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Molybdenum oxide quantum dots prepared via one-step stirring strategy and applications as fluorescent probes for pyrophosphate sensing and efficient antibacterial materials

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Molybdenum trioxide (MoO_3, AR ≥ 99.5 %), sodium fluoride (NaF, AR ≥ 98.0 %) and sodium pyrophosphate ($Na_4P_2O_7$, AR ≥ 99.0%) Ethylenediaminetetraacetic acid (EDTA, AR ≥ 99.5%), ethylenediamine (EDA, AR ≥ 98%), Sodium adenosine-5'-monophosphate ($C_{10}H_{13}N_5NaO_7P$, AR ≥ 98%), Adenosine 5'-diphosphate disodium salt ($C_{10}H_{13}N_5Na_2O_10P_2$, AR ≥ 98%), Adenosine 5'-triphosphate disodium salt ($C_{10}H_{14}N_5Na_2O_{13}P_3.2H_2O$, AR ≥ 98%), were purchased from Shanghai Macklin Biochemical Co. Ltd., China. Dimethyl sulfoxide (DMSO, AR), aluminium chloride hexahydrate ($Al_3Cl.6H_2O$, AR), potassium chloride (KCl, AR), nickel (II) nitrate hexahydrate $Ni(NO_3)_2.6H2O$, trisodium phosphate dodecahydrate ($Na_3PO_4.12H_2O$, AR), zinc nitrate hexahydrate $Zn(NO_3).6H_2O$, copper (II) nitrate trihydrate $Cu(NO_3)_2.3H_2O$ (AR), iron (III) chloride hexahydrate ($FeCl_3.6H_2O$, AR), lead (II) nitrate Pb $(NO)_2$, potassium bromide (KBr, SP), pure ethanol (C_2H_5OH) were purchased from Sinopharm Chemical Reagent Co. Ltd., China. E. coli and S. aureus are offered by Molecular Biology Laboratory Department of Biology, Qingdao University.

The PL spectra were recorded on a FS5-TCSPC-1189-0317-A3732-1 Spectrophotometer (Edinburgh, United Kingdom). The UV absorption spectra were gained from a Mapada UV-1800PC spectrophotometer (Shanghai, China). The morphology of the MoO_x QDs was observed on a JEM-2010 transmission electron microscope (JEOL Ltd., Japan) and a HITACHI UHR FE-SEM SU8010 (Tokyo, Japan), respectively. The FTIR were recorded on a Nicolet 5700 FTIR spectrometer (Thermo Electron Scientific Instruments Corp., USA). XPS data were obtained on an ESCALab 220i-XL electron spectrometer (VG Scientific, West Sussex, UK) using 300 W Al Kα radiation. The XRD was recorded on a Rigaku D-MAX 2500/PC with the Cu K α radiation (λ = 1.54056 Å) (Tokyo, Japan).

Herein, MoO_x QDs were prepared at room temperature. 1g MoO_3 powder is added into 70mL DMSO in a beaker and then treated with sonication at 300 w for 3 hours. Then the suspensionis stirred with rotor for 12 hours. Finally, the suspension is centrifuged at 8000rpm for 10min several times until clear and transparent suspension was appeared. The supernatant was collected as the as-prepared sample for further characterizations and applications.

The QY of MoO_x QDs was measured via the previously reported method[6]. Typically, quinine sulfate (literature QY: 0.54) in 0.1 M H_2SO_4 was chosen as a standard in order to minimize the re-absorption effects, the absorbance of the MoO_x QDs dispersion and quinine sulfate were kept below 0.10 and 0.05 when excited at 335 nm, respectively. Quinine sulfate was dissolved in 0.1 M H_2SO4 while the MoO_x QDs were dissolved in DMSO, respectively. The QY of the MoO_x QDs was calculated using the equation below[10]:

$$\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

Where the subscripts "ST" and "X" refer to the standard and test, respectively, "Φ" represents the QY, "Grad" stands for the slope from the plot of integrated fluorescence intensity versus absorbance, and "η" is the refractive index of the solvent.

Different metal cations (Ag^+, Al^{3+}, K^+, Mg^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+}) were chosen to assess the selectivity of the quenched PL of MoO_x QDs towards Fe^{3+}. Each metal ion with final concentration of 400 μM was added and the spectrum was measured. For the selectivity test to PPi, seven kinds of different anions including Br^-, Cl^-, F^-, NO^{3-}, PO_4^{3-}, PPi, SO_4^{2-}, EDTA, EDA, AMP, ADP, ATP were chosen to evaluate the influence of anions on the PL of MoO_x QDs-Fe^{3+} system.

E. coli and *S. aureus* were used as the model Gram (−) and Gram (+) bacteria, respectively, to evaluate the antibacterial properties of MoO_x QDs. The preserved bacteria were streaked on LB agar plate and incubated overnight at 37 °C. Following that, single colony was

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picked into liquid LB medium to purify. 1 mL of cell suspension was subcultured and harvested during exponential growth. After that, 200 mL of bacterial fluid and MoO_x QDs solution were inoculated into 3 mL LB medium and cultured for 4 hours. The cultures were centrifuged at 5000 rpm for 5 min and pellets obtained were washed three times with phosphate buffered saline (PBS, Sigma-Aldrich) (pH 7.2) to remove extracellular polymeric substances (EPS) and other growth medium constituents. The collected cell pellets were resuspended in DI and diluted to approximate cell concentration of 10^7 CFU / mL.

0.1 mL as-prepared cell suspension without washing with PBS was added to and spread on the surface of LB agar plates (90 × 15 mm) with the assistance of a sterile cotton swab, then allowed to solidify. Sterilized Oxford cups (Φ 5 mm) were then placed on the agar plates filled with 0.2 mL of MoO_x QDs and equivalent amounts of DMSO and DI were used as controls. The plates were incubated at 37 °C for 12 h.

0.2 mL cell suspension harvested during exponential growth and 0.2 mL MoO_x QDs were injected into five tubes of 3 mL liquid LB medium, respectively. The procedures were repeated with the MoO_x QDs were replaced by DMSO. All of them were cultured at 37 °C. The OD of the cell suspension was recorded every two hours.

The cell suspension used for bacteria counting method was cultured for 10 hours with the same condition for recording OD regrowth curves. The obtained suspension was diluted ten thousand times. 0.1 mL of the diluted cell suspension was spread on the surface of LB agar plates (90 × 15 mm) with the help of a sterile cotton swab. The plates were cultured for 12 hours and the numbers of the colonies were recorded.

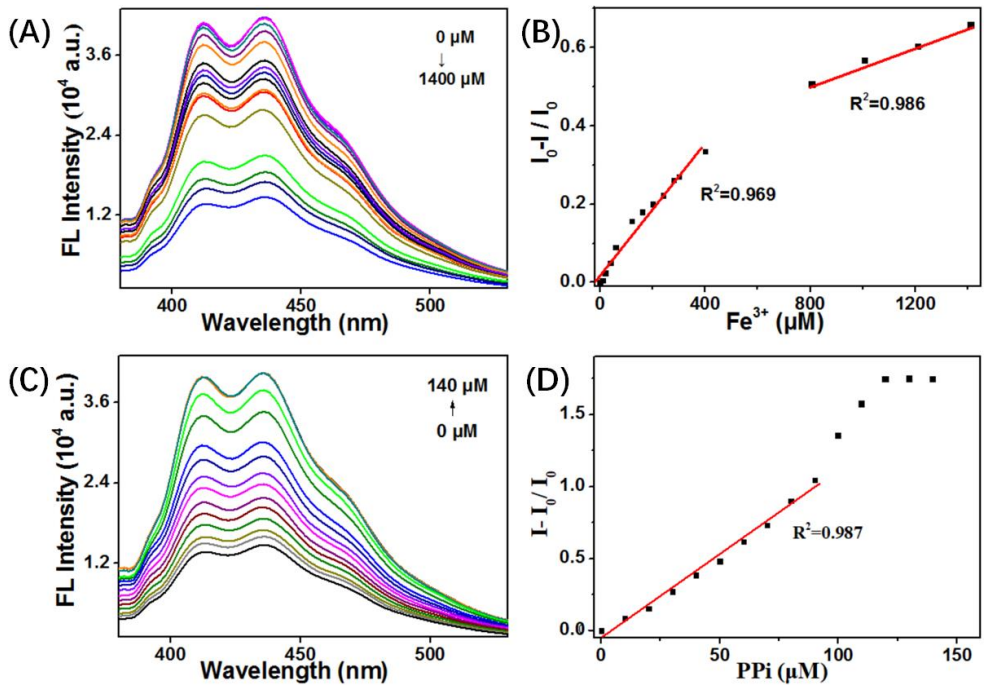

Figure S1. (A) The changes of PL spectra of MoO_x QDs in the presence of Fe^{3+} with increasing concentrations ranging from 0 to 1400 μM. (B) The linear fitting curves between PL intensity and Fe^{3+} in the ranges of 0-400 μM and 800-1400 μM, respectively. (C) The changes of PL spectra of MoO_x QDs-1400 μM Fe^{3+} in the presence of PPi with increasing concentrations ranging from 0-140 μM. (D) The linear fitting curve between FL intensity and PPi concentration ranging from 0-90 μM.

Table S1 Comparison of different Fe^{3+} detection methods.

Materials	Method	Sensitivity	Advantages	Disadvantages	References
Graphene Quantum Dots	3D graphene was synthesized by chemical vapour deposition and then it was treated with electrochemical exfoliation.	7.22 μ M	high-quality	Complex steps, high energy consumption, low sensitivity	2
4-amino-substituted 1,8-naphthalimide dye	The dye was synthesized from 4-bromo-1,8-napthalic anhy-dride via imination with ethanolamine, esterification with acrylic chloride.	unclear	excellent stability, rapid response	Tedious four steps, high cost	4
GHRP-6-Au NCs	Reduction of $HAuCl_4$ with growth hormone releasing peptide-6 (GHRP-6) acts as the reducing agent and capping ligand.	1.4μ M	high sensitivity	High cost	7
Au NCs@PTMP-PMAA	Heating the gold precusor solution in presence of PTMP-PMAA function as a reducing agent as well as a protecting agent	3.0 μM	high quantum yield	High energy consumption, long time	9
MoO_x QDs	MoO_x QDs was prepared via one-step stirring strategy.	3.3 μM	high quantum yield, low energy consumption, easily prepared	/	

Table S2 Comparison of different PPi detection methods.

Materials	Method	Sensitivity	Advantages	Shortcomings	References
Probe 1+ graphene oxide (GO) complex	Synthesis Probe 1 through three steps of complex chemical reaction then Probe 1 was added to form complexes used for PPi sensing.	2.1μM	excellent selectivity towards PPi	complex preparation process, low yield	[1]
carbon dots (CDs)/Pb^{2+} complex	CDs was synthesised by hydrothermal method and then Pb^{2+} was added to form complex which is used for PPi sensing.	54 nM	convenient, high sensitivity low-cost	high energy-consumption,	[3]
Eu(DPA)3@Lap/ Cu^{2+} complex	Eu(DPA)3@Lap was repared via ion exchange and coordination and then it was mixed with Cu^{2+} to form complex which is used for PPi sensing.	unclear	exquisite and convenient	high-cost, complex preparation process	[5]
N-doped carbon quantum dots (N-CQDs) /Fe^{3+} complex	N-CQDs was repared via a simple bottom-up electrochemical (EC) method and then Fe^{3+} was introducd to form complex which is used for PPi sensing.	0.5 μM	rapidity, simplicity, low cost, high sensitivity	complex equipment and high energy consumption	[8]
MoO_x QDs-Fe^{3+} complex	MoO_x QDs was prepared via one-step stirring strategy and then Fe^{3+} was introducd to form complex which is used for PPi sensing.	3.3 μM	simplicity, low cost, low energy consumption and cheap instrument	/	

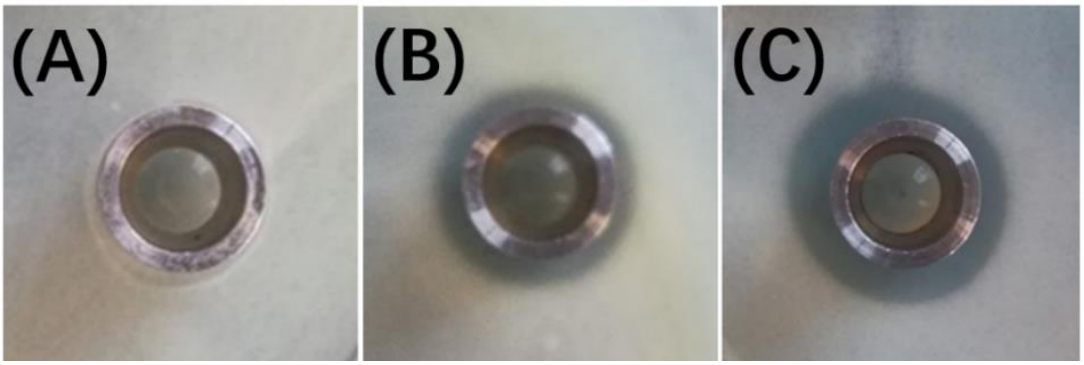

Figure S2. Images of *S. aureus* upon incubation in Oxford cups filled with (A) 0.2 mL DI [control], (B) 0.2 mL DMSO, and (C) 0.2 mL as-prepared MoO_x QDs, respectively, for 12 h at 37 °C.

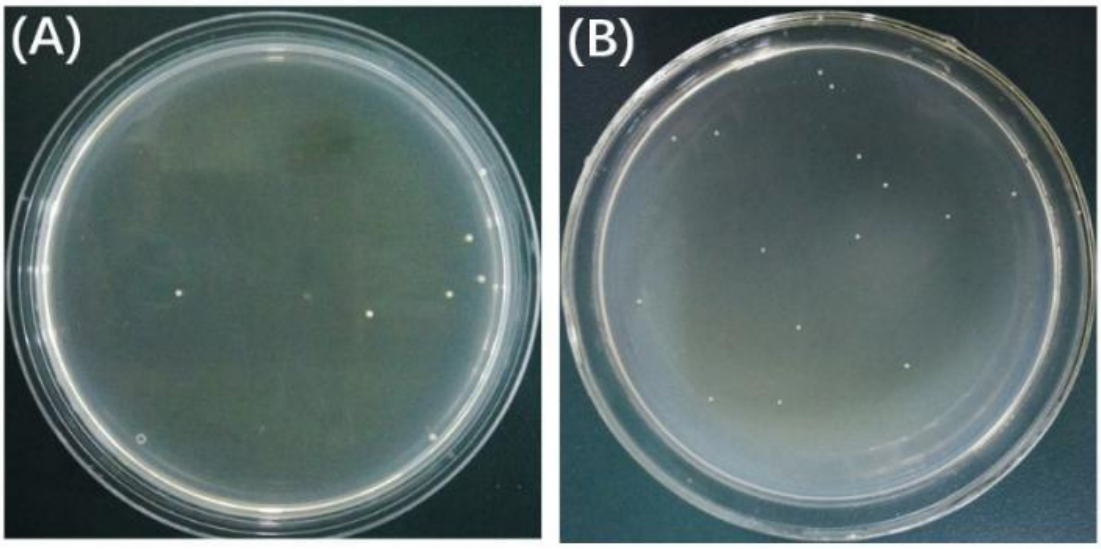

Figure S3. Photographs of plates for bacteria counting method: (A) S. aureus bacterial cells incubated with MoO_x QDs for 4 h. (B) S. aureus suspensions in DI water without MoO_x QDs were used as control, respectively.

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