In thermophoresis, with the fluid at rest, suspensions move along a gradient of temperature. In an aqueous solution, a PEG polymer suspension is depleted from the hot region and builds a concentration gradient. In this gradient, DNA polymers of different sizes can be separated. In this work the effect of the polymer structure for genomic DNA and small RNA is studied. For genome-size DNA, individual single T4 DNA is visualized and tracked in a PEG solution under a temperature gradient built by infrared laser focusing. We find that T4 DNA follows steps of depletion, ring-like localization, and accumulation patterns as the PEG volume fraction is increased. Furthermore, a coil–globule transition for DNA is observed for a large enough PEG volume fraction. This drastically affects the localization position of T4 DNA. In a similar experiment, with small RNA such as ribozymes we find that the stem–loop folding of such polymers has important consequences. The RNA polymers having a long and rigid stem accumulate, whereas a polymer with stem length less than 4 base pairs shows depletion. Such measurements emphasize the crucial contribution of the double-stranded parts of RNA for thermal separation and selection under a temperature gradient. Because huge temperature gradients are present around hydrothermal vents in the deep ocean seafloor, this process might be relevant, at the origin of life, in an RNA world hypothesis. Ribozymes could be selected from a pool of random sequences depending on the length of their stems.

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fraction (Fig. 1A; Materials and Methods). The maximum temperature difference is 5.0 K and typical temperature gradient ~0.25 K/μm. Thermophoresis depletes a PEG polymer from the hot region and builds a concentration gradient of PEG (5). As PEG concentration increased, we observed that solutes such as DNA and RNA showed depletion, ring-like localization, and accumulation (Fig. 1B) (7). We have proposed a phenomenological model that takes into account hydrodynamics effects driven by the PEG osmotic pressure gradient (7, 20–26). The equation for the flow of PEG molecule $J_{PEG}$ is

$$J_{PEG} = -D_{PEG}c_{PEG} - D^TPEG\nabla c_{PEG},$$

where $c_{PEG}$ is the PEG concentration, $D_{PEG}$ is the PEG diffusion constant, and $D^TPEG$ is the thermal diffusion constant. The PEG concentration gradient in a radius $r$ from the heated center, at steady state, yields

$$c_{PEG}(r) = c_0 \exp[-S^TPEG(T(r) - T_0)],$$

where $S^TPEG$ is the Soret coefficient, defined as $S^TPEG = D^TPEG / D_{PEG}$, $T(r)$ and $T_0$ are temperatures at $r$ and at infinity, respectively. In our experiment $S^TPEG$ is 0.064 [1/K].

Given the presence of another solute, DNA, of small volume fraction, whose concentration is $c$, it experiences thermophoresis and diffusiophoresis, the effect of the PEG concentration-dependent restoring forces (21). The flow of DNA (and RNA), $J$, can be written phenomenologically as

$$J = -Dc - cDVT + cu,$$

where $u = \frac{k_BT}{S^TPEG - 1/T}\lambda^2 c_{PEG}(r)VT$ is the diffusiophoretic velocity of DNA, $k_B$ is the Boltzmann constant, $\eta$ is the viscosity of the bulk solution, and $\lambda$ is the depth of a steric repulsion of PEG from the surface of DNA (7, 25). Given a PEG radius of gyration $R_g$, its center of mass is expelled up to a distance $R_c$ from the DNA, thus $\lambda \approx R_c$. In addition, in the presence of a temperature gradient, the thermal energy has a spatial dependence $k_BT(r)$, which leads to the

$$-1/T \text{ term in } u.$$

At steady state $J = 0$, the distribution of the DNA concentration $c(r)$ is

$$c(r) = c_0 \exp[-S^{DNA}(T(r) - T_0) + (c_{PEG} - c_{PEG}(r))/V],$$

where $c_0$ is the DNA concentration at infinity, $V = 2\pi a_l^2$ (no slip condition), and $a$ is the radius of DNA. According to this model, ring-like localization results from the interplay between thermophoresis and diffusiophoresis originating from the osmotic pressure of PEG near the surface of DNA (7). Small salt ions concentration is present in the solution, but we can neglect their effects for this localization as they have a large diffusion constant.

Fig. 2A shows the phase diagram of the localization patterns as a function of the DNA length from 250 bp up to 166 kbp of T4 DNA (T4 phage genome DNA) as a function of the PEG polymer concentration. We find that the position of the radius of accumulated dsDNA, $r(N_D)$, exhibits a nonmonotonic behavior as a function of DNA length with a minimum at $N_D = 5 \times 10^3$ base pairs (bp) (Fig. 2B). For long DNA from 5.6 to 166 kbp, the diameter of ring localization expands. For short DNA up to 5.6 kbp and RNA up to 3 kbp (7) the ring diameter decreases, following a behavior analogous to gel electrophoresis. The ring diameter decreases linearly from 2.0 to 3.5% but changes little from 3.5 to 4.5% (Fig. 2C).

**Thermophoresis of T4 DNA in a PEG Solution.** Why does dsDNA longer than 5.6 kbp show a wide range of ring-like localization?

This hints at a change in the DNA configuration that occurs around this length. The origin of this transition is the large difference in the natural length scale of the polymers: PEG has a persistence length of about $a_P = 0.35$ nm, whereas the persistence length of dsDNA is larger by two orders of magnitude: $a_P = 50$ nm (150 bp). Thus, short dsDNA of up to a few hundred base pairs stays only a few persistence lengths long and therefore is in the semiflexible regime, a rather stiff rod with a few bends surrounded by the much more flexible PEG. Only longer dsDNA strands have enough twists and turns to make a polymer blob, which geometrically defines an “inside” and an “outside” and a corresponding PEG concentration gradient. When the DNA length becomes large enough it is then compressed and moves away from the trap as if it is a small DNA. Then, the osmotic pressure of PEG of order $k_BT/a_P^2$ will overcome the resistance of the DNA blob and lead to intrachain DNA condensation (SI Appendix) (15–17, 27, 28).

To verify this hypothesis, we move to single-molecule studies of the T4 phage genome DNA. Because T4 DNA is 166 kbp long and 57 μm in end-to-end distance, this DNA is large enough to be detected and tracked as individual DNA.

**PEG Induces the Compaction of Single T4 DNA.** Single DNA visualization was performed in various PEG concentrations but in the absence of a temperature gradient. T4 DNA in a water solution buffered with Tris elongates and subsequently relaxes to an equilibrium state within a second, like a random coil. The length of T4 DNA stretching due to thermal conformational fluctuation, averaged over more than 100 molecules, was 3.5 μm. We find a transition for the mean length of stretched axis for T4 DNA as a function of PEG volume fraction (Fig. 3). The onset of compaction for T4 DNA is around 2.0% PEG volume fraction (Fig. 3A). From 2.0 to 5.0% PEG, a certain proportion of DNA is compacted into a single dot whereas others fluctuate as semiflexible polymers. Furthermore, as the PEG concentration is increased, the proportion of compacted DNA increases as in a subcritical bifurcation (15, 29).

Note that the PEG volume fraction at the onset of the coil-globule transition is close to the calculated critical volume fraction $\phi_c$ of 1.5% PEG (30, 31) but smaller than the $\phi_c$ of 4.0% obtained from the measurement by Hansen et al. (32). This difference may
come from the density of DNA in our experiment and the positive charged ions present. The condensation of T4 DNA occurs at a lower PEG concentration in the presence of small salt, called Ψ condensation (33–35).

A dsDNA blob within a PEG solution is subjected to three forces: the osmotic pressure of PEG due to the lower PEG concentration inside the blob, the osmotic pressure of the counterions that neutralize the charges along the DNA, and the elasticity of the DNA that opposes compression and extension away from the free chain gyration radius. In thermodynamic equilibrium the three corresponding pressures should balance each other. Because PEG particles are free to exchange between the blob and it surrounding, the chemical potential of the PEG is also equilibrated between the inside and the outside. Analysis of the thermodynamic equilibrium (SI Appendix) exhibits a subcritical bifurcation in the folding as a function of PEG concentration (Fig. 3B): below Ψ ≥ 2.0% the DNA is in a dilute “coil” configuration, whereas above the coil configuration coexists with a dense globular state of much smaller DNA particles. Force balance acting on the DNA determines the size of the coil and the globule. In the coil state the elasticity opposes the expansion driven by the counterion pressure, whereas in the dense globule the elasticity resists the compression due to the PEG concentration gradient.

Dynamics of Single T4 DNA in Temperature Gradient and PEG Concentration Gradients. We combine single DNA observation with the thermophoresis experiment. Single T4 DNA was clearly detected within the area of ring-like localization in 2.5% PEG solution (Fig. 4A). Individual T4 DNA was trapped under a PEG concentration and temperature gradients with observable fluctuations.

We then examined the dynamics of thermophoresis of single T4 DNA. Thermophoresis excludes DNA away from the hot region whereas a PEG concentration gradient pushes it back to the center. According to this scenario, when T4 DNA forms ring-like localization, two opposite motions, out from hot to cold in the center and from cold to hot in the periphery, occur. To test this argument we track the motion of single T4 DNA during the ring-like localization in various PEG concentrations.

Fig. 4B shows T4 DNA particle trajectories as a function of time. We detect single T4 DNA moving along a temperature gradient in 1.5%, 2.5%, and 4.0% PEG solutions (36) and show representative trajectories. In 1.5% PEG, at which ring-like localization occurs, T4 DNA exhibited thermal Brownian motion and gradually moved to the region of ring-like localization (Fig. S1). In addition, thermophoretic motion escaping from the hot region is observed in a part of T4 DNA (Fig. 4B, Left). In 2.5% PEG solution, where ring-like localization occurs, DNA is immediately expelled from the hot region, but after about 1 s DNA gradually moves from the periphery inward (Fig. 4B, Center). From 3.0 to 4.0% PEG solutions all T4 DNA move from the cold periphery to the hot center (Fig. 4B, Right). Motion from hot to cold is rarely observed. This result indicates that the force from the PEG concentration gradient occurs instantaneously and plays a dominant effect for ring-like localization, building a potential wall.

In both 2.5% and 4.0% PEG, the direction of velocity shows no correlation effects subsequent to the onset of thermophoresis but less fluctuation as T4 DNA approaches its localization position (Fig. S2). Ichikawa et al. have shown that the relaxation time for T4 DNA stretching is ~3 s (26), which is much longer than the

![Fig. 2.](image-url) Fig. 2. (A) Phase diagram of depletion, ring-like localization, and accumulation for DNA molecules from 250 to 166 kbp. (B) Ring radius r (Nφ) as a function of DNA length Nφ in 2.5% PEG solution. Lines are fitted to DNA below 5.6 kbp (purple line) and above 5.6 kbp (blue line). (C) Ring radius with respect to PEG volume fraction.

![Fig. 3.](image-url) Fig. 3. Single T4 DNA in a PEG solution. (A) Single T4 DNA molecules in 0%, 2.0%, and 5.0% PEG solutions. Scale bars, 4 μm. Actual size of compacted DNA is a few tens of nanometers (15, 16) below the diffraction limit of our epifluorescent microscope. (B) (Left) Mean sizes of coil and globule DNA as a function of PEG volume fraction. (Right) Probability distributions of the stretched axis for individual T4 DNA. Red dotted lines are fitted curves with two Gaussian functions.

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relaxation observed in our experiment. This implies that the observed fluctuation in the T4 DNA motion is dominantly Brownian.

Moreover, we found that the velocity of single T4 DNA increases from 2 to 3 μm/s, 30 μm away from the localization spot to 10 μm/s, very close to the localization region in 4.0% PEG solution (Fig. 5). The change from Brownian to persistent motion with slight acceleration indicates that the PEG potential wall is sharp close to the localization.

**Thermophoresis and Sequence Dependence of Small RNA.** In the RNA world hypothesis, tiny RNA polymers with stable sequences must emerge and be selected from a pool of random sequences. They might be the precursor motif of ribozymes. In this second part, we investigate small RNA and show that thermal gradient allows some sequence selection of small RNA.

RNA itself can also be selected in a temperature gradient in the presence of PEG of 5.0% volume fraction. The selection will depend on the RNA folding state. The stem–loop structure is the basic motif for RNA folding with a rigid double-stranded stem and a flexible loop. We show that no accumulation is observed for short poly-thymidine oligodeoxyribonucleotides (poly-T) and poly-uracil oligoribonucleotides (poly-U) where no stems are present, whereas accumulation is observed for stem length longer than 5 bp but a depletion below. The rate of accumulation increases as the stem length (number of base pairs) is quantified in Fig. 6B. We found that stem–loop RNA/DNA shows an accumulation for stem length longer than 5 bp but a depletion below. The rate of accumulation increases as the stem length elongates. Moreover, a self-acylating ribozyme with 6-bp double strand (37, 38) also exhibited an accumulation (Fig. 6B). This suggests that the length of rigid stem is important for the force generated by the PEG concentration gradient.

We examined this hypothesis by studying the thermophoresis of a folded, double-stranded RNA (dsRNA) and dsDNA in the PEG solution. A similar transition curve from depletion to accumulation above 8% was observed (Fig. 6C). However, for a conformation of 40 nucleotides poly-U ssRNA and up to 124 nucleotides poly-T ssDNA, accumulation was not observed (Fig. 6C). This provides evidence that the Soret effect in the polymer solution is sensitive to the total length of stems for an RNA or DNA polymer. The 8-bp stem length (∼2.7 nm) is comparable to the pore size of 5.0% PEG network (∼4 nm). However, the estimated size of ssRNA/ssDNA without a 40-bp stem (∼5 nm) is also comparable to the PEG network (39). This suggests that the polymer rigidity due to the presence of a double-stranded stem rather than its size affects the osmotic force from a PEG concentration gradient.

**Salt Dependence of Sequence Selection.** In addition, the accumulation is reduced as the concentration of charge salt increases and dsDNA/dsRNA uniformly distribute eventually in 500 mM NaCl or 50 mM MgCl$_2$ (Fig. 7). This suggests that charge effects such as electrostatic screening and dipole interaction between dsDNA/dsRNA and PEG mediate the accumulation, whereas the depletion of ssDNA/ssRNA become small in the presence of high concentration of salts (Fig. 7). The effective Soret coefficient at 500 mM NaCl is comparable with the offset of the Soret coefficient in water. The charge of the salt may change the size of the ssDNA and ssRNA in the measured salt concentrations because the osmotic pressure from PEG compresses RNA, whose electrostatic repulsion is significantly screened. It may also be likely to consider the change of molecule size (40, 41) to account for the change of the effective Soret coefficient for ssDNA and ssRNA.

**Conclusion.** We have described an experimental study of thermophoresis for long-genome DNA and short RNA. In the presence of another polymer of large volume fraction, those biopolymers move toward the heated center, whereas they are expelled from the hot region in a simple water solution. The PEG concentration gradient generates entropic forces on DNA counterbalancing thermophoresis. At the intermediate PEG concentration, this interplay localizes DNA as a ring-like pattern. We also observe that the size of ring-
like localization of DNA has a singular point at around 5.6 kbp. The model based on the semiflexible polymer chain surrounded by PEG molecules predicts that the effect of condensation appears at ∼10 kbp, which is in good agreement with our experiment. Indeed, our single-molecule observation confirmed that long DNA such as T4 DNA is compacted in the presence of more than 2.0% PEG, and this dramatically changes the DNA size from 3.5-μm length of the long major axis to less than 0.5 μm (15, 17). The reduction of effective DNA size may decrease the entropic force from PEG concentration gradient and result in the wider range of ring localization from 2.0 to 4.5% PEG. We also measured the local PEG concentration where T4 DNA was localized. The obtained values were correspondingly 1.8–3.5% PEG. In this PEG range DNA is condensed.

The tracking of individual single T4 DNA allowed us to analyze the dynamics of thermophoresis in a polymer solution. One key observation was the acceleration of T4 DNA when it approaches the stable point where T4 DNA localizes. The velocity of T4 DNA is proportional to the gradient of PEG concentration and it migrates toward the localization point (7). There the DNA molecule has fastest velocity and this mechanism is consistent with our observation.

In the second part we studied thermophoresis of short RNA such as a ribozyme (18, 37). Given the small radius of gyration of PEG, entropic force can act on short RNA and DNA. It leads to accumulation in a temperature gradient. The interesting result is that this entropic force overcomes thermophoresis only for a long enough stem in stem–loop RNA. Thermal separation of DNA and RNA might be relevant to molecular evolution at the origin of life: Separation of small RNA from a large library of RNA world might occur at the thermal vent. Thus, separation and accumulation are physically feasible in a temperature gradient at the early stage of life (4, 5, 7).

Intriguingly, we have shown recently that we can trap shorter RNA (1.0 kb) of a small volume fraction in a gradient of longer RNA (rRNA) of large volume fraction (Fig. S3). This result means that our finding is a general phenomenon, and does not depend on the PEG specificity.

**Materials and Methods**

**Experimental Setups for Thermophoresis.** The solution is enclosed in a polydimethylsiloxane (PDMS, Sylgard 184; Dow Corning) microchannel. A single channel 10 μm thick and 500 μm wide was closed with a cleaned glass slide to suppress thermal convection and sealed with a fast-curing epoxy (Araldite Rapid, Huntsman). The whole experiment was maintained at room temperature (T₀ = 24 °C). The temperature gradient ∇T was built by focusing an infrared laser beam (FOL1402PNJ; Furukawa Electronics, λ = 1480 nm) on the PDMS chamber with the objective lens ×32. Dieletrophoresis due to the electric field of laser light is negligible. Temperature difference at the radial distance was measured by the intensity drop of fluorescent dye 2′,7′-bis(2-
carboxyethyl)-5-(6)-carboxyfluorescein (BCECF, Molecular Probes) at 1 mM solution. Fluorescence of BCECF decreases linearly with a slope of $-1.3\%$K. Maximal temperature increase is $\Delta T_{\text{max}} = 5$ K for T4 DNA experiment and $\Delta T_{\text{max}} = 3$ K for small RNA/DNA experiment. We used smaller temperature gradient to fit to exponential distribution (see $S^\text{eff}$ in Effective Soret Coefficient for Small RNA) without ring-like localization. Small RNA can show the localization as well under a bigger temperature gradient.

Visualisation and imaging. For thermostoresis experiment, we visualized T4 DNA, small RNA, and small DNA with SYBR gold fluorescence (Molecular Probes). Fluorescence of DNA and RNA molecules was detected using an epifluorescence microscope (Olympus IX70) and recorded with the Retiga CCD camera (QImaging). SYBR gold dye depends on temperature with linear slopes of $-3.2\%$/K and $-1.1\%$/K for ssDNA/ ssRNA and dsDNA/ssDNA, respectively. We thus obtained relative concentration of DNA/RNA after the rescaling of this temperature dependence.

We tracked single T4 DNA stained by YOYO-1 instead of SYBR gold. Time-lapse movies of thermostoresis for single T4 DNA were taken at a camera set for 10 frames/s, maximum binning, region of interest of 85 $\mu$m $\times$ 85 $\mu$m. Image analysis was performed with MATLAB software (MathWorks). We measured the center of mass of single DNA from fluorescent intensity profile by fitting a 2D Gaussian distribution function $I(x, y) = I_0 + I_1 \exp \left[-(x^2 + y^2) / (2 \sigma_x^2 + 2 \sigma_y^2)\right]$ (ref. 36).

Single DNA visualization for coil-globule transition was performed using an epifluorescence microscope (Nikon; ECLIPSE) with a $\times 100$ objective lens and recorded with an electron multiplying CCD camera (Andor; iXon).

Chemical Reagents. Polyethylene glycol 10,000 (PEG) was purchased from Alfa Aesar. The powder of PEG was dissolved in 10 mM Tris-HCl buffer (pH 7.5). We mixed DNA and RNA in a PEG10000 solution at 10 mM Tris-HCl buffer solution (pH 7.4) at 100 ng/μL. After 1,000x dilution of T4 DNA solution ($\theta_{\text{DNA}} \sim 0.02\%$), we mixed YOYO-1 fluorescent dye that intercalates into base pairs in DNA with T4 DNA at a ratio of 1,000:1.

Short DNA and RNA were hybridized from oligonucleotide primers. Restriction enzymes were purchased from New England Biolabs. Short DNA and RNA were hybridized from oligonucleotide primers. Restriction enzymes were purchased from New England Biolabs. DNA, small RNA, and small DNA with SYBR gold fluorescent dye (Molecular Probes).

Effective Soret Coefficient for Small RNA. To measure the transition from depletion to accumulation for small DNA and RNA, we defined the effective Soret coefficient $S^\text{eff}$ as $c(t, \psi, T) = S^\text{eff} \left(\frac{T}{T_0} - 1\right)$. $S^\text{eff}$ is related to Eq. 4 as $S^\text{eff} = \frac{S^0 + S^c}{S^0 + S^c + S^c / \theta_{\text{DNA}}}$ (SI Appendix). $\psi$ depends on the strand length and it subsequently affects RNA accumulation. The positive sign of $S^\text{eff}$ means an accumulation, whereas a negative sign corresponds to depletion. Its value reflects the rate of accumulation or depletion.

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