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Electron Microscopy of Single Cells in Liquid for Stoichiometric Analysis of Transmembrane Proteins

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The imaging of biological systems in liquid environments with electron microscopy (EM) can provide unique information about cellular functions at the molecular scale. Conventional EM studies solid samples in vacuum, which is incompatible with the native, hydrated state of delicate biological systems, such as eukaryotic cells. Innovative techniques bridge this gap and now offer the possibility to study samples in liquids with EM [1]. Here we present an overview of three different methods that allow for EM of liquid samples with nanometer spatial resolution [2, 3]:

1. A microfluidic chamber, formed by two microchips with electron transparent windows, protects the sample from the vacuum during scanning transmission EM (STEM) (**Fig. 1A**). The microfluidic chamber can be coupled to an external pump providing a liquid flow. It is also possible to create bubbles by inducing a high electron dose to the sample and enhance the contrast due to a reduced liquid layer minimizing electron scattering by the solvent.
2. In environmental scanning EM (ESEM) the cells can be covered with a thin liquid layer in a vapor environment (**Fig. 1B**). The thickness of the liquid layer is controlled by adjusting the environmental pressure and temperature within the vacuum chamber.
3. The hydrated sample can also be encapsulated by an electron transparent membrane (e.g., graphene) and studied with conventional SEM, TEM or STEM (**Fig. 1C**). Thereby, the amount of the covering liquid is reduced to a minimum, allowing for high resolution imaging without the need for dedicated specimen holders or environmental chambers. The presence of liquid can be confirmed by inducing bubbles with the electron beam (**Fig. 2A**).

All three described methods allow for detailed analyses of numerous biological samples, without the necessity for time-consuming preparation steps like embedding or sectioning. Moreover, the formation of associated (drying) artefacts is markedly reduced. In the case of eukaryotic cells it furthermore enables the quantification and stoichiometric analysis of transmembrane proteins in the intact membrane by using specific labels with a high contrast (**Fig. 2A, B, C**). This has a high relevance for several fields of biological research addressing, e.g., ion channels or growth factor receptors. In cancer research, for example, the approach can be applied to study the direct interaction of anti-cancer drugs such as trastuzumab with HER2 [4]. With the usage of correlative fluorescence microscopy, the obtained EM results can be related to certain cellular subtypes, such as cancer stem cells, and even to certain cellular regions, e.g., membrane ruffles. Accordingly, the imaging of biological samples in their native, hydrated state, with EM is a powerful tool for life sciences [5].

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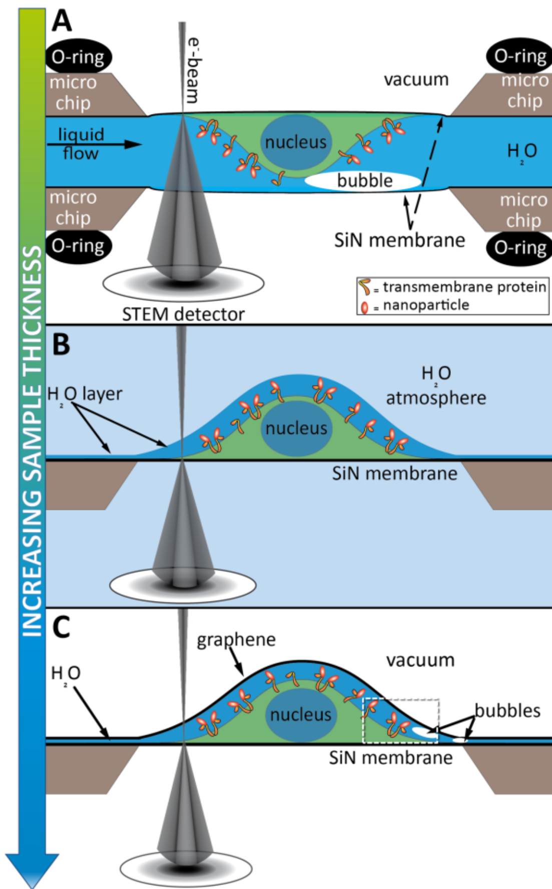
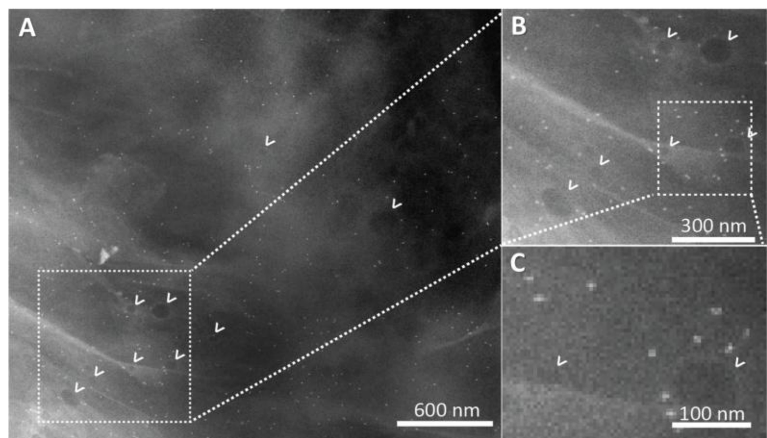


◀ **Figure 1.** Schemes depicting three methods for imaging nanoparticle-labeled transmembrane proteins on whole eukaryotic cells in water, arranged in way indicating their potential to analyze samples of increasing thickness. **A)** Cell positioned in a microfluidic chamber, formed by two microchips with a silicon nitride (SiN) membrane, as used in STEM studies. **B)** Cell on a supporting SiN membrane, covered by a thin water layer as used in ESEM. **C)** Cell covered by a layer of water and encapsulated by a thin sheet of graphene. This method can be used in SEM and (S)TEM. The dashed box symbolically indicates the cellular region of which the STEM image displayed in Figure 2) was taken.

▼ **Figure 2.** STEM image of a eukaryotic cell covered by a layer of liquid and encapsulated by a thin sheet of graphene. **A)** The flat regions of the cell appear gray, thicker cellular regions light gray. Please notice that the plasma membrane remained intact. Particular transmembrane proteins are specifically labeled by nanoparticles and clearly visible as bright spots. The presence of water is confirmed by beam-induced bubbles (arrow heads). The dashed boxes indicate the enlarged image sections in **B)** and **C)**, respectively.



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