cies. It also applies to the more common scenario of documenting newly discovered species, which (like most rediscovered species) often exist in small, isolated populations and therefore suffer from the same problems if voucher specimens are collected from the field. Field collection of individuals from small and declining populations vulnerable to extinction is also a common practice. Collection both by professional and amateur scientists has been linked to the decline or loss of a range of animal species, including Mexico's elf owl (*Micrathene whitneyi soccorroensis*) (*6*). Plants have also been affected by scientific overcollection; Norton *et al*. (*7*) cite the case of the scientific collection–driven decline and extinction of uncommon plant taxa in New Zealand over the past two centuries.

Perhaps the most powerful alternative method to collection is a series of good photographs, which can even be used to describe a species, complemented by other lines of evidence, such as molecular data and a description of a species' mating call for birds, amphibians, or insects. Advances in handheld technology have made it much easier and cheaper to identify species; most smartphones have a camera and a voice recorder sufficient to gather high-resolution images as well as an organism's call. Such nonlethal techniques were used successfully for the identification of the bird *Bugun liocichla*, a species that was newly discovered in India in 2006 (*8*). The bird's discoverer deliberately chose not to collect a voucher specimen for fear of imperiling the population; instead, a combination of photos, audio recordings, and feathers were used to distinguish the species.

In the case of rediscovered species, many were already well described, and a good-quality image should suffice. For rediscovered, rare, and newly discovered species, molecular techniques (such as skin swabbing for DNA) are an increasingly effective way to sample a specimen to confirm an identity with no or minimal harm to the organism (*9*, *10*). For this system to work, the DNA of relict populations and newly discovered species must be sequenced and the data made publicly available. This would, for example, make future population rediscoveries easier to document.

The multivariate description of a species that results from combining high-resolution photographs, sonograms (as appropriate), molecular samples, and other characteristics that do not require taking a specimen from the wild can be just as accurate as the collection of a voucher specimen without increasing the extinction risk. Clearly there remains a long-running debate over the appropriate standards for scientific description absent a voucher specimen (*11*). The benefits and costs of verification-driven specimen collection, however, should be more openly and systematically addressed by scientific societies, volunteer naturalist groups, and museums. Sharing of specimen information, including obligations to store genetic information from voucher specimens in widely accessible digital repositories, can also help to reduce the future need to collect animals from the wild.

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MATERIALS SCIENCE

Exploring the Interface of Graphene and Biology

To take advantage of the properties of graphene in biomedical applications, well-defined materials need to be matched with intended applications.

Kostas Kostarelos[1,3] and Kostya S. Novoselov[2,3]

Graphene is highly conductive, flexible, and has controllable permittivity and hydrophilicity, among its other distinctive properties (*1*, *2*). These properties could enable the development of multifunctional biomedical devices (*3*). A key issue for such applications is the determination of the possible interactions with components of the biological milieu to reveal the opportunities offered and the limitations posed. As with any other nanomaterial, biological studies of graphene should be performed with very specific, well-designed, and well-characterized types of materials with defined exposure. We outline three layers of complexity that are interconnected and need to be considered carefully in the development of graphene for use in biomedical applications: material characteristics; interactions with biological components (tissues, cells, and proteins); and biological activity outcomes.

Graphene has now been developed in many different forms in terms of shapes, sizes, chemical modifications, and other characteristics that can produce dramatically different results when studied biologically. Methods for producing graphene include direct exfoliation in organic liquids (*4*, *5*), reduction of graphene oxide (GO) (*6*), and epitaxial growth by CVD (chemical vapor deposition) on copper (*7*) or epitaxial growth on silicon carbide (*8*). The three aspects of this layer of structural complexity—the thickness, the lateral extent, and the surface functionalization of graphene—are illustrated in panel A of the figure and show how the materials produced by different methods fall in very different parts of this parameter space. These different physical and chemical characteristics dictate the suitability of a material for specific biomedical applications.

These wide discrepancies between the available graphene types will crucially determine the second layer of complexity, that of interactions of graphene with living cells and their compartments. In panel B

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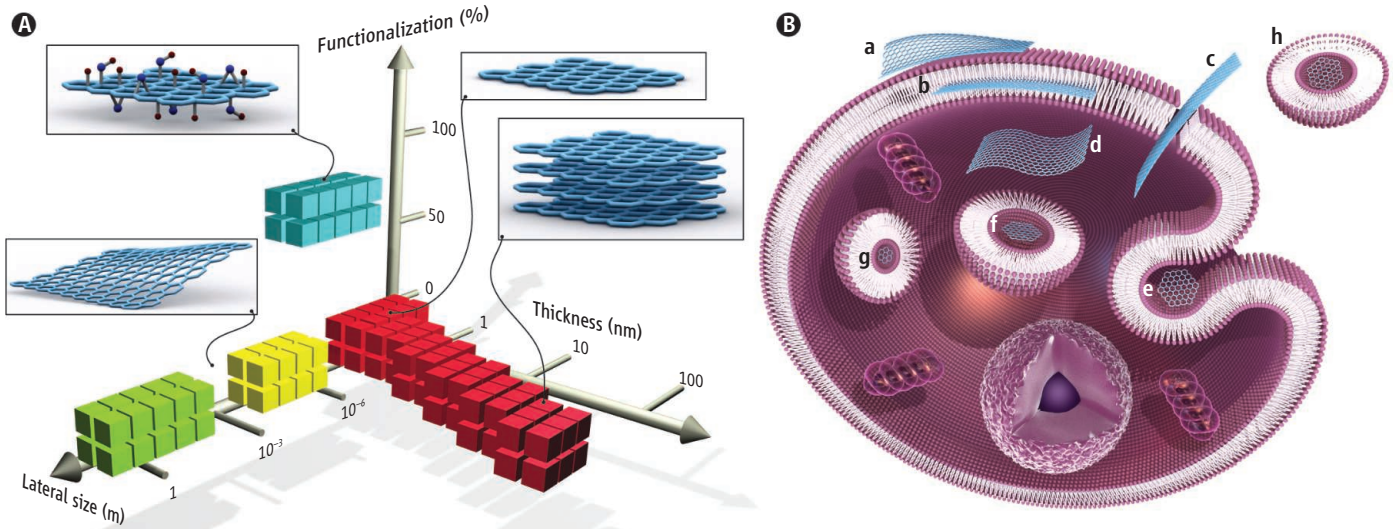

Graphene materials and their biological interactions. (**A**) A parameter space for the most widely used graphene materials can be described by the dimensions and surface functionalization of the material, the latter defined as the percentage of the carbon atoms in sp^3 hybridization. Green squares represent epitaxially grown graphene; yellow, mechanically exfoliated graphene; red, chemically exfoliated graphene; blue, graphene oxide. Note that a number of other graphene-related materials (such as graphene quantum dots and graphene nanoribbons) are also being used in experiments. (**B**) Possible interactions between graphene-related materials with cells (the graphene flakes are not to scale). (a) Adhesion onto the outer surface of the cell membrane. (b) Incorporation in between the monolayers of the plasma membrane lipid bilayer. (c) Translocation of membrane. (d) Cytoplasmic internalization. (e) Clathrin-mediated endocytosis. (f) Endosomal or phagosomal internalization. (g) Lysosomal or other perinuclear compartment localization. (h) Exosomal localization. The biological outcomes from such interactions can be considered to be either adverse or beneficial, depending on the context of the particular biomedical application. Different graphene-related materials will have different preferential mechanisms of interaction with cells and tissues that largely await discovery.

of the figure, we show in a schematic fashion some of the possible cell-graphene interactions.

Nanoflakes of chemically exfoliated graphene, micrometer-size flakes of GO, or substrate-bound CVD graphene will have dramatically different interactions and effects (if any) on live cells and tissue that can result in contradicting conclusions. Even experiments on similar, but not well-defined, materials can produce puzzling results. For instance, recently published papers on pulmonary inflammation after exposure to graphene platelets found no effects after 6 weeks (*9*) but some degree of acute (24 hours) inflammatory response (*10*).

The consequence of interactions at the cellular level will determine the third layer of complexity, that of overall biological activity outcomes. These outcomes can be adverse to the cell or tissue (e.g., fibrosis, membrane damage, or accumulation) or beneficial (e.g., facilitating intracellular transport of therapeutic or diagnostic agents, or providing antimicrobial or protective shielding). Some outcomes, such as cell activation or apoptosis, can be harmful or beneficial depending on the cell type and the intended use (in cancer therapy, harm to cells may be good; in vaccine design, activation of some parts of the immune system may be desired).

Both adverse and beneficial outcomes have been reported recently by different groups, even for similar graphene materials and cellular interactions. For example, cell internalization has been shown both as a mechanism that can lead to cell intoxication in some studies (*11*, *12*) and as a means to transport therapeutic agents intracellularly without ensuing damage in others (*13*). In another example, some recent computational and experimental studies have demonstrated that specific forms of graphene can directly interact with plasma membranes, which suggests that graphene may cause cell membrane damage (*14*, *15*). However, other studies demonstrated that interaction and binding of various graphene material types onto the mammalian plasma membrane can lead to a potentially beneficial enhancement of cell growth (*16*) or shielding effect (*17*, *18*) with no cell damage. Lastly, some types of graphene materials have been shown to physically adsorb and wrap around bacterial cell membranes, suggesting possible antibacterial activity (*19*–*21*), but this result has not been confirmed by others (*16*).

Also, the safety profile of graphene materials on interaction with living biological matter cannot be directly drawn from that for other carbon-based materials (graphitic platelets, amorphous carbon, and diamond-like carbon that have been studied for decades). These materials have properties very different from either graphene, bilayer graphene, or even few-layer graphene, and so will be their biological outcomes. Furthermore, despite some (very vague) similarities between graphene and carbon nanotubes, the former is generally not fiber-shaped, so fiber toxicology paradigms are not directly applicable (*22*). The limited number of available in vivo studies suggest that flat graphitic structures are not able to trigger the adverse (inflammatory) reaction associated with fibrous asbestos or long, rigid carbon nanotubes (*23*).

Another biological process of great importance is the biodegradation of graphene that will determine the safety profile of graphene materials from its residence time and persistence within tissues. Additionally, the kinetics of graphene degradation will define the limitations posed in relation to specific biomedical applications that may require long-term integration within the biological milieu (e.g., orthopedic or neuronal implants, catheters, wound healing agents, and corneal devices). The biodegradability of different graphene types will vary, as will the products of any biodegradation process. Some initial experimental evidence suggests that graphene can be enzymatically degradable by the oxidation activity of horseradish peroxidase (*24*) or macrophage-mediated degradation in vivo (*25*).

The development of graphene-based technologies for biomedical applications, either in the form of a device or an adminis-

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tered substance for therapeutic or diagnostic purposes, will be thoroughly scrutinized by the existing regulatory and approval framework implemented by national and international agencies. In the meantime, we urge very careful characterization and rational selection of the graphene materials to be studied in specific biological models, based on a hypothesis-driven intended biomedical purpose. Only rational, well-designed studies of graphene interactions with cells, tissues, and organisms will help guide the best choices for the use of this exciting family of materials.

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APPLIED PHYSICS

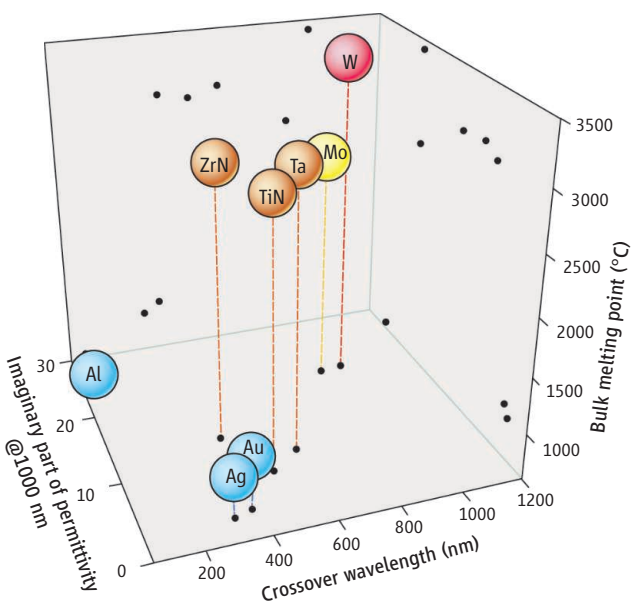
Refractory Plasmonics

Stable at high temperatures, refractory plasmonic materials could boost existing optoelectronic technologies.

Urcan Guler, Alexandra Boltasseva, Vladimir M. Shalaev

Refractory materials are defined as those with a high melting point and chemical stability at temperatures above 2000°C. Applications based on refractory materials, usually nonmetallic, span a wide range of areas including industrial furnaces, space shuttle shields, and semiconductor technology. Metals have also been studied as refractories; however, the optical properties of those metals that have been tried for high-temperature applications were not good enough to be used in plasmonic applications (these are almost entirely based on noble metals, which are not good refractories). Refractory materials that exhibit reasonably good plasmonic behavior would undoubtedly enable new devices and boost such existing applications as heat-assisted magnetic recording (HAMR) (*1*), solar/thermophotovoltaics (S/TPV) (*2*), plasmon-assisted chemical vapor deposition (*3*), solar thermoelectric generators (*4*), and nanoscale heat transfer systems (*5*).

The field of plasmonics offers the potential to greatly enhance the efficiencies of existing technologies, such as electronics and photonics, as well as to create new technological opportunities (*6*). Although several proof-of-concept studies have been reported, the realization of practical devices has been hindered by the challenges associated with the properties of noble metals—in particular, poor chemical and thermal stability and high losses (*7*, *8*). Usually listed as a problem for plasmonic applications, resistive losses result in heating of the plasmonic material, enabling a temperature rise in a confined volume around the nanostructure. Several plasmonic applications with a great potential for practical use, such as photothermal treatment (*9*) and HAMR (*1*), rely on the heating effects. Because of the local temperature rise, the mechanical and chemical stability of plasmonic nanostructures are of paramount importance; refractory plasmonic materials are therefore indispensable.



A handful of alternatives. The low melting point and softness of metals pose problems when real-world applications are considered, especially in nanostructures in which the melting point is reduced. Refractory plasmonic materials would provide a solution for high-temperature applications where corrosion and wear resistance are desired. Refractory metals exhibit plasmonic resonances mostly in the near-infrared region with relatively higher losses. Transition metal nitrides mimic the optical properties of gold and provide the superior material properties of the refractory materials. It is above the "crossover wavelength" that a material becomes plasmonic.

S/TPV technology is based on the idea of absorbing solar irradiation with a broadband absorber, which results in heating of an intermediate component and the subsequent emission of this thermal energy in a narrow spectrum for efficient absorption by the photovoltaic cell. Such devices can theoretically achieve energy conversion efficiencies up to 85% (*2*). However, the operational temperatures required for high-efficiency devices are estimated at ~1500°C, and emitter materials that can withstand prolonged exposure to such temperatures have not yet been developed. Engineered absorber and emitter photonic crystals can be fabricated with refractory metals (*10*, *11*), but achievable operational temperatures are still far below

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