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A two-dimensional many-body system with competing interactions as a model for segregation of photosystems in thylakoids of green plants

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Abstract We address the segregation of photosystems I (PSI) and II (PSII) in thylakoid membranes by means of a molecular dynamics method. We assume a two-dimensional (in-plane) problem with PSI and PSII being represented by particles with different values of negative charge. The pair interactions between particles include a screened Coulomb repulsive part and am exponentially decaying attractive part. Our modeling results suggest that the system may have a complicated phase behavior, including a quasi-crystalline phase at low ionic screening, a disordered phase and, in addition, a possible ''clotting'' agglomerate phase at high screening where the photosystems tend to clot together. The relevance of the observed phenomena to the stacking of thylakoid membranes is discussed.

Key words Lipid-protein interactions · Photosystem I · Photosystem II · Thylakoid membranes

Introduction

The organelles of plants in which all the photosynthetic activity takes places, the chloroplasts, contain membraneous bodies, thylakoids, that partly form cylindrical stacks called grana and partly form sheets called stroma lamellae. The thylakoid membranes incorporate two types of photosystems, photosystem I (PSI) and photosystem II (PSII), that are pigment-protein complexes capable of transforming light quanta energy into charge separation which, in turn, drives vital metabolic functions of the plant.

The distribution of the two photosystems, PSI and PSII, between grana and stroma lamellae is uniform, with the PSII mainly concentrated in grana and PSI in stroma (Barber 1982; Butter 1978). The role of the spatial separation of PSI and PSII is not well understood. This separation of photosystems, conventionally termed segregation, conflicts in a way with the traditional assumption of two photosystems operating in accord, because the further the two photosystems are separated, the less likely is a strong coupling between them. Also, the role and function of the grana have been the subject of many speculations, the most recent being that of physically separating a slow (PSII) and a fast (PSI) photosystem (see, e.g. Trissl and Wilhelm 1993).

The coupling between the two photosystems, as it is currently understood, takes place via two main mechanisms. The first (and the fastest) coupling mechanism implies an exciton transfer between the two photosystems sharing common antenna. This mechanism is called spillover. The regulation of the supply of light quanta of PSI and PSII via redistribution of excitons was first observed in the 1960s (see Barber 1982; Butler 1978 and references therein) by means of fluorescence spectroscopy, where the spillover phenomena manifest themselves in increasing the level of PSI fluorescence with concomitant decrease of PSII fluorescence. A number of studies combining spillover measurements and electron microscopy showed (see, e.g. Ivanov and Apostolova 1997) that the degree of spillover strongly depends on the degree of thylakoid stacking and segregation of photosystems in the membrane as well as the distances between them.

Another, much slower, mechanism is common electron transport (termed Z-scheme), that is essentially a diffusion of plastoquinone electron carriers within

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the thylakoid membrane. This diffusion process also depends strongly on the topology of the two-dimensional manifold within which it occurs (see, e.g. Dubinski and Tikhonov 1997). The degree of segregation and thylakoid membrane stacking has been proved to be highly dependent on the environmental conditions in vivo (Butler 1978) and on the experimental conditions in vitro in response to dodecylsulfate treatment (Ivanov and Apostolova 1997), heat stress (Gounaris et al. 1984; Velitchkova and Ivanov 1993), photolipase A_2 treatment (Ivanov 1991), divalent cations and trypsin (Ivanov et al. 1987; Steinbeck et al. 1979). Light scattering experiments (Dobrikova et al. 1997a, b) showed the correlation between changes in the spillover, surface charge density, and membrane fluidity. These results hint at a possible dominant role of the electrostatic interactions in segregation and stacking phenomena, as well as of kinetic properties of PSI and PSII diffusion in the membrane. These conclusions are summarized in Table 1.

The models concerning the mechanisms of cation-induced PSI-PSII segregation are conventionally classified into two groups. One is molecular recognition (MR) theory, which explains ion-dependent segregation and stacking by changes in protein structures and binding specificities (Allen 1992). Another model is surface charge (SC) theory (Barber 1982), which attributes segregation and stacking to cooperative phenomena in ensembles of electrostatically interacting lipids and photosystems. A decrease in spillover and the concomitant increase in stacking correspond to a high degree of segregation of photosystems in the membrane while randomized, i.e. a more uniform distribution of photosystems (no stacking case) corresponds to high spillover values. The above conclusions suggest some sort of cooperative phenomena, where the ordering of photosystems within the membrane becomes the result of interplay between electrostatic interactions and diffusion. It has also been shown by fast fluorescence kinetics experiments that the segregation and stacking are independent phenomena caused by two different ion-dependent mechanisms (see Stys 1995 and references therein).

The protein interactions with and within the membrane include Coulomb interactions (screened in the presence of cations), van der Waalls forces, dipole-dipole interactions, and lipid-induced protein-protein attraction (Ben-Tal et al. 1997; Kleinschmidt and Marsh 1997; Sintes and Baumgartner 1997). In particular, Sintes and Baumgartner (1997) found two types of lipid-mediated attraction between proteins embedded in a bilayer membrane: a short-range depletion-induced attraction and a long-range fluctuation-induced attraction. The presence of competing attractive and repulsive interactions can qualitatively explain the effects of divalent cations, trypsin, and high-temperature treatments on the protein-protein interactions by changing the interactions between photosystems.

Table 1 Effects of different treatments on the thylakoid membrane stacking and intersystem spillover (see text for references)

Treatment	Electrostatic repulsion strength	Membrane fluidity	Stacking	Spillover
Heat	Increase	Increase	Decrease	Increase
Tripsin	Increase	–	Decrease	Increase
Divalent cations	Decrease	–	Increase	Decrease

In the present paper we model the equilibrium state of an ensemble of photosystems in thylakoid membranes as a classical many-body system with a competing screened Coulomb repulsion and a lipid-induced attraction. Reviews of the application of molecular dynamics method to study the kinetics of liquid phases and lipid membranes are given by Allen and Tildesley (1987) and Tieleman et al. (1997). However, the most detailed models applied in the study of lipid kinetics in the membranes avail the time evolution of the system up to a few nanoseconds. The stacking and segregation phenomena manifest themselves at a time scale of 0.1–1 s and at spatial scales of micrometres, which makes the utilization of simplified effective interactions unavoidable, especially if one is to study the final distributions of photosystems at equilibrium.

A model for PSI/PSII interactions within the membrane

The use of a simplified model is backed by the fact that the segregation and stacking phenomena seem to be similar to a phase transition, so that the changes in structure are essentially macroscopic. In this case, the use of an approximate "effective" interaction is, in most cases, acceptable, if one is not preoccupied with calculating fine details of the phase transition, such as critical indices. As the correlation length increases in the vicinity of the transition point, the effective interaction is averaged over interacting domains of correlated molecules. In this case it is reasonable to assume that fine details of the interaction, including the asymmetry of interaction, are averaged out, and a rather simple effective interaction may be put in their place. In our simple model we neglect any deviations from pairwise spherically symmetric interactions that may be present in real systems due to the asymmetry of pigment-protein complexes and the entropic character of lipid-induced protein-protein interactions.

We assume that the photosystems carry negative charges of -1.6×10^{-18} C (PSI) and -1.2×10^{-18} C (PSII) and can move within a two-dimensional plaquette $0.6 \times 0.6\ \mu m^2$ (which is approximately four times the granal vesicle size) with periodic boundary conditions representing the thylakoid membrane. Data for the thylakoid membrane surface charge density (Barber 1982; Ivanov et al. 1987) confirms the correct order magnitude of these values.

We neglect the effects of membrane plane curvature on the diffusion properties of particles and take the lipid membrane to be electrically neutral (Quinn and Williams 1983). The total number of particles in the system was taken to be 800 (corresponding to a typical value of 2000–3000 particles/μm^2) with the PSII to PSI ratio being 7:3, which is close to the commonly accepted value of 2:1. We postulate that the interactions between particles include a Debye-type repulsion U_C and a lipid-induced attraction U_L (Sintes and Baumgartner 1997):

$$U_C(r) = \frac{q_1 q_2}{4\pi\varepsilon_0\varepsilon}\frac{e^{-|r|/\xi}}{r}; \quad U_L(r) = -\lambda k_B T e^{-|r|/\xi} \; ;$$
$$\xi = \sqrt{\frac{2Z^2\bar{n}e^2}{k_B T\varepsilon_0\varepsilon}} \qquad (1)$$

with $q_{1,2}$ being effective charges of the PSI and PSII particles, and r and ξ being, respectively, the interparticle distance and Debye length. Also ε represents the dielectric permeability of the medium around the membrane, $\bar{n}$ and Ze denote, respectively, the ionic strength and the charge of an ion. The quantity $k_B T$ is the temperature in energy units, λ represents the strength of attraction, and ζ is the characteristic length of the lipid-induced attraction. The fluctuation nature of this interaction makes it reasonable to measure its strength in $k_B T$ units; hence the coefficient in Eq. (1). Also we account for the diffusion, which we model by adding a random displacement component with an amplitude according to the diffusion coefficient, which we took as $D = 3.1 \times 10^{-12}$ cm^2/s at 14 °C. The viscosity of the system is considered to be high enough as to exclude acceleration terms from the equations of motion.

In our crude approximation we used an overall parameter λ that has the meaning of the effect of dependence of this interaction on temperature, geometry, photosystems' structure, and membrane lipid composition. This parameter, as well as the dielectric permeability of the medium, are hard to estimate. On one hand, the dielectric permeability of water is 50–100 depending on the conditions. On the other hand, in the vicinity of a membrane, or a pigment-protein complex, it can be much lower (10–20) (see, e.g. White and Wimley 1999). In our calculations we chose $\lambda\varepsilon$ as a free parameter, having values such that $\lambda\varepsilon \sim 1$–10. The reason for this is that with the interactions the equilibrium state depends only on the ratio of repulsion and attraction (as sum of all the forces acting on each particle equals 0). We also used Debye length, not the ionic strength, as a free parameter, because of the same reason of not knowing the exact value of ε.

Molecular dynamics method

We start with the particles randomly distributed within the plaquette. Each step of the calculation consists of moving all the particles, one by one. When a particular particle to be moved is chosen, then:

1. We calculate the resulting force of interaction of that particle with all the others in the ensemble, including their closest images in the neighboring plaquettes. The interaction cutoff distance was taken to be five times the greatest of ξ and ζ, but not greater than 0.3 μm.
2. After the x- and y-components of the resultant force are calculated, they are divided by viscosity to obtain velocity components:

   $$v_{x,y} = \frac{F_{x,y}}{\eta}$$

   where the dynamic viscosity η is calculated as:

   $$\eta = \frac{k_B T}{D}$$

3. The velocity components then multiplied by a time step to obtain the displacement components due to the interaction forces:

   $$r^i_{x,y} = v_{x,y}\Delta t$$

4. Finally, we calculated the displacement due to diffusion as:

   $$r^D_{x,y} = \sqrt{2D\Delta t}\, v_{x,y}$$

   with $v_{x,y}$ being randomly chosen from a discrete set of numbers $\{-1, 0, 1\}$.

The time step, Δt, was chosen as a trade-off between reasonably quick relaxation into the equilibrium state while, at the same time, ensuring that the elementary displacements at each step are much less than the system size. In particular, the time steps for this calculation were taken to be 10–100 μs.

The above procedure was repeated 100–500 times, until the equilibration occurred, which could be judged visually by a decrease in displacement of the particles. Then, another 50–100 runs were accomplished to achieve representative calculation of the pair correlation function. We should note here that to obtain a reasonably accurate phase diagram, more rigorous criteria of equilibration have to be employed, particularly at parameter values close to the phase boundary, as the equilibration times in those areas tend to increase drastically. However, the exact phase boundaries for our particular case represent, in our view, only academic interest, because the uncertainties in determining the interaction constants and other parameters of the biological system are quite significant.

Modeling results and discussion

In this preliminary modeling we were mostly interested in the overall visualisation of the equilibrium configu-

rations as well as calculating the pair correlation functions. The simultaneous correlation function at a given distance, r, was calculated as the average number of particles positioned within an interval $[r-\delta r,\ r+\delta r]$ away from a given particle, divided by $2\pi r\ \mathrm{d}r$ to account for the area in a circular belt described by the above interval. We did not distinguish between the different types of particles when calculating the correlation function. In the figures the PSIIs are depicted by crosses and PSIs by dots. All the data presented were obtained taking $\zeta = 0.02$ μm for the characteristic length of the attractive interaction. For each set of parameters, several runs with different starting conditions were accomplished to ensure the independence of the results on the initial distribution of particles.

Our objective was to look for possible sources and types of inhomogeneity in the spatial distribution of photosystems which could account for further membrane slicing and stacking. First we examined the low screening case (see Fig. 1), with $\lambda\varepsilon = 5$ and the Debye length $\xi = 0.006$ μm. Both the correlation function and the configuration (Fig. 1a and b, respectively) showed a highly regular "packing" of the particles in a lattice-like structure. The reason for the existence of such a phase is the dominance of the repulsive screened-Coulomb interaction.

Next we looked at the case of stronger screening (smaller ξ), taking the same value of $\lambda\varepsilon$ but with $\xi = 0.004$ μm (Fig. 2a, b). The configuration becomes visibly more disordered (Fig. 2b), which corresponds to

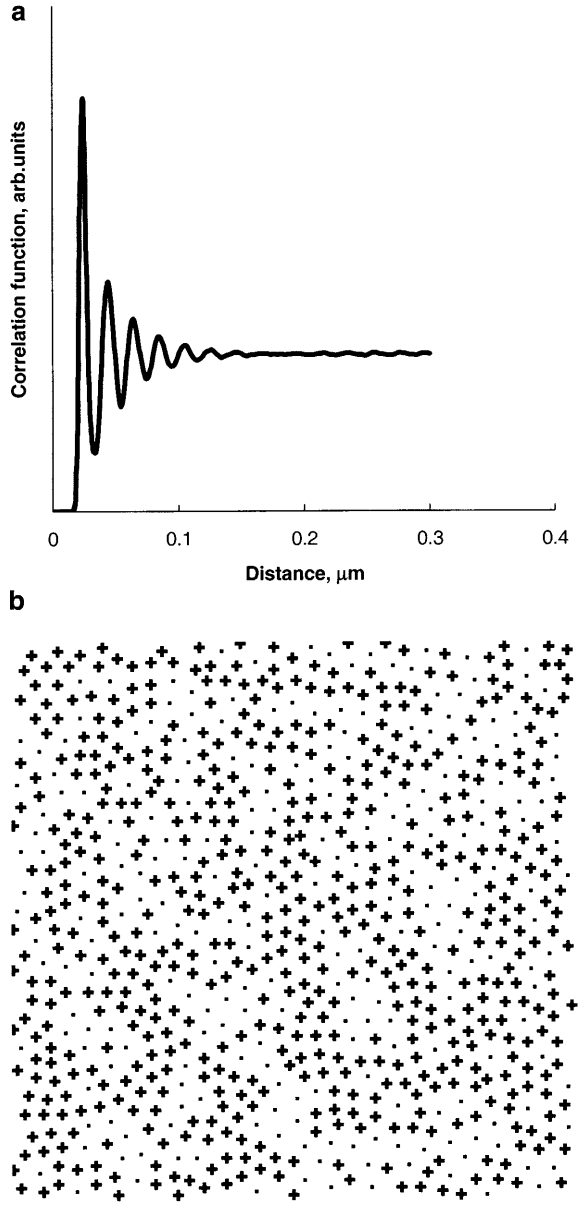


Fig. 1 Correlation function (**a**) and configuration (**b**) for the case of $\xi = 0.006$ μm and $\lambda\varepsilon = 5$ (see text for the other parameters)

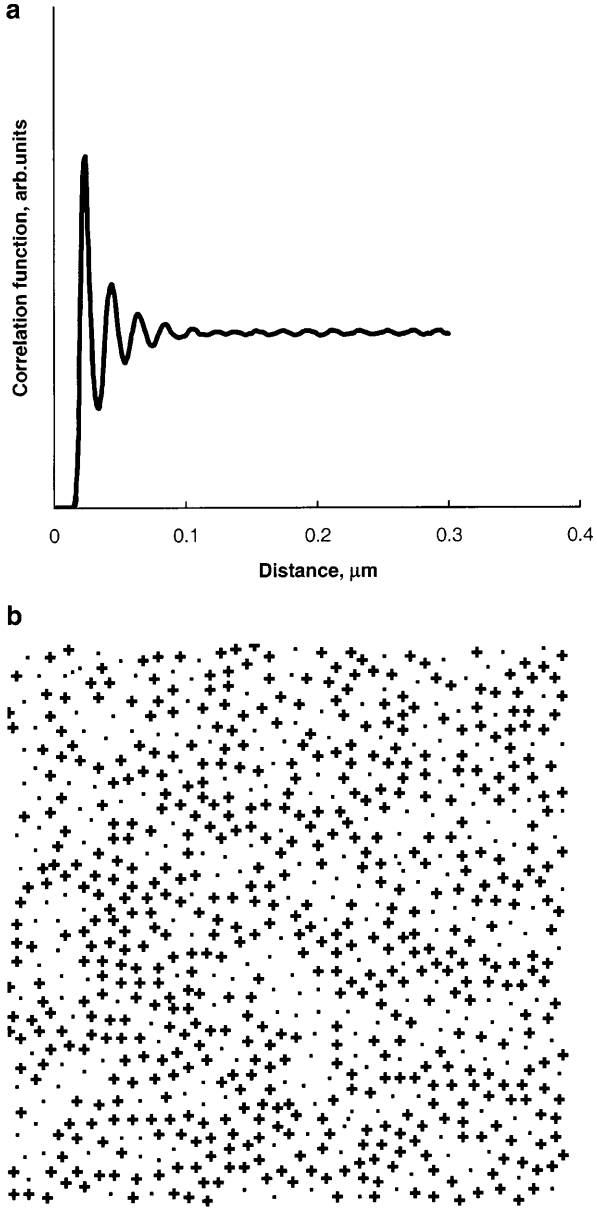
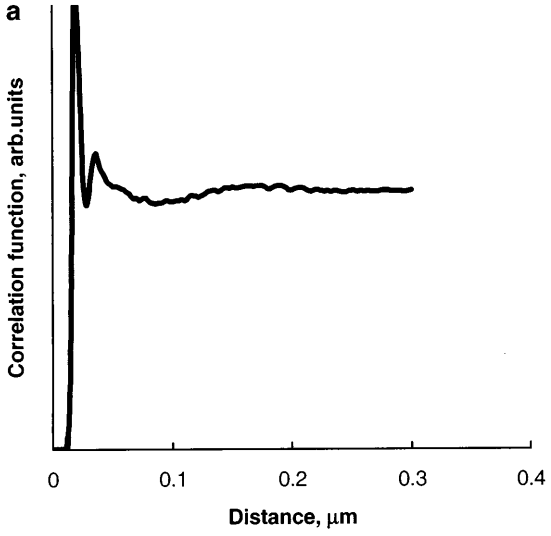


Fig. 2 Correlation function (**a**) and configuration (**b**) for the case of $\xi = 0.004$ μm and $\lambda\varepsilon = 5$ (see text for the other parameters)



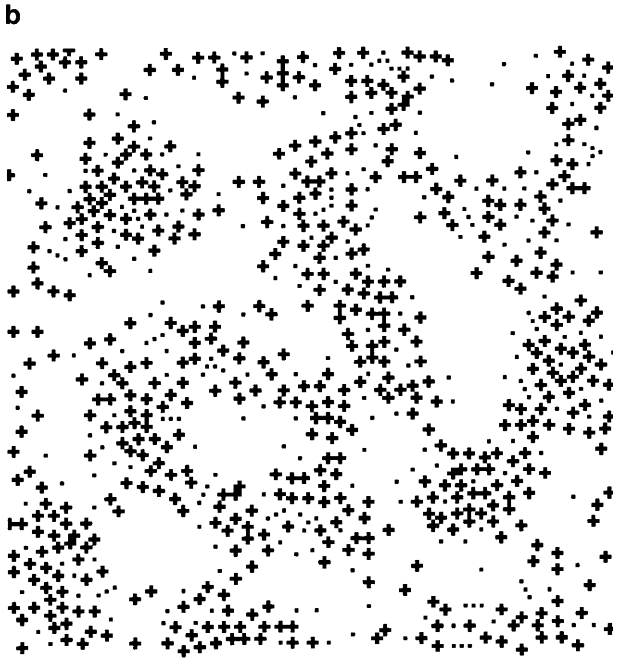


Fig. 3 Correlation function (**a**) and configuration (**b**) for the case of $\xi = 0.003$ μm and $\lambda\varepsilon = 5$ (see text for the other parameters)

a shorter correlation function decay length. A further decrease of ξ down to $\xi = 0.003$ μm leads to a drastic change in both the configuration and correlation function (Fig. 3a, b). It is seen that strongly inhomogeneous "clotting" appears as the particles agglomerate together, leaving empty spaces between the "clots". Moreover, the size of the domains (~0.1–0.2 μm) is of the same order of magnitude as the size of vesicles in the stacked granal regions of a thylakoid (~0.1–0.3 μm). This fact suggests a possible role of such "clotting" in further stacking of thylakoid membranes.

To study the clotting phenomenon further, we plotted the positions of the first (R_m) and the second (R_{m2}) maxima of the correlation function against the Debye length (Fig. 4). It is clearly seen that above

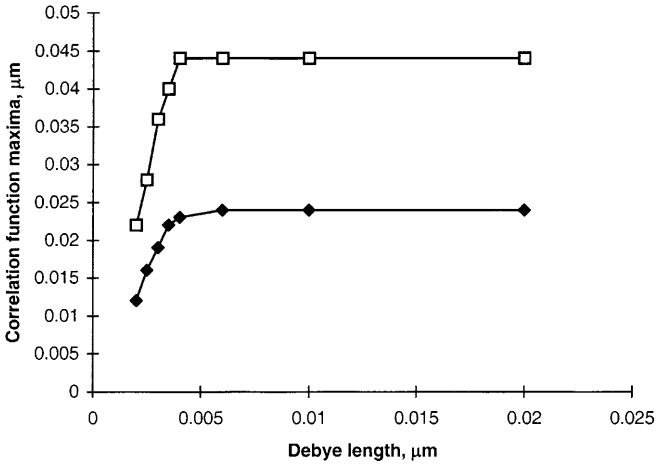


Fig. 4 Positions of the first two maxima of the correlation function as a function of Debye length ξ (see text for the other parameters). *Diamonds* R_m, *squares* R_{m2}

$\xi = 0.003$ μm the positions of the maxima do not depend on ξ, whilst at $\xi \leq 0.003$ μm a rapid decrease occurs. This suggests a critical character of this phenomenon, with the positions of the correlation function maxima being good candidates for the order parameter.

To check the role of the parameter $\lambda\varepsilon$ we also made some calculations with $\xi = 0.002$ μm for $\lambda\varepsilon = 2$ and $\lambda\varepsilon = 1$ (Figs. 5 and 6). Both calculations produced configurations which showed some signs of agglomeration, but not as pronounced as before in the case of the higher $\lambda\varepsilon$ value. This accounts for the fact that in the conditions of sufficient screening, "clotting" is dominated by the attraction strength.

Conclusions

We have shown that, depending on the relative values of parameters such as the strength of the Debye screening of the Coulomb interaction by cations in the solution, there may be three types of equilibrium state. If there is little or no screening, the system is ordered in a fashion similar to that of a crystal, with the photosystems forming an almost regular lattice. Such an even distribution of photosystems may account for the high spillover data at low ionic strengths reported (Dobrikova et al. 1997a, b).

As the screening increases, the system undergoes a disordering transition. If the screening is increased further the photosystems tend to form clusters, thus providing for the inhomogenity necessary for further stacking process. The formation of these clusters seems to depend critically on the Debye length at given values of the interaction strengths. The way that the clusters are formed in the system resembles the so-called polymorphic phase transitions in liquids, which result in the coexistence of two distinct liquid phases

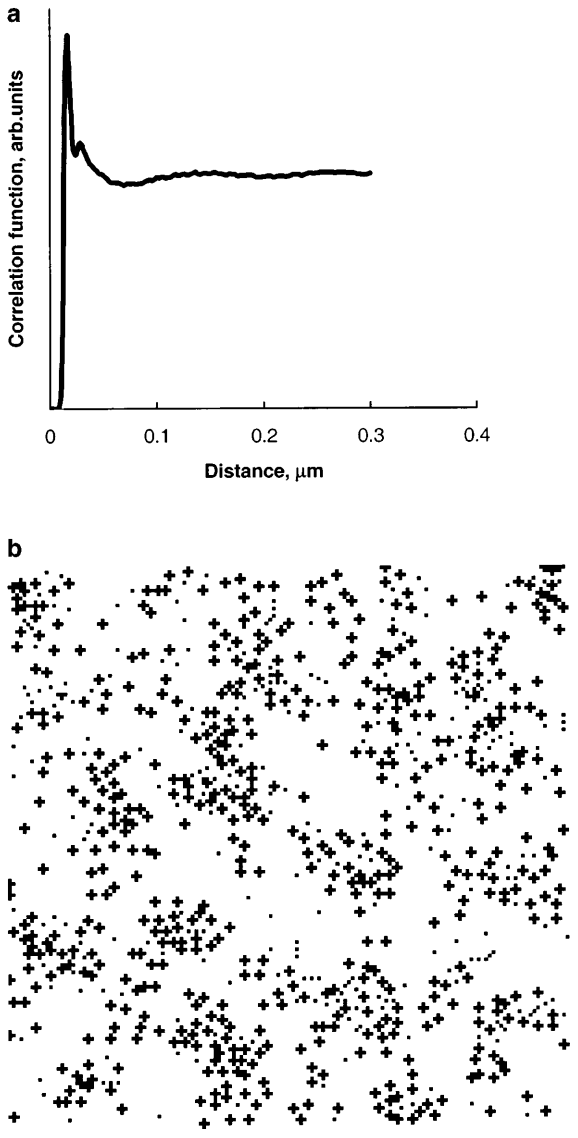


Fig. 5 Correlation function (**a**) and configuration (**b**) for the case of $\xi = 0.002$ μm and $\lambda\varepsilon = 2$ (see text for the other parameters)

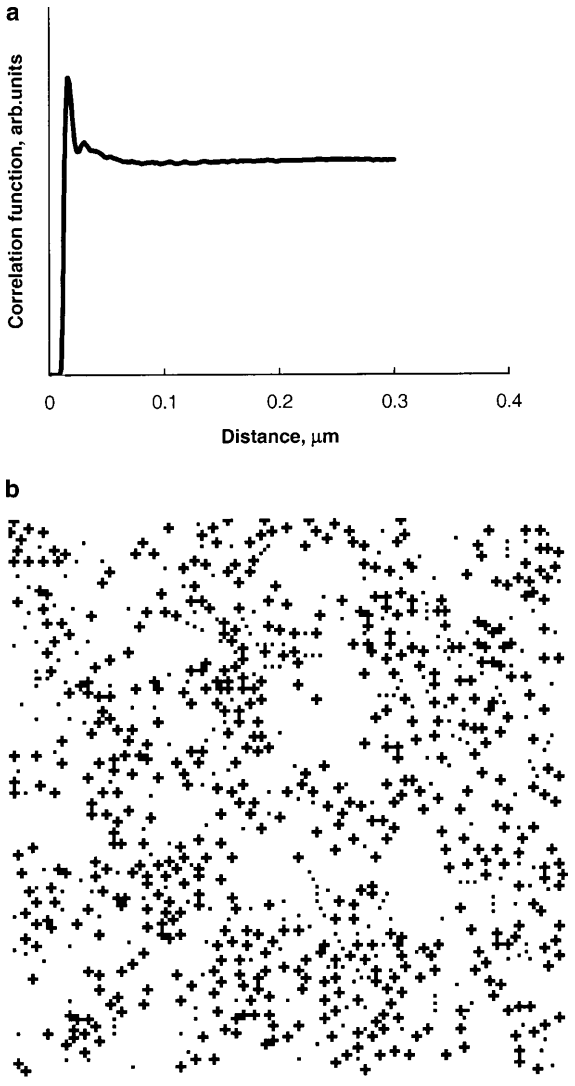


Fig. 6 Correlation function (**a**) and configuration (**b**) for the case of $\xi = 0.002$ μm and $\lambda\varepsilon = 1$ (see text for the other parameters)

differing in density (see, e.g. Poole et al. 1997 and references therein). In that work it was shown that complex pair interaction potential may cause the system to possess two different liquid phases. In our case the interaction, depending on the values of the parameters, may be completely repulsive, attractive, or competing.

Our results suggest that we can present the following hypothesis for the stacking scenario for thylakoid membranes. A change in the ionic strength of the solution around the membrane weakens the electrostatic repulsion of the photosystems. Thus an occurring "clotting" phase provides inhomogeneity in the distribution of the photosystems within the membrane. As the presence of large protein complexes affects the local curvature of a membrane (May and Ben-Shaul 1999), the clotting affects the surface free energy of the membrane, associated with the curvature and surface tension. A continuous membrane no longer provides for minimization of such free energy, and slicing occurs along the empty spaces between the clusters of photosystems. The resulting membrane "discs" close into vesicles (Ninham and Fennel Evans 1986). At the same time, boundary effects provide for segregation between the photosystems, "pushing" the stronger charge PSIs out towards vesicles' boundaries.

We were not able at this stage to obtain any definitive evidence for the segregation of the photosystems. However, the segregation itself may be caused by changes in the membrane curvature depending on the distribution of charges. To test this hypothesis, a more complicated model is needed. This model should self-consistently take into account the effects of variations in the membrane curvature on the distribution of the photosystems, and vice versa. Also, future work in sketching a phase diagram of the system as function of the parameters $\lambda\varepsilon$, ξ and ζ is planned.

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