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Stirring a fluid at low Reynolds numbers: Hydrodynamic collective effects of active proteins in biological cells

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HIGHLIGHTS

- Stirring of fluids at low Reynolds numbers by a population of active force dipoles is considered.
- Advection of passive particles in the resulting fluctuating fluid flows is discussed.
- Diffusion enhancement and drift of the particles due to such advection are investigated.
- Application of the results to problems of diffusion in biological cells is made.

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ABSTRACT

Most of the proteins in the cell, including not only molecular motors and machines, but also enzymes, are active. When ATP or other substrates are supplied, these macromolecules cyclically change their conformations. Therefore, they mechanically stir the cytoplasm and nucleoplasm, so that non-thermal fluctuating flows are produced. As we have recently shown (Mikhailov and Kapral, 2015), stochastic advection by such flows might lead to substantial diffusion enhancement of particles inside a living cell. Additionally, when gradients in the concentrations of active particles or in the ATP/substrate supply are present, chemotaxis-like drift should take place. Here, the motion of passive tracers with various sizes in a mixture of different kinds of active proteins is analyzed. Moreover, effects of hydrodynamic interactions on the motion of active proteins are explored. Theoretical results are compared with available experimental data for ATP-dependent diffusion of natural and microinjected particles in biological cells.

1. Introduction

Although it is well known that molecular motors play a variety of crucial roles in the biochemistry of the cell, and that some motors function as cargo carriers to transport material, it is usually assumed that the transport properties of most chemical species in the cell are given by the values they take in systems at equilibrium. For example, the diffusion coefficient of proteins and other species are often estimated by the Stokes–Einstein formula. Of course, biochemistry and transport in the cell do not occur in equilibrium conditions. Fuel in the form of substrates such as adenosine triphosphate (ATP) must be supplied to maintain activity. There is a growing body of experimental evidence that diffusion is enhanced in nonequilibrium environments that support protein activity [1–3]. Several mechanisms have been suggested to account for this enhancement [4–6]. For example, molecular motors walking on filaments can generate nonequilibrium forces that could modify both the cytoskeletal network and transport properties of species in the cell [4,5]. *In vitro* experiments [7,8] have shown diffusion enhancement due to simple protein enzymatic activity and possible mechanisms for this behavior have been discussed [9]. Recently we proposed another mechanism that can give rise to enhanced diffusion as a result of the nonequilibrium conformational fluctuations induced by enzymatic activity [10].

When fluids are macroscopically stirred, turbulence with high Reynolds numbers develops. In a cascade, energy is transferred from long to short wavelengths and eventually dissipated on the shortest scales. Advection of passive particles by turbulent flows is

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known to strongly affect kinetic processes; for instance, diffusion of pollutants in the air is almost entirely due to turbulent advection effects [11]. On microscopic scales typical for a biological cell, the flows are always characterized by low Reynolds numbers [12] and turbulence cannot exist. Nonetheless, fluids can still be stirred on such scales. Moreover, stirring naturally occurs whenever an active protein or other macromolecule cyclically changes its shape. Collectively, active proteins in a living cell generate non-thermal fluctuating flows that can be viewed as analogs of macroscopic turbulence on long length scales. We have shown [10] that such fluctuating flows may lead to substantial diffusion enhancement in the cytoplasm and in biomembranes. Furthermore, when gradients in concentrations of active proteins (or of ATP/substrate supply) are present, chemotaxis-like drift of passive particles will take place [10].

In the next section, a brief outline of previous results is given. After that, we consider in Section 3 the motion of tracers in a mixture of active proteins of different kinds. Here, the dependence of diffusion enhancement and drift of tracers on their size is discussed. Moreover, the evolution of the tracer concentration distribution with time is analyzed and the final stationary distribution of passive tracers in the medium with a given distribution of active proteins is constructed. In Section 4, a comparison of our theoretical results with the experimental data on the ATP-dependent diffusion phenomena in biological cells is made. Section 5 deals with collective evolution of active proteins as affected by hydrodynamic interactions between them. The paper ends with conclusions and discussion of the perspectives for future research.

2. Active proteins as hydrodynamic force dipoles

The cellular cytoplasm is crowded with active proteins of various kinds. Active proteins undergo mechanochemical conformational changes in their cyclic operation, which are induced by substrate binding and product release. In motor proteins this catalytic activity plays only an auxiliary role by inducing conformational changes that are needed for the operation of a particular protein machine. Most true enzymes also execute mechanochemical motions within their turnover cycles. Through conformational changes resulting from such motions, substrates can be brought into optimal positions for catalytic conversion to products and the release of products can be facilitated. Even the structural protein actin that forms the cytoskeleton, and therefore is present in large amounts inside a cell, undergoes ligand-induced conformational changes, with the conformations of actin monomers strongly dependent on whether ATP or ADP is bound [13]. A database of ligand-induced conformational motions in many proteins is available [14].

The change in shape of a protein is due to internal molecular forces that act on its different parts. In solution, any shape change of a macromolecule is also accompanied by forces acting on the surrounding fluid. Since inertia is negligible in the regime of low Reynolds numbers characteristic for microscales, the forces are always balanced. This means that any force applied to a particle is exactly compensated by the viscous force acting on it. Thus, the same forces which lead to conformational changes in a protein also act on the fluid around it.

If a force is locally applied to a fluid, it induces hydrodynamic flows that extend far from the point where the force was applied. Passive particles are advected by such flows; hence, application of a force at one point induces motions of particles (tracers) in the fluid at a distance from it. Hydrodynamics at low Reynolds numbers is linear and the local velocity of the flow is proportional to the applied force. The instantaneous relationship between the force $\mathbf{F}$ applied at point $\mathbf{R}$ and the flow velocity $\mathbf{V}$ at point $\mathbf{R}+\mathbf{r}$ is determined by the mobility tensor $\hat{G}(\mathbf{r})$, so that $\mathbf{V}(\mathbf{R}+\mathbf{r}) = \hat{G}(\mathbf{r})\mathbf{F}(\mathbf{R})$. In three dimensions, for sufficiently large distances r, $\hat{G}(\mathbf{r})$ is given by the Oseen tensor, which varies as $1/r$.

Suppose that two forces $\mathbf{F}$ and $-\mathbf{F}$, equal in magnitude and opposite in direction, are applied to the fluid at two points separated by vector $\mathbf{e}$ of length x along the direction of the force. The resulting flow is the superposition of the flows created by the individual forces. At large distance from the region where the forces are applied, the velocity flow is approximately given by

$$V_\alpha(\mathbf{R}+\mathbf{r}) = \sum_{\beta,\gamma=1,2,3} \frac{\partial G_{\alpha\beta}(\mathbf{r})}{\partial r_\gamma} e_\beta e_\gamma m(\mathbf{R}). \tag{1}$$

Here, $m = Fx$ defines the *force dipole*. The 3D flow field of the force dipole falls as $1/r^2$ for large r. It can be shown [15] that, at large separations, almost any object that changes its shape will create a fluid flow corresponding to some force dipole. Therefore, Eq. (1) can be applied more generally than its derivation might indicate. Note furthermore that Eq. (1) is invariant with respect to the transformation $\mathbf{e} \to -\mathbf{e}$, so that the sign of this vector can be arbitrarily chosen.

An active protein repeatedly changes its conformation and therefore it corresponds to some stochastic force dipole, $m(t)$. The time average of this force dipole vanishes, $\langle m(t)\rangle = 0$, because the conformational changes are cyclic. Thus, the fluctuating force dipoles of active proteins may be characterized by their correlation function,

$$S(t-t') = \langle m(t)m(t')\rangle. \tag{2}$$

Using this function, the integral intensity $S = \int_0^\infty S(\tau)d\tau$ and the frequency spectrum of force dipoles,

$$S(\omega) = \int_{-\infty}^{\infty} S(\tau)e^{-i\omega\tau}d\tau, \tag{3}$$

can be defined. Although the correlation functions of force dipoles depend on the specific proteins under consideration, and they should be experimentally determined or numerically computed for each type of protein, they possess some general characteristic features.

In the absence of substrate (ATP) supply, cyclic activity will disappear and the protein will be in a state of thermal equilibrium. In this state, only thermal conformational fluctuations are present and they are characterized by the equilibrium frequency spectrum $S_T(\omega)$ with intensity S_T. When the substrate (ATP) supply is switched on, the frequency spectrum and the force dipole intensity will acquire additional contributions $S_A(\omega)$ and S_A, so that

$$S(\omega) = S_T(\omega) + S_A(\omega), \quad S = S_T + S_A. \tag{4}$$

The active part S_A of the force dipole intensity should depend on the substrate (ATP) concentration, c_S, so that $S_A = S_A(c_S)$. The precise form of the concentration dependence must be determined for each protein. At low substrate concentrations, the protein must wait some length of time until a substrate molecule binds and the next cycle begins. During this waiting period, the protein is in a quasi-equilibrium state that does not contribute to the active component S_A of the force dipole intensity. Therefore, it should be expected that S_A will be proportional to c_S at low substrate concentrations. On the other hand, the force dipole activity S_A should saturate at high substrate concentrations. In this limit, the protein is able to immediately bind a new substrate molecule and begin a new catalytic cycle once the previous cycle is complete. In this case, the waiting time is vanishingly short.

Generally, the dependence of S_A on substrate concentration takes the form

$$S_A(c_s) = S_0 W(c_s) \tag{5}$$

where S_0 is the saturation value and $W(c_s)$ is a function that is proportional to the concentration at low substrate levels and reaches a saturation value of unity at high substrate concentrations.

3. Passive tracers in solution with different kinds of active proteins

Since each active protein acts as a stochastic force dipole, it generates a fluctuating flow in the fluid around it. When many active proteins of different kinds are randomly distributed in the medium, they create a fluctuating flow field that can be obtained by summing the contributions of all active proteins that are present in the system. Any passive particle (i.e., a tracer) will be advected by such fluctuating flows (note that at low Reynolds numbers the velocity of the particle always coincides with that of the local flow). Similar to the advection of a passive scalar in a turbulent flow, advection in the fluctuating flows of force dipoles should lead to enhancement of diffusion of passive particles.

Recently, we considered the problem of diffusion enhancement [10] by assuming (a) that active proteins are randomly distributed in the medium and their orientations are also random and (b) that the cycles of different protein machines are not correlated. Now, we show how these results can be generalized to describe a medium that is populated by different kinds of active proteins, each with its own substrate.

Suppose that the solution includes different kinds k of active proteins, characterized by force dipoles with intensity $S_{A,k}(c_{S,k})$ that depend on the concentration $c_{S,k}$ of substrate for that kind of protein. The concentration of active protein of kind k is c_k. The diffusion coefficient for a passive particle is given by a sum $D = D_T + D_A$. Here D_T is the equilibrium diffusion coefficient that is observed when the proteins are not active (because substrates are not supplied or the activity is chemically inhibited) and D_A is the diffusion enhancement due to active proteins.

By following the same line of arguments as in Ref. [10], we find that total local diffusion enhancement can be estimated as

$$D_A = \frac{1}{60\pi\eta^2}\sum_k \frac{S_{A,k}(c_{s,k})c_k}{l_{tracer}+l_k} \tag{6}$$

where η is the fluid viscosity and the sum is taken over all kinds k of active proteins.

The approximate result in Eq. (6) is obtained from an evaluation of an integral that diverges at short separations between the tracer and proteins by introducing a cut-off at some characteristic length. Taking into account that the tracer and protein particles cannot interpenetrate, we assumed that the cut-off length is simply given by $l_c = l_{tracer} + l_k$ where l_{tracer} is the characteristic size of the tracer and l_k is the characteristic size of the proteins of kind k. If longer-range repulsive potential interactions between such molecules are present, the effective cut-off length would increase. Furthermore, it should be kept in mind that the estimate (6) is based on the Oseen approximation for the mobility tensor that fails at short distances. More accurate approximations, such as the Rotne–Prager mobility tensor, may be used but this has not been done so far. Hence, the dependence on the sizes of tracers and proteins and the numerical prefactor of $1/60\pi$ in Eq. (6) should be regarded as rough estimates for the magnitude of the effects.

Several important observations can be made by examining Eq. (6). The result for D_A in this equation may be contrasted with the Stokes–Einstein value $D_T = k_BT(6\pi\eta l_{tracer})^{-1}$. The active contribution D_A depends on $1/\eta^2$ rather than $1/\eta$, and the temperature dependence enters only through the viscosity. Moreover, D_A is inversely proportional to the square of the solvent viscosity η, whereas the equilibrium diffusion coefficient depends on η as $D_T \propto 1/\eta$. These features indicate that we are dealing with a nonequilibrium effect where the fluctuation–dissipation theorem and the Einstein relationship do not hold.

Since $D_A \propto 1/(l_{tracer} + l_k)$, it will not depend on the nature of the tracer provided $l_k >> l_{tracer}$. However, for larger tracer particles whose sizes are comparable to those of proteins or more, the dependence on the tracer size enters into the estimate (6) through the cut-off length. According to this estimate, the diffusion enhancement should scale as $1/l_{tracer}$ when $l_{tracer} >> l_k$. If the spatial distribution of active proteins and/or the substrate (ATP) supply are not uniform, the estimate (6) holds locally and diffusion will be more strongly enhanced in regions where a large number of active proteins are located.

When gradients of the protein concentration and/or substrate supply are present, chemotaxis-like drift of tracer particles should take place, as shown in Ref. [10]. For a mixture of active proteins, we find that the local drift velocity is given by

$$\mathbf{V} = \frac{1}{30\pi\eta^2}\sum_k \frac{1}{l_{tracer}+l_k}\nabla(S_{A,k}(c_{s,k})c_k). \tag{7}$$

Similar to diffusion enhancement (6), Eq. (7) provides only an estimate for the drift. It too was obtained from the evaluation of a diverging integral by introducing a cut-off at $l_{tracer} + l_k$. Thus, both the functional form of the size dependence and the value of the numerical prefactor in Eq. (7) may differ if more accurate estimates are used.

The local drift velocity can also be expressed as

$$\mathbf{V} = \frac{1}{30\pi l_c\eta^2}\left[\sum_k S_{A,k}\nabla c_k + \sum_k \frac{\partial S_{A,k}}{\partial c_{S,k}}c_k\nabla c_{S,k}\right]. \tag{8}$$

Both gradients in the protein concentration and in the substrate (ATP) supply contribute to the local direction and magnitude of the drift. The proportionality coefficient in Eq. (8) is positive and therefore the tracers tend to drift towards regions with the higher total concentration of active proteins or to regions with higher substrate or ATP supply rate.

Note that, according to Eqs. (6) and (7) or (8), a simple relationship between the drift velocity and diffusion enhancement holds, $\mathbf{V}(\mathbf{r}) = 2\nabla D_A(\mathbf{r})$. In view of the above comments about the values of the estimates, the proportionality coefficient in this relationship cannot be taken as exact and, instead, a relationship

$$\mathbf{V}(\mathbf{r}) = v\nabla D_A(\mathbf{r}) \tag{9}$$

with some (yet to be determined) proportionality coefficient v of order unity can be instead assumed. Only under the simplest estimates in Ref. [10] do we have $v = 2$.

Evolution of the tracer distribution in the medium obeys the diffusion equation for the local tracer concentration that has the form

$$\frac{\partial n}{\partial t} = -\frac{\partial}{\partial \mathbf{r}}[\mathbf{V}(\mathbf{r})n(\mathbf{r})] + \frac{\partial^2}{\partial \mathbf{r}^2}[D(\mathbf{r})n(\mathbf{r})] \tag{10}$$

where $D(r) = D_T + D_A(\mathbf{r})$. The coefficients in this equation are given by expressions (6) and (7). They can be determined (see Ref. [10]) by tracking the motion of tracer particles. If $\mathbf{R}(t)$ is a trajectory of a tracer, we have

$$D = \lim_{t\to\infty}\frac{1}{6t}\langle(\Delta\mathbf{R}(t))^2\rangle, \qquad \mathbf{V} = \lim_{t\to\infty}\frac{1}{t}\langle\Delta\mathbf{R}(t)\rangle. \tag{11}$$

Although Eqs. (7) and (10) appear to be similar to the equations arising in the chemotaxis studies, important differences exist. According to (7), the drift velocity of a tracer does not depend on its own mobility and size for the tracers that are much smaller than active proteins. The particles drift not because there are some forces applied to them. Rather, their collective directed motion is a consequence of an advection intensity gradient in the medium. However, for large tracers, the drift velocity should depend on the inverse of their size.

If the evolution equation (10) for distribution of passive tracers in the medium is written in the standard diffusion form, it reads

$$\frac{\partial n}{\partial t} = -\frac{\partial}{\partial \mathbf{r}}\left[\left(\mathbf{V}(\mathbf{r}) - \frac{\partial D}{\partial \mathbf{r}}\right) n\right] + \frac{\partial}{\partial \mathbf{r}}\left(D(\mathbf{r})\frac{\partial n}{\partial \mathbf{r}}\right). \tag{12}$$

By taking into account the relationship (9) and that $D(\mathbf{r}) = D_T + D_A(\mathbf{r})$, we obtain

$$\frac{\partial n}{\partial t} = -\frac{\partial}{\partial \mathbf{r}}\left[(v-1)\frac{\partial D_A}{\partial \mathbf{r}} n\right] + \frac{\partial}{\partial \mathbf{r}}\left((D_T + D_A(\mathbf{r}))\frac{\partial n}{\partial \mathbf{r}}\right). \tag{13}$$

Note that if $v = 1$ the drift term disappears in this diffusion equation form.

Eq. (13) can be used to determine the stationary tracer distribution established in the medium under a heterogeneous distribution of active proteins and/or non-uniform substrate supply. Simple calculations yield

$$n(\mathbf{r}) = n_0\left(1 + \frac{D_A(\mathbf{r})}{D_T}\right)^{v-1}, \tag{14}$$

where n_0 is the normalization constant determined by the total number of tracers present in the medium.

Using the simple estimate, $v = 2$, tracers will tend to aggregate in the regions with intense protein activity where the number of active proteins is higher and/or larger amounts of the substrate (such as ATP) are supplied.

4. Comparison with experimental data

Using plausible values for the quantities that determine D_A and V in the cell, the diffusion enhancement for small tracers due to active proteins could be estimated in Ref. [10] to be about $D_A = 10^{-6}$ cm^2/s. If a protein gradient of 10% is present, the characteristic drift velocity of passive tracers could be estimated as $V = 1$ μm/s. These values indicate that the effects may well be substantial. Indeed, the equilibrium diffusion constant of small molecules in water solution is about 10^{-5} cm^2/s and the diffusion constant of proteins is about 10^{-7} cm^2/s.

In recent experiments [1], particles with sizes of several hundred nanometers were injected into biological cells and their motions were optically tracked. From such measurements, mean square displacements of microinjected particles as a function of time were determined, allowing one to estimate diffusion constants. In these investigations, activity of molecular myosin motors could be chemically inhibited and supply of ATP could be effectively eliminated. It was found [1] that the diffusion constant of tracers was reduced by only about 10% under myosin inhibition, whereas the diffusion constant fell to almost undetectable values under depletion of ATP.

These experimental results are interesting since they clearly demonstrate that myosin motors, providing a classical and well-investigated example of active intracellular proteins, are indeed responsible for substantial diffusion enhancement within the cell. In fact, our numerical estimate for the magnitude of active force dipoles was based on the known forces generated by myosin during its power stroke. However, the experiments also indicate that myosin motors alone cannot explain the sharp diffusion reduction under depletion of ATP. Other energetically activated mechanisms must be involved as well. According to our predictions, *all* active proteins in a cell should contribute, to different degrees, to the observed diffusion enhancement in the presence of ATP or other energy-bringing substrates. Additionally, the inverse dependence of the diffusion constant on the size of microinjected particles was observed [1]. Such dependence is predicted by Eq. (6) when tracer particles are much larger than proteins.

There are other experiments [2] where natural intracellular particles were optically labeled and their diffusive motions within the cell were tracked, both under physiological conditions and under ATP depletion. For the smallest tracked particles with the size of about 3 nm, no significant ATP dependence of the diffusion behavior could be observed. However, for various particles of the size of several tens of nanometers, strong diffusion reduction in the absence of ATP was clearly seen [2]. Apparently, diffusion of small particles was dominated by thermal forces, while energetically activated processes were responsible for diffusion of the particles with larger sizes.

These observations are consistent with our analytical results and numerical estimates. According to Eq. (6), diffusion enhancement should be approximately independent of the tracer size provided that it is not much larger than a protein. Moreover, the magnitude of diffusion enhancement should be about $D_A = 10^{-6}$ cm^2/s. Since the thermal diffusion constant for small particles is about 10^{-5} cm^2/s, hydrodynamic effects of active proteins should not play an important role for them. On the other hand, thermal diffusion constants for particles with the size of proteins are about 10^{-7} cm^2/s, which is significantly less than the predicted ATP-dependent diffusion enhancement. Note that, similar to the study with microinjected tracers [1], a decrease of the ATP-dependent diffusion with the particle size has also been observed in the experiments in Ref. [2].

An intriguing further observation was that, under ATP depletion, diffusion was not only substantially reduced, but also transformed to subdiffusion [2]. Thus, ATP supply could effectively "fluidize" the cytoplasm in such *in vivo* experiments. Usually, subdiffusion is caused by weak localization, or caging, of particles and such localization will probably be destroyed when strong hydrodynamic agitation through active proteins is present. The ATP-dependent subdiffusion has moreover been observed in nucleoplasm of biological cells [3]. The temperature dependence of subdiffusion was found [3] to be exponential rather than linear, indicating non-Stokes–Einstein behavior.

In *in vitro* experiments by Sen et al. [7,8], an increase of the diffusion coefficient of enzymes was observed when they were catalytically active. When the activity was suppressed by removing substrate supply the diffusion constants returned to their equilibrium values. Such experiments provide an opportunity to study the enhancement mechanism in some detail since *in vitro* systems are less complex than the biological cell and many parameters are under the control of the experimentalist. While experimental results [7,8] are qualitatively in agreement with our predictions, their quantitative analysis is difficult in the absence of knowledge of the magnitudes of the force dipoles.

5. Collective evolution of active proteins

So far, only effects of active proteins on passive tracer particles in the solution have been discussed. A question however can also been asked: Will hydrodynamic interactions affect the motion of active proteins themselves?

In this section, we assume that there is only one kind of active protein present with force dipole intensity S. Because each protein molecule can also be viewed as a tracer particle, following the arguments that led to Eq. (13), the local protein concentration satisfies c the equation

$$\frac{\partial c}{\partial t} = -\frac{\partial}{\partial \mathbf{r}}\left[(v-1)\frac{\partial D_A(c)}{\partial \mathbf{r}} c\right] + \frac{\partial}{\partial \mathbf{r}}\left((D_T + D_A(c))\frac{\partial c}{\partial \mathbf{r}}\right) \tag{15}$$

where diffusion enhancement $D_A(c)$ is given by Eq. (6) with the cut-off length twice the linear size l of a protein,

$$D_A = \frac{S_A(c_s)c}{120\pi\eta^2 l}. \tag{16}$$

Suppose further that the substrate is uniformly supplied and $S(c_s) = S = \text{const}$. Then, evolution equation (15) takes a simple form

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial \mathbf{r}}\left(D_{\mathit{eff}}(c)\frac{\partial c}{\partial \mathbf{r}}\right) \tag{17}$$

with the effective concentration-dependent diffusion coefficient

$$D_{\mathit{eff}}(c) = D_T - \frac{\upsilon - 2}{120\pi l\eta^2}Sc. \tag{18}$$

According to this result, evolution of the distribution of active proteins in a medium depends crucially on the numerical value of the proportionality coefficient υ. If we take $\upsilon = 2$, as implied by the simple estimates (6) and (7), hydrodynamic stirring by active proteins does not affect their concentration distribution: it changes with time according to the diffusion equation with equilibrium diffusion coefficient D_T, as if the proteins were not active. However, if $\upsilon > 2$ the effective diffusion coefficient decreases with the concentration c and, for sufficiently high concentrations of active proteins it becomes negative. Negative values of the diffusion coefficient signal the instability onset of the uniform protein distribution and spontaneous development of regions enriched in active proteins. Note that negative values of the effective diffusion coefficient are characteristic for the onset of spinodal decomposition in equilibrium solutions in the presence of potential interactions between the particles. In the latter case, terms with quartic spatial derivatives have to be retained, yielding the Cahn–Hilliard evolution equation that describes the phenomenon of phase separation. On the other hand, if $\upsilon < 2$, hydrodynamic interactions between active proteins only enhance their diffusion and the instability should be absent at any protein concentrations. When $\upsilon = 1$, we have $D_{\mathit{eff}}(c) = D_T + D_A(c) = D(c)$.

As has been stressed above, our present approximations do not allow us to reliably estimate the numerical value of the proportionality coefficient in Eq. (9). The value $\upsilon = 2$ that corresponds to the simple estimates lies exactly on the boundary of the instability onset for the distribution of active proteins and therefore even small deviations from this numerical value would be very important. Because of this, no definite predictions for collective evolution of active proteins can be made on the basis of the current theory. Further investigations that take potential interactions between proteins at short distances into account and the use of improved approximations for hydrodynamic interactions are required.

6. Conclusions and discussion

We have shown that any microscopic particles that repeatedly change their shapes under nonequilibrium conditions should act as active force dipoles and mechanically stir the fluid in which they are dispersed and generate non-thermal fluctuating flows. Advection by such fluctuating flows leads to diffusion enhancement of passive tracers. Moreover, persistent average flows and steady drift of tracers should take place if the activity and concentration of force dipoles are non-uniform. The effect is general and applies to all conformationally active micro-particles, natural or synthetic; it also holds for the tracers of various origins and sizes.

The cellular medium is a highly complex environment and the simple model for diffusion enhancement we constructed made no attempt to account for this complexity. It is likely that no single mechanism is responsible for the observed enhancement. Nevertheless, the mechanism we discussed here should be in operation with magnitude depending on the parameter values entering into the estimates. Further experiments and theory will help to refine these estimates.

In vitro experiments where activities of different selected groups of proteins are chemically inhibited could be carried out to determine contributions of the respective groups to diffusion in the cell. Moreover, persistent hydrodynamic flows inside a living cell can be sought. *In vitro* experiments on protein solutions can be performed to explore the effects of diffusion enhancement under well-defined and completely controlled conditions. Finally, experiments with specially prepared active colloids, where individual particles exhibit persistent shape oscillations, would be very interesting to perform.

As we have shown, collective activity of proteins gives rise to intense nonequilibrium hydrodynamic fluctuations that span the entire living cell. Such fluctuating flows are non-thermal and the fluctuation–dissipation theorem does not hold. Moreover, the frequency spectrum of such fluctuations is also generally different from that of thermal systems (note that non-thermal force spectra have been indeed observed in the experiments with living cells [1]).

Diffusion enhancement and drift of particles, that we have considered so far, are phenomena determined by only the integral intensity of non-thermal hydrodynamic fluctuations and are not sensitive to their detailed statistical properties. Other properties may depend on the details of frequency spectrum of these non-thermal fluctuations. Since such fluctuations are non-thermal, the laws of thermodynamics do not forbid that energy is extracted from them and work is performed.

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