

Instantaneous DNA Electrophoretic Mobility

Electrophoretic mobility (μ_{ep}) is defined as the velocity of charged species gained under unit electric field strength ($\mu_{ep} = U_{ep}/E$, where U_{ep} is the velocity of charged species and E is the local electric field strength). μ_{ep} reflects the moving ability of charged species under electrophoresis (EP), which depends on the physical and chemical properties of charged species and the medium (e.g., size, ion strength, viscosity, etc). It is an important parameter widely used in electrokinetics-related research and practice, including gel electrophoretic separation and electrokinetic flow control. DNA, in many cases, is the object in such applications. Its free solution mobility and relationship between mobility, buffered solutions and DNA size have been well documented. DNA molecules mostly remain in their coiled configurations in free solutions, while their molecular chains often experience coil-stretching dynamics in DNA separation applications carried out in a gel or an artificial sieving matrix. A decreased EP mobility was observed when such dynamic transitions occurred frequently in the flow system. As highly charged macromolecules, DNA chains will have more charge exposed on the surface when stretched than when coiled. However, this effect is often neglected, and a constant DNA EP mobility is widely accepted and used in DNA dynamic studies.

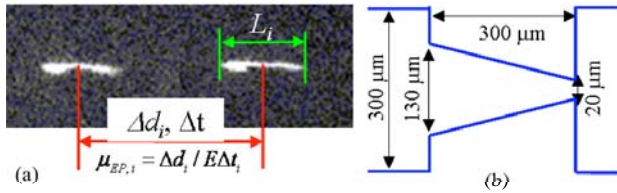


Figure 1. (a) Approach for the measurement of instantaneous electrophoretic mobility; (b) dimensions of a converging microchannel used in experiments.

Electrophoretic Mobility Measurement

There are several ways to quantify the DNA EP mobility, including the use of capillary zone electrophoresis (CZE), electrophoretic light scattering (ELS), and optical velocimetry techniques. These approaches all rely on the following equation:

$$\mu_{EP} = \Delta d / E\Delta t \quad (1)$$

where E is the local electric field strength, Δd the displacement of charged species within a time interval of Δt . Different methods quantify Δd and Δt differently. In CZE, Δd is the distance between the injection and detection locations (>10 cm) and the traveling time (Δt) is determined by the UV signal carried by DNA molecules. ELS determines Δd and Δt by measuring the Doppler shift of light scattered from moving DNA molecules. Both methods provide an average value of EP mobility from multiple DNA molecules. A recent development in microscale particle tracking velocimetry (μ PTV) can obtain instantaneous velocity of a single

charged particle. Research in single DNA dynamics enables the identification of various configurations of DNA during the coil-stretch transition. By combining these two technologies, we are able to obtain both the velocity and configuration information of individual DNA molecules in a well-defined electric field. Firstly, we tracked each individual DNA molecule and recorded its configuration at each moment. Then, by measuring the displacement of its center-of-mass in two adjacent time intervals, a transient mobility number was obtained from Equation 1 (shown in Fig. 1a). The EP mobility obtained this way is called “instantaneous EP mobility” ($\mu_{ep,i}$) in our study. Our goal is to correlate this value with DNA configuration at each moment.

Instantaneous Electrophoretic Mobility ($\mu_{ep,i}$)

DNA dynamics by electrokinetics has been carried out in various microfluidic geometries, including straight channels, converging channels, and cross-slot flows in our laboratory. It was found that both the configuration of DNA molecules and their orientation to the electric field affects the motion of DNA in a given electric field. To isolate these factors, a converging microchannel was used in this study (Fig. 1b). The focused electric field in this particular geometry overcame the relaxation of DNA molecules and allowed DNA to remain in stretched configurations along the entire contraction portion. More importantly, the stretched DNA molecules aligned in their moving direction avoided the possible influence of their orientation on DNA migration. This simplifies our EP mobility measurements.

As shown in Figure 2, $\mu_{ep,i}$ of a single DNA molecule varied substantially in the microchannel, and there is no simple relationship to correlate its value to the DNA length. In this particular case, $\mu_{ep,i}$ of DNA can vary as much as 1.5 times with the same chain length. On the other hand, chain length as different as 3 times showed the same $\mu_{ep,i}$. Collecting the $\mu_{ep,i}$ data of 50 λ -DNA molecules in the converging channel shows a very wide spread, as presented in Figure 3. Further investigation,

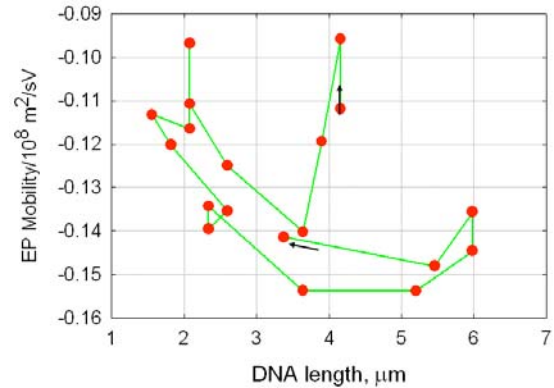


Figure 2. Instantaneous EP mobility of a λ -DNA molecule in the converging microchannel. The arrows indicate the sequential change of its configuration.

however, leads to some interesting observations. Typically, DNA with a coiled configuration (the smallest length, $\sim 1.3 \mu\text{m}$) has the lowest $\mu_{\text{ep},i}$. With an increase of its chain length, $\mu_{\text{ep},i}$ increases. The mobility data becomes highly scattered when the DNA chain length reaches $10 \mu\text{m}$.

Correlation between DNA mobility and configurations

DNA mobility was further correlated with their configurations by classifying them into several groups, as shown in Figure 3b. DNA molecules that hold the coiled configuration have the smallest EP mobility (group 1, Fig. 3b). With a slight change of its configuration from a spherical to an oval shape, $\mu_{\text{ep},i}$ increases rapidly, i.e., 7 times (group 2, Fig. 3b). However, a continuous increase of the DNA chain to a long rod shape leads to a much slower increase of $\mu_{\text{ep},i}$ (group 3, Fig. 3b). $\mu_{\text{ep},i}$ eventually levels off with a further increase of the DNA chain length (group 4, Fig. 3b). More complicated DNA configurations, such as a tadpole shape, can be considered as a combination of oval and stretching configurations. DNA with such configurations show a $\mu_{\text{ep},i}$ smaller than that with an oval shape, but larger than that with a long rod shape. Other configurations can also be treated in the same way—as the combination of several simple configurations—and their $\mu_{\text{ep},i}$ can be estimated

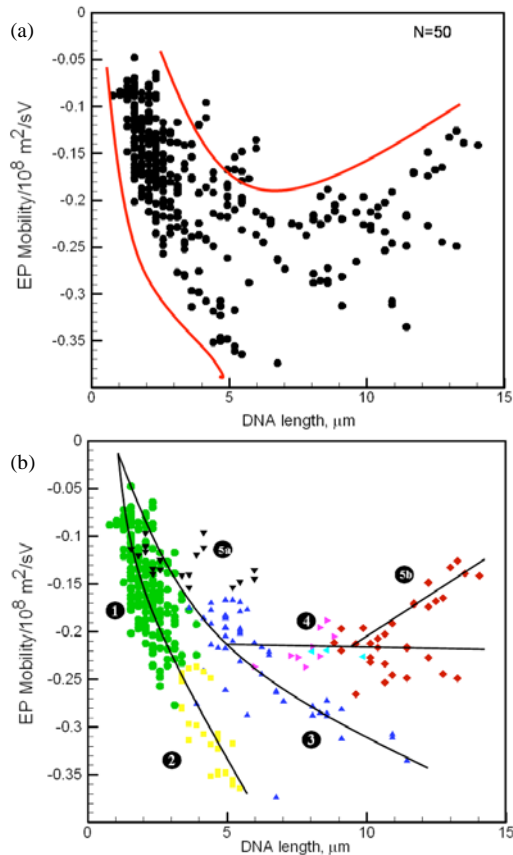


Figure 3. Instantaneous EP mobility data of 50 λ -DNA (a) as a function of DNA length and (b) as a function of DNA configuration and its configuration dynamics.

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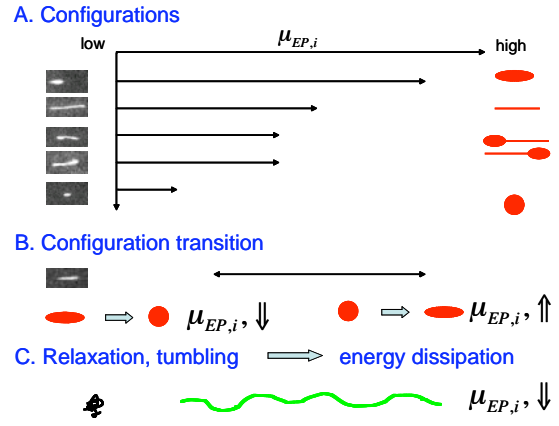


Figure 4. Instantaneous EP mobility as a function of DNA configuration and its configuration dynamics.

correspondingly. Unstable chain motion also leads to a lower $\mu_{\text{ep},i}$. As shown in Figure 3b, group 5, both tumbling (at short chain length, group 5a) and chain relaxation (at long chain length, group 5b) result in lower $\mu_{\text{ep},i}$ at the same chain length. Configuration transition also affects $\mu_{\text{ep},i}$. For example, if a DNA chain changes from a spherical shape to an oval shape, its $\mu_{\text{ep},i}$ will increase. The opposite change results in a decrease of $\mu_{\text{ep},i}$. Figure 4 summarizes the correlation between measured $\mu_{\text{ep},i}$ and observed major configurations and configuration changes.

EP mobility is often expressed as

$$\mu_{EP} = q / \xi \quad (2)$$

where q is the total effective charges carried by DNA molecules, and ξ is the total drag force contributed by the surrounding liquid. The configuration-related changes of $\mu_{\text{ep},i}$ result from the variance of either q or ξ , or both. Even though our measurements cannot distinguish the contributions from these two factors, their overall effect is reflected in the measured $\mu_{\text{ep},i}$. The stretched configuration of DNA, in particular the oval shape, may lower the drag force with a decreased chain diameter and possibly a higher effective charge because there is more surface exposure. When DNA chains become too long, too much surface area may lead to an increase of drag resistance. Eventually, these two effects reach a balance, and $\mu_{\text{ep},i}$ becomes relatively independent of the chain length (group 4, Fig. 3b). The transient relaxation of long DNA chains and tumbling of short DNA coils causes more energy dissipation and consequently lower $\mu_{\text{ep},i}$ (group 5, Fig. 3b). This is why DNA molecules with the same chain length may have different $\mu_{\text{ep},i}$ values. We are carrying out more research to quantitatively correlate the $\mu_{\text{ep},i}$ of DNA with its configuration and configuration dynamics, especially how q or ξ vary in DNA dynamics.

Publications

1. S. Wang, W.-C. Liao, X. Hu and L. J. Lee, "DNA EP mobility as a function of its configurations and configuration changes", (to be submitted).