A DIELECTRIC THIN-FILM SUBSTRATE USING GRAPHENE-INDUCED ENERGY TRANSFER FOR NANOSCALE LIPID BILAYER RESEARCH

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Abstract

In recent years, super-resolution microscopy techniques have undergone a great revolution in terms of spatial details. The enhance axial resolution is necessary for studying lipid bilayers, commonly used to mimic the structure of natural membranes with a typical thickness of 4-6 nm. Graphene-Induced Energy Transfer (GIET) has emerged as a promising technique resolving axial distances of fluorescent emitters with nanometer precision. GIET relies on the distance-dependent quenching of fluorophores by a graphene sheet acting as a planar energy aceptor. Our research aims to design substrates suited for GIET investigations on Supported Lipid Bilayers (SLBs). Specifically, we propose a nanometer-thin inorganic layer of Al2O3 by atomic layer deposition (ALD). With this approach, we aim to manipulate the electrostatic repulsion between the dipole of the DOPC headgroup and the positively charged coated Al2O3 surface to enhacend thickness of the hydration layer, with this approach can be used for GIET studies of membrane-protein binding with the potential to open the door for advanced studies in biophysics, biosensing, and elucidating the membrane-protein interactions.

Objectives

1. Design and preparation of an homogeneous thin-film substrate on a single monolayer graphene
2. Characterization of the surfaces by raman spectroscopy, AFM, contact angle goniometer
3. Characterization of SLB by GIET and FCS

Introduction

We intend to fabricate surfaces ”suitable” for the SLB studies by GIET. In GIET, the graphene surface modulates the fluorescence lifetime of emitters as a function of their distance from the graphene. Hence, the fluorescent labels attached to the headgroup of the lipid bilayer, will have different fluorescence lifetime values in the top and the bottom leaflet (Figure 1). The current surfaces used for GIET show certain disadvantages, for instance the coupling of the SLB dynamics to the underlying substrate surface. Thus, we aim to introduce a nanometer-thin inorganic layer inserted in between the graphene and the lipid bilayer. This geometry also enables the distance tuning between the membrane and the quenching surface required by GIET.

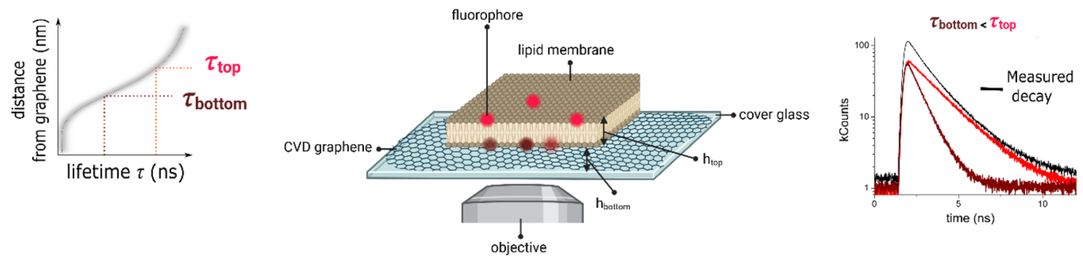

Figure 1. The basic principle of GIET approach.The fluorescence lifetime of a dye molecule is modulated by its distance from the graphene sheet. In this method (middle panel), a lipid bilayer is labeled with fluorescent probes incorporated into both the top and bottom leaflets.

Fluorophores located at the headgroup region of the lower leaflet experience stronger quenching due to their closer proximity to the graphene energy acceptor, whereas those in the upper leaflet are quenched less efficiently. Consequently, the recorded fluorescence decay exhibits a biexponential profile, corresponding to two lifetime components: top (shown in red) and bottom (in dark red) (right panel). These lifetimes can be extracted from the measured decay curve (in black, right panel) by fitting and subsequently converted into distance values using a calibration curve (left panel). This allows determination of the distances between the upper and lower leaflets relative to the graphene surface. The typical effective measurement range of the GIET technique extends up to approximately 25 nm from the graphene sheet.

As a dielectric thin-film substrate, we propose ALD Al2O3 (Figure 2). ALD will facilitate selecting the thickness of the layer which is reachable for GIET purposes. Vesicle rupture on Al2O3 occurs exclusively in the presence of high osmotic pressure and Ca2+ for SLB formation.

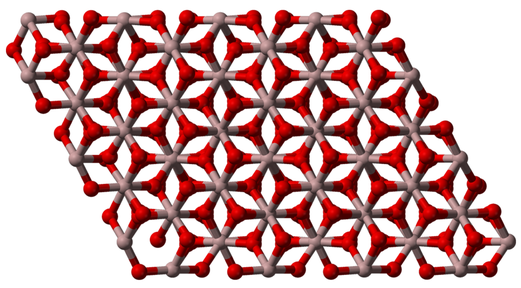

Figure 2. Aluminium oxide (Al2O3) crystal structure.

We aim to form SLBs composed of:

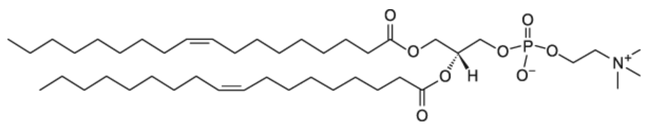

Figure 3. Chemical structure of DOPC.

Materials and methods

Contact Angle Measurements: In order to test hydrophilicity of the thin-film substrate - graphene surfaces, we used Contact Angle Goniometer technique (Figure 4). We measured the contact angle of water droplet on different surfaces (ALD Al2O3 at different cycles and temperatures), as shown in Figure 4.

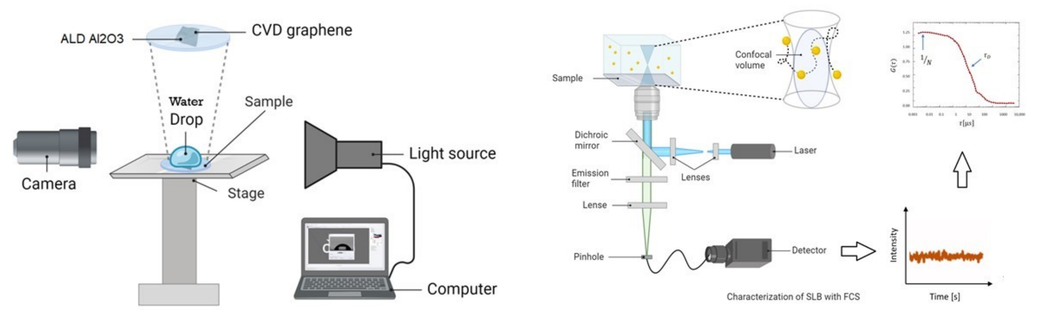

Figure 4. Contact Angle Goniometer and Fluorescence Correlation Spectroscopy.

Lifetime analysis will be performed to determine the thin-film thickness and the thickness of the bilayer via GIET approach. Moreover, lateral diffusion of fluorescent lipid analogue will be determined by FCS, which is a technique based on the statistical analysis of fluorescence intensity fluctuations, from which the diffusion coefficients can be obtained.

Results and Discussion

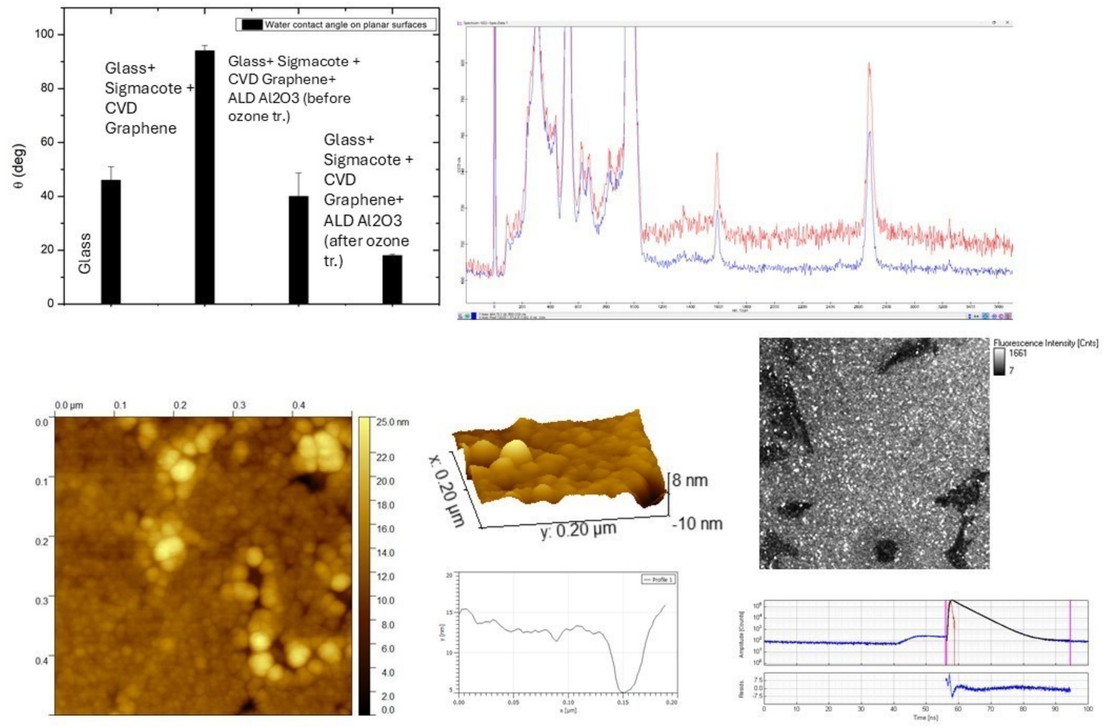

Figure 5. Top left: water contact angle measurements on glass, glass+sigmacote+CVD graphene, glass+sigmacote+CVD graphene+ALD Al2O3 (before ozone tr.), glass+sigmacote+CVD graphene+ALD Al2O3 (after ozone tr.), top right: raman spectroscopy from SiN wafer with CVD graphene+ALD Al2O3 confirming there is no change before and after atomic layer deposition. Bottom left: AFM scans on glass, ALD deposited Al2O3 and height profile showing 10 nm thickness and 3D profile, bottom right: graphene substrates coated with Al2O3 exposed to LUVs labelled with ATTO655-DPPE: Intensity image obtained for ALD Al2O3 and fluorescence decay

With the methodology mentioned above, the following results were obtained: Contact angle shows an improvement after ozone treatment. Atomic Force Microscopy (AFM) shows an homogeneous ALD Al2O3 film on graphene, defects shown might be due to nucleation sites from the graphene. Raman Spectroscopy shows no difference in CVD graphene before ALD Al2O3. Graphene substrates coated with Al2O3 exposed to LUVs labelled with ATTO655-DPPE: Intensity images obtained for ALD Al2O3 Based on the obtained results from the fluorescence decays and FCS, further optimization of the SLB formation has to be performed.

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Acknowledgements

The authors acknowledge the assistance provided by the Charles University Grant Agency (GA UK) project no. 492426