Labeled proteins in whole cells and nanoparticles in liquid studied with scanning transmission electron microscopy

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1. INTRODUCTION

Electron microscopy mostly involves the imaging of solid samples with (sub-)nano scale resolution in a vacuum environment. However, for many research questions it would be highly desirable to be able to study the samples in a more functional environment, such as in gas or in liquid. New approaches were developed in the past decade to study nanomaterials and biological samples (e.g. proteins, DNA, cells) in liquid [1]. We have used scanning transmission electron microscopy (STEM) to image specimens in a micrometers thick liquid layer [2]. A specimen, for example, an eukaryotic cell, or an assembly of nanoparticles, was placed in a microfluidic chamber with thin windows of silicon nitride (SiN), see Fig. 1A. This so-called Liquid STEM approach is capable of resolving nanoscale objects of high atomic number (Z) in low-Z liquids, resulting from the Z contrast of STEM. The spatial resolution depends on the microscope settings, the used materials, the location of the nanomaterials in the liquid, and the liquid thickness. For example, gold nanoparticles can be imaged with a resolution as high as 1 nm on top of a liquid layer of 4 micrometers thickness [3]. The same principle of STEM detection can be used for environmental scanning electron microscopy (ESEM), whereby a thin water layer is maintained in equilibrium with saturated water vapor (Fig. 1B) [4], as was introduced by Bogner et al. [5] .

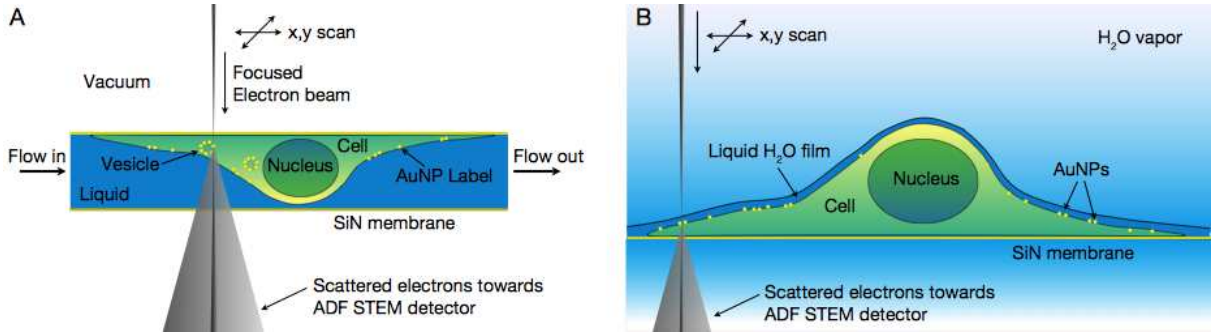

Figure 1. Principles of Liquid STEM. An eukaryotic cell is grown on a supporting SiN membrane. Proteins labeled with gold nanoparticles (AuNPs) reside in the plasma membrane. Imaging is done by scanning a focused electron beam over the cell. Transmitted electrons are recorded with the STEM detector located beneath the sample. (A) The cell is fully enclosed in a microfluidic chamber with two SiN windows for STEM. (B) The cell is maintained in a saturated water vapor atmosphere, while a thin layer of water covers the cell for Liquid STEM using ESEM. Reprinted with permission from [6].

2. RESULTS

2.1 Experimental conditions

Lung cancer cells of the type A549 were cultured on microchips with SiN membrane windows. The cells were incubated for 5 min at room temperature with a label for the epidermal growth facto receptor (EGFR) comprising of a 12 nm-diameter gold nanoparticle conjugated to epidermal growth factor (EGF) via a streptavidin-biotin bond. Cells were then fixed with glutaraldehyde, and rinsed with water. A microchip with cells was placed on the cooled stage of the electron microscope (ESEM, Quanta 400 FEG, FEI). The cells were continuously kept under a thin water film (see Fig. 1B) by regulating the stage temperature to 3° C and adjusting a chamber pressure between 720 and 720 Pa. The cells with AuNPs imaged with the STEM detector using a probe current of 0.5 nA, a beam energy of 30 keV, and a pixel-dwell time of 100 µs.

2.2 EGFR locations determined in whole cells in liquid using ESEM

Intact lung cancer cells were studied in a thin liquid layer using ESEM [4]. Fig. 2A shows and overview dark field ESEM-STEM image containing three cells. High spatial resolution was obtained in thin regions at the edge of the cell, which was impossible in the thicker cellular regions. Fig. 2B shows an image exhibiting a spatial resolution of 3 nm. Individual AuNPs are visible indicating the positions of EGFRs. EGFRs are known to form dimers or larger clusters upon activation by EGF binding. Indeed, a number of dimers and some clusters are recognized. A total of 15 whole cells were studied with ESEM-STEM [4]. The positions of a total of 1411 labels were automatically determined, and then used to calculate the distances between the labels in each image. A statistical analysis of these distances revealed a most probably center-to-center distance of the labels of 19 nm, consistent with a molecular model of the EGFR dimer with bound AuNPs.

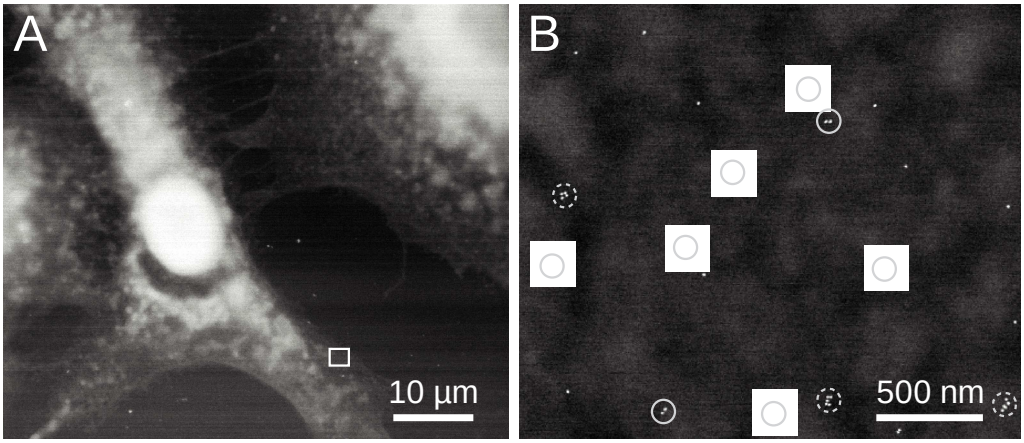

Figure 2. ESEM-STEM of a whole fixed A549 cancer cell in hydrated state. (A) Overview dark field ESEM-STEM overview image showing the contours of three cells in grey, and the thicker cellular regions in white. With permission [4]. (B) High resolution recorded at the location of the rectangle in A showing individual AuNPs, dimers (circles), and larger clusters (dashed circles).

2.3 HER2 stoichiometry studied with correlative fluorescence microscopy and ESEM-STEM

We have used quantum dots (QDs) as specific labels in recent experiments. QDs, fluorescent labels of high-Z materials, were used for correlative fluorescence microscopy and ESEM-STEM. The stoichiometric distribution HER2 was studied in breast cancer cells. HER2 dimerization was found to differ between specific functional regions of the cell membrane.

2.4 Movement and chemical processes of nanoparticles explored with Liquid STEM

The behavior of nanoparticles in liquid was studied using a microfluidic chamber for Liquid STEM at 200 keV beam energy. Various interesting effects were discovered. Most importantly, AuNPs were found to move three orders of magnitude slower than for movement in a bulk liquid. Depending on the pH and salt concentration, AuNPs aggregated, moved away or dissolved. It was also possible to grow gold dendrites. Experiments with silica nanoparticles revealed that nanoscale structures could be written on the SiN membrane, and that the scanning electron beam could be used to change the shapes of the nanoparticles.

3. CONCLUSION

The individual locations of labeled proteins can be determined within the context of an intact cell in liquid using nanoparticle labels and STEM. The unique information can be used to study stoichiometric distributions of protein complexes at the single molecule level. Liquid STEM presents a new method to study nanoscale processes in liquid, and several unexpected phenomena were observed.

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