We present molecular-level simulations of dendrimer/DNA complexes in the presence of a model cell membrane. We determine the required conditions for the complex to arrive intact at the membrane, and the lifetime of the complex as it resides attached to the membrane. Our simulations directly pertain to critical issues arising in emerging gene delivery therapeutic applications, where a molecular carrier is required to deliver DNA segments to the interior of living cells. © 2009 American Institute of Physics. [DOI: 10.1063/1.3109902]

I. INTRODUCTION

Three emerging therapeutic avenues, gene therapy, antisense therapy, and DNA vaccination, are all based on the principle of genethesis ("gene placement") (Ref. 1) as they require the delivery of intact nucleic acid segments to the nucleus of living cells.2-4 These treatment methodologies offer hope to patients of numerous currently untreatable hereditary diseases, provide new routes to fighting viral infections, and will likely propel vaccination technology to a new level of sophistication. Although currently there are several methods for delivering the nucleic acid strands to the nucleus, including mechanical pressure and electroinjection, the use of carrier molecules (vectors) offers the greatest flexibility and easiest introduction into large organisms such as humans.5 These gene vectors must be able to pack and protect the DNA, approach the cell membrane, insert into the cell, and finally release the gene into the nucleus.5 Currently the most common and efficient delivery systems having all these properties are viruses. Major safety concerns, however, such as high toxicity and immunogenicity, have limited their general use. A much less toxic, easier to produce, and more cost-effective alternative is the use of synthetic (nonviral) vectors.2 Synthetic vectors, such as cationic liposomes and cationic polymers, are also more versatile: Their chemical and physical properties, for instance, allow the packing of a large variety of gene lengths, in contrast to the capsid restricted capacity of viruses.2 These key advantages of nonviral vectors have stimulated intense clinical exploration of their utilization in genethesis. Although synthetic vectors are now routinely used in laboratories, their very low delivery efficiency remains prohibitive for their use in gene delivery. Clearly the development of safe therapies will depend strongly on improvements to the delivery efficiency of synthetic vectors. Progress in this direction will rely on advances in our understanding of the salient molecular physics involved in the formation and delivery of therapeutic complexes.

State-of-the-art computational studies in gene delivery, however, are at a very early stage and are primarily limited to pharmacokinetic models that treat all events as kinetic processes described by rate equations. Only very recently, Dinh et al.,6 presented the first attempt to capture spatial properties of the process by using a novel framework based on diffusion equations. Such models can provide valuable information regarding the criteria the complexes must meet to maximize genethesis efficiency. However, they do not address more fundamental questions such as identifying the physical and chemical properties that synthetic vectors should have in order to form adequate complexes with the desired DNA segments. Such questions can only be addressed by molecular-level numerical studies.

Here, we present the first molecular-level theoretical study of genethesis by synthetic vectors. Specifically, we will illuminate, as illustrated in Fig. 1, the processes active in cell membrane attachment and complex stability once on the membrane. Although the details will vary with the synthetic carrier, the molecular processes studied here are likely to share commonality with phenomena exhibited by other polymeric delivery formulations. We focus on dendrimer-base vectors because they possess several characteristics that make them attractive alternatives.3,4,7-11 Their monodispersed nature and regularly branched treelike connectivity give rise to compact, well-defined structures that, under the proper conditions, contain cavities.12,13 Their size is conveniently tunable by changes to their molecular weight (viz., generation of growth) and falls within the range needed to avoid macrophage capture, while permitting membrane transit. They have a large number of functional groups on their termini that may be modified for specific applications, including attaching drugs or pattern recognition moieties such as proteins for diseased cell targeting. Moreover, they are often cationic, making them suitable for entry into cellular structures that typically carry negative charge. Some chemistries, the poly(amidoamine), in particular, show acceptable cytotoxicity.14,15 Finally, they are responsive to changes in their environment, permitting the possibility of controlled release of any cargo they may carry.
II. THE MODEL

Following the work of Welch and Muthukumar,\textsuperscript{16} we represented the electrostatics by the Debye–Hückel potential, 
\[ (U_{DH}/kT) = \lambda_B e^2 \sum_{i,j=1}^{N} q_i q_j e^{-\lambda_B r_{ij}/r_{ij}}. \]
 Here, the sum runs over all pairs of the \( N \) charged beads. This includes all of the chain segments and the terminal groups on the dendrimer. The Debye length \( \lambda_D \) captures the effect of added solution salt and varies as the square root of the salt concentration. The Bjerrum length \( \lambda_B \), which is 7.1 Å in water at 25 °C, sets the length scale for the simulations. DNA is known to possess approximately 0.06 fundamental charges per base.\textsuperscript{17} Within our simulations, we set the number of charges per base \( q_i \) to 1.0, thus giving us a simulation charge unit \( e_{\text{sim}} = 0.06e \). For simplicity, we assume that the dendrimer segments hold equal charge, but opposite sign to the chain segments. The thermal energy \( kT \) is prescribed below. We employed the “finitely extensible nonlinear elastic” FENE potential to preserve the connectivity of the cationic dendrimer and oppositely charged single-stranded DNA molecule, which was treated as a linear bead chain. The FENE potential is given by 
\[ (U_F/kT) = -K(l_{\text{max}}^2 - l_0^2) \sum_{i,j=1}^{N} \log\left[1 + \left(\frac{l_j - l_0}{l_{\text{max}} - l_0}\right)^2\right] \]
where \( N \) is the number of beads in the model dendrimer or chain, \( K \) is the spring constant, \( l_j \) is the instantaneous bond length, \( l_{\text{max}} \) is the maximum bond length, and \( l_0 \) is the equilibrium bond length. To prevent bond crossing, \( l_{\text{max}} = \lambda_B \) and \( l_0 = 0.7 \lambda_B \). We set the spring constant \( K = 20.0 \lambda_B^2 \). The Morse potential, 
\[ (U_M/kT) = (\varepsilon_{a,b} kT) \sum_{i,j=1}^{N} [e^{-\alpha(r_{ij} - d_i)} - 1]^2 \]
mimicked the noncharged two-body excluded volume interactions. The indices \( a \) and \( b \) label the parameters as belonging to the chain or the dendrimer and take the values \( D \) and \( C \). The well depths for the intramolecular interactions, \( \varepsilon_{D,D} \) and \( \varepsilon_{C,C} \), were set to unity and determine the energy scale of the simulations. The intermolecular interaction well-depth \( \varepsilon_{D,C} \) was set to 0.01 \( \varepsilon_{D,D} \) to mimic a purely hard-bead model. The parameters \( a \) and \( d \) take the values \( 24/\lambda_B \) and \( 0.8 \lambda_B \), respectively. The simulations were carried out in the good solvent regime with \( kT = 0.7 \varepsilon_{D,D} \). Note that we do not include any explicit terms to introduce semiflexibility in the chain, a significant feature in double-stranded DNA. Lyulin\textit{ et al.}\textsuperscript{18} employed a similar model for their study of dendrimer-polyelectrolyte interactions.

The main purpose of this study is to elucidate the conditions for membrane attachment, the expected lifetimes of the complex, and the early-time properties of the complex on the membrane. To this end, we model the membrane as an infinite plane that interacts with the model molecules via a hard-wall repulsion and planar-averaged Debye–Hückel potential, 
\[ U_{\text{DH}}/kT = 2\pi \lambda_B K_B \rho e^{-\lambda_B \rho} \] (see Ref. 19, for example). The dimensionless (negative) surface charge density \( \sigma \) reflects the charge on the membrane due to the lipids and other components. Healthy cell membranes present a net negative charge of about 0.02 \( C/m^2 \).\textsuperscript{20} Thus, the nominal reduced charge density of a typical cell membrane in units of our simulations is \( \sigma \lambda_B^2 = 1.0 \). The membrane transit process will be studied in a future investigation with a more appropriate fluid membrane model.

This simple model neglects a number of the details that doubtlessly play important roles in the real system. Atomistic simulations by Maiti and Bagchi\textsuperscript{21} demonstrated the importance of not only counterions but also DNA sequence in the dynamics of dendrimer-DNA complexes. Coarse-grained studies of the interactions between lipid bilayers and dendrimers by Lee and Larson\textsuperscript{22–24} clearly illustrate the importance of the fluidlike nature of the membrane in the dynamics of pore formation. However, the model presented here has the merit of computational economy permitting us to capture the dominant physics within a framework that yields rich statistical information unavailable to the more detailed models. Our aim here is to survey the salient phenomena and pave the way for more detailed simulations in the future.

The mechanism of complex binding to cell membrane is of electrostatic origin: Cationic dendrimer/DNA complexes are attracted by the oppositely charged cell membrane. This process was investigated by using a bond fluctuation Monte Carlo approach. Complexes were first formed and equili-
brated for \((5-10) \times 10^6\) Monte Carlo steps (MCSs) without the model membrane (one MCS is defined as \(N\) attempted perturbations and \(N\) as the total number of particles in the simulation). Next, the complexes were placed at a distance \(z_0\) away from the membrane and the MC process begun for \(1 \times 10^6\) MCS. Coordinate samples were saved every 1000 MCS to obtain equilibrium ensemble averages. The distance \(z_0\) has to be sufficiently large to eliminate initial effects of the opposite electrostatic field on the complex stability. We confirmed that this condition is satisfied when the net force acting on the complex by the membrane is less than \(20 \lambda_B / \epsilon\), where \(\epsilon\) is the Morse well depth used to capture the non-charged segmental interactions. The lifetimes are defined as the time between surface attachment (closest dendrimer bead 1 length units above surface) and the point at which the dendrimer bead farthest away from the surface is separated from the DNA base closest to the surface by 2 length units. These lifetimes are reported in units of MCS; while Monte Carlo does not provide a quantitative dynamical measure, it is known to provide reasonable qualitative trends.

### III. NUMERICAL RESULTS

Our first goal is to ascertain the ionic strength and surface charge density conditions required for a complex to attach itself to a membrane. To accomplish this, we performed at least \(10^3\) independent simulations where an equilibrated complex was placed \(z_0\) above the surface of the model membrane and allowed to either adsorb to the surface or wander away. The probability that a given complex attached to the surface under a given set of \(\sigma \lambda_B^2\) and \(\lambda_B / \lambda_D\) conditions was calculated as the fraction of the total independent simulations that resulted in an intact complex sticking to the surface. Figure 2 shows this probability for a generation 5 dendrimer complexed to a 30-base DNA strand. Note that, unlike what has been found for the case of a single molecule attaching to a surface,19,25–27 no crisp transition is observed. We attribute this to the additional degrees of freedom allowed by the separation of the two molecules within the complex. Contrary to our expectations, the complex becomes less likely to attach to the surface as the surface charge density increases and/or as the solution ionic strength decreases. This occurs because the complexes become unstable when exposed to the polarizing field exerted by the membrane. As is known from other studies,16,19,25–27 polyelectrolytes typically exhibit a critical lower bound for the required charge density in order for complexation to occur. This arises due to the competition between the entropic drive to move freely in solution and the electrostatic forces. We observe a similar lower bound for these complexes (but focus on the upper bound in this study).

Thus, there is a window of acceptable surface charge densities and solution ionic strengths. This behavior was found to hold over the range of dendrimer generations (4–6) and DNA strands (15–60 bases) studied, as long as the total charge of the complex remains positive. Herein, we focus on complexes with only a slight positive charge excess (+18), known to be needed for efficient transfection, rather than complicate our analysis at this point with poorly understood structural variation effects.28

Our next goal is the study of the complex stability once on the cell membrane. This study is of great importance because for a successful delivery of the payload nucleic acid strand, the lifetime of the complex must exceed the finite time required to transit the membrane. In order to gain some insight into how the complex lifetime depends on membrane composition, we first study the effects varying the membrane charge density. In Fig. 3 we show the probability distribution for the lifetime of a complex of a generation 5 dendrimer and a 30-base single-stranded DNA molecule at \(\lambda_B / \lambda_D = 2\) and for three different values of \(\sigma \lambda_B^2\). We see that the most probable lifetime decreases with increasing surface charge density. This result is more clearly shown in Fig. 4(a) for a wider range of membrane charge density. However, if the average value of the lifetime is examined [see circles in Fig. 4(a)], a minimum is observed at intermediate values of \(\sigma \lambda_B^2\). This apparent disparity between the most probable and average values of lifetime can be explained by the snapshots pre-
circles correspond to the mean lifetime and squares to the most probable lifetime. It must be moderately stable. It must be stable enough to arrive at gene delivery into the nucleus. The ideal complex must only indicate that larger dendrimers will increase the efficiency of environmental conditions. However, this result does not in-

\[ \tau = \frac{\lambda_D}{\lambda_B} \]

FIG. 4. (Color) The mean and most probable lifetimes \( \tau \) in units of Monte Carlo steps for a generation 5 dendrimer complexed to a 30-base DNA strand as a function of (a) reduced surface charge density \( \sigma \lambda_B^2 \) with \( \lambda_D/\lambda_B = 2 \) and (b) reduced Debye length \( \lambda_D/\lambda_B \) with \( \sigma \lambda_B^2 = 1 \). In both subfigures, circles correspond to the mean lifetime and squares to the most probable lifetime. In (a), insert figures demonstrate representative structures of the complex in different \( \sigma \) regimes. The color coding of the beads is as in Fig. 1.

presented in Fig. 4(a). For small charge densities, we observe long lifetimes because, as seen in (i), the complex is practically unperturbed. Increasing charge density, however, results in shorter lifetimes because the electric field of the membrane critically affects the stability of the complex [see (ii)]. In the limit of high charge densities, the existence of rare long-lived “pinned” structures, such as that shown in (iii), results in longer average lifetimes. However, in this limit, the majority of the complexes dissociate easily, which reduces the most probable lifetime monotonically. Figure 4(b) shows the dependence of lifetimes on the Debye length for \( \sigma \lambda_B^2 = 1 \), approximately that of a normal cell membrane within our simulation units. We see that both average and most probable lifetimes decrease with \( \lambda_D \) due to the absence of pinned structures for this value of \( \sigma \lambda_B^2 \). We also note that for DNA of a given length to be delivered, the most probable lifetime increases with increasing dendrimer generation if the net charge of the complex remains positive, regardless of environmental conditions. However, this result does not indicate that larger dendrimers will increase the efficiency of gene delivery into the nucleus. The ideal complex must only be moderately stable. It must be stable enough to arrive at and bind to the cell membrane and fragile enough to be dissociated into the cytoplasm or nucleus in order to release the DNA molecule.

In an effort to quantify this lifetime \( \tau \) in terms of the surface charge density, solution ionic strength, and nucleic acid molecular weight, we constructed the following simple ansatz. Just prior to the escape of a linear strand, one or two tails from the chain extend perpendicularly away from the membrane surface. The dendrimer serves as an anchor to some portion in the middle of the chain and mitigates the effective local surface charge density. Indeed, we observed this in the vast majority of our simulations where escape occurred. This is clearly illustrated in Fig. 1.

The free energy density for such a chain configuration consists of two entropy terms and two electrostatic terms for the two tails. The entropy terms depend upon the number of beads in each tail and the random walk behavior of each. In the limit where the tails are stretched out the entropy terms are negligible and ignored. To the first approximation, the electrostatic and excluded volume interactions between and within the tails serve only to dictate the mass-size scaling. The only remaining, significant contribution to the free energy is due to the electrostatic repulsion that the tails experience from the membrane. Again we apply the planar-averaged Debye-Hückel potential and assume that, on average, each chain segment is obeying random walk statistics characterized by the mass-size scaling exponent \( \nu \) and is oriented perpendicular to the membrane. If one tail has \( m \) segments and the other \( N_c - m \) segments, with \( N_c \) being the total number of chain segments, the approximation free energy density is

\[ f_e = 2\pi \sigma \lambda_B \rho (\sum_{i=1}^{N_c-m} e^{-\nu l_B} + \sum_{j=1}^m e^{-\nu l_B}) \]

where \( l_B \) is the effective step length for the tails. Ignoring the inertial contributions, the equation of motion based upon this free energy density is \( \dot{\xi}_j m - dm \xi_j = 0 \) where \( \xi \) is the frictional coefficient associated with chain slippage underneath the pinning dendrimer. Assuming that \( \nu = 1 \) (consistent with neglecting the entropy terms) and \( N_c \) is large yields \( \tau = \frac{\xi \lambda_B^2}{(N_c/2)/(\nu l_B)} = \frac{1}{\sigma \lambda_B^2} \). Since we have ignored the possibility of loops, a reasonable assumption for short chains, one can view this estimate as a lower bound. As seen in the simulation results of Fig. 4, this expression captures the main effects dictating the most probable value of \( \tau \) in terms of \( \lambda_D \), \( \sigma \), and \( \lambda_B \). The solid lines in the figures represent the best fits to the data for our expression for \( \tau \). Our simple ansatz appears to faithfully capture the dependence on \( \lambda_D \), but underestimates the contribution of \( \sigma \) in the low surface charge density limit, in agreement with our assertion that we have arrived at a lower bound only.

Finally, to give insight into the mechanism by which the complexes dissociate, we show the ensemble averaged density distributions away from the surface of the membrane. Considering only the region of the parameter space where the complexes are long lived permits us to calculate meaningful equilibrium averages of the number of beads that fall in discrete layers of thickness \( 0.2/\lambda_B \) and that are parallel to the membrane surface. Figure 5 illustrates these distributions for the chain and for the dendrimer as a function of surface charge density. While the chain appears to be only slightly affected by the variations in \( \sigma \lambda_B^2 \), the dendrimer is observed...
to flatten to large extent as the surface charge density is increased. In essence, the dendrimer is squeezing out the nucleic acid strand. Note that similar conformations for dendrimers on surfaces have been reported previously in literature.29–32

IV. CONCLUSIONS

In summary, our combined analytical and computational investigation indicates that there is a finite region of parameter space in which a complex may successfully land on a cell membrane and stay intact long enough for cellular entry to occur. The surface charge densities and solution ionic strengths encountered in vitro mean that the complexes are likely to arrive intact to the surface. However, the limited lifetime of the complex on the surface and the relatively long times required for membrane transit suggest that the exponential dependence on the chain molecular weight plays a key role in determining whether a particular delivery scheme will succeed. While we have employed dendrimers in our investigation, any transport agent capable of pinning down a nucleic acid strand (such as linear polycations) should display similar behavior.

ACKNOWLEDGMENTS

This work was carried out under the auspices of the National Nuclear Security Administration of the U.S. Department of Energy at the Los Alamos National Laboratory under Contract No. DE-AC52-06NA25396. This work is supported by the U.S. Department of Energy Office of Biological and Environmental Research under work proposal number SCFY081004.

1 *Genesis* is a new term coming from "gene" and the ancient greek “thesis” (meaning placement), which we introduce to describe the delivery of genes into the cell nucleus.


