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WORKSHOP on BIOPOLYMERS: THERMODYNAMICS, KINETICS and MECHANICS of DNA, RNA and PROTEINS

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BOOK OF ABSTRACTS

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PREFACE

The purpose of the Workshop is to provide state-of-the-art perspectives on experimental, theoretical and computational approaches to this vital area and to foster discussions on the challenges and new strategies for understanding biopolymers.

Among others, the following problems will be discussed:

- **Single-molecule experiments on DNA and proteins**
- **Determination and prediction of protein structure and function**
- **DNA-protein interactions**
- **DNA structure and topology**
- **Chromosome structure**

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H.S. Chan, University of Toronto, Canada
P. De Los Rios, EPFL, Lausanne, Switzerland
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* *list of speakers as of 13 May 2005*

ABSTRACTS OF TALKS

(in alphabetical order of speaker)

ATP dependent chromatine remodeling factors

G.Lia, E. Praly, P. Milani,T. Owen-Hughes and D.Bensimon

We have studied two families of ATP dependent chromatine remodeling factors (ISWI and SWI/SNF) using single molecule manipulation tools and AFM microscopy. The evidence suggests that these factors are DNA translocases that operate as an inchworm moving on the molecule while dragging at the same time a substantial loop of DNA (few 100 bps). The issue of the induced twist in the molecule during motion of the enzyme is still an open puzzle, that we will discuss.

Long-range compaction and organization of interphase chromatin in budding yeast

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Analysis of chromosome folding in its natural state reveals a surprisingly compact chromatin structure for transcriptionally competent chromatin in live yeast cells. Using optimized *in situ* hybridization and live imaging techniques we determine compaction ratios and fiber flexibility for interphase chromatin in budding yeast. Unlike previous studies, we examine non-repetitive chromatin at intervals short enough to be meaningful for yeast chromosomes and functional domains in higher eukaryotes. We reconcile high resolution FISH data from intervals of 14- to 100-kb along single chromatids with measurements of whole chromosome arms (122 to 623 kb in length), monitored in intact cells through the targeted binding of bacterial repressors fused to GFP-derivatives. The results are interpreted with a flexible polymer model, and argue that interphase chromatin exists in a compact higher-order conformation with a persistence length of 170-220 nm and a mass density of 110-150 bp/nm. This is equivalent to 7-10 nucleosomes per 11 nm turn within a 30 nm-like fiber structure. In addition we show that chromatin fiber extension is influenced by nuclear geometry. Long-range chromosome organization is known to influence nuclear function. Budding yeast centromeres cluster near the spindle pole body, while telomeres are grouped in 5-8 perinuclear foci. Using live microscopy, we examine the relative positions of right and left telomeres of several yeast chromosomes. Integrated lac and tet operator arrays are visualized by their respective repressor fused to CFP and YFP in interphase yeast cells. The two ends of chromosomes 3 and 6 interact significantly but transiently, forming whole chromosome loops. For chromosomes 5 and 14, end-to-end interaction is less frequent, yet telomeres are closer to each other than to the centromere, suggesting that yeast chromosomes fold in a Rabl-like conformation. Mutations that disrupt telomere anchoring significantly compromise contact between two linked telomeres, but do not eliminate coordinated movement of telomere pairs. Dynamic coordination may result from both the properties of the chromatin fiber and a territorial chromosome organization.

Ligand binding to biomolecules explored by molecular simulation

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Molecular dynamics and QM/MM simulations provide very valuable insights on the interactions between drugs and their pharmaceutical targets. Here I will provide a survey of selected test cases (including membrane receptors and protein/protein complexes) which will show the power and the limitations of such techniques for the description of structural, dynamic and energetic properties of ligand/target adducts. The talk will also emphasize the role of modern tools of structural bioinformatics in molecular simulation studies.

Cooperativity Principles in Protein Folding: Desolvation is a Likely Origin of Robust Enthalpic Barriers

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Seemingly mundane phenomena in protein folding such as calorimetric cooperativity and linear chevron plots are recognized as nontrivial heteropolymer properties capable of providing fundamental insights into protein energetics. This presentation focuses on experimental data from global analyses of temperature (T) and denaturant dependence of the folding rates of small proteins that led us to an intrinsic enthalpic folding barrier hypothesis: To a good approximation, the T-dependence of folding rate under constant native stability conditions is Arrhenius. Furthermore, for a given protein, the slope of isostability folding rate versus $1/T$ is essentially independent of native stability. This hypothesis implies a simple relationship between chevron and Eyring plots of folding. By re-analyzing experimental data in the literature, we verify the predicted chevron-Eyring relationship for 14 proteins and determine their intrinsic enthalpic folding barriers, which vary approximately from 15 to 40 kcal/mol for different proteins. But these enthalpic barriers do not appear to correlate with folding rates. Intrinsic enthalpic barriers with similarly high magntidues apply as well to at least two cases of peptide-peptide and peptide-protein association, suggesting that these barriers are a hallmark of certain general and fundamental kinetic processes during folding and binding. Using a class of simplified continuum protein chain models, we show that small microscopic pairwise desolvation barriers — which are a direct consequence of the particulate nature of water — can act cooperatively to give rise to a significant overall enthalpic barrier to folding. This theoretical finding provides a physical rationalization for the high intrinsic enthalpic barriers in protein folding energetics. Ramifications for the landscape picture of folding are discussed.

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Functional dynamics of PDZ binding domains: a normal mode analysis

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PDZ (Post-synaptic density-95/discs large/zonula occludens-1) domains are relatively small (80 to 120 residues) protein binding modules central in the organization of receptor clusters and in the association of cellular proteins. Their main function is to bind C-terminals of selected proteins that are recognized through specific amino-acids in their carboxyl end. Binding is associated with a deformation of the PDZ native structure and is responsible for dynamical changes in regions not in direct contact with the target. We investigate how this deformation is related to the harmonic dynamics of the PDZ structure and show that one low-frequency collective normal mode, characterized by the concerted movements of different secondary structures, is involved in the binding process. Our results suggest that even minimal structural changes are responsible of communication between distant regions of the protein, in agreement with recent Nuclear Magnetic Resonance (NMR) experiments. Thus PDZ domains are a very clear example of how collective normal modes are able to characterize the relation between function and dynamics of proteins, and to provide indications on the precursors of binding/unbounding events.

Force-clamp studies of the folding/unfolding pathways of single proteins

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Due to the force of gravity and the need of living organisms to perform mechanical work, mechanical stretching is most likely to have played a role in the evolution of proteins. By contrast, the large changes in temperature or chemical denaturants commonly employed in protein folding studies are not found in living cells. Furthermore, chemical or thermal denaturation experiments typically define folding through changes in fluorescence of a tryptophan residue or FRET pairs. While such measurements provide accurate kinetic information, they do not reveal to what degree the folding proteins have recovered their native form.

We use force-clamp spectroscopy to follow the end-to-end length of the small protein ubiquitin during its folding reaction, at the single molecule level. Ubiquitin is first unfolded and extended at a high force, and then the stretching force is quenched resulting in a complete folding trajectory. We verify that the protein regained its native folded state by pulling again and confirming that the protein had regained its full mechanical stability. A significant advantage of the force-quench experiments is that a mechanically stretched and unfolded protein begins its folding trajectory from a well-defined state where the polypeptide is extended to the point of losing its secondary structure. Another significant advantage is that these are true single molecule measurements where the folding/unfolding trajectories are observed in individual proteins. Both the unfolding as well as the folding pathways captured by force-clamp spectroscopy are complex and very different from the simplified two state model that is commonly used to interpret such data in classical protein biochemistry. Our results point to the necessity of using statistical mechanics and single molecule thermodynamics to fully describe the extension and folding of proteins under a stretching force.

We anticipate that force-quench studies at the single protein level will become a method of choice to probe the dynamic equilibration of proteins folding against a stretching force.

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How small proteins fold

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The phage lambda switch: a case study (in biology and TPM methodology)

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After infecting a host bacterium, the bacterio-phage lambda, has alternative fates: lysogeny or lysis. In one case, viral DNA is reproduced with each cell division in a latent manner and proliferates as do the bacterium progeny, while in the other case, viral progeny are generated and the host bacterium susequently lyses releasing them. The genetic switch at the basis of this regulation involves the formation of a nucleo-protein complex of DNA and dimers of the lambda repressor that changes the overall conformation of DNA over a span of about 2400 bp. Using the tethered particle motion technique and magnetic tweezers we have characterized this conformational change. In order to do, so we have developed a novel method of analysis for TPM data that will be described.

Solvation effects and the topology-dependent folding rates of proteins

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Abstract

We study the solvation effects on the folding kinetics of proteins by means of Monte Carlo simulations on the three-dimensional cubic lattice. The proteins are modeled as self-avoiding chains with the pairwise Go interactions, in which attractions are assigned only to the native contacts. A solvation energy is given when an amino acid makes no contact with the other amino acids, i.e. when it is fully exposed to the solvent. We show that this kind of interaction, when the solvation energy is sufficiently *negative*, significantly enhances the correlation between the folding rates and the relative contact order parameter. Our results suggest that the origins of the topological determinant of the folding rates, as well as the cooperative character of the folding transition, well pertain to interaction with solvent.

Replication origin usage in the frog Xenopus: single-molecule studies on the DNA replication random completion problem.

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Eukaryotic DNA replication initiates at multiple replication origins. Initiation events are established in two steps. Before S phase, pre-replicative complexes (pre-RCs) are assembled on chromatin by loading of ORC, Cdc6, Cdt1 and MCM2-7 protein factors. During S-phase, pre-RCs are activated by two kinases, Cdc7 and the S-CDKs, that trigger DNA unwinding and assembly of active replication forks.

In Xenopus early embryos and egg extracts, S phase is very rapid (20 min) and replication initiates at ~10 kb intervals but at random with respect to DNA sequence. This raises a paradox, named the random completion problem. Given the velocity of replication forks (0.5 kb/min), two forks emanating from a single origin cannot replicate more than 20 kb of DNA within S phase. A completely random distribution of replication origins with a mean spacing of 10 kb should generate a geometric distribution of interorigin distances with a significant fraction (~0.1) of unacceptably large (>20 kb) distances, preventing replication completion in due time. One potential solution is that origins occur at random with respect to DNA sequences but are not assembled at random distances from each other. Another possibility is that much more potential origins are assembled than are actually used but that origin choice during S phase ensures a sufficiently regular pattern of initiation.

In previous works, we have studied the distribution of initiation events on single DNA molecules of plasmid and sperm nuclei replicating in egg extracts, using electron microscopy and DNA combing. We found that i) origins fire throughout S phase and at broadly distributed, not strictly regular intervals; ii) the frequency of initiation (number of initiation events per time unit per length unit of unreplicated DNA) raises strongly as S phase progresses, suggesting that potential origins are abundant and form a reservoir of yet unfired origins available at any time in S phase; it appears that multiple MCM complexes spread and can initiate replication over a large region around each ORC. Third, we observed origin interference : the firing of an origin prevents other origins from firing too close to it. Both origin interference and the increasing frequency of initiation could regularize the spacing of initiations and speed up replication completion, but the mechanistic basis remained unclear.

Here, we demonstrate that the cell cycle checkpoint kinase ATR regulates the frequency of origin firing. ATR is activated by already existing forks and downregulates origin firing. When ATR is artificially inhibited by caffeine or by specific antibodies more forks are established but they tend to stall. These results indicates a crucial role for ATR in the normal coordination and stability of replicons. ATR adapts the frequency of initiation to the number and progression rate of already existing forks, ensuring an optimum replication rate throughout S phase. The maintenance of a relatively constant number of forks despite the dwindling of unreplicated DNA and ongoing replicon merge results in the observed increase in the frequency of initiation through S phase. The high ATR activity in the vicinity of existing forks may also explain origin interference.

THE COHESIVE FLUCTUATION DYNAMICS OF PROTEINS

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Representation of molecular structures as networks of interacting elements has proven fruitful for simulating the large domain motions of large structures, which are not computationally accessible with molecular dynamics. Normal modes are obtained as the eigenmodes of the connectivity matrix. By representing proteins as a coarse-grained uniform block of material, utilizing only one representative point per residue and connecting the close residues to one another with identical springs, the normal modes of motion can be obtained. Here, the largest scale motions, which are the most important, should be the most reliable since these depend principally on the overall shape. This simple model reproduces crystallographic B factors, and when there are two different forms of the protein, a combination of the slow modes of motion corresponds closely to the transition. In a number of cases the level of detail required to reproduce the slowest motions is well below 1 point per residue. We are finding always that 1 point per 10 residues and often 1 point per 40 residues is sufficient, because the shape is the feature that is most important in determining these motions. Applications have been made to several large systems including the ribosome, where the ratchet motion between the two subunits is seen, and the details of the translation of the tRNA and mRNA can be seen. - See movies at http://ribosome.bb.iastate.edu/70SnKmode/ . A mixed coarse grained approach has also been developed.

Properties of the free energy barriers for protein folding

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The properties of free energy barriers for protein folding reactions can be probed using rate-equilibrium free energy relationships (REFERs). The most commonly applied REFERs are the use of denaturants (chevron plots) or mutations (φ-value analysis) an analyze their effect of folding kinetics and stability. Analysis of results from a large number of proteins suggested that folding proceeds over a small number of consecutive barriers with structural well-defined transition states (1). Data for the effect from other perturbations on protein folding are scarce. For tendamistat, which was shown to fold via two consecutive transition states and a high energy intermediate, we analyzed the properties of transition barriers by varying pressure and temperature. In contrast to results from denaturant-dependence, REFERs derived from these perturbations indicate Hammond behavior, which is more pronounced for the early transition state compared to the late transition state. This indicates that the barriers become narrower along the folding pathway. Further results on the properties of the folding free energy barriers will be presented that address the role of intermediates and the existence of parallel folding pathways.

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Single-Molecule Visualization of Protein-DNA Interactions

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Breaks in either one or both strands of double-stranded DNA can be repaired by a variety of recombination-dependent replicational repair processes. The biochemical mechanism of this process, and its visualization at the single-molecule level, will be discussed.

In *E. coli*, at least 25 different proteins are involved in homologous recombination: these include the RecA, RecBCD, RecF, RecG, RecJ, RecN, RecO, RecQ, RecR, RuvAB, RuvC, PriA, and SSB proteins; DNA polymerases; DNA topoisomerases; and DNA ligase, as well as the *cis*-acting recombination hotspot χ (5'-GCTGGTGG-3'). The RecBCD-pathway acts on DSBs, whereas the RecF-pathway can act to repair both DSBs and SSGs. In *S. cerevisiae*, recombinational repair of broken dsDNA requires coordination of the proteins defined by the *RAD52*-epistasis group: minimally, Mre11, Rad50, Rad51, Rad52, Rad54, Rad55, Rad57, Rad59, RPA, SGS1, SRS2 and Xrs2. These proteins also have counterparts in human cells.

We can visualize steps of this process at the single-molecule level. Individual molecules of the bipolar RecBCD helicase can be seen acting on single molecules of DNA. Detection involves optical trapping of fluorescently-tagged dsDNA attached to a polystyrene bead. Translocation is monitored by displacement of fluorescent dye from the DNA, using fluorescence microscopy. The enzyme is regulated by the DNA sequence, χ, which is recognized during translocation. We could see that RecBCD paused precisely at χ. More unexpectedly, after pausing, the enzyme continued but at about half of its initial rate. We proposed that interaction with χ uncouples one of the two motor subunits from the enzyme to produce the slower translocase. Thus, χ is a molecular throttle that controls translocation by RecBCD enzyme.

To test the long-standing hypothesis that uncoupling one of the motor subunits of the RecBCD helicase results from dissociation of the RecD motor upon recognition of χ, we tagged the RecD subunit with a fluorescent nanoparticle. Using this approach, we could visualize directly the movement of single molecules of RecBCD enzymes at speeds of 1,800 bp/sec. Individual enzymes were seen to both pause at χ and change translocation velocity, yet they retained the RecD motor. Therefore, we conclude that the RecD motor subunit remains an integral part of the translocating enzyme beyond χ, and that control of both enzymatic activity and biological function is likely mediated via conformational changes elicited by the continued binding of χ-containing ssDNA to the heterotrimeric RecBCD enzyme, as visualized previously.

Recent progress visualizing other biochemical steps of recombination at the single-molecule level will be discussed.

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Computer simulation of rare events in biological systems

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The realistic simulation of systems of biophysical interest is one of the biggest challenges in computational chemistry. The energy landscape of a biopolymer is full of local minima and the dynamics on this landscape is characterized by several time scales, ranging from the 10^{-14} seconds of the bond vibrations to the tenths of seconds associated to major rearrangements, with no clear gap in between. We recently introduced a method that seems to provide a manner to cope with this complexity[1–6], greatly enhancing the possibility to observe rare events in a short computational time. The method, that we called "metadynamics", is based on the construction of a coarse-grained non-Markovian dynamics in the space defined by a few collective coordinates. The dynamics is biased by a history-dependent potential term that, in time, fills the minima in the free energy surface, allowing the efficient exploration and the accurate determination of the free energy surface.

We will discuss the issue of accuracy control and of the optimal choice of metadynamics parameters for a given available computational time.

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DNA ejection from T5 bacteriophage under osmotic pressure

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Parametrizations of Coarse-Grain Sequence-Dependent Models of DNA Mechanics

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I will describe ongoing efforts to effect the passage from all atom Molecular Dynamic (MD) simulations of DNA fragments, which are of necessity still short both in duration of simulation and length of oligomer, to coarser grain sequence-dependent models involving rigid base, rigid base-pair, and continuum descriptions. These coarser grain models in principle allow quantitative, sequence-dependent modelling of experiments on DNA involving several tens to a few hundreds of base pairs, e.g. cyclization rates of minicircles, but detailed parametrizations of the energies in these models must be passed up from a finer resolution description.

Modelling biopolymers in a cellular context: physical aspects of DNA replication, transcription and translation

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Biopolymers in living organisms often attain structures which are markedly distinct from their *in vitro* counterparts. Quite often these have evolved as they function in a better way, and they may have been selected by taking advantage of particular phenomena which are unique to the *in vivo* situation. Here we discuss two examples taken from the cellular cycle in which we feel this has been the case.

First we discuss spatial genome organisation during replication and transcription. There is now convincing evidence that in both pro- and eu-karyots replication and transcription do not take place homogeneously in the cell or in the nucleus, but are localised in transcription and replication foci or factories, which span several nm in size. The DNA may then be spatially organised into loops which attach to these factories, bringing distant genes together so that they can bind to a local concentration of polymerases. Here we show that a simple physical mechanism based on the entropy depletion effect and relying on the molecular crowding which is found in living cells may naturally lead to the formation of factories and consequently of DNA looping. We discuss when this looping is stable or transient.

Second, we consider the dynamics of protein translation, when a nascent polypeptide is synthesised at a tracking ribosome. Although it is usually accepted that the three dimensional folded state of a protein is a consequence of equilibrium alone, here we give evidence that a non-equilibrium effect might be at work during the very first stage of folding, favouring the preferential selection of helices while the protein is in the ribosomal exit tunnel. Indeed we show that a growing self-interacting string attached to a tracking origin, modeled to resemble nascent polypeptides *in vivo*, develops helical structures which are more pronounced at the growing end. This is in line with stereochemical considerations and with a statistical analysis of native states of proteins which shows that there is a slightly larger helical propensity at the C-terminus, the growing end of a nascent protein.

A unified physics perspective on proteins

Amos Maritan

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Biophysics of the bacterial nucleoid

Theo Odijk

Complex Fluids Theory, Delft University of Technology

The nucleoid of Escherichia coli consists of supercoiled, chromosomal DNA which is crosslinked by various proteins attached to it like HU and H-NS. In the bacterial compartment it is a compact structure though it is not densely packed. A depletion theory of its compaction by cytoplasmic proteins is presented which is corroborated by microscopy experiments in which the isolated nucleoid is reduced in size by adding inert polymer. Dynamic light scattering, sedimentation and fluorescent microscopy experiments are interpreted theoretically.

Multi-scale Modeling of DNA

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The hierarchy of DNA structures, ranging from the chemical architecture of the nucleotide repeating unit to the large-scale folding and dynamics of many thousands of base pairs packaged in chromatin, calls for a hierarchy of molecular models. The presentation will highlight some of our efforts to model nucleic acids at different levels of resolution, focusing on the sequence-dependent properties of the double helix. We make use of the arrangements of neighboring base pairs and the positioning of ligands around the constituent nucleotides (A, T, G, C) in high-resolution nucleic acid structures to deduce knowledge-based potentials of sequence-dependent structure, deformations and interaction. We are currently using these functions in simulations of spontaneous DNA loop closure and in the evaluation of the nucleosomal binding affinities of DNA fragments of arbitrary sequence.

Using supercoiling sensitive genes to study topological domains in *Escherichia coli*

Lisa Postow, Brian J. Peter, Javier Arsuaga, Christine D. Hardy, Adam M. Breier, Arkady B. Khodursky, Patrick O. Brown, and Nicholas R. Cozzarelli
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The *Escherichia coli* chromosome is maintained in a negatively supercoiled state, which is required for viability. Since just one single- or double-strand break would release this negative supercoiling, it is protected through the organization of the chromosome into topological domains. These loops of DNA are thought to organize and compact the chromosome throughout DNA replication, chromosome segregation, and repair. Estimates for average *E. coli* domain length in the literature have ranged from 20 to 200 kb, depending on the method of study used. There is also evidence for the existence of topological domains in eukaryotic chromosomes.

To study topological domains throughout the genome in vivo, we generated probes to gauge relaxation in the living cell. Using microarrays of cDNAs corresponding to nearly all genes in the *E. coli* genome, we identified 306 genes that are either induced or repressed when the chromosome loses its negative supercoiling due to drugs or a mutation in the gene for the topoisomerase DNA gyrase. Of these genes, 106 were induced upon relaxation and 200 were repressed. Using these genes as local topological probes, we quantitatively measured the spread of relaxation from double-strand breaks generated in vivo, and calculated distance to the nearest domain boundary. In a complementary approach, we gently isolated chromosomes and examined the lengths of individual supercoiled loops by electron microscopy. Finally, we used these results to test various models of domain organization. We conclude that domain barriers are not placed stably at fixed sites on the chromosome, but instead appear to be randomly distributed. In addition, we find that domains are smaller than previously reported, about 10 kb on average. These results have implications for bacterial chromosome organization and compaction.

Phase transitions in DNA

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The Two Aspects of the Protein Folding Problem

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There are actually two aspects of the theoretical approach to the protein folding problem. The first is to compute the thermodynamically stable native structure, and the second is to compute the folding pathways from the unfolded to the folded native form. I will discuss the evolution of computational methodology from an all-atom representation of the polypeptide chain to a united-residue representation of the chain. Blind tests in successive CASP exercises demonstrate increasing prediction success, in computing protein structure, from one CASP test to another. As for folding pathways, two different methods are used: (1) a stochastic difference equation procedure, and (2) Lagrangian dynamics with the united-residue force field. The results of all the computations, and the methods leading to them will be discussed.

Barrier Crossing by a long chain molecule

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Escape of a particle over barrier in one or more dimensions is a well-understood problem. We consider the generalization of this to a long chain molecule, using the Rouse model for the chain. The chain is initially confined to the meta-stable side of a biased double well potential. It can escape from this side to the other by the motion of its N segments over the barrier. For a very long molecule, there are two steps in such an escape process. The first is the overcoming of the barrier by a portion of the polymer. It is found that the activation energy for this does not depend on the length of the polymer. The second is the crossing of the remaining parts of the molecule, once a portion has overcome the barrier. We argue that this takes place by a kink mechanism and that the time required for the crossing is proportional to N. We also discuss the applicability of the mechanism to the recent experiments and consider the translocation of hydrophilic polypeptides across hydrophobic pores, a process that is quite common in biological systems. Biological systems accomplish this by having a hydrophobic signal sequence at the end that goes in first. We find that for such a molecule, the transition state resembles a hook, and this is in agreement with presently accepted view in cell biology.

We also discuss briefly other rate processes in which a long chain molecule is involved. These are (a) opening of a weak link in a semi-flexible polymer (b) transport of a protein through the nuclear pore complex.

Heterogeneous Biochemistry

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The field of chemistry is commonly divided into two parts, namely homogeneous and heterogeneous chemistry. Homogeneous chemistry deals with the study of chemical processes taking place in the bulk of a homogeneous phase, such as a gaseous, a liquid or a solid phase. Heterogeneous chemistry is concerned with the investigation of chemical processes that involve more than one phase. In a similar way one can define a homogeneous and a heterogeneous biochemistry. Because water plays a central role in biochemistry, the scope of homogeneous biochemistry can be narrowed down to the study of biochemical processes taking place in the bulk of a homogeneous aqueous phase. The goal of this presentation will be to define and to explore the field of heterogeneous biochemistry. This is a broad field, as indicated for instance by the list of the states of matter (which can give rise to macroscopic phases) that we need to consider: 1) Solid states (amorphous or crystalline); 2) Liquid states (isotropic or anisotropic (mesomorphous): liquid crystals, amphiphiles); 3) Gaseous States and 4) Polymers. The following issues will be addressed:

1) *Historical background.* In contrast with the field of heterogeneous chemistry for which there exists a rich literature, the field of heterogeneous biochemistry has never been reviewed. We shall briefly recall the history of homogeneous biochemistry, starting from Buchner's solution of the Liebig-Pasteur controversy, and try to explain why it has become a central method in modern biochemistry. We shall also sketch the history of heterogeneous biochemistry, which is already discussed in the writings of Arrhenius and Bayliss. This latter history turns out to be much more intricate than the history of homogeneous biochemistry. Heterogeneous biochemistry includes for instance membrane biochemistry (a field which has been lucidly reviewed by Mitchell), but it is in no way limited to that topic.

2) *Merits and limitations of homogeneous and heterogeneous biochemistry.* Homogeneous and heterogeneous approaches of biochemistry are associated with a theoretical framework and an experimental methodology. We shall review the merits and the limitations of the two approaches: our main conclusion is that homogeneous biochemistry has strong limitations, and that there is a need to develop a heterogeneous biochemistry, both from a theoretical and an experimental perspective.

3) *Economics of heterogeneity.* Why does Nature make such an extensive use of heterogeneous systems in living organisms? A plausible hypothesis is that such systems are more efficient than homogeneous systems and have been selected for that reason during evolution. To illustrate the efficiency of heterogeneous biochemistry, we shall explain how one can increase the yield or the rate of a biochemical reaction in heterogeneous systems. The basic ideas here have been introduced by van't Hoff and Smoluchowski, and applied recently to nucleic acid biochemistry (for DNA cyclization and DNA renaturation).

Pulling a nanostring with a nanomotor: The RuvAB-DNA interaction

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The genomes of all organisms are constantly subject to insult by mutagens, UV light as well as by metabolism intermediates. Evolution has given rise to elaborate mechanisms to repair or bypass this damage, and thus enable DNA replication and cell division. As part of these mechanisms, the cell creates DNA intermediates with two homologous tracts, having a cruciform structure, called Holliday junctions. Bypass of a lesion on one of the strands entails the directed motion of the junction (branch migration), enlargening two arms of the junction at the expense of the two others. In a bacterial cell, branch migration is carried out by the RuvAB motor complex as part of the SOS genetic network of DNA repair. I present results of single-molecule experiments in which we have characterized branch migration induced by a single motor complex on a single Holliday junction. We attach one of the arms of a junction to a surface, while the opposite arm tethers a paramagnetic bead on which a force is exerted by an external pair of magnets. The motion of the bead is followed in real time through an optical microscope, as the RuvAB complex consumes energy in biological currency units of ATP. These experiments open a new window to understand the inner workings of this natural molecular motor.

Structure and Assembly of Viral Capsids based on Tiling Theory

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A vital constituent of a virus is its protein shell, called the viral capsid, that encapsulates and hence protects the viral genome. The capsids of a large number of viruses are icosahedrally symmetric, and a combination of group theory and tiling theory can thus be used to model their surface structures and predict the locations of both the protein subunits and the inter-subunit bonds.

The approach presented here generalises Caspar-Klug Theory, which is a key concept in virology for the classification and analysis of viral capsids but is not applicable to a significant number of medically important viruses. For example, the cancer causing viruses in the family of Papovaviridae fall out of the remit of Caspar-Klug Theory, but can be described within the framework of our approach. In particular, the theory presented here solves a structural puzzle concerning the surface structure of the (pseudo-) T=7 particles in the family of Papovaviridae. Moreover, it is shown in this talk that our tiling approach leads to a classification of the tubular malformations that can occur during assembly of the major capsid proteins, and accounts for the crosslinking structures that lend particular stability to the capsids.

In view of anti-viral drug design it is important to understand the assembly process of viral capsids. Since our approach predicts besides the locations of the protein subunits also the locations of the intersubunit bonds, it can be used as a basis for the construction of assembly models. This will be demonstrated for SV40, a member of the family of Papovaviridae, and the relative concentrations of the assembly intermediates are determined for assembly under equilibrium conditions. Moreover, the dominant assembly path is identified, and it is discussed how this information may be exploited in the framework of anti-viral drug design.

Supercoiled minicircle DNA as a tool to probe DNA structure and enzyme-DNA interactions

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Lk is the number of times that the two DNA strands of a double helix are interwound. In all known living cells *Lk* is lower than Lk_0 (the energetically favored *Lk*). This results in a negative value for the specific linking difference, σ ($\sigma = [(Lk-Lk_0)/Lk_0]$), which is commonly referred to as DNA negative supercoiling. DNA supercoiling influences all proteins that act on DNA except the DNA cutting restriction endnucleases, which were thought previously to be independent. The study of DNA supercoiling, and thus enzymes that act on DNA, has been hampered severely by a lack of biophysically manipulable supercoiled DNA.

Using the λ-integrase (Int) site-specific recombination system in *E. coli*, we have produced milligram quantities of minicircle DNA substrates with well-defined (from $\sigma = 0$ (relaxed) to $\sigma = -0.2$) DNA supercoil density. In this system, recombination between the two Int sites on the same high-copy plasmid results in Int-mediated conversion of the plasmid into two linked rings: a large ring and a minicircle. Recombination strongly depended upon the length between the Int sites. No recombination took place when the smallest length was ≤217 bp, suggesting that DNA site synapsis in *E. coli* requires greater flexibility than 217 bp will allow.

Normally, linked DNA circles are decatenated by the action of topoisomerase IV, resulting in loss of the small ring. Inhibition of topoisomerase IV with a fluoroquinolone traps the linked DNA. We have optimized this system for minicircle DNA in the size range of a few hundred base pairs. The DNA yields are more than two orders of magnitude greater than previously obtainable.

As determined by polyacrylamide gel electrophoresis and atomic force microscopy, >90% of the minicircles adopt a single topoisomer distribution. This is as opposed to a Gaussian spread in the large plasmids studied previously. Because they are so small, the minicircles can be used in quantitative gel shift and real-time enzyme kinetic experiments.

We first used these minicircles to revisit the question of the influence of supercoiling on restriction enzymes. In contrast to previous work with large plasmid DNAs, we find that the restriction endonuclease, EcoRV, is affected up to 10-fold by DNA supercoiling. Thus, now it can be concluded that all DNA acting enzymes are influenced by DNA supercoiling. These results call to question previous protein binding/catalysis results carried out with linear DNA substrates.

ABSTRACTS OF POSTERS

(in alphabetical order of author name)

Structural Modelling of Cyclic Nuclotide Activated Channel C-Terminal Domain

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Ion permeation is crucial for a variety of biological functions such as nervous signal transmission and osmotic regulation. Many human and animal diseases are also associated to defects in ionic channels function, the majority of them arising from mutations in the genes encoding the channel proteins. A lot of effort is still necessary to connect these mutations to the structural and functional changes causing the disorder.

Ionic channels are proteins inserted in the membrane lipid bilayer, by forming aqueous pores through the cell membrane. They allow ions to cross the hydrophobic barrier of the core membrane, guarantying to the cell a controlled exchange of ionized particles.

Different kinds of ionic channels are present in nature, differing in functional, gating and selectivity properties.

CNG channels are voltage independent, nonselective ion channels that are gated by the direct binding of cyclic nucleotides.

Native cyclic nucleotide gated (CNG) channels are heterotetramers formed by homologous subunits, generally referred to as α and β subunits. β homotetramers are functionally inactive, whereas α homotetramers form functional channels, although with distinct features with respect to native channels. In particular, α homomeric channels show currents characterized by longer openings with respect to $\alpha+\beta$ channels, which on the contrary show a flickering activity. We present two possible three-dimensional models for the tetrameric C-linker and CNBD regions for CNGA1 homotetrameric channels and generalized the results to $\alpha+\beta$ heterotetrameric and β homotetrameric channels. These models were constructed using the HCN2 crystallographic structure as a template for the C-linker domain, and either HCN2 or the CAP protein for the CNBDs. Our modeling shows that α homotetramers present stronger interactions between adjacent cyclic nucleotide binding domains, with respect to $\alpha+\beta$ channels, due to an extended loop containing K554, which forms an additional salt bridge with D588. Models of $\alpha+\beta$ heterotetramer channels have less pronounced interactions between adjacent binding domains, possibly contributing to the flickering nature of the heteromeric CNG channels.

S. cerevisae DNA toposiomerase II participates in sister chromatid junction turnover

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Physical junctions between newly replicated DNA molecules have been proposed to contribute to chromatid cohesion until chromosome segregation at anaphase. Replication dependent sister chromatid X-shaped joint molecules can be detected by two-dimensional gel electrophoresis at active origin containing regions upon replication initiation. These joint structures are able to branch migrate after replication forks and distribute along the replicon. Their formation does not require Rad51/Rad52 mediated homologous recombination events and is independent of DNA damage or replication blocks. Genetic and biochemical evidence suggests these molecules represent hemicatenate joints, rather than recombinative intermediates, that might tether sister chromatids together priming cohesion and facilitating sister chromatid mediated recombination and replication events. Additionally, these structures seem to be converted into pathological structures at replication forks in checkpoint defective cells and to participate in the replication of damaged templates by contributing to template switching an error-free replication bypass DNA lesions.

In hemicatenane structure one strand of a duplex crosses trough the other duplex creating a topological link between both DNA molecules. For these reason DNA topoisomerases are good candidates for participating in hemicatenane joints formation, migration and/or resolution. DNA toposiomerases are enzymes which catalyze trasnsient breaks in DNA strands allowing them to pass trough each other. This common mechanism allows DNA topoisomerases to regulate supercoiling state of DNA in different chromatin transactions such as DNA replication and transcription. Furthermore topoisomerases resolve knots and catenation araising in replicated chromosomes, which is needed for proper chromosome segregation in mitosis.

In *S. cerevisiae* exist two type I topoisomerase enzymes, top1 and top3 - which allow one strand passage at a time- and one type II homodimeric enzyme top2, that catalyzes transient breaks in both strands and represents the main decatenating activity. Top1 is tough to act as a replication swivel by removing positive supercoil ahead of the fork. Top2 and top1 function in removing torsional stress during DNA replication seems to overlap, as only top1top2 double mutants fail complete elongation step. Additionally top2 is required for chromatin condensation and for chromosome segregation. Top2 mutants fail to untangle their chromosomes prior to anaphase leading to genetic instability.

In order to analyze topological nature of joint structures we analyzed X-molecule dynamics at replication origins by two-dimensional gel electrophoresis in DNA topoisomerase defective strains. Our results show that X-molecule abundance is increased in top2-1 ts mutant at origin containing and adjacent regions, suggesting that topoisomerase2 activity might either counteract hemicatenane formation or participate in their resolution.

cAMP binds to the cytoplasmatic domain of HCN channels: insight from molecular dynamics simulations

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The Hyperpolarization-activated Cyclic Nucleotide-modulated cationic channels family includes in vertebrates four members (HCN1-4) involved in cardiac and neuronal pacemaker activity. They are opened by membrane hyperpolarization, while their activation is enhanced by the binding on the intracellular side of cyclic Adenosine MonoPhosphate (cAMP).

Here we investigate the molecular basis of cAMP channel modulation by performing molecular dynamics simulations of C-linker and Cyclic Nucleotide Binding Domain (CNBD) in the presence and absence of cAMP, based on the crystal structure of HCN2 from mouse [1].

We observe that the absence of ligand triggers conformational rearrangements within the CNBDs, driving this domain to a more flexible state, similarly to what described in CNBDs of other proteins [2–5]. This increased flexibility causes a rather disordered movement of the CNBDs, resulting in an inhibitory effect on the channel. In contrast, the presence of cAMP allows for a more compact and rigid configuration of the CNBDs. Moreover, this structure undergoes a harmonic oscillation of the quaternary structure in the ~12 ns timescale, which is not observed in the apoprotein. We propose that this large-scale oscillation plays an important role for channel's function. In fact, this oscillation might be coupled to a motion of the C-linker which in turn affects channel gating.

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A thorough comparison of the mechanism of action of serine proteases and aspartic proteases from molecular simulation

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Nature is believed to use different strategies to perform enzymatic reactions either by stabilizing the transition state (TS) of the reaction or by increasing the free energy of the ground state. Regarding the first, a fundamental hypothesis focuses on the existence of a preorganized electrostatic field in the active site, which stabilizes the transition state(s) of the reaction. Another proposal suggests that, in some specific cases, large scale fluctuations of the complex enhance the probability of a conformation of the active site and substrate favourable for the reaction. In this work, we perform a comparative study on two classes of hydrolases, which have been proposed to work in different manners. The first concernes serine proteases class,which are believed to work through the electrostatic stabilization of the TS. The second is the aspartic proteases (AP's), for which conformational fluctuations have been suggested to play a role. Here we provide a comparison of the simulations on these enzymes with exactly the same computational setup, classical molecular dynamics and hybrid DFT/MM calculations. This will allows us to investigate similarites and differences of structural, dynamical and electronic features, which in turn are related to the biological function. For the serine proteases, we focus on furin, a member of Subtilisin family which plays a crucial role in Alzheimer disease, and is therefore considered a relevant target for farmaceutical inhibition.For the AP's, we focus on two members of the proteins, HIV-1 PR, from human immunodeficiency virus, and human BACE, another pharmacological target for Alzheimer's disease.

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All-atom folding simulations of Small Proteins from stochastically-selected coarse-grained structures

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We discuss the application of a novel simple and efficient protocol for the theoretical study of the folding of single domain proteins from the only knowledge of primary sequence. Our approach is based on the combination of a *Monte Carlo (MC) coarse-grained conformational* search with *all-atom molecular dynamics (MD) simulations in explicit solvent*. The MC search simplifies the protein's energy landscape to identify efficiently viable starting conformations for MD. A general fine-graining algorithm is then used to reconstruct the full atomic detail of the protein. All atom MD simulations in explicit water are employed to investigate the protein's conformational evolution towards the native state. We discuss the application of this novel approach to the *Villin headpiece*, a widely used test system for folding studies, for which we obtain and maintain an *RMS deviation from the NMR structure of 2.4 Å for the core region and 4.0 Å for the whole protein*.[1,2] Finally, the analysis of the MC-MD trajectories provides insight into the dynamical evolution and docking of locally formed secondary structure elements.

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TOWARDS A UNIFIED MODEL OF THE FOLDING PROCESS OF HELICAL PROTEINS

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Finding simplified but informative models of protein folding is a major challenge facing theoreticians who are trying to manage the complexity of biomolecules. Such models are expected to possess the following major requisites: (a) sufficient specificity for sequence details without using representations at atomic level which are too demanding even for computer assisted investigations; (b) capability to describe dynamical events on time scales comparable with the folding time of the protein. The foldon diffusion-collision model (FDC model) (1,2) meets these requisites since it focuses on the minimal determinants of folding (foldons) and is an effective tool for calculating the kinetics of folding of helical proteins. The FDC model relies on a neural network to determine position and stability of foldons starting from the protein sequence. The brownian dynamics of foldons is then studied in the framework of a stochastic approach. The FDC model was tested successfully on two-state (2) and three-state (3) proteins covering a gamut of different folding mechanisms with different degree of cooperativity and modularity.

Here we summarize the results obtained so far, stressing the sensitivity of the model to the smallest possible variations in the sequence of the protein (point mutations) (4). The FDC model follows effectively the changes in the folding rate of most of the mutants examined in this work. The general conclusion is that foldons are the critical target for mutations that are intended to affect the kinetics of the folding process. Foldons can be likened to the accelerator pedals of the protein in that mutation in these regions are likely to elicit the maximal effect. The most remarkable effects are due to the death of a foldon or the birth of a new foldon.

The FDC model provides a physical interpretation of the effects of the mutations. Beside this, it turns out to be a good candidate for a unifying model of the folding of helical proteins, that is effective in describing folding processes ruled by cooperative or hierarchical mechanisms. The FDC model suggests how to build up an index of cooperativity which is useful to characterize the different modularity of the folding processes so far examined in the present work and in our previous studies (1,2,3).

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TITLE:

Preliminary studies on Apoflavodoxin with a simple Gaussian Network model.

AUTHORS:

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ABSTRACT:

We apply the Gaussian Network Model introduced by Micheletti and coworkers (PRL 87, 088102 (2001)) to the study of Apoflavodoxin, a protein that shows a three-state thermodynamic behavior under thermal denaturation, and a two-state equilibrium under chemical denaturation.
The thermal intermediate has been extensively investigated, mainly by J. Sancho's research group at BIFI, in a number of single and double-mutant experiments. Our ultimate goal is to provide some handy theoretical tool that may help rationalize the experimental results and help designing new experiments: in this perspective, the Gaussian Network Model, which is amenable of analytic solution within a self-consistent approximation, represent a very promising candidate.
Experiments suggest that the intermediate of Apoflavodoxin presents an overall structured core, with just a part of the protein being substantially unfolded: in this study, we investigate the effect that contact weakening has on the predicted thermodynamics.

Physical and genetic characterization of replication-dependent sister chromatid junctions.

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Using the two-dimensional gel electrophoresis technique we have identified X-shaped sister chromatid junctions that appear during chromosome replication at the origins. These DNA structures resemble hemicatenanes (DNA-junctions in which one strand of a duplex is wound around one strand of another duplex) and have been suggested to play a role in coupling replication with sister chromatid cohesion and in sister chromatid-mediated recombination and replication bypass processes. These joint molecules are able to branch migrate chasing replication forks and, importantly, their formation is not dependent upon recombination pathways.
Using the yeast *Saccharomyces cerevisiae* as an experimental system I'm currently trying to elucidate the molecular structure of these molecules with physical approaches. Moreover, I'm involved in searching for the genetic pathways implicated in the formation and migration of the sister chromatid junctions.

Scaling exponents and probability distributions of irreversibly adsorbed DNA

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The end-to-end distance (correlation length) exponent ν for long linear DNA molecules was determined by direct measurement of the average end-to-end distance as a function of the contour length s by means of atomic force microscopy (AFM). Linear DNA, up to 48'502 base pairs (bp), was irreversibly deposited from a solution onto silanized mica and imaged in air. Under the adsorption conditions used, the DNA is trapped onto the surface without any two-dimensional equilibration. The measured exponent is $\nu = 0.589 \pm 0.006$, in agreement with the theoretical 3D value of $\nu = 0.5880 \pm 0.0010$. The persistence length ℓ_p of DNA was estimated to be 44±3 nm, in agreement with the literature values. The distribution of the end-to-end distances for a given contour length s and the exponents characterizing the distribution were determined for different s. For s smaller or comparable to ℓ_p, a delta function like distribution was observed, while for larger s, a probability distribution of the type $x^{d-1}x^g e^{-bx^\delta}$ was observed with $g = 0.33 \pm 0.22$ and $\delta = 2.58 \pm 0.76$. These values are compared to the theoretical exponents for Self-Avoiding Walk (SAW): namely $g = \frac{\gamma-1}{\nu}$ and $\delta = (1-\nu)^{-1}$. So for $d = 2$, $g \approx 0.44$ and $\delta = 4$, while for $d = 3$, $g \approx 0.33$ and $\delta \approx 2.5$. The present data indicate that the DNA behaves on large length scales like a 3 dimensional SAW.

Calmodulin: dynamics and target recognition by molecular simulations

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Calmodulin is the most general calcium-signal translator protein, playing a keyrole for pathways dependent on variations of Ca^{2+} concentration (calcium "sparks" and "waves"). Here we use molecular dynamics and history-dependent metadynamics approaches to address fundamental issues about the protein flexibility and the binding to calmodulin's target peptides. The flexibility of calmodulin's interdomain linker, predicted by NMR data but not observed in many X-ray structures, is reproduced in MD simulation by spontaneous unwinding (G. Fiorin *et al.*, Proteins: Str., Func. and Bioinf., *in press*).
The complex of calmodulin with a target peptide is also studied by metadynamics as part of a separate project, and new conformations are observed which well correlate with experimental data. This work is done in collaboration with the group of prof. Michele Parrinello at ETHZ (Zurich, Switzerland).

Force induced triple point for interacting polymers

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Abstract

We show the existence of a force induced triple point in an interacting polymer problem that allows two zero-force thermal phase transitions. The phase diagrams for two different models of mutually attracting but self avoiding polymers are presented. One of these models has an intermediate phase and it shows a triple point. A general phase diagram with multicritical points in an extended parameter space is also discussed.

Conformational changes of DNA induced by the eukaryotic transcriptional factor NFY using single molecule microscopy

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NFY is a ubiquitary eukaryotic transcriptional factor which binds with high affinity to the CCAAT box on DNA. NFY is a heterotrimer constituted by two histon-like subunits and by a third subunit necessary for the association of the previous two and for DNA binding. Experimental evidence indicates that NFY, upon specific binding, may bend DNA by approximately 62-82°. Such a distortion of the double helix may permit transcriptional activation by other factors such as RFX and, in so doing, explain the strategic role of NFY in transcriptional regulation. Therefore, using two different single molecule techniques, namely AFM and the tethered particle motion (TPM), we studied the specific versus non-specific binding of NFY to DNA and we have characterized both the bending of the double helix due to one NFY molecule, and the concentration-dependent condensation of DNA due to non specific binding of multiple NFY molecules. Furthermore, we have investigated the interaction between NFY and RFX.

Proteomics-based Phylogenetic Studies on Lysosomal Hydrolases and Lysosomal Membrane Proteins

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Lysosomal storage disorders are a family of heritable disorders caused by the functional deficiency of lysosomal enzymes or proteins needed to degrade macromolecular substrates in the lysosome or to transport the monomeric products of degradation out of the lysosome. These questions bear on understanding molecular basis of lysosomal storage diseases resulting from defects of transporter proteins. We have employed a proteomics based approach in current investigation. The proteomic data allows for a design of antibodies to be raised in hens and collected from eggs. The goal was to prepare and characterize hen antibodies for studies of novel lysosomal membrane proteins needed to examine novel proteins even ahead of the identification of their function. The work was thus of 3 parts: (a) Constructing plasmids encoding cDNAs for Lysosomal and membrane proteins (b)Raising antibodies IgYs in hen using the DNA constructs and (c) studying biosynthesis, processing and transport of the novel proteins using the hen antibodies.

Purification of Control Antibodies (IgY) of hen origin has been standardized in our lab. cDNAs against various membrane transporters have been prepared by the German group. We have cloned the available cDNAs in 2 vectors, pCI and pcDNA3 both of which have the CMV promoter One cDNA of interest has been obtained from German Cancer Research Center (Deutsche Krebs Forschung Zentrum) and been made by Dr S. Wiemann. There is a collaborative agreement in force between Prof. Hasilik and Dr. Wiemann about the cDNA clone which Dr Wiemann generated at the DKFG.

Two out of the three vectors made by us, that is pcDNA3, pCI and pMCI have been used for expression studies in Human HEK 293 cells. We have done transfection experiments on cultured human HEK 293 cells separately with CFP(Cyan variant mutant of Green Fluorescent Protein) and YFP(Yellow variant mutant of GFP) and studied immunofluorescence using anti-Cathepsin D Ig (as control lysosomal visualization) and anti-GFP Ig (Santa Cruz Biotech Co. USA) to localize the CFP- and YFP-fusion protein. The transfection seems to be at a lower rate, say 5-10 % and later we got impovement upto 30%. We require atleast 50 % transfection efficiency to improve the is needed. We have evaluated the expression of the fusion protein, in 2 metabolic labeling experiments with the transfected HK 293 cells from the same batches in parallel. The recombinant protein was immunoprecipitated from cell lysates indicating that the cDNA for hypothetical membrane protein is indeed made and processed by the cells and is a real one. More experiments are going on in this direction.

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Single Molecule Studies of F_1-ATPase with γ-Subunit Truncations

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Motor proteins are nanometer-scale biological machines that transport cargo at the microscopic level within the biological cells. They use ATP as fuels and provide chemical energy that is finally transduced to mechanical energy. In order to understand the molecular basis of life it is necessary to unravel the underlying physical principles of these tiny natural machines. The bio molecules in the cell may show heterogeneous behavior due to various local environments or conformational states so that the best way of understanding is to study their behavior at single molecule level because in this case one can directly observe the behavior of individual molecules but only average behavior can be realized if the experiment is done over the ensemble. A single molecule of F_1-ATPase is a rotary motor in which the γ subunit rotates with respect to $\alpha_3\beta_3$ hexamer during hydrolysis of ATP. In the structure of mitochondrial F_1-ATPase (MF_1) that is superimposable with $\alpha_3\beta_3$ hexamer derived from thermophillic bacteria PS3, carboxyl (C) terminus of the γ subunit forms an α helix which fits into the hydrophobic bearing formed by the loops of $\alpha_3\beta_3$ ring. The last amino acid residues at the C-terminus of the γ subunit were suggested to play important roles. This has inspired us to study the effect of successive truncation of C-terminal amino acid residues of γ subunit of the $\alpha_3\beta_3\gamma$ complex of F_1-ATPase from thermophillic bacillus PS3 on the rotation and hydrolysis activities. The hydrolysis activity at different MgATP concentrations (2000-0.02 μM) was measured for both wild type and mutant complexes in the presence of an ATP regenerating system using a thermostatted spectrophotometer at 23 ^{0}C. The measurement reveals that hydrolysis activity decreases to 27-63% for truncation up to the 14th residues and 17-67 % up to 17th residues compared to the intact complex. Rotation of single molecules of F_1 was observed under a microscope using 0.49 μm bead duplex as a marker in the same range of ATP. It has been found that the wild type and the 14th deleted mutant generate 40 pN-nm torque while the 17th deleted mutant produces 30 pN-nm at all ATP concentrations.

Complete Phase Diagram of DNA Unzipping

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During DNA replication and RNA transcription, the double stranded DNA (dsDNA) opens up due to the force exerted by enzymes. In the case of replication this opening takes place near one of the ends, whereas for transcription it can be anywhere on the DNA[1, 2, 3]. The opening of the dsDNA by force is a cooperative phenomenon and known in the literature by the name of unzipping transition.

We study the unzipping of dsDNA by applying a pulling force at a fraction s ($0 \leq s \leq 1$) from the anchored end. The phase diagram is obtained in both the fixed force and fixed distance ensemble by using analytical and exact transfer matrix. The phase diagram is insensitive to the ensemble when the pulling force is applied at the end ($s = 1$). In contrast, the phase diagram shows strong ensemble dependence for other s. We show the existence of an eye phase which, depending on the ensemble used and the value of s, resembles with the Y-fork in replication and the transcription bubble. A triple point also exists at the intersection of three phase boundaries in fixed force ensemble[4].

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Modelling of Odorant Receptors: A Combined Bioinformatic and Simulation Approach

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Odorant receptors (ORs) constitute the largest gene family in the genome and belong to G-protein coupled receptors (GPCRs) superfamily. ORs are involved in the recognition and discrimination of numerous odorants: single OR may recognize several odorants and at the same time a single odorant may be recognized by several ORs. Unfortunately, no experimental structural data are available.

Here we used homology modelling technique in order to construct the 3D-model of mouse I7 odorant receptor based on the X-ray structure of bovine rhodopsin (Palczewski et al., Science 289 (2000) 739; Okada et al., J.Mol.Biol. 342 (2004) 571), which, although it shares low homology with all ORs, it is the only GPCR structure determined so far. Our model has been validated against mutagenesis experiment on the similar (42% of identity) mouse OR. Our calculations suggest that the binding pocket is located roughly at the center in the helical bundle, much farer from the extracellular region than in the previous proposals (Singer, Chem. Senses 25 (2000) 155; Hall et al., Chem. Senses 29 (2004) 595). The calculations provide also a functional role for a key Lys residue (Lys 164), which was proposed previously to bind OR ligands.

This work is done in collaboration with Prof. A. Menini.

HOMOLOGY MODELLING OF LARGE CONDUCTANCE CA^{2+}-AND VOLTAGE- GATED POTASSIUM CHANNEL

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Large conductance Ca^{2+}- and voltage- gated potassium channels (BK channels) are widely expressed in mammalian tissues and are necessary for normal physiological functions as muscle contractions and neurotransmitter release. Due to their ubiquitous involving in many processes, they are important target for treatment several cardiovascular, respiratory, neurological and urological diseases. BK channel is a tetramer of four identical units, each formed by seven membrane spanning helices and cytoplasmic C-terminal domains.

Our main aim is to construct the structural models of pore region in the transmembrane domain and the intracellular C-terminal domains (RCK domains - **R**egulate the **C**onductance of $\mathbf{K}^{+}$).

We used a two-step approach. First we used homology modelling to construct the models. Pore region was modelled on KcsA and MthK templates, RCK domains on E.coli and MthK C-terminal domains. Due to low sequence homology (22% and 26% for pore region, 21% and 19% for C-terminal domains) we then included all available mutational studies. Channel blockers such as Tetraethylammonium and Leiurus Quinquestriatus (Lq2, scorpion neurotoxin) are being performed and the results compared with the experimental data.

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Statistical mechanics of coil-hairpin transition in a single stranded DNA oligomer

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Abstract

A model of self-avoiding walk with suitable constraints on self-attraction is developed to describe the conformational behavior of a short RNA or a single stranded DNA molecule that forms hairpin structure and calculate the properties associated with coil-hairpin transition by enumerating all possible conformations of a chain of N monomers in two and three dimensions. The first and last five monomers of the chain have been allowed to pair and form the stem of the hairpin structure while the remaining monomers can form a loop. The coil-hairpin transition is found to be first order with large entropy change. While the rate of unzipping of the hairpin stem is found to be independent of the length of the loop and the dimensionality of the space, the rate of closing varies greatly with loop length and dimensionality of the space.

Chirality in Protein Folding

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There are several simple criteria of folding to a native state in model proteins. One of them involves crossing of a threshold value of the RMSD distance away from the native state. Another checks whether all native contacts are established, i.e. whether the interacting amino acids come closer than some characteristic distance. We use Go-like models of proteins and show that such simple criteria may prompt one to declare folding even though fragments of the resulting conformations have a wrong sense of chirality. We propose that a better condition of folding should augment the simple criteria with the requirement that most of the local values of the chirality should be nearly native. The kinetic discrepancy between the simple and compound criteria can be substantially reduced in the Go-like models by providing the Hamiltonian with a term which favors native values of the local chirality. We study the effects of this term as a function of its amplitude and compare it to other models such as with the side groups and with the angle-dependent potentials.

Conformational Preferences of the Plant Polypeptide Hormone Systemin

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Abstract

Systemin is the first polypeptide hormone isolated from plants. This 18 amino acid long peptide is supposed to bind to a specific membrane-bound receptor kinase and activate wound response in plants. Biochemical evidence points to its N-terminal 14 residues as important for binding and the C-terminal 4 residues for activation. A substitution of Pro at position 13 with Ala nearly abolishes its function. We have looked at the energy landscape and conformational preferences of systemin using replica exchange and simulated annealing molecular dynamics method and found conformational clustering which might explain the loss of function due to substitution of Pro at position 13.

Anisotropic mechanical properties of grafted microtubules

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Microtubules are stiff biopolymer filaments, formed by aggregation of tubulin dimers in a complex macromolecular architecture. The characterization of their elastic response at the single filament level is fundamental in order to understand the properties of the cytoskeleton, a complex network of protein filaments that is responsible for cell mechanics and locomotion. We use single-particle tracking to study the elastic properties of single microtubules grafted to a substrate. Thermal fluctuations of the free microtubule's end are recorded, in order to measure position distribution functions from which we calculate the persistence length of microtubules with contour lengths between 2.6 and 48 μm. We find the persistence length to vary by more than a factor of 20 over the total range of contour lengths. Our results support the hypothesis that shearing between protofilaments contributes significantly to the mechanics of microtubules. We also provide a first insight into how microtubules get their unique mechanical properties from a clever design of anisotropic molecular interactions.

Adsorption of Protein GlnB-Hs of *Herbaspirilum seropedicae* on Si (111) investigated by AFM and XPS.

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The protein GlnB-HS (GlnB of *Herbaspirillum seropedicae*) in diazotroph microorganisms signalizes levels of nitrogen, carbon and energy for a series of proteins that converts atmospheric nitrogen in ammonia, resulting in biological nitrogen fixation. Its structure was already determined by x-ray diffraction, revealing a trimer of (36kDa) with lateral cavities having hydrophilic boundaries. The subject of this investigation is the interaction of GlnB-Hs with Si (111) surface at different incubation times, protein concentration in initial solution, deposition conditions and substrate initial state. The protein solution was deposited on Si (111) and dried under controlled conditions. Atomic Force Microscope (AFM) operating in dynamic mode shows images of donut and filament types of organization, which vary in size from a few nanometers to micrometer sizes, depending on initial deposition conditions. The filament formation seems to be favored by the protein surface polarity when in contact with the silicon surface, following some specific orientation. The spin coating technique together with AFM and XPS analysis were successfully used to obtain a more uniform surface covering.

Modeling Truncated Hemoglobin vibrational dynamics

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We present a study on the near equilibrium dynamics of two small proteins in the family of truncated hemoglobins, developed under the framework of a Gaussian network approach. Effective beta carbon atoms are taken into account besides C^{α}s for all residues but glycines in the coarse-graining procedure, without leading to an increase in the degrees of freedom (βGaussian Model). Normalized covariance matrix and deformation along slowest modes with collective character are analyzed, pointing out anti-correlations between functionally relevant sites for the proteins under study. In particular we underline the functional motions of an extended tunnel-cavity system running inside the protein matrix, which provide a pathway for small ligands binding with the iron in the heme group. We give a rough estimate of the order of magnitude of the relaxation times of the slowest two overdamped modes and compare results with previous studies on globins.

Light atomic nuclei quantum mechanical effects for systems of biological interest

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There is a relevant amount of experimental data suggesting that effects due to the quantum mechanical nature of light atomic nuclei are relevant for systems of biological interest, and in particular in enzymatic reactions [1-3]. We developed a new algorithm in order to calculate and parametrize full dimensional potential energy surfaces from *ab initio* quantum chemical calculations for floppy molecular systems. Our algorithm could model systems containing up to 40/50 atoms. We have been implementing a new code based on the Path Integral Quantum Monte Carlo (PIMC) to calculate observables of physical-chemical interest taking into acccount quantum mechanical effects. We present here the initial results of our calculations for some small prototypical floppy molecular systems. The present approach will be employed to model some important enzymatic reaction's active sites.

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Protein Coarse-Grained Model with an United Atom Description of the Functional Active Region

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A new approach for exploring the free energy landscape of proteins near their native state is developed. The methology combines the advantages of coarse grained models, which usually only consider the C_α atoms of the backbone, and those of all-atoms descriptions, needed in simulating functional processes. The goal is to describe biologically-relevant events, occuring on time scales order of magnitude larger that those accessible by conventional molecular dynamics. The comparison between calculated structural properties with those obtained with MD simulations establishes the method accuracy.

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Inferring the diameter of a biopolymer from its stretching response

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Abstract

We investigate the stretching response of a thick polymer model by means of extensive stochastic simulations. The computational results are synthesized in an analytic expression that characterizes how the force versus elongation curve depends on the polymer structural parameters: its thickness and granularity (spacing of the monomers). The expression is used to analyze experimental data for the stretching of various different types of biopolymers: polypeptides, polysaccharides and nucleic acids. Besides recovering elastic parameters (such as the persistence length) that are consistent with those obtained from standard entropic models, the approach allows to extract viable estimates for the polymers diameter and granularity. This shows that the basic structural polymer features have such a profound impact on the elastic behaviour that they can be recovered with the sole input of stretching measurements.

Production Dynamics of Extracellular Protease from *Bacillus macerans* IKBM – 11, *B. licheniformis* IKBL – 17 and *B. subtilis* IKBS – 10

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ABSTRACT

Screening and isolation of proteolytic bacteria were carried out from soil samples of Ikogosi warm spring (SW, Nigeria). Eighteen isolates were positive on skim milk agar (10%) of which fifteen produced protease in culture broth. Three isolates identified as *Bacillus macerans* IKBM – 11, *Bacillus licheniformis* IKBL – 17 and *Bacillus subtilis* IKBS – 10 were selected for further study. These *Bacillus* species could grow up to 65°C within a broad pH range of 5.0 – 10.0 with an optimal growth temperature and pH at 60°C and 8.0 respectively. Protease production from *B. macerans* IKBM – 11, *B. licheniformis* IKBL – 17 and *B. subtilis* IKBS – 10 was investigated at different temperatures and pH. For the three *Bacillus* species, protease production occurred between 37°C and 65°C and pH 5.0 – 10.0. Maximum growth and maximum enzyme production was observed at 48 hours when grown in 50ml medium (pH 8.0) under shaking condition at 60°C. The results showed that *Bacillus* species under study are good producers of extracellular protease at high temperature. This might be an indication that protease produced would be thermostable. Proteases produced from the three *Bacillus* species are currently being characterized.

Keywords: Protease, proteolytic bacteria, *Bacillus macerans*, *B. licheniformis*, *B. subtilis*

Model Study of DNA Photoreactivation: Photosensitized Cleavage of Thymine Dimer Through Rapid Electron Transfer

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Abstract Irradiation of DNA with UV-B light causes the formation of mutagenic DNA lesions such as cis-syn cyclobutane pyrimidine dimers or 6-4 product. DNA photolyases are flavin-dependent repair enzymes which directly revert the mutagenic cyclobutane pyrimidine dimers into the corresponding monomers by a light-facilitated repair reaction[1,2]. In model study of photoreactivation mechanism, it is found that the splitting reaction of thymine dimer could occur by a one-electron oxidation mechanism involving appropriate oxidants such as sulfate radical anion, OH radical and excited anthraquinone[3,4]. In this work, the oxidative splitting reaction of cyclobutane dimethylpyrimidine dimer (DMTD) by excited triplet state of anthraquinone-2-sulfonate was investigated using nanosecond laser flash photolysis technique and steady-state analysis. The cycloreversion process yields the anion radical of AQS and corresponding cation radical of DMT. Triplet AQS could be quenched drastically by added DMTD and the decay kinetics of triplet AQS fit well the pseudo-first-order kinetics. The quenching rate constant of triplet AQS by DMTD was determined to be 3.53×10^9 $M^{-1}s^{-1}$, which is close to the diffusion-controlled limit in water. The yields of DMT and splitting efficiencies of DMTD were measured. Recently, the splitting processes of DMTD were also examined by using molecular fluorescence technique. Several new sensitizers with strong donating/accepting abilities were introduced. k_{sv} values of these quenching processes were obtained according to Stern-Volmer equation. The splitting mechanisms of these reactions will be further investigated by time-resolved spectroscopic technique and ab initio calculation.

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Unfolding and stretching dynamics of biopolymer's simple models

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The object of our studies is the Langevin dynamics of a simple polymer model, the so-called PH model (Polar-Hydrophobic), with different contributions to the total force field. Using Principal Components Analysis (PCA[1,2]) we get information on the essential dynamics of the model. Specifically, we analyze the dependence of the effective frequencies of the system on temperature or external force. In this way we characterize the different structures in which the polymer evolves as well as the transitions between them.

Our 2D polymer suffers the following monomer interactions, where $r_{i,j} = |\vec{r}_i - \vec{r}_j|$ and $i, j = 1, ..., N$:

$$V_1(r_{i,i+1}) = \frac{1}{2}K(r_{i,i+1} - r_0)^2$$

$$V_2(\theta_i) = \frac{1 - \cos\theta_i}{16} \qquad \cos\theta_i = \frac{(\vec{r}_i - \vec{r}_{i-1}) \cdot (\vec{r}_{i+1} - \vec{r}_i)}{r_{i,i-1} r_{i+1,i}}$$

$$V_3(r_{i,j}) = \frac{1}{r_{i,j}^{12}} - \frac{c_{i,j}}{r_{i,j}^{6}}$$

$$c_{i,j} = \frac{1}{2}(2 - 3\xi_i - 3\xi_j + 5\xi i \xi j) \qquad |i - j| > 1$$

V_3 simulates the effective interaction among H and P monomers in the presence of a solvent. Thereby, an heteropolymer is defined with a sequence of $\xi_i = 0$ (hydrophobic monomer) or $\xi_i = 1$ (polar monomer). The result is an attractive interaction when both monomers are P or H ($C_{i,j} = 1/2$ or $C_{i,j} = 1$, respectively); and a repulsive interaction in the case of different monomers ($C_{i,j} = -1/2$).

The Langevin equations are integrated by a Runge-Kutta in the Greenside-Helfand version. It let our polymer be in contact with a stochastic thermal reservoir at temperature T:

$$\dot{\vec{q}}_i(t) = \vec{p}_i \qquad \dot{\vec{p}}_i(t) = -\nabla H - \gamma \vec{p}_i + \vec{\eta}_i(t)$$

$$\langle \vec{\eta}_i(t) \rangle = \vec{0} \qquad \langle \eta_{z,i}(t) \eta_{z,j}(0) \rangle = 2\gamma K_B T \delta(t) \delta_{i,j} \qquad z=x,y$$

Along the dynamics we compute the covariance matrix ($2N \times 2N$) C. We get the principal modes (eigenvectors) and the related mean square fluctuations (eigenvalues λ_k). These will be used to define a set of effective frequencies (ω_k):

$$C_{i,j} = \Big\langle \big(q_i(t) - \langle q_i(t) \rangle_t\big)\big(q_j(t) - \langle q_j(t) \rangle_t\big) \Big\rangle_t$$

$$R^T C R = diag(\lambda_1, \lambda_2, ..., \lambda_{2N}) \qquad \omega_k = \sqrt{\frac{K_B T}{\lambda_k}}$$

The sequence [0001000100011001 1000][3] has been identified as "good folder", converging below it's transition temperature to a 'native' structure. Looking at the behaviour when it is stretched by a constant force, we find the system jumping among some different metastable minima. With PCA we studie those transitions.

In the figure the effective frequencies of a $\xi_i = 0\ \forall i$ sequence are shown for different temperatures. At low enough temperature a gap appears and the polymer behaves rigid (importance of V_3). The modes softend when we rise the temperature, now V_2 plays an important role. At high enough temperatures the system unfolds. The frequency model dependence looks like the Rouse model (gaussian chain), just V_1 is relevant.

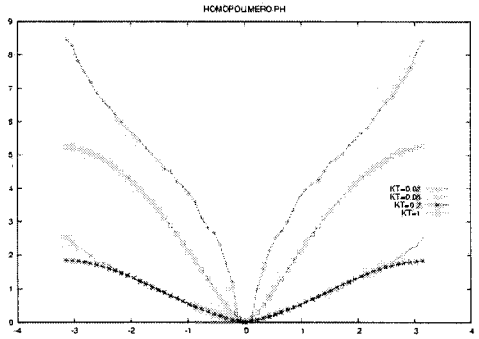

FIG. 1. Effective frequencies (Y) versus principal modes (X) for different temperatures

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Three Bead Rotating Chain model shows universality in the stretching of proteins

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A new model of proteins is introduced by inclusion of all key atoms of the protein backbone in the Freely Rotating Chain model. This model has more details than Freely Rotating Chain (FRC), but less than Four bead model. We used the average bond lengths and angles from Protein DataBank (PDB) as input parameter so the number of residues is the only variable. The model is used to study the stretching of proteins in the entropic regime and we observed that the results of our Monte Carlo simulations fitted well the experiments which suggests that the force extension plot is universal and does not depend on the side chains or primary structure of proteins.

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Mechanism of action of the CphA, a novel metallo β-lactamase.

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Resistance to β-lactam antibiotics is a major issuen in the treatment of bacterial infectious processes. The expression of β-lactamases is one of the most efficient mechanism for resistance developed by bacteria. In the last decade, there has been a growing concern on the dissemination of genes coding for a relatively recent type of beta-lactamases, the metallo-beta-lactamases (MBL's). MBL's are zinc-dependent enzymes which split in three subclasses B1, B2 and B3. Detailed X-ray structures are available for some members of the B1 and B3 classes. These families of MBLs can act by binding either one or two Zn(II) ions (mono and bizinc form of the MBLs) and they can hydrolyze structurally different substrates, including the newest generation of beta-lactam antibiotics. In contrast, B2 subclass members are only active in the monozinc form and have a remarkably greater specificity. Despite the interest in these enzymes due to the different substrate repertoire and presumably different mechanism of action, no structural data were available until now.

Hybrid Car-Parrinello QM/MM calculations together with classical molecular dynamics tools we have undertake new insights on the reaction mechanism of the hydrolysis of antibiotics promoted by a MBL. We will focus on a novel B2 class MBL, the CphA enzyme from Aeromonas hydrophila which has been specialized almost exclusively as carbapenemase. In addition, this is the first MBL for which the X-ray structure has been determined with an antibiotic molecule trapped in the active site (Garau, G., Bebrone, C., Anne, C., Galleni, M., Frere, J. M., Dideberg, O.: A Metallo-Beta-Lactamase Enzyme in Action: Crystal Structures of the Monozinc Carbapenemase Cpha and its Complex with Biapenem J.Mol.Biol. 345 pp. 785 2005). It is also the first structure of any enzyme belonging to the B2 class MBLs. The work is done in collaboration with Prof. A. Vila, from University of Rosario, Argentina.

Force Induced Unzipping of DNA

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Abstract

We have discussed the unzipping transition under the influence of external force on a dsDNA molecule. The critical force $F_c(T)$ for unzipping calculated in the constant force ensemble is found to depend on the potential parameter k which measures the stiffness associated with a single strand of DNA and on D, the well depth of the on-site potential representing the strength of hydrogen bonds in a base pair. The dependence on temperature of $F_c(T)$ is found to be $(T_D - T)^{1/2}$ (T_D being the thermal denaturation temperature) with $F_c(T_D) = 0$ and $F_c(0) = 2kD$. When a constant extension ensemble has been used the calculated average force $F(y)$ that is needed to keep one of the ends of the two strands of DNA at a fixed distance y apart is found to have a very large value for $y \approx 0.2$ Å compared to the critical force found from the constant force ensemble in all cases investigated by us. It is shown that the value of $F(y)$ at the peak depends on the value of k which measures the energy barrier associated with the reduction in DNA strand rigidity as one passes from dsDNA to ssDNA. The effect of defects on the position and height of the peak in $F(y)$ curve is investigated by replacing some of the base pairs including the one being stretched by defect base pairs. On-site potential on a defect site is represented by a potential that has only a short-range repulsion and a flat part without well of the Morse potential. The formation and behaviour of a loop of Y shape when one of the ends base pair is stretched and a bubble of ssDNA with the shape of "an eye" when a base pair far from ends is stretched are investigated.

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Twisting Semiflexible Polymers At Low and High Tensions

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Abstract

We present a theoretical treatment of DNA stretching and twisting experiments. We discuss global topological subtleties of self avoiding ribbons and provide a better understanding of the Worm Like Rod Chain (WLRC) model proposed by Bouchiat and Mezard. We describe the phenomenon of "topological untwisting" and show how it is prevented in the WLRC model. Some theoretical points regarding the WLRC model are clarified: the writhe of open curves and the use of an adjustable cutoff parameter to "regularise" the model. Our treatment brings out the precise relation between the Worm Like Chain (WLC) and the WLRC models. We propose an experiment to probe the continuous transition between the two models.

On geometry of curved bundles of perfectly packed filaments

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Abstract

It is known that the densest packing of infinite straight cylinders is hexagonal when all their axes are parallel. It evidently corresponds to hexagonal packing of disks in a plane. The hexagonal packing of tubular objects occurs in numerous instances at nano to macro scale. Among examples there are nanotubes, high density columnar hexatic liquid crystalline DNA mesophases and others. In most cases, this packing extremizes the interaction energy between filaments. If the strands are not straight, then it is still possible to form a perfect bundle which must be twistless. A strict geometrical description is given for arrangement of filaments in a curved hexagonal packing.

Of particular interest are closed bundles like DNA toroids or spools. The closure or return constraints of the bundle result in an allowable group of automorphisms of the cross-sectional hexagonal lattice. The structure of this group is explored. A condition on the writhing number of filaments in the closed bundle is formulated. Examples of an open helical-like and closed toroidal-like bundles are presented. A possible implication on condensation of DNA in toroids and its packing inside a viral capsid is discussed.

Symmetry dependence of dynamical and thermodynamical behaviour of polypeptides

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Polypeptides are model compound for the study of proteins. Vibrational dynamics of polymeric systems and polypeptides in particular is an order of magnitude more complicated as compared with simple molecules. This is because of the vibrational phase relationship of neighbouring residues. In the present communication we report such dynamical and thermodynamical study of 2 fold (β sheet), 3 fold and 4 fold (ω helix) and α helical polypeptides. A close examination of the dispersion profiles of the lowest optical and acoustical modes shows that these profiles are very characteristic of their conformational symmetry. Thus it provides us with a simple tool predict the conformational states. The heat capacities of these polypeptides in different conformational states have been evaluated from the dispersion curves via density-of-states and agrees well with the experimental data.

Molecular recognition of DNA by anthramycin: insights from molecular dynamics

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The anticancer drug anthramycin exert its antitumoral activity by covalently binding to DNA, thus inhibiting replication and transcription process. We have studied the interactions between anthramycin and a 12-mer oligonucleotide by performing classical molecular dynamics (MD) simulations. We have simulated two non covalent adducts between the postulated pre-reactive forms of the drug and the oligonucleotide, along with the covalent complex. In addition, hybrid QM/MM calculations have been performed to investigate electronic properties of the complexes. The calculations are based on the X-ray structure of the anthramycin in complex with the oligonucleotide d(CCAACGTTG*G)d(CCAACGTTG*G) [1]. Our calculations suggest that: (i) only one form of the ligand (the so called hydroxy-form [2] lies in the vicinity of the reactive center for 20 ns, while the other form (called anhydro-form) moves to the nearest site within the minor groove; (ii) both hydroxy- and anhydro-anthramycin have the electronic characteristics of pre-reactive forms. Based on these results, we propose that the anhydro form bind aspecifically DNA and subsequently move within the minor groove towards a favourable reactive site (as already found for other anticancer drugs [3] whilst the other form can bind directly to its reactive site.

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Perturbed soliton excitations in DNA molecular chain

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Abstract

Deoxyribonucleic acid or DNA is a biological macromolecule which plays an important role in the conservation and transformation of the genetic information in biological systems. The DNA molecule is considered as a complex dynamical system having many degrees of freedom, many types of internal motions and specific distribution of internal forces. The nonlinear character of the internal DNA dynamics is of great interest in the recent times and few theoretical models have been proposed to describe the nonlinear properties of DNA. Important among them is the dynamic plane-base rotator model. We study the nonlinear dynamics of DNA double helix by considering the plane-base rotator model and by taking into account the energy associated in the twisting of base pair in the helical chain which is assumed to have inhomogeneity in the stacking energy of the chain. We consider a Hamiltonian which consists of inhomogeneous stacking energy, hydrogen bonding energy and anisotropic energy; Then we construct the dynamical equation in the continuum limit using Taylor series and we obtain the perturbed sine-Gordon equation. The kink and antikink soliton solutions of the sine-Gordon equation describe an open state in the DNA double helix. The perturbed terms which are proportional to the inhomogeneity in the stacking energy introduce fluctuation in the kink and antikink. This is understood by carrying out a multiple scale perturbation analysis. We observed that the inhomogeneity in the stacking modifies the shape of the soliton by a correction of linear dispersion and also undergoes slow time change of the soliton parameter.

Strength of DNA and RNA Watson-Crick base pairs: a computational NMR study

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The characterisation of the base pair H-bond properties is an important step to rationalise the physical properties of DNA and RNA. Deuterium isotope effects (DIE) on chemical shifts are indicators of hydrogen bond strength, where larger absolute values are indicative of stronger H-bonds. Very recent NMR experiments (Vakonakis and LiWang, 2004 *J. Am. Chem. Soc.* 126:5688; *J. Biomol. NMR* 29:65) on DNA and RNA duplexes have revealed trans-hydrogen bond DIE on the chemical shifts of carbon in adenosine:thymidine (A:T) and adenosine:uracil (A:U) base pairs. In particular, these experiments showed that: i) different base pair conformations may modulate the transmission of the isotope effect across the hydrogen bond; ii) hydrogen bonds of A:U base pairs of RNA are stronger than those of A:T base pairs of DNA.

We used classical and ab-initio molecular dynamics calculations to investigate which are the key factors governing the observed DIE. Specifically, long time scale classical molecular dynamics has been used to sample conformational preferences in selected DNA and RNA duplexes, while ab-initio calculations has been used to investigate the intrinsic interaction energy of A:T and A:U base pairs and to calculated the DIE on chemical shifts.

LOW-FREQUENCY DIELECTRIC SPECTROSCOPY OF AQUEOUS SOLUTIONS

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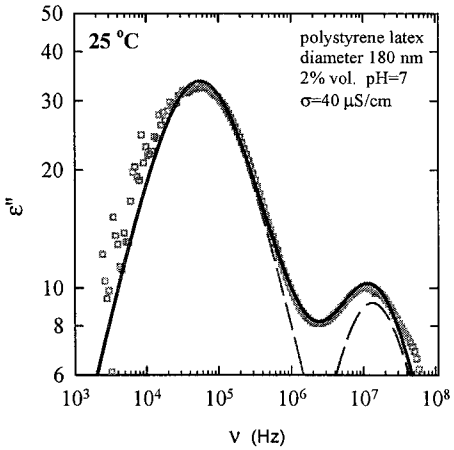
Electrical signaling and complex dynamics in soft-matter systems are of the fundamental interest in life-sciences and biotechnology. With the aim of investigating dielectric relaxation in charged systems, polyions and colloids, in aqueous environment of varying ionic strength and pH we employ the low-frequency dielectric spectroscopy (LFDS). LFDS is application specific and non-destructive technique allowing detection and quantification of polarization response of charged systems in polar and non-polar solvents. LFDS is also well established in the solid state, for investigations of the collective electronic response in the low-dimensional synthetic materials [1].

In order to apply LFDS we designed and built a chamber for measurements of complex conductivity of liquid (mostly aqueous) samples of small volume – 50-200 μL, with conductivity in the range of 2- 2000 μS/cm. Temperature stabilization is ±10 mK in the range of -20 - +60 °C. Reproducibility is 1.5% and the measured sample stability is achieved on the time scale of 2 hours. Measurements are performed by the precision impedance analyzer Agilent 4294A (range: 40 Hz - 110 MHz). However, the frequency range is limited by the electrode polarization effects, as well as by the parasitic impedances of the measurement circuit. Background subtraction methods and extensive test measurements established that the practical frequency range for detection of the dielectric modes is 0.5kHz-50 MHz.

The purpose of the present investigation was to carry out test LFDS measurements on monodispersed polystyrene latex particles suspended in low-ionic strength electrolytes. Colloidal stability of these particles is generated due to their high surface charge. This charge, along with the well-defined counterion atmosphere, leads to the well-determined polarization response, and furthermore, to the appearance of two characteristic dielectric modes, which are well-described by generalized Debye function. Accordingly, the low-frequency mode, in the kHz range, is related to the particle size, while the high-frequency one, in the MHz range, is related to the thickness of the double-layer and defined by the Debye-Hückel screening length of the electrolyte [2,3].



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DNA topological changes in the λ CI negative autoregulation

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After infection by bacteriophage λ, two possibile fates, reflecting alternative patterns of gene expression, await the bacterial host cell. Either the bacteriophage enters the lytic pathway, in which case the host cell ultimately lyses to release a burst of progeny phage, or it enters the lysogenic pathway, in which case the phage genome is stably integrated into the bacterial chromosome and normal bacteria growth continues.
The phage's ability to efficiently use these two modes of development is due to a complex gene regulatory mechanism known as "lambda's genetic switch".
The key regulator in the λ genetic switch is the CI protein, which binds as a dimer to the O_L and O_R regions of the λ genome to repress the lytic promoters during lysogeny.
Switching from lysogeny to lytic development, called prophage induction, occurs on activation of the host SOS system in response to DNA damage: RecA bound to ssDNA stimulates the self-cleavage of CI monomers, removing CI's ability to bind DNA. CI is also able of repressing the transcription of its own gene when present at high concentration. This negative autoregulation would act to limit CI concentration in the lysogenic state and permit an efficient switching into lytic development and is thought to be mediated by the formation of a DNA loop between the O_L and O_R control regions.
This mechanism of CI's negative autoregulation needs still to be proved and characterized. In particular, the contribution of the O_L operators in the formation of the loop between the O_L and O_R regions is not clear, and the kinetics and thermodinamics of this process have not been studied yet.
We performed Tethered Particle Motion measurements, first, to verify the CI induced loop formation between O_L and O_R, second, to analize the effect of the distance between the operators and third, to test the contribution of the different O_L operators in loop formation.
We recently started to implement TPM measurements with magnetic tweezers to further investigate the kinetics and thermodinamics of CI-mediated DNA loop formation and to determine the effect of DNA supercoiling on this process.

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