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A *Langevin* model of physical forces in cell volume fluctuations

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Cells interact mechanically with their physical surroundings by attaching to the extracellular matrix or other cells and contracting the cytoskeleton. Cells do so dynamically, exhibiting fluctuating contractile motion in time. In monolayers, these dynamic contractions manifest as volume fluctuations, which involve the transport of fluid in and out of the cell. An integrated understanding of cell elasticity, actively generated stresses, and fluid transport has not yet been developed. Here we apply a minimal model of these forces to cell volume fluctuation data, elucidating the dynamic behavior of cells within monolayers.

Introduction

In each type of tissue throughout the body, cells generate forces to sense the rigidity of their surroundings; cell function, fate, and survival depend on the cellular mechanical microenvironment (Discher et al., 2005; Harris et al., 1980; Pelham and Wang, 1997). The cell's F-actin cytoskeleton comprises several types of contractile elements that actively exert forces on the extracellular matrix and on neighboring cells. On surfaces, cells form stress fibers that generate traction forces (Dembo and Wang, 1999; Pelham and Wang, 1997). In monolayers, cells form equatorial actin belts that contract to generate in-plane tensile forces (Hirano et al., 1987; Parczyk et al., 1989; Schroeder, 1973). Across the cell surface, a cortical actin network

contracts to augment membrane tension and rigidity (Engler et al., 2006; Zhelev et al., 1994). An F-actin network links the apical and basolateral cell surfaces, permeates much of the cell volume, and contracts to generate cytoskeletal pre-stress, modulating the stiffness of the cell itself (Wang et al., 2002). If cells maintain a state of mechanical equilibrium, internal elements that bear compressive loads or stresses in the extracellular surroundings must balance these tensions. Internally, tension may be balanced by microtubules, which exhibit a buckling instability like slender rods under compression (Brangwynne et al., 2006). Externally, deformations in the cell's substrate and stresses in neighboring cells could balance the cell generated tension (Tambe et al., 2011; Trepat et al., 2009).

The collective migration patterns and transient stress fluctuations that exist within monolayers indicate that internally generated tensile forces within each cell are not balanced by elastic forces in their surroundings (Tambe et al., 2011). Imbalances in normal stress may drive the volumetric expansion or contraction of cells; it was recently shown in these dynamic monolayers that cells fluctuate in volume by 20% every four hours (Zehnder et al., 2015). Such volume fluctuations are facilitated by the cell's permeability; water from the cytosol can exchange with the surrounding bath or between neighboring cells through gap junctions with about the same hydraulic resistance (Bennett and Verselis, 1992; Giaume et al., 1986; Hoffmann et al., 2009; Timbs and Spring, 1996; Zehnder et al., 2015). The interplay between a minimal number of dominant forces like cell elasticity, resistance to fluid transport, and cell generated stress may determine volume fluctuations of single cells. However, this integrated set of physical forces that control cell volume fluctuations have not been investigated previously.

Here we explore the mechanics of spontaneous cell volume fluctuations in Madin Darby Canine Kidney (MDCK) epithelial monolayers. The sizes of over 1000 cells are measured and

tracked over time. We fit a simple damped harmonic oscillator model driven by a random white noise stress to the average power spectrum of cell volume fluctuations, determining the corresponding elastic and viscous parameters. We also use the fits to estimate the amplitude of the driving stress, finding a value of 6.8 kPa. These results introduce a potential method for measuring stresses and stiffness of cells grown on rigid surfaces like glass and polystyrene, on which soft substrate methods like traction force microscopy cannot be employed.

Materials and Methods

Madin Darby Canine kidney cells with green fluorescent protein (GFP) labelled histones are cultured in Dublecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin streptomycin. Histones are tagged with the triple nuclear localization signal (NLS) expression system and a gentamicin resistance sequence for cell selection. Culture medium is supplemented with 0.5 μg/mL gentamicin to continuously select for cells with fluorescent histones. Cells are incubated at 37 °C under a 5% CO_2 atmosphere. Prior to imaging, cells are seeded in 5mm diameter islands at a minimally confluent density on glass-bottomed petri dishes coated with bovine collagen type I. Dishes are imaged on an inverted light microscope with an environmental control stage maintained at 37 °C in a 5% CO_2 atmosphere. Phase contrast and FITC fluorescence images are captured in time-lapse every minute over the course of 9 hours. Exposure times are kept below 100 ms per minute and fluorescence excitation light is passed through a 10% transmitting neutral density filter to limit light exposure.

Results

We track cell positons by following fluorescent nuclei in space and time, and at each time point we compute a Voronoi tessellation map from the cell centers. The area of each cell within

the continuous layer is estimated from the area of its corresponding Voroinoi cell. In our previous work we showed that cell volume fluctuations are proportional to the fluctuations in this projected cell area (Zehnder et al., 2015). Thus, the volume fluctuation of each cell in time is defined by projected area fluctuations, given by $\delta V(t)=\left[A(t)-\langle A\rangle_t\right]/s_A$, where $A(t)$ is the projected area of a cell at any point in time, $\langle A\rangle_t$ is that cell's average area and s_A is the standard deviation of area over time. This definition of volume fluctuation is chosen to compute a normalized cell volume autocorrelation function, $C_{\delta V\text{-}\delta V}(\tau)$, which exhibits a characteristic oscillation time of approximately 4 hours, consistent with individual traces of cell volume (Zehnder et al., 2015) (Fig. 1). Errors associated with determining cell centers by their nuclei rather than from their geometric centers do not affect the analysis performed here; the results do not depend on translocation of the whole cell. However, direct measurement of volume fluctuations from the borders of over 300 cells shows that the Voronoi treatment of the cell as polygonal artificially reduces the measured amplitude of cell volume fluctuations, which we discuss further below.

To analyze cell volume fluctuations with a minimal physics-based model, we compute the power spectral density function, $S_{\delta V\text{-}\delta V}(\omega)$, by Fourier transforming $C_{\delta V\text{-}\delta V}(\tau)$. The peaked nature of the power spectrum and oscillatory nature of the correlation function are reminiscent of the behavior of a simple damped harmonic oscillator (DHO). We therefore model cell volume fluctuations with a Langevin equation in terms of normal stress and strain, given by

$$c_1\gamma_V(t)+c_2\dot{\gamma}_V(t)+c_3\ddot{\gamma}_V(t)=\sigma_N(t), \tag{1}$$

where c_1, c_2, c_3 are unknown constants and $\gamma_v(t)$ is volumetric strain defined as $\gamma_V(t)=\left[V(t)-\langle V\rangle_t\right]/\langle V\rangle_t$. $\sigma_N(t)$ is assumed to be a random white noise driving stress with

amplitude σ_o. Thus, $\sigma_N = \sigma_o f(t)$, where $f(t)$ is a delta-correlated random noise function with a constant power spectrum equal to unity, and all other terms are treated as passive mechanical responses to the driving stress. Traditionally, Langevin equations are used to incorporate the effects of random forces from Brownian solvent particles in transport equations; modern Langevin treatments model stochastic driving forces in active systems including bacteria, self-driven colloids, tissue cells, and the cytoskeleton (Marchetti et al., 2013). Here we consider the random driving stress, $\sigma_N(t)$, to be generated by stochastic contractions and relaxations of molecular motors on the cytoskeleton (Guo et al., 2014).

In equation 1, c_1 is the elastic modulus of a cell associated with volumetric strain. The molecular liquid component of the cytosol does not compress in these volume fluctuations, so c_2 is the drag coefficient associated with moving water into and out of cells which we have previously shown can occur by transport across the membrane or through gap junctions (Zehnder et al., 2015). When a cell expands or contracts within a confluent monolayer, all of the surrounding cells must move, on average, 180^0 out of phase with the central individual cell, expanding when the cell contracts and contracting when the cell expands. To capture the mechanical signals associated with this complementary motion, we include the term $c_3 \ddot{\gamma}_V(t)$, which is analogous to an inertial term. However, at the extremely slow rates of oscillation, inertia is totally negligible; this term merely approximates the out of phase chemo-mechanical signals in a cell's surroundings.

We rewrite equation 1 in terms of σ_o and $\delta V(t)$ yielding

$$c_1 \delta V(t) + c_2 \dot{\delta V}(t) + c_3 \ddot{\delta V}(t) = \frac{\langle V \rangle_t}{s_V} \sigma_o f(t). \tag{2}$$

With the equation of motion in terms of $\delta V(t)$ we define our model spectrum,

$$S_{\delta V-\delta V}(\omega)=\delta V(\omega)\delta V^{*}(\omega), \tag{3}$$

where ω is angular frequency, ~ denotes the Fourier transform, and * denotes the complex conjugate. Taking the Fourier transform of both sides of equation 2 and rearranging terms yields

$$\delta V(\omega)\left(c_1+i\omega c_2-\omega^2 c_3\right)=\frac{\langle V\rangle_t}{s_V}\sigma_o f\,. \tag{4}$$

Solving for δV in equation 4 and combining constants into common terms we compute the classic model power spectral density function of a damped harmonic oscillator,

$$S(\omega)=\frac{A}{\left(\omega_o^2-\omega^2\right)^2+\omega^2\Gamma^2} \tag{5}$$

where $A=\left(\langle V\rangle_t\,\sigma_o/s_V c_3\right)^2 f f^{*}$, $\omega_o=\sqrt{c_1/c_3}$, and $\Gamma=c_2/c_3$. We fit the model in equation 5 to the spectrum determined from the volume fluctuation data, allowing A, ω_0, and Γ to freely vary using a non-linear least squares algorithm. The fit matches the data over a range denoted by the blue line in Figure 2A with an R^2 value of 0.99 (Fig 2 A). We find A = 521 ± 45 h^{-2}, ω_0 = 3.1 ± 0.1 h^{-1}, and Γ = 12.6 ± 0.6 h^{-1}. We have also fit other model spectra to the data, in which each individual term or multiple terms in the equation (1) are dropped. All such candidate models fail to capture the experimental spectral line-shape and always predict power spectra that decay with the incorrect power.

To estimate each of the model parameters from the three fitting parameters, we relate c_2 to the hydraulic permeability of MDCK cells, k = 0.06 μm^3 kPa^{-1} s^{-1}. (Zehnder et al., 2015). c_2 is the stress per-unit strain-rate dissipated in volume fluctuations; k is the volumetric flow rate per unit applied stress. Either of these two parameters can be used in different forms of Darcy's law to describe the relationship between flow an pressure. The parameters are related by

$$c_2 = \frac{\langle V \rangle_t}{k}. \tag{6}$$

We find c_2 = 22.7 kPa h. With this estimate of c_2, all of the other model parameters can be found. c_3 is determined by $\Gamma = c_2/c_3$, yielding c_3 =1.3 kPa h^2. From ω_o and c_3 we determine the volumetric elasticity of cells, c_1 = 11.3 kPa. Finally, we compute the amplitude of the driving stress, σ_o = 6.8 kPa, determined from A and the other parameters already determined. The method of approximating individual cell volume fluctuations from Voronoi maps is found to under-estimate the amplitude of fluctuations by about 17%, which correspondingly decreases this estimate of σ_o. Experimental measurement of cell permeability, k, will allow confidence intervals to be generated for all of the model parameters estimated here.

Discussion and Conclusions

In this study we have applied a minimal physical model to experimental data of cell volume fluctuations. We have characterized the line-shape of the fluctuation spectrum and determined the physical parameters that control cell volume fluctuations and cell-cell mechanical interactions. We find that cell volume fluctuations are well characterized by a simple damped harmonic oscillator spectrum that deviates from the data at low frequencies. The simplest explanation of this deviation is that the cytoskeletal contractions driven by molecular motors and ATP hydrolysis cannot generate a white noise spectrum over time-scales of hours. Inspired by studies of the driving spectra of molecular motors in active cytoskeleton networks, we divide the experimental spectrum by the extrapolated white-noise driven spectrum to estimate the real driving spectrum (Fig. 2B) (Mizuno et al., 2007). The resulting driving spectrum is high-pass white noise, which reflects the fact that cells do not expand and contract for periods of time

longer than a few hours. This result shows that cell volume oscillations need not arise from a single sinusoidal driving force; a white noise spectrum with a low-frequency cut-off can drive cell volume fluctuations. Further exploration of this potential driving spectrum must be performed by a combination of active measurements that physically drive cell volume fluctuations, and passive measurements like those investigated here.

Our results represent a potential method for measuring cell generated stress on rigid substrates like glass. Traction force microscopy is a powerful method that enables the estimation of cell generated forces by measuring how cells deform their substrates (Dembo and Wang, 1999). Traction force microscopy cannot be performed on tissue culture polystyrene or functionalized glass surfaces because cells cannot deform these rigid materials. Here, by measuring cell volume fluctuations and estimating a hydraulic permeability for fluid transport, we have measured metrics of cell stiffness and cell generated stress. Experiments that actively indent cell layers and measure both forces and fluid displacement are needed to better determine cell permeability, which anchors the mechanical parameters inferred from our model. The volumetric modulus found here of 11.3 kPa, and the driving stress of 6.8 kPa, are about an order of magnitude larger than moduli and stresses found from cells grown on soft substrates (Tambe et al., 2011; Wang et al., 2002). However, cells grown on stiff substrates are known to exhibit a higher modulus, and the modulus typically measured is a shear modulus which will be smaller than a bulk modulus associated with volume changes (Discher et al., 2005). A combination of the method presented here and traction force microscopy could deepen our understanding of cell mechanics by accounting for resistive forces associated with fluid transport while expanding measurements to include the dynamic behavior of cells.

Conflict of interest

The authors report no conflict of interest

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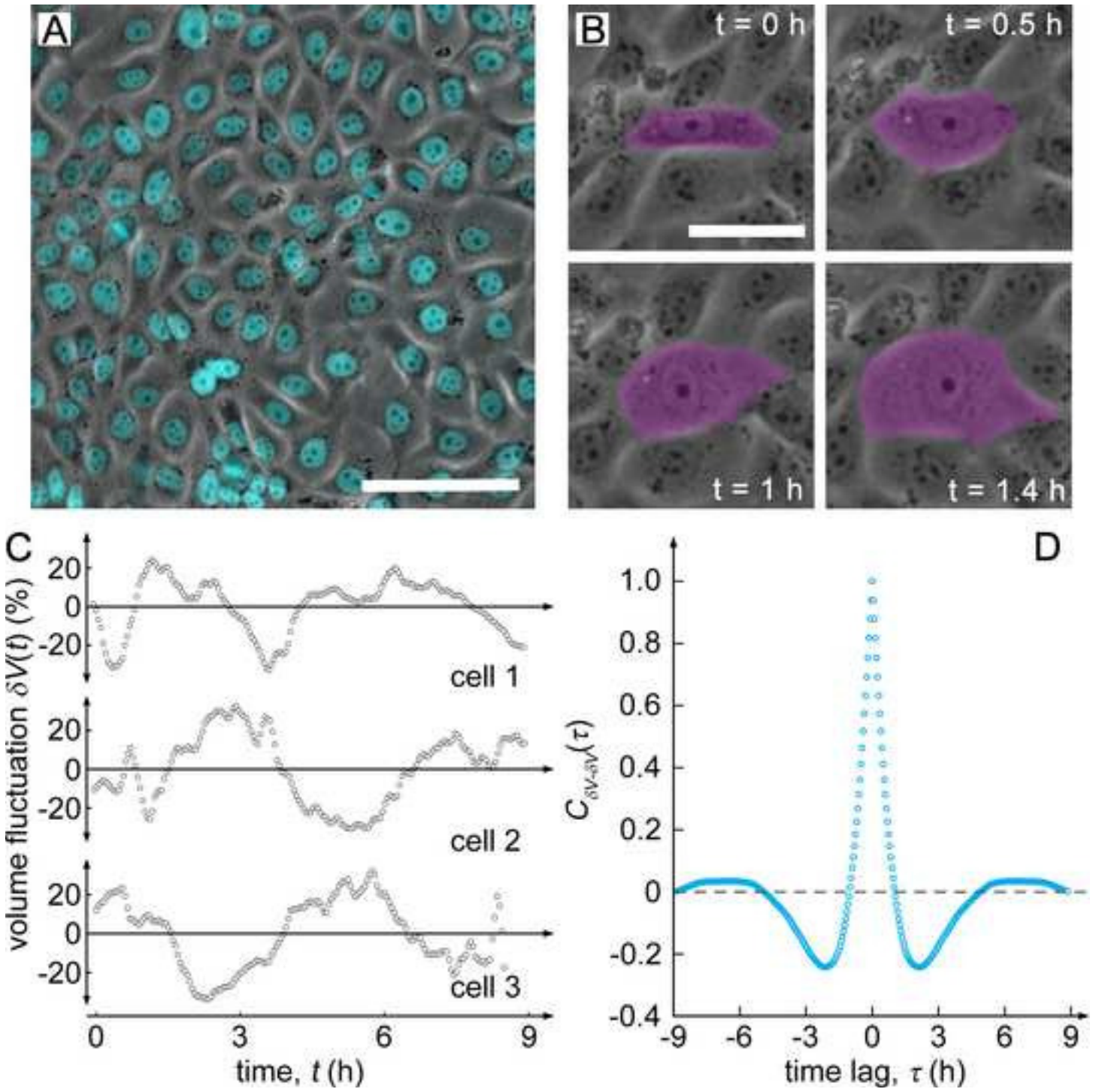
Captions

Figure 1. Cell volume fluctuations. A) Fluorescent nuclei of MDCK epithelial cells are tracked in time and space. B) Cell sizes can change significantly over short periods of time. C) Individual traces of cell volume over time for three representative cells show a representative period of between 4 and 6 hours. D) The volume-volume correlation function averaged over all cells, $C_{\delta V\text{-}\delta V}$, shows an anti-correlation peak at approximately 2 hours, suggesting a 4 hour period of oscillation (N=1038) The gray dotted line denotes zero correlation.

Figure 2. A damped harmonic oscillator model is fit to the power spectrum of volume fluctuations. A) The power spectral density function for cell volume fluctuations (data = open black circles). The blue line represents the fit of the data with the power spectrum model described in equation 5. The red line is an extension of the model beyond fitted data points. B) The rescaled spectrum of the driving stress shows a cut-off of the high frequency noise. The red line denotes σ_o = 6.8 kPa.

Figure 1



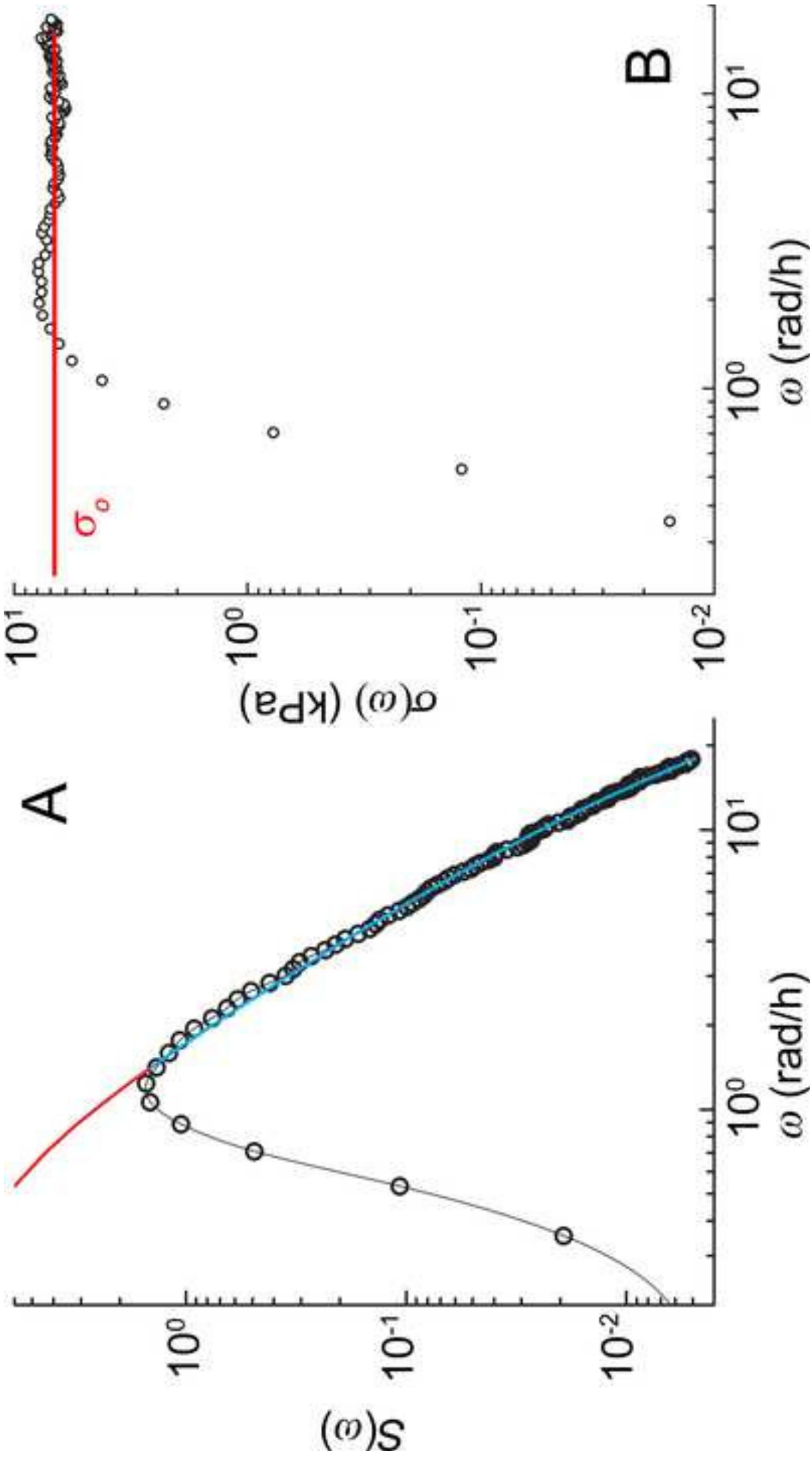

A
B
$S(\omega)$
$\sigma(\omega)$ (kPa)
σ_0
ω (rad/h)
ω (rad/h)
10^{1}
10^{0}
10^{-1}
10^{-2}

Figure 2