Poster

The cryoWriter – a new and reliable way of automated cryo-EM grid preparation

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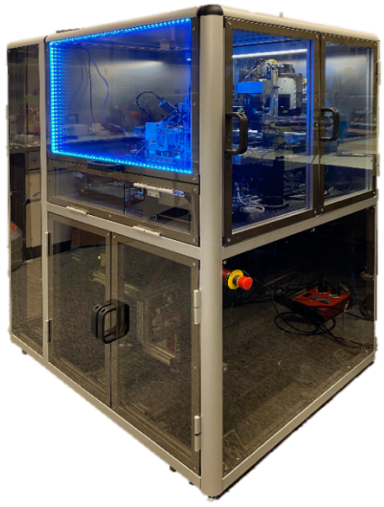
Compared to X-ray crystallography and NMR spectroscopy, cryogenic electron microscopy (cryo-EM) is still an upcoming technology to resolve protein structures. One of the bottlenecks of cryo-EM is sample preparation. Conventionally, microliter sized samples are applied on a grid, of which >99% is removed by blotting. The blotting step has limited reproducibility and can damage the sample. Here, we present our solution to tackle this challenge, the cryoWriter. The cryoWriter is a robot for automated and reproducible sample preparation in cryo-EM (Fig.1a). Using microcapillaries, the cryoWriter replaces manual pipetting tasks for grid preparation in an automated way. It applies (writes) nanoliter sized samples on a grid and vitrifies them for subsequent cryo-EM analysis. The system has been optimized for single particle analysis. Such particles can be globular, but also membrane, or filamentous proteins.
The gripper and glass capillary are at the core of the system and are key to create a flexible, automated liquid-handling system. The gripper automatically transports the transmission electron microscope (TEM) grid to all stations of the preparation workflow: It carries grids from a grid box via the on-board glow discharge unit to the writing platform. After the writing, the gripper plunges the sample into liquid ethane and puts the grid into a cryo-puck, stored for subsequent analysis by cryo-EM. It takes less than 200ms to vitrify the sample after writing. The whole procedure from grid gripping to storing takes less than 2 minutes, whereas the glow discharge step takes up most of this time.
The pipette unit handles loading the sample and writing it to the grid. Inside the pipette there is no air-water interface, leading to more reliable grid writing. It only writes a few nanoliters of sample and the conventional blotting step can be omitted. In addition, the use of pipettes provides the basis for the unprecedented modularity of the cryoWriter. For example, an inline purification step can be performed in the pipette. Importantly, the environment is precisely controlled during the entire process. A newly implemented feature allows for full access to both the relative humidity in the cryoWriter as well as the temperature of the pipette, the sample and the grid holder. As a result, the writing happens at a temperature that can be set close to the dew point. This feature has immensely enhanced the reproducibility of the process resulting in repeatedly well written grids, with tens to partially over 100 grid squares with well vitrified sample. An example of a written grid is shown in Fig.1b.



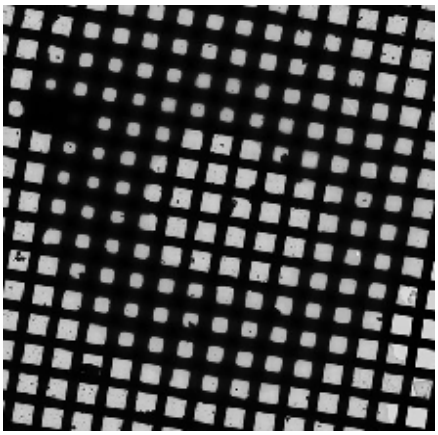


Figure 1. (a) Photograph of the cryoWriter – a fully automated sample preparation robot for cryo-EM. (b) TEM image of a grid written with the cryoWriter with high reproducibility in quality.