Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

A Study of microbially fabricated bio-conjugated quantum dots for pico-molar sensing of H_2O_2 and glucose

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CONTENT

(I) Growth media preparation and culture condition for *Pseudomonas aeruginosa*

The biological preparation of 2D MoS_2 QDs was carried out using molybdate reducing bacteria i.e. *Pseudomonas aeruginosa*, obtained from Microbial Biotechnology and Downstream Processing Laboratory, Indian Institute of Technology, Kharagpur, India. The anaerobic microbial cultivation was carried out at 37 ˚C in modified P2 medium containing 0.25g/L yeast extract and 0.4 M glucose. Sodium β glycerophosphate (1g/L) was used as a phosphorus source while Magnesium Sulphate heptahydrate (2.5g/L) and 0.01 g/L each of sodium chloride, ferrous sulphate heptahydrate and manganese sulphate monohydrate were used as a salt mixture in the media. The pH of the culture during experimental conditions i.e. 8-9 was maintained using Sodium bicarbonate (2.5 g/L). Cysteine hydrochloride monohydrate (0.2% (w/v)) was included in the media components to act as a reducing agent and the sulphur source. Sodium Molybdate dihydrate pure was obtained from Sigma-Aldrich. Molybdenum standard for Atomic Absorption Spectroscopy (AAS) was purchased from Agilent technologies.

The bacterial growth curve was plotted with absorbance at 660 nm versus time post-inoculation of *P.aeruginosa* in the growth media. Lag phase refers to the no increment in the absorbance that is directly proportional to cell growth, log phase represents the exponential growth of the cells while the stationary phase is defined as the saturated growth of the microorganism.

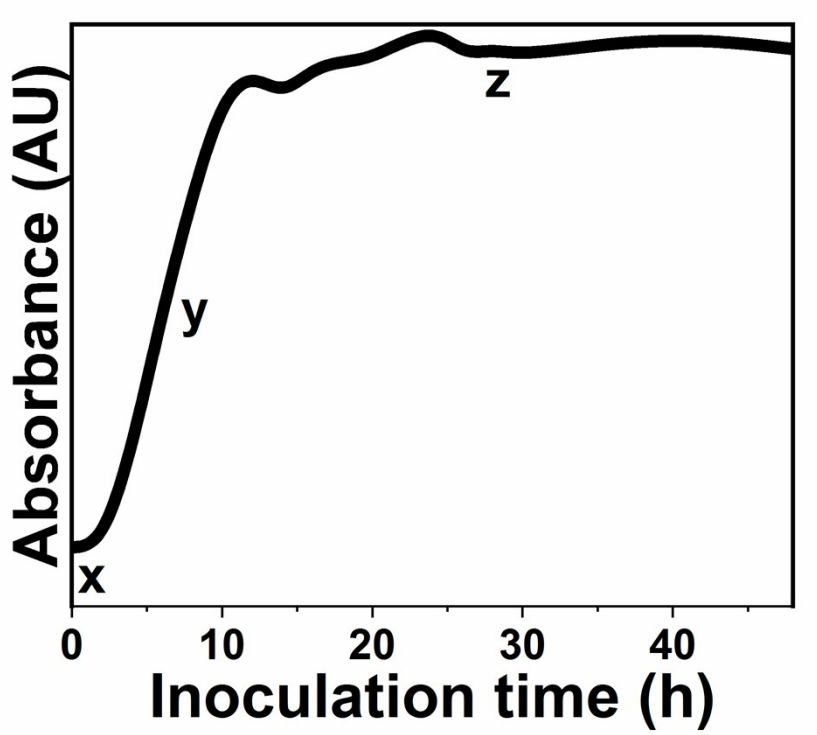

Figure S1. Growth curve obtained for *P. aeruginosa* where x= lag, y= log and z= stationary phase of growth

(II)

Table S1. Molybdenum threshold shown by *P.aeruginosa* cells

[Mo] concentration (mM)	Lag hours (h)	Colony Formation
0.5	2	Yes
1	2	Yes
2	2	Yes
4	4	Yes
6	8	Yes
8	NA	No
10	NA	No

(III)

Table S2. Mass balance for Mo removal by *P. aeruginosa*

Cysteine concentration (%)		Initial concentration (mM)	Final concentration (mM)	Percentage removal
0.2	Inoculated media	1	0.16	84%
	Uninoculated media	1	0.97	3%

(IV)

Quantum yield (Q) of the MoS_2 quantum dot, in water solution, was measured using the following relation.

$$Q = Q_{std} \times \frac{M_x}{M_{std}} \times \frac{\eta_x^2}{\eta_{std}^2} \quad (1)$$

Where M_x and η_x is the slope of the linear plot and refractive index of the solvent for MoS_2 quantum dot, respectively. Q_{std}, M_{std} and η_{std} denote the quantum yield, slope and refractive index of the standard sample (Methylene blue with quantum yield 0.52), respectively.

Calculation of Quantum yield:

Sample	**Slope**	**Refractive Index**	**Quantum yield**
Methylene Blue	0.71	1.33	0.52
MoS_2 QD	0.63	1.33	0.46

(V)

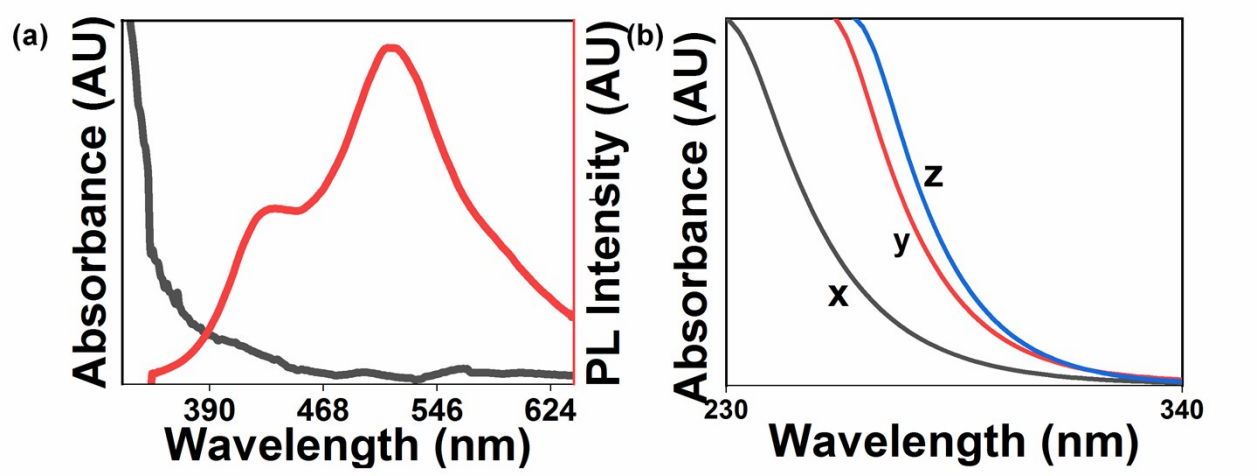

Figure S2. (a) UV-Vis spectra of H_2O_2 overlapped with PL emission spectra of MoS_2 QDs, (b) UV-Vis spectra of H_2O_2 in the MoS_2 QDs suspension at different concentration x= 10 nM, y= 20nM, z= 50 nM.

The overlap integral (*J(λ)*) characterizing the overlap between the emission spectrum of the MoS_2 QDs and the absorption spectrum of the H_2O_2 and glucose, and it can be calculated according to the following equation:

$$J = \frac{\int_0^\infty f_D(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda}{\int_0^\infty f_D(\lambda)d\lambda}$$

Where, f_D is the unnormalized emission spectrum of the donor. ε is the molar absorption coefficient of the acceptor. λ is the emission wavelength. The large spectral overlap in between donor (MoS_2 QDs) and acceptor (H_2O_2, glucose) confirms the possibility of excited energy transfer. We have also calculated the overlap integral values for H_2O_2 is 2.40×10^9 $M^{-1}cm^{-1}nm^4$

(VI)

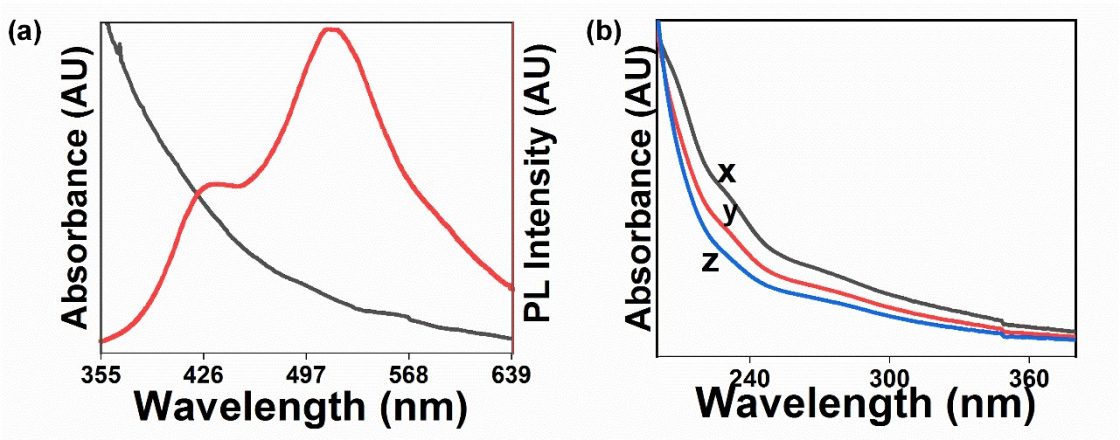

Figure S3. (a) UV-Vis spectra of glucose overlapped with PL emission spectra of MoS_2 QDs, (b) UV-Vis spectra of glucose in the MoS_2 QDs suspension at different concentration x= 10 nM, y= 20nM, z= 50 nM.

The oxidation of glucose produced D-gluconic acid and H_2O_2, where GO_x acts as an oxidant in aqueous solution. The oxidation reaction was:[4]

$$Glucose + O_2 + H_2O_2 \rightarrow D - gluconic\ acid + H_2O_2$$

The formation rate of H_2O_2 was directly proportional to the concentration of glucose added in the MoS_2 QDs solution. We observed that the PL intensity of MoS_2 QDs decreases with increasing glucose concentration. In Figure S3 (a), it was shown that the PL emission spectra of the MoS_2 QDs and the UV-Vis absorption spectra of glucose around were overlapped each other. This is the evidence for FRET phenomenon. PL intensity of MoS_2 QDs was found to be gradually decreasing with the increment of glucose molecules, because of MoS_2 QDs donates the energy, which was accepted by glucose molecules.

We have also calculated the overlap integral values for glucose is 4.11×10^{9} $M^{-1}cm^{-1}nm^{4}$.

(VII)

Table S3 Sensing performance of MoS_2 QDs

Detection	**Overlap integral** ($M^{-1}cm^{-1}nm^{4}$)	**Limit of detection (pM)**	**K_{sv} (nM^{-1})**	**R^2**	**Linear range (nM)**
Glucose	**4.11×10^{9}**	**2**	**0.02352**	**0.99849**	**0-30**
		68	**0.00710**	**0.94582**	**30-50**
H_2O_2	**2.40×10^{9}**	**43**	**0.02167**	**0.97192**	**0-30**
		87	**0.69971**	**0.95706**	**30-50**

(VIII)

Table S4 Glucose and H_2O_2 analysis in the presence of Phosphate buffer solution

Sample	Added (nM)	Found(nM)	Recovery (%)	RSD (%) (n=5)
Glucose	10	9.89	98.9	-
	20	19.85	99.25	1.01
	30	29.8	99.33	2.00
H_2O_2	10	9.45	94.5	1.07
	20	19.89	99.45	2.35
	30	29.85	99.50	1.27

(IX)

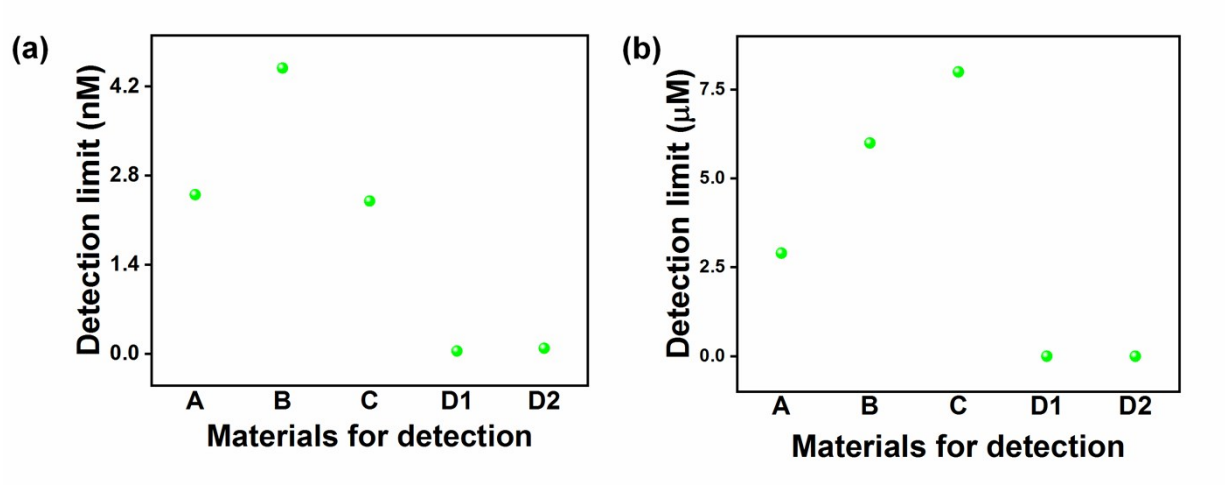

Figure S4 (a) H_2O_2 detection limit shown by materials: A= MoS_2, B= CdTe, C= WS_2, D1= MoS_2 QDs for H_2O_2 (0-30nM), and D2= MoS_2 QDs for H_2O_2 (30-50nM) (b) Glucose detection limit for materials: A= ZnO, B= Carbon QDs, C= B-doped Carbon QDs, D1 = MoS_2 QDs for glucose (0-30nM), and D2= MoS_2 QDs for glucose (30-50nM)

(X)

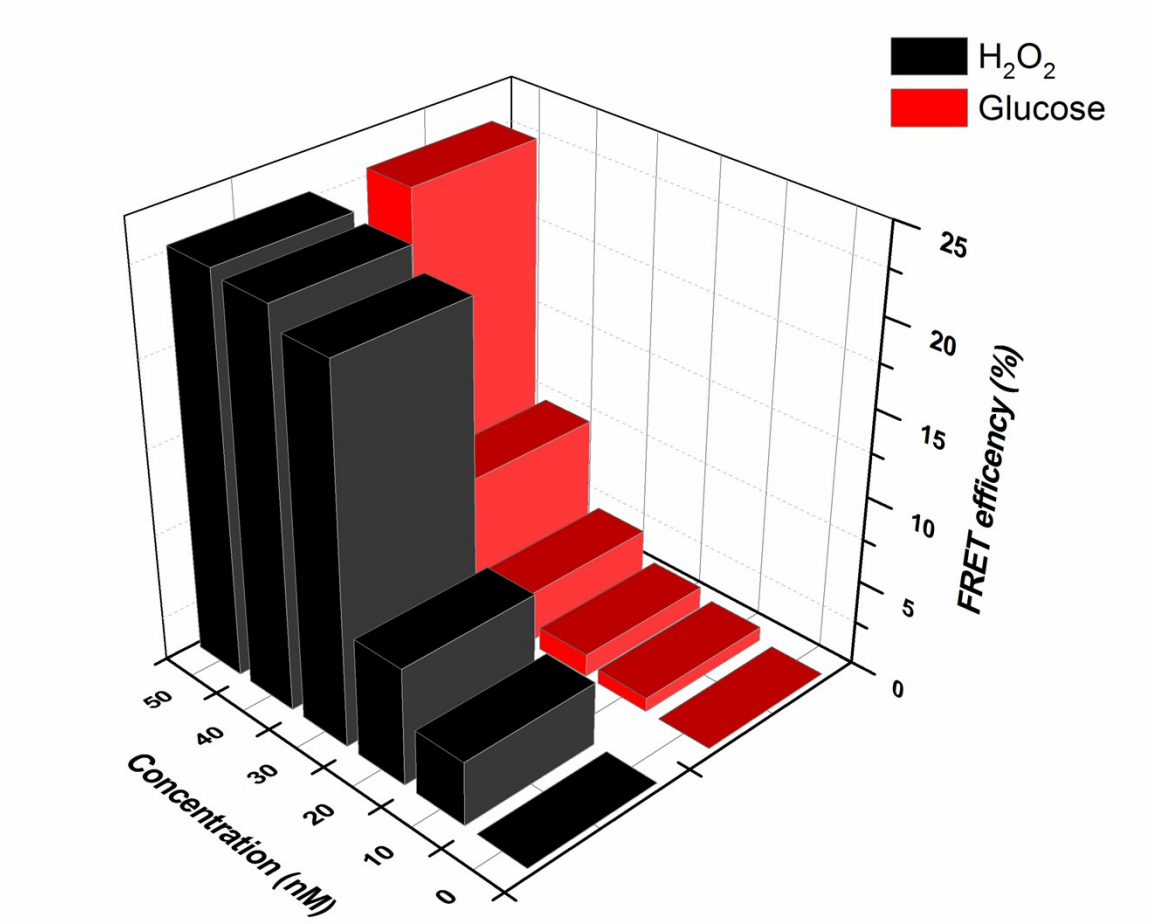

Figure S5. FRET efficiency comparison for glucose and H_2O_2

(XI)

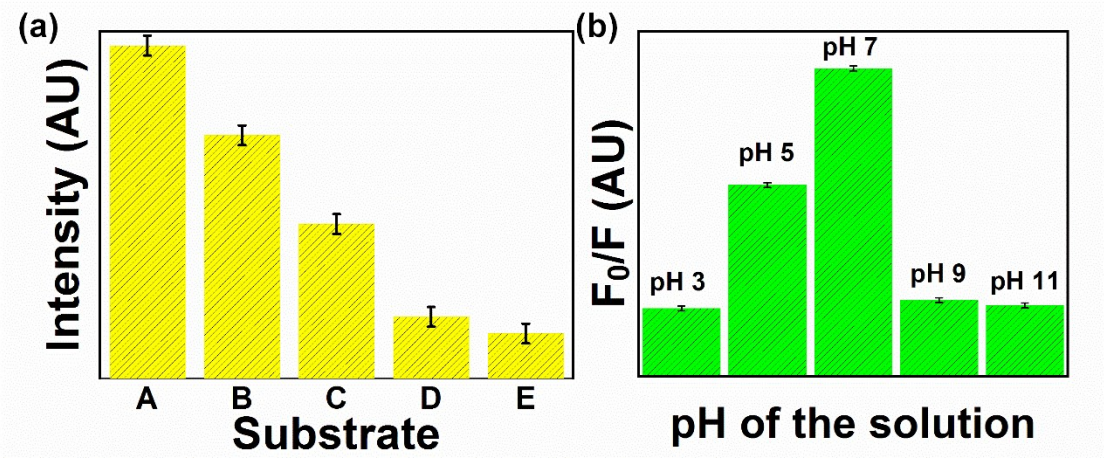

Figure S6. (a) Selectivity of the biologically produced MoS_2 QDs towards substrates: A- H_2O_2; B-Glucose; C- Sucrose; D-Galactose and E-Lactose, (b) MoS_2 QDs in the different pH parameter.

(XII)

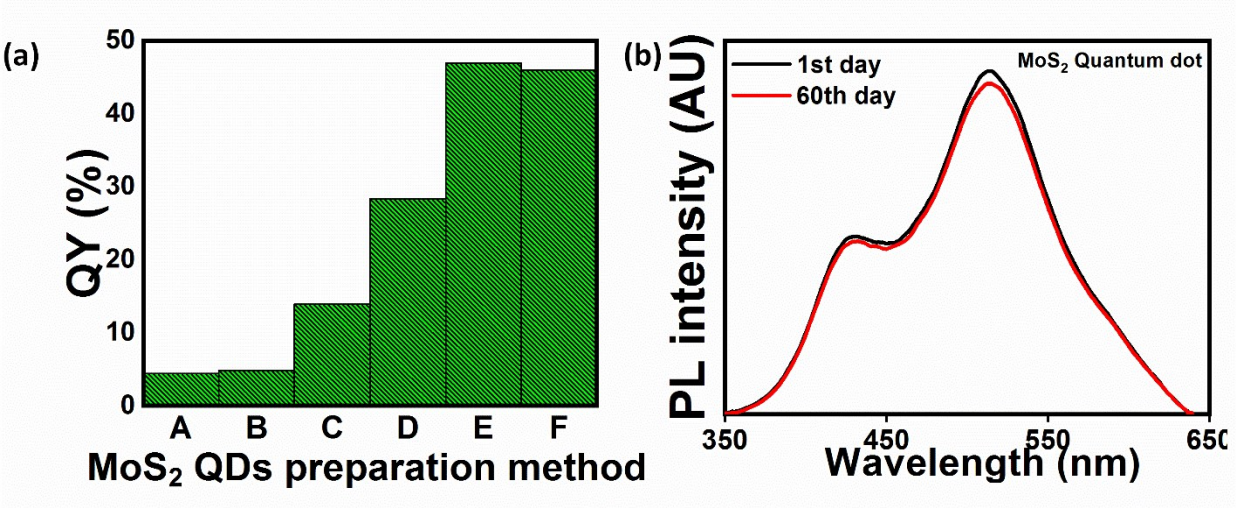

Figure S7. (a) Comparative bar chart demonstrating the QY (%) for different MoS_2 QDs preparation methods i.e. A –colloidal synthesis; B – Ultrasonic exfoliation; C –Electrochemical method; D – Ultrasonication experiment; E – Hydrothermal method and F: present method (*P.aeruginosa* mediated) and (b) QY stability shown over a period of two months.

From FigureS7 (b), it is clear that for 60 days there is no significant change in the PL intensity. It is indicated that as-prepared MoS_2 QDs are highly stable for a long time.