

Defect integrated graphene support layer for cryo-electron microscopy

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Graphene with integrated defects has great potential in a wide range of applications. By controlling the density and size of these defects and furthermore, nanopores, graphene can be used, for instance, for gas or liquid filtration applications, biodevices, DNA sequencing [1]. Graphene also serves as an excellent support film for imaging nanoscale materials due to its single atomic thickness, mechanical stability, and high conductivity [2, 3]. One of the recent interests is to adapt graphene as an electron transparent support membrane for cryo-Transmission Electron Microscopy (cryo-TEM). Currently, for single-particle cryo-TEM, biological specimens such as protein and viral particles are most often suspended in a thin free-standing ice layer. Nonetheless, for many samples, it remains a challenge to achieve a high enough concentration of samples with a uniform distribution and random orientations of the particles while maintaining high resolution in a reproducible manner. In this work, we treated graphene monolayers transferred onto TEM grids using hydrogen plasma. With different experimental conditions, hydrogenated surface, mono vacancies and a few nm nanopores are realized in a controlled way and their atomic structures are fully surveyed by aberration corrected TEM. Creation of vacancies drastically improved the hydrophilicity of the graphene surface, which allowed the formation of a thin ice layer with a uniform distribution of biological samples. The surface-treated graphene support layers were used for Cryo-TEM imaging of two test samples: a bacteriophage and a membrane protein. Parameters critical to optimal 3D-reconstruction in single particle analysis from data collected on a modified graphene grid were studied, in correlation to the detailed atomic structure of graphene.

References

[1] Liu, G., Jin, W., & Xu, N., Chemical Society Reviews, 44(15) (2015)5016-5030.

[2] Han, Yimo, et al., Proceedings of the National Academy of Sciences, 117.2 (2020) 1009-1014.

[3] Kato, Ryuichi, et al., Carbon 160 (2020)107-112.

Figures

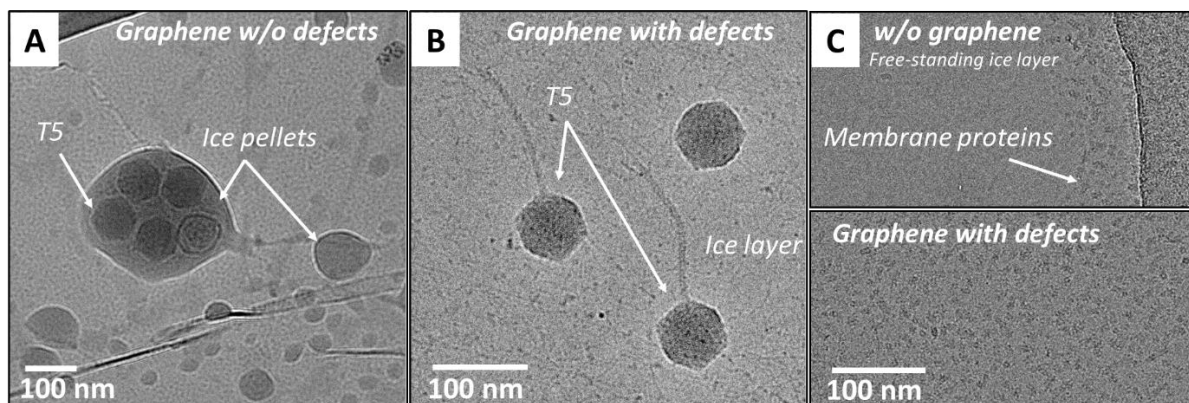


Figure: Cryo-TEM images of: Bacteriophage-T5 suspended in ice (A) on unmodified graphene showing droplets of vitrified ice, which arises from the hydrophobicity of pristine graphene, (B) graphene with vacancies with a thin homogeneous ice layer showing the hydrophilic feature of the modified graphene surface and (C) high density of membrane proteins uniformly distributed on defect integrated graphene (bottom).