Comparative Analysis of Viscosity of Complex Liquids and Cytoplasm of Mammalian Cells at the Nanoscale

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Supporting Information

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ABSTRACT: We present a scaling formula for size-dependent viscosity coefficients for proteins, polymers, and fluorescent dyes diffusing in complex liquids. The formula was used to analyze the mobilities of probes of different sizes in HeLa and Swiss 3T3 mammalian cells. This analysis unveiled in the cytoplasm two length scales: (i) the correlation length $\xi$ (approximately 5 nm in HeLa and 7 nm in Swiss 3T3 cells) and (ii) the limiting length scale that marks the crossover between nano- and macroscopic viscosity (approximately 86 nm in HeLa and 30 nm in Swiss 3T3 cells). During motion, probes smaller than $\xi$ experienced matrix viscosity: $\eta_{\text{matrix}} \approx 2.0 \text{ mPa} \cdot \text{s}$ for HeLa and 0.88 mPa·s for Swiss 3T3 cells. Probes much larger than the limiting length scale experienced macroscopic viscosity, $\eta_{\text{macro}} \approx 4.4 \times 10^{-2}$ and $2.4 \times 10^{-2}$ Pa·s for HeLa and Swiss 3T3 cells, respectively. Our results are persistent for the lengths scales from 0.14 nm to a few hundred nanometers.

KEYWORDS: Viscosity, diffusion, cytoplasm, complex liquids, scaling

The mobility of proteins and other probes is one of the main regulating factors in processes taking place in living cells. Small proteins (tens of kilodaltons with hydrodynamic radii $r_h \sim 1 \sim 5$ nm) show diffusivities orders of magnitude faster than those predicted by the Stokes–Sutherland–Einstein (SSE) equation $D = kT/6\pi\eta_{\text{macro}}r_h$ on the basis of the macroscopic viscosity $\eta_{\text{macro}}$. Also the measurements of the self-diffusion coefficients of proteins and small molecules in E. coli, HeLa, and Swiss 3T3 cells, could not be explained via the SSE equation and the macroscopic viscosity. Luby-Phelps et al. performed fluorescence recovery after photobleaching (FRAP) measurements of fluorescently labeled dextrins in Swiss 3T3 muscle cells. They described the data via the diffusion coefficient ratio (DCR), calculated as the ratio of the coefficient of self-diffusion of probes in the cytoplasm to the self-diffusion in water. They observed that DCR decreased with increasing size of the probes up to the size of 14 nm. For probes larger than 14 nm DCR had an approximately constant values. FRAP measurements performed on CHO cells by the Verkman group suggested that translational mobility of green fluorescence protein (GFP) was determined mostly by the concentration of cellular obstacles. Lukacs studied the mobility of DNA fragments (from 21 up to 6000 bp) in the cytoplasm of HeLa cells. Fragments longer than 3000 bp presented unexpectedly low mobility. On the contrary in a nucleus, diffusion of all investigated DNA fragments (from 21 up to 6000 bp) was strongly hindered and did not change with size of diffusing entities. Dauty et al. compared self-diffusion coefficients (SDC) of fluorescently labeled DNA fragments diffusing in the solutions of polymerized actin with SDC obtained in vivo in HeLa cells. They postulated that it was the cytoskeletal network of actin filaments that hindered the diffusion of probes in the cytoplasm. Studies on viscoelastic properties of polymerized actin solutions were reported in numerous works. Most of them, however, consider length scales of probes from 1 to 100 nm. These studies showed that the viscoelastic properties of the actin network are determined by the amount of bundles and cross-linkers connecting actin filaments. Additionally, various theoretical models mimicking the cytoskeleton behavior have been proposed. Also an opposite approach (biomaterials which mimic polymers) has been established recently.

The development of a theory relating the mobility of solutes in a cytoplasm to their hydrodynamic radii and to the cytoplasmic viscosity is important because mobilities of biomolecules influence the transport, signaling, metabolism, and life cycle of living
cells.\textsuperscript{15} On the other hand studies on diffusion of solutes in different systems such as polymer solutions have also been done.\textsuperscript{16–29} Schachman et al.\textsuperscript{16} showed that small and large probes sedimenting in a solution of DNA differ dramatically in sedimentation coefficients. Currently most researchers\textsuperscript{17–30} accept an empirical ansatz for the relation between the viscosity experienced by the nanoscopic probes and their hydrodynamic radius \(r_p\)

\[\eta = \eta_0 \exp\left(K p^a c^c\right)\]  

(1)

Here \(\eta_0\) is the viscosity of the solvent (i.e., water), \(K\) is a constant, and \(c\) denotes the concentration of the polymer. \(\mu\) and \(\nu\) are exponents whose values vary from system to system.\textsuperscript{29} In our previous paper,\textsuperscript{31} we have shown that eq 1 may be replaced by a more general expression describing viscosity.

\[\frac{\eta}{\eta_0} = \exp\left(\frac{d}{\xi}\right)^a\]  

(2)

\[\frac{\eta}{\eta_0} = \exp\left(\frac{R_g}{\xi}\right)^a\]  

(3)

Here \(\xi\) is the correlation length interpreted as the average distance between the point of entanglement in the polymer network, \(R_g\) is the radius of gyration of the polymer coil, and \(d\) is the diameter of the probe. The physical meaning of the exponent \(a\) (a constant of order 1) is still under discussion, and as was shown by Odijk\textsuperscript{32}\textsuperscript{32} it may vary from system to system. \(\xi\) is a function of the concentration of the polymer and of \(R_g\)\textsuperscript{32}

\[\xi = b\,R_g\left(\frac{c}{c^*}\right)^\beta\]  

(4)

Here \(b \sim 1\), \(c^*\) is the overlap concentration defined for flexible polymers as

\[c^* = \frac{M_w}{4/3\pi R_g^3 N_A}\]  

(5)

and \(R_g\) is a gyration radius of the polymer. The value of the \(\beta\) exponent depends on type of the solvent. In a good solvent \(\beta = -0.75\), while in \(\theta\) solvent \(\beta = -1.\textsuperscript{33} \) Equations 2 and 3 are valid only in two separate regimes: for \(d \ll R_g\) or for \(d \gg R_g\). Equations 2 and 3 provide a uniform description of viscosity experienced by nanoprobes (eq 2) and macroscopic viscosity experienced by the macroscopic probe (eq 3). Additionally one can expect a crossover length scale above which the probe experiences the macroscopic viscosity of the solution. Equation 1 transforms into the form of eq 2 with the constant \(K = (2bR_g c^*)^{-a}\), \(\mu = a\) and \(\nu = \beta a\).

Here we generalize the description of viscosity further to all sizes of probes including probes of radius comparable to the radius of cosolute (polymer or micelle). We also show that the viscosity experienced by the probe of radius \(r_p\) depends on the hydrodynamic radius of polymer coils, \(R_h\), and not as in eq 3 on their radius of gyration. \(d\) in eq 2 should be interpreted as hydrodynamic radius of probes \(r_p\) and not as their diameter. We develop a scaling law of viscosity of flexible polymer solutions. We also reanalyze the data of the viscosity of solutions of rigid micelles\textsuperscript{3} according to developed scaling law. Finally we use our model to analyze published diffusion coefficients of solutes diffusing in the cytoplasm of two different types of mammalian cells (HeLa and Swiss 3T3).\textsuperscript{4,6,35–38}

We have shown previously\textsuperscript{31,34} that the Stokes–Sutherland–Einstein relation can describe diffusion at the nano-scale \((D = kT(6\pi\eta(r_p)r_p))\) if one uses the scale-dependent viscosity, \(\eta(r_p)\). We use this relation to calculate the viscosity experienced by the probe from measurements of its self-diffusion coefficient

\[\frac{\eta}{\eta_0} = \frac{D_0}{D}\]  

(6)

where \(\eta\) is the viscosity experienced by the probe, \(\eta_0\) is the viscosity of the solvent, \(D\) is the self-diffusion coefficient of the probe in the studied solution, and \(D_0\) is the diffusion constant of the same probe in a pure solvent.

According to eq 6 we determined the viscosity of aqueous solutions of poly(ethylene glycol) (PEG) and of solutions of polystyrene (PS) in acetonaphene. Properties of these polymers are listed in Table 1. Hydrodynamic (\(R_h\)) and gyration (\(R_g\)) radii of PEG were calculated according to the relations\textsuperscript{39} \(R_h = 0.0215M_w^{0.58}\) and \(R_g = 0.0145M_w^{0.57}\) where \(M_w\) is the number average molecular weight of the polymer obtained from gel permeation chromatography. Hydrodynamic and gyration radii of PS were determined according to the procedure described elsewhere.\textsuperscript{40} In addition, we studied the viscosity of solutions of rigid micelles of surfactant (hexa(ethylene glycol) monododecyl ether, C\textsubscript{12}E\textsubscript{8}).\textsuperscript{34} For concentrations of C\textsubscript{12}E\textsubscript{8} lower than 35% and at room temperature the micelles are \(L \approx 23\) nm in length corresponding to an equivalent hydrodynamic radius \(R_h \approx 5.3\) nm.\textsuperscript{31} Diffusion coefficients of the probes were measured with fluorescence correlation spectroscopy technique (for details see Supporting Information). We used Rhodamine B \((r_p = 0.58\) nm) and lysozyme \((r_p = 1.9\) nm) labeled with TAMRA (carboxytetramethylrhodamine) as probes for the solutions of PEG. In the solutions of PS 220000 in the acetonaphene we used N-(2,6-disoproplyphenyl)-9-(p-styryl)perylene-3,4-dicarboximide (PMI) \((r_p = 0.53\) nm) and PMI-labeled polystyrene (PMI-PS 34000 and PMI-PS 255000, cf. Table 1). The viscosity of surfactant solutions (for experimental details see Supporting Information) was probed with water \((r_p = 0.14\) nm), TAMRA \((r_p = 0.85\) nm), and TAMRA labeled proteins such as lysozyme \((r_p = 1.9\) nm), chymotrypsinogen \((r_p = 2.6\) nm), ovalbumin \((r_p = 3.4\) nm), bovine serum albumin \((r_p = 4.2\) nm), and apoforin

<table>
<thead>
<tr>
<th>symbol</th>
<th>(M_w) or (M_n) (g/mol)</th>
<th>(R_h) (nm)</th>
<th>(R_g) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG 400</td>
<td>325</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>PEG 6000</td>
<td>3461</td>
<td>2.4</td>
<td>1.5</td>
</tr>
<tr>
<td>PEG 20000</td>
<td>10944</td>
<td>4.7</td>
<td>2.9</td>
</tr>
<tr>
<td>PEG 35000</td>
<td>15040</td>
<td>5.7</td>
<td>3.5</td>
</tr>
<tr>
<td>PEG 600000</td>
<td>276862</td>
<td>30.8</td>
<td>18.3</td>
</tr>
<tr>
<td>PEO 8000000</td>
<td>7019190</td>
<td>201</td>
<td>115.8</td>
</tr>
<tr>
<td>PS 34000</td>
<td>34000</td>
<td>5.8</td>
<td>4.4</td>
</tr>
<tr>
<td>PS 220000</td>
<td>220000</td>
<td>14.9</td>
<td>11.5</td>
</tr>
<tr>
<td>PS 255000</td>
<td>255000</td>
<td>16.3</td>
<td>12.5</td>
</tr>
</tbody>
</table>

\(M_w\) Number averaged molecular weight \(M_w\) of PEG, weight averaged molecular weights \(M_w\) of PS, gyration \(R_g\), and hydrodynamic \(R_h\) radii.
Viscosity of the complex liquid depends on the concentration of objects which create the fluid (polymer coils or micelles). Viscosity experienced by the probe diffusing in the complex liquid is affected not only by the concentration but also by the size of the diffusant itself. In order to visualize differences in the viscosities experienced by the different probes, in Figure 1 we plot the viscosities as a function of the polymer or of the surfactant concentration for different ratios of hydrodynamic radii \( r_p/R_h \). We measured the viscosity in two solutions for different ratios of \( r_p/R_h \): one composed of PEG 400 (\( R_h = 0.4 \) nm) and of PEG 20000 (\( R_h = 2.9 \) nm) with lysozyme (\( r_p = 1.9 \) nm) as the probe (Figure 1a). When the \( r_p < R_h \) (\( r_p/R_h < 1 \)) the probe diffused with nanoscopic viscosity lower than the macroscopic viscosity of the solution. The same probe in polymer solution composed of the smaller polymer coils (i.e., \( r_p > R_h \) and \( r_p/R_h > 1 \)) experienced the macroscopic viscosity. In the PS 220000 solutions (\( R_h = 11.5 \) nm), probes such as PMI (\( r_p = 0.53 \) nm) or fluorescently labeled polystyrene, PMI-PS 34000 (with PMI as fluorophore), experienced much lower viscosity than the macroscopic one (Figure 1b). On the other hand \( r_p \) of the PMI-PS 255000 was larger than the hydrodynamic radius of the matrix obstacles. Surprisingly, PMI-PS 255000 in PS 220000 solutions (\( r_p > R_h \) and \( r_p/R_h \approx 1 \)) experienced a viscosity lower than the macroscopic viscosity of the solution. This result showed that the crossover between the two regimes (nano- and macroscopic viscosity) could not be described with a sharp threshold determined by the radii of the probe and of the obstacles (i.e., nanoscopic viscosity) according to the empirical equation below:

\[
\eta = \eta_0 \exp \left( \frac{R_{\text{eff}}}{\xi} \right)^a
\]

(7)

Table 2. Hydrodynamic Radii of Obstacles (\( R_h \)), Probes (\( r_p \)), \( r_p/R_h \) and the Effective Hydrodynamic Radii \( R_{\text{eff}} \) (eq 8) for Different Systems Shown in Figure 1

<table>
<thead>
<tr>
<th>matrix/probe</th>
<th>( R_h ) (nm)</th>
<th>( r_p ) (nm)</th>
<th>( r_p/R_h )</th>
<th>( R_{\text{eff}} ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG 20000, macroscopic viscosity</td>
<td>2.9</td>
<td>( \infty )</td>
<td>( \approx )</td>
<td>2.9</td>
</tr>
<tr>
<td>PEG 20000/lysozyme</td>
<td>2.9</td>
<td>1.9</td>
<td>0.66</td>
<td>1.6</td>
</tr>
<tr>
<td>PEG 400, macroscopic viscosity</td>
<td>0.4</td>
<td>( \infty )</td>
<td>( \approx )</td>
<td>0.4</td>
</tr>
<tr>
<td>PEG 400/lysozyme</td>
<td>0.4</td>
<td>1.9</td>
<td>4.75</td>
<td>4.0</td>
</tr>
<tr>
<td>PS 220000, macroscopic viscosity</td>
<td>11.5</td>
<td>( \infty )</td>
<td>( \approx )</td>
<td>11.5</td>
</tr>
<tr>
<td>PS 220000/PMI</td>
<td>11.5</td>
<td>0.5</td>
<td>0.04</td>
<td>0.5</td>
</tr>
<tr>
<td>PS 220000/PMI-PS 34000</td>
<td>11.5</td>
<td>4.5</td>
<td>0.4</td>
<td>4.2</td>
</tr>
<tr>
<td>PS 220000/PMI-PS 255000</td>
<td>11.5</td>
<td>12.5</td>
<td>1.09</td>
<td>8.5</td>
</tr>
<tr>
<td>C12E6, macroscopic viscosity</td>
<td>5.3</td>
<td>( \infty )</td>
<td>( \approx )</td>
<td>5.3</td>
</tr>
<tr>
<td>C12E6/lysozyme</td>
<td>5.3</td>
<td>1.9</td>
<td>0.36</td>
<td>1.8</td>
</tr>
<tr>
<td>C12E6/apoferritin</td>
<td>5.3</td>
<td>6.9</td>
<td>1.3</td>
<td>4.2</td>
</tr>
<tr>
<td>C12E6/particles</td>
<td>5.3</td>
<td>57</td>
<td>10.75</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Figure 1. Viscosities of PEG (a), PS (b) and C12E6 (c) solutions as a function of concentration measured with different nanoprobe. In panel a, we present macroscopic viscosity of low molecular weight oligmer PEG 400 (\( \eta \), \( r_p/R_h = \infty \), \( R_h = 4.75 \) nm, \( r_p = 1.9 \) nm), macroscopic viscosity of PEG 20000 (\( \bullet \), \( r_p/R_h = \approx \), \( R_h = 2.9 \) nm) and one measured by diffusion of lysozyme in PEG 20000 (\( \triangle \), \( r_p/R_h = 0.66 \), \( r_p = 1.9 \) nm). In panel b we show viscosity measured with different probes for solutions of PS 220000 in acetonaphene. As probes we use: PMI (\( \triangle \), \( r_p/R_h = 0.09 \), \( r_p = 0.53 \) nm), PMI-PS 34000 (\( \bullet \), \( r_p/R_h = 0.4 \), \( r_p = 4.45 \) nm), and PMI-PS 255000 (\( \bigcirc \), \( r_p/R_h = 1.09 \), \( r_p = 12.5 \) nm). Macroscopic viscosity of PS 220000 matrix is represented by \( \square \) (\( r_p/R_h = \approx \), \( R_h = 11.5 \) nm). Panel c shows viscosity of aqueous solutions of rigid micelles C12E6. Macroscopic viscosity (\( \bullet \), \( r_p/R_h = \approx \), \( R_h = 5.3 \) nm) and viscosities measured with different probes: fluorescent particles (\( \bigcirc \), \( r_p/R_h = 10.75 \), \( R_h = 57 \) nm), apoferritin (\( \triangle \), \( r_p/R_h = 1.3 \), \( r_p = 6.9 \) nm), and lysozyme (\( \triangle \), \( r_p/R_h = 0.36 \), \( r_p = 1.9 \) nm).
Here \( R_{\text{eff}} \) is an effective hydrodynamic radius related to hydrodynamic radii of the probe \( r_p \) and of the polymer/micelle, \( R_0 \):

\[
R_{\text{eff}}^{-2} = R_0^{-2} + r_p^{-2}
\]  

In eq 7, \( a \) is an exponent of the order of 1 and has same meaning as in eqs 2 and 3. In the limit of \( r_p \gg R_0 \), eq 7 becomes eq 3 with \( R_0 \) replaced by \( R_0 \). In the opposite limit (\( r_p \ll R_0 \)) eq 7 becomes eq 2 with \( d \) replaced by \( r_p \). The novelty of eq 7 is in the combination of hydrodynamic properties of the probe (\( r_p \)) and cosolute (\( R_0 \)) with the structural property of the network (\( \xi \)). Equations 2 and 3 reflect only structural properties of the matrix liquid (gyration radius \( R_0 \) and \( \xi \)). In addition, eqs 2 and 3 exhibit a discontinuity since one boundary refers to the radius of gyration and the second to the diameter of the probe. In contrast eq 7 provides a smooth transition between the nano- and macroscopic scale. In Table 2 we present effective radii \( R_{\text{eff}} \) for different probes presented in Figure 1. Now we can formulate the crossover criterion, i.e., a criterion which states at what length scale the probes start to experience the macroscopic viscosity. The probe experiences macroscopic viscosity when \( R_{\text{eff}} \approx R_0 \). For practical reasons when \( r_p > 4R_0 \), the viscosity experienced by the probe is very close (within 1–5%) to the macroscopic viscosity according to eq 8.

In Figure 2 we present viscosity values scaled with the factor \( \frac{R_{\text{eff}}}{\xi} \) (in PEG, Figure 2a, in PS, Figure 2b). In all plots the data followed the same master curve defined by eq 7, same for the macroscopic probes (falling ball viscometer) and for the nano-probes (proteins or dyes). The scaling law describes the viscosity experienced by the proteins (compact structures similar to hard spheres) and labeled polymer coils (entangling with polymer coils — matrix obstacles) whose motion in concentrated solutions of polymers was used to be described with the reptation model.42 We tested the universality of our scaling law by applying it to solutions of elongated rigid micelles as shown in Figure 2c. In the studied system, above critical micelle concentration the surfactants aggregate forming elongated, ellipsoidal micelles of length \( L \).41 Further increase of concentration causes the micelles to overlap. In this system the correlation length \( \xi \) (eq 4) is defined by the distance between two touching points of micelles. It is a similar definition to the one used in polymer solutions where \( \xi \) is defined as the distance between entangle points of polymer coils. In micellar systems we defined correlation length as in eq 4. Namely \( \xi = bL(c/\rho)^{\beta} \) where \( L \) is the length of the micelles. The overlap concentration in the solution of rigid micelles (with length much larger than their diameter) is given by \( \rho^* = 3A_mM_w/(N_aL^3) \), where \( A_m \) is an average number of surfactants in a micelle (aggregation number) and \( M_w \) is the molecular weight of the surfactant. The value of \( \beta = -1 \) for solution of micelles guarantees that \( \xi \) does not depend on micellar length (similarly as \( \xi \) in polymer solutions does not depend on the molecular weight of the polymers).35 The viscosity data scaled with \( R_{\text{eff}}/\xi \) for the micellar solution (Figure 2c) follows the same curve given by eq 7 as in the case of the polymer solutions.

Each of the systems described above had only one type of crowding agent (objects which crowd the environment, micelles or polymers in our case) that formed the complex liquid. Viscosity in these systems depended on two length scales: correlation length \( \xi \) and an effective hydrodynamic radius \( R_{\text{eff}} \). More generally, complex liquids (including cytoplasm in cells) may comprise many types of crowding agents that differ in size and shape. In cells apart from the cytoplasm and actin filaments one can find lots of cytoplasmic proteins and other macromolecules and organelles which crowd the environment.43 In vitro studies show that the self-diffusion of small (few to tens of nanometers) probes in cells is hindered by the cytoskeleton network.44 On the other hand studies of diffusion of proteins in protein solutions45 suggest that viscosity of such solutions may be only slightly higher than the viscosity of water. Therefore in cells we could expect that diffusion of probes will depend on viscosity which is a product of both viscosities (viscosity of the protein solution and of the viscosity of the cytoskeleton network). In this case we expect that the effective viscosity of the system

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Scaled viscosities (same as in Figure 1) of PEG, PS, and C12E6 solutions divided by the viscosity of water at 25 °C (relative viscosity). In panel a we show scaling plot for the logarithm of relative viscosity measured for different probes in aqueous solutions of PEG. In panel b we show scaling plot obtained for PS 220000 solutions in acetophenone. In both panels (a and b), all data follow the same curve given by eq 7 with parameters \( a = 0.62 \pm 0.02 \) and \( b = 0.24 \pm 0.02 \) (cf. eq 4) for PEG solutions and \( a = 0.71 \pm 0.1 \) and \( b = 0.43 \pm 0.02 \) for solutions of the PS. In panel c we show scaled relative viscosities of aqueous solutions of C12E6 consisting of rigid micelles. All data follow the same curve given by eq 7 with parameters \( a = 0.87 \pm 0.02 \) and \( b = 1.18 \pm 0.04 \).}
\end{figure}
should depend on the properties of all types of crowding agents. In particular we expect that the protein solution exhibits viscosity $\eta_{\text{matrix}}$ and that the actin filament network is responsible for large change of viscosity according to the exponential factor $\exp[(R_{\text{eff}}/\xi)^a]$ (similarly as for complex liquids). We assume that the viscosity of the composite solution of proteins and actin filaments can be described by the following scaling law

$$
\eta = A\eta_0 \exp\left[(R_{\text{eff}}/\xi)^a\right]
$$

where $\eta_{\text{matrix}} = A\eta_0 R_{\text{eff}}$ is defined by eq 8, and $A$ is a constant of the order of 1.

We reanalyze the data of diffusion in a cytoplasm of HeLa cells for various probes: DNA, water, EGFP, DNA fragments and nanodiamonds. We also reanalyzed diffusion data of fluorescent dye and dextrans in Swiss 3T3 mammalian cells. Variables such as hydrodynamic radius of the probes ($r_p$) were partially available in the literature. However, for the analysis of diffusion of DNA, the hydrodynamic radius of DNA was determined separately. Robertson et al. studied diffusion of linear DNA fragments in buffer solutions. From those data we determined an empirical equation for the hydrodynamic radius of DNA fragment as a function of its molecular weight.

$$
r_p = 0.024 M_w^{0.57} \text{ (nm)}
$$

$r_p$ obtained from eq 10 is the hydrodynamic radius of the sphere equivalent to the DNA fragment. Hydrodynamic radii and viscosities experienced by all probes together with the cell type are listed in Table 3. For fitting of viscosity of the cytoplasm data, we combined eqs 8 and 9 to obtain the following form of the scaling law

$$
\ln\left(\frac{\eta}{\eta_0}\right) = \ln(A) + \left(\frac{L^2}{R_h^2} + \frac{\xi^2}{r_p^2}\right)^{-a/2}
$$

Here $\xi$, $A$, $a$, and $R_h$ (equivalent hydrodynamic radius of obstacles) were fitting parameters. In Figure 3 we show the viscosity experienced by the probes listed in Table 3. We fit the data obtained for living cells with eq 11. We found that in HeLa cells the viscosity of the matrix $\eta_{\text{matrix}} = A\eta_0 = (1.3 \pm 0.3)\eta_{\text{water}}$. Correlation length $\xi = 5 \pm 4$ nm, $R_h \approx 86$ nm, and $a = 0.49 \pm 0.22$. For Swiss 3T3 cells we obtained $\eta_{\text{matrix}} = (2.9 \pm 0.6)\eta_{\text{water}}$, $\xi = 7 \pm 2$ nm, $R_h \approx 30$ nm, and $a = 0.62 \pm 0.37$.

![Figure 3](image-url)
Dauty et al. who measured the filaments to range from 100 to 500 nm. In addition, our analysis yields macroscopic viscosities for both cell types. For HeLa cells $\eta_{\text{macro}} \approx 64\eta_{\text{water}} \approx 4.4 \times 10^{-2}$ Pa·s since for Swiss 3T3 cells $\eta_{\text{macro}} \approx 34\eta_{\text{water}} \approx 2.4 \times 10^{-2}$ Pa·s. These values are in agreement with the literature data of the cytoplasmic viscosity in an amoeba Dictyostelium discoideum cells ($5 \times 10^{-2}$ Pa·s). According to the criterion that was proposed for complex liquids the probe will experience macroscopic viscosity when $r_p \geq 4R_0$. One can estimate minimal hydrodynamic radius of the probe which would experience the macroscopic viscosity of the cytoplasm of HeLa and Swiss 3T3 cells. For HeLa cells hydrodynamic radius of such probe should exceed 350 nm while for Swiss 3T3 cells $r_p \geq 120$ nm.

The data shown in Figure 3 reflect a universal trend of viscosity dependence on hydrodynamic radius of the probe. The data obtained in the cytoplasm of mammalian cells are different from those in polymer or surfactant solutions in one respect: in the cytoplasm both $\xi$ and $R_0$ are fixed. In the polymer or in the surfactant solution we vary $\xi$ by changing the concentration of the polymer coils or surfactant micelles. $R_0$ is controlled by changes of the molecular weight of the polymer. We represent data for complex liquids (polymers and micelles) in the most general form shown in Figure 2. The scaling law (eq 7) is based on the ratio $R_{\text{eff}}/\xi$. Therefore correct determination of the scaling form may be achieved by use one of two different ways. One of them is to change the hydrodynamic radius of the probe under $\xi = \text{constant}$. This is the method used for mammalian cells, because by definition, $\xi$ is fixed. Another way is to change the molecular weight of the polymer, hydrodynamic radius of the probe, and the concentration of the polymer or of the micelles. This method was used for polymer and surfactant solutions.

For big probes ($r_p \gg R_0$) changes in the viscosity experienced by the probe are negligible when two probes of different sizes are compared. However in order to obtain precise results in biological systems, measurements for all length scales ($r_p < \xi < \xi < r_p < R_0$, $r_p \gg R_0$) are needed. This is not the case for synthetic systems where values of $R_{\text{eff}}/\xi$ can be controlled by all variables ($r_p$, $\xi$, and $R_0$). Furthermore measurements at the nanoscale for complex liquids might be not necessary in order to determine the scaling formula. In particular one can measure the macroscopic viscosity, and whole needed range of $R_{\text{eff}}/\xi$ values can be covered by changes of the concentration only.

Our work is complementary to the work of Arcizet et al. who proposed to probe the cytoplasm at the micrometer scale and distinguish between active and passive transport. In our work we probed hydrodynamic radii below 100 nm. One can propose an experiment to probe all length scales (from nano to macro) and to determine, for investigated cell strain, scaling of viscosity across all length scales. Such experiment should be based on single molecule measurements (e.g., FCS) to probe nanoscopic length scale and on single particle tracking to probe microscopic scale. Additionally submicrometer length scale (>100 nm) can be probed with the technique described by Wirtz. In all techniques however, at which hydrodynamic radius of the probe is smaller than a few hundred nanometers, results should be analyzed with scale dependent viscosity taken into account.

In our model we did not consider viscoelastic effects. We suppose that when the probe size exceeds a certain value, elasticity of the cytoplasm may affect the probe’s motion. The interior of a cell is a gel, and below a certain length scale, motion is controlled by purely viscous effects. We also did not account for possible effects of electrostatic charge that may also influence the diffusion of microscopic probes. We performed our measurements on synthetic systems for nonionic polymers and surfactants which eliminate the problem of electrostatic interactions between the probe and the matrix. Additionally in living cells charge should also not influence the diffusion of the probes, because the Debye’s screening length for living cells is short (less than 1 nm), significantly below the value of $\xi$.

In summary we showed that viscosity depends strongly on the hydrodynamic radius of the probe (the scale of flow). Therefore all measurements of viscosity, diffusion, or electrophoretic mobility (via dynamic light scattering, capillary electrophoresis, chromatography, and many others) in complex liquids should be treated very carefully when the probes are nanoscopic. The SSE equation can be applied to complex liquids at all length scales, with a proper account for the viscosity dependence on $R_{\text{eff}}$ (eq 8).

Additionally viscosity experienced by the probes of a given $r_p$ in a solution comprising obstacles of $R_0 = r_p$ (polymers in polymers or micelle in micellar solution) is lower than the macroscopic viscosity of the solution. Furthermore if the scaling form of the viscosity is known in a given system, one can use the SSE equation with the scale dependent viscosity to predict the self-diffusion coefficient for any probe of known hydrodynamic radius. As far as we know, this is the first model of viscosity that takes into account and unites both the relationship between the probe and the obstacles ($R_{\text{eff}}$, eq 8) as well as the structural properties of the matrix ($\delta(R_{\text{eff}})$ or $\delta(L_0$)), introduced by Langevin and Rondelez. In addition, our results show that using simple self-diffusion measurements performed in living cells for a series of probes, it is possible to estimate such variables as the matrix and the macroscopic viscosity. Our investigations of viscosity of cytoplasm of living cells should be followed by further studies to extend our understanding of the internal structure of cells.

**ASSOCIATED CONTENT**

Supporting Information. Materials and methods and description of the experimental details. This material is available free of charge via the Internet at http://pubs.acs.org/.

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