

Mechanical Profiling of Biopolymer Condensates through Acoustic Trapping

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Characterizing the mechanical properties of single colloids is a central problem in soft matter physics. It also plays a key role in cell biology through biopolymer condensates, which function as membraneless compartments. Such systems can also malfunction, leading to the onset of a number of diseases, including many neurodegenerative diseases; the functional and pathological condensates are commonly differentiated by their mechanical signature. Comprehensive understanding of mechanical properties of biopolymer condensates has, however, remained challenging. In this study, we demonstrate that acoustic trapping can be used to probe the bulk modulus of biopolymer condensates in a contactless manner. We find that acoustic fields exert an acoustic radiation force on condensates, leading to their migration to a trapping point where acoustic potential energy is minimized. Furthermore, our results show that the positional fluctuation of a condensate undergoing Brownian motion in an acoustic potential well can be used as a probe for its bulk modulus. Using this framework, we infer changes in the bulk modulus of polyadenylic acid condensates in response to variations in ionic strength. Our results show that acoustic trapping opens up a path to characterize the bulk modulus of soft colloids in a noninvasive manner with applications in biology, materials science, and beyond.

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I. INTRODUCTION

Characterizing the mechanical properties of soft colloids is a key issue in soft matter physics, and its significance has

recently been highlighted in cell biology by the emergence of biopolymer condensates composed of proteins and/or nucleic acids [1]. These condensates play crucial roles in regulating a range of physiological functions within cells [1–3]. They exhibit liquidlike properties, allowing them to dynamically assemble and disassemble in response to cellular signals [4,5]. On the other hand, certain proteins have been shown to form liquidlike condensates that can undergo an aging process, transitioning into a more solidlike state, which has been implicated in the pathology of neurodegenerative diseases [6–8]. Since the mechanical properties of condensates, such as fluidity and stiffness, underlie their biological functions and/or pathological malfunctions, their research has attracted

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considerable attention in the fields of life science and biophysics [9,10].

For example, Jawerth *et al.* analyzed viscoelastic properties of FUS and PGL-3 condensates during their aging process and demonstrated that, while the morphology of protein condensates remained unchanged, their low-frequency viscosity, a mechanical property, increases with age [8]. The observation that condensate mechanical properties are highly sensitive to aging underscores the importance of characterizing mechanical properties of biopolymer condensates for understanding their aging behavior. For analyzing their mechanical properties, bulk rheological measurements are often infeasible because the available sample volume is orders of magnitude smaller than that required for conventional rheometers. Moreover, condensate size itself can influence phase behavior and aging pathways; for example, surface-initiated solidification in FUS condensates and size-dependent aging products in Sup35NM have been reported [11,12]. These findings highlight the need for measurements in microscale to accurately probe the mechanical properties of biopolymer condensates. Various techniques, including micropipette aspiration [13,14] and optical-tweezer-based microrheology [15], have been developed and applied for quantifying the mechanical properties of protein condensates, such as viscosity, surface tension, and complex shear moduli [8,16,17]. These approaches have provided valuable insights into the complex mechanical behavior of condensates and have been instrumental in elucidating their viscoelastic nature.

From the standpoint of elasticity theory, an isotropic material is characterized by two fundamentally independent elastic moduli: the shear modulus and the bulk modulus [18]. As mentioned, the frequency dependence of complex shear moduli has been widely characterized using established techniques [8,16,17]. However, the bulk modulus of condensates has been less explored, despite providing complementary information to other viscoelastic parameters and contributing to a more complete description of their material properties. Brillouin microscopy has recently enabled optical probing of the longitudinal-wave modulus of condensates, which closely approximates the bulk modulus for liquidlike materials [19–21]. However, because of the intrinsically weak scattering signal, this method often requires high-intensity laser illumination, which may perturb the condensate properties through local heating [22]. Developing noninvasive approaches capable of directly measuring the bulk modulus would provide complementary information and further advance our understanding of condensate mechanics.

Here, we focus on acoustic trapping as an analytical tool for characterizing the bulk modulus of biopolymer condensates. Acoustic trapping [23,24], analogous to optical trapping [25,26], utilizes the acoustic radiation force, which acts on microparticles within a standing pressure field [27]. The acoustic radiation force arises from the interference between the standing acoustic field and the field scattered by a particle suspended within it [27]. Its magnitude depends on the acoustic contrast factor, defined by differences in mass density and bulk modulus between the particle and the surrounding medium. This nature provides a capability to probe the bulk modulus of meso- to microscale soft colloids. Moreover, acoustic trapping is inherently biocompatible, as it does

not involve focused laser irradiation. This biocompatibility has enabled the manipulation of cells [28,29], extracellular vesicles [30], and embryos [31] without compromising their viability [32], suggesting the potential applicability of acoustic trapping to the study of biopolymer condensates. In this context, acoustic trapping could provide complementary information to existing techniques such as microrheology [16] and Brillouin microscopy [19]. Previous work has demonstrated the feasibility of using acoustic trapping to manipulate phase-separated droplets. For example, Tian *et al.* employed acoustic traps to control the localization of coacervate droplets [33], and subsequent studies further explored related applications [34,35]. However, acoustic trapping has not yet been applied to quantify the bulk modulus of biopolymer condensates.

In this study, we suggest a framework to probe the bulk modulus of microscale biopolymer condensates through acoustic trapping from their positional fluctuations. For acoustic trapping, we fabricate acoustic devices composed of a microfluidic channel and piezoelectric substrate to generate surface acoustic waves (SAWs) with frequencies of ≈ 35 and 50 MHz, thereby creating an acoustic potential field in the sample solution containing condensates [Figs. 1(a) and 1(b)]. The acoustic potential field exerts an acoustic radiation force on the condensates [Fig. 1(b)]. We show that polyadenylic acid (poly-rA) condensates can be trapped with a trapping stiffness of tens to hundreds of fN/ μm and aligned into a lattice pattern corresponding to the acoustic potential field in a contactless manner. By trapping two condensates in a single acoustic potential well, we can induce their coalescence events to analyze the inverse capillary velocity [4]. Finally, we present a framework focusing on the positional fluctuations of the Brownian motion of condensates as a probe of their bulk modulus. We theoretically describe the positional fluctuation of acoustically trapped condensates and use this approach to investigate the change in the bulk modulus of poly-rA condensates in response to ionic strength.

II. RESULTS

A. Acoustic trapping of biopolymer condensates

We constructed an acoustic device composed of a 128° Y-cut lithium niobate (LN) substrate and a polydimethylsiloxane (PDMS) microfluidic channel. The PDMS channel possesses a square chamber with a volume of $500 \times 500 \times 53 \mu\text{m}^3$. The LN substrate features two pairs of interdigital transducers (IDTs) that are orthogonal to each other. By positioning the PDMS chamber at the intersection of these two pairs of IDTs and applying an alternating voltage to the IDTs [Fig. 1(a) and Supplemental Material (SM) Sec. 1 [36]], a two-dimensional (2D) standing pressure field can be generated in the sample solution.

When a particle with a bulk modulus and mass density different from those of the surrounding liquid medium is placed in a standing pressure field, an acoustic radiation force is exerted on the particle [Fig. 1(b)]. Theoretically, the acoustic radiation force $\mathbf{F}$ acting on a spherical particle in an inviscid

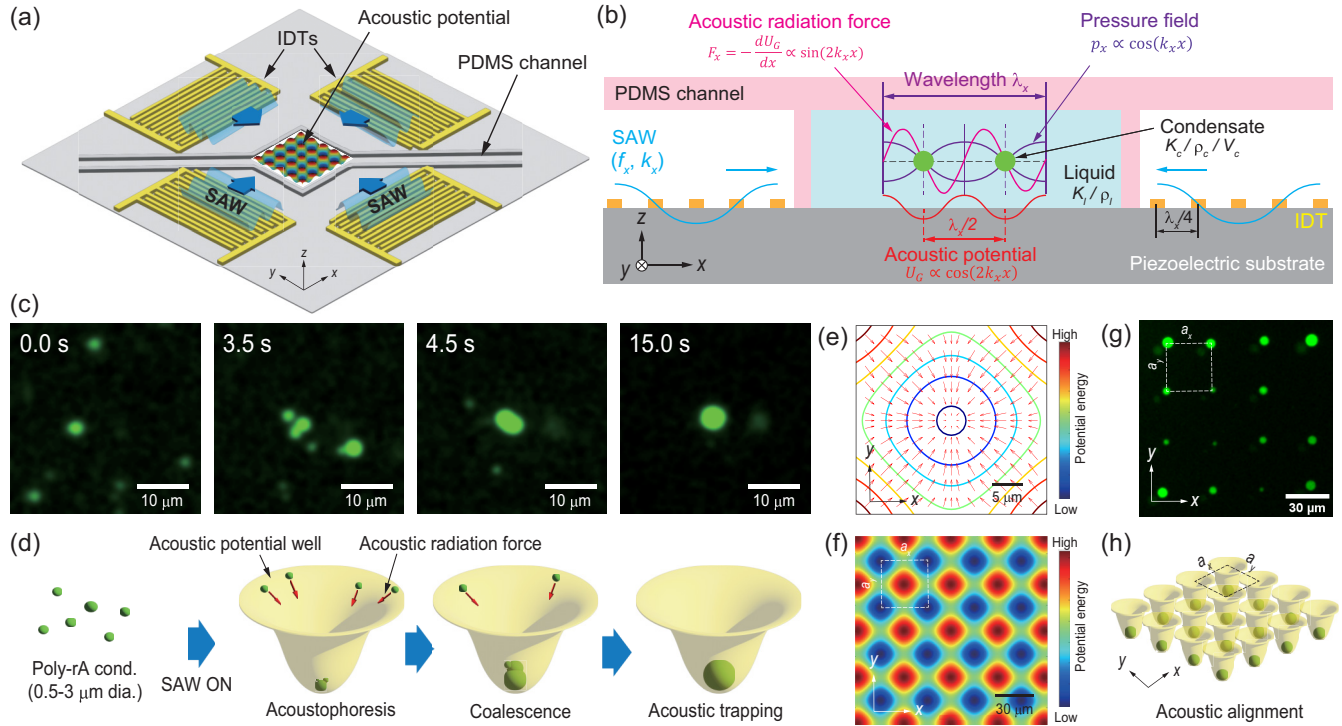


FIG. 1. Acoustic trapping of biopolymer condensates. (a) Schematic illustration of the acoustic device developed in this study. By applying an alternating voltage to interdigital transducers (IDTs), IDTs emit surface acoustic waves (SAWs). The SAWs create a two-dimensional (2D) acoustic potential within the PDMS microfluidic chamber. (b) Working principle of the acoustic device for trapping condensates. The SAWs produce a standing acoustic-pressure field p_x in the sample solution, resulting in the acoustic potential field U_G and an acoustic radiation force F_x that acts on condensates. (c) Fluorescent images (SM Movie S2 [36]) and (d) schematic illustration of poly-rA condensates trapped by acoustic radiation force. Scale bars: 10 μm . (e) Acoustic potential (colored lines) and acoustic radiation force (red arrows) in the 2D acoustic field with $f_{x,y} = 49.6$ and 49.7 MHz. Scale bar: 5 μm . (f) Calculated acoustic potential field formed by the 2D acoustic field with $f_{x,y} = 49.6$ and 49.7 MHz. Scale bar: 30 μm . (g) Experimental fluorescence image and (h) schematic illustration of poly-rA condensates aligned by the 2D acoustic field with $f_{x,y} = 49.6$ and 49.7 MHz. Scale bar: 30 μm . The experimental and simulation conditions used here are summarized in SM Tables S-A and S-B [36].

fluid can be expressed as the gradient of the Gor'kov acoustic potential U_G [43,44] given by

$$U_G = V_c \left\{ \frac{f_1}{2K_m} \langle p^2 \rangle - \frac{3f_2 \rho_m}{4} \langle \mathbf{v}^2 \rangle \right\}, \quad (1)$$

$$\mathbf{F} = -\nabla U_G. \quad (2)$$

Here, $f_1 = 1 - \frac{K_m}{K_c}$ and $f_2 = \frac{2(\rho_c - \rho_m)}{2\rho_c + \rho_m}$, where K_m and K_c are the bulk moduli of the liquid medium and the particle (or condensate), respectively, and ρ_m and ρ_c are their mass densities. V_c denotes the volume of the particle. $\langle p^2 \rangle$ and $\langle \mathbf{v}^2 \rangle$ are the time-averaged pressure and velocity field squared, respectively.

In the one-dimensional (1D) case [Fig. 1(b)], the SAWs emitted from a pair of the IDTs along the x axis create a standing pressure field $p_x = A_x \cos(k_x x) \cos(2\pi f_x t)$ in the liquid medium. Here, A_x , k_x , and f_x are the amplitude of the pressure field, the wave number, and the frequency of the SAW along the x axis, respectively. Under this pressure field, the velocity field is derived as $v_x = \frac{A_x}{\rho_m v_R^x} \sin(k_x x) \sin(2\pi f_x t)$ using the relationship $\frac{\partial p_x}{\partial x} = -\rho_m \frac{\partial v_x}{\partial t}$, where v_R^x denotes the phase velocity of the SAW propagating along the x axis. By substituting p_x and v_x into Eqs. (1) and (2), we obtain the

acoustic potential and acoustic radiation force along the x axis in the 1D case as

$$U_G = A_x^2 V_c \{C_- + C_+ \cos(2k_x x)\}, \quad (3)$$

$$F_x = -\frac{dU_G}{dx} = 2A_x^2 C_+ k_x V_c \sin(2k_x x), \quad (4)$$

where $C_{\pm} = \frac{f_1}{8K_m} \pm \frac{3f_2}{16\rho_m(v_R^x)^2}$. For soft colloids dispersed in an aqueous solution, C_+ is typically positive, as the density and bulk modulus of the particle are higher than those of the liquid medium ($\rho_c > \rho_m$ and $K_c > K_m$) [45]. This results in the migration of the particles, a phenomenon known as acoustophoresis [46], to the pressure nodes. Consequently, microparticles are aligned along the x axis with a spacing equal to half the wavelength of the SAW [Fig. 1(b)].

The working principle of acoustic trapping in the 2D case is essentially the same as in the 1D case. By introducing a second standing pressure field along the y axis, which is perpendicular to the x axis, given by $p_y = A_y \cos(k_y y) \cos(2\pi f_y t)$, we can calculate the 2D acoustic potential field. Here, A_y , k_y , and f_y represent the amplitude of the pressure field, the wave number, and the frequency of the SAW along the y axis, respectively. By using $\langle p^2 \rangle = \langle (p_x + p_y)^2 \rangle$ and

$\langle \mathbf{v}^2 \rangle = \langle v_x^2 + v_y^2 \rangle$, we can derive the time-averaged pressure and velocity field squared as

$$\langle p^2 \rangle = \frac{1}{2} \{ A_x^2 \cos^2(k_x x) + A_y^2 \cos^2(k_y y) + 2A_x A_y \cos(k_x x) \cos(k_y y) \delta(f_x - f_y) \}, \quad (5)$$

$$\langle \mathbf{v}^2 \rangle = \frac{1}{2\rho_m^2} \left\{ \frac{A_x^2}{(v_R^x)^2} \sin^2(k_x x) + \frac{A_y^2}{(v_R^y)^2} \sin^2(k_y y) \right\}, \quad (6)$$

where $\delta(\cdot)$ is the Kronecker delta function, and v_R^y denotes the phase velocity of the SAW propagating along the y axis. The 2D acoustic potential and acoustic radiation force can be calculated by substituting Eqs. (5) and (6) into Eqs. (1) and (2). We experimentally confirmed that the 2D acoustic field predicted theoretically is formed in our experimental setup using polystyrene particles with an average diameter of 1.0 μm (SM Sec. 2 and Movie S1 [36]).

In this study, we used poly-rA condensates as our target system because their phase behavior is highly sensitive to salt concentration: poly-rA undergoes phase separation upon the addition of sufficient NaCl [47,48]. We therefore expected that varying the salt concentration would also modulate the mechanical properties of the condensates, making poly-rA a convenient system for demonstrating the proof of concept.

By adding 1 M NaCl to a 1.0-mg/mL poly-rA solution in 50-mM HEPES buffer (pH 7.4), we obtained poly-rA condensates with diameters ranging from 0.5 to 3 μm before the acoustic field was applied [Fig. 1(c)]. By generating a 2D standing acoustic field, the dispersed poly-rA condensates migrate toward a trapping point via acoustic radiation force [Figs. 1(c) and 1(d) and SM Movie S2 [36]]. This migration results in the coalescence of individual condensates into a larger, single condensate, which remains trapped. This observation shows that the acoustic field exerts the acoustic radiation force on the poly-rA condensates, driving them toward the trapping point where the acoustic potential energy is minimized [Fig. 1(e)].

Figure 1(f) depicts the theoretical acoustic potential field generated by the 2D acoustic pressure field with frequencies of $f_{x,y} = 49.6$ and 49.7 MHz. The acoustic potential exhibits a lattice structure with a lattice constant of $a_{x,y} \approx 36.1 \mu\text{m}$. When the frequencies of two SAWs are the same, a peculiar pattern of the acoustic potential, which is different from a lattice one, is observed (SM Sec. 3 [36]). Therefore, the frequencies of two SAWs used in this study have a small difference to generate a lattice structure. In the experiment, a similar lattice structure of poly-rA condensates was observed, with lattice constants of $a_{x,y} = 34.2 \pm 1.3$ and $34.2 \pm 1.6 \mu\text{m}$ [Figs. 1(g) and 1(h)], in which the variation of the lattice constant is presumably induced by imperfections in the fabrication of the IDTs. The experimentally determined lattice constant was in close agreement with the theoretical value, providing robust evidence that the acoustic radiation force can simultaneously trap multiple biomolecular condensates in a contactless manner. The trapped condensates in the lattice are categorized into three types in a long-term observation (SM Movie S3): (i) condensates that are stably trapped and fluctuate around the trapping point, (ii) condensates that gradually drift and eventually settle at the trapping point, and (iii)

condensates that remain immobile due to adhesion to the channel wall. These three types can be clearly distinguished by their trajectories. For the analysis of the bulk modulus below, we used only type (i) condensates.

B. Acoustically induced coalescence of condensates

The inverse capillary velocity, which quantifies the ratio between the interfacial tension and viscosity of the condensates [49,50], can be analyzed by examining the kinetics of the coalescence events for condensates with various diameters. This parameter is useful for evaluating the liquidlike properties of biomolecular condensates [51] and is commonly employed to monitor the transition from liquidlike to solidlike states as the condensates age [52].

In the experiment, two poly-rA condensates initially separated by approximately 20 μm were brought together at a single trapping point, where they subsequently merged into a single condensate within $t = 60$ s [Figs. 2(a) and 2(b) and SM Movie S4 [36]]. The time-course plot of the aspect ratio of the condensate during the coalescence event demonstrates an exponential relaxation behavior [Fig. 2(c)]. The experimental data are well fitted with the function $f(t) = A \exp(-t/\tau) + 1$, where A is a fitting parameter, and τ denotes the relaxation time [53]. These results indicate the feasibility of monitoring coalescence within acoustic traps.

The relaxation time of coalescence, τ , can be expressed as $\tau = \bar{d}_c(\eta_c/\gamma_c)$, where $\bar{d}_c$ is the average diameter of the two condensates, and η_c and γ_c represent the viscosity and interfacial tension of the condensates, respectively [4]. This relationship allows for determining the inverse capillary velocity, η_c/γ_c . Figure 2(d) presents the correlation between the relaxation time and the average diameter for 15 independent coalescence events. The linear fit shows a good correlation with the experimental data, yielding a Pearson correlation coefficient of $|R| = 0.92$. We calculated the inverse capillary velocity from this fit to be $2.34 \pm 0.27 \text{ s}/\mu\text{m}$ for poly-rA condensates formed from 1.0 mg/mL RNA and 1 M NaCl. These results demonstrate that acoustic trapping can effectively induce the coalescence of condensates by bringing two condensates together at a trapping point, facilitating the analysis of the inverse capillary velocity.

C. Brownian motion of acoustically trapped condensates

The condensates trapped within the acoustic field exhibit confined Brownian motion in the acoustic potential well. By experimentally tracking the trajectory of this Brownian motion [Fig. 3(a)], we quantified its positional fluctuations, σ , by measuring the standard deviation of the spatial distribution of the condensate centers. An increase in the applied voltage to the IDTs resulted in more pronounced confinement of the Brownian motion of the poly-rA condensates and thus smaller positional fluctuations [Fig. 3(b)]. The acoustic trapping stiffness on poly-rA condensates can be determined using the equipartition theorem of energy [54], given by $\kappa = 2k_B T/\sigma^2$, where κ , k_B , and T denote the trapping stiffness, the Boltzmann constant, and the absolute temperature, respectively. For poly-rA condensates with a diameter of $3.16 \pm 0.03 \mu\text{m}$, the trapping stiffness is tens to hundreds of fN/ μm [Fig. 3(b)].

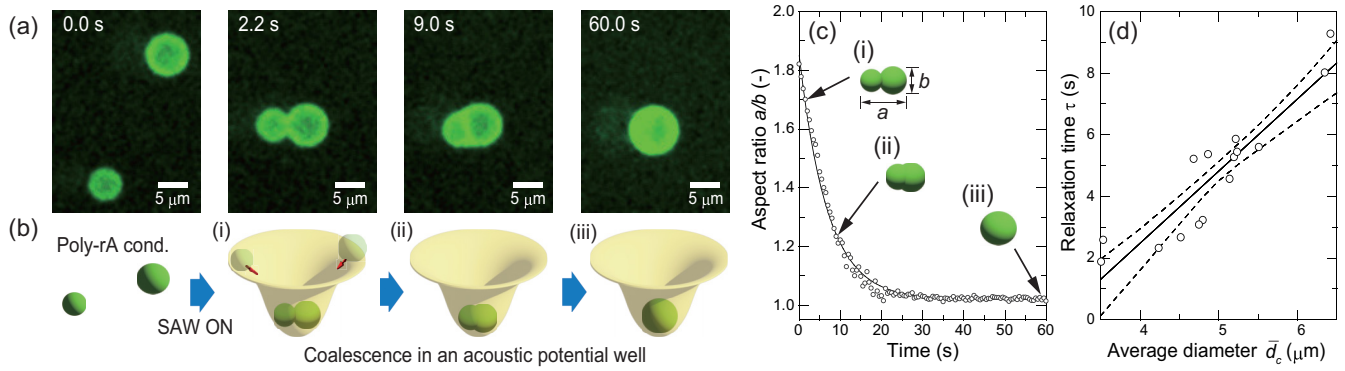


FIG. 2. Analysis of the inverse capillary velocity by acoustic trapping. (a) Fluorescent images (SM Movie S4 [36]) and (b) schematic illustration of the acoustically induced coalescence event between two poly-rA condensates. Scale bars: 5 μm. (c) Time-course plots of the aspect ratio of poly-rA condensates (circles) during the coalescence event, with a fitted curve to determine the relaxation time (solid curve). (d) Relaxation times of coalescence for poly-rA condensates of different average diameters ($n = 15$). The solid and dotted lines represent an average of the linear fit and its 95% confidence interval, respectively. The slope indicates the inverse capillary velocity of poly-rA condensates, $\eta_c/\gamma_c = 2.34 \pm 0.27$ s/μm. The experimental condition used here is summarized in SM Table S-A [36].

The trapping stiffness corresponds to the curvature of the acoustic potential near its minima. The higher the applied voltage to the IDTs, the greater the amplitude of the acoustic field [27], resulting in the steeper curvature of the potential in proximity to the minima [Fig. 3(c)]. The magnitude of this positional fluctuation is governed by the interplay between thermal energy, $k_B T$, and the potential energy created by the standing acoustic waves, U_G [Fig. 3(d)]. Thus, the more pronounced confinement of the Brownian motion of the condensates in the acoustic potential well can be understood by the steeper curvature of the acoustic potential at higher acoustic pressure.

In theory, the Brownian motion of a micrometer-sized condensate in an acoustic potential well can be described by the overdamped Langevin equation with an acoustic potential term [43,44,55]:

$$0 = -\xi \frac{d\mathbf{x}(t)}{dt} + \mathbf{R}(t) - \nabla U_G. \quad (7)$$

Here, $\mathbf{x}(t)$ represents the position of the center of the condensate, and $\mathbf{R}(t)$ denotes the stochastic force corresponding to thermal fluctuation. The friction constant ξ is given by $\xi = 3\pi\eta_m d_c$, where d_c and η_m are the diameter of the condensate and the viscosity of the liquid medium, respectively. The stochastic force $\mathbf{R}(t)$ satisfies the relationships $\langle \mathbf{R}(t) \rangle = 0$ and $\langle \mathbf{R}(t) \cdot \mathbf{R}(t') \rangle = 4\xi k_B T \delta(t - t')$.

It is noteworthy that the acoustic potential includes a term related to the bulk modulus of the trapped condensate [Eq. (1)], suggesting that the bulk modulus influences the positional fluctuation of the condensate. This is essentially different from optical trapping, which does not include mechanical information about a condensate, as the trapping force acting on a condensate is determined by the difference in the refractive indices between a condensate and the surrounding liquid. We conducted Langevin dynamics simulations of Brownian motion trajectories for acoustically trapped condensates with a diameter of $d_c = 3$ μm, varying their bulk moduli while keeping other parameters constant [Figs. 3(e) and 3(f)]. The simulated trajectories were calculated for condensates with bulk moduli of $K_c = 2.5$ and 4.5 GPa, respectively, under

identical acoustic fields. The condensate with the higher bulk modulus [Fig. 3(f)] exhibits less fluctuation compared to the one with the lower bulk modulus [Fig. 3(e)]. These results reflect the steeper curvature of the acoustic potential on the stiffer condensate than the softer one [Fig. 3(g)]. The acoustic radiation force results from the interference between the incoming wave and the wave scattered by the particle in the acoustic field [27]. Stiffer condensates scatter the incoming waves more significantly than softer ones due to the greater mismatch in bulk modulus between the condensate and the liquid medium. This increased amplitude of the scattered wave enhances the acoustic radiation force acting on the stiffer condensates, leading to more confined Brownian motion in the acoustic potential well.

D. Framework for estimating the bulk modulus of acoustically trapped objects from positional fluctuations

We propose a framework to estimate the bulk modulus of particles or condensates trapped in an acoustic potential well from their positional fluctuations by combining experimental measurements with Langevin-dynamics simulations. The central idea is that objects with different bulk moduli exhibit distinct diameter-fluctuation relationships [Fig. 3(h)].

In the experiment, condensates dispersed in a surrounding medium are trapped in an acoustic potential well. We record their trajectories and measure their diameters to obtain an experimental diameter-fluctuation dataset. A sufficiently long observation time is required to estimate positional fluctuations reliably. Based on experimental and numerical validation (SM Sec. 4 [36]), we analyze trajectories of 300 s. Condensate diameters are obtained by image analysis (see Sec. V). In parallel, we generate synthetic diameter-fluctuation datasets using Langevin-dynamics simulations over a range of bulk moduli. We then compute the mean-squared error (MSE) between the experimental and simulated datasets as a function of bulk modulus, and take the modulus that minimizes the MSE as the estimated bulk modulus (see Sec. V).

To validate that this framework can quantitatively probe the bulk modulus of microscale objects, we performed

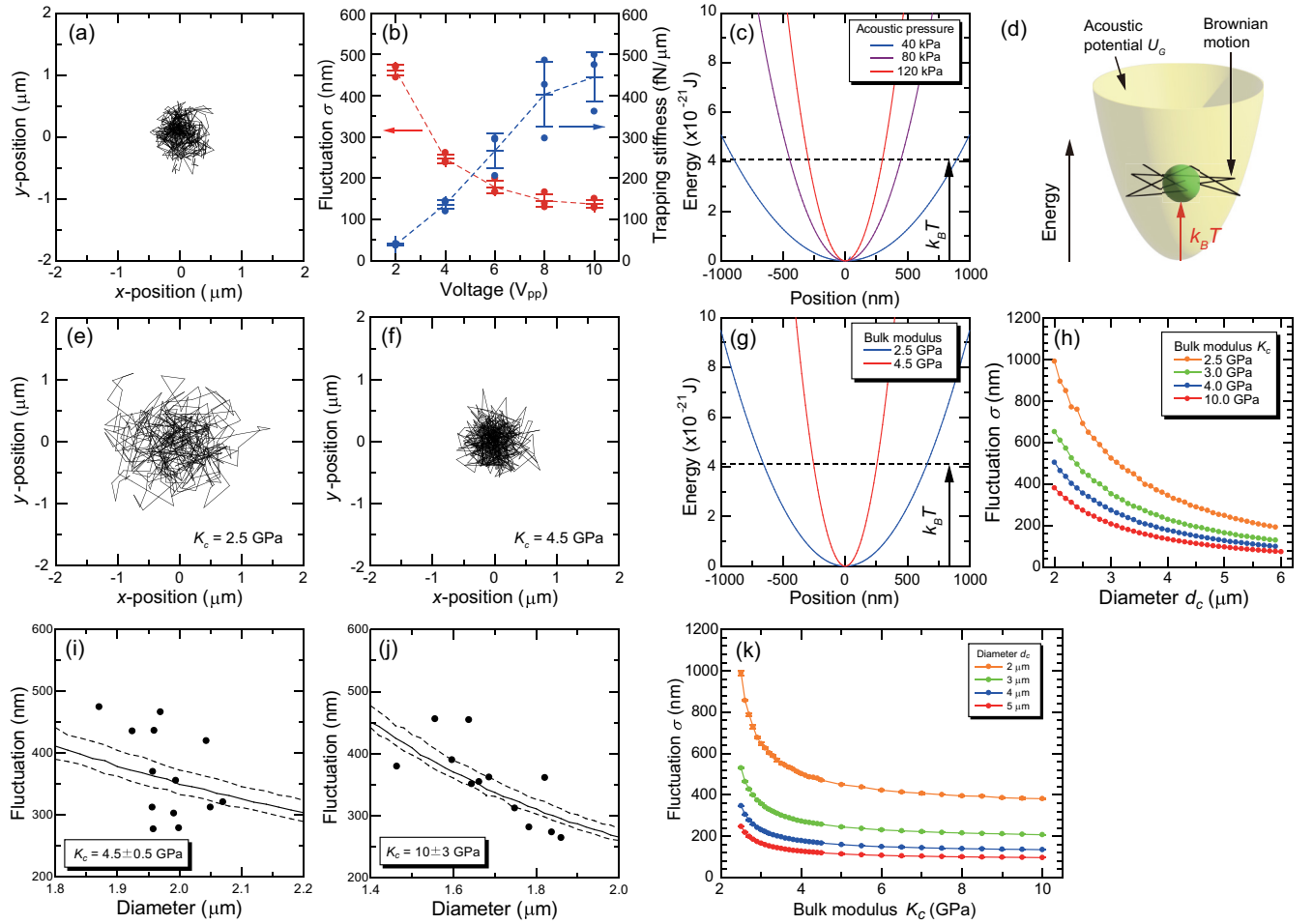


FIG. 3. Brownian motion of a single condensate in an acoustic potential well. (a) Experimental trajectory of the Brownian motion of the poly-rA condensate with a diameter of $3.16 \pm 0.03 \mu\text{m}$ trapped by acoustic frequencies of $f_{x,y} = 35.2$ and 35.3 MHz and the applied voltage of $4 V_{pp}$. Here, V_{pp} stands for voltage peak-to-peak in the unit of volt, representing the difference between the maximum positive and maximum negative values of a waveform. (b) Positional fluctuation of the Brownian motion of the acoustically trapped poly-rA condensates with a diameter of $3.16 \pm 0.03 \mu\text{m}$ (red plots) and trapping stiffness (blue plots) calculated from the fluctuation with various voltages applied on the IDTs. Here, the measurements of the positional fluctuations were performed by scanning the applied voltage from 2 to $10 V_{pp}$ with the increment of $2 V_{pp}$. The scan was repeated three times for the same condensate, and the average and standard deviation of the positional fluctuations were calculated using data among three independent scans ($n = 15000$). Error bars denote the standard deviation among three independent measurements. (c) Computed acoustic potentials under various acoustic pressures. (d) Schematic illustration of the Brownian motion of a condensate in an acoustic potential well, determined by the interplay between the thermal energy $k_B T$ and acoustic potential U_G . (e), (f) Trajectories of the Brownian motion of acoustically trapped condensates with bulk moduli of (e) $K_c = 2.5$ GPa and (f) $K_c = 4.5$ GPa, from the Langevin dynamics simulation. Although the Langevin dynamics simulations were performed with a time step of $100 \mu\text{s}$, trajectories are shown with a time step of 0.1 s for visualization. (g) Computed acoustic potentials for condensates with various bulk moduli. (h) Simulated diameter dependency of the positional fluctuations of particles with various bulk moduli. (i), (j) The measurements of positional fluctuations of (i) PS and (j) SiO₂ particles. The solid and dashed lines are simulated fluctuations using the mean and mean $\pm$ standard deviation bulk modulus for each analysis, respectively. (k) Change in the positional fluctuations depending on the bulk modulus of condensates. The central value and error bars denote the mean and standard deviation among five independent simulations. The experimental and simulation conditions used here are summarized in SM Tables S-A–S-C [36]).

experiments using polystyrene (PS) and silica (SiO₂) microparticles as reference materials. For quantitative evaluation, we assume that the commercially available microparticles have mass densities and bulk moduli comparable to their bulk values: $\rho_c = 1060 \text{ kg/m}^3$ and $K_c = 4.55 \text{ GPa}$ for PS [45], and $\rho_c = 2200 \text{ kg/m}^3$ and $K_c = 36.3 \text{ GPa}$ for SiO₂ [56]. Because the

particles are fluorescently labeled, their properties may deviate slightly from those of pristine PS and SiO₂.

The positional fluctuations of these particles were measured in an acoustic potential well generated by an acoustic field with amplitudes and frequencies of $A_{x,y} = 135 \pm 14 \text{ kPa}$ and $f_{x,y} = 35.2$ and 35.3 MHz . The acoustic pressures $A_{x,y}$ were calibrated in a separate experiment, showing that

an applied voltage with the peak-to-peak amplitude of 1 V corresponds to an acoustic pressure of 27 ± 3 kPa (SM Sec. 5 [36]). We measured the positional fluctuations of $\approx 2\text{-}\mu\text{m}$ PS and SiO_2 particles and constructed the corresponding diameter-fluctuation datasets [Figs. 3(i) and 3(j)]. To minimize wall-induced artifacts, we analyzed only trajectories of particles located near the center of the square region of the microfluidic channel; trajectories acquired near the walls exhibited larger deviations, consistent with hydrodynamic and/or acoustic wall effects (SM Sec. 6).

By calculating the MSEs between the Langevin-dynamics simulations and the experimental datasets, we obtained inferred bulk moduli of $K_c = 4.5 \pm 0.5$ GPa for PS and $K_c = 10 \pm 3$ GPa for SiO_2 [Figs. 3(i) and 3(j)]. Uncertainties are reported as standard deviations obtained from bootstrap resampling (see Sec. V). The agreement for PS demonstrates that the framework can extract bulk moduli in a relatively soft regime relevant to polymeric materials. For SiO_2 , the inferred value is substantially lower than the bulk reference, indicating that the present fluctuation-based metric becomes weakly informative for very stiff objects.

This behavior is expected from the underlying physics. The acoustic radiation force is governed by the contrast in bulk modulus between the object and the surrounding medium. Accordingly, positional fluctuations are most sensitive to changes in bulk modulus when the object modulus is comparable to that of the liquid medium ($K_m \approx 2.2$ GPa for water). When the object is much stiffer than the medium, the contrast effectively saturates and the fluctuations become insensitive to further increases in modulus [Fig. 3(k)]. Thus, while the approach is not designed to precisely quantify high bulk moduli (e.g., ceramics and metals), it is well positioned to characterize the bulk modulus of soft materials.

Consistent with this interpretation, simulations further predict an accuracy and precision of bulk-modulus estimation on the order of $\approx 5\%$ in the modulus range relevant to PS (SM Sec. 7 [36]). These results suggest that the method is particularly well suited for soft microscale objects with bulk moduli comparable to those of liquids, including polymer microspheres and, presumably, phase-separated biopolymer condensates.

We also quantified how uncertainties in related parameters propagate to the inferred bulk modulus (SM Sec. 7 [36]). This reveals that the dominant contributions arise from experimental variation in acoustic pressure among different potential wells and analytical uncertainty in particle and condensate diameter determination. Additional effects, such as particle-wall interactions, can further broaden the measured fluctuations. Overall, despite these practical error sources, the framework maintains useful accuracy and precision for soft particles and condensates, with a typical uncertainty floor of at least $\approx 5\%$ under our experimental conditions.

It should be noted that the Gor'kov acoustic potential [Eq. (1)] is derived under the assumption that the liquid medium is inviscid, so that the results are unaffected by viscosity in our simulation model. It has been reported that when the thickness of the viscous boundary layer $\delta = \sqrt{\eta_m/\pi\rho_m f}$ is much smaller than the diameter of the trapped particles, the effects of viscosity are negligible [57]. In our experimental conditions, the δ value is less than 100 nm, so the effects

of viscosity on the acoustic radiation force and resultant positional fluctuations are insignificant. This fact is further confirmed by the Langevin dynamics simulation using the viscous correction, showing that the effects of the viscosity are negligible (SM Sec. 8 [36]) [57].

E. Analysis of bulk moduli of poly-rA condensates

We then employed the framework for evaluating the bulk moduli of poly-rA condensates formed under various ionic strength. To determine the solution conditions for forming poly-rA condensates, we obtained a high-resolution phase diagram for the phase separation of poly-rA using a droplet microfluidic platform [58,59] [Fig. 4(a)]. Briefly, we generated approximately 30 000 water-oil droplets with varying salt and polymer concentrations using a microfluidic device and examined which conditions led to poly-rA phase separation through microscopic observation [insets of Fig. 4(a)]. Subsequently, poly-rA condensates formed under three different environments were trapped in acoustic potential wells, and their fluctuations in the acoustic potential well were plotted as a function of the condensate diameter. We confirmed that the diameters of the condensates reached equilibrium after a certain period (SM Sec. 9 [36]), except in cases where condensates accidentally underwent coalescence during the 300-s observation. Furthermore, the poly-rA condensates may undergo time-dependent aging, which can change the bulk modulus as a function of time. Therefore, we incubated the condensates overnight to equilibrate its diameter and composition and treated the condensate diameter as constant during the observation period and excluded data from condensates that coalesced with others. Finally, their bulk moduli were analyzed using Langevin dynamics simulations [Fig. 4(b)].

Under environmental conditions with poly-rA and NaCl concentrations of 0.5 g/L and 1.5 M [Fig. 4(b)(i)], respectively, the bulk modulus of the condensates was determined to be $K_c = 3.9 \pm 0.2$ GPa. The statistical values are evaluated by the bootstrap method (SM Sec. 10 [36]). When the NaCl concentration was reduced while keeping the poly-rA concentration constant [Fig. 4(b)(ii)], the bulk modulus decreased to $K_c = 3.1 \pm 0.1$ GPa. Increasing the poly-rA concentration to 2.0 g/L while maintaining the NaCl concentration at 1.5 M [Fig. 4(b)(iii)] resulted in a bulk modulus of $K_c = 4.4 \pm 0.3$ GPa, which is a closer value to that of poly-rA condensates formed at the high salt concentration. These results indicate that the bulk modulus of poly-rA condensates is more sensitive to changes in NaCl concentration than changes in poly-rA concentration. The possible source causing the change in the diameter-fluctuation datasets is further discussed, such as statistical fluke and salt-dependent change in the mass density of the condensate (SM Sec. 11). These support that our framework successfully captured the salt-dependent change in the bulk modulus of the poly-rA condensates through the measurement of the positional fluctuations.

As shown in the phase diagram [Fig. 4(a)], the binodal phase boundary is nearly parallel to the poly-rA concentration axis, indicating that the phase separation behavior of poly-rA primarily depends on the NaCl concentration. We also performed fluorescence recovery after photobleaching (FRAP) [60] assays [Fig. 4(c)]

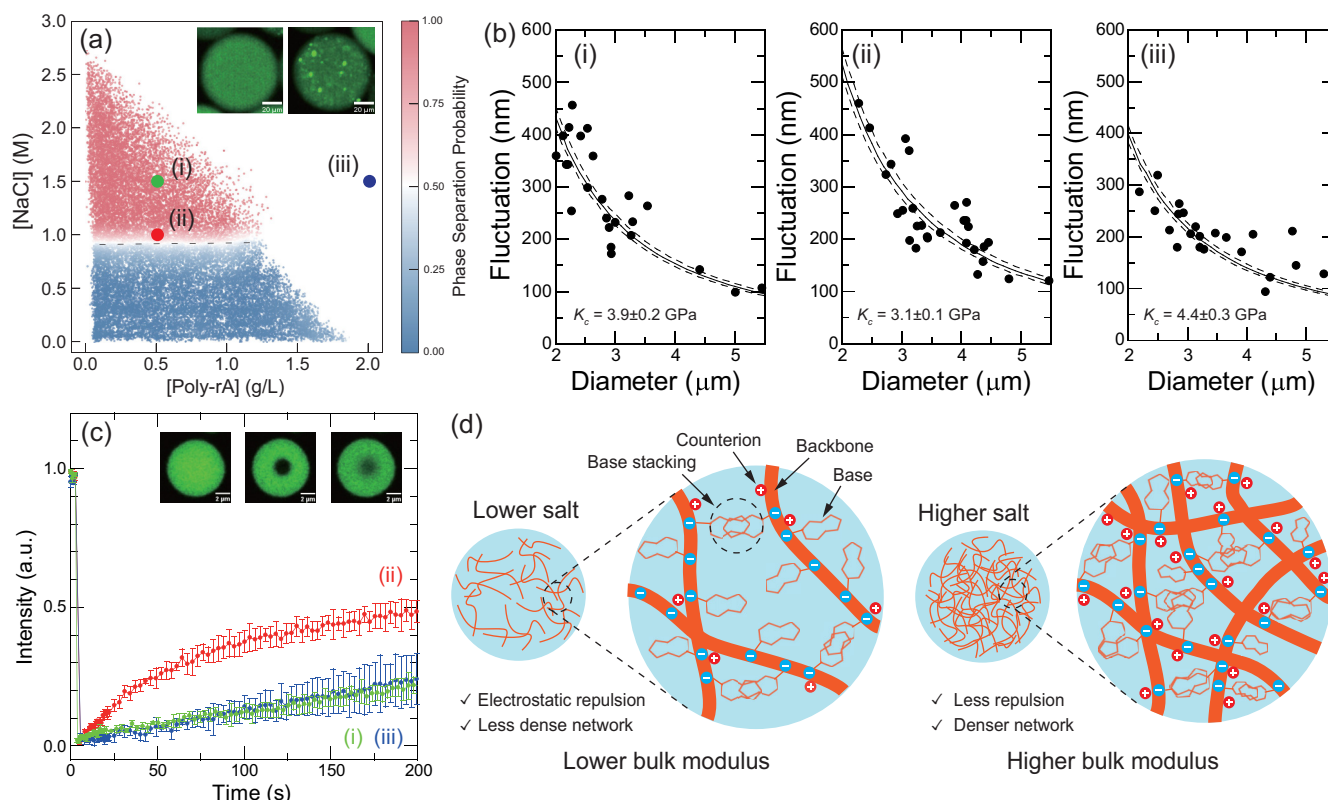


FIG. 4. Change in the bulk modulus of poly-rA condensates under different environments. (a) High-resolution phase diagram of poly-rA phase separation with various salt and polymer concentrations obtained using the droplet microfluidics platform ($n = 30\,546$). The dashed line is a guide to the eye for the phase boundary. Insets show the fluorescent images of water-in-oil droplets without (left) and with phase separation (right). Scale bars: $20\,\mu\text{m}$. (b) Analysis on bulk moduli of poly-rA condensates formed under poly-rA and NaCl concentrations of (i) $0.5\,\text{g/L}$ and $1.5\,\text{M}$ ($n = 25$), (ii) $0.5\,\text{g/L}$ and $1.0\,\text{M}$ ($n = 28$), and (iii) $2.0\,\text{g/L}$ and $1.5\,\text{M}$ ($n = 24$). The solid and broken lines show the theoretical fitting lines of the average and 95% confidence interval of determined bulk moduli, respectively. (c) FRAP curves of poly-rA condensates formed under various solution conditions. The insets show the fluorescence image of the condensate, formed with poly-rA and NaCl concentrations of $0.5\,\text{g/L}$ and $1.0\,\text{M}$, immediately before (left) and after (middle) bleaching of the dye and after $200\,\text{s}$ (right). Scale bars: $2\,\mu\text{m}$. Error bars denote the standard deviation among three independent measurements. In panels (b) and (c), the numbers i–iii indicate the solution conditions shown in the phase diagram in panel (a). (d) Schematic illustration of the proposed interaction network within poly-rA condensates with various salt concentrations. The experimental and simulation conditions used here are summarized in SM Tables S-A–S-C [36].

to monitor the mobility of small client molecules (poly-rU-Cy5) within the condensates. Condensates formed under $1.5\,\text{M}$ NaCl exhibited similar recovery curves regardless of poly-rA concentration, with approximately 25% of fluorescence recovering after $200\,\text{s}$. In contrast, condensates formed under lower NaCl concentrations showed higher mobility of the client molecules, as indicated by faster recovery in the FRAP curves. These results suggest that poly-rA condensates formed under these conditions allow higher mobility of small client molecules, which may correlate with lower effective bulk moduli.

III. DISCUSSION

In soft matter, such as gels and rubbers, it is well known that the bulk modulus is significantly larger than the tensile and shear moduli. The bulk modulus is typically $1\,\text{GPa}$, while the tensile and shear moduli generally range from 10 to $100\,\text{kPa}$ [61]. This disparity arises from the different mechanisms underlying the restoring forces against compression and

tensile deformation. Under tensile stress, the restoring force in rubbers primarily comes from entropic effects, where polymer molecules resist stretching to maintain their chain configurations to the greatest extent possible. In contrast, the restoring force against compression mainly results from short-range intermolecular interactions between interacting groups [62]. Therefore, the denser the interaction network within soft materials, the higher the bulk modulus.

Our experimental results indicate that poly-rA condensates formed at higher salt concentrations exhibit higher bulk moduli than those formed at lower salt concentrations. Previous research has shown that the phase separation of poly-rA molecules is primarily driven by intermolecular base stacking [63]. Although we did not directly measure changes in internal RNA or salt concentrations within the condensates, at higher salt concentrations, the intermolecular electrostatic repulsion, which originates from the negative charges on the backbone phosphate groups, can be reduced due to electrostatic shielding by counterions [Fig. 4(d)]. This suggests that this reduction in repulsion could facilitate the formation of a denser

base-stacking network within the condensates, potentially resulting in an increased bulk modulus [Fig. 4(d)]. Therefore, measuring the bulk modulus of biomolecular condensates provides valuable insights into the interaction networks within these structures.

The storage elastic moduli, G' , of liquidlike biopolymer condensates have been reported to range from $G' \approx 0.1$ to 10 Pa [15,64], significantly lower than typical gels or rubbers. In such systems, macroscopic deformation is primarily governed by surface tension rather than elasticity, making accurate measurements of their elastic properties inherently challenging. Thus, utilizing acoustic trapping to measure the bulk modulus presents an approach for profiling the elastic behavior of mesoscale condensates at the single-particle level. This method provides a framework for enhancing our understanding of the interaction networks within condensates, opening a path to a more comprehensive understanding of condensate material properties.

In cellular environments, biopolymer condensates are likely to be exposed to external force fields with timescales typically ranging from seconds to hours, due to the dynamic remodeling of cytoskeletal and adhesion structures, such as focal adhesion maturation and actin turnover [65]. These timescales are much longer than those imposed by the acoustic fields with frequencies on the order of 10 MHz used in this study. The bulk modulus analyzed here corresponds to the real part of the complex bulk modulus at the 10 -MHz frequency. Since elastic moduli of dense polymer solutions exhibit intricate frequency dependence [66], it is not straightforward to extrapolate the bulk modulus measured at the MHz range to the biologically relevant Hz to mHz range. This introduces a limitation in interpreting the measured bulk moduli as representing the material properties at biologically relevant timescales. Nevertheless, such high-frequency measurements still provide meaningful material information: They quantify the intrinsic compressibility of the condensate network in the limit where structural rearrangements cannot follow the imposed deformation. This offers a complementary perspective to low-frequency rheological behavior and helps constrain the range of possible mechanical behaviors across timescales.

IV. CONCLUSION

In this study, we demonstrated that acoustic trapping can be used to profile the mechanical properties of biopolymer condensates with precision and without physical contact. Our results showed that the 2D acoustic field generated by SAWs with frequencies of ≈ 50 MHz efficiently trapped and aligned poly-rA condensates based on the acoustic radiation force in a contactless manner. We presented the framework for noninvasive measurements of the mechanical properties of condensates by using the positional fluctuation of acoustically trapped condensates as a probe of their bulk modulus. The framework was applied to infer changes in the bulk modulus of poly-rA condensates in response to variations in ionic strength, which presumably originates from the changes in interaction networks within the condensates. This approach not only provides a powerful tool for probing the bulk modulus of soft colloids but also holds significant potential for applications across biology, materials science, and other disciplines.

V. MATERIALS AND METHODS

A. Preparation of poly-rA condensates

Lyophilized poly-rA with a chain length of 2100 – $10\,000$ nucleotides was purchased from Merck (1010862001). The lyophilized poly-rA was dissolved into nuclease-free water (AM9937, Thermo Fisher Scientific) to be the concentration of ≈ 10.0 g/L, determined by an absorbance measurement. Then, the poly-rA solution was mixed with HEPES buffer (pH 7.0) and poly-uracil labeled with cyanine-5 (poly-rU-Cy5), which is purchased from Sigma Aldrich via the custom-oligo synthesis service. The final concentrations of poly-rA, poly-rU-Cy5, and HEPES buffer are 0.5 mg/mL, 10 μ g/mL, and 50 mM, respectively. Here, the poly-rU-Cy5 comprises 15 uracil molecules and one cyanine-5 moiety ($MW \approx 5$ kDa). The molar concentrations of poly-rA and poly-rU-Cy5 are 0.4 – 1.8 and 2.0 μ M, respectively, which correspond to the molar ratio of $1:1$ – $1:5$. After mixing them well, the phase separation was induced by adding NaCl.

B. Fabrication of piezoelectric chips for acoustic tweezers

Four IDTs were fabricated on a piezoelectric 128° Y-cut LN substrate with a thickness of 0.35 mm. An Al thin film with a thickness of ≈ 300 nm was formed on the LN substrate by means of radio-frequency magnetron sputtering. A photoresist (AZ P4210, Merck) layer was spin coated on the Al layer and exposed to UV light using a photomask. The pattern of the IDTs was formed by developing the photoresist layer (AZ developer, Merck) and subsequently removing the unnecessary Al thin film by chemical etching (Mixed acid Al etchant, Kanto Chemical Co., Inc.). After removing the remaining photoresist on the IDTs by a remover reagent (AZ Remover100, Merck), a silicon dioxide layer with a thickness of ≈ 200 nm was formed on the substrate for protecting the IDTs.

The fabricated LN chip possesses two pairs of IDTs, which are orthogonal to each other. The direction of each IDT is 45° rotated from the x axis of the crystal because the 128° Y-cut LN shows an anisotropy of a sound velocity of the Rayleigh wave in a plane. Consequently, we obtain the four equivalent IDTs with the sound velocity of the Rayleigh wave of $v_R = 3590$ m/s [67]. The width of the electrode and the gap between the electrodes were designed using this sound velocity.

C. Fabrication of microfluidic channels

The microfluidic channel was designed using AUTOCAD and printed on the acetate film photomask (Micro Lithography). The SU-8 photoresist (SU-8 3050, KAYAKU Advanced Materials Inc.) was spin coated on a polished silicon wafer. After baking the silicon wafer at 95°C for 20 min, the photomask was put on the top of the wafer, and then the wafer was exposed to UV light. The microfluidic pattern was developed by soaking the wafer in propylene glycol methyl ether acetate (Sigma Aldrich). After drying and washing the SU-8 mold, a mixture of the PDMS elastomer and crosslinking agent (Sylgard184, Dow Corning) was poured on the SU-8 mold and incubated at 65°C for 3 h for polymerization of PDMS. The PDMS channel was cut from the mold using a scalpel and used for the experiments.

D. Acoustic trapping experiments

The fabricated LN chip and PDMS microchannels were washed by isopropanol using a sonication bath for 5 min and dried at 65 °C for 15 min. The LN chip and PDMS microchannels were assembled under a microscope for alignment. The surface of the chip and microchannel were treated by oxygen plasma (500 s; 80% power, Femto, Diener Electronics). The microchannel was filled with 1% (w/v) polyvinyl alcohol (PVA) solution for 15 min, and then the PVA solution was removed by incubating the device on a hotplate with 115 °C for 1 h. After that, the channel was thoroughly washed by flushing ultrapure water for 15 min. These procedures are performed to make the surface of the microchannel hydrophilic [68] to prevent poly-rA condensates from wetting the channel surface. The sample solution was introduced inside the microfluidic channel using the syringe pump (Cetoni, Nemesys S) with the flow rate of 100 $\mu\text{L}/\text{h}$. The alternative voltage whose frequency matches the resonant frequency of the IDTs was generated using a two-channel function generator (RS components, RSDG 6022X) and applied to the IDTs using needle-type contact probes (Harwin, P25-0423). All experiments were performed in a laboratory maintained at approximately 25 °C.

E. Validation experiments using solid microparticles

To validate our framework, we measured the positional fluctuations of acoustically trapped PS and SiO₂ microparticles with nominal diameters of approximately 2 μm . The microspheres were purchased from Abvigen (PS: ABWG-21-0200; SiO₂: ASFG-21-0200). The particles were dispersed in deionized water at a 1:100 dilution and introduced into the microfluidic channel using a syringe pump. After allowing the background flow to reach equilibrium, the particles were exposed to an acoustic field with frequencies of $f_{x,y} = 35.2$ and 35.3 MHz and pressures of $A_{x,y} = 135$ kPa. The positional fluctuations of individual particles were recorded for 300 s.

F. Analysis of the diameter of particles/condensates from fluorescent images

For the diameter measurement, time-lapse fluorescence images were analyzed using a custom script written in MATLAB (version R2023b). Each frame was processed independently as follows. First, images were smoothed using a two-dimensional Gaussian filter to reduce noise. The brightest pixel in the smoothed image was identified, and a square region of interest (ROI) (± 5 μm) centered on this position was extracted from the original image. The intensity-weighted centroid within the ROI was then calculated to determine the particle center with subpixel accuracy. To estimate particle diameter, one-dimensional fluorescence intensity profiles were sampled along multiple directions (5° to 175° in 20° increments) passing through the centroid analyzed above. For each direction, a line profile was computed by averaging over a finite width (± 3 pixels) perpendicular to the sampling direction. The resulting profiles were baseline corrected and normalized to their maximum intensity to mitigate the effects of photobleaching. Each profile was fitted using an

error-function-based edge model:

$$I(x) = p_1 + p_2 \times \frac{1}{2} \left[\operatorname{erf} \left(\frac{x + p_3/2}{\sqrt{2} p_4} \right) - \operatorname{erf} \left(\frac{x - p_3/2}{\sqrt{2} p_4} \right) \right], \quad (8)$$

where p_1 is the baseline offset, p_2 is the amplitude, p_3 corresponds to the object diameter, and p_4 represents the standard deviation of the Gaussian point-spread function.

G. Tracking analysis of condensate trajectory

Poly-rA condensates were visualized by a fluorescence microscope (CAIRN, OpenFrame Microscope) using a 40 $\times$ objective lens (Nikon, Plan Fluor 40 $\times$). The fluorescence images were acquired using a sCOMS camera (2560 $\times$ 2160 pixels, Andor Neo) with the pixel size of 160 nm/pixel. For the measurement of fluctuation of the Brownian motion, 1000 fluorescence images of identical condensates were sequentially acquired with the exposure time and frame rate of 10 ms and 10 fps, respectively. The acquired images were processed using the Trackmate algorithm [69], a plug-in of IMAGEJ software, to acquire the trajectory of the Brownian motion. The diameter of each condensate was determined through the identical process with that used for the solid microspheres mentioned above.

To calculate the fluctuation from the trajectory, we determined one axis a , which minimizes the standard deviation of the distribution of the condensate position along that axis, and another axis b , which is perpendicular to a axis. We calculated the standard deviations along two axes, σ_a and σ_b . Then, the averaged fluctuation σ was obtained as $\sigma = \sqrt{\sigma_a \sigma_b}$. This operation corresponds to converting an ellipse with a minor and major axis of σ_a and σ_b into a circle with the equivalent area. This coordinate rotation is a purely mathematical procedure introduced to reduce the inherently two-dimensional fluctuation to an effective one-dimensional measure. It does not carry any physical significance and is independent of the camera coordinate system.

H. Langevin dynamics simulation

For the Langevin dynamics simulation of the 2D Brownian motion of the condensate in an acoustic potential well, we used governing equations, Eqs. (1), (2), and (5)–(7), and the used parameters for each simulation are summarized in SM Tables S-B and S-C [36]. The simulation was performed by converting Eq. (7) into a discrete form with a time step of 100 μs using the MATLAB software (version R2023b). The acoustic potential field was calculated on a uniform mesh with a spacing of 10 nm, with the mesh center located at the acoustic potential minimum. The simulation domain was a 20 $\times$ 20 μm^2 square. The acoustic potential and the corresponding acoustic radiation force acting on the condensate at arbitrary positions were then obtained by linear interpolation between the two nearest mesh points along each spatial axis.

I. Determination of bulk moduli of poly-rA condensates

The poly-rA condensates were trapped in the acoustic field with the frequencies and pressures of $f_{x,y} = 35.2$ and 35.3 MHz and $A_{x,y} = 135$ kPa, respectively. After

the equilibrium of background flow, the fluctuations of the Brownian motion of ≈ 25 condensates with various diameters were recorded. We then generated a dataset from Langevin dynamics simulations to obtain fluctuation-diameter curves while systematically varying the bulk modulus. We next calculated the MSEs between the experimental and simulated data as a function of the imposed bulk modulus. The modulus value that minimized the MSE was taken as the bulk modulus of the condensate. To estimate the uncertainty of the determined bulk modulus, the bootstrap method [70] was employed with the iteration of 50 000, respectively. The uncertainties in the bulk modulus were reported by the standard deviation among 50 000 iterations. The parameters used in this analysis are shown in SM Sec. 10 and Tables S-B and S-C [36].

J. High-resolution phase diagram obtained by PhaseScan

The microfluidic devices for acquiring the high-resolution phase diagram were fabricated by casting PDMS (Sylgard 184 kit; Dow Corning) on a master wafer, curing it at 65 °C for 60 min, peeling it off, punching the holes for inlets and outlets, and bonding it to a 1-mm-thick glass slide (Epremedia) after oxygen plasma activation in a plasma oven (Diener Femto, 40% power for 30 s). Subsequently, hydrophobic treatment of the channels of the microfluidic device was performed. The channels were filled with 1% v/v trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma-Aldrich) in HFE-7500 fluorinated oil (3M Novec engineered fluid) solution and incubated for 1–2 min. After the incubation, channels were washed with HFE-7500 fluorinated oil and dried under airflow.

Phase diagrams were constructed using the semiautomated microfluidic platform PhaseScan [58,59]. Briefly, poly-rA and NaCl stock solutions were mixed at various mass ratios and encapsulated in water-in-oil droplets and individual microenvironments using a microfluidic device. Fluorescence images of droplets in the observation chamber of the microfluidic device were taken using an epifluorescence microscope (Cairn Research) equipped with a 10 $\times$ objective lens (Nikon CFI Plan Fluor 10 $\times$, NA 0.3) and analyzed via automated image analysis script to detect condensates and to determine RNA concentrations in individual droplets. The data were plotted as a color-coded scatter plot, where each point represents an

individual microfluidic droplet, and color represents the phase separation probability (1 or dark red, phase separated; 0 or dark blue, mixed) assigned to the droplet. This probability was calculated by averaging the phase state assignment of neighboring droplets. The radius of the neighborhood was defined as a percentage of data range (5%).

K. FRAP assay

FRAP was performed using the Stellaris 5 confocal microscope equipped with a 63 $\times$ oil immersion objective lens (Leica HC PL APO 63 $\times$ /1.40 Oil CS2, NA 1.4). A 488-nm argon laser at 100% power was used to bleach a disk-shaped 1- μm^2 area in the center of condensates. The half-lives of fluorescence recovery, tau, were obtained by analyzing FRAP kymographs using the built-in software. We performed the measurements for poly-rA droplets with diameters of approximately 3–5 μm and report the average and standard deviation of 3 repeats for each condition.

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K.N., T.S., and T.P.J.K. designed and managed this research project. K.N., T.S., and L.L.G. established the experimental setup and protocols. K.N., Y.H., T.S., and N.A.E. acquired experimental data and analyzed them. K.N., T.S., L.L.G., N.A.E., H.O., and T.P.J.K. discussed the experimental results and wrote the manuscript.

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