The observation of square ice in graphene questioned

ARISING FROM G. Algara-Siller *et al. Nature* **519,** 443–445 (2015); doi:10.1038/nature14295

Algara-Siller *et al.*[1] reported the observation of a new phase of water—'square ice'—sandwiched between two graphene layers at room temperature. Their key evidence consists of transmission electron microscope (TEM) images of a square lattice from small encapsulated crystals, the detection of oxygen from relatively large regions containing such crystals and molecular dynamics (MD) simulations indicating 'square ice' formation inside hydrophobic nanochannels. Here we propose that the reported experimental data can be better explained by salt (for example, NaCl) contaminants precipitating as nanocrystals in the dried-out graphene liquid cells[2] and common oxide contaminants in graphene. Consequently, we question the observation of room-temperature 'square ice'. There is a Reply to this Brief Communication Arising by Algara-Siller, G. *et al. Nature* **528,** http://dx.doi.org/10.1038/nature16149 (2015) relating to the electron energy-loss spectra (EELS) and a Reply by Wang, F. C. *et al. Nature* **528,** http://dx.doi.org/10.1038/nature16146 (2015) relating to the MD simulations.

The TEM images and the dynamics of the reported 'square ice' under electron irradiation bear a considerable resemblance to those we have observed of NaCl nanoplatelets in graphene and dried-out graphene liquid cells. Such NaCl nanoplatelets usually orient along the [100] direction, displaying a square lattice with a spacing of approximately 2.8 Å (Fig. 1a, b, d); the corresponding fast Fourier transform (FFT) matches the reported[1] diffraction data. Edge termination, dislocations and grain-boundary structures within the lattice, and the dynamics of

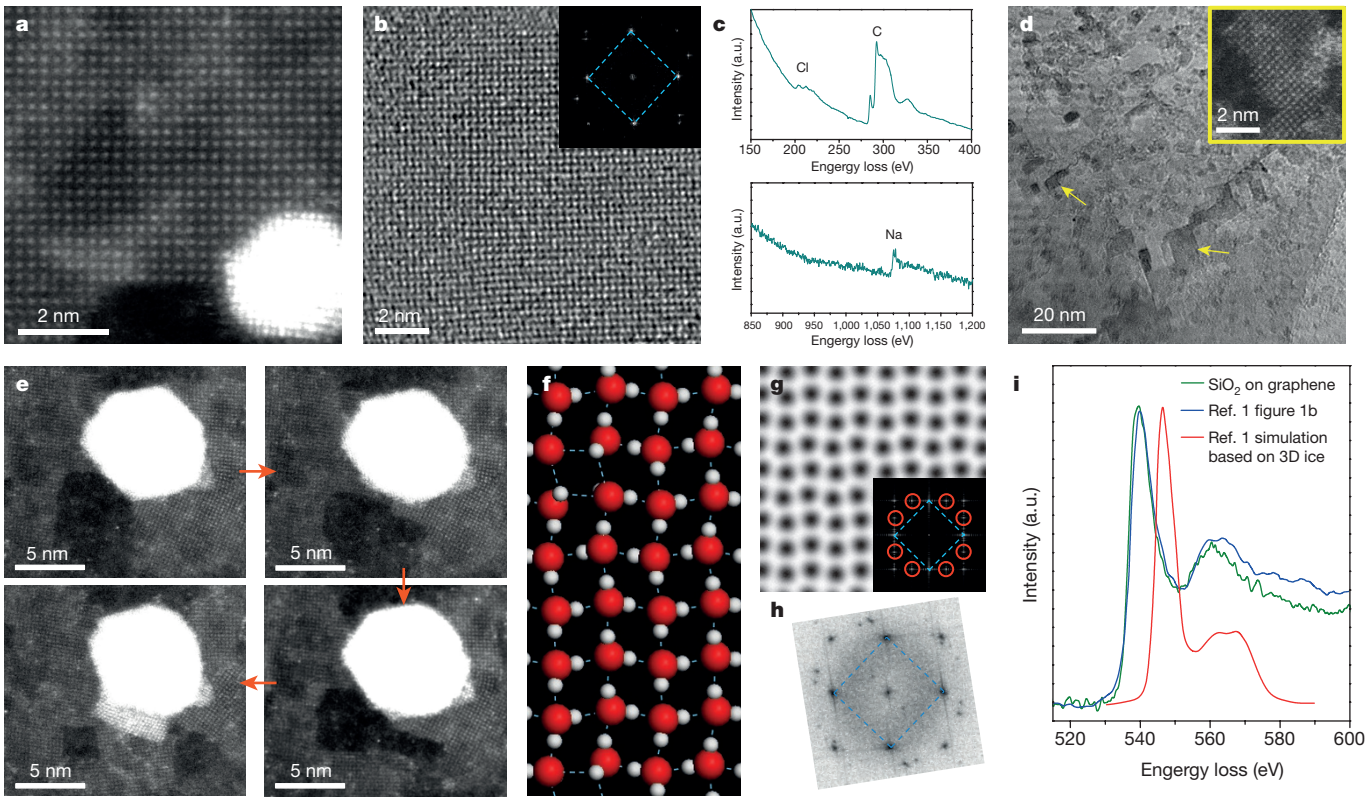

Figure 1 | Structure and analysis of NaCl nanoplatelets and reported 'square ice'. a, Scanning TEM annular dark field (STEM-ADF) image of a NaCl nanoplatelet in graphene. **b**, STEM bright field image (main panel) and the FFT (inset) of a NaCl nanoplatelet. The blue dashed lines highlight the four equivalent {200} planes with 90° interplanar angle. **c**, EELS acquired from a 4 nm × 4 nm region of the NaCl nanoplatelet. The peaks corresponding to chlorine (Cl), carbon (C, from graphene) and sodium (Na) are labelled. a.u., arbitrary units. **d**, TEM bright field image of a dried-out graphene liquid cell containing NaCl residuals (indicated by the yellow arrows) that exhibit chequered patterns similar to those reported in ref. 1. Inset, ADF image taken from a small region of a typical chequered pattern. **e**, Sequential (indicated by the red arrows) STEM-ADF images showing the reconstruction of NaCl crystals under electron beam irradiation, as seen by the constant change of the outline of the thin NaCl crystal. **f**, Ice structure in the graphene layers calculated using DFT. Graphene layers are not shown. Red circles denote oxygen; white circles denote hydrogen; blue dashed lines represent hydrogen bonds. **g**, Simulated image (main panel) and the corresponding FFT (inset) of monolayer 'square ice' based on the structure calculated using DFT. The red circles highlight the additional diffraction spots arising from the rhombic, zig-zag structure; the blue dashed lines highlight the square structure. **h**, FFT adapted from figure 1a in ref. 1; the blue dashed lines highlight the square structure. **i**, Comparison of the experimental oxygen K-edge EELS from '2D ice' (ref. 1) and the corresponding simulated EELS (from 3D ice, adapted from ref. 9 and reported in ref. 1) with our experimental oxygen K-edge EELS from SiO_x particles on graphene.

NaCl nanoplatelets under electron irradiation (Fig. 1e), also resemble what is reported in ref. 1. EELS collected from 4 nm × 4 nm regions inside the nanocrystals have clear chlorine and sodium peaks (Fig. 1c) and indicate the presence of a trace amount of oxygen due to contamination, confirming that these crystals are NaCl.

Although the MD simulations of monolayer water molecules in graphene nanocapillaries reveal a structure similar to that seen in the TEM images of ref. 1, the simulated graphene-confined 2D ice crystals do not have a perfectly square structure, but instead exhibit a zig-zag arrangement of water molecules (figure 2d in ref. 1). In our density functional theory (DFT) calculations (Fig. 1f) and MD simulations, we find that densely packed water molecules inserted between two graphene sheets as a perfect square lattice undergo relaxation to yield a rhombic, zig-zag structure. This rhombic structure, found previously in simulations of 2D water-molecule structures[3,4], is sometimes referred to as 'square ice' to indicate the fourfold coordination of water molecules as opposed to the threefold coordination in conventional ice; to our knowledge, 'square ice' has not previously been used to indicate square symmetry[3,4].

The zig-zag structure reduces the symmetry and generates additional spots in the electron diffraction pattern and FFT (Fig. 1g). The FFT of the reported[1] TEM image (taken over 1 s) lacks these extra spots (Fig. 1h), indicating that the image was generated from a crystal with higher apparent symmetry than that of the ice structure presented in the reported[1] MD snapshot (taken over 1 fs) or seen in our DFT and MD calculations (Fig. 1f). It might be argued that lattice vibrations average out the positions of the oxygen atoms to yield a square lattice; however, the averaged oxygen positions in our MD snapshots taken over 200 ps still have a rhombic structure. In the absence of a demonstration that averaging over macroscopic timescales produces a square lattice, the reported[1] MD simulations do not seem to support the observation of 'square ice'.

We conclude that crystals with a highly symmetric rock-salt structure[5] and atomic column spacing of 2.82 Å, such as NaCl, better account for the TEM images and the corresponding diffraction data presented in ref. 1 than does 'square ice'; the NaCl structure also explains the observed stacking at larger crystal thickness that the reported[1] MD simulation was unable to reproduce.

An experimental oxygen K-edge EELS taken from an area with a diameter of about 100 nm containing the much smaller 'square ice' crystals (about 10 nm in diameter) was also compared[1] to a calculated oxygen K-edge EELS of 3D ice, revealing the spectra to be "qualitatively similar", but with a peak shift of approximately 6 eV. The differences were attributed[1] to those between 3D and '2D square ice'; however, Fig. 1i demonstrates that the reported[1] experimental oxygen K-edge EELS is in nearly perfect agreement with the oxygen K-edge spectrum of SiO_2, a common contaminant in graphene samples. Given that Algara-Siller *et al.*[1] reported the presence of silicon EELS signals throughout their sample, the reported oxygen signal might well have a large contribution from contaminants and thus should not be considered evidence of ice. Furthermore, the EELS data provided in ref. 1 do not rule out the presence of other elements such as sodium and chlorine, which have weak edges or edges outside typical spectrum ranges.

In conclusion, we believe that the experimental data presented by Algara-Siller *et al.*[1] neither provide definitive evidence for the existence of 'square ice', nor agree with their reported theory. We suggest that accidental contamination with NaCl (or another salt with similar structure) and subsequent salt nanocrystal formation better explains the reported experimental data than does 'square ice'. Salts are hygroscopic and so would be associated with any water left in the graphene liquid cells and would precipitate upon water evaporation under electron beam irradiation. Further experimental and theoretical studies are required to assess the existence of 'square ice' in graphene nanochannels.

Methods

Graphene liquid cells containing 0.06 M NaCl solution were prepared using the method described in ref. 2. The liquid cells were dried out inside the TEM by exposing them to high-electron-dose-rate illumination, which generates bubbles and causes the liquid cells to burst. The dried-out cells prepared with dilute NaCl solution have a considerable amount of visible residue, whereas graphene liquid cells prepared with de-ionized water have no visible residue after drying.

High-resolution TEM image simulation (Fig. 1g) was performed on our DFT-calculated structure using the MacTempasX software (http://www.totalresolution.com/MacTempasX.htm) and on the structural model reported in the MD snapshot (figure 2d in ref. 1) using codes provided in ref. 6; very similar results were obtained. Parameters for image simulation are: accelerating voltage, 80 kV; spherical aberration C_s, 30 μm; defocus, −13.4 nm.

Both *ab initio* MD and classic MD simulations were performed to test whether the zig-zag structure of the water molecules could be smoothed to yield a square pattern over a certain length of time. For the *ab initio* MD simulation with a PBE (Perdew–Burke–Ernzerhof) functional[7], 36 water molecules were constrained between two graphene layers at a higher density than that of water at 4 °C and 1 atm (and similar to that in ref. 3). The average positions of oxygen atoms over 2 ps (2,000 timesteps) remain in a rhombic pattern. The classic MD simulation was run using the COMPASS force field[8], and 144 water molecules were constrained between two graphene layers. One oxygen atom was fixed to avoid a translational motion. The overall trajectories of oxygen atoms over 200 ps (200,000 timesteps) still have a noticeable zig-zag structure.

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Algara-Siller *et al.* reply

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In the accompanying Comment[1], Zhou *et al.* showed that a NaCl solution captured between graphene sheets leads to the formation of few-layer crystals of NaCl with similar structure and lattice constant as for the 'square ice' we described[2]. They suggest that our samples were accidentally contaminated with NaCl or another salt and that the oxygen K-edge in our electron energy-loss spectra (EELS) originates from oxide contaminants on graphene.

We emphasize that at no point were our samples in proximity to NaCl or other salts. All our spectra were obtained in diffraction mode with an effective diameter of 100 nm, not high-resolution imaging mode in which individual crystals may be selected, to decrease the electron dose and allow longer acquisition times. In our EELS analysis, we focused on the energy window in which the oxygen peak was expected; the full energy spectrum comparing regions with and without ice crystals was not acquired in all cases. Unfortunately, this means that we cannot retrospectively prove the absence of NaCl. Nevertheless, following our new simulations of transmission electron microscope (TEM) images of NaCl, the difference in contrast between sodium and chlorine should be visible under our imaging conditions in the case of a mono- or trilayer crystal with a half unit cell. We do not find corresponding differences in contrast in any of our experimental images.

We agree with Zhou *et al.*[1] that our oxygen K spectrum in figure 1b in ref. 2 probably has a contribution from silicon oxide, but we believe this contribution is small. There is disagreement in the literature regarding the peak shape and exact position of the oxygen K-edge for ice. In our paper[2], we compared the experimental oxygen K-edge (figure 1b, main oxygen K peak at 540 eV) with a simulated spectrum[3] for which the main peak is shifted by approximately 6 eV compared to our experiment. However, other calculations[4,5] of oxygen K spectra for hexagonal and cubic ice give the oxygen K peak at 540 eV, in agreement with the spectrum in figure 1b in ref. 2. In addition, in our unprocessed oxygen K spectrum, a pre-edge shoulder is seen that is very similar to those in refs 4 and 5. Unfortunately, these weak features are not visible in figure 1b in ref. 2, owing to smoothing of the raw spectrum. Only EELS in high-resolution imaging mode selecting individual crystals (or scanning TEM-EELS) could unambiguously distinguish such features.

In view of the above, further experiments are needed to rule out the contamination hypothesis.

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Wang *et al.* reply

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In the accompanying Comment[1], Zhou *et al.* reproduced our[2] molecular dynamics (MD) results and pointed out that the simulated 2D ice is slightly rhomboidal, in contrast to the square lattice seen in the transmission electron microscope (TEM) images[2]. We were aware of this disagreement, but did not discuss it in ref. 2 for the following reasons. First, previous MD simulations[3,4] have reported 'square ice', although it remains unclear whether this ice is different to the distorted lattice we found[2]. Second, and more importantly, we were convinced that the simulated, slightly rhomboidal structures should be observed experimentally as square ice.

Indeed, our MD snapshots[2] (and those presented in ref. 1) show substantial disorder. Each realization is metastable, and the finite temperature is expected to move such defects through the crystal lattice. Our simulations show that this happens on a timescale of tens of nanoseconds for nanometre-sized ice crystals, much longer than the time used by Zhou *et al.*[1], but much shorter than the time needed to obtain experimental images (about 1 s). To simulate this time-averaging effect, we created a number of intermittent states (such as that shown in figure 2d in ref. 2) and superimposed them, keeping the positions of only the edge molecules fixed to simulate the confinement. We found that the slightly rhomboidal lattice averaged out into one that is indistinguishable from a perfect square (not shown in ref. 2).

Finally, perfectly square ice discussed in ref. 2 was subsequently found to be the most stable configuration using first-principle analyses[5,6]. Therefore, we maintain that square ice can theoretically occur in hydrophobic nanocapillaries, in agreement with the experiment[2].

R. R. Nair and I. V. Grigorieva support this Reply, but did not contribute to the part of research that was addressed in the accompanying Comment.

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