P-D1-05

STRUCTURAL STUDIES ON CHROMATIN SPREADS PRODUCED BY ISOTONIC LYSIS

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Purpose: Isotonic lysis is a new method that allows the production of decondensed chromatin structures from interphase nuclei and metaphase chromosomes, preserving to some extent the relationships existing in the condensed chromatin. In this study, we used (immuno) electron microscopy (EM), FISH, and scanning force microscopy (SFM) to examine the properties of the chromatin structures produced by isotonic lysis. **Methods:** Chromatin of PBS-suspended cells and metaphase chromosomes were spread onto glass coverslips or EM grids by isotonic lysis. Human autoimmune sera and gold conjugated second antibodies were used for immuno EM. For FISH, biotinylated DNA probes specific to pericentromeric repetitive sequences of chromosomes 1 and 13/21 were used. Some specimens were dryed after FISH and subjected to SFM, followed by rehydration and fluorescence microscopy. **Results:** The smallest chromatin fibres were showed to be 25-30 nm wide by EM and 40-65 nm wide / 7-8 nm high by SFM. In more condensed regions, large bundles of fibres were revealed. Immuno EM showed that nonhistone proteins remained in the chromatin after isotonic lysis. FISH signals apperared as fluorescent dots along the decondensed chromatin fibres. By the comparison of fluorescent microscopic pictures of DAPI counterstained, hybridised spreads and their SFM images, specific chromatin domains, including those hybridized to the probes, could be idnetified in the topographic contrast of SFM. Condensed pericentromeric regions were 50-150 nm higher than their surroundings, and FISH labeled fibres also appeared higher and wider. **Conclusion:** The study of chromatin spreads produced by isotonic lysis could provide further information on chromatin structure. In situ hybridization of these spreads allows for the ultrastructural investigation of specific chromatin regions.

P-D1-06

Counterion Induced Bundle Formation of Charged Biopolymers. Effects of Crowding on Bundling and Connection with Liquid Crystalline Order-A Unified Perspective

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Polyvalent cations induce lateral aggregation of DNA, F-actin, microtubules, and viruses such as the filamentous phage fd and tobacco mosaic virus (TMV). Such general effects are due to the common polyelectrolyte nature of these anionic biopolymers, and their nonspecific binding to ligands carrying several net opposite charges. In addition to shielding the repulsive charges on the macromolecular surface, an attractive interaction between the charged biopolymers may be induced by counterion fluctuation, or by lateral redistribution of the shared counterion cloud. A balance of forces thereby allows the reversible formation of bundles containing either single types of filaments or mixtures of different filament types.

Molecular crowding in solution by the biopolymers and other inert solutes such as polyethyleneglycol (PEG) or noninteracting proteins can also facilitate the lateral aggregation of rodlike polyelectrolytes. This type of bundling is entropically driven, and experimentally it manifests different features from the cation-induced bundle formation, including an opposite dependence on the solution ionic strength and polyelectrolyte concentration.

Suspensions of rodlike or semiflexible macromolecules including all the bio-polymers mentioned above have been observed to form orientationally ordered liquid crystalline phases at high concentrations in solutions containing neither polyvalent cations nor any other polymer. Modifications of the Onsager theory based on the excluded volume effect suffice to explain this general phenomenon. However, consideration of solute-solute and solute-solvent interactions may lead to a unified understanding of these distinct, yet intricately related effects which jointly may play pivotal functional roles in forming the arrays of bundled filaments found in many cell types.

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FUNCTIONALIZED LIPID TUBULES: A TOOL FOR HELICAL CRYSTALLIZATION OF PROTEINS

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Purpose: We have discovered a functionalized (biotin) lipid molecule forming supramolecular assemblies of tubular morphology. These tubules present properties of molecular recognition for proteins (streptavidin) and of helical crystallization of proteins. Our aim was to understand which part of the lipid molecule was responsible for the formation of the tubular supramolecular structures. **Methods:** The supramolecular structures formed in aqueous solutions by DODA-EO2-biotin and 7 related lipids were investigated by (cryo)-electron microscopy. Binding of streptavidin to the lipid structures was studied by TEM and by PAGE. **Results:** The lipid DODA-EO2-biotin forms unilamellar tubular structures of constant diameter (27 nm) and μm length.These functionalized tubes bind streptavidin by molecular recognition. Streptavidin assembles into ordered helical arrays, the order extending up to 1.5 nm resolution. None of the other lipid molecules formed tubes. **Conclusions:** The presence of the biotin moiety is strictly required for the formation of tubular structures. We anticipate that other tubular structures with chosen functionalities can be designed in a rational manner, opening potentially powerful areas for molecular structure determination by electron crystallography.

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COMPUTER SIMULATION OF MESO-SCALE PHENOMENA: PATTERN FORMATION IN BIOMIMICKING COMPLEX FLUIDS.

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We investigated the application of dynamic mean-field density functional methods to pattern formation in self organizing systems. at molecular length scales (nm). In particular, we compare simulation results with experiment for di-octadecyl-amine (DODA) self assemblies. These assemblies serve as precursor to lipid tubes and find application in 2D protein crystalization.

Our method describes slowly diffusing systems. The driving thermodynamic force is the intrinsic chemical potential, which is calculated using a generalized Ginzburg-Landau approach. Inter-molecular interactions are modelled by mean-field, intra-molecular interactions are modelled by a Gaussian-chain model. The optimal Gaussian representation for DODA was found by fitting the single chain structure factor to Monte-Carlo results.

We simulated the behavior of DODA in water and found a rich variety of meso-scale structures as a function of the volume fraction. Similar structures are also found in experiments. Hydrodynamic effects are discussed.