Patterned Functionalization of Graphene specimen supports for CryoEM with a Low-energy Plasma Instrument

Katerina Naydenova, Mathew Peet¹ and Christopher J. Russo¹*

¹ MRC Laboratory of Molecular Biology, Cambridge, United Kingdom
* Corresponding author: crusso@mrc-lmb.cam.ac.uk

Single particle cryoEM can provide an atomic resolution structure from any biological macromolecular complex. Still, in practice the success of structure determination is often determined by the successful preparation of a vitrified specimen suitable for high resolution imaging in the electron microscope. This process is often difficult because the complex interacts with the surfaces present in the specimen support structure. We recently combined the ultrastable gold specimen support described previously [1] with a new graphene synthesis and transfer method to create a monolayer graphene on gold specimen support with many desirable properties for cryoEM [2].

Here we will describe an instrument (Figure 1) and method for decorating the graphene on the specimen support with a variety of covalent modifications in a rapid and simple process. The instrument comprises a vacuum chamber with an RF plasma generator in a remote configuration, a special specimen holder described below, a chemical injection manifold to introduce chemical precursors into the reaction chamber, and an UV-Vis optical spectrometer which can monitor the plasma in real time. By using helium (which is inert and has a low atomic number) as the primary plasma gas with a remote plasma generator, sputter damage and other changes to the graphene were prevented. This is in contrast to typical residual air glow discharge systems used for cryoEM which create a plasma with sufficient energy to destroy the suspended monolayer graphene even with short exposures. We tested several precursor chemicals with different terminal reactive groups: 1-pentanethiol, amylamine, hexanoic acid, acetone, 4-pentylphenol, and water. In principle this method of functionalization can be used with any small compound provided it has a vapor pressure sufficient to inject into the chamber.

We also designed a new non-contact specimen mask to pattern multiple functionalizations across the graphene surface of a single 3 mm support, allowing one to simultaneously test multiple conditions for the same biological specimen on a single grid. Multiple exposures allow multi-axis patterning and serial patterning of the same region with multiple chemicals. At the typical plasma process pressure of ~1 Torr, and with ≈50 μm mask-to-surface spacing, the resolution of this patterning method is approximately 50 μm or 1 grid square. With more complicated masks, a distinct functionalization for each of the hundreds of grid squares on a grid is possible; smoother surface patterning transitions or gradient functionalization can also be achieved by increasing the distance between the mask and the graphene surface.

We found that first modifying the graphene with a low energy hydrogen plasma [3], allowed us to control the hydrophobicity of the support, largely independent of the functionalization compound. This proved important since several of the functional groups of interest actually increased the contact angle of the support when added. Different functionalizations resulted in different orientation distributions for the same specimen on different regions of a single grid (Figure 2.). Examples of structures determined on these supports are given.

References:
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**Figure 1.** Covalent graphene functionalization and patterning with a low-energy helium plasma. (a) Design of an inductively coupled plasma instrument for covalent functionalization of graphene. (b) The slotted grid holder positions reliably and precisely up to 10 grids under a single mask plate. The retractable shutter is used to control the exposure time independent of establishing a stable plasma reaction condition in the chamber. (c) A non-contact knife-edge mask design for patterning of graphene grids with plasma-assisted functionalizations. Masks with arbitrary shapes are possible.

**Figure 2.** Panels (a-d) show representative micrographs of 20S proteasomes on graphene in ice taken from the four quadrants of a single patterned multifunctional graphene grid. The quadrant of the grid from which the micrograph was collected is highlighted in insets. Scale bar is 500 Å.