Materials science

Isotopes tracked on a sub-nanometre scale

Jordan A. Hachtel

Measurements of atomic vibrations can now identify chemical isotopes on a sub-nanometre scale in an electron microscope. An innovative approach makes use of this resolution to build and track isotopic domains. **See p.68**

The isotopes of an atom have the same number of protons, but different numbers of neutrons. Changing the neutron count changes the mass of an atom, but it doesn't have a pronounced effect on its chemical properties. As a result, isotopes feature in a host of techniques used in biological and materials sciences, where they function as atomic-scale labels that can track specific compounds in heterogeneous samples and can trace chemical-reaction pathways. To make the most of isotopic labelling, isotopes must be detected with high spatial resolution. On page 68, Senga *et al.*[1] report that, using a specialized electron microscopy technique, they have identified isotopic labels with sub-nanometre spatial resolution, allowing the behaviour of the labelled atoms to be revealed as they move around the crystal.

There are two fundamental ways to identify isotopic labels. The most direct method is mass spectrometry, in which a material is broken down into individual atoms or molecules, given electrical charge, then electrically accelerated towards a detector that measures the mass-to-charge ratio. Some mass spectrometry techniques, such as atom probe tomography, can achieve sub-nanometre spatial resolution[2]. However, such techniques require the atoms and molecules to be separated from the material in a controlled manner. This limits the types of sample that can be analysed, and allows only a single measurement per sample.

The alternative approach uses vibrational spectroscopy. Specific arrangements and types of chemical bonds in a material produce atomic oscillations that give rise to collective vibrations (called phonons or vibrational modes), and the frequency of these vibrations depends directly on the mass of the individual atoms involved in the bond. Thus, isotopic labels can be identified through shifts in the frequency of the vibrational modes. A wide range of spectroscopy techniques can measure these vibrational frequencies, using photons, electrons or neutrons, without destroying the sample. This means that vibrational spectroscopy can be applied to a much wider variety of samples than can mass spectrometry, and that many measurements can be made, greatly increasing the versatility of the approach.

The vibrational spectroscopy techniques with the highest spatial resolution originally involved scanning-tip optical spectroscopy, which uses the metal-coated tip of an atomic force microscope or of a scanning tunnelling microscope. The tip creates a highly localized interaction with the material, which, in certain circumstances, enables vibrational spectroscopy with up to sub-nanometre resolution[3]. But, in practice, these methods are limited by the nature of the interaction between the tip and the sample, and artefacts can occur in samples that have uneven surfaces; this usually restricts the spatial resolution to between 5 and 10 nanometres.

However, a breakthrough came in 2014 with an innovative design[4] for a type of device called an electron monochromator, which is used in scanning transmission electron microscopy to reduce the spread of energies present in the electron beam to an extremely narrow band. Use of this new monochromator design enabled the spectral resolution of a technique known as electron energy-loss spectroscopy to reach single-digit millielectronvolt resolution. It reached a level sufficient to measure vibrational modes, while retaining sub-nanometre spatial resolution. Since then, many studies have used this approach to report exceptionally high spatial resolution in systems of nanoparticles[5,6], superlattices[7], interfaces[8,9], planar defects[10] – and even single atoms[11].

The spectral resolution of monochromated electron energy-loss spectroscopy is sufficient to distinguish between the vibrational modes of different isotopic labels[12]. However, to demonstrate that the technique's high spatial resolution can be used for isotopic analysis, a sample is required that contains isotopic differences on a sub-nanometre scale. Here is where Senga and colleagues' approach truly advances the field.

The team studied cracks occurring in large flakes of graphene, which is a single layer of carbon atoms (Fig. 1a). Such cracks self-heal under electron irradiation[13], because the small amount of residual gas left in the high vacuum of the electron microscope acts as a kind of thread to stitch the cracks back together. The carbon in the residual gas is the dominant naturally occurring isotope (^{12}C), so Senga *et al.* used isotopically enriched ^{13}C-graphene

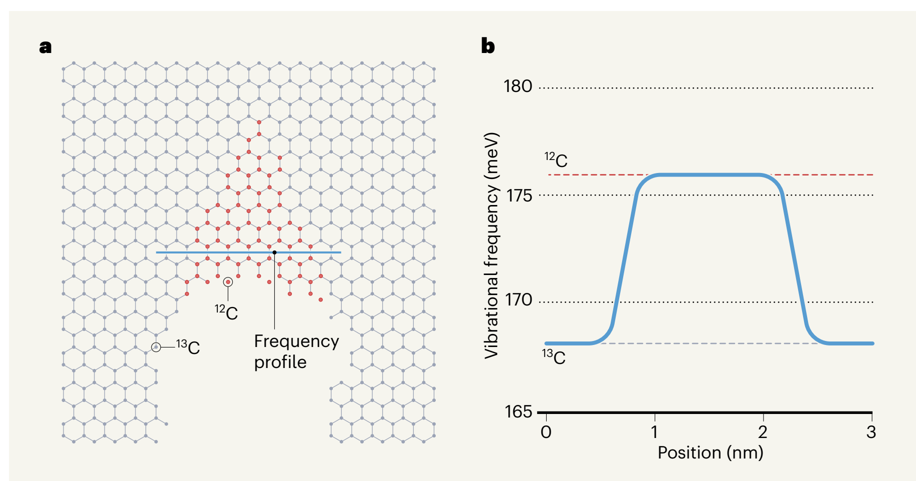

Figure 1 | Building and measuring nanoscale isotopic domains in an electron microscope. Cracks in graphene (a single layer of carbon atoms) can be healed under irradiation from an electron microscope, by carbon atoms from the residual gas left in the high vacuum of the microscope. **a**, Senga *et al.*[1] used this approach to heal cracks occurring in graphene flakes made from the carbon isotope ^{13}C, which has one neutron more than the naturally occurring ^{12}C isotope. The carbon in the residual gas was ^{12}C, so the healing process created nanoscale domains of ^{12}C in a ^{13}C-graphene crystal. **b**, The authors then used a technique called monochromated electron energy-loss spectroscopy to detect subtle changes in the vibrational frequency of the carbon atoms caused by the added mass of the extra neutron, achieving sub-nanometre spatial resolution. Positions in the frequency profile shown in **b** correspond to locations along the blue line shown in **a**.

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as a base and allowed the cracks to be stitched back together with ^{12}C-graphene to create a localized patch of isotopic heterogeneity. The regions in which the cracks healed were only a few nanometres wide, and each contained a sharp interface between ^{12}C and ^{13}C isotopes. This inspired methodology can rigorously and reproducibly generate samples on which nanoscale isotopic analysis can be performed using monochromated electron energy-loss spectroscopy in a scanning transmission electron microscope (Fig. 1b).

There are also challenges associated with the use of graphene: the signal from such a thin sample is extremely low, and the vibrational shifts are three times smaller than the energy resolution of the instrument. The authors overcame these limitations through careful data analysis and thorough validation. By modelling the potential vibrations in graphene, they were able to unambiguously distinguish the ^{12}C-graphene vibrational frequency on the ^{13}C background. Moreover, they eliminated defects as a potential cause of the measured shift by performing control tests on ^{13}C-graphene flakes that had a high concentration of defects, and on ^{12}C-healed cracks on ^{12}C-graphene bases, neither of which produced a shift comparable to the one measured at the isotopic interface.

More crucially, Senga *et al.* performed the measurement multiple times on the same sample, which offered unique insight into isotopic dynamics in graphene. When they heated the sample at high temperatures for two hours and then repeated the measurement, they observed that the localized domain of ^{12}C had vanished – the isotopes had diffused into the surrounding material and only the ^{13}C signal remained. This observation allowed the authors to directly measure the diffusion of carbon atoms in the homogeneous graphene lattice. Previously, such measurements have been limited to materials with defects and impurities[14], but vibrational electron energy-loss spectroscopy enables isotopic labels to be tracked in a pristine crystal.

Senga and colleagues' work signals the advent of nanometre-scale isotopic analysis in the electron microscope. It has major implications for the study of biological materials in which isotopic labels are used to track chemical-reaction pathways, because it brings unprecedented length scales to these tried-and-tested techniques. Furthermore, the technique opens avenues for exploration using electron microscopy, such as the study of heterostructures consisting of materials containing different isotopes, or *in situ* nanoscale isotopic tracking during heating or application of an electric voltage.

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Neurobiology

Visual identity isn't a light decision for all neurons

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When mouse pups first open their eyes, what they see shapes neuronal connectivity. A study shows that this visual experience has cell-type-specific effects, acting only on a subset of malleable neurons.

Environmental stimuli have a crucial role in shaping a developing organism's behaviour. Birds readily accept foster parents — even human ones — provided they see them at birth[1]. Cats raised in an environment consisting only of vertical stripes are later unable to see horizontal lines[2]. Even (brainless) bacteria must react to changes in environmental lactose to keep on thriving and dividing[3]. Distinguishing inborn (nature) from acquired (nurture) facets of development is thus a fundamental quest in biology, and particularly so in the developing brain. But, despite decades of research, we do not understand precisely how the environment shapes the brain at the molecular, cellular and circuit levels. Writing in *Cell*, Cheng *et al.*[4] take on this challenge by investigating how postnatal visual experience affects distinct neuronal cell types in the maturing mouse brain.

Mouse pups open their eyes and start exploring their environment at around two weeks of age. Cheng and colleagues collected cells from the brains of mice at 6 time points between 8 and 38 days after birth. The group focused on the primary visual cortex — a part of the brain that integrates information coming from the eyes. They isolated thousands of cell nuclei from this region in each animal and sequenced their RNA, which codes for the cells' proteins. They then used advanced bioinformatics to classify the cells on the basis of the types of gene that they expressed[5], producing a cell atlas for the primary visual cortex.

Cortical neurons are organized into layers. Deep-layer neurons connect deep within the brain to reach the spinal cord, whereas superficial-layer neurons interconnect to form intracortical networks that integrate sensory and motor information[6]. Cheng and colleagues' atlas indicated that neurons in the superficial layer refine their identities after birth, whereas deep-layer neurons do not.

Next, the authors asked how being reared in the dark would affect the development of these visual cortical neurons. The researchers kept mice in a dark environment from 21 to 28 or 38 days of age (a period essential for vision-dependent brain development[7]). They then compared the gene-expression profiles of light- and dark-reared mouse pups. They found that different types of visual cortical neuron have different sensitivities to visual experience. Some cell populations relied on ocular input to mature and differentiate, whereas others were unaffected by an absence of light in the environment.

Specifically, Cheng *et al.* showed that neurons in the superficial layer are particularly susceptible to visual input. In the absence of light, superficial-layer neurons remained immature, such that crisply defined cell subtypes failed to emerge. By contrast, deep-layer neurons remained impervious to visual deprivation and matured adequately (Fig. 1). The authors found that re-exposure to light after ten days of darkness partially restored the maturation of superficial-layer neurons. This indicates that lack of visual stimulation does not irreversibly jam neuronal specification; rather, neuronal identity remains sensitive to light over long periods of time.

Single-nucleus RNA sequencing provides information about the expression of thousands

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