

# Gromacs Workshop at CSC, Finland

27<sup>th</sup> February - 1<sup>st</sup> March 2007  
(26<sup>th</sup> Pre Workshop tutorial)

## Book of Abstracts



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## Lecturers

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Erik Lindahl

<http://lindahl.sbc.su.se/>

David van der Spoel

<http://folding.bmc.uu.se>

## Contributed talks

Soren Enemark

Maxim Fedorov

Tomas Kubar

Perttu Niemelä

## Workshop program

time	Monday 26.2. Pre workshop tutorial
13:15-13:30	Registration
13:30-14:15	<b>Introduction to molecular modeling:</b> Theoretical framework, modeling levels, limitations and possibilities, systems and methods (Erik) ( <a href="#">Slides, pdf</a> )
14:15-14:30	Coffee
14:30-15:15	<b>Molecular dynamics simulations &amp; Gromacs introduction:</b> Force fields, integrators, control of temperature and pressure (Berk) ( <a href="#">Slides, pdf</a> )
15:30-16:30	<b>Hands-on simulation &amp; analysis:</b> First a simple liquid (water), and then a protein solvated in water ( <a href="#">Hands-on files, html/tar</a> )
17:00-	Mixer

time	Tuesday 27.2. Workshop day 1
09:00-09:15	Registration
09:15-10:10	<b>Replica exchange and kinetic analysis</b> (David) ( <a href="#">Slides, pdf</a> )
10:10-10:40	Coffee
10:40-11:35	<b>Free energy calculations</b> (Berk) ( <a href="#">Slides, pdf</a> )

time	Tuesday 27.2. Workshop day 1
	<b>Contributed talks</b>
11:35-12:30	<b>Tomas Kubar:</b> "Free-energy calculations and the study of DNA..ligand interactions" ( <a href="#">Slides, ppt</a> )
	<b>Maxim Fedorov:</b> "What effects do salts have on biopolymers?" ( <a href="#">Slides, ppt</a> )
12:30-13:30	Lunch
	<b>hands-on:</b> Analysis tools ( <a href="#">Hands-on files, html</a> )
13:30-16:30	<b>hands-on:</b> Potential of mean force calculations ( <a href="#">hands-on files, tar</a> )
	<b>hands-on:</b> Free energy of solvation ( <a href="#">Hands-on files, tar</a> )
16:30-17:30	<b>Poster Session</b>

time	Wednesday 28.2. Workshop day 2
09:15-10:10	<b>Improving your Gromacs skills.</b> How to speed up convergence? (David) ( <a href="#">Slides, pdf</a> )
10:10-10:40	Coffee
10:40-11:35	<b>Membrane simulations</b> (Erik) ( <a href="#">Slides, pdf</a> )
	<b>Contributed talks</b>
11:35-12:30	<b>Søren Enemark</b> "Tubulin Monomer Mechanical Properties obtained by simulating Atomic Force Microscopy experiments using Molecular Dynamics" ( <a href="#">Slides, ppt</a> )
	<b>Perttu Niemela:</b> "Assessing the Nature of Lipid Raft Membranes" ( <a href="#">Slides, ppt</a> )
12:30-13:30	Lunch
	<b>hands-on:</b> Analysis tools ( <a href="#">Hands-on files, html</a> )
13:30-16:30	<b>hands-on:</b> Potential of mean force calculations ( <a href="#">hands-on files, tar</a> )
	<b>hands-on:</b> The pull package ( <a href="#">Hands-on files, tar</a> )
~20:00-	<b>Dinner in Östrabotnia</b>

time	Thursday 1.3. Workshop day 3
09:15-10:10	<b>Gromacs development.</b> Installing the CVS version and using advanced features (Erik) ( <a href="#">Slides, pdf</a> )
10:10-10:40	Coffee
10:40-11:35	<b>Building your own Linux cluster</b> for Gromacs (and other programs) (Erik) ( <a href="#">Slides, pdf</a> )
11:35-12:30	<b>Birds-of-a-Feather:</b> ask about the Gromacs, the Universe, and Everything.
12:30-13:30	Lunch
	<b>hands-on:</b> Parallel simulations in practise ( <a href="#">Hands-on files, html</a> )
13:30-16:30	<b>hands-on:</b> The gory details: Understanding and hacking the Gromacs source ( <a href="#">Hands-on files, html</a> )
	<b>hands-on:</b> Help with your own code or simulations
16:30	Workshop over

## Participants

Cesar Araujo	University of Oulu
James Bell	University of Cambridge
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Grzegorz Domanski	Max-Planck
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Alex Rodriguez	Universitat de Barcelona
Jennifer Ryder	Cambridge University
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Donatas Zmuidinavicius	Cambridge University

### **Organizers**

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Johanna Blomqvist	CSC
Nino Runeberg	CSC

## Elastic constant evaluation of collagen $\alpha_1$ and $\alpha_{II}$ helices by means of Molecular Dynamics

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**INTRODUCTION.** Knowledge of the mechanical behaviour of collagen molecules is critical for understanding the mechanical properties of collagen fibrils that constitute the main architectural building block of a number of connective tissue. In this research, we studied 30-residues-long sequences of both  $\alpha_1$  and  $\alpha_{II}$  collagen helices by performing stretching simulations in order to assess their elastic properties.

**MODELS AND METHODS.** Collagen  $\alpha_1$  and  $\alpha_{II}$  sequences were obtained from RCSB Protein Data Bank, and from them we built two strands composed of the first 10 N-terminal triplets, using the software developed by Rainey and Goh<sup>1</sup>. Both chains were then solvated, relaxed via energy minimisation and subsequently heated and equilibrated at 300 K. Stretching was achieved by attaching the first  $C_\alpha$  to a spring kept fixed, while the last  $C_\alpha$  was linked to an axial-moving spring. At last, we calculated the stiffness of the  $\alpha$ -helices as the second-derivative of the molecule potential energy, monitored during the dynamic simulations.

**RESULTS.** We found that the mechanical behaviour of the two strands of collagen  $\alpha$  helices is similar, and that it can be subdivided into two regions. In the first one, we estimated an elastic constant of 322 and 100  $\text{kJmol}^{-1}\text{nm}^{-2}$  respectively for  $\alpha_1$  and  $\alpha_{II}$ . When the chains are fully elongated, energy rise quickly due to angle deformation, leading to a higher stiffness, around 3000  $\text{kJmol}^{-1}\text{nm}^{-2}$  for both chains.

**DISCUSSION.** Results show that molecular modelling is a powerful tool in examining biological molecules at scales not accessible to experimental investigation. This research is intended as a case study preliminary to the computational assessment of Young modulus of human collagen molecule in both physiological and pathological forms. Long-term goals are a) the bottom-up construction of a micro-mechanical model of tendons and b) to understand the mechanism underlying some collagen-related pathologies such as Osteogenesis Imperfecta.

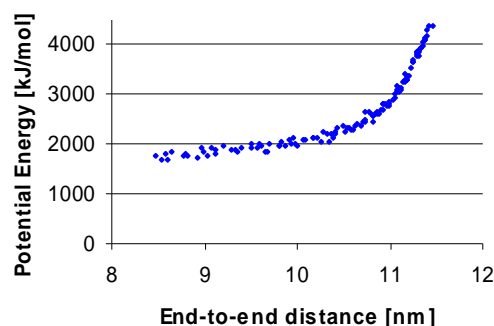


Fig. 1 – Potential energy of  $\alpha_1$  chain during the stretching simulation

1. Rainey, J.K.; Goh, M.C. *Bioinformatics*, **2004**, *20*(15), 2458-9.

## **Computer Simulations of Protein-Peptide Complexes Using the Myosin Light Chain Protein as a Model**

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The Mlc1p protein from the budding yeast *Saccharomyces cerevisiae* is a Calmodulin-like protein that interacts with IQ-motif peptides located at the yeasts myosin neck. We present molecular dynamics simulations of the Mlc1p-IQ2 and the Mlc1p-IQ4 complexes, following their relaxation in a physiological salt solution. During the simulations, the Mlc1p-IQ4 complex underwent a complicated modulation process, which involves bending of the angles between the alpha-helices of the protein, breaking of the alpha-helical structure of the IQ4 peptide into two sections, and formation of new contact points between the protein and the peptide. In contrast to the Mlc1p-IQ4 complex, the Mlc1p-IQ2 complex relaxed without losing its main packing features, exhibiting a limited conformational change throughout its simulation. We performed a comprehensive detailed comparison between the structure and the dynamics of the Mlc1p-IQ complexes and an extensive interaction free energy analysis. The latter included an analysis of the various forces operating on the protein-peptide complexes by indicating their specific contributions. Based on the molecular dynamics simulations of both Mlc1p-IQ complexes and the free IQ peptides, a model for the light chain-binding domain of myosin V was constructed. At the core of our suggested model stands the notion that the light chain-binding domain is a dynamic cellular entity, and hence the proposed model incorporates the ability of the Mlc1p protein and the IQ peptides to flex and curve in a mutual manner. The study, which portrays a computerized journey, starting from molecular dynamics simulations and ending at physiological insights concerning myosin V, exemplifies that cooperation between crystallographers and biophysicists may contribute to a better understanding of structure-function relationship.



Figure 1: The Mlc1p-IQ4 complex after 12-ns of MD simulation.

### References

1. Ganoth, A., E. Nachliel, R. Friedman, and M. Gutman. *Proteins*, 2006, 64:133-146.
2. Ganoth, A., R. Friedman, E. Nachliel, and M. Gutman. *Biophys J* 2006. 91:2436-2450.

## **Molecular modeling of the interaction between kinesin motor domain and $\alpha\beta$ -tubulin**

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Kinesins are motor proteins involved in the transport of organelles along microtubules. The metabolic energy that drives kinesins is provided in the form of ATP; the energy released by its hydrolysis is converted into direct movement after kinesin binds strongly to the microtubule. To clarify the nucleotide depending binding mode, we studied the interaction properties of the complex between tubulin dimer and kinesin motor domain in ATP and ADP state, by means of molecular dynamics (MD) approach, using Gromacs code.

The atomic structures of the analysed protein was taken from RCSB Protein Data Bank (1IA0.pdb and 1VFX.pdb). Each analysed system was refined, arranged in a rectangular box, minimized in vacuum and solvated by adding explicit water molecules. After a further optimization, each system was heated up to 300K and equilibrated.

The study of the interaction properties of each complex was carried out mimicking an AFM experiment. The tubulin dimer was kept frozen while the kinesin was moved by means of a spring that was attached to its center of mass. Interaction energy vs. intermolecular distance data points around the minimum were fitted using a 3rd-order polynomial function. The interaction force was calculated as the first order derivative of the energy fitting curve.

Results of the simulations show that, in case of performing AFM simulations with a pulling rate of  $10^{-3}$  nm/ps and a stiffness constant of  $3000 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{nm}^{-2}$ , kinesin in ATP state has a higher affinity for the tubulin dimer. The minima of interaction energy are  $-1414.15 \text{ kJ/mol}$  and  $-1194.63 \text{ kJ/mol}$ , corresponding to the complex between tubulin dimer and kinesin in ATP and ADP state, respectively. The maximum interaction force between kinesin in ATP state and tubulin dimer is 1.2 nN, that is greater than the one calculated for the complex with kinesin in ADP state which is characterized by a maximum interaction force of 0.7 nN.

These results are consistent with the observations of Kikkawa et al.<sup>1</sup> which noted that the nucleotide-induced conformational changes influence the potential energy of the complex, and enable tighter contact between the kinesin in ATP state and microtubule.

**Acknowledgments** - This research has been supported by the EST Marie Curie programme contract number MEST-CT-2004-504465 and by the Active Biomics STREP project contract number NMP4-CT-2004-516989.

1. Kikkawa, M., Sablin, E.P., Okada, Y., Yajima, H., Fletterick, R.J., Hirokawa, N., *Nature*, **2001**, *411*, pp.439-445.

## Tubulin Monomers Interaction Study by Molecular Dynamic Simulations

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The basic unit in microtubules (MTs) is  $\alpha\beta$ -tubulin, a hetero-dimer consisting of an  $\alpha$ - and  $\beta$ -tubulin monomer. In the MT  $\alpha\beta$ -tubulins are arranged with head-to-tail motif in protofilaments which bind together laterally along the MT's long axis. Previous experimental investigations have showed high correlations between MTs' properties such as dynamic instability and interactions between monomers as they are kept inside the MT [1]. However, the topic is still under debate.

Molecular Dynamic (MD) method can allow a better understanding of the interaction between adjacent monomers from a nanoscopic point of view. The investigation was carried out with the software GROMACS 3.3.1 using GROMOS96 43a1 force field and a cut-off of 1 nm for non-bond interactions. The atomic structure of  $\alpha\beta$ -tubulin (1TUB.pdb) was arranged in a box, explicitly solvated and neutralized by adding  $\text{Na}^+$  ions. The system was energy minimized, then heated to 300 K for 35 ps, and finally equilibrated for 800 ps. Starting from the 800 ps equilibrated structure four different molecular systems were generated, each consisting of two monomers in water: the intra-dimer ( $\alpha\beta$ -interaction), inter-dimer ( $\beta\alpha$ -interaction) and lateral-interaction ( $\alpha\alpha$ - and  $\beta\beta$ -interaction). For all the molecular systems several configurations were generated with different distances between the centres of mass of the two monomers. For each distance the mean interaction potential energy ( $V_{int}$ ) was obtained by running a MD simulation for at least 100 ps. The mean value of  $V_{int}$  between the two monomers as a function of the mean value of the monomer-to-monomer distance ( $d$ ) was then fitted with a 3<sup>rd</sup> order polynomial function. The interaction force ( $F_{int}$ ) and the elastic constant ( $k_{int}$ ) were directly calculated by deriving the potential energy function found for each molecular system.

Preliminary results for interactions between neighbouring monomers showed elastic constants along the protofilament (45 N/m for the intra-dimer interface and 18 N/m for the inter-dimer interface) more rigid than elastic constants calculated for lateral interfaces (11 and 15 N/m).

The work represents a preliminary step of a MT mesoscale model aimed at correlations of MT's physical properties with hierarchical organization and mechanical properties of tubulin monomers.

Acknowledgements - This research was supported by the EST contract number MEST-CT-2004-504465 and by the Active BIOMICS STREP project contract number NMP4-CT-2004-516989.

1. Howard, J., **2001**, *Mechanics of Motor Proteins and The Cytoskeleton*, Sinauer, Sunderland.

## Tubulin Monomer Mechanical Properties obtained by simulating Atomic Force Microscopy experiments using Molecular Dynamics

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Microtubules (MTs) are fundamental elements of the cytoskeleton in eukaryotic cells. Their unique mechanical properties depend both on the MT's basic units, namely the  $\alpha$ - and  $\beta$ -tubulin monomers, and the way these monomers are arranged in the MT. The mechanical characteristics of the individual monomers may, therefore, be used to obtain new insight into the microtubule tensile properties.

In the present work we use molecular dynamics to evaluate the elastic constants of each monomer in the directions of the inter-monomer interactions. Molecular models of  $\alpha$ - and  $\beta$ -tubulin were developed from the TUB.pdb structure and placed in an explicit water environment. Simulations were performed by mimicking experiments carried out with atomic force microscopy. The results, showing elastic constants of 3.4-3.9 N/m for  $\alpha$ -tubulin and 1.8-2.4 N/m for  $\beta$ -tubulin, are discussed in relation to the structural changes and the simulation parameters imposed. Further and higher level modelling is possible by exchanging the atomic level description with the key mechanical features.

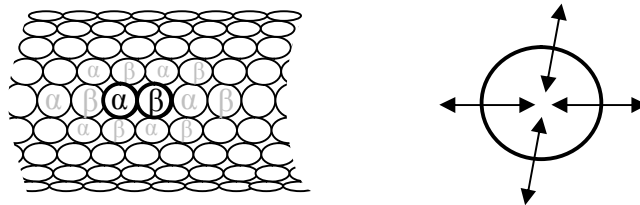


Figure 2. *Left:* Small part of a microtubule showing the  $\alpha$ - and  $\beta$ -tubulin monomers and how they are arranged within microtubule. *Right:* Scheme of a monomer (circle), i.e.,  $\alpha$ - or  $\beta$ -tubulin, shown together with directions of inter-monomer interactions (arrows).

## Structure and Dynamics of the N-terminal Loop of PsbQ from Photosystem II of *Spinacia oleracea*

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Infrared and Raman spectroscopy were applied to identify restraints for the structure determination of the 20 amino acid loop between two beta-sheets of the N-terminal region of the PsbQ protein of the oxygen evolving complex of photosystem II from *Spinacia oleracea* by restraint-based homology modeling. One of the initial models has shown a stable fold of the loop in a 20 ns molecular dynamics simulation that is in accordance with spectroscopic data. Cleavage of the first 12 amino acids leads to a permanent drift in the root means square deviation of the protein backbone and induces major structural changes.

The probable binding site of PsbQ to the complex could be formed by the lysyl rich region of the helix bundle and the N-terminal loop region around Asp24 and thus would contain a large positively charged region and a small negatively one. We hypothesize that after binding to PSII the loop loses its high flexibility and bends in the direction of Lys96 with Thr20 and Glu21 interacting with this residue and so burying it under the accessible surface (Fig. 1). Thus Lys96 could probably behave as a molecular hook holding Glu21 by a salt bridge.<sup>1</sup>

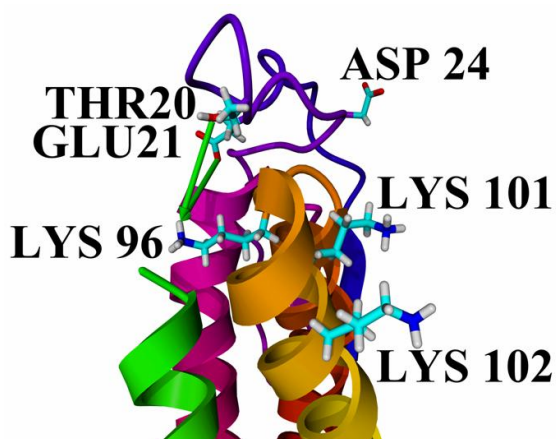


Figure 1: Lys96, one of the four lysyl residues which are probably orientated to the luminal facing intrinsic proteins of PSII, lies on the opposite side as are the other three lysyl residues and the conserved loop residue Asp24, in a distance from 6–12 Å and 7–13 Å to the loop residues Thr20 and Glu21, respectively, in the MD simulation

1. Ristvejova, J., Kopecky, V., Sovova, Z., Balsera, M., Arellano, J.B., Green, M., Ettrich, R., *Biochem. Biophys. Res. Comm.*, **2006**, 345 (1), 287-291.

## **Molecular dynamics simulations of the amyloid-beta peptide binding alcohol dehydrogenase (ABAD) enzyme**

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Amyloid-beta (A $\beta$ ) peptide binding alcohol dehydrogenase (ABAD) is a homotetrameric enzyme which has function in the mitochondria, where it participates in several distinct metabolic processes, including, the oxidation of fatty acids and branched-chain amino acids, sex steroid metabolism, and oxidation of steroid modulators of GABA<sub>A</sub> receptors [1,2]. ABAD has attracted considerable attention due to its ability to interact with A $\beta$  and mediate the cytotoxic effects of this peptide inside the mitochondrial compartment [2,3]. The dynamical and structural properties of ABAD have been investigated in aqueous solution using molecular dynamics simulations. The starting structures were obtained from the crystal structure of the ABAD complexed with a small inhibitor and simulations of the tetramer, the two types of dimers and the monomer were done with and without the ligand. The stability of the different structures was evaluated and a detailed analysis of the atomic fluctuations was conducted. Essential dynamics analysis was employed to characterize the large-scale collective motions of ABAD.

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## In Silico Design Of New Affinities On Periplasmic Binding Proteins And Its Evaluation By Molecular Dynamics

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The development in computer algorithms to evaluate interaction energy and protein stability from three-dimensional structure has generated important breakthroughs in structure design and de novo catalysis in proteins (1, 2). These methods offer a new scope in ligand-protein or protein-protein interaction redesign. Our model system is the L-lysine, L-arginine and L-ornithine periplasmic binding protein from *Salmonella typhimurium* (LAO protein).

LAO belongs to a protein family that presents specific affinity to different ligands: aminoacids, carbohydrates, carboxylic acids, ions and oligopeptides. Taking advantage of this characteristic, the central aim of our Project is to modify the ligand specificity using *in silico* methods in order to generate new LAO protein-ligand affinities.

In the first phase of design we used the Rosetta program to generate *in silico* mutations of the aminoacids around the LAO binding site. This step was made in order to improve their interaction parameters to aromatic aminoacids. The search was made by evaluating the Rosetta parameters: stability energy of the protein and the ligand interaction energy. Given the fact that this protein suffers a conformational change to a closed form, the second phase of the design consisted in analyzing the open/closed equilibrium by molecular dynamics using the GROMACS suite. When we used the open form of the wild type protein with and without ligand in different 10 nanoseconds dynamics we observed a shift to the closed form implying a favourable LAO-ligand interaction.

In the case of a simulation of the non native ligand tyrosine at the LAO binding site is apparently not favourable, given the fact that this aminoacid was ejected from the binding site during the trajectory.

From these results, we believe that simulations of our mutants in the presence of their "new" ligands will offer insight to better choose those to perform bench experiments.

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## Water molecules in nanotubes: A molecular dynamics study

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Recent experiments have demonstrated that solvated nanotubes could be separated according to their diameter in centrifugation experiments<sup>12</sup> but unexpected order of diameters is obtained. This could be explained by considering the nanotube's environment : not only the mass of the carbon atoms contributes to the overall system density but also their environment, in particular water molecules that are trapped in the nanotube.

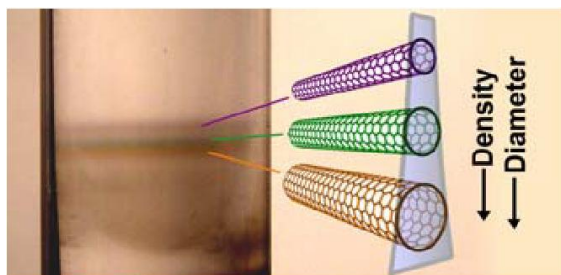


Figure 3: Experiment picture. <sup>1</sup>

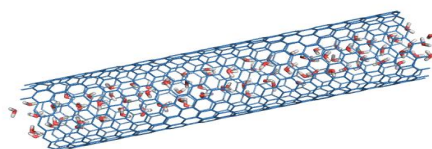


Figure 2: Snapshot of a simulation

Recent studies<sup>3</sup> have demonstrated unusual arrangements of water molecules confined in carbon nanotubes and large deviations in the hydrogen-bonding network of such molecules in the nanotube in comparison to bulk water. The density of water trapped in the nanotube will deviate significantly from the bulk water under the same macroscopic conditions. Using Gromacs, we have studied the behavior and density water molecules trapped in different carbon nanotubes, ranging in diameter from 0.7 nm to 3 nm. Employing various models for the dielectric environment of the solvated tubes we find conditions, which are favorable for nanotube separation and which are in agreement with experimental observations.

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## Free-energy calculations and the study of DNA..ligand interactions

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This contribution deals with two topics:

### Free energy calculations with controlled uncertainty

The molecular dynamics-thermodynamic integration approach is a widely used tool to calculate free energy. In most applications, the major issue of this method consists in the ill-defined accuracy of the result. A scheme of efficient control of uncertainty<sup>1</sup> has been implemented in Gromacs by ourselves. We present the calculations of (i) binding free-energy difference of DNA ligands<sup>2</sup> and (ii) folding free-energy difference of a thermostable protein and its mutants<sup>3</sup>.

### The modes of ligand binding to DNA

We study the binding of several derivatives of the anti-cancer drug ellipticine to DNA both in the intercalative mode and to the minor groove<sup>4</sup>. The diversity of ligands in terms of charge and polarity provides the study with a more general significance. We have used various Gromacs tools to investigate the energetics and dynamics of DNA..ligand complexes. Interesting features of these complexes appeared, namely the general sequence preference of the minor-groove binder and the change of DNA flexibility upon ligand binding.

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## **Feeding on Human Blood: MD Simulations Reveal how a Parasite gets ready to eat Haemoglobin**

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Malaria is a fatal mosquito-borne disease which is caused by the Plasmodium parasite. Plasmodia infects red blood cells, in order to degrade haemoglobin as a nutritional source. Plasmeprins (PMs) are aspartic proteases (protein-degrading enzymes), which are utilised by the Plasmodia for the initiation of haemoglobin breakdown. PM II is the most studied of the PMs. As other aspartic proteases, it is translated as an inactive zymogen (proenzyme). The zymogen is activated by a cleavage of its N-terminal prosegment under acidic conditions. The crystal structure of pro-PM II shows that in the inactive zymogen, the active site is found in an open conformation which does not allow the catalytic process to take place. The atomistic mechanism, by which PM II undergoes the conformational change that forms the active enzyme upon the cleavage of the prosegment (displayed in Figure 1) is hitherto unknown. We used MD simulations of different stages of pro-PM to elucidate the mechanism by which the enzyme shifts between open and closed conformations. Spontaneous formation of key aspects of the haemoglobin binding site was evident in the simulations. Shedding light on this transformation mechanism is interesting by itself and may be proven useful for the development of anti-malarial drugs.

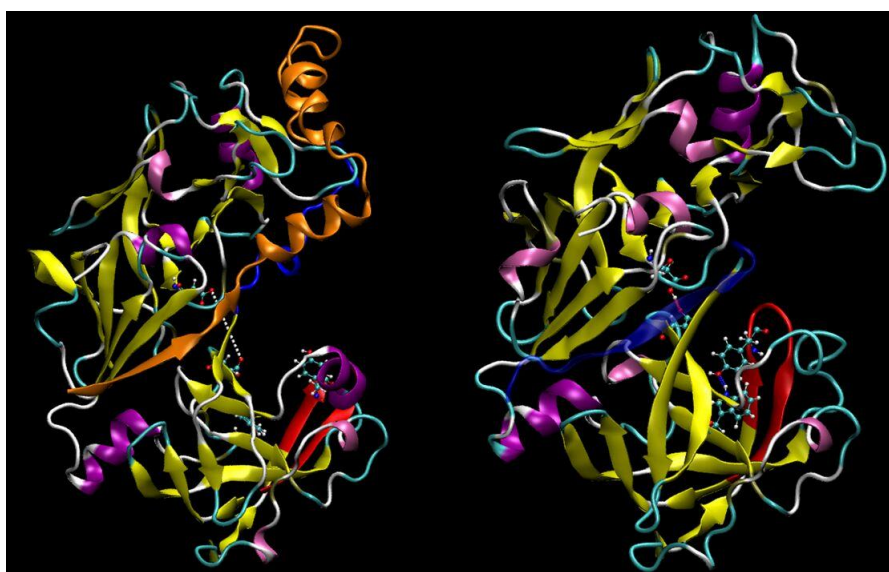


Figure 4: The proenzyme (left) and the active enzyme (right). Note the location of the two catalytic asp residues, which are not adjacent in the proenzyme, while forming a h-bond in the active enzyme.

## What effects do salts have on biopolymers?

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Studies of biomolecular hydration have more than a hundred year history. Nevertheless, there are still many unanswered questions in this area, one of them is: 'What is the influence of different salts on the conformation of a macromolecular solute?'. Indeed, the structural and thermodynamic properties of biopolymers are strongly influenced by cosolvents and cosolutes. There are many processes relevant for modern biomolecular science and technology which are influenced by the concentration and type of salts present, including: biopolymer solubility, biopolymer denaturation temperatures, enzyme activity, biopolymer swelling, growth rates of bacteria, and stability of protein macroaggregates.

In this work we are trying to gain insight on the molecular mechanisms of the salt effects on the conformational stability of biopolymers with use of modern fully atomistic Molecular Dynamics simulations techniques. Such 'in silico' approach allows one to obtain very realistic data on the time and scale resolutions that are unavailable for both 'in vitro' and 'in vivo' experimental techniques.

In general, there are three main effects of salts on the macromolecular conformation:

- i. **Debye-Huckel** screening effect;
- ii. **Electroselectivity**: specific interaction with charges by the ion-pair formation.
- iii. **Hofmeister effect** - disruption of water structure which consequently results in an increase or decrease in the hydrophobic interaction of proteins and biomolecules.

If the Debye-Huckel screening has the major contribution, the effect of various ions will be determined only by the ionic strength of solution. If the specific interactions with charges are of the most importance, the effects of different ions should follow the electroselectivity series of the salts toward anion-exchange resins. The significance of the changes in hydrophobic interactions can be determined by comparing the effects of different ions with the Hofmeister series.

To validate these hypotheses we performed long timescale molecular dynamic simulations of several polypeptides and small proteins in different alkali halide solutions varying concentrations of the salts. The results have been compared with available experimental data and simulations in bulk water solution.

From our results we conclude that all these three effects are present but their 'weights' vary with concentrations and type of salts. The 'right answer' depends from the protein primary structure and the particular conditions of ionic solution (concentration, sort of ions, etc). Therefore, the popular hypothesis of preferential ion exclusion from the protein-water interface has to be revisited by the forthcoming combined theoretical and computational studies. In principal, all these effects (Debye-Huckel screening, electroselectivity and Hofmeister effects) are important and one cannot neglect none of them a priori. But for any given secondary structure of a macromolecule one can find its own 'breakers' and 'stabilizers', knowing basic principles of ion-water, ion – ion and ion – molecule interactions.

## Assessing the Nature of Lipid Raft Membranes

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Sphingomyelin (SM) molecules are, together with phosphatidylcholines (PC) and cholesterol, most important lipid components of cellular plasma membranes. Sphingomyelin shares many common features with PC, but the higher hydrogen bonding capacity and the higher degree of saturation of the fatty acyl chains makes SM likely to interact with cholesterol<sup>1</sup>. This interaction has been suggested to be the driving force in formation of lateral domains called rafts<sup>2</sup>, which might have an exceptionally important functional role through sorting and regulating the activity of membrane proteins. However, the exact nature of these interactions and the characteristics of possible domains in living cells have remained rather unclear, largely due to limitations in experimental methods.

We are carrying out computational work to determine properties of lipid bilayers related to SM. We have recently utilized atom scale molecular dynamics simulations to study the properties of pure SM bilayers<sup>3,4</sup>, the molecular interactions between the suggested key lipid components in raft formation<sup>5</sup> and compared the bulk properties of raft and non-raft membranes<sup>6</sup>. The results have yielded biologically important conclusions, for example the properties of the lipid environment have been shown to be able to affect the functionality of membrane proteins.

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