1030
doi:10.1017/S1431927620016724

Microsc. Microanal. 26 (Suppl 2), 2020

Monolayer Graphene-covered Grids Enable 2.6-Å Single-particle Cryo-EM Reconstruction of 52-kDa Streptavidin

Yimo Han[1], Xiao Fan[2], Haozhe Wang[3], Fang Zhao[2], Christopher Tully[2], Jing Kong[3], Nan Yao[2] and Nieng Yan[2]

[1]Rice University, Houston, Texas, United States, [2]Princeton University, Princeton, New Jersey, United States, [3]Massachusetts Institute of Technology, Boston, Massachusetts, United States

Single-particle cryogenic electron microscopy (cryo-EM) represents the cutting-edge technology for the structural determination of biomacromolecules. However, technical challenges associated with cryo-EM sample preparation limit its ability from achieving higher resolution for a broader range of targets, especially for small proteins. In order to improve the sample quality, new designs of the cryo-EM grids, aimed at preserving thin, uniform vitrified ice and improving protein adsorption, have been considered a promising approach to achieving a higher resolution with the minimal amount of materials and data.

Here, we demonstrate a 2.6-Å single-particle reconstruction of 52-kDa streptavidin (**Fig. 1**) [1-3]. This is so far the highest resolution structure of the smallest protein by cryo-EM. The structure was achieved by using a high-yield, monolayer graphene-supporting cryo-EM grid, which allows for effective protein adsorption and thin vitrified ice [1]. We fabricated graphene cryo-EM grids by transferring continuous monolayer graphene from its original substrate, a copper foil, to a Quantifoil holey carbon Au grid using an organic molecule-assisted transfer method (**Fig. 2a**). We employed UV/ozone, which uses ultraviolet (UV) irradiation to generate a small amount of ozone gas to gently oxidize sample surfaces (**Fig. 2b**). UV/ozone has the advantage of adding functional groups to graphitic surfaces at a slow, and thus, controllable rate, therefore, fine-tuning the surface properties of graphene (**Fig. 2c**). Using this approach, we have achieved uniform and clean monolayer graphene with a high yield of suspended areas (~99% on average) (**Fig. 2d**). In addition to streptavidin, graphene grids also effectively increase the density of other examined soluble, membrane, and lipoproteins (**Fig. 2e-i**), affording the opportunity for structural investigation of challenging proteins which cannot be produced in large quantity.

In summary, we developed a robust approach to produce graphene-covered grids for cryo-EM and achieved a 2.6-Å structure of 52-kDa streptavidin. Our technique paves the way for higher quality and more general cryo-EM sample preparation for near-atomic resolution cryo-EM. In addition, our method employs only simple tools that most structural biology laboratories can access. We expect our method to benefit the cryo-EM community by improving the sample preparation process [5].

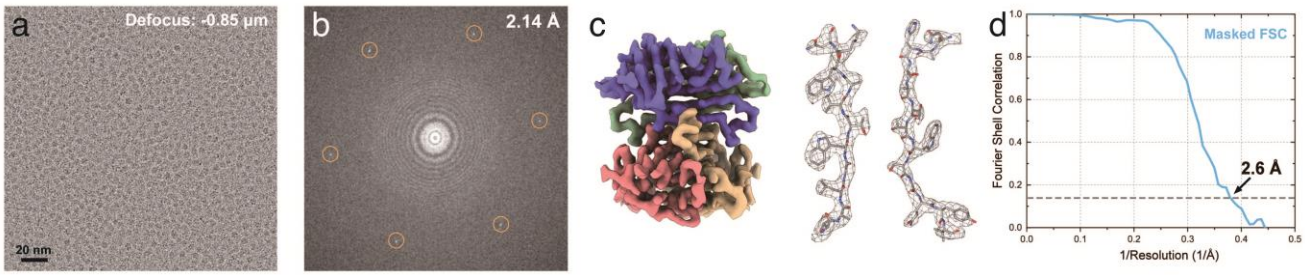

Figure 1. Single-particle cryo-EM reconstruction of 52-kDa streptavidin at a 2.6-Å resolution. a, Cryo-EM micrograph of 52-kDa streptavidin particles with a small defocus value (-0.85 µm). **b,** Fourier

transform of **a** with graphene reciprocal lattice circled in orange. **c,** Single-particle reconstruction of streptavidin at 2.6-Å resolution [2,3]. **d,** Gold standard FSC (criteria 0.143) curve of the masked map.

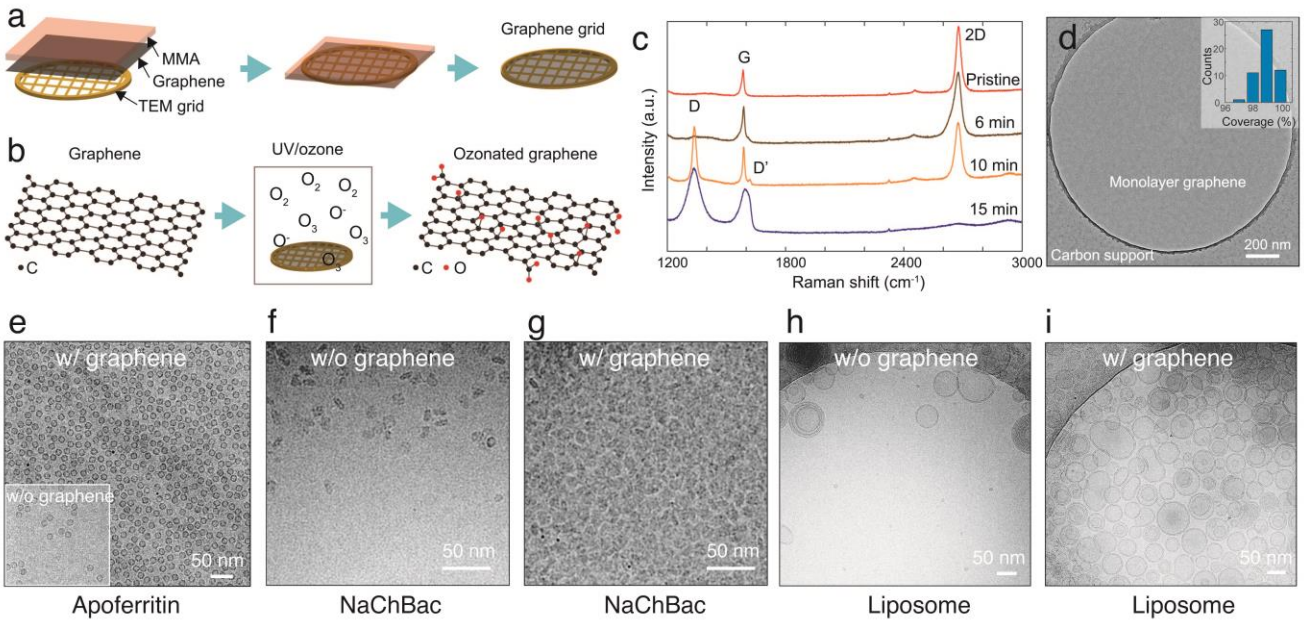

Figure 2. Fabrication and applications of high-yield monolayer graphene grids for cryo-EM. a, Schematic summary of the grid fabrication process (more details in ref. 1). **b,** Schematic of graphene surface treatment by UV/ozone, which can convert graphene to hydrophilic oxygenated graphene derivatives. **c,** Raman spectroscopy shows that 10-15 min UV/ozone treatment can effectively functionalize graphene surface. **d,** TEM image of suspended graphene over a hole in the Quantifoil Au grid. The inset displays the statistics of graphene yield (99% on average). **e,** Cryo-EM micrograph of apoferritin on graphene grids, compared to the same sample on holey carbon grids (inset). **f-g,** Cryo-EM micrographs of a bacterial sodium channel (NaChBac) on holey carbon grids (**f**) and graphene grids (**g**). **h-i,** Cryo-EM micrographs of liposomes on holey carbon grids (**h**) and graphene grids (**i**).

References

[1] Y. Han *et al.* PNAS, **117** (2020), p. 1099-1014.
[2] Y. Han, X. Fan, and N. Yan, EMD-20907 (2019).
[3] Y. Han, X. Fan, and N. Yan, EMPIAR-10335 (2019).
[4] Funded by the Dean for Research Innovation Fund for New Ideas in the Natural Sciences from Princeton University.
[5] Thank the Princeton Institute for the Science and Technology of Materials (PRISM).

https://doi.org/10.1017/S1431927620016724 Published online by Cambridge University Press