Diffusional Dynamics of Cytochrome c Molecules in the Presence of a Charged Surface

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Online publication date: 06 November 2003
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ABSTRACT

A new Brownian dynamics code was developed that is capable of computing trajectories of several spherical particles in the presence of a charged planar surface. The code takes into account electrostatic, van der Waals, and hydrodynamic interactions. In this work we describe the methods used in the program and show results from calculations for cytochrome c molecules interacting with a negatively charged lipid bilayer. This system is of particular biological interest since these molecules play a major role as electron carriers, e.g., in photosynthesis. The shape and charge distribution of cytochrome c molecules can be well approximated as spherical particles with an embedded monopole and dipole and can therefore easily be handled by the program. That level of approximation makes it possible to study large systems with many (up to 100) particles over time scales up to milliseconds, which would be computationally too expensive using detailed atomistic models.

Key Words: Brownian dynamics; Diffusion; Cytochrome c; Lipid membrane; Hydrodynamic interaction.

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INTRODUCTION

Diffusion is the basic mechanism of many important processes in biological cells. Examples are the transport of metabolic substrates and products between different enzymes, protein-protein association in signal transduction chains, or the diffusion of the neurotransmitter acetylcholine through the synaptic cleft to initiate membrane depolarization. Also, the passage of ions through membrane channels and even certain aspects of protein folding[1] may be described as diffusional processes. Experimentally, the role of diffusional steps is often measured indirectly by varying the viscosity of the solvent or directly by fluorescence recovery after photobleaching (FRAP). The computational method of choice to study diffusion processes are stochastic dynamic or Brownian dynamics simulations. Several algorithms have been proposed,[2,3] and are implemented in a number of computer codes. Brownian dynamics simulations are very common in colloidal systems where one particle is typically of 1–5 μm size and the surface can be treated as uniform. Proteins, on the other hand, have typical diameters of several nanometers, and details of their molecular surfaces are often important.

Binding water-soluble positively charged cytochrome c molecules to negatively charged phospholipid bilayers with embedded membrane proteins is an important process in photosynthesis and respiration. In purple bacteria, cytochrome c molecules are enclosed in tubular membranes of ca. 1 μm length and 0.5 μm diameter and transport electrons between two membrane proteins, the cytochrome bc1 complex and the bacterial reaction center. In bacterial mitochondria, cytochrome c diffuses in the 20 nm wide inner-membrane space between outer and inner membranes again transporting electrons between two membrane proteins of the respiratory chain, the cytochrome bc1 complex and cytochrome c oxidase. To execute this function, cytochrome c is built as a large molecular dipole with a positive net charge. While positively charged Lysine amino acids are evenly distributed over its entire molecular surface, surrounding the surface-exposed porphyrin, the negatively charged Aspartic and Glutamic amino acids are preferentially arranged on one side. It is currently unknown whether reduced cytochrome c diffuses along the membrane to deliver its electron to the next binding partner or whether it returns to a solution pool after reduction. The former case, a preferential 2D diffusion, is reminiscent of the process of "substrate channeling" between subsequent enzymes of a biochemical pathway that may be mediated by complementary charges on the protein surfaces.[4] Before studying the diffusion of cytochrome c in complex geometries and with embedded membrane proteins, we started looking at the binding of cytochrome c molecules to negatively charged surfaces that mimic phospholipid bilayer membranes.

The classical relationship for the electrostatic potential at a distance from a biomembrane is the Gouy-Chapman law; see Eq. (16). This simple relationship is a special case of the linearized Poisson-Boltzmann equation and works surprisingly well. At physiological ionic strength of ca. 100 mM, the Debye length is in the order of 1 nm. The charged membrane is very effectively shielded by a corresponding cloud of oppositely charged counter ions.

The binding of charged cytochrome c molecules to phospholipid bilayer membranes was carefully studied by experiment.[5] By varying the lipid composition of negative DOPG lipids and zwitterionic DOPC lipids between 100:0 and 0:100
mixtures, the negative surface charge of the membrane can be varied. The lipid-protein mixtures were equilibrated for 48 hours, and the concentrations of soluble protein were then determined by spectrophotometric extinction after ultracentrifugation. For total concentrations of 0–40 $\mu$M, up to 7 $\mu$M were bound at the membrane for the highest surface charge and 90mM ionic strength. The authors note that the binding of cytochrome $c$ to DOPG:DOPC membranes was found to be considerably greater than that predicted for a homogenous lipid mixture. This was explained by a redistribution (partial demixing) of the lipids in the vicinity of bound protein. Another important factor is possible structural transitions of bound cytochrome $c$. Excessive equilibration may stabilize a different protein conformation from that found in solution.

Juffer and coworkers recently studied the adsorption of the 8-residue peptide somatostatin to a POPC bilayer using molecular dynamics simulations.\(^\text{[6]}\) Atomistic modelling of protein:membrane assembly, however, is currently unfeasible due to computational limitations. Molecular dynamics simulations of systems with 100,000 atoms are limited to 10 $\mu$s in length, while this is also the typical range of relaxation times of the counter ion sphere. One would also need to account for possible changes of hydration states of protein side chains and lipid head groups, partial penetration of the protein into the bilayer, bending of the membrane towards or away from the protein, possible inclusion of a fine water layer between membrane and protein, and so on. In short, there are myriads of unsolved problems related to the theoretical treatment of peptide and protein binding to phospholipid membranes. One should therefore not expect a perfect agreement between simulation and experiment at the current stage. For example, Lin and coworkers recently found that adding up to four layers of explicit water molecules significantly alters the electrostatic potentials at a distance from a biomembrane that are computed by solving the Poisson-Boltzmann equation.\(^\text{[7]}\)

It seems therefore advantageous to try using a coarser description of the system. The first stochastic dynamics simulations of a cellular system was reported in 1996 by Bicout and Field\(^\text{[8]}\) who simulated the dynamics of ribosomes, tRNAs, and proteins. In their model, no hydrodynamic interactions were included. An important problem for computing hydrodynamic interactions is the unfavorable scaling of the approach with the number of moving particles. Northrup\(^\text{[9]}\) simulated the encounter of cytochrome $c$ with cytochrome $c$ peroxidase using simplified models of cytochrome $c$—a monopole charge with dipole charges of equal sign on both sides.

We are not aware of prior Brownian dynamics simulations of protein-membrane systems. By using a simplified simulation model, we investigated how dipolar, positively charged particles move in the surroundings of an oppositely charged surface. The interaction between the particles and with the membrane was described by a screened Coulombic potential and by a short-range van der Waals potential. In our model we do not account for possible deformations of the membrane, partial penetration of cytochrome $c$ into the bilayer, or membrane deformations to increase or decrease the contact surface.

The simulation time step used in the simulations was 10 $ps$ according to the limitations given by the iteration algorithm; see Eq. (3). The total simulation times are in the range of 10 $\mu$s to 1 $ms$. In the following sections we present the design of a new Brownian dynamics simulation program and results from various simulations performed to study concentration profiles, diffusion coefficients, and angular orientation effects of the particle dipoles.
METHODS

Equation of Motion and Algorithm

The Brownian motion of \( N \) particles can be described by \( 6N \) Langevin equations, \( 3N \) for translation and for rotation, respectively:\[^{10}\

\[
m_i \frac{d(v_i)_k}{dt} = - \sum_{j=1}^{N} \sum_{l=1}^{3} \left[ \left( \left( (T)_{ij} \right)_{kl} \cdot (v_j)_l \right) + \left( \left( (T^{TR})_{ij} \right)_{kl} \cdot (\omega_j)_l \right) \right] + (F_i)_k \\
+ \sum_{j=1}^{6N} \alpha_{ij} \cdot f_j, 1 \leq i \leq N, 1 \leq k \leq 3
\]

(1)

and

\[
I_{ik} \frac{d(\omega_i)_k}{dt} = - \sum_{j=1}^{N} \sum_{l=1}^{3} \left[ \left( \left( (R)_{ij} \right)_{kl} \cdot (v_j)_l \right) + \left( \left( (R^{TR})_{ij} \right)_{kl} \cdot (\omega_j)_l \right) \right] + (T_i)_k \\
+ \sum_{j=1}^{6N} \alpha_{(3N+3(i-1)+k)}j \cdot f_j, 1 \leq i \leq N, 1 \leq k \leq 3
\]

(2)

where \((v_i)_k = \frac{d(x_i)_k}{dt}\) is the \( k \)th component of particle \( i \)’s velocity and \((\omega_i)_k = \frac{d(\theta_i)_k}{dt}\) is the \( k \)th component of particle \( i \)’s angular velocity (rotation about \( k \)th axis). The mass of particle \( i \) is denoted by \( m_i \), and \( I_{ik} \) is the \( ik \)th element of the inertia tensor. The \( \Gamma \) are the translational (T), rotational (R), and coupled translational-rotational (TR) friction matrices (for index notation see the section “Hydrodynamic Interaction”) \((F_i)_k\) and \((T_i)_k\) are the \( k \)th components of the external force and torque on particle \( i \), respectively. The terms \( \alpha_{ij} f_j \) describe forces and torques due to random collisions of fluid molecules with the particles. The numbers \( f_j \) are Gaussian variables with mean value \( \langle f_j \rangle = 0 \) and covariance \( \langle f_j(0) f_j(t) \rangle = 2\delta_{ij}\delta(t) \). Finally, the \( \alpha_{ij} \) are connected to the friction matrices by 

\[
\Gamma_{ij} = \beta \sum_{l=1}^{6N} \alpha_{il} \cdot \alpha_{jl}
\]

Starting with the translational Langevin equation, Ermak and McCammon derived an iteration algorithm\[^{23}\

for the spatial coordinates of the particles that is valid for time steps \( \Delta t \gg \frac{b}{m D_0} \), with \( b = 1/kT \). Here, \( D_0 = kT/6\pi \eta a \) is the free diffusion coefficient of a single sphere with radius \( a \). This time scale is called the diffusive regime, which is much larger than the momentum relaxation time. Thus, the algorithm gives the position displacements after each time step \( \Delta t \):

\[
(r_i)_k = (r_i^0)_k + \sum_{j=1}^{N} \sum_{l=1}^{3} \frac{\partial \left( (D^T)_{ij} \right)_l}{\partial (r_j)_l} \Delta t + \beta \sum_{j=1}^{N} \sum_{l=1}^{3} \left( (D^{TR})_{ij} \right)_l (F_j^0)_l \Delta t \\
+ \left( R_i(D^T,\Delta t) \right)_k, 1 \leq i \leq N, 1 \leq k \leq 3
\]

(3)

The superscript 0 indicates variables to be evaluated at the beginning of each time step, \( D^T \) is the translational diffusion matrix, and \((R_i(D^T,\Delta t))_k\) are random displacements according to the last term in the translational Langevin equation with
mean value \( \langle R_i(D^T, 0, \Delta t) \rangle = 0 \) and variance-covariance \( \langle (R_i(D^T, 0, \Delta t) - R_j(D^T, 0, \Delta t))^2 \rangle = 2(\Delta^2 D_{ij}) \). Based on this approach a generalized algorithm also taking into account the coupling between translational and rotational movement was developed in.\(^{[10]}\)

\[
(r_i)_k = (r_i^0)_k + \sum_{j=1}^{N} \sum_{l=1}^{3} \frac{\partial \left( (D^T)_{ij} \right)}{\partial (r_j)_l} \Delta t + \sum_{j=1}^{N} \sum_{l=1}^{3} \frac{\partial \left( (D^{RT})_{ij} \right)}{\partial (\varphi_j)_l} \Delta t
\]

\[
+ \beta \sum_{j=1}^{N} \sum_{l=1}^{3} \left( (D^T)_{ij} \right) (F^0)_{ij} \Delta t + \beta \sum_{j=1}^{N} \sum_{l=1}^{3} \left( (D^{RT})_{ij} \right) (T^0)_{ij} \Delta t
\]

\[
+ (R_i(D^0, \Delta t) - R_j(D^0, \Delta t))_k, 1 \leq i \leq N, 1 \leq k \leq 3
\]

(4)

and

\[
(\varphi_i)_k = (\varphi_i^0)_k + \sum_{j=1}^{N} \sum_{l=1}^{3} \frac{\partial \left( (D^{RT})_{ij} \right)}{\partial (r_j)_l} \Delta t + \sum_{j=1}^{N} \sum_{l=1}^{3} \frac{\partial \left( (D^{RT})_{ij} \right)}{\partial (\varphi_j)_l} \Delta t
\]

\[
+ \beta \sum_{j=1}^{N} \sum_{l=1}^{3} \left( (D^{RT})_{ij} \right) (F^0)_{ij} \Delta t + \beta \sum_{j=1}^{N} \sum_{l=1}^{3} \left( (D^{RT})_{ij} \right) (T^0)_{ij} \Delta t
\]

\[
+ (R_i(D^0, \Delta t) - R_j(D^0, \Delta t))_k, 1 \leq i \leq N, 1 \leq k \leq 3
\]

(5)

These two equations can be written in the following compact form by using the generalized 6\(N\) dimensional vectors:

\[
y = ((r_1)_1, (r_1)_2, (r_1)_3, \ldots, (r_N)_3, (\varphi_1)_1, (\varphi_1)_2, (\varphi_1)_3, \ldots, (\varphi_N)_3)
\]

(6)

and

\[
f = ((F_1)_1, (F_1)_2, (F_1)_3, \ldots, (F_N)_3, (T_1)_1, (T_1)_2, (T_1)_3, \ldots, (T_N)_3)
\]

(7)

yielding

\[
y_i = y_i^0 + \sum_{j=1}^{6N} \frac{\partial D_{ij}^0}{\partial y_i} \Delta t + \beta \sum_{j=1}^{6N} D_{ij}^0 \cdot f_j^0 \Delta t + R_i(D^0, \Delta t)
\]

(8)

The 6\(N\)-dimensional random displacements have mean values \( \langle R_i (D^0, \Delta t) \rangle = 0 \) and variance-covariance \( \langle R_i (D^0, \Delta t), R_j (D^0, \Delta t) \rangle = 2D_{ij}^0 \Delta t \).

**FORCES**

**Hydrodynamic Interaction**

The hydrodynamic interaction (see e.g. Ref. [11]) between particles is described by the friction or diffusion matrices related to each other by \( D_{ij} = \beta^{-1}(\Gamma^{-1})_{ij} \). The
grand diffusion matrix \( D \) contains four sub-matrices \( D_T \), \( D_{TR} \), \( D_{RT} \), \( D_R \), and each of these is a \( 3N \times 3N \) matrix containing \( N^2 \) 3 \( \times \) 3 “sub-sub-matrices.” Thus, \( D \) itself is a \( 6N \times 6N \) matrix:

\[
D = \begin{pmatrix}
D_T & D_{TR} \\
D_{RT} & D_R
\end{pmatrix}
\]  \hspace{1cm} (9)

To clarify the convention used here, i.e., \( D_R \) without any indices denotes the \( 3N \times 3N \) rotational diffusion matrix, so that \((D_R)_{ji}\) with \((i, j) \in [1, N]\) is its \((i, j)\)-th 3 \( \times \) 3 sub-matrix. Then, finally, \(( (D_R)_{ji} )_{kl}\) with \((k, l) \in [1,3]\) is its \((k, l)\)-th component. A general scheme to evaluate diffusion tensors of an arbitrary number of spheres, immersed in a viscous fluid, is presented in Ref. [12]. The following tensor approximations were used in our simulations.

Translation \( D_T \) (Rotne-Prager Tensor approximation):

\[
((D_T)_{ji})_{kl} = D_0 \left\{ \delta_{ij} \delta_{kl} + (1 - \delta_{ij}) \left[ \frac{3}{4} a \frac{1}{r_{ij}} \left( \delta_{kl} + \langle \mathbf{r}_{ij} \rangle_k \cdot \langle \mathbf{r}_{ij} \rangle_l \right) \\
+ \frac{1}{2} \left( \frac{a}{r_{ij}} \right)^3 \left[ \delta_{kl} - 3 \langle \mathbf{r}_{ij} \rangle_k \cdot \langle \mathbf{r}_{ij} \rangle_l \right] \right\}
\]  \hspace{1cm} (10)

Rotation \( D_R \):

\[
((D_R)_{ji})_{kl} = D_0 \left\{ \frac{3}{4a^2} \delta_{ij} \delta_{kl} + (1 - \delta_{ij}) \frac{3a}{8r_{ij}} (3 \langle \mathbf{r}_{ij} \rangle_k \cdot \langle \mathbf{r}_{ij} \rangle_l - \delta_{kl}) \right\}
\]  \hspace{1cm} (11)

Translation-Rotation \( D_{TR} \) and \( D_{RT} \):

\[
((D_{TR})_{ji})_{kl} = -((D_{RT})_{ji})_{kl} = \frac{3aD_0}{4r_{ij}^2} (1 - \delta_{ij}) \sum_{m=1}^{3} e_{k,m} \langle \mathbf{r}_{ij} \rangle_m
\]  \hspace{1cm} (12)

Here, \( \delta_{ij} \) denotes the Kronecker delta, which equals 1 for \( i = j \) and 0 otherwise. The total antisymmetric third rank tensor \( e_{ijk} \) is defined by \( e_{ijk} = e_i^j \cdot (e_j^k \times e_k^i) \), where \( e_i^j \) is the unit vector in direction of axis \( i \). Interactions due to interparticle distances are taken into account up to order \( r^{-3} \). The free diffusion coefficient of a single sphere with radius \( a \simeq 1.6 \text{ nm}^{13} \) is \( D_0 \simeq 1.5 \cdot 10^{-10} \text{ m}^2/\text{s} \).

**Electrostatic Interaction**

The electrostatic interaction between diffusing particles is described here by the dipolar sphere model (DSM). In this model a cytochrome c molecule is represented as a sphere containing a monopole charge in the center and two dipole charges...
embedded inside the sphere surface 1.5 nm away from the center. The interaction potential energy between two such spheres is then given by the following screened Coulomb/Debye potential:

\[
W_{12} = \frac{1}{4\pi\varepsilon_0} \sum_{i=1}^{3} \sum_{j=1}^{3} q_i q_j e^{-\kappa(r_{ij}-B_{ij})} \frac{1}{(1 + \kappa B_{ij})^2} \]  \hspace{1cm} (13)

\( r_{ij} \) is the distance between charge \( i \) of protein 1 and charge \( j \) of protein 2. \( B_{ij} \) is an empirical distance shifting factor, taking into account the finite ion size of the surrounding fluid.\(^{[14]} \) It is given by the distances \( b_i \), by which the charges are imbedded inside the protein surface: \( B_{ij} = b_i + b_j \). The strength of the exponential screening term that mimics the screening of interactions between charges of counter ions not modelled explicitly is determined by the Debye length \( l_D = 1/\kappa \). The resulting force between two proteins and the torque with respect to the center of mass of a sphere are then:

\[
\vec{F}_{12} = -\nabla W_{12} = \frac{1}{4\pi\varepsilon_0} \sum_{i=1}^{3} \sum_{j=1}^{3} q_i q_j \left( \frac{\kappa}{(1 + \kappa B_{ij})^2} \frac{1}{r_{ij}^2} \right) + \frac{1}{(1 + \kappa B_{ij})^3} \left( \vec{r}_{ij} \times \vec{e}^{-\kappa(r_{ij}-B_{ij})} \right)
\]

and

\[
\vec{T}_{\text{cms}} = \frac{1}{4\pi\varepsilon_0} \sum_{i=1}^{3} (\vec{r}_i - \vec{r}_{\text{cms}}) \times \sum_{j=1}^{3} q_i q_j \left( \frac{\kappa}{(1 + \kappa B_{ij})^2} \frac{1}{r_{ij}^2} \right) + \frac{1}{(1 + \kappa B_{ij})^3} \left( \vec{r}_{ij} \times \vec{e}^{-\kappa(r_{ij}-B_{ij})} \right)
\]

Also to be considered are electrostatic interactions between the diffusing particles and the charged membrane surface. According to the solution of the linearized Poisson-

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**Table 1.** System parameters used in the simulations.

<table>
<thead>
<tr>
<th>General</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Box side length [nm]</td>
<td>Debye length [nm]</td>
</tr>
<tr>
<td><strong>cyt c</strong> Membrane</td>
<td></td>
</tr>
<tr>
<td>Radius [nm]</td>
<td>1.66</td>
</tr>
<tr>
<td>Mass [kD]</td>
<td>12.5</td>
</tr>
<tr>
<td>Monopole</td>
<td>+7.5e</td>
</tr>
<tr>
<td>Dipole moment [e nm]</td>
<td>5.2</td>
</tr>
<tr>
<td>vdW minimum energy [kT]</td>
<td>-1</td>
</tr>
<tr>
<td>vdW minimum distance [Å]</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Boltzmann equation, the Gouy-Chapman law, the potential of a charge density $\sigma$ surrounded by a fluid with dielectric constant $\varepsilon$ and Debye length $l_D = 1/\kappa$ is given by

$$j = j_0 e^{-z/l_D}$$

at a vertical distance $z$ from the plane. The potential value on the surface is $j_0 = \sigma l_D \varepsilon$, and Eq. (16) is strictly valid for $|q_0| \ll RT/F \approx 25 \text{mV}$, where $F$ is the Faraday constant. For the highest charge density used in the simulations, we have $|q_0| \approx 300 \text{mV}$. (See Table 1.) Summation over all charges in the proteins as in Eqs. (14) and (15) leads to the force and torque on the molecules to be used in the simulations.

**Van der Waals Interaction**

Hamaker\(^\text{[15]}\) derived formulas for the interaction energy and force between two spherical particles with radii $R_1$, $R_2$ and constant atom densities $\rho_1$, $\rho_2$ as well for the interaction of a sphere and a planar surface. He assumed an attractive interaction potential proportional to $r^{-6}$ between each pair of atoms in the particles. Here we used his integration method also to find formulas for a short-range repulsive potential proportional to $r^{-12}$. Generally, for potentials $r^{-n}$ the energy is

$$E = -\int_{V_1} \int_{V_2} C r^{-n} dV_1 dV_2$$

where $C$ is a constant depending essentially on the particle atom densities, $V_i$ is the volume of sphere $i$, and $r$ is the distance between $dV_1$ and $dV_2$. For particles at a distance $d = R-R_1-R_2$, Hamaker obtained

$$E_{np-np}^{n=6} (x) = -A^2 \frac{1}{12} \left[ \frac{y}{x^2 + xy + x} + \frac{y}{x^2 + xy + x + y} + 2 \ln \left( \frac{x^2 + xy + x}{x^2 + xy + x + y} \right) \right]$$

using the definitions $x = \frac{d}{2R_1}$ and $y = \frac{R_2}{R_1}$. (See Figure 1.) The interaction between a sphere and a surface plane then immediately follows by making sphere 2 infinitely large ($y \to \infty$):

$$E_{np-np}^{n=6} (x) = -A \frac{1}{12} \left[ \frac{1}{x} + \frac{1}{x + 1} + 2 \ln \left( \frac{x}{x + 1} \right) \right]$$

![Figure 1. Definition of contact distance $d$ between a sphere and a planar surface and two spheres.](image-url)
A and $\tilde{A}$ are empirical parameters that determine the strength of the attractive forces of a sphere and a plane, respectively. In the same way, we derived analogous equations for the $r^{-12}$ potential:

$$E_{sp-sp}^{n=12}(x) = \frac{B^2}{1260R_0^6} \left( \frac{1}{1+y+2x} \left[ \frac{1}{160} \left( \frac{1}{(1+y+x)^3} - \frac{1}{(1+x)^3} - \frac{1}{(y+x)^3} + \frac{1}{x^3} \right) \right] 
- \frac{1}{384} (-5 + y + 2x) \left( \frac{1}{(1+y+x)^6} - \frac{1}{(1+x)^6} \right) 
+ \frac{1}{384} (7 + y + 2x) \left( \frac{1}{(y+x)^6} - \frac{1}{x^6} \right) + \frac{1}{128} (2+y+2x) \times \left( \frac{1}{(1+y+x)^7} + \frac{1}{(1+x)^7} \right) - \frac{1}{128} (y+2x) \left( \frac{1}{(y+x)^7} - \frac{1}{x^7} \right) \right)$$

and

$$E_{sp-pl}^{n=12} = \frac{BB}{161280R_0^6} \left[ \frac{1}{3(1+x)^6} - \frac{1}{3x^6} + \frac{1}{(1+x)^6} + \frac{1}{x^6} \right]$$

$B$ and $\tilde{B}$ are empirical parameters that determine the strength of the repulsive forces of a sphere and a plane, respectively. Similar formulas were derived by Bicout and Field \[8\] (although not given in their paper) and were fitted for convenience by cubic splines. Figure 2 shows the energy curves for the van der Waals interaction. The well-depth was deliberately set to $-1kT$.

**Figure 2.** Distance dependence of the van der Waals interaction between two spheres (solid line) and between a sphere and the surface (dashed line).
PROGRAM

The simulations described below were performed with a new Brownian dynamics code. This program was developed with C++ in an object-oriented fashion to ensure easy maintenance and upgradeability. Several classes represent different constituents of the simulation system. A protein is modelled by a class called Protein with a special child class MovingProtein, which represents the cytochrome c molecules. The periplasma is designed by a class Periplasm, which contains a variable number of MovingProteins and the properties of the fluid in the cell. Membrane is the membrane class and describes the surface charge and the membrane potentials. There are also classes for creating random numbers, for representing the grand diffusion tensor, and, finally, for the algorithms used, called RandomNumbers, DiffusionTensor, and Engine, respectively.

SIMULATION SYSTEM

The simulation parameters are summarized in Table 1. For the computation of van der Waals interactions, the potentials are linearized (constant forces) at short distances \( \leq x_0 = dR_0 \), in order to avoid very high repulsive forces when the particles try to penetrate the membrane surface or each other. In the case of particle-particle interactions, for all distances smaller than the empirical value \( x_0 = 0.095 \), the repulsive force

![Simulation box illustrating the use of periodic boundary conditions and the evaluation of interactions. In the case of reflecting boundaries, particles are reflected from the side and upper walls by a random repulsion smaller than the radius of the particles.](image_url)

**Figure 3.** Simulation box illustrating the use of periodic boundary conditions and the evaluation of interactions. In the case of reflecting boundaries, particles are reflected from the side and upper walls by a random repulsion smaller than the radius of the particles.
value corresponding to this distance is chosen. In the case of particle-membrane interaction, the corresponding distance is $x_0 = 0.11$. The way we calculate forces is illustrated in Figure 3. For all interactions between Brownian particles, we use a cutoff with radius $boxLength/2$ in case of periodic boundary conditions. (For reflecting boundary conditions no cutoff was used.) Thus, only interactions with the closest image particles are taken into account. For small boxes this is not a good approximation in the case of hydrodynamic interaction. Hence, for all simulations we choose a rather large simulation box with side lengths at least one order of magnitude larger than the linear dimensions of the particles. The parameters for the moving particles are taken from [14] and mimic horse heart cytochrome $c$ molecules. The Debye length of 1 nm corresponds to an ionic strength of 90 mM.

RESULTS AND DISCUSSION

The computed trajectories were used to study the following system properties:

1. concentration profiles
2. diffusion coefficients
3. angular orientations

Concentration Profiles

The probability distribution of Brownian particles along the $z$-axis is shown in Figure 4. The probability to find particles close to the membrane is higher for a larger surface charge density that yields a very sharp peak at the position of the most favorable interaction energy. Since periodic boundary conditions are only used in $x$-$y$ direction, there is hardly any influence of the use of the boundary conditions on the concentration profile in $z$ direction. This is different for the profiles in $x$ or $y$ direction, since particles close to the walls interact with their image particles for periodic boundary conditions. The concentration profile parallel to the membrane is shown in Figure 5. The concentration profiles seem well converged. For reflecting boundary conditions, ca. 20% higher populations are observed at the system walls. Here the charged particles minimize the repulsive interactions with other moving particles that carry the same positive charge.

We also compared simulations with different cytochrome $c$ concentrations (see Figure 6) and calculated “bulk” concentrations, i.e., how many particles are effectively to be found in solution at distances from the membrane $z \geq 5$ nm. These values are shown in Table 2. The more particles present, the larger the bulk concentrations relative to the concentrations close to the membrane. The ratio decreases from 1:20 for $N = 1$ to 1:12 for $N = 40$.

About 40–50% of the particles seem to cluster at the membrane surface. In the experimental setup of Ref. [5], the dimensions of the system are, of course, much larger, and the volume of the Gouy-Chapman layer is very small compared to the total volume. Yet, it was also observed experimentally that a high percentage of added cytochrome $c$ molecules bound at the fully charged lipid membrane. [5]
Figure 4. The relative distribution of particles is shown as a function of the distance from the membrane. For the calculation of the frequencies, the box was divided into bins of 0.001 nm lengths. Thus, the relative frequencies of finding particles in intervals with this length are shown. The frequencies (also referred to as probabilities later) are normalized to 1. These plots were obtained from a simulation of 16 particles in a 100 nm box, which corresponds to a cytochrome c concentration of 32 μM. The simulation time was ca. 0.1 ms and took 168 h on a 1 GHz PC with 256 Mb memory. The lower panel shows the sum of van der Waals and electrostatic interaction between moving particles and the membrane.

Figure 5. The relative particle distribution in x-direction parallel to the membrane surface (16 particles in a 100 nm box, see Figure 4) is shown. Here, larger bins of 0.1 nm length were used. Thus, the values here are probabilities of finding particles between distance and distance + 0.1 nm. The coordinate origin is placed at the lower-left corner of the box, i.e., x = y = z = 50 nm refers to the center of the box.
The diffusion coefficient and its time dependence were computed using the Einstein relation

\[
D(t) = \frac{1}{6Nt} \sum_{i=1}^{N} \left( \bar{r}_i(t) - \bar{r}_i(0) \right)^2
\]

for various time intervals \(t\).

Figure 7 shows such a diffusion measurement for different simulations. The diffusion coefficient of a single free sphere is ca. 30–50% larger than for a 40 μM concentration. Furthermore, for a high surface charge density, particles are more restricted in their movement than for a low surface charge density because they only diffuse in two dimensions when they are bound at the surface. Therefore, the diffusion coefficient is smaller in the first case. The curves also show the difference between

**Table 2.** Total, membrane, and bulk particle numbers and concentrations. \(p_{>5 \text{ nm}}\) is the probability of finding particles at distances larger than 5 nm, i.e., bulk refers to particle numbers and concentrations for this region. Equally, the subscript <5 nm refers to values close to the membrane.

<table>
<thead>
<tr>
<th>(N_{\text{tot}})</th>
<th>(c_{\text{tot}}[\mu M])</th>
<th>(p_{&lt;5 \text{ nm}})</th>
<th>(N_{\text{membrane}})</th>
<th>(c_{\text{membrane}}[\mu M])</th>
<th>(p_{&gt;5 \text{ nm}})</th>
<th>(N_{\text{bulk}})</th>
<th>(c_{\text{bulk}}[\mu M])</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.51</td>
<td>0.51</td>
<td>20.4</td>
<td>0.49</td>
<td>0.49</td>
<td>1.03</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.45</td>
<td>0.9</td>
<td>36</td>
<td>0.55</td>
<td>1.1</td>
<td>2.32</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>0.38</td>
<td>2.28</td>
<td>91.2</td>
<td>0.62</td>
<td>3.72</td>
<td>7.83</td>
</tr>
<tr>
<td>30</td>
<td>60</td>
<td>0.38</td>
<td>11.4</td>
<td>456</td>
<td>0.62</td>
<td>18.6</td>
<td>39.16</td>
</tr>
<tr>
<td>40</td>
<td>80</td>
<td>0.39</td>
<td>15.6</td>
<td>624</td>
<td>0.61</td>
<td>24.4</td>
<td>51.37</td>
</tr>
</tbody>
</table>
simulations with and without hydrodynamics. For these simulations, which correspond to a cytochrome c concentration of 40 \( \mu M \), hydrodynamics reduces the diffusion coefficient by 1% at most.

For long time intervals the diffusion coefficient decreases. This effect is due to finite size effects of the simulation box. It is illustrated in Figure 8 where the parallel and the perpendicular diffusion coefficients are plotted. The two components were computed as

\[
D_p(t) = \frac{1}{4Nt} \sum_{i=1}^{N} \left[ (x_i(t) - x_i(0))^2 + (y_i(t) - y_i(0))^2 \right] \\
D_z(t) = \frac{1}{2Nt} \sum_{i=1}^{N} (z_i(t) - z_i(0))^2
\]

As shown in Figure 8, diffusion perpendicular to the membrane is significantly slower than parallel to the membrane. However, the decrease occurs in the \( x-y \)-components where periodic boundary conditions are used, not in the perpendicular \( z \)-component. Diffusion in the \( x-y \) plane is essentially free diffusion. This shows again that the solutions are sufficiently dilute so that dynamic properties are hardly affected by interparticle interactions. The leveling off occurs roughly at 0.4 \( \mu s \), which is about the time a free particle needs to cross a quarter of the box (\( t = \frac{\text{box} / 4}{6D_{\parallel}} \)).

Angular Orientations

Since rotational motion was explicitly considered, one should expect some kind of preferential orientation of the particles when they come close to the membrane. This
Effect was studied by averaging over the angle between the particles’ dipole and an axis perpendicular to the membrane surface. Figure 9 shows that for small distances from the surface the cosine of the depicted angle tends towards \( \frac{1}{C_0} \). This corresponds to an angle of \( \frac{180}{C_176} \), which means that the positive dipole charge, on average, points in the direction of the negatively charged membrane. At distances beyond the Debye length, the particles have no preferred orientation.

This computed property may be most affected by the simplicity of the particle representation. Note that the particle diameter is 3.3 \( \text{nm} \), while the electrostatic field

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**Figure 8.** Comparison between total, parallel, and perpendicular diffusion coefficients with respect to the membrane surface for the same simulation as in Figure 7 with hydrodynamic interactions.

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**Figure 9.** Preferential orientation of the dipolar particles with respect to the charged surface as a function of their distance from the membrane. Plotted is the average cosine value of the angle between the particles’ dipoles and an axis perpendicular to the membrane surface. The average was taken over all particles, using bins of 0.1 \( \text{nm} \) length. The same data were analyzed as in Figure 4.
changes over much smaller distances. Therefore, both poles of its dipole feel very different electrostatic fields.

In future work we plan to investigate the effect of representing the cytochrome c molecules in more detail with 10–20 suitably adjusted surface charges.

**Limitations**

The depth of the attractive van der Waals potential between cytochrome c particles and membrane, and between two cytochrome c particles was set to $E_{\text{min}} = -kT$ without further justification. We will, in future work, treat these two parameters as adjustable to reproduce experimental data for cytochrome c adsorption to phospholipid membranes.\(^5\) For the moment, $E_{\text{min}} = -kT$ and minima distances of $x_{\text{min}} = 0.1$ for cytochrome c—cytochrome c interaction and $x_{\text{min}} = 0.2$ for cytochrome c—membrane interaction (corresponding to $d_{\text{min}} = 0.33$ nm and $d_{\text{min}} = 0.66$ nm, respectively) seemed to yield quite reasonable results.

The Gouy-Chapman law is a solution of the linearized Poisson-Boltzmann equation and should be used primarily for potentials $\ll 25$ mV. At higher potentials, the solution of the full, non-linearized Poisson-Boltzmann equation,\(^{16}\)

$$
\varphi = \frac{2}{c} \ln \left( \frac{e^{c \varphi_0} + 1 + (e^{c \varphi_0} - 1)e^{-kz}}{e^{c \varphi_0} + 1 - (e^{c \varphi_0} - 1)e^{-kz}} \right) = \frac{4}{c} \text{artanh} \left( \frac{c}{4 \varphi_0} e^{-kz} \right)
$$

with $c = \frac{\sqrt{2} \varepsilon_0}{\varepsilon}$ and $\varphi$ the valency of the electrolyte should be advantageous. Again, we will test the effect of using this more accurate description in future work. However, as noted by one reviewer, one could then question the mixed level of description: screened Coulomb potential between particles as a solution of the linearized Poisson-Boltzmann equation versus nonlinearized Poisson-Boltzmann equation between particles and membrane.

The treatment of hydrodynamic interactions in this work does not include interactions of the diffusing particles with the membrane. Therefore, the diffusion at close distance from the surface may be currently overestimated. Such effects were studied in Ref. [17] by multipole expansion of the hydrodynamic force densities induced on spherical particles and by image representations to account for the fluid boundary. Because it was observed here that hydrodynamic interactions between diffusing particles had only a very little effect on the diffusion coefficient, we believe that this omission has a minor effect on the computed properties. However, such surface effects should have a much more pronounced effect in narrow, confined geometries. On the other hand, hydrodynamic interactions are long range in contrast to the screened electrostatics and van der Waals interactions in our model system, and a proper treatment of hydrodynamic forces under periodic boundary conditions should involve Ewald summation techniques.\(^{18}\) Because it was not clear how to implement Ewald techniques for mobility tensors for systems with two-dimensional periodicity, we used rather large simulation boxes. After all, biological structures do not show any periodicity, and such systems will be our main interest in the future.

Another effect not included in our present model is the reduction of the protein diffusion coefficient when the proteins are bound to the membrane due to friction caused by atomic contacts or entangled protein side chains. Therefore, particles absorbed at the membrane currently do not lose as much translational entropy as they do “in real life.” We suspect that this may be the primary reason for the relatively
high values obtained for the concentration of absorbed particles. In future work, both
van der Waals and a z-dependent friction force in x-y direction need to be fine-tuned to
reproduce experimental data.

SUMMARY AND CONCLUSIONS

A new Brownian dynamics simulation program was presented to study the
diffusion of several charged spherical molecules in the presence of a charged planar
surface. The simple properties computed appear reasonably converged on time scales
between 20 μs and 1 ms. By performing simulations with varying particle numbers, one
could observe deviations from the “ideal gas behaviour” in simulations with a single
particle. The code can handle up to about 100 Brownian particles in reasonable CPU
times because the computation of the hydrodynamic interaction and standard Cholesky
factorization to obtain multivariate random displacements scales as \( N^3 \), where \( N \) is
the number of diffusing particles.\(^{[19]}\) As expected, the membrane charge density
significantly affects the probability of finding particles at a certain distance from the
surface. The profiles showed sharp peaks very close to the membrane. The apparent
diffusion coefficient was shown to be smaller for systems with higher surface charge
density due to a higher proportion of particles adsorbed to the surface. For the
relatively low particle concentrations studied so far, hydrodynamic interaction plays a
minor role for the diffusional behavior of the molecules. Preferential orientation of the
particle dipoles towards the negatively charged membrane occurs only within distances
of the Debye length. This could be of interest with respect to the long-range
electrostatic steering—incoming cytochrome c molecules have a preferential preorientation
when binding to their electron transfer partners cytochrome c oxidase and the
bacterial reaction center.

Bacterial cells are very crowded environments.\(^{[20]}\) To investigate the effects of this
so-called “macromolecular crowding,” in future simulations, we plan to take into
account some kind of dummy particles interacting with the particles of interest, e.g.,
only via van der Waals and hydrodynamics. In contrast to what was found here,
crowding effects should significantly reduce diffusion rates. For example, measurement
of diffusion of green fluorescent protein in \( E. \ coli \) cytoplasm showed that it is slowed
11-fold in comparison to diffusion in water.\(^{[20]}\)

ACKNOWLEDGMENTS

This work was supported by a grant by the Klaus Tschira Stiftung GmbH,
Heidelberg. We also want to thank Dagmar Flöck, Markus Elsner, Tomaso Frigato, and
Michael Hutter for helpful discussions, Vidya Chandran for proofreading, and the two
referees for very valuable comments.

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Received April 30, 2002
Accepted July 18, 2002