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and Liposomes**

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ADVANCES IN PLANAR LIPID BILAYERS AND LIPOSOMES

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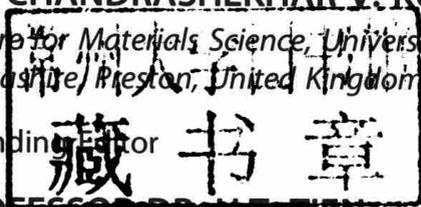
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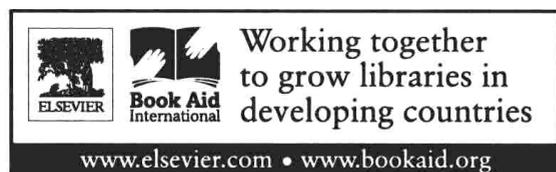
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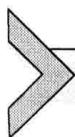
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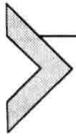
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CONTENTS

<i>Contributors</i>	<i>ix</i>
1. Charged Particles can Mediate Attraction Between Equally Charged Membranes—Theoretical Study	1
Šárka Perutková	
1. Biological Systems in Electrolyte Solutions	2
2. Theoretical Basis	5
3. Theoretical Methods and Simulation	12
4. Application of Methods to Different Biological Systems	22
5. General Discussion	42
6. Conclusions	45
References	46
2. Cationic Liposomes as Model Nonviral Vectors for Pulmonary Delivery of DNA	53
Abdelbary Elhissi, Israr Ul Hassan, Antigoni Papapanou, Nimrah Zeb, and Kevin M. G. Taylor	
1. Introduction	54
2. Viral Versus Nonviral Vectors of Genes	54
3. Cellular Uptake of Lipoplexes	56
4. Gene Therapy via Pulmonary Delivery	56
5. DNA Expression in the Lung	58
6. Factors Influencing Expression of Gene in the Lung Using Lipoplexes	59
7. Novel Strategies for Pulmonary Delivery of Genes	62
8. Conclusions	63
References	63
3. Giant Unilamellar Vesicles and Suspended Nanobilayers as Model Systems for Biophysical Research	67
Oliver J. Pambos and Ulrich F. Keyser	
1. Introduction	68
2. Giant Unilamellar Vesicles	69
3. Case Study: Nanobilayers on Glass Supports	77
4. Summary	86

Acknowledgments	86
References	87
4. Hydrophobically Modified Chitosan: Solution Properties by Rheology and Light Scattering	91
Aarti S. Shedge and Manohar V. Badiger	
1. Introduction	92
2. Experimental	94
3. Results and Discussion	96
4. Conclusions	101
Acknowledgments	101
References	101
5. Structural Transitions in Lipid Membranes: Mechanism for Cell-Penetrating Peptides	103
Abhijit Mishra	
1. Introduction	104
2. Structure of Lipids	105
3. Polymorphism of Lipids in Aqueous Solution	107
4. Cubic Phases in Lipids	111
5. Characterization of Lipid Phases	113
6. Cell-Penetrating Peptides	115
7. Proposed Cellular Uptake Mechanisms	116
8. Experimental Methods	119
9. Peptide–Lipid Interactions	123
10. Conclusion	130
References	131
6. Biomimetic Liposome Model Systems to Study Cell Shape Control by the Cytoskeleton	139
Feng-Ching Tsai, Sophie Roth, Marileen Dogterom, and Gijsje Hendrika Koenderink	
1. Introduction	140
2. Creating Cell-Sized Liposomes Encapsulating Cytoskeletal Polymers	144
3. Toward Complex Cytoskeletal Model Systems	153
4. Conclusion and Perspective	159
Acknowledgments	160
References	160

7. Role of Blood Sampling in Assessment of Concentration of Extracellular Nanovesicles in Isolates from Peripheral Blood	175
Roman Štukelj, Ines Hribar Ignaščenko, Sonja Peternelj, Magda Peruško, Tatjana Blažič, Manca Pajnič, Špela Bračun Vnuk, Vid Šuštar, Apolonija Bedina Zavec, Karin Schara, Rado Janša, and Veronika Kralj-Iglič	
1. Introduction	176
2. Material and Methods	179
3. Theory	180
4. Experimental Results on the Free Flow	182
5. Discussion	184
References	187
 <i>Index</i>	 191



Charged Particles can Mediate Attraction Between Equally Charged Membranes— Theoretical Study

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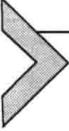
Contents

1. Biological Systems in Electrolyte Solutions	2
1.1 Cell membrane structure and its electric properties	2
2. Theoretical Basis	5
2.1 Electric double layer theory	5
2.2 Some important parameters in the electrostatics of membranes	10
3. Theoretical Methods and Simulation	12
3.1 Poisson–Boltzmann equation with additional boundary conditions	12
3.2 Functional density theory for spherical counterions and co-ions	14
3.3 Monte Carlo simulations	18
4. Application of Methods to Different Biological Systems	22
4.1 Attraction between negatively charged vesicles in contact with a solution of plasma proteins	22
4.2 Electrostatic interactions between osteoblasts and an implant surface	27
4.3 Influence of ionic strength on β_2 glycoprotein I-induced vesicle agglutination	33
5. General Discussion	42
6. Conclusions	45
References	46

Abstract

Electrostatic interactions are one of the leading interactions in all biological systems. In this chapter, we present a possible mechanism which can lead to attractive interaction between two like-charged biological surfaces. We show three different modeled systems of charged biological surfaces in the solution containing charged macro-ionic particles which were inspired by experimental observations. In our proposed mechanism, we take into consideration the orientational entropy of the system. Namely, when the

macro-ionic particles in solution have distinctive internal distribution of charge, the additional degrees of freedom for rotation are involved in the entropic contribution to the total free energy. The contribution is negative and thus lowers the free energy. Under certain conditions, this can lead to attractive force between like-charged surfaces. In the study, we use analytical as well as numerical methods—modifications of Poisson–Boltzmann equation and Monte Carlo simulations. We studied rod-like and spherical geometry of particles which can mediate the attraction. The macro-ionic particles can represent different kinds of proteins or various metallic nanoparticles in aqueous surrounding.



1. BIOLOGICAL SYSTEMS IN ELECTROLYTE SOLUTIONS

Cells, macromolecules, and all other components in biological systems are embedded in aqueous media (Fig. 1.1) with a high dielectric constant ($\epsilon_r \cong 80$). The salt concentration of the electrolyte in the cell's environment is around 0.15–0.2 mol/l [1,2]. The electrostatic properties of such electrolyte solutions have a considerable effect on the different processes occurring in biological systems. The functioning of various processes (transport through ion channels, DNA–protein interactions, action and mechanism of biological molecules, etc.) would fail to work without them. Because of their long-range character, electrostatic interactions are of fundamental importance in understanding many biological processes. Many membrane properties such as structure, rigidity, and dynamics are directly affected by electrostatics [1].

1.1. Cell membrane structure and its electric properties

The main function of the biological membrane is to enclose the cell and the interior of the cell's organelles and separate them from their surroundings, both in prokaryotes and eukaryotes. The backbone of a biological membrane is built up of membrane lipids self-assembled into the lipid bilayer. The forces holding the bilayer together originate from the amphiphatic nature of membrane lipids. The heads of membrane lipids are polar (hydrophilic) and the tails (or chains) are nonpolar (hydrophobic). The hydrophobic forces lead membrane lipids to hide their tails from water and, on the other hand, to expose their heads to water (Fig. 1.2). Other components of the biological membrane are various kinds of proteins (integral proteins, peripheral proteins, structural proteins, etc.), glycoproteins, and glycolipids embedded in the lipid bilayer.

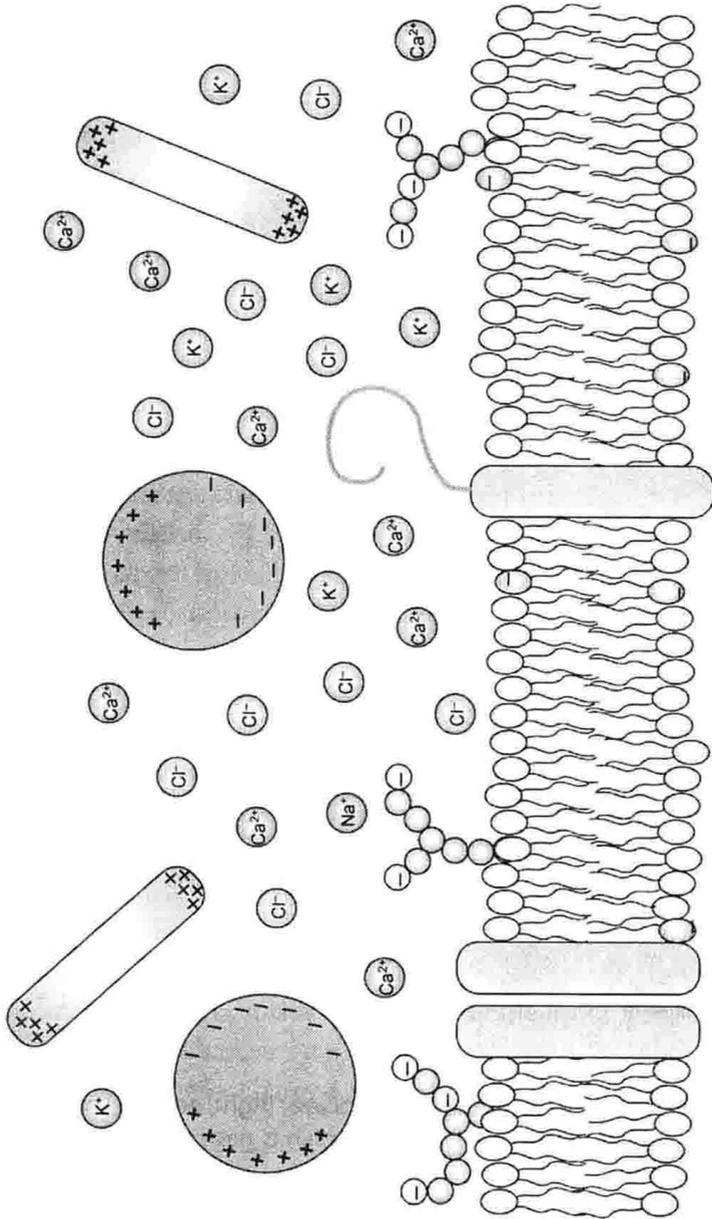


Figure 1.1 Schematic figure: all biological components are immersed in electrolyte solution. The interplay of electrostatic interactions between all of the components is of fundamental importance.

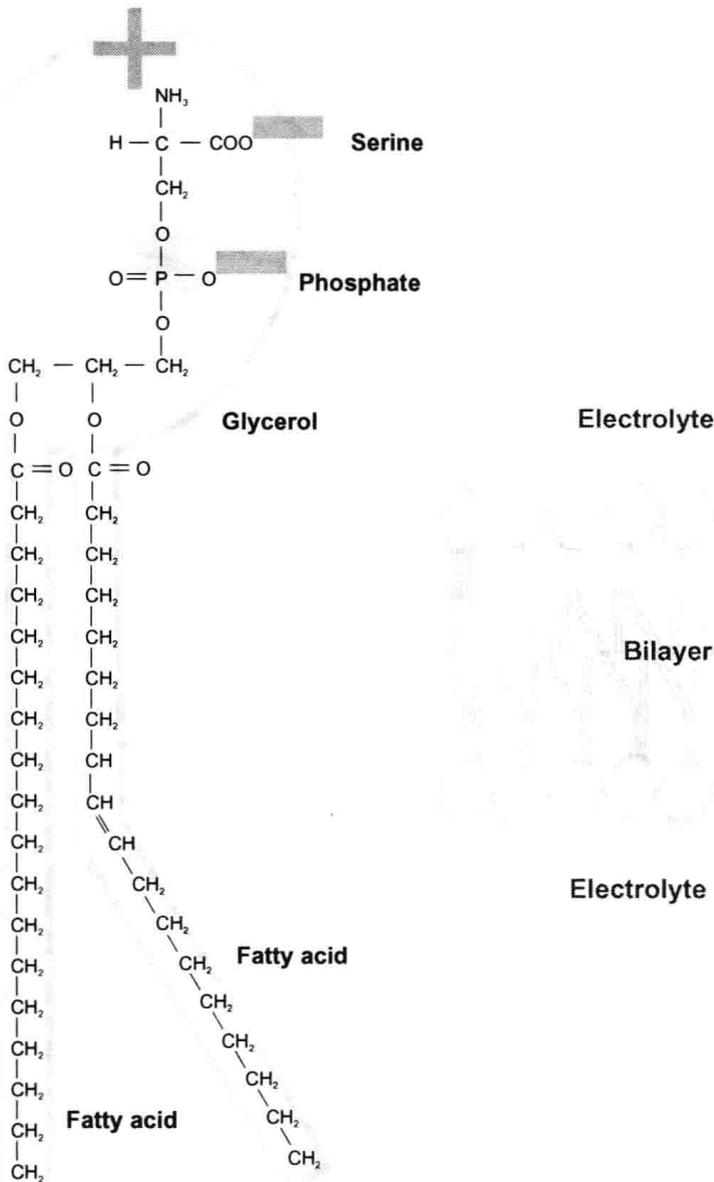


Figure 1.2 Schematic figure of a phospholipid bilayer (right) and the chemical structure of a single phospholipid (phosphatidylserine) which is negatively charged (left).

There exist various kinds of cell membrane lipids. One of the most important groups are phospholipids. The head of a phospholipid can be neutral with a nonzero dipole moment or negatively charged. It is rather mysterious that nature choose not to create positively charged membrane lipids. These can only be made artificially (e.g., a surfactant sterylamine is positively charged).

Neutral (zwitterionic) lipid heads have a dipole moment because the phosphate group carries a negative charge and the alcohol (or aminoalcohol) which esterifies the phosphate group usually has a positive charge. Zwitterionic lipids include, for example, phosphatidylcholines, phosphatidylethanolamines, sphingomyelin, and others. However, some alcohols esterifying the negatively charged phosphate group are neutral and thus the resulting charge of the lipid headgroup is negative (Fig. 1.2). The most known negatively charged phospholipids are phosphatidylserines, phosphatidylinositols, and phosphatidylglycerols (cardiolipin). The ratio between neutral and negatively charged lipids in plasma membranes is approximately 9:1 [3]. Phosphatidylserines are present, for example, in the inner part of the red blood cell membrane. Cardiolipin, which has four fatty acid chains, is very abundant in inner mitochondrial membranes. Though phosphatidylinositol is a minor component in biological membranes, it plays an important role in guiding membrane traffic and cell signaling [4].

Because of the presence of negatively charged lipids, the whole bilayer can also be negatively charged. The maximum surface charge density of a lipid bilayer is around -0.3 As/m^2 if the whole bilayer is composed only of negatively charged lipids [5]. In nature, however, the surface charge density of phospholipid bilayers is considerably lower (according to the ratio between negatively charged and neutral lipids in membranes). Negatively charged lipids usually occupy the inner part of the membrane.

Besides the negative charge from the lipid bilayer, the whole cellular surface can also be treated as uniformly charged because of the thick layer of negatively charged glycoproteins covering the plasmatic membrane. The average negative fixed charge on the cell surface is of the order of -0.001 As/m^2 [6].

As it was already mentioned, charged surfaces in biological systems can consist of macromolecules such as DNA, whole cells, liposomes created from a mixture of neutral and charged membrane lipids, surfaces of implants, and others. In this chapter, we focus on negatively charged liposome–liposome and negatively charged implant–cell interactions.



2. THEORETICAL BASIS

2.1. Electric double layer theory

The interaction between charged surfaces in an electrolyte solution is governed by the interplay between the electrostatic forces on the surfaces and the entropy of ions moving between them due to their thermal motion.

Entropy favors a uniform distribution of ions. On the other hand, electrostatics prefers all counterions to be clustered at the surface and all co-ions moved far away from it. The basic theory describing these interactions is the theory of the electric double layer. According to this theory, freely moving counterions from the solution are distributed mainly near the charged surfaces (without binding to them) and thus screen the surface electric potential, whereas co-ions are mostly depleted from the surfaces, as is described in Fig. 1.3.

The electric double layer has been the subject of extensive study since the pioneering work of Gouy [7] and Chapman [8] around 1910. The so-called Poisson–Boltzmann theory (PB theory) is the basic mean-field theory which relates the electric potential to the spatial distribution of charges, subjecting to the principles of statistical physics.

The basic problem which can be solved within PB theory is shown schematically in Fig. 1.3. Two planar infinitely large equally charged surfaces are in contact with a 1:1 electrolyte solution (e.g., NaCl) with surface separation D , surface charge density σ , and bulk concentration of ions n_s (in units $1/\text{m}^3$). The electrostatic potential is constant in the yz plane and varies only with x . The so-called Poisson–Boltzmann equation for a 1:1 electrolyte when $\varphi(x, y, z) = \varphi(x)$ is [1,9,10]:

$$\frac{d^2\varphi(x)}{dx^2} = \frac{2n_s e_0}{\varepsilon_r \varepsilon_0} \sinh \frac{e_0 \varphi(x)}{kT}, \quad (1.1)$$

where e_0 is the elementary charge, k the Boltzmann constant, T the thermodynamic temperature, and ε_0 and ε_r the vacuum permittivity and the relative permittivity, respectively. Equation (1.1) can be solved for some approximate cases (e.g., in linearized form) analytically [1]. Without simplifications, it can be solved only numerically. The solution of the equation depends on the boundary conditions at the two charged surfaces. The boundary conditions are derived from the condition of electroneutrality of the whole system:

$$\frac{d\varphi}{dx}(x=0) - \frac{\sigma}{\varepsilon_r \varepsilon_0} \quad \text{and} \quad \frac{d\varphi}{dx}(x=D) = + \frac{\sigma}{\varepsilon_r \varepsilon_0} \quad (1.2)$$

From PB theory, the pressure between two surfaces can be derived. The pressure is uniform across the gap between the surfaces and depends only on the total concentration of ions in the midplane position $\sum_i n_{mi}(D)$ and on the bulk ion concentration $\sum_i n_{mi}(\infty)$ for a certain value of D [1,10]:

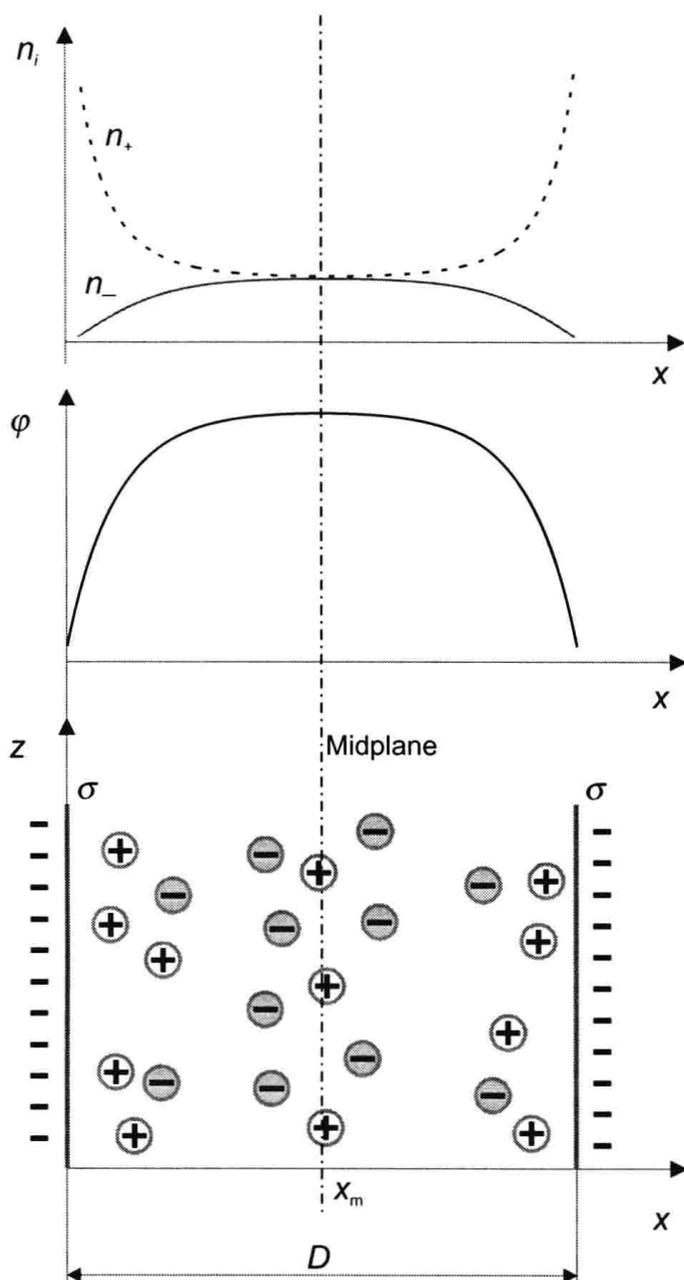


Figure 1.3 Schematic figure of the standard electric double layer problem. Two equally charged surfaces are immersed in electrolyte solution. The number density distribution of counterions and co-ions and the electric potential calculated from Eq. (1.1) are also shown.