

Direct Observation of Transitions between Surface-Dominated and Bulk Diffusion Regimes in Nanochannels

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The diffusion of charged proteins in liquid-filled nanometer-sized apertures with charged surfaces has been investigated with fluorescence correlation spectroscopy (FCS). Based on a two-dimensional (2D) multicomponent diffusion model, key parameters such as the number of molecules diffusing freely inside the nanochannel or interacting with the surfaces, together with the specific diffusion parameters, could be extracted. Different regimes of diffusion have been observed and described by a model, which takes into account the steric exclusion, the reversible surface adsorption of the biomolecules, and the exclusion-enrichment effect that is due to the charge of the proteins and the ionic strength of the solution. Conditions where the diffusion of proteins through nanoconfined spaces can be of the same magnitude as in the bulk were both predicted and experimentally verified.

Recent technological advances in nanofluidics have contributed to new insights in the understanding of fluid transport in (and close to) nanometer-sized objects with at least one characteristic dimension of <100 nm.¹ This research in nanofluidics is particularly well-suited for the detection, identification, and dynamic study of single molecules under various conditions. Nanofluidics phenomena² such as electrokinetic effects that are due to the electrical double layer (EDL), the induced redistribution of the electrical potential including the associated surface charge density³ and surface conductance,⁴ and the exclusion-enrichment effect⁵ are some of the well-known properties that affect the particular diffusion of biomolecules in highly confined spaces. During the 1980s, the steric exclusion was observed by measuring the hindered transport of neutral spheres through pores, giving a deeper understanding of the effects of solute concentration on partitioning and transport coefficients.^{6–8} An even stronger decrease of the effective diffusion coefficient of proteins diffusing

through nanochannels, because of interactions with surfaces, has been recently reported.^{9–12} However, accurate models of the concentration partitioning and of the diffusion reduction under such conditions are still missing.

In this study, we supply a theoretical model that describes the concentration of proteins inside nanoconfinements, taking into account the steric exclusion, the presence of reversibly adsorbed molecules on surfaces, and the exclusion-enrichment effect. Based on single molecule detection experiments by fluorescence correlation spectroscopy (FCS), we demonstrate the different diffusion regimes governed by the solution characteristics (ionic strength, pH, charge and concentration of proteins, etc.) and provide evidence for our proposed diffusion model. Therefore, this study presents a new step for a deeper understanding of the transport of charged molecules in nanofluidics.

The experimental setup is depicted in Figure 1a; the nanochannel device used in the experiment consisted of two microchannels cross-linked by a thin nanochannel (with the following dimensions: height, $h = 50$ nm; width, $w = 10$ μ m; length, $l = 30$ μ m). Solutions that contain fluorescently labeled proteins are injected in both microchannels and measurements are performed as soon as the system is at equilibrium, meaning that all channels are completely filled. External negative pressure applied to the outlet ports allow a precise fine-tuning of the flows in each microchannel, as well as in the nanochannel.

Theory. Effective Concentration in a Nanochannel. For solutes that are large enough to behave as hydrodynamic particles, the constrained space of a nanochannel causes the molecular friction coefficient to exceed its maximum value and leads to steric exclusion. This type of hindered diffusion has been extensively studied with neutral spheres in cylindrical nanopores.^{8,13–16} The equilibrium partitioning of solute concentration between that

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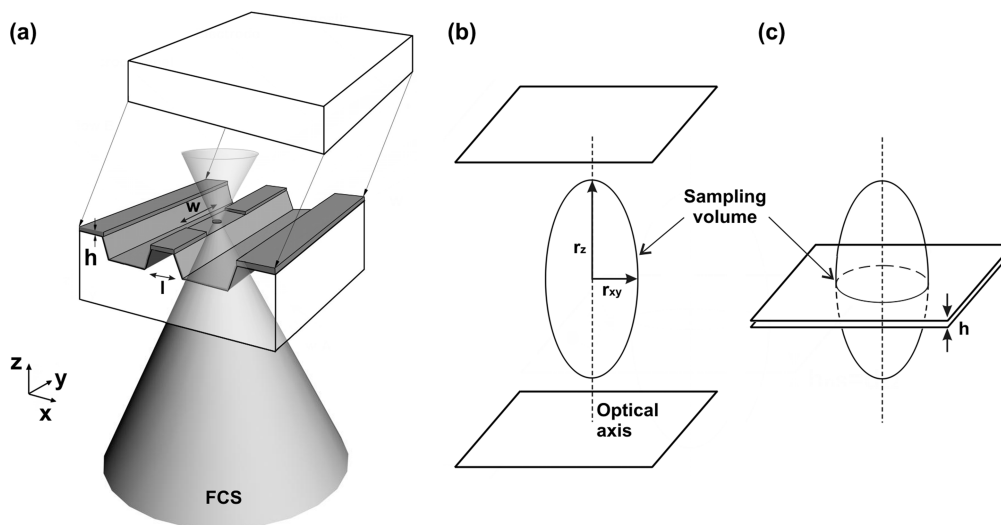


Figure 1. Nanofluidic device using fluorescence correlation spectroscopy (FCS) for the detection of proteins: (a) three-dimensional (3D) schematic of the nanofluidic system (not to scale), which is comprised of two microchannels linked by a nanochannel (a 50-nm amorphous silicon layer, sandwiched between two Pyrex wafers, defines the height of the nanochannel); (b) FCS detection volume in the case of 3D freely diffusing biomolecules; and (c) FCS detection volume of 2D confined diffusing molecules between two walls (when $h_{\text{ns}} \ll 2r_z$), such as in the case of the nanochannel. The ellipsoidal detection volume, which has a height of $2r_z$ and width of $2r_{xy}$, is approximated by a cylindrical volume of height h and radius r_{xy} in panel (c).

inside and outside a nanoslit, assuming there is no surface close to the measurement volume outside the nanochannel, is given by^{8,17}

$$\Phi = \frac{\langle c_{\text{ns}} \rangle}{c_0} = \int_0^{(1-\lambda)} \exp\left(-\frac{E(\beta)}{k_B T}\right) d\beta \quad (1)$$

where $\langle c_{\text{ns}} \rangle$ is the mean concentration of solute in the nanochannel and c_0 is the concentration of solute in the bulk. The parameter λ is defined as the ratio of the solute diameter d_s to the nanochannel height h ($\lambda = d_s/h$) and $\beta = (2z/h) - 1$ is the dimensionless z position (z is the projection axis parallel to the height). E is a potential that describes the long-range interactions between the solute and the nanochannel walls, which, here, are assumed to be dependent only on the z -position; k_B is the Boltzmann constant and T is the temperature (in Kelvin). Equation 1 indicates that, when the size of the particles is similar to the height of the channel, a decrease in the solute concentration is expected within the nanochannel.

In microfluidics and nanofluidics, the surface-to-volume ratio is high and the total concentration inside the confined space is a function of the number of molecules in the bulk and the number of molecules adsorbed on the nanochannel surfaces. The Langmuir isotherm model,¹⁸ which describes reversible adsorption, can be used to define the concentration in the nanochannel, taking into account both the molecules in the free-diffusion space (bulk) of the nanochannel and those adsorbed on the walls:

$$\langle c_{\text{ns}} \rangle = \left(1 + 2 \frac{\Gamma_0}{N_A h}\right) c_0 \Phi \quad (2)$$

where N_A is the Avogadro constant. The effective steady-state surface concentration (Γ_0) is a function of the full coverage

surface concentration Γ_{max} and is also governed by the binding and dissociation constants k_{on} and k_{off} :

$$\Gamma_0 = \left(\frac{k_{\text{on}} c_0}{k_{\text{on}} c_0 + k_{\text{off}}}\right) \Gamma_{\text{max}} \quad (3)$$

The bulk-surface partitioning cofactor (χ) is defined as

$$\chi = 1 + 2 \left(\frac{\Gamma_0}{N_A h}\right) = 1 + \left(\frac{2\Gamma_{\text{max}}}{N_A h}\right) \left(\frac{k_{\text{on}}}{k_{\text{on}} c_0 + k_{\text{off}}}\right) \quad (4)$$

To take into account the electrostatic interactions between charged molecules and charged surfaces, as well as the interactions of the solute with the electrical double layer (EDL), depending on the ionic strength of the solution c_i , a model that was given by Plecis et al.,⁵ describing the exclusion-enrichment effect, was used. This model introduces a partitioning coefficient (Φ_β), which takes into account the electrostatic effects and the interactions with the EDL but ignores the steric exclusion that is due to the solute size, as well as the adsorption effect:

$$\Phi_\beta = \frac{1}{h} \int_0^h \exp\left(-\frac{q\Psi(z)}{k_B T}\right) dz \quad (5)$$

where q is the net charge of the solute and $\psi(z)$ is the Debye–Hückel approximation for the electrical potential between two infinite planar surfaces.¹⁹

$$\psi(z) = \zeta \frac{\cosh\{(h/2) - z\}/\lambda_D}{\cosh[h/(2\lambda_D)]} \quad (6)$$

where ζ is the zeta potential and λ_D the EDL thickness that corresponds to the Debye screening length, in a 1:1 electrolyte solution:⁵

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$$\lambda_D = \sqrt{\frac{\varepsilon_0 \varepsilon_r R T}{2 F^2 c_i}} \quad (7)$$

where ε_0 is the permittivity of a vacuum, ε_r the dielectric constant of the medium, R the molar gas constant, and F the Faraday constant.

Combining these contributions (adsorption, steric exclusion, and electrostatic interactions) by combining eqs 1, 4, and 5, the equilibrium partitioning coefficient for solute concentration between that inside and outside a nanochannel Φ_{ns} can be defined as

$$\Phi_{\text{ns}} = \frac{\langle c_{\text{ns}} \rangle}{c_0} = \frac{\chi}{h} \int_0^{h(1-\lambda)} \exp\left(-\frac{q\Psi(z)}{k_B T}\right) dz \quad (8)$$

When the solute is not charged, as is the case at the isoelectrical point (pI) for proteins, the charge is zero ($q = 0$) and eq 8 is reduced to

$$\Phi_{\text{ns}} = (1 - \lambda) \left[1 + \left(\frac{2\Gamma_{\text{max}}}{N_A h} \right) \left(\frac{k_{\text{on}}}{k_{\text{on}} c_0 + k_{\text{off}}} \right) \right] \quad (9)$$

Effective Diffusion in Nanoconfinement. In nanoconfined spaces, solute molecules undergo diffusion because of Brownian motion, but they can also adsorb at the channel walls, because of the high surface-to-volume ratio of the nanoconfinement. The Stokes–Einstein equation²⁰ can be used to estimate the bulk diffusion coefficient D_{bulk} of spherical particles of radius r_s (and by extension of globular proteins) in aqueous solutions:

$$D_{\text{bulk}} = \frac{k_B T}{6\pi\eta r_s} \quad (10)$$

where η is the solvent viscosity. The diffusion time (τ_{bulk}) is related to the diffusion coefficient (D_{bulk}) by the expression²¹

$$\tau_{\text{bulk}} = \frac{r_{xy}^2}{4D_{\text{bulk}}} \quad (11)$$

where r_{xy} is the radius of the observed cylindrical volume. Proteins may also adsorb on surfaces, which can result in an increased diffusion time through a given volume. The Langmuir isotherm model gives an expression of the time of interaction τ_{surf} .^{22,23}

$$\tau_{\text{surf}} = \frac{1}{k_{\text{off}}} \quad (12)$$

The effective diffusion time (τ_{eff}) can be expressed as a weighted arithmetic mean of the diffusion times of freely

diffusing molecules in the bulk and at reversibly adsorbed molecules on the surfaces:

$$\tau_{\text{eff}} = \frac{N_{\text{bulk}}\tau_{\text{bulk}} + N_{\text{surf}}\tau_{\text{surf}}}{N_{\text{bulk}} + N_{\text{surf}}} \quad (13)$$

The effective diffusion time τ_{eff} can be determined with our FCS measurements. The effective diffusion coefficient D_{eff} is related to τ_{eff} by the expression

$$D_{\text{eff}} = \frac{r_{xy}^2}{4\tau_{\text{eff}}} \quad (14)$$

Overall, these are the dominant contributions to an effective diffusion coefficient that is strongly decreased when measuring the diffusion of proteins in nanoconfinements.^{9,12,24}

Fluorescence Correlation Spectroscopy. Fluorescence correlation spectroscopy (FCS) allows one access across the fluctuation analysis of fluorescently labeled single biomolecules static and dynamic molecular parameters such as the mean number of molecules and their diffusion behavior.^{21,25–27} The classical FCS model for a free 3D diffusion of a single molecular component (ignoring the triplet population) is given by the following relationship:²⁸

$$G(\tau) = \frac{1}{N} \left(1 - \frac{I_B}{I_{\text{tot}}} \right)^2 \frac{1}{1 + (\tau/\tau_D)} \sqrt{\frac{1}{1 + \omega^2(\tau/\tau_D)}} + 1 \quad (15)$$

where $G(\tau)$ is the autocorrelation function (ACF), N the number of molecules in the sampling volume, and τ_D the characteristic time of diffusion across the illuminated sampling volume. The factor $[1 - (I_B/I_{\text{tot}})]^2$ corrects for an uncorrelated background intensity I_B , with I_{tot} being the total intensity, including I_B .²⁹ The aspect ratio ω of the sampling volume is defined as the ratio between the height $2r_z$ and the width $2r_{xy}$ of the detection volume $\omega = r_z/r_{xy}$. In a nanochannel, the detection volume is approximated by a cylinder of small height, compared to its diameter. Consequently, $r_z \ll r_{xy}$ and ω approaches zero: the square-root term can then be neglected, leading to a 2D FCS model.²⁵ Figures 1b and 1c show the difference between the FCS detection volumes of freely 3D diffusing particles (Figure 1b) and those of confined 2D diffusing particles inside a nanochannel (Figure 1c).

Our proposed FCS model for the measurements inside the nanochannel integrates a 2D multicomponent diffusion model with two different populations of particles: biomolecules that diffuse freely through the nanochannel (N_{bulk} , τ_{bulk}) and biomolecules that interact with the channel surfaces (N_{surf} , τ_{surf}):

$$G(\tau) = \left(1 - \frac{I_B}{I_{\text{tot}}} \right)^2 \left\{ \frac{N_{\text{bulk}}}{(N_{\text{bulk}} + N_{\text{surf}})^2} \left[\frac{1}{1 + (\tau/\tau_{\text{bulk}})} \right] + \frac{N_{\text{surf}}}{(N_{\text{bulk}} + N_{\text{surf}})^2} \left[\frac{1}{1 + (\tau/\tau_{\text{surf}})^\alpha} \right] \right\} + 1 \quad (16)$$

where the coefficient α is a constant retention parameter that is dependent mainly on the geometry of the nanochannel. This

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phenomenological retention parameter takes into account the modified diffusion behavior of reversibly adsorbed particles. The best fittings of autocorrelation curves have allowed one to determine that α is an exponential term, in a similar way to the obstruction diffusion parameter, which is widely used in anomalous diffusion in biology.^{30,31} In this first model, the triplet state population has been ignored; however, it will be considered in detail in a forthcoming publication. Because of the low concentration of charged biomolecules, interactions between particles have been neglected.

EXPERIMENTAL SECTION

Device Microfabrication. Cleanroom microfabrication processes have been used to produce the fluidic chips on which the experiments have been carried out. Microchannels have been wet-etched on a 200 μm thick Pyrex wafer using standard lift-off techniques, and a 50 nm layer of amorphous silicon, used to define the height of the nanochannel was sputtered and structured by plasma etching. A second Pyrex wafer that contained access holes (drilled by sandblasting) was anodically bonded onto the first wafer. Finally, the wafers were diced into individual chips. The nanochannel had the following dimensions: height, $h = 50$ nm; width, $w = 10$ μm ; and length, $l = 30$ μm .

The solutions that contained proteins were injected in the microchannels through the inlets. The liquids were driven by air aspiration that was controlled using air-pressure regulators (Bellfram Corp., Newell, WV) from 0 to -800 mbar. For all measurements, flow conditions have been chosen where there is no flow across the nanochannel.

Measurement Instrument. The FCS experiments were performed with a ConfoCor microscope (Carl Zeiss, Jena). The laser beam size in the sample was adjusted by varying the laser beam size in front of the microscope (Objective Olympus UAp0 340; NA 1.15; 40 $\times$ water immersion). The measured spot size was $r_{xy} = 420$ nm at a wavelength of 632.8 nm (HeNe). The nanofluidic setup was placed on an xyz table with a resolution of 1 μm . The autocorrelation (ALV correlator) was transferred to a computer and was analyzed with FCS Access ConfoCor Control Program 1.2.1.1 (Zeiss) and custom-made Matlab code R2006a (MathWorks, Inc., Natick, MA).

Materials. To investigate and characterize the hindered diffusion of proteins, we used wheat germ agglutinin labeled with Alexa Fluor 633 (WGA, from Molecular Probes). WGA has a molecular mass of 38 kDa, an isoelectric point of $\text{pI} \sim 4$, and a free diffusion coefficient in water of $D_{\text{bulk}} = 7.6 (\pm 0.3) \times 10^{-11}$ m^2/s .³²

These WGA proteins have been added to potassium chloride (KCl) solutions, which have been prepared with different dilutions

(10^{-5} M to 10^{-1} M) via the addition of deionized water (18 M Ω cm). The concentration of the WGA proteins is 50 nM in all prepared solutions, which were adjusted to pH 7 via the addition of a small amount of HCl. For all experiments, no pretreatment was performed on the chip in which the solutions have been directly injected. All solutions were prepared and degassed before use at room temperature ($T = 25$ $^{\circ}\text{C}$).

RESULTS AND DISCUSSION

A series of experiments have been conducted to evaluate the number of molecules and the diffusion time of the WGA proteins at two different locations: (i) inside the reservoirs, to measure the bulk values (away from chip surfaces), and (ii) inside the nanochannel, to measure the diffusion parameters inside the channel.

After filling the microchannels, the solution entered the nanochannel by capillarity, resulting in a slow change of fluorescence intensity inside the nanochannel. All measurements have been started after the fluorescence intensity attained a steady state.

3D Diffusion of WGA. The fluorescence fluctuations of a 50 nM solution of WGA proteins placed on-chip, in one of the reservoirs, were measured with the FCS setup, and a diffusion time of $\tau_{\text{bulk}} = 0.6 (\pm 0.02)$ ms was obtained by fitting the experimental data to eq 15, because this bulk experiment corresponds to the standard 3D free diffusion of a single component. From this value of τ_{bulk} , a diffusion coefficient of $D_{\text{bulk}} = 7.35 (\pm 0.3) \times 10^{-11}$ m^2/s was obtained from eq 14 with $r_{xy} = 420$ nm, which corresponds to the radius of the detection area. This value is very similar to the diffusion coefficient of WGA in water that was reported by Munson et al.³² ($D_{\text{bulk}} = 7.6 (\pm 0.3) \times 10^{-11}$ m^2/s).

Exclusion of Proteins at Low Ionic Strength. The curve in Figure 2a shows the mean fluorescent intensity (I_{tot}) measured under different ionic conditions in the 50-nm-high nanochannel. Pyrex surfaces in contact with aqueous solutions are negatively charged, which electrostatically repulses negatively charged WGA proteins. A strong decrease in I_{tot} is observed when increasing the EDL by reducing the ionic concentration in the nanochannel, which is coherent with eq 8. In Figure 2a, eq 8 is fitted on the experimental data points with $d_s = 4$ nm, $q = -e$, and $\zeta = 30 \times 10^{-3} \log(c_i)$, as measured by Kosmulski et al.³³ For ionic concentration values that are $<10^{-4}$ M, the EDL overlap (EDLO) is observed; only the uncorrelated background intensity was measured, which corresponds to the exclusion of the proteins from the nanochannel.

2D Diffusion of WGA in the 50-nm-High Nanochannel. The autocorrelation curves obtained when WGA proteins are diffusing inside the nanochannel for different values of the ionic strength of the solution are presented in Figure 2b. Because of the fact that all experiments are performed with identical nanochannels, at a constant value of pH and using solutions of 50 nM WGA proteins, while the ionic concentration is varied, eq 16 can be used to fit each set of experimental data points, with τ_{bulk} , τ_{surf} , and α being constant parameters. The values of $\tau_{\text{bulk}} = 0.6 (\pm 0.02)$ ms was measured independently, as presented in a previous paragraph, and values of $\tau_{\text{surf}} = 10 (\pm 2)$ ms and $\alpha = 0.45 \pm 0.1$ were obtained from a fitting of all FCS measurements inside the nanochannel.

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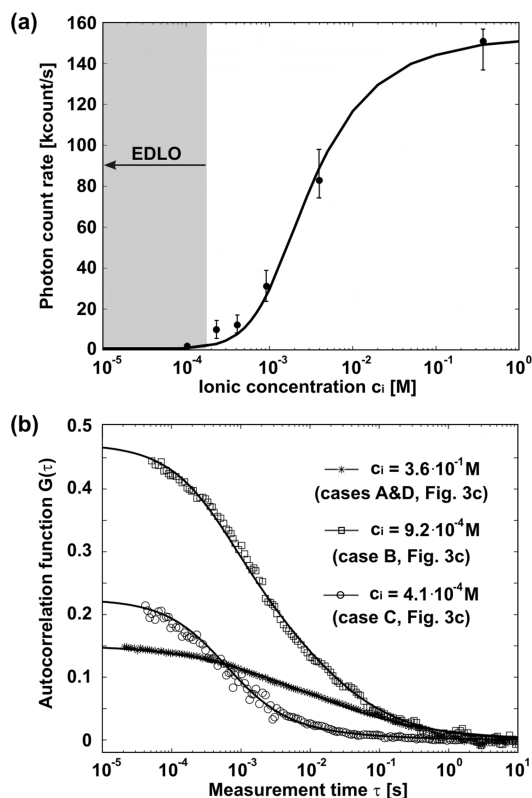


Figure 2. Measurements of fluorescent proteins. (a) Photon count rate of 50 nM WGA at different ionic strengths (c_i) in a 50-nm-high nanochannel. The dots represent experimental measurements, whereas the line is a fitting obtained with eq 8. Pyrex surfaces are negatively charged, which excludes negatively charged WGA proteins from the nanochannel. When electrical double layer overlap (EDLO) occurs, a total exclusion of proteins from the nanochannel can be observed. (b) Autocorrelation functions of fluorescence fluctuations of 38 kDa wheat germ agglutinin with Alexa Fluor 633 in a 50-nm-high nanochannel for solutions with different ionic concentrations at pH 7. Fitting using eq 16 with $\tau_{\text{bulk}} = 0.6$ ms, $\tau_{\text{surf}} = 10$ ms, and $\alpha = 0.45$ as fixed parameters gives $N_{\text{bulk}} = 0.2$ and $N_{\text{surf}} = 5.4$ for $c_i = 3.6 \times 10^{-1}$ M, $N_{\text{bulk}} = 0.5$ and $N_{\text{surf}} = 0.6$ for $c_i = 9.2 \times 10^{-4}$ M, and $N_{\text{bulk}} = 0.9$ and $N_{\text{surf}} = 0.1$ for $c_i = 4.1 \times 10^{-4}$ M.

With all other parameters being kept constant in eq 16, the fit of the data to the autocorrelation function allows one to estimate the values of N_{bulk} and N_{surf} , which correspond to the average number of WGA proteins diffusing freely or interacting with the nanochannel walls, respectively. A good agreement between the measurements and eq 16 has been obtained in all cases, as shown in Figure 2b for three values of the ionic concentration.

Figure 3 shows the evolution of the estimated values of N_{bulk} and N_{surf} , as a function of the ionic strength of the solution.

At high ionic strength (for $c_i > 10^{-1}$ M), a large number of proteins are present in the nanochannel and most of them interact with the walls (N_{bulk} is close to zero). This situation is schematically described in Figure 4a: As the ionic concentration is high, the EDL is small and does not prevent the interaction between the proteins and the walls.

When the ionic strength of the solution decreases from 10^{-1} M to 4×10^{-4} M, the number of proteins present in the nanochannel strongly decreases. The EDL induces an electrostatic repulsion of the negatively charged WGA proteins, and this effect is amplified as the EDL becomes larger. As depicted

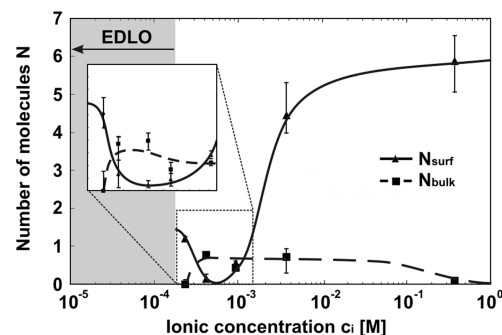


Figure 3. Number of WGA proteins diffusing in a 50-nm-high nanochannel, at different ionic strengths, obtained by fitting the autocorrelation functions with eq 16. N_{bulk} represents the number of molecules that do not interact with the surfaces ($\tau_{\text{bulk}} = 0.6 \pm 0.02$ ms) and N_{surf} represents the molecules that interact with surfaces and get retarded ($\tau_{\text{surf}} = 10 \pm 2$ ms, $\alpha = 0.45 \pm 0.1$). Experimental data were performed at pH 7 and are represented by marks; lines are plotted by interpolating these data and are present to guide the eye of the reader.

in Figure 4b, the number of proteins reaching the walls decreases while the number of molecules diffusing freely in the bulk of the nanochannel increases.

For values of the ionic strength close to 4×10^{-4} M, the number of molecules that interact with the walls is close to zero. As presented in Figure 4c, the proteins are kept away from the channel walls by the large dimension of the EDL. However, because there is no EDL overlap, a small, but significant, amount of proteins enters the nanochannel.

When the ionic strength decreases further, the number of molecules that interact with the surfaces increases again, while the number of molecules diffusing in the bulk of the nanochannel decreases to zero. As presented in Figure 4d, this corresponds to a case where there is not yet an overlap of the EDL. Only a small number of proteins can enter the nanochannel, but they are no longer confined to the center by electrostatic forces and consequently interact with the surfaces. In that case, the autocorrelation functions measured were similar to those measured for high ionic strength.

Finally, for ionic strengths of $<10^{-4}$ M, there is an exclusion of the proteins from the nanochannel, because there is an overlap of the EDL.

Figure 5 shows the effective times of diffusion τ_{eff} , which have been calculated with eq 13, using N_{bulk} and N_{surf} values that have been reported in Figure 3. As expected, at high ionic strength, τ_{eff} is similar to τ_{surf} , because all proteins interact with the walls of the nanochannel, because of the limited extension of the EDL and the large surface-to-volume ratio of the channel. When increasing the EDL, the effective time of diffusion decreases until reaching a minimal value close to τ_{bulk} . This corresponds to the case depicted in Figure 4c, where the proteins do not reach the walls of the nanochannel anymore, because of the size of the EDL, resulting in a diffusion of proteins almost as fast as that in the bulk, under such specific ionic conditions. Finally, at very low ionic concentrations (similar to the EDLO), the rare proteins that may enter the nanochannel have a diffusion time that is similar to τ_{surf} .

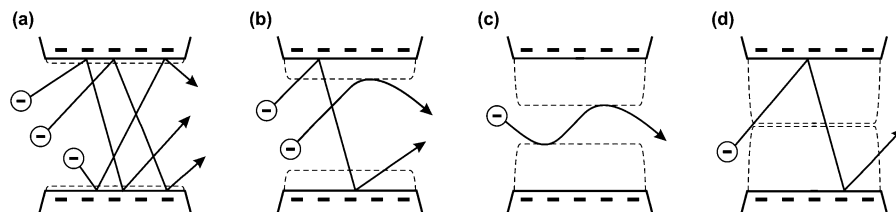


Figure 4. Schematics of the typical behavior of negatively charged biomolecules inside a nanochannel with negatively charged surfaces, at different ionic strengths. Our model is based on two different populations of molecules: the molecules that never touch the pore walls (N_{bulk} , τ_{bulk}) and those that adsorb on the surfaces and get retarded (N_{surf} , τ_{surf}): (a) at high ionic concentrations, the electrical double layer (EDL, represented by a dashed line) is thin and does not prevent most molecules from interacting with the surfaces ($N_{\text{bulk}} \rightarrow 0$); (b) when the ionic concentration decreases, the EDL increases and some molecules do not interact with the surfaces anymore (as the EDL increases, the number of molecules that interact with the surfaces decreases); (c) when the EDL is large enough, most of the molecules do not interact with the surfaces ($N_{\text{surf}} \rightarrow 0$); and (d) when the channel is just not closed by the EDL, very few molecules enter in the channel and interact again with the surfaces.

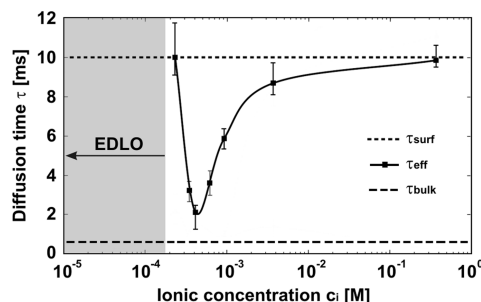


Figure 5. Effective diffusion times for WGA proteins in a 50-nm-high nanochannel at different ionic strengths. Marks represent the effective diffusion times calculated with eq 13 from measurements of Figure 3. The line is plotted by interpolating these data and is present to guide the eye of the reader. The dashed and dotted lines represent the values of the fixed parameters $\tau_{\text{bulk}} = 0.6$ ms and $\tau_{\text{surf}} = 10$ ms, respectively.

CONCLUSION

Many technologies have recently been developed to exploit the exclusion or the enrichment of particles in a confined area, particularly for biotechnology applications;³⁴ however, diffusion through such artificial structures is still largely misunderstood.

In this work, we have demonstrated that the concentration and diffusion of hindered proteins can be measured directly inside a nanochannel with a fluorescence correlation spectroscopy (FCS) measurement unit. Our model for the prediction of the concentration in a nanochannel takes into account the steric exclusion, as

well as interactions of biomolecules with the surfaces and the exclusion-enrichment effect, which is due to the electrical double layer and to the charge of the proteins. We propose a model for the FCS autocorrelation functions, which have been used to estimate the number of molecules that diffuse freely inside the nanochannel and the number of molecules interacting with the surfaces. The measurements performed at different ionic strengths showed different regimes of diffusion and highlighted that, for a specific electrical double layer (EDL) thickness, the diffusion of proteins through a nanochannel can be almost as high as that in the bulk.

The models presented in this paper that were used to predict the concentration and diffusion of proteins in nanoconfined spaces can be easily used to obtain a better understanding of on-chip, membranes separation, or proteomic processes. We believe that they will help to get a better understanding of fundamental nanofluidic physics to develop further biological and biomedical applications.

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