

SCANNING NANOPORE MICROSCOPY

A new tool for cell signalling research

The combination of a nanopore and an atomic force microscope allows stochastic sensing of secreted molecules and the activity of ion channels in arbitrary locations both inside and outside of a cell.

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Cell signalling is a fundamental process in all organisms and is the primary mechanism for coordinating cell activities in living tissue. The secretion of signalling molecules — primarily small proteins and ions — from donor cells triggers a biochemical response in the acceptor cells, allowing them to react to changes in their microenvironment. It is well established that many of the common diseases, including cancer, diabetes and autoimmune diseases, are associated with erroneous cell signalling interactions. Hence, our ability to directly sense and quantify signalling molecules from individual cells holds paramount fundamental and practical importance. To date, however, the arsenal of experimental techniques for localized, direct sensing of secreted biomolecules with high sensitivity is limited. Patch-clamping, developed in the late 1970s by Nobel laureates Erwin Neher and Bert Sakmann, made it possible to record the activity of individual ion channels embedded in the cell's membrane, consequently transforming the neurosciences. However, this classical technique has not permitted sensing of individual secreted macromolecules, such as proteins or nucleic acids. In the late 1990s, nanopore biosensing emerged as a new method for sensing of unlabelled single RNA and DNA molecules^{1–3} and was later expanded to protein sensing. Now, writing in *Nature Nanotechnology*, Aramesh and colleagues report a clever marriage between nanopore biosensors and a scanning atomic force microscope (AFM) to sense secreted biomolecules while scanning the cell membrane, thereby mapping its secretory activity⁴.

Developed in the 1980s, AFM utilizes a highly flexible cantilever and force-generating piezo transducers to poke soft objects, such as the membrane of a live cell, while measuring the deflection of the cantilever tip to report the amount of force applied at any given point⁵. Scanning of the AFM tip over the sample's three-dimensional landscape is used to create a force-based image of the object with a spatial resolution below 10 nm. Nanopore sensing

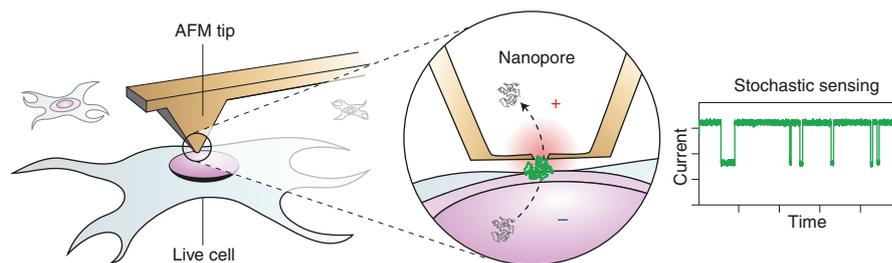


Fig. 1 | Schematic of the AFM/nanopore tip in operation. A nanopore probe made at the centre of a flattened AFM tip enables in situ stochastic sensing of secreted biomolecules when used to scan the surface of live cells.

is an entirely different method. Developed primarily for single-DNA-molecule sequencing⁶, it relies on the application of electrophoretic forces in order to thread and progressively translocate long biopolymers through a protein pore or a nanoscale aperture formed in an ultrathin insulating membrane. As the DNA molecules translocate through the nanopore, a time-dependent measurement of the ion current flowing through the nanopore reports on the biomolecule local structure or nucleotide composition. AFM and nanopore sensing are based on different physical principles; however, they both operate at the nanoscale level, and, importantly, can be used for the sensing of unlabelled biomolecules. Hence, the combination of the two methods, demonstrated in the report by Aramesh and colleagues, is very powerful, particularly for studying cell-secreted biomolecules in situ.

To realize a combined AFM/nanopore instrument, the researchers utilize advanced ion-milling technology to flatten the apex of the AFM tip, forming a small nanopore of 5 to 20 nm at the centre of the flattened membrane (Fig. 1). The scanning AFM tip, now equipped with a nanopore sensor, enables monitoring of live-cell activity using simultaneous force and ion current measurements. An extracellular recording from a single live fibroblast cell reveals two types of time-dependent ion current events, exhibiting either long and deep, or shorter and shallower events. The two

event types are attributed to either secreted fibronectin proteins or potential changes in the vicinity of the cell membrane, most probably due to ion channel activity. Interestingly, recordings from fibronectin-knockout mouse embryonic fibroblast cells display a much-reduced occurrence of the long event type, without a significant change in the occurrence of shorter events. The shorter events, which are attributed to ion channel clusters localized in the cell, can be suppressed when imaging CRISPR/Cas9 knockout HEK293 cells for the Piezo1 ion channels. On the other hand, transiently transfected HEK293 cells with overexpressing Piezo1 channels exhibit a five times larger population of short events, further supporting the correlation of these events to the cell's ion channel activity. Moreover, the ability to scan with the AFM/nanopore tip facilitates the creation of spatially resolved heat maps of the membrane of a living cell according to its ion current activity.

In addition to its ability to scan and localize the nanopore at different positions across the cell, the AFM tip can also be used to apply desired forces locally. This latter feature is utilized to perform intracellular recording of the cell's activity. The authors show that applying forces in the range of 150–250 nN is enough to penetrate through the HEK cell membrane, without penetrating through the nucleolus. Current leakage through the outer cell membrane

is sufficiently high to permit ion current recording in the cytoplasm, as one of the nanopore electrodes is placed in the extracellular environment. Interestingly, placing the nanopore near the surface of the nuclear membrane results in a large number of translocation events, presumably from molecules and objects coming from the nucleolus. This direct measurement of molecules secreted from the cell's nucleolus beautifully illustrates the ability of the combined scanning AFM/nanopore tool to temporally resolve biomolecule secretion events and map their locations in a live cell.

Understanding the biomolecular mechanisms associated with cell signalling would undoubtedly benefit from the

development of new techniques able to resolve individual secretion events in live cells. A nanopore scanning microscope is an important step in this direction. Although many challenges remain ahead, for example, significantly increasing the sensor's ability to identify varying proteins with high efficiency and specificity, the work done by Aramesh and colleagues — that is, augmenting the nanopore capability from a stationary sensor to a movable nanodevice that can be selectively positioned and made to operate near and inside single live cells — is a noticeable leap. □

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