Electronic Supporting Information

Graphene Oxide Single Sheets as Substrate for High Resolution cryoTEM

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Supporting Materials:

Materials and Methods

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Materials and Methods

Materials. Salmon Sperm DNA (~300 bp/molecule; sodium salt) was kindly provided by Nichiro Corporation (Yokosuka-shi, Kanagawa prefecture, Japan) and was used as-received without further purification. Solutions (2.5 mg/mL) were prepared using a phosphate buffer at pH 7.4 . (8 gr NaCl, 1.4 gr Na_2HPO_4, 0.2 gr KCl, 0.24 gr KH_2PO_4 were mixed in 1000 mL ultrapure water 18 MΩ cm^{-1}). All chemicals were used as-received without further purification. Graphene oxide (GOx) and the silica nanoparticles were synthesized as reported previously.[1, 2]

Cryogenic Transmission Electron Microscopy. Samples were vitrified on R2/2 Quantifoil TEM grids (Quantifoil Micro Tools GmbH, Jena, Germany) after 5 minutes of stabilization time using an automated vitrification robot (FEI Vitrobot™ Mark III, FEI

Company). Graphene oxide layers were applied by diluting a 0.01 mg/mL solution 2 times prior to vigorous stirring. Before application of the GOx solution, the TEM grids were hydrophilized by glow discharge using a Cressington 208 carbon-coater for 40 seconds. The GOx films were invisible in bright field TEM imaging, even at high defocus values. However, electron diffraction confirmed their presence by revealing the typical hexagonal spot pattern also observed for graphene with a corresponding *d*-spacing to a C-C distance of 1.42 Å (Figure S1).[3] The spots in the diffraction pattern were slightly diffuse, which was attributed to a variation in the direction of the surface normal with respect to the electron beam due to local curvature in the GOx surface.[4] By recording patterns under different tilt angles and comparing the relative spot intensities we could distinguish monolayers from multilayers, and established that approximately 40% of the grid surface was covered with The TEM grids that were used without GOx monolayers were hydrophilized in a similar procedure before vitrification. A 3 μL drop of the solution (as well for the DNA solution as for the suspension of silica in water) was applied to a Quantifoil grid in the environmental chamber (operating at 25 °C with 99% relative humidity) of the Vitrobot and the grid was blotted with two filter papers to remove the excess liquid. For conventional cryoTEM images, ultrapure water was used in the humidifier and the blotting time was 3 seconds. Grids pretreated with GOx were used to image the silica nanoparticles and for these grids a mixture of 20% (v/v) IPA in ultrapure water was used in the humidifier. The grids were blotted twice for 7 seconds after a stabilization time of 5 seconds at 99% relative humidity. After blotting, another 5 seconds at 99% relative humidity was used. Subsequently, the grid was plunged into liquid ethane which was maintained at approximately –183 °C. The vitrified sample was then transferred to the TU/e cryoTITAN (FEI, FEG, 300 kV, Gatan Energy Filter, 2k x 2k Gatan CCD camera) and kept at liquid nitrogen temperatures at all times. Imaging was performed using low

dose mode, typically with defocus values of −1600 nm and −1000 nm. Images were acquired with an exposure time of 1 second at a dose rate of 100 $e^{-}Å^{-2}s^{-1}$. Imaging on the GOx prepared grids was done using a parallel beam with an illuminated area between 550 – 600 nm (nanoprobe mode) at the same dose rate.

Contrast analysis. Line scans were performed using intensity profiles in Digital Micrograph, using the same pixel dimensions (length × width is 80 × 20 pixels) for all images. Analysis was performed on multiple ribbons, but the presented values reflect only the clearly identifiable ribbons.

Contrast transfer functions (CTF) Calculations. CTF's were calculated by CTF Explorer v0.999a for the FEI Titan operating at 300 kV, an energy spread of 0.6 eV (focal spread 3.61 nm) and a semi-angle convergence of 0.3 mrad. Lens instabilities were set to 0.5 ppm with a C_s aberration of 2.7 mm.

Measuring the inelastic mean free path (IMFP). The elastic cross–section σ_{el} and inelastic cross–section σ_{in} (nm^2) are given by[5]:

$$\sigma_{el} = \frac{1.4 \cdot 10^{-6} Z^{\frac{3}{2}}}{\beta^2}\left[1 - \frac{0.26Z}{137\beta}\right]$$

$$\sigma_{in} = \frac{1.5 \cdot 10^{-6} Z^{\frac{1}{2}}}{\beta^2} \ln \frac{\beta^2\left(U_0 + mc^2\right)}{10}$$

where β is the ratio between the velocity of the electron and light (β^2=1−[mc^2/(U_0+mc^2)]^2), Z is atomic number, U_0 is the incident electron energy, and mc^2 is the rest energy of an electron. At U_0 = 300 kV, the elastic cross–section and inelastic cross–section for hydrogen (Z_H = 1) and oxygen (Z_O = 8) are

	Elastic σ_{el}	Inelastic σ_{in}
Hydrogen (Z = 1)	σ_{Hel}= 2.32·10^{-6}	σ_{Hin}= 2.69·10^{-5}
Oxygen (Z = 8)	σ_{Oel}= 5.15·10^{-5}	σ_{Oin}= 7.60·10^{-5}

Therefore, the total elastic cross–section and inelastic cross–section for ice (H_2O) are given by:

$$\sigma_{el} = 2\cdot\sigma_{Hel}+\sigma_{Oel} = 5.61\cdot10^{-5}\ \text{nm}^2\ ;\ \sigma_{in} = 2\cdot\sigma_{Hin}+\sigma_{Oin} = 1.30\cdot10^{-4}\ \text{nm}^2$$

The mean free path (inelastic Λ_{in} and elastic Λ_{el}) is related to the density ρ (ρ_{H2O} = 0.93 g/cm^3), and the elastic and inelastic cross–section,

$$\Lambda_{el} = M_w/\rho N_A\sigma_{el} = 572.80\ \text{nm},\ ;\ \Lambda_{in} = M_w/\rho N_A\sigma_{in} = 247.95\ \text{nm}$$

where M_w is the molar mass, and N_A is Avogadro's number.

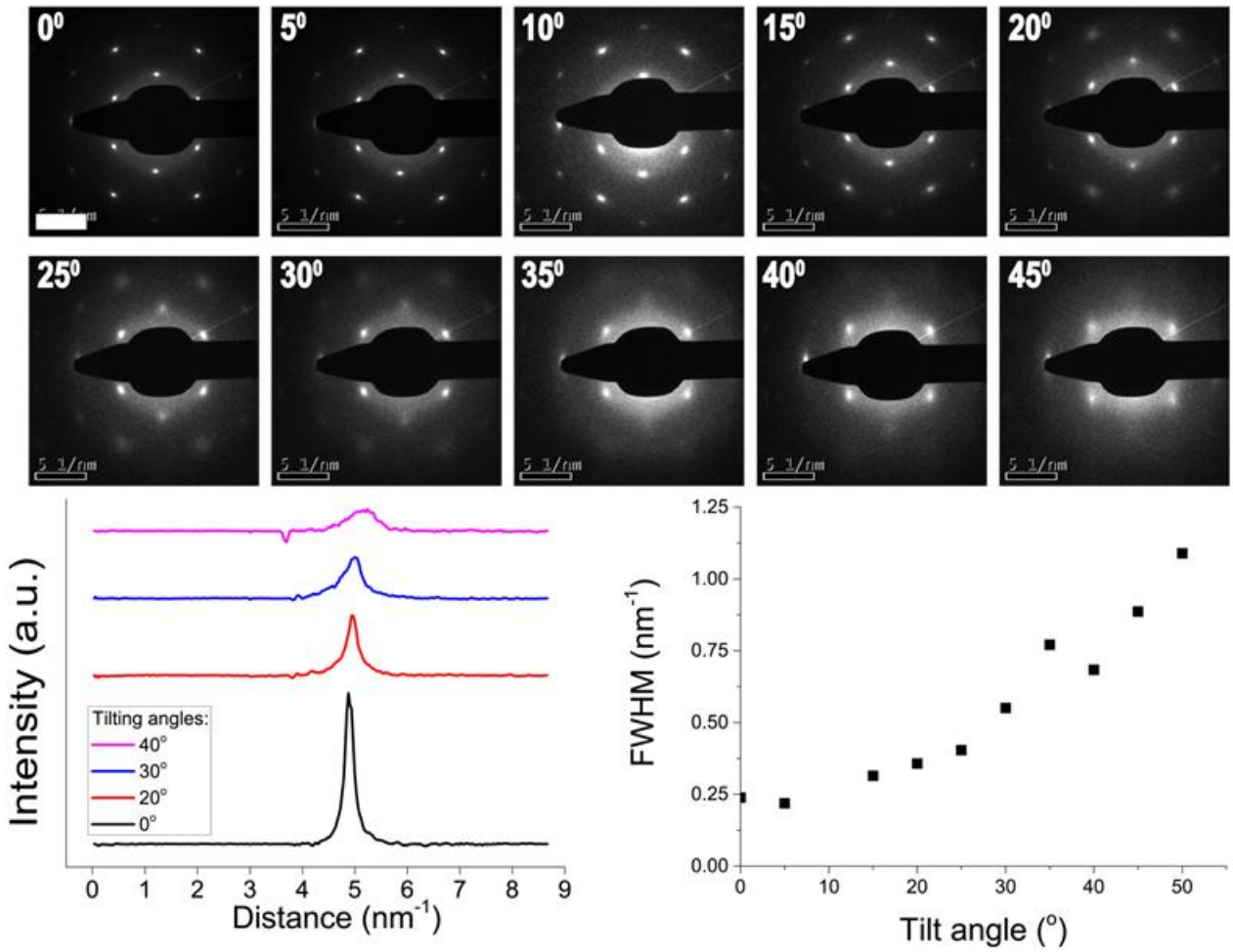

Figure S1: tilting of a GOx monolayer in electron diffraction mode. Electron diffraction patterns of a GOx monolayer with the corresponding integration over the intensities of the (0 −1 1 0) peak.

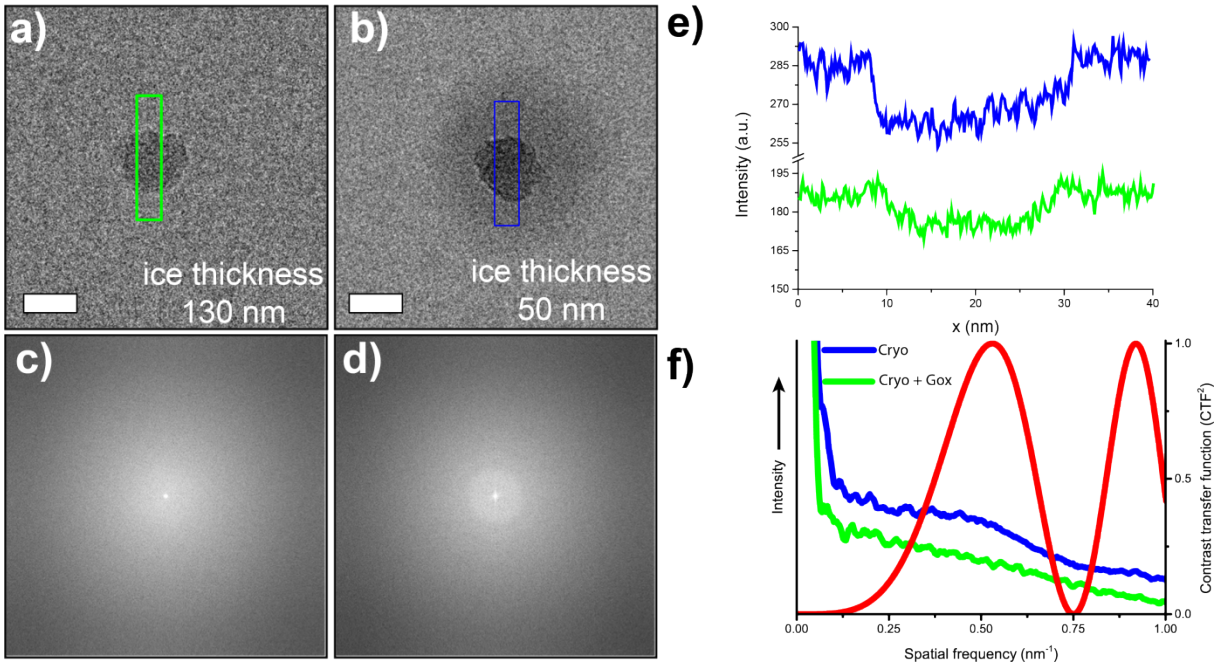

Figure S2: Contrast increase in thinner films. CryoTEM images of silica nanoparticles prepared on (a) a conventional TEM grid and (b) on a graphene oxide substrate with (c,d) the corresponding FFT's, respectively. The intensity profiles are given in (e) to illustrate the contrast enhancement. The yellow arrow in (d) indicates the first maximum in the contrast transfer function (CTF) calculated in (f) for the FEI Titan operating at 300 kV at 61,000x magnification, with a spherical lens aberration of 2.7 mm. Scale bars are 20 nm.

References

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