Supplementary Material

for

"Distinct Relaxation Timescales of Neurites Revealed by Rate-dependent Indentation, Relaxation and Micro-rheology Tests"

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# These two authors have the equal contribution to the paper.

Note 1—gluing bead to the AFM cantilever:

To glue or coat the bead to AFM cantilever, the following steps are conducted:

Step 1: Dilute the microsphere solution by 10 times, put a droplet of the diluted solution on cover glass and then spread the glue (Dymax OP) close to it.

Step 2: use AFM microscope to dip the tip on glue and quickly retract the cantilever.

Step 3, move and press the cantilever over an isolated bead for 5 minutes.

The processed AFM probe/cantilever should be stored in a clean incubator for 12 hours before actual indention test. More details can be found in JPK manual book (*https://www.jpk.com/app-technotes-img/AFM/pdf/jpk-tech-spheres-on-tip.14-1.pdf*).

Note 2—neural cell dissection and culture:

Primary cortical neurons are obtained from the embryonic 17-day-old Sprague-Dawley Rats provided by Laboratory of Neurodegenerative Diseases (The University of Hong Kong). Specifically, the meninges and blood attached to the brain tissue were removed during dissection. After that, special medium was used to culture neuron cells that cannot proliferate. The composition of the medium as well as its low serum concentration also inhibits the proliferation of other types (such as glia and fibroblast) of cells. Finally, 5-fluorodeoxyuridine,

a toxin that kills proliferating cells was added in the medium one day after seeding. After maintaining the culture in the incubator for 7 days, only neurons (having the unique morphology with dark soma and extensive neurites forming network) can be observed under the light microscope.

Note 3—simulation details

FEM model
All simulations were implemented in the commercial FEM analysis package ABAQUS. Since the indentation location picked was far away from the soma, the neurite can be treated as cylinder (Fig. 2a) and only half of it was considered in the simulation due to symmetry. Both the spherical indenter and substrate were simplified as rigid bodies for their large elastic moduli relative to neurites. The neurites were modeled as the generalized Maxwell viscoelastic material (see more details in 2.3 Finite Element Model).

Step, interaction and load settings
In our simulations, we set the contact mode between the indenter and neurite as well as between the neurite and substrate as "hard contact", that is neither friction nor adhesion was considered. In addition, since the concerned deformation is not small (the maximum indentation depth could reach ~ 50% of neurite diameter), we considered the large deformation in FEM calculation by switching on geometric nonlinear rule "Nlgeom" in ABAQUS. Finally, the loading conditions (such as loading rate, oscillation amplitude and frequency) were chosen to be consistent with those adopted in our experiments.

Data processing
The total reaction forces on the indenter were output from ABAQUS by self-built-in XY plot function and were then further processed in MATLAB. To better compare the simulated relaxation curve with experiment, all force curves were normalized by its value at the beginning of the relaxation stage.

Note 4—comparison with power law rheology

It must be pointed out that the response of live cells during the creep or rheology test has been reported to often follow a power law, with the corresponding creep function $J(t)$ and the complex modulus $G_e(w)$ scaling with time and driving frequency as $J(t) \sim t^\alpha$ and $|G_e(w)| \sim w^\alpha$ where α typically ranges from 0.2 to 0.25 [1, 2]. Interestingly, in our relaxation test, the apparent modulus (or the total indentation force) of neurite was found to follow a similar relationship: $E(t) \sim 0.24t^{-0.25}$, refer to Fig. S.1. However, such scaling law breaks down in short timescales (i.e. when $t < 10^{-2}$ s). In addition, since it is difficult to connect the power law exponent with different biological contributors, this issue is not further pursued here.
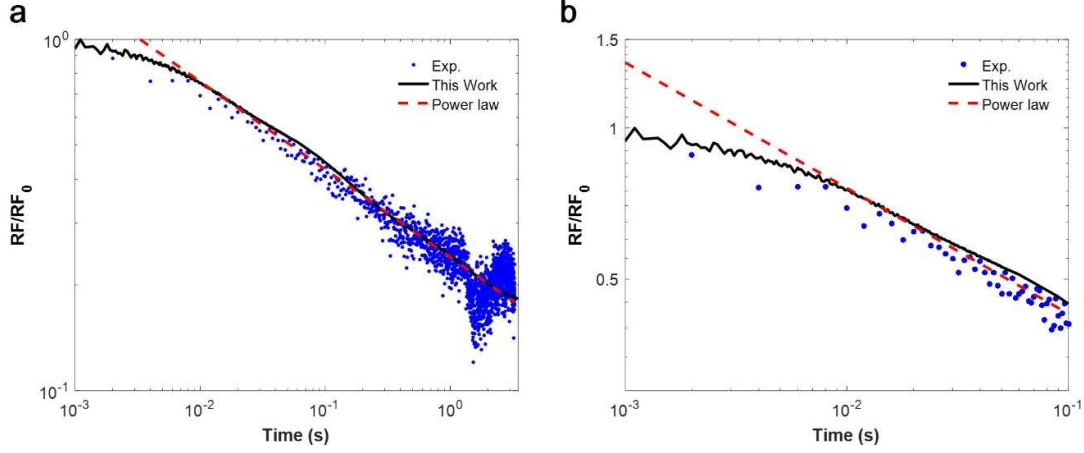
Figure S.1 (a) Good agreement between the relaxation test data (blue dots) and predictions from the power law rheology (red dashed line) and three relaxation timescales model (black solid line) can be achieved over the range of 10^{-2}~3.5s. **(b)** Power law predictions deviate significantly from the experimental data in short timescales while the model proposed in this study remains accurate.

Note 5—converting data from time to frequency domain:

Following the classical Hertz contact theory, the dynamic complex modulus can be defined as [2, 3]:

$$G' + iG'' = \alpha \cdot \left[\frac{F(\omega)}{\delta(\omega)} - i\omega b(0)\right], \tag{S1}$$

whereas the factor α accounts for the contact geometry. For a pyramid shape indenter in contact with a flat surface, $\alpha = (1 - v)/(3\delta_0 \, tan\theta)$ with δ_0, v and θ being the indentation depth, the Poisson's ratio of the sample and the tip angle, respectively [2]. On the other hand, $\alpha = (1 - v)/4(\delta_0 \, R_0)^{1/2}$ for a spherical indenter with radius R_0 [4]. Since thin cylindrical neurites were indented by a spherical tip in the present study, neither formula listed above can be applied. Nevertheless, the term $\frac{F(\omega)}{\delta(\omega)} - i\omega b(0)$ can still be used to characterize the dynamic properties of cells.

In the present study, both the driving displacement and force response (in the time domain) are obtained from AFM micro-rheology [2, 4]. These signals can then be converted to the frequency domain via Fourier transformation as:

$$F(\omega) = \int_0^\infty F(t)e^{-2\pi it\omega}dt, \ \delta(\omega) = \int_0^\infty \delta(t)e^{-2\pi it\omega}dt. \tag{S2}$$

Here $F(\omega)$ and $\delta(\omega)$ are the recorded force response and driving displacement signal in frequency domain. Physically, $|F(\omega)|$ and $|\delta(\omega)|$ represent the oscillation amplitude under driving frequency ω, while $arg(F(\omega))$ and $arg(\delta(\omega))$ correspond to their phases. The position where $F(\omega)$ reaches its maximum (red cross in Fig. S2(b)) is chosen as the dominant

oscillating frequency of the force response while contributions from other frequencies can be treated as noises (which can be neglected). As shown in Fig. S2(a), the fitted force response (containing only the dominant oscillating frequency term) match well with the recorded data.
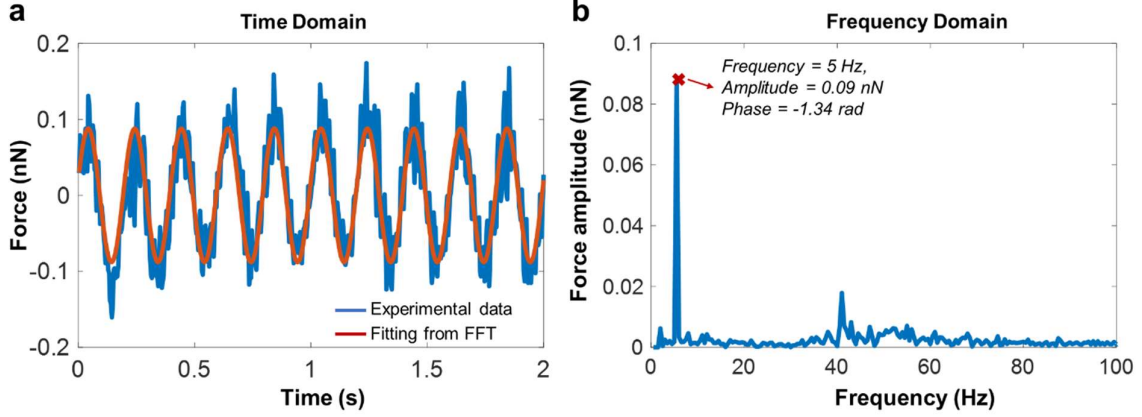
Figure S.2 (a) The force response signal under a driving frequency of 5 Hz. Blue line corresponds to the recorded data while the red line represents the fitted force response where only the dominant oscillating frequency term is included. **(b)** Recorded force response in the frequency domain (i.e. $F(\omega)$) obtained from Fast Fourier Transform (FFT). The dominant oscillating frequency (of the force response) and corresponding amplitude/phase can all be extracted from $F(\omega)$.

Note 6—correction for hydrodynamic drag

Micro-rheology tests were conducted in the viscous medium where hydrodynamic drag can influence the measurement resulted. Based on the work by Alcaraz and co-workers [5], such effect can be characterized by

$$H_d(f) = 2\pi i f b(h) \tag{S3}$$

where $b(h)$ represents the hydrodynamic drag factor taking the form

$$b(h) = \frac{6\pi\eta a_{eff}{}^2}{h + h_{eff}}. \tag{S4}$$

Here $\eta \sim 1$ mPa·s is the viscosity of the culture fluid (i.e, close to pure water), while h represents the tip-substrate separation. a_{eff} and h_{eff} correspond to the effective cantilever radius and height of the tip, respectively. To estimate the values of these parameters, we conducted the AFM rheology tests in pure water under different driving frequencies and tip-substrate separations. By fitting Eq. (S3) and (S4) to the these calibration data, the values of

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a_{eff} and h_{eff} can be obtained.

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