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Multiplexed Biomimetic Lipid Membranes on Graphene by Dip-Pen Nanolithography

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The application of graphene in sensor devices depends on the ability to appropriately functionalize the pristine graphene. Here we show the direct writing of tailored phospholipid membranes on graphene using dip-pen nanolithography. The membranes are stable in aqueous environments and we observe electronic doping of graphene by charged phospholipids. As a proof of principle, we demonstrate the specific binding of streptavidin to biotin-functionalized membranes. The combination of atomic force microscopy and binding experiments yields a consistent model for the layer organization within phospholipid stacks on graphene.

The graphene flakes used in this work were prepared by micromechanical cleavage of natural graphite at the surface of oxidized Si wafers with a surface layer of silicon dioxide.[1] The number of layers of the fabricated flakes was confirmed by a combination of optical contrast,[2] Raman spectroscopy [3] and AFM measurements [1].

The schematic writing process is shown in Fig. 1. 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) was used as the main component of the phospholipid inks used in L-DPN functionalization of the graphene. To this carrier, 1 mol% of 1,2-dioleoyl-sn-glycero-3- phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Liss Rhod PE), 5 mol% of either 1,2-dioleoyl-snglycero-3-phosphoethanolamine-N-(cap biotinyl) (Biotinyl Cap PE) or 1,2-dioleoyl-sn-glycero-3-phosphate (DOPA) were added, yielding three different functional ink mixtures. The Rhod-PE being fluorescent gives an easy detectable marker for optical control of the lithographic outcome, at least in case of writing on silicon dioxide. The Biotin-PE can act as a model for active sensor elements by its high affinity to binding streptavidin [4], a concept widely used in biotechnology for the linking and immobilization of various proteins and other bioactive compounds. The third lipid mixture with DOPA was used to probe the effect of negative charge doping on the graphene.

Phospholipids exhibit higher mobility on graphene compared with the commonly used silicon dioxide substrate, leading to well-spread uniform membranes. To establish the dependence of lipid transfer and feature size on writing speed and humidity, dot patterns with different tip-substrate contact time were written at different humidity. The transfer characteristics for lipids to graphene substrates observed in our experiments follow the diffusion models established for the transport of thiols on gold by DPN.

The charge transfer interaction between lipids and graphene and the integrity of the graphene during the writing process were confirmed by Raman spectroscopy. When the graphene is covered by the lipid membrane, the G and 2D peaks downshift closer to the charge neutrality point (become less p-doped), the G peak broadens and the 2D to G intensity ratio increases, all of which are consistent with electron transfer from the n-doping DOPA in the lipid membrane. No defect related D peak is observed in the

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graphene before and after the L-DPN writing, indicating that the lithography process does not damage the graphene.

Although the phospholipids are found to be more mobile on the graphene substrate, allowing for uniform spreading, the formed membranes are still stable enough to allow for immersion into liquid and binding of analytes from solution. More importantly, it is demonstrated that multiplexing of different membrane compositions in close proximity can be achieved on graphene by L-DPN, enabling the crucial control over spatial distribution and chemical composition lacking in self-assembled membranes yielded by vesicle fusion. On the basis of the current results and taking into account appropriate measures to control spreading in immersion, for example, by tailoring graphene devices with distinct graphene stripes or applying chemical barriers in forms of polymers or photoresist, L-DPN-generated membranes could become active elements in graphene-based sensor devices.

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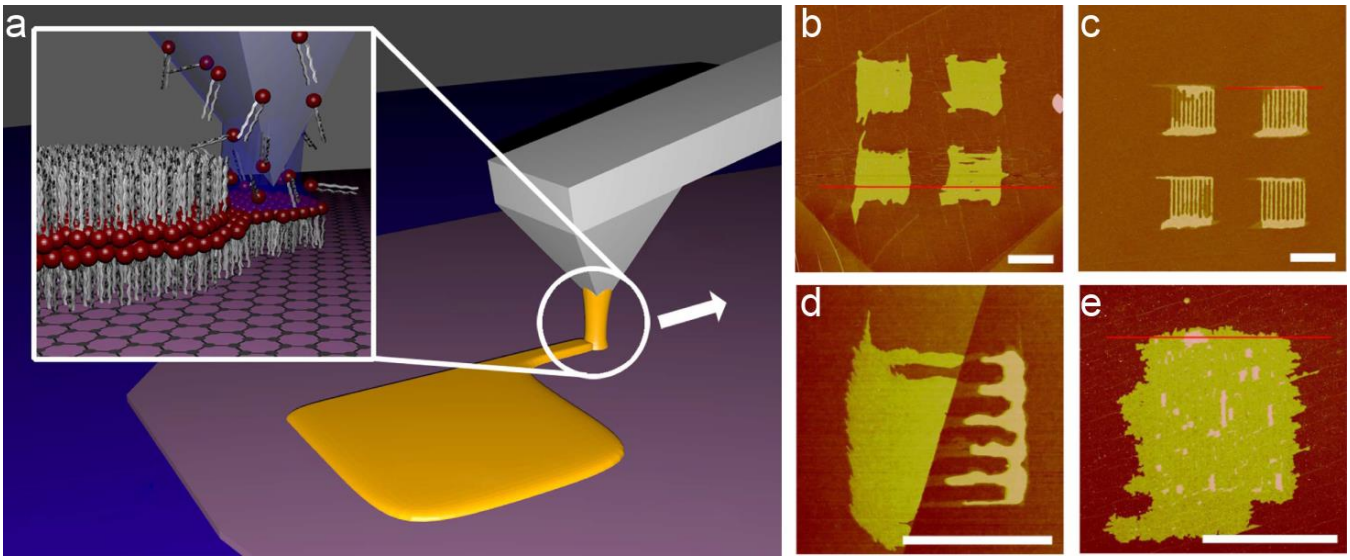

Figure 1. (a) Schematics of DPN writing process. (b-e) AFM images of phospholipid membranes on graphene and silicon dioxide. (b) Four 5x5 μm^2 phospholipid patches on graphene. (c) Patches written under the same writing conditions but on neighboring silicon dioxide area. (d) Close up of a patch written directly on the edge of a graphene flake and (e) image of a membrane written onto graphene with some residues of the exfoliation process present on the graphene before the lipid DPN process.

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