

# 1 Introduction to Charged Macromolecules

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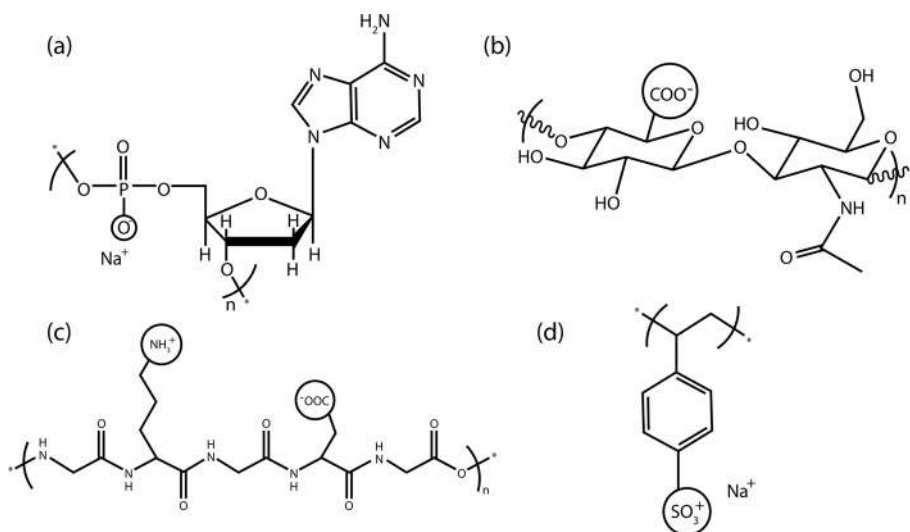
## 1.1 General Premise

Charged macromolecules are everywhere in nature. Examples in biological contexts are deoxyribonucleic acid (DNA), ribonucleic acid (RNA), polysaccharides, and proteins, which are dispersed in aqueous solutions usually under crowded conditions. These macromolecules have existed since the dawn of life on earth some billions of years ago, as they constitute the makeup of various organisms and their functions. There is also a plethora of synthetic charged macromolecules such as polyacrylic acid, poly(styrene sulfonate), and poly(vinyl pyridinium) salts, which are of significance in the materials world. These charged macromolecules have led to water-based materials with amazing attributes in the healthcare industry and biotechnology. A proper understanding of the structure and dynamics of charged macromolecules and their collections is a difficult challenge due to multiple forces acting simultaneously over long distances. In this overview chapter, we shall present the key conceptual features responsible for the behavior of charged macromolecules and some tantalizing experimental data exhibited by charged macromolecules in water.

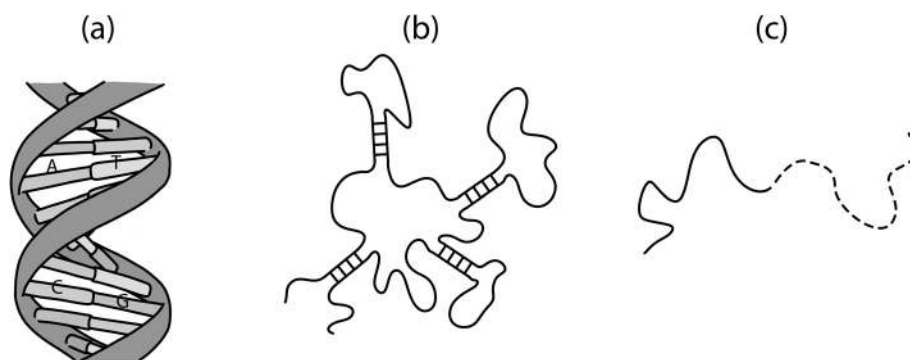
Charged macromolecules are made up of many electrically charged monomers that are connected contiguously into long polymer chains. The examples of single-stranded DNA (ssDNA), a polysaccharide, a polypeptide, and poly(styrene sulfonate) are illustrated in Fig. 1.1. Each repeat unit bears a charge as indicated by the circles in the figure.

We begin with the nomenclature of these macromolecules. We shall refer to the charged macromolecules made up of identical repeat units as **homo polyelectrolytes**. Typically, the skeleton of such polyelectrolytes is flexible under physiologically relevant conditions. Therefore, these are called **flexible polyelectrolytes**. In contrast to homo polyelectrolytes, **hetero polyelectrolytes** are composed of more than one kind of charged repeat unit. Classic examples are DNA and RNA. The incipient characteristic of these information-carrying charged macromolecules is their ability to form hydrogen bonds among the bases of the various repeat units, as illustrated in Fig. 1.2a for double-stranded DNA (dsDNA) and in Fig. 1.2b for single-stranded RNA (ssRNA). The base pairing in dsDNA results in the formation of double helices where the continuous backbones are stiff, in contrast to flexible ssDNA. Such molecules are called **semiflexible polyelectrolytes**, and they can even be rod-like if their contour

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**Figure 1.1** Chemical structures of (a) poly(deoxyadenylic acid), (b) the polysaccharide hyaluronate, (c) a polypeptide, and (d) poly(styrene sulfonate), showing the contiguous chain connectivity of charged repeat units. The charged groups are indicated by circles.



**Figure 1.2** (a) Base pairing due to hydrogen bonding in dsDNA results in a stiff polyelectrolyte. (b) Base pairing in ssRNA results in a branched polyelectrolyte. (c) Diblock copolymer of poly(styrene sulfonate) (solid curve), which is charged, and uncharged poly(ethylene oxide) (broken curve).

length is short. On the other hand, base pairing within a single chain, as in ssRNA depicted in Fig. 1.2b, results in highly branched structures. In such structures, the hydrogen-bonded stems function as branch points. In addition, the branch points in a branched architecture can arise from either chemical cross-linking or physical association between several chemical groups. Such branched molecules either with physical association or chemical cross-linking are labeled as **branched polyelectrolytes**. In terms of synthetic polyelectrolytes, the simplest example of a hetero polyelectrolyte is a diblock copolymer with one block being a polyelectrolyte and the other block



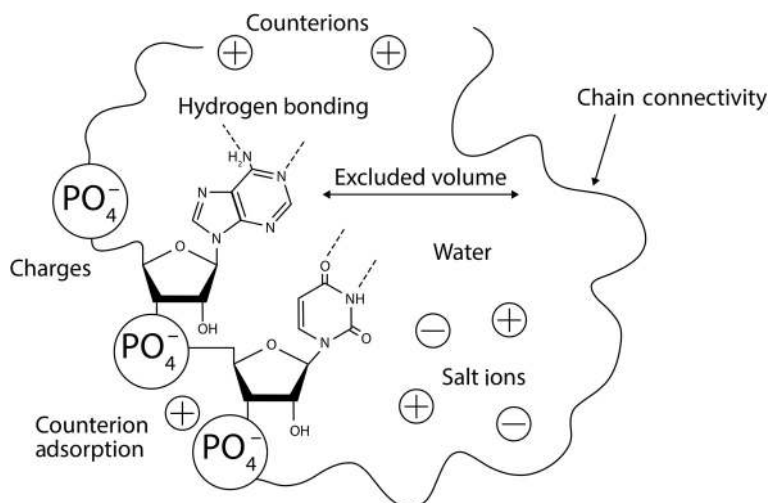
**Figure 1.3** (a) Folded state of the human  $\gamma$ -D crystallin protein. (b) An example of intrinsically disordered proteins.

being an uncharged polymer, as illustrated in Fig. 1.2c for the poly(styrene sulfonate-co-ethylene oxide) diblock copolymer. Such synthetic systems offer a broad range of opportunities for formulating novel self-assembling structures of technological relevance.

In the examples of Figs. 1.1a, 1.1b, and 1.1d, all repeat units bear charges of the same sign (as in the phosphate group in Fig. 1.1a). If a polymer is made up of positively charged repeat units, it is called a polycation, and if it is made up of negatively charged repeat units, it is called a polyanion. There is another class of charged macromolecules, as shown in Fig. 1.1c, where each molecule has both positive and negative charges in a certain sequencing order, as exemplified by proteins. Macromolecules containing both positive and negative charges are known as **polyampholytes**. Depending on the extent and sequence of positive and negative charges and their tunability by the ionic environment through pH, salt concentration, and hydrophobicity, such molecules can either appropriately fold into stable tertiary structures (Fig. 1.3a) or lack this ability by forming intrinsically disordered proteins (Fig. 1.3b).

For polyelectrolytes and polyampholytes, ionization equilibria of the repeat units in the polymer sequences dictate where the charges ought to be present at a given pH and salt concentration. A prescription of specific charge locations along the chain contour is referred to as **quenched** charge distribution. In reality, however, the charge on a particular repeat unit can fluctuate between on and off over a period of time depending on the local electrostatic environment. As a result, the charges on the polymer can be imagined to be smeared over the chain backbone. Such a description is called **annealed** charge distribution. Almost all theoretical formulations on charged macromolecules treat only this class of charged macromolecules.

The above descriptions of polyelectrolytes are only for the backbone of the chains, which cannot exist on their own, since the accumulation of similar charges on one molecule would be energetically prohibitive. Because the whole polyelectrolyte solution must be electrically neutral in equilibrium, there is an equal number of oppositely charged ions, called **counterions**, to balance the number of ionized groups of the polyelectrolyte chains. Depending on the temperature, the amount and identity of added salt, and the polarizability of the dipolar aqueous background medium, small ions hover around the polyelectrolyte chains to variable degrees. Furthermore, hydrogen



**Figure 1.4** Sketch of a portion of ssRNA in salty water emphasizing the simultaneous presence of various factors: chain connectivity, charges on the repeat units, counterions, salt ions, ion-pair formation between charged repeat units and the counterions, polarizability of water, hydrogen bonding, and van der Waals type excluded volume interaction.

bonding, structural reorganization of water, and short-ranged van der Waals interactions (called **excluded volume interactions**) are also present. As an example, a small portion of an isolated ssRNA molecule in salty water is sketched in Fig. 1.4. The system is endowed with the omnipresent electrostatic interaction between charges (phosphate groups, counterions, and salt ions), hydrogen bonding, dipolar interactions, and excluded volume interactions. The confluence of all of these attributes in addition to the topological character of chain connectivity is responsible for the structure and dynamics of the system. This description of the various forces is not unique to only ssRNA portrayed in Fig. 1.4 but is ubiquitous to all charged macromolecules in aqueous solutions.

In general, charged macromolecules dispersed in aqueous solutions are strongly correlated. Every molecule interacts with every other molecule in the entire system. The various structural and functional properties of solutions of macromolecules are collective behaviors involving essentially all molecules in the system mediated by its electrolyte background. The origin of the various distinct properties of charged macromolecules, which are qualitatively different from those of uncharged polymers, lies in the long-ranged electrostatic interactions among the various molecules and ionic constituents of the system. Modulations of these Coulomb forces by van der Waals type interactions are responsible for the collective properties of charged macromolecules. In crowded environments, as are common in many biological situations, the system appears as a Coulomb soup. The structure of charged macromolecules, their self-assembly into large-scale mesoscopic structures, and the movement of the various macromolecules in such a Coulomb soup form the subject of this book.

## 1.2 Chain Connectivity and Long-Ranged Topological Correlation

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**Table 1.1** Both polymeric and ionic properties control polyelectrolyte precipitation

Solute	BaCl <sub>2</sub>	AlCl <sub>3</sub>
NaPSS	Yes	Yes
ClSO <sub>3</sub> H	Yes	No
<i>p</i> -Toluenesulfonate	Yes	No

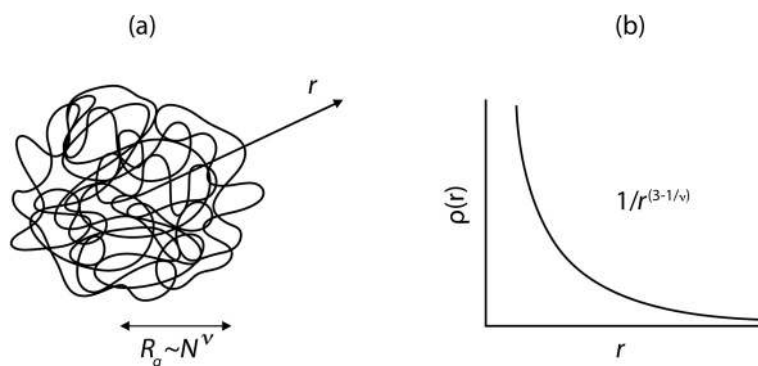
The behavior of charged macromolecules in solutions cannot be separately attributed to either solely polymeric properties or solely ionic properties. As an example, consider separate aqueous solutions of sodium poly(styrene sulfonate) (NaPSS), chlorosulfonic acid (ClSO<sub>3</sub>H), and *p*-toluenesulfonate, each containing either BaCl<sub>2</sub> or AlCl<sub>3</sub> at pH 3.2 and room temperature [Narh & Keller (1993)]. Except for the solutions of chlorosulfonic acid and *p*-toluenesulfonate containing AlCl<sub>3</sub>, all other solutions yield solid precipitates under these experimental conditions, as given in Table 1.1 (where “Yes” and “No” indicate the presence and absence of a precipitate, respectively). The ionic groups of NaPSS, ClSO<sub>3</sub>H, and *p*-toluenesulfonate are essentially the same, while NaPSS is a polymer and the other two are monomers. As shown in Table 1.1 under the column of BaCl<sub>2</sub>, precipitation in these three solutions indicates that the precipitation is an ionic property and that the polymer nature of NaPSS is not critically relevant. On the other hand, NaPSS precipitates in AlCl<sub>3</sub> solutions, whereas ClSO<sub>3</sub>H and *p*-toluenesulfonate do not, as shown in Table 1.1 under the column of AlCl<sub>3</sub>. This indicates that precipitation in AlCl<sub>3</sub> solutions is essentially a polymer property. Thus, polyelectrolyte behavior must be a combination of both polymeric and ionic properties. We briefly introduce the basic features of polymeric and ionic properties in the following two sections, before deeply dwelling on them in the following chapters.

### 1.2 Chain Connectivity and Long-Ranged Topological Correlation

Consider an uncharged large flexible macromolecule of  $N$  repeat units in isolation. On average, it adopts a coil-like conformation, as depicted in Fig. 1.5a, due to chain connectivity. It appears as a rough sphere. A measure of its physical size is its radius of gyration  $R_g$ . In general, the dependence of  $R_g$  on  $N$  (which is proportional to the molecular weight of the polymer) is given by the proportionality relation [de Gennes (1979)],

$$R_g \sim N^\nu, \quad (1.2.1)$$

where  $\nu$  is called the size exponent. The value of  $\nu$  depends on the chemical details of the repeat units of the polymer and the solvent, and temperature, whose roles are collectively called **excluded volume interactions**. The range of values of  $\nu$  is between 1/3 and 1, corresponding to the extreme limits of globule-like and rod-like conformations for the chain. The local monomer density  $\rho(r)$  at radial distance  $r$  from



**Figure 1.5** (a) Random coil conformation of an uncharged flexible chain with the radius of gyration  $R_g$ , which depends on the number of repeat units  $N$  as  $R_g \sim N^\nu$ , where ( $1/3 < \nu < 1$ ). (b) Long-ranged topological correlation due to chain connectivity, as shown by the dependence of local monomer density  $\rho(r)$  on the radial distance  $r$ .

the center of mass of the chain decays with  $r$  as an inverse power law [de Gennes (1979)],

$$\rho(r) \sim \frac{1}{r^{3-1/\nu}}, \quad (1.2.2)$$

as sketched in Fig. 1.5b. The value of the exponent in the power-law decay depends on the value of  $\nu$ . For example, for a flexible chain without consideration of excluded volume interactions,  $\nu = 1/2$ , and hence  $r$  dependence of  $\rho(r)$  is long-ranged,

$$\rho(r) \sim \frac{1}{r}. \quad (1.2.3)$$

This long-ranged correlation of monomer density is entirely due to chain connectivity. Hence, it is called the topological correlation of the chain. The quantitative nature of the topological correlation of the chain given in Eq. (1.2.2) is modified by both the excluded volume interactions and electrostatic interactions as represented through the size exponent  $\nu$ .

### 1.3 Scales of Energy, Length, and Time in Charged Systems

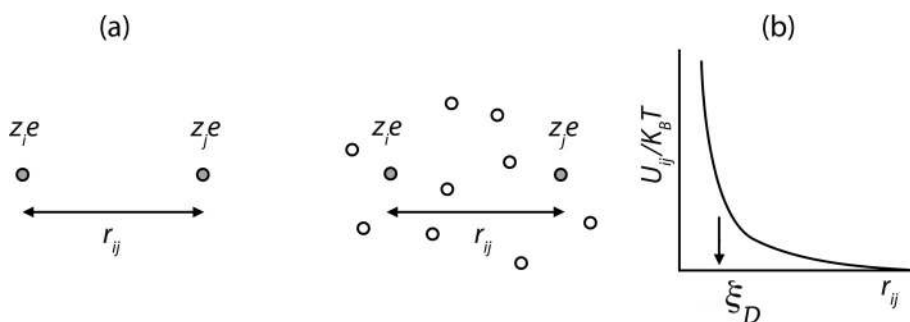
**Energy:** Consider the electrostatic interaction energy  $U_{ij}$  between a pair of ions with charges  $z_i e$  and  $z_j e$  separated by a distance  $r_{ij}$  (Fig. 1.6a) in a medium with a uniform dielectric constant  $\epsilon$  (in the absence of any other charges). This is given by Coulomb's law,

$$\frac{U_{ij}}{k_B T} = z_i z_j \frac{e^2}{4\pi\epsilon_0 \epsilon k_B T r_{ij}}, \quad (1.3.1)$$

where  $k_B T$  is the Boltzmann constant times the absolute temperature,  $z_i$  is the valency of the  $i$ th ion,  $e$  is the electronic charge, and  $\epsilon_0$  is the permittivity of vacuum. The

## 1.3 Scales of Energy, Length, and Time in Charged Systems

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**Figure 1.6** (a) The interaction energy between two ions of charges  $z_i e$  and  $z_j e$  in the absence of any other charges in their neighborhood is the long-ranged Coulomb interaction energy. (b) In the presence of other ions, shown as open circles, the electrostatic interaction energy between two ions is a screened Coulomb energy, also known as the Debye–Hückel electrostatic energy. The range  $\xi_D$  over which the electrostatic interaction decays by  $1/e$  is the Debye length.

middle factor in Eq. (1.3.1) has the dimension of length and is called the **Bjerrum length**  $\ell_B$ ,

$$\ell_B \equiv \frac{e^2}{4\pi\epsilon_0\epsilon k_B T}. \quad (1.3.2)$$

If the separation distance  $r_{ij}$  is comparable to  $\ell_B$ , then the electrostatic interaction energy of two monovalent ions ( $z_i = 1 = z_j$ ) is comparable to the thermal energy  $k_B T$ , as is evident from Eq. (1.3.1). For oppositely charged ions, if the distance between them is shorter than  $\ell_B$ , then the attraction is stronger than  $k_B T$ , and the ions are more likely to be ion pairs instead of completely dissociated ions. The Bjerrum length sets the scale for energy. It is inversely proportional to  $\epsilon T$ , where  $\epsilon$  itself is temperature dependent. For aqueous solutions at 25°C, the Bjerrum length (with  $\epsilon = 80$ ) is

$$\ell_B \approx 0.7 \text{ nm}. \quad (1.3.3)$$

**Length:** When other ions are present in the solution, the electrostatic interaction energy between a pair of ions is no longer the Coulomb energy given by Eq. (1.3.1). Instead, it is a screened Coulomb energy. According to the approximate theory of Debye and Hückel for electrolyte solutions, the pairwise interaction energy between two ions of charges  $z_i e$  and  $z_j e$  in an electrolyte solution is given by the screened Coulomb energy,

$$\frac{U_{ij}}{k_B T} = z_i z_j \ell_B \frac{e^{-\kappa r_{ij}}}{r_{ij}}, \quad (1.3.4)$$

where

$$\kappa^2 = \frac{e^2}{\epsilon_0 \epsilon k_B T} \sum_i z_i^2 n_{i0}, \quad (1.3.5)$$



where  $n_{i0}$  is the average number concentration of the  $i$ th ion. While the strength of the electrostatic interaction between ions is given by the Bjerrum length, its range is given by  $\kappa^{-1}$ , known as the **Debye length**  $\xi_D \equiv \kappa^{-1}$ ,

$$\xi_D = \left( \frac{e^2}{\epsilon_0 \epsilon k_B T} \sum_i z_i^2 n_{i0} \right)^{-1/2}. \quad (1.3.6)$$

The range decreases if  $\epsilon T$  is reduced and/or if the electrolyte concentration is increased. For monovalent salts in water ( $\epsilon = 80$  at  $25^\circ\text{C}$ ), the Debye length is

$$\xi_D \simeq \frac{0.3}{\sqrt{c_s}} \text{ nm}, \quad (1.3.7)$$

where  $c_s$  is the salt concentration in units of moles per liter. The screened potential (Debye–Hückel potential) given by Eq. (1.3.4) is only approximate. Nevertheless, the Debye length is a fundamental scale of length in the treatment of polyelectrolyte solutions. For interionic distances larger than  $\xi_D$ , the electrostatic interaction energy between any pair of ions is weak; for distances shorter than  $\xi_D$ , the electrostatic interaction energy is strong. Thus, the Debye length denotes the range of distance over which a pair of ions interact significantly between them, as sketched in Fig. 1.6b.

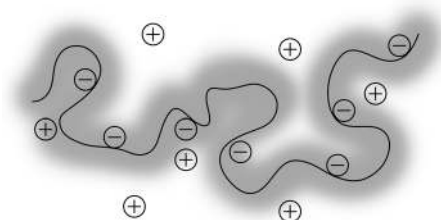
The above two fundamental parameters ( $\ell_B$  and  $\xi_D$ ) have very important consequences in the physics of solutions of all kinds of charged macromolecules. Consider a polyelectrolyte solution at a finite concentration. Although the polyelectrolyte molecule is fully ionizable, the chance that a counterion would be near a monomer and be bound to the polymer is finite, if the distance between the monomer and the counterion is less than  $\ell_B$ . In particular, the effective dielectric constant in the neighborhood of the chain backbone can be quite low and hence the binding energy is very high, as can be seen from Eq. (1.3.1).

As a result, a certain number of counterions are bound to the polymer as depicted in Fig. 1.7, and the naked charge of the molecule is never realizable in equilibrium at finite polyelectrolyte concentrations and ambient temperatures. Furthermore, when a counterion is bound to the monomer of the polymer, a dipole is formed. Such dipoles are only temporary but can be sufficiently long-lived to interact with other dipoles formed along the chain contour. These dipole–dipole interactions can be quite strong compared to  $k_B T$ . For example, for two freely rotating dipoles  $\mathbf{p}_1$  and  $\mathbf{p}_2$  of unit charge separated by the distance  $r$  in an electrolyte solution, the angularly averaged interaction energy is attractive (Appendix 3),

$$\frac{U_{\text{dipole-dipole}}(r)}{k_B T} = -\frac{p_1^2 p_2^2 \ell_B^2}{3r^6} e^{-2\kappa r} \left[ 1 + 2\kappa r + \frac{5}{3}(\kappa r)^2 + \frac{2}{3}(\kappa r)^3 + \frac{1}{6}(\kappa r)^4 \right]. \quad (1.3.8)$$

For typical physiological values of monovalent salt concentrations of about 150 mM and a typical length of the ion pairs, the effective pairwise interaction energy is about  $10 k_B T$ . The dipole–dipole interaction energy can therefore be much higher than the thermal energy. In view of such strong attraction energies associated with dipole–dipole pairs, we readily anticipate that some segments of the chains would cling together due to the formation of quadrupoles and the rest of the chains would repel





**Figure 1.7** Counterions can adsorb on chain backbone forming temporary dipoles. The shaded area denotes the local environment around the oil-like chain backbone where the local dielectric constant can be substantially lower than the bulk value away from the chain.

each other. Naturally, such a scenario can result in nonuniform structures and even attraction between similarly charged polymers.

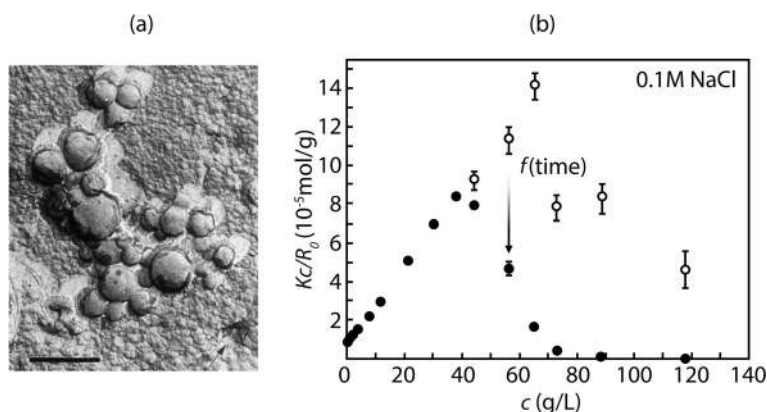
**Time:** Another message conveyed by Fig. 1.7 is that there is a hierarchy of time scales: diffusion time for a free counterion, lifetime of an adsorbed counterion, segmental relaxation time, characteristic relaxation time for the whole polymer, characteristic time for the relaxation of the collection of dipole pairs, entanglement time, etc. As a result, the dynamics of polyelectrolyte chains and the transport properties of polyelectrolyte solutions exhibit very rich phenomenology. In qualitative terms, sometimes the large macromolecules follow the motion of small free counterions, and some other times, the small ions hover over the large macromolecules, both by maintaining electroneutrality over a reasonably small volume.

## 1.4 Confluence of Electrostatic and Topological Correlations

The cooperative features of chain connectivity and electrostatic interactions among all charged species in the system lead to amazing properties as evident in various biological processes in life itself. Many challenges arise in a fundamental understanding of the behavior of charged macromolecules. We now briefly mention a few representative examples of some of the enigmatic properties displayed by charged macromolecules.

### 1.4.1 Similarly Charged Macromolecules Can Attract Each Other

As the polymer concentration of dsDNA of molar mass  $10^5$  Da in a 0.1 M NaCl solution is increased, the macromolecules can aggregate into large clusters of micrometer size and settle down at the bottom of the solution over a period of time [Wissenburg *et al.* (1995)]. This phenomenon is seen through cryoelectron microscopy (Fig. 1.8a) and light scattering (Fig. 1.8b). In Fig. 1.8b, the ordinate is proportional to the inverse of scattered intensity from the solution and the abscissa is the concentration of dsDNA. At a DNA concentration of about 40 g/L or above, thermodynamic instability sets



**Figure 1.8** dsDNA molecules can aggregate although each molecule bears the same negative charge. (a) Electron micrograph for a 73 g/L DNA solution at 0.01 M NaCl. The bar corresponds to 300 nm. (b) Inverse scattering intensity versus DNA concentration  $c$  at 0.1 M NaCl. Thermodynamic instability and precipitation occur above a threshold value of  $c$ , and depend on time history,  $f(\text{time})$ . Open symbols denote the condition after filtration of the precipitate and the filled symbols denote the condition before filtration [Wissenburg *et al.* (1995)].

in and the solution is not homogeneous anymore. The precipitation depends on time history,  $f(\text{time})$ , as indicated in Fig. 1.8b. This clumping phenomenon is surprising because dsDNA molecules are all similarly charged and are expected to repel each other to remain fully dispersed in the solution. Even more remarkably, the instability occurs at lower DNA concentrations if the salt concentration is lower where the electrostatic repulsion among the polymer chains is expected to be higher.

### 1.4.2 Ordinary–Extraordinary Transition

In dilute solutions of uncharged macromolecules, the measured diffusion coefficient  $D$  using the dynamic light-scattering technique follows the Stokes–Einstein law, enabling accurate characterization of the macromolecule in terms of its hydrodynamic radius  $R_h$  (which is proportional to the radius of gyration  $R_g$ ),

$$D = \frac{k_B T}{6\pi\eta_0 R_h}, \quad (1.4.1)$$

where  $\eta_0$  is the viscosity of the solvent. This methodology fails in the case of charged macromolecules when the concentration  $c_s$  of the added low molar mass salt in the solution is not high. As  $c_s$  is reduced, the value of  $D$  increases [Lin *et al.* (1978)] (Fig. 1.9a), contrary to the expectation that the chain would be bigger due to electrostatic repulsion among the charged repeat units at lower salt concentrations, and hence from Eq. (1.4.1)  $D$  would be smaller. In spite of this counterintuitive result, the increase in  $D$  as  $c_s$  is decreased is known as the “ordinary” or “fast” diffusive behavior. In addition, upon further decrease in  $c_s$ , a new “slow” diffusive mode of relaxation