

Graphene Enclosure Facilitates Single-Molecule Analysis of ErbB2 Receptors in Intact, Hydrated Eukaryotic Cells by Electron Microscopy

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Although membrane receptors mediate important cellular functions, such as cell growth, division or cell death, techniques for the analysis of their distribution and assembly in the intact cell membrane of fully hydrated cells are limited. For the visual examination of molecular structures, electron microscopy (EM) represents the gold standard. Yet, conventional high resolution EM is based on the investigation of thin, solid sections of cells and typically does not reveal the membrane proteins in intact cells. Light microscopy, on the other hand, does not provide sufficient spatial resolution to resolve the subunits of protein complexes as needed to elucidate their functional state. A new approach is presented by liquid-phase electron microscopy [1]. In this study, we introduced a graphene liquid-enclosure to study the assembly of the growth factor receptor ErbB2 in intact, hydrated breast cancer cells by scanning EM (SEM) and scanning transmission EM (STEM). ErbB2 is overexpressed in about 30% of all breast cancer cases and targeted by the antibody trastuzumab [2]. The high number of primary and acquired drug resistance (~ 70%) underlines the need for a reliable biomarker in the clinic, as well as a better understanding of underlying molecular mechanisms [3].

We used mono- and bilayer graphene to enclose hydrated SKBR3 breast cancer cells grown on silicon microchips with silicon nitride membrane windows (Fig. 1a). ErbB2 proteins were labeled with quantum dot (QD) nanoparticles bound to specific anti-ErbB2 Affibodies. The cells were subjected to quantitative analysis with correlative light microscopy and high resolution EM. Cellular regions with high expression of ErbB2 emitted a red fluorescence at 655 nm (Fig. 1b). Inspection of the samples in SEM (10 kV) provided information about the integrity of the graphene enclosure in the area of interest (Fig. 1c). The subsequent investigation of the graphene covered regions with STEM (200 kV) provided a resolution of < 3 nm and allowed for identification of single, paired and clustered QDs (Fig. 1d-f). Using this microscopy approach, we analysed the QD distribution in two different cellular regions, namely cell edges (CE) and cell-cell-connections (CC), compared to the background (BG). Statistical analysis of the radial distances between two labels revealed a high number of ErbB2 pairs in CE and CC with a median distance of about 20 nm which we interpreted based on our recent findings as functionally active homodimers, compared to the random distribution of BG [4] (data not shown). The CCs, which can facilitate intercellular communication by transferring membrane parts between cells [5], show the highest number of ErbB2 homodimers, suggesting activation of down-stream signalling pathways in these structures.

In summary, we utilized a graphene enclosure of hydrated cells to analyse the distribution of ErbB2 membrane receptors with high resolution STEM. We found a considerable increase in the number of homodimers on cell-cell-connections, indicating a participation of ErbB2 receptors in inter-cellular communication in ErbB2 overexpressing breast cancer. Besides this novel finding, our method provides

a versatile technique for studying distribution patterns of membrane proteins in eukaryotic cells at the single cell *and* single molecule level [4].

References:

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 [7] We thank Justus Hermannsdörfer, Ulrike Korf, and Diana Peckys for research and discussions, and Eduard Arzt for his support through INM. The Research was in part supported by the Leibniz Competition 2014. R.S.W. acknowledges a Research Fellowship from St. John's College, Cambridge and a Marie Skłodowska-Curie Individual Fellowship (Global) under grant ARTIST (no. 656870) from the European Union's Horizon 2020 research and innovation program.

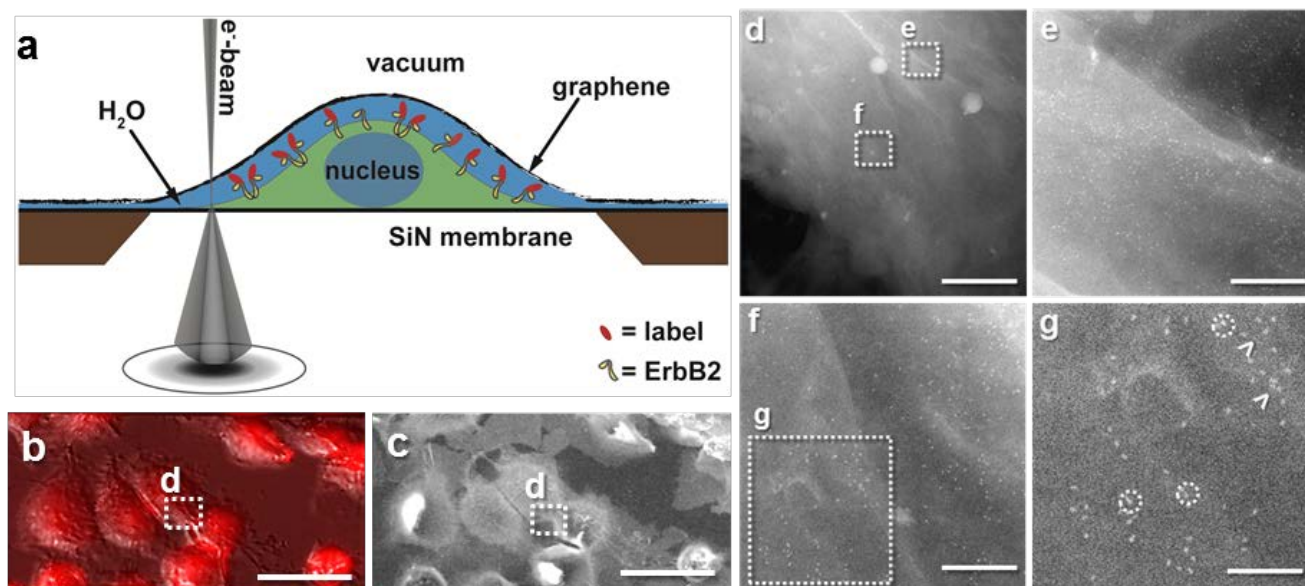


Figure 1. Electron microscopic imaging of QD-labeled ErbB2 membrane proteins in intact, hydrated breast cancer cells. a) Schematic of experimental approach. b) Fluorescence image of graphene covered SKBR3 cells, grown on a silicon microchip and cells labeled with QDs emitting red fluorescence. The dotted box marks area of correlative STEM images in d). c) SEM (10 kV) image of corresponding regions showing graphene covering most of the imaged area. White regions indicate electric charging, due to cracks in the graphene cover. d)-g) High resolution dark-field STEM (200 kV) images showing details as marked in c) and d) with white, dotted boxes, respectively. In e)-g), QDs appear as white bullet-shaped dots. In the enlarged area shown in g), several examples for pairs of ErbB2 labels, possibly indicating signaling active ErbB2 homodimers, are marked with white, dotted circles. Scale bars: b, c: 30 μ m, d: 4 μ m, e: 350 nm, f: 240 nm, g: 120 nm. Panel a) reprinted from [6] with permission, copyright 2016 Cambridge University Press.