Blocking of an ion channel by a highly charged drug: Modeling the effects of applied voltage, electrolyte concentration, and drug concentration

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We present a simple physical model to estimate the blocked pore probability of an ion channel that can be blocked by a highly charged drug in solution. The model is inspired by recent experimental work on the blocking of the PA_{63} channel, involved in the anthrax toxin infection, by a highly charged drug [Karginov *et al.* PNAS **102**, 15075 (2005)]. The drug binding to the pore is highly specific but the strong dependence of blocking on the applied voltage and electrolyte concentration suggests that long range electrostatic interactions are important. Since basic electrostatic concepts rather than detailed molecular models are considered, the microscopic details of the channel blocking are ignored, although the model captures most of the qualitative characteristics of the problem.

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I. INTRODUCTION

Ion channels allow selective transport across cell membranes [1]. Recent theoretical studies have addressed the origin of the channel selectivity and blocking using molecular simulations [1–3]. These simulations provide atomistic level pictures that can relate structural information to observed phenomena, but they usually involve relatively short time scales. On the other hand, low-resolution approaches based on continuum and kinetic models [1,4–6] introduce severe simplifications concerning structural aspects and channel states and transitions but provide useful information on channel ionic selectivity since electrophysiological experiments are conducted over long times compared to those on an atomic scale. Atomic simulations give results coherent with classical concepts traditionally used by membrane electrophysiologists in a number of cases [1].

We present a simple model to estimate the blocked pore probability of an ion channel that can be blocked by a highly charged drug in a solution. The model is inspired by recent experimental work on the blocking of the PA_{63} channel, involved in the anthrax toxin infection, by a highly charged drug (Karginov *et al.*, Ref. [7]). Although the drug binding to the pore is highly specific, the strong dependence of blocking on the applied voltage and electrolyte concentration suggests that long range electrostatic interactions are important. Basic electrostatic concepts rather than detailed molecular models are considered. Therefore, the (high-resolution) aspects of the complementary, high affinity docking [7] of the drug to the pore blockage sites are disregarded. Likewise, we consider only the drug blocking from the cis side (the side where the drug is added; it is the left side in Fig. 1 later), ignoring both the asymmetry effects associated with the pore entrance side and the voltage-dependent relief of the drug [7,8]. Finally, the complex kinetics of the channel is also ignored [7]. However, useful qualitative conclusions can still be obtained.

The model employs some ideas previously introduced in Ref. [9], although we concentrate on the electrolyte concentration effects instead of analyzing thoroughly the voltage-dependent blockage. Moreover, we study the following questions not considered in this reference: the explicit calculation of the average potential difference between the external solution and the pore (due to the charges of the pore-lining residues) in terms of the pore fixed charge concentration, the pH dependence of this concentration and the drug charge number, and, finally, the effect of the lipid charges on the drug blocking. Note also that we use a rather detailed Poisson-Boltzmann formalism to obtain explicitly the potential profile in the pore (see Figs. 1 and 2 later), in contrast to

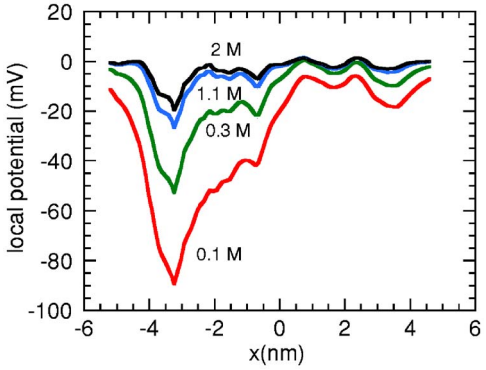

FIG. 1. (Color online) Local potential vs axial position along PA_{63} prepore for the electrolyte concentrations c_e in the curves.

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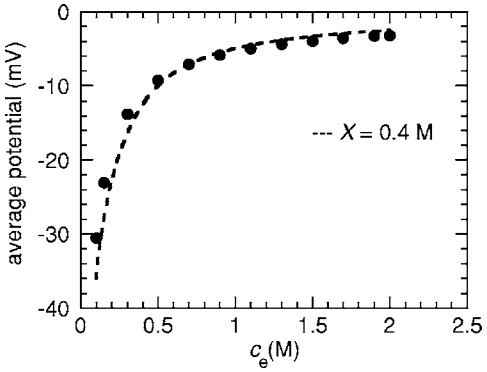

FIG. 2. Electric potential vs c_e. The points correspond to the potential averaged over the whole pore for each c_e. The curve corresponds to Eq. (3) (multiplied by $-RT/F \approx -25$ mV) for the effective concentration $X=0.4$ M of net negative charge in the pore-lining residues.

Ref. [9] where a more phenomenological site binding approach suited to the voltage-dependent blockage analyzed there was used. The Poisson-Boltzmann formalism is based on a continuum approach. Although continuum theories have been criticized for the case of narrow ion channels [10], they are useful approximate tools for larger nanometer scaled pores in which the pore radius is greater than the Debye length of the electrolyte [6,11–13]. In this context, the results to be obtained could also find application for the modeling of analyte blocking in biosensors based on nanopores [11–13].

II. THEORETICAL MODEL

We assume that the open (unblocked) and blocked pore probabilities can approximately be obtained using the single site binding equations:

$$P_{\text{open}} = \frac{1}{1 + k_e c_A}, \tag{1a}$$

$$P_{\text{blocked}} = \frac{k_e c_A}{1 + k_e c_A}, \tag{1b}$$

where $k_e(\text{M}^{-1})$ is the effective binding constant and $c_A(\text{M})$ is the blocker (drug) concentration in the external solution. This binding constant depends on the electrical potential difference between the site in the cis vestibule of the pore and the external solution,

$$k_e = k e^{z(|\phi_p| + \alpha V)}, \tag{2}$$

where k is the binding constant when this potential difference is zero, α is the "electrical distance" (in fact, a number in the range $0 \leqslant \alpha \leqslant 1$) to the blockage site, and z is the drug charge number. The potential difference between the pore and the solution has two contributions: $V \geqslant 0$ is the potential applied at the cis side where the cationic drug is added and $|\phi_p|$ is the absolute value of the equilibrium potential difference between the pore and the external solution when $V=0$. The latter potential difference is negative because the charges of the pore-lining residues are predominantly negative [7]. Note that all potentials in Eq. (2) are dimensionless (1 unit corresponds to $RT/F \approx 25$ mV at 20 °C, where R is the gas constant, T is the absolute temperature, and F is the Faraday constant). Equations (1) and (2) assume a quasiequilibrium approach for the external solution and the cis vestibule solution where the binding site is presumably located [7,8].

Within the above quasiequilibrium approach, $|\phi_p|$ can be approximated by the Donnan potential [14]:

$$|\phi_p| \approx \ln\left[\frac{X}{2c_e} + \sqrt{\left(\frac{X}{2c_e}\right)^2 + 1}\right], \tag{3}$$

where $X(\text{M})$ is the molar concentration of the net amount of negative charges of the pore-lining residues [15] and c_e is the electrolyte (e.g., KCl) concentration. Equation (3) implicitly assumes that the electrolyte ions determine the equilibrium potential difference (note that $c_e \approx 0.1-1\ \text{M} \gg 10^{-8}-10^{-6}\ \text{M} \approx c_A$ in the experiments of Ref. [7]). One could argue that the effective drug concentration in the pore might be much higher than c_A because of its high charge number. However, since the pore and drug diameters are similar [7], the binding of the drug is likely to occur shortly after pore entrance, and we cannot assume a macroscopic number of free to move (unbound) drug molecules within the pore in equilibrium with those in the external solution. Therefore, it should be the partition of a number of small, mobile electrolyte ions between the pore and the external solution that determines the value of $|\phi_p|$ seen by the drug in absence of site blocking.

Substituting Eqs. (2) and (3) into Eqs. (1a) and (1b),

$$P_{\text{open}} = \frac{1}{1 + kc_A\left\{\left[\frac{X}{2c_e} + \sqrt{\left(\frac{X}{2c_e}\right)^2 + 1}\right] e^{\alpha V}\right\}^z}, \tag{4a}$$

$$P_{\text{blocked}} = 1 - P_{\text{open}}. \tag{4b}$$

Equations (4a) and (4b) allow us to estimate the open and blocked pore probabilities as a function of the externally controlled parameters (drug concentration, electrolyte concentration, and applied voltage) if we know the drug-channel interaction parameters (drug charge number and binding constant, electrical distance, and concentration of the net charge of the pore-lining residues). Hopefully, these parameters could be extracted from high-resolution molecular simulations [2,3], although it is usually the case that first order estimations can be obtained by using approximated models [4–6]. Equations (3) and (4a) show the effect of the electrolyte concentration on the Debye screening of the pore lumen fixed charges. A decreased concentration of mobile charges leads to a higher value of the potential in Eq. (3) and, in turn, to a higher blocked pore probability. This should be expected, since lower electrolyte concentrations lead to higher Debye lengths and, therefore, to less effective Debye screening (in the sense that larger distances are now needed to electrostatically screen the pore fixed charges).

Since this model aims to rationalize general qualitative results rather than study quantitatively a particular experimental case, we estimate the characteristic parameters of the problem as follows. As a first approximation to the inserted

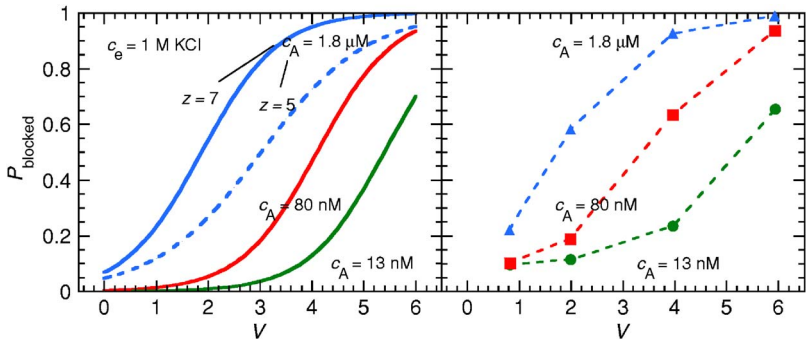

FIG. 3. (Color online) Left figure: theoretical P_{blocked} vs V (1 unit corresponds to $RT/F \approx 25$ mV) curves for $c_e=1$ M and the three drug concentrations c_A in the curves. The values of the characteristic parameters in Eqs. (4a) and (4b) are given in the text. All curves were obtained with $z=7$ (and also with $z=5$ for $c_A=1.8$ μM). Right figure: experimental data from Ref. [7].

channel, the pdb file of the PA_{63} prepore (code 1TZO) was downloaded from the protein data bank repository [16,17]. Hydrogens were added to the file and the full structure was rotated to make the x axis coincide with the symmetry axis of the heptamer (in the new orientation, Lys24 had negative coordinates and Arg107 had positive coordinates). We then employed the program University of Houston Brownian Dynamics (UHBD) [18] for computing the electrostatic potential around the protein at pH=7 and different electrolyte concentrations using the linearized Poisson-Boltzmann equation with an ion exclusion layer of 2.5 Å. The initial grid spacing was set to 2.5 Å, with two further focusing steps of spacing 1.0 and 0.5 Å, respectively. The potential was subsequently averaged over the pore cross section by resolving the channel length in slices 1 Å thick. This procedure gives us an estimation of the average potential at each axial position as well as the local surface area of the pore lumen.

Some arguments for the applicability of the Poisson-Boltzmann model are in order. First, the pore is relatively wide through most of its axial length (the PA_{63} prepore internal diameter is 20–35 Å; see [7] as well as Refs. [11] [25] and [26] therein). Second, the minimum diameter above only spans a few angstrom across the pore while the major part of the channel is significantly wider. Finally, this minimum value is of the same order of magnitude of the highest Debye length obtained for the lowest experimental concentration. Therefore, the average potential difference between the external solution and the pore could be approximately calculated using a continuum treatment, at least for qualitative purposes. These treatments have proven to be useful also for synthetic nanometer-scale pores [11–13].

The electric potential vs the axial position along the pore is shown in Fig. 1 for several electrolyte concentrations c_e. There is a clear potential well at approximately $\alpha=0.2$ from the cis side whose axial position does not change with c_e (note also other secondary potential wells close to the trans side). This presumed site is much closer to the cis than to the trans side, according to the fact that most blockers are more effective from the cis side [7,8] (the vestibule of the channel on the cis side is reported to contain the negatively charged residues involved in the binding of several compounds [19]). Although the above value of α may seem reasonable, we emphasize that the model calculations critically depend on this parameter in the exponential of Eqs. (4a) and (4b). Clearly, the depth of the potential well changes with the electrolyte concentration. From the data in Fig. 1, we compute a second average to obtain the (concentration dependent) mean potential characteristic of the pore. This electric potential averaged over the whole pore is shown in Fig. 2 (circles) as a function of c_e. We assume that this should be the potential influencing the drug entry from the external solution into the pore. The theoretical points (circles) of Fig. 2 are then compared with the curve for the quasiequilibrium potential of Eq. (3) to obtain the approximate value $X=0.4$ M for the molar concentration of the net amount of negative charges in the pore-lining residues.

The use of the linearized Poisson-Boltzmann equation requires some justification. We have obtained also the potential over the whole pore using the full, nonlinearized Poisson-Boltzmann equation [20] (data not shown) and found the same qualitative trends as in Fig. 2, probably because of both the relatively wide pore considered as well as the fact that our approximated treatment involves a potential averaged first over the pore cross section (Fig. 1) and second over the pore axis (Fig. 2) for each electrolyte concentration c_e. This average potential is lower than RT/F=25 mV for $c_e>0.2$ M, which covers most of the concentration range in Fig. 2. Therefore, the results in Fig. 2 are essentially valid for most of the experimental conditions, being consistent with the low-resolution approach used here.

III. RESULTS AND DISCUSSION

In most of the calculations, we take $z=7$ (the charge number of the drug in Ref. [7]), $X=0.4$ M [from the comparison between the averaged Poisson-Boltzmann potential and Eq. (3); see Fig. 2], and $k=10^4$ M^{-1} (the dissociation constants for tetraalkylammonium ions reported in Ref. [9] are in the range 3.7–1600 μM, which gives association constants in the range $0.6\times10^3-3\times10^5$ M^{-1}). Note that this binding constant is the only parameter that has not been estimated independently in the model.

Figure 3 shows the P_{blocked} vs V curves for $c_e=1$ M and three values of c_A. For small applied potentials, the blocked pore probability increases almost linearly with V, the higher the drug concentration the higher the slope. For high enough V, however, the sigmoidal character of the curves becomes evident except for low c_A where the drug concentration at the pore entrance can still be rate limiting, and the s-shaped curve is not fully developed. The curves were obtained with $z=7$, although the charge number $z=5$ is also considered for $c_A=1.8$ μM to emphasize the importance of the drug charge number on the blocking process (note that z appears in the exponent of Eqs. (4a) and (4b)). The theoretical results in

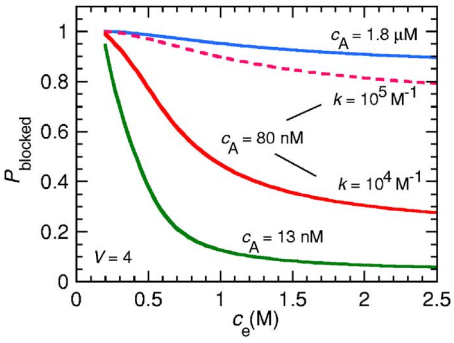

FIG. 4. (Color online) P_{blocked} vs c_e curves for $V=4$ and the same values of c_A as in Fig. 3. The curves were obtained with $k=10^4$ M^{-1} (and also with $k=10^5$ M^{-1} for $c_A=80$ nM).

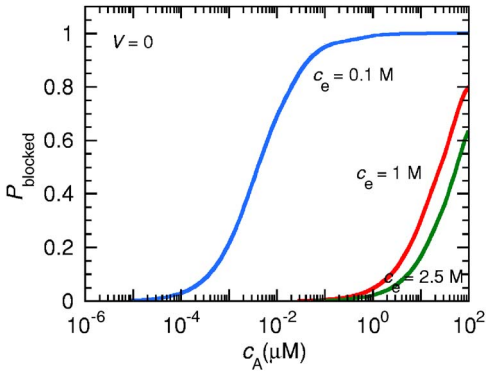

FIG. 5. (Color online) P_{blocked} vs c_A curves for $V=0$ and three values of c_e.

Fig. 3 qualitatively reproduce the experimental trends in Fig. 3(A) of Ref. [7] (also shown in Fig. 3 here). However, we believe that the limited number of microscopic characteristics included in the model precludes its use for quantitative purposes and have therefore not attempted any fitting of the experimental data (see also Fig. 6 later).

Figure 4 considers the effect of electrolyte concentration on the electrostatic pore blocking by the drug. The P_{blocked} vs c_e curves are calculated for $V=4$ (100 mV, approximately) and three values of c_A. The influence of the Debye screening on the blocking is evident [see Eq. (4a)], especially in the case of low drug concentrations. All curves were obtained with the specific binding constant $k=10^4$ M^{-1}, although the constant $k=10^5$ M^{-1} is also considered for $c_A=80$ nM to emphasize that increasing the drug-pore specific interaction can partly compensate for the decreased blocking observed at high electrolyte concentration. The involvement of electrostatic interactions in the blocking is strongly suggested by Figs. 3(B), 3(C), and 4(B) of Ref. [7] where the experimental dependence of blockage parameters on electrolyte concentration is shown. The influence of the solution electrolyte concentration on these interactions has also been noted for other positively charged ligands [15].

Figure 5 shows the P_{blocked} vs c_A curves for $V=0$ and three values [7] of c_e. The curves can be considered as the theoretical binding isotherms [see Eq. (1b)] of the drug to the channel and constitute a measure of the fraction of normalized conductance that is blocked [9]. The marked influence of the electrolyte concentration on the binding shows the importance of electrostatics on the pore blocking. This effect is clearly seen in Fig. 6 where the P_{open} vs c_A curves for $V=0.8$ (20 mV, approximately) are shown for the three values of c_e in Fig. 5. For small drug concentrations, the open pore probability is close to unity (unblocked pore), except for $c_e=0.1$ M where the blocking of the pore is noticeable even at relatively low values of c_A, a fact of physiological relevance [7]. For high enough drug concentrations, P_{open} decreases to zero following sigmoidal curves. However, this decrease is significantly delayed at high electrolyte concentrations due to the electrolyte screening effects on the pore blocking. Fig. 4(B) of Ref. [7] shows the experimental normalized conductance of the channel as a function of c_A parametrically in c_e. Since this dimensionless conductance should be proportional to P_{open}, we can compare the experimental data of Fig. 4(B) of Ref. [7] (also shown in Fig. 6 here) with the theoretical results. Clearly, there are some quantitative discrepancies between the theoretical and the experimental values of the inhibitory drug concentration and, in particular, between the $c_e=1$ M experimental and theoretical curves. This could indicate that the intuitive ideas considered here cannot give a quantitative description of the problem [note in particular that the potential in Eq. (3) is given by the net charge of the whole pore rather than by the local charge distribution at the presumed binding site]. However, it is remarkable that the model calculations reproduce some of the observed phenomena using only a reduced number of basic concepts [see Eqs. (4a) and (4b)].

The lipid charge is also mentioned in Ref. [7] as one of the factors influencing strongly the drug binding and pore blocking, although no experimental data are shown. A direct

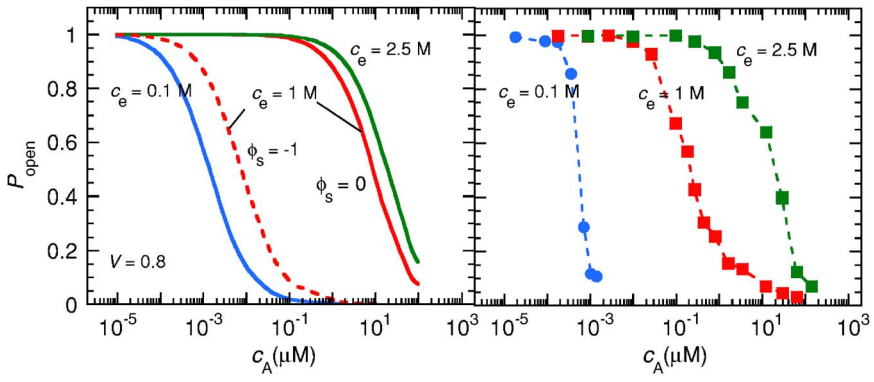

FIG. 6. (Color online) Left figure: theoretical P_{open} vs c_A curves for $V=0.8$ and three values of c_e. The curves were obtained with $\phi_s=0$ (and also with $\phi_s=-1$ for $c_e=1$ M). Right figure: experimental data from Ref. [7].

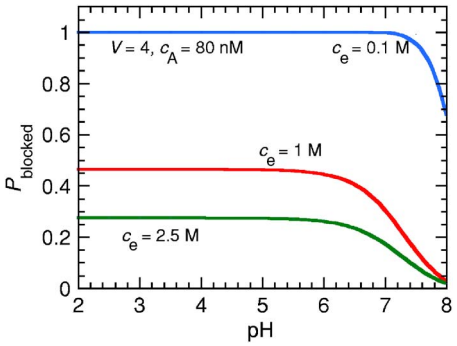

FIG. 7. (Color online) P_{blocked} vs pH curves for $V=4$, $c_A=80$ nM, and three values of c_e. The drug charge number z changes with pH (see text).

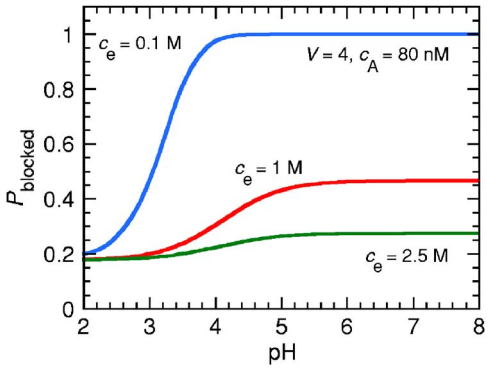

FIG. 8. (Color online) P_{blocked} vs pH curves for $V=4$, $c_A=80$ nM, and three values of c_e. The concentration of fixed negative charge X changes with pH (see text).

extension of the model suggests that the (highly charged) cationic drug concentration at the pore entrance could be significantly increased with respect to the bulk concentration c_A (and therefore the blocking effect enhanced) if the lipids are negatively charged. In this case, the model must incorporate the effect of the surface potential ϕ_s created by the surface charge density σ of the lipids on the effective drug concentration at the pore entrance [21,22]. To show the effect of the lipid charges, we rather arbitrarily substitute $c_A\mathbf{e}^{-z\phi_s}$ for c_A in Eq. (2) and ignore any change in the potential of Eq. (3) as a first approximation. The resulting curve for the cases $\phi_s=0$ and $\phi_s=-1$ (−25 mV, approximately) with $c_e=1$ M is shown in Fig. 6. The pore blocking is clearly enhanced in the latter case because of the local increase in the concentration of the charged drug close to the lipid surface [21,22]. This increase can partly compensate for the diffusion limited rate at which the drug can get from solution to the channel entrance [8,23]. Therefore, when using charged lipids, the electrolyte concentration can modulate both the drug interaction with the pore lumen charges [see Eq. (3) and Fig. 2] and the drug interaction with the lipid charges (potential ϕ_s depends on c_e), which offers additional possibilities to tune the binding in the case of highly charged drugs.

Finally, since the charge groups in the channel and the drug have different dissociation equilibria, experiments conducted over a range of pH values should also be useful to identify the electrostatic effects involved in the blocking. In principle, the dissociation equilibria equations could be used to calculate X and z (and also σ if the lipids were charged) at each pH. Figure 7 shows the P_{blocked} vs pH curves for $V=4$ and three values of c_e. The curves were obtained with $c_A=80$ nM, $X=0.4$ M and the charge number dissociation equilibrium $z=7/(1+10^{\text{pH}-\text{pK}_a})$ with $\text{pK}_a=8$. Figure 8 shows also these curves for the same c_A and c_e values of Fig. 7, but now we take $z=7$ and assume the highly idealized dissociation equilibrium $X=0.4/(1+10^{\text{pK}_a-\text{pH}})$ for the concentration of net negative charge in the pore lumen, with $\text{pK}_a=4$. Since the electrical charges modulate the interaction between the drug and the channel, pore blocking should very sensitive to high and low pH values, although it is likely that these values cause also structural changes in the channel.

IV. CONCLUSIONS

A simple continuum model to study the blocking of an ion channel by a highly charged drug in solution has been presented and applied to the case of the PA_{63} prepore, involved in the anthrax toxin infection [Karginov *et al.*, *PNAS* (**102**, 15075, 2005)]. In this case, the strong dependence of blocking on the applied voltage and electrolyte concentration suggests that long range electrostatic interactions are important. Qualitative results useful for the understanding of the pore blocked probability are obtained as a function of the applied voltage, drug charge and concentration, pH, and electrolyte solution concentration. Although the details of the channel blocking are clearly much more complicated [7] and a high resolution, molecular approach of the drug binding would be desirable for quantitative purposes, the present low-resolution description appears to capture some significant characteristics of the problem. Moreover, the results obtained could also find application in the modeling of analyte blocking in biosensors based on nanopores.

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