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Technical Note

Vitrification after multiple rounds of sample application and blotting improves particle density on cryo-electron microscopy grids.

Joost Snijder, Andrew J. Borst, Annie Dosey, Alexandra C. Walls, Anika Burrell, Vijay S. Reddy, Justin M. Kollman, David Veessler

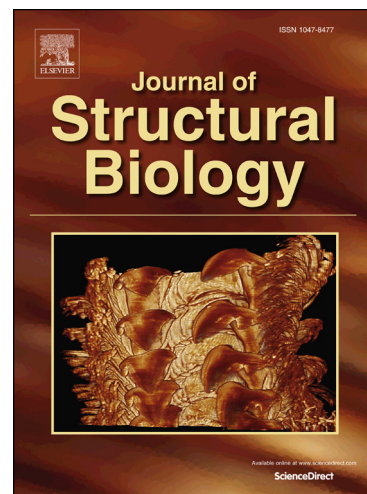
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Vitrification after multiple rounds of sample application and blotting improves particle density on cryo-electron microscopy grids.

Joost Snijder¹, Andrew J. Borst¹, Annie Dosey¹, Alexandra C. Walls¹, Anika Burrell¹, Vijay S. Reddy², Justin M. Kollman¹, David Veessler¹

¹Department of Biochemistry, University of Washington, Seattle, Washington, USA.

²Department of Integrative Computational and Structural Biology, The Scripps research Institute, La Jolla, California, USA.

Correspondence to: dveessler@uw.edu

Single particle cryo-electron microscopy (cryoEM) is becoming widely adopted as a tool for structural characterization of biomolecules at near-atomic resolution. Vitrification of the sample to obtain a dense distribution of particles within a single field of view remains a major bottleneck for the success of such experiments. Here, we describe a simple and cost-effective method to increase the density of frozen-hydrated particles on grids with holey carbon support films. It relies on performing multiple rounds of sample application and blotting prior to plunge freezing in liquid ethane. We show that this approach is generally applicable and significantly increases particle density for a range of samples, such as small protein complexes, viruses and filamentous assemblies. The method is versatile, easy to implement, minimizes sample requirements and can enable characterization of samples that would otherwise resist structural studies using single particle cryoEM.

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