Interaction between charged membrane surfaces mediated by charged nanoparticles

J. Pavlic[1,2], A. Iglic[1], V. Kralj-Iglic[3], K. Bohinc[1,2]

[1] Faculty of Electrical Engineering, Trzaska 25, 1000 Ljubljana, Slovenia
[2] University College for Health Studies, Poljanska 26a, 1000 Ljubljana, Slovenia
[3] Faculty of Medicine, Lipiceva 2, Ljubljana, Slovenia

Abstract— **The interaction between charged membrane surfaces, separated by a solution containing charged nanoparticles was studied experimentally and theoretically. The non-local theory for the nanoparticles was developed where finite size of nanoparticles and spatial distribution of charge within a particle were taken into account. It was shown that for large enough membrane surface charge densities and large enough dimensions of nanoparticles, the force between equally charged membranes may be attractive due to spatially distributed charges within the nanoparticles.**

Keywords— **Nano particles, charge density, membrane surface**

I. INTRODUCTION

In biology, there are many phenomena which motivate the studies of electrostatic interaction between charged macroions in the electrolyte solution. The condensation of DNA can be induced by the presence of multivalent counterions [1, 2], and corresponds to the packing of DNA in viruses. The complexation of DNA with positively charged colloidal particles [3, 4] was observed, which corresponds to nucleosome core particles and the basic fiber of chromatin. Network formation in actin solutions [5] is the consequence of the attractive interactions between cytoskeletal filamentous actin molecules mediated by multivalent ions. The aggregation of rod-like M13 viruses is induced by divalent tunable diamin ions [6].

In this work we study interaction between negatively charged membrane surfaces of giant phospholipid vesicles in the sugar solution containing multivalent rod-like ions. The negative charge of the phospholipid bilayers was generated by a certain proportion of the phospholpid cardiolipin while the multivalent cations are represented by Spermidine and Spermine. Adhesion of phospholipid vesicles due to the presence of Spermidine or Sperimine in the solution was observed. To describe the observed features, a theoretical model was constructed where the phospholipid membranes are described as infinite flat surfaces bearing uniformly distributed charge while the Spermidine and Spermine are considered as rod-like counterions. Due to the specific shape of these molecules which bear charge at their ends, it is taken that the charge distribution within Spermidine and Spermine is represented by two effective charges e, separated by a distance l. The system is described by the non-local theory of the electric double layer, where the shape and the orientational restrictions of the rod-like ions is considered.

II. MATERIALS AND METHODS

A. Spermidine and Spermine polyamine molecules

Polyamines Spermidine and Spermine (Fig. 1) were purchased from Sigma-Aldrich. The Spermidine is tri-valent, while the Spermine has the valency of four. They are positively charged (amino groups contribute to the charge). Spermidine and Spermine were obtained in powder and were dissolved in distilled water to the final concentration of 2mg/ml.
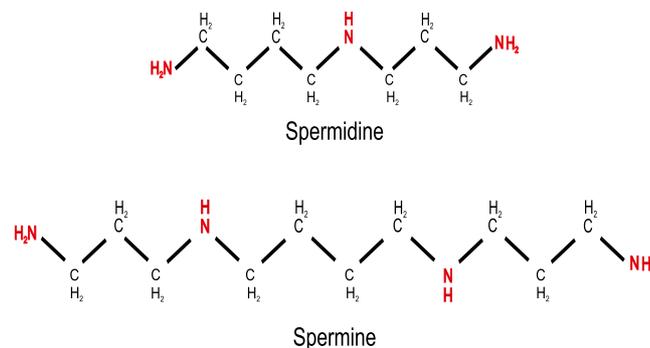

Fig. 1 Schematic presentation of Spermidine and Spermine polyamines.

B. Giant phospholipid vesicles (GPV)

GPVs were prepared at room temperature (23°C) by the modified electroformation method [7]. The synthetic lipids cardiolipin (1,1'2,2'-tetraoleoyl cardiolipin), POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), and cholesterol were purchased from Avanti Polar Lipids, Inc. Ap-

propriate volumes of POPC, cardiolipin and cholesterol, all dissolved in a 2:1 chloroform/methanol mixture, were combined in a glass jar and thoroughly mixed. POPC, cholesterol and cardiolipin were mixed in appropriate proportions such as 2:2:1. Volume of 20 μl of lipid mixture was applied to the platinum electrodes. The solvent was allowed to evaporate in a low vacuum for 2 hours. The coated electrodes were placed in the electroformation chamber which was then filled with 3 ml of 0.2 M sucrose solution. An AC electric voltage with an amplitude of 5 V and a frequency of 10 Hz was applied to the electrodes for 2 hours, which was followed by 2.5 V and 5 Hz for 15 minutes, 2.5 V and 2.5 Hz for 15 minutes and finally 1 V and 1 Hz for 15 minutes. The content was rinsed out of the electroformation chamber with 5 ml of 0.2 M glucose and stored in plastic test tubes at 4°C. The vesicles were left for sedimentation under gravity for one day and were then used for a series of experiments.

C. Observation

Vesicles were observed by an inverted microscope Zeiss Axiovert 200 with phase contrast optics and recorded by the Sony XC-77CE video camera. The solution containing vesicles was placed into observation chamber made from cover glasses and sealed with grease. The larger (bottom) cover glass was covered by two smaller cover glasses, each having a small semicircular part removed at one side. Covering the bottom glass by two opposing cover glasses formed a circular hole in the middle of the observation chamber. In all experiments the solution of vesicles (45 μl) was placed in the observation chamber. The solution containing the substance under investigation (Spermidine or Spermine) was added into circular opening in the middle of the observation chamber.

III. Experiment

The solution of GPVs contains a heterogeneous population of vesicles of different shapes and sizes. The vesicles are subject to thermal fluctuations of their shape. Few minutes after the addition of either Spermidine or Spermine to the solution with GPVs the thermal fluctuations of vesicles diminish while the vesicles adhere to each other and to the ground. Further away from the site of insertion of Spermidine or Spermine, the process of adhesion may take up to 30 min. Vesicles slowly approach each other, but once acquiring a small distance they adhere to each other.

The complexes formed by the adhesion of charged giant phospholipid vesicles after the addition of Spermidine and Spermine is shown in (Fig. 2) and (Fig. 3), respectively.
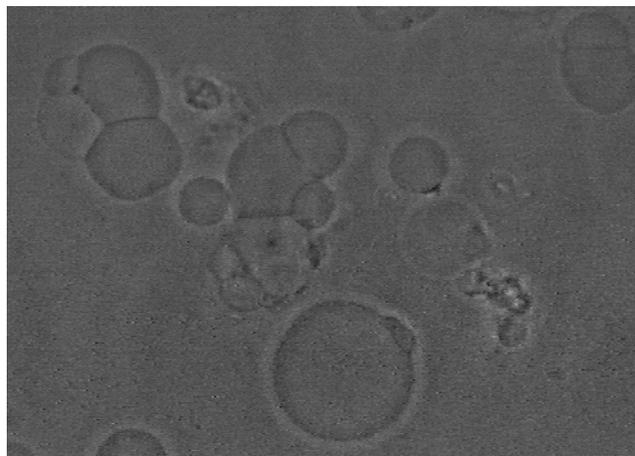

Fig. 2 The solution of giant phospholipid vesicles containing 20% weight ratio of cardiolipin few minutes after the addition of Spermidine to the solution with vesicles.
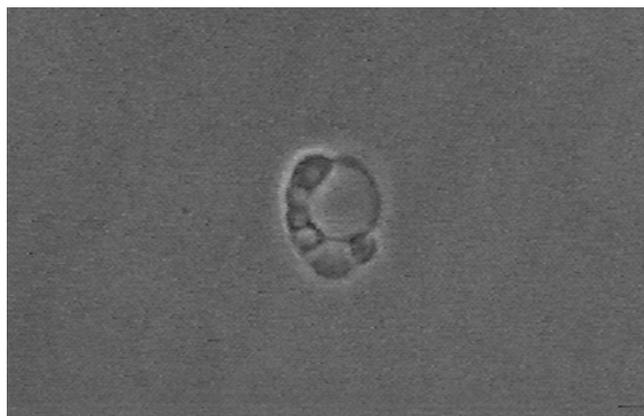

Fig. 3 A complex of liposomes formed by adhesion of giant phospholipid vesicles containing 20% weight ratio of cardiolipin few minutes after the addition of Spermine to the solution with vesicles.

IV. Mathematical model

We consider an aqueous electrolyte solution containing divalent rod-like ions. The solution is sandwiched between two equally charged planar surfaces with surface charge density σ. The two surfaces of area A are separated by a distance D. The description of the system is based on the non-local theory of the electric double layer where the rod-like ions are characterized by positional and orientational degrees of freedom. The energy is therefore stored in the electrostatic field as well as in the translational and orientational entropy of the rod-like ions. The electrostatic free energy of the system is

$$\frac{F}{AkT} = \frac{1}{8\pi l_B} \int_0^D \Psi'^2 dx + \int_0^D [n(x)\ln[v_0 n(x)] - n(x)]dx +$$

$$\int_0^D n(x) < p(l\,|\,x)[\ln p(l\,|\,x) + U(x,l)] > dx + \tag{1}$$

$$\int_0^D n(x)\lambda(x)[< p(l\,|\,x) > -1]dx + \mu \int_0^D [-2n(x) - \frac{2\sigma}{De}]dx$$

where Ψ is the reduced electrostatic potential, μ is the reduced chemical potential, $U(x,l)$ is the external reduced potential of the charged wall, $n(x)$ is the local concentration of reference charges of multivalent rod-like ions, $p(l|x)$ is the conditional probability density describing the position of the second charge on the rod-like counterion if the first charge is located at x, $l_B = e^2/4\pi\epsilon kT$ is the Bjerrum length, ϵ is the dielectric constant of water, k is the Boltzmann constant, T is the absolute temperature, v_0 is the volume of the divalent ion while $< ... >$ indicates the averaging over all possible orientations. Two constraints are added to the free energy describing the normalization condition for the probability density and the electro-neutrality of the system. The equilibrium state of the system is obtained by the minimization of the free energy (1). The variational problem is stated by means of integro-differential equation which is solved numerically. As a result we obtain the consistently related equilibrium positional and orientational distribution functions for the counterions and the equilibrium free energy of the system. The dependence of the equilibrium free energy on the distance between the charged surfaces reveals the nature of the interaction between the surfaces. If the free energy increases with increasing distance between the surfaces, then the force between the surfaces is attractive. The equilibrium distance between the surfaces is obtained at the mininum of the dependence of the free energy on the distance D.

The free energy of the system as a function of the distance between two negatively charged surfaces is shown in (Fig. 4).

For large enough σ the free energy first decreases with increasing distance D, reaches a minimum and then increases. For small σ the free energy monotonously decreases with increasing distance D. The minimum of the free energy is more pronounced for longer rod-like ions. The insets show a scheme of the most probable orientation of rod-like ions at minimal free energy.

V. DISCUSSION AND CONCLUSIONS

The presented model provides a simplified analysis of the problem. In this paper we studied the interaction between negatively charged surfaces mediated by positively charged tri-valent Spermidine and four-valent Spermine.

The experiments showed the adhesion of GPVs after the addition of Spermidine and Spermine. This means that even though the surfaces are negatively charged they are attracted to each other. In order to better understand the experimental effects we considered the theoretical model, where the Spermidine and Spermine are considered as rod-like ions [8] and GPVs are described by two equal planar surfaces. The theory confirmed the attraction of negatively charged surfaces mediated by charged nano particles.

The attraction between equally charged surfaces originates from correlations between the multivalent counterions, which are not considered in the mean field Poisson-Boltzmann theory [9]. Theoretically, the Monte Carlo (MC) simulations of Guldbrand et al. [10] have first confirmed the existence of attraction between equally charged surfaces immersed into the solution composed of divalent ions in the limit of high surface charge density, which were originally predicted by Oosawa [11]. Further, MC simulations show that the attractive interaction between equally charged surfaces may arise for high surface charge density, low temperature, low relative permittivity and polyvalent counterions [12]. Also the anisotropic hypernetted chain approximation within the primitive electrolyte model for divalent ions was used [13, 14], where the ions were described as charged hard spheres immersed in a continuum dielectric medium. At moderate surface-surface distances and high surface charge density the attraction between the equally charged surfaces was found.
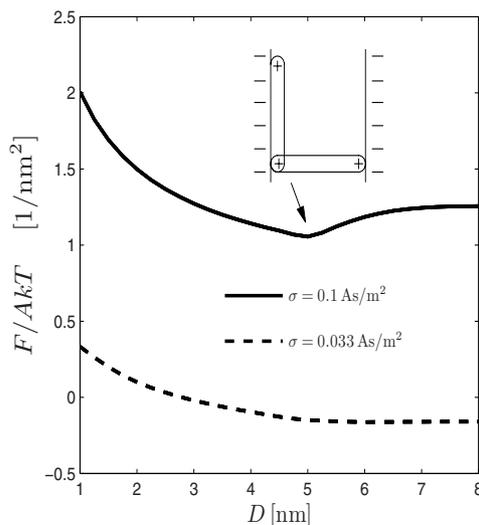

Fig. 4 The free energy as a function of the distance between two equally charged surfaces. The model parameter is l = 5 nm.

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Author: Janez Pavlič
Institute: University College for Health Studies
Street: Poljanska 26a
City: Ljubljana
Country: Slovenia
Email: janez.pavlic@fe.uni-lj.si