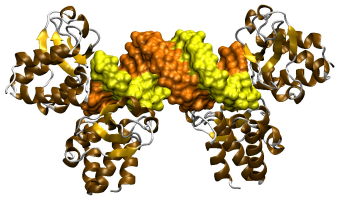
Dynamics of Protein-Nucleic Acid Interactions: Integrating Simulations with Experiments



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Summary:

Protein-nucleic acid interactions play a key role in numerous biological processes, especially those related to replication, transcription, and translation. The structural information about many protein-nucleic acid complexes has become available during the last decade, but detailed understanding of their dynamic features is still lacking. Understanding the dynamics of interactions of these complexes is crucial for the development of therapeutic approaches interfering with, e.g., viral life cycle or bacterial translation.

The aim of this workshop was to bring together scientists who perform experimental and/or computational studies on protein-nucleic acid systems to discuss the aspects of the dynamics of these systems and to increase the communication between different communities. Particular goals were to discuss how to integrate experimental and computational efforts that often involve different time scales and

resolutions and to exchange information about latest developments in computational methodology. Furthermore, we aimed to discuss a wide range of protein-nucleic acid complexes to develop a more general understanding of common biophysical principles and methodological approaches taken to understand such problems.

Description of the scientific content and discussion at the event

The program consisted of presentations discussing the experiments and simulations of nucleic acids, protein-RNA, and protein-DNA complexes. From the point of view of computational methodology a diverse set of techniques was covered including all-atomic molecular dynamics, Brownian dynamics, coarse-grained models, as well as normal mode analysis and Monte Carlo algorithms. The experimental techniques included NMR, X-ray crystallography, time-resolved hydroxyl radical footprinting, single molecule FRET studies, and cryo-EM. The talks allowed for extensive discussions of the merits of different computational methodologies.

The two macromolecular complexes that were given more attention in terms of the number of talks were the nucleosome – the basic unit of the chromatin, and the ribosome – the protein synthesis machinery. The first is the protein-DNA complex and the latter is an even larger protein-RNA complex. The ribosome dynamics was described by all-atom molecular dynamics simulations and included the efforts to simulate the movement of the tRNA through the ribosome. The dynamics of the entire ribosome was characterized by Karissa Sanbonmatsu and Hisashi Ishida. Also, Lennart Nilsson discussed how certain nucleotide modifications affect the tRNA codon-mRNA anticodon interactions. Holger Gohlke presented the dynamics of the ribosomal exit tunnel from the constraint counting on topological network approach. Sarah Woodson has shown how the 30S subunit self-assembles based on the hydroxyl radical footprinting studies and single molecule fluorescence spectroscopy. Her group found that the 30S proteins influence the rRNA interactions distant from their binding sites, as well as the hierarchy and rates of protein-induced conformational changes.

The talks on the DNA organization spanned a large spatiotemporal scale from the nucleosome to chromatin and chromosomes. The interactions of the core of the nucleosome with the linker-histone protein were discussed by Rebecca Wade. With the Brownian Dynamics technique her group identified the linker histone binding site on the nucleosome. Arya Gaurav, apart from studying the histone tail H4 – nucleosome interactions by all-atom molecular docking, has been developing a low-resolution lattice model of chromosomes. Michael Levitt developed a method to predict the effect of DNA methylation on nucleosome occupancy. The protocol is based on the crystal structure of the nucleosome and all-atom empirical potential energy functions. The histone-like nucleoid structuring protein responsible for the organization of DNA in bacteria was the topic of Jocelyne Vreede's talk. The experiments showed two conformations and with molecular dynamics simulations she aimed to elucidate which is the correct one.

Another system that was covered in detail was DNA mismatch repair. Dorothy Erie showed the results of single molecule fluorescence resonance energy transfer (FRET) studies showing the dynamics of the mismatch DNA upon MutS protein binding and also the changes in interactions of MutS and DNA during ATP binding and hydrolysis. Joyce Lebbink showed the SPR analysis and mass spectrometry used to elucidate the dynamics of MutS on DNA and how this dynamics activates the repair in a mismatch-dependent manner.

Protein interfaces that bind nucleic acids were analyzed in detail by Nilesh Banavali based on statistical information from the experimentally derived protein-nucleic acid complexes. These analyses showed base preference for certain amino acids. Also, the dynamics and free energy profiles of base flipping have been discussed. The recognition of a specific sequence between the protein and DNA is still not fully understood; Simone Furini presented the molecular dynamics approach to this problem based on two examples lactose repressor protein and the Cro repressor. The recognition is connected with the rates of the diffusion of proteins toward DNA. The problems of diffusion toward the DNA target, protein search, and protein movement along the DNA were presented by Anatoly Kolomeisky. NMR experiments give also invaluable insight into the recognition phenomenon by providing the solution structures of protein-nucleic acid complexes. Frederic Allain presented the

research on RNA binding proteins (RBP) that are involved in RNA alternative splicing and RNA editing. Interestingly, the structures of proteins containing the same RNA recognition motif showed different mode of recognition.

Other important proteins in RNA processing are the Sm proteins, evolutionarily conserved superfamily. Cameron Mura has shown the crystallographic and biochemical studies on the Sm protein - RNA complexes, performed in order to understand their structure, dynamics and function. Other systems that were presented were Dicer, an RNase III enzyme that cleaves double stranded RNAs and pre-micro RNAs into short double stranded siRNAs (Joanna Sarzyńska), archeal initiation factor IF2, a GTPase involved in translation (Thomas Simonson), aminoacyl-tRNA synthetase (Claude Sauter, Thomas Simonson), and topoisomerase I (Ioan Andricioaei).

The mechanics of DNA and DNA motion in protein-DNA complexes were also given attention. Molecular dynamics studies on the relaxation of DNA supercoil by topoisomerase were presented by Ioan Andricioaei, the unwinding of single stranded DNA from the ssDNA binding protein by Aleksei Aksimentiev.

Many interesting discussions took place. One was on the time scales that are available to study experimentally and computationally. It seems that there is still a large gap between the achieved experimental and computational time scales since molecular dynamics simulations can give the dynamics only up to a microsecond that is still orders of magnitude smaller than what can be observed in experiments.

The workshop included two poster sessions on the first and second day of the workshop. Fourteen posters were presented and there were discussions by the poster boards. The posters were displayed both by graduate students (PhD students) and post-doctoral researchers. About half of the posters presented the results of molecular dynamics simulations of nucleic acids or protein-nucleic-acid systems.

Assessment of the results and impact of the event on the future direction of the field

Binding of proteins to nucleic acids is a dynamic process, therefore, the methodology to predict the dynamics of nucleic acids and conformational changes upon protein binding were thoroughly discussed. The main outcome of the workshop was that even though the force fields used in the molecular dynamics simulations of nucleic acids have been improved, still tests are needed on longer than hundreds of nanoseconds time scales. The speakers showed the recent all-atomic force field improvements for nucleic acids both in Charmm and Amber. In case of Amber the parameters for the *chi* dihedral torsion have been changed in order to properly account for the anti and high-anti conformations of nucleotides (Thomas Cheatham, Jiri Sponer). In case of Charmm, Alexander MacKerell presented the force field optimization for RNA that includes proper treatment of the 2'OH. Since RNA can assume a wide range of non-canonical conformations, the force field issues are revealed mostly in the simulations of RNA systems. Pascal Auffinger has shown that the parameters for some ions fail in case of high ionic strength. Overall, the strengths and weaknesses of the two most common force fields Amber and Charmm were discussed. The future directions in improving the force fields must focus on the data obtained for longer molecular dynamics simulations. Also, the work nowadays focuses on the development of force fields for proteins and DNA or RNA separately but the question still unsolved is which force field to use in atomic simulations of protein-nucleic acid complexes. There were also discussions regarding the performance of molecular dynamics codes on GPU.

A question yet unsolved but brought forward during the discussion on the force field issues was how to compare the results with experiments if time scales are too short. Two talks (by Pemra Doruker and Filip Lankas) covered also the coarse-grained approached to study the dynamics. These reduced molecular models naturally increase the spatiotemporal scales of the simulation but have also other drawbacks and cannot compare with the all-atomic level of detail. Also, Jocelyne Vreede presented the results of replica-exchange molecular dynamics, an enhanced sampling technique.

A range of software to determine the 3D structural information and model nucleic acid systems was presented. Janusz Bujnicki described both the homology based and statistics based tools for RNA structure prediction that were developed in his laboratory. He emphasized that performing the sequence alignment for RNA is more difficult than for proteins. Pascal Auffinger presented tools to derive useful information from the structures of RNA and DNA deposited in the Protein Data Bank. The new tools to derive 3D structures for RNA are needed and analysis of the available structural data can help in designing better software for this purpose.

The workshop was an excellent place to start new collaborations between experimentalists and theoreticians, as well as between theoreticians who study similar systems with different methods. The workshop had 28 speakers, 12 speakers were from the US, one from Japan, and 15 from Europe. Many of them did not know each other in person before the workshop. The talks were of very high quality. The workshop's topic received great attention and we received many requests for attendance long after the official deadline. The room at ETH was full. We had to refuse some applications due to the limit on the number of participants.

Final programme of the meeting

Day 1 - September, 14 2011

- 9:00 to 9:35 - **Aleksei Aksimentiev**
  Molecular mechanics of ssDNA unwinding from SSB

- 9:35 to 10:10 - **Simone Furini**
  All-atom molecular dynamics simulations of specific and nonspecific binding of repressor proteins to DNA

- 10:10 to 10:40 - Coffee Break

- 10:40 to 11:15 - **Anatoly Kolomeisky**
  Physical-chemical aspects of protein-dna interactions: mechanisms of facilitated target search

- 11:15 to 11:50 - **Joyce Lebbink**
  Mechanisms of dna mismatch repair: formation and function of the atp-activated muts sliding clamp

12:00 to 13:30 - Lunch

- 13:30 to 14:05 - **Filip Lankas**
  Indirect readout of modified and unmodified dna: the role of conformational entropy at the rigid base level

- 14:05 to 14:40 - **Janusz Bujnicki**
  New methods for 3D modeling of rnas and rna-protein complexes

- 14:40 to 15:15 - **Claude Sauter**
  Structure, function and dynamics of aminoacyl-trna synthetases

- 15:15 to 16:00 - Poster Session and Coffee Break

- 16:00 to 16:35 - **Alexander MacKerell**
  Role of the 2'OH on RNA conformational heterogeneity; relevance to nucleic acid force field optimization

- 16:35 to 17:10 - **Pemra Doruker**
  Inferring functional dynamics of protein-nucleic acid complexes via elastic network models

- 17:10 to 17:45 - **Ioan Andricioaei**
  Simulations of DNA motion in topoisomerase I and through a bacteriophage portal

Day 2 - September, 15 2011

- 9:00 to 9:35 - **Thomas Simonson**
  Free energy simulations of nucleotide-protein binding: two illustrations related to genetic code translation

- 9:35 to 10:10 - **Nilesh Banavali**
  Component interactions, metastable states, and structure-function questions at protein interfaces with nucleic acids

- 10:10 to 10:40 - Coffee Break

- 10:40 to 11:15 - **Thomas Cheatham III**
  Nucleic acid and protein force field assessment and implications for simulation of protein-nucleic acid complexes with amber

- 11:15 to 11:50 - **Jocelyne Vreede**
  The effect of salt on the H-NS dimerization domain

12:00 to 13:30 – Lunch

- 13:30 to 14:05 - **Jiri Sponer**
  Improving the capability of contemporary atomistic simulations of nucleic acids: recent refinements of the AMBER force field.

- 14:05 to 14:40 - **Joanna Sarzynska**
  Processing of double stranded RNAs by dicer-like proteins in plants

- 14:40 to 15:15 - **Pascal Auffinger**
  Exploration of structural databases: for a better understanding of nucleic acid structure and solvation

- 15:15 to 16:00 – Poster Session and Coffee Break

- 16:00 to 16:35 - **Frédéric Allain**
  Insight into RNA splicing and editing mechanisms from the NMR structures of protein-RNA complexes

- 16:35 to 17:10 - **Rebecca Wade**
  Simulation of linker histone-nucleosome interactions

- 17:10 to 17:45 - **Arya Gaurav**
  Computational modeling of DNA organization: from nucleosomes to chromatin to chromosomes

- 19:30 to 22:00 – Dinner

Day 3 - September, 16 2011

- 9:00 to 9:35 - **Michael Levitt**
  Computational epigenomics:DNA methylation effect on nucleosome occupancy from first principles

- 9:35 to 10:10 - **Dorothy Erie**
  Single-molecule fluorescence studies of the initiation of DNA mismatch repair

- 10:10 to 10:40 - Coffee Break

- 10:40 to 11:15 - **Cameron Mura**
  The RNA-associated Sm protein family

- 11:15 to 11:50 - **Hisashi Ishida**
  Energy landscape of tRNA translocation through ribosome analysed by electron microscopy density maps and molecular dynamics simulations

12:00 to 13:30 – Lunch

- 13:30 to 14:05 - **Lennart Nilsson**
  Nucleotide modifications and tRNA anticodon- mRNA codon interactions on the ribosome

- 14:05 to 14:40 - **Holger Gohlke**
  Statics of the ribosomal exit tunnel

- 14:40 to 15:15 - **Sarah Woodson**
  Remodeling protein-RNA interactions during ribosome assembly

- 15:15 to 15:50 - **Karissa Sanbonmatsu**
  Integrating simulation and experiment: movement of tRNA through the ribosome