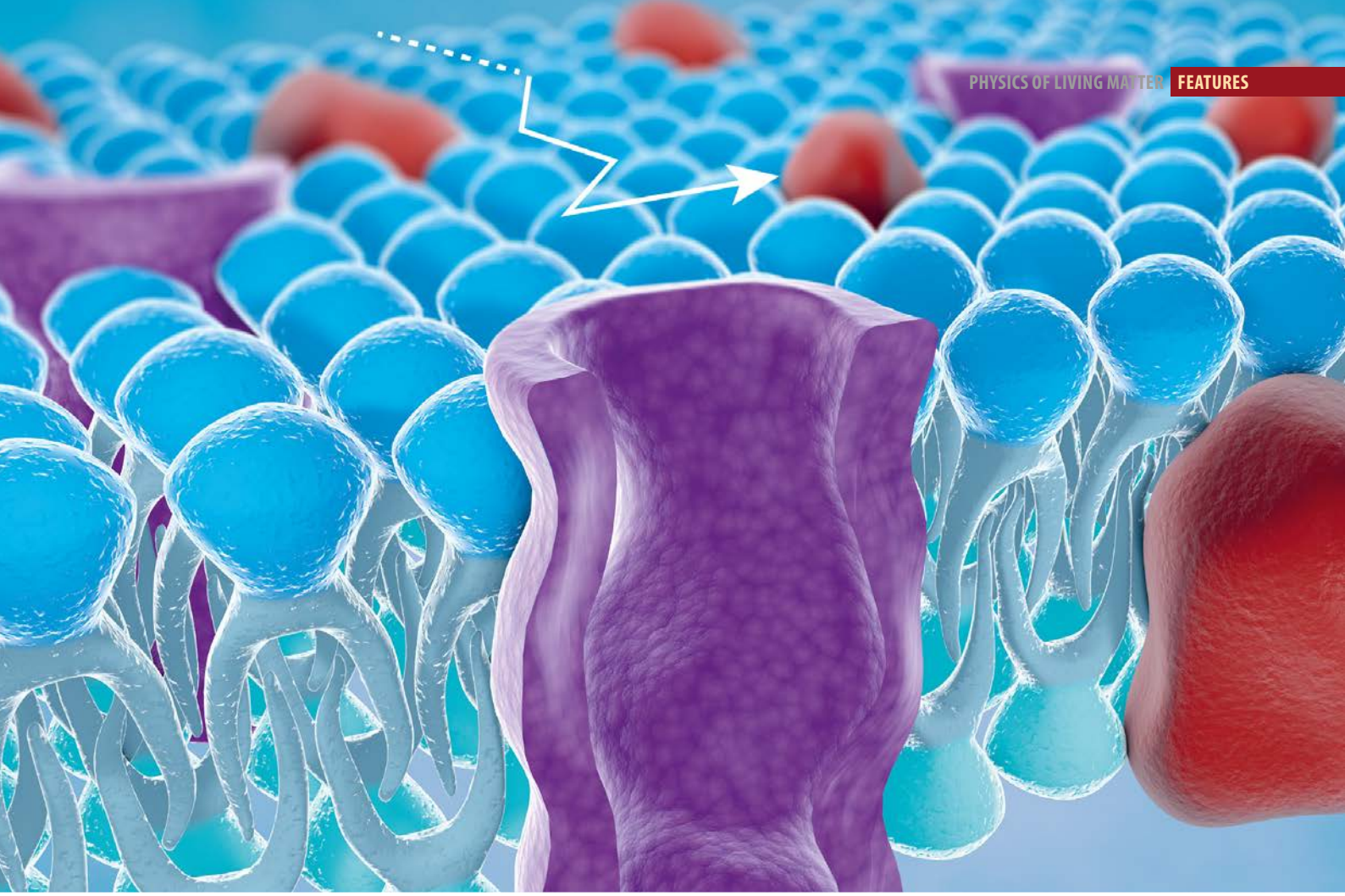

FROM SINGLE PARTICLE MOTION TO STRUCTURE OF BIOLOGICAL SYSTEMS

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Single molecule and single particle microscopy opened the door to observing dynamical processes in noisy living systems. Recent studies demonstrate how the stochastic motion of tracer particles can also provide us with information about the structure and flow properties of active and complex biological systems.

The realization that microscopic particles suspended in fluid move randomly dates back to Robert Brown, a botanist who first observed this phenomenon when watching plant pollen immersed in water under the microscope in 1827. This motion was named Brownian motion and its origin, collisions between water molecules and the suspended particles, was suggested first in 1905 by Albert Einstein [1]. In 1926 Jean Perrin received the Nobel Prize in Physics for using quantitative measurements of Brownian motion as evidence of the discontinuous nature of matter. In his experiments, Jean Perrin recorded the stochastic trajectories of diffusing particles and related the auto-correlation of their motion to their diffusion constant (D), $\langle \Delta \vec{x}^2 \rangle = 4Dt$. The diffusion constant of particles performing Brownian motion in a simple fluid was found to be inversely proportional to the viscosity and linearly proportional to the temperature of the fluid.

The motion of a microscopic particle in a fluid excites long-range flows that affect the motion of other particles immersed in the same fluid. The cross

Gels of actin and myosin reconstituted in the lab from pure proteins have been studied extensively as a model system for active biomaterials.

correlation in motion of two particles embedded in the same fluid arises from such flows, and is proportional to temperature and viscosity in a similar manner to the diffusion constant.

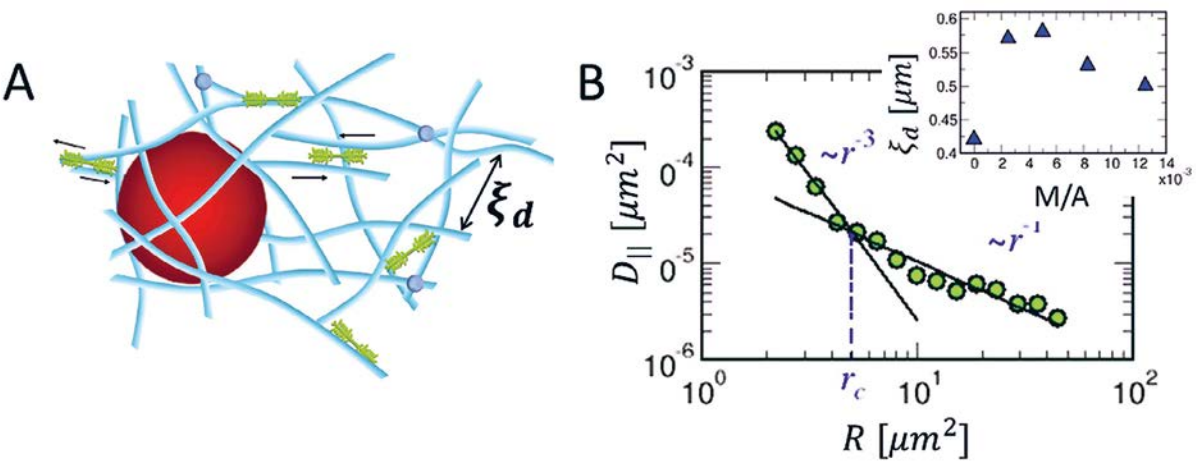
Thermally induced motion, therefore, enables such particles to explore their surroundings and interact with each other over large distances. Turned around, by monitoring the autocorrelation and cross correlation in the motion of colloidal particles, it is possible to characterise the fluid in which they are immersed. The cross correlation in the diffusive motion of two particles at a distance R is defined as $D_{\parallel,\perp} = \langle \Delta\vec{x}_i \, \Delta\vec{x}_j \, \delta(|\vec{x}_{ij}|-R)\rangle_{ij}$ in the parallel and perpendicular direction to the vector connecting their centre. This observation is the basis of the field of microrheology [2]; a method to characterise the mechanical properties of materials using extremely small samples of less than a few microliters.

The application of microrheology to characterise biological systems is challenging. First, biological systems are not in thermal equilibrium and therefore the fluctuation of a tracer particle, or biomolecules such as proteins, do not arise solely from thermal motion. This implies that the diffusion constant does not relate to the viscosity of the fluid through the temperature. Second, biological materials, such as the membrane or the skeleton of a cell, are examples of complex fluids, which are materials that respond to mechanical perturbation with a combination of viscous flow and elastic resistance. Complex fluids are comprised of mesoscopic building blocks, such as the protein chains of the cytoskeleton or the membranal proteins of the cell membrane.

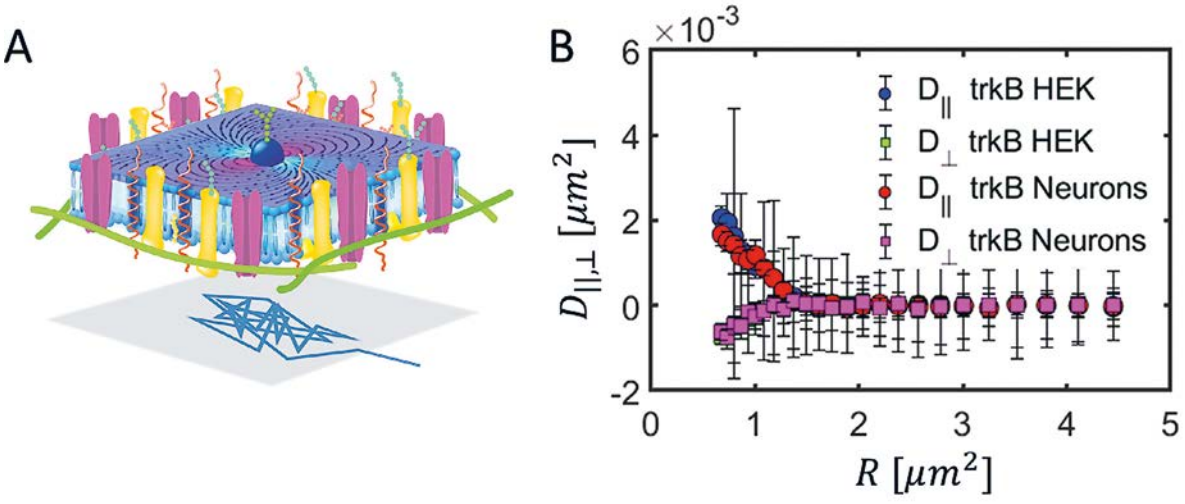
A tracer particle diffusing in a complex-fluid and exploring its neighborhood is affected by the local structure of the fluid. This is reflected in the type of diffusion that it undergoes. Moreover, if the fluid has active components such as molecular motors, the tracer will be affected by them as well. Identifying all the physical processes underlying the motion of tracer particles in such complex conditions remains an ongoing challenge in physics. However, comprehensive analysis tools exist for identifying to leading order structural and dynamical effects have been developed [3]. On the other hand, the flow field a tracer particle produces is affected by the structure of the complex fluid in a completely different manner. Since the functional form of its decay with distance is governed by momentum and mass conservation laws [4], it depends significantly on the interparticle distance. For example, at large inter-particle distances the correlated diffusion reflects the bulk properties of the complex fluid compared to the fluid's typical structural features. The question then arises, what can we infer from the fluctuation of a tracer particle in biological systems?

Structural features affect flow in biological gels

One of the ubiquitous proteins in cells is actin, the polymer that acts to maintain the shape of the cell, that helps to resist external stresses and plays a major role in cell motion. In the cell, actin self-organises into many different structures, one of which is a network supporting the outer membrane of the cell. Myosin II is a molecular motor that can slide two actin filaments one against the other by consuming chemical energy and converting it to motion (Fig. 1A). Gels of actin and myosin reconstituted in the lab from pure proteins have been studied



▲ **FIG. 1:** The structure of actin and myosin networks. **A** Illustration of an actin network (light blue) with myosin motors (green), cross linkers (circles) and a large tracer particle (red sphere). **B** A distinct cross-over in the decay rate of the correlated diffusion is observed at r_c. From r_c we extract the mesh size of the network ξ_d and plot it as a function of myosin to actin (M/A) concentration (inset).



◂ **FIG. 1: A** suggested model for the structure inducing flow arrest in the plasma membrane. Proteins are transiently caged in compartments made by actin binding proteins (blue trajectory). The flow field they induce is suppressed by the same dense fence of proteins. **B** Correlated motion is completely suppressed at a distance of 1.5 μm.

extensively as a model system for active biomaterials. The large difference, *i.e.* two orders of magnitude, between the mechanical properties inferred from the single particle diffusion and the correlated diffusion are a signature of the complex structure of these gels [4].

We examine a range of actin networks with varying typical mesh size. Single particle trajectories allow us to characterise the local environment of the particle and specifically the network structure in terms of the network pores. The correlated motion of tracer particles allows us to measure the flow field at different length scales (Fig. 1B). At intermediate distances, the correlations and induced flow field decay fast ($\sim 1/r^3$) with distance. At asymptotically large distances they decay slowly as $1/r$. The typical network mesh size is related to the cross-over distance, r_c, through the ratio of auto to cross correlated motion of the tracers [5].

In the presence of myosin motors, the fluctuations of the actin network include both thermal and active contributions [6]. Nonetheless, these complex fluctuations induce a similar type of random motion that provides the opportunity to measure the local structure of the actin network and induced flow field within them. The same telltale of the distinct transition in the correlated diffusion of tracer particles is observed in such acto-myosin networks and demonstrates the change in structure due to the introduction of the myosin motors to the network (inset Fig. 1B).

Flow arrest in the plasma membrane

The plasma membrane forms the interface between a cell and its environment. It controls the incoming traffic of signals and nutrients and the outflux of signals and waste. The plasma membrane is made of a bilayer of phospholipids, molecules that have a hydrophilic charged head and hydrophobic tails. The complex function of the membrane relies on embedded proteins that constitute approximately half of the mass of the membrane. It is also supported by and transiently connected through proteins to the underlying actin network.

Measurements of the motion of fluorescently tagged single molecules within the plasma membrane reveal caged motion (Fig. 2A). Moreover, the correlated motion shows that the membrane flow is local (Fig. 2B) [7]. Combined, these results support the "picket and fence" model with a high density of proteins forming the fences (Fig. 1A).

Conclusions

The combination of the auto and cross correlation in the motion of tracer particles in biological systems provides insight into details of their structure and flow properties, even far from equilibrium. Using this technique, we were able to show that the mechanical response of the cell is local, which supports the notion of the importance and location selectivity of mechanical signals at the cellular level.

About the author

Yael Roichman is a professor of Physics and Chemistry in Tel Aviv University. She is an expert in microscopy, holographic optical trapping, and single particle tracking, which she uses to study biophysics, soft matter, and non-equilibrium statistical mechanics.

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