BE/APh 161: Physical Biology of the Cell

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15 The cell as a material

The cell as a whole behaves like a **viscoelastic material**. By viscoelastic, we mean the the cell has properties that are both fluid-like and solid-like. As a reminder, the stress/strain relationship for a solid is

$$\sigma = E\varepsilon, \tag{15.1}$$

where ε is the strain, σ is the stress, and E is the Young's modulus. That is to say that the stress is directly proportional to the strain, at least for small stresses/strains. Nonlinearities start to become important for larger stresses or strains.

Conversely, the stress is proportional to the *strain rate* for a viscous fluid.

$$\sigma = \eta\,\dot{\varepsilon}, \tag{15.2}$$

where the overdot signifies time differentiation. This makes sense if we consider that stress counteracts viscous dissipation due to velocity gradients. You may recall from our discussion of elastic beams that the strain is the normalized displacement of material. As discussed in section 5.3 and 5.4 of *PBoC2*, the strain is given by the spatial derivative of the displacements u; $\varepsilon = \Delta a/a_0 = \partial u/\partial x$ in one dimension. Then,

$$\dot{\varepsilon} = \frac{\partial}{\partial t}\frac{\partial u}{\partial x} = \frac{\partial}{\partial x}\frac{\partial u}{\partial t} = \frac{\partial v}{\partial x}, \tag{15.3}$$

where v is the local velocity at which the material is moving, equal to the time derivative of the displacement. So, the strain rate is equal to the velocity gradient, which is proportional to the stress in a viscous fluid.

15.1 Storage and loss moduli

Imagine the following thought experiment. A material (either a cell, or something like a reconstituted actin network) is subjected to a periodic stress with frequency ω and amplitude σ_0.

$$\sigma(t) = \sigma_0 \sin \omega t, \tag{15.4}$$

After some time, the strain will also be periodic, with amplitude ε_0 and frequency ω. However, it will not necessarily be in phase with the stress, so we define a phase shift δ.

$$\varepsilon(t) = \bar{\varepsilon} + \varepsilon_0 \sin(\omega t - \delta), \tag{15.5}$$

where $\bar{\varepsilon}$ is the baseline strain from the oscillation. If $\delta = 0$, then $\sigma \propto \varepsilon$, so the material behaves like an elastic solid.[14] If $\delta = \pi/2$, then

$$\varepsilon(t) = \bar{\varepsilon} + \varepsilon_0 \sin(\omega t - \delta) = \bar{\varepsilon} + \varepsilon_0 \cos \omega t. \tag{15.6}$$

In this case, then $\sigma(t) \propto \dot{\varepsilon}(t)$, so the material behaves like a viscous solid. For phase shifts in between, the material behaves both like a solid (strain in phase with the stress) and like a viscous fluid (strain out of phase with the stress). We can define parameters to describe the solid-like and fluid-like responses

[14] I am being loose with the $\propto$ symbol here. There is an additive constant, $\bar{\varepsilon}$, but that constant is zero for purely elastic responses, as we will see later in the response for a Maxwell material.

of a material to stress. These parameters are respectively the **storage and loss moduli**. They are defined in terms of the amplitudes of the stress and strain amplitudes and the phase shift δ. The are

$$\text{storage modulus} = E' = \frac{\sigma_0}{\varepsilon_0} \cos\delta \tag{15.7}$$

$$\text{loss modulus} = E'' = \frac{\sigma_0}{\varepsilon_0} \sin\delta\,. \tag{15.8}$$

Note that the storage and loss moduli are sometimes denoted respectively as G' and G''. They are in general both frequency dependent. They can be measured empirically. Typically the stress is imposed (so σ_0 is known), and the strain is measured. The storage modulus is a measure of the solid-like response and the loss modulus is a measure of the viscous-like response. They are sometimes referred to as elastic and viscous moduli for that reason. Note that these moduli are *not* the Young's modulus and viscosity of the material. They are defined by equations (15.7) and (15.8). How they relate to other parameters is dependent on how we choose to model the material, which is the subject of the next lecture.

15.2 Doing the "thought" experiment with reconstituted actin

The thought experiment of applying a periodic stress to a material is possible via several means. The amplitude and phase of the strain response is measured, enabling determination of the storage and loss module to characterize the material.

Chaudhuri and coworkers did a clever experiment in which they grew an actin network on the tip of an atomic force microscope. The network grew to a surface, and then they could move the surface up and down at set frequencies and measure the strain by observing the deflection of the AFM cantilever. (See Fig. 20).

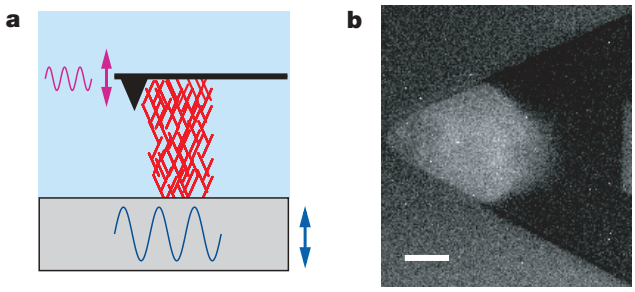

Figure 20: a) Schematic of experimental setup. b) Fluorescence image of an actin network growing on a cantilever. Scale bar is 10 μm. Figure taken from Chaudhuri, Parekh, and Fletcher, *Nature*, **445**, 295–298, 2007.

A typical stress/strain temporal profile from the experiment is shown in Fig. 21. The strain lags the stress slightly, indicating that the actin network is predominantly, though not purely, elastic.

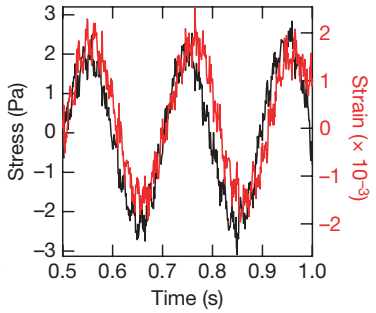

Figure 21: A typical stress-strain profile, taken with 5 Hz forcing frequency. Figure taken from Chaudhuri, Parekh, and Fletcher, *Nature*, **445**, 295–298, 2007.

The frequency can be varied by adjusting the movement of the surface. For each frequency, the storage and loss moduli can be measured. The result is shown in Fig. 22. At low frequency, the loss modulus does not depend on frequency, but at higher frequency it grows with frequency. The storage modulus shows power law behavior, $E' \sim \nu^a$, where ν is the frequency and a is the power law exponent. The maximum likelihood estimate[15] puts the value of the exponent a to be about 0.13. Many cellular materials behave in this way, and I emphasize again that the molecular details of how this comes about are not immediately obvious nor ascertainable in this experiment. Materials are often described by the power law behavior of the storage modulus and by plots such as these, and they are useful for comparison.

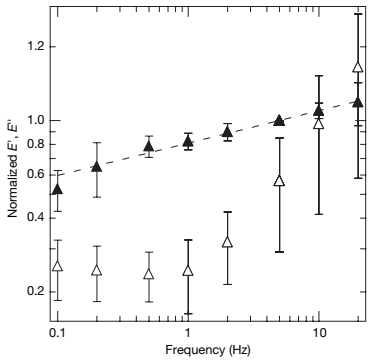

Figure 22: The storage (filled symbols) and loss (open symbols) moduli measured for various frequencies. The values are normalized to the average storage modulus at 5 Hz, which was approximately 985 Pa. Though not clearly stated in the paper, I believe the imposed stress magnitude was about 2 Pa. Figure taken from Chaudhuri, Parekh, and Fletcher, *Nature*, **445**, 295–298, 2007.

The storage modulus of the actin network is about 10^3 Pa, which is similar to that observed for cell cortices, as measured by pushing a large magnetic bead against the outside of a cell. Fabry et al. (*PRL*, **87**, 148102, 2001) found that the cortical storage modulus is about 10^3-10^4 Pa in human airway smooth muscle cells.

[15] I am not actually sure this is a maximum likelihood estimate because the statistical procedures in the paper were not detailed enough.

While the frequency sweep gave a picture of the power law nature of the storage modulus, Chaudhury and coworkers also did a sweep of imposed stress with the frequency fixed at 5 Hz. The results are displayed in Fig. 23. At low stress, the observed moduli of the actin network did not change. At these low stresses, compression will tend to straighten out the wiggles in the fibers, resulting in a primarily entropic response, like the entropic springs we encountered when we studied polymer pulling. As the applied stress grows, these fluctuations are already pulled out. Filaments running orthogonally to the surface serve as struts, while those running parallel to the surface are stretched as the material deforms. This strong resistance to stretching results in **stress stiffening**; the storage modulus grows with applied stress. This happens above about 15 Pa. However, when the stress becomes very large, close to 230 Pa, the filaments start to buckle, which can result in **stress softening**. The buckled filaments can no longer push effectively against the surface, and the storage modulus gets smaller with greater stress.

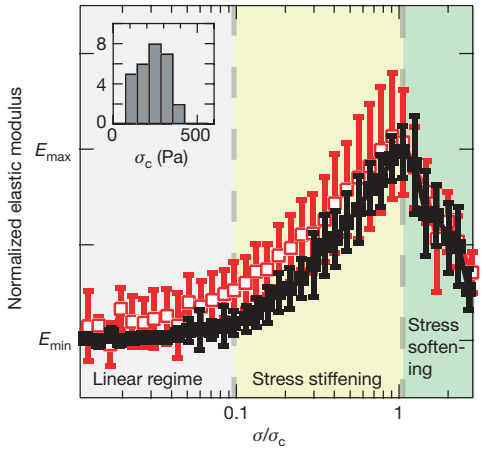

Figure 23: The storage modulus (averaged over many experiments) for a range of imposed stress magnitudes. The experiment starts with low stress and then ramps up to the maximal imposed stress of about 600 Pa. The resulting storage modulus was calculated through the stress sweep and is plotted in black. Then, the imposed stress amplitude was decreased, the storage modulus calculated, and plotted in red. the stress is normalized to the critical stress where stress softening starts to occur, around 230 Pa. The inset shows a histogram of the observe critical imposed stress, σ_c. Figure taken from Chaudhuri, Parekh, and Fletcher, *Nature*, **445**, 295–298, 2007.

The red curve, going from high stress to low stress, does not overlap with the black curve, giving an apparent hysteresis. This is likely due to the fact that this actin network is grown in *Xenopus* extract and is still dynamically changing during the course of the experiment. It can therefore experience greater crosslinking and perhaps greater density as the experiment progresses.

Importantly, this simple experiment exposes some key features of actin networks.

- They are viscoelastic, with a strong elastic component at high frequencies.
- They exhibit strain stiffening and strain softening.
- They are dynamic.

15.3 Microrheological studies of cytoplasmic viscoelasticity

Microrheology is a technique wherein micron-sized tracer beads are injected into a possibly viscoelastic material. The rheological properties of the material are ascertained by monitoring the motion of the beads.

For example, imagine we track the position of a bead moving through a viscous material. It should diffuse via a random walk. From the track of the bead's position over time we can compute a mean square displacement, $\langle r^2 \rangle$. Since the imaging is typically done in two dimensions, the mean square displacement is related to the diffusion coefficient as we have worked out previously in class,

$$\langle (\mathbf{r}(t+\tau) - \mathbf{r}(t)) \cdot (\mathbf{r}(t+\tau) - \mathbf{r}(t)) \rangle \equiv \langle r^2(\tau) \rangle = 4D\tau. \tag{15.9}$$

Then, if we plot the mean square displacement versus time and perform a regression, we can get a value for the diffusion coefficient D. For a purely viscous medium, we can work out the viscosity from the calculated D using the Stokes-Einstein-Sutherland relation,

$$D = \frac{k_B T}{6\pi\eta a}, \tag{15.10}$$

where a is the radius of the bead.

Conversely, if the bead were in a purely elastic medium and we tracked it, the mean square displacement is independent of time,

$$\langle r^2 \rangle = \frac{k_B T}{K}, \tag{15.11}$$

where K is a spring constant related to the Young's modulus E of the medium by $K = 3\pi Ea$ when the Poisson ratio is zero.[16]

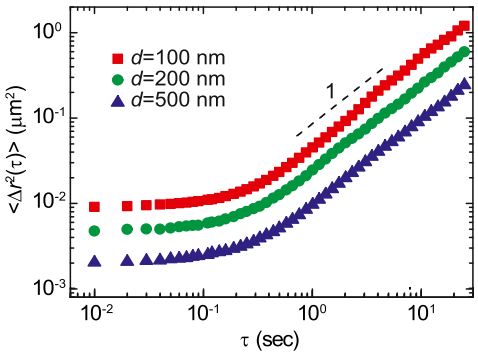

Figure 24: Mean squared displacement of passively tracked beads injected into A7 melanoma cells. Figure taken from Guo, et al., *Cell*, **158**, 822–832, 2014.

So, if we inject beads into a viscoelastic medium, we might expect elastic-like behavior on short time scales and viscous-like behavior on long time scales. We would then get $\langle r^2(\tau) \rangle \approx$ constant for small τ. For large τ, we expect $\langle r^2(\tau) \rangle \sim \tau$. This kind of experiment has been done many times. Shown in Fig. 24 are results of injecting passive beads of different diameter into A7 melanoma cells. At short times, the mean square displacement curve is flat, transitioning to a slope of unity at time

[16] This result comes from a generalization of the Stokes relation that we will not go through here.

scales beyond one second. The gap between the curves is commensurate with the diffusion coefficient varying like d^{-1}, where d is the diameter of the bead. We can read the diffusion coefficients from the plot, with $D \approx 2.5 \times 10^{-3}$ μm^2/s for the 500 nm beads. Using the Stokes-Einstein Sutherland relation, this gives a viscosity of $\eta \approx 0.4$ Pa-s, about two and a half orders of magnitude more viscous than water. This is a value typically reported for cytoplasmic viscosity. But *please read the next section!*

15.3.1 The cytoplasm is neither viscous nor passive

This interpretation of the experiment is wrong! At least it is wrong for the cytoplasm of these cells, which have active forces in them due to motor protein activity, polymerization, etc. Guo and coworkers performed the same experiment in the same cell types treated with blebbistatin, which inhibits myosin activity. As shown in Fig. 25, the mean square displacement curves shift rightward, showing inhibited motion. Inhibiting myosin may change the structure of the cytoplasm by changing the crosslinking of filaments, so we may expect to see a shift in the dynamics. Guo and coworkers went step further and depleted ATP using sodium azide and 2-deoxyglucose. The result is the solid lines in Fig. 25. The beads barely move at all in the absence of ATP. Together, these results imply the *active* forces, driven by energy consuming processes in the cell are moving the beads. The movement of the beads is *not* by thermal diffusion, so the Stokes-Einstein-Sutherland relation is cannot be validly applied to this experiment.

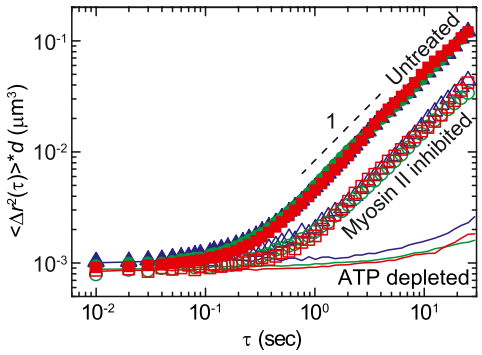

Figure 25: Mean squared displacement of passively tracked beads injected into A7 melanoma cells, including cells treated with blebbistatin (open symbols) and with ATP depleting agents sodium axide and 2-deoxyglucose (solid lines). Red, green, and blue symbols respectively are for beads of 100, 200, and 500 nm in diameter. The mean squared displacement if multiplied by the diameters to collapse the curves. Figure taken from Guo, et al., *Cell*, **158**, 822–832, 2014.

15.3.2 Active microrheology

In the experiments we have just describe, the tracer beads are allowed to passively move around the cytoplasm. By "passive," I mean that the experimenter is not exerting a force on the bead. The beads, as we have just argued, are being actively moved around by nonequilibrium processes in the cell, but the technique is called passive microrheology when the experimenter does not move the bead.

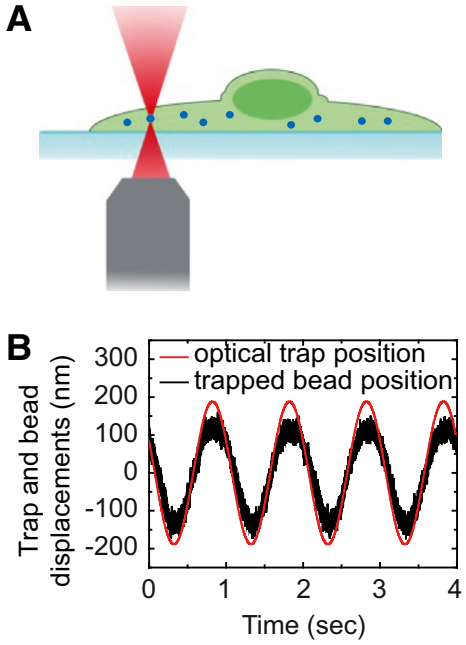

Figure 26: A) Schematic for a cytoplasmic active microrheology experiment using an optical trap and tracer particles. B) Trap and bead displacement from oscillatory forcing of the bead at 1 Hz frequency. Figure taken from Guo, et al., *Cell*, **158**, 822–832, 2014.

An alternative approach is to mechanically move the tracer particles within the cell. Optical traps, schematically shown in Fig. 26A, provide a great way to do this. The trap can be calibrated so that the force, and therefore stress, exerted can be deduced from the trap displacement and bead position. The bead position can also be used to infer the displacement of the surrounding cytoplasm, giving the stress-strain relationship. We can then infer the storage and loss modulus from these measurements. From the plot in Fig. 26B, we see that the bead position moves closely with the trap, implying that the cytoplasm is predominantly elastic. The result for A7 cells under various treatment conditions is shown in Fig. 27. The elastic modulus is dominant, and shows a power law with $E' \sim \nu^{0.15}$, in agreement with the results found with reconstituted actin networks we discussed earlier in this lecture.

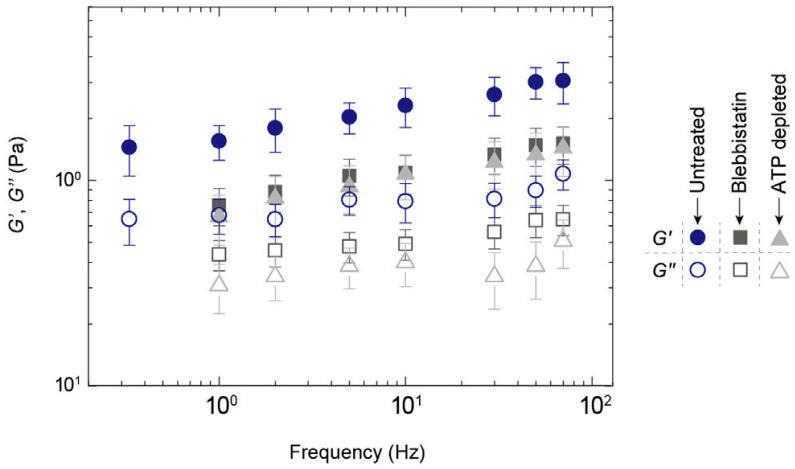

Figure 27: Frequency-dependent storage (G') and loss (G'') moduli in A7 cells under various treatments. Figure taken from Guo, et al., *Cell*, **158**, 822–832, 2014.

Note that the storage modulus of the cytoplasm in these cells is of order 1 Pa, in contrast to a storage modulus of about 1000 Pa for the actin network. The cortex is much more dense with actin than the cytoplasm, but the cytoplasm nonetheless can behave like an elastic gel, albeit a must less stiff one.

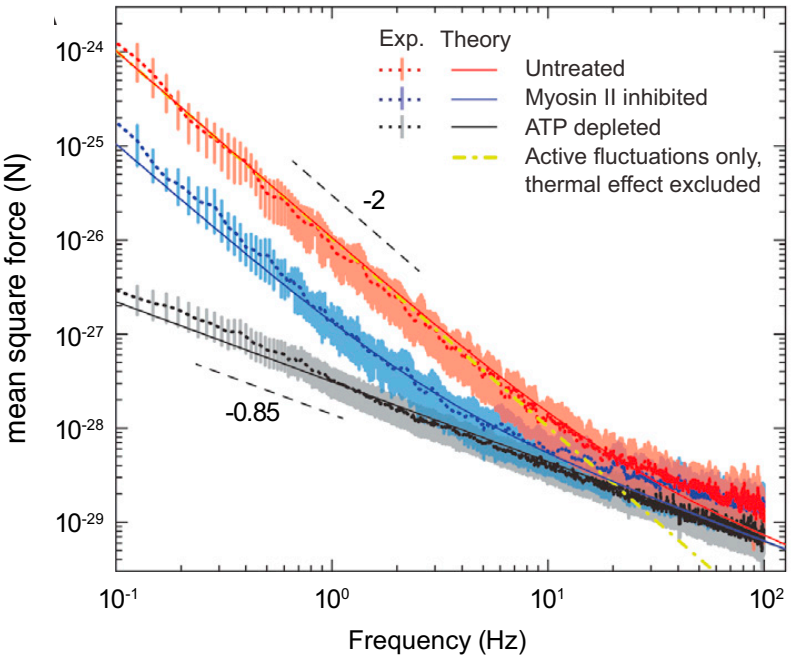

Figure 28: Frequency-dependent mean square forces for in A7 cells under various treatments. Figure taken from Guo, et al., *Cell*, **158**, 822–832, 2014.

The active microrehology enables determination of the elastic properties of the cytoplasm, from which forces present on passive beads may be inferred. The forces are directional and stochastic, and

on average cancel out (at least for an isotropic cytoplasm). We therefore need to compute the mean square force. Specifically, $\langle (F(\nu))^2 \rangle = |K(\nu)|^2 \langle r^2(\nu) \rangle$, where ν is the frequency of forcing. Since we can know the spring constant K from the active microrheological experiments ($K = 3\pi E' d$), we can track passive beads to infer forces. The result is show in Fig. 28. For small frequency, we get a mean square force that scales like ν^{-2}, consistent with movement of the bead due to active forces within the cytoplasm. For high frequencies, that is at faster time scales than active forces can be exerted, the force has weaker dependence on frequency, indicative of thermal fluctuations rattling a bead in an elastic cage.