Effective charge and free energy of DNA inside an ion channel

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(Received 21 July 2006; revised manuscript received 3 November 2006; published 15 February 2007)

Translocation of a single stranded DNA (ssDNA) through an α-hemolysin channel in a lipid membrane driven by applied transmembrane voltage V was extensively studied recently. While the bare charge of the ssDNA piece inside the channel is approximately 12 (in units of electron charge) measurements of different effective charges resulted in values between one and two. We explain these challenging observations by a large self-energy of a charge in the narrow water filled gap between ssDNA and channel walls, related to large difference between dielectric constants of water and lipid, and calculate effective charges of ssDNA. We start from the most fundamental stall charge q_s, which determines the force $F_s=q_sV/L$ stalling DNA against the voltage V (L is the length of the channel). We show that the stall charge q_s is proportional to the ion current blocked by DNA, which is small due to the self-energy barrier. Large voltage V reduces the capture barrier which DNA molecule should overcome in order to enter the channel by $|q_c|V$, where q_c is the effective capture charge. We expressed it through the stall charge q_s. We also relate the stall charge q_s to two other effective charges measured for ssDNA with a hairpin in the back end: the charge q_u responsible for reduction of the barrier for unzipping of the hairpin and the charge q_e responsible for DNA escape in the direction of hairpin against the voltage. At small V we explain reduction of the capture barrier with the salt concentration.

DOI: 10.1103/PhysRevE.75.021906 PACS number(s): 87.15.Tt, 82.39.Jn, 87.14.Gg, 87.16.Uv

I. INTRODUCTION

A DNA molecule in a water solution carries negative charges. With the help of applied voltage, it can translocate through a wide enough ion channel located in a lipid membrane [1–9] or through a solid state nanopore in a semiconductor film [10–12]. An intensively studied example is the translocation of a single stranded DNA (ssDNA) molecule through an α-hemolysin (α-HL) channel [1–9]. With the average internal diameter $\sim$1.7 nm the channel is wide enough for ssDNA molecules, but is too narrow for a double helix. To be specific below we always talk about the experimental data for this system. Our theory is also applicable to a double helix DNA translocating through a narrow nanopore [10–12], but there is less quantitative data for this case.

In order to study the translocation experimentally, the electric current through the channel is observed under voltage V, applied between two vessels of salty water on both sides of the membrane. Due to large conductivity of the bulk solution practically all the voltage drops on the membrane. When a ssDNA is added to the negative voltage side it is dragged into the channel by the voltage (Fig. 1). When the ssDNA molecule is in the channel as shown in Fig. 1 the ion current is blocked, and the blocked current I_b is much smaller than the open pore current I_0 without DNA in it,

$$I_b \simeq 0.1 I_0. \tag{1}$$

Translocation events in a single channel can be studied by monitoring the current. It was argued recently that the steric mechanism of strong current blockage is amplified by the increase electrostatic self-energy of an ion in water passage narrowed by DNA [9] because DNA and lipids have much smaller dielectric constants than water and, therefore, the electric field lines of an ion in the space between DNA and the lipid are squeezed in the channel increasing the ion self-energy. This idea was borrowed from the physics of narrow ion channels without DNA [13].

Besides the blocked current, one can measure the time between two successive translocation events, τ, or the capture rate $R_c=1/\tau$ of DNA molecules into the channel. It is natural to compare the observed value of R_c with the diffusion limited rate R_D of ssDNA capture. This comparison shows that $R_c \ll R_D$. For instance [4], the typical R_c is in the range of $0.01-10\ s^{-1}$ at applied voltage 50–200 mV and ssDNA concentration 0.9 μM, while $R_D \sim 100\ s^{-1}$. The ratio R_c/R_D may be as small as 10^{-6} if one extrapolates the experimental data to $V=0$. So there must be a large barrier $\sim 14 k_B T$ for ssDNA capture. We return to the nature of this barrier in the end of this introduction, but first we concentrate on the challenging question of the voltage effect on this barrier.

The capture rate at zero voltage $R_c(0)$ is so small that all experiments are actually done with a large applied voltage $V=50-200$ mV. The voltage pulls DNA into the channel and reduces the barrier for DNA capture. It was found [1,3,4] that the capture rate is

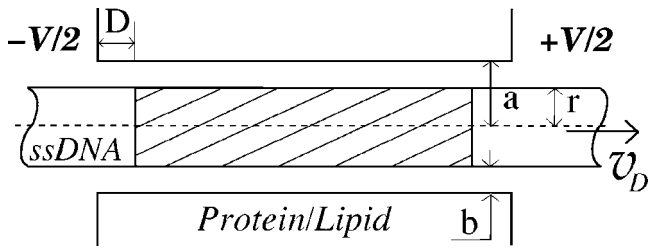

FIG. 1. The side view of the membrane and the channel with captured DNA. All DNA phosphates in the shaded part are neutralized by K^+ ions. The contact layer on each end has the length D. The arrow shows the direction of DNA motion.

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$$R_c(V) = R_c(0)\exp(-q_c V/k_B T), \tag{2}$$

where $q_c=-1.9e$ is an effective "capture" charge, and e is the proton charge. Apparently for ssDNA in α-HL channel $|q_c|$ is much smaller than the absolute value of the total DNA charge in the channel $-N_0 e \simeq -12e$ and this is why large voltage $V \gg k_B T/e$ is necessary [2] in order to make the capture rate observable.

Why is the capture charge of DNA so small and what does it depend upon? How is the capture charge related to the stall charge defining the stall force F_s, which one should apply to DNA occupying the whole length of the channel (Fig. 1) to stall it against the voltage V? (This, for example, can be done with the help of laser tweezers [11,14].) In other words, $F_s = -F_p$, where F_p is the force, with which the voltage V pulls the stalled DNA. We write F_s as

$$F_s = -q_s E, \tag{3}$$

where the electric field $E=-V/L$ and q_s is the stall effective charge. The charge q_s seem to be the simplest and the most fundamental effective charge one can introduce for DNA. Is it different from q_c? If yes, which one is larger? How are these two charges related to unzipping and escape charges which describe ssDNA with a hairpin at the end (see definitions below)?

Inspired by all these challenges in this paper we use for DNA the simplest model of a rigid cylinder charged by the pointlike surface charges (phosphates located on the spiralling backbone) and moving coaxially through a cylindrical tunnel filled by salty water (Fig. 1). The model of rigid cylinder should be good for a double helix DNA in a narrow cylindrical semiconductor pore. For ssDNA such model gets some support from the known tendency of ssDNA stuck to its bases [15] in a bulk solution. It is natural to expect that this tendency is enhanced inside the channel. One may say that the case of ssDNA in α-HL channel pushes our model too close to the molecular limit. Nevertheless, we will show that our results for effective charges are in a reasonable agreement with experiment. We believe that this happens because these results are practically model independent.

The plan of this paper is as follows. In Sec. II we discuss our model and the electrostatics of the channel. We concentrate on the role of the enhanced self-energy of a salt ion in the narrow water-filled space between DNA and internal walls. First, we argue that DNA in the channel is almost perfectly neutralized and, second, salt cations are bound to DNA charges. Then we introduce narrow charged contact layers near the end of DNA and qualitatively explain the electrostatic mechanism of the current blockage.

In Sec. III we calculate the stall force F_s and the stall effective charge q_s. Our main result is that q_s is proportional to the ratio of currents in blocked and opened channels

$$q_s \simeq -eN_0 \frac{I_b}{I_0}. \tag{4}$$

Using Eq. (1) we get that for ssDNA in α-HL channel stall charge $q_s \sim -1e$. Intuitively this is clear because if the blocked current were exactly zero this would mean that counter ions are stuck on DNA and, therefore, compensate the DNA charge, so that the net pulling charge would vanish.

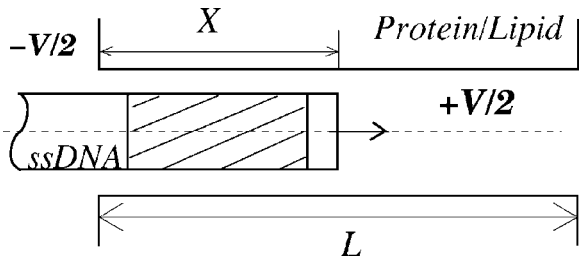

FIG. 2. The side view of a ssDNA molecule entering the channel.

In Secs. IV and V we show that the stall charge is the fundamental charge so that all other charges can be expressed in terms of it. In Sec. IV in order to find the capture charge q_c we calculate the pulling force $F_p(X)$ and the stalling force $F_s(X)=-F_p(X)$ for the partial penetration of ssDNA into the channel to the depth $X<L$ (see Fig. 2). Then the correction to the capture barrier is calculated as a work which the electric field $E=-V/X$ does slowly pulling DNA into the whole channel. In order to obtain the voltage correction $-q_c V$ to the minimum work necessary to overcome the capture barrier we integrate the pulling force over X. The resulting capture charge q_c is larger than the stall charge q_s. The reason is that the self-energy barrier becomes smaller for a shorter channel.

In Sec. V we discuss effective charges, which have to do with release rate of ssDNA, when it is trapped in the channel due to a hairpin in the back end (see Fig. 3). There are two ways for such a DNA molecule to leave the channel: DNA can get unzipped by pulling electric field and leave to the right or DNA can escape against the pulling force of the electric field to the left. The former route dominates at large voltages, while the latter one dominates at smaller ones.

It was found [5,6] that the unzipping rate exponentially grows with the voltage as

$$R_u(V) = R_u(0)\exp(|q_u|V/k_B T). \tag{5}$$

We show that the "unzipping" effective charge $q_u = q_s(M/N_0)$, where M is the number of base pairs in the

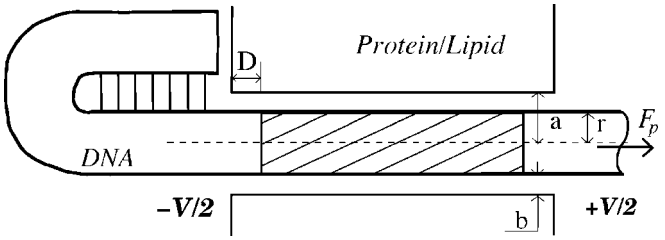

FIG. 3. Unzipping of the DNA hairpin with the help of the voltage induced pulling force F_p results in DNA translocation through the channel to the right. Alternatively DNA can escape against electric field to the left. The hairpin is shown schematically with the bound base pairs presented by short straight lines. There are M base pairs in the hairpin, N_0 bases in the channel, and K bases in the tail to the right of the channel.

hairpin. For use in Ref. [6] $M \simeq 10$ and $N_0 \simeq 12$, Eq. (5) gives $q_u \approx q_s \approx -1e$ in agreement with Refs. [5,6].

The rate of alternative escape against the voltage should exponentially decrease with V,

$$R_e(V) = R_e(0)\exp(-q_e V/k_B T). \quad (6)$$

Here q_e is the fourth effective charge, which we call the "escape" charge. We show in Sec. V that $q_e = |q_s|(K/N_0)$, where K is number of bases in the ssDNA tail on the right-hand side of the membrane when escape begins.

In Sec. VI we are concerned with the nature of the capture rate barrier. Reduction of the conformation entropy due to confinement of the DNA piece in the channel was suggested as a natural explanation for the capture rate barrier [7]. Indeed, ssDNA molecules in the bulk solution are rather flexible, with the persistence length about $p \simeq 1.4$ nm [16] at 1 M KCl. The channel length $L \simeq 5$ nm, so it holds $N_p = L/p \simeq 3.5$ persistence lengths of ssDNA during translocation, and their undulations are restricted by the channel. The large entropic barrier due to this effect is $N_p k_B T \Delta s$, where Δs is the loss of entropy for one persistence length in the channel. Using $\Delta s \sim 2$ we get $\sim 7k_B T$ for this barrier.

An additional entropy loss comes from free tails of DNA outside the channel [17]. When one end of the DNA chain is anchored onto the wall, the Gaussian chain has the free energy $\frac{1}{2}\ln M_p$, where M_p is the length of the tail in persistence lengths. The ssDNA molecules used in experiments [1,4] are relatively short ($\leq$40 bases), and two tails can give only a barrier $\sim 1-2k_B T$. All the losses of conformation entropy together can explain a substantial part of the estimated barrier $\sim 14k_B T$ extrapolated to zero voltage at salt concentration 1 M KCl.

However, they cannot explain the observed dependence of the capture rate on the salt concentration c. Indeed, the persistence length of ssDNA decreases when c increases [16], making the conformation barrier larger, while in the experiment the capture rate grows with c [9]. So there must be another kind of barrier with the opposite c dependence.

In Sec. VI we suggest a mechanism for such a barrier. We argue that when a DNA molecule enters the channel, the screening cloud is squeezed in the narrow water-filled space surrounding the DNA. Due to this compression the total free energy of DNA and ions is higher for DNA in the channel than for DNA in the bulk. This barrier decreases with c because entropy of screening atmosphere in the bulk solution decreases. Using even more simplified model of DNA as uniformly charge cylinder and the Poisson-Boltzmann approximation we show that this barrier is in qualitative agreement with the observed dependence R_c on the salt concentration c.

In Sec. VII we conclude with the summary of our results.

II. NEUTRALIZATION OF DNA IN THE CHANNEL AND THE CONTACT POTENTIAL

We assume the ssDNA molecule is a rigid cylinder coaxial with the channel. The inner radius of the α-HL channel is $a \simeq 0.85$ nm, and the radius of the ssDNA molecule is $r \simeq 0.5$ nm (Fig. 1). Salt ions are located in the water-filled space between them, with thickness $b \simeq 0.35$ nm. The length of the channel is $L \simeq 5$ nm. Such a model is even more appropriate for double helix DNA in a wider (say 4 nm in diameter) solid state nanopore [10,12].

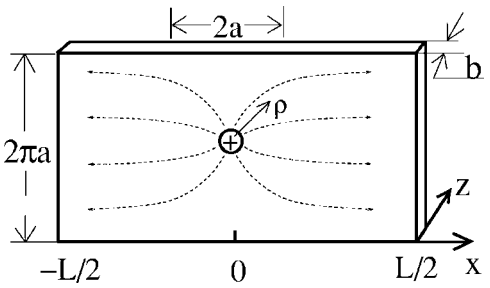

FIG. 4. An unfolded view of the water-filled space containing an extra K^+ ion. One side of the water-filled space between ssDNA and the channel walls is cut by a radial half-plane starting from the channel axis and the cut is unfolded to make the water filled space flat. Dashed lines represent the electric field lines of the charge. At $\rho < a$ this electric field spreads in all directions and becomes uniform far from the charge.

The dielectric constant of the channel and the ssDNA molecule ($\kappa' \sim 2$) is much smaller than that of water ($\kappa \simeq 80$). So if ssDNA is neutralized by cations and there is an extra charge e at the point x located in the thin water-filled space between the channel internal wall, the electric field lines starting from this charge are squeezed in the thin layer (Fig. 4). This results in a high self-energy $U(x)$ of the charge [13,18].

In order to calculate $U(x)$ we write $U(x) = e\phi(x)/2$, where $\phi(x)$ is the electrostatic potential created by the extra charge at position x. If ρ is the distance from the charge e, electric field is two dimensional at $\rho < a$ (see Fig. 4), and becomes uniform at larger distance $\rho > a$. Our numerical calculation in the limit of infinite ratio κ/κ', when all electric lines stay in the channel and at $a/b \gg 1$ can be well approximated by the following expression:

$$U(x) = U_1(x) + U_2(x) = \frac{e^2}{\kappa b}\left[\frac{L}{4a}\left(1 - \frac{4x^2}{L^2}\right) + \ln\frac{a}{b}\right]. \quad (7)$$

The origin of the two terms in Eq. (7) is illustrated in Fig. 4 for $x=0$. At $b < \rho < a$ the electric field of the central charge gradually spreads over all azimuthal angles in the whole water-filled space decaying as $E = 2e/\kappa\rho b$. This leads to the two-dimensional potential $\phi(x) = (2e/\kappa b)\ln(a/b)$ and produces the constant term $U_2(x)$ in Eq. (7). [Equation (7) works when the charge is not too close to the channel ends, $L/2 - |x| > a$. However, when the charge is at channel ends Eq. (7) is no longer valid. Instead the electric field lines are attracted to the bulk solution, and $U_2(x)$ vanishes.] On the other hand, $U_1(0)$ is created by the one-dimensional uniform electric field at distances $\rho > a$, which according to Gauss' theorem is $E_0 = e/\kappa ab$. For $|x| > 0$ the electric field at the closer end is stronger than that of the other end, therefore $U_1(x)$ decreases parabolically with $|x|$, and vanishes at the channel ends [19]. For $L=5$ nm, $a=0.85$ nm, and $b=0.35$ nm, Eq. (7) gives $U_1(0) = 2.9k_B T$ and $U_2(0) = 1.7k_B T$

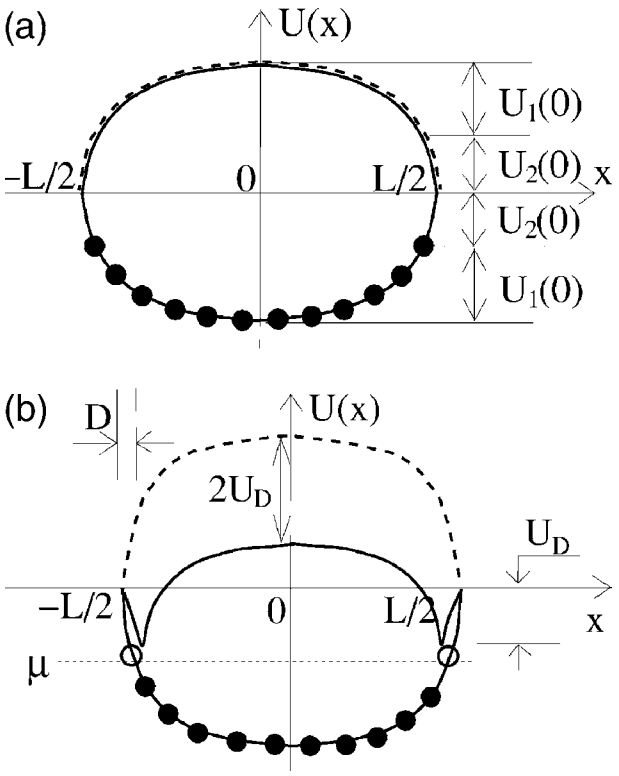

FIG. 5. Energy band diagram for K^+ ions (solid lines) and Cl^- ions (dashed lines). The lower band represents the energy of the cations bound to DNA phosphates (the self-energy necessary to create a vacancy with sign minus). The empty upper bands show the self-energy of the extra salt cation (solid line) and anions (dashed line) entering the channel. (a) In the absence of the contact layers ($c > c_D$). (b) With contact layers of the width D creating contact electrostatic potential $-U_D$. Vacant phosphates are shown by empty circles. The chemical potential μ of K^+ ions in the system is shown by the thin dotted line.

where T is the room temperature. The total barrier $U(x)$ of an extra K^+ or Cl^- ion is shown in Fig. 5 by the upper thick line [20].

Now recall that there are K^+ ions bound to ssDNA phosphates in the channel. Each of them can be removed to the bulk solution creating a vacancy. The energy penalty for this process is close to the penalty for placing an extra ion in the same place. Thus, energies of bound K^+ ions are $-U_1(x) - U_2$ and can be shown by the lower full curve of Fig. 5 as a reflection of the upper one with respect of the x axis. Vacancies must overcome the barrier $U(0)$ to cross the channel. Using an analogy with semiconductors, we can say that extra K^+ ions play the role of electrons in the conduction band, while vacancies play the role of holes in the valence band. The peculiar result of electric field confinement in the water-filled space is that the energy gap $2[U_1(x)+U_2]$ has the maximum at $x=0$ (Fig. 5). In the above discussion we ignored entropy effects [18,19], which in principle can reduce self-energy barriers [21].

The most important conclusion from the above discussion is that the large self-energy of extra charges deep inside the channel leads to very accurate neutralization of DNA by salt cations. Such nearly perfect neutralization was observed in computer simulations [22] of the channel.

When salt concentration in the bulk solution c is smaller than the characteristic concentration of K cations c_D in DNA occupied channel, some K cations close to the channel ends can escape to the bulk in order to enjoy larger entropy in the solution. As a result there are negative phosphate charges in the layer of width D at each end, and the screening (positive) charge in the adjacent layers of the bulk solution. These double layers (capacitors) of the width D (see Fig. 1) produce the contact potential $-U_D$, where

$$U_D = k_B T \ln(c_D/c) \tag{8}$$

in the channel, and prevent remaining K cations from leaving the channel. The contact potential appears because negative charges in the channel are immobile (belong to practically static DNA). In this sense this contact potential is similar to Donnan potential appearing on membranes permeable only for one sign of ions. This contact potential is also similar to the contact potential at the junction between a p-type doped and an intrinsic semiconductor.

The contact potential moves down energies of both bands of extra cations and vacancies, while bending these bands up in the very ends [Fig. 5(b)]. On the other hand, the energy band of an extra anion [shown in Fig. 5(b) by the dashed line] is moved up by the contact potential. [Without contact potential this band coincided with the energy band of an extra cation as shown in Fig. 5(a).] This leads to the total exclusion of anions from the channel. Such exclusion was also noticed in Ref. [22].

At $c > c_D$ both additional cations and anions freely enter the channel in equal number from the bulk in order to equilibrate their concentration. This does not lead to the contact potential because this process does leave behind layers of fixed charges. At $c > c_D$ ion current through the channel should be due to both extra salt cations and anions and, therefore, should be proportional to salt concentration c. On the other hand, at $c < c_D$ the transport is due to cations only and current is roughly independent on c, because U_D grows with decreasing c and the contact potential $-U_D$ reduces the barrier $U(0)$ (compensating for decreasing c). One can say that at small c transport is only due to cations neutralizing DNA. For example, one of them residing near the right end of the channel may go through the channel to the left end, while another cation from the left solution replaces it.

These ideas provide reasonable interpretation for the experimental data [9] for the blocked ion $I_b(c)$ as a function of salt concentration c. It was found that $I_b(c) \propto c$ at $c \geq 1$ M, while at $c < 1$ M the current $I_b(c)$ weakly depends on c. We interpret this data as evidence that $c_D \sim 1$ M. [See more about the experimental data for $I_b(c)$ and their explanation in Ref. [9].] We are not trying here to estimate c_D microscopically because this would require dealing with ion sizes.

III. EFFECTIVE CHARGE OF THE STALL FORCE

The effective charge q_s is defined by Eq. (3) for the force F_s necessary to stall ssDNA, when the ssDNA occupies the whole channel (see Fig. 1). We show below that the stall charge q_s is proportional to the blocked current I_b. Let us

concentrate on the case $c<c_D$, when anions (Cl^-) are excluded from the channel and all blocked ion current is due to cations.

Let us assume that external electric field, $E=-V/L$, is applied in the direction opposite to the x axis. It generates a force $-N_0eE$ on N_0 ssDNA charges in the channel moving DNA along the x axis. The opposite force N_0eE acts on the cations. If the average drift velocity of DNA is v_D, and the average drift velocity of cations inside the channel is v_c we can write two momentum balance equations of steady state viscous motion for v_c and v_D,

$$N_0eE = k_c v_c + k_{cD}(v_c - v_D), \tag{9}$$

$$-N_0eE + F_a = k_D v_D + k_{cD}(v_D - v_c). \tag{10}$$

Here F_a is the additional nonelectrostatic force applied to ssDNA along $-x$ (in the direction opposite to DNA motion), k_c and k_{cD} are friction coefficients of N_0 cations with the channel walls and with DNA, respectively, while k_D is the friction coefficient of DNA with the channel walls.

Although water is not included in these equations explicitly, it is water viscosity that provides the transfer of momentum between moving DNA and cations and from them to the walls. If DNA is long (compared to N_0), the friction force of free ends with the water is also included in k_D. Note that voltage drops on the membrane, so that electric field acts only on N_0 phosphates and N_0 cations.

Equations (9) and (10) give nontrivial predictions. First, when $F_a=0$, addition of Eqs. (9) and (10) gives

$$v_D = -k_c v_c / k_D. \tag{11}$$

So the average drift velocity of ssDNA is proportional to average drift velocity of cations. In experiment both v_c and v_D are small, so that the linear in v_c and v_D approximation is justified. Formally, Eq. (11) looks like a drag of DNA by cations. Actually, this is an antidrag, because the coefficient in Eq. (11) is negative. The reason for the antidrag is simple: if k_c or v_c were equal to zero, the N_0 cations would transfer all momentum they receive from the electric field to the DNA molecule. Then DNA would not move at all. The transfer of negative cations momentum to walls makes the net force applied to DNA positive. Thus, DNA moves in x direction only when cations move in the $-x$ direction.

Second, when $F_a=F_s$, by the definition the applied force stalls the ssDNA setting $v_D=0$. Adding Eqs. (9) and (10) at $v_D=0$ we find the stalling force

$$F_s = k_c v_c, \tag{12}$$

where strictly speaking v_c is the average drift velocity of cations at $v_D=0$. Actually in all experiments $v_D \ll v_c$, which means k_D is very large. Thus, one can use v_c from experiments where $F_a=0$. This allows us to express v_c through blocked current I_b, which may be written as

$$I_b = n e v_c, \tag{13}$$

where $n=N_0/L$ is the linear density of cations in the channel. Both I_b and v_c are small because of electrostatic barrier for ion motion in the blocked channel. Indeed, most of the time all cations are bound to DNA phosphates. Only rarely a cation goes through the middle of the channel contributing both into the current and the drift velocity v_c.

Combining Eqs. (12), (3), and (13) we arrive at

$$q_s = -k_c I_b / neE. \tag{14}$$

We can exclude E from Eq. (14) using the equation for the current of the open channel

$$I_0 = n_0 e v_c^0 = n_0 e^2 E / 6\pi\eta R. \tag{15}$$

Here $v_c^0 = eE/6\pi\eta R$ is the drift velocity of a cation in the open channel, $n_0=2c\pi a^2$ is a number of cations and anions per unit length of the open channel (we assume that they have the same radius R and use Stokes formula for viscous resistance force because $R \ll a$). Combining Eqs. (14) and (15) we get

$$q_s = -\frac{k_c e}{6\pi\eta R}\frac{n_0 I_b}{n I_0}. \tag{16}$$

The last step is to calculate k_c. When the cation moves in the blocked channel it transfers the force eE half to the wall and half to the DNA. We write the force on the wall as $6\pi\eta R v_c f(R/b)$. If the cation size is small enough $R \ll b$ the Stokes formula is applicable and $f(R/b)=1/2$. If the cation is large so that $b-2R \ll b$ one arrives at the asymptotic estimate $f(R/b)=\beta \ln \frac{2R}{b-2R}$, where according to Ref. [23] the coefficient $\beta=8/5$. Here we illustrate the origin of this logarithmic expression by the following simple derivation leading to slightly different $\beta=3/2$. Let us locally approximate both the DNA surface and internal walls of the channel as static parallel plane walls at $z=0$ and $z=b$ (see Fig. 4). Let us assume that the center of the ball is moving in the plane $z=b/2$ with velocity v_c. When the ball is at $x=y=0$ and $z$axis is a polar axis of the ball let us cut the spherical surface of the ball by a big number of coaxial cylinders with the axis z and radiuses ρ in the range $0<\rho<R$. We arrive at a number of rings on the surface of the ball. Let us estimate the force to the external wall as a sum over close to the rings. A distance of the ring with the radius $\rho \ll R$ to the wall is $h(\rho)=(b/2)-R+\rho^2/2R$, the local gradient of velocity is $\sim v_c/h$, the effective area of the ring is $2\pi\rho d\rho$ and the total momentum transfer rate (force) is

$$2\pi\eta\int_0^R \frac{v_c \rho d\rho}{h(\rho)} \simeq 4\pi v_c \eta R \int_{b/2-R}^R \frac{d\rho}{\rho} = 4\pi v_c \eta R \ln\frac{2R}{b-2R}. \tag{17}$$

This leads to $f(R/b)\simeq\beta\ln\frac{2R}{b-2R}$ with $\beta=2/3$ for a cation in the middle plane $z=b/2$. For a cation closer to the external wall at finite $b/2<z<b-R$ we get $\ln\frac{2R}{b-R-z}$ instead of $\ln\frac{2R}{b-2R}$. Because dependence on z is only logarithmic, averaging of f over the cation positions z makes a negligible change to β. Assuming that all N_0 cations move with average drift velocity v_c we find that the total force cations exerted on the wall is $6\pi N_0 \eta R v_c f(R/b)$. This gives $k_c = 6\pi N_0 \eta R f(R/b)$ and

$$q_s = -eN_0 \frac{n_0 I_b}{n I_0} f(R/b). \tag{18}$$

For ssDNA in α-HL channel using $\beta=5/8$ and a rude estimate $\ln \frac{2R}{b-2R} \sim 1.6$ we arrive at $f(R/b) \simeq 1$. The ratio $n_0/n \sim 1$ at the typical salt concentration $c=1$ M. Thus, we arrive at Eq. (4) and $q_s \simeq -1e$.

This may look surprising because the net DNA charges of the DNA contact layers near the two channel ends together are definitely larger than e. These charges, however, do not move with DNA and do not contribute to the pulling DNA force and to the effective charge q_s of the stall force.

Up to now we dealt with the case of relatively small concentrations of salt, $c < c_D$, when linear concentration of cations n inside the blocked channel is fixed and anions are excluded from the channel. One can show that at $c > c_D$ when current is due to both cations and anions in Eq. (18), one should drop the ratio n_0/n.

We are not aware of any direct measurement of q_s, for ssDNA in α-HL channel. In the next two sections we show how one can express the capture charge q_c, the unzipping charge q_u and the escape charge q_e through the stall charge q_s.

IV. EFFECTIVE CHARGE OF THE CAPTURE RATE

In the preceding section we focused on the stall force F_s or pulling force $F_p=-F_s$ in the situation, when the ssDNA already occupies the whole channel (Fig. 1). In order to calculate the effect of the applied voltage on the capture rate, we turn to the pulling force $F_p(X)$ in the situation, when the ssDNA penetrates only a fraction of the channel X/L (Fig. 2). The length of DNA in the channel X changes from 0 to L while DNA enters the channel, and the work done by the force pulling ssDNA is

$$-q_c V = \int_0^L F_p(X) dX. \tag{19}$$

This work reduces the free energy barrier of the capture rate (see Fig. 6). Below we assume that all voltage drops on the blocked part of the channel, because the unblocked part of the channel is so wide that its resistance is much smaller than that of the blocked part. Then, similarly to Eq. (14), the force $F_p(X)$ can be expressed through the blocked current of the partially blocked state $I_b(X)$,

$$F_p(X) = -\frac{k_c I_b(X)}{ne}. \tag{20}$$

The force $F_p(X)$ is larger than $F_p(L)$, because a shorter blocked channel leads to a larger current $|I_b(X)| > |I_b(L)|$ at a given voltage V. The main reason is the smaller electrostatic barrier for the traversing cations.

Combining Eqs. (19), (20), and (14) we arrive at a simple result

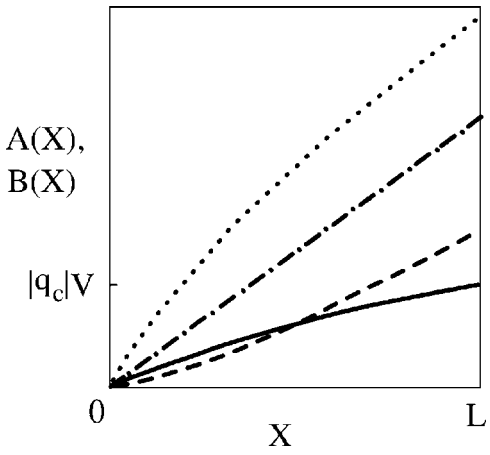

FIG. 6. Schematic plots of several free energy barriers $B(X)$ for the partial (to the length X) DNA capture. The barrier for DNA capture without electric field is nearly linear (dashed-dotted line). Also shown are the barriers $B(X)$ for the direction in which V helps to capture DNA (dashed line) and for the direction in which V hinders the capture (dotted line). The work $A(X)$ done by the applied potential V is shown by the solid line.

$$\frac{q_c}{q_s} = \frac{1}{L} \int_0^L \frac{I_b(X)}{I_b(L)} dX. \tag{21}$$

In order to calculate this ratio for ssDNA in α-HL channel we use the experimental data [2] for the blocked current $I_b(N)$ as a function of the total DNA length (in bases), when it is shorter than $N_0=12$, namely $4 \le N \le 12$. We assume that $I_b(N)$ can be used for $I_b(X)$ in Eq. (21). In this way we obtain $q_c/q_s \approx 2.5 \pm 1.0$ somewhat larger than the experimental value $q_c/q_s \approx 1.9e/(1.1e) = 1.7$. We emphasize that in agreement with our arguments both theoretical and experimental values are substantially larger than e.

In Fig. 6 we illustrate the difference between capture barriers without a voltage, along the voltage pulling force and against this force.

V. VOLTAGE ENHANCED HAIRPIN UNZIPPING AND ESCAPE AGAINST THE VOLTAGE

In this section we deviate from the capture rate theory and discuss relationship between unzipping and escape effective charges q_u and q_e defined in the Introduction and the fundamental stall charge q_s. The charge q_u determines the voltage dependence of the release rate of ssDNA, when it is trapped in the channel by an intentionally designed double helix DNA hairpin at the end [see Eq. (5)]. Double helix DNA of the hairpin is too thick to go through the α-HL channel. Thus, unzipping of the hairpin is necessary in order to release DNA from the channel to the right (Fig. 3).

In experiment [6] ssDNA was first inserted into the α-HL channel and then kept in by a relatively low voltage. The voltage was increased to the large value V at time t_0 and probability that DNA is still in the channel at the time t_0+t was measured. This probability behaves as $\exp(-R_u t)$ defining the rate of unzipping R_u in Eq. (5).

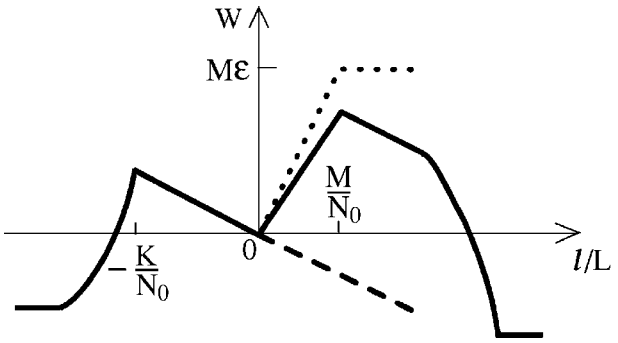

FIG. 7. Free energy W of ssDNA with a hairpin as a function of displacement l of DNA (solid line). Positive l correspond to unzipping and release to the right, and for this case l is defined as the length of DNA passing by the right end of the channel starting from original configuration of Fig. 3. Negative l corresponds to the DNA escape in the direction of hairpin, for this case $-l$ is defined as the length of DNA passing by the left end of the channel (Fig. 3). The dotted line is the energy of broken base pairs, the dashed line is the energy $W=-F_p l=q_s V l/L$ of the voltage induced pulling force. Relative height of the barriers for unzipping and escape apparently depends on the magnitude of the voltage V.

The rate R_u was found [6] to grow with the voltage according to Eq. (5). This equation defines unzipping charge q_u. We would like to show that $q_u=q_s(M/N_0)$, where M is the number of base pairs in the hairpin. (In the experiment [6] $M=7,9,10$.)

Indeed, unzipping would raise DNA eventually to the barrier $M\varepsilon$, where ε is energy of one base pair. This happens when DNA moves along the x axis by M bases or by the length $l_{\max}=ML/N_0$. Corresponding potential energy is shown in Fig. 7 by dotted line as a function of displacement $l>0$ of the DNA. The electric field reduces the barrier. After unzipping and moving $2M$ hairpin bases through the channel, DNA slides down the capture barrier (see Fig. 6) and eventually reaches a constant energy plateau in the bulk of the right solution (Fig. 7).

In order to evaluate the correction to the barrier we should assume that DNA moves very slowly and calculate the work of the pulling force $F_p=-q_s(V/L)$, along the displacement $l_{\max}=ML/N_0$. This gives the correction to the barrier $F_s l_{\max}=|q_s|(M/N)V=|q_u|V$, and, therefore

$$q_u = q_s \frac{M}{N_0}. \tag{22}$$

We estimated above that $q_s\sim -1e$. In experiments [6] $M/N_0\sim 1$ and we get $q_u\simeq -1e$. This is close to $q_u\simeq -1.1e$ found in Ref. [6].

Our derivation is valid when the unzipping barrier corrected by voltage V is still much larger than k_BT. In this case even with applied force, unzipping randomly alternates with zipping, while the saddle point corresponding to the totally unzipped hairpin is being reached in equilibrium way. To our mind, this condition is satisfied in the original experiment [6] and therefore, agreement of our theory with its results can be expected [24,25]. More complicated problem of unzipping of several hairpins of RNA was discussed recently [26].

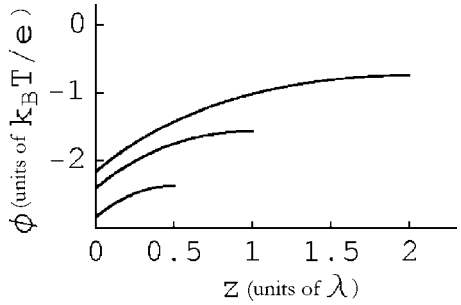

FIG. 8. The potential ϕ as a function of z for $b/\lambda=0.5$, 1, and 2 (from bottom to top), at ion concentration $ce^2/(l_B\sigma^2)=1$. Here the Gouy-Chapman length $\lambda=k_BT\kappa/(2\pi\sigma e)$ is the unit of distance.

Let us switch to the escape effective charge. Suppose DNA with a hairpin is brought to the channel by a pulling voltage (Fig. 3). Let us assume that the single stranded part of DNA has N_0+K bases, so that K bases have already arrived to the bulk solution opposite to the hairpin. Let us keep DNA in the channel by an applied voltage V, which is so small that the unzipping rate is smaller than the rate of escape in the direction opposite to the pulling force. The rate of such alternative escape should behave according to Eq. (6) and dominate at small enough voltages. We want to show that the escape charge $q_e=|q_s|(K/N_0)$. The free energy profile W for the escape process (negative l) is shown in Fig. 7 together with the unzipping process (positive l). The pulling force preventing the escape is $F_p=|q_s|V/L$. DNA is climbing up against this force until all the DNA fits in the channel (no tail in the bulk solution opposite to hairpin). Starting from this point DNA slides down the capture barrier before reaching the energy plateau, when all DNA arrives to the left solution (see Fig. 6). Therefore, displacement at which F_p works is $l^*_{\max}=-LK/N_0$ and the escape barrier is $|l^*_{\max}|F_p=|q_s|(K/N_0)V$ or

$$q_e = |q_s|\frac{K}{N_0}. \tag{23}$$

The only measurement of q_e we know was done in Ref. [8]. It resulted in roughly speaking 4 times larger value of $|q_s|$ than we estimated here, but this result should be taken with caution, because surprisingly in this experiment I_b has unconventional sign. We hope that measurements of q_e will be repeated.

VI. ELECTROSTATIC FREE ENERGY BARRIER FOR DNA CAPTURE AT LOW VOLTAGES

In this section we return to the capture rate at a small voltage V and concentrate on the difference between free energies of screening atmospheres of a rodlike DNA molecule in the channel and in the bulk solution, contributing into the capture rate barrier. We deal with the neutral channel, for which self-energy plays no role. Therefore, in the first approximation one can neglect discreteness of charges, assuming the DNA surface charges are uniformly smeared

and the distribution of ions obeys Poisson-Boltzmann equation. To simplify calculation we look on unfolded DNA surface as a uniformly charged plane at $z=0$ and the unfolded inner channel wall as another neutral plane at $z=b$ (Fig. 4). We calculate the free energy price to push the neutral plane from infinity to the distance b compressing the screening cloud. It is easy to verify that the price grows as the distance between the planes b decreases. The fact that the DNA surface is cylindrical is not important when the wall separation b is smaller than the DNA radius r. We label the charge density of DNA surface as $-\sigma$, the mean field electric potential as ϕ.

To find the free energy of the system we need to study $\phi(z)$ for a given wall separation b. Far from the channel ends ϕ depends only on z, and the Poisson-Boltzmann equation for such a system for $0<z<b$ is

$$\frac{d^2\phi}{dz^2} = \frac{8\pi ec}{\kappa}\sinh\left(\frac{e\phi}{k_BT}\right), \tag{24}$$

It should be solved with the boundary conditions which follows from the overall neutrality and the absence of charges at $z<0$ and $z>b$,

$$\left.\frac{d\phi}{dz}\right|_{z=b} = 0, \qquad \left.\frac{d\phi}{dz}\right|_{z=0} = \frac{4\pi\sigma}{\kappa}. \tag{25}$$

The integration of Eq. (24) gives

$$\frac{d\phi}{dz} = \sqrt{\frac{16\pi k_BTc}{\kappa}\cosh\left(\frac{e\phi}{k_BT}\right) + A}. \tag{26}$$

With the help of the second boundary condition (25) we can write A in the form $A=\left(\frac{4\pi\sigma}{\kappa}\right)^2 - \frac{16\pi k_BTc}{\kappa}\cosh\left(\frac{e\phi(0)}{k_BT}\right)$, and calculate numerically

$$z(\phi) = \int_{\phi(0)}^{\phi} \frac{d\psi}{\sqrt{\frac{16\pi k_BTc}{\kappa}\left[\cosh\left(\frac{e\psi}{k_BT}\right) - \cosh\left(\frac{e\phi(0)}{k_BT}\right)\right] + \left(\frac{4\pi\sigma}{\kappa}\right)^2}}. \tag{27}$$

Numerical inversion of Eq. (27) gives $\phi(z)$. Then the only remaining parameter, the constant $\phi(0)$ in the integral Eq. (27) is determined using the first boundary condition (25). In this way we can find the potential $\phi(z)$ for any given wall separation b. A few examples are shown in Fig. 8.

We emphasize that the results obtained so far are valid for the neutralized main part of the ssDNA in the channel. They are not applicable to the DNA charges near the end of the channel, which lose their counter ions to the bulk solution forming two contact layers. It is the contact potential $-U_D$ that makes the potential $\phi(z)$ in the neutral part of the channel negative even at $z=b$ (see Fig. 8). This is not surprising, because through Eq. (26) the potential $\phi(z)$ is related to c, which is in turn directly related to U_D by Eq. (8).

The total free energy of the system per unit area of DNA surface can be calculated as

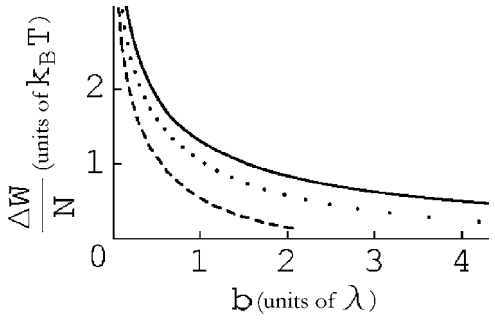

FIG. 9. Free energy of the capture barrier per one screened DNA charge in the channel as a function of b (in units of λ) for $c=0$ (solid line), $ce^2/(l_B\sigma^2)=0.1$ (dotted line), and $ce^2/(l_B\sigma^2)=1$ (dashed line).

$$\frac{W}{\text{area}} = \frac{-\sigma}{2}\phi(0) + \sum_{\pm}\int_0^b \left(\frac{\pm e}{2}\phi(z) - k_BT\ln\frac{c}{c_{\pm}}\right)c_{\pm}dz. \tag{28}$$

The Boltzmann distribution

$$c_{\pm} = c\exp\left(-\frac{\pm e\phi(z)}{k_BT}\right) \tag{29}$$

reduces Eq. (28) to

$$\frac{W}{\text{area}} = c\int_0^b \sinh\left(\frac{e\phi(z)}{k_BT}\right)e\phi(z)dz - \frac{\sigma}{2}\phi(0). \tag{30}$$

One can find the free energy at any given b and plot it choosing $b=\infty$ as the reference point (Fig. 9).

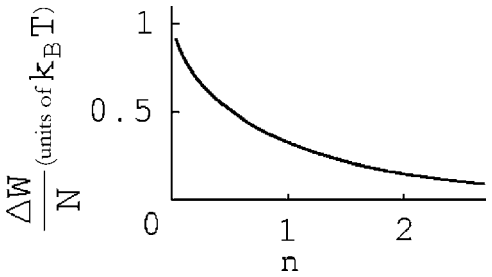

FIG. 10. Free energy barrier per one of screened DNA charge in the channel as a function of the dimensionless salt concentration $n=ce^2/(l_B\sigma^2)$, for the wall separation $b=1.4\lambda$.

For ssDNA threading through α-HL channel with $\sigma = Ne/[\pi(r+a)L]$, the Gouy-Chapman length $\lambda = \frac{k_B T \kappa}{2\pi\sigma e}$, the salt concentration c in units of $l_B\sigma^2/e^2$, and free energy per area in units of $k_BT\sigma/e$. Here $l_B = \frac{e^2}{\kappa k_B T}$ is the Bjerrum length (for water at the room temperature $l_B = 0.7$ nm).

For the system of ssDNA threading through α-HL channel, the Gouy-Chapman length λ is about 0.25 nm, while the wall separation is about 0.35 nm. The free energy barrier as a function of salt concentration in this example is shown by the solid line in Fig. 10.

We see that for the salt concentration $c=1$ M or $n = ce^2/(l_B\sigma^2) = 1.37$, the barrier is about $\Delta W = 0.24 k_B T N \simeq 2.4 k_B T$. Here $N = N_0 - 2N_D$ is number screened DNA phosphates in the channel and N_D is number of phosphates in each contact layer. We estimated that $N_D \sim 1$ and therefore, $N = 12 - 2 = 10$ in the range of salt concentrations between 0.25 M and $c=1$ M. For smaller salt concentration $c = 0.5$ M and $c=0.25$ M, the barriers are $4.3 k_B T$ and $6.0 k_B T$. The smaller c the larger barrier. This barrier being added to conformation entropy barrier of ssDNA discussed in the Introduction may invert dependence of the total barrier on the salt concentration and explain the observed growth of the capture rate with the salt concentration.

VII. CONCLUSION

In this paper we evaluate effective charges of DNA responsible for the pulling and stall forces, for the voltage affected capture rate, as well as for voltage induced unzipping ssDNA with a hairpin and for its escape against the pulling force of the electric field. The stall charge q_s is the most fundamental one, measurements of the other three charges can be used to evaluate it.

The main result of this paper is the linear equation connecting the stall charge with the blocked ion current I_b. In the simplest form of Eq. (18) it is applicable only for relatively small concentration of salt, $c < c_D$, when blocked current is due to neutralizing cations only and, therefore, roughly speaking is salt concentration independent. This equation is based only on momentum conservation, does not depend on the mechanism of the ion current blockage or specific model of DNA and, therefore, has a high degree of universality.

We also find a new kind of the barrier for DNA capture, which can explain the puzzling growth of the low voltage capture rate $R_c(0)$ with the salt concentration c. We show that such a barrier results from squeezing of the screening cloud of DNA when DNA enters the channel.

The focus of this paper is on ion channels or nanopores barely permitting DNA translocation. Our theory can be applied to ssDNA translocating through a solid state nanopore with diameter comparable to α-hemolysin [12]. It should also work for a double helix DNA in a solid state nanopore, which diameter $2a \leq 3$ nm only slightly exceeds the diameter of DNA (2 nm). For a wider pore with $2a \geq 4$ nm the self-energy electrostatic barrier vanishes and one can use purely hydrodynamic theory of the stall charge [11,27].

ACKNOWLEDGMENTS

The authors are grateful to R. Bundschuh, A. Yu. Grosberg, A. Kamenev, S. G. Lemay, A. Meller, Y. Rabin, and A. M. Tsvelik for useful discussions. One of the authors (B. S.) is also grateful for hospitality to KITP and ACP, where this paper was finished.

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pens with characteristic length $\xi=6.8a$. ssDNA occupies roughly speaking 40% of the channel cross-section area. This leads to 20% smaller effective radius of the water filled channel and correspondingly to somewhat stronger leakage and smaller $\xi\sim 5.5a$. Leakage of electric field reduces the electrostatic barrier [18] by the factor $(2\xi/L)\tanh(L/2\xi)\simeq 0.88$. Below we neglect difference between this factor and unity and ignore all the leakage effects.

[21] The entropy effect is, however, much weaker than in the one-dimensional case studied in Refs. [18,19]. As we see in Sec. II in the α-HL channel blocked by ssDNA the self-energy has a local logarithmic contribution $U_2(0)$ of the two-dimensional origin. It leads to additional small factors in the important dimensionless constants α and γ in Refs. [18,19], reduces them to small enough values, and as a result the electrostatic barrier survives. For wider nanopores with diameter $a\sim 4$ nm blocked by a double helix DNA [12] with the diameter 2 nm, the width of the water-filled space between DNA and channel wall $b=1$ nm is larger than l_B. In this case, $U_2(0)$ is not important and at $c=1$ M the electrostatic barrier should vanish [18,19]. Indeed, the blocked ion current $I_b=0.63I_0$ is not too far from the fraction of the blocked cross section.

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