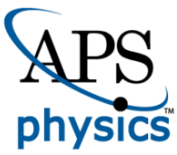

Thermal Separation: Interplay between the Soret Effect and Entropic Force Gradient

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Thermal separation: Interplay between the Soret effect and entropic force gradient

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Thermophoresis, the Soret effect, depletes a high concentration of a PEG polymer solution from the hot region and builds a concentration gradient. In such a solution, solutes of small concentration experience thermophoresis and PEG concentration-dependent restoring forces. We report using focused laser heating and varying the PEG concentration one observes geometrical localizations of solutes like DNA and RNA into patterns such as ring. For DNA up to 5.6kbp as a solute, the ring size decreases following a behavior analogous to a gel electrophoresis separation. Above 5.6kbp the ring diameter increases with the DNA length. Mixtures of DNA and RNA can be separated as well as different RNA lengths. Separation of colloids of different sizes is also observed. The experiments might be relevant for the separation of small RNA ribozymes in early stage of life.

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Introduction.

A suspension bombarded by water molecules, the solutes, experiences random instantaneous forces but zero average force. But in a temperature gradient, with the solute at rest, a suspension will move with a velocity proportional to the temperature gradient due to the Soret effect [1]. Experiments, using light induced heating, have measured transport of rigid particles and DNA in liquids along a temperature gradient [2][3][4]. They have stimulated theoretical studies of hydrodynamic stress and surface forces in such states [5][6].

In particular, in a water solution at room temperature, a large volume fraction of the PEG (polyethylene glycol) polymer will lead to an exponential concentration of PEG, away from the hot region. It has been shown that in such a state, a small concentration of microbeads accumulates at the hot spot, thus reversing the effect present without the polymer [7]. The restoring force that brings beads back to the hot region has many theoretical origins [5][8][9], but they are all based on the presence of the PEG gradient. For rigid objects the force is associated with a depletion zone around the object [7]. The word “diffusiophoresis”, which is transport phenomenon of a solute in a gradient of another solute concentration [8][10], has been introduced to define those forces.

The main focus of this Letter is on the bio-molecular polymers DNA and RNA under a temperature gradient. We show, for the first time, that size separation of DNA or RNA is feasible in the presence of a PEG gradient, with a behavior similar to gel electrophoresis. We also show that short and long DNA has a very different scaling behavior. By varying the concentration of PEG, while keeping a large volume fraction ϕ_{PEG}>1%, a suspension of DNA or RNA of small volume fraction positions itself at various distances from the hot spot. Those distances are a function of the DNA or RNA lengths. DNA and RNA are compressible objects, each with a radius of gyration R_g. In a PEG gradient, the change in compressibility for short DNA or RNA and the shape instability for long DNA seem to be the driving forces.

Thermal transport of bio-molecules has applications for DNA amplification [11] and binding kinetics [12]. Also such separation of biomolecules may have been used in the evolution of primitive life, particularly in deep ocean thermal vents, where temperature

gradients are present. In the presence of large libraries of RNA polymers, sorting of small RNA, like ribozymes, becomes essential.

Experimental Setup.

The solution was entrapped in a chamber of 10μm thickness and 500μm width made by standard soft lithography techniques. This small thickness suppresses the onset of thermal convection. Single channels made of silicone elastomer (PDMS, Sylgard 184, Dow Corning) closed with a glass slide base were filled with the water solution and sealed with a curing epoxy (Araldite Rapid). The chamber was observed with an epifluorescence microscope (Olympus, IX70) (Fig.1a) and a 40X objective. The temperature of the microscope stage was kept at room temperature ($T_\infty = 24 \pm 1$°C). To create a temperature gradient by laser heating, the condenser of the microscope was replaced by a focusing system (8mm collimator lens and objective lens 32X) connected to a fibered infrared laser (FOL1402PNJ, λ=1480nm, $P_{\max}$ =200mW, Furukawa Electronics) (Fig.1a). The temperature profiles $\Delta T(r) = T(r) - T_\infty$ vary along the radial distance r from the hot spot (Fig.1b).

Thermophoresis of a single solute, PEG.

We used PEG conjugated with fluorescent dye (NANOCS, Rhodamine-PEG10000) dispersed in Tris pH7 buffer with a PEG volume fraction of bulk solution ϕ_{PEG} (ϕ_{PEG}=1, 2, 3, 4, 5%). Beyond the critical volume fraction ϕ^*_{PEG}=1.2%, the polymer is in a semi dilute regime [13][14]. Laser heating (∇T=0.25 K/μm, $\Delta T_{\max}$ =5 K) [15] expelled the polymer from the hot region (Fig.1c). The phenomenological equation for the flow J is

$$J = -D^{PEG}\nabla c - cD_T^{PEG}\nabla T \qquad (1).$$

D^{PEG} is PEG's diffusion coefficient, c is the PEG concentration, and D_T^{PEG} is the thermal diffusion coefficient. In steady state ($J = 0$), $c(r) = c_\infty \exp\left[-S_T^{PEG}\Delta T(r)\right]$ with the Soret coefficient $S_T^{PEG} \equiv D_T^{PEG}/D^{PEG}$ and c_∞ the PEG concentration at the infinity. We fitted the profiles of PEG volume fraction $\phi_{PEG}(r) = c(r)/\rho$ starting from different initial ϕ_{PEG} (Fig.1c) [16][17]. An exponential fit with $\Delta T(r)$ deduced from Fig.1b gives $S_T^{PEG} = 0.064 \pm 0.002$ K^{-1} [18]. Similar measurements for fluorescent polystyrene beads, DNA, and RNA in buffer give: $S_T^{beads} = 4.6 \pm 0.1$ K^{-1} (0.5μm beads), $S_T^{DNA} = 0.38 \pm 0.05$ K^{-1} (5.6kbp linear DNA), and $S_T^{RNA} = 0.13 \pm 0.03$ K^{-1} (3.0kb RNA).

Thermal separation of DNA and RNA in a PEG solution.

We first analyzed thermophoresis of double strand DNA of small volume fraction (ϕ_{DNA} =0.01%) in the presence of non-fluorescent PEG10000 of large volume fraction such that DNA does not affect the PEG distribution. DNA was visualized by SYBR green [16]. We observed a new behavior: ring-like localization of 5.6kbp DNA within ϕ_{PEG} =1.5% to 2.5% (Fig.2a). In addition, DNA concentrated in the center above ϕ_{PEG}>3.0% while it is depleted from the heated region for ϕ_{PEG} below 1.0% (Fig.2a). The phase diagram of DNA lengths N vs. PEG volume fractions is presented in Fig.2b. Each dot corresponds to a measured value. Intriguingly, for long DNA such as 48.5kbp lambda DNA, the regime of ring localization is expanded to 4.5% PEG. This is not related to a change in D_T^{DNA} which remains almost constant (D_T^{DNA} =1.1 $\mu m^2.s^{-1}.K^{-1}$ for 5.6kbp DNA, and $D_T^{DNA} \propto N^{-0.08}$) [19][20].

From 250 bp up to 48.5 kbp, a ring-like localization was observed at the intermediate PEG volume fraction close to 2.5% (Fig.2b) [21]. To study the dependence of ring-like localization against DNA size, we measured the radius of the ring, R_r, in this 2.5% PEG solution (Fig.2c) [22]. R_r reaches a minimum for 5.6kbp DNA and decreases monotonically as $R_r \propto N^{-0.3}$ below 5.6kbp (Fig.2c, green line). Shorter DNA localizes farther from the heated center. Such a size-dependent localization is similar to gel electrophoresis of DNA [23]. But for long DNA above 5.6kbp, it increases as $R_r \propto N^{0.5}$ (Fig.2c, red line). This surprising reversal might be caused by the unfolding of long DNA chains induced by strong drag forces exerted by PEG polymer matrix. Such an unfolding process may be similar to the reptation mechanism observed in electrophoresis [24], and the anomalous mobility measured for large DNA (>10kbp).

We also measured how $\phi_{PEG}(R_r)$ at the peak of the fluorescent ring varies with DNA length [22]. For short DNA, the dependence of $\phi_{PEG}(R_r)$ on DNA length N is a monotonically decreasing non-linear function (Fig.2d).

The same experiment performed on RNA fragments labeled with Alexa Fluor-UTP shows that RNA of various sizes from 0.1kb to 3.0kb diluted in PEG solutions exhibited also the 3 regimes, depletion, localization, accumulation, as ϕ_{PEG} was increased

(Fig.3a). The measured value of $\phi_{PEG}(R_r)$ at the peak decreases as RNA becomes longer (Fig.3b).

This size-dependence motivates us to separate two RNA of different sizes. We mixed two RNA polymers (ϕ_{RNA}=0.01% 3kb RNA labeled by Alexa-546-UTP and ϕ_{RNA}=0.05% 0.1kb transfer RNA labeled by Ribo-Green) in a PEG solution (ϕ_{PEG}=2.0%). In a temperature gradient, the visualization by two-color imaging clearly showed that long RNA accumulated at the center while short RNA localized to form a ring (Fig.3c).

Furthermore, Fig.2d and Fig.3b suggest that long RNA localizes at smaller $\phi_{PEG}(R_r)$ than short DNA, thus RNA and DNA are also separable. We mixed 3kb RNA labeled with Alexa-488-UTP and 250bp DNA labeled by TAMRA in 3.0% PEG solution. The segregated localization of RNA from DNA was observed (Fig.3d). It is thus possible to separate 3 different polymers (DNA, RNA, PEG) in a temperature gradient.

Thermal separation of colloidal beads

It has been shown that rigid beads in a PEG6000 solution greater than 2.0% concentration accumulated in the hot region [7]. We examined ring-like localization of a dilute suspension of carboxylated fluorescent polystyrene beads (ϕ_{beads}=0.1%, 0.5μm diameter, Molecular probes F8893) in a PEG10000 solution of large volume fraction. Ring-like localization of beads was observed at intermediate volume fractions 1.5% $\leq \phi_{PEG} \leq$2.5% (Fig.4a). The plot of $\phi_{PEG}(R_r)$ monotonically decreases as a function of beads size d, following approximately $\phi_{PEG}(R_r) \propto d^{-0.3}$ (Fig.4b, solid curve). This relation indicates that mixed beads are separable. We mixed beads having two different diameters (red: 0.5μm ϕ_{beads}=0.05%, green: 0.1μm ϕ_{beads}=0.001%) with PEG. Upon local laser heating, for ϕ_{PEG}=2.5%, big beads accumulate while small beads form a ring (Fig.4c) showing size separation.

Theoretical interpretation of localization

To capture the mechanism of ring-like localization, let us consider a theoretical model of diffusiophoresis for beads [7]. The osmotic pressure gradient of PEG occurring within the depletion layer at the surface of beads results in shear stress that makes beads migrate against the Soret effect [5][7][8][10]. Taking into account diffusiophoresis with no-slip condition, the flux of beads J obeys

$$J = c^b u - D\nabla c^b - c^b D_T \nabla T \quad (2)$$

where c^b, D, D_T are the beads concentration, diffusion coefficient, and thermal diffusion coefficient respectively. The first term in the Eq.(2) takes into account of the diffusiophoresis under PEG concentration gradient, with the diffusiophoretic velocity of beads $u(r) \approx \frac{k_B T}{3\eta} S_T^{PEG} \lambda^2 c_{PEG}(r) \nabla T(r)$ where λ is the depletion depth of PEG from the beads surface, η is the viscosity of the solution, k_B is Boltzmann constant, and $c_{PEG}(r)$ is the local concentration of PEG [7][8]. $c_{PEG}(r)$ follows $\rho\phi_{PEG}(r) = \rho\phi_{PEG} \exp\left[-S_T^{PEG} \Delta T(r)\right]$ where $\phi_{PEG}(r)$ is the volume fraction of PEG and its density ρ. Solving Eq.(2) in a steady state $J = 0$, we obtain the distribution of c^b as $c^b(r) = c_\infty^b \exp\left[-S_T^{beads} \Delta T(r) + \rho V\left(\phi_\infty - \phi_{PEG}(r)\right)\right]$ with $V = \pi\lambda^2 d$ where d is the beads diameter and c_∞^b the beads concentration at the infinity. Because $\Delta T(r)$ varies in space, $\ln(c^b(r)/c_\infty^b)$ has a local maximum point at $\Delta T_C = \frac{1}{S_T^{PEG}} \ln \frac{\rho\phi_\infty V S_T^{PEG}}{S_T^{beads}}$. Thus ring-like localization of beads occurs at $\phi_{PEG}(R_r) = \phi_\infty \exp\left[-S_T^{PEG} \Delta T_C\right]$. We note that further investigations, e.g. the change of viscosity due to PEG gradients, would be required to understand the physical mechanism underlying the nonlinear relation between $\phi_{PEG}(R_r)$ and d that makes two different sized beads separable.

On the other hand, DNA and RNA do not have solid interface and are compressible molecules. The gradient of compression of these polymers could be relevant to the generation of a restoring entropic force in gradients of temperature and PEG concentration. A full description, beyond the scope of the present paper, would require an extensive analysis of the microscopic forces acting on a DNA chain in this regime.

Conclusion.

In this study, we used PEG as the polymer of large volume fraction imposing separation of solutes. The generation of non-equilibrium entropic forces by polymer gradients is a general phenomenon. Our observation is the first demonstration of how polymer migrates in the mesh of another polymer under a temperature gradient. We also found that small RNA can accumulate by the gradient of another RNA of large volume fraction in a temperature gradient [26]. It might be relevant for the origin of life scenario: the separation and accumulation of DNA and RNA might be possible in a temperature gradient supplied from thermal vent. This comes in addition to past

experiment where DNA accumulation and amplification are feasible under thermal convection [11]. Furthermore, the size-dependent localization of polymer mixtures offers new perspectives for non-equilibrium polymer dynamics. For small polymer sizes, the behavior is classical, but when the polymer size exceeds a certain threshold the reptation mechanism seems relevant. The possibility there to sort large genomes is practically useful. Finally, a one-dimensional shallow gradient will realize a better separation resolution [27], and the integration within a microfluidic device may offer novel applications in biophysics [10][28].

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***Figure Captions*.**

Figure 1: (a) Experimental setup. The sample is observed by epi-fluorescence microscopy. **(b)** Visualization of the temperature gradient. A fluorescent dye (BCECF 1mM concentration) was used as a temperature reporter. The fluorescence intensity map is used to determine the radial temperature profile $\Delta T(r)$ (power P=30mW and hot region extension ~50μm). The temperature gradient curve fits a Gaussian distribution (black line). **(c)** PEG profiles for various initial dilutions (1% to 5%). fraction profiles $\phi_{PEG}(r)$ are deduced from the fluorescence intensity of Rhodamine-PEG. Black lines are fitted curves using $\phi_{PEG}(r) = \phi_{\infty} \exp\left[-S_T^{PEG} \Delta T(r)\right]$.

Figure 2: (a) Thermophoresis of 5.6kbp DNA in PEG solutions. Depletion, and accumulation were observed. Scale bar 35μm. The plot presents normalized DNA concentration vs radial distance. **(b)** Phase diagram of depletion (red square), ring-like localization (green circle), and accumulation (blue triangle) for DNA. Each square, circle and triangle represents a measurement. **(c)** Ring radius R_r as a function of length in 2.5% PEG solution. **(d)** PEG volume fraction $\phi_{PEG}(R_r)$ at the peak of localization radius R_r is plotted versus DNA length from 250bp to 5.6kbp. ϕ_{PEG} of solution is 2.5%.

Figure 3: (a) Thermophoresis of 3.0kb RNA in PEG solutions. **(b)** PEG volume $\phi_{PEG}(R_r)$ versus RNA length. ϕ_{PEG} of bulk solution is 2.0%. **(c)** Separation of two RNA polymers (Red: 3.0kb, and Green: 0.1kb) in 2.0% PEG. **(d)** Separation of RNA (Red, 3.0kb) and DNA (Green, 250bp) in 3.0% PEG. Scale bar 35μm.

Figure 4: (a) Thermophoresis of 0.5μm beads in PEG solutions. **(b)** $\phi_{PEG}(R_r)$ vs beads diameter d. ϕ_{PEG} of bulk solutions that are close to the onset of localization 2.0% for 0.1, 0.2, 0.5μm, 3.0% for 40nm, 4.5% for 20nm beads. **(c)** Separation of 0.5μm (Red) and 0.1μm (Green) beads in 2.5% PEG. Scale bar 35μm**.**

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[15] We visualize temperature increase by the drop of fluorescence of BCECF. The fluorescence intensity is -1.3%/K at a peripheral temperature of 24℃.

[16] Rhodamine-PEG, SYBR green dye were sensitive to temperature as -0.8%/K and -1.0%/K. The decrease of fluorescence was rescaled.

[17] The volume fraction ϕ_{PEG} is calculated from the conversion of $\phi_{PEG} = c_{PEG}/\rho$ where the density of PEG powder (ρ=1.08g/cm^3) in the experiment and its concentration c_{PEG}.

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[19] We estimated D of solutes (DNA, RNA, and beads) using the time for recovery. After depletion of solute by great temperature gradient, the solute diffuses back as $c(r,t) = A \cdot erfc\left[(r_0 - r)/\sigma(t)\right] + B$. We fitted this function to fluorescent intensity profile to obtain σ and determined D by fitting $\sigma = \sqrt{4Dt}$.

[20] Fit on the density profiles of beads with Eq.1 gives S_T. Using D measured by [19] and the expression of $S_T = D_T/D$, we obtained D_T. Typical values are S_T^{DNA} =0.38 K^{-1} and D_T^{DNA} =1.1 μ m^2.s^{-1}.K^{-1} (5.6kbp DNA), S_T^{beads}=0.83 K^{-1} and D_T^{beads} =3.4 μm^2.s^{-1}.K^{-1} (0.1μm beads), and S_T^{beads}=4.6 K^{-1} and D_T^{beads}=3.8 μm^2.s^{-1}.K^{-1} (0.5μm beads). D_T^{beads} is unaffected by the size of beads.

[21] We obtained 250bp and 1kbp DNA by PCR, 2.6 and 5.6kbp of linear plasmids by EcoR1, 21kbp is a fragment of lambda DNA.

[22] The radius R_r is defined as the distance from the center of hot region to the peak of ring-like localization. $\phi_{PEG}(R_r)$ is deduced from the plot of $\phi_{PEG}(r)$ in Fig.1c.

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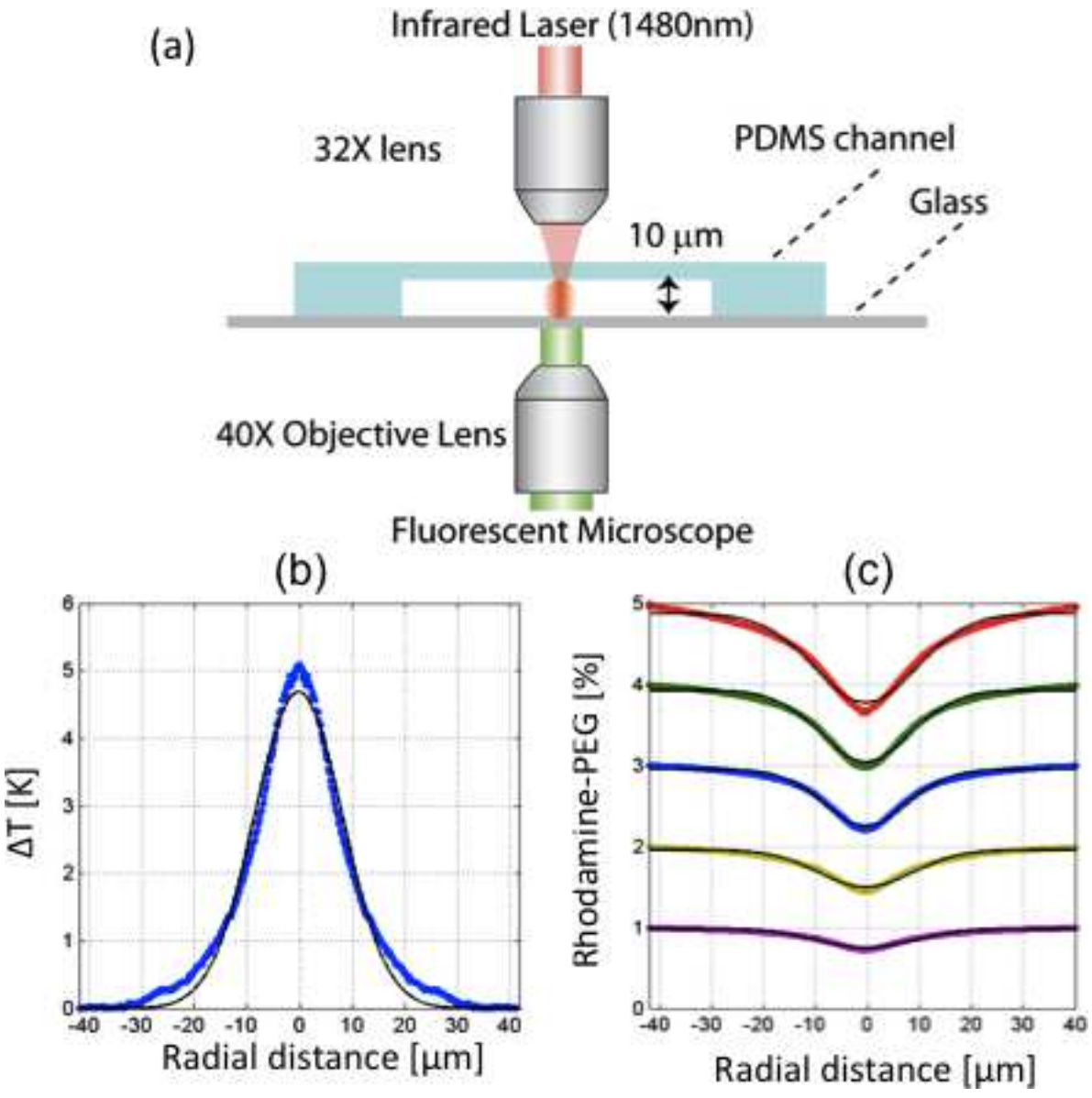


Figure 1 LZ12245 16JUN2011

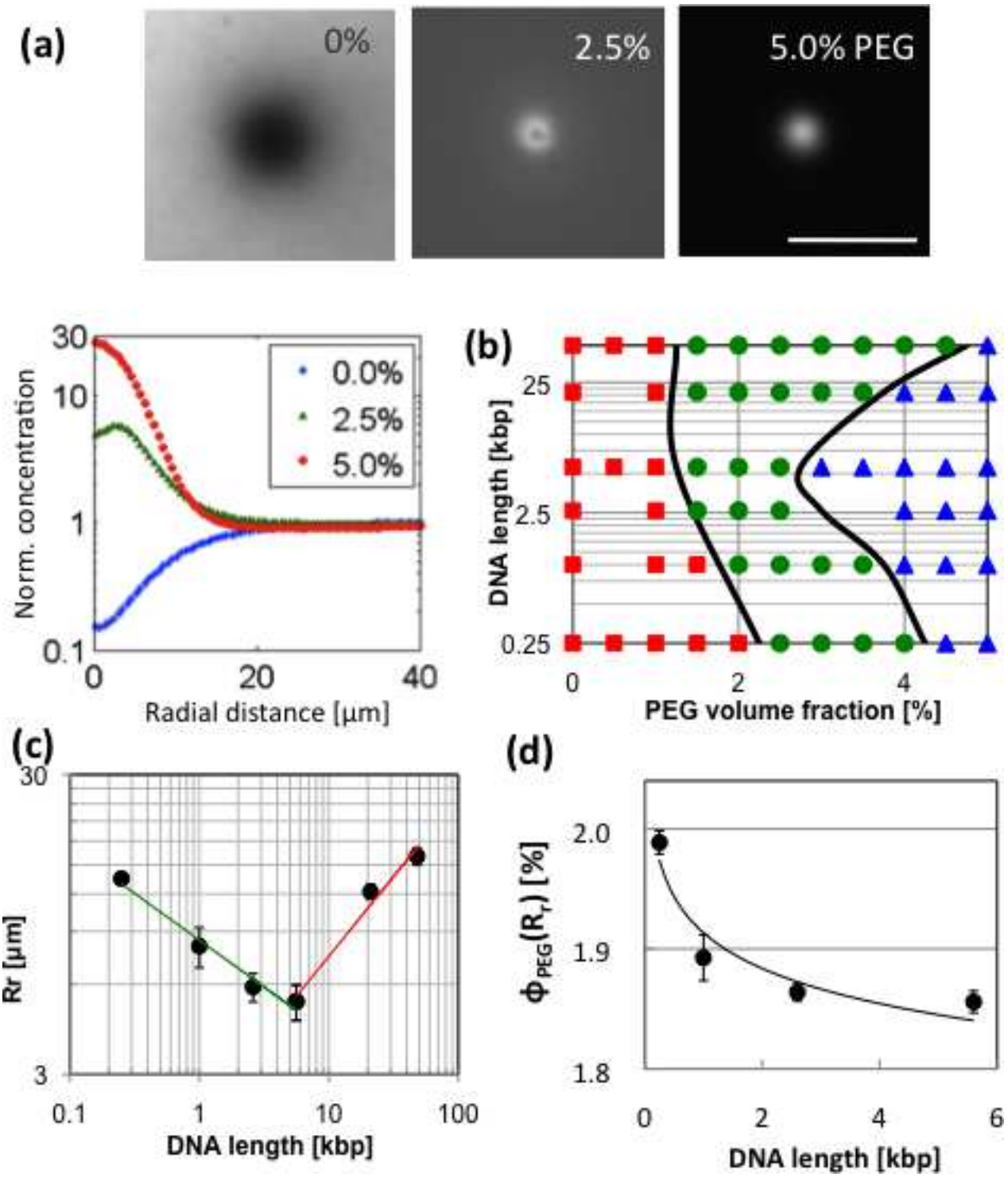
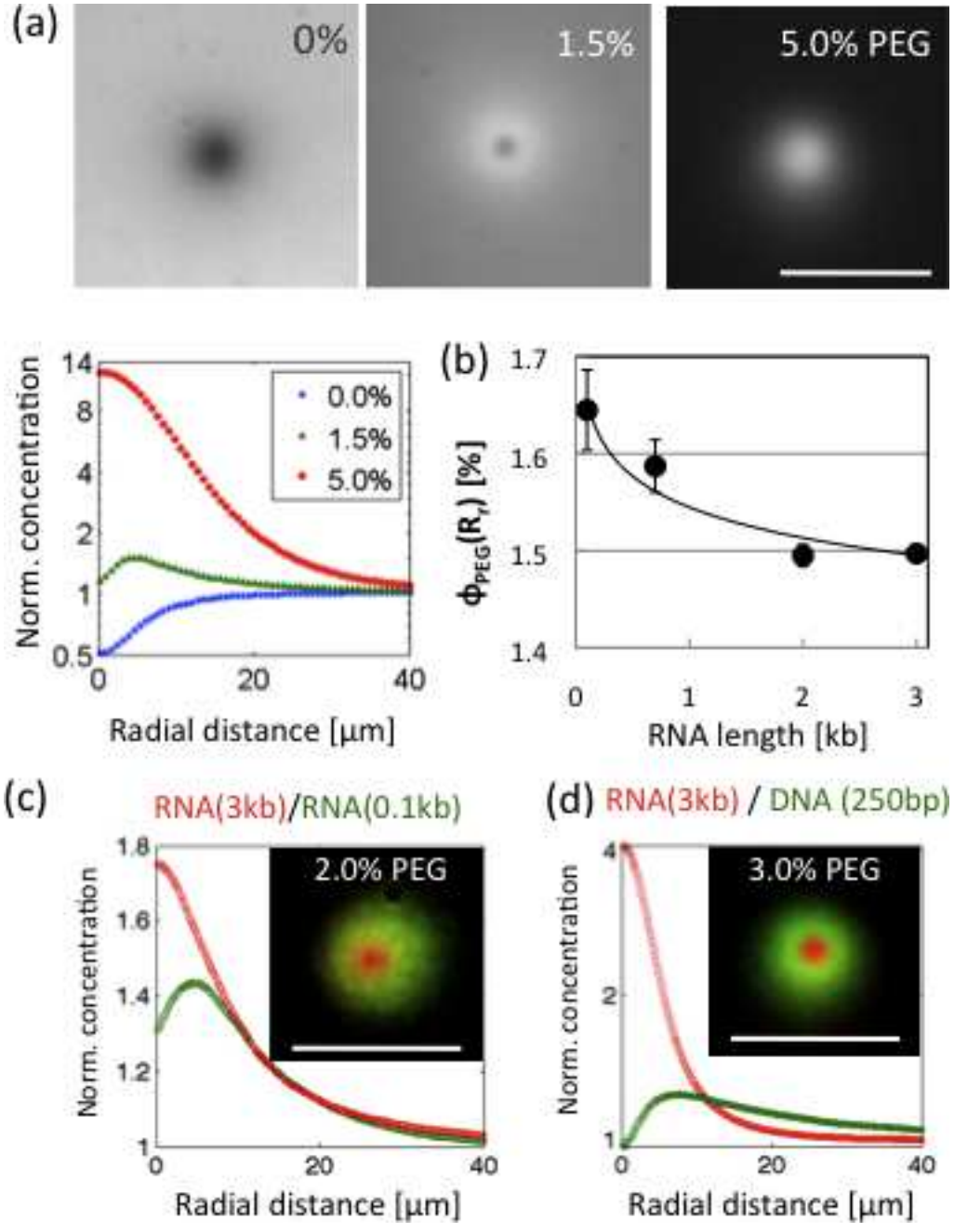


Figure 2 LZ12245 16JUN2011



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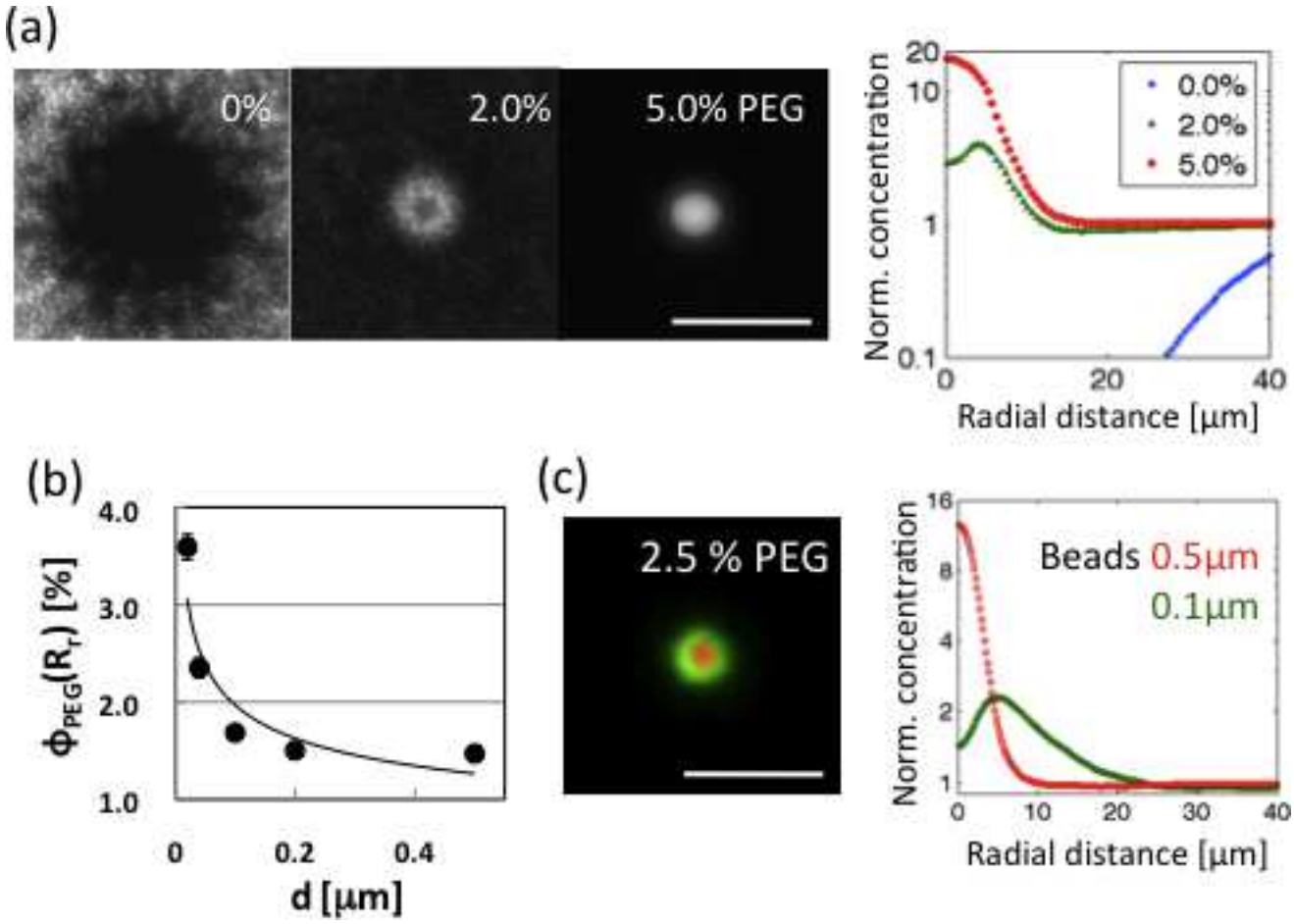

Figure 4 LZ12245 16JUN2011