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Exploring the Synergies of Single-Molecule Fluorescence and 2D Materials Coupled by DNA

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The world of 2D materials is steadily growing, with numerous researchers attempting to discover, elucidate, and exploit their properties. Approaches relying on the detection of single fluorescent molecules offer a set of advantages, for instance, high sensitivity and specificity, that allow the drawing of conclusions with unprecedented precision. Herein, it is argued how the study of 2D materials benefits from fluorescence-based single-molecule modalities, and vice versa. A special focus is placed on DNA, serving as a versatile adaptor when anchoring single dye molecules to 2D materials. The existing literature on the fruitful combination of the two fields is reviewed, and an outlook on the additional synergies that can be created between them provided.

1. Introduction

2D materials have prompted tremendous interest in science and industry since the discovery of graphene.[1] Over the last couple of years, the family of 2D materials has grown substantially, and several 2D materials have become commercially available in various forms, such as mono- or multilayers, powders, crystals, flakes, etc. Although some 2D materials are still in their infancy, scientists envision this family of materials disrupting numerous industrial sectors and becoming part of our everyday lives during the next few decades.[2–5] Graphene, for example, is now found in batteries, inks for printable electronics, photodetectors, and chemical and biological sensors.[6–8] With the expansion of the 2D material family exhibiting a wide spectrum of unusual properties, the opportunities are all encompassing for both basic science and application-driven pursuits. Moreover, the capabilities of applications are further extended by the integration of other 2D materials, bulk materials, or soft matter, which results in complex coupling effects.

Our motivation to combine 2D materials with single-molecule fluorescence techniques coupled by DNA is twofold: first, DNA-based supramolecular assemblies on 2D materials can be tailored for biophysics, bioimaging, and biosensing by harnessing the materials as a transducer platform in single-molecule fluorescence studies.[9,10] Second, the addressability of DNA nanostructures can be utilized to place single fluorescent molecules at well-defined positions on top of the materials and study their optical and electronic properties. Moreover, single-molecule fluorescence allows investigating the presence and spatial distribution of different kinds of material defects with nanometer-scale resolution. Additionally, the respective methods are highly sensitive and non-invasive, thus representing a promising platform to study 2D materials under ambient conditions. The choice of DNA as a linker is also advantageous from a practical point of view since it is widely available and has a well-studied chemistry that enables easy access to functionalized strands.

While experimental studies combining fluorescent dye molecules and 2D materials have been primarily carried out in ensemble spectroscopy, single-molecule fluorescence techniques provide valuable information that is otherwise averaged out.[11–15] For example, unique photoluminescent properties of graphene quantum dots were observed by single-molecule fluorescence that were not discernible in previous ensemble experiments.[16] Furthermore, single-molecule fluorescence allowed to prove the distance dependence of the energy transfer from fluorescent dyes to graphene, obtaining a separation with 50% energy transfer at unprecedented precision and a substantially higher value than previously reported.[17] Yet, from our perspective, the research in the area has been hindered by insufficient communication between the single-molecule fluorescence and 2D materials communities. Bridging the gap between these fields would be strongly synergistic: on the one hand, by using single-molecule fluorescence methods, the performance of devices based on 2D materials is enhanced, for example, by gaining spatial information and avoiding averaging

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effects. On the other hand, single-molecule experiments can be used to investigate the 2D materials' properties and the presence of defects with a great level of detail. Moreover, those approaches allow for exploring the mechanisms underlying the interplay among DNA, single fluorescent molecules, and 2D materials. Here, single-molecule fluorescence methods are proposed as complementary modalities that provide a distinct perspective on the molecular behavior and interactions at the interface of a material. By combining different techniques such as atomic force microscopy (AFM), scanning tunneling microscopy, and single-molecule fluorescence methods, researchers can gain a more complete understanding of the material's properties, such as its surface chemistry, reactivity, and energy-transfer properties, with a high level of spatial and temporal resolution.

Due to the overwhelming literature content about 2D materials, we have selected five groups of materials to discuss herein; namely, graphene, graphene oxide, hexagonal boron nitride, transition metal dichalcogenides (mainly MoS_2 and WS_2), and MXenes. We step into the current knowledge of DNA-2D material interactions, followed by a brief introduction of single-molecule fluorescence techniques. We stress the synergies by elaborating on how different fields can benefit. Finally, we close with an outlook on the challenges and possible future development.

2. 2D Materials

2.1. Graphene

In 2004, graphene, a material theoretically predicted to be unstable under ambient conditions, was experimentally isolated for the first time via mechanical exfoliation.[1] It was a breakthrough for basic research, and soon graphene entered nearly every field of science due to its impressive list of properties.[5] Without electrical contact, graphene is a zero-bandgap semiconductor, and its charge carriers obey a linear dispersion relation. As a consequence, it manifests wavelength-independent light absorption across the visible-near IR regions, with an absorbance defined as $\pi\alpha \approx 2.3\%$, where $\alpha \approx 1/137$ is the fine-structure constant.[18] With these characteristics, graphene behaves as a unique acceptor system that has been exploited in biosensing applications.[19–22] Nonetheless, for fields such as electronics and optoelectronics, the flexibility to engineer the energy bandgap is highly advantageous. Another supreme phenomenon broadly utilized is the ambipolar electric field effect, which enables tuning the electronic properties of graphene and is especially critical for the development of graphene field effect transistors (GFETs), photovoltaics, photodetection, energy conversion, or light modulation devices. The high-charge carrier's mobility finds applications in all sorts of optoelectronic devices and GFET-based sensors.[23,24] Finally, high transparency, together with the aforementioned properties, is broadly applied in the development of coating materials, touch screens, flexible solar cells, and biological imaging. Today, graphene incessantly has a solid position in science and slowly advances into commercial applications.[25,26] Nevertheless, the high-yield and cost-free production on a larger scale is still challenging, and for many applications, graphene is replaced by graphene nanoflakes or graphene oxide.

2.2. Graphene Oxide

The history of graphite and graphene oxide (GO) dates back to the 19th century, when they were obtained for the first time by prolonged oxidation of graphite.[27] Nowadays, GO is commonly obtained using Hummers' method.[28–30] It carries oxygen-containing functional groups, mainly hydroxyl, carbonyl, epoxide, and carboxyl groups, which disrupt the sp^2 carbon network and entail the insulating character of GO, in contrast to highly conductive graphene.[31] It is probably the most studied 2D material, gathering attention as a water-processable material similar to graphene and as a precursor for reduced GO (rGO) production, which was supposed to act as a cheaper alternative for graphene.[32] Although the efficiency of the reduction process could never reach the point that rGO behaved exactly as graphene, especially for areas larger than tens of micrometers,[33] it possesses advantages, such as the flexibility with the choice of the reducing agent, the controlled degree of the reduction, and the eventual incorporation of other moieties within the lattice.[34] GO, as an independent material, has also found applications in various research fields, for instance in environmental and biomedical applications, sensing, composites, batteries, or solar cells.[31,35]

2.3. Hexagonal Boron Nitride

Hexagonal boron nitride (hBN) has an almost identical crystal structure as graphene, with alternately arranged boron and nitrogen atoms. Due to its high reflectivity in the visible light range, it is also called "white graphene".[36] It gained much interest as a wide-bandgap semiconductor that is chemically inert and highly temperature-resistant. This is particularly interesting for in-plane and stacked van der Waals heterostructures, where hBN serves as an atomically flat substrate, insulator, or barrier/tunneling layer.[37,38] hBN has been extensively studied for applications in field-effect transistors, tunneling devices, optoelectronic, and photoelectric devices.[36] Nevertheless, due to the latest advances in fabricating, doping, and merging hBN with other materials to form nanocomposites, it has also been used in several other applications related to environmental sensing, catalysis, energy storage and conversion, membrane separation, as well as thermal and chemical protection coatings.[39]

2.4. Transition Metal Dichalcogenides

2D transition metal dichalcogenides (TMDs) are a family of atomically thin semiconductors with two chalcogen atoms (S, Se, or Te) interconnected by a transition metal atom (Mo, W, etc.).[40] The composition of TMDs determines their crystal structure, which, unlike graphene and hBN, reveals three atomic layers (a sandwich-like structure) with strong in-plane bonding and weak out-of-plane interactions. The energy bandgap of TMDs is typically between 1 and 2 eV. The presence of the intrinsic bandgap and the resulting optoelectronic properties determine the directions for exploring TMDs for photonics, spintronics, optoelectronics, transistors, photodetectors, etc.[40,41] Yet, also applications in sensing, biosensing, DNA sequencing, and biomedicine

were found.[42,43] Herein, we will mainly focus on the most studied members of the family, MoS_2 and WS_2.

2.5. MXenes

Another group of 2D materials are MXenes, which were first reported by Naguib et al., in 2011.[44] MXenes are part of a large family of hydrophilic multielement materials with the formula $M_{n+1}X_nT_x$ (M: early transition metal; X: carbon or nitrogen; T_x: O, OH, F, and/or Cl surface terminations). They are synthesized by applying a strong etchant, for example, hydrofluoric acid, on a so-called MAX phase, where "A" stands for the main groups III or IV of the periodic system. MXenes raised much interest because of their metallic conductivity and intercalation properties, as well as their tunable surface chemistry, clay-like adsorptive properties, and biocompatibility.[45–48] By now, MXenes have been applied in energy storage, electronics, electromagnetic shielding, biomedical, and environment-related applications.[49]

2.6. Homo- and Heterostructures

Better understanding and growing interest in 2D materials naturally raised the question of expanding the field by creating homo- and heterostructures.[50] They are formed in vertical (layer-by-layer) or lateral (so-called in-plane) architectures. While the first strategy is commonly used and several layered structures have been created employing this approach, the second one remains challenging due to difficulties in "stitching" two materials. Engineering homo- and heterostructures, on the one hand, opens possibilities to explore new properties and phenomena, while, on the other hand, it may improve the performance of their components. With the effort of theoretical and experimental scientists, the world of 2D homo- and heterostructures has greatly expanded over the last decade. Discussing them in detail is out of the scope of this article; nevertheless, we would like to encourage the reader to explore the field with the comprehensive review articles available.[51–54]

3. Interactions of DNA with 2D Materials

We suggest the use of DNA as a linker to 2D materials. DNA is an important anchoring molecule because of its spatial dimensions and well-documented behavior.[68,69] Modifications of DNA employing a wide range of moieties are often commercially available while being affordable. By doing so, DNA offers a simple means of coupling, particularly for single fluorescent dye molecules, which are the focus of this perspective piece.[70,71] Thanks to existing findings, the interaction of DNA with 2D materials is partially understood. For example, we know that mostly non-covalent forces drive supramolecular self-assembly. To this day, nanopore-based DNA sequencing devices[72] and biosensing platforms[73,74] are the primary applications of biointerfaces. This has led to the development of a whole new area of study dedicated to elucidating the mechanisms underlying these interactions, which have almost entirely been elicited by theoretical simulations. Experimental approaches are difficult to implement and, as a result, are less prevalent. Examples include studies based on adsorption and desorption experiments monitored by material-induced fluorescence quenching,[75] isothermal calorimetry,[76,77] and AFM.[78]

From a materials sciences' point of view, DNA—short for deoxyribonucleic acid—is a negatively charged polymer. The primary structure of DNA is made of four different nitrogenous nucleobases (adenine [A], cytosine [C], guanine [G], and thymine [T]), which are held by a sugar molecule and are interconnected by phosphate groups. The covalent linkage between sugar and phosphate groups forms the so-called sugar-phosphate backbone of the single-stranded DNA polymer. Non-covalent hydrogen bonds between complementary nucleobases (A with T and C with G) of two single strands lead to the formation of a double helix. This structure is stabilized by stacking interactions among neighboring nucleobases (**Figure 1**a). A planar projection of the double-stranded DNA (dsDNA) helix reveals alternating smaller and larger gaps in between two single strands. Those are denoted as minor and major grooves, respectively.[79] The single-stranded DNA (ssDNA) polymer is flexible with a persistence length of ≈2 nm, and hence it is typically coiled up.[80] Instead, dsDNA is rigid, with a persistence length of ≈50 nm.[81] The DNA polymer is generally hydrophilic due to its sugar-phosphate backbone. However, the nucleobases allow for hydrophobic interactions, especially by base stacking, and to some degree in the grooves of the double helix. Because of the phosphate groups, DNA has a negative net charge and is a polyelectrolyte that can be employed in layer-by-layer assemblies.[82] With respect to its electrical conductivity, the most important mechanism is charge transfer by hole transport along the nucleobases, with a dominant role of the guanosine nucleoside that has the highest oxidation potential.[83,84] DNA encodes the genetic information in organisms, providing the instructions to build the proteins that maintain life. Regardless of its various applications, the polymer is designed for information storage and transfer. Despite several protective mechanisms, even subtle modifications in DNA's primary structure can alter this information.

DNA has become the central material for bottom-up synthetic biology aiming at artificially creating life-like functions.[85] This does not only build on influencing the genetics of organisms, but also on the prebiotic side with the construction of complex and functional nanostructures using the DNA-origami technique and similar approaches.[86] The specificity and simplicity of the DNA-origami technique using DNA like a LEGO brick-like building block to create complex 3D nanoscale structures led to its broad application in nanofabrication, biosensing, and drug delivery.[87] This is only possible as the DNA-origami technique enables the realization of stable and precise structures at the nanometer scale, thereby making it possibile to manufacture nanostructures. Within this text, we refer to different nucleic acid structures, differentiated by the following terminology: DNA refers either to its single-stranded (ssDNA) or to its double-stranded form (dsDNA). DNA nanostructure refers to nanoscale structures made of DNA self-assemblies, and DNA-origami nanostructures are a specific example of the latter.

In this section, we briefly review the basics of the interactions between DNA and 2D materials, as well as the approaches to attach more complex DNA nanostructures, such as systems fabricated with the DNA-origami technique. For an in-depth analysis

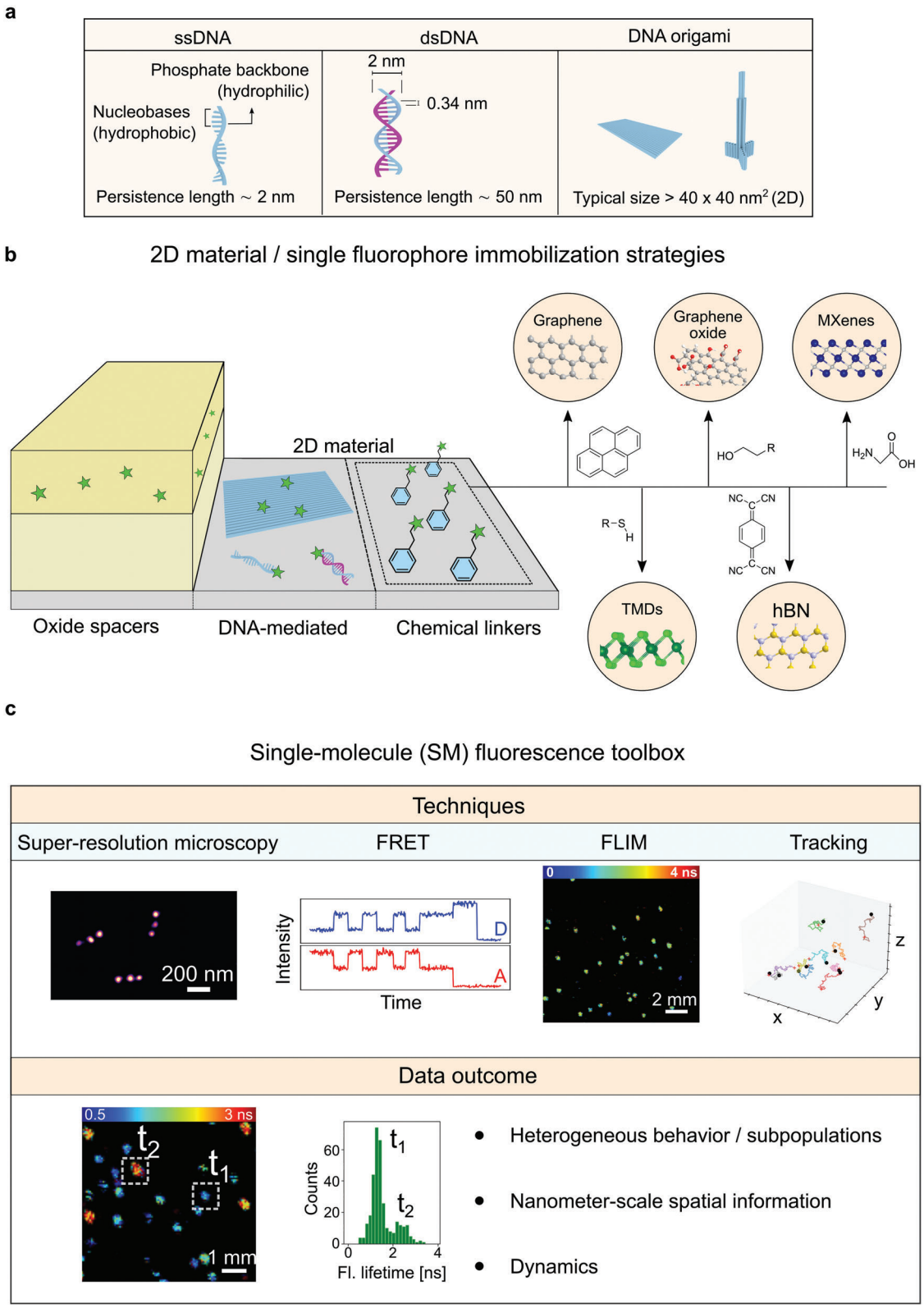

Figure 1. Controlled placement of dye molecules onto 2D materials for single-molecule fluorescence studies. a) Summary of the main physicochemical properties of single-stranded DNA, double-stranded DNA (dsDNA) and DNA-origami nanostructures. The cylinders in the scheme displaying DNA-origami nanostructures represent dsDNA. b) Sketch showing three approaches linking 2D materials and single fluorescent molecules, namely the use of oxide layers as spacers, the use of DNA molecules as mediators, and the linkage using chemical connectors. The right side elaborates on the linkage by coupling specific molecules, highlighting five examples for the selected groups of 2D materials. c) Summary of single-molecule fluorescence techniques

of these interactions, the reader is referred to more comprehensive reviews on this topic.[74,88–90]

3.1. Spontaneous Assembly of DNA onto 2D Materials

As a polymer, DNA adsorbs through multiple interaction points or segments throughout its chain. As a result, the sub-additive effect of enthalpy changes is high in magnitude.[77] The strong non-covalent DNA-2D material interaction is exemplified by the high affinity of the ssDNA-graphene system. The contact of ssDNA with graphene drives both hydrophobic and van der Waals interactions with purine and pyrimidine nucleobases via π–π stacking, rendering the hydrophilic sugar-phosphate backbone oriented away from the surface.[91] An early report based on isothermal calorimetry revealed that the relative interaction energies of isolated nucleobases in alkaline media with graphene dispersions decrease in the order guanine > adenine > cytosine ≥ thymine, the latter two being interchangeable and the strongest being −13.45 kcal mol^{-1}.[76] More recently, it has been computationally shown that ssDNA lies flat on the graphene surface with an average interaction energy as high as −23 kcal mol^{-1} per nucleotide, which is even stronger on hBN, for example, −29 kcal mol^{-1} per nucleotide.[92] In other findings, boron atoms have been found to play a crucial role in the alignment of the nucleobases, explaining why hBN has a higher affinity for DNA than graphene, both of which rely on the stacking of the nucleobases on the surface.[93] The strong interactions observed with boron nitride have been exploited in nanopore sequencing work, slowing down the DNA translocation time and providing enough time resolution for DNA sequencing.[94] The interactions of graphene/hBN with dsDNA differ from those of ssDNA as the nucleobases are shielded within the duplex. It has been shown that the stacked bases compete with π–π stacking on graphene.[95,96] In general, this differential affinity of ss- and dsDNA is non-surprisingly found for most 2D systems; however, the interaction mechanisms and contrast levels differ.

Introducing oxygen-rich moieties on these low-dimensional surfaces drives more complex interaction behaviors with nucleic acids. The interactions of graphene oxide with DNA involve a dynamic interplay between three contributions. i) Hydrogen bonds of the nucleobases with epoxy and hydroxyl groups that decorate the basal plane, as well as carboxyl, carbonyl, phenol, lactone, and quinone groups lining up on the edges. ii) π–π stacking occurs on the graphenic regions containing unoxidized sp^2 hybridized carbon. iii) Cation bridges of the phosphate backbone of ss- and dsDNA with the oxidized regions are formed in the presence of divalent ions, that is, commonly Mg^{2+}.[91,95] It has been revealed, both computationally and experimentally, that the degree and way in which DNA adsorbs onto graphene oxide vary according to the level of oxidation of the material.[97,98] In addition, it affects the balance between hydrogen bonding and π–π interactions, strongly impacting the biosensing performance of the material.[91,98,99]

Newer materials, such as TMDs and MXenes, have been found to interact more weakly with DNA than graphene-like materials.[100] An experimental adsorption/desorption study conducted with MoS_2 and WS_2 revealed that van der Waals forces play a key role in the adsorption mechanism of DNA oligonucleotides.[89] In another study, the average interaction energy of polynucleotides with MoS_2 was calculated to be five times weaker than that of graphene.[101] Moreover, dsDNA fails to adsorb onto a MoS_2 surface, tending to detach from it and diffuse into the bulk solution.[102] This strong dsDNA repulsion was exploited in a mix-and-read homogeneous biosensor platform.[103] In the case of MXenes, the negatively charged phosphate groups serve as anchors for DNA through cation bridges.[45,100] Researchers observed that only divalent cations induce adsorption of ssDNA in Ti_2C-MXenes, as evidenced by fluorescence quenching following the order $Mn^{2+} > Ni^{2+} > Ca^{2+} > Mg^{2+}$, where Mn^{2+} showed the highest adsorption efficiency.[100] Additionally, both ss- and dsDNA are capable of adsorbing onto MXenes because of the phosphate backbone-mediated interaction.

3.2. Controlled Immobilization of DNA Nanostructures on 2D Materials

The spontaneous assembly of ss- and dsDNA on 2D materials has brought about a wide range of applications. Going one step further, DNA nanostructures provide a superior degree of control that, together with single-molecule techniques, makes them an ideal platform for high-resolution studies of both material and biochemical systems. The DNA-origami technique, which takes advantage of DNA's inherent programmability, is an ideal way to generate these nanostructures. As a result, we can exert nanometer-level distance control as well as stoichiometric control over the inclusion of moieties of interest.[104] To make the best use of the programmability of DNA-origami nanostructures, they must be immobilized in a controlled manner on 2D materials using specific linkers for non-covalent and covalent chemistries. Pyrene is possibly the most important non-covalent linker for connecting graphene to biomolecules. Our group has leveraged the pyrene-graphene systems' intrinsic stacking interaction to build interfaces with DNA-origami nanostructures and graphene. Additionally, single-stranded extensions can be employed to immobilize those structures, building on the non-covalent interactions previously introduced.[105,106] As there are studies where distortions of DNA-origami nanostructures on graphene have been reported,[107,108] it is crucial to perform control experiments to verify that the integrity of the former is conserved. Naturally, the choice of an anchoring method will

and features of the main data outcome. Super-resolution microscopy exploits the transition between bright and dark states that take place at the level of single fluorescent molecules to overcome the diffraction barrier.[55–60] Single-molecule Förster resonance energy transfer (FRET) provides distance information between a fluorescent donor and acceptor dye molecule in the range of 110 nm,[61,62] and it has been widely used to study biomolecular interactions, and structural and conformational changes in proteins.[63,64] In single-molecule fluorescence lifetime imaging microscopy (FLIM), sparse single emitters are imaged and the fluorescence lifetime of each molecule is obtained.[65,66] These temporally resolved techniques allow studying the nanoscale surroundings of each fluorescent dye molecule, as well as gaining information about possible energy-transfer processes.[17] In single-molecule tracking, the position of a single fluorescent dye molecule is followed over time, which provides key information about its diffusion properties.[67] The introduced techniques provide varying information, as depicted in the lower part.

Table 1. Overview of the most-common, covalent functionalization strategies for the site-specific linkage of DNA on different 2D materials.

Material	Covalent linkage strategies
Graphene[112,116–120]	• Addition of free radicals to bind to the sp^2 carbon atoms • Addition of dienophiles to bind to carbon-carbon bonds • Nucleophilic addition reaction
Graphene oxide[112,116–119,121]	• Same as for graphene • Silanization (Si–O–C bond is formed) and etherification to attach to –OH groups • Fischer esterification reaction or Williamson ether synthesis, where carbodiimides are used as a coupling agent to bind with –COOH groups • Nucleophilic ring-opening reaction to bind epoxide groups
Hexagonal boron nitride[116,122]	• Radical reactions (carbene and nitrene intermediate) • Steam-mediated hydroxylation • Hydrothermal functionalization (hydroxyl and fluorine) • Introduction of hydroxyl, amino, ether, amine, arcyl, alkyl, or halogen groups, as well as heteroatoms (C and O)
Transition metal dichalcogenides (MoS_2/WS_2)[116,123,124]	• Ligand conjugation of functional group (from organic molecules) by linkage of i) chalcogen atoms ii) vacant sites of chalcogen atoms • Addition of molecules with thiol groups, favorably bond with sulfur vacancies of MoS_2/WS_2 • Hydrothermal functionalization (carboxyl and thiol ligands)
MXenes[110,116,125,126]	• Amine-silane-functionalization for further coupling with –COOH terminated biomolecules • Glycine-mediated chemisorption via N–Ti bonds

depend on the specific surface chemistry of the 2D material. For graphene, hydrophobic-driven interactions are the norm. However, for hydrophilic materials, other strategies must be deployed. For example, DNA-origami nanostructures have been interfaced on GO flakes via salt bridges with divalent cations. In our lab, we make use of glycine-modified DNA nanostructures for their controlled placement on MXene surfaces in the absence of divalent cations to prevent non-specific binding. The use of glycine is based on prior works on the interactions of amino acids with MXenes[109] and the use of glycine-MXene composites in energy storage.[110]

While non-covalent functionalization allows for the specific placement of DNA nanostructures on 2D materials without disrupting the underlying lattice or significantly altering the electronic behavior,[111,112] covalent approaches alternatively provide site-specific linkage with high chemical resistance. We listed the most common covalent strategies in **Table 1**. In Figure 1b, we further visualize the linkage of nanostructures on top of 2D materials. Remarkably, all the covalent and non-covalent strategies described can be used not only to attach DNA molecules to the surface, but also to other kinds of entities or materials,[113–115] including fluorescent molecules. For an even more comprehensive description of covalent functionalization of 2D materials, the reader is referred to recent reviews on the topic.[111,116]

4. Single-Molecule Fluorescence Detection

Single-molecule techniques were pioneered three decades ago[127] and have revolutionized the way of doing science in many fields of research. The main feature of these techniques is that they permit the study of each individual molecule present in an unsynchronized molecular population. Thus, they allow detecting heterogeneous behaviors that, otherwise, equalize in ensemble measurements. Single-molecule methods differ in the readout signal used for detection; this can be fluorescence, surface-enhanced Raman scattering, an electrochemical response, scattered electrons, or the resistive force in AFM experiments.[128] Here, we will focus our attention on single-molecule fluorescence methods, as many applications involving DNA and 2D materials have a fluorescence readout. Fluorescence techniques are non-invasive and do not depend on any mechanical interaction. However, fluorescence imposes limitations, with many samples being in a dark or dim state, thereby requiring fluorescent labeling, potentially altering the entity of interest.[129] Nonetheless, the labeling can be an advantage as well, since it allows specifically labeling a molecular target, even if it is present in a crowded environment surrounded by other molecular species. Another disadvantage is that certain materials suffer from autofluorescence, deteriorating the signal-to-background level, which can be critical in single-molecule measurements.[130]

Regarding sensing applications, single-molecule fluorescence emerges as an attractive option because it lets us observe molecular systems with the highest possible sensitivity. However, optical single-molecule detection has some intrinsic limitations, such as a limited dynamic concentration range.[131] For example, measuring targets below the picomolar concentration is challenging because significantly enlarged observation volumes are needed to increase the probability of detecting single target molecules within a reasonable period. However, a larger observation volume impairs the signal-to-noise ratio in such a way that single-molecule detection is no longer possible. Different strategies have been explored to overcome these limitations, such as the preconcentration of targets by implementing capturing schemes. In this regard, DNA-origami nanoantennas proved to be an excellent platform to both capture specific targets and improve the signal-to-noise ratio through fluorescence enhancement.[132]

Single-molecule fluorescence offers a powerful toolbox that is exploited in multiple scientific fields. In Figure 1c, we summarize some of the main single-molecule techniques available nowadays. Single-molecule fluorescence eliminates averaging effects and allows identifying heterogeneous subpopulations. When combined with imaging techniques, single-molecule fluorescence provides valuable spatial information, which enables, for example, the study of the optical and electronic properties of the materials on its edges or the distinction of pristine from defective areas at the nanometer scale. Moreover, the interactions between 2D materials and fluorophores extend the capabilities of single-molecule-based techniques. For example, the energy transfer from single dye molecules to graphene yields the distance between the fluorophore and the surface with sub-nanometer precision.[9,10] Next, we discuss different avenues in which single-molecule fluorescence techniques can be combined with 2D materials and show some successful examples. **Figure 2**a schematically portrays two categories that we propose to help understand the synergy: on the one hand, the characterization of 2D materials with single-molecule sensitivity; and on the other hand, the extension of the capabilities of single-molecule fluorescence methods. In Figures 2b–g, recent examples are presented.

5. Synergies between 2D Materials and Single-Molecule Fluorescence

In this section, we explore the synergies that can emerge from the combination of 2D materials and single-molecule fluorescence techniques. We argue for its benefits while recognizing the existing knowledge that is crucial for those studies. First, we discuss how certain features of materials can be studied with single-molecule fluorescence methods. Second, we dig into how the presence of 2D materials can enhance the capabilities of single-molecule fluorescence techniques.

5.1. Single-Molecule Fluorescence Sensitivity to Study Materials

Materials are often heterogeneous in both morphology and functionality. In some cases, materials have active regions that are in close proximity (nanometer scale) to inactive areas, and this can be studied with single-molecule experiments. For example, heterogeneous catalytic reactions converting fluorogenic substrates have been widely used to investigate the spatio-temporal properties of such reactions on nanoparticles as well as on 1D and 2D nanocrystals.[133] Single-molecule fluorescence microscopy serves as a vital approach to studying the presence and distribution of defects in 2D materials. Recently, super-resolution microscopy was used to detect and analyze sulfur-deficient point defects, grain boundaries, and line defects in flakes of MoS_2 and WS_2 (Figure 2b).[134] In this work, the authors claim a localization precision of 15 nm, which is compatible with the size of the smallest features observed in the inset of Figure 2b. Similarly, defects in hBN were uncovered with ≈11 nm resolution[135] (Figure 2c). Furthermore, antibunching measurements allowed to identify defective sites in MoS_2 by deciphering the emitter species.[136] It is instructive to consider the many works on resolving defects, making a case for how relevant it is to make them visible under ambient or even biologically compatible conditions.

The preparation of high-quality and homogeneous single-layer substrates remains a permanent challenge. Here, single-molecule studies can be used as a strategy for benchmarking new sample preparation protocols. In a recent work, time-resolved fluorescence measurements and DNA-origami nanotechnology were combined to screen the quality of the graphene lattice.[137] Single fluorophores were positioned at predefined sites of the nanostructure in such a way that the distance to the substrate was controlled with sub-nanometer accuracy. Exploiting the distance (d)-dependent energy transfer from single dye molecules to graphene, a process that follows a d^{-4} law and has a characteristic distance d_0 of ≈18 nm,[17] maps of fluorescence lifetime and intensity were obtained that revealed how the energy-transfer process occurred in different graphene areas. While high-quality graphene provided a narrow distribution of fluorescence lifetimes, the presence of holes, wrinkles, or defects was spatially identified by detecting molecules with unexpected fluorescence lifetime values and/or a broadened distribution of the spectroscopic properties[137] (Figure 2d). This also allowed using graphene sensors more accurately, for example, by attaching a reference dye molecule to the sensor in order to locally verify the quality of graphene. Consequently, it is possible to avoid defective areas of the sensor platform. Yet, it is not only about resolving defects, but we are also enabled to learn about their origin and the respective underlying physics.[138] In this respect, another example is mentioned that reveals the charge transport characteristics at solid–liquid interfaces by single-molecule localization microscopy on hBN.[139] MXenes have also been used as fluorescence quenchers for sensing applications in ensemble measurements.[140] However, for MXenes being in an earlier stage of development and understanding compared to other 2D materials, a thorough study based on single fluorescent molecules would give a profound insight into the material properties, that is, the quenching mechanism, its distance relation, and spectral dependencies.

Graphene offers many advantages compared to other materials regarding the quenching of fluorescence. Most of all, it can be used as a broadband fluorescence quencher due to its constant absorption across the visible spectral range. There are multiple studies demonstrating its optoelectronic properties also at the single-molecule level, which provide in-depth understanding about the mechanisms of the energy transfer and its distance dependency, with a sensitive range up to ≈40 nm.[17] Though many works use graphene oxide (GO) instead of graphene since, in terms of fluorescence quenching, it behaves similarly, but the material is cheaper. Although GO has other advantages, such as water solubility and rich chemistry due to the presence of oxygen-containing functional groups, there are no systematic studies on the distance dependency of the fluorescence quenching in the presence of GO. This is mainly due to the flaky nature of GO, having a small surface area, and the inability to easily determine the number of GO layers. Lacking an appropriate calibration curve, it is not possible to calculate the distance between a fluorescent dye molecule and GO based on lifetime or intensity measurements.[141] Another material used as a fluorescence quencher is gold. Although gold is not a 2D material, we mention

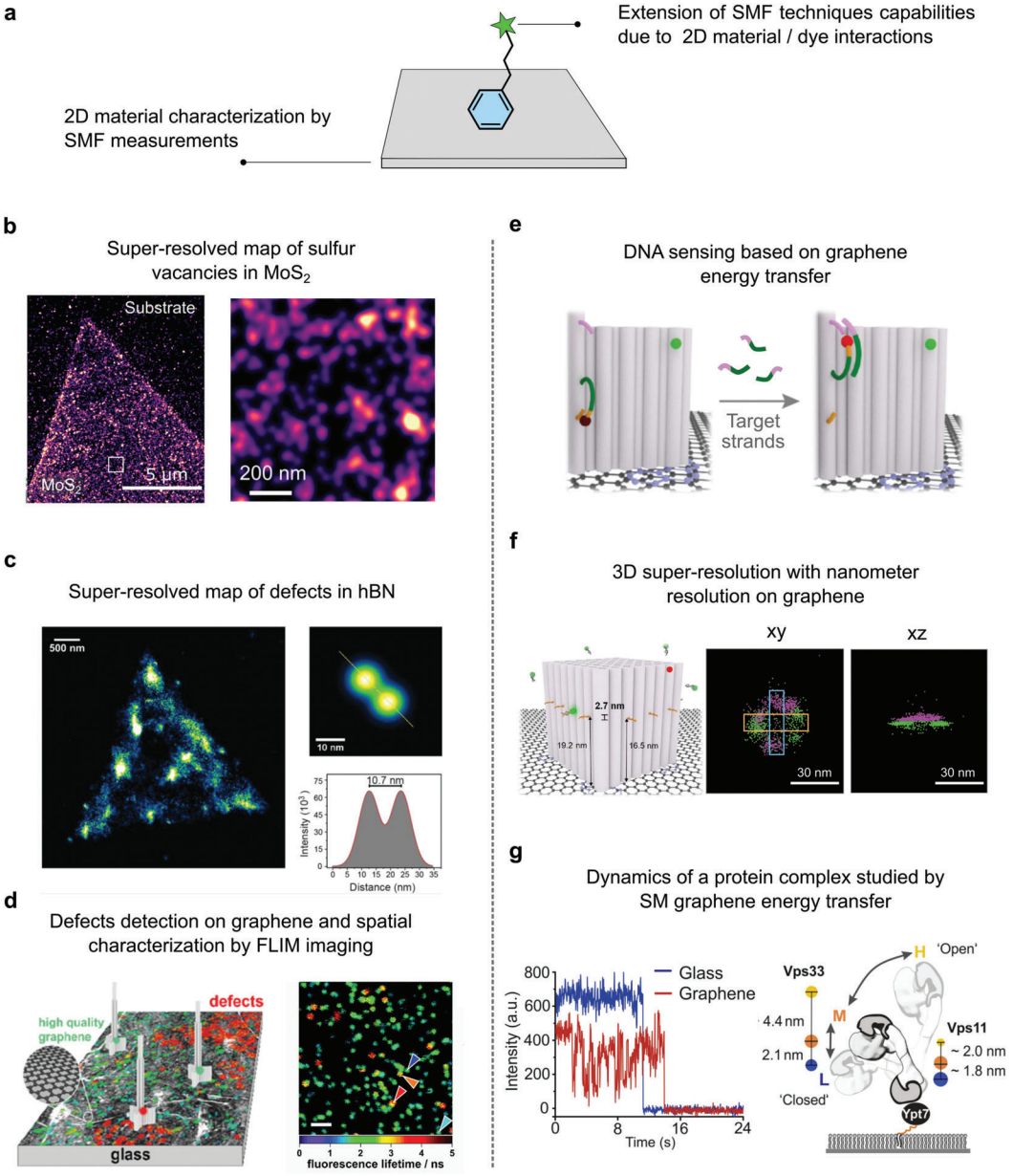

Figure 2. a) The combination of single-molecule fluorescence (SMF) and 2D materials offers a wide range of benefits for the study of materials (b–d), and the entities on top (e–g). For 2D materials, the super-resolved detection of defective sites under ambient conditions is especially relevant. Three examples show: b) MoS_2 as a typical TMD,[134] c) hBN,[135] and d) graphene.[137] On the other hand, 2D materials provide key information, for example, demonstrated for: e) biosensing,[9] f) super-resolution imaging,[9] or g) biophysical studies.[145] Images in (b): Reproduced with permission.[134] Copyright 2022, The Authors, published by American Chemical Society. Images in (c): Reproduced with permission.[135] Copyright 2018, American Chemical Society. Images in (d): Reproduced with permission.[137] Copyright 2021, American Chemical Society. Images in (e,f): Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International license (https://creativecommons.org/licenses/by/4.0).[9] Copyright 2021, The Authors, published by Wiley-VCH. Image in (g): Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International license (https://creativecommons.org/licenses/by/4.0).[145] Copyright 2021. The Authors, published by eLife Sciences Publications, Ltd.

it because the distance dependency of the fluorescence quenching of a thin gold layer is well studied. For example, a gold layer of 10 nm quenches the fluorescence up to ≈100 nm, making it suitable for cellular applications that require a larger effective range.[142,143] Nevertheless, a 10 nm layer of gold is significantly less transparent than a single sheet of graphene, and the detected background is higher, which is crucial for single-molecule measurements. There are several transparent conductors, with indium–tin oxide (ITO) being a well-known and commonly used representative. However, the quenching properties of ITO are strongly dependent on its thickness; even for a 17 nm-thick layer, it is relatively short-ranged with only 10 nm effective fluorescence quenching distance.[144] Additionally, the precise coating remains challenging.

5.2. 2D Materials Adding Information to Single-Molecule Fluorescence Experiments

Understanding the interactions between 2D materials and fluorescent dye molecules is a key to improving the performance of single-molecule based techniques. In a recent publication by our group, we show how the distance-dependent energy transfer from single dye molecules to graphene paves the way toward many types of applications[9] (Figure 2e,f). For example, we performed super-resolution microscopy measurements using DNA-origami nanostructures on graphene. In these experiments, the axial position of the detected fluorescent molecules was directly obtained by measuring the energy transfer from each dye molecule to graphene. Consequently, we were able to achieve axial resolutions as low as ≈3 nm. Furthermore, this high localization precision was also used to perform single-molecule tracking experiments and demonstrate biosensing assays. In another work, graphene energy transfer (GET) was applied to study dynamic conformational changes in protein complexes at membranes, demonstrating that there is plenty of room for biological applications[145] (Figure 2g). However, the 2D material might not only be a handy substrate, providing additional information about the specimen on top, but also alter it, as reported for the electroporation of cells on graphene[146] or the axon elongation of neurons growing on the 2D carbon allotrope.[147]

While the sensitive range for GET lies between 0 and 40 nm, other materials that undergo energy-transfer processes are sensitive in different distance ranges and, thus, are suitable to detect interactions, movements, or dynamic processes taking place there. While graphene has been thoroughly studied for almost two decades by now, and its properties are well known, for other, less explored, 2D materials, we are still witnessing how their properties are studied and rationalized. In this regard, there is a need to characterize the distance dependence of fluorescence quenching, understand the quenching mechanism, and exploit it for various applications. Moreover, it is foreseeable that the sensitive range for fluorescence quenching can be tuned by controlling the number of layers of a given material as well as by chemical or electrical doping. In this regard, especially, the electro-optical properties of 2D materials offer high potential. By doing this, for instance, the quenching of fluorescence is switched on and off, or plasmonic effects are introduced.[148–150]

Since the energy-transfer efficiency is derived from the fluorescence intensity or fluorescence lifetime of individual dye molecules, it is critical to calculate an appropriate energy-transfer calibration curve to obtain accurate results. There are different strategies for positioning dye molecules at controlled distances.[144,151] Among them, DNA-origami nanopositioners are a noteworthy option, as they enable to place single molecules with sub-nanometer accuracy in all three dimensions.[17,104,152,153] Compared to approaches where the calibration is performed using layers of oxides as spacers,[143,154] the implementation of DNA-origami nanostructures provides an aqueous environment for the reference dye molecules that is similar to the one they have in many sensing assays. Further, using DNA nanostructures allows placing reference dyes at specific heights in almost punctate spots while leaving the rest of the surface available to perform measurements. This cannot be achieved by depositing multiple oxide layers for calibration purposes.

6. Future Challenges

The synergistic combination of 2D materials and single-molecule approaches has proven to be fruitful in many senses. We have shown recent examples where these two worlds successfully merged to either shed light on materials' properties or extend the capabilities of single-molecule fluorescence techniques. Given the large number of devices based on DNA and 2D materials, we put a special focus on how single-molecule fluorescence measurements are performed on these hybrid systems.

Understanding the molecular interactions between DNA and 2D materials should be one of the first goals prior to application. However, studying these constructs is challenging and often addressed through theoretical approaches. Due to computing power limitations, these studies are typically limited to timescales of tens to hundreds of nanoseconds. More experiments are required to complement theory, and here is where single-molecule techniques frequently provide valuable insight. For example, they enable us to study not only the orientation of biomolecules on 2D materials but also more complex phenomena such as stretching, bending, or diffusion under varying stimuli. In particular, heterostructures have the potential to induce interesting effects on the biophysics of DNA, as demonstrated by computational studies.[92,155] To perform single-molecule studies in this direction, controlling the positioning of DNA molecules close to the material surface is crucial. As discussed above, chemical connectors offer an opportunity to achieve this goal. Regarding this challenge, DNA-origami nanostructures are a unique tool because they provide means for multiple functionalities, which are organized in a predefined manner at the nanometer scale. Super-resolution methods based on the localization of single fluorescent molecules hold strong potential to be applied in materials science. When combined with 2D materials, they provide key information about the spatial distribution of defects at the nanoscale, even if they are densely packed. Alternatively, the distance-dependent energy-transfer process enables one to decipher the axial position of the fluorophore and perform 3D super-resolution experiments on the material surface. Recent advances have demonstrated that localizing single dye molecules with Ångström precision is possible, which would allow studying processes in the material or mapping defects with true molecular spatial resolution.[156] Furthermore, if high spatial and temporal resolution are simultaneously required, the so-called MINFLUX technique[160] or its pulsed-interleaved version, p-MINFLUX,[10,158] are the methods of choice.[159] Understanding dynamic processes will gain in importance, resulting in a growing number of tracking applications.[159] Toward this end, unveiling fast conformational changes will be one step.[160]

While the proposed combination of different fields can be beneficial, it also introduces the challenges and drawbacks of each field. For example, an impending challenge with 2D materials is the complex and costly synthesis. Currently, industrial-scale production providing the required quality for fundamental research is not possible, constituting a major bottleneck.[161,162] Here, hBN and MXenes represent prime examples of the problem of producing large-area surfaces while maintaining high quality. As another issue, the optical detection of single fluorescent molecules requires an efficient collection of emitted photons, which is often synonymous to demanding a transparent substrate. The

necessary transfer of the 2D material introduces further uncertainties, as reported for graphene.[163] Moreover, single-molecule fluorescence techniques require expensive and sophisticated setups. Operating them requires well-trained staff for data acquisition and handling. Additionally, single-molecule fluorescence studies restrict the choice of dyes, imposing difficulties when working at shorter wavelengths. However, there are efforts to relax these constraints, with a special focus on alternative equipment.[164,165] Despite these drawbacks, we foresee an increasing number of applications and more 2D materials being exploited for complex biological experiments, aided by single-molecule techniques. In the future, we will find more and more 2D materials in everyday life. We aim to make a case for the insights we gain at the single-molecule level and encourage researchers in the materials science field to reinforce links with the single-molecule community. Given the recent efforts and technical developments aiming to decrease the technical complexity of single-molecule fluorescence setups, we envision that it will become possible to detect single fluorescent molecules individually on a 2D material even without highly specialized equipment.

Over the past few years, we have recognized the potential of 2D materials such as graphene as an increasingly useful tool for our single-molecule fluorescence measurements. Interfacing this material with DNA-origami nanostructures has recently enabled a plethora of exciting applications, from biosensing and biophysics to the fine elucidation of biological structures with super-resolution microscopy. Furthermore, this platform is now allowing us to unravel intricate biorecognition phenomena with unprecedented sensitivity, creating powerful opportunities in structural biology. Yet, while single-molecule fluorescence techniques have been historically tightly associated with biology, there is also potential in interfacing such technologies with materials science to answer important questions in that field. For example, we have seen how to utilize DNA nanostructures and the robustness of graphene's energy-transfer properties to probe the in situ quality of graphene monolayers as well as to understand the effect of multilayers on the energy transfer. Beyond the realm of graphene, we have also begun to interface other 2D materials, such as MXene flakes or hBN–graphene heterostructures.

We believe that the combination of 2D materials, DNA-mediated interactions, and single-molecule fluorescence techniques yields a powerful interdisciplinary tool for both fundamental and applied scientific pursuits, many of which are yet to be explored.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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[1] K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang, S. V. Dubonos, I. V. Grigorieva, A. A. Firsov, *Science* **2004**, *306*, 666.
[2] X. Cai, Y. Luo, B. Liu, H.-M. Cheng, *Chem. Soc. Rev.* **2018**, *47*, 6224.
[3] A. K. Geim, *Science* **2009**, *324*, 1530.
[4] Y. Liu, X. Duan, H.-J. Shin, S. Park, Yu Huang, X. Duan, *Nature* **2021**, *591*, 43.
[5] K. S. Novoselov, V. I. Fal'Ko, L. Colombo, P. R. Gellert, M. G. Schwab, K. Kim, *Nature* **2012**, *490*, 192.
[6] Chemicals & Materials, *Graphene – A Global Market Overview.* **2023**.
[7] The Graphene Council, *The 2020 Graphene Survey.* www.thegraphenecouncil.org **2021**.
[8] T. Barkan, *Nat. Nanotechnol.* **2019**, *14*, 904.
[9] I. Kamińska, J. Bohlen, R. Yaadav, P. Schüler, M. Raab, T. Schröder, J. Zähringer, K. Zielonka, S. Krause, P. Tinnefeld, *Adv. Mater.* **2021**, *33*, 2101099 .
[10] J. Zähringer, F. Cole, J. Bohlen, F. Steiner, I. Kamińska, P. Tinnefeld, *Light: Sci. Appl.* **2023**, *12*, 70.
[11] W. E. Moerner, *J. Phys. Chem. B* **2002**, *106*, 910.
[12] W. E. Moerner, D. P. Fromm, *Rev. Sci. Instrum.* **2003**, *74*, 3597.
[13] L. Von Diezmann, Y. Shechtman, W. E. Moerner, *Chem. Rev.* **2017**, *117*, 7244.
[14] P. Tamarat, A. Maali, B. Lounis, M. Orrit, *J. Phys. Chem.* **2000**, *104*, 1.
[15] W. E. Moerner, M. Orrit, *Science* **1999**, *283*, 1670.
[16] Q. Xu, Qi Zhou, Z. Hua, Qi Xue, C. Zhang, X. Wang, D. Pan, M. Xiao, *ACS Nano* **2013**, *7*, 10654.
[17] I. Kaminska, J. Bohlen, S. Rocchetti, F. Selbach, G. P. Acuna, P. Tinnefeld, *Nano Lett.* **2019**, *19*, 4257.
[18] R. R. Nair, P. Blake, A. N. Grigorenko, K. S. Novoselov, T. J. Booth, T. Stauber, N. M. R. Peres, A. K. Geim, *Science* **2008**, *320*, 1308.
[19] C. Zhu, D. Du, Y. Lin, *2D Mater.* **2015**, *2*, 032004 .
[20] X. Deng, H. Tang, J. Jiang, *Anal. Bioanal. Chem.* **2014**, *406*, 6903.
[21] H. Zhang, H. Zhang, A. Aldalbahi, X. Zuo, C. Fan, X. Mi, *Biosens. Bioelectron.* **2017**, *89*, 96.
[22] M. Pumera, *Mater. Today* **2011**, *14*, 308.
[23] P. Zhan, J. Wang, Z.-G. Wang, B. Ding, *Small* **2014**, *10*, 399.
[24] A. Béraud, M. Sauvage, C. M. Bazán, M. Tie, A. Bencherif, D. Bouilly, *Analyst* **2021**, *146*, 403.
[25] Y. Zhu, H. Ji, H.-M. Cheng, R. S. Ruoff, *Natl. Sci. Rev.* **2018**, *5*, 90.

[26] R. Ye, J. M. Tour, *ACS Nano* **2019**, *13*, 10872.
[27] B. C. Brodie, *Philos. Trans. R. Soc. London* **1859**, *149*, 249.
[28] W. S. Hummers, R. E. Offeman, *J. Am. Chem. Soc.* **1958**, *80*, 1339.
[29] D. C. Marcano, D. V. Kosynkin, J. M. Berlin, A. Sinitskii, Z. Sun, A. Slesarev, L. B. Alemany, W. Lu, J. M. Tour, *ACS Nano* **2010**, *4*, 4806.
[30] N. I. Zaaba, K. L. Foo, U. Hashim, S. J. Tan, W.-W. Liu, C. H. Voon, *Procedia Eng.* **2017**, *184*, 469.
[31] Y. Zhu, S. Murali, W. Cai, X. Li, W. Suk, J. R. Potts, R. S. Ruoff, *Adv. Mater.* **2010**, *22*, 3906.
[32] A. T. Dideikin, A. Y. Vul', *Front. Phys.* **2019**, *6*, 00149.
[33] C. Backes, A. M. Abdelkader, C. Alonso, A. Andrieux-Ledier, R. Arenal, J. Azpeitia, N. Balakrishnan, L. Banszerus, J. Barjon, R. Bartali, S. Bellani, C. Berger, R. Berger, M. M. B. Ortega, C. Bernard, P. H. Beton, A. Beyer, A. Bianco, P. Bøggild, F. Bonaccorso, G. B. Barin, C. Botas, R. A. Bueno, D. Carriazo, A. Castellanos-Gomez, M. Christian, A. Ciesielski, T. Ciuk, M. T. Cole, J. Coleman, et al., *2D Mater.* **2020**, *7*, 022001.
[34] R. Tarcan, O. Todor-Boer, I. Petrovai, C. Leordean, S. Astilean, I. Botiz, *J. Mater. Chem. C* **2020**, *8*, 1198.
[35] D. J. Joshi, J. R. Koduru, N. I. Malek, C. M. Hussain, S. K. Kailasa, *TrAC Trends Anal. Chem.* **2021**, *144*, 116448.
[36] J. D. Caldwell, I. Aharonovich, G. Cassabois, J. H. Edgar, B. Gil, D. N. Basov, *Nat. Rev. Mater.* **2019**, *4*, 552.
[37] M. Xu, T. Liang, M. Shi, H. Chen, *Chem. Rev.* **2013**, *113*, 3766.
[38] C. R. Dean, A. F. Young, I. Meric, C. Lee, L. Wang, S. Sorgenfrei, K. Watanabe, T. Taniguchi, P. Kim, K. L. Shepard, J. Hone, *Nat. Nanotechnol.* **2010**, *5*, 722.
[39] S. Roy, X. Zhang, A. B. Puthirath, A. Meiyazhagan, S. Bhattacharyya, M. M. Rahman, G. Babu, S. Susarla, S. K. Saju, M. K. Tran, L. M. Sassi, M. A. S. R. Saadi, J. Lai, O. Sahin, S. M. Sajadi, B. Dharmarajan, D. Salpekar, N. Chakingal, A. Baburaj, X. Shuai, A. Adumbumkulath, K. A. Miller, J. M. Gayle, A. Ajnsztajn, T. Prasankumar, V. V. J. Harikrishnan, V. Ojha, H. Kannan, A. Z. Khater, Z. Zhu, et al., *Adv. Mater.* **2021**, *33*, 2101589.
[40] S. Manzeli, D. Ovchinnikov, D. Pasquier, O. V. Yazyev, A. Kis, *Nat. Rev. Mater.* **2017**, *2*, 17033.
[41] K. F. Mak, J. Shan, *Nat. Photonics* **2016**, *10*, 216.
[42] H. Hu, A. Zavabeti, H. Quan, W. Zhu, H. Wei, D. Chen, J. Z. Ou, *Biosens. Bioelectron.* **2019**, *142*, 111573 .
[43] S. Zhu, L. Gong, J. Xie, Z. Gu, Y. Zhao, *Small Methods* **2017**, *1*, 1700220.
[44] M. Naguib, M. Kurtoglu, V. Presser, J. Lu, J. Niu, M. Heon, L. Hultman, Y. Gogotsi, M. W. Barsoum, *Adv. Mater.* **2011**, *23*, 4248.
[45] C. L. Manzanares-Palenzuela, A. M. Pourrahimi, J. Gonzalez-Julian, Z. Sofer, M. Pykal, M. Otyepka, M. Pumera, *Chem. Sci.* **2019**, *10*, 10010.
[46] A. Zamhuri, G. P. Lim, N. L. Ma, K. S. Tee, C. F. Soon, *Biomed. Eng. Online* **2021**, *20*, 33.
[47] Y. Gogotsi, B. Anasori, *ACS Nano* **2019**, *13*, 8491.
[48] F. Meng, M. Seredych, C. Chen, V. Gura, S. Mikhalovsky, S. Sandeman, G. Ingavle, T. Ozulumba, L. Miao, B. Anasori, Y. Gogotsi, *ACS Nano* **2018**, *12*, 10518.
[49] A. Vahidmohammadi, J. Rosen, Y. Gogotsi, *Science* **2021**, *372*, 1165.
[50] A. K. Geim, I. V. Grigorieva, *Nature* **2013**, *499*, 419.
[51] P. V. Pham, S. C. Bodepudi, K. Shehzad, Y. Liu, Y. Xu, B. Yu, X. Duan, *Chem. Rev.* **2022**, *122*, 6514.
[52] R. Zhang, M. Li, L. Li, Z. Wei, F. Jiao, D. Geng, W. Hu, *Adv. Funct. Mater.* **2021**, *31*, 2102049.
[53] L. Liu, D. Peng, Q. Ma, Z. Jiang, J. Wang, J. Qian, *Nano-Micro Lett.* **2019**, *11*, 13.
[54] J. Wang, Z. Li, H. Chen, G. Deng, X. Niu, *Nano-Micro Lett.* **2019**, *11*, 48.
[55] S. W. Hell, *Science* **2007**, *316*, 1153.
[56] S. W. Hell, J. Wichmann, *Opt. Lett.* **1994**, *19*, 780.
[57] E. Betzig, G. H. Patterson, R. Sougrat, O. W Lindwasser, S. Olenych, J. S. Bonifacino, M. W. Davidson, J. Lippincott-Schwartz, H. F. Hess, *Science* **2006**, *313*, 1642.
[58] M. J. Rust, M. Bates, X. Zhuang, *Nat. Methods* **2006**, *3*, 793.
[59] M. Heilemann, S. Van De Linde, M. Schüttpelz, R. Kasper, B. Seefeldt, A. Mukherjee, P. Tinnefeld, M. Sauer, *Angew. Chem., Int. Ed.* **2008**, *47*, 6172.
[60] R. Jungmann, C. Steinhauer, M. Scheible, A. Kuzyk, P. Tinnefeld, F. C. Simmel, *Nano Lett.* **2010**, *10*, 4756.
[61] R. Roy, S. Hohng, T. Ha, *Nat. Methods* **2008**, *5*, 507.
[62] B. Hellenkamp, S. Schmid, O. Doroshenko, O. Opanasyuk, R. Kühnemuth, S. Rezaei Adariani, B. Ambrose, M. Aznauryan, A. Barth, V. Birkedal, M. E. Bowen, H. Chen, T. Cordes, T. Eilert, C. Fijen, C. Gebhardt, M. Götz, G. Gouridis, E. Gratton, T. Ha, P. Hao, C. A. Hanke, A. Hartmann, J. Hendrix, L. L. Hildebrandt, V. Hirschfeld, J. Hohlbein, B. Hua, C. G. Hübner, E. Kallis, et al., *Nat. Methods* **2018**, *15*, 669.
[63] B. Schuler, W. A. Eaton, *Curr. Opin. Struct. Biol.* **2008**, *18*, 16.
[64] H. Mazal, G. Haran, *Curr. Opin. Biomed. Eng.* **2019**, *12*, 8.
[65] P. Tinnefeld, V. Buschmann, D.-P. Herten, K.-T. Han, M. Sauer, *Single Mol.* **2000**, *1*, 215.
[66] M. Heilemann, D. P. Herten, R. Heintzmann, C. Cremer, C. Müller, P. Tinnefeld, K. D. Weston, J. Wolfrum, M. Sauer, *Anal. Chem.* **2002**, *74*, 3511.
[67] A. Dupont, D. C. Lamb, *Nanoscale* **2011**, *3*, 4532 .
[68] N. Z. Fantoni, A. H. El-Sagheer, T. Brown, *Chem. Rev.* **2021**, *121*, 7122.
[69] Y. Dong, C. Yao, Yi Zhu, Lu Yang, D. Luo, D. Yang, *Chem. Rev.* **2020**, *120*, 9420.
[70] M. Madsen, K. V. Gothelf, *Chem. Rev.* **2019**, *119*, 6384.
[71] Y. Hu, C. M. Niemeyer, *Adv. Mater.* **2019**, *31*, 1970190.
[72] D. B. Wells, M. Belkin, J. Comer, A. Aksimentiev, *Nano Lett.* **2012**, *12*, 4117.
[73] C.-H. Lu, H.-H. Yang, C.-L. Zhu, Xi Chen, G.-N. Chen, *Angew. Chem., Int. Ed.* **2009**, *48*, 4785.
[74] N. S. Green, M. L. Norton, *Anal. Chim. Acta* **2015**, *853*, 127.
[75] M. P. Kushalkar, B. Liu, J. Liu, *Langmuir* **2020**, *36*, 11183.
[76] N. Varghese, U. Mogera, A. Govindaraj, A. Das, P. K. Maiti, A. K. Sood, C. N. R. Rao, *ChemPhysChem* **2009**, *10*, 206.
[77] S. V. Ranganathan, K. Halvorsen, C. A. Myers, N. M. Robertson, M. V. Yigit, A. A. Chen, *Langmuir* **2016**, *32*, 6028.
[78] B. S Husale, S. Sahoo, A. Radenovic, F. Traversi, P. Annibale, A. Kis, *Langmuir* **2010**, *26*, 18078.
[79] R. R. Sinden, in *DNA Structure and Function*, Elsevier, Amsterdam **1994**.
[80] E. Roth, A. Glick Azaria, O. Girshevitz, A. Bitler, Y. Garini, *Nano Lett.* **2018**, *18*, 6703.
[81] Q. Du, C. Smith, N. Shiffeldrim, M. Vologodskaia, A. Vologodskii, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5397.
[82] C. Jewell, D. Lynn, *Adv. Drug Delivery Rev.* **2008**, *60*, 979.
[83] C. Dekker, M. Ratner, *Phys. World* **2001**, *14*, 29.
[84] S. Doose, H. Neuweiler, M. Sauer, *ChemPhysChem* **2009**, *10*, 1389.
[85] K. Göpfrich, I. Platzman, J. P. Spatz, *Trends Biotechnol.* **2018**, *36*, 938.
[86] C. E. Castro, F. Kilchherr, Do-N Kim, E. L. Shiao, T. Wauer, P. Wortmann, M. Bathe, H. Dietz, *Nat. Methods* **2011**, *8*, 221.
[87] P. Wang, T. A. Meyer, V. Pan, P. K. Dutta, Y. Ke, *Chem* **2017**, *2*, 359.
[88] S. H. Chen, D. R. Bell, B. Luan, *Adv. Drug Delivery Rev.* **2022**, *186*, 114336.
[89] C. Lu, Y. Liu, Y. Ying, J. Liu, *Langmuir* **2017**, *33*, 630.
[90] N. Asefifeyzabadi, P. K. Das, A. H. Onorimuo, G. Durocher, M. H. Shamsi, *RSC Adv.* **2021**, *11*, 28332.
[91] H. S Kim, B. L. Farmer, Y. G. Yingling, *Adv. Mater. Interfaces* **2017**, *4*, 1601168.

[92] B. Luan, R. Zhou, *Nat. Commun.* **2019**, *10*, 4610.
[93] Q. Lin, X. Zou, G. Zhou, R. Liu, J. Wu, J. Li, W. Duan, *Phys. Chem. Chem. Phys.* **2011**, *13*, 12225.
[94] S. Liu, Bo Lu, Q. Zhao, Ji Li, T. Gao, Y. Chen, Y. Zhang, Z. Liu, Z. Fan, F. Yang, L. You, D. Yu, *Adv. Mater.* **2013**, *25*, 4549.
[95] S. Muraru, C. G. Samoila, E. I. Slusanschi, J. S. Burns, M. Ionita, *Coatings* **2020**, *10*, 289.
[96] A. K. Manna, S. K. Pati, *J. Mater. Chem. B* **2013**, *1*, 91.
[97] K. Kim, J. Guo, Z. Liang, D. Fan, *Adv. Funct. Mater.* **2018**, *28*, 1705867.
[98] B. J. Hong, Z. An, O. C. Compton, S. T. Nguyen, *Small* **2012**, *8*, 2469.
[99] C. L Manzanares Palenzuela, A. M. Pourrahimi, Z. Sofer, M. Pumera, *ACS Omega* **2019**, *4*, 1611.
[100] Z. Huang, B. Liu, J. Liu, *Langmuir* **2019**, *35*, 9858.
[101] L. Liang, W. Hu, Z. Xue, J.-W. Shen, *FlatChem.* **2017**, *2*, 8.
[102] Hu Qiu, A. Sarathy, K. Schulten, J.-P. Leburton, *npj 2D Mater. Appl.* **2017**, *1*, 3.
[103] C. Zhu, Z. Zeng, H. Li, F. Li, C. Fan, H. Zhang, *J. Am. Chem. Soc.* **2013**, *135*, 5998.
[104] M. Scheckenbach, J. Bauer, J. Zähringer, F. Selbach, P. Tinnefeld, *APL Mater.* **2020**, *8*, 110902.
[105] A. Mangalum, M. Rahman, M. L. Norton, *J. Am. Chem. Soc.* **2013**, *135*, 2451.
[106] L. Tang, Y. Wang, Y. Liu, J. Li, *ACS Nano* **2011**, *5*, 3817.
[107] Y. Kabiri, A. N. Ananth, J. Van Der Torre, A. Katan, J.-Y. Hong, S. Malladi, J. Kong, H. Zandbergen, C. Dekker, *Small* **2017**, *13*, 1700876.
[108] N. S. Green, P. H. Q. Pham, D. T. Crow, P. J. Burke, M. L. Norton, *Mater. Res. Express* **2018**, *5*, 045035.
[109] J. D. Gouveia, G. Novell-Leruth, P. M. L. S. Reis, F. Viñes, F. Illas, J. R. B. Gomes, *ACS Appl. Bio Mater.* **2020**, *3*, 5913.
[110] C. Chen, M. Boota, P. Urbankowski, B. Anasori, L. Miao, J. Jiang, Y. Gogotsi, *J. Mater. Chem. A* **2018**, *6*, 4617.
[111] A. R. Brill, E. Koren, G. De Ruiter, *J. Mater. Chem. C* **2021**, *9*, 11569.
[112] V. Georgakilas, J. N. Tiwari, K. C Kemp, J. A. Perman, A. B. Bourlinos, K. S. Kim, R. Zboril, *Chem. Rev.* **2016**, *116*, 5464.
[113] F. Dumur, *Eur. Polym. J.* **2020**, *126*, 109564 .
[114] M. Ahangarpour, I. Kavianinia, P. W. R. Harris, M. A. Brimble, *Chem. Soc. Rev.* **2021**, *50*, 898.
[115] A. Gräwe, V. Stein, *Trends Biotechnol.* **2021**, *39*, 731.
[116] J. H. Jeong, S. Kang, N. Kim, R. Joshi, G.-H. Lee, *Phys. Chem. Chem. Phys.* **2022**, *24*, 10684.
[117] I. A. Vacchi, C. Ménard-Moyon, A. Bianco, *Phys. Sci. Rev.* **2017**, *2*, 20160103.
[118] A. Criado, M. Melchionna, S. Marchesan, M. Prato, *Angew. Chem., Int. Ed.* **2015**, *54*, 10734.
[119] J. Park, M. Yan, *Acc. Chem. Res.* **2013**, *46*, 181.
[120] H. Yang, F. Li, C. Shan, D. Han, Q. Zhang, Li Niu, A. Ivaska, *J. Mater. Chem.* **2009**, *19*, 4632.
[121] S. Georgitsopoulou, N. D. Stola, A. Bakandritsos, V. Georgakilas, *Surf. Interfaces* **2021**, *26*, 101320.
[122] Q. Weng, X. Wang, X. Wang, Y. Bando, D. Golberg, *Chem. Soc. Rev.* **2016**, *45*, 3989.
[123] J. Azadmanjiri, P. Kumar, V. K. Srivastava, Z. Sofer, *ACS Appl. Nano Mater* **2020**, *3*, 3116.
[124] B. L. Li, M. I. Setyawati, L. Chen, J. Xie, K. Ariga, C.-T. Lim, S. Garaj, D. T. Leong, *ACS Appl. Mater. Interfaces* **2017**, *9*, 15286.
[125] H. Riazi, M. Anayee, K. Hantanasirisakul, A. A. Shamsabadi, B. Anasori, Y. Gogotsi, M. Soroush, *Adv. Mater. Interfaces* **2020**, *7*, 1902008.
[126] R. Fang, C. Lu, A. Chen, K. Wang, H. Huang, Y. Gan, C. Liang, J. Zhang, X. Tao, Y. Xia, W. Zhang, *ChemSusChem* **2020**, *13*, 1409.
[127] W. E. Moerner, L. Kador, *Phys. Rev. Lett.* **1989**, *62*, 2535.
[128] S. Mohapatra, C.-T. Lin, X. A. Feng, A. Basu, T. Ha, *Chem. Rev.* **2019**, *120*, 36.
[129] J. R. Lakowicz in *Principles of Fluorescence Spectroscopy*, Springer, Berlin, Germany **2006**.
[130] S. Wang, W. X. Ren, Ji-T Hou, M. Won, J. An, X. Chen, J. Shu, J. S. Kim, *Chem. Soc. Rev.* **2021**, *50*, 8887.
[131] P. Holzmeister, G. P. Acuna, D. Grohmann, P. Tinnefeld, *Chem. Soc. Rev.* **2014**, *43*, 1014.
[132] V. Glembockyte, L. Grabenhorst, K. Trofymchuk, P. Tinnefeld, *Acc. Chem. Res.* **2021**, *54*, 3338.
[133] P. Chen, X. Zhou, N. M. Andoy, K.-S. Han, E. Choudhary, N. Zou, G. Chen, H. Shen, *Chem. Soc. Rev.* **2014**, *43*, 1107.
[134] M. Zhang, M. Lihter, T.-H. Chen, M. Macha, A. Rayabharam, K. Banjac, Y. Zhao, Z. Wang, J. Zhang, J. Comtet, N. R. Aluru, M. Lingenfelder, A. Kis, A. Radenovic, *ACS Nano* **2021**, *15*, 7168.
[135] J. Feng, H. Deschout, S. Caneva, S. Hofmann, I. Lončarić, P. Lazić, A. Radenovic, *Nano Lett.* **2018**, *18*, 1739.
[136] J. Klein, L. Sigl, S. Gyger, K. Barthelmi, M. Florian, S. Rey, T. Taniguchi, K. Watanabe, F. Jahnke, C. Kastl, V. Zwiller, K. D. Jöns, K. Müller, U. Wurstbauer, J. J. Finley, A. W. Holleitner, *ACS Photonics* **2021**, *8*, 669.
[137] S. Krause, E. Ploetz, J. Bohlen, P. Schüler, R. Yaadav, F. Selbach, F. Steiner, I. Kamińska, P. Tinnefeld, *ACS Nano* **2021**, *15*, 6430 .
[138] J. Comtet, E. Glushkov, V. Navikas, J. Feng, V. Babenko, S. Hofmann, K. Watanabe, T. Taniguchi, A. Radenovic, *Nano Lett.* **2019**, *19*, 2516.
[139] J. Comtet, B. Grosjean, E. Glushkov, A. Avsar, K. Watanabe, T. Taniguchi, R. Vuilleumier, M.-L. Bocquet, A. Radenovic, *Nat. Nanotechnol.* **2020**, *15*, 598.
[140] X. Zhu, Y. Zhang, M. Liu, Y. Liu, *Biosens. Bioelectron.* **2021**, *171*, 112730 .
[141] X. Xiao, Y. Zhang, L. Zhou, B. Li, L. Gu, *Nanomaterials* **2022**, *12*, 2444.
[142] S. Isbaner, N. Karedla, I. Kaminska, D. Ruhlandt, M. Raab, J. Bohlen, A. Chizhik, I. Gregor, P. Tinnefeld, J. Enderlein, R. Tsukanov, *Nano Lett.* **2018**, *18*, 2616.
[143] A. Ghosh, A. I. Chizhik, N. Karedla, J. Enderlein, *Nat. Protoc.* **2021**, *16*, 3695.
[144] R. J. Moerland, J. P. Hoogenboom, *Optica* **2016**, *3*, 112 .
[145] N. Füllbrunn, Z. Li, L. Jorde, C. P. Richter, R. Kurre, L. Langemeyer, C. Yu, C. Meyer, J. Enderlein, C. Ungermann, J. Piehler, C. You, *eLife* **2021**, *10*, e62501.
[146] S. Moon, W. Li, M. Hauser, Ke Xu, *ACS Nano* **2020**, *14*, 5609.
[147] D. Convertino, F. Fabbri, N. Mishra, M. Mainardi, V. Cappello, G. Testa, S. Capsoni, L. Albertazzi, S. Luin, L. Marchetti, C. Coletti, *Nano Lett.* **2020**, *20*, 3633.
[148] F. Wang, Y. Zhang, C. Tian, C. Girit, A. Zettl, M. Crommie, Y. R Shen, *Science* **2008**, *320*, 206.
[149] K. J. Tielrooij, L. Orona, A. Ferrier, M. Badioli, G. Navickaite, S. Coop, S. Nanot, B. Kalinic, T. Cesca, L. Gaudreau, Q. Ma, A. Centeno, A. Pesquera, A. Zurutuza, H. De Riedmatten, P. Goldner, F. J. García De Abajo, P. Jarillo-Herrero, F. H. L. Koppens, *Nat. Phys.* **2015**, *11*, 281.
[150] O. Salihoglu, N. Kakenov, O. Balci, S. Balci, C. Kocabas, *Sci. Rep.* **2016**, *6*, 33911.
[151] A. Ghosh, A. Sharma, A. I. Chizhik, S. Isbaner, D. Ruhlandt, R. Tsukanov, I. Gregor, N. Karedla, J. Enderlein, *Nat. Photonics* **2019**, *13*, 860.
[152] J. J. Schmied, A. Gietl, P. Holzmeister, C. Forthmann, C. Steinhauer, T. Dammeyer, P. Tinnefeld, *Nat. Methods* **2012**, *9*, 1133.
[153] J. J. Schmied, M. Raab, C. Forthmann, E. Pibiri, B. Wünsch, T. Dammeyer, P. Tinnefeld, *Nat. Protoc.* **2014**, *9*, 1367.

[154] S. O. Raja, A. I. Chizhik, C. F. Schmidt, J. Enderlein, A. Ghosh, *Nano Lett.* **2021**, *21*, 8244.
[155] Z. He, R. Zhou, *Nanoscale* **2020**, *12*, 13822.
[156] M. Weber, H. Von Der Emde, M. Leutenegger, P. Gunkel, S. Sambandan, T. A. Khan, J. Keller-Findeisen, V. C. Cordes, S. W. Hell, *Nat. Biotechnol.* **2022**, *41*, 569.
[157] F. Balzarotti, Y. Eilers, K. C. Gwosch, A. H. Gynnå, V. Westphal, F. D. Stefani, J. Elf, S. W. Hell, *Science* **2017**, *355*, 606.
[158] L. A. Masullo, F. Steiner, J. Zähringer, L. F. Lopez, J. Bohlen, L. Richter, F. Cole, P. Tinnefeld, F. D. Stefani, *Nano Lett.* **2021**, *21*, 840.
[159] P. Zdańkowski, L. F. Lopez, G. P. Acuna, F. D. Stefani, *ACS Photonics* **2022**, *9*, 3777.
[160] T. Schröder, J. Bohlen, S. E. Ochmann, P. Schüler, S. Krause, D. C. Lamb, P. Tinnefeld, *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e221189612.
[161] S. H. Choi, S. J. Yun, Y. S. Won, C. S. Oh, S. M. Kim, K. K. Kim, Y. H. Lee, *Nat. Commun.* **2022**, *13*, 1484.
[162] M. C. Lemme, D. Akinwande, C. Huyghebaert, C. Stampfer, *Nat. Commun.* **2022**, *13*, 1392.
[163] P. Bøggild, *Nat. Commun.* **2023**, *14*, 1126.
[164] K. Trofymchuk, V. Glembockyte, L. Grabenhorst, F. Steiner, C. Vietz, C. Close, M. Pfeiffer, L. Richter, M. L. Schütte, F. Selbach, R. Yaadav, J. Zähringer, Q. Wei, A. Ozcan, B. Lalkens, G. P. Acuna, P. Tinnefeld, *Nat. Commun.* **2021**, *12*, 950.
[165] B. Ambrose, J. M. Baxter, J. Cully, M. Willmott, E. M. Steele, B. C. Bateman, M. L. Martin-Fernandez, A. Cadby, J. Shewring, M. Aaldering, T. D. Craggs, *Nat. Commun.* **2020**, *11*, 5641.

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