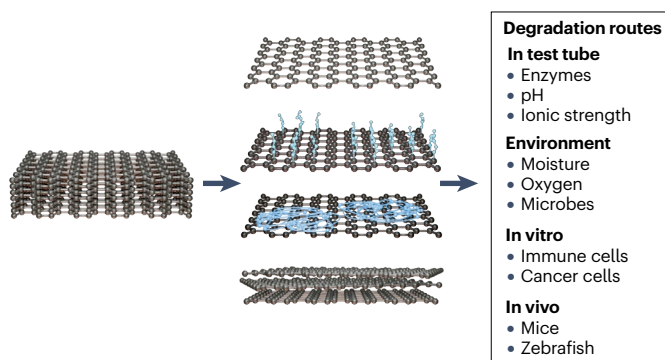


# Biological and environmental degradation of two-dimensional materials

Sandra Vranic<sup>1,2,7</sup>, Rajendra Kurapati<sup>3,7</sup>, Kostas Kostarelos<sup>2,4,5</sup>✉ & Alberto Bianco<sup>6</sup>✉

## Abstract

As the use of two-dimensional materials continues to grow, so too does the need to understand the environmental and biological impact of such materials. Degradation is a critical step in the life cycle of any material, but the majority of such knowledge is obtained from test tube and in vitro studies. Therefore, there remains a gap in understanding the degradability of two-dimensional materials in complex systems (in vivo) and in different ambient environments. In this Review, we highlight the need for more data-driven studies on the degradation of two-dimensional materials, including their kinetics, by-products, stability and possible downstream effects. Although challenging, building an understanding of the degradation profiles of different advanced materials in various environments at the chemical and molecular level is essential.



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A full list of affiliations appears at the end of the paper. ✉ e-mail: [kostas.kostarelos@manchester.ac.uk](mailto:kostas.kostarelos@manchester.ac.uk); [a.bianco@ibmc-cnrs.unistra.fr](mailto:a.bianco@ibmc-cnrs.unistra.fr)

## Introduction

The development of advanced materials, including those based on graphene and other two-dimensional materials (2DMs), must align with the requirements for sustainability and a manageable total product life cycle<sup>1</sup>. Both the acute and longer-term environmental and biological health impacts from exposure or accumulation of such materials have to be determined in parallel, if not before, their mass production. At present, only exposure of a specific material and the risk and safety limitations to human health are usually considered. However, another facet that must be considered in the development and adoption journey of an advanced material, which is commonly ignored or considered of less importance, is degradability.

The development and mass adoption of plastic materials over the past century is clearly illustrative of the environmental and health issues that will need to be addressed before the next generation of materials reach mass industrialized use. Data-driven approaches are critical for understanding the degradation trends and kinetics of plastics, by linking abiotic and biotic degradation behaviours in different environments with physical properties and molecular structures<sup>2,3</sup>. Such analyses can reveal a hierarchy of predictors to quantify surface erosion, as well as combinations of features (like glass transition temperature and hydrophobicity) to classify plastics into fast, medium and slow degradation categories<sup>2</sup>. Mapping novel materials in relation to plastics using *in silico* and machine learning tools can provide an invaluable tool in prediction and regulatory framework design. An initial proposition in this direction for 2DMs (not including graphene-based materials) has been proposed by Shukla et al., who used a data-driven analysis to classify materials into ‘biosoluble’, ‘biodegradable’ and ‘persistent’ categories, based on literature-reported experimental and theoretical degradation studies<sup>4</sup>.

In an attempt to provide a framework of degradation, we have identified four ‘degradation environments’ for 2DMs: 1) in test tube degradation, incubating the material with enzymes, buffers at different pH, salts, ions and protein compositions, or in simulated body fluids; 2) environmental degradation, outside of an organism, such as degradation by ambient oxygen, moisture and sunlight, and by bacteria and fungi in the environment; 3) *in vitro* degradation, mainly within mammalian cells; and 4) *in vivo* degradation, within living mammalian (such as rodents) and non-mammalian (such as zebrafish) species (Fig. 1a).

In the context of this article, 2DMs include the graphene-family materials (GFMs), transition metal dichalcogenides (TMDCs) (such as MoS<sub>2</sub> and WS<sub>2</sub>), non-metallic hexagonal boron nitride (h-BN), graphitic carbon nitride (g-C<sub>3</sub>N<sub>4</sub>), monoelemental Xenes (such as phosphorene and borophene), transition metal carbides, nitrides and carbonitrides called MXenes (such as Ti<sub>3</sub>C<sub>2</sub> and Ti<sub>2</sub>N), and transition metal oxides (such as MnO<sub>2</sub> and TiO<sub>2</sub>) (Fig. 1b). In general, 2DMs show higher reactivity towards oxidative conditions than their bulk counterparts owing to their higher surface area and structural distortions<sup>5</sup>. Therefore, interrogating the chemical stability of exfoliated 2DMs is necessary to understand their behaviour and applications. The intrinsic chemical structure and the strength of the bonds of the respective 2DMs (such as C–C, C–O, P–P, B–N and S–Mo–S bonds), the nature of the chemical functionalization (covalent or non-covalent), and the thickness of materials (single or consisting of a few layers) are the main factors that determine whether a material undergoes degradation under environmental and test tube conditions<sup>6,7</sup>. Furthermore, the crystalline phase of 2DMs (such as 2H phase or 1T phase in case of TMDCs) is also crucial for assessing their chemical stability.

In the first part of this article, we discuss the impact of the structure and surface properties (including functionalization) of 2DMs on their degradation by test tube and environmental conditions, including the mechanisms of degradation, when available<sup>8–12</sup>. We then summarize the efforts to understand the degradability of 2DMs in living systems (in mammalian cells and *in vivo* within living organisms), discuss the by-products of degradation and provide an outlook of the challenges and need to determine the characteristics of degradation before mass-scale use becomes prevalent.

## In test tube degradation of 2DMs

A large body of work on 2DM degradation has studied in test tube degradation, which involves treating the material with isolated oxidative enzymes or peroxidases (from living organisms), simulated body fluids (such as gastric juices), or environmental degradation conditions. In test tube degradation studies are particularly valuable to decipher the mechanisms and kinetics of material degradation and interrogate interactions with specific molecules and molecular combinations.

### Graphene-based materials

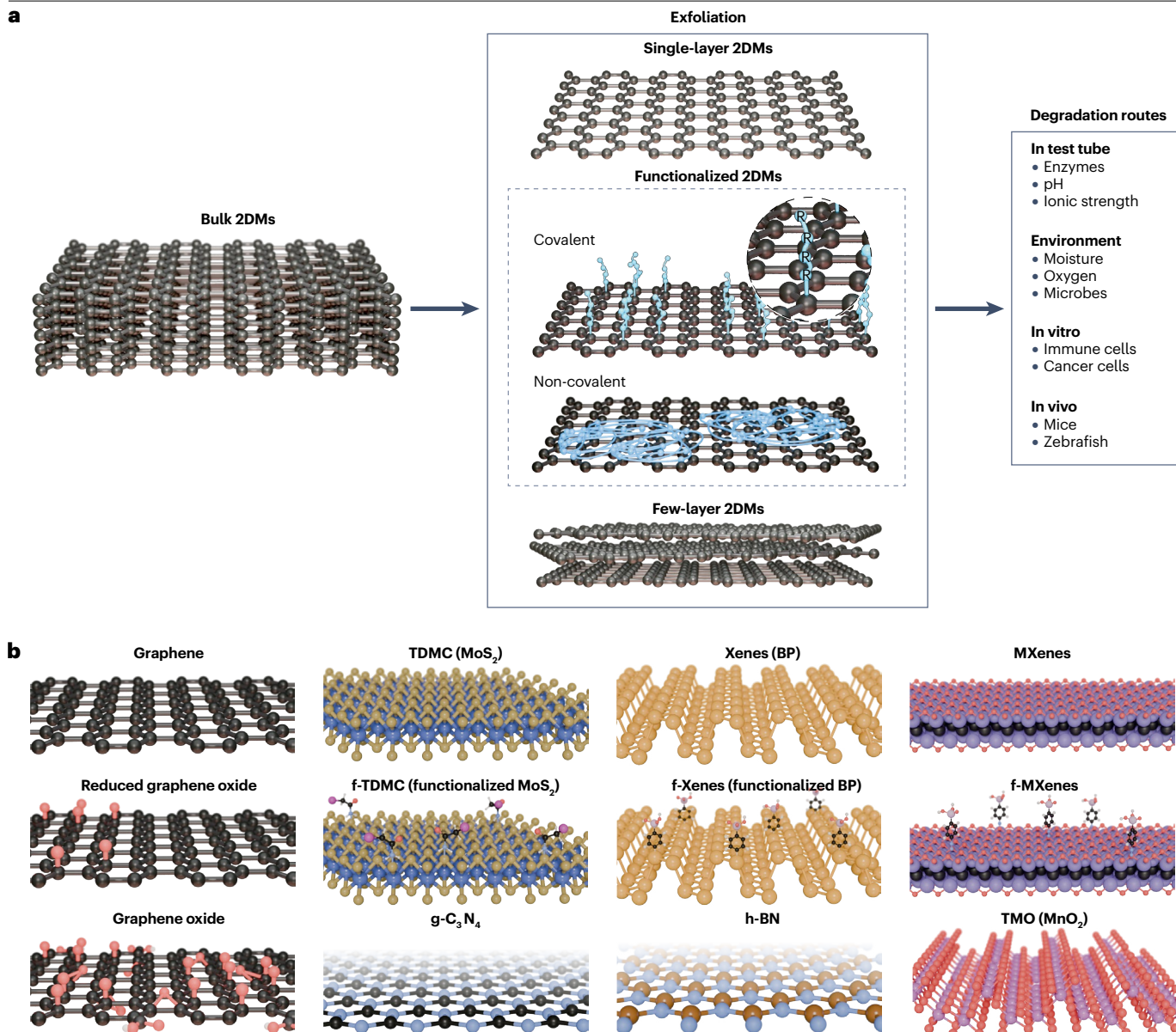
Graphene exhibits a higher chemical resistance to environmental oxidation than graphene oxide (GO) owing to its extended *sp*<sup>2</sup>-hybridized carbon network. GO comprises small graphitic areas within an extended amorphous structure, in turn made of *sp*<sup>3</sup>-hybridized carbons and oxygenated functional groups<sup>8</sup>. GO can undergo biodegradation in test tube when treated with horseradish peroxidase (HRP), whereas reduced GO (rGO) is inert to this enzyme (see Fig. 2a and Supplementary Table 1 for more details)<sup>10</sup>. Interestingly, non-covalent functionalization of GO with bovine serum albumin (BSA) and polyethylene glycol can prevent the degradability of GO by HRP. By contrast, the covalent functionalization of GO with BSA and polyethylene glycol bearing a cleavable disulphide linker facilitates degradability, most probably due to further structural defects introduced in the graphene lattice during covalent bond formation<sup>13</sup>.

We have previously proposed that degradation-by-design can be achieved for different types of carbon nanomaterials, including GO<sup>14</sup>. According to such strategies, the degradability of GO can be enhanced or accelerated by covalent functionalization with appropriate bioactive molecules, such as catechol and coumarin. This further demonstrates the importance of surface modifications (by non-covalent coating or chemical functionalization) of GFMs as tools to tune their biodegradability kinetics.

GO was also shown to be degraded by human myeloperoxidase (hMPO)<sup>15</sup>, eosinophil peroxidase (EPO)<sup>16</sup> and lignin peroxidase (LPO, from fungi)<sup>17</sup>. Graphene, few-layer graphene (FLG) and graphene nanoribbons showed only partial degradability by hMPO due to them having stronger chemical and structural resistance than GO<sup>18–21</sup>. Artificial enzymes (such as a complex of hemin with G-quadruplex) mimicking peroxidases could also degrade GO similarly to the action of HRP<sup>22–24</sup>.

In addition to the peroxidase-mediated degradation (MPO, EPO, LPO, HRP), other *in test tube* studies have been performed, such as GO incubation with peroxyxynitrite radical (OONO<sup>•</sup>) generators like xanthine/xanthine oxidase or propylamine propylamine NONOate mimicking the microenvironment in activated M1 macrophages<sup>25,26</sup>. In this case, GO resulted fully degraded within 5 days of treatment with *in situ*-produced OONO<sup>•</sup>, confirming the potent ability of the reactive oxygen species found in immune cells to degrade GO<sup>26</sup>.

An alternative *in test tube* approach for degradation has been explored to help understand the biotransformation of 2DMs mimicking



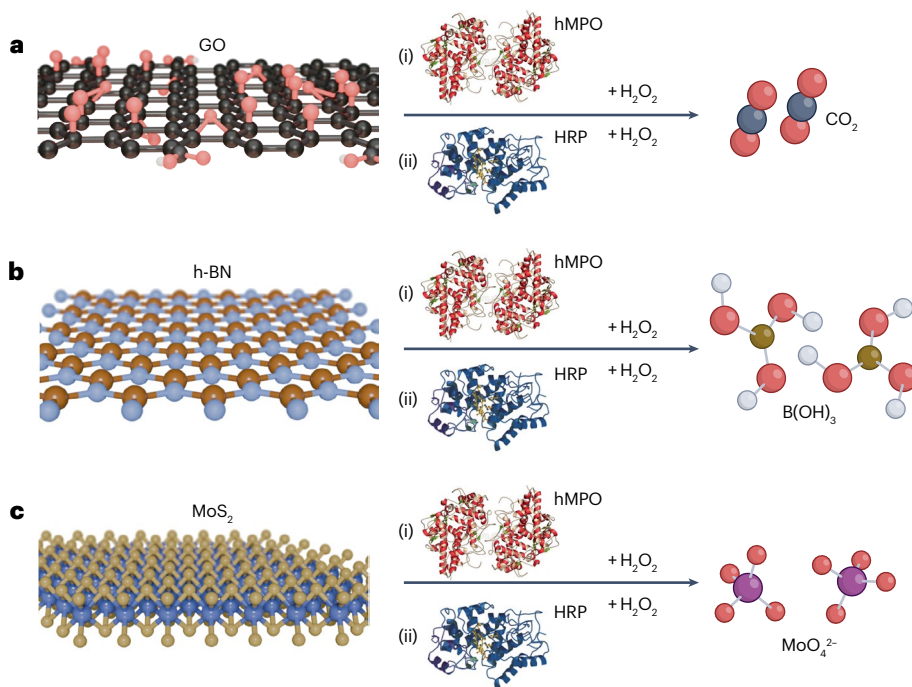
**Fig. 1 | Biological and environmental degradation routes of 2D materials.**  
**a**, Following the exfoliation of bulk two-dimensional materials (2DMs) and their categorization into pristine (single or few layers) or surface-functionalized (covalent modification with different types of functional groups, symbolized by R blobs bound to the material through different types of linkers, symbolized by the curved line, or non-covalent functionalization with polymers, proteins or polysaccharides, symbolized by the curled line), a material can be degraded in four different environments, including test tube degradation (mimicking body or ambient oxidative conditions), environment degradation (involving atmospheric oxygen, moisture, sunlight and microbial species, such as bacteria and fungi), in vitro degradation using mainly mammalian cells, and in vivo model

degradation using rodent and zebrafish models. **b**, Chemical structures of 2DMs including the main graphene family materials (graphene, reduced graphene oxide and graphene oxide), inorganic 2D materials, such as pristine transition metal dichalcogenides (TDMCs) and covalently functionalized TDMCs (f-TDMC) conjugated with acetamide ( $-\text{CH}_2-\text{CO}-\text{NH}_2$ ) through a S–C bond, hexagonal boron–nitride (h-BN), graphitic carbon nitride (g-C<sub>3</sub>N<sub>4</sub>), mono-elemental Xenes (BP) and covalently functionalized Xene BP (f-BP) with aryl diazonium salts, transition metal carbides or metal nitrides (MXenes, such as Ti<sub>3</sub>C<sub>2</sub>TX), functionalized MXenes (f-MXenes), and transition metal oxides (TMO, such as MnO<sub>2</sub>). Element colour coding: C, black; O, red; S, yellow; Mo, blue; N, cyan; B, brown; P, golden yellow; H, white; metals (Mn or Ti), silver, cyan or purple.

different environments of living tissue, by simulating the gastrointestinal (GI) tract fluids, human lung fluid and blood plasma<sup>27,28</sup>. The biotransformation of GO was studied when exposed to simulated lung fluids, such as Gamble's solution, mimicking the interaction between

GO and the interstitial fluid deep within a healthy lung, and artificial lysosomal fluid simulating phagocytosis by lung macrophages. Surprisingly, in those studies, GO was reported to undergo substantial chemical transformation to become rGO<sup>29</sup>. Human ingestion of 2DMs





**Fig. 2 | In test tube model biodegradation of 2D materials.** In a typical experiment, the 2D material (for example, graphene oxide (GO), hexagonal boron nitride (h-BN) and  $\text{MoS}_2$ ) is dispersed in aqueous buffer incubated with a peroxidase enzyme (for example, horseradish peroxidase (HRP) or human myeloperoxidase (hMPO)) in the presence of a low-concentration of hydrogen peroxide. **a**, The oxidation reaction of GO occurring in the test tube leads to the formation of  $\text{CO}_2$  (liberated in the atmosphere). **b**, The oxidation reaction of h-BN occurring in the test tube leads to the formation of soluble  $\text{B(OH)}_3$ . **c**, The oxidation reaction of  $\text{MoS}_2$  occurring in the test tube leads to the formation of soluble  $\text{MoO}_4^{2-}$  ions.

was simulated by exposing GFMs to the digestive environments found in the GI system (salivary, gastric and duodenal fluids, along with bile and  $\text{NaHCO}_3$  to mimic the intestinal tract)<sup>30,31</sup>. Agglomeration and folding of the materials was observed, as well as chemical doping effects and a degree of reduction. However, no chemical oxidation and no degradation occurred for either GO or FLG.

Finally, the effect of pH has also been investigated in test tube<sup>32,33</sup>. In particular, an acidic pH ( $\leq 2$ ) simulating the stomach (or intestinal tract) environment is vital for understanding the oxidation stability of orally exposed 2DMs<sup>30</sup>. In this regard, exposure to such acidic solutions resulted in a minor increase of the C/O ratio for both GO or graphene nanoplatelets (aggregated graphene sheets) according to X-ray photoelectron spectroscopy analyses, suggesting a degree of surface reduction taking place.

## Inorganic 2D materials

Transition metal dichalcogenides (such as  $\text{MoS}_2$ ,  $\text{WS}_2$ ,  $\text{MoSe}_2$ ,  $\text{MoTe}_2$  and  $\text{WSe}_2$ ) are some of the most widely studied 2DMs. As with GFMs, in test tube approaches have been utilized to understand the biodegradability of inorganic 2DMs, most commonly by incubation with enzymes obtained from mammalian cells. Although  $\text{MoS}_2$  was completely degraded by hMPO, covalent functionalization that introduced S–C bonds (Fig. 1b) could reduce the degradation rate<sup>34</sup>. h-BN and g- $\text{C}_3\text{N}_4$  have displayed the strongest chemical resistance to oxidation among all other GFMs and TMDs<sup>5,8</sup>. The B–N bonds in h-BN are in a covalent network, and the degradation of h-BN could occur more readily by disruption of the B–N bond through B–O bond formation upon treatment with hMPO, leading to boric acid as the final product<sup>35–37</sup> (Fig. 2b).

Transition metal oxides are another interesting class of 2DMs that includes  $\text{MnO}_2$  (Fig. 1b),  $\text{MoO}_3$ , other molybdenum oxides ( $\text{MoO}_x$ ) and  $\text{TiO}_2$  (ref. 38). In test tube studies have shown that the reduction of

$\text{Mn}^{4+}$  in  $\text{MnO}_2$  to  $\text{Mn}^{2+}$  ions occurs through the action of intracellular glutathione<sup>39,40</sup>, whereas  $\text{MoO}_x$  exhibits pH-dependent degradability<sup>41,42</sup> (see also Supplementary Table 3 for more details). Studies exposing inorganic 2DMs such as  $\text{MoS}_2$  and MXenes (such as  $\text{Ti}_3\text{C}_2\text{X}$ ) to simulated tissue fluids are currently lacking. Until now, only the biodegradation of  $\text{Nb}_2\text{C}$ , another MXene material, was studied using hMPO in in test tube<sup>43</sup>. However, given that inorganic 2DMs are chemically less robust than GFMs, it is anticipated that they would undergo rapid oxidation, leading to degradation in physiological fluids<sup>34,44</sup>.

Indeed, some inorganic 2DMs, such as  $\text{MoS}_2$  and black phosphorus sheets, have been reported to undergo pH-dependent oxidation and degradation<sup>45</sup>.  $\text{MoS}_2$  sheets showed oxygen-induced degradation under ambient conditions. This process is accelerated as the pH changes from acidic to alkaline, and this acceleration is explained by the sheets achieving a better colloidal dispersion at basic pH<sup>46</sup>. Similarly, layered black phosphorus sheets underwent rapid degradation in alkaline conditions compared with acidic conditions, owing to the oxidation of the P–P bonds by hydroxide ions to form P–O bonds<sup>45</sup>. Other 2DMs like germanium telluride sheets showed more rapid degradation at pH 2 than at pH 8, as rapid oxidation of Te took place<sup>47</sup>.

## Environmental degradation of 2DMs

Environmental degradation of 2DMs is achieved either by natural conditions (air, sunlight, water) or under the effect of the organisms, such as primary decomposers (bacteria, fungi) and some insects. According to current understanding, environmental degradation does not need to involve ingestion or uptake of 2DMs by organisms, but will happen outside of the biological system.

## Degradation by natural ambient conditions

The effect of water, humidity and air exposure on GFMs has not been thoroughly studied because it was hypothesized that these conditions

induce negligible changes. GO is the most surface-reactive GFM, and it gradually degrades in ambient air and water, with sunlight able to promote its transformation into oxidized polyaromatic hydrocarbons (PAHs) owing to the presence of photoreactive oxygen groups on GO's surface<sup>48</sup>. TMDCs were also found to undergo faster degradation in ambient conditions (air and moisture) than GFMs, as a result of their defect sites and grain boundaries<sup>49</sup>. For example, MoS<sub>2</sub> in ambient air underwent slow oxidation to form MoO<sub>3</sub>, and it degraded to soluble MoO<sub>4</sub><sup>2-</sup> and SO<sub>4</sub><sup>2-</sup> ions in aqueous solutions<sup>6,46,49</sup> (Fig. 2c and Supplementary Table 2). Degradation of TMDCs has also been shown to be dependent on their crystalline phase. Group VI metallic 1T-phase crystals (including MoS<sub>2</sub>) are chemically unstable with faster oxidation over the 2H-phase TMDCs<sup>36,46,50,51</sup>. Similar to chalcogenides, selenides (such as WSe<sub>2</sub> and MoSe<sub>2</sub>) and tellurides (such as MoTe<sub>2</sub> and WTe<sub>2</sub>) can also be oxidized in ambient air<sup>6,52,53</sup>.

Overall, the chemical stability of TMDCs is gradually reduced by varying the chalcogen atoms from sulfur to selenium to tellurium, in correlation with the decrease of their electronegativity. This results in higher electron transfer from the metal (M) to oxygen, forming more stable and stronger M–O bonds<sup>6</sup>. For the same type of dichalcogenides, WX<sub>2</sub> (X = S, Se), the W–S bond is stronger than the W–Se bond, whereas for the same chalcogen (X), WX<sub>2</sub> showed higher oxidation than MoX<sub>2</sub>, because W has a reducibility greater than Mo<sup>6</sup>. Non-covalent surface modification (such as coating with polymers, h-BN or alumina) and covalent functionalization (for example with alkyl halides forming S–C bonds) of TMDCs considerably improved their chemical stability in ambient/aqueous conditions<sup>6,49,54</sup> (Supplementary Table 2). Similarly, g-C<sub>3</sub>N<sub>4</sub> has been reported to be resistant to environmental oxidation due to the presence of the tris-triazine units, providing sp<sup>2</sup>-hybridization of N–C bonds, leading to higher stability<sup>55,56</sup>.

Unlike graphene (sp<sup>2</sup>-hybridized), 2D Xenes made of Si, Ge and Sn have a buckled hexagonal honeycomb geometry with large interatomic distances and mixed sp<sup>2</sup>–sp<sup>3</sup> hybridization, whereas phosphorene (also black phosphorus) has a puckered geometry (sp<sup>3</sup>-hybridized)<sup>57</sup>. The structural arrangement of the atoms into buckled or puckered geometries causes high surface sensitivity to ambient moisture and oxygen, resulting in the rapid oxidation of 2D Xenes, with borophene being the most chemically stable of these species<sup>57,58</sup>. In the case of phosphorene, P lone pairs are responsible for its rapid oxidation, readily reacting with ambient oxygen to form phosphorus oxides and phosphorus ions (such as PO<sub>3</sub><sup>3-</sup> or PO<sub>4</sub><sup>3-</sup>)<sup>45,59,60</sup> (Supplementary Table 3). Hydroxide ions are even more powerful than H<sub>2</sub>O at breaking P–P bonds, enabling P–O–P bond formation. An even faster degradation occurred under visible light irradiation<sup>45,60,61</sup>. Much like TMDCs, the covalent functionalization of black phosphorus (Fig. 1b) forming P–C or P–N bonds suppresses the fast oxidation of the said species in air<sup>62</sup>. The same is true for the covalent functionalization of other Xenes (such as silicene, germanene and stannene) via fluorination and hydrogenation to obtain ligand-terminated Xenes (termed Xenes), which are also more resistant to degradation. In these cases, the ligands directly couple to half-filled p<sub>z</sub> orbitals of Xenes, converting π-orbitals states to σ-orbital states<sup>57,58,63,64</sup>. The presence of σ-orbitals improved the environmental stability of the Xenes over Xenes, given that π-orbitals are more prone to be affected by ambient conditions in the external environment<sup>58</sup>.

In recent years, MXenes have emerged as new 2DMs for their attractive physicochemical properties, especially their hydrophilicity and their surface reactivity<sup>65,66</sup>. However, the poor thermodynamic stability of MXenes is a serious issue, as MXenes can easily undergo degradation in ambient atmosphere<sup>67,68</sup>. Indeed, the incomplete bonds of the edged

metal atoms facilitate their oxidation to metal oxides by oxygen and water in air (for example, Ti<sub>3</sub>C<sub>2</sub> transforms to TiO<sub>2</sub> and amorphous C)<sup>67,69</sup> (Supplementary Table 3). The oxidation kinetics of MXenes depend on pH, ionic strength, concentration, size of the sheets and type of surface terminal atoms. For example, in alkaline pH, hydroxide ions can deprotonate the hydroxyl groups at the surface of Ti<sub>3</sub>C<sub>2</sub>OH forming Ti<sub>3</sub>C<sub>2</sub>O<sup>-</sup> and accelerating the oxidation<sup>70–72</sup>. MXenes with M=O terminating groups are more stable than analogues with M–OH terminations, as higher energy is required to create Ti vacancies in the former case<sup>72</sup>. Similar to Xenes, the covalent functionalization of MXenes (Fig. 1b) improves their chemical stability<sup>73–75</sup>. The edge capping of MXenes with organic or inorganic ligands (including hydrazine, polyanionic salts or sodium L-ascorbate) is an effective approach to prevent rapid oxidation<sup>67,76–78</sup>.

The non-covalent (for example with polyethylene glycol) and covalent functionalization increase also the chemical stability of another family of inorganic 2DMs, namely transition metal oxides<sup>79</sup>. 2D TiO<sub>2</sub> sheets have shown excellent air stability owing to the strong Ti–O bonds<sup>80</sup>.

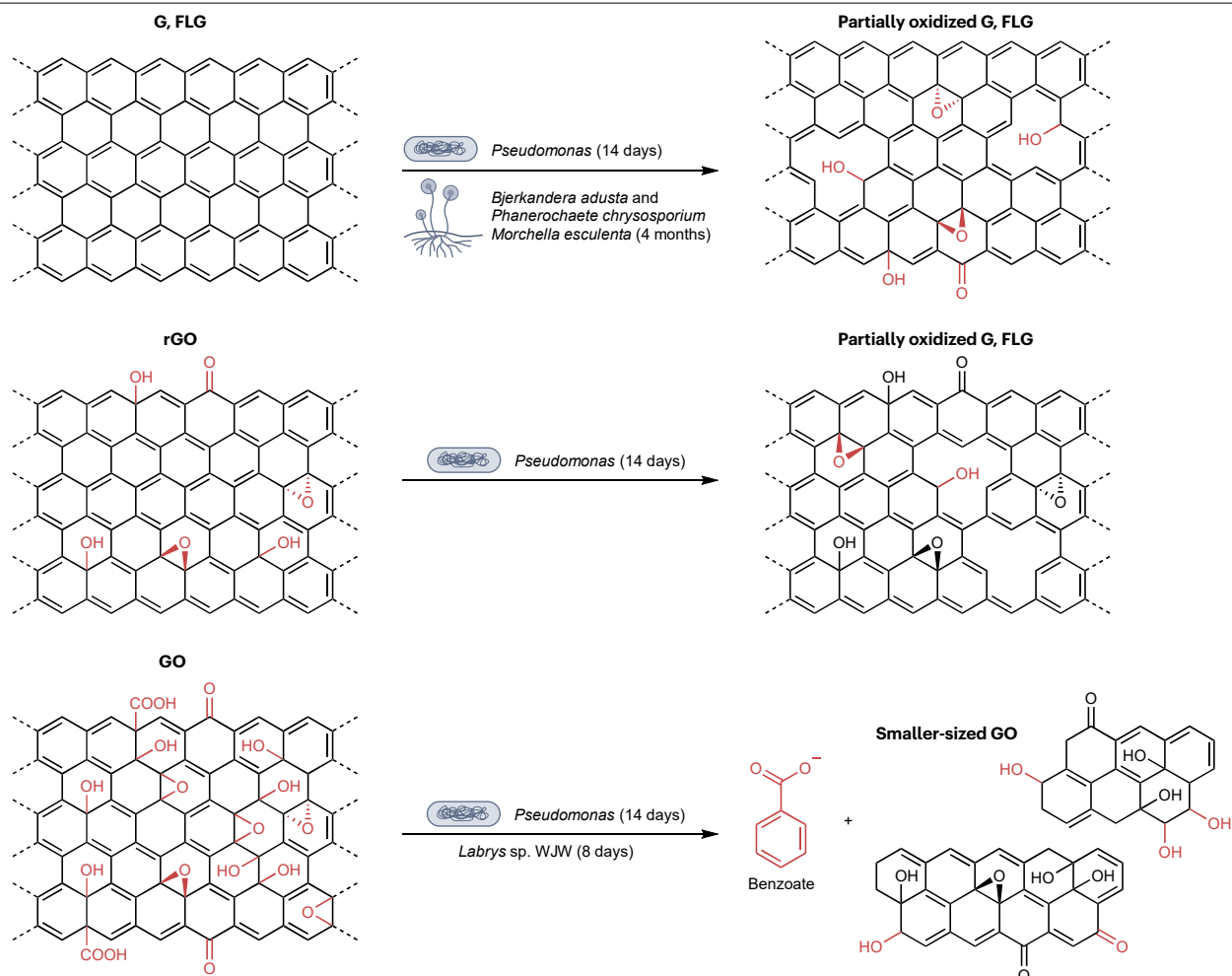
Overall, a controlled synthesis, minimizing the exposure to oxygen and moisture, and storage in an inert atmosphere at low temperature can limit, if not suppress, the oxidation process of 2DM materials<sup>72</sup>.

## Biodegradation by primary decomposers

It is essential to understand the biodegradability of 2DMs in the soil in order to assess their overall environmental degradation. Degradation of GFMs by both types of primary decomposers (bacteria and fungi) has been found to depend on the oxidation state of the material (Fig. 3). Naphthalene-degrading bacteria can oxidize and degrade various graphitic materials through an electron transfer mechanism occurring when the microorganisms come in contact with the material<sup>81</sup>. Graphite, GO and rGO showed different rates of oxidation and degradation after being incubated with *Pseudomonas* bacterial strain isolated from the soil in a graphite mine for 14 days (Fig. 3). Graphite and rGO were partially oxidized and holes appeared in their structure, whereas GO was broken down to smaller sheets and small molecules. Consistent with this, biodegradation of GO by the *Labrys* sp. WJW bacterium strain, isolated from natural soil, resulted in the formation of holes and functional group changes that led to full degradation to benzoate, through phenol and benzoic acid intermediates, after 8 days<sup>82</sup> (Fig. 3).

Degradation of FLG leading to fragments of oxidized graphene sheets by white-rot basidiomycetes (such as *Bjerkandera adusta* and *Phanerochaete chrysosporium*) and soil saprotrophic ascomycetes (such as *Morchella esculenta*) was studied over a period of 4 months<sup>12</sup> (Fig. 3). All fungi were able to oxidize FLG to a GO-like material through mechanisms that involved pH decrease, secretion of degrading enzymes such as lignin peroxidase, and release of H<sub>2</sub>O<sub>2</sub> and laccase/ peroxidase (Fig. 3), suggesting that FLG released into terrestrial environment would likely be oxidized by soil microflora.

Studies conducted so far have indicated that GFMs are biodegradable by primary soil decomposers through oxidation, and carbon-containing degradation products could be used to support growth of the bacteria, thus entering the food chain<sup>82</sup>. A recent study looked at the degradation of GO by yellow mealworms (*Tenebrio molitor* larvae), and it concluded that insects could eat and digest a piece of GO film (1.5 × 1.5 cm) consisting of layers of GO nanosheets in 15 days. Degradation was performed mainly by gut microbes and extracellular enzymes, leading to partial transformation of GO into CO<sub>2</sub> and incorporation into the biomass<sup>83</sup>. Beyond graphene and similar materials,



**Fig. 3 | Biodegradation of graphene-family materials (GFMs) by primary decomposers (bacteria and fungi).** Biodegradation of GFMs occurs through the oxidation in the soil, without the need for the uptake of the material by decomposers. Bacteria (*Pseudomonas*) were found to partially oxidize graphite (G) and reduced graphene oxide (rGO) after 14 days<sup>81</sup>, whereas graphene oxide (GO) is either broken down into smaller-sized sheets after 14 days

(by *Pseudomonas*)<sup>81</sup> or fully oxidized to benzoate molecules, through phenols and benzoic acid intermediates, after 8 days of incubation with *Labrys* sp. WJW<sup>82</sup>. Different fungal strains oxidize and degrade few-layer graphene (FLG) to GO-like material through a decrease in pH, production of H<sub>2</sub>O<sub>2</sub> and secretion of the enzyme lignin peroxidase<sup>12</sup>.

the role of primary decomposers in degradation of 2DMs has not yet been investigated.

Anaerobic oxidation or degradation is another important route to consider in the biotransformation process of 2D materials. Recent works have shown that GO undergoes reduction, that is forming rGO, under anoxic conditions by removing GO oxygenated functional groups<sup>84</sup>. The reduction of GO can also be triggered by the presence of reducing agents that are ubiquitous in the environment, such as sulphides, ferrous ions and reducing sugars. However, similar studies are still needed for other 2DMs.

## Biodegradation in model cellular systems (in vitro)

Determining the capability of cells to internalize 2DMs, their intracellular localization, kinetics and eventual fate is of critical importance. Non-degraded or slowly degrading material may be retained for prolonged periods of time within cells, ultimately leading to

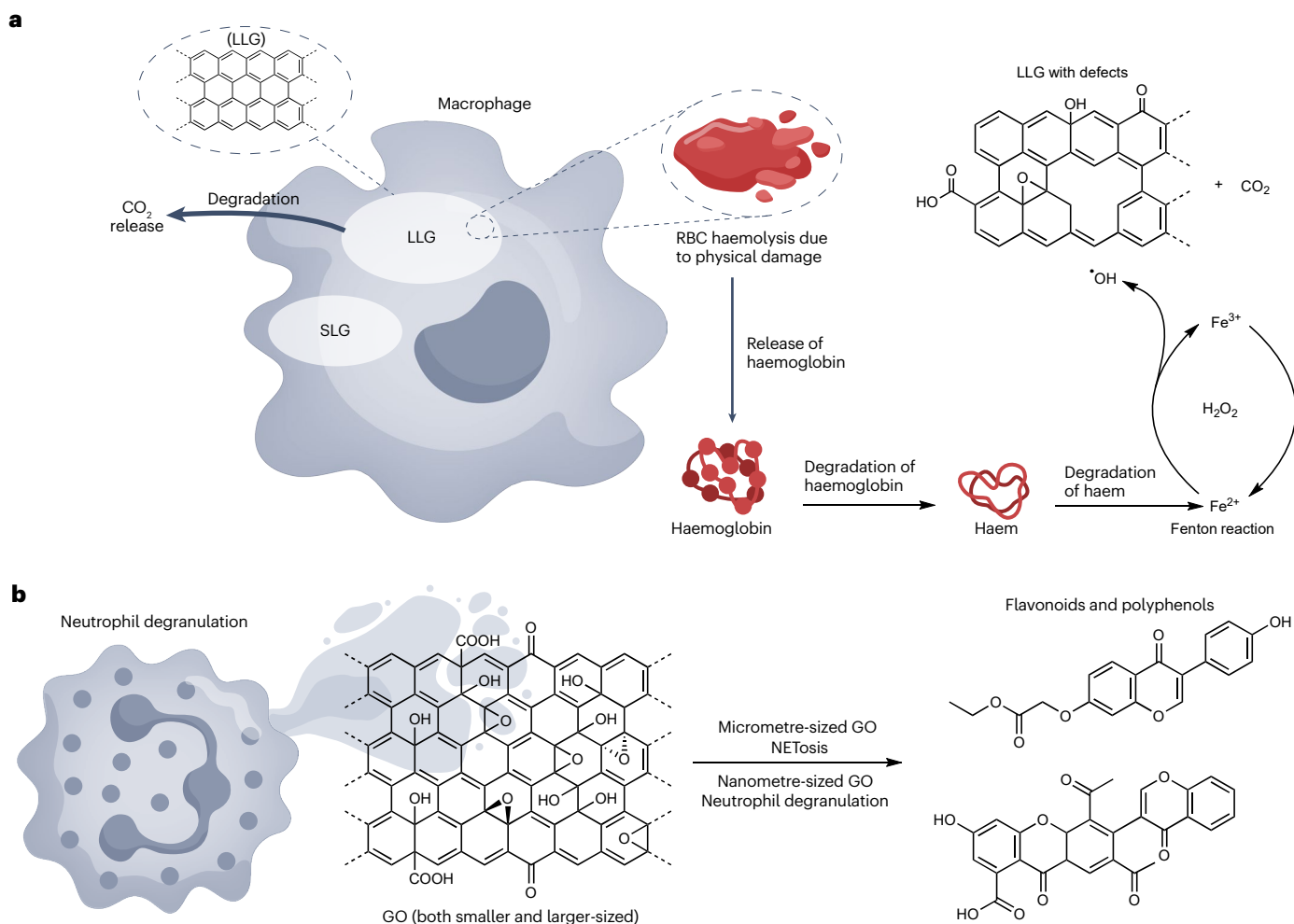
accumulation in vital organs. In vitro degradation of GFMs has predominantly been studied using mammalian cells of the immune system, such as neutrophils and macrophages (see Fig. 4 and Supplementary Table 1 for more details). A distinct mechanism of degradation has been reported for these two cell types. Macrophages typically degrade GFMs intracellularly in the phagosomes (cytoplasmic vesicles designed to digest extracellular particles, microbes or cells)<sup>25,85</sup>. Interestingly, when co-cultured with red blood cells, Kupffer cells (resident liver macrophages) degraded <sup>14</sup>C-labelled large layered graphene through enhanced erythrophagocytosis (naturally occurring removal of erythrocytes in the liver and iron recycling), resulting in the degradation of haemoglobin into haem and a rise in iron concentration in cells<sup>85</sup> (Fig. 4a). The increased iron concentration triggered a Fenton reaction to generate the hydroxyl radical, facilitating the degradation of larger particles into large layered graphene with defects and <sup>14</sup>CO<sub>2</sub>. Even though the study is comprehensive, the materials studied are

substantially larger and thicker than typical GFMs. Small layered graphene on the other hand was not following this degradation route and mainly remained in the phagosomes.

Neutrophils, on the other hand, were found to produce neutrophil extracellular traps (NETs) and secrete degradative enzymes, some of which are investigated in test tube studies. This resulted in the GFMs being degraded extracellularly and in a size-dependent manner<sup>86</sup> (Fig. 4b). A micrometre-sized GO induces NETosis (a lytic form of regulated cell death of neutrophils) by releasing NETs, whereas a nanometre-sized GO elicits neutrophil degranulation (extracellular secretion of degradative enzymes). Degradation products of the GFMs were identified as biocompatible flavonoids and polyphenols.

Generally, very few studies have followed the intracellular fate of GFMs for a prolonged period of time, so there is a knowledge gap on the molecular identity of the final degradation products in situ (within cells) and their impact on cell functions<sup>87</sup>. For instance, human

primary macrophages have been reported to degrade graphdiyne oxide using inducible nitric oxide synthase (iNOS), leading to almost complete degradation after 72 h. Interestingly, the degradation was observed in pro-inflammatory M1-polarized macrophages, the cells typically responsible for the clearance of microorganisms, whereas anti-inflammatory M2-polarized macrophages did not degrade graphdiyne oxide. Furthermore, the degradation of graphdiyne oxide in the M1 macrophages led to the modulation of cytokine expression, which could trigger downstream effects on various biological processes<sup>25</sup>. Another study reported the degradation of GO by neutrophils, and the impact of degraded GO on bronchial epithelial cell line BEAS-2B has also been evaluated<sup>88</sup>. GO was found to be non-cytotoxic, and it did not elicit any DNA damage, indicating that neutrophils can degrade GO and that the biodegraded GO is non-toxic for human lung cells. The cellular effects of GFM degradation products after exposure to simulated tissue fluids have also been explored<sup>28,30,31</sup>. Although GFMs (GO and FLG)



**Fig. 4 | In vitro degradation of graphene-family materials.** In vitro degradation occurs intracellularly in macrophages (part **a**) and extracellularly by neutrophils (part **b**). **a**, Graphene materials with different lateral dimensions were localized in phagosomes. Large layered graphene (LLG) was degraded through erythrophagocytosis via Fenton reaction, leading to complete degradation<sup>85</sup>. Briefly, haemolysis of erythrocytes (RBCs) by LLG causes the release of free haem, which is further processed by the Kupffer cells (liver macrophages). This catalyses the degradation of LLG into CO<sub>2</sub> by generating hydroxy radicals from hydrogen

peroxide through the haem-based Fenton reaction. Small layered graphene (SLG) was not degraded by this mechanism and remained in phagosomes. **b**, Neutrophils produce neutrophil extracellular trap (NET) and secrete oxidative enzymes, hence degrading graphene oxide (GO) sheets extracellularly and in a size-dependent manner. The micrometre-sized GO induces NETosis (a lytic form of regulated cell death of neutrophils) by releasing NET, whereas nanometre-sized GO elicits neutrophil degranulation. Degradation products were identified as biocompatible flavonoids and polyphenols<sup>86</sup>.



exposed to gastrointestinal (GI) tract fluids showed no acute adverse effect on cells of the intestinal epithelium, the biodegradation of GO to reduced GO in simulated lung fluids affected the pattern of interactions with pulmonary macrophages<sup>27</sup>.

Other types of 2DMs have not been studied as systematically as GFMs<sup>32</sup>. Few studies report intracellular fate and biodegradation of MoS<sub>2</sub> nanosheets. One of these studies confirmed degradation of MoS<sub>2</sub> by macrophages within phagosomes, in the regions containing lysozyme (a digestive enzyme)<sup>89</sup>. However, the products of degradation have not been identified. In another study, MoS<sub>2</sub> and chemically functionalized MoS<sub>2</sub> were degraded in test tube by human (hMPO) and environmental (HRP) peroxidases, and the degraded and partially degraded products were used to expose cancer cells. Cancer cell viability was reduced, suggesting cytotoxic responses to high concentrations of the degradation products<sup>34</sup>.

The process of biodegradation of 2DMs by mammalian cells still remains unclear and will warrant more systematic studies, particularly in relation to longitudinal internalization, chronic kinetics of clearance and degradation, and the accumulated impact of such cellular processes. This need is even more pertinent for 2DMs beyond graphene, given that much fewer studies currently exist as many more types of materials with different chemical constituents are becoming prevalent.

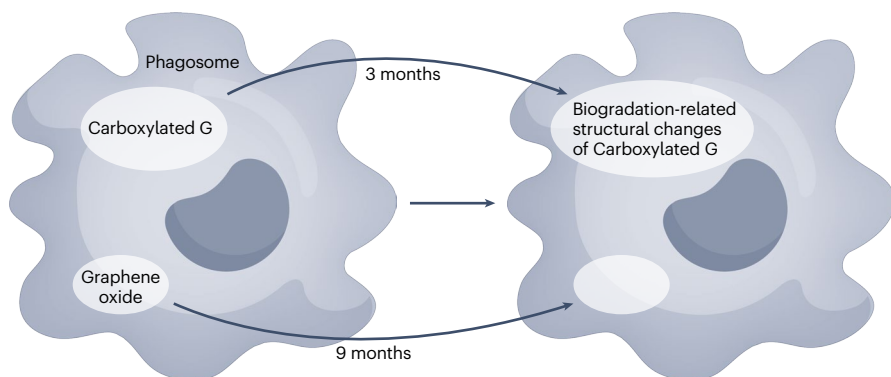
## Biodegradation in living organisms (in vivo)

A limited number of studies have investigated 2DM degradation in vivo, and most of them have focused on understanding the biodegradability of GFMs. Generally, most studies have been performed in mice and are

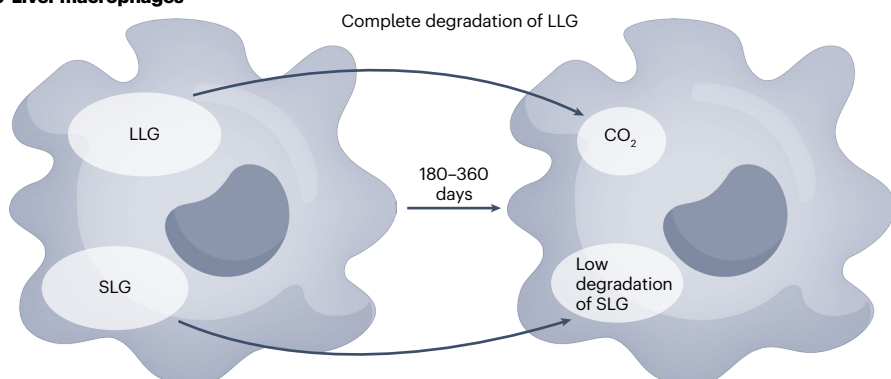
reporting the capture and biodegradation of materials by organ-specific macrophages (Fig. 5). The degradation of carboxyl-functionalized graphene of lateral size ~200 nm was studied in a mouse model following intravenous administration. Large graphene aggregates of sizes up to 10 µm could be detected in macrophages in various organs 24 h after injection. Confocal Raman imaging identified the gradual structural changes in functionalized graphene over a period of 3 months in the macrophages of the lung, liver, kidney and spleen (Fig. 5a). However, the specific mechanism involved in the degradation of the materials was not assessed<sup>90</sup>.

Our groups have also studied the effects of intravenous administrations of high-purity and very thin ‘medical grade’ GO nanosheets of different lateral dimensions. Following the minority fraction of injected dose that localized in the spleen, the GO material fate and its biological impact on tissue function over a 9-month period were evaluated<sup>91,92</sup>. Splenic macrophages were found to be the predominant cell type responsible for the capture, internalization, retention and subsequent intracellular biodegradation of the GO sheets. Furthermore, the translocation of the same GO nanosheets was explored via the nose-to-brain route, upon nasal instillation of the material in mice. Elemental and confocal Raman analyses detected trace amounts of GO in the brain in a size-dependent manner. The smallest GO sheets had the greatest access to the brain (in terms of both quantity and coverage). Raman mapping and immunofluorescence studies showed that GO resided in microglia and that trace quantities of GO were maintained over 1 month but underwent biodegradation-driven structural changes.

### a Microglia, kidney, lung, liver and spleen macrophages



### b Liver macrophages



### Fig. 5 | In vivo biodegradation of graphene-family materials.

In vivo, macrophages capture GFMs and either partially (for carboxylated and small layered graphene in microglia, liver, lung, spleen and kidney resident macrophages) or completely degrade them intracellularly (for large layered graphene and graphene oxide in liver and splenic macrophages, respectively). **a**, Carboxylated graphene (Carboxylated G) was found to undergo gradual structural changes over a period of 3 months in the macrophages of the lung, liver, kidney and spleen, after injection into a mouse model followed by confocal Raman imaging<sup>90</sup>. The intravenous administrations of ‘medical grade’ graphene oxide (GO) in a mouse model resulted in the accumulation of the injected GO in the spleen over a 9-month period. Splenic macrophages were found to degrade the GO, so that GO lost its detectable graphitic crystalline structure, as confirmed by confocal Raman and transmission electron microscopy<sup>92</sup>. **b**, Following intravenous injection, <sup>14</sup>C-labelled few-layer graphenes were detected mostly in the liver (over 1 year), where large layered graphene sheets (LLG) were degraded to CO<sub>2</sub> by Kupffer cells (resident liver macrophages), whereas small layered graphene sheets (SLG) were predominantly non-degraded<sup>85</sup>.



In a thorough work on biodegradation of GFMs (Fig. 5b),  $^{14}\text{C}$ -labelled GFMs of different lateral dimensions were found to mainly accumulate in the liver and were detected for up to 1 year, with larger particles being degraded into  $^{14}\text{CO}_2$  by Kupffer cells (resident liver macrophages). By contrast,  $^{14}\text{C}$ -labelled small layered graphene was not degraded *in vivo*.

Finally, the biodegradation of GO in the zebrafish GI tract has been recently investigated, focusing on the inducible nitric oxide synthase (iNOS) pathway responsible for the production of the nitric oxide (NO) in the GI tract. A *nos2a*-knockout zebrafish (zebrafish lacking the gene encoding iNOS) and a wild-type zebrafish were compared with each other to determine the mechanism by which GO biodegradation occurred in the GI tract<sup>26</sup>. The results indicated that the predominant process leading to GO degradation was nitric oxide-dependent.

Most importantly, the biotransformation and biodegradation of 2DMs beyond graphene is almost unknown. Kinetics of their degradation *in vivo*, chemical identity of degradation products, and their effects on tissues and organs remain essentially unexplored. The connection between physicochemical properties of advanced 2DMs, their interactions with biomolecules in the body and biodegradation are still to be investigated. This class of nanomaterials deserves particular attention, especially as these materials contain trace elements essential for the functioning of enzymes involved in metabolic processes. To emphasize this point, it was demonstrated that, after intravenous injection, human serum albumin-coated  $\text{MoS}_2$  nanodots localized in the liver and spleen, where their biotransformation led to the incorporation of molybdenum into enzymes such as aldehyde oxidase and xanthine oxidoreductase, increasing their metabolic activities in the liver. These enzymes are involved in the metabolism of anticancer drugs, and they responsible for the production of nitric oxide, an important factor for tumour progression<sup>93</sup>. This study emphasizes the importance of identifying the products of biodegradation of 2D NMs containing such trace elements as well as their downstream biological, metabolic and physiological effects.

## By-products from 2DM degradation

Assessing the degradation kinetics of 2DM, identifying the by-products, and understanding the long-term biological effects of intermediate and final products of degradation are essential to determine possible limitations for their biomedical, industrial use and their disposal requirements<sup>41</sup>. In general, enzymatic oxidation of GFMs produces  $\text{CO}_2$  as a final product, along with different types of polycyclic aromatic hydrocarbons with various oxygen groups<sup>20</sup>. In earlier studies, oxidized products (PAHs) of carbon nanotubes showed cytotoxicity to human cells and induced DNA damage<sup>20</sup>. However, MPO-mediated-degradation by-products (resulting in a mixture of partially degraded sheets of GO) of GO were not found to be toxic or genotoxic to human lung cells<sup>88</sup>. Further *in vivo* degradation and more sophisticated assays could reveal more details on the impact of the resulting PAHs.

The stability of many inorganic 2D materials was much lower than that of the GFMs owing to the rapid reaction of the former with ambient oxygen and moisture, even in the absence of oxidative enzymes (such as hMPO and HRP)<sup>8</sup>. 2DMs like black phosphorus, silicene, MXenes ( $\text{Ti}_3\text{C}_2$ ) and 1T-phase  $\text{MoS}_2$  were found to degrade rapidly at the air and in humidity. However, compared with GFMs, the degradation by-products of inorganic 2DMs are predicted to be simpler (such as  $\text{MoO}_3$  and  $\text{PO}_4^{3-}$  ions) unlike the mixture of PAHs reported for GO (shown in Supplementary Table 2). For example,  $\text{MoO}_4^{2-}$  and  $\text{SO}_4^{2-}$  ions are the by-products of  $\text{MoS}_2$  degradation, whereas  $\text{TiO}_2$  and amorphous

carbon residues are the oxidized by-products of silicene and  $\text{Ti}_3\text{C}_2$ , respectively<sup>4</sup>. Furthermore, as expected from their structural resemblance with graphene, g- $\text{C}_3\text{N}_4$  and h-BN showed much higher oxidation resistance to enzymatic degradation than typical inorganic 2DMs, although the cytotoxicity of by-products like  $\text{B}_2\text{O}_3$  were not studied<sup>35,94</sup>. The degradation fragments and the excretion of heavy elements (including Mo, W and Nb) from the biotransformation of 2DMs into different organisms will require a thorough investigation<sup>20,95</sup>.

Time-resolved X-ray photoelectron spectroscopy or inductively coupled plasma mass spectrometry have not been able to provide accurate quantitative data on the chemical species obtained from the degradation process, such as soluble ions like  $\text{MoO}_4^{2-}$ , which can only be assessed qualitatively or semi-quantitatively<sup>96</sup>. Indeed, the quantitative elucidation of biodegradation products of 2DMs, particularly *in vivo*, is essential to understand the relation between biodegradation and toxicity<sup>96,97</sup>. More recently, the synchrotron radiation-based X-ray absorption near-edge structure technique was utilized to assess the accurate information of the chemical valence state of 2H-phase  $\text{MoS}_2$  during its degradation<sup>93</sup>. The obtained results revealed that  $\text{MoS}_2$  was oxidized in the liver by converting Mo(IV) to Mo(VI), and the intermediate form of Mo(V) accounted for 44.6% of total molybdenum, which might be binding with amino acid residues (cysteine, serine or tyrosine) of molybdenum enzymes.

## Conclusions and outlook

Understanding the environmental and biological degradability of 2DMs, including identification of intermediates and their degradation by-products, will offer great confidence for adoption in biomedical and industrial applications. Oxidation state, chemical bonds, covalent and non-covalent surface functionalizations, and chemical stability are key factors that govern the degradability of 2DMs in different environments. Degradation is typically achieved through oxidation, followed by the breakdown of the sheet-like structure of 2DMs into smaller parts. It is crucial that materials have a high resistance to oxidation at room temperature if they are to be used for electronic and other device-based applications.

Recently, the effect of long-term environmental exposure of graphene-coated steel panels began in France in collaboration with AGM (Applied Graphene Materials) and will continue for 2 years<sup>98</sup>. Studying longitudinally such graphene-based products under environmental exposures (moisture, ambient air, heat and sunlight) will inform further on their stability and long-term performance. Such environmental stability and degradation studies for different technologies consisting of 2DMs will be very interesting to undertake and allow us to determine any limitations from the standpoint of the practical use of 2DM-containing products.

In terms of novel 2DMs, although MXenes have very promising material properties, their chemical instability is a primary concern. Their exposed metal atoms, especially on the edges, lead to fast degradation and oxidation. Therefore, new types of MXene carbides (including  $\text{TiC}_2$  and  $\text{NbC}$ ) with a lower ratio of metal atoms to C atoms are being developed. Their chemical stability and electronic properties should be substantially enhanced<sup>99</sup>; however, some reports indicate that the mechanism of oxidation for MXenes remains unclear<sup>100</sup>. Moreover, novel and high-efficiency methods to prevent rapid oxidation of MXenes must be developed, which is highly challenging, particularly in consideration of biomedical products<sup>67,72,101,102</sup>. The discovery of novel 2DMs will continue, as in the case of red phosphorous sheets exfoliated from bulk material demonstrating much higher chemical stability than

black phosphorus sheets in ambient conditions<sup>103</sup>. Another need and opportunity identified is in data-driven studies to offer some form of predictive modelling on the kinetics of degradation of different 2DMs and establish links between chemical properties, degradation environments and degradation kinetics. This could allow a much needed classification of 2DMs based on their degradation rate that could inform future use and in silico predictive tools for the next generation of advanced 2D materials.

In relation to the understanding the degradation of 2DMs within living organisms<sup>104</sup>, long-term in vivo studies that include toxicity, tissue retention profiles, kinetics of excretion, effects of intermediate and final products of degradation, especially for 2DM beyond GFMs, are seriously lacking. Particular attention should be paid to the biodegradation of 2DMs that are administered or ingested orally. Despite recent efforts, the impact of the enzymes from various components of the GI tract (such as, salivary, gastric and intestinal) on degradability of 2DMs is not fully understood<sup>105</sup>. Interrogating the toxicity and health risk of 2DMs by oral exposure is essential, as these nanomaterials could impact or alter the microbiota of the gut, and damage intestinal structures and function<sup>106,107</sup>. For instance, a report revealed that nano-MoS<sub>2</sub> exhibited higher intestinal inflammation than micro-MoS<sub>2</sub> in mice models. However, both types of MoS<sub>2</sub> altered the metabolic profile of the intestine and its microbial community<sup>108</sup>.

The development of physiologically relevant advanced human in vitro models will be very useful for the systematic evaluation of the biodegradation of 2DMs. This includes 3D human cell culture models or organoids that mimic human tissue architecture and cellular complexity found in key organs responsible for degradation of 2DMs (spleen, liver, lungs, digestive system). The use of such advanced in vitro models is already widespread for drug screening and basic nanotoxicological research<sup>109,110</sup>. However, to achieve the biological relevance and complexity needed for the assessment of the biodegradation of 2DMs, existing advanced in vitro systems (such as human stem cell-derived organoids) should be complemented with the immune system component, as these are critical cell types, in most cases mainly responsible for the capture and degradation of 2DMs. These systems should also be optimized and supplemented by microfluidics and organ-on-a-chip matrices to account for physiologically relevant flow conditions and best reflect the dynamic interactions of 2DMs with complex tissues.

Another challenge identified is that most studies on the biodegradation of 2DMs overlooked the role of the biomolecular corona. The formation of a protein corona is inevitable in different environmental conditions, with interesting downstream effects, such as altering interaction and processing of the 2DMs with immune cells and possibly affecting the kinetics of their degradability<sup>111,112</sup>.

Generally, more advanced techniques are needed to obtain more insightful and accurate information on the processes of 2DM degradation, such as the use of synchrotron radiation-based X-ray absorption for tracking the chemical valence of elements in the 2DMs (such as MoS<sub>2</sub>)<sup>93</sup>. More recently, electrical impedance spectroscopy was used to understand the oxidation of g-C<sub>3</sub>N<sub>4</sub> and determine the changes in the material properties, including surface chemistry and chemical composition<sup>94</sup>. In general, electrical impedance spectroscopy is used for assessing the oxidation or corrosion of metals, but it could be applied to other 2DMs as well. However, the characterization of even the more well-studied GFMs in a living biological system remains still very challenging. Limitations of the current analytical techniques (such as X-ray photoelectron spectroscopy, X-ray fluorescence and inductively coupled plasma mass spectrometry) and methods, due to the lower

contrast for carbon and oxygen (compared with metals) are further compromised by the intense background signals from the carbon-rich and oxygen-rich biological environments. Moreover, the dynamic nature of the degradation processes in situ requires analytical tools with continuous, longitudinal read-outs to afford the detection and monitoring of degradation by-products that are generally of transient stability. On a broader context, studying the biodegradability of 2DMs along their life cycle will also become critical as more industries adopt such materials. Life cycle analysis (LCA) of the degradation products originated from 2DMs in the environment are missing, no doubt due to the limited existing use cases. Addressing the exposure of degraded 2DMs in a LCA framework taking into account the evolution of the material from use to the end of life should be taken into consideration to ensure their safe and sustainable use. Overall safety of 2DMs will not only be determined by hazard assessment of pristine materials. The potential risks of 2DMs and their derived by-products may vary substantially across their life cycle. Although a provisional set of LCA recommendations for a more comprehensive assessment of nanomaterials and their application cases has been proposed<sup>113</sup>, analysis of the end-of-life degradation by-products for these nanomaterials has not been in the scope of such reports. Only a few studies exploring the impact of GFMs using a comprehensive LCA approach exist, and there are no studies on 2DMs beyond graphene<sup>1,114</sup>. Additional studies on 2DMs to identify the stages along their life cycle with the potential risk for occupational exposure and safety concerns (including the environmental impact of the degradation products) are therefore warranted.

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## Author contributions

S.V. and R.K. researched data for the article, and prepared the figures and the tables. All authors contributed substantially to discussion of the content. All authors wrote the article, reviewed and edited the manuscript before submission.

## Competing interests

The authors declare no competing interests.

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<sup>1</sup>Nano-Cell Biology Lab, Division of Cell Matrix Biology & Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK. <sup>2</sup>Centre for Nanotechnology in Medicine, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK. <sup>3</sup>School of Chemistry, Indian Institute of Science Education and Research Thiruvananthapuram, Thiruvananthapuram, Kerala, India. <sup>4</sup>Nanomedicine Lab, Catalan Institute of Nanoscience and Nanotechnology (ICN2) Campus UAB Bellaterra, Barcelona, Spain. <sup>5</sup>Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain. <sup>6</sup>CNRS, Immunology, Immunopathology and Therapeutic Chemistry, UPR 3572, University of Strasbourg, ISIS, Strasbourg, France. <sup>7</sup>These authors contributed equally: Sandra Vranic, Rajendra Kurapati.