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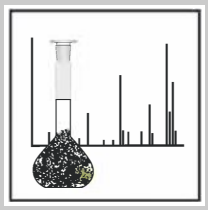

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Microfluidics and Electron Microscopy: A Powerful Couple

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Cryogenic electron microscopy (cryo-EM) enables the determination of protein structures without crystallization using a single particle analysis (SPA) approach. Unfortunately, classical protein preparation strategies are a bottleneck in this workflow. Furthermore, EM is rarely used as an analytical tool. Here we present a modular, microfluidic toolchain called *cryoWriter* for EM sample preparation. We will discuss (i) microfluidic protein isolation coupled to EM specimen preparation for structure determination, and, (ii) single-cell lysis connected to EM sample preparation for the proteome-wide detection of structural rearrangement of protein complexes.

In SPA, a thin layer of isolated protein complexes in vitrified ice is imaged. Only several thousand to a few million recorded particles are needed to calculate a high-resolution structure. We show that this amount of protein can be prepared using our microfluidic approach. We isolated the human proteasome 20S from < 1 µL cell lysate using a combination of immuno extraction and photo elution.[1] The structures of all 14 subunits of the proteasome 20S at 3.5 Å resolution are shown. Additionally, the tobacco mosaic virus (TMV, added as resolution control) was resolved at 1.9 Å.

The microfluidic EM sample preparation system can be directly combined with a single-cell lysis device. The cell is disrupted by electroporation, and the cell content is aspirated into the microcapillary and subsequently used for lossless EM grid preparation.[2] Finally, the total cell lysate is imaged by EM. A *differential visual proteomics* algorithm allows for the detection of rearranged protein complexes on a proteome-wide scale.[3] While this method is still in its infancy, we demonstrate the power of microfluidic sample preparation combined with the single-molecule detection limit of EM.

We show the application of microfluidic sample preparation methods for high-resolution structure determination and single-cell nano analytics.

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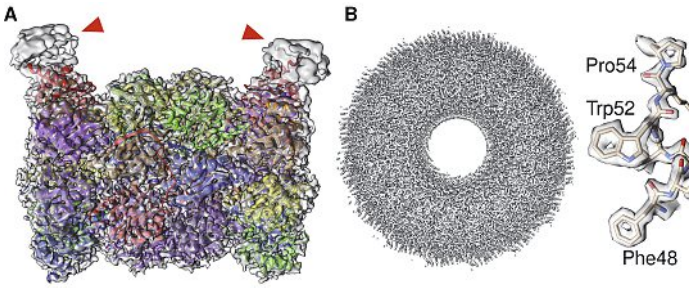
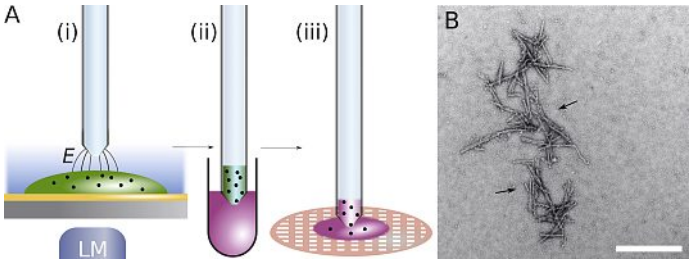


Microfluidic protein isolation and sample preparation for high-resolution cryo-EM.[1] A) Isolation of the human proteasome 20S from less than 1 µL cell lysate. Resolution at 3.5Å. An atomic model for all 14 subunits was built. Antibody fragments (red arrowheads) were used for the isolation of the endogenous protein. B) TMV. Resolution at 1.9 Å, note the holes in the aromatic rings.



Single-cell lysis and lossless EM sample preparation for *differential visual proteomics*.[2,3] A) Principles: (i) Single-cell lysis observed by light-microscopy; aspiration of lysate into microcapillary. (ii) Conditioning of the 3 to 5 nL lysate plug. (iii) Dispensing on EM sample-carrier. B) Typical image of the cell lysate. Here, amyloid fragments (arrows) are visible from a single neuron-like cell (scalebar: 500 nm).

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