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Supplementary information

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Supplementary Information

Graphene- and Metal-Induced Energy Transfer for single-molecule imaging and live cell nanoscopy with (sub)-nanometre axial resolution

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Supplementary note 1: MIET and GIET substrate preparation

MIET substrates were prepared by vapour deposition of a semitransparent gold film onto a plasma cleaned glass coverslip (thickness 170 µm) with the aid of an electron beam source (Univex 350, Leybold). Thickness of the metallic film was monitored using an oscillating quartz unit. For GIET substrates, monolayer graphene-coated glass coverslips were purchased from Graphene Supermarket, New York, USA and dielectric SiO_2 spacers of desired thickness were evaporated onto these substrates.

Supplementary note 2: Defocused imaging of single molecules

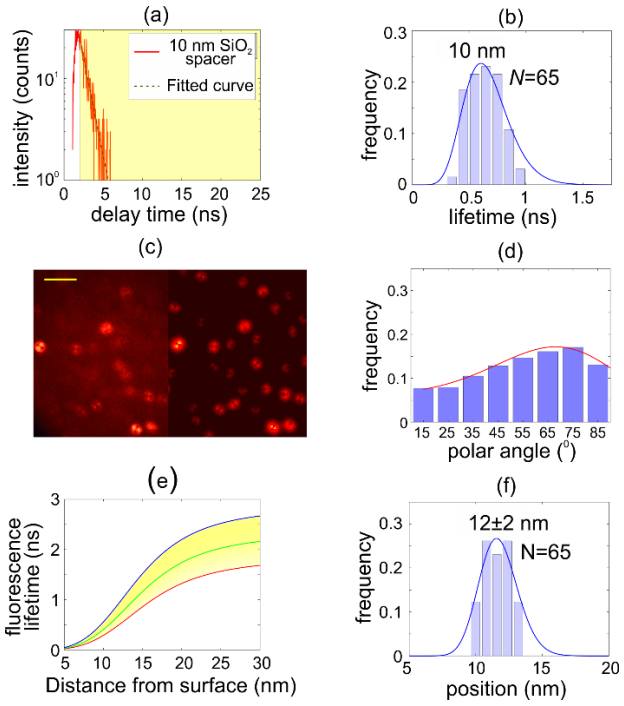
Widefield defocused imaging[1] was utilized for determining the dipole orientation of single molecules immobilized on a GIET substrate. In this vein, we spin-coated 10 µl of a 100 pM aqueous solution of Atto655 NHS ester on a GIET substrate coated with 20 nm silica spacer. Widefield fluorescence images of single dye molecules (see figure 2C in the main text) were recorded using an emCCD camera (iXon DU-885K, Andor Technology, Ireland). For excitation of dye molecules, a linearly polarized diode (PhoxX 647, 140 mW, Omicron Laserage, Germany) with λ_{exc} = 647 nm was used. The laser beam was shifted across the back aperture of the objective lens (UApoN 100XOTIRF, 1.49 N.A., Olympus) to create a TIRF illumination. Average laser intensity was 200 W/cm^2. Emission was collected by the same objective and then passed through the dichroic mirror (Di01-R405/488/561/635, Semrock) before being refocused onto the camera chip. The camera was cooled down to −80° C, preamp-gain was set to 3.7, and an em-gain of 20 were chosen. Imaging was performed with an exposure time of 10 seconds. For acquiring defocused images, the objective was shifted about ∼1 µm towards the sample.

Supplementary note 3: Determining orientation of fluorophores on GUVs

Widefield fluorescence images of dye-tagged GUVs were collected using a home-built microscope. Excitation was done with a linearly polarized laser beam (PhoxX 647, 140 mW, Omicron Laserage, Germany) and fluorescence emission was imaged using an emCCD camera (iXon DU-885K, Andor Technology, Ireland). The core idea of this experiment was to identify high emission intensity regions in the image, providing information on fluorophore orientation with respect to the bilayer of the GUV [2]. Figure 5B in the main text visualizes a typical fluorescent image of a GUV demonstrating the dye molecules are oriented parallel with respect to the plane of bilayer.

Supplementary note 4: Axial localization of single Atto488 molecules using GIET imaging

We have also demonstrated GIET imaging using a fluorophore exhibiting fluorescence excitation and emission in the blue-green spectral region. For this purpose, we localized single molecules of the dye Atto488 (see Supplementary Figure 1) spin coated on the top of a GIET substrate having a dielectric silica spacer 10 nm thick. Fluorescence lifetime measurements and defocused imaging were done following the same procedure as described for Atto655 in the main text (see figure 4 in the main text). The lifetime-versus-distance calibration curve for Atto488 was calculated using free-space lifetime (τ_0) 3.6 ns, quantum yield (ϕ) of 0.74 from a previously published report[3]. An axial distance of 12 ± 2 nm was determined, a bias of ~2 nm originates most likely from roughness of the substrate and local quantum yield variations of adsorbed molecules.



Supplementary Figure 1: Axial localization of single Atto488 molecules using GIET (a) TCSPC histogram for a single Atto488 emitter. The shaded region is used for a maximum likelihood mono-exponential tail fit of the fluorescence decay time. (b) Lifetime distributions as obtained by tail-fitting the decay curves of individual molecules. Numbers above a distribution indicate how many molecules contributed to the corresponding distribution. Solid lines represent fits of a general Gamma distribution to the histograms. (c) Measured defocused single molecule images (left) for Atto488 molecules deposited on SiO_2, and theoretically fitted defocused images (right). Scale bar is 1 µm. (d) Bar histogram of molecular orientation distribution (angle towards the vertical axes) as extracted from the defocused images shown in (c). (e) Calculated GIET calibration curves: Blue and red curves show the GIET calibration curves which one would have for purely horizontally or vertically oriented molecules. The yellow density plot shows a distribution of GIET curves where the shading reflects the weight proportional to the orientation distribution from panel (d). The green curve is the corresponding orientation averaged GIET calibration curve used for subsequently converting experimental lifetime to distance values. (f) Distance distributions as obtained by converting the lifetime values from panel (b) into distance values by using the green GIET calibration curve from panel (e). Figure adapted with permission[4].

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