

Release of Proteins from Nanochannel Delivery Systems: a Coupled Many-Scale Simulation - Experimental Investigation

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Abstract. Transport and surface interactions of proteins in nanopore membranes play a key role in many processes of biomedical importance. Although the use of porous materials provides a large surface-to-volume ratio, the efficiency of the operations is often determined by transport behavior, and this is complicated by the fact that transport paths (i.e., the pores) are frequently of molecular dimensions. Under these conditions, wall effects become significant, with the mobility of molecules being affected by hydrodynamic interactions between protein molecules and the wall. Modeling of transport in pores is normally carried out at the continuum level, making use of such parameters as hindrance coefficients; these in turn are typically estimated using continuum methods applied at the level of individual diffusing particles. In this work we coupled experimental evidences to many-scale molecular simulations for the analysis of hen egg-white lysozyme adsorption/diffusion through a microfabricated silicon membrane, having pores of nanometric size in only one dimension. Our joint efforts allowed us a) to elucidate the specific mechanisms of interaction between the biopolymer and the silicon surface, and b) to derive molecular energetic and structural parameters to be employed in the formulation of a mathematical model of diffusion, thus filling the gap between the nano- and the macroscale.

Introduction

The rapid convergence of nanotechnology and biotechnology has generated a need for designing tools that can incorporate sufficiently detailed models of the nanoscale molecular phenomena with overarching transport and related effects in microfluidic devices. Currently, no unique software is available that can perform this task. This void is sought to be filled by our recent efforts, whose innovative aspects include the development of a novel many-scale simulation tool for integrated nanosystem design, analysis and optimization based on a three-tiered modeling approach consisting of (a) molecular models (b) atomistic molecular dynamics simulations and (c) dynamical models of protein transport at the continuum scale. The generalized three-level modeling paradigm under development integrates nanoscale effects of arbitrary biosystems accurately, efficiently, and seamlessly using continuum models carrying molecular information. The first step in the paradigm is to perform molecular simulations to elucidate the essential molecular behavior. Next, selected molecular parameters are derived from these calculations. Finally, the information from the atomistic models is coupled to the continuum model. The validity of this approach is demonstrated in this paper through a proof-of-concept study of the non-Fickian release of a protein (hen egg-

white lysozyme, LYS) through microfabricated silicon membranes [1], consisting of arrays of parallel rectangular channels. The channels have a width of 45 μm , and their length is 4 μm . The microfabrication process allows to precisely tuning the channel height to 13 nm. Accordingly, these membranes have pores of nanometric size in only one dimension, thus differing from the majority of the cases analyzed in literature, where a cylindrical pore configuration is usually considered, leading to non-Fickian diffusion phenomena, like single file diffusion [1], different from the one investigated in the present work.

Materials and methods

We carried out atomistic molecular dynamics (MD) simulations according to the following schema [3]: (a) direct energy minimizations of the protein close to the silicon surface, and (b) MD runs with explicit solvation over 10 ns of time using the Amber 7.0 molecular platform [4]. The first procedure should correspond, in principle, to the initial adsorption stage, but it should also yield the preferred conformations if the adsorption process is either under kinetic control, or at large surface coverage. Conversely, the second procedure should yield the best overall conformation on a clean surface with the largest interaction energy under thermodynamic control. Starting from the work of Peskir [5], we then developed a mathematical model of biomolecular diffusion through nanochannels to provide a reasonable interpretation of the observed non-Fickian phenomenon [6]. Importantly, the model depends on two molecular parameters which are obtained from the MD simulations described above. The model was implemented in the Matlab-Simulink™ software environment[7] to run virtual experiments by means of pc-based simulations at the continuum scale. The experimental measurements of the contact angle between LYS and a silicon surface were obtained by dipping a silicon wafer into hydrofluoric acid to remove the native oxide layer. The wafer was then immersed in a lysozyme solution (5mg/mL in PBS) at room temperature for two days. The wafer was finally rinsed and dried under nitrogen current. A micropipette was used to place a solution drop of 5 μL onto the surface, and the contact angle was calculated by fitting the shape of sessile drop. The reported values are averaged over three repeated experiments. Pure Millipore water was used for comparison.

The AFM imaging of adsorbed LYS was obtained with a Veeco Nanoscope instrument (Digital Instruments, Santa Barbara, CA), using an NP-S20 tip in the tapping mode. The applied force was always minimized, so as not to deform the soft protein layer.

Results and discussion

The average contact angle of water on the LYS-covered silicon surface was 55°, whilst that obtained in the case of clean Si surface was 73°. These values, in line with similar literature evidences for other proteins absorbed onto hydrophobic surfaces, clearly indicate that the LYS layer is still quite hydrophobic.

The AFM image, topography, and cross sectional profile along the line in topography were obtained simultaneously, and are shown in Figure 1. Several, important information can be deduced from these AFM evidences. Indeed, Figure 1 shows that the coverage of the silicon surface by LYS seems to be complete. Interestingly, the height of the adsorbed protein lies around 3 nm, with some peaks around 4-5 nm, as can be seen on the profile in Figure 1. These dimensions are approximately equal to the diameter of the protein in solution (3×3×4.5 nm), which constitute a favorable sign for nondenaturation of LYS upon adsorption. Further, the relative frequencies of appearance of the smaller peaks with respect to the higher ones seem to support an edge-on prevalent configuration of adsorbed LYS molecules.

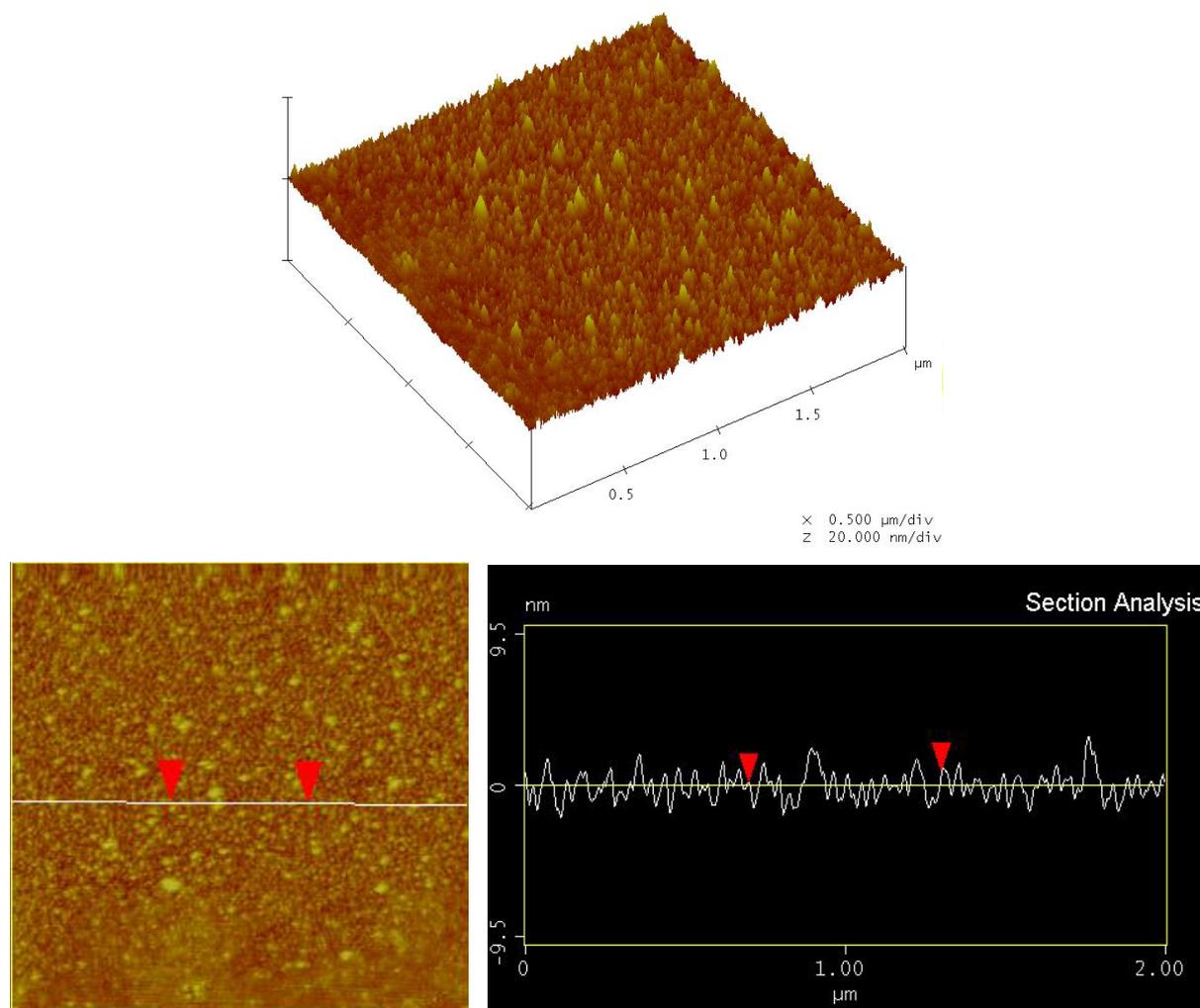


Figure 1. AFM image (top), topography (bottom left) and cross sectional profile along the line in topography (bottom, left) of the silicon surface after adsorption of LYS from solution.

In harmony with the AFM experimental finding, Figure 2 illustrates the evaluated change in solvent accessible surface (SAS) [7] for an isolated LYS and the LYS/surface system, respectively, as obtained from our MD simulations. Here, the polar, nonpolar, and total SAS of the protein are computed as they were represented alone in the bulk water. Thus, a decrease in the SAS value would not indicate contacts between LYS and surface, with subsequent exclusion of water, but rather a compaction of the biomacromolecular structure with a consequent reduction in its surface area. Contrarily, an increment in the SAS value should represent the unfolding of the protein to expose previously buried amino acids. As we can see from this Figure, no meaningful variation can be envisaged in the total SAS, as well as in its components, both for the control protein and for the system including the silicon surface. This can be taken as an indication that the silicon surface does not induce any substantial protein unfolding over the surface itself during the course of the MD simulation. Furthermore, the curves in Figure 4 suggests that the changes in RMSDs reflect only relative motion about a stable core protein, as expected for ‘hard’ proteins such as LYS, as opposed to any significant changes in the global protein conformation. From the MD simulation of the LYS alone in water we also calculated the molecular volume of the protein, $V_{\text{vdw,LYS}}$, by averaging the value of the van der Waals volume of different saved snapshots over the equilibrated trajectory. The resultant value is $V_{\text{vdw,LYS}} = 16651 \text{ \AA}^3$.

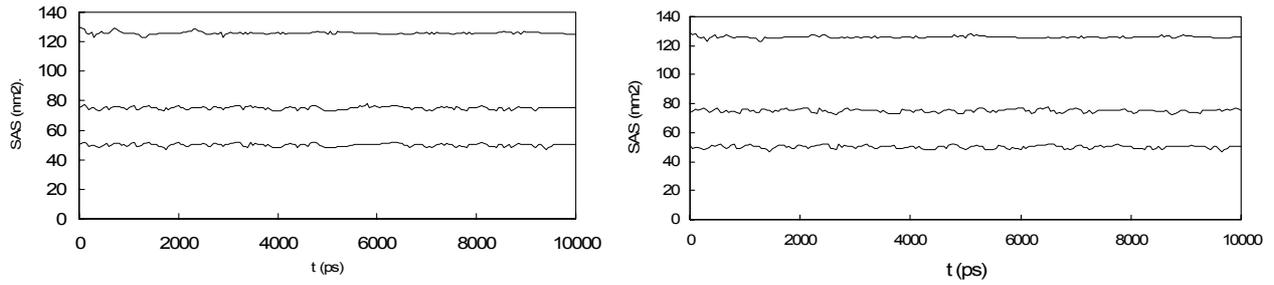


Figure 2. Change in total (top data line), nonpolar (middle data line), and polar (bottom data line) SAS of LYS in bulk water (left), and over the silicon surface (right).

We then determined the energy of attraction between a lysozyme chain adsorbed onto the silicon surface and another, free lysozyme molecule. For the sake of simplicity, we considered that the free LYS molecule would sit on top of the bound one upon adsorption. Each simulation, again carried out by keeping the silicon surface atoms static, was repeated six times. The distance of the free LYS from the bound LYS was then changed, and the simulation repeated until 19 different protein/protein distances were evaluated. The resulting interaction energies were then averaged, and the corresponding standard deviations were recorded. Figure 3 shows a plot of the interaction energies on the free LYS as a function of position from the LYS adsorbed onto the surface. As we can see, the interaction energy follows the standard particle dispersion interaction pattern: a very repulsive potential at close distances, followed by an attractive region, and ending with forces approaching zero at long distances. In details, the energy becomes negligible at distances greater than 5-6 Å, is at a minimum at approximately 2.5 Å, and become increasingly repulsive as the free polypeptide approaches the bound protein. The average calculated value at the minimum is equal to $E_{\min} = -93.93$ kJ/mol.

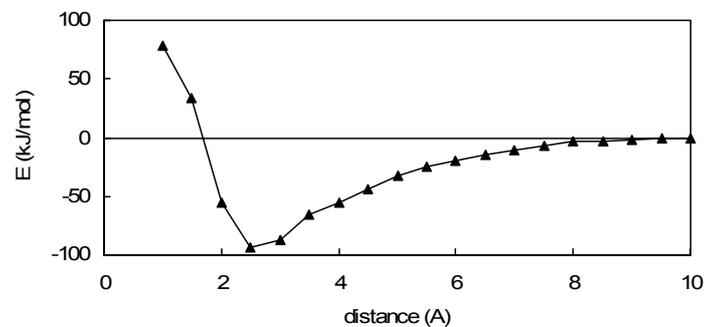


Figure 3. Free/bound LYS interaction energy as a function of position from the LYS molecule bound to the silicon surface.

To proceed to the third level of our multiscale procedure, we can start by recalling that classical diffusion theory is based on the Einstein's relation, which expresses the diffusion coefficient of spherical Brownian particles in a solution as:

$$D_{AB} = \frac{kT}{6\pi r\eta}. \quad (1)$$

where k is the Boltzmann constant, T the temperature, r the radius of the solute particles, and η the viscosity coefficient of the liquid. In order to derive a more general model, one needs to take into account the fact that the mixture does not satisfy the hypothesis of ideal gas law. When the state of Brownian particles in the Einstein argument deviates from the ideal gas law, it assumes the form of the van der Waals equation:

$$\left(p + \frac{a}{\gamma^2} \right) (\gamma - b) = RT. \quad (2)$$

where p is the pressure, R is the universal gas constant, and $\gamma = V/n$ is the molar volume, V being the total volume and n the number of moles. It is well known that the constants a and b have a physical interpretation: The term a/γ^2 represents the additional positive pressure caused by the presence of other solute particles, as a consequence of the long-range attractive forces; the constant b , instead, represents the volume occupied by the gas molecules, so that the term $(\gamma - b)$ represents the effective “free volume”. We have then exploited the work of Peskir [5], who derived from equation (2) the generalized expression for the diffusion coefficient (compare with equation (1)):

$$\tilde{D}_{AB} = \frac{kT}{6\pi r\eta} \left[\frac{1}{(1 - b_0 v)^2} - \frac{2a_0}{kT} v \right]. \quad (3)$$

where $v = v(t,x)$ is the average number of Brownian particles at position x at time t , $a_0 = a/N_0^2$, $b_0 = b/N_0$, and N_0 is Avogadro’s number. Note that the generalized coefficient \tilde{D}_{AB} recovers the Einstein coefficient D_{AB} when $a = b = 0$. Expression (3) yields the following relation:

$$\frac{\partial v}{\partial t} = \left(\frac{kT}{6\pi r\eta} \frac{2b_0}{(1 - b_0 v)^3} - \frac{2a_0}{6\pi r\eta} \right) \left(\frac{\partial v}{\partial x} \right)^2 + \left(\frac{kT}{6\pi r\eta} \frac{1}{(1 - b_0 v)^2} - \frac{2a_0}{6\pi r\eta} v \right) \frac{\partial^2 v}{\partial x^2}. \quad (4)$$

which is a generalized diffusion law. A finite-element model has been built upon the relation (4), reported in details in [5].

In order to predict the release behavior of LYS from the nanochannel membrane, we simply equated the parameter b to the lysozyme molar volume. The parameter E_0 was calculated, according to its definition [x] as the potential energy of interaction exerted on the free lysozyme molecule by the bound one at minimum. In details, since the van der Waals molecular volume of lysozyme $V_{vdW,LYS} = 16651 \text{ \AA}^3$, the parameter b is equal to:

$$b = V_{vdW,LYS} \times N_0 = 0.0100 \text{ m}^3. \quad (5)$$

The value of the interaction parameter a is then calculated as [2]:

$$a = -\frac{E_0 V N_A^2}{2N_m} = 6.95 \times 10^3 \text{ Pa m}^6. \quad (6)$$

in which the number N_m of interacting molecule is 2 (i.e., the bound and free LYS molecules), and the total volume is set equal to the volume of the simulation cell: $V = 4.91 \times 10^{-25} \text{ m}^3$. The value of E_0 is obtained from the minimum of the potential energy of interaction curve between the free and bound LYS molecules, as explained above: $E_{\min} = -93.93 \text{ kJ/mol}$, as $E_0 = E_{\min}/N_0 = -1.56 \times 10^{-19} \text{ J/molecule}$.

The release profile obtained for lysozyme by our model is shown in Figure 4, together with the experimental data, and the profile corresponding to a Fickian release. From Figure 4 it is evident how the diffusion profile estimated by Fick law is completely different from the experimental one. Further, in the same figure it is also reported the diffusion profile of the model obtained by selecting the parameters a and b which better fit, according to a mean square error policy, the experimental data. It is worth noting that the release curve obtained from the model using the a and b parameters obtained from our molecular modeling approach works even better than the model derived from the data fitting for a period covering the first 20-25 days (actually our model fits almost perfectly the experimental data for the first 20 days). Moreover, the last part of the experimental curve is not completely reliable, due to the offset caused by the experimental errors which cumulate day after day; this could explain the discrepancies between the proposed model and the data after day 25.

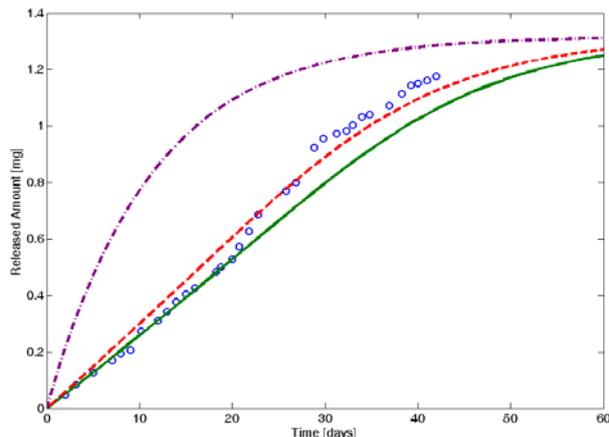


Figure 4. LYS mass flux through a 13 nm pore height membrane: experimental data (○), Fick's law prediction (---), model based simulation with parameters derived by fitting (---), and model based simulation with parameters derived by molecular dynamics simulation (continuous line).

Conclusions

In conclusion, in this work we have formulated a multiscale simulation approach to the biomacromolecule diffusion in nanochannels, based on computer simulations spanning from the atomic world to the continuum dimension. Long regarded as a purely mathematical subject, molecular multiscale modeling can now be considered as most relevant for its physical significance. Molecular engineering is indeed entering a new era, characterized by an unprecedented control over chemical reaction, as well as product molecular architecture, conformations and morphology. Molecular multiscale modeling thus provides a sort of unifying set of principles to understand and interpret the behavior of seemingly different systems on common grounds. In other terms, it provides a common language that enables practitioners of molecular engineering to approach problems in areas as diverse as, for instance, chemical sensing, microfluidics, genetics, and last but not least, nanomechanical microdevices such as that experimentally employed in this study. Therefore the main contribution of this paper is that of establishing a sort of *ab-initio* methodology which allows predicting the kinetics of biomolecular diffusion through membrane nanochannels without requiring, in principle, expensive laboratory experiments.

References

1. Q. Wei, C. Bechinger, P. Leiderer, Single-File Diffusion of Colloids in One-Dimensional Channels, *Science* Vol. 287 (2000) p.625.
2. F. Martin, R. Walczak, A. Boiarski, M. Cohen, T. West, C. Cosentino and M. Ferrari: *J. Control. Release* Vol. 102 (2005), p.123.
3. S. Pricl, M. Ferrone, M. Fermeglia, F. Amato, C. Cosentino, M.M.C. Cheng, R. Walczak and M. Ferrari: *Nanoletters* submitted (2006).
4. D.A Case, D.A. Pearlman, J.W. Caldwell, et al.: *AMBER 7* (2002), University of California, San Francisco, CA, USA.
5. G. Peskir: *Stoch. Models* Vol. 19 (2003), p. 383.
6. F. Amato, C Cosentino, S Pricl, M. Ferrone, M. Fermeglia, M.M.C. Cheng, R. Walczak, and M Ferrari: *Nanoletters* submitted (2006).
7. *Simulink user's guide: Dynamic system simulation for MATLAB* (The MathWorks, Inc., Natick, MA, USA, 1997).
8. B. Lee and F.M. Richards: *J. Mol. Biol.* Vol. 55 (1971), p.379.