

Magnetic micromanipulation in the living cell

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During the diverse processes that govern the survival and development of living organisms, each cell must react continuously, in a perfectly orchestrated manner, to a variety of internal and external constraints. The mechanical properties of the cell membrane are fundamental in this respect: on the one hand, resistance to deformation allows the cell to control its shape and to resist high shear stresses, while, on the other hand, membrane tension governs exchanges with the extracellular environment. Furthermore, all cells possess a dynamic inner cytoskeleton—a three-dimensional network composed of three sorts of protein filaments. The cytoskeleton serves as an active mechanical support for the cell body and contributes to cell shape maintenance. It controls chromosome migration during cell division, offers a "railway network" for intracellular trafficking, and contributes to the formation of membrane extrusions necessary for cell movement.

Cellular functions and physical properties (membrane rigidity and tension, cytoskeleton elasticity, cytoplasmic viscoelasticity, etc.) thus appear to be intricately related. A new challenge for the biophysicist is therefore to develop micromanipulations that mechanically deform the living cell in a controlled manner and thereby gain insights into the cellular machinery. These deformations may target the whole cell or involve local constraints applied to the cell surface. The micropipette aspiration technique is by far the most widely used method for manipulating the overall shape of individual cells [1]. This approach has been used to explore the rheological behavior of blood cells in suspension, to characterize the resistance of their membranes, and to model the mechanical responses of endothelial cells and fibroblasts. More recently, new designs were made to stretch a single cell either between two microplates to which it was adherent, or using an optical stretcher which takes advantage of the optical deformability of cells. Local perturbations can be generated on the plasma membrane by using microneedles or atomic force microscopy (AFM) probes. Finally, it is through the use of microbeads attached to the cell membrane and manipulated by means of magnetic [2] or optical tweezers that some interesting data have been obtained in recent years, notably on the transfer of forces between the inner cytoskeleton and the extracellular matrix, and the local characteristics of elastic and viscoelastic modules and the tension of the external cell membrane and cortical cytoskeleton.

Yet even these locally applied techniques mechanically disturb the plasma membrane, potentially altering the cell machinery which itself is submitted to a variety of physiological constraints. In the 1980s the intracellular medium started to be considered as a viscoelastic matrix contributing to cytoplasmic organization, cell movements, intracellular exchanges, and cell shape. Biophysical methods available to probe the local organization of the intracellular environment remain rare, however, because of the experimental

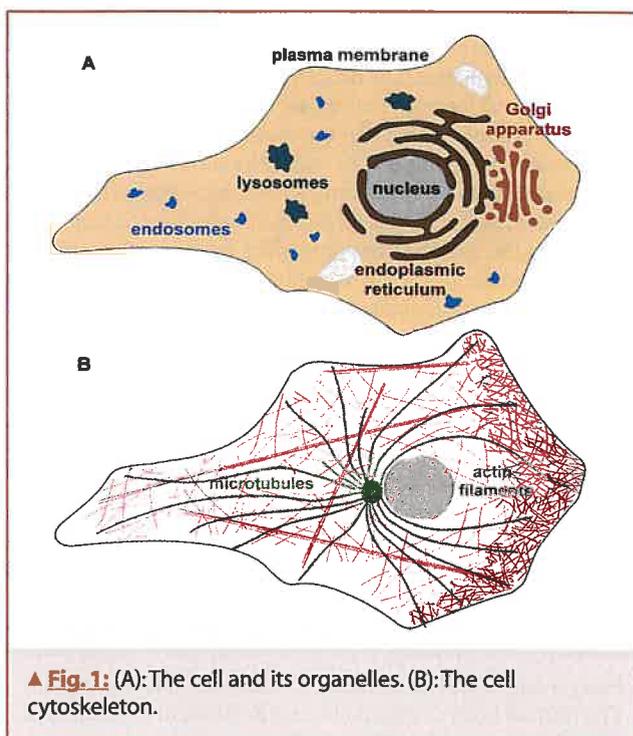
difficulties inherent in introducing a probe into a living cell and manipulating it in a "non invasive" manner. Two principal approaches have been developed. The first approach (passive microrheology without external force application, see box 2) involves studying the spontaneous displacement of intracellular granules [3] or internalized microbeads [4] in order to deduce local rheologic properties. In the second approach (active microrheology, see box 2), magnetic microbeads engulfed by macrophages are submitted to a magnetic force or couple, and their response offers information on the viscoelastic modules of the surrounding medium [5]. However, all these approaches necessitate the used of particular cellular models (specialized cells capable of phagocytosing micron-size objects or containing readily identifiable rigid granules), meaning that current intracellular microrheology techniques cannot be applied to all cell types. Furthermore, while these methods can be used to probe the dynamics of intracellular objects or to apply precise constraints, they are unsuitable for exploring the dynamics of intracellular membranes. Yet these membranes represent 98% of the total membrane surface area of a given cell, forming enclosed aqueous compartments with specialized functions. Macromolecules are intensively trafficked between these different compartments, involving processes of mem-

Let's talk about cells

Every living organism is made of millions of millions of cells. The human body contains about 200 different types of cells which have various functions and life times. For instance, we can mention the blood cells (erythrocytes, leucocytes, ...) that circulate throughout the body via the circulatory system, the endothelial cells that form the lining of all blood vessels, the tissue cells that are the bricks of the organs, the fibroblasts associated with healing wounds and the macrophages able to recognize (and then ingest) foreigners.

The cell is a highly organized small factory (Figure 1A). The nucleus is the head office and contains the DNA which carries the genetic information. The plasma membrane is fencing the cell. The space between the nucleus and the plasma membrane is called the cytoplasm. In the cytoplasm can be found the cell specialized sub-units called organelles. For instance, the endoplasmic reticulum organelle is the production center for proteins and lipids which are then addressed to the sorting center, the Golgi apparatus. Internalization of small materials (<0.1µm) through the cell plasma membrane is called the endocytosis. When larger materials (1µm or more) enter the cell, the process is called the phagocytosis. Organelles known as endosomes (in the case of the endocytosis) or phagosomes (in the case of phagocytosis) conveys the newly ingested materials and delivers them to other organelles where they will be used. Unuseful materials are sent to the organelles called lysosomes which are the cell waste centers.

The cell has a structure which could appear as a mixture of a skeleton and a musculature (Figure 1B). This structure is called cytoskeleton and gives the cell its shape, cohesion, ability to move and internal organization. The cytoskeleton is composed of a dense network of protein filaments. Its most important components are actin filaments and microtubules. Both are involved in shaping the cell. Moreover, actin filaments are essential to the movement generation, enabling the cell to crawl, whereas microtubules are the cell conveyer belts, enabling intracellular transport. This cytoskeleton confers to the cell outstanding mechanical properties. In particular, the cell content appears as a viscoelastic body, meaning it is at the same time a viscous liquid and an elastic solid.



brane fusion, fission and budding, and this is crucial for cell survival. Each membrane compartment possesses a specific lipid and protein composition conferring the mechanical properties required to support these dynamic exchanges. Hence characterizing the mechanical properties of these intracellular membranes appears fundamental. However, while plasma membrane tension and deformation have been widely studied, there has as yet been no way of constraining or deforming intracellular membranes in a controlled manner.

We have developed a new method designed to apply, both locally and non invasively, mechanical constraints inside the living cell. We use anionic magnetic nanoparticles which, thanks to their spontaneous interaction with the outer cell membrane, follow the endocytosis pathway and become concentrated in pre-existing intracellular membrane-bound vesicles known as endosomes. This renders these compartments magnetic, and allows them to be manipulated within the cytoplasmic environment by applying an external magnetic field. A precise constraint can thus be applied to the endosome itself or to its microenvironment. This enables us not only to deform the endosome membrane and thereby to deduce its bending stiffness and resting tension, but also to form small chains of magnetic endosomes whose dynamic behavior in the externally applied magnetic field reflects the intracellular architecture. This is, to our knowledge, the first time one could deform in living cell intracellular organelles. Besides the magnetic endosome appear for now as the unique probe that can be obtained in any living cell to explore the local mechanical properties of the cell intracellular microenvironment. The originality and biological relevance of this magnetic probing method lie in the fact that endocytosis is used by all cells to communicate with their environment.

Endocytosis of magnetic nanoparticles [6]

We first showed that, after adsorbing to the cell membrane, anionic magnetic nanoparticles are trafficked to intracellular compartments via the endocytosis mechanism used by cells to internalize extracellular substances. Figure 2 shows the different steps of nanoparticle endocytosis, as seen under the electron microscope. The electron-dense nanoparticles appear as small black spots. They begin by

attaching to the cell membrane in small aggregates (figure 2a). A portion of the membrane then invaginates (figure 2b) to form a membrane-bound vesicle less than 100 nm in diameter, which moves into the intracellular space (figure 2c). The vesicle then releases its contents into preformed membrane compartments (endosomes), in which the nanoparticles gradually accumulate (figure 2d). Each of these "magnetic endosome" forms an ideal probe for intracellular magnetic micromanipulation.

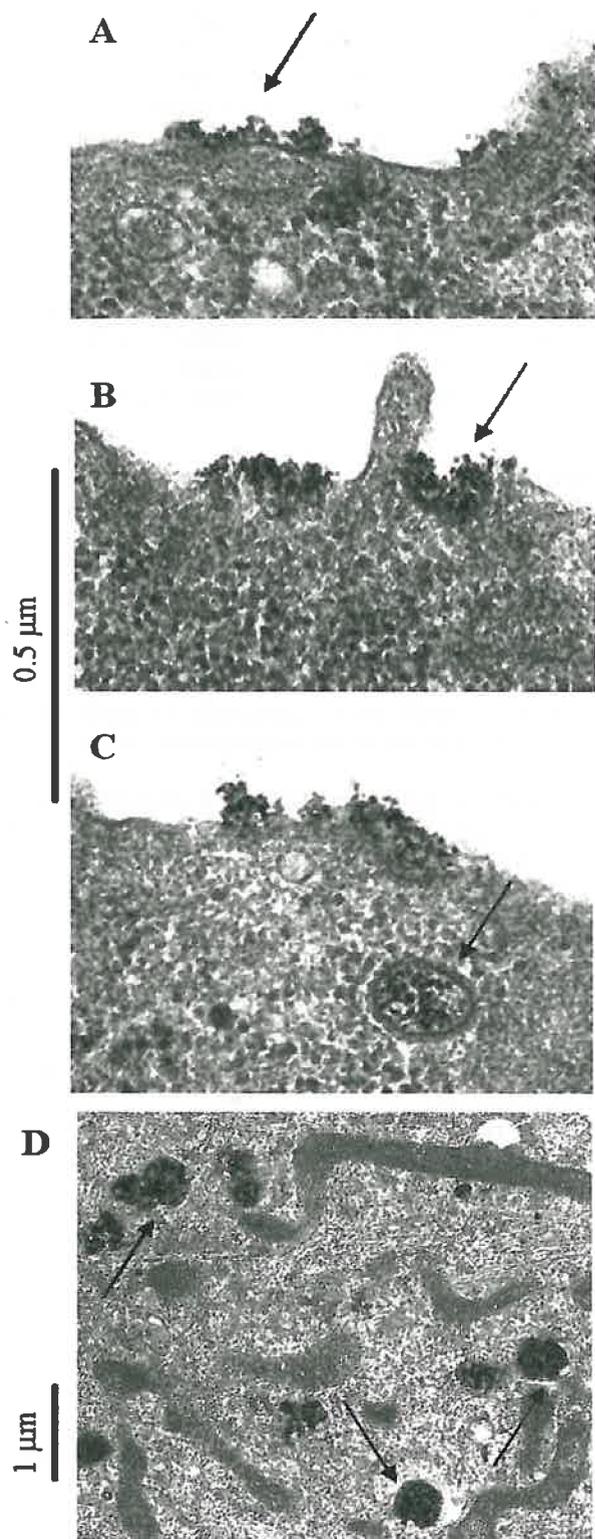
Controlled intracellular membrane deformation [7]

By using this approach we were able to apply controlled constraints to intracellular membranes of a living cell, for the first time to our knowledge. Magnetization of the fluid contents of the endosome deforms the endosome along the direction of the applied field, under the influence of magnetic surface force effects. Only electronic microscopy can reveal the structure and morphology of the membranes of these submicronic compartments. Figure 3a shows nanoparticle-loaded endosomes that have not yet been submitted to a magnetic field. The contours of the endosome are undulated, showing that the membrane is flexible and flaccid. If we could film these endosomes, we would see their membranes agitated by thermal fluctuations and exploring different shape configurations. Under the effect of a uniform magnetic field (see figure 3b), the endosome appears to elongate in the direction of the field, while conserving a wavy, irregular membrane. To quantify this deformation, we averaged a large number of instantaneous shapes, each representing a different state of fluctuation. Figure 3c shows, for several different magnetic field intensities, the contour of the averaged endosome, superposed on the merged image of all the shapes analyzed. In zero field conditions the averaged contour is a circle; in other words the endosome is isotropic. Under a magnetic field, the averaged endosome gradually turns into a prolate ellipsoid whose long axis matches the direction of the magnetic field. The deformation of the endosome membrane is then perfectly described in terms of the reduction in membrane undulations. We were thus able to demonstrate that endosomes appear to be flaccid with significant resting tension (compared to plasma membrane), but low bending stiffness. This important resting tension will probably trigger fusion events, whereas the low bending stiffness should be related to the peculiar protein-lipid composition of endosomal membranes.

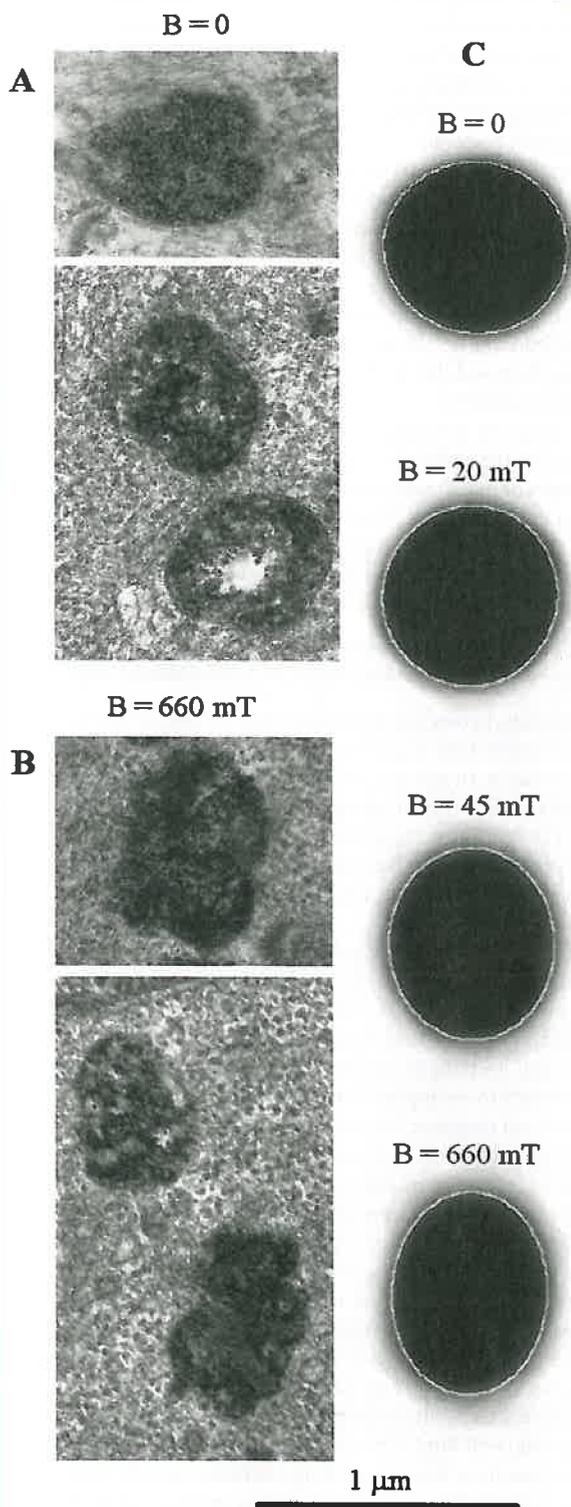
Viscoelastic architecture surrounding endosomes [8]

These magnetic endosomes also interact with one another under the effect of the dipolar magnetic force, forming small cohesive chains inside the cell (figure 4a). We were able to make these chains of magnetic endosomes rotate, and could thereby obtain information on the viscoelastic properties of their microenvironment after developing a new microrheology technique (see box 2). In this way we could map intracellular viscoelasticity within the vicinity of magnetic endosome chains located in various parts of the cell. We found that viscoelasticity in the vicinity of endosome chains situated close to the nucleus resulted from close cooperation between the different networks of cytoskeleton filaments (microtubules and actin filaments): viscoelasticity was high in untreated cells, and fell to a similar extent when one of the filament types was selectively depolymerized. Viscoelasticity in the vicinity of chains located some distance from the nucleus was about one order of magnitude lower and did not depend on the presence of microtubules or actin filaments.

The probed cellular architecture depends closely on the biological nature of the probe. Endosome mobility between the plasma membrane and the perinuclear region is crucial for all recycling and degradation pathways. In keeping with our measurements, mobile endosomes must move in a weakly viscoelastic environment.



▲ **Fig. 2:** Electron micrographs showing (black arrows): (A) adsorption of magnetic nanoparticles (black spots) to the cell outer membrane; (B-C) the first steps of endocytosis: membrane invagination and formation of early vesicles; (D) subsequent formation of micronic intracellular compartments (late endosomes and lysosomes) containing abundant nanoparticles.



▲ **Fig. 3:** Subcellular magnetic micromanipulations. (A): Electron microscopic studies of the undulating nature of the magnetic endosome membrane when no magnetic field is applied. (B): Under the effect of a magnetic field (B), the endosome membrane remains irregular and wavy, while the endosome elongates in the direction of the field. (C): Quantification of the deformation: averaged shape of endosomes at different intensities of applied field B.

features

In contrast, endosome mobility is reduced in the perinuclear region by a dense viscoelastic matrix created by the two subnetworks of actin and microtubules. Both types of filament thus appear to be involved in stabilizing late endosomes and lysosomes in the perinuclear region, thereby facilitating fusion events and regulating the degradation pathway.

These studies are part of broader investigations of local and global cellular responses to mechanical constraints. Much current work is based on applying mechanical constraints to the whole cell or to its membrane, while methods allowing constraints to be applied locally and non invasively inside a living cell are still in their infancy. Our initial experimental micromanipulations of intracellular organelles should lead to a better understanding of intracellular dynamics by offering the possibility to modulate physical parameters that control biological activities:

-Resistance to deformation of membranes of compartments involved in the endocytosis pathway may contribute to controlling exchanges of macromolecules and lipids between the different intracellular compartments. These exchanges indeed require membrane fusion/fission, and budding/fusion of vesicles with the membranes of the different compartments—processes that are dependent on the mechanical properties of the membranes concerned.

Rotational microrheology

In classical rheological approaches the sample is macroscopically perturbed by a controlled constraint or an imposed deformation. In new microrheology techniques developed over the last decade the sample is deformed locally by the displacement of micron-scale probes. These techniques can be used to study the rheological behavior of living cells (volume about 10^5 mm^3). Two complementary approaches have proven particularly useful:

1. "Passive" techniques based on the observation of the Brownian movement of probing beads dispersed in a fluid. Rheological properties are deduced indirectly by applying the fluctuation-dissipation theorem.
2. "Active" techniques use the translational response of micron-scale beads to an applied force. The force is produced either by an external magnetic field (magnetic tweezers) or by a focalized laser (optical tweezers). Our rotational microrheology technique involves the use of an active rotating probe. The use of translated probes encounters problems when the probe is very small: applied magnetic or optical forces proportional to the probe volume must counteract viscoelastic forces that depend only on the probe diameter. In contrast, rotational dynamics depend only on the aspect ratio of the probe.

We first developed local macroscopic probes in translation (magnetic beads 1 mm in diameter) and in rotation (nickel needles 0.5 mm long) in order to compare their dynamics within a linear Maxwell fluid. The use of a rotating probe to deduce the viscoelasticity of the surrounding medium was fully validated in these experiments, and we then used a microscopic probe consisting of chains of magnetic 650nm-diameter particles, in the same viscoelastic fluid. Two types of measurements were made to validate and calibrate the measurement of viscoelasticity at the microscopic scale, namely a global measurement (based on light diffusion) of the frequency response of the particle chains to an oscillating magnetic field; and a local (microscopic) measurement of the response of a chain to the field. It is the second approach that we adopted to mechanically probe the interior of living cells.

Quantification of the spatial organization of actin filaments and microtubules would throw light on the cooperation between these two polymer types and their role in intracellular dynamics. The magnetic endosome can be considered both as a probe inserted within a viscoelastic matrix whose properties reflect local polymer organization, and as an effector interacting with cytoskeleton components through associated molecular motors. Thus, movements of endosomes piloted by an external magnetic field could offer signatures of specific molecular mechanisms.

The magnetic endosome is an innovative tool for probing the properties of intracellular membranes and biological polymers that form the intracellular matrix. This approach may offer answers to questions on the intracellular transport, on the mechanisms underlying exchanges between compartments of the endocytosis pathway, and on the cooperation between cytoskeleton component filaments. ■

About the authors

Jean-Claude Bacri is professor of Physics at the University Paris 7 since 1985 and is head of a research group concerning physical properties of magnetic colloids (ferrofluids). He is one of the most knowledgeable ferrofluid researcher in ferrohydrodynamics.

Florence Gazeau and **Claire Wilhelm** received their PhD in Physics in 1997 and 2002 and are researchers at Centre National de La Recherche Scientifique (CNRS) since 1998 and 2003, respectively. They are developing biomedical applications of magnetic nanoparticles for cell mechanics, cellular imaging (using MRI) and tumor therapy.

Jean-Claude Bacri, Florence Gazeau and **Claire Wilhelm** have joined the group "Physique du Vivant (Physics of Life)" of the laboratory "Matière et Systèmes Complexes" of the University Paris 7 (CNRS, UMR7057).

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