Regulation of DNA conformations and dynamics in flows with hybrid field microfluidics

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Visualizing single DNA dynamics in flow provides a wealth of physical insights in biophysics and complex flow study. However, large signal fluctuations, generated from diversified conformations, deformation history dependent dynamics and flow induced stochastic tumbling, often frustrate its wide adoption in single molecule and polymer flow study. We use a hybrid field microfluidic (HFM) approach, in which an electric field is imposed at desired locations and appropriate moments to balance the flow stress on charged molecules, to effectively regulate the initial conformations and the deformation dynamics of macromolecules in flow. With λ -DNA and a steady laminar shear flow as the model system, we herein studied the performance of HFM on regulating DNA trapping, relaxation, coil-stretch transition, and accumulation. DNA molecules were found to get captured in the focused planes when motions caused by flow, and the electric field were balanced. The trapped macromolecules relaxed in two different routes while eventually became more uniform in size and globule conformations. When removing the electric field, the sudden stretching dynamics of DNA molecules exhibited a more pronounced extension overshoot in their transient response under a true step function of flow stress while similar behaviors to what other pioneering work in steady shear flow. Such regulation strategies could be useful to control the conformations of other important macromolecules (e.g., proteins) and help better reveal their molecular dynamics. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4762852>]

I. INTRODUCTION

The study of single molecule dynamics offers great promise in genomic analysis, molecule sensing, biophysics, biomechanics, and polymer processing.^{1–8} Deformations of free or tethered macromolecules have been explored using various external forces including atomic force microscopy,^{9,10} optical or magnetic tweezers,^{11–13} fluid flow,^{14,15} and electrokinetics.^{16,17} Among these studies, the coil-stretch dynamics of long DNA in fluid flows offers a wealth of physical insights in complex flow study.^{18–29} In hydrodynamic flows, DNA molecules exhibit various conformations and continuously experience tumbling, stretching, and coiling in a stochastic manner.^{30,31} Considering the strong dependence of macromolecule dynamics to their flow-induced deformation history, single stable conformation is often desired. Regulation of long DNA conformations and their transitions has been predicted in simulation^{32–34} and done experimentally on a single molecule basis.^{35,36} However, it is still very difficult to experimentally maintain a large population of DNA molecules in one or limited number of well-defined conformations for reasonably long time (e.g., multiple Hencky strain). Currently, the conformational fluctuations are often auto-correlated with statistical tools or largely suppressed by

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studying DNA responses in startup flows.^{37–40} The former often demands complicated mathematical analysis while the latter involves the intrinsic coupling of flow development and DNA coil-stretch dynamics. We here present a new hybrid field microfluidics (HFM) approach, employing both hydrodynamic forces and an electric field, to regulate DNA initial conformations, their deformation dynamics, and accumulation at appropriate locations and moments. By switching on or off an appropriate electric bias, charged molecules can be conveniently trapped, concentrated, or released without expensive facility or extra conjugation steps.

II. HFM WORKING PRINCIPLES

In HFM, a pressure-driven flow (PDF) is run all the time or in a programmed manner, while an electric field is imposed only at desired moments (Figures 1(a)–1(c)). The electrodes are arranged in such a way that electrophoresis on charged objects has an opposite direction to flow upon the application of an electric bias. If in a straight channel, this contributes an uniform “backward” velocity component on charged objects. When superimposed on the “forward” velocity component from the pressure-driven flow (with a parabolic velocity profile if in a steady laminar flow), the charged objects exhibit a bi-directional velocity profile.⁴¹ At some locations, the magnitude of these two velocity components is equal and the passing charged objects will get trapped. When captured in such non-disturbance environment, soft objects such as DNA will start having their molecular chains relaxing to highly packed conformations regardless their various initial conformations. Varying the electric field strength (or the flow rate) could further change the trapping locations in the transverse direction (Figure 1(d)). If suddenly removing the electric field, the external forces on trapped

FIG. 1. (a) Schematic of the working principles of HFM, (b) a typical application scheme for an electric bias in HFM, (c) regulation the conformations and dynamics (trapping, concentration, and sudden stretching) of λ -DNA molecules in HFM, and (d) schematic of the trapping location regulation (enhanced online). [URL: <http://dx.doi.org/10.1063/1.4762852.1>]

macromolecules will become imbalanced and the fluid flow alone will cause quick coil-stretch transitions of these molecule chains in a way similar to that in a startup flow (see Figure 1(c)). Better than in startup flows, the sudden stretching in HFM has much shorter response time for the use of an electric signal trigger ($\sim 0.02\text{--}5\ \mu\text{s}$). Moreover, because all trapped macromolecules are held to similar sizes and conformations, the coil-stretch transitions in HFM are minimally affected by their conformation fluctuations and/or deformation history. In addition, such DNA dynamics happens in a well-defined flow field, in which the flow development and the flow-induced conformation transitions of macromolecules are wisely decoupled. For example, in a common control strategy shown in Figure 1(b), the captured DNA chains will first relax and wind up into the globule shape with uniform size and then respond to a step flow stress. When combined with different flow fields, more sophisticated tests may also be done to better reveal many flow-induced molecular dynamics phenomena, particularly for non-Newtonian fluids. Since electrical signals can be conveniently switched on/off, the same regulation can be executed multiple times or even for a frequency test.

Herein, we focus on the feasibility demonstration of HFM for λ -DNA trapping and conformation regulation in a steady laminar shear flow. The choice of this model system benefits from its relatively simple analysis methodology and the convenience in comparison with other pioneer work.

III. MATERIALS AND METHODS

A. HFM platform design and fabrication

The core component of the HFM platform is a straight microchannel (the length, $L = 5\text{ mm}$, the width, $W = 20\ \mu\text{m}$, and the depth, $H = 32\ \mu\text{m}$), as shown in Figure 2. Considering the dimensions of λ -DNA (with the radius of gyration or $R_g \sim 0.73\ \mu\text{m}$ and the fully stretched contour length or $L_{DNA} \sim 22\ \mu\text{m}$ after YOYO-1 labeling^{18,19}), this provides ratios of the size and the contour length of λ -DNA to the characteristic length of the flow channel to be ~ 0.04 (R_g/W) and 1.1 (L_{DNA}/W), respectively. The $20\text{-}\mu\text{m}$ channel is connected to two fluid receiving reservoirs (as inlet and outlet) through a large, straight connecting channel ($W = 100\ \mu\text{m}$ and $L = 5\text{ mm}$) on each end.

The HFM platform was fabricated by MEMS technologies. Briefly, a Si (100) wafer was patterned with photoresist (PR), and the pattern was transferred to the Si substrate via deep reactive ion etching (DRIE) system. The DRIE etching process was performed through a Bosch process with a gas mixture of SF_6 and C_4F_8 . The actual depth of microchannels was found to be $32\ \mu\text{m}$. The particle desorption mass spectrometry (PDMS) chips were then made by casting the mixture of 10:1 ratio (by weight) of PDMS precursor and curing agent (Sylgard 184, Dow Corning) by soft lithography and sealed to a glass slide after O_2 plasma treatment. The channel surface was further poly(ethylene glycol) (PEG)-coated to suppress potential electroosmosis effect.⁴²

FIG. 2. (a) Schematic of a HFM device with the channel dimensions labelled; “Site A” and “Site B” are the two locations we measured DNA accumulation in HFM; (b) microscopic images from both top view and cross-section view of a HFM channel at the two imaging locations in the experiment. Their relative locations are also marked in (a). The scale bars are $50\ \mu\text{m}$.

B. HFM operation

When operating HFM, the fluid flow was maintained by controlling the liquid level difference between the two 3-mm fluid reservoirs where Pt wires (250- μm in diameter) were immersed as electrodes to avoid unnecessary electrochemical reactions. A DC power supply from EMCO high voltage corporation (Sutter Creek, CA) was used to provide an appropriate electric field when needed. The electric voltage was applied via a home-made digital trigger to control the turn-on/off moments, which requires $\sim 0.02\text{--}5\ \mu\text{s}$ to establish an appropriate electric field.

C. DNA dynamics visualization

A dilute solution ($\sim 0.03\ \mu\text{g/ml}$, about 10^{-4} of the overlapping concentration, at which the macromolecules completely fill the space but not overlapping) of λ -DNA (New England Biolab, Inc) in 10 mM TE buffer ($\text{pH} = 8.0$) with 4% β -mercaptoethanol was used as the experimental fluid. DNA molecules were labeled with YOYO-1 dyes at a dye-to-base-pair molar ratio of 1:5. Glucose oxidase and catalase were added to the fluid (10 mM TE buffer) to significantly slow down potential oxidative damage.^{18,19,39} In order to increase the shear stress, the bulk viscosity of the DNA solution was adjusted to 30 cp by adding 18% w/w glucose and 40% w/w sucrose.

The HFM chip was mounted on the stage of an inverted epi-fluorescence microscope (TE2000U, Nikon, Japan). The dynamics of DNA was observed through a $60\times/1.3\text{NA}$ oil immersion objectives and recorded with a 12-bit, monochrome CCD camera (CoolSNAP fx, Roper Scientific®, Tempe, AZ) with a frame rate >25 frames/s.

When recording DNA coil-stretch dynamics, our observation window (“site B” in Figure 2(a)) was placed 3.5 mm away from the entry of the 20- μm channel (the junction of the 20- μm microchannels and the 130- μm connection channel, marking as “site A” in Figure 2(a)). Considering the pre-stretching effect around the junction,^{34,43} we measured the instantaneous contour length of ~ 200 DNA molecules within 10 s after turning off the electric bias. Estimated from the DNA migration velocity and our observation location, DNA molecules analyzed in this time window should have been trapped inside the 20- μm channel upstream prior to the removal of the electric field. This effectively excludes those DNA molecules, which have experienced pre-stretching when passing the sudden contraction junction.

D. DNA dynamics analysis

The longest relaxation time (τ) of λ -DNA was estimated via an empirical formula,⁴⁴ which was $\sim 2.82\text{ s}^{-1}$ in our cases. The center-of-mass velocity of DNA at the presence of hydrodynamic flow alone was measured at various transverse locations and then fitted with,

$$U_x(y, z) = \frac{1}{2\eta} \left(-\frac{dp}{dx} \right) \left[\left(\frac{H}{2} \right)^2 - z^2 + H^2 \sum_{n=1}^{\infty} \frac{(-1)^n}{\alpha_n^3} \times \frac{\cosh(2\alpha_n y/H) \cos(2\alpha_n z/H)}{\cosh(\alpha_n W/H)} \right], \quad (1)$$

where $\alpha_n = (2n-1)\pi/2$, $(-dp/dx)$ is the pressure gradient, η is the viscosity, H and W are the height and width of the straight channel.⁴⁵ The velocity data of DNA molecules which were once captured at $\sim 4.2\ \mu\text{m}$ away from the bottom channel surface ($\sim 1/5$ of $L_{\lambda\text{-DNA}}$).⁴⁶ Under a pressure gradient of $\sim 3.5\text{ mm H}_2\text{O}$, the average shear rate in that specific viewing plane was $\sim 5.2\text{ s}^{-1}$ and this gives the Weissenberg number (Wi) as ~ 15 .

IV. RESULTS AND DISCUSSION

A. DNA trapping and relaxation

We first evaluated the feasibility of HFM for DNA trapping and relaxation. Prior to trap, a steady shear flow was maintained, so that all DNA molecules were freely tumbling, coiling, or stretching with various lengths and conformations.^{30,31} Upon applying an electric field (e.g., 147 V/cm), DNA molecules in the focused planes started getting trapped in flow, attributing to

the balance of motions caused by flow and the electric field. Most trapping sites on DNA chains were close to the anode side, suggesting that more or less charge re-distributions occurred within segments of individual molecules when they fell in the trapping plane. The polarization (non-uniform charge distribution) is attributed to both the electric field as well as the flow field: the electric field attracts the negatively charged groups on DNA segments to move against the field (towards the anode side) while the flow stress tends to stretch the DNA chain to a more extended state (Figure 3(a)). The former causes local segment packaging and charge accumulation on one side while the latter tends to unpack segments and has these charges dispersed along the DNA chain. The establishment of a trapping depends on the dominant of these two effects and the resulting conformational reorganization situation on each specific DNA chain. Therefore, not all molecules got trapped immediately and some were found to freely pass the observing window in the first few seconds. Occasionally, a few trapped molecules also escaped from their original capture sites while quickly got captured again somewhere downstream. The occurrence of these unusual events results from the strong flow extension on those unstable packaged DNA segments. As the initial extended DNA chain is large enough to have some segments fall outside the trapping plane, these segments “feel” unbalanced forces. The flow stress tends to continuously stretch them until its rotation component helps align the DNA chain to the flow direction. So if a trapping is not fully established, a large extension tendency of DNA chains could pull the entire molecule out of the trapping plane and break the holding. However, if the DNA chain rotates and aligns to the flow direction quickly, the extension force will be cancelled by the electrophoresis. As the consequence, the trapped DNA chains are less affected by the field outside the trapping planes in HFM and their chances to escape will be largely reduced. It was recently found that an electric field could also cause rotations of the DNA chains when they fall in a non-uniform local field.³⁴ Such “local effect” could help collapse DNA segments in more packed states.^{34,47} This explains our observation on the gradual disappearance of these unsteady behaviors when the HFM trapping continued.

After getting captured, macromolecules started relaxing until reaching the globule conformations. Two different routes of relaxation dynamics were found. The first type is similar to what had been reported:³⁵ a short, fast relaxation stage followed by a long, slow relaxation stage (Figure 3(b)). This type of relaxation occurred for molecules whose initial extension was

FIG. 3. (a) Schematic of how a DNA chain is polarized, trapped or escape, and relax in HFM; (b) and (c) Time serial snapshots of the relaxation dynamics of an intermediated-stretched (b) and an over-stretched λ -DNA (c). (d) Extension versus residence time for ~ 80 λ -DNA molecules. The red and blue lines in (d) are from the individual DNA molecules shown in (b) and (c), respectively.

much less than its full contour length. In this two-stage relaxation process, a DNA chain first coiled from the captured end and then gradually wound up the rest segments towards this end until forming a ball. When the initial DNA length was close to or over its contour length, the other end of DNA chain might also curl up into a ball during the relaxation (Figure 3(c)), showing a dumbbell intermediate conformation. The molecule continued relaxing from both ends similarly and the two coiled balls gradually moved towards each other, accompanied with some rotational motions. Eventually, these two balls merged and further packed into one with a smaller size. Due to the optical limit, it is hard to tell how these two balls merged and further packed. We suspect that the chain rotations were accompanied by the unwinding of one coil, which re-packed onto the other one. More advanced experimental and/or simulations tools are needed to better reveal the mechanism of such unusual relaxations.^{48–50}

The instantaneous contour length of ~ 80 DNA molecules was measured during their relaxation (Figure 3(d)). Despite the large variations on the actual relaxation dynamics, all DNA chains eventually formed similar conformations in HFM, which are quite different from those presenting in still fluid: λ -DNA molecules trapped in HFM were packed more tightly and had more uniform size and nearly globule shape, unlike in still liquid, molecules still exhibited various sizes and shapes (Figure 4(a)). To quantify such conformation difference, the radius of gyration of λ -DNA molecules and the offset of DNA conformations from the sphere shape (represents as the ratio between the major and minor axes of molecules when treating as ellipse objects, a/b) were measured, respectively. In these measurements, a point spread function (PSF) was considered to correct the size measuring errors, which were caused by fluorescence signals of the optical system (Note: the apparent size of DNA in fluorescence images is larger than its actual value).⁵¹ As shown in Figure 4(b), we got an average radius of gyration (R_g) of λ -DNA $0.89 \pm 0.20 \mu\text{m}$ in still liquid and $0.63 \pm 0.13 \mu\text{m}$ when trapped in HFM. Note: the measured R_g value of λ -DNA in still liquid of our experimental fluids is slight larger than the well adopted value (i.e., $0.73 \mu\text{m}$) obtained from diffusivity measurement.^{18,19} This does lead to a negligible increase of the R_g/W value in our system. Nevertheless, our R_g measurements indicate clearly that the size of fully packed λ -DNA molecules in HFM is much smaller than those in still liquid. In addition, as shown in Figure 4(c), DNA molecules trapped in HFM are nearly isotropic (with $a/b = 1.18 \pm 0.16$) when compared to those coiled in still liquid (with $a/b = 1.93 \pm 0.66$).

Collapse of flexible molecules from coil-to-globule conformations has also been found under an uniform alternative current (AC) electric field on weak polyelectrolytes, such as poly(2-vinyl pyridine) or PVP, whose backbone charges are mobile.⁵² However, as strong polyelectrolytes, DNA molecules, whose charges immobile on their backbone, their chain condensation in a DC electric field is quite different and more complicated: the electric polarization is always coupled with the reorganization of their chain segments as described early while its mechanism is not yet fully understood. According to some Langevin dynamics simulation results, the change of local salt concentration could contribute to the conformation changes

FIG. 4. (a) Images of instantaneous conformations of DNA in still liquid and trapped in HFM, (b) histograms of the radius of gyration, and (c) histograms of DNA conformations away from coiled ball shape (represents as the ratio between the major and minor axes when treating molecules as ellipse objects, a/b) of λ -DNA molecules, which stay in still liquid and are trapped by HFM. Ball shape ($a/b = 1$) and a large a/b value means more anisotropic conformations. The measuring approaches used in (b) and (c) are similar to what were used in Ref. 51. The scale bars are $5 \mu\text{m}$.

(e.g., unfolding) of polyelectrolytes under a strong DC field.⁵³ We suspect that the observed tightly packed conformations of DNA in HFM might be attributed to the change of local ion strength around DNA segments because of electrohydrodynamic effects. But this argument needs further evidences to confirm.

B. Manipulation of the trapping location and concentration

The trapping plane in HFM can be adjusted by varying the electric field strength (or the flow rate). As demonstrated in Figures 5(a) and 5(b), the actual trapping location was shifted from close to the channel wall to $\sim 4.2 \mu\text{m}$ towards the channel centerline by increasing the overall electric bias from 60 V to 100 V. In Figure 5(a), some DNA molecules were focused so close to the channel bottom that they were partially attached to the solid surface and experienced some tethered stretching when released in flow. The trappings occurred everywhere along the entire flow channel and their locations vary spatially. As shown in Figure 5(c), DNA molecules in another viewing plane far away from the trapping location were found captured near the channel wall.

In HFM, charged molecules not only get trapped and relaxed but also accumulate around the trapping sites. As shown in Figure 5(d), a rapid increase of the trapped DNA molecule number was found in the first 15 s upon the establishment of an appropriate electric field, both at the sudden contraction location, “site A,” and our major viewing location, “Site B.” The sudden contraction site got more DNA molecules accumulated because of its special converging geometry (details discussion could be found in Ref. 41). Note: in the enhanced Fig. 1(c) online movie, a large number of stretched DNA molecules were seen to pass the viewing window a while later after the removal of the electric field. Those DNA molecules are the same ones, which were initially trapped at the sudden contraction location (“site A”) when the electric bias was on. The disappearance of “unstable trapping” events could contribute somewhat to this molecule accumulation. However, the major increase is believed from molecule immigrations from adjacent layers. The molecule accumulation slowed down gradually when the HFM trapping continued and approached a plateau if given enough time. The slowdown might come from the depletion of free DNA molecules and/or the change of local electric and molecule interactions in adjacent layers. This feature allows for capturing and concentrating molecules at desired locations, such as near the channel wall (i.e., the depletion layer^{44,54}) to study hydrodynamic interactions.

FIG. 5. (a)-(c) Snapshots of DNA in various trapping planes: near the bottom surface (a) and at $4.2 \mu\text{m}$ from the bottom surface (b) and (c) with the viewing window at the trapping plane (b) and near the flow channel centerline (c). (d) The accumulation effect of HFM. The bold red line and bold green line in (d) represent the instantaneous number of DNA trapped in the viewing window at Site ‘B’ and ‘Site A’ in Figure 2, respectively. The thin blue line presents the instantaneous number of DNA passing the viewing window in a shear flow alone. The scale bars are $10 \mu\text{m}$.

C. DNA sudden coil-stretch dynamics

HFM can also be conveniently used to study the coil-stretch dynamics in flow with uniform globule initial conformations. As shown in Figure 6(a), a steady shear flow was maintained at the beginning. An electric field (147 V/cm) was then switched on and maintained for sufficient

FIG. 6. The ensemble average fractional extension of ~ 200 λ -DNA when switching from “flow field” alone to “hybrid field” (a) and 60 λ -DNA molecules after the sudden removal of the electric field (c). (b) Time serial snapshots of an individual λ -DNA molecule from (c). Time is reset to zero when switching. (d) Re-plotting the transient response of DNA in HFM after adding a step flow stress ($Wi=15$) together with data from other researchers at various Wi .³⁸ (e) Plot of the dimensionless of power spectral density PSD of the extension fluctuation for DNA molecules in HFM in steady shear flow ($Wi=15$). The brown solid line is for a Lorentzian function with $PSD \sim 1/(a + f^2)$ where a is a constant, close to that of $Wi=0$. The scale bar is $5 \mu m$.

time (e.g., 15 s) to allow DNA molecules to get trapped and fully relaxed. The electric bias was then removed suddenly, causing instant imbalance of the external forces on those trapped DNA molecules. Under only the flow stress, their tightly packed chains experienced sudden deformation (i.e., stretching), analogous to release coiled DNA molecules in a steady shear flow. Different from single DNA dynamics study in startup flows, in which both macromolecules and flow were in their own deformation development,^{37–40} these two process dynamics are de-coupled in HFM: the flow is well developed while DNA molecules are executed a step flow stress. Unlike in other steady flows, in which DNA chains have various initial conformations and extensions, molecules in HFM were stretched from uniform size and nearly globule conformations to more extended states (Figure 6 and Ref. 46). Figure 6(b) showed the dynamics of an individual DNA molecule from a coiled conformation to extended conformations in response to a step shear stress (~ 0.16 Pa). For this specific DNA, it took ~ 1.25 s to reach 65% extension of its full length and then gradually returned to common chain deformation dynamics. Changes of the ensemble average extension ($\langle x/L \rangle$) of ~ 60 λ -DNA molecules in a steady shear flow after the sudden removal of the electric field was also calculated and shown in Figure 6(c). With a Weissenberg number of ~ 15 , the ensemble average extension of DNA molecules increased rapidly at the first 4–5 s (with a strain of ~ 0 –25) and reached its peak ($\sim 55\%$) at a strain of ~ 20 –25. The sudden stretching influence of HFM slowly diminished afterwards and the ensemble extension of λ -DNA molecules gradually recovered to the steady-state value ($\sim 35\%$). To make further comparison with other pioneering work, the same set of data was re-plotted by adding data from Babcock *et al.* measured for diluted λ -DNA solution in a startup shear flow under various Wi (Figure 6(d)).³⁸ The extension overshoot was clearly shown for the transient response of DNA in HFM at $Wi = 15$, much pronounced than the Babcock's result at $Wi = 19$ and 38. As the extension overshoot in shear flow was believed as the result of the initial chain expansion prior to their alignment to flow, this suggests that DNA chains in HFM had a large portion in more extended states than that in a start-up shear flow during their transient response to flow stress. Such large average extension is attributed to their monotonic initial conformations (tightly packed globule shape) and the application of a true step function of the flow stress.

We also examined the extension fluctuation of DNA chains in steady shear flow (for strain in excess of 50). The power spectral density per unit time (PSD) was calculated using a fast Fourier transform (FFT). The extension data was first subtracted by their mean and then multiple with a Welch window function. The PSD for all data was normalized according to Parseval's theorem and plotted versus frequency in dimensionless unit. The extension fluctuations of DNA chains in HFM in steady shear flow shows similar spectral trend with what others observed early (Figure 6(e)):^{37–40} a fast decline in the intermediate frequencies followed by a modest decay at the high frequencies with no distinct peaks in the entire power spectra. This suggests that the stretching and contraction of DNA chains with HFM in steady shear flow is similar to those starting with random initial conformations. The contribution of monotonic initial conformations (i.e., size and shape) to DNA deformation dynamics is largely limited to their transient response to flow stress.

V. CONCLUSIONS

In summary, we successfully demonstrated a new HFM approach to conveniently manipulate the conformations and coil-stretch dynamics of charged macromolecules with a combination of flow and an electric field. Charged macromolecules, such as λ -DNA, were trapped, coiled, and accumulated at appropriate moments and desired locations in a continuous flow with the hybrid field while deformed from uniform initial conformations when suddenly removing the electric field. The same regulation strategy might be useful for mechanics study of other charged macromolecules (e.g., protein unfolding/refolding).

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