Chromatin Hydrodynamics: Supporting Material

Robijn Bruinsma[1], Alexander Y.Grosberg[2], Yitzhak Rabin[3], Alexandra Zidovska[4,5]

[1] Department of Physics and Astronomy, University of California, Los Angeles, CA, U.S.A.
[2] Department of Physics and Center for Soft Matter Research, New York University, New York, NY, U.S.A.
[3] Department of Physics, Bar Ilan University, Ramat Gan, Israel
[4] Department of Systems Biology, Harvard Medical School, Boston, MA, U.S.A.
[5] School of Engineering and Applied Sciences, Department of Physics, Harvard University, Cambridge, MA, U.S.A.

S.1. Hydrodynamic Equations of Motion and Linear Response

In this supplementary section we apply the Doi-Onuki method (Ref. (22) in the main text) to construct the two-component hydrodynamic equations for polymer solutions and gels including the linear response of such systems to perturbations.

In general, the relaxation equations for a thermodynamic system close to equilibrium can be phrased as the condition that the Rayleigh Function for the time-dependent thermodynamic quantities $x_i(t)$, $(i = 1, 2, ..n)$

$$\mathcal{R} = W + \sum_i \frac{\partial F}{\partial x_i}\left(\frac{dx_i}{dt}\right) \qquad (S1)$$

must be minimized with respect to $\frac{dx_i}{dt}$ for fixed x_i (see, for instance, Refs. (23,24) in the main text; see also Ref. (25) for further details of the derivation and its applicability limits). The dissipation function W is quadratic in the generalized velocities $\frac{dx_i}{dt}$ while F is the thermodynamic free energy. The first part of the Rayleigh Function is the energy dissipated per unit time, and the second part the work performed per unit time by the thermodynamic forces. For a polymer network, the thermodynamic variables are the local monomer concentration $\phi(\vec{r})$ and the coarse-grained displacement field $\vec{u}^p(\vec{r})$ of the polymer molecules. For polymer solutions that are not cross-linked, such as chromatin solution, the displacement field is not truly a proper thermodynamic quantity. However, for a visco-elastic medium, the displacement description is maintained and the polymer solution is treated as a polymer network in the limit that the zero-frequency shear modulus vanishes. Only time-derivatives of the displacement variables should enter in the final hydrodynamic equations in that case.

In the hydrodynamic description, energy dissipation is dominated by friction associated with relative flow of polymer and solvent, in which case the dissipation function takes the form

$$W = \frac{1}{2}\int d^3r\, \zeta\,(\vec{v}^p - \vec{v}^s)^2 \ , \qquad (S2)$$

with ζ the friction coefficient per unit volume, $\vec{v}^s$ the solvent flow velocity, and $\vec{v}^p = d\vec{u}^p/dt$.

As regards the second term of the Rayleigh function (1), the free energy F of the polymer network or solution is composed of two terms. The first term is

$$F_\phi = \int d^3r\, f(\phi(\vec{r})) \qquad (S3)$$

with ϕ the polymer concentration and $f(\phi)$ the equilibrium concentration-dependent free energy density of the polymer network. After imposing the continuity equation

$$\frac{\partial \phi}{\partial t} + \vec{\nabla}\cdot\phi\vec{v}^p = 0 \ , \qquad (S4)$$

F_ϕ is found to contribute a term

$$\frac{dF_\phi}{dt} = -\int d^3r\left(\frac{\partial f}{\partial \phi}\vec{\nabla}\cdot(\phi\vec{v}^p)\right) \qquad (S5)$$

to the Rayleigh Function. This term accounts for interactions between the polymer monomers with each other and with the surrounding solvent molecules. The second term is the polymer elastic free energy F_p, which contributes to the Rayleigh Function a term of the form

$$\frac{dF_p}{dt} = -\int d^3r\left(\sigma_{\alpha\beta}(\vec{r})\frac{\partial v^p{}_\alpha}{\partial r_\beta}\right) \ . \qquad (S6)$$

Here, $\sigma_{\alpha\beta}$ is the symmetric traceless shear stress tensor of the polymer network. In the main text, the form of the stress tensor for a viscoelastic solution is discussed. Here it will be left unspecified. The part of the stress tensor associated with swelling/compression of the network that has a finite trace is absorbed in the first, concentration dependent, part of the free energy.

The polymer system is compressible: if locally the polymer concentration increases then this is compensated by solvent drainage from the region. The combined polymer-solvent at fixed ϕ will however be assumed to be incompressible. The incompressibility condition

$$\vec{\nabla}\left((1-\phi)\vec{v}^s + \phi\vec{v}^p\right) = 0 \ , \qquad (S7)$$

is included in the Rayleigh Function by introducing the hydrodynamic pressure $P(\vec{r})$, acting as a Lagrange multiplier. Collecting all terms gives

$$\mathcal{R} = \int d^3r\left(\frac{1}{2}\zeta(\vec{v}^p - \vec{v}^s)^2 - \frac{\partial f}{\partial \phi}\vec{\nabla}\cdot(\phi v^p) - \right.$$
$$\left. -\sigma_{\alpha\beta}\frac{\partial v^p{}_\alpha}{\partial r_\beta} - P\vec{\nabla}\left((1-\phi)\vec{v}^s + \phi\vec{v}^p\right)\right) \ . \qquad (S8)$$

Finally, in order to apply linear response theory, infinitesimal, time-dependent "fields" $h_i(t)$ are introduced that are thermodynamically conjugate to the thermodynamic parameters x_i in the sense that $dW = \sum_i h_i dx_i$ is the work done by the external fields for an infinitesimal change dx_i of the thermodynamic parameters. The change in the Hamiltonian of the system is of the form $\Delta H = -\sum_i h_i x_i$. The time-dependent fields induce time-dependent changes in the thermodynamic parameters:

$$\delta x_i(t) = \int_{-\infty}^{t} dt' \chi_{ij}(t - t') h_j(t') . \tag{S9}$$

where $\chi_{ij}(\tau)$ is the dynamical susceptibility (response function). The Fluctuation-Dissipation (FD) theorem states that the imaginary part of the frequency Fourier transform of the dynamical susceptibility is related to the autocorrelation matrix of the thermodynamic parameters by

$$\text{Im}\left[\chi_{ij}(\omega)\right] = \frac{\omega}{2k_B T} S_{ij}(\omega) , \tag{S10}$$

where $S_{ij}(t - t') \equiv \langle x_i(t) x_j(t') \rangle$.

For the present case, we introduce an infinitesimal, externally imposed, time-dependent chemical potential $\mu^{(\text{ex})}(\vec{r}, t)$ for monomers that is conjugate to the concentration field and an infinitesimal, externally imposed stress tensor $\sigma_{\alpha\beta}^{(\text{ex})}(\vec{r}, t)$ that is conjugate to the strain tensor of the polymer network. These fields contribute a term to the Rayleigh Function of the form

$$\delta\mathcal{R} = \int d^3 r \left(-\mu^{(\text{ex})}(\vec{r}, t) \vec{\nabla} \cdot (\phi v^p) - \right.$$
$$\left. -\sigma_{\alpha\beta}^{(\text{ex})}(\vec{r}, t) \frac{\partial v^p_{\alpha}}{\partial r_{\beta}} \right) . \tag{S11}$$

Minimizing the Rayleigh Function gives

$$\zeta(\vec{v}^p - \vec{v}^s) = \vec{\nabla}\left(\overleftrightarrow{\sigma} + \overleftrightarrow{\sigma}^{(\text{ex})}\right) - \vec{\nabla}\Pi - \phi\vec{\nabla}\mu^{(\text{ex})} - \phi\vec{\nabla}P , \tag{S12}$$

$$\zeta(\vec{v}^s - \vec{v}^p) = -(1 - \phi)\vec{\nabla}P . \tag{S13}$$

The linearized version of these equation are used in the main text, where $\phi_0 \mu^{(\text{ex})}(\vec{r}, t)$ is replaced by the source term $\alpha(\vec{r}, t)$ for scalar events and the externally imposed stress $\sigma^{(\text{ex})}$ by the active stress $\sigma^{(\text{ac})}$ for vector events. As shown in the main text, if the fluctuation modes are decomposed into transverse and longitudinal components then the external chemical potential only generates longitudinal modes and the traceless external stress tensor only transverse components. The cross-correlation matrix is thus diagonal. The diagonal components of the dynamical susceptibility are given in the main text, and the final application of the FD theorem is straightforward.

S.2. Green's Function Derivation for a Scalar Event

Assume a scalar event takes place at the origin at time $t = 0$. The linearized force balance equation is

$$\zeta(\vec{v}^p - \vec{v}^s) = \vec{\nabla}\overleftrightarrow{\sigma} - K\vec{\nabla}\delta\phi$$
$$- \phi_0\vec{\nabla}P + s\vec{\nabla}\delta(\vec{r})g(t) . \tag{S14}$$

In a few lines of algebra, this yields

$$\frac{\partial\delta\phi}{\partial t} = D\nabla^2\delta\phi - \tilde{s}\nabla^2\delta(\vec{r})g(t) - \frac{(1 - \phi_0)^2}{\zeta/\phi_0}\vec{\nabla}\cdot\left(\vec{\nabla}\overleftrightarrow{\sigma}\right) , \tag{S15}$$

with $D = K\frac{(1-\phi_0)^2}{\zeta/\phi_0}$ and $\tilde{s} = s\frac{(1-\phi_0)^2}{\zeta/\phi_0}$. We can identify D as the *collective diffusion constant*. This equation can be viewed as defining a *Greens function* for scalar events.

As a concrete example, assume that passive chromatin behaves like a simple Newtonian fluid. The stress tensor term $\vec{\nabla}\cdot\left(\vec{\nabla}\overleftrightarrow{\sigma}\right)$ then reduces to $\eta\nabla^2\left(\vec{\nabla}\cdot\vec{v}^p\right)$ with η the viscosity. After applying the continuity equation to eliminate the flow, one obtains:

$$\left(1 - \frac{(1 - \phi_0)^2}{\zeta}\eta\nabla^2\right)\frac{\partial\delta\phi}{\partial t} \simeq D\nabla^2\delta\phi - \tilde{s}\nabla^2\delta(\vec{r})g(t) . \tag{S16}$$

The term $\frac{(1-\phi_0)^2}{\zeta}\eta\nabla^2$ is small compared to one for wavenumbers $q\xi \ll \sqrt{\eta_0/\eta}$. If one assumes that to be the case then the remaining equation is:

$$\frac{\partial\delta\phi}{\partial t} \simeq D\nabla^2\delta\phi - \tilde{s}\nabla^2\delta(\vec{r})g(t) . \tag{S17}$$

If the local change in solubility is permanent, with $g(t) \sim \theta(t)$, then the solution of the equation leads to a Green's function:

$$\delta\phi(\vec{r}, t > 0) \simeq \frac{\tilde{s}}{D}\left[\delta(\vec{r}) - \frac{e^{-r^2/4Dt}}{(4\pi Dt)^{3/2}}\right] . \tag{S18}$$

This describes a localized change in concentration at the origin, equal to $(s/K)\delta(\vec{r})$ together with a transient diffusion pulse that travels outwards from the origin. The volume integral over the concentration variation is zero: no new material is introduced by the change in solubility as is physically correct. The linearization condition $\delta\phi \ll \phi_0$ is satisfied provided $s \ll K\xi^3$.

S.3. Derivation of the dynamic susceptibility

To deal with a distribution of $\mathcal{M}_s$ scalar events, write the linearized equations of motion in Fourier transformed form:

$$\zeta(\vec{v}^p - \vec{v}^s) = i\vec{q}\cdot\left(\overleftrightarrow{\sigma} + \overleftrightarrow{\sigma}^{(\text{ac})}\right) - i\vec{q}K\delta\phi - i\phi_0\vec{q}P . \tag{S19}$$

Here, $\sigma_{ij}^{(\mathrm{ac})}(\vec{q}, \omega) = \delta_{ij}\alpha(\vec{q}, \omega)$ with

$$\alpha(\vec{q}, \omega)) = g(\omega) \sum_{k=1}^{\mathcal{M}_s} s_k e^{i\left(\omega t_k - \vec{q}\cdot\vec{R}_k\right)} \,, \qquad \text{(S20)}$$

where $g(\omega)$ is the Fourier transform of $g(t)$, the function that defines the time course of scalar event. For instance, if $g(t) = \theta(t)e^{-t/\tau_a}$ (which means, $g(t)$ is zero at $t < 0$, starts at $t = 0$, and falls off exponentially with characteristic time τ_a at $t > 0$), then $g_\omega = \frac{\tau_a}{1 - i\omega\tau_a}$. Darcy's Law reduces to:

$$\zeta\left(\vec{v}^s - \vec{v}^p\right) = -i(1 - \phi_0)\vec{q}P \,, \qquad \text{(S21)}$$

while the incompressibility condition becomes:

$$i\vec{q}\cdot\left((1 - \phi_0)\vec{v}^s + \phi_0\vec{v}^p\right) = 0 \,, \qquad \text{(S22)}$$

and the continuity equation:

$$-\omega\delta\phi + \phi_0\vec{q}\cdot\vec{v}^p = 0 \,. \qquad \text{(S23)}$$

Next, define $v_\parallel^p = \vec{q}\cdot\vec{v}/q$ to be the longitudinal component of the polymer flow field. Eliminating pressure and longitudinal solvent flow leads to an expression for the concentration fluctuations in the hydrodynamic limit:

$$\delta\phi(\vec{q}, \omega) = \chi(q, \omega)\alpha(\vec{q}, \omega) \,, \qquad \text{(S24)}$$

where

$$\chi(q, \omega) = \frac{iq^2\phi_0}{\omega\left[\frac{\zeta}{(1-\phi_0)^2} + \frac{4}{3}E(q,\omega)q^2\right] + iq^2K\phi_0} \qquad \text{(S25)}$$

is the *dynamic susceptibility*. Note, that in the limit of zero frequency, $\chi(q, \omega = 0)$ equals the static osmotic susceptibility $1/K$, as it should. The dynamic susceptibility has pole singularities in the lower half of the complex plane at $\omega = -i\Gamma(q)$ with $\Gamma(q)$ the relaxation rate of a collective mode of the system. In section S.7, the specific case of the Maxwell Fluid (MF) is discussed, which has two such collective modes, corresponding to hybridized collective diffusion and stress relaxation.

S.4. Vector Activity Derivation

Vector activity in general represents action of a set of force dipoles. Each force dipole is composed of a pair of collinear opposing forces $f\hat{n}$ and $-f\hat{n}$ exerted in two different points separated by a microscopic distance a. Here $\hat{n}$ is a unit vector marking the direction of force dipole; at the same time vector $a\hat{n}$ connects the points where two forces are applied (such that two forces forming a dipole generate no torque). If $\vec{R}$ is position vector of a center-point of the force dipole, then two forces are exerted in points $\vec{R} - (a/2)\hat{n}$ and $\vec{R} + (a/2)\hat{n}$. In reality there are more than one vector source, and their total

contribution to the divergence of stress tensor (i.e., applied force per unit volume) reads

$$\vec{\nabla}\cdot\overleftrightarrow{\sigma}^{(\mathrm{ac})}(\vec{r}, t) = \sum_{k=1}^{\mathcal{M}_v} f_k g(t - t_k)\times$$
$$\times\left[\delta\left(\vec{r} - \vec{R}_k - \frac{a_k}{2}\hat{n}_k\right) + \delta\left(\vec{r} - \vec{R}_k + \frac{a_k}{2}\hat{n}_k\right)\right] \,, \qquad \text{(S26)}$$

where $g(t)$ describes time development of the event, starting at time t_k for event k, while $\mathcal{M}_v$ the number of vector events. For the case of nucleosome rearrangement events, a would have the typical size of a spacer length between nucleosomes. Given that distance a (any a_k) is microscopic, we can write

$$\delta\left(\vec{r} - \vec{R}_k \pm \frac{a_k}{2}\hat{n}_k\right) \simeq \delta\left(\vec{r} - \vec{R}_k\right) \pm \frac{a_k}{2}\hat{n}_k\vec{\nabla}\delta\left(\vec{r} - \vec{R}_k\right) \,. \qquad \text{(S27)}$$

Combining now two of such δ-functions in formula (26), we arrive at

$$\vec{\nabla}\cdot\overleftrightarrow{\sigma}^{(\mathrm{ac})}(\vec{r}, t) = \sum_{k=1}^{\mathcal{M}_v} f_k a_k \hat{n}_k(\hat{n}_k\cdot\vec{\nabla})\delta\left(\vec{r} - \vec{R}_k\right)g(t - t_k) \,. \qquad \text{(S28)}$$

If all forces are the same, $f_k = f$, and all distances are the same, $a_k = a$, then we arrive at the result used in the main text (Eq. 19).

S.5. Greens Function: Vector Event

First consider the case of a vector event with long event duration in a medium that can be described as a Netwonian fluid. The source term is then the sum of two time-independent δ-function forces. Each produces a flow field of the form

$$\vec{v}(\vec{r}) = f\,\hat{n}\cdot\overleftrightarrow{S}(\vec{r})/8\pi\eta \,, \qquad \text{(S29)}$$

where

$$S_{ij}(\vec{r}) = \frac{\delta_{ij}}{r} + \frac{r_i r_j}{r^3} \qquad \text{(S30)}$$

is the Oseen tensor. The combined dipole flow field has the form:

$$\vec{v}(\vec{r}) = fa\,(\hat{n}\cdot\vec{\nabla})\,(\hat{n}\cdot\overleftrightarrow{S}(\vec{r}))/8\pi\eta \,. \qquad \text{(S31)}$$

The pattern of flow lines is that of extensional flow oriented along the force direction.

For the case of a general linear viscoelastic medium, it is useful to define the transverse projection operator $\overleftrightarrow{P}$. In q-space, the projection operator is defined as $P_{ij}(\vec{q}) = \delta_{ij} - \hat{q}_i\hat{q}_j$, where $\hat{q} = \vec{q}/q$ and $\hat{q}_i = q_i/q$. Apply this projection operator to Eqs. 1 and 2 for two-fluid hydrodynamics. This leads to $\vec{v}_\perp^p = \vec{v}_\perp^s$ and

$$E*\nabla^2\vec{v}_\perp = fa\overleftrightarrow{P}\cdot\hat{n}(\hat{n}\cdot\vec{\nabla})\delta(\vec{r})g(t) \,. \qquad \text{(S32)}$$

The $*$ symbol stands for the convolution of the space-time memory kernel E with the transverse polymer velocity field

(indicated from here on as the flow field itself). After Fourier transform, the flow field produced by the vector event is:

$$\vec{v}_\perp(\vec{q}, \omega) = i f a g(\omega) \frac{\overleftrightarrow{P}(\vec{q}) \cdot \hat{n}(\hat{n} \cdot \vec{q})}{q^2 E(q, \omega)} \ . \qquad (S33)$$

The Green's function is the reverse Fourier Transform.

S.6. FSD and PSD

S.6.1 Derivation

In this section we discuss the relationship between the FSD, as measured experimentally, and the PSD that is a convenient quantity to compute theoretically. We start from the advective equation of motion of a labelled site of the chromatin moving in the surrounding hydrodynamic flow field

$$\frac{d\vec{r}(t)}{dt} = \vec{v}_p(\vec{r}(t), t) \qquad (S34)$$

The tracer displacement between successive measurements is

$$\frac{\vec{r}(t + \Delta t) - \vec{r}(t)}{\Delta t} = \int_t^{t + \Delta t} \vec{v}(\vec{r}(t'), t') \frac{dt'}{\Delta t} \qquad (S35)$$

(where we suppressed index p for brevity). Perform a mode expansion of the flow field, according to the Fourier transform convention Eq. 4:

$$\frac{\vec{r}(t + \Delta t) - \vec{r}(t)}{\Delta t} =$$
$$\int_t^{t + \Delta t} \left[\frac{\sum_{\vec{p}} \int_{-\infty}^\infty \vec{v}(\vec{p}, \omega) e^{-i(\vec{p} \cdot \vec{r}(t') + \omega t')} \frac{d\omega}{2\pi}}{\Delta V} \right] \frac{dt'}{\Delta t} \qquad (S36)$$

Multiply by $e^{i\vec{q} \cdot \vec{r}_i(t)}$ and do the summation over $i = 1, 2, \ldots, N$ tracer particles:

$$\vec{F}(\vec{q}, \Delta t) =$$
$$\sum_i^N \int_t^{t + \Delta t} \left[\frac{\sum_{\vec{p}} \int_{-\infty}^\infty \vec{v}(\vec{p}, \omega) e^{i(\vec{q} \cdot \vec{r}_i(t) - \vec{p} \cdot \vec{r}_i(t') - \omega t')} \frac{d\omega}{2\pi}}{\Delta V} \right] \frac{dt'}{\Delta t} \ .$$
$$\qquad (S37)$$

If the particle displacement over time Δt is such that we can replace $\vec{q} \cdot \vec{r}_i(t') \simeq \vec{q} \cdot \vec{r}_i(t)$, and if the tracers are uniformly distributed over the sample, then only the $\vec{p} = \vec{q}$ mode survives in the sum $\sum_i^N e^{i((\vec{q} - \vec{p}) \cdot \vec{r}_i(t))} \simeq N \delta_{\vec{p}, \vec{q}}$ provided that $1/q$ is large compared at the distance between labelled particles but small compared at the nucleus size: $N/\Delta V \gg q^3 \gg 1/\Delta V$. Under these assumptions:

$$\vec{F}(\vec{q}, \Delta t) = \frac{N}{\Delta V} \int_{-\infty}^\infty \vec{v}(\vec{q}, \omega) \left[\int_t^{t + \Delta t} e^{-i\omega t'} \frac{dt'}{\Delta t} \right] \frac{d\omega}{2\pi}$$
$$= \frac{N}{\Delta V} \int_{-\infty}^\infty \vec{v}(\vec{q}, \omega) e^{-i\omega t} \frac{1 - e^{-i\omega \Delta t}}{i\omega \Delta t} \frac{d\omega}{2\pi} \qquad (S38)$$

If $\langle \vec{v} \rangle = 0$ and $\langle \vec{F} \rangle = 0$, one has to look at the second moment. Multiplying by the complex conjugate and doing the ensemble average gives for $S(q, \Delta t) = \left\langle \left| \vec{F}(q, \Delta t) \right|^2 \right\rangle / N$,

$$S(q, \Delta t) \simeq \frac{N}{\Delta V} \int \frac{1 - \cos \omega \Delta t}{(\omega \Delta t)^2} \left(\vec{v}^2 \right)_{\vec{q}, \omega} \frac{d\omega}{\pi} \qquad (S39)$$

which is the relation between FSD and power spectral densities used in the main text (Eq. 32).

S.6.2 Limiting cases

We conclude by discussing a few limiting cases. In the limit that Δt is small compared to the inverse of all characteristic frequencies, the FSD goes to a constant:

$$S(q, \Delta t \to 0) \simeq \frac{N}{\Delta V} \int \left(\vec{v}^2 \right)_{\vec{q}, \omega} \frac{d\omega}{2\pi} \qquad (S40)$$

In the limit that Δt is large compared to the inverse of all characteristic frequencies, the FSD reduces to

$$S(q, \Delta t) \simeq \frac{N}{\Delta V} \frac{\left(\vec{v}^2 \right)_{\vec{q}, 0}}{\Delta t} \ , \qquad (S41)$$

the flow spectral density drops as $1/\Delta t$. In the limit of q large compared to the inverse of the spacing between labelled sites, the result is most easily derived directly from the definition of $\vec{F}(\vec{q}, \Delta t)$, Eq. 31 in the main text:

$$S(q, \Delta t) \simeq \frac{\left\langle \Delta r^2 \right\rangle}{\Delta t^2} \ , \qquad (S42)$$

with $\left\langle \Delta r^2 \right\rangle$ the mean square displacement of a single labelled genome site after a time Δt. If single-site dynamics is characterized by a power law of the form $\left\langle \Delta r^2 \right\rangle \propto \Delta t^\alpha$ in the limit of large Δt then the FSD would be proportional to $N/\Delta t^{2-\alpha}$ or large Δt. Here, α is less than one for subdiffusion and α is greater than one for superdiffusion. If the labelled sites would remain localized, which would be the case for a gel, then α would be zero.

S.7. FSD of a Maxwell Fluid

In this section we compute the FSD of a Maxwell Fluid for passive and active fluctuations. A Maxwell Fluid has a complex viscosity $E(\omega) = \frac{\eta}{1 - i\omega\tau}$, independent of wavenumber, with τ the stress relaxation time.

S.7.1 Thermal Fluctuations

According to the fluctuation-dissipation theorem, the amplitude of thermal transverse velocity fluctuations depends on the assumed rheology only through the factor $\text{Re} \left[\frac{1}{E(q, \omega)} \right]$.

This equals $1/\eta$ both for a Newtonian Fluid and for a Maxwell Fluid so the spectra of transverse velocity fluctuations of a Maxwell Fluid is that of a Newtonian Fluid:

$$S_\perp(q, \Delta t) \simeq [c] \frac{4 k_B T}{q^2 \eta \Delta t} \ . \qquad (S43)$$

The FSD for longitudinal velocity fluctuations is more complex. The dynamic susceptibility for the concentration fluctuations of a Maxwell Fluid is

$$\chi(q, \omega) = \frac{i q^2 \phi_0}{\omega \left[\frac{\zeta}{(1-\phi_0)^2} + \frac{4}{3} \frac{\eta q^2}{1 - i\omega\tau} \right] + i q^2 K \phi_0} \ . \qquad (S44)$$

The dispersion relation of the relaxation rates associated with different modes of the system is determined by the poles of the dynamical susceptibility, found by setting the inverse of the dynamical susceptibility to zero. Introducing dimensionless wavenumbers and a dimensionless osmotic modulus through,

$$Q^2 = \frac{4}{3} \frac{\eta q^2}{\zeta} (1 - \phi_0)^2 \qquad (S45a)$$

$$\frac{1}{\kappa} = \frac{4}{3} \frac{\eta}{\tau K \phi_0} \qquad (S45b)$$

the mode dispersion $\omega = -i\Gamma(Q)$ must be the solution of the quadratic equation

$$(\tau\Gamma)^2 - \tau\Gamma \left[1 + Q^2(\kappa + 1) \right] + Q^2\kappa = 0 \ , \qquad (S46)$$

or, explicitly,

$$\Gamma_1(Q) = \frac{1 + Q^2(\kappa+1)}{2\tau} - \frac{\sqrt{[1 + Q^2(1-\kappa)]^2 + 4\kappa Q^4}}{2\tau}$$
$$(S47a)$$

$$\Gamma_2(Q) = \frac{1 + Q^2(\kappa+1)}{2\tau} + \frac{\sqrt{[1 + Q^2(1-\kappa)]^2 + 4\kappa Q^4}}{2\tau}$$
$$(S47b)$$

The two branches are separated by a gap, as shown schematically in Fig. 1. For small wavenumbers, the lower branch approaches Dq^2, the relaxation rate for collective diffusion with a collective diffusion coefficient $D = K\phi_0(1 - \phi_0)^2/\zeta$ that does not depend on the viscosity or the stress relaxation time. For large wavenumbers, the lower branch saturates at $\Gamma_1\tau = \frac{\kappa}{1+\kappa}$. Concentration fluctuations are most pronounced at a consolute point where the osmotic modulus K vanishes. The relaxation rate of the lower branch goes to zero ("critical slowing down") in that limit for any wavenumber. The upper branch reduces to the relaxation rate $1/\tau$ for shear stress in the small wavenumber limit. For large wavenumbers, the upper branch approaches $D_2 q^2$ with a diffusion coefficient $D_2 = (1 - \phi_0)^2 \left[\frac{4}{3}\eta + K\tau\phi_0 \right] / \zeta\tau$ that now does depend on the viscosity and the stress relaxation time.
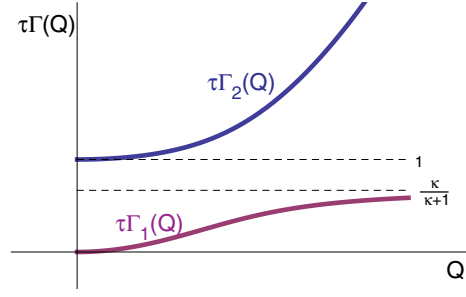

Figure S1 Relaxation rate $\Gamma(Q)$ of a Maxwell Fluid. The lower branch $\Gamma_1(Q)$ represents collective diffusion in the long wavelength limit with a collective diffusion coefficient $D = 3K\phi_0/4\eta$. In the limit that the osmotic modulus K vanishes, the lower branch relaxation rate goes to zero for all wavelengths. The upper branch represents stress relaxation for long wavelengths and collective diffusion for short wavelengths.
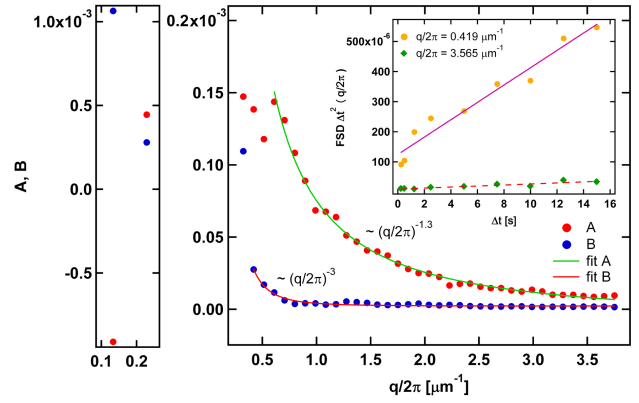

Figure S2 Fit coefficients $A(q)$ and $B(q)$ of the fitting form Eq. 33 for the case of ATP-consuming cells. *Inset:* FSD multiplied by Δt^2 of wildtype (ATP-consuming) cells for two particular fixed values of q as a function of Δt; solid and dashed lines: linear regression fit. In addition to the Fig. 5 of the main text, we show here left panel which reports data for very small q where completely different scale on the vertical axis has to be used.

The longitudinal contribution to the FSD takes the form

$$S_{||}(q, \Delta t) = [c] \frac{2 k_B T \tau (1 - \phi_0)^2}{\phi_0 \zeta \Delta t^2} \times$$
$$\frac{\left(\frac{1}{\Gamma_1} - \tau \right) \left[1 - e^{-\Gamma_1 \Delta t} \right] - \left(\frac{1}{\Gamma_2} - \tau \right) \left[1 - e^{-\Gamma_2 \Delta t} \right]}{\Gamma_2 - \Gamma_1} \ .$$
$$(S48)$$

The full FSD is the sum of the longitudinal and transverse contributions:

$$S(q, \Delta t) = S_{||}(q, \Delta t) + S_\perp(q, \Delta t) \ . \qquad (S49)$$

S.7.2   Active Vectorial Fluctuations

The FSD for transverse velocity fluctuations in the presence of active vectorial sources in a Maxwell Fluid has the form

$$S_\perp(q, \Delta t) \simeq \frac{[c]}{q^2 \eta^2 \pi} \times$$
$$\int \left(\vec{\beta}^2\right)_{\vec{q},\omega} \left(1 + (\omega\tau)^2\right) \frac{1 - \cos \omega \Delta t}{(\omega \Delta t)^2} d\omega \quad \text{(S50)}$$

This depends in general on the form of the active structure factor. We will consider the case in which there are no spatial or temporal correlations between the active events and where the direction of the active forces is random in space. In that case (see Eq. 30), $\left(\vec{\beta}^2\right)_{\vec{q},\omega} = \frac{2}{15} \frac{m(fa)^2 \tau_a^2}{(\omega\tau_a)^2 + 1}$ with $m = \mathcal{M}/\Delta V \Delta T$ the number of active vector events per unit volume per unit time. This results in a FSD

$$S_\perp(q, \Delta t) = \frac{2}{15} [c] \frac{m(fa)^2 \tau \tau_a^2}{q^2 \eta^2 \Delta t^2} \times$$
$$\left[\left(\frac{\tau}{\tau_a} - \frac{\tau_a}{\tau}\right) \left(1 - e^{-\Delta t/\tau_a}\right) + \frac{\Delta t}{\tau}\right] \quad \text{(S51)}$$

In the two limits of Δt large and small to τ_a this expression is proportional to $1/(q^2 \Delta t)$ and thus of the same form as the equilibrium FSD.

S.7.3   Active Scalar Fluctuations

The FSD for active scalar sources in a Maxwell Fluid is

$$S_{||}(q, \Delta t) \simeq [c] \frac{q^2 (1 - \phi_0)^4}{\pi \zeta^2 \Delta t^2} \times$$
$$\int (\alpha^2)_{\vec{q},\omega} \frac{(1 - \cos \omega \Delta t)(1 + (\omega\tau)^2)}{|(\omega + i\Gamma_1)(\omega + i\Gamma_2)|^2} d\omega \quad \text{(S52)}$$

In the absence of correlations between active events (Eq. 14), $(\alpha^2)_{\vec{q},\omega} = \frac{m\langle s^2 \rangle \tau_a^2}{(\omega\tau_a)^2 + 1}$ with $m = \mathcal{M}/\Delta V \Delta T$ the number of scalar events per unit time per unit volume:

$$S_{||}(q, \Delta t) \simeq [c] \frac{m \langle s^2 \rangle q^2 (1 - \phi_0)^4 \tau_a^2}{\zeta^2 \tau^2 \Delta t^2} \times$$
$$\left[\frac{\left(1 - (\Gamma_2 \tau)^2\right) \left(1 - e^{-\Gamma_2 \Delta t}\right)}{\Gamma_2 \left[\Gamma_1^2 - \Gamma_2^2\right] \left[1 - (\Gamma_2 \tau_a)^2\right]} + \right.$$
$$\frac{\left(1 - (\Gamma_1 \tau)^2\right) \left(1 - e^{-\Gamma_1 \Delta t}\right)}{\Gamma_1 \left[\Gamma_2^2 - \Gamma_1^2\right] \left[1 - (\Gamma_1 \tau_a)^2\right]} + \quad \text{(S53)}$$
$$\left. \frac{\tau_a \left(\tau_a^2 - \tau^2\right) \left(1 - e^{-\Delta t/\tau_a}\right)}{\left[1 - (\Gamma_2 \tau_a)^2\right] \left[1 - (\Gamma_1 \tau_a)^2\right]} \right]$$

The FSD is proportional to $1/\Delta t$ for Δt small compared to the three relaxation times τ_a, $1/\Gamma_2$, and $1/\Gamma_1$. In the long-time limit that Δt is large compared to these three relaxation

times, the FSD is dominated by the slowest of the three relaxation rates. If the slowest relaxation rate is collective diffusion - the only gapless mode - then for long wavelengths

$$S_{||}(q, \Delta t) \simeq [c] \frac{m \langle s^2 \rangle (1 - \phi_0)^4 \tau_a^2}{\zeta^2 \Delta t^2 D} , \quad \text{(S54)}$$

with $D = K\phi_0(1 - \phi_0)^2/\zeta$ the collective diffusion coefficient. The FSD depends on Δt as $1/\Delta t^2$ but it is independent of wavenumber. If the duration of the active event is the longest time scale then the FSD

$$S_{||}(q, \Delta t) \simeq [c] \frac{m \langle s^2 \rangle (1 - \phi_0)^4 q^2 \tau_a^5}{\zeta^2 \Delta t^2} \quad \text{(S55)}$$

is proportional to q^2.