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Stochastic optical active rheology

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We demonstrate a stochastic based method for performing active rheology using optical tweezers. By monitoring the displacement of an embedded particle in response to stochastic optical forces, a rapid estimate of the frequency dependent shear moduli of a sample is achieved in the range of 10^{-1}–10^{3} Hz. We utilize the method to probe linear viscoelastic properties of hydrogels at varied cross-linker concentrations. Combined with fluorescence imaging, our method demonstrates non-linear changes of bond strength between T cell receptors and an antigenic peptide due to force-induced cell activation. © *2012 American Institute of Physics*. [http://dx.doi.org/10.1063/1.4737159]

Soft materials such as polymers and biological samples are viscoelastic. Their viscoelastic properties are characterized by a complex shear modulus (G). The real and imaginary parts of G are the storage (G') and loss shear modulus (G''), respectively, representing the energy stored and dissipated in response to an external force. Traditionally, material properties have been estimated by commercial rheometers, in which a specimen, located between two plates, is subjected to an oscillatory shear stress applied by rotating one of the plates. However, probing time-scales are limited by the inertial effects of the lab frame device, allowing measurement of only up to tens of Hz. In addition, large sample volumes are required for reliable measurement, and local, microscale, mechanical properties are difficult to capture.

Microrheology techniques developed in the past two decades can measure mechanical properties in a much smaller volume and over a broader range of frequencies.[1] Typically, the frequency-dependent shear modulus is derived by tracking the thermally driven motions of particles embedded in the specimen, with measurable time scales extended to 10^{4} Hz.[2] Due to their volume and temporal advantages, microrheology techniques have been broadly used to estimate the viscoelastic properties of cross-linked polymers, living cells, and cytoskeletal proteins.[3–5] However, for a given driving force, the passive movement of a particle is inversely proportional to the stiffness of a material. Thus, measurable stiffness is limited by detection resolution. For example, a thermally driven micron-sized bead in a 10 Pa · s solution moves by only 130 nm after 10 s, which would require sub-nanometer resolution for proper measurement at 1 kHz. Interpretation can also be complicated depending on sample homogeneity and particle size relative to the structure's length scale. Two-point microrheology overcomes some shortcomings of single-particle methods by measuring the correlated motion of pairs of particles, which is independent of the size, shape, and coupling between particles and the sample. These factors reduce contribution from sample drift.[6] Nonetheless, passive microrheology techniques always probe within linear viscoelastic regimes due to the fluctuation-dissipation theorem.

Recent measurements for colloidal gels and biological samples have revealed nonlinear viscoelastic properties that depend on the magnitude of applied force.[7,8] Both living cells and *in vitro* reconstituted actin networks undergoing external deformation exhibit strain hardening and softening.[9,10] Underlying these observations are a number of potential force-induced contributions such as unbinding/unfolding of cross-linkers, buckling of stretched actin filaments, and network reorganization by motor proteins. Internally/externally generated mechanical forces can significantly remodel cell structures through cellular functions, such as migration, adhesion, and apoptosis. Measurements that use external forces are advantageous over passive methods for elucidating active cellular functions such as protein assembly and cell signaling. However, active techniques which typically use an oscillating force to obtain frequency-dependent material properties were limited to sequential measurements of only a few sinusoidal inputs.

Here, we develop an active stochastic rheology technique using optical tweezers and demonstrate its utility in a hydrogel system that exhibits linear material properties. The technique is further demonstrated by tracking non-linear changes in the bond strength of cell receptor-ligand interactions subjected to external forces. Stochastic forces are applied by moving an optical trap with an acousto-optic deflector following a white noise input. Separate position sensitive devices simultaneously monitor bead displacement and trap position. With stochastic input, our developed technique

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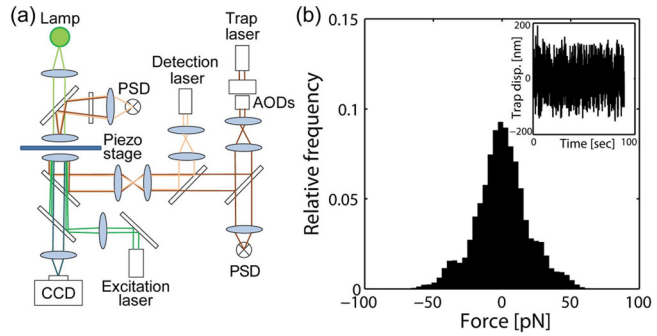

FIG. 1. (a) Optical layout of optical tweezers for rheological measurements. (b) Stochastic forces are generated by displacing the trapping laser (inset) with a Gaussian random distribution.

can obtain both G' and G'' over a wide frequency range, 10^{-1}–10^{3} Hz, from a single measurement lasting one minute. The measurements for both "near sol-gel transition" and "gel" state hydrogels are compared with the results obtained from a rheometer. Finally, rheological measurements with parallel fluorescence imaging demonstrate strengthened interactions between T cell receptors and antigenic peptides bound to a major histocompatibility complex molecule (pMHC) that results from force-triggered T cell activation. We call this technique stochastic optical active rheology (SOAR).

SOAR was developed using a custom-built optical trap described previously,[11] with some modifications. Briefly, three separate lasers are integrated for trapping (1064 nm), detection (975 nm), and fluorescence excitation (532 nm). The trap position is controlled by acousto-optic deflectors (AODs) and directly monitored by a position sensitive device (PSD). A collinear detection laser allows conventional tracking of the trapped particle on a second PSD [Fig. 1(a)]. Both the trapping laser and bead positions are detected at 3 kHz. The sample position is controlled using a piezoelectric stage. Trap stiffness and bead position are calibrated using standard procedures.[12] Once a single bead is centered in the detection zone, stochastic forces are applied by displacing the trapping laser using AODs [Fig. 1(b)] with a computer generated white noise input. The applied force was confirmed as an uncorrelated random signal by analyzing its autocorrelation. The mechanical response of the bead is determined by the stiffness of the trapping laser and the viscoelastic properties of the sample.

The force $F(t)$ applied to a system is calculated by multiplying the trap displacement by its stiffness. The complex response function $\alpha(f) = \alpha'(f) + i\alpha''(f)$ is defined as the Fourier transform $X(f)$ of the bead displacement x(t) divided by the Fourier transform $F(f)$ of the force F(t).

$$\alpha(f) = X(f)/F(f). \tag{1}$$

The generalized Stokes-Einstein relation provides a connection between the frequency-dependent complex shear modulus $G(f) = G'(f) + iG''(f)$ and $\alpha(f)$

$$G(f) = \frac{1}{6\pi a\,\alpha(f)}, \tag{2}$$

where a is the radius of the bead. Assuming the trapping laser acts as a purely elastic spring, the frequency-dependent complex shear modulus of the sample, G_{sample}, is calculated by subtracting the elastic component of the trap from the apparent G[13]

$$G_{sample} = G_{apparent} - \frac{k}{6\pi a}, \tag{3}$$

where k is the stiffness of the trap.

Using the developed method, the mechanical properties of polyacrylamide samples were measured. Polyacrylamide was chosen as a model system because (1) its mechanical properties exhibit linear characteristics independent of applied strain,[8] (2) its rheological properties can be modulated by adjusting the concentrations of acrylamide monomer and methylenebisacrylamide cross-linker, and (3) its cross-linked network mimics many biological structures.[14,15] Samples are prepared by gently mixing acrylamide monomers with methylenebisacrylamide and then adding 0.005% (w/v) polystyrene beads. The acrylamide polymerization is catalyzed by 0.2% (w/v) N,N,N′,N′-Tetramethylethylenediamine with 0.5% ammonium persulfate.

The viscoelastic properties of polyacrylamide gels (3% acrylamide/0.02% bisacrylamide) were measured with trap stiffness varying from 0.3 pN/nm to 0.7 pN/nm. The power spectral density of the bead displacements in response to stochastic random forces followed a Lorentzian profile showing that the motion was overdamped (data not shown). The

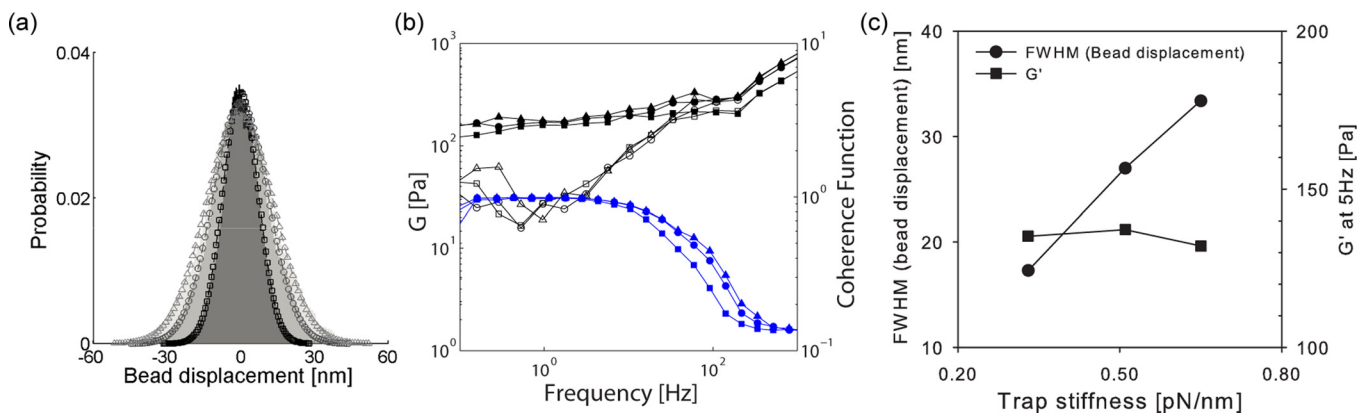

FIG. 2. Stochastic rheology measurements with varied trap stiffness. (a) Bead displacements as a function of trapping laser stiffness [0.33 pN/nm (square), 0.51 pN/nm (circle), 0.65 pN/nm (triangle)]. (b) Measured G' (black, closed) and G'' (black, open) as a function of the trapping laser stiffness [0.33 pN/nm (square), 0.51 pN/nm (circle), 0.65 pN/nm (triangle)]. Coherence functions (blue, closed) between trap and bead displacements. (c) While the full width at half maximum (FWHM) of the bead displacement increased with trapping stiffness, the storage shear moduli exhibited similar values.

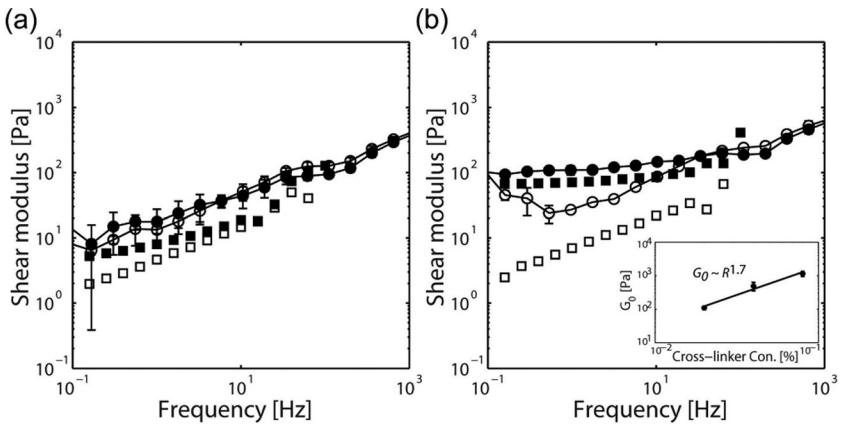

FIG. 3. G' (closed) and G'' (open) shear modulus of 3% polyacrylamide in "near sol-gel transition" (a, 0.01% cross-linker) and "gel" (b, 0.02% cross-linker) states measured by SOAR (circles) and a bulk rheometer (squares). (Inset) The plateau storage shear moduli (G_0) observed in gel state polyacrylamide as a function of cross-linker concentration (R).

average bead displacement increased proportionally with trap stiffness [Fig. 2(a)]. However, the calculated G' and G'' values were unchanged and independent of trap stiffness. We used the coherence function to examine the linearity of the responses. At frequencies <10 Hz in which G' exhibited plateau values, the coherence defined as the cross-spectral density divided by the autospectral density of trap and bead displacements was close to 1, indicating linear sample characteristics [Fig. 2(b)]. Measurements of G at varied trap stiffness produced consistent results demonstrating the method's accuracy [Fig. 2(c)].

SOAR was used to investigate the effect of cross-linker concentration on the mechanical properties of polyacrylamide for a given acrylamide concentration of 3%. When the cross-linker concentration was less than 0.02%, values of G' and G'' were similar over the entire range of frequencies, showing an increase with frequency (f) as $G \sim f^{0.4}$ [Fig. 3(a)]. Once the sol-gel transition occurs at ~0.02% cross-linker concentration, the storage shear modulus becomes less dependent on frequency [Fig. 3(b)]. G' dominates G'' at frequencies lower than the relaxation frequency of ~30 Hz, where G' is equal to G''. We define a plateau storage modulus G_0 as the value at 2 Hz for the "gel" state polyacrylamide with a cross-linker concentration $\geq 0.02\%$. The magnitude of G_0 varies one order of magnitude with a 4 fold increase in cross-linker concentration, scaling as $G_0 \sim$ cross-linker$^{1.7}$ [Fig. 3(b) inset]. This suggests that the cross-linkers form a tight and rigid network.

Experimental results from SOAR were compared with those obtained by a strain-controlled rheometer (AR-G2, TA Instruments) using a 40 mm diameter parallel plate geometry, 140 μm gap, and strain maintained below 4%. As shown in Figures 3(a) and 3(b), bulk measurements probed the "near sol-gel transition" and "gel" characteristics of polyacrylamide with 0.01% and 0.02% cross-linkers, respectively. The measured shear moduli are similar to those obtained from SOAR.

SOAR was used to measure the dynamic responses of T cells to mechanical stimulation. The specific interaction between the T cell receptor (TCR) and an antigenic peptide bound to a pMHC triggers activation. This initiates a cascade of downstream signaling events, including early phase calcium flux to the cytoplasm. N15 TCR expressing T cells specific for the peptide ligand bound to the MHC molecule were purified.[16] To monitor real-time TCR signaling, cells were incubated with a cell-permeable calcium dye (Calcium Orange, Invitrogen) for 30 min. The cells were transferred into a custom-built chamber and allowed to adhere to a 2% L-lysine coated cover glass for 15 min at 37 °C. After blocking the chamber surfaces with 1 mg/ml casein solution for 5 min, 1 μm diameter pMHC-coated beads were loaded for rheological measurements. A single bead was captured and

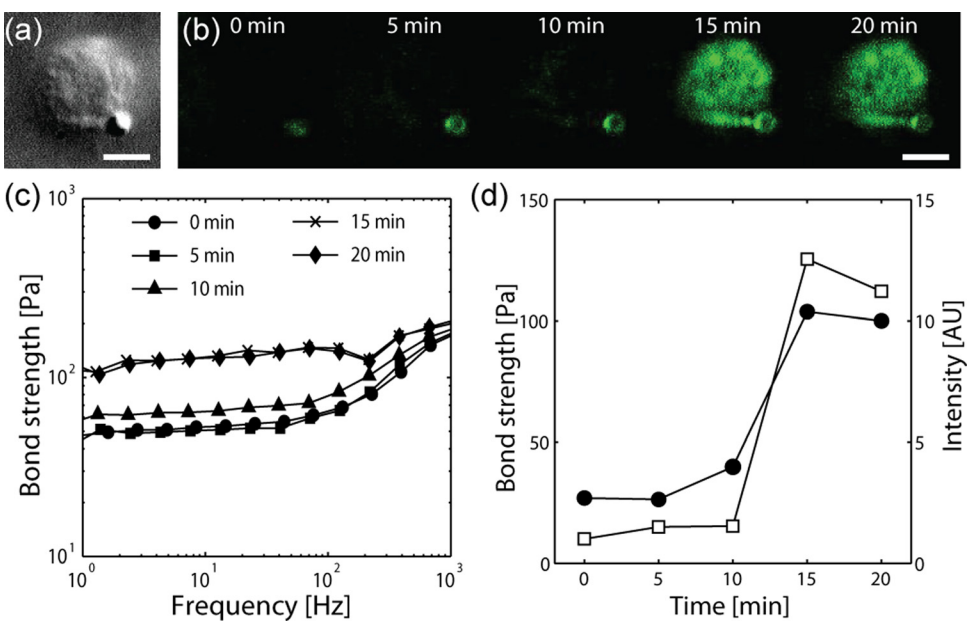

FIG. 4. Rheological measurements combined with fluorescence imaging (scale bar: 2 μm). (a) A pMHC-coated bead bound to a single T cell. (b) Fluorescence images of calcium dye showing that T cells are activated by mechanical stimulation applied by SOAR. (c) Adhesion strength between pMHC and T cell receptors estimated by SOAR. (d) Changes of bond strength (solid circle) and internal calcium flux (open square) as a function of time.

positioned against a surface bound T-cell. Upon attachment [Fig. 4(a)], stochastic random forces were applied. Adhesion strength was measured every 5 minutes using the developed method. The width of the applied force distributions was ~23 pN. Calcium dye was simultaneously imaged to monitor intracellular calcium flux[17] [Fig. 4(b)]. As indicated by increases in storage shear modulus, adhesion between the pMHC coated bead and the T cell membrane becomes stiffer [Fig. 4(c)] over the ~20 min experiment. The calcium signal also increased simultaneously with the modulus as tracked through the active stochastic measurements [Fig. 4(d)]. The observed increase in the strength of the attachment may be explained by either actin polymerization near the activated site or by the formation of multiple TCR-pMHC linkages, as was previously reported upon signal initiation.[18]

We developed an active stochastic rheology technique using optical tweezers. This active measurement is advantageous for characterizing the mechanical properties of a sample in which the Brownian motion of a thermally fluctuating particle is difficult to detect. By monitoring the mechanical responses of an embedded bead to externally applied stochastic forces, a frequency-dependent shear modulus is obtained in a short measurement time. Measurement accuracy was demonstrated by comparing the results with those obtained from bulk rheology. As deformation of a specimen is caused by displacement of the bead captured by the trapping laser, maximum deformation is limited by the size of the detection region and zone over which a calibrated force can be applied, which in our instrument is a circular area with a diameter of ~500 nm for 1 μm beads. However, to extend maximal deformation our experimental setup can be modified to include other detection schemes and strain can be applied by displacing the sample stage, allowing study of strain hardening or softening behaviors that can be observed at a larger deformation. The maximal stiffness that can be measured with SOAR can be estimated by balancing the potential energy of optical tweezers with the elastic energy of a material. In our stochastic measurement, energy generated by optical tweezers ($=\frac{1}{2}\, k\, \langle r^2 \rangle$) is limited by the trap stiffness (k) and the variance of trap displacement ($\langle r^2 \rangle$). Elastic energy of a material ($\approx GaL^2$, where G is a shear modulus, a microsphere radius, L bead displacement) is limited by the detection resolution in optical tweezers. Assuming k, $\langle r^2 \rangle$, a, and L are 1 pN/nm, $(50\,\text{nm})^2$, 500 nm, and 20 nm, respectively, the maximum measurable G would be ~6 kPa.

Using rheology measurements with simultaneous fluorescence imaging, we demonstrated that the adhesion strength between a T cell membrane and pMHC increases as mechanical forces activate the cell. Because the application of forces to pMHC-TCRs induces T cell activation, the bond strength also increases. The strengthening might be due to structural reorganization of the cell or clustering of TCR complexes. Further studies about how force-induced activation depends on magnitude and type of applied force will be useful in elucidating the underlying molecular mechanism. Our rheology technique based on the optical trapping method is useful for characterizing mechanical properties at a user-defined location, magnitude, and orientation. Due to these advantages, we believe that this method can also be applied to studies where measurement time and force application are critical, including cell signaling via mechanosensors.

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