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Determination of Condensate Material Properties from Droplet Deformation

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Abstract

I derive theoretical results that relate the effective spring constant, χ , of protein droplets to their material properties. χ , defined as the ratio between a uniaxial applied force and the extent of the corresponding deformation, can be measured by optical tweezers. When the deformation is static, where the applied force is balanced by the interfacial tension of the droplet, the spring constant allows for the determination of the surface tension (γ). When a sinusoidal force is applied (at frequency ω), the dynamic spring constant $\chi(\omega)$ is related to both γ and the complex shear modulus, $G(\omega)$ of the droplet. The results derived here enable accurate extraction of γ and $G(\omega)$ from $\chi(\omega)$ data.

Graphical Abstract

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INTRODUCTION

Biomolecular condensates, formed by phase separation, are upending our understanding in much of biology. Condensates often appear as droplets with diameters of a few microns. Many theoretical and computational studies have focused on the thermodynamic properties such as the coexistence curves between droplet and bulk phases.^{1–6} However, biomolecular condensates have complex macromolecular compositions (often with hundreds of species).⁷ The macromolecular components can form multiple dense phases that are demixed; material properties including surface tension and viscosity appear to be important driving forces of demixing.^{8–9} Moreover, the material states of condensates often evolve over time, both for normal cellular functions and as aberrant transitions.¹⁰ The time evolution is also partly driven by material properties. Experimental measurements of material properties are crucial for deepening understanding of condensates. Both fluorescence microscopy^{8, 11–12} and optical tweezers (OT)^{13–16} have proven useful in dealing with the micron sizes of droplets. Analyzing the experimental data typically require a theoretical model for the statics or dynamics of the material states of condensates. Here I solve a theoretical model for analyzing data from an elegant OT-based experiment introduced by Jawerth et al.¹⁴

In this experiment (Fig. 1a), two small beads are optically trapped at the opposite caps of a protein droplet and sinusoidally driven (with frequency ω). The uniaxial, opposite forces exerted by the beads lead to deformation of the droplet shape. The ratio in amplitude between force and deformation defines an effective spring constant, $\chi(\omega)$. Along with their experiment, Jawerth et al. also introduced a theoretical model, where motions of the fluids inside and outside the droplet are described by the Navier-Stokes equations. The goal of the theoretical model is to relate the measured $\chi(\omega)$ to material properties including interfacial tension γ and complex shear modulus $G(\omega)$. They obtained only an approximate solution. Here I present the full solution.

I also propose a static version of the OT-based experiment. In this version, a pair of constant, opposite forces is applied at the poles of a droplet; the experiment thus measures a static spring constant χ_0 , which I show is proportional to the surface tension. Moreover, when surface tension dominates over shear modulus, i.e., $\gamma/R \gg G(\omega)$ (R denoting the droplet radius), $\chi(\omega)$ becomes equal to χ_0 .

THEORETICAL MODEL

I. Model Under Static Forces

Consider a droplet deformed by a pair of opposite forces applied at the caps of a protein droplet (Fig. 1). I assume the system to be axisymmetric, and use spherical coordinates (r, θ, ϕ) for any position inside the droplet; the origin is at the center of the droplet. Following Jawerth,¹⁴ the applied forces are modeled in the form of an external pressure

$$p_{\text{ex}}(\theta) = p_{\text{ex}0}[\Theta(\theta_0 - \theta) + \Theta(\theta - \pi + \theta_0)] \quad [1]$$

where $\Theta(x)$ is the Heaviside step function (equals 1 if $x > 0$ and 0 otherwise), $p_{\text{ex}0}$ is the magnitude of the external pressure, and θ_0 represents the size of the caps where the pressure

is applied (Fig. 1b). The shape of the droplet is determined by the balance between the external force and the surface tension. This balance leads to the Young-Laplace equation,

$$\gamma \nabla \cdot \mathbf{n} = p - p_{\text{ex}} \quad [2]$$

where $\mathbf{n}$ is the outward unit normal vector, $\nabla \cdot \mathbf{n}$ represents twice the mean curvature, and p is the interior pressure, which is a constant.

In the absence of the external pressure, the solution of eq [2] is a spherical shape for the droplet, with radius R related to the surface tension and interior pressure via

$$p_0 = \frac{2\gamma}{R} \quad [3]$$

Note that a constant p_{ex} does not change the spherical shape; it can simply be absorbed into the interior pressure, i.e., subtracted from the latter.

I limit to a $p_{\text{ex}0}$ that only causes a small perturbation of the droplet from its spherical shape. Let this perturbation along the radial direction be $f(\theta)$. The new shape is specified by

$$r = R + f(\theta) \quad [4]$$

The shape function, which is defined to be 0 for all positions on the droplet surface, can be identified as

$$S(\mathbf{x}) = r - [R + f(\theta)] \quad [5]$$

The normal vector can be expressed as

$$\mathbf{n} = \frac{\nabla S}{|\nabla S|} \quad [6]$$

To the first order in f , the normal vector is given by

$$\mathbf{n} = \mathbf{e}_r - \nabla f = \mathbf{e}_r - \frac{f'}{R} \mathbf{e}_\theta \quad [7]$$

where a prime means derivative. The divergence of $\mathbf{n}$ is

$$\nabla \cdot \mathbf{n} = \frac{2}{R} - \frac{2f}{R^2} - \frac{1}{R^2 \sin \theta} \frac{d}{d\theta} (\sin \theta f') \quad [8]$$

Eq [2] thus becomes

$$\frac{1}{\sin \theta} \frac{d}{d\theta} (\sin \theta f') + 2f - 2R = \frac{R^2}{\gamma} (p_{\text{ex}} - p) \quad [9]$$

This equation can be solved by expanding both $f(\theta)$ and $p_{\text{ex}}(\theta)$ in Legendre polynomials:

$$f(\theta) = \sum_{l=0}^{\infty} A_l P_l(\cos \theta) \quad [10a]$$

$$p_{\text{ex}}(\theta) = p_{\text{ex}0} \sum_{l=0}^{\infty} C_l(\theta_0) P_l(\cos \theta) \quad [10b]$$

For the latter, the expansion coefficients are

$$C_l(\theta_0) = \begin{cases} P_{l-1}(\cos \theta_0) - P_{l+1}(\cos \theta_0), & l \text{ even} \\ 0, & l \text{ odd} \end{cases} \quad [11]$$

Substituting eqs [10a] and [10b] into eq [9], one finds

$$p = \frac{2\gamma}{R} + p_{\text{ex}0}(1 - \cos \theta_0) \quad [12]$$

$$f(\theta) = -\frac{p_{\text{ex}0}R^2}{\gamma} \sum_{l \geq 2; l \text{ even}} \frac{C_l(\theta_0)}{(l-1)(l+2)} P_l(\cos \theta) \quad [13]$$

The shape perturbation caused by the optical stress of a laser beam (such as that operating in OT) was recently solved.¹⁷

The external pressure causes a compression at either pole,

$$X_0 = f(0) = -\frac{p_{\text{ex}0}R^2}{\gamma} \sum_{l \geq 2; l \text{ even}} \frac{C_l(\theta_0)}{(l-1)(l+2)} \quad [14]$$

The force exerted on either pole is

$$F_0 = -p_{\text{ex}0}\pi R^2(1 - \cos^2 \theta_0) \quad [15]$$

This is obtained by integrating over each cap the axial component (i.e., along the pole-to-pole direction) of the force due to the external pressure. The net force in the lateral direction is zero due to axial symmetry. In contrast, Jawerth et al.¹⁴ integrated the external pressure over each cap and obtained $p_{\text{ex}0}2\pi R^2(1 - \cos \theta_0)$ for the force. For small θ_0 , their result agrees with mine to the third order in θ_0 . The negative sign indicates that the force is directed inward; the sign of X_0 is also negative (corresponding to compression). If $p_{\text{ex}0}$ were negative, the applied force F_0 would be directed outward, and X_0 would be positive and correspond to stretching.[†] Moreover, the magnitude of X_0 is proportional to the magnitude of F_0 . So indeed the droplet behaves like a spring. The effective spring constant is

[†]Although technically pressure can only be positive, one can assume that a positive constant external pressure has been absorbed into the interior pressure. Then p_{ex} is a perturbation on this constant external pressure, and can thus be both positive and negative.

$$\chi_0 \equiv \frac{F_0}{X_0} \quad [16a]$$

$$= \frac{\pi\gamma(1 - \cos^2 \theta_0)}{\sum_{l \geq 2; l \text{ even}} \frac{C_l(\theta_0)}{(l-1)(l+2)}} \equiv \pi\alpha_0(\theta_0)\gamma \quad [16b]$$

This definition of the spring constant is the same as the counterpart in a spring pressed on both ends by opposite forces, but is larger by a factor of 2 than the one used by Jawerth.¹⁴

Eq [16b] is valid as long as the droplet does not have a permanent elastic modulus (i.e., the elastic modulus $G'(\omega) = 0$ at $\omega = 0$; see the next section for more about $G'(\omega)$). If $G'(0) \neq 0$, then an elastic restoring force also comes into play. However, there has not yet been any reports of a permanent elastic modulus for bimolecular condensates.

II. Model Under Sinusoidal Forces

Now consider the case where the external pressure varies sinusoidally in time:

$$p_{\text{ex}}(\theta, t) = \delta p_{\text{ex}}(\theta) e^{i\omega t} \quad [17a]$$

$$\delta p_{\text{ex}}(\theta) = \delta p_{\text{ex}0} [\Theta(\theta_0 - \theta) + \Theta(\theta - \pi + \theta_0)] \quad [17b]$$

For convenience, time dependence is expressed in the complex form, but it is understood that only the real part of any time-dependent variable should be taken. In response to the sinusoidal external pressure, the interior pressure is also oscillatory:

$$p(\mathbf{x}, t) = p_0(\mathbf{x}) + \delta p(\mathbf{x}) e^{i\omega t} \quad [18]$$

where $p_0(\mathbf{x})$ is a time-independent component, and $\delta p(\mathbf{x})$ is the amplitude of the time-dependent component. Similarly, the fluid displacement field can be written as

$$\mathbf{u}(\mathbf{x}, t) = \mathbf{u}(\mathbf{x}) e^{i\omega t} \quad [19]$$

Accordingly the velocity field is

$$\mathbf{v}(\mathbf{x}, t) = i\omega \mathbf{u}(\mathbf{x}) e^{i\omega t} \quad [20]$$

The constitutive relation for the stress tensor of a Newtonian fluid, which leads to the Navier Stokes equations, now takes the form

$$\tilde{\tau} = -p\tilde{\mathbf{I}} + i\omega\eta [\nabla \mathbf{u} + (\nabla \mathbf{u})^T] \quad [21]$$

where η is the viscosity of the interior fluid, and the superscript “T” denotes transpose. For a viscoelastic fluid, the coefficient in the preceding equation is the complex shear modulus $G(\omega) = G'(\omega) + iG''(\omega)$, with both a real part $G'(\omega)$ (the elastic or storage modulus) and an

imaginary part $G''(\omega)$ (the viscous or loss modulus); the viscosity corresponds to the ratio $G''(\omega)/\omega$ in the low-frequency limit. The constitutive relation becomes

$$\tilde{\tau} = -p\tilde{\mathbf{I}} + G(\omega)[\nabla\mathbf{u} + (\nabla\mathbf{u})^T] \quad [22]$$

The Navier-Stokes equations for incompressible, high-viscosity fluids take the form

$$G(\omega)\nabla^2\mathbf{u} - \nabla p = 0 \quad [23]$$

$$\nabla \cdot \mathbf{u} = 0 \quad [24]$$

I again assume a small perturbation of the droplet shape, with the radial distance (see eq [4])

$$r = R + f(\theta)e^{i\omega t} \quad [25]$$

which is now time-dependent. The shape function now becomes

$$S(\mathbf{x}, t) = r - [R + f(\theta)e^{i\omega t}] \quad [26]$$

Because fluid elements on the droplet surface move at the local fluid velocity, one boundary condition is¹⁸

$$\frac{\partial S}{\partial t} + \mathbf{v} \cdot \nabla S = 0 \quad [27]$$

To the first order in f , this boundary condition yields

$$u_r = f \text{ at } r = R \quad [28]$$

Subscripts r and θ denote the components of the displacement vector.

Additional boundary conditions come from force balance at the droplet surface:

$$\mathbf{n} \cdot \tilde{\tau} \cdot \mathbf{t} = 0 \quad [29]$$

$$\mathbf{n} \cdot \tilde{\tau} \cdot \mathbf{n} = -p_{\text{ex}} - \gamma \nabla \cdot \mathbf{n} \quad [30]$$

where $\mathbf{t}$ denotes a tangential direction. For the present problem, these lead to

$$\frac{\partial u_r}{\partial \theta} + r \frac{\partial u_\theta}{\partial r} - u_\theta = 0 \text{ at } r = R \quad [31]$$

$$\begin{aligned} -p_0(\mathbf{x}) - \delta p(\mathbf{x})e^{i\omega t} + 2G(\omega)\frac{\partial u_r}{\partial r}e^{i\omega t} = -\delta p_{\text{ex}}(\theta)e^{i\omega t} - \frac{2\gamma}{R} \\ + \frac{2\gamma f}{R^2}e^{i\omega t} + \frac{\gamma}{R^2 \sin \theta} \frac{d}{d\theta} \left(\sin \theta \frac{df}{d\theta} \right) e^{i\omega t} \text{ at } r = R \end{aligned} \quad [32a]$$

In the last equation, the only time-independent terms are $p_0(\mathbf{x})$ on the left-hand side and $2\gamma/R$ on the right-hand side. Therefore $p_0(\mathbf{x}) = 2\gamma/R$, which is the solution of the unperturbed droplet (see eq [2]). Removing the two time-independent terms and using eq [28], one obtains

$$-\delta p(\mathbf{x}) + 2G(\omega)\frac{\partial u_r}{\partial r} = -\delta p_{\text{ex}}(\theta) + \frac{2\gamma u_r}{R^2} + \frac{\gamma}{R^2 \sin \theta} \frac{\partial}{\partial \theta} \left(\sin \theta \frac{\partial u_r}{\partial \theta} \right) \text{ at } r = R \quad [32b]$$

To solve the foregoing fluid dynamics problem, I use the stream function $\Psi(r, \theta)$. The solution of the Navier-Stokes equations has the form¹⁸

$$\Psi = \sum_{l \geq 2; l \text{ even}} (A_l r^{l+3} + B_l r^{l+1}) Q_l(\cos \theta) \quad [33]$$

where A_l and B_l are coefficients to be determined by the boundary conditions, and $Q_l(x)$ are polynomials related to the more familiar Legendre polynomials $P_l(x)$ via

$$Q_l(x) = \int_{-1}^x dx' P_l(x') \quad [34]$$

Using the relation

$$\frac{dP_{l+1}(x)}{dx} - \frac{dP_{l-1}(x)}{dx} = (2l+1)P_l(x) \quad [35]$$

one finds

$$Q_l(x) = \frac{P_{l+1}(x) - P_{l-1}(x)}{2l+1} \quad [36]$$

The components of the displacement vector and the pressure are given by

$$i\omega u_r = v_r = -\frac{1}{r^2} \frac{\partial \Psi}{\partial \cos \theta} \quad [37a]$$

$$= - \sum_{l \geq 2; l \text{ even}} (A_l r^{l+1} + B_l r^{l-1}) P_l(\cos \theta) \quad [37b]$$

$$i\omega u_\theta = v_\theta = -\frac{1}{r \sin \theta} \frac{\partial \Psi}{\partial r} \quad [38a]$$

$$= - \sum_{l \geq 2; l \text{ even}} [A_l(l+3)r^{l+1} + B_l(l+1)r^{l-1}] \frac{Q_l(\cos \theta)}{\sin \theta} \quad [38b]$$

$$\frac{i\omega\delta p}{2G(\omega)} = - \sum_{l \geq 2; l \text{ even}} A_l \left(2 + \frac{3}{l}\right) r^l P_l(\cos \theta) \quad [39]$$

Substituting eqs [37b] and [38b] into eq [31] leads to

$$\bar{A}_l l(l+2) + \bar{B}_l (l+1)(l-1) = 0 \quad [40]$$

where $\bar{A}_l = A_l R^l$ and $\bar{B}_l = B_l R^{l-2}$. Similarly, using eqs [37b] and [39] in [32b] leads to

$$\begin{aligned} & \left[2G(\omega) \frac{l^2 - l - 3}{l} + \frac{\gamma}{R} (l-1)(l+2) \right] \bar{A}_l \\ & + \left[2G(\omega)(l-1) + \frac{\gamma}{R} (l-1)(l+2) \right] \bar{B}_l = i\omega\delta p_{\text{ex}0} C_l(\theta_0) \end{aligned} \quad [41]$$

where $C_l(\theta_0)$ is given by eq [11]. The results for $\bar{A}_l$ and $\bar{B}_l$ are:

$$\bar{A}_l = - \frac{i\omega\delta p_{\text{ex}0} C_l(\theta_0)}{2G(\omega) \frac{2l^2 + 4l + 3}{l(l+1)} + \frac{\gamma}{R} \frac{(l+2)(2l+1)}{l+1}} \quad [42a]$$

$$\bar{B}_l = \frac{i\omega\delta p_{\text{ex}0} C_l(\theta_0)}{2G(\omega) \frac{(l-1)(2l^2 + 4l + 3)}{l^2(l+2)} + \frac{\gamma}{R} \frac{(l-1)(2l+1)}{l}} \quad [42b]$$

The first few coefficients are

$$\bar{A}_2 = - \frac{i15\omega\delta p_{\text{ex}0} \cos\theta_0 \sin^2\theta_0}{2(19G(\omega) + 20\frac{\gamma}{R})} \quad [43a]$$

$$\bar{B}_2 = \frac{i20\omega\delta p_{\text{ex}0} \cos\theta_0 \sin^2\theta_0}{19G(\omega) + 20\frac{\gamma}{R}} \quad [43b]$$

$$\bar{A}_4 = - \frac{i15\omega\delta p_{\text{ex}0} \cos\theta_0 \sin^2\theta_0 (1 + 7 \cos 2\theta_0)}{8(17G(\omega) + 36\frac{\gamma}{R})} \quad [43c]$$

$$\bar{B}_4 = \frac{i3\omega\delta p_{\text{ex}0} \cos\theta_0 \sin^2\theta_0 (1 + 7 \cos 2\theta_0)}{17G(\omega) + 36\frac{\gamma}{R}} \quad [43d]$$

These first coefficients are the same as those listed by Jawerth et al.,¹⁴ but these authors did not find a general expression for arbitrary l and truncated the summation at $l = 10$.

Let us now look at the dynamic spring constant. Analogous to eq [15], the amplitude of the force exerted on either pole is

$$F_0 = \delta p_{\text{ex}0} \pi R^2 (1 - \cos^2 \theta_0) \quad [44]$$

Noting eq [28], one finds the amplitude of the droplet deformation at the poles as

$$X_0(\omega) = -u_r(R, 0) \quad [45a]$$

$$= \frac{R}{i\omega} \sum_{l \geq 2; l \text{ even}} (\bar{A}_l + \bar{B}_l) \quad [45b]$$

The dynamic spring constant is thus

$$\chi(\omega) \equiv \frac{F_0}{X_0(\omega)} \quad [46a]$$

$$= \frac{\pi R (1 - \cos^2 \theta_0)}{\sum_{l \geq 2; l \text{ even}} \frac{C_l(\theta_0)}{2G(\omega) \frac{(l-1)(2l^2 + 4l + 3)}{l(2l+1)} + \frac{\gamma}{R}(l-1)(l+2)}} \quad [46b]$$

If $\gamma/R \gg G(\omega)$, eq [46b] reduces to

$$\chi(\omega) \approx \frac{\pi \gamma (1 - \cos^2 \theta_0)}{\sum_{l \geq 2; l \text{ even}} \frac{C_l(\theta_0)}{(l-1)(l+2)}} \quad [47]$$

which is identical to χ_0 (eq [16b]), the spring constant due to a static external force. Therefore in the surface tension-dominated regime, the response of the droplet to a sinusoidal force is identical to that to a static force. A special situation where $\gamma/R \gg G(\omega)$ holds is at very low frequency, since as we assume $G(0) = 0$ for biomolecular condensates. Thus $\chi(0) = \chi_0$. Eq [47] states that $\chi(\omega) = \chi_0$ at nonzero frequencies as long as $\gamma/R \gg G(\omega)$. In the opposite limit $\gamma/R \ll G(\omega)$, one has

$$\chi(\omega) \approx \frac{2\pi R G(\omega) (1 - \cos^2 \theta_0)}{\sum_{l \geq 2; l \text{ even}} \frac{C_l(\theta_0)}{\frac{(l-1)(2l^2 + 4l + 3)}{l(2l+1)}}} \equiv 2\pi \alpha_1(\theta_0) R G(\omega) \quad [48]$$

Summations of the kind in eqs [16b] and [46b] have appeared in the context of diffusion-controlled bimolecular reactions, where a ligand binds to a site modeled as an absorbing patch on a spherical target.^{19–20} For small patches, these summations are notoriously slow to converge, and the slow convergence has in fact led to an interesting prediction on how an ligand-target interaction potential affects the binding rate.²⁰ In the present context, the cap size θ_0 is typically less than 0.1 and indeed very small.¹⁴ Then given the slow convergence

of the summations, truncating the summations at $l = 10$ can generate serious errors. Indeed, as I demonstrate next, such truncation lead to qualitatively incorrect predictions for the dependence of χ_0 and $\chi(\omega)$ on cap size and to different extents of underestimation for γ and $G(\omega)$. Table 1 presents a summary of the contrast and comparison between Jawerth et al. and the present work.

RESULTS AND DISCUSSION

Extraction of surface tension from static spring constant.

In the static experiment that I propose, the applied force is balanced by surface tension. As given by eq [16b], the static spring constant χ_0 is proportional to the surface tension γ , thereby allowing for a direct determination of the latter. This direct determination of γ is very useful since, as will be seen below, extracting both γ and $G(\omega)$ from the dynamic spring constant alone can be a challenge.

The proportionality constant $\pi\alpha_0$ in eq [16b] depends on the cap size θ_0 . Based on their approximate solution, Jawerth et al.¹⁴ proposed a quadratic fitting function:

$$\pi\alpha_0 \approx 2(1.25 + 4.36\theta_0^2) \quad [49]$$

which predicts a nonzero value, 1.26, for α_0 at $\theta_0 = 0$. I verified this fit by limiting the summation in eq [16b] to $l = 10$ (Fig. 2, open circles and dashed curve). However, as noted in the preceding section, this summation is very slow to converge for small θ_0 . Converged results, obtained by summing up to $l = 100,000$, for θ_0 from 0.001 to 0.5 show a very different dependence on θ_0 (Fig. 2, filled circles). First off, $\alpha_0 \rightarrow 0$ as $\theta_0 \rightarrow 0$. Correspondingly $1/\alpha_0$ diverges at $\theta_0 = 0$. The following fitting function:

$$1/\alpha_0 \approx -0.50 \ln \theta_0 + 0.34 \quad [50]$$

represents well the results for θ_0 from 0.001 to 0.5 (Fig. 2, dotted line). At $\theta_0 = 0.01$, Jawerth et al.'s formula would overestimate α_0 by a factor of 2.1 and underestimate γ by the same factor.

Extraction of $G(\omega)$ from $\chi(\omega)$ at negligible γ .

At high frequencies, $G(\omega)$ is large and the effect of surface tension is negligible, then the dynamic spring constant is proportional to the complex shear modulus (see eq [48]). Jawerth et al. again found a quadratic function for the proportionality constant:

$$\pi\alpha_1 \approx 0.58 + 3.42\theta_0^2 \quad [51]$$

which predicts a nonzero value, 0.18, for α_0 , at $\theta_0 = 0$ (Fig. 3, open circles and dashed curve). However, converged results for α_1 , goes to 0 at $\theta_0 = 0$, and is equal to θ_0 for small θ_0 :

$$\alpha_1 \approx \theta_0 \quad [52]$$

(Fig. 3, filled circles and dotted line). At $\theta_0 = 0.01$, Jawerth et al.'s formula would overestimate α_1 , by a factor of 18.4 and underestimate $G(\omega)$ by the same factor.

As noted in the preceding section, summations similar to the one in eq [48] have appeared in the context of diffusion-controlled binding reactions.^{19–20} For a small θ_0 , binding to a spherical cap is equivalent to binding to a circular disk on a plane. By solving these two problems and equating the results, one finds[‡]

$$\sum_{l=0}^{\infty} \frac{C_l(\theta_0)}{l+1} \approx 2\theta_0 \quad [53]$$

The convergence of this summation requires the contributions of many terms with large l . Two consequences of large l then allow for the generalization of eq [53] to the summation in eq [48]. First,

$$\frac{(l-1)(2l^2 + 4l + 3)}{l(2l+1)} \approx l \quad [54]$$

Second, the sum of even terms and sum of odd terms become approximately equal. With these considerations, one finds

$$\sum_{l \geq 2; l \text{ even}} \frac{\frac{C_l(\theta_0)}{(l-1)(2l^2 + 4l + 3)}}{l(2l+1)} \approx \theta_0 \quad [55]$$

This result leads to eq [52].

Eq [54] indicates that the coefficient of $G(\omega)$ in eq [46b] scales as l , but the coefficient of γ/R scales as l^2 . Therefore the summation when only γ/R is present converges faster than the counterpart when only $G(\omega)$ is present. This explains why the error caused by truncation at $l = 10$ is much larger for α_1 than for α_0 . The disparate errors mean that γ and $G(\omega)$ would be underestimated to different extents, and hence their ratio would be incorrectly predicted. This ratio is related to the speed of droplet fusion,^{13, 16} as further explained below.

Extraction of $G(\omega)$ at intermediate to high frequencies.

Extraction of γ and $G(\omega)$ from $\chi(\omega)$ becomes complicated when both γ and $G(\omega)$ have to be taken into consideration. $G(\omega)$ and $\chi(\omega)$ both have real and imaginary parts (denoted by prime and double prime, respectively), and hence one is trying to extract three quantities [i.e., γ , $G'(\omega)$, and $G''(\omega)$] from only two knowns [i.e., $\chi'(\omega)$ and $\chi''(\omega)$], and do so for a range of frequencies.

[‡]The problems are solved by the so-called constant-flux approximation,¹⁸ where one assumes that the flux to the binding site is a constant and determines this constant by approximately satisfying the absorbing boundary condition on the binding site. In the original version of the approximation, the absorbing boundary condition was replaced by the stipulation that the ligand concentration was 0 when averaged over the binding site. To obtain eq [53], I used a variant of the foregoing approximation, where the ligand concentration is set to 0 only at the center of the binding site.

To illustrate the complexity, let us consider the situation where γ/R is not negligible but still small relative to $G(\omega)$, as happens at intermediate frequencies. Then one can try an expansion like

$$\chi(\omega) \approx 2\pi\alpha_1(\theta_0)RG(\omega) + 2\pi\alpha_2(\theta_0)\gamma \quad [56]$$

By plotting $\chi/2\pi RG - \alpha_1$, against γ/GR (assuming real values for G), one can find α_2 by a linear fit (Fig. 4). The resulting values of α_2 are approximately 0.53 at $\theta_0 = 0.1$ and 0.49 at $\theta_0 = 0.01$. The corresponding α_1 , values are 0.103 and 0.010, respectively. In comparison, Jawerth et al.'s approximation gives 0.58 and 0.56 for α_2 , and 0.196 and 0.185 for α_1 . The relative errors for α_1 , are much greater than for α_2 , again meaning different extents of underestimation for γ and $G(\omega)$.

Even though eq [56] separates $\chi(\omega)$ into contributions by γ and $G(\omega)$, it does not allow γ and $G(\omega)$ to be determined simultaneously. However, if γ is separately determined from the static experiment, eq [56] then allows for easy extraction of $G(\omega)$.

Extraction of $G''(\omega)$ at negligible $G'(\omega)$.

Biomolecular condensates at low frequencies should have $G''(\omega) \gg G'(\omega)$. Note that this condition is satisfied at all frequencies by Newtonian fluids, where $G'(\omega) = 0$ and $G''(\omega) = \omega\eta$. I now explain how to find $G''(\omega)$ when $G'(\omega)$ is neglected and γ is already determined.

The real and imaginary parts of $\chi(\omega)$ in eq [46b] can be found as

$$\chi'(\omega) = \frac{\pi R(1 - \cos^2 \theta_0)H'(\omega)}{[H'(\omega)]^2 + [H''(\omega)]^2} \quad [57a]$$

$$\chi''(\omega) = \frac{\pi R(1 - \cos^2 \theta_0)H''(\omega)}{[H'(\omega)]^2 + [H''(\omega)]^2} \quad [57b]$$

where

$$H'(\omega) = \sum_{l \geq 2; l \text{ even}} \frac{C_l(\theta_0)D_l(\omega)}{[D_l(\omega)]^2 + [E_l(\omega)]^2} \quad [58a]$$

$$H''(\omega) = \sum_{l \geq 2; l \text{ even}} \frac{C_l(\theta_0)E_l(\omega)}{[D_l(\omega)]^2 + [E_l(\omega)]^2} \quad [58b]$$

The $D_l(\omega)$ and $E_l(\omega)$ terms are

$$D_l(\omega) = F_{1;l} + F_{2;l}G'(\omega) \quad [59a]$$

$$E_l(\omega) = F_{2;l}G''(\omega) \quad [59b]$$

with

$$F_{1;l} = \frac{\gamma}{R}(l-1)(l+2) \quad [60a]$$

$$F_{2;l} = \frac{2(l-1)(2l^2 + 4l + 3)}{l(2l+1)} \quad [60b]$$

When $G'(\omega) = 0$ and γ is given, one can calculate $G''(\omega)$ for a range of $G'(\omega)$ values, and fit the dependence of $G''(\omega)$ on $G'(\omega)$ to a polynomial. Then any measured value of $\chi''(\omega)$ can be fed to this polynomial to determine $G''(\omega)$. For forthcoming reference, I denote this solution as $G_0''(\omega)$. An example of such a $G_0''(\omega)$ determination is shown in Fig. 5.

The zero-frequency limit of $G''(\omega)/\omega$ is the viscosity η . Modeling protein droplets as Newtonian fluids, one can show that the speed of the fusion between two droplets is proportional to γ/η .¹⁶ The static and dynamic versions of the OT-based deformation experiment together can measure both γ and η , and thereby indirectly determine the fusion speed.

Extraction of $G(\omega)$ at low frequencies.

The preceding result obtained assuming $G'(\omega) = 0$ can be further improved, by using it as the reference for a perturbative solution of $G(\omega)$ at low frequencies. To that end, I write

$$G'(\omega) = 0 + \delta G'(\omega) \quad [61a]$$

$$G''(\omega) = G_{0''}(\omega) + \delta G''(\omega) \quad [61b]$$

and look for the first-order corrections $\delta G'(\omega)$ and $\delta G''(\omega)$. As the first step, eqs [57a] and [57b] can be inverted to find

$$H'(\omega) = \frac{\pi R(1 - \cos^2 \theta_0) \chi'(\omega)}{[\chi'(\omega)]^2 + [\chi''(\omega)]^2} \quad [62a]$$

$$H''(\omega) = \frac{\pi R(1 - \cos^2 \theta_0) \chi''(\omega)}{[\chi'(\omega)]^2 + [\chi''(\omega)]^2} \quad [62b]$$

Then substituting eqs [61a] and [61b] into eqs [58a] and [58b] (via eqs [59a] and [59b]), and expanding the right-hand sides to first order in $\delta G'(\omega)$ and $\delta G''(\omega)$, one finds

$$A\delta G'(\omega) + B\delta G''(\omega) = \Delta H'(\omega) \quad [63a]$$

$$B\delta G'(\omega) - A\delta G''(\omega) = \Delta H''(\omega) \quad [63b]$$

where

$$A = \sum_{l \geq 2; l \text{ even}} \frac{C_l(\theta_0) F_{2;l} \left\{ F_{1;l}^2 - [F_{2;l} G_{0''}(\omega)]^2 \right\}}{\left\{ F_{1;l}^2 + [F_{2;l} G_{0''}(\omega)]^2 \right\}^2} \quad [64a]$$

$$B = \sum_{l \geq 2; l \text{ even}} \frac{2C_l(\theta_0) F_{1;l} F_{2;l}^2 G_{0''}(\omega)}{\left\{ F_{1;l}^2 + [F_{2;l} G_{0''}(\omega)]^2 \right\}^2} \quad [64b]$$

$$\Delta H'(\omega) = \sum_{l \geq 2; l \text{ even}} \frac{C_l(\theta_0) F_{1;l}}{F_{1;l}^2 + [F_{2;l} G_{0''}(\omega)]^2} - H'(\omega) \quad [64c]$$

$$\Delta H''(\omega) = \sum_{l \geq 2; l \text{ even}} \frac{C_l(\theta_0) F_{2;l} G_{0''}(\omega)}{F_{1;l}^2 + [F_{2;l} G_{0''}(\omega)]^2} - H''(\omega) \quad [64d]$$

Note that $H''(\omega)$ would be 0 if $G_0''(\omega)$ is an exact solution of eq [58b] obtained with $G'(\omega) = 0$. However, $H''(\omega)$ may not be strictly 0 if $G_0''(\omega)$ is predicted from a polynomial fit as outlined above (see Fig. 5). Solving the linear eqs [63a] and [63b] leads to

$$\delta G'(\omega) = \frac{A \Delta H'(\omega) + B \Delta H''(\omega)}{A^2 + B^2} \quad [65a]$$

$$\delta G''(\omega) = \frac{B \Delta H'(\omega) - A \Delta H''(\omega)}{A^2 + B^2} \quad [65b]$$

Complete extraction of $G(\omega)$.

Finally I demonstrate that the procedure outlined above for extracting $G(\omega)$ at intermediate to high frequencies and the one at low frequencies can be combined for a complete extraction of $G(\omega)$. I consider the so-called Jeffreys model for the complex shear modulus

$$G(\omega) = i\omega \left(\eta_0 + \frac{\eta_1}{1 + i\omega\tau_R} \right) \quad [66]$$

which arises from a complex viscosity with two components: one, with amplitude η_0 , is frequency-independent; the other, with amplitude η_1 , has a frequency dependence due to relaxation at a time constant τ_R . The dynamic spring constant $\chi(\omega)$ generated by this $G(\omega)$ is shown in Fig. 6a, and is similar to the experimental data of Jawerth et al. for the PGL-3 protein droplet.

Fig. 6b compares the $G(\omega)$ extracted from the $\chi(\omega)$, using the procedures at intermediate to high frequencies (red curves) and at low frequencies (blue curves), against the original $G(\omega)$ (circles). The extracted results agree with the original $G(\omega)$ at the respective frequency ranges. Importantly, the two different extractions cover overlapping frequencies, where they

agree well with each other. So their combination indeed allows for a complete extraction of $G(\omega)$.

In summary, I have presented a full solution to a theoretical model for analyzing OT-based droplet deformation in order to determine material properties of biomolecular condensates and also proposed a static version of the deformation experiment (see Table 1). The theoretical model enables determination of the surface tension γ directly from the static spring constant χ_0 . I also describe procedures that allow for a complete extraction of the complex shear modulus $G(\omega)$ from the dynamic spring constant $\chi(\omega)$. The full solution of the theoretical model has dependences of χ_0 and $\chi(\omega)$ on the cap size θ_0 that are qualitatively different from those predicted by the approximate solution of Jawerth et al. This analytical solution should also be useful for validating numerical methods for solving related fluid dynamics problems.

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Figure 1. Illustration of the OT-based experiment. (a) Two optically trapped beads (yellow) apply stretching or compressing forces at the poles of a protein droplet (gray). (b) Cross section showing the deformation of the droplet (black boundary) from its original spherical shape (cyan dash).

Figure 2. Proportionality constant for relating γ to χ_0 . Open circles and dashed curve present results from Jawerth et al.'s approximation; filled circles and dotted line present results from the full solution.

Figure 3.

Proportionality constant for relating $G(\omega)$ to $\chi(\omega)$ when γ is negligible. Open circles and dashed curve present results from Jawerth et al.'s approximation; filled circles and dotted line present results from the full solution. Inset: results for $0.1 \leq \theta_0 \leq 0.5$ presented on a linear scale.

Figure 4. Plots to determine $\alpha_2(\theta_0)$. Points are results calculated for the two θ_0 indicated in the legend; lines are linear fits.

Figure 5.

Relation between $G_0''(\omega)$ and $\chi''(\omega)$. Points are calculated according to eq [57b], for $\theta_0 = 0.0893$, $R = 5.6 \mu\text{m}$, and $\gamma = 6.2 \mu\text{N/m}$. Superimposed on the points is the following polynomial: $y = -4.69 \times 10^{-9}x^5 + 1.37 \times 10^{-6}x^4 - 1.53 \times 10^{-4}x^3 + 7.91 \times 10^{-3}x^2 + 1.42 \times 10^{-1}x$, obtained by fitting.

Figure 6.

Complete extraction of $G(\omega)$ from $\chi(\omega)$. (a) Curves show $\chi(\omega)$ for a droplet with $\theta_0 = 0.0893$, $R = 5.6 \mu\text{m}$, $\gamma = 6.2 \mu\text{N/m}$, and $G(\omega)$ of eq [66] at $\eta_0 = 0.5 \text{ Pa s}$, $\eta_1 = 1.5 \text{ Pa s}$, and $\tau_R = 5 \text{ ms}$. Circles show the experimental data of Jawerth et al. for the PGL-3 protein droplet at 75 mM KCl. (b) Original and extracted $G(\omega)$. The original $G(\omega)$ of eq [66] is shown as circles. $G(\omega)$ extracted at intermediate to high frequencies according to eq [56], with $\alpha_1 = 0.0917$ and $\alpha_2 = 0.5265$, is shown as red curves; that extracted at low frequencies according to eqs [65a] and [65b] is shown in blue. Insets: zoom into the overlap region. In all panels, solid curves and filled circles display real parts of $\chi(\omega)$ and $G(\omega)$, while dashed curves and unfilled circles display the corresponding imaginary parts.

Table 1.

Contrast and comparison between Jawerth et al. and the present work

	Advance	Solution of theoretical model	Extraction of $G(\omega)$
Jawerth et al.	Introduced dynamic version of OT-based experiment and theoretical model	<i>Partial</i> : summation only up to $l = 10$	<i>Incomplete</i> : (i) unable to extract $G'(\omega)$ at small ω ; (ii) difficult to extract both $G(\omega)$ and γ
Present work	Proposes static version of experiment to separately determine surface tension	<i>Full</i> : general expression given for any l	<i>Complete</i> : (i) extraction of both $G'(\omega)$ and $G''(\omega)$ at any ω ; (ii) static experiment allows independent determination of γ