Cluster phases of membrane proteins

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A physical scenario accounting for the existence of size-limited submicrometric domains in cell membranes is proposed. It is based on the numerical investigation of the counterpart, in lipidic membranes where proteins are diffusing, of the recently discovered cluster phases in colloidal suspensions. I demonstrate that the interactions between proteins, namely short-range attraction and longer-range repulsion, make possible the existence of stable small clusters. The consequences are explored in terms of membrane organization and diffusion properties. The connection with lipid rafts is discussed and the apparent protein diffusion coefficient as a function of their concentration is analyzed.

I. INTRODUCTION

The question of membrane functional organization is a key issue in modern cell biology. The central problematics is to establish the relationship between (dynamical) organization and functions of the different constituents of the membranes. In this context, many microscopy techniques are implemented to have access, with the highest possible spatial and temporal resolutions, to the distribution and dynamics of membrane proteins and lipids, in particular to their diffusive properties in connection with their crowded environment.

Using these techniques, there exist a large variety of situations, in live cells or in model membranes, where membrane proteins are found in oligomers or small clusters. This co-localization is supposed to facilitate encounters of partners in different processes, such as complexes involved in signal transduction. Lipid rafts are usually invoked to account for co-localization. These membrane submicrometric domains enriched in certain lipids (e.g. cholesterol and sphingolipids) are supposed to recruit proteins having a higher affinity for their composition. They are believed to ensue from a lipidic micro-phase separation. However, a consensus has not yet been reached to explain why the separation process stops and domains remain size-limited. And understanding the relationship between these structural patterns and diffusion of their constituents remains topical.

At the same time, there is an increasing interest in colloid science for systems presenting a cluster phase. It is the fruit of a competition between a short-range attraction (e.g. a depletion interaction) which favors clustering, and a longer-range repulsion (e.g. electrostatic) which prevents a complete phase separation, because when clusters grow, their repulsion also grows and the repulsion barrier cannot be passed by thermal activation anymore. The result is an equilibrium phase with small clusters of concentration-dependent size. It has been suggested that the existence of cluster phases is not a singular behavior in a specific system. They indeed occur in different physical systems (e.g. colloids, star polymers, proteins) and for a large variety of interactions. Such patterns have already been experimentally observed or simulated in 2D or 3D.

I propose, as an alternative paradigm, to re-interpret the aggregation of membrane proteins in terms of two-dimensional cluster phases, because membrane proteins, like colloids, interact with energies of the order of magnitude of the physiological thermal energy $k_BT$ ($T \approx 310\mathrm{K}$). I discuss in this framework the mechanism driving the formation of so-called rafts. I propose that proteins spontaneously congregating in small clusters instead of constituting a “gas” of independently diffusing inclusions, they promote the formation of nano-domains in membranes. The sub-micrometric, limited size of these nano-domains now appears naturally in this scenario, as a result of the competition between attraction and repulsion. By contrast to the lipid raft scenario where domains result from a lipidic micro-phase separation and then recruit specific proteins, the present mechanism proposes that domain formation is mainly driven by protein interactions. Recent experiments in live cells support this point of view that protein-protein interactions are necessary to induce clustering, whereas lipids alone are not sufficient in the system studied.

I also relate the protein diffusive properties as a function of their concentration to the limited size of clusters, itself depending on concentration. I demonstrate that the mean long-term diffusion coefficient $D$ of proteins decreases when the mean cluster size grows: $D \propto 1/\langle n \rangle$ where $\langle n \rangle$ is the mean cluster size (its number of proteins). I also anticipate that $\langle n \rangle$ grows with the protein concentration $\phi$. Thus $D$ also depends on $\phi$. From this point of view, contact is made with prior experiments, where $D$ was found to decrease like $1/\sqrt{\phi}$ or that were interpreted by appealing to such a behavior. I propose a simple scenario leading to this law $D \sim 1/\phi$, requiring that $\langle n \rangle \propto \phi$. Previous attempts to address this issue, based on low $\phi$ expansions of $D$, are not adapted to catch the physical mechanisms capable of accounting for this behavior.

To give substance to and to support this cluster-phase scenario, I first discuss available experimental data. In Ref. [13], proteoliposomes of egg lecithin and bacteri-
orthodopsin (BR) are observed by freeze-fracture electron microscopy. BR is found to aggregate in small clusters (called “particles”), the mean size of which, \( \langle n \rangle \), depends on BR concentration \( \phi \). In Table I (column 5), it is found that \( \langle n \rangle \propto \phi \) for \( 1/\phi \in [90, 300] \) (in mol./mol. lipid to protein ratio), in complete agreement with my arguments. In addition, the Fig. 4 of this reference shows unambiguously that this behavior comes from the bimodal character of the cluster size distribution: the balance between monomers \((n = 1)\) and multimers \((n > 1)\) make possible the cluster density to be nearly constant while the density of protein grows. I will confirm below that it is exactly what is observed in simulations. Moreover, the diffusion constant \( D \) of BR is precisely found to decay like \( 1/\phi \) in the same interval in Ref. 31, as anticipated above. At the end of this article, I shall propose experiments to validate definitely this scenario.

II. SHORT AND LONGER-RANGE INTERACTIONS

There exist several short-range attractive forces between proteins embedded in membranes, each with a range of a few nanometers and a binding energy of order \( k_B T \). They first of all consist of a depletion interaction due to the 2D osmotic pressure of lipids on proteins, which tends to bring them closer when they are about a nanometer apart [29]. There also exist hydrophobic mismatch interactions between proteins, the hydrophobic core of which does not match the width of the membrane [2]. The energy cost of the subsequent membrane deformation increases with the distance between two identical proteins, thus resulting in an attractive force. The energy scale is of order \( k_B T \) [28]. In membranes with several lipid species, another scenario leads to attractive forces: proteins recruit in their neighborhood lipids which match best their hydrophobic core. The closer the proteins, the more energetically favorable the configuration. Binding energies are also of order \( k_B T \) or larger [2, 29]. A protein-driven mechanism for domain formation invoking such forces has been proposed [2, 29], but the limited domain size due to additional repulsive forces has not been discussed in this context.

Membrane inclusions are also affected by longer-range repulsive forces. Electrostatic repulsion between like charged proteins is usually considered as negligible because it is screened beyond a few nanometers in physiological conditions: at physiological ionic strength \( I_\nu \sim 0.1 \) M, the Debye screening length is of the order of 1 nm [32]. Only proteins with (unreasonable) charges of several hundreds of elementary charges can give a repulsion of a fraction of \( k_B T \) at 10 nm. By contrast, there exist specific repulsions due to the deformation that proteins impose on the membrane when they are not strictly speaking cylindrical inclusions but conical ones or peripheral proteins [25]. For example, using the formulae of this reference for transmembrane proteins with a moderate contact angle of 10°, one finds that the repulsive energy barrier at 10 nm is 0.10 \( k_B T \) for a typical bending rigidity \( \kappa = 100k_B T \). For example, rhodopsin has a contact angle larger than 10° [33].

Thus the ingredients for the existence of cluster phases are present in assemblies of membrane proteins and cluster phases should generically exist in cell membranes. Below, I shall take a typical binding energy of \(-4k_B T\) [17] and an energy barrier of 0.1 \( k_B T \) at about 10 nm.

III. CLUSTER DIFFUSION

First I study the diffusive properties of particles in cluster phases. I consider an isolated cluster of \( n \) proteins, modeled as an assembly of interacting Langevin particles with “bare” diffusion coefficient \( D_0 \) (the diffusion coefficient at vanishing concentration). The center of mass of the assembly diffuses with a coefficient \( D_0/n \), because the clusters considered are not rigid entities but loosely bound, fluctuating ones in which the proteins diffuse [2] (this property will be confirmed below). If clusters interact weakly because they are sufficiently far away (Fig. 1), the long-term diffusion coefficient of each protein of the cluster is also equal to \( D_0/n \) [3]. If clusters contain \( \langle n \rangle \) proteins on average (counting a monomer as a cluster with \( n = 1 \)), then the mean long-term diffusion coefficient \( D = D_0/\langle n \rangle \): if the system contains \( N \) proteins

\[
D = \frac{1}{N} \sum_{c=1}^{N_c} n(c) \frac{D_0}{n(c)},
\]

where \( N_c = N/\langle n \rangle \) denotes the number of clusters, because a cluster \( c \) contains \( n(c) \) proteins that diffuse each with a diffusion coefficient \( D_0/n(c) \). Thus \( D = D_0 N_c/N = D_0/\langle n \rangle \). If \( R \) is the average cluster radius, then \( D \propto 1/R^2 \). Such an experimental behavior of \( D \) with cluster size has already been observed [4], but has been left unexplained.

Now, in 3D, it has been shown analytically [18, 21, 23], and measured experimentally [16, 17, 19], that \( \langle n \rangle \) is proportional to the particle concentration \( \phi \). My purpose here is not to demonstrate such a relation, but simply to remark its validity in a wide range of situations in 3D and to anticipate its equivalent in 2D. In addition to the numerical evidence presented below, further calculations, appealing for example to the theory of micellization [34], will be necessary to confirm this last point. They go beyond the scope of the present numerical paper. If \( \langle n \rangle \propto \phi \), then the effective diffusion coefficient of proteins in a cluster phase is inversely proportional to their concentration:

\[
D = \text{Const.}/\phi.
\]
a potential shape already studied in detail \cite{21,22,24}: the typical cluster diameter. Therefore I have focused on range, weaker repulsion extending on a range larger than the binding energy between two proteins is 

The parameters are chosen so that, as required above, the binding energy between two proteins is \(-4 k_B T\) and the energy barrier is 0.1 \(k_B T\). The following values fulfill this requirement: \(\varepsilon_a = 32 k_B T\) and \(\varepsilon_r = 0.3 k_B T\); 

\(1/\gamma_a = 2\) nm and \(1/\gamma_r = 16\) nm. In spite of the high value of \(\varepsilon_a\), the binding energy is low because the attractive part is cut at \(r = d_0\) due to the hard core repulsion (Inset of Fig. 2). This hard-core diameter is chosen as \(d_0 = 4\) nm, the typical diameter of a protein of average molecular weight \cite{13}. The proteins are given a “bare” diffusion coefficient \(D_0 = 1\) \(\mu m^2/s\) \cite{30,36}: at each Monte Carlo step (MCStep), a randomly chosen protein attempts to move a distance \(\delta r\) forward in a randomly chosen direction; Here \(\delta r = 1\) Å \(\ll d_0\); With this \(\delta r\), the acceptance rate of MCSteps is larger than 60 \%, even at the highest densities considered; The time step between two MCSteps is \(\delta t = \delta r^2/(4D_0) = 2.5\) ns. A Monte Carlo sweep corresponds to \(N\) MCSteps. The simulation time is chosen so that error bars on \(D\) and \(\langle n \rangle\) are smaller than 10 \% (more than \(10^7\) sweeps, \textit{i.e.} 30 ms of real time). The protein average long-term diffusion coefficient \(D\) is measured at different concentrations \(\phi = N d_0^2/a^2\) (\(a\) is the size of the box with periodic conditions in which the proteins diffuse). By long-term, it is meant at time-scales larger than the time needed to diffuse inside the clusters (typically 0.1 ms). The measures are performed after a long equilibration period. To be sure that equilibrium has indeed been reached, I simulate two systems with initial configuration chosen as (a) a random one where proteins later coalesce to form clusters; (b) a completely condensed state where all proteins belong to the same big cluster which later splits into smaller ones and gas. Equilibrium is considered to have been reached when both systems (a) and (b) are qualitatively identical (same number of multimeric clusters). The Monte Carlo time needed is generally shorter than \(10^8\) sweeps (however, see \cite{35}). Note that the time needed to reach equilibrium in (a) is rather long because after a transient period where small clusters appear \textit{via} a bimodal-like decomposition, larger clusters are formed by evaporation of the smaller ones. Evaporation is the result of escape of single proteins from the clusters, one after the other. The energy barrier to evaporate a single protein being of several \(k_B T\), this is a slow process \cite{35}.

![Two snapshots of the cluster phase at \(\phi = 0.1\) (\(N = 200\) proteins, box side \(a = 0.25\) \(\mu m\), periodic boundary conditions); The time delay between both snapshots is 0.5 ms. Clusters diffuse slowly and appear non-rigid, deformable and fluctuating.](image.png)

**FIG. 1:** Two snapshots of the cluster phase at \(\phi = 0.1\) (\(N = 200\) proteins, box side \(a = 0.25\) \(\mu m\), periodic boundary conditions); The time delay between both snapshots is 0.5 ms. Clusters diffuse slowly and appear non-rigid, deformable and fluctuating.

IV. MONTE CARLO SIMULATIONS

\textit{A priori}, these mechanisms should be valid only at low \(\phi\), where separate clusters interact weakly and diffuse independently. To test further their relevance in 2D and at higher \(\phi\), I have performed Monte Carlo simulations of systems of \(N = 100\) particles (\(N = 200\) for the highest density considered \(\phi = 0.1\)), interacting \textit{via} pairwise potentials displaying a hard-core repulsion, a short-range attraction and a longer-range repulsion \cite{24,21}. I have chosen physically and biologically relevant parameters as justified above. I have observed a strong robustness of the cluster phase with respect to the potential shape. I have tested repulsive terms decaying linearly, algebraically (like \(1/r^2\) or \(1/r^4\) \cite{27}) or exponentially \cite{21} with the distance \(r\) between molecules, as well as attractive ones varying exponentially or linearly with \(r\). In all cases, a cluster phase exists at equilibrium (\textit{i.e.} after very long runs) for wide ranges of parameters. Clusters co-exist with a gas of monomers, of density depending on potential and (weakly) on density \(\phi\) (as observed experimentally in Ref. \cite{13}). What is important is not the precise shape of the potential but the existence of a short-range attraction of a few \(k_B T\) and of a longer-range, weaker repulsion extending on a range larger than the typical cluster diameter. Therefore I have focused on a potential shape already studied in detail \cite{21,22,24}:

\[
U(r) = -\varepsilon_a \exp(-\gamma_a r) + \varepsilon_r \exp(-\gamma_r r). \tag{3}
\]

The parameters are chosen so that, as required above, the binding energy between two proteins is \(-4 k_B T\) and the energy barrier is 0.1 \(k_B T\). The following values fulfill this requirement: \(\varepsilon_a = 32 k_B T\) and \(\varepsilon_r = 0.3 k_B T\); 

\(1/\gamma_a = 2\) nm and \(1/\gamma_r = 16\) nm. In spite of the high value of \(\varepsilon_a\), the binding energy is low because the attractive part is cut at \(r = d_0\) due to the hard core repulsion (Inset of Fig. 2). This hard-core diameter is chosen as \(d_0 = 4\) nm, the typical diameter of a protein of average molecular weight \cite{13}. The proteins are given a “bare” diffusion coefficient \(D_0 = 1\) \(\mu m^2/s\) \cite{30,36}: at each Monte Carlo step (MCStep), a randomly chosen protein attempts to move a distance \(\delta r\) forward in a randomly chosen direction; Here \(\delta r = 1\) Å \(\ll d_0\); With this \(\delta r\), the acceptance rate of MCSteps is larger than 60 \%, even at the highest densities considered; The time step between two MCSteps is \(\delta t = \delta r^2/(4D_0) = 2.5\) ns. A Monte Carlo sweep corresponds to \(N\) MCSteps. The simulation time is chosen so that error bars on \(D\) and \(\langle n \rangle\) are smaller than 10 \% (more than \(10^7\) sweeps, \textit{i.e.} 30 ms of real time). The protein average long-term diffusion coefficient \(D\) is measured at different concentrations \(\phi = N d_0^2/a^2\) (\(a\) is the size of the box with periodic conditions in which the proteins diffuse). By long-term, it is meant at time-scales larger than the time needed to diffuse inside the clusters (typically 0.1 ms). The measures are performed after a long equilibration period. To be sure that equilibrium has indeed been reached, I simulate two systems with initial configuration chosen as (a) a random one where proteins later coalesce to form clusters; (b) a completely condensed state where all proteins belong to the same big cluster which later splits into smaller ones and gas. Equilibrium is considered to have been reached when both systems (a) and (b) are qualitatively identical (same number of multimeric clusters). The Monte Carlo time needed is generally shorter than \(10^8\) sweeps (however, see \cite{35}). Note that the time needed to reach equilibrium in (a) is rather long because after a transient period where small clusters appear \textit{via} a bimodal-like decomposition, larger clusters are formed by evaporation of the smaller ones. Evaporation is the result of escape of single proteins from the clusters, one after the other. The energy barrier to evaporate a single protein being of several \(k_B T\), this is a slow process \cite{35}.

V. RESULTS AND DISCUSSION

As \(\phi\) was increased, \(D\) decreased dramatically, as expected (Fig. 2). One observes that \(D \approx \text{Const.}/\phi\) over nearly two decades, thus confirming the hypothesis that cluster phases can account for this behavior. This law had been observed 25 years ago \cite{30}, without receiving a full explanation, apart from arguments invoking “crowding effects” or “aggregation” reminiscent of the clustering mechanism discussed here, but unable to predict quantitatively the dependence of \(D\) on \(\phi\) \cite{30}. The diffusion coefficient at typical cell membrane protein concentration (\(\phi \sim 0.1\)) appears to be reduced by a factor larger than 10, which is the decrease of \(D\) observed in cell membranes as compared to the same diffusion coefficient \(D_0\) in model membranes at low \(\phi\) \cite{6,30}. One can also see in Fig. 2 that \(\langle n \rangle\) is proportional to \(\phi\) at low concentration, as expected. Interestingly, at higher \(\phi\), the data for \(\langle n \rangle\) still follow this law for this choice of potential. In addition, the relation \(D \approx D_0/(\langle n \rangle)\) holds on all the range of concentrations studied. This demonstrates that the in-
interactions between clusters are negligible and that they diffuse independently at the time scale considered.

As it was observed experimentally (Fig. 4 of Ref. [13]), the cluster distributions obtained in simulations are bimodal on all the range of concentrations where $\langle n \rangle \propto \phi$. A gas of monomers ($n = 1$) coexists with large multimers ($n > 1$), the distribution of which is Gaussian around a typical size $n^*$. With the parameters chosen here, there are virtually no small multimers (dimers, trimers, etc.). These distributions are illustrated in Fig. 3 at different concentrations $\phi$. The relation $\langle n \rangle \propto \phi$ then comes from a subtle balance between monomers and multimers: as $\phi$ increases, the density of monomers is essentially constant while large clusters capture additional proteins.

Once formed, clusters appear to be non-rigid, deformable (as illustrated on the two consecutive snapshots in Fig. 1), they can be seen as liquid droplets, that reorganize rapidly; proteins diffuse inside the clusters with measured short-term diffusion coefficients larger than 0.01 $\mu$m$^2$/s and long-lived, as anticipated above. By long-lived, it is meant that clusters are stable at the time-scale of the simulations (about 30 ms). However, some proteins constantly leave the clusters (via the evaporation process discussed above), diffuse freely in the gas phase and are captured later by another cluster. Rare events of clusters being disintegrated or nucleating spontaneously in the gas have been observed. Therefore clusters are certainly not stable at long time scales (seconds or minutes) and are only transitory.

I have also observed in simulations that small modulations of the parameters $\varepsilon$ or $\gamma$ in $U(r)$, can lead to segregation [1, 12, 26], if two (or more) groups of proteins (A’s and B’s, which are not necessarily identical in the same group) are present in the simulation and if A-A and B-B interactions are slightly favored as compared to A-B ones, then the proteins segregate. There are A-rich and B-rich clusters because even though it is entropically unfavorable, it is energetically favorable. For instance, an A-B binding energy 10% smaller than equal A-A and B-B ones suffices to ensure segregation. At the biological level, this implies that groups of proteins which show a slight tendency to associate, because of their physico-chemical properties, would segregate in distinct clusters (see [12]). This mechanism might play an important role in sorting together proteins that must congregate to perform biological functions [1, 3]. This point will be quantified in future investigations [34].

The existence of cluster phases in live cells would mean that proteins spontaneously congregate in small clusters of a few entities or a few tens of entities in the plasma membrane instead of constituting a “gas” of independently diffusing inclusions, thus promoting nano-domains in plasmic membranes. By contrast to the lipid raft scenario where domains result from a lipidic micro-phase separation and then recruit specific proteins, the present mechanism proposes that domain formation is mainly driven by protein interactions (even though lipids do play an important role in the effective forces). Note that this scenario does not exclude a concomitant recruitment by protein clusters of specific lipids having a higher affinity for those proteins (and which participate in the effective attractions [26]), thus reconciling my hypothesis with the observation of detergent-resistant membrane fractions [14]. This mechanism, by constraining an increasing fraction of lipids to diffuse slowly (with clusters) as $\phi$ increases, could also explain why the diffusion constant of lipids decreases significantly when the con-
centration of proteins increases \[30\].

In spite of the evidence provided above, the existence of protein clusters has to be confirmed definitively at the experimental level. Even though such clusters have already been observed by freeze-fracture electron microscopy \[13, 30\], their existence must be explored by different techniques in a wider range of situations, in cell and model membranes. Near-field scanning microscopy is an ideal tool because it is able to identify individual proteins after immobilizing the membrane onto an adequate substrate. Counting numbers of proteins in clusters is then in principle possible \[8\]. Such experiments would be able to investigate the dependency of cluster sizes on protein concentration, as well as the correlation between \(n\) and \(D\). Recently, high-frequency single-particle tracking has demonstrated that proteins are confined in nano-domains in the plasma membrane of live cells \[8, 29\], of typical diameter a few tens of nanometers. An appealing hypothesis is that these nano-domains are the clusters under consideration. For proteins several nanometers apart, the previous size would correspond to clusters containing a few tens of proteins, in agreement with the previous simulations. The confirmation of this hypothesis would provide additional evidence of cluster phases in live cells.

I have proposed in this paper a paradigm leading to the formation of size-limited nano-domains in cell and model membranes. This scenario, based on reasonable hypotheses about the energy and length scales in biological membranes, sheds light on several so far open issues in cell biology: (i) it provides a mechanism for the limited size of nano-domains; (ii) it gives a complete qualitative justification for former experiments in model membranes \[13, 30\]; (iii) it proposes a simple explanation leading to the proportionality law \(D \propto \phi\). If cluster phases were to be experimentally confirmed in model membranes and in live cells, it would mean that, by physical mechanisms, proteins generally gather in small assemblies in biological membranes, thus shedding new light on membrane functional processes.

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[1] D.M Engelman, Nature **438**, 578 (2005).
[2] O.G. Mouritsen, Life - as a matter of fat (Springer, Berlin, Germany, 2005).
[3] M.A. Davare, et al., Science **293**, 98 (2001).
[4] N. Kahya, D.A. Wiersma, B. Poolman, and D. Hoerkstra, J. Biol. Chem. **277**, 39304 (2002).
[5] F. Daumas, et al., Biophys. J. **84**, 356 (2003).
[6] K. Murase, et al., Biophys. J. **86**, 4075 (2004).
[7] A. Bodnár, et al., J. Int. Immunol. **15**, 331 (2003).
[8] D. Fotiades et al., Nature **421**, 127 (2003); R.P. Conçalves, et al., Nature Methods **3**, 1007 (2006).
[9] L.M. Veenhoff, E.H.M.L. Heuberger, and B. Poolman, Trends Biochem. Sci. **27**, 242 (2002).
[10] P. Sharma, et al., Cell **116**, 577 (2004).
[11] M. Benveniste, et al., J. Cell. Biol. **106**, 1903 (1988).
[12] J.J. Sieber, et al., Biophys. J. **90**, 2843 (2006).
[13] T. Gulik-Krzywicki, M. Seigneuret, and J.L. Rigaud, J. Biol. Chem. **262**, 15580 (1987).
[14] M. Eddin, Annu. Rev. Biophys. Biomol. Struct. **32**, 257 (2003).
[15] Fielding, C.J. (Editor). Lipid Rafts and Caveolae: From Membrane Biophysics to Cell Biology, (Wiley, Weinheim, Germany, 2006).
[16] A. Stradner, et al., Nature **432**, 492 (2004).
[17] P.N. Segrè, et al., Phys. Rev. Lett. **86**, 6042 (2001).
[18] J. Groenewold and W.K. Kegel, J. Phys. Chem. B **105**, 11702 (2001).
[19] H. Sedgwick, S.U. Egelhaaf, and W.C.K. Poon, J. Phys.: Condens. Matter **16**, S4913 (2004).
[20] E. Stiatakis, et al., Europhys. Lett. **72**, 664 (2005).
[21] R.P. Sear and W.M. Gelbart, J. Chem. Phys. **110**, 4582 (1999).
[22] R.P. Sear, et al., Phys. Rev. E **59**, R6255 (1999).
[23] Y. Liu, W.R. Chen, and S.H. Chen, J. Chem. Phys. **122**, 044507 (2005).
[24] A. Imperio and L. Reatto, J. Phys.: Condens. Matter **16**, S3759 (2004).
[25] M. Seul and D. Andelman, Science **267**, 476 (1995).
[26] A. Borodich, I. Rojdestvenski, and M. Cottam, Biophys. J. **85**, 774 (2003).
[27] M. Goulian, R. Bruinsma, and P. Pincus, Europhys. Lett. **22**, 145 (1993); J.B. Fournier and P.G. Dommermes, Europhys. Lett. **39**, 681 (1997).
[28] C. Nielsen, M. Goulian, and O.S. Andersen, Biophys. J. **74**, 1966 (1998).
[29] T. Gil, et al., Biochim. Biophys. Acta **1376**, 245 (1998).
[30] R. Peters and R.J. Cherry, Proc. Natl. Acad. Sci. USA **79**, 4317 (1982).
[31] D.S. Dean and A. Lefèvre, Phys. Rev. E **69**, 061111 (2004).
[32] R.J. Hunter, Foundations of colloidal science, Vol. 1. (Clerendon Press, Oxford, 1989).
[33] J. Deisenhofer, et al., J. Mol. Biol. **246**, 429 (1999).
[34] L. Foret and N. Destainville (in preparation).
[35] However for \(\phi = 0.1\), systems (a) and (b) are note qualitatively identical after 2.10\(^7\) sweeps (7 big clusters vs 4 big ones). Thus in Fig. 2 two values for \(1/(n)\) are plotted, the higher one corresponding to system (a). For \(D/D_0\), both values are identical within 10 \% error bars.
[36] This particular value of \(D_0\) is chosen for the sake of simplicity and because it has the correct order of magnitude. However, all diffusion constants below will be given in units of \(D_0\) and its precise value is irrelevant. Furthermore, fixing \(D_0\) and the the space scale via \(d_0\) imposes the time scale. Thus all times calculated will be rough approximates.