Criticality and Self-assembly in Biological Ionic Fluids

Ghee Hwee Lai

December 13, 2005

Abstract

Electrostatics in aqueous media pervade biological systems. Interactions within a collection of highly-charged macro-ions in an aqueous environment are very complex and often exhibit counter-intuitive critical effects, whose manifestations govern the self-organization and activity of living systems. This paper attempts to give a broad overview of work done on the behaviour of long rod-like highly-charged biological macromolecules as well as possible far-reaching applications.
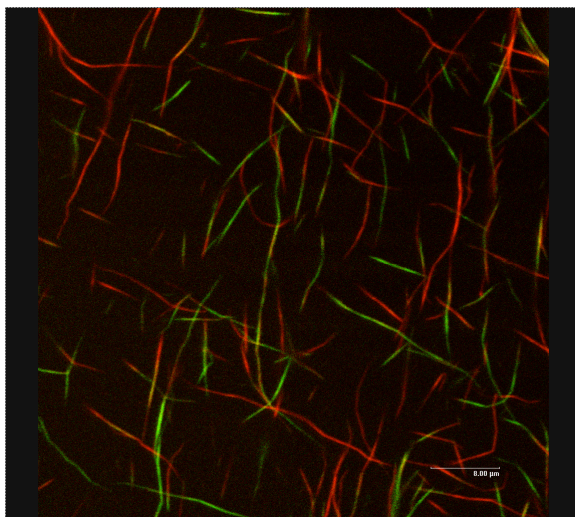
Figure 1: Self-assembled actin bundles in divalent salt solution [1].

1   introduction and background

This paper is not about scaling laws or critical exponents. Rather, I hope to elucidate the importance of phase transitions in biological fluids. In fact, a casual glance at biological systems probably does little to inspire thoughts on critical phenomena. However, it has been suspected that phase separation and transition influence the composition and fluidity of lipid bilayers, and may play a role in the cell membrane during the formation and fusion of buds and vesicles. Another example would be phase transitions in protein folding. The focus of this paper is to understand the electrostatic interactions between highly charged macro-ions in an aqueous solution – an unifying feature of biological systems, and possibly find ways to tailor the critical behaviour for medical applications.

A polyelectrolyte is an ionic polymer which, when dissolved in polar solvents, dissociates into a highly-charged macro-ion and small mobile counterions. With the electrostatic repulsion of the uncompensated charges along the macro-ion, the chain is stretched out in a rod-like configuration. Indeed, most important biolomolecules (nucleic acids and proteins) and bio-structures (cell membrane and extra-cellular protein networks) are highly-charged objects in aqueous environment. For example, DNA has an effective density of one negative charge every 0.17nm of its length, while F-actin has about one negative

charge every 2.5nm. They need to be charged to avoid precipitation and phase separation at the high physiological concentrations in cells. Under physiological pH conditions, the high charge gives rise to strong repulsions between neighbouring macro-ions in a simple (monovalent) salt solution.

Interest in stiff polyelectrolyte systems is growing, because in such systems under certain conditions, we see net attractive interactions between like-charged macro-ions, a counter-intuitive result that strongly affects the system structure! Many experiments show that multivalent counterions can mediate attraction between like-charged polyelectrolytes and induce condensation into compact states/bundles, while the same polyelectrolytes repel one another in solutions of monovalent salts.

The problem of aqueous electrostatics has been pursued since more than seventy years ago, when Debye and Hückel published a theory of the thermodynamics of strong electrolytes [3]. Mean field theories such as the Poisson-Boltzmann formalism are often used, but such mean-field treatment inescapably leads to repulsive forces [2], even after considering the screening effect of counterions condensation on the rod-like polyelectrolytes [4]. An attractive interaction capable of competing with the residual Coulombic repulsion of the polyelectrolytic backbones (with compensated charge densities) is needed. Nonetheless, in order to understand the origin of such attraction, we need to start with the mean field Poisson-Boltzmann (PB) theory.

2   theory

Due to the changing landscape of theory in counterion condensation, a broad survey of various corrected mean-field theories are discussed without going deep into their derivations. In mean-field PB theory, the mean-field approximation together with Gauss' law gives the Poisson-Boltzmann (PB) equation [5]:

$$\nabla \cdot (\varepsilon(x)\nabla\varphi(x)) = -4\pi e \sum_i c_i^\infty z_i e^{-z_i e\varphi(x)/k_B T}, \tag{1}$$

where z_i is the valency for each charge i and the normalisation c_i^∞ is the concentration of charged species i far away from any "fixed" charges and is usually the bulk ion concentration before the introduction of the macro-ions.

For a $z : -z$ salt such as NaCl and assuming uniform ε, we end up with

$$\nabla^2\varphi(x) = \frac{-4\pi ezc^\infty}{\varepsilon}\left(e^{-ez\varphi(x)/k_BT} - e^{ez\varphi(x)/k_BT}\right)$$
$$= \frac{8\pi ezc^\infty}{\varepsilon}sinh(ez\varphi(x)/k_BT) \tag{2}$$

For large distances, the PB equation reduces to the well-known Debye-Hückel (DH) equation [3]:

$$\nabla^2\varphi(x) = \kappa^2\varphi(x), \tag{3}$$

where the inverse screening length

$$\kappa \equiv \sqrt{\frac{4\pi e^2}{\varepsilon k_BT}\sum_i z_i^2 c_i^\infty} \equiv \sqrt{4\pi l_B\sum_i z_i^2 c_i^\infty}, \quad l_B \equiv \frac{e^2}{\varepsilon k_BT} \text{ (Bjerrum length)}$$

$$\tag{4}$$

In PB theory, counterions have a two-fold effect [2]. Firstly, as mentioned earlier, these counterions can condense onto the macro-ions and renormalise the effective charge density. Secondly, they give rise to an exponentially decaying potential at large distances, in contrast with the monotonic-inverse relation with spacing for salt-free environments. Notice that the interaction between identical macro-ion remains repulsive, albeit reduced. The structure and dynamics of the condensed counterions surrounding the polyelectrolytes must be controlling this interactions.

In the late 1960s, the Oosawa-Manning [6, 7, 8] theory of counterion condensation was developed and postulates that the self-assembly of these polyelectrolytes is primarily driven by electrostatic interactions and the entropic gains derived upon release of bound counterions. The behaviour of counterions is strongly influenced by dimensionality [9]. In d dimensions, a D-dimensional macro-ion of size $L_\parallel$ attracts a counterion at a perpendicular distance $L_\perp$, with a Coulomb energy that goes like $E_c \sim \frac{1}{L_\perp{}^{d-D-2}}$. On the other hand, entropy of a particle confined in such a box can be obtained as $S \sim ln(L_\parallel{}^D L_\perp{}^{d-D})$. Minimizing the free energy $F = E_c - TS$ with respect to $L_\perp$ and neglecting inter-counterion repulsions, we obtain the following results:

For $d < D+2$, there is a stable finite distance at which counterions prefer to stay from the macro-ion to minimize energy, or in simpler terms, condense.

For $d > D + 2$, the counterions prefer to be free to gain entropy.

For $d = D + 2$, a counterion condensation (1st-order) transition takes place.

Thus, for charged planes, the counterions are always condensed while for an isolated spherical macro-ion, the counterions prefer to be free. The interesting case with a phase transition is a system of 1-D macro-ions (with polyvalent counterions), which describes the high aspect ratio, rod-like polyelectrolytes.

Consider the approach of two rod-like polyelectrolytes. During this process, the counterions may rearrange their positions and this correlated separation of charges can lead to an attraction. Several different mechanisms may result in a correlated charge separation [4].

1. Thermal fluctuations can induce instantaneous charge separations, which intercorrelate on the two objects, leading to an attraction akin to van der Waals interaction. Manning [7] used a dimensionless quantity ξ (the "Manning parameter"): $\xi = \frac{l_B}{b}$, where $l_B = \frac{e^2}{\varepsilon k_B T}$ is the Bjerrum length, the distance at which Coulombic interaction is comparable to thermal energy $k_B T$, and b is the line density of the fixed charges along the macro-ion. Hence, the Manning parameter can be identified as the ratio of the characteristic monovalent electrostatic energy to the thermal energy. Manning's criterion postulates that counterion condensation occurs when the distance between charges, b, is small enough that ξ exceeds a critical value.

   Oosawa [6] obtained an approximate expression for the force per unit length $f(R)$ between two parallel charged rods, in the limit of large distances R (Z is the counterion valency):

   $$f(R) \approx k_B T \left(\frac{1}{Z^2 l_B R} - \frac{(Z\xi)^2}{1 + (Z\xi)^2} \frac{1}{R^2} + \dots \right). \qquad (5)$$

   The first term is the ξ-independent PB repulsion while the second term describes the effect of correlated long-wavelength thermal fluctuations to lowest order. Notice that crossover from repulsion to attraction as a function of R takes place when R is less than the Bjerrum length l_B – we see the possibility for aggregation! These interactions are long-ranged.

2. At low temperatures, short-ranged electrostatic correlations between the counterions of the two clouds (attracted by two macro-ions) may form a self-ordered 2-D Wigner crystal, which leads to an attractive interaction with a range set by the lattice spacing [10, 11]. The low

temperature threshold depends strongly on the valence Z of the counterions. It is possible that remnants of a Wigner crystal may survive for multivalent counterions.

3. Specific binding of counterions to the polyelectrolytes can account for attraction [12]. We focus mainly on non-specific interactions in this paper.

4. The polyelectrolytes/macro-ions can be over-charged by the condensing counterions, due to a gain in correlation energy rather than counterion release. Consequently, when two such macro-ions are brought into close proximity in a neutralising solution, the counterion clouds can be asymmetric, leading, at any instance, to one overcharged and undercharged macro-ion. This behaviour leads to a long range Coulombic attraction between the two decorated macro-ions [13].

3   experiment and simulation

A universal phase behaviour is observed for all polyplexes with a critical multivalent salt concentration required to induce the discontinuous phase transition. For DNA literature, aggregation and condensation are different but closely related: aggregation refers to the attraction of multiple chains into a compact structure while condensation usually refers to the collapse of a single long DNA strand. In both cases, a variety of tri- and tetravalent counterions can induce aggregation and condensation of DNA, among them the polyamines spermidine (3^+) and spermine (4^+) [14]. For F-actin, divalent ions are sufficient to induce bundling at low salt concentrations [15]. Similar results are observed for other rod-like highly-charged systems like bacteriophages [17].

A broad variety of experimental methods are used to study these mixed polyelectrolyte-multivalent ion systems:
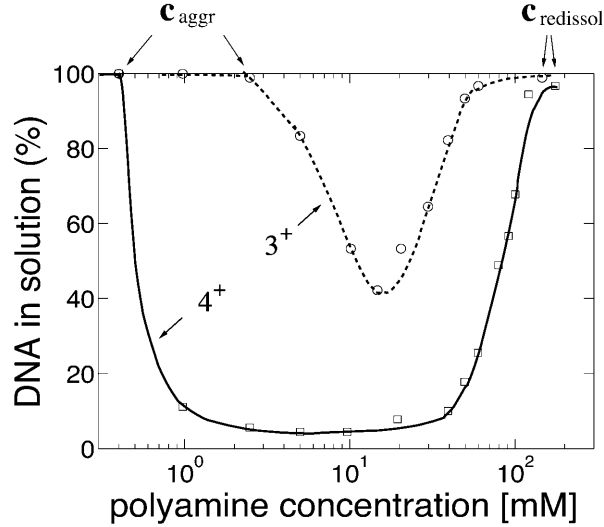
FIGURE 2 Percent of solubilized DNA, as function of polyamine concentration. Squares, spermine; circles, spermidine. Solid and dashed lines are guides for the eye. DNA and NaCl concentrations are 3 mM and 25 mM, respectively. Below the aggregation threshold, c_{aggr}, and above the redissolution threshold, $c_{redissol}$, all the DNA is dissolved. The data is adapted from Pelta et al. (1996b).

Figure 2: Pelta et al. (1996) [14]

Pelta et al. [14] studied DNA precipation under varying polyamine concentrations by measuring the amount of left-over DNA remaining in the bulk solution – the mixture was vortexed and the amount of DNA in the supernatant was determined by two methods: measurement of absorbance at 260nm or the radioactivity of ^{32}P-labeled DNA. As the multivalent ion concentration is raised above a certain threshold (c_{aggr}), DNA segments begin to aggregate and precipate from the solution (See Figure 2). This threshold for the first transition depends quite sensitively on various parameters, including the total polyelectrolyte concentration and the monovalent salt concentration. Further addition of multivalent ions eventually reverses the aggregation at much higher concentration $c_{redissol}$. The redissolution threshold is almost independent on the DNA concentration and is attributed to the screening effect of the multivalent counterions. The phenomena of precipation and resolubilization are experimentally fully reversible [16].
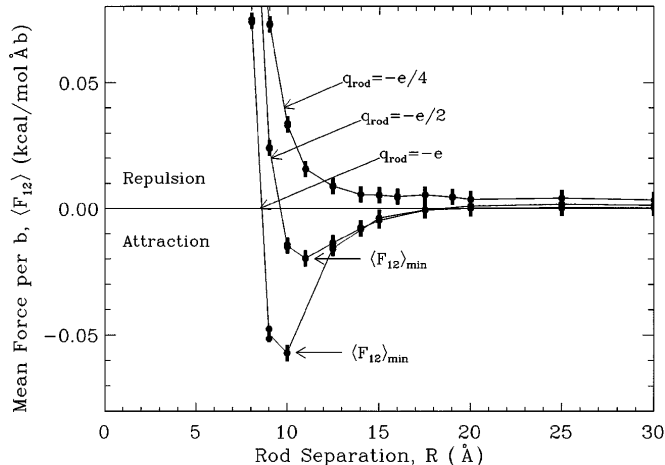
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FIG. 3. Mean force $\langle F_{12} \rangle$ (per length b) between two parallel charged rods, with divalent counterions, as a function of the rod separation distance R for temperature $T \approx 300$ K, ionic radius $r_0 \approx 4.2$ Å, system size L_z, and different values of the rod charge q_{rod} (per 1.7 Å). The effective Manning parameter $Z\xi = |q_{\mathrm{rod}}|q_{\mathrm{ion}}/\varepsilon b k_B T$ and system size are respectively: $Z\xi = 2.1$ and $L_z = 544$ Å, with $q_{\mathrm{rod}} = -e$. The estimated statistical uncertainty is always less than indicated by the error bars.

Figure 3: Grøbech-Jensen et al. BD-simulations [10].

The long-range interactions and spatial inhomogeneity meant that finite-temperature Coulomb systems of macro-ions in solution are difficult to model with molecular dynamics and Monte Carlo methods. However, recent years have seen several successful studies that match closely in terms of qualitative features with experiments.

Grøbech-Jensen et al. [10] reported on their results of long-time Brownian-dynamics simulations of electrostatic interactions between two rigid polyelectrolyte rods and a plot of the mean force between rods is reproduced (See Figure 3). Notice that for high charge density polyelectrolytes, there is a regime of attraction between two regimes of repulsion. The sharp slope at the first cross-over hints at a sharp transition whereas the very gradual slope at the rear implies a more gradual transition. The structure is evident in actual experimental results (see Figure 5). However, it is still unclear which
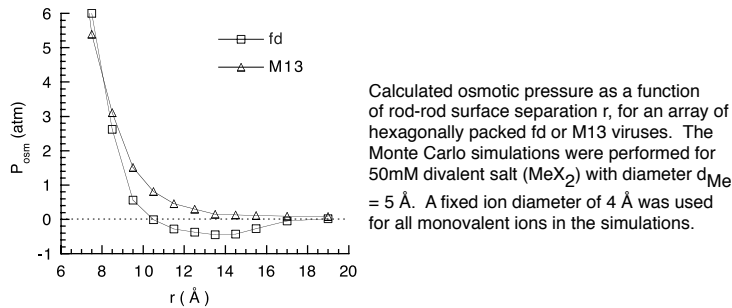
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Calculated osmotic pressure as a function of rod-rod surface separation r, for an array of hexagonally packed fd or M13 viruses. The Monte Carlo simulations were performed for 50mM divalent salt (MeX$_2$) with diameter d$_{Me}$ = 5 Å. A fixed ion diameter of 4 Å was used for all monovalent ions in the simulations.

Figure 4: Tang et al. (2002). The solvent water is treated as a dielectic continuum in the qualitative Monte Carlo simulation [17].
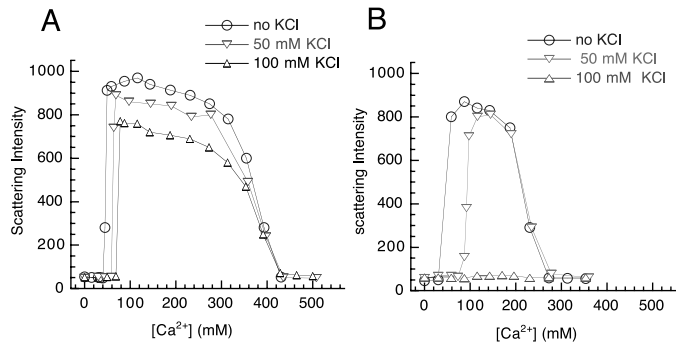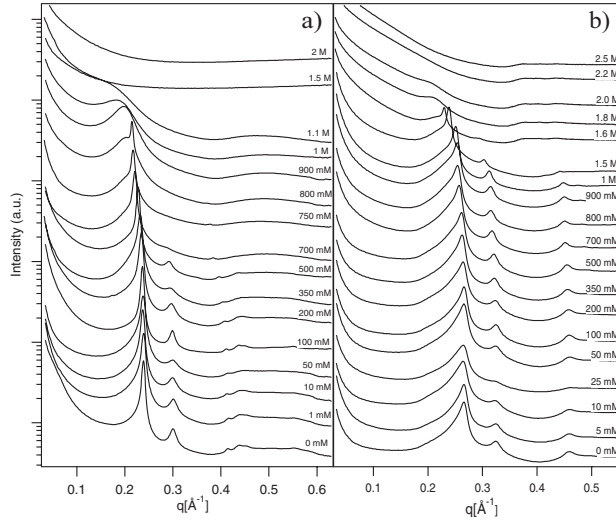

Figure 5: Bundle formation and subsequent redissolution in excess Ca^{2+}. The experimental results are shown for both (A) fd and (B) M13, each with 3 concentrations of monovalent salt KCl. (Tang et al. [17])

of the counterion correlation theories portray the true picture.

Tang et al. (2002) also reported comparisons between Monte-Carlo simulations and actual experiments on bundling/aggregation of either fd or M13 viruses in the presence of divalent metal ions [17]. The osmotic pressure is calculated as a function of rod-rod separation for different divalent salt concentrations (Figure 4) and mirrors the force profile obtained by Grøbech-Jensen et al.. Bundling efficiency varies with different divalent alkali-earth metal ions ($Ca^{2+} > Mg^{2+} > Ba^{2+} > Sr^{2+}$). Also, bundling and redissolution are observed for both fd and M13 viruses (See Figure 5 above), once again highlighting the universality of this critical phenomena. The resolubi-

Synchroton SAXS intensity profiles for (a) poly-L-lysine(PL)-DNA mixture and (b) poly-L-arginine(PA)-DNA mixture as a function of the external monovalent NaCl salt concentration. At low NaCl concentrations, the peaks for hexagonal lattice are observed. With increasing salt concentration, a salt-induced transition is observed where we see coexistence of two states. Further addition of salt flattens the intensity profile and indicates a dilute phase with no aggregation.

Figure 6: Synchroton SAXS, DeRouchey et al (2005).

lization phenomena was actually predicted by a recent analytical approach by Perel and Shklovskii [18].

Very recently, DeRouchey, Netz and Rädler (2005) [19] investigated the internal structure of DNA-polycation complexes by small-angle X-ray scattering (SAXS). Instead of varying the polycation concentration, they started with an excess (but fixed ratio) of polycations and instead varied the monovalent ion concentration. A salt-induced melting (dissolution) is observed (See the changing profiles in Figure 6), indicating a change from the tight bundle phase to coexistence and finally to a dilute dispersed phase.

4   conclusions

There is still plenty room for discovery and analysis before theory and experiment align quantitatively. Most simulations and theories only offer a correct broad qualitative picture. Why is all these interesting? Such reversible electrostatic self-assembly behaviour has important implications as charged polymeric systems become increasingly prevalent in industrial, biotechnological and medical applications. Elucidating the behaviour of DNA condensation allows us to understand how nature generates and uses condensates to store and protect genetic information [20]. This could pave the way towards developing effective gene delivery methods, in which the DNA compaction can be made fast, effective and easily reversible without damaging the DNA helix.

Experiments have shown that the critical behaviour can be controlled via the nature of counterion, the pH(which affects the line-charge density of the polyelectrolytes as well as the polycation) and the concentrations of monovalent ions as well as the multivalent counterions (polycations). Together with these different variables to tailor the critical behaviour, the non-specificity of the interactions involved during the phase transitions entails a universality that allows the possibility of wide-ranging applications, ranging from membrane and surface coating technologies (which may, for example, improve water-purification) to the understanding of cystic fibrosis, a genetic disease (the mucus is a mixture of DNA, F-actin and other polyelectrolytes and salts).

I have avoided mentioning other exciting advances in the field as well. Overcharging of polyelectrolytes has been observed experimentally via electrophoresis [21]. The phase diagram for rod-like polyelectrolytes is actually rather complex and may involve coexistence with lamellar phases [22]. There is also evidence of a charged density wave along condensed F-actin [23]. Future work should probably include precise scaling law studies carried out around the critical points of the polyelectrolyte-polycation system to throw light on the relevance of the various correlation-interaction hypotheses.

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