

Chapter 9

Nanobiosciences: New Ideas and Tools for Investigating and Manipulating Living Systems

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Abstract Nanoscience has reached a point today where we can begin to create hybrid objects at the interface between nanomaterials and the life sciences. These objects combine advanced physical properties, viz., optical, mechanical, magnetic, etc., with the remarkable reactivity of biological molecules. In particular, they can be used as probes or micromanipulation tools, from the molecular and cellular scale right up to the scale of organisms. This should bring us new insights into the organisation of living things, and new prospects for diagnosis and therapy in nanomedicine.

The beginning of the twenty-first century was marked by an encounter between two major scientific disciplines: on the one hand, materials science, which studies the elaboration, structure, and properties of matter made up of inert entities, and on the other, the life sciences which seek to understand the function—and indeed the malfunction—of living systems. Materials science traditionally borrows from physics, chemistry, and mechanics, while the life sciences are associated with disciplines like biology, biochemistry, and medicine.

To a large extent, this encounter has been catalysed by the rapid expansion of nanoscience over the past fifteen years. Today, it has become possible to synthesise nanomaterials wherein not only the size (from a few nanometres to a few hundred nanometres), but also the shape and composition can be very tightly controlled. Quantum dots, nanotubes, nanowires, and nanoribbons are but a few examples of nano-objects that can now be prepared down to the finest detail (see Parts I and II of this book). They exhibit unique optical, electric, mechanical, and magnetic properties depending on the materials they are made from. And what is striking is that the nanometric scale is precisely that of the objects making up living systems like proteins, nucleic acids, macromolecular assemblies, viruses, and so on. These biological molecules, while they often manifest less spectacular physical characteristics than nanomaterials, nevertheless exhibit quite remarkable properties of chemical reactivity and specificity. Indeed, these properties, optimised by nature during the long process of evolution, are often impossible to reproduce in synthetic systems.

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Thanks to this convergence of size at the nanometric scale, nanomaterials and biological molecules can be combined to make hybrid objects with quite unique physical, chemical, and biological characteristics. Naturally, this merger between the life sciences and materials science cannot be carried out without raising some difficulties. Building together objects as different as a semiconducting nanowire and a protein involves sophisticated chemical techniques. And exploiting them in a biological or medical context means controlling their reactivity in the living environment. Actually detecting them in complex environments like cells, living tissue, or living organisms is often a considerable challenge, requiring advanced physical methods. Nanobioscience is therefore by its very definition a cross-disciplinary field whose potential can only be realised by bringing together knowhow from a range of backgrounds.

The examples given below, while necessarily a subjective choice in some ways, should serve to illustrate some of the techniques of nanoscience used in biology, and in particular their ability to probe and manipulate living systems with unprecedented sensitivity. However, the contribution of nanoscience is not limited merely to novel techniques. Since they make available new quantitative measurements that were inaccessible to conventional tools, nano-objects can reveal unexpected aspects of the biological world and by doing so call into question some of our preconceptions about the organisation and dynamics of living systems.

9.1 Nano-Objects as Functional Probes for Nanoscale Exploration of Living Systems

One of the most common applications of nano-objects in a living environment is the functional probe. The best example is when the nano-object is used as a sensor in a biological environment. We can thus measure the local characteristics of the environment through changes in the physical properties of the nano-object itself. In many cases, we use changes in the intensity or spectrum of the optical response of a nanoparticle to measure biochemical quantities such as ion concentrations [1], or physical parameters such as the temperature [2] or pressure. Note that these really are local measurements, on the scale of the nano-object, so we can study the biological environment with nanometric resolution. In other cases, a measurement of electrical conductivity is used to detect molecules or biological entities such as viruses through their interaction with the probe [3]. Probes can also be targeted, labelling some particular molecule of interest. Provided they do not perturb the molecules to which they are attached, such probes then serve to locate them within some living sample, to measure their functional activity, or to determine their concentration.

The transformation of a nanomaterial into a functional probe is often a major challenge. To ensure that it has the necessary specificity for chemical detection or targeting, its surface must be modified by grafting reactive biological molecules or chemical groups onto it. Such a functionalisation chemistry is a delicate matter, but it is essential for making this kind of measurement. A detailed description can be found in Sect. 5.4 of Chap. 5, dealing with nanochemistry.

In the case just described where nano-objects are used to probe the environment or to label biomolecules, the role of nanoscience is not just conceptual. However, the physicochemical characteristics of the nano-objects make them choice systems for increasing sensitivity or specificity and thereby carrying out experiments that would be difficult or even impossible using conventional methods. An important and even emblematic example illustrating the impact of nano-objects in the life sciences is provided by semiconductor nanocrystals known as quantum dots [4] (see Sect. 2.3 of Chap. 2 on nanophysics). These are nanoparticles with sizes typically in the range 2–20 nm. Not only can their dimensions be adjusted to within a few atomic layers, but their shape, e.g., spherical or threadlike, can also be very closely specified. Since they are made from a semiconducting material such as cadmium selenide, these nanoparticles have fluorescence properties. On the nanoscale, the optical response is determined by quantum effects. So for example, the fluorescence spectrum will depend on the size of the nanoparticle, shifting from the blue toward the red as the radius increases.

Moreover, it has been known for fifteen or more years how to functionalise quantum dots so as to make them compatible with biological media. They can thus be used as multicolour fluorescent probes, much brighter and more photostable than organic markers. The quantum dots, which act like little nanoscale light bulbs, provide the possibility of ultrasensitive detection and have largely contributed to the development of a new field of study: the imaging of single proteins in living media [5].

9.2 Tracking Single Biomolecules in the Cell

On the molecular scale, objects move in a very different way to what we see in our own macroscopic world. Under the effects of thermal agitation and diffusion, molecules are in a permanent state of motion. In order to understand the functional architecture of the cell, it is essential to be able to describe these motions and analyse their role in molecular interactions. One particularly fruitful approach is to track the motions of individually labelled proteins.

When tracking molecular motions in this way, the signal from the probe attached to the relevant protein must be recorded in a sequence of images. The experimental challenge is to actually detect the signal from a single marker against the background noise produced within a living sample. The use of bright probes like nanoparticles is then often a decisive advantage. There are two important features of such measurements. On the one hand, the position of a molecule can be determined with an accuracy that depends only on the signal-to-noise ratio and can in practice reach about 10 nm. Plotting the trajectory of a protein thus amounts to exploring the cell medium on a nanometric scale. On the other hand, the motion of biological molecules is governed partly by diffusion and partly by their interactions with molecular partners. In many cases, one thus has direct access to the kinetics of these interactions in the context of the living cell.

A particularly striking illustration of these experimental techniques concerns glycine receptors, membrane proteins involved in the transmission of inhibitory signals in neuronal cells (see Fig. 9.1a, b, and c). To track their motion, a quantum dot is coupled with an antibody which recognises an extracellular domain of the protein [6]. When their trajectories are recorded, these proteins are found to be extremely mobile, much more than one would expect. They diffuse in the neuronal membrane and end up in the synapses, where they interact with molecular partners. However, these are transient interactions and the receptors subsequently escape from the synapses to resume their exploration of the membrane. Such observations raise a fundamental question: given that the synapses constitute key structures in the connections between nerve cells, playing an important role in the fundamental processes of learning and memory, how can they maintain their functional stability when the molecular components making them up undergo a permanent process of association and dissociation?

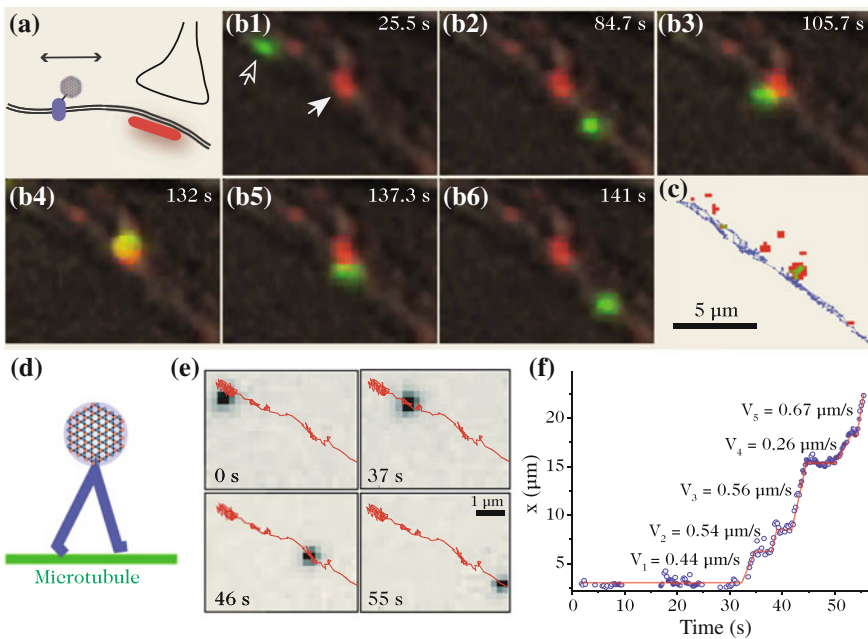


Fig. 9.1 Single-molecule visualisation using nanoprobes. **a** Diagram of a receptor (blue) labelled by a quantum dot moving in the neuronal membrane. The receptor can stabilise at the synapse by interacting with scaffold proteins (red). **b1–b6** Image sequence showing the motion of a single receptor (green) relative to a synaptic site (red). **c** Reconstruction of the trajectory (blue). Green points correspond to the part of the trajectory during which the receptor is trapped at the synapse. **d** Diagram of the motor protein kinesin, labelled by a quantum dot and moving along a microtubule filament. **e** Image sequence showing the motion of the motor in the cytoplasm of a living cell. **f** Analysis of the motion along the trajectory, showing alternating phases of directed motion and pauses. **b–d** Adapted from [7], © 2007 Elsevier, **e** and **f** adapted from [8], © 2006 American Chemical Society, reproduced with permission

Tracking measurements are not restricted to membrane proteins. Single molecules can also be monitored in the cytoplasm inside living cells. A particularly fascinating case concerns motor proteins. These are proteins that can transform chemical energy in the form of adenosine triphosphate (ATP) into mechanical energy. Such motors operate with extraordinary efficiency, close to 100%, well beyond what can be reproduced by chemical synthesis. For example, kinesin, a molecular motor which moves along the microtubule cytoskeleton, is involved in many intracellular transport mechanisms (see Fig. 9.1d). By marking it with a quantum dot, its motion can be tracked within the cell [8]. It is observed to move in a directed manner along a microtubule at a speed of about $0.5 \mu\text{m/s}$. However, this displacement phase lasts only a certain time. After a fraction of a second, the motor detaches itself from the microtubule and diffuses freely for a while before reconnecting with the cytoskeleton (see Fig. 9.1e and f).

While fluorescence imaging methods remain the most common, there are other detection modes for imaging single molecules. It is in fact a feature of nanobioscience that the biological systems investigated and the questions raised regularly require the design of novel nanoprobe and the development of ever more elaborate measurement tools. As an example, gold nanoparticles can be used for single-molecule detection. These nanoparticles do not fluoresce but are nevertheless effective light scatterers, with spectral properties that can be modulated by changing their size and shape (see Sect. 5.5.1 of Chap. 5). To increase sensitivity and stand out above the background signal from other cell structures, an elegant approach is to combine the scattering properties of gold nanoparticles with a photoinduced modulation of their temperature. This photothermal method allows a highly specific measurement and accurate tracking of the object to which the gold nanoparticle is attached [9].

Single-molecule experiments provide direct access to the many specificities of biological media, something that would be difficult to apprehend from collective measurements involving molecular ensembles. To begin with, the cell environment turns out to be extremely inhomogeneous and in permanent evolution. Moreover, the interactions between molecular partners are governed by stochastic rather than deterministic processes. This leads to considerable variability in molecular behaviour. It is largely an open question as to how cells can function so effectively despite, or perhaps thanks to, this permanent molecular noise, and this is an active subject of research today.

Quite generally, tracking a molecule in a cell and measuring the kinetics of its interactions with molecular partners is an efficient way to understand the reaction–diffusion processes governing the reactivity of molecular species. Thanks to nano-objects and the sensitivity of the observations they make possible, biochemical measurements can be carried out *in situ* in the cell. This represents a considerable advantage over *in vitro* measurements, where it is often difficult to reproduce the heterogeneity, size constraints, and molecular crowding of cell environments.

9.3 Manipulating Living Systems on the Molecular and Cellular Scales

Nano-objects are not just exceptional tools for observing living systems. They can also be used to manipulate and perturb biological systems on the molecular and cellular scales. To do this, a whole range of micromanipulation tools have been developed over the past twenty years [10]. Exploiting the optical or magnetic properties of nano-objects, forces can be applied on a scale from a fraction of a piconewton to several tens of nanonewtons. In this way, one can probe the relationship between mechanical properties and biological activity at the molecular level, or control the events that trigger cell processes.

The *in vitro* micromanipulation of biological molecules can be illustrated by the example of optical tweezers, introduced in Sect. 2.4 of Chap. 2 on nanophysics. A light intensity gradient is created on the scale of the wavelength (of the order of $1\ \mu\text{m}$, see Fig. 9.2a) by strongly focusing a laser in an aqueous medium. Particles with sizes in the range 100–1 000 nm can be trapped near the focal point. When these particles are functionalised with biological molecules, we may also apply forces to these molecules in order to study their mechanical response. For example, by pulling on a DNA molecule grafted at the same time onto a glass surface and onto the trapped particle, we may measure the elasticity of DNA (see Fig. 9.2a left).

This is also a useful way of studying interactions between DNA and proteins [11]. For example, RNA polymerase, the enzyme that synthesises RNA from nucleotides, acts locally on its substrate by separating the double helix structure there. In doing so, it modifies the mechanical properties of the DNA and these modifications can be detected through the motion of the particle, which thus acts here as a force transducer (see Fig. 9.2a right). The sensitivity of these measurements is so good that we may now detect effects on the scale of a single base pair, i.e., at distances of barely 0.3 nm.

Micromanipulation *in vitro* is not restricted to DNA–protein interactions. Using optical or magnetic tweezers, or an atomic force microscope, we can study the properties of many systems, including kinesins and myosins, molecular motors which act on microtubules and actin filaments, respectively (see Fig. 9.2b–d). Globally speaking, these experiments reveal the complex relationship between the mechanics of molecular systems and their biochemical reactivity.

The response to mechanical stresses is a central issue in cell biology. Using nanoparticle micromanipulation tools, the effects of mechanical forces can be very precisely measured. Hence, by pulling on a nanoparticle attached to the cell membrane, we can study mechanical transduction mechanisms, i.e., those biological processes whereby the cell detects mechanical perturbation and responds.

Nano-objects can be used to control cell signalling, without necessarily inducing any mechanical perturbation. For example, small paramagnetic nanoparticles ($\sim 30\text{ nm}$) can be coupled specifically to membrane receptors. Under the effect of a constant magnetic field, these nanoparticles acquire a magnetic dipole. The dipole–dipole interaction induces aggregation of the particles (see Fig. 9.2b), and thus also of the receptors to which they are attached, thereby activating signalling channels

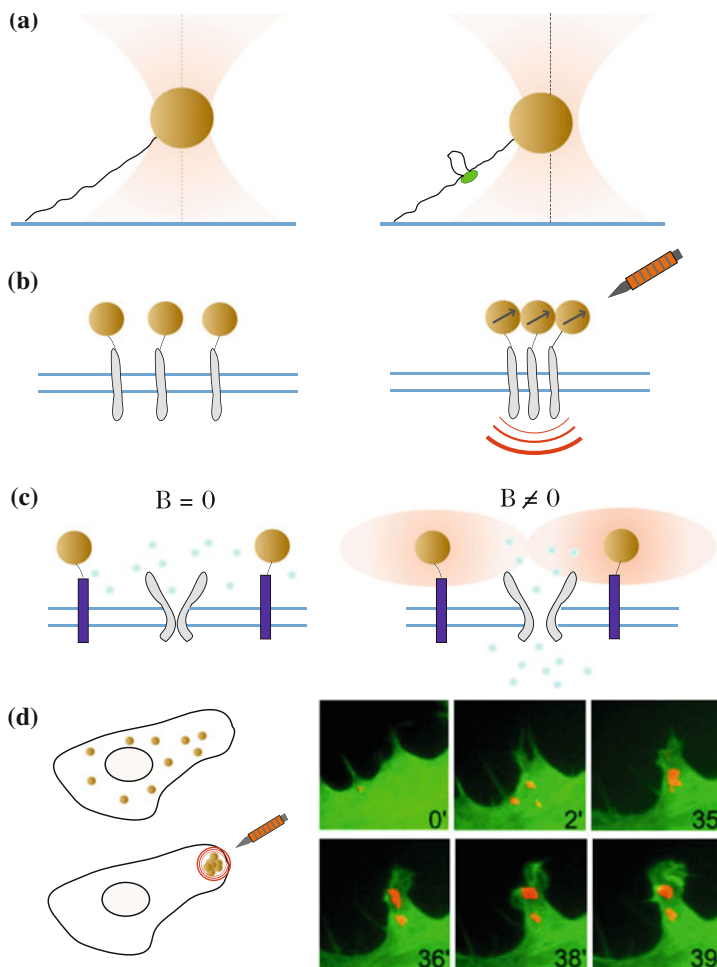


Fig. 9.2 Examples of micromanipulation on the molecular and cellular scale. **a** *Left* A focused laser beam (*pink*) traps a nanoparticle. The latter can be coupled to a DNA molecule which is itself attached to a glass surface and which can thus be put under tension. *Right* When a protein (*green*) interacts mechanically with the DNA, the motion of the nanoparticle can be used to determine the forces generated. **b** *Left* Magnetic nanoparticles are coupled with membrane proteins. *Right* In the presence of a static field, these nanoparticles interact and the aggregation thereby induced triggers a biochemical signal. **c** *Left* Magnetic nanoparticles are coupled with membrane proteins. *Right* A radio frequency magnetic field B causes the nanoparticles to heat up, causing the heat-sensitive membrane channels to open. **d** *Left* Nanoparticles functionalised with signalling proteins are injected into a cell. By means of a magnetic force, they are then localised in a particular region of the cell where their accumulation triggers a response from the cell. *Right* Image sequence illustrating this process. Nanobeads (*red*) are carried to the cell membrane where they induce the formation of protrusions. The time is indicated in minutes. Adapted from [12], © 2013 Nature Publishing Group

[13]. Alternatively, a radio frequency magnetic field can be applied instead of a static one (see Fig. 9.2c). The effect is to heat the nanoparticles and this local temperature rise can be exploited to activate heat-sensitive receptors which themselves trigger cascades of intracellular signals [14]. Another approach is to introduce nanoparticles functionalised with signalling proteins directly into the cytoplasm (see Fig. 9.2d). Using magnetic tweezers, they can then be localised in specific regions of the cell, whence it becomes possible to measure the response of the cell to a biochemical perturbation in different local cell environments [12].

Quite generally, whether it be through mechanical effects or direct activation of signalling channels, nano-objects provide a way to apply spatially and temporally controlled perturbations. They are thus invaluable tools for the development of a new discipline, systems biology, which seeks to understand the integrated properties of cell systems. By analogy with the study of electrical circuits, the aim is to measure the cell response (output) to a controlled stimulation (input). Determining the input–output relation is an important step in figuring out the functioning of the molecular circuits which handle information transfer within the cell.

9.4 Nano-Objects for Diagnosis and Therapy: The Prospect of Nanomedicine

Applications of nano-objects in the life sciences are not limited to molecular and cell biology. Indeed, more and more uses are being found for them in the field of medicine. These applications, described in more detail in Chap. 10, are today gathered under the heading of nanomedicine.

The sensitivity of diagnostic imaging methods can thus be improved by exploiting the special physical properties of nanoparticles. For example, we know today how to synthesise quantum dots or carbon nanotubes emitting in the near-infrared [15]. In this spectral range, biological media are relatively transparent, so such objects can be used to image tissues in depth. Another possibility is to use magnetic nanoparticles as contrast agents in magnetic resonance imaging (MRI) [16]. In most cases, nanoparticles are functionalised in such a way that they target particular regions of the organism such as a tumour or inflammation, while they are carefully passivated to ensure that they do not interact with healthy tissues. Achieving high specificity in the recognition mechanisms of the nano-objects, a process that is already complex in cell samples, often represents a much more considerable challenge in the medical context, where the nano-objects have to circulate in the vascular system.

Medical applications of nano-objects concern not only detection and diagnosis, but also therapy. One of the most attractive prospects in nanomedicine is the use of nanosystems as multipurpose platforms, combining a contrast mechanism with targeting and therapeutic functions. This approach, known as theranostics, aims to combine diagnosis and therapy. It forms a natural component in the development of personalised medicine, which seeks to customise treatments. The therapeutic action

can be implemented in a targeted way, with localised drug release (see Chap. 11), thereby improving the efficiency of the treatment and reducing the side-effects of the drugs on healthy cells. Physical mechanisms can also be exploited in thermal therapy, where nanoparticles are heated up by irradiating them with light [17] or, as above, by application of a radio frequency magnetic field [18]. The rise in temperature can kill targeted cells, and in particular, tumour cells, in which the nanoparticles have accumulated.

Despite the many positive aspects of nanobioscience, it also raises some questions and a certain apprehension. For the moment, we do not have a good understanding of the mechanisms governing the interactions between nanoparticles and living organisms, nor the degradation of nanoparticles in such a context. Given the growing number of applications and the high economic stakes, it is crucial to assess the potential toxicity of nano-objects and their general effects on health (see Chap. 12). The high chemical reactivity of nanoparticles is also likely to have environmental consequences (see Chap. 13). Whatever the situation, it is essential to be able to precisely specify the dose beyond which exposure becomes a risk. On the other hand, this is often a delicate matter given the sensitive dependence of toxic effects on physico-chemical parameters such as size, shape, composition, and surface characteristics of nanoparticles, not to mention the type of exposure.

9.5 Conclusion and Prospects

Nanoscience has become an important factor in the development of both fundamental and applied biomedical research. The potential of nanoscience in the life sciences was stressed [19] during the launch in the United States of the project known as *Brain Research through Advancing Innovative Neurotechnologies* (BRAIN) in the spring of 2013. This project, announced at the same time as the *Human Brain Project* in the European Community, commits a considerable investment to improving our understanding of neuronal mechanisms in the human brain. To achieve this, a great many tools will be needed to visualise and monitor neuronal activity, either optically or electrically. Eminent research scientists in the field have pointed out that nanoscience will have a key role to play, thanks to its capacity to design systems on the molecular or subcellular scale, interfaced with biological systems while communicating with the macroscopic world [19].

To end, we may quote Sydney Brenner, Nobel Prize for Medicine in 2002 and one of the founding fathers of molecular biology and developmental genetics: “Progress in science depends on new techniques, new discoveries and new ideas, probably in that order.” This introduction to nanobioscience together with the next four chapters is an invitation to discover the rich panoply of innovative techniques available today for researchers and engineers in a range of disciplines, describing some of the results already obtained here. It is our hope that the reader will find a source of inspiration for the new ideas which will one day lie at the very heart of many areas of science.

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