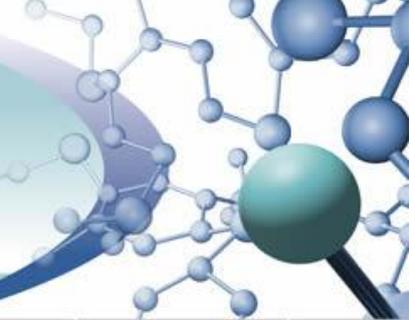


International Symposium on Polyelectrolytes

⊖ ⊕ ⊖ From Basic Science to Biological Applications

Research Workshop of the Israel Science Foundation

Ein Gedi, Israel, January 20-23, 2014



Examining Role of Different Macromolecules In Biological Lubrication

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The ultralow friction coefficients between the articular cartilage surfaces in human hips or knees play an important role in supporting human daily activities. Breakdown of this lubrication can lead to osteoarthritis, which is one of the common joint dysfunction and affects a very large portion of the population in the world. Hyaluronic acid (HA) is present at the surface of articular cartilage at high concentrations. We are extending earlier work to investigate HA using a surface force balance (SFB), by constructing bHA-avidin-mica coating, and in addition examining how complexing of these layers with different materials may affect their lubrication. Results will be presented at the meeting.



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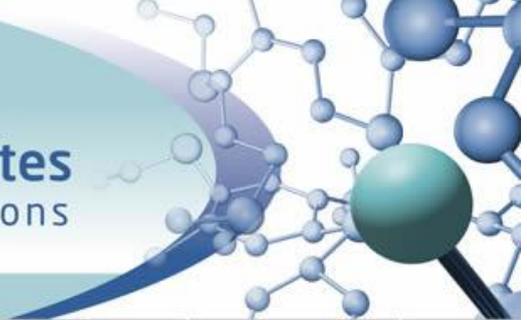


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Examining Role of Different Macromolecules In Biological Lubrication

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The Phosphatidylcholine (PC) unit is the most common head group of the phospholipids constituting artificial liposomes and of lipids extracted from the synovial fluid, and is the group exposed at the outer surface of liposomes composed of these lipids. Poly [2-(Methacryloyloxy) ethyl Phosphoryl Choline] (pMPC) is a unique biocompatible polymer that contains the PC unit as a zwitterionic side-group on the backbone of the polymer chain [1]. Brushes of the biomimetic pMPC were grown on macroinitiator-coated mica surfaces in an oxygen free aqueous solution by using Atom Transfer Radical Polymerization (ATRP) for surface force balance (SFB) measurements [2]. Shear force measurements yielded friction coefficients in the order of $\mu \sim 10^{-4}$ - 10^{-5} at compressions of up to ~ 10 MPa (100 atmospheres) across water and 0.2M salt solutions [3], and moreover we were able to characterize the brush height, adsorbance and surface density as a function of the growth conditions. We attribute this excellent lubrication performance to the hydration of the pMPC, with each PC side-group being hydrated by up to 15 or more water molecules behaving fluidly under shear motion.

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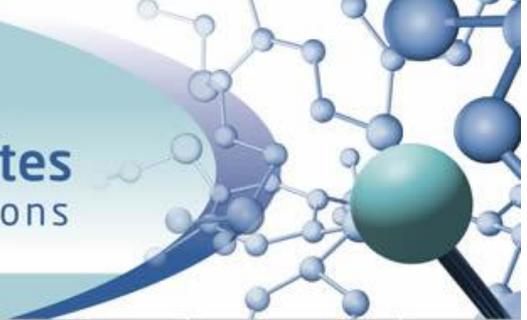


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Swelling Characteristics Of Electroactive Composite Systems Based On Acrylamide, Acrylic Acid And Polyaniline

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Recently, works are being widely carried out on the conducting polymers, so-called synthetic metals, to create nodes of fuel cells [1-3]. A number of works indicate the possibility of using polymers with electron-proton conductivity, such as polyaniline (PANI), to improve the effectiveness of platinum catalysts [4]. High level of electronic conductivity in combination with proton conductivity was indicated in polyaniline, which is critical in electrochemical processes associated with the simultaneous transport of protons and electrons. However, poor mechanical properties, poor water solubility and high melting point of polyaniline are a deterrent to use them.

In order to obtain elastic materials based on brittle polyaniline synthesis of composition systems was carried out by immobilizing the aniline in a hydrogel matrix of acrylamide (AAM) and acrylic acid (AA) of 3:1 mol/mol, respectively, which provides the required strength of material. The aim of this work was to study the kinetics of swelling of electroactive composites in water and aqueous media with different pH.

Synthesis of AAM-AA/PANI composite consisted of two stages, the first of which was receiving the polymer network of acrylamide and acrylic acid monomer and sorption of monomer by hydrogels of different concentrations (0.05-0.5 mol/L), and the second stage was polymerization of aniline by solution of ammonium peroxydisulfate (APS). The weight content of electrically conductive component, polyaniline, determined gravimetrically showed that the increasing of concentration of aniline from 0.05 to 0.15 mol/L leads to an increase in the content of conductive polymer in studied composite systems. When the concentration of aniline is 0.175-0.5 mol/L, the decrease of the content of polyaniline, which suggests the occurrence of changes in the structure of the mesh matrix AAM-AA during the formation of an electrically conductive component in it.

Degree of swelling in water and acid was measured and swelling kinetics of the pH value was studied for all of the composites. Since the degree of swelling of composites with a high concentration of polyaniline (0.05-0.5 mol/L) was significantly higher than initials, which were 0.14-0.2 g/g vs. 0.09 g/g of hydrogels without polyaniline. This proves the assumption of destruction of gel base of composition systems underwent to polymerization using APS [4]. Decrease of swelling ability of composites was noticed with increasing of concentration of aniline (0.4-0.5 mol/L). Perhaps this is due to the mechanical properties, i.e. brittleness of conductive gel.

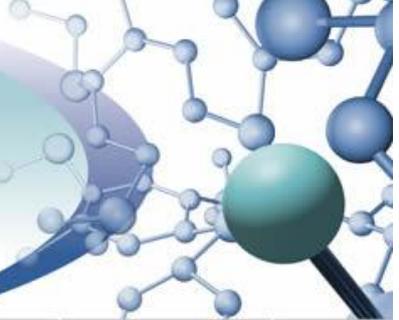
It was shown that the degree of swelling of electroactive hydrogels sharply increases in alkaline media with pH increase, it is also possible due to the destruction of the mesh structure.

It was found that the nature and concentration of the conductive polymer has a significant impact on the structure of hydrogels of acrylamide and acrylic acid.

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Ion transport in polyelectrolyte-modified nanopores

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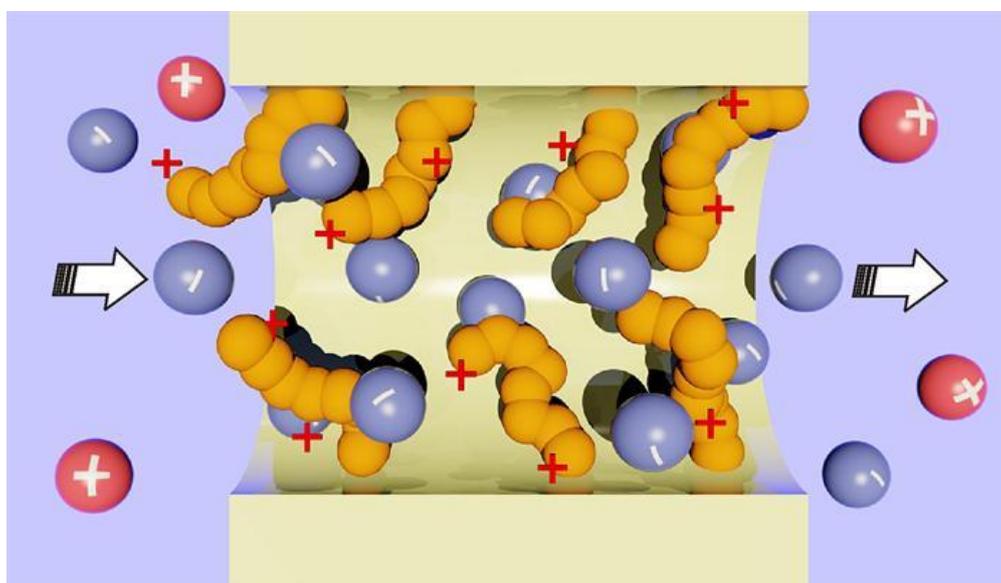
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This work introduces a molecular theory to study ion transport in nanochannels and short nanopores modified by end-grafted polyelectrolytes. The theory explicitly considers the coupling between the conformations of the polyelectrolyte chains, the electrostatic and non-electrostatic interactions, the shape, charge and volume of all molecular species in the system and the presence of ion currents. In particular, it describes the non-equilibrium organization of the polyelectrolyte layer and ion concentrations due to the presence of ion fluxes in the system.

Calculation results are presented for a short cylindrical nanopore with inner walls modified by an end-grafted polyelectrolyte layer. Ion current flows through this system upon applying a potential between the electrodes located in the reservoirs. In the very low applied potential regime, where the distribution of polyelectrolyte and ions is similar to that in equilibrium, a simple analytical model accounts for the conductance in excellent agreement with the calculations of the full non-equilibrium molecular theory. On the other hand, for a large applied potential bias the theory predicts a dramatic reorganization of the polyelectrolyte chains and the ions. This reorganization leads to non-linear (non-ohmic) current-potential curves. Moreover, it is shown that the morphology of the polyelectrolyte layer can be controlled by the direction and magnitude of the ion-fluxes.

The theory was also applied to model a neutral bare pore with outer walls modified by polyelectrolyte end-grafted layers of opposite charge. This system rectifies ionic currents: the conductivity when the positively charged polyelectrolytes face the negatively charged electrode (*on* state) is larger than when they face the positively charged electrode (*off* state). Ionic rectification is a desired property of nanopores and nanochannels as it enables applications in sensing and energy transduction. Our work shows that current rectification can be achieved in short neutral cylindrical nanopores by modification of the outer walls; this strategy is appealing for experiments since the outer walls can be chemically modified before drilling the pore. The effect of the pore dimensions and charge density of the outer walls on current rectification were analyzed. An analytical model was introduced that predicts the current-potential curves in excellent agreement with the non-equilibrium molecular theory calculations.



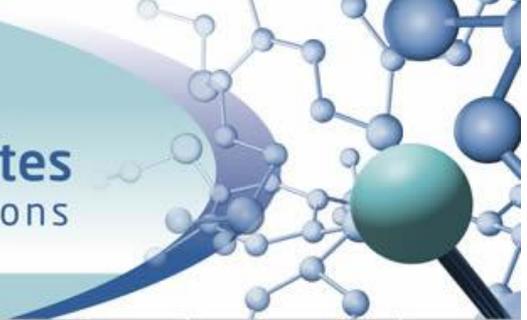


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Folding And Aggregation Of Proteins In The Presence Of Amphiphilic Polyelectrolytes

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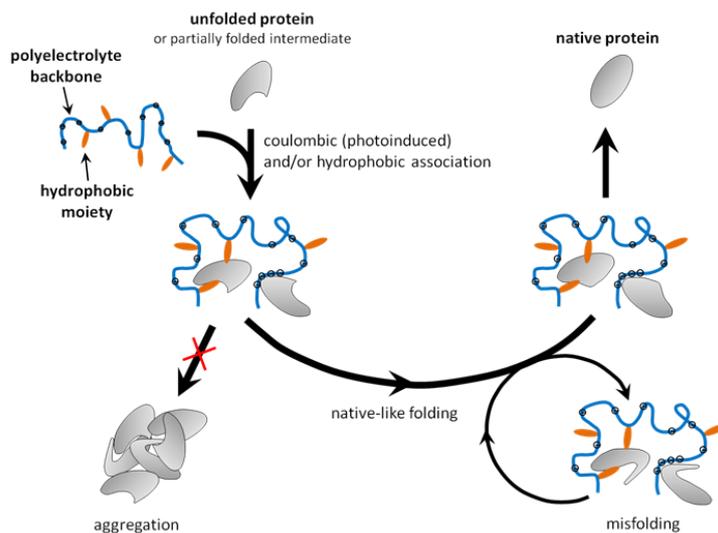
(4) WPI International Center for Materials Nanoarchitectonics (MANA), National Institute for Materials Science, Japan

Biopharmaceutical protein products constitute the fastest growing class of human drugs. The development of these engineered proteins is yet hampered by their natural propensity towards irreversible aggregation particularly critical during refolding steps. A usual mean to prevent such aggregation relies on reversible immobilization (on chromatography columns), which limits the quantities that are manipulated, or on the addition of osmolytes (detergents, short polyols or ionizable amino acids) at high concentrations, which is costly and complicates *in vivo* injection or may bias *in vitro* tests. The use of polymers, and specifically of polyelectrolytes, may circumvent such limitations by combining features of both the immobilization and the osmolyte effects. Due to multipoint binding, including hydrophobic and coulombic reversible associations, macromolecules have significantly enhanced affinities for native proteins compared to small molecules and complex formation can prevent interprotein aggregation without significant perturbation of activity [1]. Non-toxic salt additives trigger in addition the release of proteins from polyelectrolyte complexes [2]. For these reasons, polyelectrolytes appear promising for improvement of refolding yields and it is of importance to study competitions between protein folding and protein:polymer association at intermediate refolding steps (while the hydrophobic residues are gradually buried into the protein and the charged hydrophilic ones are gathered at the protein's surface).

We studied cytochrome c, a representative basic protein whose folding upon gradual crease of urea concentration is well documented, and antibody scFv fragments, as a model of complex proteins of high therapeutic value. To discriminate between the hydrophobic and coulombic contributions to protein:polymer interaction, we considered poly(acrylic acid) derivatives modified to contain a small fraction (<25mol%) of hydrophobic moieties. *In situ* variations of either charge density or hydrophobicity were obtained by introducing light-sensitive photo-ionizable hydroxyazobenzene groups or neutral azobenzene ones [3,4]. Circular dichroism and intrinsic fluorescence were used to characterize the protein folding. Fluorescence correlation spectroscopy and capillary electrophoresis were implemented to characterize the polymer:protein complexes along the refolding pathway.

In the case of scFv fragments, the efficient protection of polyanions at a weight ratio as low as 1:1 (*i.e.* typically at micromolar concentrations) was confirmed by native-like folding with no aggregation. Surprisingly, we found that the charge of the polyanions is of utmost importance in their interaction with the partially folded intermediates. The predominant contribution of coulombic interactions was confirmed by the disappearance of association at high ionic strength [5]. Electrostatic modeling of scFv surface showed positively-charged patches which may be responsible for the interaction with polyanions. Coulombic binding also appeared to be the predominant driving force that facilitate unfolding of cytochrome c in water/urea solutions. Upon exposure to light, photo-ionization was shown to affect the degree of unfolding, whereas photo-switch of the azobenzene with no ionization was ineffective at perturbing the protein, confirming that protein handling and control of folding can be achieved by playing with the coulombic interaction and photoionization of chromophores [6].





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Electrospinning Polyelectrolyte Complex: Ph Responsive Fibers

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Polyelectrolytes are good candidates as active polymers. Thin multilayers of Polyelectrolytes complex demonstrate isotropic switching properties in response to pH external stimuli. Example to such complex is PAA (poly acrylic acid) and PAH (poly allylamine hydrochloride). Switchable properties are achievable due to switchable intermolecular interactions – shifting from ionic interactions to hydrogen bonds depending on surrounding. We propose to enhance interactions between those polyelectrolytes by fabricating quasi 1D nanofibers using electrospinning. Electrospinning is a kinetic process in which fibers comprising aligned and highly confined chains are formed under high electric field. It is assumed that this chains' packaging will enhance intermolecular interactions along the fibers axis, resulting in enhancement of anisotropic physical properties of the responsive fibers. Polymer solution of 1:1 PAA/PAH was successfully electrospun resulting in fibers with diameters ranging from 300 to 800nm. Differential scanning calorimetry analysis of the fibers showed complete miscibility of the polymers. Anisotropic swelling of 600% increase in fiber diameter was observed when shifting from neutral to acidic aqueous surrounding. Electrospun responsive fibers can be implemented into various biological and industrial systems due to their high sensitivity and unique structure.



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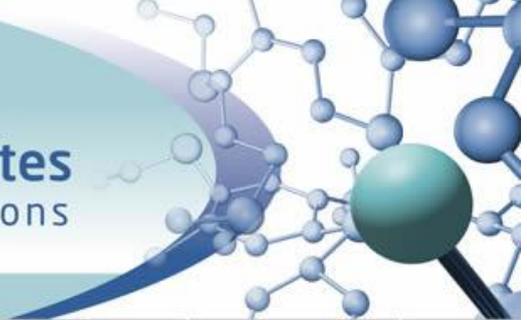


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Structures and interactions of intermediate-filament complexes

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Intermediate filaments (IFs) are one of three components of the cytoskeletal system, along with actin and microtubules. IFs are rather versatile: their expression patterns are cell- and tissue-type specific, providing each major cell type with a specific set of IF proteins specially designed to mechanically support its needs. The study of IFs interactions is key for understanding the IF network's structure, stability and functions. In order to understand the way molecular alterations in the different IF proteins translates to designated supramolecular interactions, in our research we study neuronal IF protein structures and interactions. The main role recognized for neuronal IF is to increase the axonal caliber, thereby increasing nerve conduction velocity. Our study includes the three NFs subunit proteins (NF-L/NF-M/NF-H) and α -internexin.

The IF hydrogel's structures and interactions are examined using our in-house small angle X-ray scattering (SAXS) device and using 3rd generation synchrotron sources. The study of how supramolecular forces such as electrostatic, hydrophilic/phobic, and H-bonding are affecting on the above mentioned proteins and networks is achieved by tuning salt concentrations, pH, osmotic pressure, and temperature.



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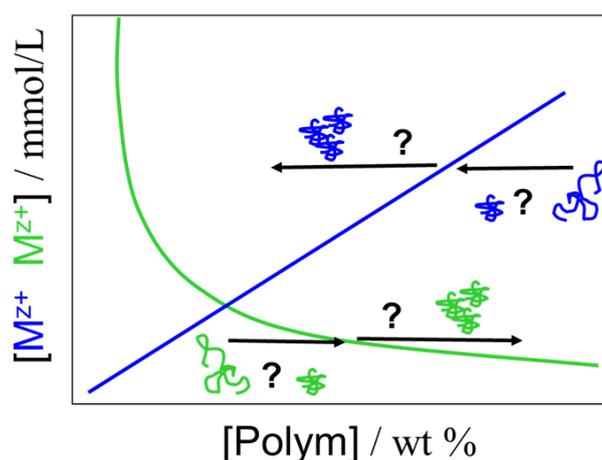
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Specific Interactions of Cations with Polystyrenesulfonate in Dilute Solution and the Impact on the Phase Behaviour

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Anionic polyelectrolyte chains interact specifically with a number of metal cations in dilute solution. These interactions initially induce a collapse of the coils and cause a precipitation of the corresponding metal salt once a characteristic threshold is crossed. Two different types of thresholds could be identified. One threshold follows a phase boundary, which indicates a growing number of the metal cations required to precipitate the polyelectrolyte with increasing polyelectrolyte concentration and which thus corresponds to a stoichiometric amount of metal cations necessary to precipitate the respective amount of polyelectrolyte chains. This trend is called S-threshold.¹ The second type of behavior, denoted as M-threshold, obeys the law of mass action, where the amount of metal cations necessary to precipitate the polyelectrolyte decreases with increasing concentration of polyelectrolyte.² A particularly well investigated system in this field is anionic polyacrylates (PA) and the salts with various specifically interacting cations. Anionic PA chains exhibit an S-threshold with alkaline earth cations in dilute aqueous solution. Upon approaching this threshold, the PA chains collapse to compact particles, which start to aggregate once the threshold is crossed.³ Similar mechanistic details from other types of polyelectrolytes are still lacking as does the knowledge on the behavior of polyelectrolyte chains upon approaching and crossing the M-threshold. Also, the question whether anionic chains behave similar at an M-threshold (or an S-threshold), independent of its chemical nature is an entirely unsolved problem. It is these open questions, which shall be addressed in the investigation of polyelectrolytes in the presence of multivalent cations. To this end, experiments with polystyrenesulfonate (PSS) in 0.1 M NaCl-solution in the presence of Ba^{2+} , Fe^{3+} - and Al^{3+} -ions were carried out in order to significantly broaden our knowledge on the phase behavior of polyelectrolytes with specifically interacting cations and morphological transformations induced close to the precipitation threshold. Care had been taken that the systems under consideration also include examples which exhibit an S-threshold.⁴ The experiments comprised simple turbidity measurements to establish phase diagrams as well as detailed light scattering close to the phase boundary in order to reveal structural information on single coils and aggregates at the respective boundary.



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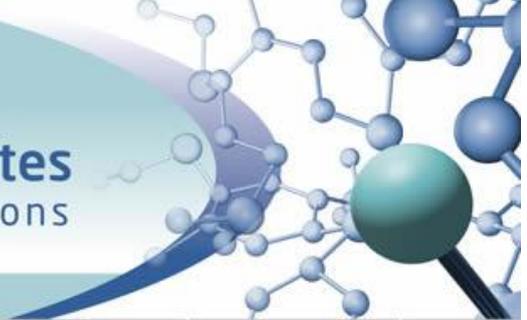


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Modeling The Effect Of Adsorbed Polyelectrolytes On The Potential Of Charged Particles For Electrophoretic Deposition Aspects

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The electrophoretic-deposition (EPD) method was tested in our electrochemistry research group to fabricate pristine and composite ceramic-polymer membranes for application in planar and 3D microbattery configurations. The goal of this work is to gain a phenomenological understanding of the EPD process for a system of charged colloidal particle covered with a polyelectrolyte (PE).

As a key factor for the different aspects of EPD (e.g. electrophoretic mobility, ion conductivity and particles interactions) is the electric potential, we developed a model for it on the basis of the Poisson-Boltzmann (PB) relation. To be close to experiment, the model addresses the physical parameters of the system used in our lab i.e. Yttria-Stabilized-Zirconia (YSZ) nanoparticles covered with polyethylenimine (PEI) as a dispersant in ethanol [1]. We simulate a set of scenarios in attempt to present the influence of the pH, surface potential, PE density, and the PE brush length on the electric potential and charge distribution profiles as a function of distance from the solid-surface/brush interface, where 'brush' is one of the possible PE configurations. Our results enabled us to draw conclusions on the effect of a PE additive on the suspension stability, as well as the morphology and electrokinetics of the electrophoretically deposited PE-stabilized particles.

In this work a fast and easy-to-operate simulator was derived, which enables flexibility in choosing any set of parameters that satisfies the model assumptions. Special algorithm was derived for PE brush with lengths smaller or comparable with the screening length. In addition, full analytical expression for the brush/bulk interfaces potential (V_s) and the Donnan potential (V_D) were first presented in this study, which increases the accuracy of our model for a pH-dependent PE brush, unlike former studies [2]. The novelty of our model with respect of previously developed models [3, 4] is the combination of charged solid-surface ('bare' particle) with a charged brush-like PE layer ('soft' particle).

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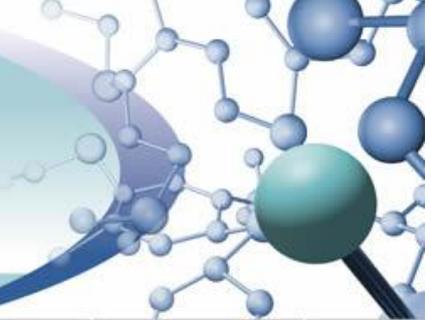


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Complexes of Charged Lipids and Oppositely Charged Polyelectrolytes Characterized by Cryo-TEM

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In recent years there has been rising interest in the study of the mechanisms governing the complexation of polyelectrolytes and oppositely charged lipids. This is due to their interesting and still not completely understood phenomenology, and more so to the increased awareness of their potential for innovative applications in nano-medicine and nano-biotechnology, as in gene-delivery.

In this work we used direct-imaging cryo-TEM to study the structural evolution on the nano-level, as the charge-ratio between a negative polyelectrolyte and a cationic lipid is changed. Charge ratio (CR) is the ratio between the negatively charged groups of the polymer and the positively charged groups of the lipids. This system was composed of reduced bis(11-ferrocenylundecyl)-dimethylammonium bromide (BFDMA), a positively charged synthetic double-tailed cationic lipid and sodium poly(acrylic acid) (NaPAA), a negatively charged polyelectrolyte.

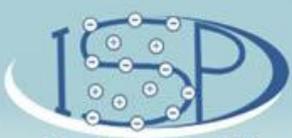
We observed different nanostructures with changing CR. First, solution of pure BFDMA (1mM) had only polydispersed population of large unilamellar vesicles, 200 nm – 1 μ m, in diameter. Upon addition of appropriate amount of PAA to the BFDMA solution to give varying values of CR, we saw formation of the multilamellar complexes. The unilamellar vesicles start to add on more layers of cationic lipid, where the polyelectrolyte is sandwiched in the middle screening the electrostatic repulsion. As CR approached unity, the structures tended to aggregate and form larger complexes, since each complex has a neutral charge, which allows them to get in close proximity. Once they are close, they are attracted to each other by short-range interactions. Beyond CR of 1, aggregated clusters are not seen, and the structure average size decreases. However, only at CR of 2 we observed structures that are faceted. At other CR > 1 values, round multilamellar liposomes were dominant structure in solution.

Another system under current investigation is the system of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and NaPAA. We would like to find out whether the trends described above hold in another type of cationic lipid.



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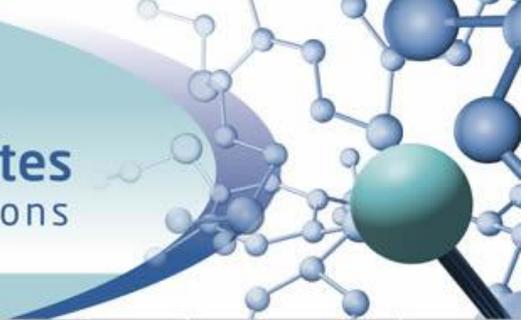


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Unraveling and eliminating dissipation mechanisms in contacts of polymer-bearing surfaces

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Polymer brushes are well known to lubricate high-pressure contacts, because they can sustain a high normal load while maintaining low friction at the interface. Depending on the contact-geometry, direction of motion and brush characteristics, different dissipation mechanisms dominate the friction forces. For example, in a parallel plate geometry the interdigitation of the opposing polymers determines the lubricity, while for spherical star polymers in relative motion, viscoelastic deformation governs the energy dissipation. We discuss the relative importance of the dissipation channels for real contacts and show via molecular dynamics simulations and atomic force microscopy measurements that, by using an asymmetric contact of two immiscible polymer brush systems, the important dissipation-mechanisms – interdigitation and capillary break-up – can be eliminated. Moreover, viscoelastic deformation is strongly reduced. For such immiscible polymer brush systems, we find that the friction upon sliding is a few orders of magnitude lower than for symmetric miscible contacts. Our developed system therefore holds great potential for application in industry.



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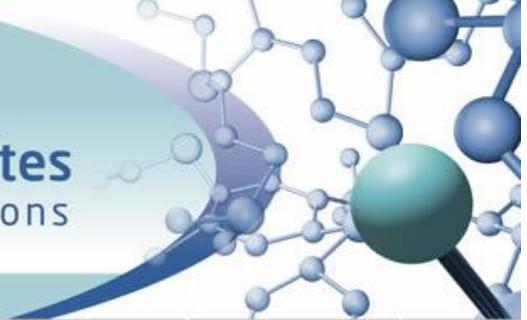


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Effect Of Counter-Ion Exchange Between Charged Macromolecules

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We study the effect of the counter-ion exchange between charged macromolecules on their interactions and also on the dielectric response of the macromolecule solutions. We show, by scaling theory and Monte Carlo simulations, that the counter-ion exchange between macromolecules induces like-charge attraction, which have originated from monopole-monopole interactions, in the highly charged limit. Moreover, we find that the dynamical time scale of the counter-ion exchange in a polyelectrolyte (PE) solution is responsible for the dielectric response in so-called the super low frequency (SLF) regime, which was found and suggested in recent simulation work [S. Fischer and R. R. Netz, *The European Physical Journal E*, 36, 117 (2013)]. Our results show the effect of thermally activated barrier-hopping of counter-ions on the relaxation time of the SLF mode.



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