Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

for

"DNA translocation to giant unilamellar vesicles during electroporation is independent of DNA size"

Shaurya Sachdev, Aswin Muralidharan, Dipendra K. Choudhary, Dayinta L. Perrier, Lea Rems[#], Michiel T. Kreutzer and Pouyan E. Boukany*

Department of Chemical Engineering, Delft University of Technology, van der Maasweg 9, 2629 HZ, Delft, The Netherlands

[#]*Present Address: Science for Life Laboratory, Tomtebodavägen 23, SE-171 65 Solna, Sweden*

*Email: p.e.boukany@tudelft.nl

Section 1: Determination of uptake time and the slope of normalized intensity (I/I_o) vs. time during uptake of DNA by the GUV

In figure 2 (e) of the main text, apart from the final translocation efficiency $(I/I_o)_f$, the time for DNA uptake (t_{uptake}), and the slope of the curve (I/I_o) vs. time (s) during the application of electric field pulses were extracted. Before showing the time of DNA uptake and the slope corresponding to each DNA size, the process of calculating these quantities is explained below for a representative experiment corresponding to a DNA size of 100 bp (bp=base pairs).

Figure 2 (e) in the main text is re-plotted below in Figure S1 (a) to describe how the step increment and time were calculated during DNA uptake. The black dotted line represents the normalized mean fluorescence intensity of DNA molecules (I/I_o). The mean intensity of DNA molecules inside the GUV is I and the mean intensity of DNA molecules outside the GUV is I_o. The filled blue points connected by a solid red line represents the smoothed data of (I/I_o). The difference in normalized mean fluorescence intensity was calculated as $\Delta(I/I_o)_t = (I/I_o)_t - (I/I_o)_{t-\Delta t}$, for each time step using the smoothed data, and is plotted in figure S1 (b) as filled black points. A Gaussian curve shown as a solid red line was fitted to this data. The fitted function was $\Delta(I/I_o)_t = Aexp(-0.5(t-t_o)^2/\sigma^2)$, where A, t_o, and σ are the fitting parameters. σ represents the standard deviation of the Gaussian, and the time of DNA uptake is estimated to be $t_{uptake} \sim 3\sigma$. The full width at half maximum ($FWHM$), was calculated as $FWHM = 2\sqrt{2\ln(2)}\,\sigma$. The times when the Gaussian curve attains a value of $\Delta(I/I_o)_{max}/2$, where $\Delta(I/I_o)_{max}$ is the maximum of Gaussian is shown as t_1 and t_2, such that $FWHM = t_2 - t_1$.The smoothed data of (I/I_o) of Figure S1 (a) is plotted again in Figure S1 (c) from times t_1 to t_2. A straight line is fitted through this curve in order to find the slope of the curve (I/I_o) vs. time (s). The fitted function was $(I/I_o) = mt + c$, where m represents the slope and c represents the intercept.

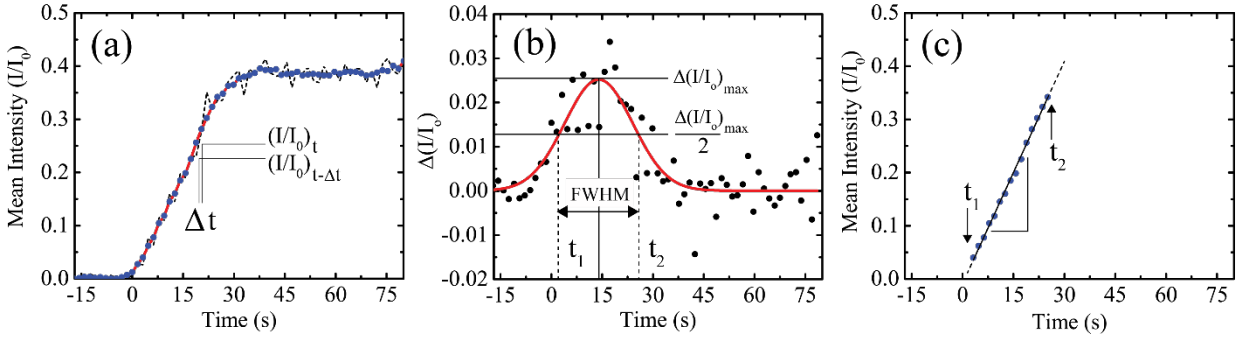

Figure S1: Process of calculating the time of DNA uptake and the slope of DNA uptake curve (DNA size = 100 bp). (a) Dashed line represents the normalized meant intensity (I/I_o) of DNA molecules inside the GUV as a function of time. This is the same as shown in Figure 2 (e) of the main text. Filled blue circles connected by a solid red line representing the smoothed data. Consecutive normalized mean intensities are marked as $(I/Io)_t$ and $(I/Io)_{t-\Delta t}$ where Δt is the time between consecutive measurements or frames. (b) $\Delta(I/I_o)_t$ as a function of time (s). $\Delta(I/I_o)_t$ is calculated as $\Delta(I/I_o)_t = (I/I_o)_t - (I/I_o)_{t-\Delta t}$ from the smoothed data in (a). Shown in red is the fitted Gaussian curve $\Delta(I/I_o)_t = Aexp(-0.5(t-t_o)^2/\sigma^2)$, where A, t_o and σ are the fitting parameters and σ represents the standard deviation of the gaussian. The values of the parameters are A=0.025, t_o=13.98 s and σ=10.06. The standard error for A, t_o and σ are 0.0020, 0.86 and 1.02 respectively, as obtained from the best fit. t_1 and t_2 represent the times when $\Delta(I/I_o)_t$attains a value of $\Delta(I/I_o)_{max}/2$ where $\Delta(I/I_o)_{max}$ is the maximum of the gaussian. $t_2 - t_1$ represents the full width at half maximum ($FWHM$). (c) (I/I_o) vs. time (s) as shown in (a), however for times between t_1 and t_2 only. A straight line $(I/I_o) = mt + c$ is fitted through the data, where m (slope) and c (intercept) are the fitting parameters. m=0.014 s^{-1} and c=-0.010 with standard errors of 2.08x10^{-4} s^{-1} and 0.003 respectively.

The time during which DNA uptake was observed $t_{uptake} \sim 3\sigma$, as shown in Figure S1 (b), was calculated for each experiment and for each DNA size and is plotted in Figure S2 (a) as a scatter. On the same curve, a solid red line is plotted representing the time during which electric field pulses were applied. Since 10 pulses were applied, each of 5 ms duration and at a frequency 0.33 Hz, therefore this line is plotted at t = 30.05 s.

Similarly, the slope of the curve, $m = d(I/I_o)/dt$, was calculated for each experiment and for each DNA size and is plotted in Figure S2 (b) as a scatter.

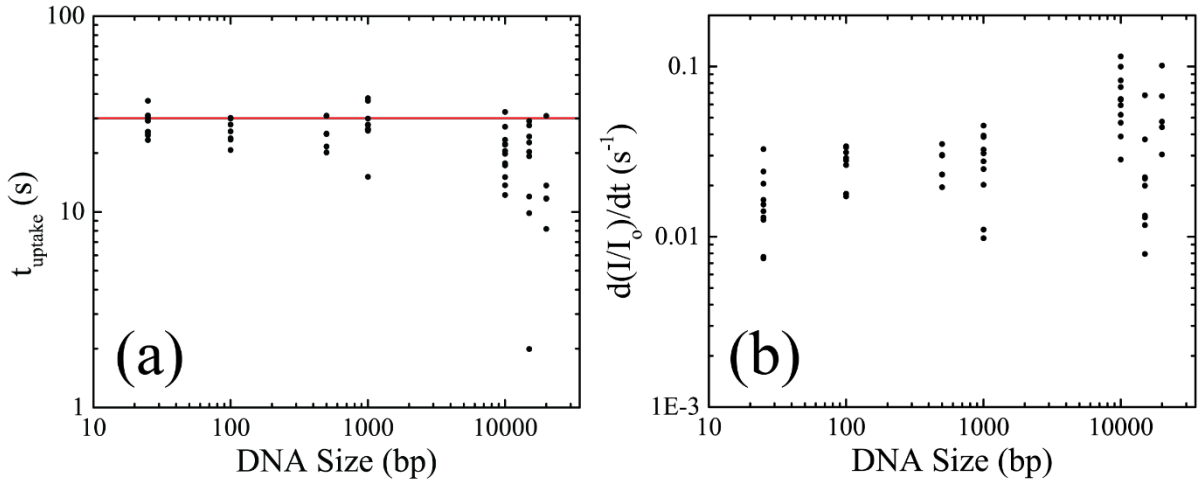

Figure S2: (a) Time during which DNA uptake was observed (t_{uptake}) potted as a scatter for each DNA size. Each data point (filled black circles) corresponds to a single experiment. Solid red line shown represents the time during which pulses were applied. 10 pulses were applied, each of 5 ms duration and at a frequency of 0.33 Hz, hence the solid line is drawn at t=30.05 s. (b) Slope $m = d(I/I_o)/dt$, as shown in Figure S1 (c) plotted for each DNA size. Each data point (filled black circles) corresponds to a single experiment.

Section 2: Electrophoretic mobilities in different buffers

To calculate the theoretical prediction plotted in Figure 3 (b) of the main text corresponding to Tris-acetate EDTA (TAE) buffer, the electrophoretic mobilities of DNA molecules (μ) used in equation 1 of main text, were obtained from Stellwagen *et al.* (1997) [1]. These values are listed in table ST1 below.

Table ST1: Electrophoretic mobility of DNA molecules of different sizes in TAE buffer [1].

DNA Size (bp)	Electrophoretic Mobility (μ) ($m^2s^{-1}V^{-1}$)
25	3.36×10^{-8}
100	3.67×10^{-8}
500	3.75×10^{-8}
1000	3.75×10^{-8}
10000	3.75×10^{-8}
15000	3.75×10^{-8}
20000	3.75×10^{-8}

For low conductive buffers, the electrophoretic mobility of dsA5 (20 bp) DNA molecule was estimated to be $\mu = 4.60 \times 10^{-8}$ $m^2s^{-1}V^{-1}$ [2]. Considering this to be the value of electrophoretic mobility for a 25 bp DNA molecule in a low conductive buffer, this corresponds to an increase of 1.24×10^{-8} $m^2s^{-1}V^{-1}$ in the electrophoretic mobility from TAE buffers (Table S1). A similar increase in in electrophoretic mobility was observed for both dsA5 (20 bp) and pUC19 (2686 bp) DNA molecules upon reducing the conductivity of the buffer [3]. Therefore, considering the same increase in the electrophoretic mobility of DNA molecules of all sizes used in this work, the electrophoretic mobilities corresponding to low conductive glucose/sucrose buffers are listed in Table ST2 below.

Table ST2: Electrophoretic mobilities of DNA molecules in low conductive glucose/sucrose buffers.

DNA Size (bp)	Electrophoretic Mobility ($m^2s^{-1}V^{-1}$)
25	4.60×10^{-8}
100	4.91×10^{-8}
500	5.99×10^{-8}
1000	5.99×10^{-8}
10000	5.99×10^{-8}
15000	5.99×10^{-8}
20000	5.99×10^{-8}

Section 3: COMSOL Simulations of electric field through the electro-pore

To estimate the electric flux through a pore on a giant unilamellar vesicle (GUV) placed in an electric field, $E_{app,}$ finite element numerical calculations were carried out using COMSOL Multiphysics. The numerical calculations were carried out in two dimensions assuming the system is axisymmetric. The GUV is modelled as a spherical shell of radius R as seen in Figure S3. The electrical conductivity of aqueous solution inside the GUV is σ_i. The GUV is placed in an aqueous solution of electrical conductivity σ_e represented as a rectangle of dimensions 100 µm × 50 µm. It is assumed that aqueous solution inside the GUV and the external medium mix after the formation of the pore and hence σ_i and σ_e are equal. The size of electro-pore is parameterized as a spherical cap of angle θ on the membrane on the sides facing both anode and the cathode.

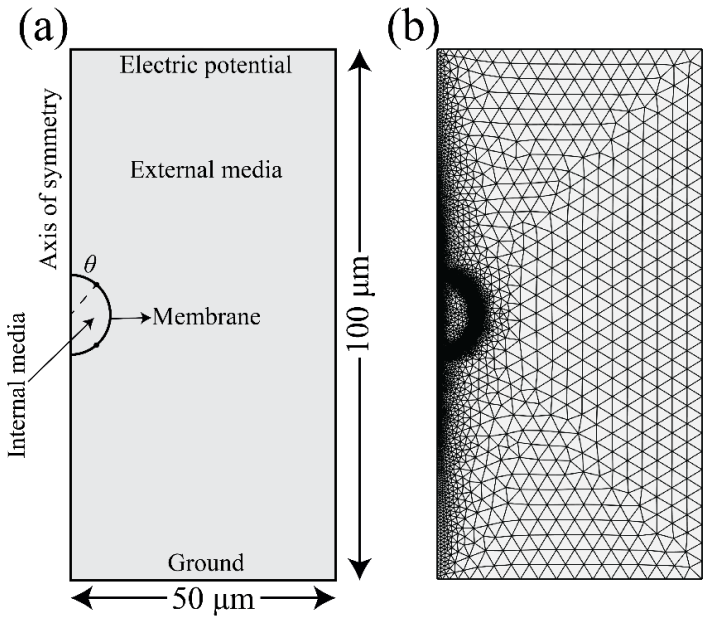

Figure S3 (a) Geometry of the domain used for finite element numerical calculations. The GUV is modelled as the semicircle enveloped by the membrane and the pore is modelled as the spherical cap of angle θ on the membrane. The external medium is modelled as the rectangle of dimensions 100 µm × 50 µm. The top and bottom sides of the rectangle are modelled as electrodes such that an electric field E_{app} exists between the two sides. The axis of symmetry is labelled on the figure. (b) The mesh that is used for numerical calculation of electric potential. The domain is discretized in to approximately 70000 elements through free triangulated meshing in COMSOL Multiphysics. The minimum mesh size is close to the edges of the pores with a mesh size of $5x10^{-11}$ m. The maximum mesh size is $1x10^{-9}$ m.

To compute the electric potential distribution in the system, Laplace's equation SEQ 1 (shown below) for the electric potential is solved numerically using the *Electric currents(ec)* module.

$$\nabla.\sigma\nabla V = 0 \qquad \text{(SEQ 1)}$$

The GUV membrane is modelled as a thin high resistance region using contact impedance boundary condition [4].

$$\boldsymbol{n}.\boldsymbol{J_1} = \frac{\sigma_m}{d_m}(V_1 - V_2) \qquad \text{(SEQ 2)}$$

$$\boldsymbol{n}.\boldsymbol{J_2} = \frac{\sigma_m}{d_m}(V_2 - V_1) \qquad \text{(SEQ 3)}$$

Here, $\boldsymbol{n}$ is the unit normal vector to the boundary surface, $\boldsymbol{J}$ is the electric current density, V_1 is the electric potential inside the GUV and V_2 is the electric potential on the exterior of the GUV, σ_m is the membrane conductivity and d_m is the thickness of the membrane.

After the values of electric potential is obtained, the electric field $\boldsymbol{E}$ is determined by: -

$$\boldsymbol{E} = -\boldsymbol{\nabla} V \qquad \text{(SEQ 4)}$$

Table ST3: Model parameters used for numerical calculations.

Symbol	Parameter	Value
R	Vesicle radius	15 µm
d_m	Membrane thickness	4 nm
σ_e	External conductivity	1.5×10^{-4} S/m
σ_i	Internal conductivity	1.5×10^{-4} S/m
σ_m	Membrane conductivity	1×10^{-9} S/m
E_{app}	Applied electric field	45 kV/m

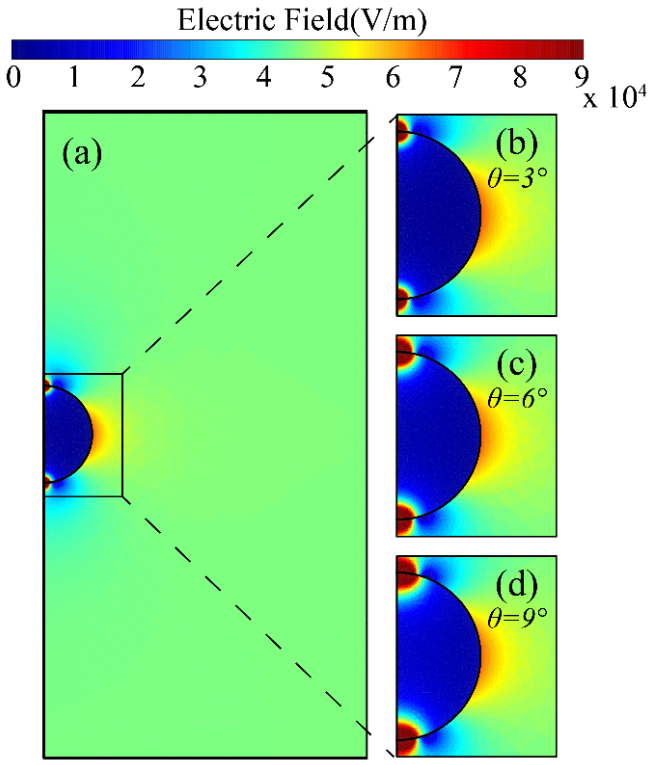

Figure S4: (a) Electric field distribution in the geometry show in Figure S3. The applied electric field is 45 kV/m and the radius of the GUV is 15 µm. (b), (c), (d) The electric field distributions in a small region enveloping the GUV for angle of electropore, θ=3°, 6°, 9°. The electric field is higher at the pores and low inside the GUV.

The electric flux through the pore is then calculated numerically by integrating the electric field on the surface of the electro-pore ($\int \boldsymbol{E} \cdot \boldsymbol{dS} = \boldsymbol{f}(\boldsymbol{\theta}) = \boldsymbol{f}'(\boldsymbol{0})\boldsymbol{\theta}$). The electric flux is then calculated as a function of the angle θ.

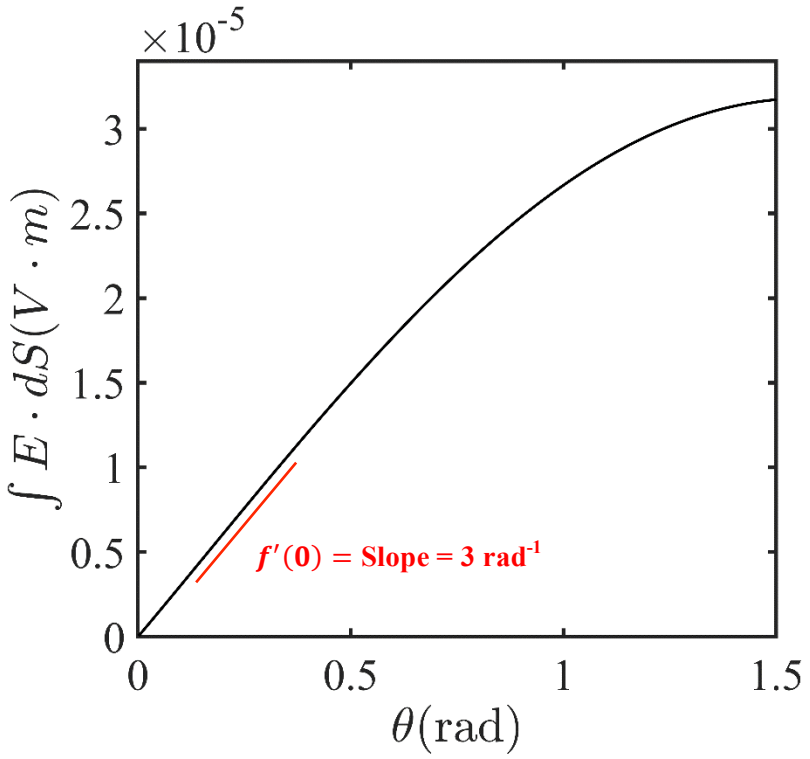

Figure S5: Electric flux across the electropore ($f(\theta) = f'(0)\theta$) given as a function of angle of electropore, θ. The internal and external conductivities are equal and are equal to 0.15 mS/cm [5]. The conductivity of membrane is equal to 1×10^{-8} mS/cm [6]. The applied electric field across two electrodes is equal to 45 kV/m and the radius of the vesicle is equal to 15 µm.

Section 4: Radius of gyration

The contour length of DNA (L) can be estimated as $L = (no.of\ base\ pairs) \times 0.34\ nm$, since the inter bp distance is ~ 0.34 nm [7]. The persistence length (l_p) of DNA in physiological salt conditions is $l_p \sim 50\ nm$ (150 bp), however it increases with decreasing salt concentrations to $l_p \sim 150\ nm$ (~450 bp) [8]. Therefore, DNA molecules of size 25 bp and 100 bp behave as rod-like molecules (l_p>L), and DNA molecules of size 1000 bp, 10000 bp, 150000 bp and 20000 bp behave as coil-like molecules (L>l_p). The DNA molecules of size 500 bp have are assumed to behave either as coil-like molecules if $l_p \sim 150\ bp$, or rod-like molecules if $l_p \sim 450\ bp$.

In order to calculate the radius of gyration ($R_{g,\mathrm{rod}}$) for rod-like molecules (l_p>L), the following equation (SEQ 5) was used [9].

$$R_{g,rod} = \sqrt{\frac{L^2}{12}} \quad \text{(SEQ 5)}$$

In order to calculate the radius of gyration ($R_{g,\mathrm{coil}}$) for coil like molecules (L>l_p), the following equation (SEQ 6) was used (end-to-end length for the Kratky-Porod model) [10].

$$R_{g,coil} = \sqrt{2l_pL - 2l_p^2\left[1 - exp\left(-\frac{L}{l_p}\right)\right]} \quad \text{(SEQ 6)}$$

The radius of gyration according is estimated according to the above equations (SEQ 5 and SEQ 6) and is shown in Table ST4 below. For rod-like DNA molecules (25 bp, 100 bp), equation SEQ5 was used to estimate R_g. For coil-like DNA molecules (1000 bp, 10000 bp, 15000 bp and 20000 bp), 3 different values of persistence length l_p= 50 nm, 100 nm and 150 nm were used to estimate R_g according to equation SEQ 6. To estimate radius of gyration for DNA molecules of size 500 bp, both equations (SEQ 5 and SEQ6) were used.

Table ST4: Radius of gyration (R_g) values for different DNA sizes used.

DNA Size (bp)	**Contour Length (L) (nm)**	**Equation**	$\boldsymbol{R_{g,rod}}$ **(nm)**	$\boldsymbol{R_{g,coil}}$ **(nm)** l_p= 50 nm	$\boldsymbol{R_{g,coil}}$ **(nm)** l_p= 100 nm	$\boldsymbol{R_{g,coil}}$ **(nm)** l_p= 150 nm
25	8.5	SEQ 5	2.4			
100	34	SEQ 5	9.8			
500	170	SEQ 5/6	49.1	110.3	132.9	143.1
1000	340	SEQ 6		170.3	220.6	248.3
10000	3400	SEQ 6		578.8	812.4	987.4
15000	5100	SEQ 6		710.6	1000.0	1218.6
20000	6800	SEQ 6		821.6	1157.6	1412.4

References

[1] Stellwagen, N.C., Gelfi, C. and Righetti, P.G., 1997. The free solution mobility of DNA. Biopolymers: Original Research on Biomolecules, 42(6), pp.687-703.

[2] Stellwagen, E. and Stellwagen, N.C., 2003. Probing the electrostatic shielding of DNA with capillary electrophoresis. Biophysical journal, 84(3), pp.1855-1866.

[3] Stellwagen, E. and Stellwagen, N.C., 2002. The free solution mobility of DNA in Tris□acetate□EDTA buffers of different concentrations, with and without added NaCl. Electrophoresis, 23(12), pp.1935-1941.

[4] Rems, L., Ušaj, M., Kandušer, M., Reberšek, M., Miklavčič, D. and Pucihar, G., 2013. Cell electrofusion using nanosecond electric pulses. Scientific reports, 3, p.3382.

[5] Portet, T., Favard, C., Teissié, J., Dean, D.S. and Rols, M.P., 2011. Insights into the mechanisms of electromediated gene delivery and application to the loading of giant vesicles with negatively charged macromolecules. Soft Matter, 7(8), pp.3872-3881.

[6] Perrier, D.L., Rems, L. and Boukany, P.E., 2017. Lipid vesicles in pulsed electric fields: fundamental principles of the membrane response and its biomedical applications. Advances in colloid and interface science, 249, pp.248-271.

[7] Mandelkern, M., Elias, J. G., Eden, D., & Crothers, D. M. 1981. The dimensions of DNA in solution. Journal of molecular biology, 152(1), 153-161.

[8] Pan, S., At Nguyen, D., Sridhar, T., Sunthar, P., & Ravi Prakash, J. 2014. Universal solvent quality crossover of the zero shear rate viscosity of semidilute DNA solutions. Journal of Rheology, 58(2), 339-368.

[9] Rubinstein, M. and Colby, R.H., 2003. Polymer physics (Vol. 23). New York: Oxford university press.

[10] Kirby, B.J., 2010. Micro-and nanoscale fluid mechanics: transport in microfluidic devices. Cambridge university press.