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Towards Microsecond Time-Resolved Cryo-Electron Microscopy

Jonathan M. Voss[1], Oliver F. Harder[1], Gabriele Bongiovanni[1], Pavel K. Olshin[1], Marcel Drabbels[1] and Ulrich J. Lorenz[1*]

[1.] Laboratory of Molecular Nanodynamics, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland
* Corresponding author: ulrich.lorenz@epfl.ch

Cryo-electron microscopy (cryo-EM) is rapidly becoming the dominant method in structural biology. Yet its time resolution is currently insufficient to directly observe the dynamics of proteins, leaving our understanding of these nanoscale machines fundamentally incomplete. So-called time-resolved cryo-EM achieves a time-resolution of a few milliseconds, which is notably too slow to study the dynamics of many proteins whose relevant motions typically occur on the microsecond timescale. We have recently demonstrated a novel approach to time-resolved cryo-EM that affords microsecond time resolution.[1-3] Our method (Fig. 1) involves melting a cryo sample with a laser beam (a), which allows dynamics of the embedded particles to occur in liquid once a suitable stimulus is provided (b), for example by inducing a temperature jump or by releasing a caged compound. While the dynamics occur, the heating laser is switched off at a well-defined point in time, causing the sample to rapidly recool (c), so that it vitrifies and traps the particles in their transient configurations (d), in which they can subsequently be imaged (e).

We demonstrate the viability of this approach and show that cryo samples can be melted for durations of tens of microseconds or longer and successfully revitrified thereafter. Stroboscopic time-resolved experiments as well as heat transfer simulations shows that after the end of the laser pulse, the sample cools with a rate of almost 10^8 K/s, which is two orders of magnitude faster than required to achieve vitrification. Moreover, this allows particles to be rapidly trapped in a transient configuration. We conclude that our experiment offers a time resolution of 5 µs or better.

A proof-of-principle experiment (Fig. 2) demonstrates that our method allows particle dynamics to occur in liquid phase during the short time window created by laser melting, before the particles are subsequently trapped in transient states during rapid revitrification. We make use of the well-known fact that proteins incur electron beam damage during cryo imaging. It is commonly believed that the vitreous ice matrix counteracts this damage and preserves the protein structure by fixing fragments in place. However, melting the sample should allow the particles to unravel once they find themselves in a liquid environment. This is exactly what we observe in our experiment. When we image a cryo sample of apoferritin (a) and subsequently melt and revitrify it with a 15 µs laser pulse (b), we find that particles in the areas previously exposed to the electron beam have disassembled and have been trapped in partially unraveled states (d,e). At the same time, particles that have not incurred beam damage are left intact (c). Single-particle reconstructions of revitrified samples are indistinguishable from those of conventional samples, confirming that the process leaves the particles undamaged. Moreover, they do not undergo any structural changes.

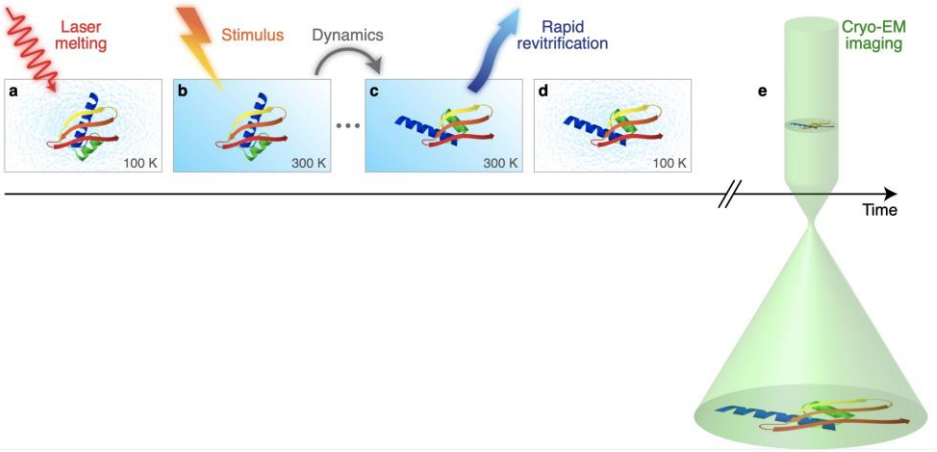

Figure 1. Experimental concept for microsecond time-resolved cryo-EM. (a) A cryo sample is melted in situ with a heating laser. (b) Once the sample is liquid and has reached room temperature, dynamics of the embedded particles are induced with an external stimulus. (c) As the particle undergoes conformational changes, the heating laser is switched off, so that the sample rapidly cools and revitrifies. (d) The particle is trapped in its transient configuration and can be subsequently imaged with conventional cryo-EM techniques (e). (Adapted from Ref. [1]).

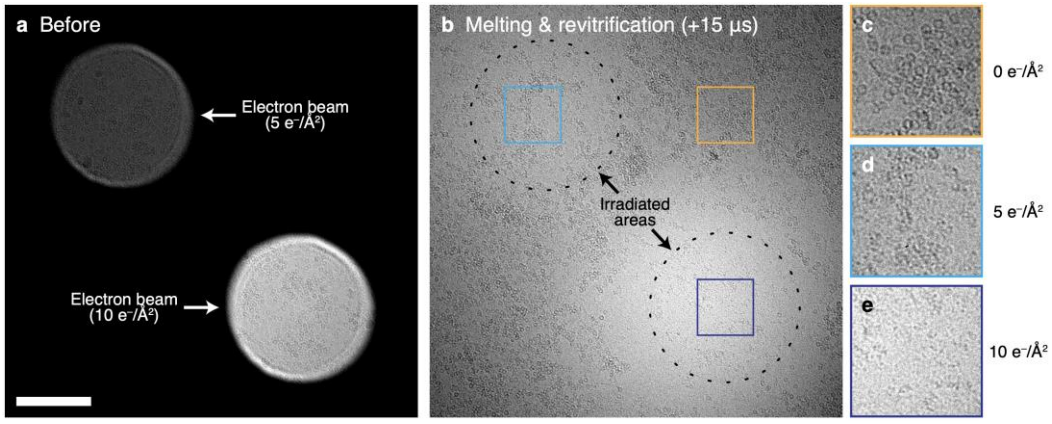

Figure 2. Proof-of-principle demonstration of microsecond time-resolved cryo-EM. (a) Micrograph of a cryo sample of apoferritin on a holey gold film. Only the top left and bottom right areas are exposed with a dose of 5 and 10 electrons/Å^2, respectively. Scale bar, 200 nm. (b) The sample is melted *in situ* with a 15 µs laser pulse and revitrifies. (c) The particles not illuminated with the electron beam prior to melting and revitrification remain intact. In contrast, those previously damaged with the electron beam have unraveled in liquid and upon revitrification, have been trapped in partially disassembled configurations (d, e). (Adapted from Ref. [2]).

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