

The Electrostatics of DNA-Lipid Intercation

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Introduction

DNA is an information storage device of living organisms made from a polymer. Due to its high charge density it shows a whole zoo of physical phenomena, when immersed in salt water containing other substances of biological relevance. This behavior is attributed to electrostatic, Van-der-Waals and entropic forces [1, 2, 3]. A good understanding of the principles behind the effects is necessary for successful applications in medicine and biology.

Research on synthetic polymers has been done for a long time. A theoretical framework for the calculation of properties like correlation length and free energy has been established, which is as far as possible independent of the specific molecular structure. But for biopolymers some questions arise, to which this theory will give no answer. One will not search for a general theory of the physics of all biopolymers. Instead one will try to go from the design requirements of their biological function to their implementation. We are doing a task, which has in a technical context the name "reverse engineering".

This work is further complicated by the fact, that some approximations for the theory of synthetic polymers are no longer valid. The structure of the biological polymers is far more complicated, they are highly charged and their natural environment, salt water, contains charges as well. We have to deal with a delicate balance of different forces, which have all a strength in the order of the thermal energy, except the covalent bond. This is not only a challenge for analytical calculations, but also for simulations because of the long range electrostatic interactions.

The Structure of DNA

So how we are going to deal with this challenge? Well, let's have first a look at the structure of the most fascinating biopolymer: DNA. Deoxyribonucleic acid

(DNA) is made of two complementary chains of nucleotides. The two strands are held together by hydrogen bonds, while the backbone of the chain itself is made of ribose molecules linked together by phosphodiester bonds. The information is stored on a base which is covalently bound to the ribose.

This configuration leads to the famous double helix: Each phosphodiester bond is negatively charged and therefore hydrophilic. In contrast the bases are hydrophobic. This leads to an attraction of successive base pairs, if immersed in water. The distance between the base pairs is larger than their thickness. But since the backbone can rotate freely through the connection between ribose and phosphodiester, the chains twist around each other to minimise the exposure of hydrophobic base pairs and maximise at the same time the exposure of hydrophil phosphodiester bonds.

Beside the fact that through the helical structure it is quite stiff, DNA is far from being a static molecule. Thermal fluctuations leads to a persistence length of about 50 nm. The persistence length describes the length where the correlation between two points along a polymer is lost, because thermal energy is able to bend the chain to any conformation. Further more the fitting of the base pair stack in the middle of the DNA double helix is determined by the base sequence, which leads to roll, slide and twist between successive pairs. Since there are several configurations, which are close to the energy minimum, a statistical distribution of conformations for each combination of successive basepairs exists. However, the complexity of the corresponding RST model avoided until now research on the structural, elastic and static mechanics properties.

The Properties of DNA

What are the reasons for the high charge density of DNA? The interior of organisms, especially that of the nucleus of eucaryotic cells, is very crowded. If now neutral macro molecules would come close to

each other, they would feel the attractive Van-der-Waals force and aggregate. Their biological function is then very likely to be lost. To prevent this, the negative charges give rise to a repulsive force. To make this principle work, all biopolymers have to carry charges of the same sign. Nature has chosen for negative charges on all biopolymers and membranes.

The interior of cells contains not only a zoo of charged proteins, it mainly contains water with a salt concentration around 0.1 M of NaCl (physiological condition). This leads to a more complex situation. Water will lower the coulombic interaction by nearly two orders of magnitude ($\epsilon \simeq 80$) making the interaction comparable to thermal energy. Ions from the salt water will interact with charges on the macromolecules.

Lipids

Lipids are defined as the water-insoluble molecules in cells that are soluble in organic solvents [4]. Fatty acids are an important examples of lipids, because they are the basis for all kinds of membranes found in cells. These thin sheets are largely composed from molecules with a hydrophilic head, which can also be charged and a hydrophobic tail. If immersed in sufficiently high concentration in water, these lipids will form a bilayer or micelles to minimize the exposure of hydrophobic tails. To satisfy also the borders of the bilayer, they can form a sealed compartment called vesicle. Since the lipids are free to move in the membrane, it is a two dimensional fluid. Spontaneous flipping from one layer to the other is a very rare process. The fluidity is determined by the temperature and the shape of the tail. Shorter tails and a high number of double bonds in the hydrophobic part decrease the interaction between the fatty acids and therefore increases the fluidity of the membrane.

Energy Scales in Biochemistry

For a better understanding of the phenomena in biological systems, we should have a look at the energies involved. A suitable scale is the thermal energy of a particle at room temperature. The strongest connection between atoms is the covalent bond, which is through his energy of $100k_B T$ essentially never broken by thermal fluctuations. The universal energy source to drive endothermic processes in living organisms is the hydrolysis of ATP to ADP. This frees about $10k_B T$. The binding energy of an hydrogen bond is about $3k_B T$, while the energy of two elementary charges or typical Van-

der-Waals interaction in water and hydrophobic interactions is in the order of $k_B T$. Essentially all these forces can be tracked down to electrostatic or electrodynamic interaction.

Structural integrity of proteins is kept by the strong interaction of a covalent bonds. The weak interactions dominate folding, connection of subunits, signaling and information transfer. The strength of the binding can be scaled by simply adjusting the number of bonds. This allows nature to set the kinetics of protein interaction and their arrangement, because it can be set sufficiently high to not allow thermal fluctuations not to break it and keep it still low enough to disrupt it easily if required signaling.

Aqueous Electrostatics

For the study of protein-DNA interaction it is not necessary to rely on highly specific assumptions concerning the atomic structure. Instead it is possible to develop an elegant general description of aqueous electrostatics.

For this theory we need some assumptions: The highly charged macromolecules (macro-ions) are placed in water at fixed positions and water is treated as a continuous medium of polar molecules, characterized by a large dielectric constant in the order of 80 for room temperature. This continuum model implies, that we are dealing with length scales significant larger than a water molecule. Poisson's Law will then provide the electrostatic potential ϕ :

$$\nabla^2 \phi = -\frac{4\pi}{\epsilon} \rho$$

where ρ is the charge density, which includes the fixed macro-ions and the mobile small ions. The boundary condition is, that the charges of the macro-ions are located on their surface. This allows us to restrict the treatment to the space in between the macro-ions. For the "small" salt ions we assume a Boltzmann distribution:

$$c_i(\vec{r}) = c_i e^{-\frac{e^- z_i \phi(\vec{r})}{k_B T}}$$

with

$$\rho_{ions}(\vec{r}) = e^- \sum_i z_i c_i(\vec{r})$$

we get to a second-order non-linear differential equation for the electrical potential with a boundary condition called Poisson-Boltzmann equation (PB). The condition of charge neutrality requires that the charge density of the salt solution far from the macro-ions vanishes. The very difficult PB equation has only been

solved for a few special cases. However, for high temperature the exponential function can be linearized. This leads to the Debye-Hückel (DH) equation, which is far much easier to solve than the PB equation. In the DH theory the important Debye screening length

$$\kappa = \sqrt{\frac{4\pi(e^-)^2}{\epsilon k_B T} \sum_i z_i^2 c_i}$$

appears. It describes the typical decay length scale of the potential.

To learn more about the electrostatics of cylindrical macro-ions like DNA, they are approximated as an infinitely long, negatively charged rod. The solution for the salt free case has a characteristic voltage scale $-\lambda/\epsilon$ with $\lambda = -e^-/d_q$ the charge per unit length. The comparison with the thermal energy gives the Manning Parameter $\xi = l_B/d_q$, where l_B is the Bjerrum length, which describes the distance at which the coulombic energy between two charges equals their thermal energy.

$$\frac{(e^-)^2}{\epsilon l_B} = k_B T$$

The numbers for DNA yield $\xi \approx 5$, so electrostatic energy dominates over thermal energy near the surface of DNA.

The natural environment of DNA contains salt. If we allow for sufficient high temperatures, the DH theory is applicable. The K_0 Bessel function fulfills the equation and boundary conditions. It follows, that there is a cloud of so called counter-ions with opposite charge of the rod is compensating its charge. The rest of the ions are called ‘‘co-ions’’. But under physiological conditions the DH theory’s approximation is only valid far away from the rod, where thermal energy is large compared to the electrostatic energy. The appearing integration constant needs still to be determined by the full PB theory. A work around to this problem is the introduction of an effective charge. The effective charge turns out to be significant lower than the real charge, because of efficient shielding by the counter-ions. Oosawa theory allows to estimate the renormalized charge, by dividing the counter-ions into a class of ‘‘condensed’’ ions, which are strongly associated with the rod (known as the ‘‘Manning cloud’’) and a second population further away in the Debye cloud. With the requirement of chemical equilibrium between the two populations follows the surprising conclusion, that the renormalized charge is independent of the bare charge, unless the Manning parameter is less than one.

If one now calculates the free energy using an analytical PB solution for an infinite rod in the limit of large ξ , the charging free energy is positive! It can be explained through the dominating contribution of the entropy loss of the counter-ions confined to a small cloud around the rod.

The above presented mean-field theory fails when the finite size of particles or correlations become important. For example very close to the surface of macro-ions the finite size of counter-ions leads to oscillations in their density. PB theory also fails for two charged rods in water containing polyvalent salts. Simulations and the analysis of simple models suggests an attractive force at low temperature exists, because the counter-ions form a two dimensional crystal through short range electrostatic correlations [5, 6]. Experimentally the condensation of DNA in the presence of low concentrations of polyvalent salt ions has been observed.

Counter-ion release leading to charge reversal, can be described within PB theory [7]. One example for this phenomena is the warping of DNA around histones. But the effect has also been seen for the complexation of a charged ball by a charged chain without counter-ions [8]. So the mechanism that drives spontaneous overcharging can be both entropic and enthalpic. Overcharging has also been reported for DNA-cationic liposome complexes [2]. In this case it is caused by short range correlations between the ions at high ion densities and closely related to the correlation-induced attraction between charged rods mentioned above.

DNA-Lipid Interaction

The outstanding perspectives of gene therapy has led to a large number of publications concerned with experimental [2, 3, 9] and theoretical [10, 11] issues related to DNA-lipid complexes. Since in living organisms membrane and DNA carry both a negative charge to prevent them from complexing, the use of positive charged lipids for the gene transfer allows easy preparation of the complex and supports also delivery through the membrane.

Experimental work on the structure of the complexes discovered a wide range of different arrangements, depending on concentration of DNA, positive charged lipid and neutral lipid. Lasic et al. [3] employed x-ray and cryo electron microscopy. Rädler et al. [2] used optical microscopy and x-ray diffraction in a more systematic study. They found various liquid crystal-type structures, reminiscent of lamellar and hexagonal states

formed in pure lipid or pure DNA solutions at high concentration. On semi-macroscopic length scales the mixing of DNA and lipid produces globules of complexes with a diameter of around $1\ \mu\text{m}$. The inner structures have a size ranging from 1 to 100 nm. Above, below and at the isoelectric point a lamellar package was found, which consists of a stack of alternating lipid bilayers and monolayers of aligned DNA. Remarkably, around the isoelectric point the spacing between the DNA increases from 2.45 to 5.71 nm as a function of lipid dilution. The spacing does not diverge as the concentration of DNA approaches zero. The distance in between two bilayers is just the diameter of the DNA rod plus a hydration shell. Polarization microscopy showed that distinct globules were birefringent, indicating their liquid crystal nature. Lightscattering experiments showed, that the largest complexes were found at the isoelectric point, in agreement with the idea of charge-stabilization.

In a solution of DNA with cationic and neutral lipids, which contains also lipids that prefers a negative curvature at the water interface, a inverted hexagonal phase is encountered. This structure performs better in gene therapy applications. These systems are because of their geometry in local electrical neutrality. There is no selfadjusting parameter, leading to a system always at its isoelectric point.

The aim of both theoretical work mentioned above is to give a model for the lamellar structure discovered by Rädler et al. They use similar models: Edge effects are ignored, so the lattice of DNA monolayers with lipid bilayers spreads out infinitely. The DNA is taken as an infinite cylindrical charged rod, the lipid bilayer as a perfect planar with constant thickness. Elastic deformations are ignored for all components. But both authors allow for spatial inhomogeneities in the membrane surface charge density, in response to interactions with the anionic DNA. Through its translational symmetry along the rods, the problem reduces to two dimensions. For the analysis Bruinsma [10] relies on analytical methods and is therefore restricted to low cationic lipid contents and no added salt, while Harries et al. [11] employs also numerical methods¹.

Both publications reproduce the three phases: Complexes with an excess of DNA in solution, an isoelectric point of full complexation and complexes with an excess of lipid in solution. While Bruinsma finds an in-

stability of the isoelectric point leading to an affinity for the uptake of DNA or lipid, Harries et al. denies such a singularity of the distance between the DNA strands. Also the effects of counter-ion release appear more pronounced in the analytical discussion. Beside this a qualitative explanation of the structure with PB theory has been shown by the authors.

Gene Therapy

In principle gene therapy is simple: putting corrective genetic material into cells alleviates the symptoms of disease [12]. But in practice, considerable obstacles have emerged. The main problem are the delivery systems (vectors). An ideal vector should be available in high concentration for a high infection rate, easy and relayable to produce, specific with respect to the location on the host chromosome or a stable outside the chromosome, target only a desired type of cell and contain no components that cause an immune response. Such a vector is currently not available, but all these properties are found in disparate delivery systems.

Viral systems comes along with an elaborate machinery for infection and the addition of DNA, but are of cause subject to a strong immune response. Another point is the limited number of transported base pairs and they might also cause several safety problems. Lipid-DNA systems are in contrast unlimited in size, easy to produce and save, but they show a short duration of expression and dependend of the exact method poor integration into the host chromosome. Their exact mechanisms are unknown and the transfection efficiencies vary by up to two orders of magnitude between different cell lines [2]. However, biochemical research showed, that the DNA-lipid complex undergoes a significant change on its way through the cell, while the details remain unclear [13]. It has been proposed, that when the complex gets to the outer membrane of a cell, they will fuse and mix their fatty acids. Subsequently the environment of the DNA undergoes charges reversal because the cell membrane is negatively charged like the DNA and unlike the lipids from the complex. Then the DNA is released into the cell interior, with the possibility to get into the nucleus.

¹As mentioned above the PB theory involves a difficult non-linear differential equation, making the use of approximations or numerical methods necessary.

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