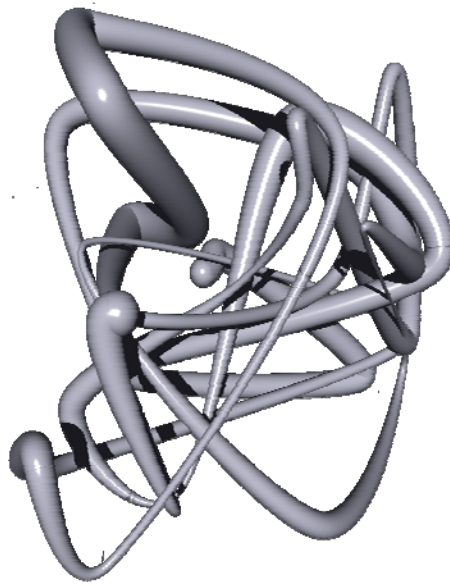


Christmas Biophysics Workshop 2006



Institute of Physics, Zagreb, Croatia
Lecture Hall in the "Mladen Paić" Building
Monday, December 18th 2006

Cosupported by

- ✦ Project 003015 "Systems of reduced dimensionality: from synthetic organic to biomaterials"
- ✦ Institute of Physics, Zagreb
- ✦ Croatian Biophysical Society

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Tentative Program

Time	Speaker / event
10:00-10:15	Opening of the Workshop
10:15-10:55	Bojan Žagrović
11:05-11:45	Antonio Šiber
11:55-12:35	Ana Smith
12:45-13:25	Primož Zihlerl
13:25-13:55	--break--
13:55-14:35	Rudi Podgornik
14:45-15:25	Jure Dobnikar
15:35-16:15	Tomislav Vuletić
16:25-17:30	Round table - open discussion

List of speakers and abstracts

1. Bojan Žagrović

Physical Chemistry Institute, ETH, Hoenggerberg HCI, 8093, Zurich, Switzerland

Unusual compactness of flexible peptides: from a model unfolded state to isolated α -helices

Most experimental observables used to characterize the structure of flexible peptides, such as CD and NMR, typically provide only short-range, sequence-local structural information which is both time- and ensemble-averaged. Here, we report two examples in which the structure of polypeptides based on such local measurements is seemingly at odds with their long-range structure.

The first example deals with an alanine-based peptide, Ace-(Daba)₂-(Ala)₇-(Orn)₂-NH₂. This molecule has previously been studied as a model for the unfolded state of proteins under folding conditions, and is believed to adopt polyproline type II (PPII) helical fold based on NMR and CD measurements. Using small-angle X-ray scattering (SAXS) we have determined the radius of gyration of this peptide to be $7.4 \pm 0.5 \text{ \AA}$, which is significantly less than the value expected from an ideal PPII helix in solution (13.1 \AA). To further study this contradiction, we have employed the Folding@Home distributed computing cluster and molecular dynamics simulations using six different variants of the AMBER force field and the GROMOS 53A6 force field. However, in all cases the simulated ensembles underestimate the PPII content, while in most cases also overestimating the experimental radius of gyration. The conformational model that we propose is that of a very flexible structure which on the level of individual residues explores a wide basin around the ideal PPII geometry but is never, or only rarely, in the ideal extended PPII helical conformation.

In the second example, we have used SAXS to measure the radii of gyration of a series of alanine-based α -helix-forming peptides of the composition Ace-(AAKAA)_n-GY-NH₂, $n = 2$ to 7 . The radii of gyration that we measure are, except for the 12-mer, significantly lower than the radii of gyration of the equivalent ideal α -helices or helices with frayed ends. For example, the measured radius of gyration of the 37-mer is $14.2 \pm 0.6 \text{ \AA}$ which is to be compared with the radius of gyration of an ideal 37-mer α -helix of 17.6 \AA . Attempts are made to analyze the origin of this discrepancy in terms of the analytical Zimm-Bragg-Nagai (ZBN) theory as well as distributed computing explicit solvent molecular dynamics simulations using two variants of the AMBER force field. The ZBN theory predicts markedly larger radii of gyration than the ones measured, even when the persistence length of the disordered parts in the chain is taken to be extremely short. Similarly, the molecular dynamics simulations give

inaccurate values of the radii of gyration of the molecules and/or their helical content. We conclude that even at the short sequence-lengths looked at here (≤ 37 amino acids) these α -helical peptides behave as fluctuating semi-broken rods rather than straight cylinders with frayed ends.

2. Antonio Šiber

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Shapes and energetics of bucky-assemblies

The icosadeltahedral design, usually attributed to Buckminster Fuller due to his constructions of geodesic domes, is found to be one of the hallmarks of shapes at the nanometer scale. Probably the best known examples are carbon icosahedral fullerenes and carbon nanotubes. A large portion of viruses also exhibits the icosadeltahedral design known in the literature as the Caspar-Klug quasiequivalent construction. Clathrin protein assemblies also clearly display the patterns of pentagons and hexagons which is a common characteristic of all the bucky-shapes. For a nanoscientist, it is certainly interesting to see how this pattern emerges from the microscopic interactions between the elementary constituents of the shape (carbon atoms or proteins) - this could open the path to construction of bottom-up, self-assembling structures (as viruses apparently are). I shall discuss some results regarding the energetics of bucky-assemblies. I shall show how the elementary application of the theory of elasticity can help in this respect, and how it can be profitably applied both to generalized fullerenes and viruses. A very concrete result of the study is hopefully reliable estimate of the elastic parameters (Young's modulus and bending rigidity) of viral protein coatings (capsids).

3. Ana Smith

Growth of adhesion-domains in membranes under force is passive and enhanced by the receptor mobility

Cell adhesion is a two step process: the formation of bonds between receptor and their counter-receptors at random sites is followed by intracellular coupling of the actin cytoskeleton to adhered receptors. Under an external detaching force, an adherent cell exhibits enlargement of adhesion domains and concomitant stiffening of the cytoskeleton, apparently implying that these two effects are inexorably linked. However, in experiments with living cells, it is not possible to distinguish between direct force-induced effects in the adhesive zone and indirect effects

associated with initiation of intracellular signalling. We circumvented this issue by constructing a model system that contains only the membrane-related ingredients of adhesion. We show here, using a vesicle/supported-bilayer system that adhesion is controlled by the ability of one or both binding partners to undergo lateral diffusion. By constructing two-dimensional maps of inter-membrane distance fluctuations, recorded with a micro-interferometric technique, we pioneer the identification of adhesion clusters smaller than the optical resolution. We, for the first time observe a strengthening of adhesion domains due to passive reorganization of bonds under an unbinding force applied via magnetic tweezers. We explain this result on the basis of theoretical thermodynamic calculations. The results imply that a mechanism of force-induced adhesion-strengthening can be envisaged without the direct involvement of the cytoskeleton or the active regulative mechanisms and points to fundamental differences between cell-cell and cell-tissue interactions.

4. Primož Ziherl

*Department of Theoretical Physics, Jozef Stefan Institute,
Jamova 39, SI-1000 Ljubljana, Slovenia*

Aggregates of lipid vesicles

The theoretical model of lipid vesicles based on the membrane bending energy can be extended by an effective adhesion term to describe aggregates of these artificial objects which reproduce much of the morphology of simple biological cells. The most salient mechanical features of aggregates become apparent in an aggregate as simple as the doublet: The contact zone is flat at small adhesion strengths, whereas at large strength it is sigmoidally curved with one evagination and a matching invagination. The calculated doublet shapes are in good agreement with the experimentally observed erythrocyte aggregates, and the sigmoidal contact zone explains certain aspects of formation of multicellular aggregates such as erythrocyte rouleaux. Similar behavior is found in two-dimensional vesicles where a variational approach can be used to analyze the complete stability diagram, which includes free vesicles and both columnar and sheet-like infinite aggregates. These results elucidate the geometry of quasi-twodimensional tissues such as epithelia.

5. Rudi Podgornik

*Department of Theoretical Physics, Jozef Stefan Institute,
Jamova 39, SI-1000 Ljubljana, Slovenia*

A model of genome packing in simple viruses

I will present a nematic nanodrop model of DNA packing in a spherical capsid. I will formulate the equilibrium conditions in terms of the measure bulk osmotic pressure in DNA arrays and use it to extract the osmotic loading curves of the capsid. The obtained loading curves will be compared with experiments. Special attention will be payed to the ionic effects in the loading curves.

6. Jure Dobnikar

*Department of Theoretical Physics, Jozef Stefan Institute,
Jamova 39, SI-1000 Ljubljana, Slovenia*

Huge Many-body interactions in colloidal systems

The colloid-colloid interactions in charge stabilized colloidal suspensions are mediated via microions (electrolyte) and are therefore inherently *many-body* in nature. Simplified descriptions in terms of pairwise additive effective interactions thus prove to be insufficient. In recent years a number of experimental and theoretical studies showed that many-body effects are essential for complete understanding of colloidal interactions.

I will present our numerical studies based on solving the nonlinear Poisson-Boltzmann equation in three dimensions. The method has been applied to study the following problems:

- ▣ Coupling the Poisson-Boltzmann solver to the Brownian dynamics simulations, we have studied the solid liquid phase transition and showed that many-body interactions are necessary to describe the melting line at low densities and low salt concentrations.
- ▣ Three-body interactions have been directly measured (experimentally) and calculated and a qualitative agreement has been obtained.
- ▣ The forces on each colloid in a system of 6000 colloids have been evaluated by means of full Poisson-Boltzmann solution and directly compared to the effective forces predicted by the cell model and Jellium model approximation. A reasonable agreement has been observed, again with some deviations only explainable by many-body interactions.
- ▣ The elastic constants have been measured and numerically calculated in 3D colloidal fcc crystals and the Cauchy relation has been shown to be violated, which is a fingerprint of non pairwise additive potentials.

7. Tomislav Vuletić

Institute of Physics, Bijenička 46, 10000 Zagreb, Croatia

Dielectric relaxation of aqueous DNA

Detailed spectroscopic (40 Hz - 110 MHz) study of complex dielectric function of aqueous solutions of native (genomic) DNA molecules will be presented. A study was performed for a rather broad range of added salt, 0.01 – 5 mM and DNA concentrations, 0.01 – 10 mg/mL (semi-dilute solutions). For the first time with a single setup (developed in our laboratory) two broad relaxation modes were observed, low (LF) and high (HF) frequency ones, in kHz and MHz ranges, respectively [1]. The corresponding relaxation times τ were then related to the length scales L , via simple diffusion relation $L^2 \sim D\tau$.

The observed length scales were then identified with different length scales previously described in theories of polymers and polyelectrolytes [2], thus providing lacking experimental insight in the behavior of DNA as a polyelectrolyte.

The dielectric strength of the modes [3], when rescaled by the respective length scale and DNA concentrations, provided further insight by allowing us to argue that the free DNA counterions are primarily active in the HF relaxation, while the condensed counterions play a role only in the LF relaxation [4].

Finally, we put our results in perspective regarding the integrity of the double stranded form of DNA at low salt conditions. That is, we have come to the conclusion that unzipping of DNA in the denaturing environment of low salt is probably at most local and complete separation of the strands at semi-dilute solutions is never really accomplished. This issue will be addressed in our further studies.

[1] S. Tomić et al., Phys.Rev.Lett. **97**, 098303 (2006).

[2] A.V.Dobrynin et al., Prog.Polym.Sci. **30**, 1049 (2005).

[3] S. Tomić et al., cond-mat/0602255

[4] G.S. Manning, J.Chem.Phys. **51**, 924 (1969) ; F. Oosawa, Polyelectrolytes (1971).