Electronic Supplementary Information

Detection and screening of basic amino acids using the luminescence switching of WS_2 nanosheets- Ag_2O nanoparticles composite

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Experimental

Materials and Characterizations

Tungsten disulphide (WS_2) and sodium hydroxide (NaOH) were purchased from Merck India Ltd. L-Arginine, L-Aspartic acid, Creatinine, Glycine, L-Glutamic acid, L(+)-Glutamine, L-Isoleucine, L-Methionine, L-Proline, L-Phenylalanine, L-Serine, L-Tryptophan, L-Valine were purchased from Spectrochem Pvt Ltd, India. DL-Homocysteine L-Cysteine and ubiquitin were obtained from Sigma Aldrich, USA, Cysteamine hydrochloride was purchased from Otto Chemical Reagents, India. L-Histidine (98%) was procured from Avra Synthesis Pvt Ltd, India. L-Aspargine monohydrate, L-(+) Lysine and Alanine were purchased from TCI Chemicals, India.

UV-visible spectra were recorded using a Carry 100 UV-visible spectrometer. All steady state fluorescence measurements (excitation and emission) were carried out using a FluoroMax-4C Spectrofluorometer (Horiba Instruments, USA). Both excitation and emission slit widths were fixed at 5 nm with an integration time of 0.1 ns. Time resolved fluorescence measurements were performed using time-correlated single-photon counting (TCSPC). TCSPC measurements were performed at excitation wavelength of 344 nm and decay profiles were collected at 450 nm. Transmission electron microscopy (TEM) images were obtained using a JEOL 2100 instrument and the atomic force microscope (AFM) images were taken using an Agilent 5500 scanning probe microscope (non-contact mode). Raman spectra were measured in a Renishaw confocal Raman microscope using 532 nm laser. X-ray photoelectron spectroscopy and (XPS) studies were performed using an Omicron ESCA probe spectrometer with polychromatic Mg Kα radiation. Mass spectra were recorded using a Bruker Q-TOF (COMPACT) mass spectrometer. A 2% acetic acid in 1:1 mixture of methanol-water mixture was used as the electrospray solvent.

Sensor Applications of WS_2 nanosheet -Ag NPs Nanocomposite

2 mL of WS_2 nanosheet -Ag_2O NP nanocomposite solution was utilized as the sensor solution in all the experiments. To this solution, 10 µL of 10^{-3} M Lys, His and Arg solution was added stepwise and fluorescence measurements were conducted without a waiting period.

The selectivity of the present sensor solution was tested using 100 µL of a 1 mM solution of various analytes. We used amino acids such as Alanine, L-Arginine, L-Aspartic acid, L-Aspargine monohydrate, Creatinine, L-Cysteine, DL-Homocysteine, Glycine, L-Glutamic acid, L(+)-Glutamine, L-Histidine, Leucine, L-Isoleucine, L-(+) Lysine, L-Methionine, L-Proline, L-Phenylalanine, L-serine, L-tryptophan, L-Valine, several metal ions (Na^+, K^+, Mg^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+} and Cd^{2+}) common biologically relevant molecules such as glutathione (GSH), Dopamine, Adenine, thiourea were used for selectivity studies. The excitation wavelength was 360 nm for all the sensing experiments.

Real Sample Analysis

Blood and urine samples were collected from healthy persons. In blood sample anti-coagulants were added and both blood and urine samles were diluted. Spiked samples were prepared by adding different concentrations of lysine into the diluted blood sample. Subsequently, aliquots of these solutions (10 µL each) were added to 2.5 mL of WS_2 NSs-Ag NPs solution and mixed thoroughly, and the PL emission was taken at an excitation wavelength of 360 nm. All experiments were performed in compliance with the institute's policy on animal use and ethics.

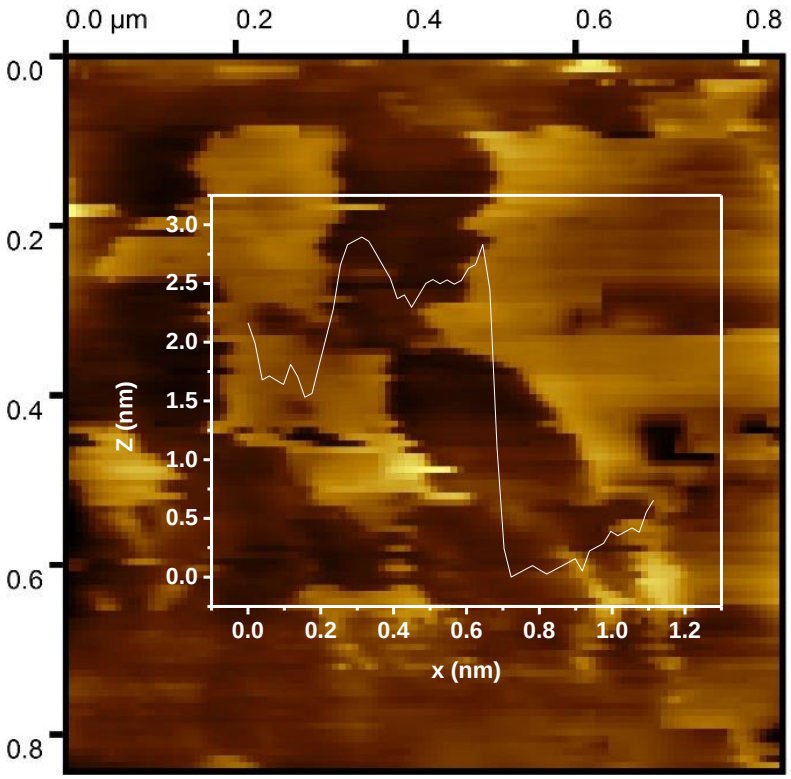


Figure S1: AFM micrograph of WS_2 nanosheet showing thickness of ~2.8 nm.

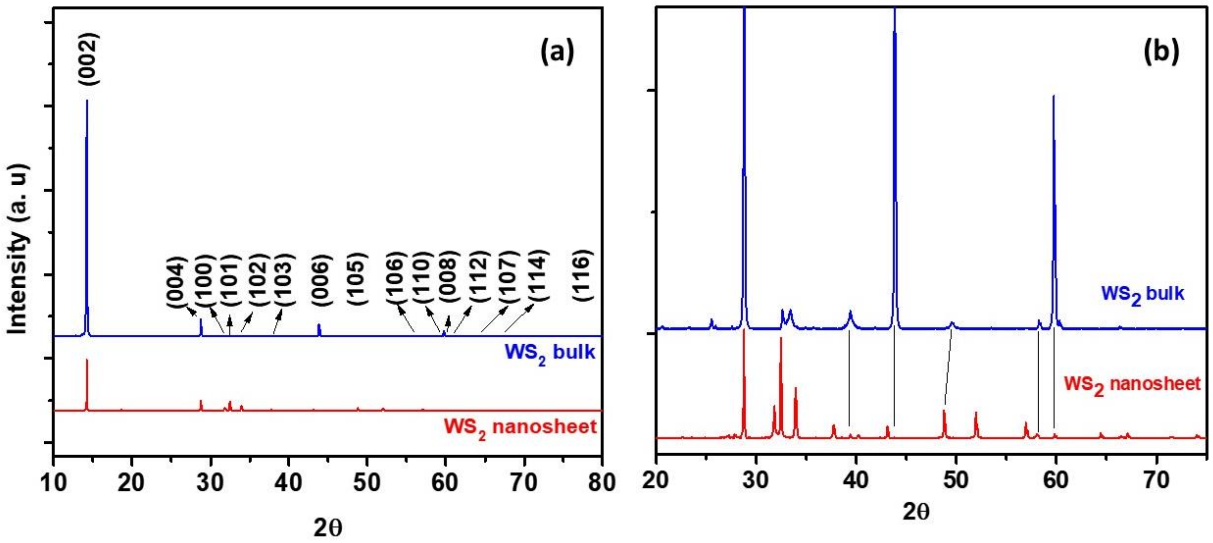


Figure S2: (a) XRD pattern of WS_2 nanosheets (red spectrum), with commercial WS_2 powder as reference (blue spectrum). (b) shows the zoomed view of diffraction in the 20-80° region.

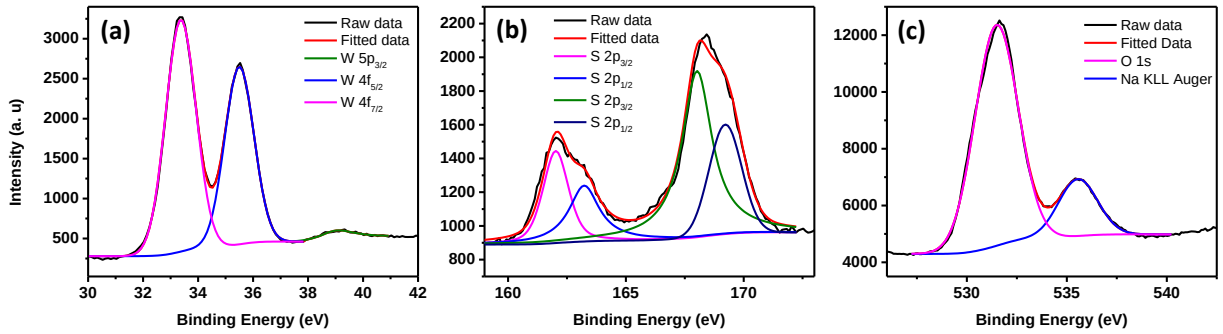


Figure S3: High-resolution XPS spectra of W, S, and O region of WS_2 NSs. (NaOH used in the hydrothermal reaction is the reason for the presence of Na peak in (c)).

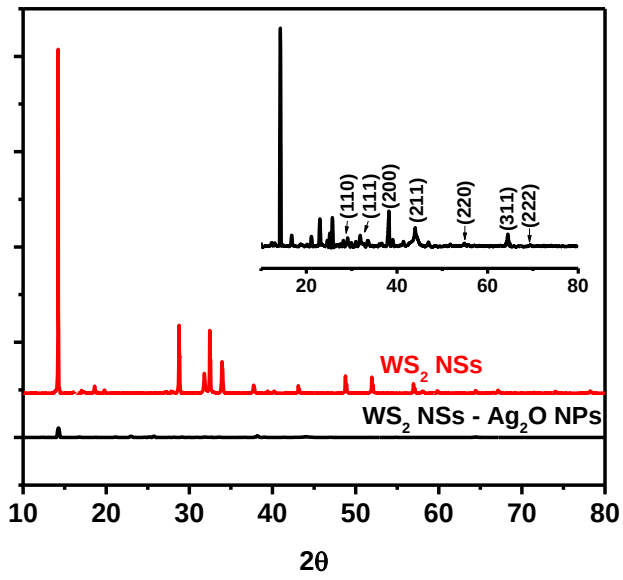


Figure S4: XRD patterns of WS_2 nanosheets (red spectrum) and that of WS_2 NSs-Ag_2O NPs nanocomposite (black). The inset shows the zoomed image of WS_2 NSs-Ag_2O NPs nanocomposite, showing peaks corresponding to the formation of Ag_2O NPs.

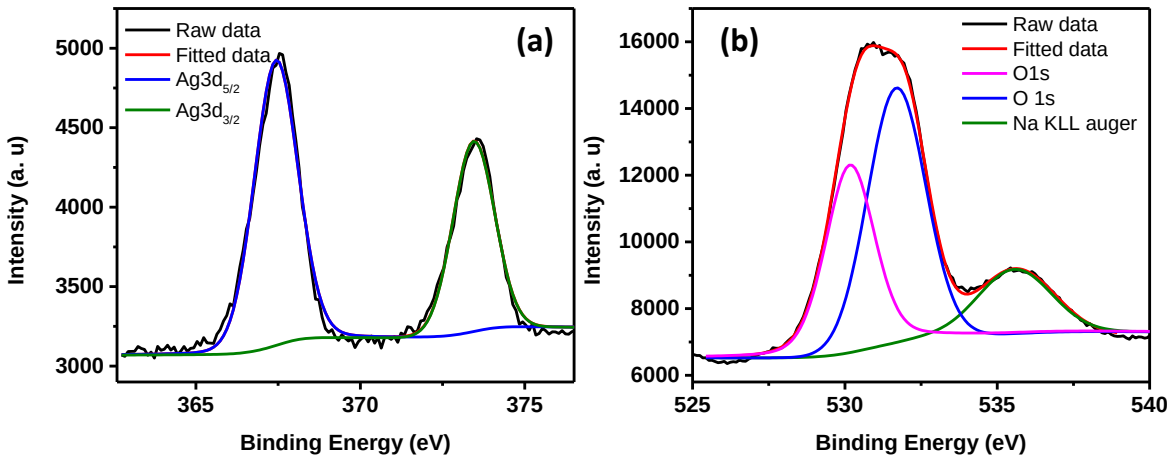


Figure S5: High-resolution XPS peaks of Ag 3d and O 1s of WS_2 NSs-Ag_2O NPs nanocomposite.

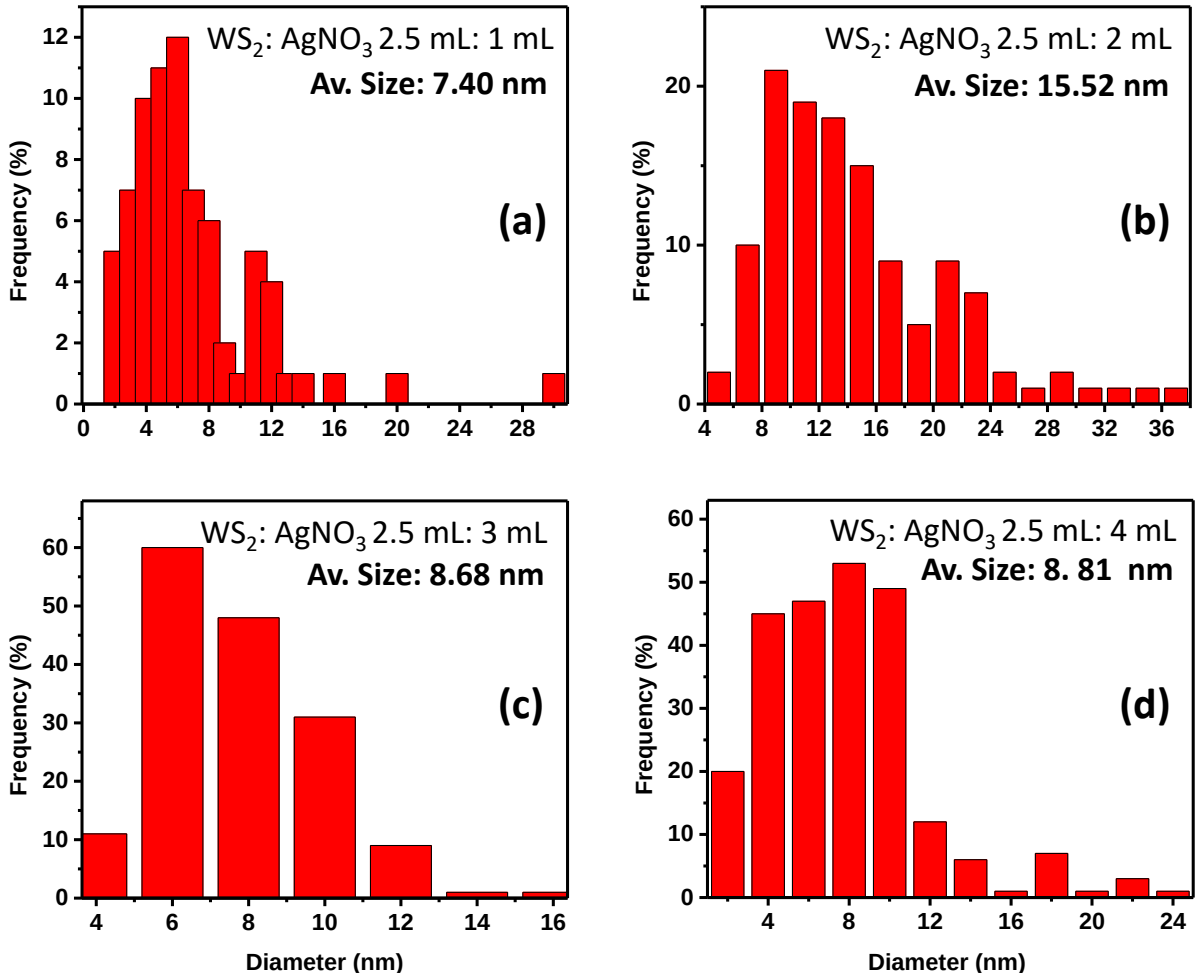


Figure S6: Size histogram obtained from TEM images of Ag NPs formed at different volume ratios of WS_2 NSs and Ag NO_3.

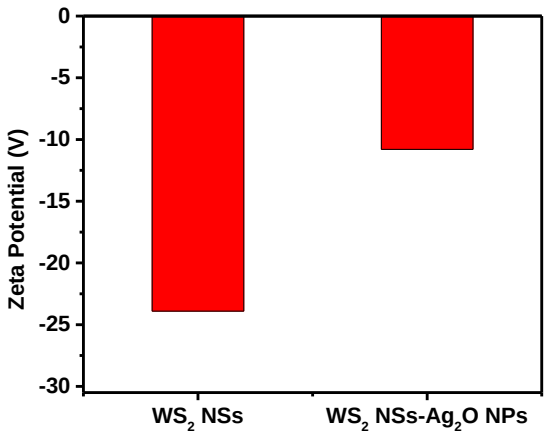


Figure S7: Zeta potential values of WS_2 NSs and WS_2 NSs -Ag_2O NPs composite. The charge on the surface of nanocomposite is also negative, which endorse that the formed Ag_2O NPs are of negatively charged.

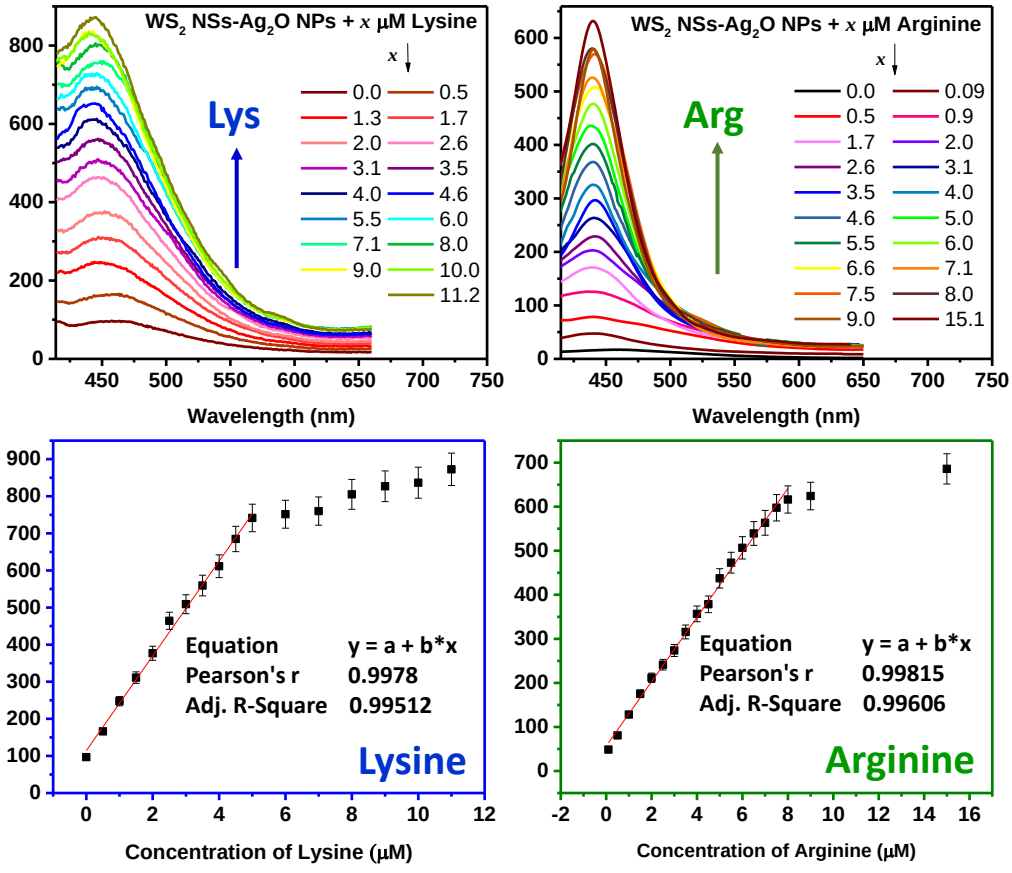


Figure S8: Response of Lys and Arg towards fluorescence emission of WS_2 NSs-Ag_2O NPs nanocomposite at pH 7. The calibration plots of the sensor solution for these two AAs, showing a linear range from 0.5- 6.6 µM, and 0.09- 8.2 µM concentrations of Lys and Arg, respectively, at pH 7.

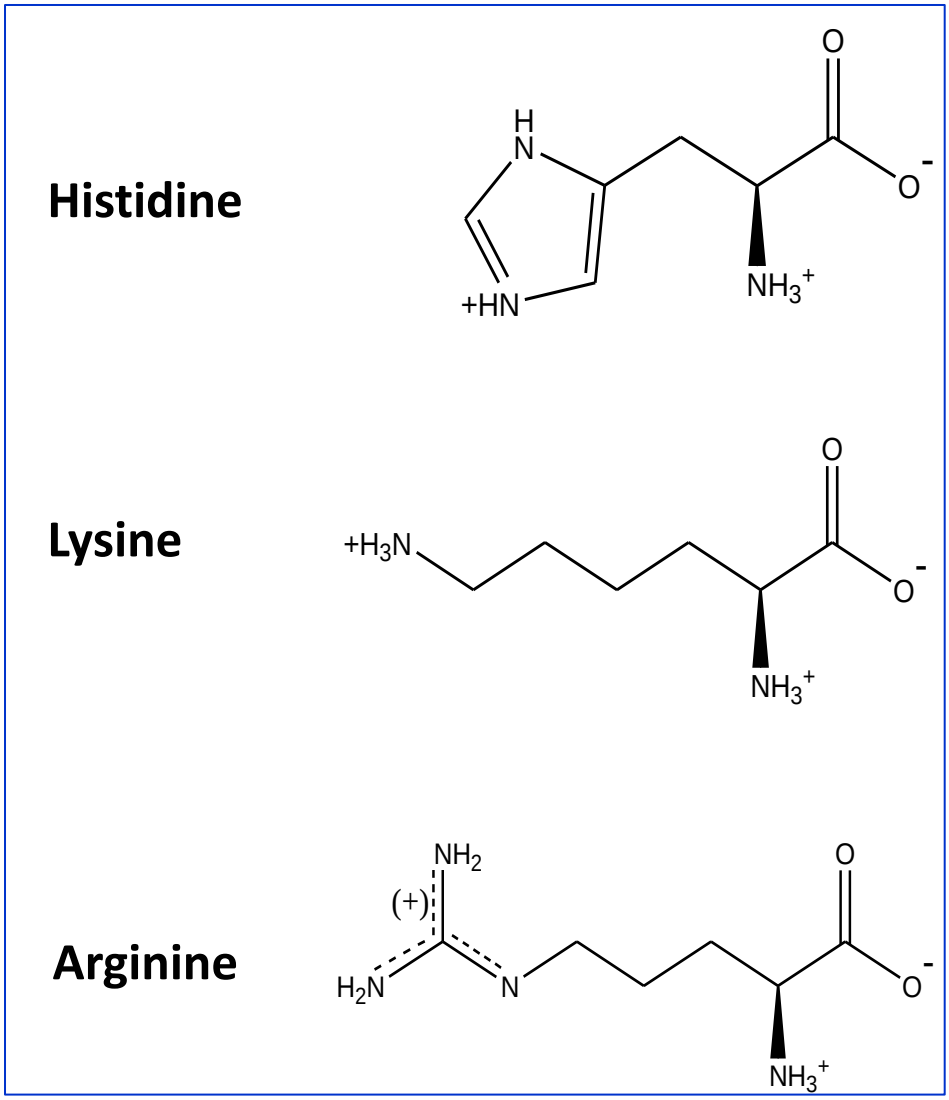


Figure S9: Structure of His, Lys and Arg, below their respective isoelectric point.

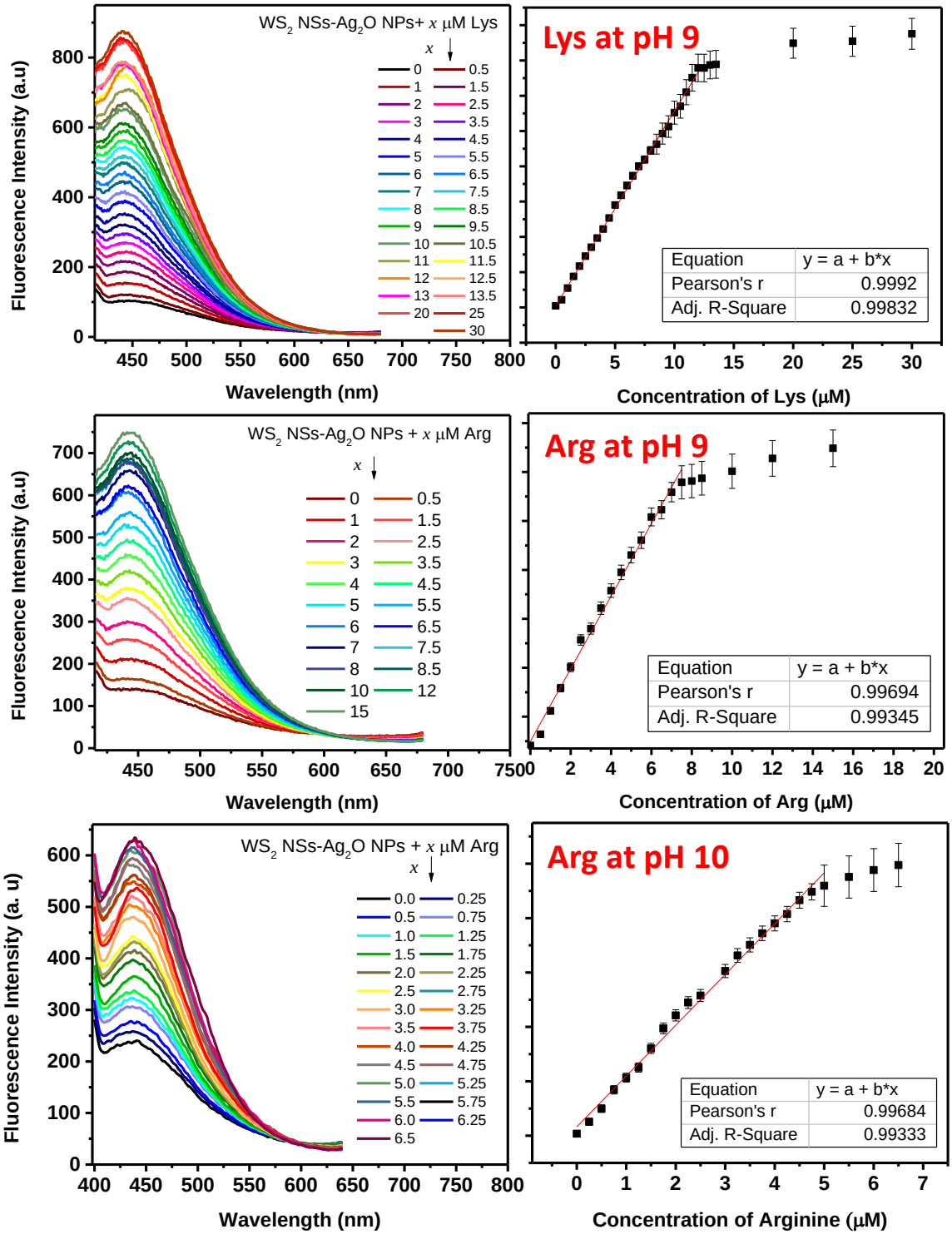


Figure S10: Response of Lys and Arg towards fluorescence emission of WS_2 NSs-Ag_2O NPs nanocomposite and corresponding calibration plots at pH 9 and 10.

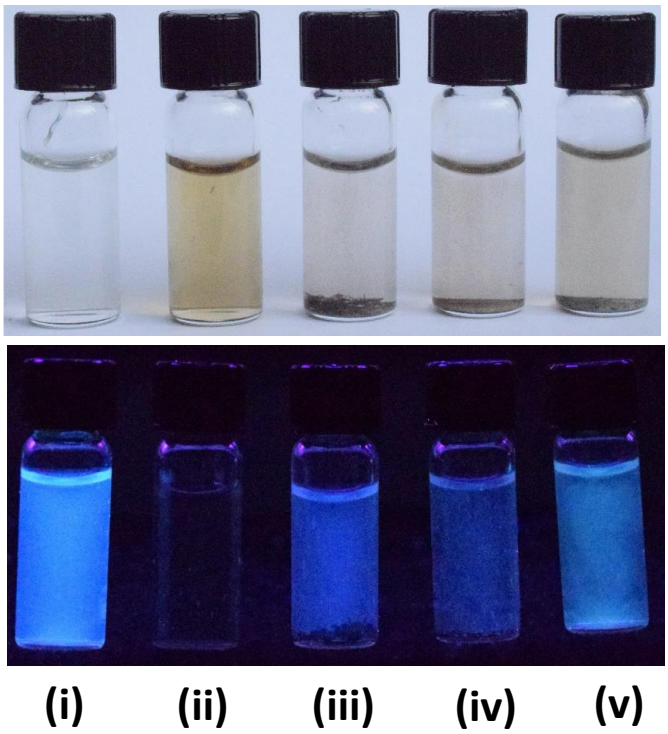


Figure S11: Photographs of WS_2 NSs (i), WS_2 NSs-Ag_2O NPs nanocomposite (ii), and the nanocomposite solution containing Lys (iii), His (iv) and Arg (v) under visible and UV light. The concentration of Lys His and Arg respectively are 10, 12 and 15 µM.

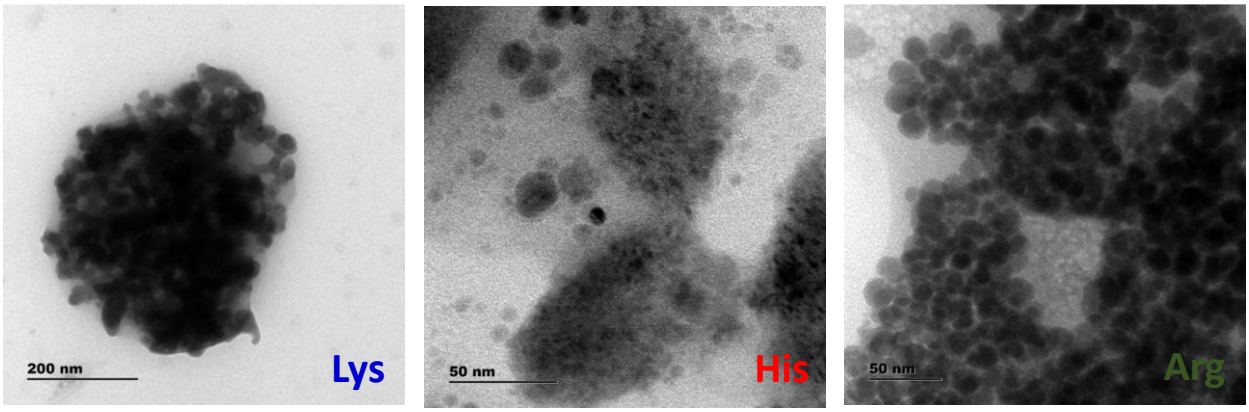


Figure S12: TEM images of WS_2 NSs-Ag_2O NPs nanocomposite upon interaction with Lys, His and Arg at pH 7

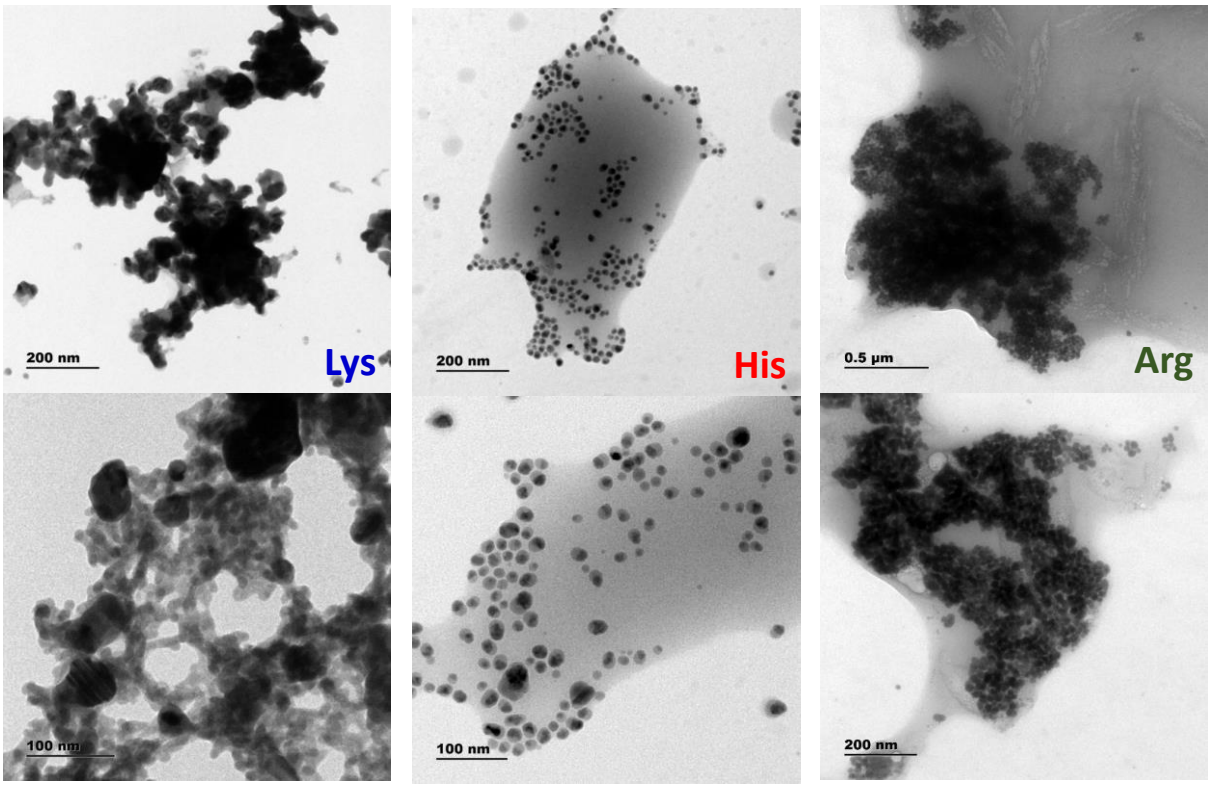


Figure S13: TEM images of WS_2 NSs-Ag_2O NPs nanocomposite upon interaction with Lys, His and Arg at pH 9, showing no aggregation of AgNP with His.

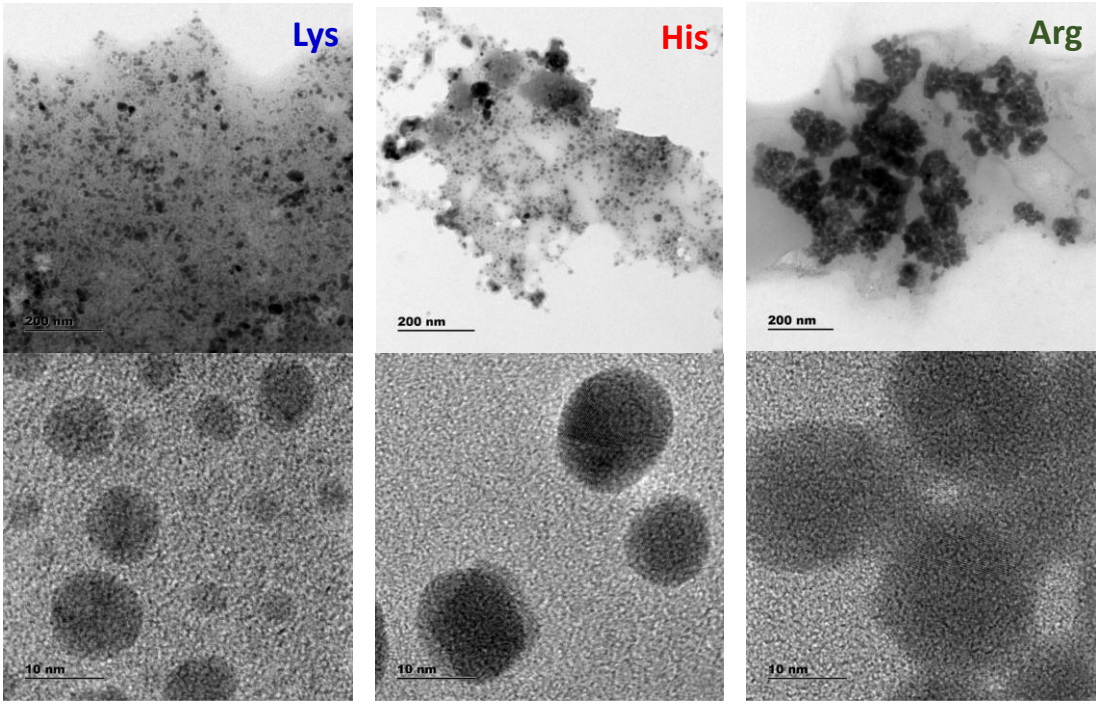


Figure S14: TEM images of WS_2 NSs-Ag_2O NPs nanocomposite upon interaction with Lys, His and Arg at pH 10, showing aggregation with only Arg.

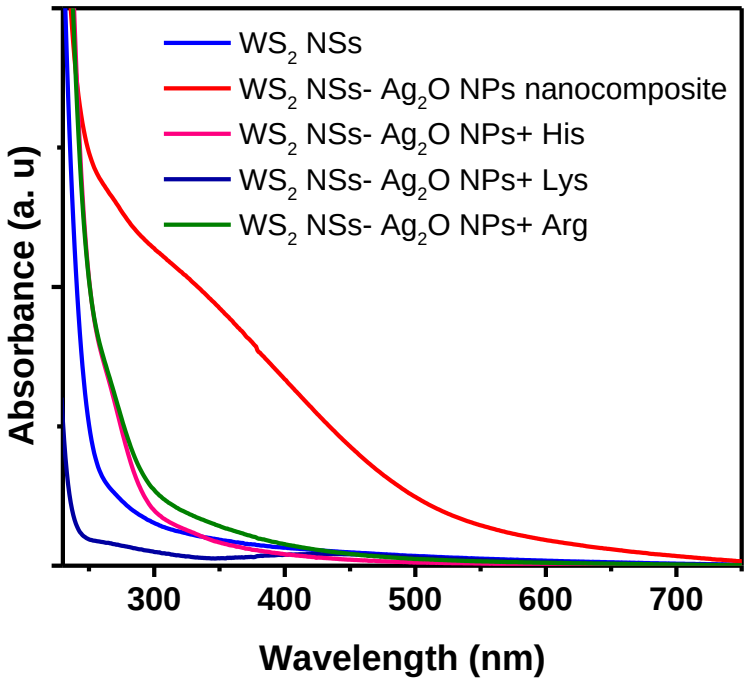


Figure S15: UV-Visible absorption spectra of WS_2 NS, WS_2 NSs-Ag_2O NPs nanocomposite, WS_2 NSs-Ag_2O NPs nanocomposite with Lys, His and Arg.

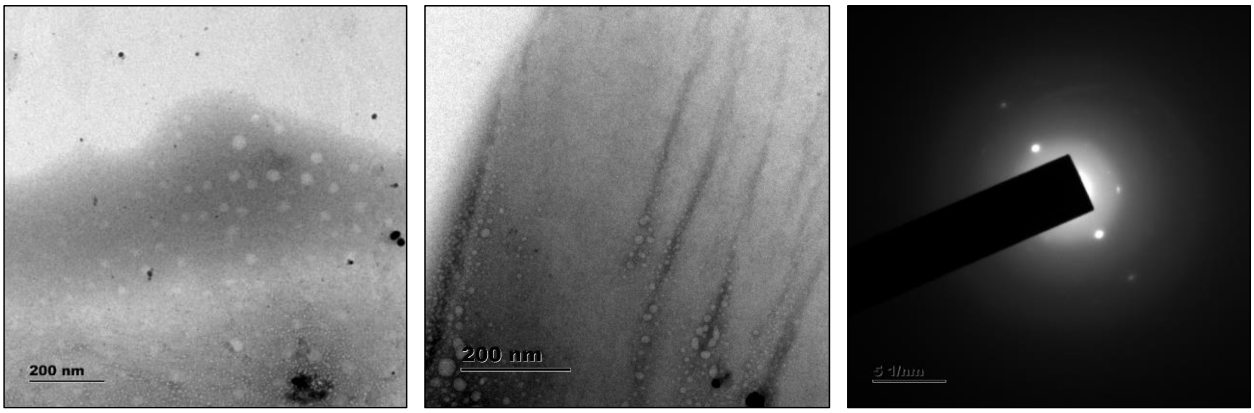


Figure S16: TEM image of colorless solution obtained after sedimentation of Ag_2O NPs upon addition of excess amount (15 µM) of Arg at pH 10.

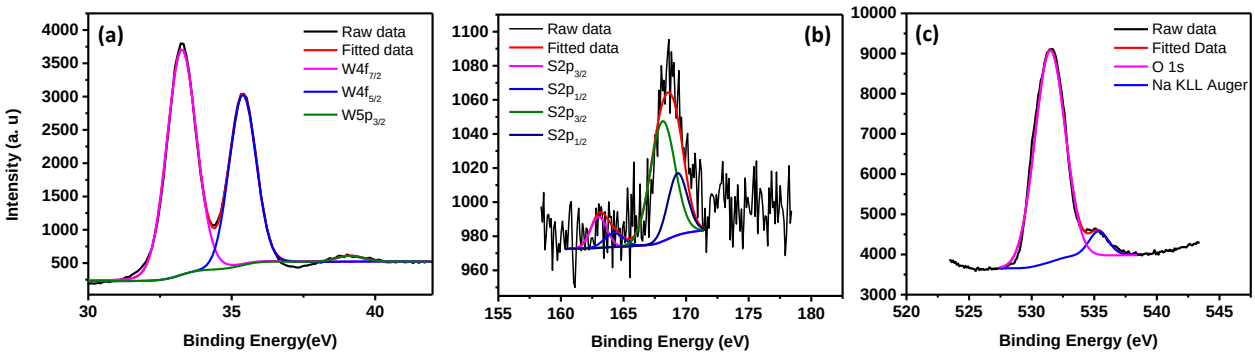


Figure S 17: High-resolution XPS peaks of (a) W, (b) S and (c) O of the clear solution (supernatant) obtained after the full aggregation Ag_2O NPs upon treated with excess AAs.

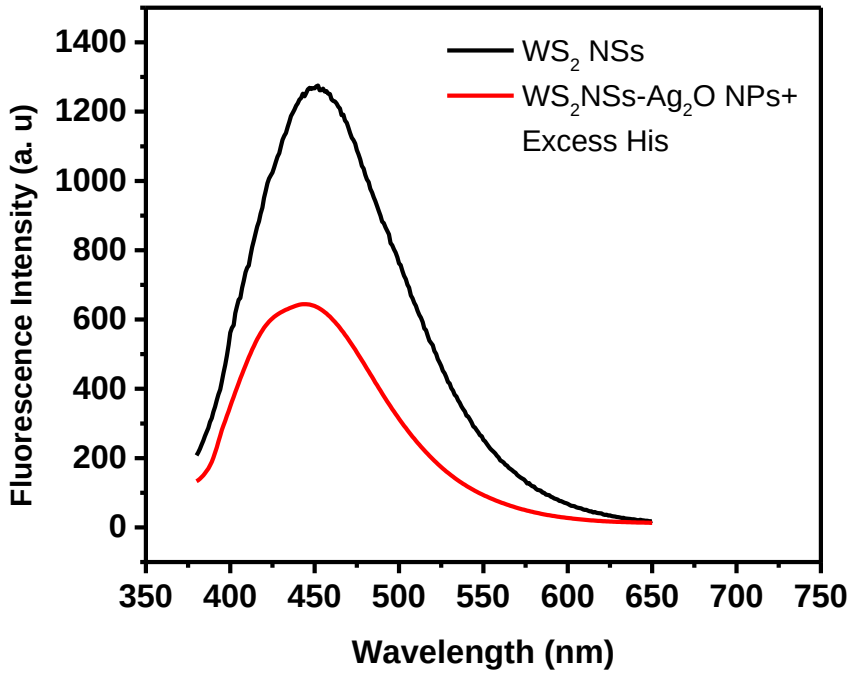
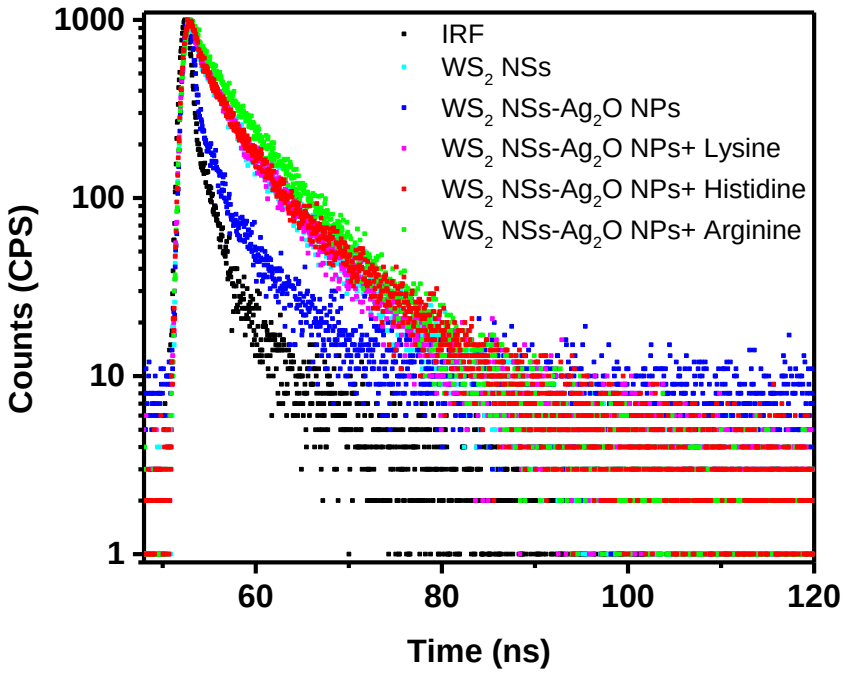


Figure S 18: PL spectra of WS_2 NS (black spectrum) and solution obtained after the aggregation of Ag_2O NP upon addition of excess AA.



System	τ_1 (ns)	α_1	τ_2 (ns)	α_2	τ_3 (ns)	$\alpha 3$	$\langle\tau\rangle$ (ns)	χ^2
WS_2 NSs	9.1	54.44	2.8	45.56	-	-	7.8	0.98
WS_2 NSs+Ag_2O NPs	6.2	19.62	0.13	80.38	-	-	5.7	1.2
WS_2 NSs+Ag_2O NPs+Lys	8.1	30.27	2.8	40.32	0.5	29.40	6.2	0.9
WS_2 NSs+Ag_2O NPs+His	8.9	31.74	3.9	45.79	0.7	22.46	6.7	0.8
WS_2 NSs+Ag_2O NPs+Arg	8.6	34.90	2.8	35.69	0.5	29.41	6.9	0.9

Figure S19: Lifetime spectra of WS_2 NSs, WS_2-Ag_2O NPs nanocomposite, WS_2-Ag_2O NPs nanocomposite with Lys, His and Arg. Table showing the lifetime of WS_2 NSs, WS_2 NSs-Ag_2O NPs nanocomposite, WS_2 NSs-Ag_2O NPs nanocomposite with Lys, His and Arg (the pH was 7). A 3 mL of 0.2 mM Ag NO_3 solution was added to 2.5 mL WS_2 solution. The volume and concentrations of amino acids were 0.5 mL of 0.1 mM His, and Lys, 0.8 mL of 0.1 mM Arg, respectively.

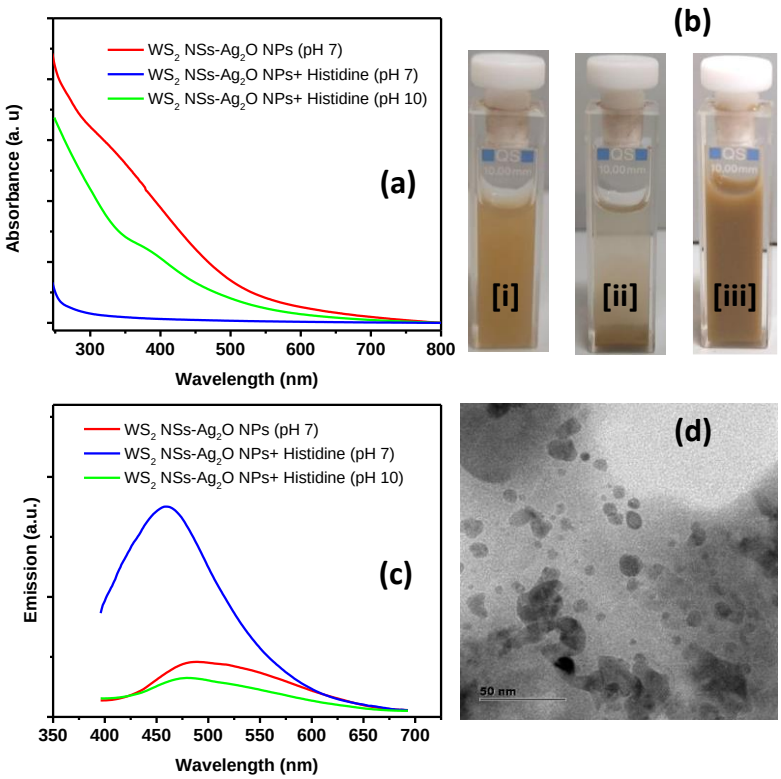


Figure S20: (a) UV-Visible spectra of [i] WS_2 NSs- Ag_2O NPs at pH 7, [ii] WS_2 NSs- Ag_2O NPs in the presence of histidine at pH 7 [iii] shows the UV-visible spectrum of the same solution given in [ii] after changing the pH to 10. (b) show the photographs of the solution mention in (a) as [i], [ii] and [iii]. (c) PL emission spectra of [i], [ii] and [iii]. (d) TEM images of separated Ag_2O nanoparticles after increasing the pH to 10.

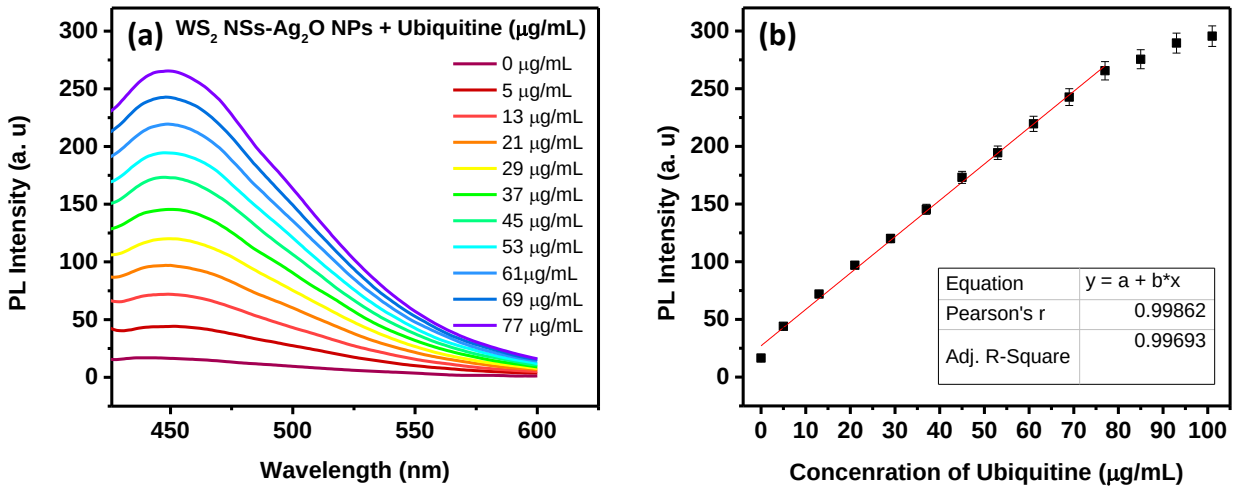


Figure S21: a) Fluorescence response of WS_2 NSs-Ag_2O NPs nanocomposite with various concentrations of ubiquitin b) Dynamic range of the sensor. Each trial is repeated three times and deviation from the mean value is represented as error bars.

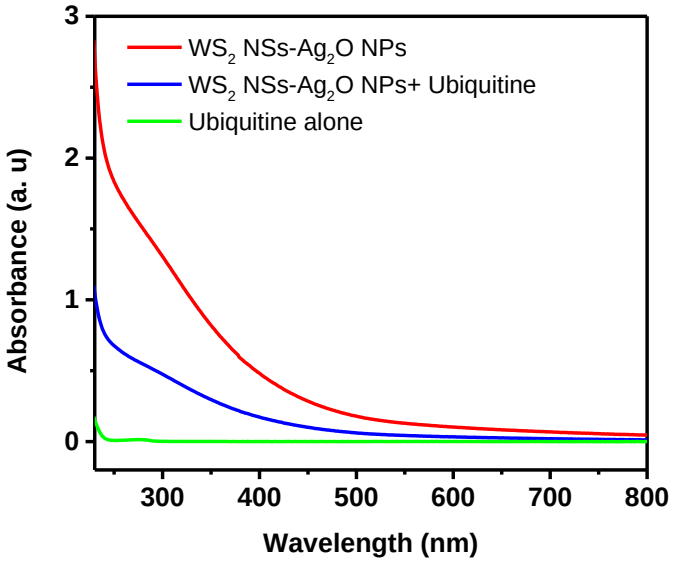
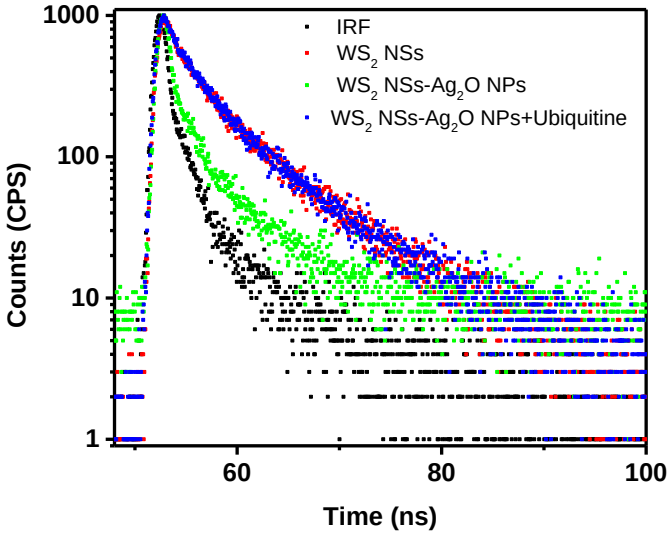


Figure S22: UV-Vis absorption spetra of WS_2-Ag_2O NPs nanocomposite, WS_2-Ag_2O NPs nanocomposite with ubiquitin and ubiquitin alone. The SPR peak of Ag_2O nanoparticles has been found to be diminished in the presence of ubiquitine, which shows the annihilation of nanoparticles in the presence of protein as well.



System	τ_1 (ns)	α_1	τ_2 (ns)	α_2	<τ> (ns)	χ^2
WS_2 NSs	9.1	54.44	2.8	45.56	7.8	0.98
WS_2 NSs+Ag_2O NPs	6.2	19.62	0.13	80.38	5.7	1.2
WS_2 NSs+Ag_2O NPs + Ubiquitin	8.5	56.37	2.32	43.63	7.4	0.87

Figure S23: Lifetime spectra of WS_2 NSs, WS_2-Ag_2O NPs nanocomposite, WS_2 NSs-Ag_2O NPs nanocomposite with ubiquitin. Table showing the lifetime of WS_2 NSs, WS_2 NSs-Ag_2O NPs

nanocomposite, WS_2 NSs-Ag_2O NPs nanocomposite with ubiquitin. A 3 mL of 0.2 mM Ag NO_3 solution was added to 2.5 mL WS_2 solution. The concentration of protein was 77 µg/mL.

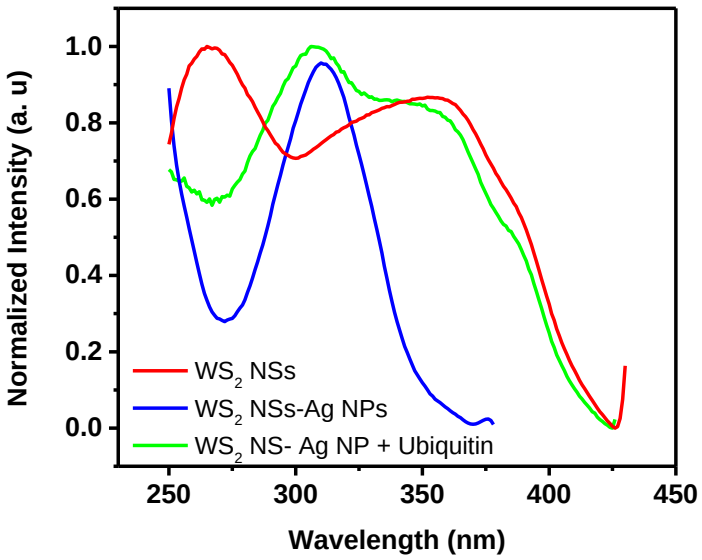


Figure S24: Excitation spectra of WS_2 NSs, WS_2-Ag_2O NPs nanocomposite, WS_2 NSs-Ag_2O NPs nanocomposite with ubiquitin.

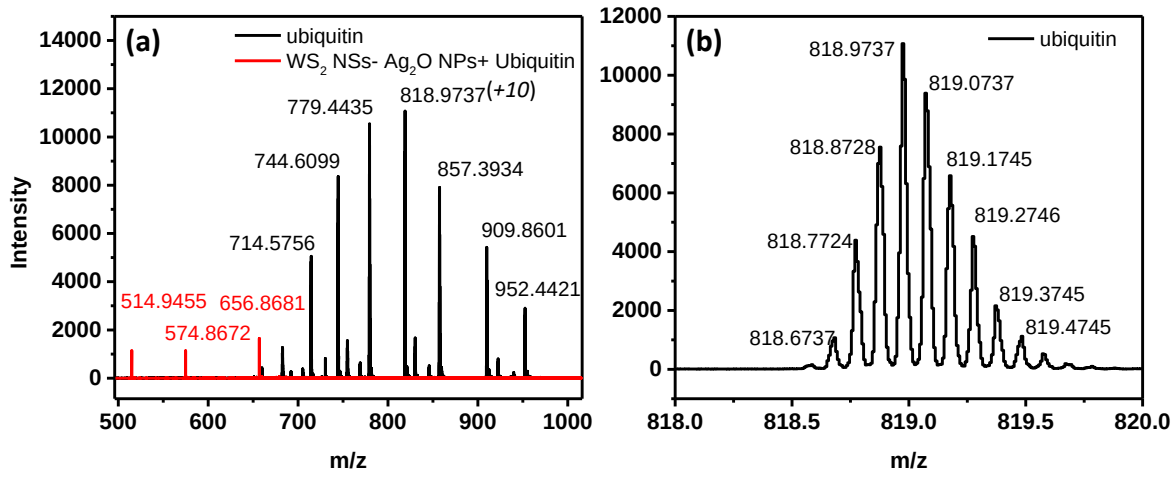


Figure S25: (a) Mass spectra of ubiquitin (black trace) and WS_2 NSs-Ag_2O NPs nanocomposite with ubiquitin (red trace). Mode: Positive; solvent: 2% acetic acid in 1:1 methanol:HPLC water mixture. The charge state of ubiquitin peaks is +10. The red trace shows the mass spectrum of WS_2 NSs-Ag_2O NPs + ubiquitin and no ubiquitin characteristic peaks are seen. **(b)** Zoomed spectra of base peak m/z 818.97 of pure ubiquitin with charge state +10.

Table S1: Selectivity coefficient for aminoacids at different pH

Interfering ions/molecules	pH 7	pH 9	pH 10
Glycine	2.13	1.93	1.52
Lysine	1.49	1.31	1.48
Serine	2.09	1.84	1.56
Leucine	2.14	1.81	1.55
Valine	2.17	1.95	1.45
Isoleucine	2.11	1.93	1.55
Alanine	2.07	1.95	1.55
Phenyl Alanine	2.08	1.94	1.53
proline	2.13	1.96	1.55
Threonine	2.04	1.95	1.55
Cysteine	2.16	1.88	1.52
Methionine	2.08	1.80	1.56
Histidine	1.05	1.64	1.47
glutamic acid	2.13	1.93	1.54
Glutamine	2.21	1.87	1.54
Aspartic acid	2.03	1.96	1.54
Aspargine	2.17	1.94	1.56
Na (I)	2.29	2.02	1.59
K (I)	2.31	2.03	1.60
Fe (II)	2.31	2.29	1.62
Fe (III)	2.34	2.19	1.63

Table S2: Table showing the dynamic range of His (at pH 7), Lys (at pH 7, 9) and Arg (at pH7, 9 and 10)

Amino Acid	**pH**	**Dynamic range**
His	7.0	0.5 – 7.0 µM
Lys	7.0	0.5 – 5.0 µM
Arg	7.0	0.1 – 8.0 µM
Lys	9.0	0.5 – 12.0 µM
Arg	9.0	0.5 – 7.5 µM
Arg	10.0	0.5 – 5.5 µM

Table S3: Zeta potential values of WS_2 NS, WS_2 NS- Ag NP and WS_2 NS- Ag NP with ubiquitin

System	Potential (V)
WS_2 NS	-24.6
WS_2 NSs-Ag_2O NPs (pH 7)	-10.8
WS_2 NSs-Ag_2O NPs (pH 9)	-23.9
WS_2 NSs-Ag_2O NPs (pH 10)	-35.2
WS_2 NSs- Ag_2O NPs with Ubiquitine	-18.3