Biosensors based on Two Dimensional MoS_2

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ABSTRACT: The unique properties of two dimensional molybdenum disulfide (2D MoS2) have so far led to immense research regarding this material's fundamentals, applications and, more recently, its potentials for biosensing. 2D MoS2 has properties that make it of great interest for developing biosensors. These properties include large surface area, tunable energy band diagrams, a comparatively high electron mobility, photoluminescence, liquid media stability, relatively low toxicity and intercalatable morphologies. In this review, the current progress on 2D MoS2 based biosensors is presented and the prospects for future possibilities of expanding its applications for a variety of biosensing applications are discussed.

Hexagonal molybdenum disulfide (2H MoS_2) is a stratified crystal. The planes, comprising the crystal structure of 2H MoS_2, have thicknesses equal to the unit cell of this material that are held together by van der Waals forces. Each plane of MoS_2 is made of molybdenum atoms sandwiched between sulfur atoms (Figure 1). When exfoliated into one or limited number of layers, two dimensional MoS_2 (2D MoS_2) demonstrates unique electronic, optical, mechanical and chemical characteristics.[1-3] 2D MoS_2 shows remarkable properties that also makes it advantageous for biosensing applications.[4-5] When grown into planes with relatively large lateral dimensions, 2D MoS_2 planes are ended onto basal surfaces with no dangling bonds. As a result, these large planes are particularly stable in liquid and oxygen containing gaseous media, which facilitate their efficient incorporation into biosensing structures.[6-7] In nanoflake morphology, when the surface to thickness ratio is reduced, 2D MoS_2 edges and corners can be engineered to either molybdenum or sulfur terminations. The molybdenum termination gives the opportunity to possibly use the metallic properties of these sites when required.

Similar to graphene and other 2D materials, 2D MoS_2 offers large surface areas that enhance its biosensing performance. However as will be discussed later, due to the existence of suitable bandgap, the overall sensitivity of devices based on 2D MoS_2 is much larger than graphene and graphene oxides which have either no or small bandgap.[8] Many stoichiometric 2D oxides in comparison, have large bandgaps that require relatively high applied energy for their electronic band structure modulation.[1]

As 2D MoS_2 is atomically thin, upon interactions with a target bio material, its whole thickness is affected.[9] Historically 2D MoS_2 has been commonly used as a lubricating material.[1] As a result, its intercalation science has experienced several decades of intensive research and therefore the relevant chemistry has been thoroughly described. In comparison to other 2D counterparts, only 2D clays have been studied to that extent for their intercalation properties.[10] However, 2D clays are generally not suitable for establishing functional materials for active biosensing.[11]

For promoting specific reactions on the surface of 2D MoS_2 and enabling biosensing, establishing particular bonds and targeting specific biomaterials can be achieved using different functionalization methods_ENREF_4.[12-13] Inherently, the surface properties of 2D MoS_2 is very different to those of graphene and its oxides. MoS_2 surface energy is unique and is a strong function of its sulfide deficiency.[14] Functionalization of basal surfaces and sulfur ended edges of 2D MoS_2 can be challenging for which several methods have already been proposed, and will be briefly presented in this paper.

Various methods have been proposed for synthesizing 2D MoS_2,[3, 7, 15-16] and as such, it is always possible to find the right technique suitable for any biosensing application. Similarly, different methods have been developed for tuning electronic band structure of 2D MoS_2. This is in contrast to the significant challenges that are faced for modulating the electronic band structures of graphene and graphene oxides.[17] The electronic energy states of 2D MoS_2 can be adjusted ranging from semiconducting, in its intrinsic state, to fully conducting when it is transformed into the 1T MoS_2 phase (with octahedral unit cells Figure 1 d and e),[18] making this material suitable for almost many biosensing conditions. In particular, layered 2D MoS_2 is

compatible with standard electrochemical systems, which are commonly used as the working electrodes of biosensors.[7] In contrast, many of the other 2D materials do not offer such concomitant electronic and chemical properties.[19]

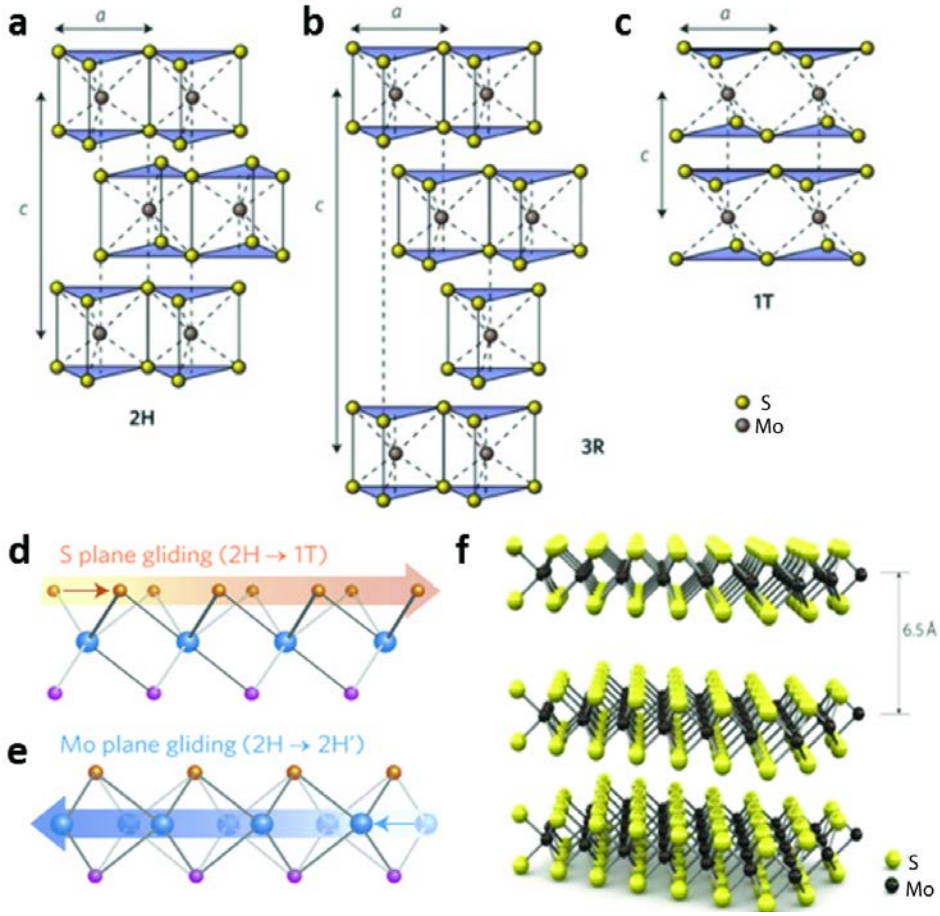

Figure 1. Schematic diagram of single-layered MoS_2 of: **(a)** 2H (hexagonal symmetry, two layers per repeat unit cell, trigonal prismatic coordination). **(b)** 3R (rhombohedral symmetry, three layers per repeat unit cell, trigonal prismatic coordination). **(c)** 1T (tetragonal symmetry, one layer per repeat unit cell, octahedral coordination). Figure 1a-c were reprinted with permission from Ref 3. Copyright 2012 Nature Publishing Group. **(d)** The S plane glides over a distance equivalent to (a = 3.16 Å) and occupies the HC site of the 2H hexagon, which results in a 2H right arrow 1T phase transition. **(e)** Gliding of the Mo plane results in a 2H right arrow 2H′ transition. Figure 1d-e were reprinted with permission from Ref 18. Copyright 2014 Nature Publishing Group. **(f)** Three-dimensional representation of the structure of MoS_2. Single layers, 6.5 Å thick, can be extracted using scotch tape-based micromechanical cleavage. Reprinted with permission from Ref 20. Copyright 2011 Nature Publishing Group.

2D MoS_2 can be easily, and at an ultrahigh capacity, intercalated *via* various ions [21-22] and_ENREF_8 a variety of bio organic molecules that assist in establishing biosensing systems. In a one layer configuration, 2D MoS_2 has a direct bandgap and hence shows photoluminescence (PL), allowing the formation of optical biosensing templates. This PL that appears in the visible range can also be strongly modulated upon bio interactions and its wavelength is compatible with the low-cost standard optical systems.[23-25] Such properties are only seen in a limited number of 2D materials.[19] Additionally, 2D MoS_2 has highly tunable vibrational and optical characteristics, which are useful for biosensing.[26-27]_ENREF_9

Something that is also of interest in biosensing is the relatively low toxicity of intrinsic 2D MoS_2 in comparison to many other nano materials, in particular of graphene and graphene oxides.[28] This is important to assure that target bio analytes are not affected in biosensing and safety measures are met. This property also allows the uptake of 2D MoS_2 into live cells without significantly deteriorating their viability for establishing *in vivo* biosensors.

Here, we present, analyze and discuss some of the major characteristics of 2D MoS_2 which are important for biosensing. We also describe methods for synthesizing, functionalizing and incorporating this 2D material into biosensors.

Electronic, optical and vibrational characteristics

The electronic band structure of 2H MoS_2 is strongly governed by the *d*-orbital of molybdenum.[29] The four electrons from the molybdenum fill the bonding states

around them and the surfaces of the layers are terminated by the lone-pair electrons.[4] The hybridization of *d*-orbital of 2H MoS_2 results in the indirect-to-direct bandgap transition (Figure 2 a and b) when exfoliated into a single layer.[26, 30] In the bulk form of stratified 2H MoS_2, the conduction band minimum is located between the Γ and K points, while the valence band maximum is located at the Γ point, which constitute a bandgap of ~1.29 eV.[31] Reducing the number of layers result in the upward shift of this conduction band. However as the conduction band states at the K point are mainly due to the *d*-orbitals of the molybdenum atoms, they remain relatively unaffected by interlayer interactions (the minimum conduction band edge is located at ~-4.1 eV with reference to the vacuum). This means that the direct band gap at the K point only increases slightly by reducing the number of layers. This results in a direct band gap of ~1.9 eV at K point in one layer MoS_2 (the max valence band edge is located at ~-6 eV with reference to the vacuum

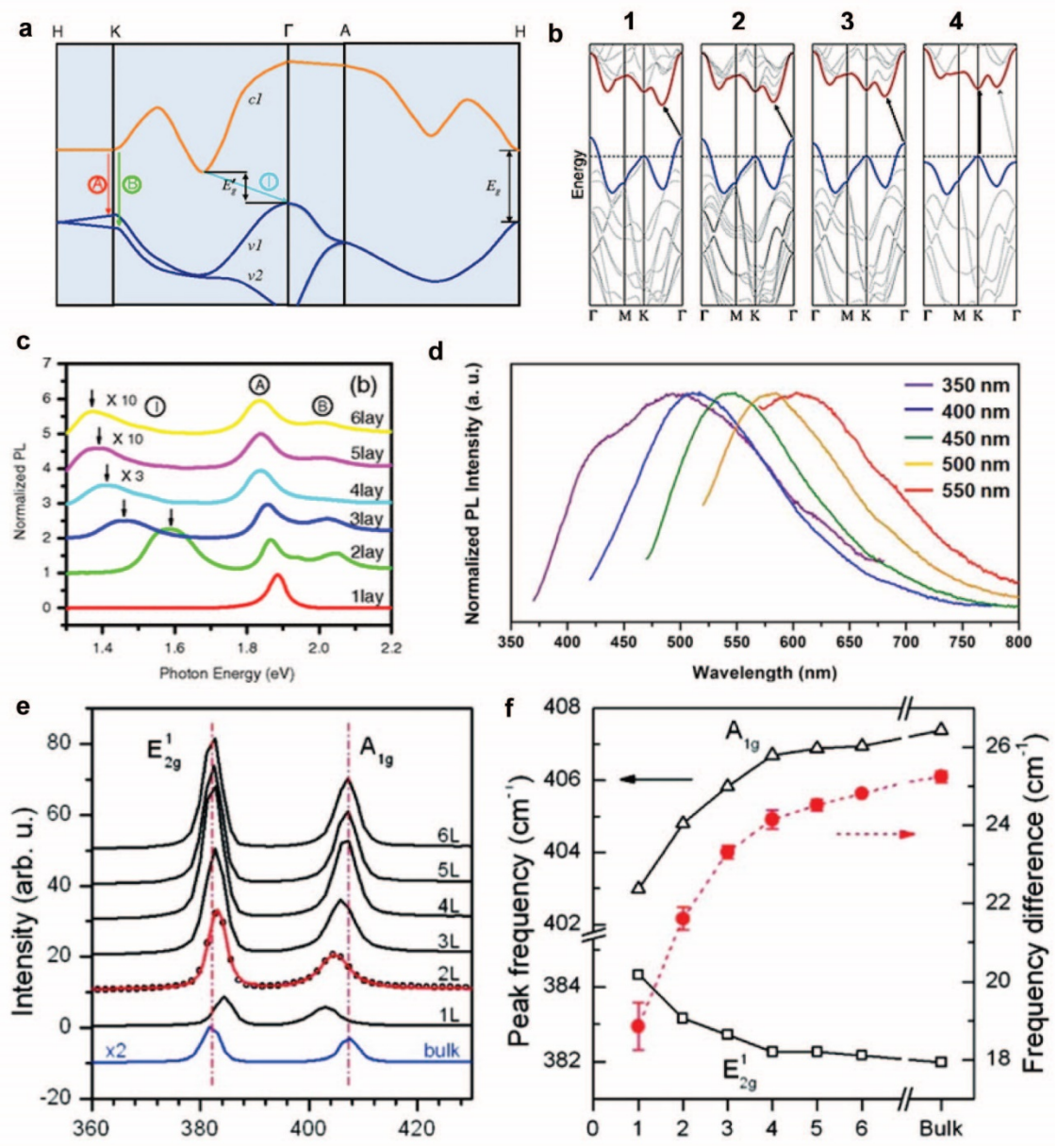

Figure 2. **(a)** Calculated band diagram for stratified 2H MoS_2 that contains both direct and indirect band gaps. Along the K point, the A and B excitons as well as the I peak of indirect transition are presented. **(b)** Calculated band structures of (1) bulk, (2) four layers, (3) two layers, and (4) monolayer MoS_2. Reprinted with permission from Ref 30. Copyright 2010 American Chemical Society. **(c)** PL spectra of 2D MoS_2 planes. Figure 2a and c were reprinted with permission from Ref 26. Copyright 2010 American Physical Society. **(d)** PL spectra of suspended quasi-2D MoS_2 nanoflakes at different excitation wavelengths (350, 400, 450, 500, and 550 nm). Reprinted with permission from Ref 21. Copyright 2014 American Chemical Society. **(e)** Raman spectra of a few layered and bulk MoS_2. **(f)** Peak positions of E^1_{2g} and A_{1g} Raman modes and their difference as a function of layer thickness. Figure 2e-f were reprinted with permission from Ref 5. Copyright 2010 American Chemical Society.

It has been shown that one layer MoS_2 is able to offer the on/off ratio of $>10^8$ when incorporated into field effect transistor configurations.[20] This large value is certainly advantageous in establishing biosensors, as any slight change in the electron channel area can be efficiently used for forming highly sensitive biosensors. The effective mass of electrons at the K point of 2D in MoS_2 has been estimated as $0.48m_e$.[31-32] The free carrier scattering in 2D MoS_2 is dominated by their interactions with acoustic phonons at temperatures lower than 100 K and optical phonons above such temperatures.[32] The highest free carrier mobility has been estimated ~400 cm^2/Vs at room temperature. In reality, gaining such free carrier mobilities without high permittivity layers is not possible. A carrier lifetime of ~100 ps and diffusion coefficient of ~20 cm^2/s have been shown for 2D MoS_2.[33] Both carrier lifetime and diffusion coefficient are suitable numbers to establish field effect and electrochemical based biosensors.

2D MoS_2 electronic band structure characteristics give rise to certain excitonic and fluorescent properties that can be efficiently used for biosensing. 2D MoS_2 PL spectrum shows two exciton peaks of A (~1.92 eV) and B (~2.08 eV) at the K point (Figure 2a–c).[26, 30] These peaks are suggested to appear due to valence band spin orbit splitting by ~145 meV.[34] Two extra exciton peaks of C and D also exist. The introduction of excess electrons causes a split of the exciton A peak, which is associated to the emergence of negative trions of high binding energies.[26] Interestingly, this splitting occurs at room temperature which is also significant for optical biosensing observations. The existence of PL can be specially designed fluorescence tagging as a possibility for surveillance, tracing and sensing of biological components. 2D MoS_2 can also be successfully bound to other fluorophores. Specially functionalized 2D MoS_2 with relatively small lateral dimensions, which can be uptaken by cells, can be applied for bio imaging and consequently biosensing using a variety of conventional and near field optical microscopy techniques (Figure 2 d).[35]

2D MoS_2 shows strong vibrational characteristics. This material has four Raman-active modes (E_{1g}, E^1_{2g}, A_{1g}, and E^2_{2g}). It also demonstrates two IR-active modes (A_{2u} and E_{1u}).[36-37] E^1_{2g} is an in-plane mode, attributed to the opposite vibration of two S atoms with respect to Mo, and A_{1g} mode is in an out-of-plane vibration of only S atoms in opposite directions. Reducing the number of layers result in the red and blue shifts of E^1_{2g} and A_{1g} modes, respectively (Figure 2 e and f).[5] Due to its out-of-plane nature, the A_{1g} mode is very sensitive to adsorbates on the MoS_2 surface and charge exchanges.[38] The Raman and IR active modes are also function of lateral dimensions, permittivity of the environment, defects and intercalation properties. As such, observations and investigations of the vibrational modes are suitable for a variety of biosensing applications *via* Raman and FTIR spectroscopies.[27]

Synthesis techniques

Methods for synthesizing 2D MoS_2 compromise a wide range, including those that exfoliate thin layers from bulk 2H MoS_2 or others that vapor/liquid phase deposit the single layers of MoS_2 directly on a substrate.

Similar to graphene, some of the most common approaches for obtaining 2D MoS_2 are still based on mechanical exfoliation using adhesion tapes.[39] Such methods generally result in the highest-quality monolayers of MoS_2 on any arbitrary substrate.[40] However, low yield and random landing of the 2D MoS_2 flakes on substrates are problematic, especially for large scale production.[3] Mechanical exfoliation methods are particularly useful for establishing field effect based biosensors.

A variety of gas/vapor chemical deposition methods have been developed for wafer scale growth of 2D MoS_2.[41-43] Many of these techniques result in 2D MoS_2 in triangular morphologies with side dimensions as large as several hundreds of μm.[41] The chemical deposition methods generally start with forming a very thin layer of organic/inorganic precursor of 2D MoS_2 followed by a series of annealing and sulfurization steps.[43-44] Ammonium tetrathiomolybdate and molybdenum oxides are amongst the common precursors which are used in one or two-step deposition/annealing processes.[41] The sulfurization step is generally conducted above 500 °C using H_2S gas or sulfur vapor.[41] The current challenge is to obtain wafer scale high quality and homogenous 2D MoS_2 layer. The crystallinity and other qualities of the substrate play an important role is the success of the deposition process. While substrates such as sapphire yield the best outcomes, SiO_2 covered Si wafers as well as silica glass or conductive glass substrates are the most on demand bases for developing biosensors. [8, 45-46]

Liquid exfoliation techniques are suitable for obtaining suspensions of 2D MoS_2. These suspensions are particularly useful for optical and vibrational biosensing applications, which are compatible with microfluidic systems.[47] Some of the most high yielding liquid exfoliation methods are based on lithium intercalation techniques.[48] However, these processes are generally hazardous and require long durations. Microwave assisted processes have been proposed to reduce the reaction time.[49] Lithiation can also be electrochemically implemented but it limits the yield.[50] Such processes generally need refluxing to remove the ionic residues in order to make the final product suitable for biosensing applications.

There are also liquid exfoliation methods which are based on high power sonication with the assistance of materials that enhance physical surface adhesion on stratified MoS_2. Colman *et al* have proposed liquid exfoliation methods based on shear force effects,[51-53] which are assisted by surfactants or organic solvents. Advantageously, these methods can be readily used in forming 2D MoS_2 suspensions in organic solvents compatible with biosensing processes. However, such liquid phase processes still leave residues on 2D flakes. Several centrifuge steps can assist in removing the remnants. Also hexane treatment

and annealing at optimum temperatures can help.[35] Obtaining a clean surface is especially important for biosensing in which specific fuctionalizations are required to make the surface more specific to any specific target bio analyte.

The readers are referred to other review papers[1, 3] for more information on the synthesis of 2D MoS_2.

Functionalization

For the development of biosensors, after the synthesis of 2D MoS_2, these thin sheets should be functionalized to respond to specific bio targets. Surface functionalization result in charge displacement and formation of surface dipoles that can cause dramatic effects on the electronic band structures of 2D MoS_2.[54] There are already many reports on the functionalization of basal surface of 2D MoS_2 *via* standard surface methods. In practice, many of those reports are incorrect as not paying attention that no dangling bonds and very large surface of MoS_2 with van der Waals affinity promote physisorption. It means that molecules tend to lie flat on the surface of MoS_2 rather than establishing covalent or strong ionic bonds. Such physisorption reactions seem to have a very low selectivity in nature. However, if they are used intelligently, the physisorbed layer onto MoS_2 can be engineered to form the base an assay that results in a high selectivity surface for a particular target bio analyte.

It has been suggested that basal planes of MoS_2 can be functionalized using silane and thiol based methods, which are commonly applied for modifying oxide and chalcogenides surfaces.[55-56] Such methods strongly rely on the formation of hydroxyl of thiol groups on the surface. In an ideal world, there is no dangling bond on the basal surface of MoS_2 and such groups can only efficiently adhere onto molybdenum, emerging from disrupted sulfur bonds. This means that effective surface functionalizations *via* those methods depend on the presence of sulfur deficiencies. The edges of 2D MoS_2 flakes are either terminated by metal or chalcogen atoms, depending on conditions of synthesis.[29] In addition, low-coordination step-edges and kinks can also be formed that induce altered local surface effects/energies.[57] Generally reducing the lateral dimensions of 2D MoS_2 flakes encourage the domination of such properties. The missing coordination at the edges, steps and kinks give rise to metallic states that can be used for incorporating functional groups, including hydroxyls and thiols.[58] Alternative methods can also be used. Disulfide bonds in the form of R-S-S-C can be established by coupling of two thiol groups for immobilizing organic entities such as various proteins. Acid compounds containing -SOH groups can also bind to proteins and carbohydrates and partially act as catalysts or intermediates for bio interactions.

Many other methods such as *in situ* reduction of metal ions, esterfication, ring-opening polymerization and free radical polymerization can also be used. There are also more recent reports on covalently bond amide and methyl moieties onto sulfur-based metallic 1T MoS_2.[59] Very interestingly, a change from metallic 1T to semiconducting 2H was observed after this procedure.

Functionalizations also play important roles in stabilizing 2D MoS_2 in ionic solutions by reducing the nonspecific dangling bonds. Ionic solutions such as phosphate buffer salines are the base of biosensing,[60-61] as such stable suspensions of 2D MoS_2 are critical.

It is important to consider that 2D MoS_2 is a stable materials in photo activated reactions.[62] As such many optical grafting technologies can be implemented for functionalization of the surface of MoS_2 without deteriorating its properties.

Catalysis

Enhancing the catalytic performance of 2D MoS_2 surface is an important property for augmenting bio reactions and also longevity of biosensors (surface poisoning). Catalytic strength of intrinsic 2D MoS_2 is not as large as metallic materials, but advantageously shows strong resistance to poisoning.[63] 2D MoS_2 catalytic properties can be based on either dark electron transfer (hydrogenation reaction) or photochemical processes or a simultaneous combination of both. It is suggested that the rim sites of 2D MoS_2 predominately cause the hydrogenation, while the metallic edges catalyze organic entities.[48]

Intercalation

2D MoS_2 is a popular material for H^+ and Li^+ intercalation as an efficient base for energy storage.[64-65] Intercalation is also potentially an important process in MoS_2 based biosensors.[66] When bio targets are brought into the vicinity of 2D MoS_2 (made of more than two layers), a portion of these materials are adsorbed onto the surface and the rest can be intercalated (reversibly included into the layered crystal).[48] The competition between the surface adsorption and intercalation depend on the type and intensity of the applied energy. It also depends on the nature of adsorbates/intercalants and their surface energies with respect to the surface and interlayer binding energies of 2D MoS_2.

Particles can be incorporated within the interlayer spacings through direct intercalation[48] and exfoliation–restacking.[48, 55] The intercalation processes of MoS_2 are generally topotactic, in which interlaminar distances change. The interlayer expansion measurements can provide relevant information regarding the nature of inserted phase. Intercalation may induce drastic changes in the electronics, optics and transport properties of 2D MoS_2. Engineering of these properties is potentially beneficial for biosensing.

There are many reports on processes for the intercalation of ionic and organic entities into MoS_2.[22, 49, 67-68] However, interaction of biological components, such as amino acids and DNA strands, frequently appearing in biosensing processes yet to be reported.

Toxicity and cell labeling

In general, toxicity can be an important issue for biosensing. While toxicity does not seem to have a direct impact on sensing organic analytes, low toxicity is important for keeping the viability of cells under biosensing investigations. 2D MoS_2 is both itself fluorescence and can also be successfully adhered to fluorophores. As a result, it can be used for tagging and imaging different cell organs.[4, 69] 2D MoS_2 flakes with small lateral dimensions can be uptaken by live cells and used in high contrast cell-targeted labeling for biosensing and imaging applications (Figure 3).[70]

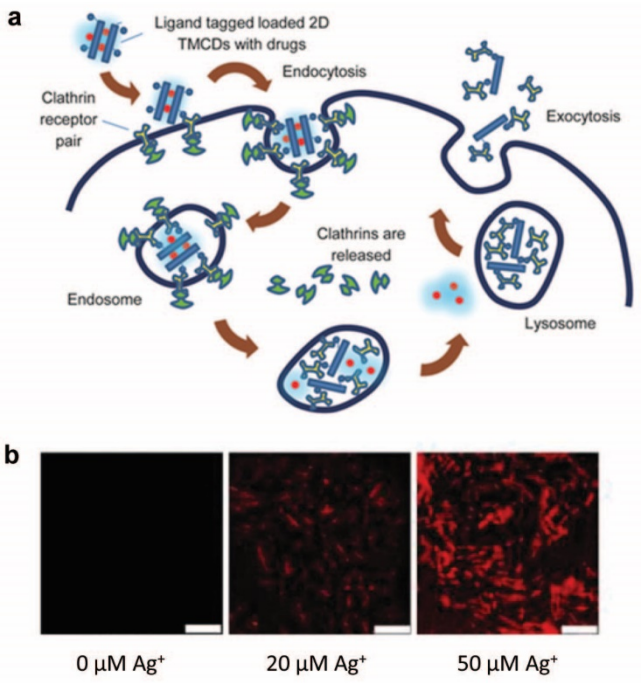

Figure 3. (a) Schematic illustrations of layered 2D MoS_2 flake uptake and drug delivery concept: layered 2D MoS_2 is loaded with drugs (or fluorophores) and conjugated with a ligand allowing cellular uptake. The ligand on the 2D MoS_2 anchors to the receptor-clathrin pair facilitating clathrinmediated endocytosis. The loaded 2D MoS_2 enters the cell within a vesicle. It may stain special cell organelles or in later stages transport and release a drug. Reprinted with permission from Ref 4. Copyright 2015 John Wiley & Sons Publishing Company. **(b)** Confocal fluorescence microscopy of *E. coli* stained with MoS_2 – rhodamine B isothiocyanate (RhoBS) nanoprobes. *E. coli* were cultured with 0, 20 and 50 μM Ag^+ ions and added to MoS_2–RhoBS before imaging. Scale bar = 7.5 μm. Reprinted with permission from Ref [69]. Copyright 2015 American Chemical Society.

It is commonly believed that multi layered MoS_2 has a low toxicity. However, depending on exfoliation parameters, defect density and chemical compositions, the toxicity of 2D MoS_2 alters. The investigations of toxicity of few-layered MoS_2 by *in vitro* colorimetric assays for a cell viability have found that it shows low toxicity profile towards lung cancer cells.[28] It has been demonstrated that by decreasing the number of layers in 2D MoS_2, the toxicity increases, which is attributed to the enhanced surface area, defects and edges.[71] When testing toxicity of nanomaterials, one shall exercise caution as the nanomaterial may also interact with viability of markers in assays.[28] Still more studies should be conducted to understand the differences between the toxicity of 1T and 2H (metallic and semiconducting, respectively) 2D MoS_2 as well as the effect of defects, kinks and edges.

Biosensors

2D MoS_2 is increasingly becoming a popular material for biosensing applications and a significant number of publications have emerged regarding its incorporation into biosensors in recent years. As already discussed in detail, this material's high surface-to-volume ratio and layered structure can accommodate large capacity of chemical/bio species. 2D MoS_2 has functionalization diversity, desirable optical and electronic properties as well as unique vibrational characterizations and shows certain advantages for establishing biosensors.[4, 72-74]

Current biosensors based on 2DMoS_2 can be categorized into several types including electrode based devices, electrodeless optical systems and reverse electro luminescent systems, which are discussed in the following sections.

Electrode based biosensors

Relying on the incorporation of electrodes, one category of 2D MoS_2 biosensors is based on field effect transducing platforms. Such devices relate the changes of charge or mass, on the surface of 2D MoS_2, to the changes in the size and area of the underneath resistive channel and hence transconductance changes.[8] The added charge (ionic entities or electron transfer) on the surface can also change the threshold voltage of the device for which an adaptation of the Nernst equation maybe implemented for assessing the concentration of the target analyte. Field effect based sensors using 2D MoS_2 have been shown for label free biosensing and employed for sensing proteins, DNA and other biochemical components.[8, 45-46] A representation of such biosensors is shown in Figure 4 a–d, in which 2D MoS_2 is placed between drain and source electrodes. Sarkar *et al* have shown the impressive sensitivity of the 2D MoS_2 based biosensors that stems from their suitable bandgap and high on/off ratio as field effect templates (Figure 4 e–i).[8]

They demonstrated ultrasensitive, and specific to selected proteins, biosensing with a sensitivity exceeding 200 even at femtomolar concentrations. In comparison to graphene based devices, their 2D MoS_2 based field effect biosensor showed an impressive ~75 fold better response. Additionally, their 2D MoS_2 based field effect pH sensor also achieved a sensitivity of >700 for a pH change by one unit.[8]

In field effect biosensors, very often a reference electrode, such as Ag/AgCl type, is used as the suspended

gate. The application of the reference electrode can be particularly important as it helps the formation of a double electrical layer on the surface of the 2D MoS_2 channel that assists the fast and efficient formation of ionic species on the channel surface, hence reducing the response time and sensitivity. Presence of a top or back gate voltage can also be helpful to assure that the transistor is biased into switched on mode during biosensing.[75] The bias can assist in forming the electrical double layer in order to reduce the need for a suspended reference electrode. A discussion on differences between the top and back gate biasing for biosensing can be found in the work of Nam *et al*.[76]

Functionalization of 2D MoS_2 is very important to achieve the selectivity and sensitivity needed for field effect biosensing. Lee *et al* [75] used prostate specific antigens to obtain pico levels of sensitivities without a reference electrode. They also obtained single stranded DNA detection limit of 10 femto moles at 17 mV change for each orders of magnitude of concentration change.[77] Nam *et al* [78] investigated an anti-human antibody for detecting specific proteins in femto molar ranges of the response time of 10 mins or more, depending on the concentration.

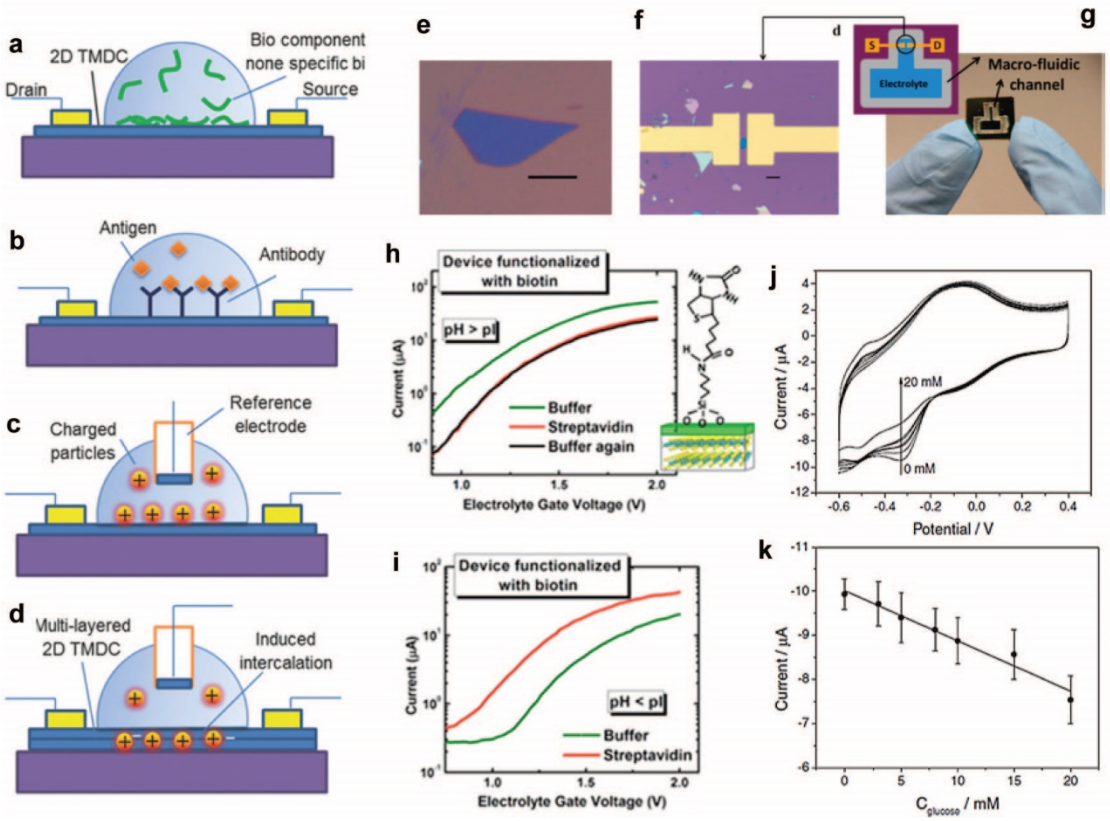

Figure 4. 2D MoS_2 based field effect biosensors in response to adsorbates according to: **(a)** Non-specific binding, **(b)** Lock and key concept (antibody-antigen interaction in this example), **(c)** With a reference electrode to produce the electrical double layer and **(d)** With a reference electrode, and multiple layer 2D MoS_2, to induce intercalation. Reprinted with permission from Ref 4. Copyright 2015 John Wiley & Sons Publishing Company. **(e)** Optical image of a MoS_2 flake SiO_2/Si substrate. Scale bar, 10 μm. **(f)** Optical image of the MoS_2 field effect biosensor. Scale bar, 10 μm. **(g)** Image and schematic diagram (inset figure) of the chip with the biosensor device and microfluidics. **(h)** Output characteristics of 2D MoS_2 field effect based biosensors functionalized with biotin: first measured in phosphate buffer. Addition of streptavidin solution (10 μM) leads to decrease in current due to the negative charge of the protein, as the pH of the solution is more than the isoelectric point of streptavidin. The device is then measured again in pure buffer, leading to no significant change. **(i)** The same device after the addition of streptavidin solution (10 μM) at a pH of 4.75, which is less than the isoelectric point of streptavidin, leading to an increase in current consistent with the positive charge of the protein. Figure 4e-i were reprinted with permission from Ref 8. Copyright 2014 American Chemical Society. **(j)** Cyclic voltammograms of different concentrations of glucose (0, 3, 5, 8, 10, 15, and 20 mM) onto MoS_2-chitosan-glucose oxidase electrodes at the scan rate of 50 mVs^{-1} and **(k)** its corresponding calibration curve. Figure 4j-k were reprinted with permission from Ref 7. Copyright 2012 John Wiley & Sons Publishing Company.

Other type of commonly used 2D MoS_2 based biosensors, which rely on electrodes, are electrochemical types. They are designed to sense bio related ionic species generated in redox reactions, in a procedure that is promoted by the electric field from a reference electrode. In these systems, the working electrode is covered with 2D MoS_2 and the reference electrode is generally the Ag/AgCl type. A counter electrode is also commonly used in such systems that help in separating the applications and measurements of the applied voltage and current. Wu *et al* [7]

presented one of the first reports of such sensors for 2D MoS_2 (Figure 4 j and k). They used a film of this material on the working electrode and demonstrated its rapid electron transfer rate in selected redox systems for glucose and dopamine sensing. Electrochemical systems based on 2D MoS_2 have also been incorporated for DNA sensing in nano molar ranges.[79-80] Loo *et al* [79] demonstrated DNA hybridization in electrochemical systems for sensing anomalies such as those in Alzheimer's disease. It is important to consider that like many other 2D materials with large and flat surface morphologies, affinity of 2D MoS_2 towards single-stranded DNA is more than double-stranded DNA. Electrochemical sensing using 2D MoS_2 has also been employed for H_2O_2 sensing, a by-product of many oxidative cell reactions, with detection limits in nano molar ranges.[81-82]

Electrodeless optical biosensors

The PL of 2D MoS_2 can be quenched upon the immobilization of biomaterials onto its surface and/or interaction with them. Such changes in the PL have been associated with charge exchanges as well as electronic energy transfer (EET) and resonance energy transfer (RET) phenomena (Figure 5 a).[23-25] However, other effects such as changes of the surface roughness and permittivity can also alter PL in such biosensors and their role should be considered.

Another popular approach for optical sensing is the indirect PL quenching of biomaterials with tagged fluorophores (Figure 5 b). Immobilization of such tagged biomaterials onto the surface of 2D MoS_2 may produce a reversible quenching and [45] tagged aptamers have been engineered for specific biosensing applications according to this concept.[83]

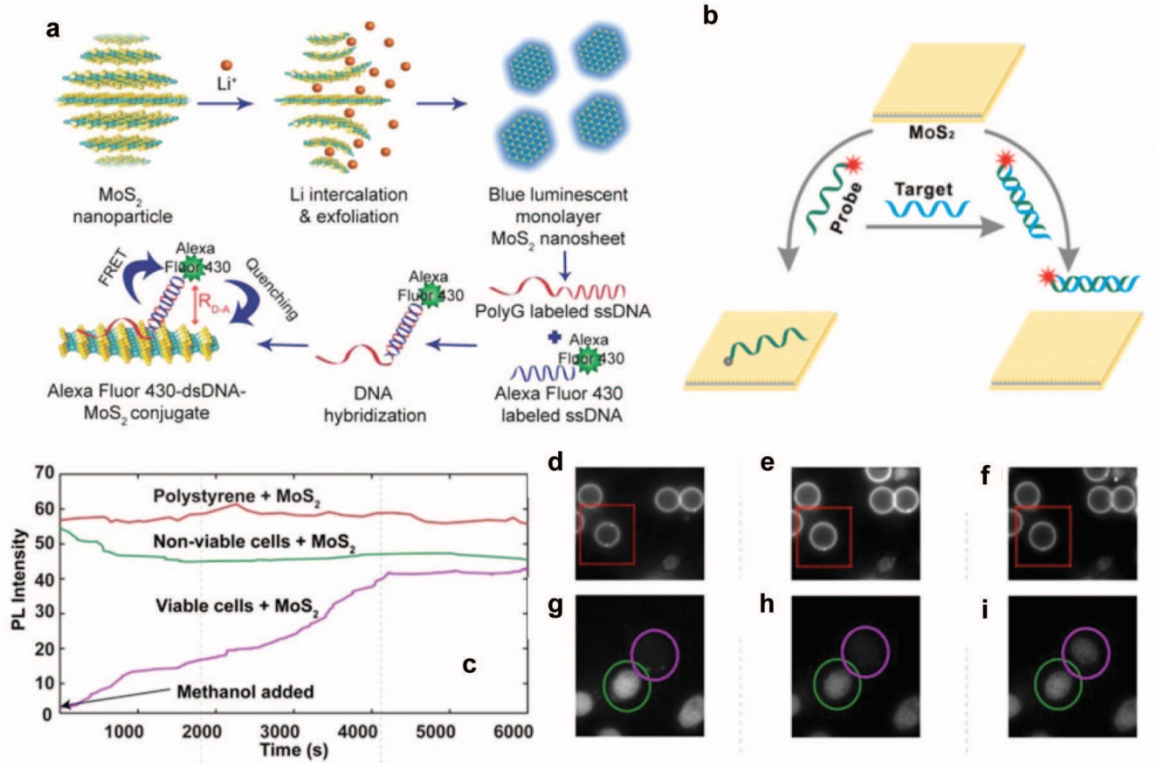

Figure 5. (a) Optical sensors based on the PL generated by MoS_2: Schematics for constructing the RET pair using the blue luminescent monolayered MoS_2 nanosheets and the Alexa Fluor 430 which are separated by the dsDNA. Reprinted with permission from Ref 23. Copyright 2014 John Wiley & Sons Publishing Company. **(b)** Incorporation of a tagged PL fluorophore: 2D MoS_2 adsorb dye-labeled single-stranded DNA probe *via* the van der Waals force and then quench the fluorescence of the dye. In contrast for double-stranded DNA, nucleobases are buried between the densely negatively charged helical phosphate backbones, and as a result their interaction with MoS_2 is so weak. Reprinted with permission from Ref 45. Copyright 2013 American Chemical Society. **(c)** PL response of quasi-2D MoS_2 nanoflake-coated polystyrene particles, non-viable and viable cells monitored over time for up to 6000 s after the addition of methanol. The corresponding fluorescent images of quasi-2D MoS_2 nanoflake-coated polystyrene particles (red square) after **(d)** 0, **(e)** 3000, and **(f)** 6000 s, respectively. The corresponding fluorescent images of quasi-2D MoS_2 nanoflake-coated nonviable (green circle) and viable cells (pink circle) after **(g)** 0, **(h)** 3000, and **(i)** 6000 s, respectively. Figure 5c-i were reprinted with permission from Ref 21. Copyright 2014 American Chemical Society.

Very recently, it has been shown that plasmonics of highly doped 2D MoS_2 can also be used for biosensing.[84] Adhesion of the biomaterials onto the surface of 2D flakes changes the plasmon resonance peaks due to charge exchange and surface permittivity changes. Doping is a critical step in producing the metallic 1T phase of MoS_2 that shifts the plasmon resonance peaks into the visible and near infrared light regions. Naturally, because of low carrier concentration of intrinsic 2D MoS_2, plasmon resonances appear in THz,[85] which is still useful for biosensing

but practically difficult in terms of accessibility to instruments for measurements in such a wavelength range.

Reverse electroluminescent biosensors

Intercalation of biologically important ions such as H^+, Li^+, Na^+ and K^+ can reduce the bandgap of 2D MoS_2 and eventually, at high intercalation concentration levels, transform 2H MoS_2 into the metallic 1T phase. As such, these intercalation processes quench the PL at different levels. However, it is important to consider that always an electric field (*via* an applied voltage) is needed to assist in the procedure as the intercalation does not occur nationally. The electric field can be applied *via* external reference electrodes.[21-22, 84] Interestingly, even the voltage across the bi-lipid membranes of a cell can provide a sufficient electric field for the intercalation to occur. The concept has been used for sensing glucose based on peroxidase-like activity on 2D MoS_2 (Figure 5 c–i).[21] Irradiation to IR or visible light can also assist in the intercalation process.[86]

Other biosensing concepts

In addition to the different types of biosensors based on pure 2D MoS_2, which were presented in the previous sections, this material has also been reported in composites for biosensing applications. Such biosensors have been developed based on composites with inorganic nanoparticles (both metals and metallic compounds), graphene, carbon nanotubes and a number of polymers.[58, 87-94] ENREF_76 To gain success, the composite components should be intelligently selected and well-engineered together with 2D MoS_2 in order to offer the enhancement for biosensing. It is suggested that synergy between 2D MoS_2 and the added components,[64, 95] the junction effects,[96-100] catalytic properties[101-102] and effective charge transfer[103-104] can tune and enhance sensitivity and selectivity of such composite biosensors.

In biosensing, what is very important is the accurate control of the liquid flow, directing the bio analytes onto the surface of 2D MoS_2. The incorporation of microfluidics certainly help in achieving compatibility with the low dimensional 2D MoS_2 biosensing systems and their eventual incorporation into practical and low cost devices that can be deployed to the market. To achieve such goals, microfluidics for PL[105] and field effect[46, 78] based biosensing using 2D MoS_2 have been shown.

Another recent application using 2D MoS_2 is in DNA sequencing. In such systems nanopores are formed into the plane of 2D MoS_2. The translocation of the DNA strands into these pores generates digital steps that correspond to the charge type on the strand. These 2D MoS_2 based nanoporous systems offer signal-to-noise ratios almost one order of magnitude superior to that of graphene.[106]

It is also worth mentioning that 2D MoS_2 shows specific bio gas sensing properties due to the physisorption of selected gases onto its surface, which are associated to the dipole effect of the gas species. Biologically important gas species such as NO,[107] NO_2,[108] and NH_3[109] as well as several organic vapors have been shown to physisorbed onto the surface of 2D MoS_2, with or without functionalized surfaces.[110-111] Membranes with embedded 2D MoS_2 can also be used for increasing the selectivity to specific gas species.[112]

Conclusion and future directions

A comprehensive review of what has so far been achieved and developed using 2D MoS_2 based biosensors was presented in this paper. We illustrated the key properties of 2D MoS_2 that provide unique opportunities for biosensing. We also demonstrated and analyzed the most recent advances based on biosensors that incorporate 2D MoS_2. However, there are still plenty of biosensing concepts, structures and applications that can be potentially studied.

Investigations regarding 2D MoS_2 for enzymatic exchanges and other protein based systems should be expanded. Such biosensing reactions can also fully use the intercalation effects seen in 2D MoS_2.

A fundamental remaining issue is to understand more about the influences of the defects, dopants and lateral dimensions/thickness on the bio properties of 2D MoS_2. Knowing that the defects, kinks and edges introduce localized metallic regions and also changes in the electronic band diagram of 2D MoS_2, it is important to fundamentally understand them in order to more efficiency implement such properties for engineering efficient biosensors.

Making novel composites of 2D MoS_2 by incorporating yet to be investigated chemical components (e.g. metal oxides or other metallic compounds) and forming organic compounds with a variety of conductive and non-conductive polymers are all important topics that should be fully studied for biosensing. It is also worth mentioning the new concept of bio compatible inks for optical biosensing and bio surveillance should be considered. Due to remarkable optical properties and stability of 2D MoS_2 flakes, they are ideal as the semiconducting or metallic component of bio inks.

So far only a limited number of templating platforms have been used for incorporating 2D MoS_2 in order to establish biosensors. Many other platforms can be used that are compatible with this material for which the incorporation of 2D MoS_2 may potentially result in exciting biosensing capabilities. This includes, but not limited to, surface plasmon resonance (SPR) and acoustic based templates. Advantageously, SPR systems are intrinsically compatible with layered materials. Many of the efficient SPR systems are covered with metallic gold as the surface plasmon confinement layer. In an alternative case, metallic 1T MoS_2 can offer a biocompatible layer, also providing a great opportunity to obtain high Q SPR biosensors. 2D MoS_2 can be potentially incorporated onto acoustic wave sensing templates such as surface acoustic wave (SAW) devices, standard cantilevers, and quartz crystal micro-

balances. The surfaces of such acoustic transducers show high compatibility with MoS_2. Additionally, 2D MoS_2 can accommodate both mass and charge within its layers and on its surface that can both produce electroacoustic responses.

2D MoS_2 has also a relatively large strain limit and a high elastic modulus.[113-114] As a result, this 2D material can be directly used for establishing the most sensitive cantilever biosensors with extraordinary low detection limits, possibly for the detection of single molecules.[114-115] Very Recently, strong piezoelectric properties have been observed for odd layered 2D MoS_2 (2D MoS_2 made of 1, 3, 5,... number of layers), which is the strongest for single layer MoS_2 most sensitive cantilever biosensors with extraordinary low detection.[115] As a result, the properties of odd layered 2D MoS_2 can be potentially employed to develop highly sensitive cantilever based mass detectors for biological components.

Thermal effect can also be used for bio calorimetry sensors. The thermal conductivity of multilayered 2D MoS_2 is extremely large along the planar directions and low in the direction normal to the planes. As a result, accurate structures for spatially confining and releasing heat from biosensing applications can be made based on 2D MoS_2.

Altogether 2D MoS_2 has still much more to offer to biosensing. It is expected that a large number of new biosensing concepts to be discovered and developed in the next decade to provide more opportunities for researchers working in relevant areas and also for industries *via* the rapid uptake of this material into biosensing systems.

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