Electronic Supplementary Information (ESI) for RSC Advances.

Green synthesis of luminescent and defect-free bio-nanosheets of MoS_2: interfacing two-dimensional crystals with hydrophobins

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Extraction of Vmh2 from *Pleurotus Ostreatus* mycelia

White-rot fungus, *P. ostreatus* (Jacq.: Fr.) Kummer (type: Florida; ATCC No. MYA-2306) was maintained at 4 °C through periodic transfer on potato dextrose agar (Difco) plates in the presence of 0.5% yeast extract. Mycelia were inoculated in 1L flasks containing 500 mL of potato-dextrose broth (24 g L^{-1}) supplemented with 0.5% yeast extract, grown at 28 °C in shaken mode (150 rpm). After 10 days of fungal growth, mycelia were separated by filtration through gauze, treated twice with 2% sodium dodecyl sulfate (SDS) in a boiling water bath for 10 min, washed several times with water and once with 60% ethanol to completely remove the detergent. The residue was dried under nitrogen, grinded and treated with 100% trifluoroacetic acid (TFA) in a water bath sonicator (Elmasonic S30, Elma) for 30 min, and centrifuged (10 min at 3200 g).

The supernatant was dried, and then lipids were extracted in a mixture of water–methanol–chloroform 2:2:1 v/v (5 min in bath sonicator). After centrifugation, proteins appeared as a solid aggregate at the interface between the water–methanol and the chloroform phases. They were recovered by liquid phase removal. The aggregated protein was dried, treated with TFA for 30 minutes in bath sonicator, re-dried, and dissolved in 80% ethanol. The sample was centrifuged (90 min at 12 000 g) and ethanol was removed from the supernatant under vacuum at 40 °C using rotavapor and the material was freeze-dried, treated with TFA as above described, and dissolved again in 60% ethanol.

Exfoliation and biological functionalization. MoS_2 powder

MoS_2 powder (Aldrich, 69860, particle size ~6 µm, density 5.06 g mL^{-1} at 25 °C), was exfoliated in 60% ethanol aqueous solution (5 mg in 6.25 mL) using a tip sonicator (Branson digital sonifier, inbuilt power meter output power 40W) for 90 minutes in cylindrical glass tubes (13mm diameter, 10 cm height, rounded bottom). The

temperature of the dispersion during sonication was controlled in an ice-water bath. After sonication, Vmh2 was added to fractions of the exfoliated dispersion to reach a variable final concentration of Vmh2, 2÷75 μg mL^{-1}, and a fixed concentration of material, 200 μg mL^{-1} of MoS_2. The solutions were mixed by sonicating for 10 minutes in a bath sonicator (Elma; Elmasonic S30). The samples were left to settle down at room temperature and imaged during a period of 10 weeks.

Controlled centrifugation

This technique of size selection is an important step for efficient production of monolayer enriched dispersions. The unexfoliated crystallites were removed with low centrifugal force, 40 g for 45 minutes and the dispersion produced contains nanosheets with a wide distribution of size and thickness and a small percentage of monolayers population with varying lateral sizes. The supernatant is then centrifuged with a higher force of 150 g for 45 minutes and the pellet was removed. This pellet contains nanosheets of larger size distribution which tends to sediment due to their bulky size. However, the supernatant is separated and again centrifuged with a higher centrifugal force of 600 g for 45 minutes and finally the associated supernatant is proceeded with the highest centrifugal force of 2400 g for 45 minutes.

Exfoliation and Size Selection: MoS_2 crystals

MoS_2 crystals (Graphene supermarket, average area = 0.7 cm^2, 99% purity), were exfoliated in 60% ethanol aqueous solution (5 mg in 5 mL) using a tip sonicator (Sonics vibra cell ultrasonics processor, inbuilt power meter output power 18 W) for 2 h in cylindrical glass tubes (13 mm diameter, 10 cm height, rounded bottom). The temperature of the dispersion during sonication was controlled in an ice-water bath. After sonication, Vmh2 was added to fractions of the exfoliated dispersion to reach a variable final concentration of Vmh2, 0, 25, 50, 75, 100

μg mL^{-1}, and a fixed concentration of material, 500 μg mL^{-1} of MoS_2 and the solutions were mixed by sonication for 10 minutes in a bath sonicator (Elma; Elmasonic S30). Serial centrifugations were carried out (Eppendorf Centrifuge 5810R, Rotor F-34-6-38) for 45 minutes each step, at 40 g, 150 g, 600 g and 2400 g analyzing the supernatants.

UV-Vis Analysis

Optical extinction spectra were acquired on Jasco V-530 UV–VIS spectrophotometer using 1 cm optics quartz cuvettes. The spectra of all the samples were analyzed to determine the mean number of layers and mean lateral size of the nanosheets by metrics as reported by Backes and colleagues.[1]

ζ-potential

ζ potential measurements were carried out on a Malvern Zetasizer Nano ZSP with irradiation from 633nm He-Ne laser. The samples were injected in capillary cells and the electrophoretic mobility (μ) was measured using a combination of electrophoresis and laser Doppler velocimetry techniques as reported elsewhere.[2] The ζ-potential was estimated from the electrophoretic mobility data through the Henry's equation. However, because of the particular solvent-sample relationship, the Henry's function was approximated to both the Huckel and Smoluchowsky limits offering upper and lower bounds of the possible ζ-potential values, Figure 1b.[2] The specific monitoring of flakes in the mixture of free protein and protein-MoS_2 mixture was possible because in the electrophoretic analysis, which is based on the combination of electrophoresis and laser Doppler velocimetry techniques,[3] the signal is originated from the scattered laser light. Indeed, in the presence of the exfoliated flakes, which are more than one order of magnitude larger than proteins, the signal of the proteins is negligible and

only the flakes are observed. All the measurements were conducted at 25°C at the natural pH of the protein-MoS_2 solution.

AFM analysis

The topographic analysis has been carried out by employing a commercial Atomic Force Microscope (Witec Alpha SNOM RAS 300). Liquid phase exfoliated nanosheets of MoS_2 were deposited onto silicon substrates (SiO_2 on Silicon). The MoS_2 nanosheets are weakly bound to the substrate and the AFM tip can drag particles across the surface leading to blurred images with poor resolution. In order to avoid such a disadvantages, our measurements were performed with AFM operating in intermittent contact regime (tapping mode), with cantilevers of 75 kHz resonant frequencies and k = 2.8 N/m spring constant. We have chosen large free amplitude and set-point values as high as possible in order to image our samples with small tip-sample interaction forces (in repulsive regime). We have recorded simultaneously phase images along with the surface topography. Since the phase shift (between the free oscillation and the oscillation while the tip is in contact with the surface) depends on the viscoelastic properties of the sample as well as on the adhesive potential, the phase images outline domains of varying material properties.

Additional AFM images of biofunctionalized MoS_2 are shown in fig. S4. At high concentration, the protein covers the flakes and a self-assembled layer is formed from which we can estimate a relative height of about 3-4 nm.

Confocal Raman and Photoluminescence spectroscopy and few layer metrics

Confocal Raman spectroscopy has been performed by exploiting the excitation wavelength at 488 nm of a solid-state laser source. The laser was focused on the samples through a lens objective (NA = 0.75, 50 x magnification) with a spot size of about 350 nm. Emission from the sample was collected by the same optics, was filtered to

eliminate any residue from the pump beam and focused on the 50 µm core of an optical fiber (acting as confocal pinhole). The emission was sent to a spectrometer coupled to a high sensitivity EMCCD (Electron multiplying charge-coupled device). The sample was raster scanned and an entire Raman or Photoluminescence spectrum was collected at every point.

As test experiment, we have exfoliated by mechanical tape method flakes of MoS_2 starting from the same bulk material and we have obtained micron-sized flakes of MoS_2 with occurrence of single layer flake (fig. S5). We have verified the displacement of the frequencies of the two Raman vibrational modes with the varying thickness. The single layer corresponds to the top-left region (the brightest one in the inset image) and presents $\Delta f = 18\ cm^{-1}$.

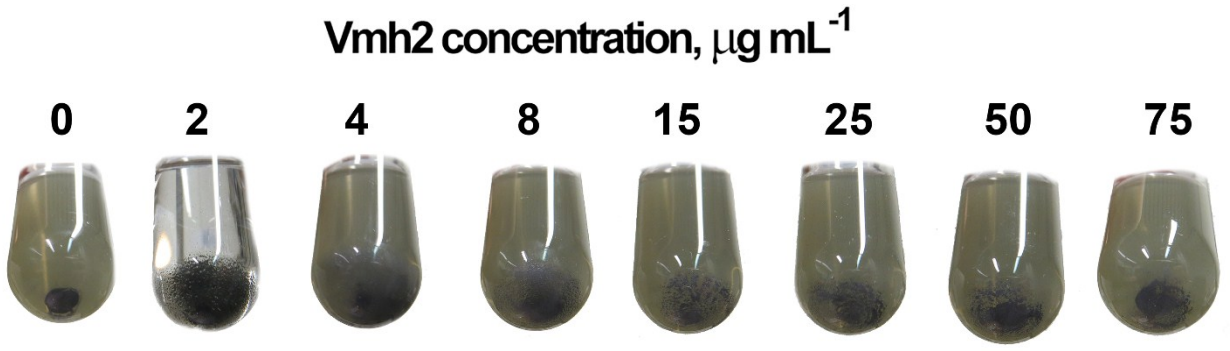

Figure S1 Stability of liquid phase exfoliated MoS_2 samples at different concentrations of hydrophobin Vmh2, imaged after 10 hours of sedimentation.

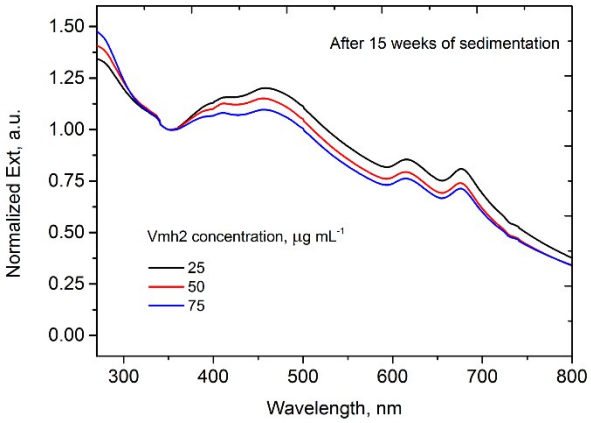

Figure S2 Normalized extinction spectra of MoS_2 samples at different concentrations of hydrophobin, after 15 weeks of sedimentation. 25 µg mL^{-1} Vmh2 / 16 µg mL^{-1} MoS_2; 50 µg mL^{-1} Vmh2 / 16 µg mL^{-1} MoS_2; 75 µg mL^{-1} Vmh2 / 14 µg mL^{-1} MoS_2.

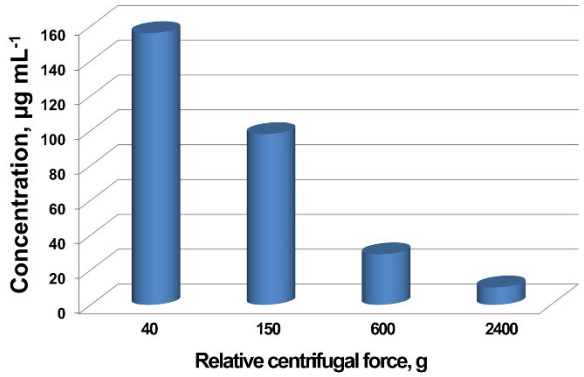

Figure S3 Concentration of exfoliated MoS_2 samples after step-wise centrifugation till 2400g.

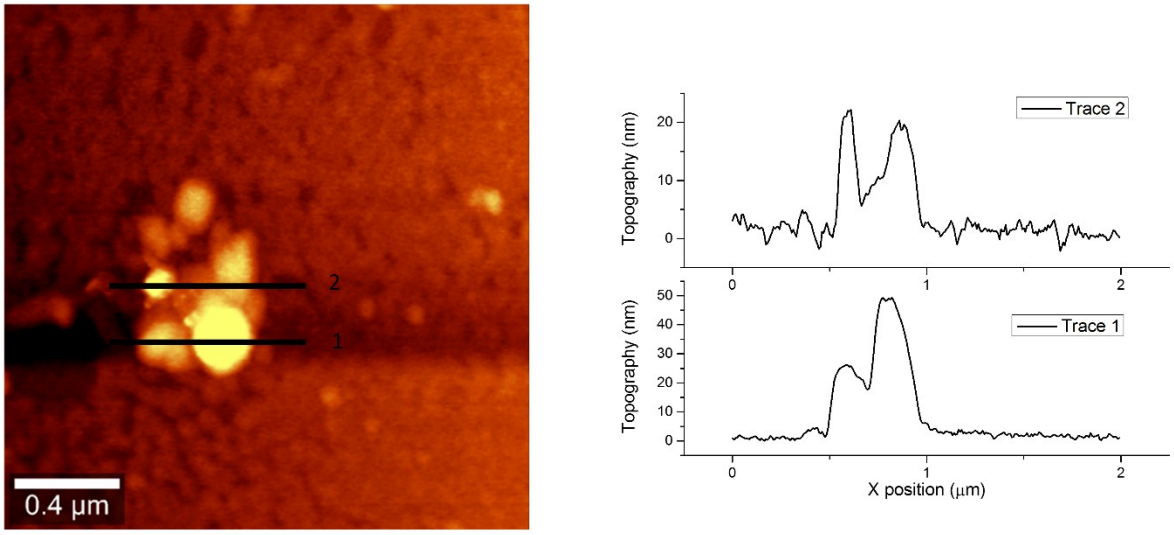

Figure S4: Atomic Force Microscopy and height analysis for exfoliated MoS_2 with HFB functionalization: (a) AFM scan of bio-MoS_2 with high HFB concentration. A layer of self-assembled HFB is evident with height about 3-4 nm height (b) the topographic trace shows presence of clusters of several nm surrounding the material.

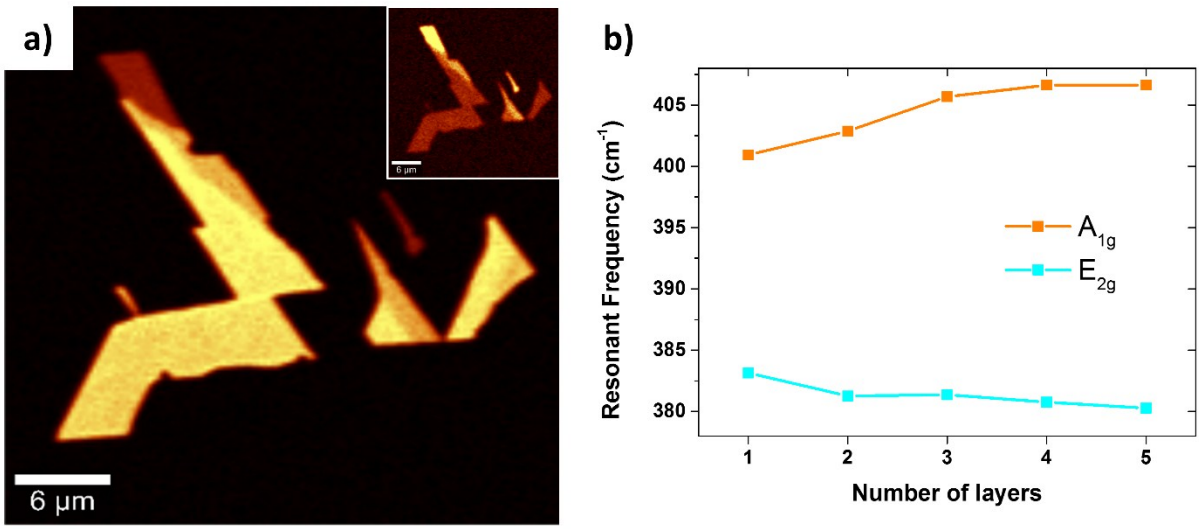

Figure S5: Raman spectroscopy and imaging: (a) Raman image of the integrated signal in the spectral region 370-420 cm^{-1}, the inset reports the corresponding photoluminescence image in the spectral region 620-700nm (b) Spectral analysis shows variation of the Raman spectrum with number of layers. The positions of the two main peaks (E^1_{2g} and A_{1g}) are reported in the graph.

Table S1 ζ-potential, Mean Number of Layers per flake and Mean Lateral size per flake estimated by Electrophoretic mobility, UV-Vis spectroscopy or Dynamic Light Scattering, for liquid phase exfoliated MoS_2 after centrifugation at 2400 g in the presence of Vmh2 at various concentrations.

	Vmh2 concentration, μg mL^{-1}				
	0	**25**	**50**	**75**	**100**
ζ-potential, mV, Smoluchowski approximation.[2]	-22.5 ±0.5	+23.9 ±0.6	+27.8 ±0.6	+30.6 ±0.2	+31.5 ±0.6
Mean No. of layers, estimated by UV-Vis[1]	2.6	2.9	2.9	2.9	2.9
Mean Lateral Size and range, nm, estimated by DLS[4]	100 (44÷348)	130 (56÷462)	97 (42÷334)	100 (44÷348)	90 (39÷307)
Mean Lateral Size, nm, estimated by UV-Vis[1]	97	100	96	97	89

Exfoliation and biological functionalization: WS_2

WS_2 powder (Aldrich, 243639, particle size ~2 μm, 99% purity, density 7.5 g mL^{-1} at 25 °C), was exfoliated in 60% ethanol aqueous solution (7.5 mg in 6.25 mL) using a tip sonicator (Branson digital sonifier, inbuilt power meter output power 40W) for 90 minutes or 4 hours in cylindrical glass tubes (13mm diameter, 10 cm height, rounded bottom). The temperature of the dispersion during sonication was controlled in an ice-water bath. After sonication, Vmh2 was added to fractions of the exfoliated dispersion to reach a variable final concentration of hydrophobin, 2÷75 μg mL^{-1}, using a fixed concentration of material, 300 μg mL^{-1} of WS_2, and the solutions were mixed by sonicating for 10 minutes in a bath sonicator (Elma; Elmasonic S30). The samples were left to settle down at room temperature and imaged during a period of 6 weeks.

Exfoliation and Size Selection: WS_2

WS_2 powder (Aldrich, 243639, particle size ~2 μm, 99% purity, density 7.5 g mL^{-1} at 25 °C), was exfoliated in 60% ethanol aqueous solution (7.5 mg in 5 mL) using a tip sonicator (Sonics vibra cell ultrasonics processor, inbuilt power meter output power 18W) for 120 min in cylindrical glass tubes (13mm diameter, 10 cm height, rounded bottom). The temperature of the dispersion during sonication was controlled in an ice-water bath. After sonication, Vmh2 was added to fractions of the exfoliated dispersion to reach a variable final concentration of hydrophobin, 0, 75, 100 μg mL^{-1}, using a fixed concentration of material, 800 μg mL^{-1} of WS_2 and the solutions were mixed by sonication for 10 minutes in a bath sonicator (Elma; Elmasonic S30). Vmh2 concentration was chosen on the basis of the stability experiments, figure S6. Serial centrifugations were carried out (Eppendorf Centrifuge 5810R, Rotor F-34-6-38) for 45 minutes each step, at 40 g, 150 g, 600 g and 2400 g analyzing the supernatants.

Production of few layer bio-MoS_2

Synthesis of biofunctionalized WS_2 and dispersion stability

The colloidal stability of WS_2 sheets in function of time and Vmh2 concentration is displayed in Figure S6. In the absence of Vmh2 and 2 μg mL^{-1}, the sample was stable over 1 week, while at lower concentrations of Vmh2 a quick formation of aggregates and precipitation occurred as observed to the naked eye. When the concentration of Vmh2 increased, the stability of the dispersion has gradually improved. However, at 75 μg mL^{-1} of Vmh2, the dispersion is quite stable up to 6 weeks. It is worth to note that the dispersion stability of WS_2 is affected by sonication time much more than MoS_2.

Particle size selection

After sonication, small aliquots of the obtained dispersions were serially centrifuged at 40, 150, 600 and 2400 g for 45 minutes each in presence of Vmh2 at the concentrations which lie in the stability range, i.e. Vmh2 > 50 μg mL^{-1} or in its absence. After every step of centrifugation, the resultant supernatant was proceeded with the next step of centrifugation which makes it more refined in size and thickness. The extinction spectra of the WS_2 samples (supernatant) were acquired after every step of centrifugation and compared with the previous steps, plotted in Figure S7.

On analysing the optical spectra by UV-VIS spectrophotometry of the fractions produced, we estimated the mean number of layers $<\bar{N}>$ per flake, figure S8. As the centrifugal force was increased, the A-exciton peak shifted towards lower wavelengths, signifying reduction in number of layers and lateral size of nanosheets and leading to few-layer enriched dispersions.[5]

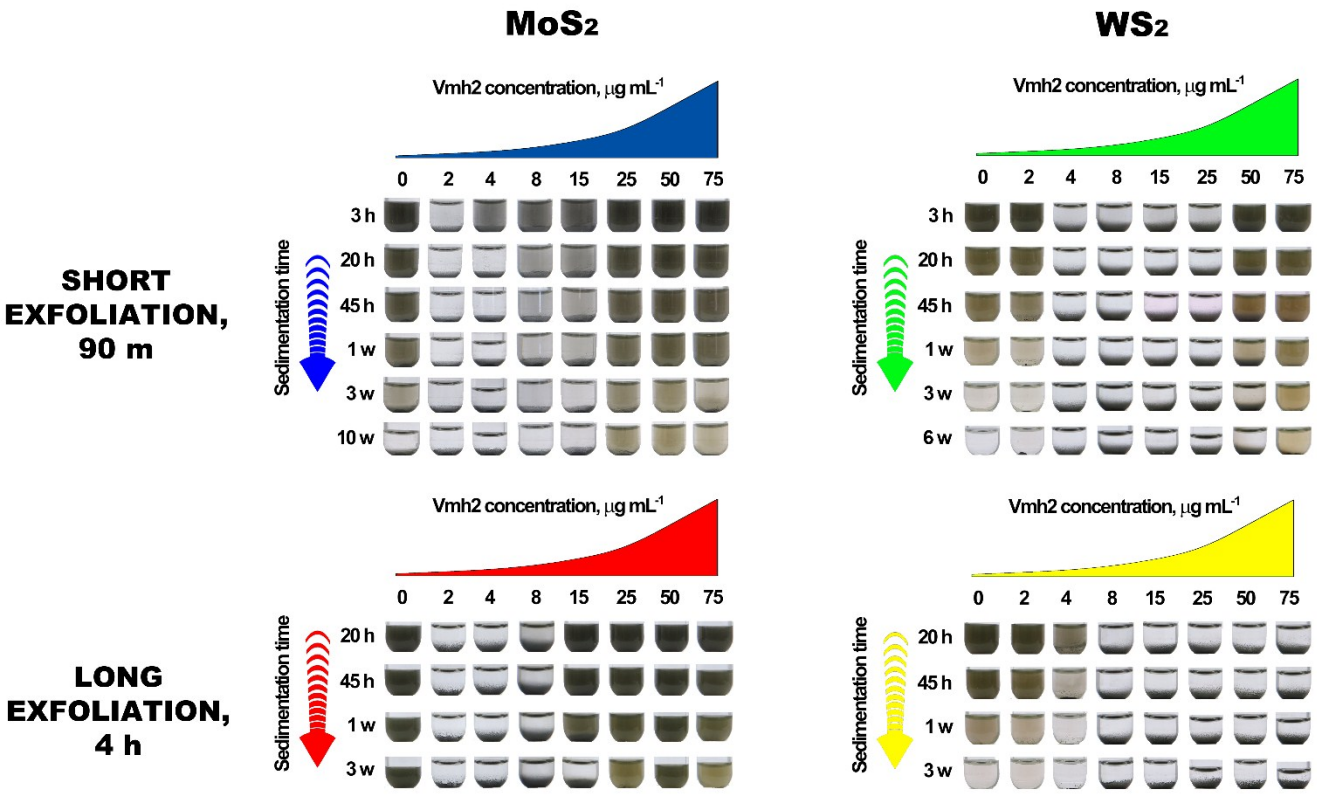

Figure S6 Dispersion stability of the exfoliated MoS_2 and WS_2 samples dependent on the concentration of Vmh2.

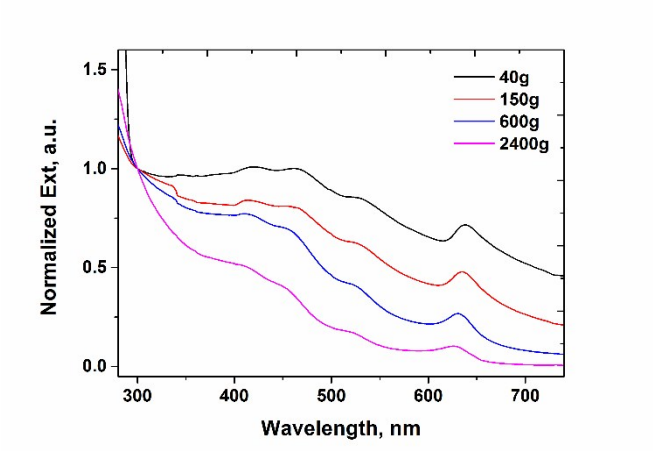

Figure S7: Normalized extinction spectra of WS_2 samples exfoliated and mixed with 100 µg mL^{-1} Vmh2, at different steps of centrifugation.

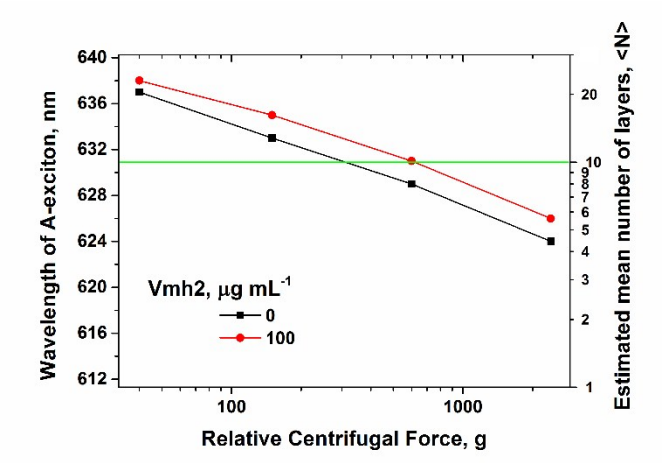

Figure S8: Wavelength of A-exciton of the centrifuged samples of WS_2 and estimation of mean number of layers. Accuracy of thickness estimation by metric is better than 20% if <N> is below 10.[5]

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