montel et al., Biophys. J *93*, 566 (2007)]. Using a linear energetic model for the distribution of DNA complexed length, we extract the net wrapping energy of DNA onto the histone octamer, and compare it to previous studies. Similar mapping of the nucleosome position and DNA complexed length is used to quantify the impact of ATP-activated remodeling and sliding on nucleosomes. The results

suggest experimental insights into the processivity and directionality of the RSC motor on nucleosomes.

2886-Pos The Sequence-specific Mechanical Stability Of Nucleosomes Formed With Alpha-satellite DNA Analyzed By Force-spectroscopy

Malte Bussiek[1], Christian Hoischen[2], Stephan Diekmann[2], Vinod Subramaniam[1], Martin Bennink[1]

[1] *University of Twente, Enschede, The Netherlands*
[2] *Fritz-Lipmann Institute, Jena, Germany.*

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The sequence-specific mechanical stability of nucleosomes formed with alpha-satellite DNA analyzed by force-spectroscopy

Repetitive DNA, referred to as satellite DNA, is a typical component of constitutive heterochromatin, the most condensed chromatin fraction of eukaryotic cells. Particularly stable DNA-histone interactions represent one assumed mechanism of this chromatin compaction. Examples of species with intrinsically curved satellites support the view that, by their sequence, satellites contribute to this process. It is however impossible to conclude such function in general because of an extensive heterogeneity of satellites in terms of nucleotide composition, repeat length and extent of intrinsic curvature. Satellite DNA from primate species is referred to alpha-satellite DNA (AS) and has a repeat length of 172 bp.

We are applying force-extension measurements using optical tweezers to determine if nucleosomes formed with AS are characterized by a higher mechanical stability compared with random sequence DNA. The sequence is isolated from African green monkey. We find that rupture forces of single AS-nucleosomes are significantly higher than of random sequence nucleosomes. In addition, we asked which peculiar sequence-dependent mechanical properties may be responsible for the higher nucleosomal stability. Electrophoretic analysis indicates levels of intrinsic curvature slightly exceeding that of control DNAs. Whereas force-extension measurements reveal a high degree of bendedness, indicating a feature not recognized by curvature analyis. In a next step, atomic force microscopy measurements are planned with the aim to map the locations of high bendedness within the AS (curvature/bendability).

2887-Pos AFM Studies On The Mechanism Of Nucleosome Remodeling By RSC

Martijn de Jager[1], Joke van Vugt[2], Colin Logie[2], John van Noort[1]

[1] *BF/LION, Leiden University, Leiden, The Netherlands*
[2] *NCMLS, Radboud University, Nijmegen, The Netherlands.*

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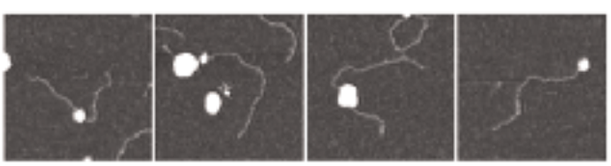
ATP-dependent remodelers continuously modify the structure of chromatin to accommodate genome transactions. In this study we used Atomic Force Microscopy to visualize individual RSC complexes on single nucleosome substrates. Multiple lobes of the RSC complex were resolved in close agreement with previously reported EM tomographs. A strong preference of RSC binding to DNA ends over bare DNA and nucleosomes was observed. Upon incubation with ATP, nucleosomes were displaced from the central positioning element towards the ends of the DNA. From complexes trapped during remodeling and the distribution of nucleosomes before and after remodeling the mechanism of remodeling could be deduced, in which RSC binds to a DNA end, translocates along DNA and drags the nucleosome with it. The mechanism was quantitatively tested using Monte Carlo simulations. From the simulations the processivity of DNA translocation by RSC was fitted to be 1 kb.



2888-Pos Probing The Coupling Between Transcription Hubs And Cellular Architecture Using Magnetic Force Induced Perturbations Within Living Cells

Feroz M. Hameed[1], K. Venkatesan Iyer[1], G.V. Shivashankar[1,2]

[1] *National Centre for Biological Sciences - TIFR, Bangalore, India*
[2] *Raman Research Institute, Bangalore, India.*

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The 3D organization of the cell nucleus is found to be a critical determinant of genome function. Gene locus positions and transcription hubs are physically repositioned within the cell nucleus during gene expression and yet the underlying mechanisms are unclear. Notably mechanical cues are found to alter gene expression programs suggesting a strong coupling between cellular architecture and transcription control within living cells. To investigate this coupling, we have developed a magnetic force apparatus to induce mechanical perturbations within living cells. For this 100 nm magnetic beads are non-specifically adhered onto the plasma membrane of mammalian cells. The application of a mechanical force by a tip-based electromagnet induces stretching of the cytoskeletal network resulting in physical decoupling of the nuclear architecture leading to nuclear shrinkage and chromatin reorganization. Further, forces applied on 1 μm diameter magnetic beads, microinjected into the cell nucleus, triggered an ATP-dependent active remodeling of chromatin assembly. The local unfolding of chromatin also resulted in dramatic alterations in functional nuclear organization as mapped using fluorescence anisotropy imaging. In addition, repositioning of the magnetic bead in the cell nucleus leads to the displacement of the fluorescent tagged UTP enriched transcription hubs in the direction of the external force. Taken together these data evidence a hardwired cellular architecture suggesting a structural platform to integrate mechano-signaling cues to transcription sites within living cells.

2889-Pos Computer Modeling and Electron Microscopy Reveal New Linker-Histone and Physiological-Salt Induced Conformational Transitions in Chromatin

Gaurav Arya[1], Tamar Schlick[2], Sergei Grigoryev[3]

[1] *University of California, San Diego, La Jolla, CA, USA*
[2] *New York University, New York, NY, USA*
[3] *Penn State University, Hershey, PA, USA.*

Board B192

A universal step in the organization of double-stranded DNA in eukaryotes is its association with histone proteins to form the chromatin fiber. The exact structure of chromatin is still debatable and two limiting models have emerged over the years. In the zigzag model, adjacent nucleosomes lie on opposite sides of the chromatin axis and the connecting linker DNA is straight or gently bent. In the solenoid model consecutive nucleosomes lie side by side along chromatin periphery and the connecting linker DNA is sharply bent and never crosses the chromatin axis. Here we use computer modeling/simulations and electron microscopy (EM) to elucidate the internal structure of chromatin under different conditions. Specifically, we extend our earlier mesoscale oligonucleosome model to now incorporate the linker histone H1/H5. We also develop a new EM-based technique that indirectly captures the internal arrangement of nucleosomes in the fiber by measuring the histone tail mediated internucleosomal interaction pattern. We demonstrate using both approaches that oligonucleosomes exhibit markedly different equilibrium conformations depending upon the concentration and valency of solution cations and the presence of the linker histones. Oligonucleosomes condensed at physiological concentrations of monovalent cations (Na^+) have predominantly straight linkers and a two-start helical arrangement of nucleosomes consistent with the zigzag model. However, the number of bent linker DNAs is significantly increased in chromatin condensed by divalent cations (Mg^{2+}) such that in its most condensed form the number of the bent linkers surpasses that of straight linkers, consistent only with a model that combines elements of both the zigzag and solenoid model, i.e., with interchanging bent and straight linker DNAs.

2890-Pos Higher-order Folding of Chromatin and the Random Loop Model

Manfred Bohn, Dieter W. Heermann

Institute for Theoretical Physics, Heidelberg University, Heidelberg, Germany.

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Remarkably little is known about the higher-order folding motifs of the chromatin fiber inside the cell nucleus. Folding depends among others on local gene density and transcriptional activity and plays an important role in gene regulation. Strikingly, at fiber lengths above 5 to 10 Mb the measured mean square distance between any two points on the chromatin fiber is independent of polymer length. The formation of loops on different length scales seems to play an important role in transcriptional regulation. We analyze the data with respect to different polymer models on short fiber lengths. For long fiber length above 10 Mb, where simple models fail in explaining experimental results, we propose a polymer model that explains the leveling off by means of random looping. We derive an analytical expression for the mean square displacement between two arbitrary beads. A detailed investigation of this model shows that only a small number of loops on all scales are necessary to fit experimental data.

2890.01-Pos Improving the Sensitivity of Tobramycin Beacon Aptamers

Jeff E. Vandenengel, Daniel P. Morse

United States Naval Academy, Annapolis, MD, USA.

Board B193.01

Beacon Aptamers are modified nucleic acid molecules that change conformation and emit light upon binding to a specific target molecule. A beacon aptamer has a fluorophore attached to one end of the molecule and a quencher attached to the other end. In the absence of the target, the molecule adopts a stem-loop conformation in which the 5′ and 3′ ends are paired. In this conformation, fluorescence is efficiently quenched. Upon binding to the target, the fluorophore moves away from the quencher causing an increase in the fluorescence intensity.

In theory, beacon aptamers can provide a simple, sensitive, and specific assay for any ligand so there is great interest in their efficient production. We previously developed a method for the direct selection of RNA beacon aptamers from a randomized sequence pool. The method was tested by using the antibiotic tobramycin as the target (1). Two RNA sequence families emerged from the selection. When these RNAs were converted into beacon aptamers, a modest but reproducible increase in fluorescence intensity was detected in the presence of tobramycin but not in the presence of a related antibiotic. In addition, an RNA that was not enriched by the selection procedure did not respond to tobramycin.

Here, we report the initial results of experiments designed to produce tobramycin beacon aptamers with improved sensitivity. This is being done by partial randomization of the previously selected beacon aptamers followed by re-selection.

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2890.02-Pos Utilizing Steady-State and Time-Resolved Fluorescence to Probe DNA/Gata Binding Interactions

Jacquelyn D. Lewis, Nora E. Kuiper, Lydia R. Hartsell, Brent P. Krueger, Michael J. Pikaart

Hope College, Holland, MI, USA.

Board B193.02

The recruitment of transcription factor proteins is an essential step in the regulation of many processes including hematopoiesis–the production of red blood cells. One critical transcription factor in this process, GATA-1, binds to the DNA sequence (A/T)GATA(A/G) via a zinc finger. The specificity of this interaction is governed by several key amino-acid/nucleotide contacts, which were identified using gel electrophoresis (Mott, Bassman, and Pikaart, *BBRC 316* (2004) 910–917). These studies are being extended using steady-state and time-resolved fluorescence anisotropy to characterize the thermodynamics of the GATA-1/DNA interaction in a solution-phase environment. Results of anisotropy studies of fluorescent probes in solution, probes conjugated to DNA, and fluorescently-labeled DNA bound with Gata protein are presented and compared to previous results of gel electrophoresis.

2890.03-Pos The Mitochondrial Genome Encodes Small RNAs

Seungil Ro, Chanjae Park, Rui Song, Kenton M. Sanders, Wei Yan

Physiology and Cell Biology, University of Nevada Medical School, Reno, NV, USA.

Board B193.03

Mammalian mitochondrial genome encodes 2 rRNAs, 22 tRNAs, and 13 polypeptide genes that are essential for protein synthesis and ATP production.

Here we report the identification of 12 small non-coding RNAs that are transcribed from the mitochondrial genome in mice. In addition, the genome encodes five Piwi-interacting RNAs (piRNAs) and one microRNA (miRNAs). The 12 mitochondria-derived small RNAs are a bit larger (∼34 nt) than miRNAs and piRNAs and they display some structural commonalities that are distinct from any of the known small RNA species. We, therefore, named these novel small RNAs mitochondrial small RNAs (misRNAs). Most of the misRNAs are mapped to either the 5′ or the 3′ ends of the known mitochondrial genes, but some are derived from the light chain of the mitochondrial DNA where no known genes are located. We further show that these misRNAs and their corresponding residing transcripts (tRNAs, rRNAs or peptide-coding mRNAs) are mainly located in the cytosolic rather than the mitochondrial fraction. We also identified eight human orthologs of misRNAs, which we predicted based on the mouse misRNAs, and we verified their expression in human tissues. Since these misRNAs possess sequences either identical or complimentary to their residing tRNAs, rRNAs, or mRNAs, they may have a role in the regulation of mitochondrial gene expression and thus may be involved in human mitochondrial diseases.

2890.04-Pos Free Energy Profiles of DNA-nanoparticle Interactions by Umbrella Sampling Simulations

Kathleen M. Mills, Mark Banaszak Holl, Bradford G. Orr, Ioan Andricioaei

University of Michigan, Ann Arbor, MI, USA.

Board B193.04

Polyamidoamine (PAMAM) dendrimers, reported by Tomalia in 1985 (1), show great promise as potential delivery systems for gene therapy (2,3,4). However, the nature of the interaction between dendrimers and DNA is not fully understood. While it has been shown that DNA binds PAMAM dendrimers (5,6,7), the molecular details of this interaction cannot be probed using experimental techniques. In order to better understand these interactions, umbrella sampling simulations were run using NAMD on a generation 3 PAMAM dendrimer and a 24bp double-stranded DNA. Free energy profiles were calculated for the interaction between DNA and dendrimers with amine terminations as well as dendrimers with mixed amine-acetamide terminations. We find that the DNA deforms considerably upon interaction with the amine-terminated dendrimer. Substantially less deformation was seen in the interactions between DNA and dendrimers with mixed amine-acetamide terminations.

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2890.05-Pos Towards Large-scale Integrated Nucleic Acid Logic Circuits

Georg Seelig

Caltech, Pasadena, CA, USA.

Board B193.05

To systematically create complex yet reliable circuits, electrical engineers use digital logic, wherein gates and subcircuits are composed modularly and signal restoration prevents signal degradation. We have adapted such engineering ideas to the design and experimental implementation of DNA-based digital logic circuits that operate in an aqueous environment.

We demonstrate AND, OR, and NOT gates, signal restoration, amplification, feedback, and composition of logic gates into multi-

layered circuits. Biological nucleic acids such as microRNAs can serve as inputs, and circuits work reliably in a background of unrelated nucleic acids, suggesting applications to the analysis of complex biological samples.

Motions of Cell Surface Molecules

2891-Pos Membrane Diffusion Measured Using Fluorescence Correlation Spectroscopy in Polarized S. cerevisiae

Travis I. Moore, Theodore Hazlett, Enrico Gratton, Tau-Mu Yi

Univ. California at Irvine, Irvine, CA, USA.

Board B194

The cellular membrane not only serves as the interface and barrier between the extracellular environment and intracellular biochemical processes, but also a dynamic organelle where proteins are embedded, recruited and organized, in many cases free to diffuse around the cell surface creating a homogeneous protein concentration.

During polarization this uniformity is broken and localization of some proteins changes resulting in their localization becoming polarized. To explore the role of diffusion and how changes of a proteinâ™s diffusion rate may be involved in polarization, Fluorescence Correlation Spectroscopy (FCS) was utilized to measure membrane diffusion in the budding yeast, S. cerevisiae, during mating projection formation. Slow diffusion rates were determined for cytosolic and membrane bound (EGFPmem) EGFP which were consistent with previously reported values obtained using FRAP [1]. a-cells were exposed to varying concentrations of Î±-factor (0, 10, 100, 1000 nM) and localization of EGFPmem and the Î±-factor receptor (Ste2-EGFP) after the formation of a projection were observed. Localization of the Ste2-EGFP was highly polarized whereas localization of EGFPmem was only slightly weighted toward the projection. This may be the result of increased membrane order in the projection [2]. Diffusion rates of both Ste2-EGFP and EGFPmem were measured at the front and back of the polarized cell.

Future work will incorporate these measured diffusion rates as well as FCS derived molecule numbers into mathematical models of yeast cell polarization. EG&TH supported by NIH RR003155

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2892-Pos Cholesterol Mediates Heterogeneity in a Non-Equilibrium Lipid Membrane Model

Andrew P. Paradis[1], Susan R. McKay[1], Samuel T. Hess[1,2]

[1] *Department of Physics and Astronomy, University of Maine, Orono, ME, USA*
[2] *Institute for Molecular Biophysics, University of Maine, Orono, ME, USA.*

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Cellular membranes show strong heterogeneity in their lateral organization, forming domains of various sizes. However, direct observations of the interactions between individual lipids and cholesterol have been challenging to obtain experimentally. Here, we simulate cellular membranes using a two-dimensional triangular lattice with the Monte Carlo method, including interactions between cholesterol, saturated and unsaturated lipids. In addition, simplified endo- and exocytosis events keep the system far from equilibrium. Results confirm that cholesterol plays the role of mediator between unsaturated and saturated lipids, consistent with experimental observations. Higher cholesterol concentrations lead to a weaker temperature dependence of the average energy per bond. At lower temperatures, under some conditions we also observe ordered cholesterol super-lattice structures within saturated domains. Finally, the endo- and exocytosis lead to a broader range of domain sizes, which we have quantified using Ripley's K-test. These non-equilibrium simulations of the direct interactions between cholesterol and lipids provide a more realistic picture of cellular membranes on the molecular scale, which may allow significant predictions to be made as a function of membrane composition.

2893-Pos Mobility Of Tsr, TatB And PhoR In The Inner Membrane Of Escherichia Coli

Dongmyung Oh, Yang Yu, Yi Ju, Barry Wanner, Ken Ritchie

Purdue University, West Lafayette, IN, USA

Board B196

Many transmembrane and membrane associated proteins display non-random distributions, such as clustering into finite-sized domains or localization to specific regions, in both prokaryotic and eukaryotic cells. In many cases it is believed that these non-random distributions are essential for, or at least facilitate, function. Here we present observations of the distribution and mobility of three transmembrane proteins that show distinct localization patterns in the inner membrane of *Escherichia coli.*

1. The serine chemoreceptor, Tsr, labeled by the yellow fluorescent protein variant Venus, which is found primarily at the poles. Single molecule imaging of the mobility Tsr reveals a slow/immobile fraction (93% of the observed molecules) with an average diffusion coefficient D = 0.0125 ± 0.0024 $\mu m^2/s$