The role of chromatin conformations in diffusional transport of chromatin-binding proteins: Cartesian lattice simulations

Annika Wedemeier,1 Ting Zhang,2 Holger Merlitz,2,3 Chen-Xu Wu,2 and Jörg Langowski1,a
1Deutsches Krebsforschungszentrum, D-69120 Heidelberg, Germany
2Softmatter Laboratory, Department of Physics and ITPA, Xiamen University, Xiamen 361005, People’s Republic of China
3Leibniz-Institut für Polymerforschung Dresden, 01069 Dresden, Germany

(Received 18 December 2007; accepted 18 February 2008; published online 15 April 2008)

In this paper, a lattice model for the diffusional transport of chromatin-binding particles in the interphase cell nucleus is proposed. Sliding effects are studied in dense networks of chromatin fibers created by three different methods: Randomly distributed, noninterconnected obstacles, a random walk chain model with an attractive step potential, and a self-avoiding random walk chain model with a hard repulsive core and attractive surroundings. By comparing a discrete and continuous version of the random walk chain model, we demonstrate that lattice discretization does not alter the diffusion of chromatin-binding particles. The influence of conformational properties of the fiber network on the particle sliding is investigated in detail while varying occupation volume, sliding probability, chain length, and persistence length. It is observed that adjacency of the monomers, the excluded volume effect incorporated in the self-avoiding random walk model, and the persistence length affect the chromatin-binding particle diffusion. It is demonstrated that sliding particles sense local chain structures. When plotting the diffusion coefficient as a function of the accessible volume for diffusing particles, the data fall onto master curves depending on the persistence length. However, once intersegment transfer is involved, chromatin-binding proteins no longer perceive local chain structures. © 2008 American Institute of Physics. [DOI: 10.1063/1.2895048]

I. INTRODUCTION

DNA is organized into higher order structure to accommodate the genome within the spatial confines of the cell nucleus. 5%–12% of the nucleus are filled with the dense network of chromatin fibers.1 Establishment and alteration of global and local chromatin states are modulated by the combined action of a multitude of chromatin-binding proteins.2 Essentially, all of the biological functions of DNA are performed by proteins that interact with specific DNA sequences.3,4 These reactions are diffusion controlled.

Due to their functional significance, chromatin-binding proteins have been extensively characterized and their dynamics in the living cell has been studied by in vivo microscopy techniques.5,6 The major part of these data is of qualitative nature and quantitative parameters such as binding rates are missing.

Moreover, it is not yet clear how proteins bind to the chain and displace themselves within the chromatin network. Several mechanisms, differing in detail, have been proposed.7,8 All of them essentially involve two steps: The binding to a random nonspecific DNA site and the diffusion (sliding) along the DNA chain. These two steps may be reiterated many times before proteins actually find their target, since the sliding is occasionally interrupted by dissociation.

In general, the diffusional transport of chromatin-binding proteins in the living cell nucleus is only rudimentarily understood. In particular, it is still a matter of extensive discussions to what extent the macromolecular mobility is affected by structural components of the nucleus,9 i.e., it is not known how the sliding process of a protein along the DNA or the chromatin fiber is influenced by its local structure. Furthermore, it is not clear in detail to what extent the sliding mechanism alters the character of the diffusional transport of proteins in the cell nucleus. This transport is fundamentally different from the normal kind of diffusion which a molecule undergoes in a homogeneous fluid where the mean square displacement behaves linear in time \( r^2(t) = 6Dt \) with \( D \) as the diffusion coefficient. The motion of molecules in the cell nucleus is strongly influenced by the dense network of chromatin fibers due to steric obstruction and transient binding. Fluorescence correlation spectroscopy (FCS) studies have shown obstructed diffusion of autofluorescent proteins.9,10 Obstructed diffusion or subdiffusion is characterized by \( r^2(t) \sim t^\alpha \) with the anomalous diffusion coefficient \( \alpha < 1 \).

This paper develops a theoretical description of network diffusion of chromatin-binding particles in the interphase cell nucleus. Such descriptions have already been given;11,12 however, they do not incorporate realistic structures of the chromatin fibers. In the following, the chromatin fibers are approximated by flexible polymer chains. Chromatin-binding proteins diffusing through the fiber network are referred to as particles or walkers.

In the present paper, we investigate in detail how the diffusion coefficient of chromatin-binding particles depends on the protein-chromatin affinity, the three dimensional ge-
ometry of the chromatin fiber network and density in the nucleus. Particularly, we study the influence of structural properties of the fibers such as persistence length and contour length on the diffusion coefficient of chromatin-binding particles. At the same time, the anomaly of the transport process of chromatin-binding particles is investigated and it is shown that sliding activities cause transient subdiffusion.

To minimize computational time and effort, the chromatin network in the interphase cell nucleus is simulated using a lattice model. In a first approach, we create crowded environments with polymer chains constructed by a random walk (RW) on the lattice without excluded volume. A second method creates self-avoiding random walks (SAW) of well-equilibrated polymer chains with excluded volume interactions and more realistic static properties such as end-to-end distance. These chains are simulated on the lattice by applying a simplified version of the bond fluctuation method, the single site model, in combination with a Metropolis Monte Carlo procedure. Both methods, with RW and SAW chain models, are compared to a third test system consisting of disconnected, randomly distributed obstacles.

In Sec. II, the lattice for chain construction and particle diffusion is presented. In addition, the test system is described. Section III introduces the RW chain model with an attractive step potential for the discrete and continuous space. We test the validity of the lattice approach by comparing the diffusion coefficient of particles in the discrete and the continuous model. After presenting the results on particle diffusion in the RW chain system and the test system, we conclude that the latter one is insufficient for a description of transport processes in the interphase cell nucleus.

In Sec. IV, the SAW chain model is introduced, which exhibits important features of a real biological fiber network such as a hard repulsive core with attractive surroundings. The chain simulation algorithm, a combination of the bond fluctuation method (BFM) and MC procedure, is presented briefly. A detailed description can be found in Ref. 15. It is demonstrated that sliding causes transient subdiffusion. After stating the results on particle diffusion and comparing them to the RW chain model, it is concluded that the SAW chain model with a hard repulsive core comes closer to the situation in the cell nucleus and thus yields more realistic diffusion coefficients. The simulation results agree with the experimental findings. Finally, it is found that sliding particles sense the local chain structure as long as no intersegment transfer is involved in the sliding mechanism. Chromatin-binding particles perceive the persistence length of the fiber network. This is not the case for particles which freely diffuse in the cell nucleus, as found in earlier work. Section V summarizes the obtained results.

II. MODELING VOLUME

The model is contained in a $100 \times 100 \times 100$ cubic lattice. To prevent boundary effects due to the lattice walls, periodic boundary conditions are applied both for the particle motion and the chain construction.

A. Particle movement

In our model systems, a particle is implemented as one occupied lattice site. Since a rotation mechanism of the particles is not integrated in the model, we omit investigations of particles larger than one occupied site as we did in Ref. 15. In the continuous model, the walker was pointlike. The movement of a particle is modeled by a random walk. If a particle collides with a chain it may bind with a certain sliding probability $p_{\text{slide}}$. Otherwise, it is reflected. Implementation of sliding is discussed in the following. The initial position of the particle is sampled randomly among nonoccupied lattice sites. Then, RWs of different duration are carried out.

B. Test system with disconnected obstacles

To study the influence of chain connectivity on particle diffusion, a test system is introduced with randomly distributed obstacles, occupying between 5% and 15% of the lattice sites.

III. RANDOM WALK CHAIN SYSTEMS

In this system, the chromatin fibers are created by a standard RW without excluded volume effects, i.e., allowing self-intersection. This approach is more realistic than the set of disconnected obstacles of the test system, since here the monomers are interconnected to create fibers. The RW system is set up in both lattice and continuum models, in order to justify the accuracy of the lattice approximation during a sliding process.

A. Chain simulation

In the discrete model, the chromatin fibers were realized by chains of occupied connected lattice sites chosen by RWs on the $100 \times 100 \times 100$ lattice with a persistence length of $l_p=5$. In the continuous model, the modeling volume was defined by a box of edge-length 100 with periodic boundaries, containing a chain of given length with a persistence length of $l_p=4.45$ implemented by a RW. The particular choice of the persistence lengths in the different systems will be discussed later. To simulate a persistent chain in the lattice model, five neighboring sites pointing in the same direction were chosen randomly as a starting segment of the chain and occupied. During each random walk step one segment consisting of five occupied neighboring lattice sites pointing all in the same, randomly chosen, direction was connected to the previous chain segment. This results in a persistence length of $l_p=5$. The eight lattice sites surrounding each of the chosen occupied sites were also occupied to create a persistent chain with a cross sectional area of nine lattice points. The total number of occupied lattice sites yields the occupation volume or chain density. The occupation volume fraction of the chain is denoted by $\alpha_{\text{chain}}$. Simulations of RWs with chain lengths of 10 000–500 000 were carried out, yielding chain densities between 0.05% and 60%.

In the continuous model, a persistent chain was created with an interaction radius of $R_{\text{chain}}=1.5$ using a RW with a step size of 4.45, which also defined the segment length.
The total occupation volume of the chain was computed via MC integration, i.e., \( n = 10^5 \) points were distributed randomly inside the system of volume \( V \), and the number of points hitting the chain \( n_c \) was determined to yield the chain volume \( V_{\text{chain}} = n_c \times V / n \).

### B. Simplified model for diffusion of chromatin-binding proteins

The walker-chain interaction was defined as an attractive step potential

\[
U(s) = \begin{cases} 
-E_0, & s \leq r_{\text{chain}} \\
0, & \text{else} 
\end{cases} 
\]  

(1)

where \( s \) is the shortest distance between walker and chain. The cross-sectional area of nine lattice points yields \( r_{\text{chain}} = \sqrt{2} \). Once the walker entered the region of attractive potential, it was in bound mode. Whenever the walker was about to leave this region (i.e., to dissociate from the chain) to turn into free diffusion mode, the Metropolis condition was applied, i.e., the walker was allowed to pass with a probability \( p_{\text{slide}} = 1 - e^{-E_0 / k_B T} \)

(2)

shall be denoted as sliding probability. Different sliding probabilities, \( p_{\text{slide}} = 1 - 2^{-l}, l \in \{1, \ldots, 11\} \), are tested. If one move was rejected due to the Metropolis condition, the walker remained at the same position and one time step was counted. This wait cycle is required to assure that \( D_1 = D_3 \), i.e., the diffusion coefficients coincide in both free diffusion and sliding modes. In the continuum model, the walker was allowed to diffuse inside the cylindrical tube of radius \( R_{\text{chain}} \) which formed the attractive region of the chain. If the Metropolis condition did not allow the walker to pass through the boundary of the chain, it was reflected back inside the tube. Here, identical step-sizes inside and outside the chain delivered the condition \( D_1 = D_3 \).

It is important to note that \( p_{\text{slide}} \) defines the equilibrium constant \( K \) of the two phases, the free and the nonspecifically bound protein according to

\[
K = \frac{p}{c} = \frac{V_{\text{chain}}}{L} \left( \frac{1}{1 - p_{\text{slide}}} - 1 \right),
\]

(3)

where \( c \) is the concentration of free proteins, \( p \) is the linear density of bound proteins, \( V_{\text{chain}} \) denotes chain density, the number of lattice sites which belong to the chain, and \( L \) is the chain length. It should be noted that we make the following assumptions during binding simulation.

- The walker can be adsorbed on any site of the chain, it binds nonspecifically.
- The sliding probability \( p_{\text{slide}} \) is the same everywhere on the chain.
- 1D diffusion along the chain is as fast as 3D (free) diffusion.
- The chain remains immobile.

- Cross-links are allowed but with significantly reduced probability: A protein which is sliding along the chromatin fiber has to first jump off the chain (which causes an energy penalty) before being able to attach to another segment which is located nearby or even “crossing.” As a result, the probabilities to enter from segment \( i \) to \( i+1 \) or \( i-1 \) (where there is no energy barrier) are different from the probabilities of entering another non-adjacent segment. The energy barrier is the same as the one for leaving the chain, i.e., \( 1 - p_{\text{slide}} \). In Sec. IV C 4, this condition was dropped and a free intersegmental hopping was allowed.

The excluded volume of the chain is defined via the Mayer f-function as

\[
V_{\text{ex}} = \int_v \left( 1 - \exp \left[ -\frac{U(s(r))}{k_B T} \right] \right) dr
\]

(4)

\[
= V_{\text{chain}} \left( 1 - \frac{1}{1 - p_{\text{slide}}} \right),
\]

(5)

and depends on the energy of the walker \( U(s) \), as defined by Eq. (1), and hence the implementation of the binding potential between walker and chain. In the following, volume fractions are denoted by \( \sigma \).

The accessible volume parameter is defined as

\[
\sigma_{\text{eff}} = 1 - \sigma_{\text{ex}}
\]

(6)

\[
= 1 - \sigma_{\text{chain}} \left( 1 - \frac{1}{1 - p_{\text{slide}}} \right).
\]

(7)

For the excluded volume defined in Eq. (5), \( V_{\text{ex}} \leq 0 \) holds, and for the corresponding accessible volume parameter \( \sigma_{\text{eff}} \geq 1 \), which needs some explanation.

First, with the definition of the step potential in Eq. (1) and of \( p_{\text{slide}} \) in Eq. (2), \( p_{\text{slide}} \geq 0 \) holds only for attractive or zero interaction potential. The consequence is that the probability for the walker to be on the chain is either equal to that for being in free solution (\( p_{\text{slide}} \) = 0) or larger than that (\( p_{\text{slide}} > 0 \)). In the former case, the effective excluded volume is zero, while for \( p_{\text{slide}} > 0 \), the walker concentration free in solution is decreased below the \( V_{\text{ex}} \) = 0 case. This gives rise to the effective “negative” excluded volume and an accessible volume parameter \( \sigma_{\text{eff}} \geq 1 \).

The sliding probability \( p_{\text{slide}} \) determines the fraction of time \( \tau \) which the walker spends sliding along the chain. In the following, let \( N_1 \) be the number of time steps which the walker spends in free diffusion mode, and \( N_2 \) the number of time steps which the walker slides along the chain, i.e., the time the walker is in bound state. \( N_1 + N_2 \) denotes the total number of time steps of the diffusion process of the particle. \( \tau \) is numerically obtained via

\[
\tau_{\text{numerical}} = \frac{N_2}{N_1 + N_2}.
\]

(8)

Analytically, \( \tau \) can be predicted dependent on the given sliding probability.
\[ \tau_{\text{theor}} = \frac{\sigma_{\text{chain}} \left( 1 - \frac{1}{1 - p_{\text{slide}}} \right)}{1 - \left( \sigma_{\text{chain}} - \frac{1}{1 - p_{\text{slide}}} \right)}. \] (9)

The coincidence of Eqs. (8) and (9) was verified during the simulations, thereby confirming that the system had reached its chemical equilibrium and that the statistics was sufficient to obtain accurate numerical results (data not shown).

C. Comparability of lattice model and continuum model

In order to compare consistently the results of the lattice model with simulations of the continuum model, a couple of conditions have to be satisfied. First of all, the step size of the walker was different in both models. The lattice model enforces a step size of one lattice unit. The continuum model requires the step size to be small compared to the chain diameter to avoid finite size effects, so that a step size of \( dr = 0.5 \) was chosen (with a chain diameter of \( 2R_{\text{chain}} = 3 \)). This naturally delivered different diffusion coefficients \( D_a = dr^2/6 \) for both models in the case of free diffusion. Therefore, all diffusion coefficients had to be normalized with the free diffusion coefficient of the respective model.

Another rescaling was required for the segment lengths for both models to deliver a consistent sliding behavior for a given walker-chain affinity. As shown in our earlier work,16 the sliding length can consistently (i.e., invariant of the step size) be defined as

\[ \xi = \sqrt{\frac{D_2}{D_3}} \frac{K}{2\pi} \] (10)

where \( D_1 \) and \( D_3 \) are the diffusion coefficients in binding mode [one dimension (1D)] and free diffusion [three dimension (3D)], respectively, and \( K \) is the equilibrium constant [Eq. (3)]. In our model, \( D_1 = D_3 \) holds (see model assumptions in Sec. III B) so that Eq. (10) simplifies to \( \xi = \sqrt{K/2\pi} \). With the definition of \( K \) in Eq. (3), different model dependent equilibrium constants are obtained,

\[ K_c = \pi R_{\text{chain}}^2 \left( \frac{1}{1 - p_{\text{slide}}} - 1 \right), \] (11)

for the continuum model and

\[ K_l = a^2 \left( \frac{1}{1 - p_{\text{slide}}} - 1 \right) \] (12)

for the lattice model. \( a \) is the width of the chain in lattice units. The ratio of sliding lengths in continuum model and lattice model is then

\[ \frac{\xi_c}{\xi_l} = \sqrt{\frac{K_c}{K_l}} = \frac{R_{\text{chain}}}{a} \frac{\tau_{\text{theor}}}{\tau}. \] (13)

With \( R_{\text{chain}} = 1.5 \) and \( a = 3 \), we obtain \( \xi_c/\xi_l = 0.89 \), i.e., the average sliding lengths in both models differ (at any fixed walker-chain affinity) by a factor of 0.89 as a result of the different geometric shapes of the chain segments. To achieve consistency between both models, the segment lengths needed to be normalized accordingly, so that for a certain value of \( p_{\text{slide}} \), the protein was sliding exactly over one persistence length. The choice of our segment lengths, \( l_p = 4.45 \) and \( l_p = 5 \) for the continuum model and the lattice model, respectively, satisfied this condition, and a sliding probability of \( p_{\text{slide}} = 0.946 \) then yielded an average sliding length which coincided with the corresponding segment length in both models.

D. Results

For each run, one particle was randomly initialized inside the simulation volume. The 200 000 RW steps were carried out and the motion of the walker was monitored. This procedure was repeated 5000 times and the data were averaged. Figure 1(a) shows the dependence of the diffusion coefficient on the chain density fraction \( \sigma_{\text{chain}} \) and on the sliding probability \( p_{\text{slide}} \) in the lattice model and in the continuous model. The results of both models, continuum (solid symbols) and lattice (blank symbols), agree very well at low and high sliding probabilities (upper panel). The simulations in the system of disconnected obstacles (crosses) yielded diffusion coefficients which were somewhat lower when compared to the chain systems at the same affinity (triangles). The nonadjacency of the occupied lattice sites in

![Figure 1](http://jcp.aip.org/jcp/copyright.jsp)
the test system was responsible for that: At a given value of the occupation volume, the obstacles of the test system are more homogeneously distributed than the RW chains. This property is yielding a larger value for the effective occupation volume so that the walkers easily bind to the obstacles. Once in bound state, they can only move around that single occupied lattice site, i.e., a transport in terms of sliding is impossible.

Figure 1(b) shows that results for different walker-chain affinities roughly fall onto a single curve once the diffusion coefficients are plotted as a function of the accessible volume parameter. An approximate power law dependence is seen in this logarithmic plot. This result reveals that in both models the effect of either increasing the chain density or the sliding probability (i.e., the effective volume of the chain) is equivalent within a certain range of parameters. Furthermore,

$$D \sim \sigma_{\text{eff}}^k,$$

where $k$ is the scaling exponent of the power law. For the RW chain model $k = -0.74$ was found [Fig. 1(b)].

E. Conclusion: Random walk chain system

Due to the very good agreement of the results obtained with the discrete and the continuous model, we conclude that lattice discretization does not affect the characteristic properties of the diffusional transport of chromatin-binding proteins, which is of interest in this work. This equivalence was already found in an earlier work in which, however, the sliding was not yet implemented. The results presented here imply that the lattice model is also able to account for the protein transport in sliding mode, in exact correspondence to the continuum model, once the system parameters are accordingly normalized. Thus, for the rest of our investigations, the lattice model, being about two orders of magnitude faster than a corresponding continuum model, is employed. However, the adjacency of the chain monomers has an impact of the sliding effect of particles [see Fig. 1(a)]. Hence, the test system, consisting of randomly distributed occupied lattice sites, is not a reasonable approximation to the sliding and transport of proteins in a crowded environment inside the cell nucleus.

IV. SELF-AVOIDING RANDOM WALK CHAIN SYSTEM

In a realistic model, another feature of the chain must be implemented: The chromatin chain is not allowed to intersect itself. That was considered in the SAW chain system. This system incorporates two important characteristics of chromatin fibers in biological systems which are not considered in the RW chain model. In the SAW chain system, chains are created by a self-avoiding RW, i.e., the excluded volume effect is taken into consideration. The chains are well equilibrated, relaxed, and exhibit a given persistence length reflecting a certain stiffness of the fiber. A hard repulsive core is incorporated in the sliding model.

Results on diffusion of particles which may bind to and slide along the chains in this system to those of the RW chain system were compared. The dependence of the sliding process on the excluded volume effect and on the persistence length became visible.

A. Chain simulation

The chromatin fibers are modeled by chains consisting of monomers connected by segments. Monomers are represented by occupied lattice sites. Neighboring occupied lattice sites reflect neighboring monomers in the chains. No lattice site is allowed to be occupied more than once. Possible bonds between two adjacent monomers are given by the set of all component permutations and sign inversions $P$ of two bond vectors, $P(1, 0, 0) \cup P(1, 1, 0)$, inducing a bond length of 1 or $\sqrt{2}$. The initial state for the MC process consists of chains folded into cubes (see Ref. 15). The energy model for the stiffness of the chromatin fibers includes a bending potential $E^{b}$. This potential is computed as

$$E^{b} = \gamma \sum_{i=1}^{N-2} \theta_i^2,$$

where $N$ stands for the number of chain monomers, $\theta_i$ is the angular displacement of bond $i$ relative to bond $i+1$, and $\gamma$, in units of $k_{B}T$, is the bending rigidity constant and is directly related to the persistence length $l_p$ of a fiber. $\gamma$ is determined by the procedure described by Jian et al. This combined procedure applied to a typical start conformation of cubic compact chains yields a dense network of chains.

B. Model for diffusional transport of chromatin-binding proteins

The walker-chain interaction is applied using the attractive step potential

$$U(s) = \begin{cases} -E_0, & s < \sqrt{2} \\ 0, & \text{else} \end{cases}$$

where $s$ is the shortest distance between walker and chain. In contrast to Eq. (1), the chain segment now features a hard repulsive core potential, surrounded by an attractive layer. The walker may bind to the chain with a given sliding probability $p_{\text{slide}}$ if the distance of the walkers current position to the chain, i.e., an occupied lattice site, is either 1 or $\sqrt{2}$. The sliding probability $p_{\text{slide}}$ is defined in Eq. (2). Once in the binding mode, the particle slides directly along the chain with probability $p_{\text{slide}}$. Different sliding probabilities, $p_{\text{slide}} = 1 - 2^{-l}$, $l \in \{1, \ldots, 11\}$, are tested. A step away from the chain during which the particle does not dissociate counts to the waiting time, i.e., in that case, the particle does not move at all. Otherwise, if the particle does not bind, it freely diffuses. If $p_{\text{slide}} = 0$, the particle freely diffuses at all time steps. The hard repulsive core potential prohibits movement of the particle to occupied lattice sites. If the particle collides with a chain, it is reflected to the last visited site. Thus, the diffusional behavior of the particle depends on two competing modes: The binding mode and the free diffusion mode.
It should be noted that during binding simulation we make the following modeling assumptions.

- The walker can be adsorbed on any site of the chain, it binds nonspecifically.
- The sliding probability $p_{\text{slide}}$ is the same everywhere on the chain.
- 1D diffusion along the chain is as fast as 3D (free) diffusion.
- The chain remains immobile.
- Cross-links are allowed but with significantly reduced probability: A protein which is sliding along the chromatin fiber has to first jump off the chain (which causes an energy penalty) before being able to attach to another segment which is located nearby or even crossing. As a result, the probabilities to enter from segment $i$ to $i+1$ or $i-1$ (where there is no energy barrier) are different from the probabilities of entering another nonadjacent segment. The energy barrier is the same as the one for leaving the chain, i.e., $1-p_{\text{slide}}$. In Sec. IV C 4, this condition was dropped and a free intersegmental hopping was allowed.

The excluded volume of the chain is obtained via Eq. (4)

$$V_{\text{ex}} = V_{\text{chain}} + V_{\text{bind sites}} \left(1 - \frac{1}{1-p_{\text{slide}}} \right),$$

(17)

$V_{\text{chain}}$ denotes the chain density, the number of occupied lattice sites. $V_{\text{bind sites}}$ denotes the volume of all binding sites. An unoccupied lattice site is defined as a binding site if the distance of this site to the nearest occupied lattice site is less or equal $\sqrt{2}$, i.e., if the lattice site lies in the binding radius of the chain.

The accessible volume parameter is defined as

$$\sigma_{\text{eff}} = 1 - \sigma_{\text{ex}}$$

(18)

$$= 1 - \sigma_{\text{chain}} - \sigma_{\text{bind sites}} \left(1 - \frac{1}{1-p_{\text{slide}}} \right).$$

(19)

In this model, the fraction of time $\tau$, which the walker spends sliding along the chain, can be predicted analytically via

$$\tau_{\text{theor}} = \frac{\sigma_{\text{bind sites}} \left(1 - \frac{1}{1-p_{\text{slide}}} \right)}{1 - \left(\sigma_{\text{chain}} + \sigma_{\text{bind sites}} \left(1 - \frac{1}{1-p_{\text{slide}}} \right)\right)}.$$  

(20)

The consistency of Eqs. (8) and (20) was verified during the simulations, thereby confirming that the system had reached its chemical equilibrium and that the statistics was sufficient to obtain accurate numerical results (data not shown).

C. Results

One particle was randomly initialized in the lattice. 200 000 random walk steps were carried out and the motion of the walker was monitored. This procedure was repeated 5000 times and the data were averaged. The diffusion coefficient $D$ was determined for long times ($t > 100 000$ time steps), where normal diffusion is observed.

1. Particle-chain interactions cause transient subdiffusion

For all investigated sliding probabilities $p_{\text{slide}} > 0$, transient subdiffusion is observed in which there is a crossover from subdiffusion (anomalous diffusion) at short times to normal diffusion at long times

$$(r^2(t)) = \begin{cases} t^a & \text{for } t \ll \tau_{\text{cr}} \\ t & \text{for } t \gg \tau_{\text{cr}} \end{cases},$$

(21)

where $\tau_{\text{cr}}$ is the transition time. The transition time depends on the homogeneity of the chain structure. The more homogeneously the chains are distributed, the smaller $\tau_{\text{cr}}$ and the lower the anomaly parameter $\alpha$ (Fig. 2). To emphasize the crossover, the asymptotic time dependence is normalized out and $(r^2(t))/t$ versus $t$ is plotted on a logarithmic scale. Then, anomalous diffusion gives a straight line of slope $\alpha > 1$, normal diffusion gives a horizontal line, and the intersection of these lines gives the crossover time. In the test system (disconnected obstacles) $\tau_{\text{cr}} \approx 100$ time steps, in the SAW system $\tau_{\text{cr}} \approx 1000$ time steps, and in the RW system $\tau_{\text{cr}} \approx 11 000$ time steps, with a chain density of 6.4% in all three systems.

2. Particle diffusion in various environments

Binding events slow down the overall mobility of the protein. The stronger the protein-chromatin interactions, the slower the diffusion coefficient (Fig. 3). Although it is known that one dimensional diffusion reduces the time to search for a specific binding site on the chain contour (facilitated diffusion), the sliding process globally causes a slow down of the diffusion coefficient.

The nonadjacency of the occupied lattice sites in the test system can be made responsible for a decreased diffusion coefficient compared to the RW chain system and the SAW chain system (Fig. 3). Once a particle binds to one of the
disconnected sites, it is only able to move around that obstacle while in binding mode. In both other systems, the chains are longer, yielding a higher diffusion coefficient. Besides chain length, the diffusion coefficient depends on the homogeneity of the fiber geometry. Disconnected obstacles are more homogeneously distributed than the RW and SAW chains. Thus, the number of time steps a particle spends in free diffusion mode until it collides with an occupied lattice site is smaller in the test system compared to the RW chain system and the SAW chain system. In contrast, the RW chains not only create regions of high density but also leave areas of low density where it is unlikely for the particle to collide with the chain. This yields the highest diffusion coefficient in all three systems. In case of strong binding interactions, these differences in the three systems are reduced (solid symbols).

A variation of chain length of sufficiently long chromatin fibers ($N \in \{50, 250, 500\}$), while keeping the chain density constant, did not affect the diffusion coefficient. However, once shorter fibers were involved (with $N < 50$) a reduction of $D$ was observed [Fig. 4(a)]. This is easily understandable, considering the fact that with decreasing chain length the chance for the walker to hit one of its end points is increasing. In such a case, the walker has no other option left but to reverse its direction of walk and hence to slow down its effective speed of transport. With $N=1$, we are approaching the limit of disconnected obstacles which was discussed above.

Next, simulations were carried out with 46 long fibers of length $N \in \{10^3, 11^3, 12^3\}$, similar to chromatin fiber territories in the cell nucleus. Here, the persistence length $l_p$ was modified in order to study how particle diffusion is influenced by local variations of the fiber conformations. It was found that the diffusion is accelerated with increasing persistence length [Fig. 4(b)]. This is quite a remarkable result since in earlier diffusion simulations, which did not include the sliding mode, the walker was found to be insensitive to the chains’ persistence length. After rescaling with respect to the accessible volume parameter, the data, diffusion coefficients dependent on different chain densities, different chain lengths and different sliding probabilities fall on separate master curves for different persistence lengths (Fig. 5). This implies that proteins with a certain affinity to chromatin can be used to probe the persistence length of the fiber inside the nucleus. For $p_{slide} > 0$, the resulting curves are fitted by a power law,

$$D \sim \sigma_{eff}^k.$$  \hspace{1cm} (22)

The larger the persistence length, the larger is the scaling exponent $k$, the higher is the diffusion coefficient. For the data of the SAW system with $l_p=3$, $k=-0.55$ is found (Fig. 5), for the data of the SAW system with $l_p=0.5$ $k=-0.66$ and for the data of the RW system (normal random walk without given persistence length) $k=-0.86$. For $p_{slide}=0$, the data points are fitted by a straight line reflecting the results found in Ref. 15: The diffusion coefficient is proportional to the accessible volume.
3. Comparison to experimental data

For transient subdiffusion, a special kind of anomalous diffusion, experimental evidence exists.\textsuperscript{19,20} Experimental fluorescence recovery after photobleaching recovery kinetics of chromatin-associated proteins have proven a slowdown of the overall mobility of a chromatin-binding protein.\textsuperscript{21,22}

Anomalous diffusion due to binding has been reported in experiments, e.g., in earlier work from our own group\textsuperscript{9} we could show by FCS that the diffusion of the fluorescent protein enhanced green fluorescent protein in the nuclei of living cells is anomalous. One possible interpretation of the subdiffusive behavior was binding (or trapping) with a broad distribution of binding affinities. Later, we developed a theory to interpret simultaneously FCS and microphotolysis experiments.\textsuperscript{23} Other authors showed anomalous subdiffusion as a consequence of cytoplasmic crowding,\textsuperscript{24} and also for membrane proteins.\textsuperscript{25,26} Theoretical studies\textsuperscript{27} also showed transient subdiffusion due to binding.

In Ref. \textsuperscript{27} the transport of nucleosome core particles (NCPs) in semidilute DNA solutions was investigated. NCPs can change their conformation depending on the salt concentration of the solution.\textsuperscript{28} The histone tails of the proteins entangle at high salt concentrated solution. The entanglement increases the interaction between the NCPs and the DNA. As shown within our numerical studies presented in the previous subsection, Mangenot et al.\textsuperscript{27} found a slowdown of the diffusion coefficient for high salt concentration. Moreover, with constant salt concentration but increasing DNA network concentration the diffusion coefficient decreases. The higher the salt concentration, the less pronounced is this effect.

4. Protein hopping-intersegment transfer

In the previous studied models, we assumed that sliding (1D diffusion) of a particle along one chain is separated by periods of three dimensional diffusion. Intersegment transfer of chromatin-binding proteins is another mechanism that can be involved in the diffusional transport of these proteins. Assuming that two segments of different chromatin fibers (chains) come close to each other, a particle sliding along one segment can hop to the segment of the neighboring chain with the sliding probability \(p_{\text{slide}}\). Especially for proteins which have multiple binding sites, this hopping mechanism may play a role \textit{in vivo}.\textsuperscript{11} There exists experimental evidence for intersegment transfer: The transcription factor tetrmeric LacI, which has two DNA binding sites, travels along DNA through 1D diffusion and intersegment transfer. It is not yet clear whether transcription factors with only one binding site can perform intersegment transfer.\textsuperscript{11}

To demonstrate that the existence of intersegment transfer has a significant influence on the diffusional transport of chromatin-binding proteins, a model for the hopping mechanism is briefly described and results are discussed.

Compared to the model presented in Sec. IV B the following changes are made. The particle is allowed to transfer between different chains (if their segments come close together) while in bound state. For the particle, the chains are not distinguishable. In the case of a constant chain density, the diffusion coefficient decreases with increasing sliding probability. Hence, the modeling of diffusional transport with intersegmental transfer supports the experimental finding: The mobility of particles is slowed down with increasing chromatin-protein interactions. At low sliding probability \((p_{\text{slide}} < 0.75)\), the diffusion coefficient decreases with increasing chain densities. For \(p_{\text{slide}} = 0.75\), the diffusion coefficient remains constant while the chain density is increased. Higher sliding probabilities \((p_{\text{slide}} > 0.75)\) lead to an increased diffusion coefficient while increasing the chain density [Fig. 6(a)]. The higher the chain density, the higher is the number of crosslinks in the system. The hopping mechanism is responsible for the fact that diffusion of particles in networks of short chains is faster than that in networks of long chains [Fig. 6(b)]. Shorter chains are more homogeneously distributed enabling the particle to easily hop to a neighboring chain. For high sliding probabilities \((p_{\text{slide}} > 0.75)\), the diffusion coefficient increases with increasing persistence length showing the same effect as in the model without intersegment transfer; the diffusion becomes faster with increasing persistence length [Fig. 6(c)].

The differences in the diffusion coefficients due to different conformational properties are properly accounted for after rescaling with respect to the accessible volume parameter. After rescaling all data fall onto master curves with different slopes corresponding to different sliding probabilities (Fig. 7). This result demonstrates that in fact different chain densities and conformations affect the sliding process of particles but these differences can be projected into a single parameter, the accessible volume. The diffusion coefficients for different persistence lengths but constant sliding probabilities fall on the same curve. This is in contrast to Fig. 5, where intersegment hopping was suppressed. Proteins exhibiting only one binding site are able to scan the structure of the chain in a more exact way than proteins with multiple binding sites which are more likely to move via intersegment transfer.
V. SUMMARY

The present work was intended to shed some light on the modeling of the diffusional transport of chromatin-binding proteins in the cell nucleus. First of all we have demonstrated that such a process can be described with a lattice model of the nucleus and a random walk of the chromatin-binding proteins. The validity of this approach was checked using a straight comparison with the corresponding continuum model. Compared to the continuum model, the lattice model with the advantage of having a finite number of states delivers a substantial speed up: Chain conformations are simulated much faster with the BFM Ref. 15, and the same holds for the transport of chain-binding particles.

Three different models were tested to create a crowded environment of chromatin fibers in the cell nucleus yielding different structural properties of the chains. One of them was made of randomly distributed and disconnected obstacles, the second one a RW chain and finally the rather realistic SAW chain model with excluded volume and persistence length.

In several systematic simulations, including different sliding probabilities of the walker, it was shown that conformational variations of the crowded environment led to significant changes of the diffusion coefficients. First of all, as seen in experiments, protein-chromatin interactions generally decrease the diffusion coefficient. This even holds for different sliding mechanisms as proved within our set of simula-

FIG. 6. (Color online) Diffusion coefficient of chromatin-binding particles undergoing intersegment transfer as a function of (a) chain density fraction (constant chain length \(N=50\)), (b) chain length \(N\) (constant chain density of 6.4%), (c) persistence length \(l_p\) (constant chain density of 4.6%), and different sliding probabilities \(p_{\text{slide}}\). Solid symbols: Triangles, \(p_{\text{slide}}=1-2^{-2}=0.75\); circles, \(p_{\text{slide}}=1-2^{-4}=0.9375\); and squares, \(p_{\text{slide}}=1-2^{-11}=0.9995\). Blank circles: No intersegment transfer \(p_{\text{slide}}=1-2^{-2}=0.75\), solid symbols, \(p_{\text{slide}}=1-2^{-4}=0.9375\). blank symbols, \(p_{\text{slide}}=1-2^{-11}=0.9995\).

FIG. 7. (Color online) Diffusion coefficient of chromatin-binding particles undergoing intersegment transfer as a function of the accessible volume parameter. Squares, different volume fractions (6.4%,8%,10%,12.5%); circles, different chain lengths \(N \in \{1,25,50,250,500\}\); and triangles, different persistence lengths \(l_p \in \{0.5,1,2,3\}\). (a) Blank symbols, \(p_{\text{slide}}=1-2^{-2}=0.75\), solid symbols, \(p_{\text{slide}}=1-2^{-4}=0.9375\). (b) \(p_{\text{slide}}=1-2^{-11}=0.9995\).
tions, with and without intersegment transfer. Without intersegment transfer we observed that a larger chain density is slowing down the diffusion coefficient (Fig. 3). The same observation was made without protein-chromatin interactions (see Ref. 15). There, we marked this finding as trivial which is not longer true if a protein-chromatin affinity comes into play: Proteins with multiple binding sites may hop between chains during bound state. This intersegment transfer may lead to an increased diffusion coefficient while increasing the chain density [Fig. 6(a)]. The finding of subdiffusion during diffusional transport of chromatin-binding particles (Fig. 2) agreed with experimental data. So far no experimental data are available which cover protein diffusion as a function of structural properties of the chromatin fibers, such like contour length and persistence length.

The more homogeneously the obstacles were distributed in the nonintersegment transfer model, the lower were the observed diffusion coefficients (Fig. 3). The opposite was found with the intersegment transfer model assuming a high sliding probability: The more homogeneously the obstacles are distributed, the higher is the diffusion coefficient (Fig. 6). A more homogeneous structure facilitates the hopping process of a protein. In both models an increasing persistence length of the chains lead to an increasing diffusion coefficient in case of a high protein-chromatin affinity [Figs. 4(b) and 6(c)].

These effects could be accounted for with the concept of the accessible volume of the system: Once the diffusion was plotted as a function of the accessible volume parameter, the data from the intersegment transfer model fell onto master curves with different slopes corresponding to different sliding probabilities (Fig. 7). An effect of conformational variations on the diffusion could be eliminated. Regarding the nonintersegment transfer model, the data fell onto different master curves, each curve corresponding to different persistence length (Fig. 5). A protein with one binding site, which is allowed to hop between chains with significantly reduced probability, would sense the local structure of the chain much better than proteins with multiple binding sites.

Studying protein dynamics in living cells is ongoing experimental work, however, it is not yet clear which binding mechanisms are preferred by special proteins and how they work in detail. Experimental data could prove that sliding mechanisms without waiting time can be found: Once bound to their target sites, many enzymes catalyze reactions on DNA that involve the progressive motion of the protein along the DNA in steps of one or more base pairs.29 Through theories exist allowing estimates of diffusion-controlled reaction times of a protein, i.e., the time a protein needs to find its target.16 However, it is still unknown to what extend the reaction time is controlled by the conformational properties of the fiber network.

We believe that the model of diffusional transport of chromatin-binding proteins presented here is feasible to clarify these questions with the help of further systematic simulations.

ACKNOWLEDGMENTS

One of the authors (A.W.) thanks the Institute for Mathematics and its Applications (IMA), MN, for their hospitality during a research stay paid by funds provided by the National Science Foundation. In addition, A.W. thanks S. Kunkel for fruitful discussions in the initial phase of this work.