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# Classification Framework for Graphene-Based Materials

Peter Wick,\* Anna E. Louw-Gaume, Melanie Kucki, Harald F. Krug, Kostas Kostarelos, Bengt Fadeel, Kenneth A. Dawson, Anna Salvati, Ester Vázquez, Laura Ballerini, Mauro Tretiach, Fabio Benfenati, Emmanuel Flahaut, Laury Gauthier, Maurizio Prato, and Alberto Bianco

classification · graphene · nanotechnology · structure–activity relationship · toxicology

## 1. Introduction

Graphene is the enabling material of the 21st century and there are high expectations for its potential applications. A clear and consistent system describing the various derivatives of graphene promotes a precise vocabulary for the family of graphene-based materials. This will be a prerequisite, for example, to understand structure–activity relationships in the context of human health and safety and to avoid generalizations about the capabilities and limitations of graphene-based materials. Within the European Union's GRAPHENE Flagship project, three physical-chemical descriptors specific for graphene were defined to assist in the classification of graphene-based materials.

## 2. Graphene, a Carbon Material with Great Potential

“Carbon, the basis of all known life on earth, has surprised us once again.” This statement stems from the press release by the Royal Swedish Academy of Sciences regarding their decision to award the 2010 Nobel Prize in Physics “for the

groundbreaking experiments regarding the two-dimensional material graphene”.<sup>[1]</sup>

In just 10 years graphene has become a lodestar for researchers all over the world. While the media is boosting the public profile of graphene through the reference to “miracle material of the 21st century”,<sup>[2,3]</sup> the number of scientific papers on graphene also exceeded 3000 per year in 2010.<sup>[4]</sup> The commercial interest in graphene is also reflected by a recent review of the patent landscape of graphene by the UK Intellectual Property office. Graphene patent applications have doubled between 2010 and 2012 and that there has been an order of magnitude difference in the yearly publication figures over the last five years, with a total of 8416 patents worldwide by February 2013.<sup>[5]</sup> It is anticipated that graphene will withstand the normal seven-step sequence for any new technology: hope–hype–boom–bust–disillusionment–shake-out–profitability and meet expectations for profitability even faster than the other carbon allotropes.<sup>[6]</sup> Graphene is expected to be at the focus of even greater interest for industrial applications when mass-produced graphene will have the same outstanding performance as the best samples produced in research laboratories.<sup>[3]</sup>

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Recently, a roadmap for graphene was published, highlighting its future path in the fields of electronics, photonics, composite materials, energy generation and storage, sensors, metrology, and biomedicine.<sup>[3]</sup> However, to realize such potentials, the health and environmental impact of the family of graphene materials should be thoroughly evaluated. Although this area will profit from existing knowledge and concepts for the nanosafety hazard and risk assessment of other nanomaterials, there are still various hurdles to overcome.<sup>[7-9]</sup>

### 3. Confusion and Inconsistency in Naming the Family of Graphene-Based Materials

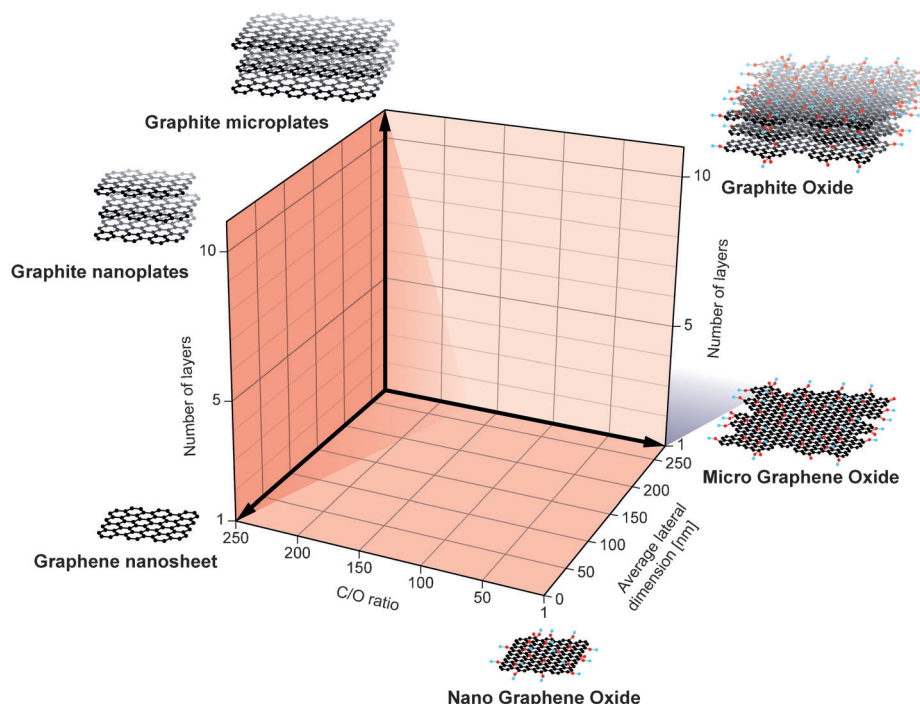
One concern in graphene research is that, similar to carbon nanotubes, the term graphene is used in a generic manner and not in a precise way by scientists to describe many graphene-based materials (GBMs) they have synthesized and studied.<sup>[10]</sup> The inconsistency in naming arises not only in connection with the use of graphene for isolated single-atom-thick sheets, but also by its reference to related two-dimensional sheetlike and flake carbon forms.<sup>[10]</sup> Thus, a clear, consistent, and widely accepted system of describing and naming the various derivatives of graphene still needs to be developed. Solving this standardization and nomenclature issue will be of paramount importance to avoid misleading understanding and interpretation amongst all the stakeholders (i.e., researchers, industry, governments, and in particular, regulatory authorities), for whom the science-based assessment of graphene toxicity and environmental, health, and safety concerns carries priority.<sup>[11]</sup>



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### 4. Classification Approach for Graphene-Based Materials

Recently, the first nomenclature for 2D carbon forms was published and the motivation for this article was given by the statement “*Precise names promote precise ideas*”.<sup>[10]</sup> Underlying this attempt towards a more rational graphene nomenclature is a set of definitions based on the fact that graphene materials should be defined by morphological descriptors. For example, typical dimensions could be described by graphene-specific variables such as thickness (layer number) and lateral size.<sup>[4,10]</sup> In this Essay we would like to build on previous work that attempted to highlight the importance of graphene structural characteristics as determinants of their implications in health and safety. We present an approach (depicted in



**Figure 1.** Classification grid for the categorization of different graphene types according to three fundamental GBM properties: number of graphene layers, average lateral dimension, and atomic carbon/oxygen ratio. The different materials drawn at the six corners of the box represent the ideal cases according to the lateral dimensions and the number of layers reported in the literature. The values of the three axes are related to the GBMs at the nanoscale, but it is feasible to expand the values to the microscale.

Figure 1) that supports the structural notation of GBMs, a term in line with that proposed previously.<sup>[10]</sup>

Our nomenclature model considers the number of graphene layers, the average lateral size, and the carbon-to-oxygen (C/O) atomic ratio as the three fundamental properties that cover the largest set of current graphene materials encountered in practice and that are the subject of biological testing and of biosafety concern. The two morphological characteristics are included because GBMs consist of not only single-layer graphenes but also few-layer graphenes (i.e., 2–10 layers), graphene oxide (GO, normally a single layer), reduced graphene oxide (rGO; normally a single layer), graphene nanosheets, ultrafine graphite (i.e., more than 10

graphene sheets but below 100 nm in thickness), graphene ribbons, and graphene dots.<sup>[9,10]</sup> Furthermore, it has been emphasized recently that little is known about the possible differences in the biological behavior of graphene types having different layer numbers and lateral sizes and these topics deserve thorough analysis.<sup>[9]</sup>

The addition of the C/O ratio as a functional attribute can be justified by the fact that GBMs are both structurally and chemically heterogeneous. The family of GBMs includes materials with widely variable surface oxygen content and thus, surface chemistry can influence biocompatible dispersion potential and colloidal behavior.<sup>[4,9,12]</sup> For example, GO and rGO are emerging as popular materials in nanocarbon research, not only as carbon building blocks for biomedical applications but also as starting materials to produce graphene-based materials.<sup>[10,11]</sup>

Our classification framework provides a starting point for the categorization of distinct graphene types within a grid arrangement according to three easy-to-measure and quantifiable characteristics. It is also important to state at this stage that future studies on graphene's biological significance might reveal other important health and safety assessment criteria for graphene. Our proposed methodology could be validated by biologists and nanotoxicologists working with graphene in order to understand relationships between graphene physicochemical characteristics and safety considerations.<sup>[10,11]</sup> Validation of the proposed methodology will also be given high priority by authors of this article, as members of the GRAPHENE Flagship project. The role of the GRAPHENE Flagship project in the determination of GBM structure–safety relationships is highlighted below.

**GBM Structure–Safety Relationships: The Role of the EU GRAPHENE Flagship:** The GRAPHENE Flagship is a 10-year project and the European Union's biggest research initiative ever with a budget of one billion EURO (<http://graphene-flagship.eu/>). This project is tasked to take graphene from the realm of academic laboratories into European society in the space of ten years and to generate economic growth, new jobs, and new opportunities.<sup>[13]</sup> A specific research program of the flagship (and in which authors of this article participate) is intended to reveal the relationships between the material structure and toxicological functions for different types of graphene materials. The adoption of a clear nomenclature framework to reflect the different structural and chemical characteristics of GBMs will greatly help in this endeavor. In combination with the adoption of benchmarked biological assays that offer reliably specific toxicological endpoints, we envision a platform by which all graphene materials will be assessed.

## 5. Biological and Toxicological Relevance of Fundamental Properties of GBMs

The biological significance of GBM properties such as layer number, lateral dimension, surface chemistry, surface area, and material purity have already been highlighted.<sup>[4,8,9]</sup>

The layer number determines the thickness, the specific surface area, and bending elasticity with expected outputs such as higher adsorptive capacity for GBM-type molecules when the layer number decreases and increased stiffness/rigidity during cellular interactions when the material thickness increases. As the lateral sizes of GBMs span several orders of magnitude, from the nanoscale to the microscale (i.e., 10 nm up to >20  $\mu\text{m}$ ), it is important to specify the lateral dimension since this parameter determines the maximum size and degree of deformability of the material which are key variables for cellular uptake, renal clearance, transport across the blood–brain barrier, and many other biological interactions that depend on particle size.<sup>[4,14,15]</sup>

The understanding of the biological relevance of the C/O atomic ratio requires a more detailed examination. From a surface science perspective, it should be considered that members of the GBM family do not have a standard surface. For example, pristine graphene and GO differ in their surface hydrophobicity/hydrophilicity. For the former, the surface is hydrophobic while for GO, surfaces consist of hydrophobic islands with hydrophilic regions showing various degrees of basal reactivity. Graphene oxide could be considered as derivatized graphene with a myriad of oxygen functionalities due to the introduction of carbonyl, hydroxy, and epoxy groups on the planar surfaces and edges of the carbon sheets during graphite oxide exfoliation. Therefore, graphite oxidation endows single- or few-layered GO with the great advantages of improved solubility or dispersibility in aqueous solutions and reasonable colloidal stability.<sup>[11,12]</sup> Since the coverage with oxygen atoms varies depending on the degree of oxidation during the preparation of GO, it is imperative to understand that these production processes will contribute to the inhomogeneity of the final GO product. Typically, GO with chemical compositions corresponding to a C/O ratio of 4:1 to 2:1 are produced. Graphene can also be transformed into reduced rGO and this will increase the C/O ratio to approximately 12:1 but values as large as 246:1 have recently been reported.<sup>[9,12,16]</sup>

The significance of investigating the three fundamental properties should also be viewed in terms of the relationship between physicochemical characteristics and the health and environmental risks of any nanomaterial. The importance of these characteristics for ecological sustainability is noteworthy as these characteristics might modulate GBM–organism interactions and thus the transfer and impact of GBMs through the food chain and ecosystems.<sup>[17]</sup> A comparison study that reviewed toxicological aspects of GO in relation to its synthesis techniques found that thickness and lateral dimensions are the structural properties that vary the most.<sup>[7]</sup> In analogy to the requirement that graphene sheets must be of an appropriate size (i.e., size tuning of the lateral dimension) to suitably interface with biological systems, evidence is accumulating that cell viability and toxicity responses can be modulated by controlling the GBM surface oxygen content.<sup>[4,18,19]</sup>

Although we wish to highlight the biological significance of these three graphene-specific properties, it is important to also consider in toxicological assays other generic evaluation criteria, as is valid for most other nanomaterials. For example,

sample impurities in graphene samples might cause non-specific interference in toxicological studies<sup>[4]</sup> and, in addition, once GBMs are introduced into a living system, a new biological “identity” may be adopted, as determined by the biomolecules that adsorb to the material surface.<sup>[20]</sup> Graphene offers a large available surface for the adsorption of proteins and other biomolecules and this should also be taken into account when attempting to determine/resolve the overall health, safety, and toxicity impact of GBMs.

## 6. Experimental Methodologies to Measure the Three Classification Parameters

Table 1 summarizes the basic analytical techniques for the measurement of the three fundamental GBM classification parameters of interest. Suitable references are also included which provide a detailed and thorough description of appropriate experimental methodologies to study these properties, and it is important that researchers are well-trained in the appropriate methodologies and incorporate more than one testing method in their characterization efforts.

**Table 1:** Analytical techniques for the classification of GBMs.<sup>[a]</sup>

GBM-specific property and suitable analytical tools for the classification of GBMs	
Number of layers (thickness)	TEM <sup>[21]</sup> AFM <sup>[21]</sup> Raman spectroscopy <sup>[21,22]</sup> Optical absorbance measurements <sup>[23]</sup>
Lateral size	TEM <sup>[21]</sup> SEM <sup>[21]</sup> AFM <sup>[24]</sup>
Atomic C/O ratio	XPS <sup>[13,16]</sup> Elemental analysis (ICP-MS) <sup>[25]</sup>

[a] TEM: transmission electron microscopy, SEM: scanning electron microscopy, AFM: atomic force microscopy, ICP-MS: inductively coupled plasma mass spectrometry.

## 7. Summary and Outlook

Consensus is accumulating that clarity in the nomenclature of GBMs is needed. The proposed classification framework, as an initial reference system for graphene biologists/toxicologists, will help to determine the role of the three physical/chemical properties on the health and safety profile of GBMs. It will contribute to the avoidance of generalizations about the capabilities and limitations of GBMs that can raise false expectations and unnecessary safety concerns.<sup>[12]</sup> Other benefits that might stem from the adoption of the classification and mapping approach are summarized in Table 2.

There are indeed numerous long-term benefits for all players in the graphene-biological community if diligence is shown by all in characterizing their materials and describing them according to layer number, lateral size, and surface chemistry rather than using ad hoc sample names.<sup>[4]</sup> Finally, it

**Table 2:** Potential benefits associated with adoption of GBM nomenclature.

- Improved vocabulary/coordinated terminology for structural characterization
- Classification within the proposed grid prevents ad hoc naming
- Better comparisons between carbon allotropes
- Fitting the “molecule of interest” into the classification grid and its position determines which reference GBM should be included in biological experiments
- Standard analytical methodology for benchmarking of materials
- Future comparative studies possible through minimal material characterization
- Aid in structure–activity analogies for predictive toxicology
- Support the peer-review process
- Clear-cut guidelines for regulatory purposes

is important to keep in mind that the set of critical nanosafety evaluation criteria for GBMs will grow in the future in order to reach a consensus on the health and environmental safety risks of graphene smart materials such as functionalized GBMs.

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