Notes about Kirkwood-Schumaker interactions

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Abstract

It is known that the overall charge of a protein can change as the molecule approaches a charged object like another protein or a cell membrane. This mechanism was formalized using a statistical mechanical framework. It was shown that Kirkwood-Schumaker interactions increases the attraction between protein molecules. The protein charge capacitance is a function of pH, salt concentration, and the number of titrating residues.

Contents

1 Introduction

Electrostatic interactions of proteins with small ligands, with DNA, or with a membrane surface, as well as the interaction between two or more protein molecules have some peculiar properties, different from these in interactions between smaller organic molecules. Because of (de)protonation of amino acid side groups and dissociation of chargeable molecular moieties such as N- and C-terminals, behaviour of proteins can not be analysed with the assumption of a constant charge[3].

It was shown by Kirkwood and Shumaker, that extremely long-ranged attractive interactions occur between proteins in an aqueous solution close to the point of zero charge[1, 2]. The Kirkwood-Shumaker interaction is very different from the van der Waals interactions, that originate only from dipolar fluctuations and act between electro-neutral bodies. So the Kirkwood-Shumaker interaction does not exist for organic molecules with a strictly fixed charge distribution[4]. So Kirkwood-Shumaker interaction exist only in systems which have a non-zero capacitance and where the net charge is not a constant but depends on the dissociation processes[5].

We can see that the effective charge on the protein surface, is regulated and responds to the local solution conditions: pH, electrostatic potential, salt concentration, spatial dielectric constant profile and the presence of other vicinal charged groups[7]. Recently it was shown, using detailed Monte-Carlo simulations, that indeed there exists an interaction between proteins which has the same features as the original approximate form of the Kirkwood-Shumaker interaction[3].

First dependence of Kirkwood-Shumaker forces on whether and how the protein charge can respond to the local electrostatic potential was formalized by Ninham and Parsegian in their theory of charge regulation[8].

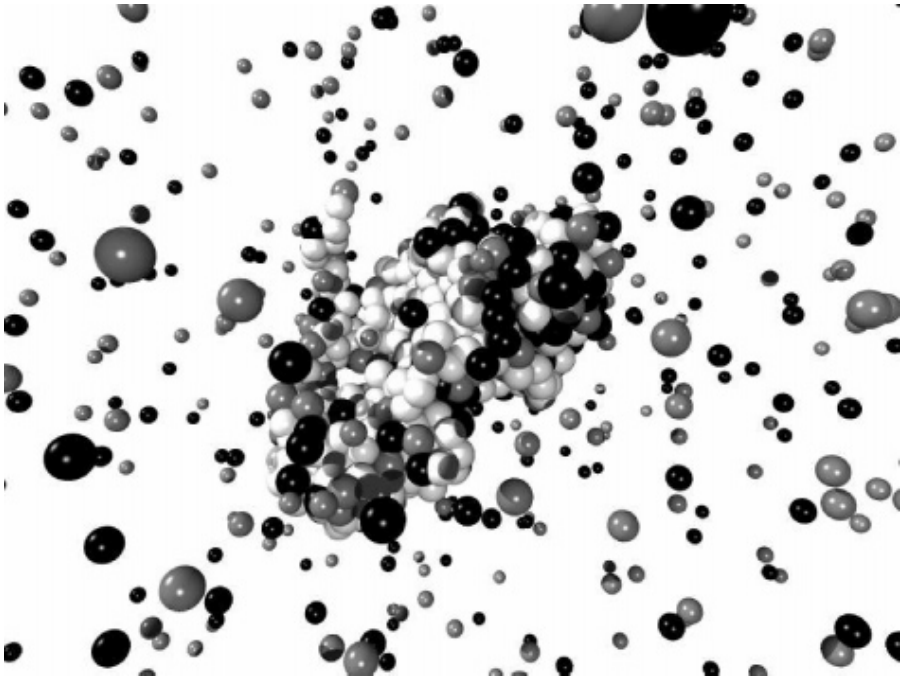

Figure 1: Schematic picture of the model system with the protein and salt solution enclosed in a cell. Image is borrowed from[3]

2 Length scales

If we compare the thermal energy $k_BT = \beta^{-1}$ with inter-ionic Coulomb interactions, we can introduce so-called Bjerrum length: $l_B = \frac{e^2}{4\pi\varepsilon_0\varepsilon_r\ k_BT}$. Systems with small l_B behave like gases, and with large l_B behave liquid- or solid-like.

In the case of ionic fluid consisting of counterions of charge valency q at a charged interface of uniform surface charge density $-\sigma e_0$, the Gouy-Chapman length $\mu = \frac{1}{2\pi q l_B|\sigma|}$ determines the strength of the thermal energy with respect to the electrostatic interaction with the surface.[6]

Also sometimes people define the electrostatic coupling parameter as:

$$\Xi = \frac{q^2 l_B}{\mu} = 2\pi q^3 l_B^2 |\sigma| \tag{1}$$

If Ξ is small enough, then the neutralizing counterions next to a charged surface form a diffuse gas-like phase. But in the case of large Ξ, the ionic cloud is reduced from a threedimensional (3D) layer to a quasi-two-dimensional (2D) sheet.[6]

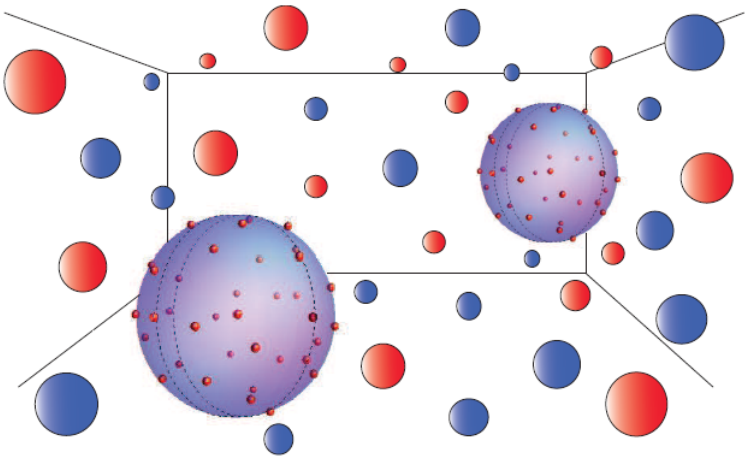

Figure 2: Two charge-regulated ions immersed in 1:1 salt solution. Image is borrowed from[4]

3 Statistical Mechanical derivation of protein charge polarizability

First it is nessecary to derive an expression for charge capacitance of protein, because Kirkwood-Schumaker interaction is basicaly Coulomb interaction between molecules but for non-fixed in time charge distribution on protein. The fluctuating charge of a protein may under certain circumstances contribute significantly to the net interaction between two proteins.

Following [3] we will start by considering two proteins in a salt solution, each described by the charge distributions $[\mathbf{r}_i, q_i]$ and $[\mathbf{r}_j, q_j]$, respectively. Let us define $\mathbf{R}$ - vector between the mass centra of the distributions, $U(R)$ - the interaction between the two charge distributions (depend only on magnitude of $\mathbf{R}$). Thus free energy F can be written as:

$$\beta F(R) = -\ln\langle e^{-\beta U(R)}\rangle \approx -\ln\{1 - \langle\beta U(R)\rangle + \frac{1}{2}\langle(\beta U(R))^2\rangle\} \quad (2)$$

Hear and $\langle ...\rangle$ denotes an average over the *single* isolated protein in salt solution. It runs over all orientations and ionization states of the protein as well as over the positions of all salt particles. This means that the calculated averages will depend on both the salt and protein concentrations and pH.

For small $\beta U(R)$ we obtain:

$$\beta F(R) \approx \langle\beta U(R)\rangle - \frac{1}{2}\{\langle(\beta U(R))^2\rangle + \langle\beta U(R)\rangle^2\} \quad (3)$$

Then using ordinary expression for Coulomb interaction and expand-

ing it into multipole expansion we get:

$$\beta F(R) \approx \frac{l_B \langle Q_A \rangle \langle Q_B \rangle}{R} - \frac{l_B^2}{2R^2}[\langle Q_A^2 \rangle \langle Q_B^2 \rangle - \langle Q_A \rangle^2 \langle Q_B \rangle^2] \tag{4}$$

Here we denoted Bjerrum length: $l_B = \frac{e^2}{4\pi\varepsilon_0\varepsilon_r\ k_B T}$. We now can define a "charge polarizability" or capacitance, C, that quantifies the charge fluctuations of the protein $C \equiv \langle Q^2 \rangle - \langle Q \rangle^2$, then:

$$\beta F(R) \approx \frac{l_B \langle Q_A \rangle \langle Q_B \rangle}{R} - \frac{l_B^2}{2R^2}[C_A C_B + C_A \langle Q_B \rangle^2 + C_B \langle Q_A \rangle^2] \tag{5}$$

The first term is the direct electrostatic interaction, and the second terms are the induced charge-induced charge interaction. If the proteins is at pH=pI (molecule carries no net electrical charge) then $\langle Q \rangle = 0$ and

$$\beta F(R) \approx -\frac{l_B^2 C^2}{2R^2} \tag{6}$$

4 Derivation of Kirkwood-Schumaker interactions

Following original paper of J. Kirkwood and J.B. Shumaker[2] we will start from a Hamiltonian of two point-like macroions in the aqueous solvent:

$$H(e_1, e_2; \mathbf{r}_1, \mathbf{r}_2) = \frac{e_1^2}{2C_1} + \frac{e_2^2}{2C_2} + \frac{e_1 e_2}{4\pi\varepsilon\varepsilon_0 |\mathbf{r}_1 - \mathbf{r}_2|} \tag{7}$$

The free energy for this system with charge fluctuations is obtained by integrating the Boltzmann weight of the Hamiltonian with respect to unconstrained fluctuations in e_1 and e_2 and is thus can be written as:

$$\beta F(R) = -\ln \int\int de_1 de_2 e^{-\beta H} \simeq -C_1 C_2 G(R) \tag{8}$$

where $G(R) = \frac{1}{4\pi\varepsilon\varepsilon_0 R}$ and $R = |\mathbf{r}_1 - \mathbf{r}_2|$. Useful to note that interaction energy scales as R^{-2}. Following the paper of Nataša Adžić and Rudolf Podgornik[4] we can also derive the Kirkwood-Schumaker force per unit area for the specific geometry of two planar surface at separation D, bearing fluctuating charge groups:

$$p = -\frac{\partial}{\partial D} \int_D^\infty 2\pi R F(R) dR \simeq D^{-1} \tag{9}$$

As we can see, the decay is slower then in the case of van der Waals interactions between either two semi-infinite media or two thin layers, that scale as D^2 and D^5 respectively. The Kirkwood-Schumaker forces correspond to **monopolar** fluctuations and thus follow a different scaling either between point particles, R^{-3}, or between fluctuating surface layers, D^1, then in the case of **dipolar** fluctuations.

5 Non universality of Kirkwood-Schumaker forces

Kirkwood-Schumaker forces depend on whether and how the protein charge can respond to the local electrostatic potential, a salient property of dissociable charge groups that is usually referred to as charge regulation. It was first shown by Ninham and Parsegian in their paper [8].

It is the most prominent difference with van der Waals interactions which are universal forces.

They considered two planar surfaces separated by a distance. These surfaces bear acidic ionizable groups at a density $\frac{1}{S}$, where S is the area of surface per acidic group. A fraction α of these groups will be dissociated so that the surface charge density equal $\sigma = -\frac{e\alpha}{S}$. The ionic solution between the surfaces is in equilibrium with a reservoir solution containing monovalent anions, and monovalent and divalent cations.

Given S, b, the ionizable group dissociation constant Z, and the pH and ion concentrations of the reservoir solution, they require the degree of dissociation α, the electrostatic potential field $\psi(x)$, and the pH and ion distribution between the charged surfaces.

They take the ionic concentrations in the reservoir to be: $n_{-}^{(r)} = n$,

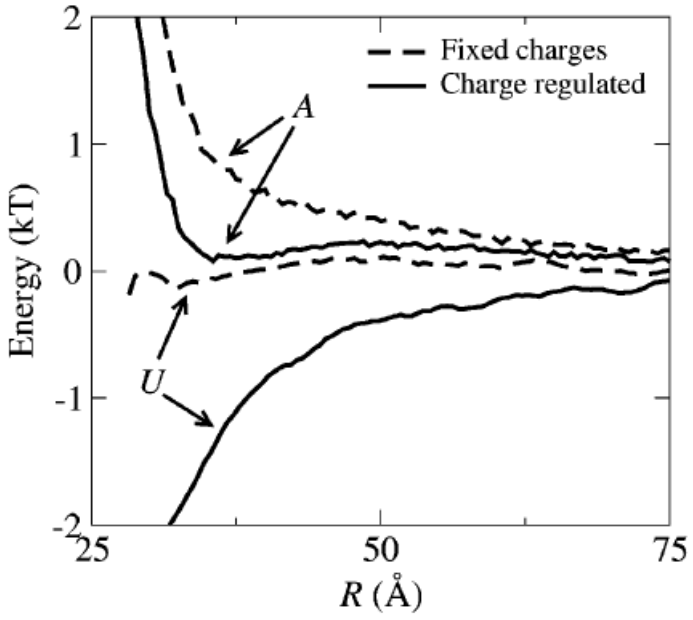

Figure 3: Simulated energy (U) and free energy (A) of the interaction between calbindin and lysozyme at pH 4 for a protein model with fixed charges and one with charge regulation. The amino acid model is used, and the salt concentration is 6 mM. Image is borrowed from[7]

$n_{+}^{(r)} = n(1-\eta)$ and $n_{++}^{(r)} = n\frac{\eta}{2}$, for monovalent anions and cations, and divalent cations respectively, where $0 < \eta < 1$. The ion concentrations between the charged surfaces are governed by a Boltzmann distribution.

Then $n_{-}^{(r)} = ne^{e\beta\psi}$, $n_{+}^{(r)} = n(1-\eta)e^{-e\beta\psi}$ and $n_{++}^{(r)} = n\frac{\eta}{2}e^{-2e\beta\psi}$. Thus they wrote Poisson equation:

$$\frac{d^2\psi(x)}{dx^2} = -\frac{4\pi ne}{\varepsilon}[-e^{e\beta\psi} + (1-\eta)e^{-e\beta\psi} + \eta e^{-2e\beta\psi}] \tag{10}$$

, where ε is the dielectric constant of the medium which is supposed independent of salt concentration.

Ninham and Parsegian obtained analytical solution of this problem in terms of elliptic functions. Then they numerically estimated of electrostatic potentials and electrostatic repulsion using the expansions of the elliptic function solutions. Their illustrative calculations show that even the small amount of divalent cation present in physiologic solutions has a dramatic effect to reduce the repulsive electrostatic force between ionizable surfaces and to alter the pH and mobile ion distribution near the surfaces.

6 Other important works

In the paper of Adžić and Podgornik[4] was proposed the following classification that concerns the fluctuating dipole contribution. They introduce KS interaction of type 1 (monopole proton charge fluctuations) [2] and KS interaction of type 2 (dipole moment fluctuations) [1], both in fact due to proton fluctuations at the dissociation sites of the macroion. The dipole moment fluctuation contributes a remarkably large increment to the dielectric constant of water, that is usually in fact negative (is decreased) for simple salts [12].

The total fluctuation interaction is composed of the fluctuating monopolar charge contribution, and the fluctuating dipole contribution, described by the total excess polarizability and is as a whole dominant when the average charge is vanishing, i.e. close to the point-of-zero-charge of the macroion. Obviously this generalized KS interaction contains the monopolar and dipolar fluctuation contributions in a highly non-additive way and could in fact go through a local maximum as a function of the separation $|\mathbf{R}_1 - \mathbf{R}_2|$.

Also it worth mentioning an interesting work of Steven L. Carnie and Derek Y.C. Chan[9], in which they use the linearized Poisson-Boltzmann theory in order to calculate the electrical double layer interaction free energy between two parallel charged plates for the case in which charge regulation due to the dissociation of surface groups may be modelled by the linearized regulation model that specifies a linear relationship between the surface charge and the surface potential.

Steven L. Carnie and Derek Y.C. Chan showed[9] that the linearized DebyeHückel model describe good enough the double layer interaction between colloidal particles at low surface potential. It is very good because it is much more difficult to solve the full nonlinear Poisson-Bolzmann equation.

Pujar and Zydney performed[10] theoretical calculations for the electrostatic potential for a spherical particle in a cylindrical pore, accounting for this charge regulation phenomenon using a linearized form of the charge regulation boundary condition. The equilibrium partition coefficient in the pore was then evaluated from the free energy of interaction. Specific calculations were provided for the protein *bovine serum albumin*, with the charge regulation parameters evaluated from a model for the detailed protein charge characteristics. They also compare the model predictions with experimental data for the bovine serum albumin sieving coefficient at different pH and ionic strength.

7 Discussion and possible alternative approach

Until now all calculations of charge regulation were done classically, not using quantum mechanics. It is quite reasonable approximation for this problem. The very notion of Kirkwood-Schumaker interaction implies to long range thermal fluctuation forces that decay much slower than the ubiquitous van der Waals interactions.

But I think it would be important to calculate impact of quantum mechanics on Kirkwood-Schumaker forces, and to see how it changes. It will be important for the case of mesoscopic geometry and special kinds of boundary conditions.

The second issue but closely related to the first one is to combine protein-protein dynamical charge distributed Kirkwood-Schumaker forces with Casimir force. Casimir effect arises from a quantized electromagnetic field and is both relativistic and quantum phenomena. Actually, Kirkwood-Schumaker interaction corresponds to the zero frequency van der Waals (also called thermal Casimir) interaction. A thermal Casimir force arise due to thermal rather than quantum fluctuations of the electromagnetic field at finite temperature.

According to Bose-Einstein statistics, the population of an electromagnetic field mode of frequency ω at temperature T is

$$n(\omega) = \frac{1}{2} + \frac{1}{e^{\frac{\hbar\omega}{k_B T}} - 1} \tag{11}$$

where the first term describes the zero-point quantum fluctuations, and the second term is the thermal population for a gas of photons. This second term gives rise to a finite-temperature term in the Casimir force. At small separations the thermal force is much smaller than the zero-point force, but for large separations the thermal force dominates. An analogous force between an atom and a surface is called thermal Casimir-Polder force.[11]

8 Conclusion

Electrostatic interactions of proteins with ligands, DNA, or with a membrane surface, as well as the interaction between two or more protein molecules are different from these in interactions between smaller organic molecules. Because of (de)protonation of amino acid side groups and dissociation of chargeable molecular moieties such as N- and C-terminals, behaviour of proteins can not be analysed with the assumption of a constant charge[3].

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