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Molecular Propulsion: Chemical Sensing and Chemotaxis of DNA Driven by RNA Polymerase

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RNA polymerase (RNAP) is one of the best characterized motor proteins which transcribes on a DNA template while presenting unique motor properties.[1] The powerful tracking ability of RNAP underlies devices supporting nanoscale motion control on planar substrates.[2] Nevertheless existing experimental systems that track protein motor activities are restricted to tethering the substrate or the motor and, therefore, cannot support observation of autonomous movement modulated by the motor in free solution. Although autonomous control has been reported by metallic, reactive colloids,[3] it has not been demonstrated with biomolecules under physiological conditions. Here we show the first experimental evidence that a molecular complex consisting of just a DNA template and associated RNAPs displays chemokinetic motion driven by transcription substrate NTPs. Furthermore this molecular complex exhibits biased migration into a concentration gradient of NTPs for mimicking cellular chemotaxis.

We studied a robust transcription system using T7 RNAP and a rod-like 310 bp DNA template bearing a T7 transcription promoter sequence. For detection, the upstream portion from the promoter of the template was fluorescently labeled. Apparent diffusion of DNA was quantified by Fluorescence Recovery After Photobleaching (FRAP).[4] The diffusion coefficient (D) for the 310 bp DNA in transcription buffer without Mg^{2+} was 15.3 $\mu m^2/s$, which is 18.6% slower than the 18.8 $\mu m^2/s$ value obtained from theoretical calculations *via* Broersma's equations.[5] We attribute this difference to impedance of DNA motion by the gel matrix (added to eliminate convection) and fluorochrome labels attached to the template.

We evaluated the motility of the DNA−RNAP complex in the presence of substrate NTPs as compared to controls prepared from the same aliquots of reagents, but without NTPs substrates (Figure 1). We observed a systematic increase (25.2% on average) of apparent diffusion of DNA when transcription is enabled. Our control showed that DNA diffusion is invariant over the ionic strength range examined in our experiments. An increase in ionic strength by monovalent Li^+ (included in added NTPs) should not significantly affect diffusivity. Therefore, RNAP motor activity must be the major cause that renders the faster movement of DNA, a phenomenon we describe as "Molecular Propulsion." While transcribing on the DNA track, the RNAP motor vigorously pushes and pulls on the template DNA against fluid viscous drag, affecting hydrodynamic interactions between DNA and surrounding water layers. A fraction of the free energy released by the transcription reaction cycle is converted into powering the transient conformational changes of DNA.[6,7] Because the free energy liberated[8] significantly exceeds the energy of thermal fluctuation (k_BT), apparently the RNAP action generates sufficient hydrodynamic interaction for coupling transcriptional actions into autonomous motion of DNA for overcoming Brownian diffusion. Consequently untethered DNA becomes self-propelled and displays a molecular version of chemical sensing imitating cellular chemokinesis.

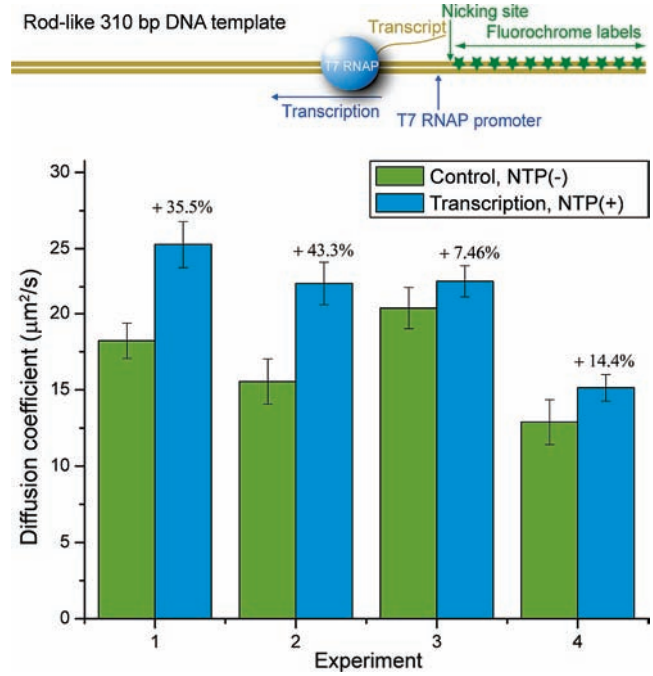

Figure 1. FRAP measurement of chemokinesis by T7 RNAP transcription. Error bar: standard deviation. Percentage increase in the apparent diffusion coefficient compared to Brownian diffusion is shown. We further examined transcription elongation and initiation as possible governing factors in Molecular Propulsion (Figure 2). By adding 3′-dNTPs to the NTPs at 1:100 ratio, the length of transcription elongation is truncated from the runoff full length (227 nt) to roughly 100 nt. Comparisons of apparent DNA diffusion between runoff transcription and 100 nt transcription showed no significant difference beyond experimental fluctuation. Increasing the ratio of 3′-dNTP vs NTP to 1:10 traps the majority of transcription complexes in the initiation stage. Here greater DNA motility relative to runoff transcription was observed (∼15%). This result suggests that RNAP action during the initiation stage may strongly propel the template where a scrunching machine mechanism[6] transiently distorts the template. In another experiment using NTP substrates without the Mg^{2+} cofactor, DNA also showed an enhanced apparent diffusion (∼15%) above runoff transcription, which indicates that bound RNAP retains propulsion activity induced by NTP substrates even though productive RNA synthesis is prohibited. Presumably, the initiation complex undergoes a dynamic transition between conformations in the absence of the stabilizing magnesium.[9]

We then tested whether chemokinesis by RNAP Molecular Propulsion becomes chemotactic within a concentration gradient

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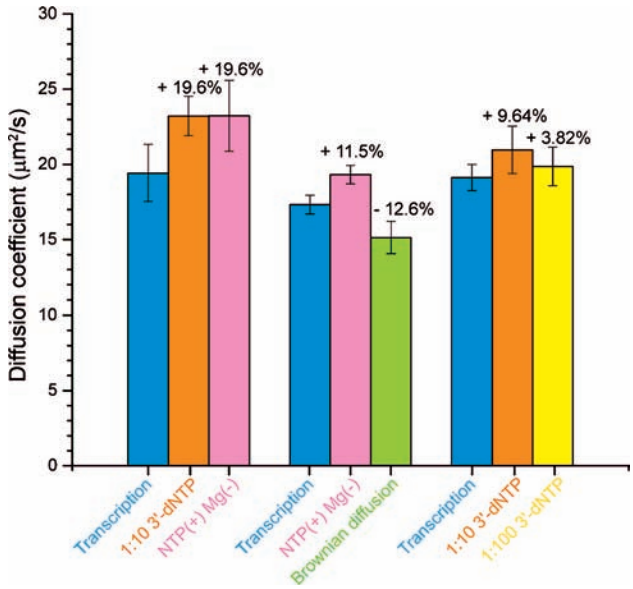

Figure 2. Dependence of diffusivity on elongation and initiation: comparison with runoff transcription. Percentage difference of diffusion coefficient compared to the runoff transcription within each set is noted. Error bar: standard deviation.

of NTPs. We constructed a device made of polydimethylsiloxane (PDMS) to establish an NTP gradient. We show that chemical gradients patterned in this device persist throughout the course of our chemotaxis experiments (Supporting Information).

We loaded two of the four peripheral ports with NTPs while leaving the others unchanged with just transcription buffer as controls. After loading (2 h), when the NTP gradient was established, the center hub was loaded with fluorescently labeled DNA template mixed with T7 RNAP in transcription buffer. Migration of DNA template toward each port was followed by time course imaging of fluorescence profiles in the device. The amount of DNA in all ports increases with time as the DNA template migrates into the ports due to diffusion. Meanwhile, Molecular Propulsion becomes activated on DNA−RNAP complexes that encounter the NTP gradient, yielding additional autonomous movement. The temporal increase of DNA amount in each port due to DNA migration toward the gradient is plotted and compared to the control as the "bias" (Figure 3).

Greater amounts of DNA template accumulated in the ports loaded with NTPs were observed throughout the course of our experiments, demonstrating chemotaxis. Slightly negative biases observed at the two earliest time points may have resulted from anomalous DNA motion upon loading, as well as error in fluorescence measurement especially for low level signals. The two curves diverge with time, showing a collective 20% bias at the end of the time course. The rate of bias increase is maximal in the initial stage of the experiment when the amount of substrate, degree of gradient, and RNAP enzyme activity are optimal. Our results demonstrate that Molecular Propulsion of DNA mediated by RNAP transcription responds to geometric inhomogeneity of chemical substrates in the solution. Resembling active chemotaxis in cellular transport, Molecular Propulsion engendered net DNA transport toward a greater concentration of transcriptional substrates within a patterned gradient.

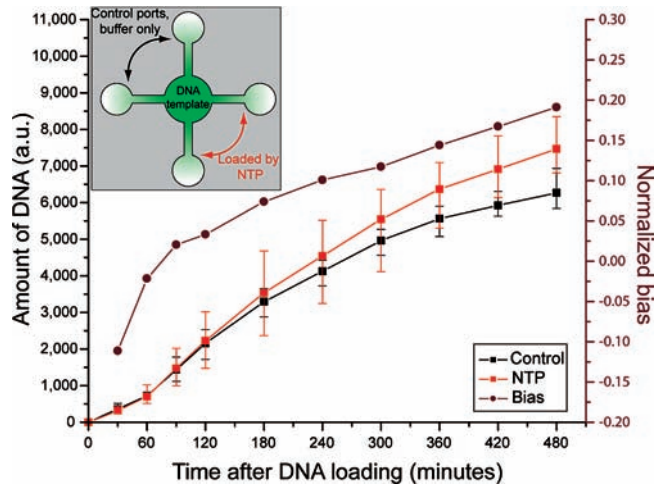

Figure 3. DNA chemotaxis: biased DNA transport toward NTP gradient. Error bar: range. Bias [normalized, $(F_{NTP} - F_{control})/F_{control}$; purple line] reveals enhanced DNA transport toward the NTP gradient, demonstrating DNA chemotaxis mediated by transcription.

We have shown here that a protein motor directionally guides the net transport of DNA template molecules in response to external chemical signals. Given the need for new experimental systems that enable acquisition of very large, complex data sets, Molecular Propulsion will support massively parallel assays where each individual molecular complex in a population regulated by experimental factors reconfigures autonomously and becomes a discrete experiment. As such, the simple approach presented here points the way toward greatly miniaturized and localized "intelligent" control of experimental systems[10,11] by virtue of the intrinsic properties of the comprised molecular components.

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Supporting Information Available: Supporting methods and results. This material is available free of charge via the Internet at http://pubs.acs.org.

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