Workshop on Nanomechanics of Biomolecules

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Center Stephano Franscini

List of Talks

Prof. Ralf Everaers
Laboratoire de Physique
Ecole normale supérieure de Lyon
Lyon, France

"Linking rigid base-pair potentials to indirect readout and mesoscale elasticity of DNA"

The sequence dependent structure and elasticity of DNA play an important role in gene regulation: locally through the indirect readout contribution to the binding affinities of proteins, on larger scales by mediating interactions between competing or cooperating DNA binding proteins via looping.
Locally, DNA is often described on the level of rigid base—pair (bp) steps. Corresponding elastic potentials have been obtained from all--atom MD simulation and from high--resolution crystal structures. In the first part of the talk we focus on indirect readout. We present a computationally inexpensive local marker for elastically optimized subsequences in protein--DNA co-crystals and, using the example of the bacteriophage 434 repressor, we investigate how predictions for relative affinities mediated by indirect readout depend on the chosen parametrization.
In the second part of the talk, we present a systematic coarse--graining of the rigid bp model to the Marko-Siggia worm-like chain description of DNA. We calculate the mean values of the resulting structural and elastic worm--like chain parameters as well as their variability for random sequence chains. We find that the available microscopic elastic potentials predict similar mesoscopic parameter sets. The thermal bending and twisting persistence lengths computed from MD data are 42 and 48 nm, respectively. The static persistence lengths which reflect the structural randomness are generally much higher, in agreement with cyclization experiments.

Prof. Richard James
University of Minnesota
Minneapolis, USA

"Lessons on structure from the structure of viruses"

As the most primitive organisms, occupying the gray area between the living and

nonliving, viruses are the least complex biological system. One can begin to think about them in a mathematical way, while still being at some level faithful to biochemical processes. We make some observations about their structure, formalizing in mathematical terms some rules-of-construction discovered by Watson and Crick and Caspar and Klug. We call the resulting structures “objective structures”. It is then seen that objective structures include many of the most important organic and inorganic structures studied in science today: the capsids, necks, tails and baseplates of many viruses, the cilia of some bacteria, DNA octahedra, actin, GroES/GroEL and a great many other protein molecules, C_60, carbon nanotubes and certain severely bent and twisted atomic scale beams. The rules defining them have rigorous quantum mechanical origins. We develop a methodology for computing such structures and their properties. Some of the nonperiodic structures revealed by the formulas exhibit beautifully subtle relations of symmetry. This common mathematical structure paves the way toward many interesting calculations for such structures. We discuss three such lines of research: 1) simplified first-principles calculations of the energy of objective structures, 2) “Objective MD”, a simplified method of exact molecular dynamics for such structures, and 3) a proposal for a new method of x-ray determination of the detailed atomic configuration of these structures, that would eliminate the need for crystallization and could possibly be done in-vivo.

Prof. Robert L. Jernigan
L. H. Baker Center for Bioinformatics & Biological Statistics
Iowa State University
Ames, USA

"Simple Models for Deriving Motions from Static Protein Structures"

Models representing large molecular structures as rubbery bodies in the form of networks are proving fruitful for simulating the large domain motions of structures, which are not computationally readily accessible with molecular dynamics. The normal modes of motion are obtained from structures by representing proteins as coarse-grained blocks of uniform material, utilizing only one representative point per residue and connecting the closest residues with identical springs. The largest domain motions, which are the most important, are the most reliable to compute, and these depend principally on the overall shape, and hence do not require extremely high resolution structures. The motions from this simple model agree well with those represented by the crystallographic B factors. In addition, in cases where there are large numbers of structures of the same protein, the principal components of the aligned structures agree with the directions of the most important normal modes, providing an important experimental confirmation. When there are two different forms of the protein, a combination of a few of the slowest modes of motion corresponds closely to the direction of the transition. In a number of cases the level of detail required to reproduce the slow large domain motions is well below 1 point for each residue. We find that coarse graining up to the level of 1 point for each sequential 40 residues provides a sufficiently detailed representation for many

globular proteins, again because the shape is the feature most important for determining its motions. We have also been making elastic networks for atoms but find only small gains with these models over the coarse-grained models. A mixed coarse grained approach has also been developed wherein some parts of the structure are given in atomic detail and the remainder in less detail. These lead to the strong conclusion that the large domain motions control many of the finer-level atomic motions relating to function. The ribosome, for example, exerts an extremely tight control over the structures at the functional sites of its components. The extent of such control depends in general, of course, on the extent of cooperativity of interactions within a protein structure - an issue not yet fully resolved.

Prof. John E. Johnson
Department of Molecular Biology
The Scripps Research Institute
La Jolla, USA

"Biophysical Analysis of Virus Particles and their Maturation: Insights into Elegantly Programmed Nano Machines"

The capsid of the lambda-like bacteriophage HK97 was investigated by crystallography, electron cryomicroscopy and image reconstruction (cryoEM), solution x-ray scattering, single molecule fluorescence, H/D exchange and biochemistry. A detailed kinetic profile was established for the dramatic maturation process where 420 copies of the 281 residue capsid protein, arranged with T=7laevo icosahedral symmetry, expands from the 500Å Prohead II particle, determined to 5.2Å resolution by crystallography(1), to the 660Å Head II particle, determined at 3.5Å resolution(2). Maturation of virions is driven by packaging dsDNA into Prohead II that contains a dodecameric portal at one pentavalent site of the icosahedral capsid, however, a closely similar process can be examined from expressed capsid protein that spontaneously assembles into portal-less capsids in the E. coli expression system(3). The stages of expansion were determined to occur in two steps; prohead II to Expansion Intermediate I (~580Å) is triggered by lowering the pH from 7 to 4 and the transition has no populated intermediates. The half-life for the transition of the ensemble is about 4 minutes at this pH and the particle expansions occur stochastically(4). Single particle analysis shows that the actual transition takes about one second. Expansion Intermediate I is capable of forming spontaneous, auto-catalytic, isopeptide cross-links between the side chains of lysine 169 and aspargine 356. The cross-links form with a half-life (as judged by gel electrophoresis) of hours to days at pH 4, but with a half-life of about 4 minutes if the pH is raised to 7(5). When roughly half the cross-links have formed (a quorum), a second transition occurs to the “balloon” state, again, with no detectable intermediates in an ensemble experiment(6). The final maturation requires a unique set of 60 cross-links that involve subunits at the 5-fold axes that participate in hexameric ring formation(7).
The mature virion of the P22 phage was determined with an asymmetric cryoEM reconstruction that permitted visualization of the dodecameric portal and dsDNA

within the particle. The structure suggests that the portal is a sensor for headful packaging and that it undergoes a dramatic conformational change when the dsDNA is wrapped tightly around it. The DNA is wrapped as a solenoid within the particle suggesting that the first portion entering may be “tied down” to the portal where the dsDNA is highly ordered(8).

1 Gertsman et. al. 2006 The structure of a virus assembly intermediate: bacteriophage HK97 prohead II. J. Mol. Biol. Submitted
2. Wikoff et. al. 2000. Topologically linked protein rings in the bacteriophage HK97 capsid. Science 289:2129-2133.
3. Wikoff et. al. 2006. Time-resolved molecular dynamics of bacteriophage HK97 capsid maturation interpreted by electron cryo-microscopy and X-ray crystallography. J Struct Biol 153:300-6.
4. Lee et. al. 2005. Cooperative reorganization of a 420 subunit virus capsid. J Mol Biol 352:723-35.
5. Gan et. al. 2004. Control of crosslinking by quaternary structure changes during bacteriophage HK97 maturation. Mol Cell 14:559-69.
6. Lee et. al. 2006. A quorum of covalent cross-links in HK97 bacteriophage capsids spring-loads a conformational switch. J. Mol. Biol. Submitted
7. Gan et. al. 2006. Capsid conformational sampling in HK97 maturation visualized by X-ray crystallography and cryo-EM. Structure submitted
8. Lander et. al. 2006. The structure of an infectious P22 virion shows the signal for headful DNA packaging. Science 312:1791-5.

Prof. Jané Kondev
Brandeis University
Waltham, USA

"Physics of chromosome structure and organization in vivo"

Chromosomes are the physical carriers of genes. Their attributes, such as location in the cell, packing density, and mobility, affect gene expression. The emerging view of chromosome organization is that particular genes are associated with specific spatial addresses within the cell. First uncovered in eukaryotes, this form of spatial organization of chromosomes has also been recently found in bacteria. I will discuss how by combining experimentation in vivo with theoretical modeling, we are beginning to address, in a quantitative manner, the structure and organization of yeast chromosomes and the role of chromosome tethering in mating-type switching.

Prof. Richard Lavery
Laboratoire de Biochimie Théorique, CNRS UPR9080
Institut de Biologie Physico-Chimique
Paris, France

"Heterogeneous molecular mechanics and recognition"

Are biological macromolecules soft machines? Yes, in that their structures depend on weak, non-covalent interactions. However, "soft" doesn't imply homogeneous. In order to function as machines, biomacromolecules must also react in specific ways to external stimuli. This implies that they should have heterogeneous internal mechanics. Data in support of heterogeneity is accumulating both from experiment and from molecular simulations at various degrees of resolution. Since macromolecules often have to deform to carry out their biological functions, heterogeneity can be exploited in the broad range of recognition processes which occur within the cell. This heterogeneity can also be probed by artificially inducing macromolecular deformations. I will present some of our recent studies in this area on proteins and their complexes.

Prof. John H. Maddocks
EPFL FSB IMB LCVMM
Lausanne, Switzerland

"Continuum Models of DNA Looping Probability"
(joint work with O. Penrose and L. Cotta-Ramusino)

A simplified, but precise, model of the probability of loop formation in DNA, including cyclization, is formulated in terms of a continuum, sequence-dependent model of the DNA double helix. The leading order term in this probability is a Boltzmann factor weighted with the energy of the minimum energy configuration satisfying the two point boundary conditions corresponding to looping or cyclization. Here we show that a correction for entropic or fluctuation effects can be computed in terms of the volume of a certain Jacobi field satisfying linearized equations at the minimum energy configuration.

Prof. Rob Phillips
California Institute of Technology
Pasadena, USA

"Tightly Bent DNA is a Fact of Life"

Tightly bent DNA plays a role in processes as diverse as the packing of DNA in viruses, prokaryotes and eukaryotes to transcriptional regulation. The goal of this talk will be to highlight some fascinating examples of tightly bent DNA and how the physics of such DNA impacts biological function.

Prof. Yitzhak Rabin
Department of Physiks
Bar-Ilan University
Ramat Gan, Israel

"Wormlike loops and Fourier knots"

Every smooth closed curve can be represented by a suitable Fourier sum as a function of an arbitrary parameter τ . We show that the ensemble of curves generated by randomly chosen Fourier coefficients with amplitudes inversely proportional to spatial frequency (with a smooth exponential cutoff) can be accurately mapped on the physical ensemble of inextensible worm-like polymer loops. The τ → s mapping of the curve parameter τ on the arc length s of the inextensible polymer is achieved at the expense of coupling all Fourier harmonics in a non-trivial fashion. We characterize the obtained ensemble of conformations by looking at tangent–tangent and position–position correlations. Measures of correlation on the scale of the entire loop yield a larger persistence length than that calculated from the tangent–tangent correlation function at small length scales. The topological properties of the ensemble, randomly generated worm-like loops, are shown to be similar to those of other polymer models.

Prof. Robert Schleif
Johns Hopkins University
Department of Biology
Baltimore, USA

"An In-depth Discussion of a Recent Experiment: Using DNA as a Tape Measure to Triangulate a Protein"

This talk will focus on the structure and mechanism of action of the gene regulatory protein AraC from the bacterium Escherichia coli.
It is sensible for cells to potentiate a metabolic pathway for the utilization of a “food source” only when that food is available. Hence, it is no surprise that in the absence of the sugar L-arabinose, cells repress the expression of the proteins that could allow its utilization. In the presence of arabinose, synthesis of these proteins is induced. AraC protein lies at the heart of these repression and induction phenomena. How the protein performs these opposing actions and how we have learned this information will likely constitute much of the talk and related discussion.
AraC protein is a transducer whose DNA binding abilities are modulated by the presence of arabinose. When it binds to DNA in one way, it forms a DNA loop and represses the synthesis of the arabinose utilization proteins. When AraC binds to DNA in a different way, it facilitates synthesis of these proteins. The issue then is: How does the binding of arabinose to AraC bring about these changes in DNA binding?
AraC protein possesses a domain that binds arabinose, a domain that binds DNA, and a flexible arm that carries information between the two domains. Reaching the

next level in our understanding of the underlying mechanisms of this system requires knowing more precisely where the DNA binding domain is located with respect to the arabinose binding domain and arm.
Conventional structure determination methods have been unsuccessful in determining the structure of the full-sized AraC protein, although the structures of the individual domains have been determined (one with high accuracy and one with low accuracy). A recently performed experiment utilized the DNA binding properties of AraC and families of DNA molecules to measure three distances between points in AraC. A structure consistent with these distances provides approximate locations of the domains and suggests additional biochemical experiments. These are currently being tested.

Prof. Christof Schütte
Freie Universität Berlin
FB Mathematik und Informatik
Institut für Mathematik II
Berlin, Germany

"Infering nano-mechanical models for poly-petides from MD-simulations"

Full-scale MD-simulations of poly-peptides resolve much more degrees of freedom than any nano-mechanical model will normally do. However, the time-scales that are accessible within MD-simulations are much shorten than those typically of interest for nano-mechanical models. The talk will address the question of how one might be able to infer optimal nano-mechanical models of the dynamics of poly-peptides from MD-simulations. The resulting concepts will be illustrated by means of application to some B-DNA 16-mer.

Prof. Julie Theriot
Dept of Biochemistry
Stanford University
Stanford, USA

"How do actin filament assembly dynamics and mechanics determine speed and direction in cell motility?"

Polymerizing networks of actin filaments generate significant mechanical forces that can be used by cells to change shape and to move. Magnitude, speed and efficiency of force generation by actin filament assembly all depend on biochemical conditions and the geometry of filament organization. We have performed experiments to measure speed and force over different spatial scales relevant to cell motility. At small spatial scales, using optical traps to produce a load force, we find

that bundles of parallel actin filaments (~8 filaments/bundle) are stalled at forces expected to stall a single filament. At larger spatial scales, using modified AFM cantilevers to produce load, we find that dendritically branched networks of filaments are able to effectively cooperate but that there are significant history-dependent effects influencing the relationship between network growth speed and force generation. In biological systems that use this mechanism for movement, there are significant individual-to-individual variations that can only be explained by history-dependent mechanisms.

Prof. Jose Vilar
Computational Biology Center
Memorial Sloan-Kettering Cancer Center
New York, USA

"Stochastic dynamics of protein-DNA complexe"

The formation and regulation of macromolecular complexes provides the backbone of most cellular processes, including gene regulation and signal transduction. The inherent complexity of assembling macromolecular structures makes current theoretical and computational methods strongly limited for understanding how the physical interactions between cellular components give rise to systemic properties of cells. Here we present a stochastic approach to study the dynamics of networks formed by macromolecular complexes in terms of the molecular interactions of their components. Exploiting key thermodynamic concepts, this approach makes it possible to both estimate reaction rates and incorporate the resulting assembly dynamics into the stochastic kinetics of cellular networks. As prototype systems, we consider the lac operon and phage lambda induction switches, which rely on the formation of DNA loops by proteins and on the integration of these protein–DNA complexes into intracellular networks. This cross-scale approach offers an effective starting point to move forward from network diagrams, such as those of protein–protein and DNA–protein interaction networks, to the actual dynamics of cellular processes.

Elizabeth Villa
TCBG / Beckman Institute
University of Illinois
Urbana, USA

"Multiscale modeling of protein-DNA complexes"

A multiscale approach to modeling complexes between proteins and looped DNA will be presented. The approach combines a coarse-grained model of DNA based on the classical theory of elasticity, with an all-atom description of proteins and protein-DNA interfaces. The two levels of description are interconnected and interact with each

other during a molecular dynamics simulation. The multiscale method was employed to model and simulate the complex structure of a DNA loop with the lac repressor, an E. coli protein that acts as a genetic switch by forcing DNA into a loop structure, thereby inhibiting the expression of three genes involved in lactose uptake and metabolism. The results of the multiscale simulations reveal in detail the structural dynamics of the protein-DNA complex. The strain arising from the interaction with the DNA loop is absorbed by the DNA-binding head groups of the lac repressor, while the rest of the protein remains structurally unchanged. Interestingly, the protein conformation is primarily stabilized by two salt bridges that form only when the protein is subject to strain, and, therefore, cannot be observed in the crystal structure. The detailed molecular picture provided by the simulations put forward novel interpretations of experimental data, and help design further experiments.

Prof. Jonathan Widom
Molecular Biology and Cell Biology Department
Northwestern University
Department of Chemistry
Evanston, USA

"A genomic code for Nucleosome Positioning"

Eukaryotic genomes are packaged into nucleosome particles that occlude the DNA from interacting with most DNA binding proteins. Nucleosomes have higher affinity for particular DNA sequences, reflecting the ability of the sequence to bend sharply, as required by the nucleosome structure. However, it is not known whether these sequence preferences have a significant influence on nucleosome position in vivo, and thus regulate the access of other proteins to DNA. We isolated nucleosome-bound sequences at high resolution from yeast and used these sequences in a new computational approach to construct and validate experimentally a nucleosome–DNA interaction model, and to predict the genome-wide organization of nucleosomes. Our results demonstrate that genomes encode an intrinsic nucleosome organization and that this intrinsic organization can explain ~50% of the in vivo nucleosome positions. This nucleosome positioning code may facilitate specific chromosome functions including transcription factor binding, transcription initiation, and even remodelling of the nucleosomes themselves.

Dr. Paul Wiggins
White Head Institute
Nine Cambridge Center
Cambridge, UK

"DNA: more than its sequence"
(The hidden mechanics of DNA)

DNA bending is a ubiquitous structural motif in cellular processes as diverse as

chromosomal DNA packaging to gene regulation. In addition to its biological relevance, understanding the conformation of double-stranded DNA is theoretically tractable to a much greater extent than the conformations of either proteins or single-stranded nucleic acids. DNA mechanics has therefore become an important proving ground for understanding the molecular machines responsible for cellular function on a quantitative level.

Despite several decades of extensive work, recent experimental studies of DNA bending, relevant for describing biological DNA conformations, have yielded a number of seemingly inconsistent results. In particular, there is evidence that sharply-bent DNA may be significantly more flexible than expected from the classic measurements of DNA flexibility.

I will first present some recently published calculations that demonstrate that, if such a high-curvature flexibility exists, it is hidden from all but one of the classic assays that measure DNA flexibility. In the second part of the talk, I will show our recently submitted results from the first experiments to directly and quantitatively measure the elastic properties of DNA on the 5 nm length scale. We find that the elastic response of DNA is significantly weaker than expected at high-curvature. These results predict that the free energy budget for protein-induced DNA bending may be significantly smaller than previously estimated.

Prof. Ir. Gijs J.L.Wuite
Vrije Universiteit
Division of Physics & Astronomy / FEW
Amsterdam, The Netherlands

"A singular view of DNA transactions"

The genetic information of an organism is encoded in the base pair sequence of its DNA. Many specialized proteins are involved in organizing, preserving and processing the vast amounts of information on the DNA. In order to do this swiftly and correctly these proteins have to move quickly and accurately along and/or around the DNA constantly rearranging it. In order to elucidate these processes we perform single-molecule experiments on model systems such as restriction enzymes, bacterial gene regulators and repair proteins. The data we use to extract the forces, energies and mechanochemistry driving these dynamic transactions. The results obtained from these model systems are then generalized and though to be applicable to many DNA-protein interactions.

Prof. Krystyna Zakrzewska
Laboratoire de Biochimie Théorique, CNRS UPR9080
Institut de Biologie Physico-Chimique
Paris, France

"Towards understanding the thermodynamics of protein-DNA binding"

A theoretical analysis of the thermodynamics of complexation is presented for 13 DNA-protein complexes for which structures and experimental binding data are known. The set of complexes was chosen to cover a variety of different protein binding motifs and a wide range of enthalpy and entropy values. Free energy contributions based on Amber force field calculations and on accessible surface area terms are used to estimate both enthalpy and entropy values. We show that these functions reproduce well the experimental data, although certain terms differ considerably from those used in standard approaches such as the so-called MM/PBSA method. The results suggest that the terms employed contain most of the physics behind the process of protein-DNA binding and they will hopefully be useful in analyzing other biologically relevant complexes.