Supplementary Information

Ratiometric Immunoassays Built from Synergistic Photonic Absorption of Size-Diverse Semiconducting MoS_2 Nanostructures

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Materials. Molybdenum sulfide (MoS_2 crystalline powder, <2 μm, 99%), sodium cholate (98%) were obtained from Sigma-Aldrich Co. (USA). 2-amino-2-(hydroxymethyl)-1,3-propanediol (tris), and agarose were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Other chemicals were of analytical reagent

grade and purchased from Kelong Chem. Ltd. Co. (Chongqing, China). Ultrapure water (18.2 MΩ cm^{-1}) was used throughout the whole experiment. Recombinant human carcino-embryonic antigen (CEA), and prostate-specific antigen (PSA) were purchased from ProSpec-Tang TechnoGene Ltd. Bovine serum albumin (BSA), and anti-CEA polyclonal antibody were obtained from BBI life science (Shanghai, China). Thrombin and adenosine triphosphate (ATP) were purchased from Sangon Biotech. Co. Ltd (Shanghai, China). Tris-acetate-EDTA (TAE) buffer solution was self-made. A portion of powder containing 48.2 g tris crystals and 7.44 g ethylene diamine tetraacetic acid (EDTA) were totally dissolved into 800 mL ultrapure water. After that, a port of 11.4 mL glacial acetic acid was added into the mixture and fully stirred. 1 mM sodium hydrate was used to adjusted the pH value of mixture until 8.0. Finally, the volume of mixture was calibrate to 1000 mL to prepare 10× TAE buffer solution. For gel electrophoresis, the 10× TAE buffer solution was diluted 50 times to obtain 0.2× TAE running buffer solution.

Instruments. The KQ-400B ultrasonic bath (400 W, Kun Shan Ultrasonic Instruments Co., Ltd., China) was adopted for the liquid-exfoliation of MoS_2 crystals. The photonic absorption spectra of layered MoS_2 were recorded on a UV-2450 UV-vis spectrophotomer (Shimadzu, Japan) at room temperature. Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) measurements were performed on a Tecnai G2 F20-STWIN transmission electron microscope (FEI, USA) operated at 200 kV. The nanoscale morphologies of layered MoS_2 were characterized using a dimension icon atomic force microscopy (AFM, Bruker, Germany). The samples were obtained through dropping nanomaterials aqueous dispersion onto the carbon grids and drying under ambient conditions. The size fractionation based on differential centrifugation was conducted using a TGL-16M high-speed refrigerated centrifuge (Xiangyi, China). The gel electrophoresis measurements were conducted using a basic electrophoresis apparatus (Bio-Rad, USA). The electrophoresis chamber of horizontal mid-size gel system (Sangon, China) was utilized to present the agarose gel.

Liquid-exfoliated strategy. Layered MoS_2 nanosheets were obtained through sonication-assisted liquid-phase exfoliation of bulk MoS_2 crystals in a surfactant aqueous solution, which was developed based on the work of Coleman and coworkers. In this assay, a portion of 400 mL mixed aqueous dispersion, containing 5 mg mL^{-1} MoS_2 bulk powders and 1.5 mg mL^{-1} sodium cholate, was sonicated (Bath Sonicator, 400 W) at room temperature for 20 h, resulting in the formation of black dispersion. Subsequently, the supernatant consisting of exfoliated MoS_2 with various lateral sizes and thicknesses, and surfactant, was collected to get the long-term stored stocking solutions for further experiments.

Differential centrifugation for size fractionation. The exfoliated MoS_2 stocking solution was collected, and size fractionation of layered MoS_2 was based on the differential centrifugation (DC) routes in the presence of surfactant. First of all, the exfoliated MoS_2 dispersion was centrifuged at low speed (1,500 rpm) for 30 min and then supernatant with green-yellow color was collected to remove the unexfoliated crystals. To achieve size screening, the surfactant-containing supernatant of exfoliated MoS_2 was centrifuged at 2,000 rpm for further 30 min and sediments of 2k-MoS_2 were collected. The separated supernatant is subsequently transferred to next-round centrifugation. Using 6,000 rpm for 30 min, the collection of sediments to get fractionated LE MoS_2 samples of 6k-MoS_2. Similar to above fractionation operation, the supernatant is then used for the collection of 10k-MoS_2, when the high centrifuge speeds of 10,000 rpm were applied, respectively. After the collection of sediments, they are re-dispersed in ultrapure water to get the fractionated samples dispersions.

Theory simulation method. First-principles electronic structure calculations were carried out within the generalized gradient approximation (GGA) in the Perdew-Burke-Ernzerhof form. The interactions between ions and electrons were described by the projector-augmented wave method with a cutoff energy of 600 eV. Uniform G-centered k-points meshes with a resolution of $2\pi * 0.03$ $Å^{-1}$ and Methfessel-Paxton electronic smearing were adopted for the integration in the Brillouin zone. These

settings ensure convergence of the total energies to within 1 meV per atom. Structure relaxation proceeded until all forces on atoms were less than 1 meV $Å^{-1}$ and the total stress tensor was within 0.01 GPa of the target value.

Ratiometric optical immunoassay. Briefly, a portion of 10 μL antibody (500 μg mL^{-1}) was individually added to 1.5 mL of 10k-MoS_2 aqueous solution (100 μg mL^{-1}). The mixtures were incubated and gently shook at 37 ℃ for 2 h and then blocked by 30 μL of 1% (m/v) BSA in phosphate buffer solution (10 mM PBS, NaH_2PO_4/Na_2HPO_4, 138 mM NaCl, pH 7.4) at 37 ℃ for further 2 h. In order to collect the purified antibody/10k-MoS_2, subsequently, the treated samples were centrifugated at 10000 rpm for 20 min at 4 ℃ and the supernatant was abandoned. The collected sediments of antibody/10k-MoS_2 were then re-dispersed in 10 mM PBS solution for further experiments. A portion of 490 μL stocking anti-CEA/10k-MoS_2 solution, containing 10 mM PBS (pH 7.4), was mixed with a 10 μL CEA solution at 37 ℃ for diverse incubation times using dry bath. For the quantitative analysis, the concentration of CEA is diverse, while that of anti-CEA/10k-MoS_2 is fixed to be about 10 μg mL^{-1} (the concentration refers to that of 10k-MoS_2). Subsequently, the photonic absorption spectra were recorded from wavelengths of 900 to 350 nm. The scan sweep is 0.1 nm.

Gel electrophoresis measurements. A portion of 1.2 g agarose powders was dissolved in 400 mL 0.2× tris-acetate-EDTA (TAE) buffer solution, and the mixture was subsequently heated to 95 ℃ to get the well-dispersed solution. After the agarose aqueous solution (0.3% in mass fraction) was cooled to 60 ℃, the mixed solution was poured into a horizontal electrophoresis chamber (12 wells, 15 cm×10 cm). Utimately, the final agarose gel was formed until the temperature of solution was cooled to 25 ℃. After the samples have been added on the loading zone of agarose gel, the whole gel electrophoresis system began to run in 0.2× TAE running buffer

solution. The applied voltages and times were modulated to be 50 V and 50 min, respectively.

Table S1. The yields and absorption peaks of fractionated LE MoS_2 samples, respectively.

Sample	Yield (%)	Wavelength (nm)		
		Peak A	Peak B	Peak D
2k-MoS_2	2.31	673.8	610.9	413.8
4k-MoS_2	4.57	669.2	607.9	406.7
6k-MoS_2	2.18	663.4	603.8	393.8
8k-MoS_2	1.69	660.9	602.9	390.6
10k-MoS_2	0.92	656.8	601.3	388.4

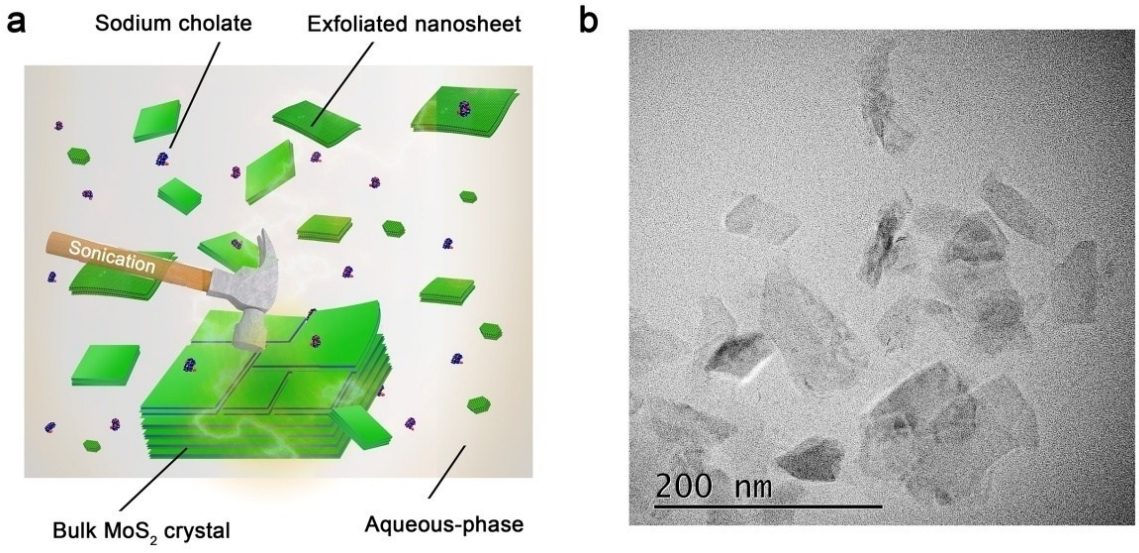

Fig. S1 (a) The scheme shows the liquid exfoliation of MoS_2 crystals for layered nanoarchitectures preparation. (b) A transmission electron microscopy image of layered MoS_2 nanoarchitectures from the liquid-exfoliated strategy.

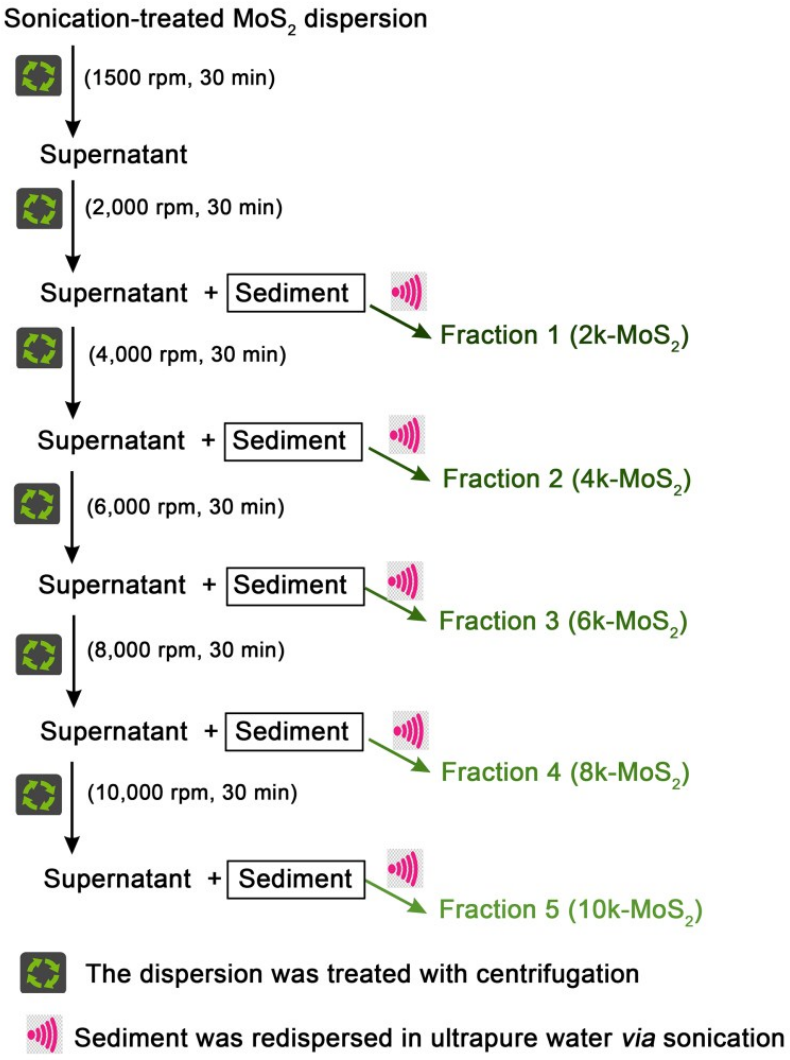

Fig. S2 The process scheme shows the step-to-step fractionation of LE MoS_2 based on differential centrifugation treatments.

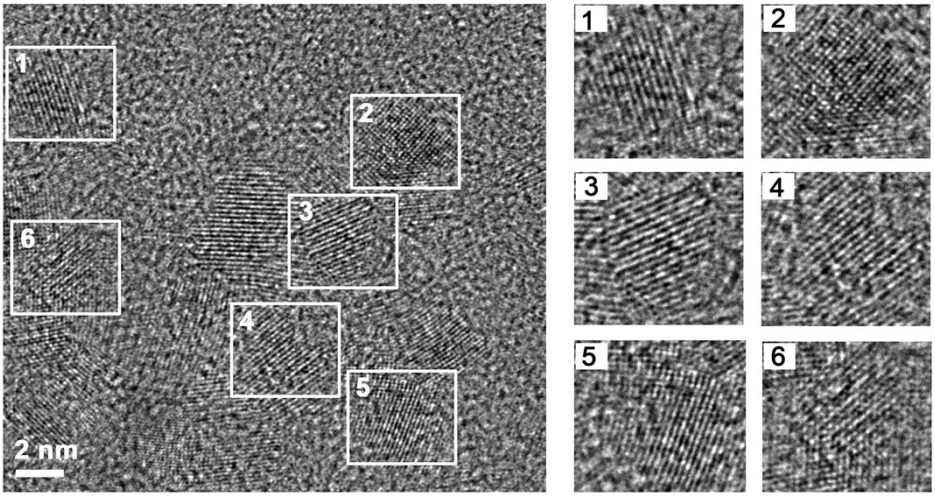

Fig. S3 HRTEM image of LE MoS_2 indicating that ultrasmall MoS_2 nanodots existed in exfoliated nanoarchitecture samples. The crystal lattices of individual nanodots are estimated to be ~0.27 nm.

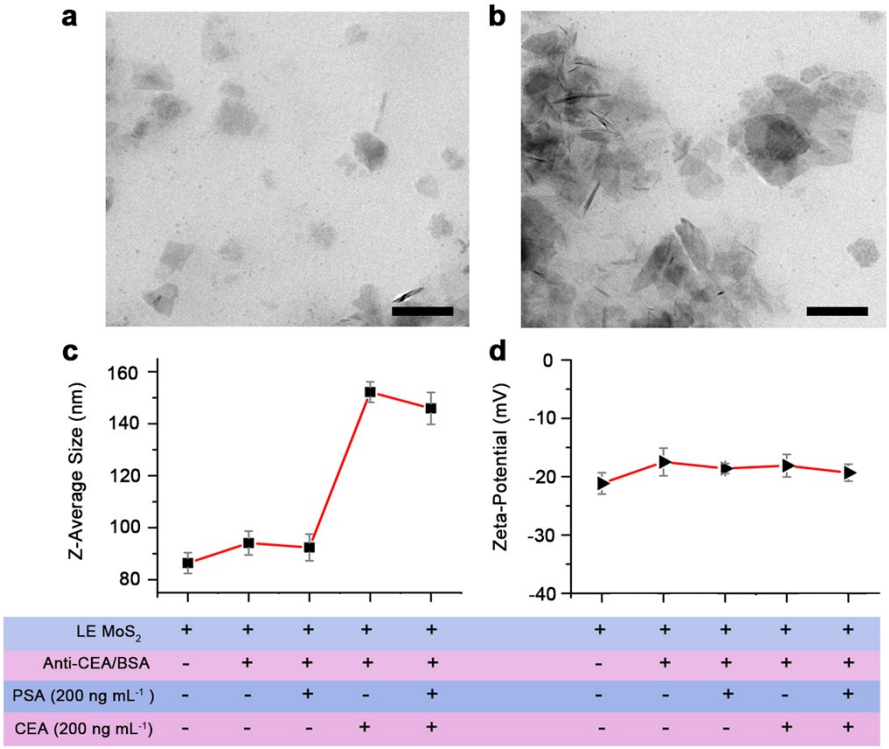

Fig. S4 TEM images of anti-CEA/BSA/MoS_2 before (a) and after (b) the incubation of CEA. The concentration of CEA is 200 ng mL^{-1}. The scale bar is 100 nm. Z-average sizes and zeta-potential values of diverse MoS_2 samples from DLS measurements.

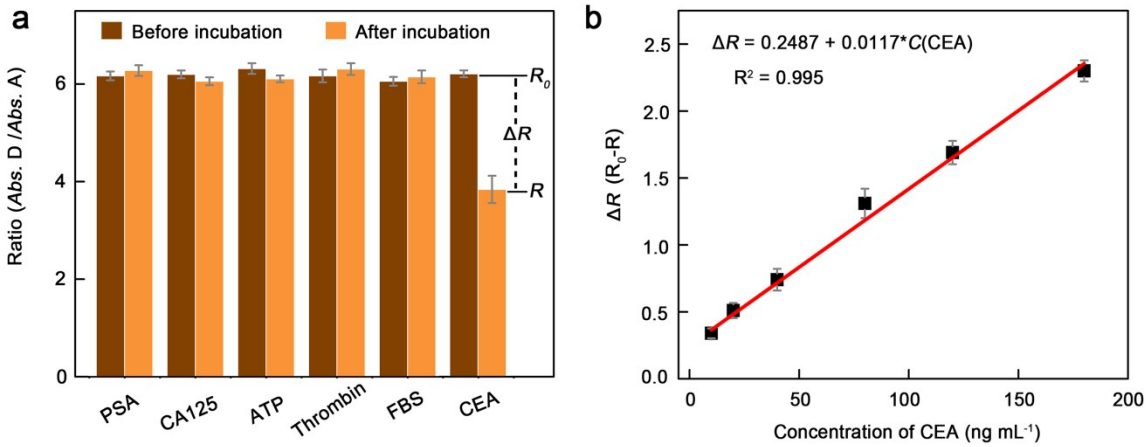

Fig. S5 (a) The selective responses of proposed ratiometric absorption sensing. The abs. ratio of peak D to peak A in the sample of anti-CEA/10k-MoS_2 before and after the incubation of different substances, including prostate-specific antigen (PSA), CA125, adenosine triphosphate (ATP), thrombin, FBS (fetal bovine serum). The concentration of CEA is 200 ng mL^{-1}, and other substances are 1000 ng mL^{-1}. (b) The absorbance ratio changes are linear to corresponding concentrations of CEA. The linear equation could be expressed as $\Delta R = 0.2487 + 0.0117 * C$ (CEA); $R^2 = 0.995$. The concentration range of CEA is from 5.0 to 180.0 ng mL^{-1}.

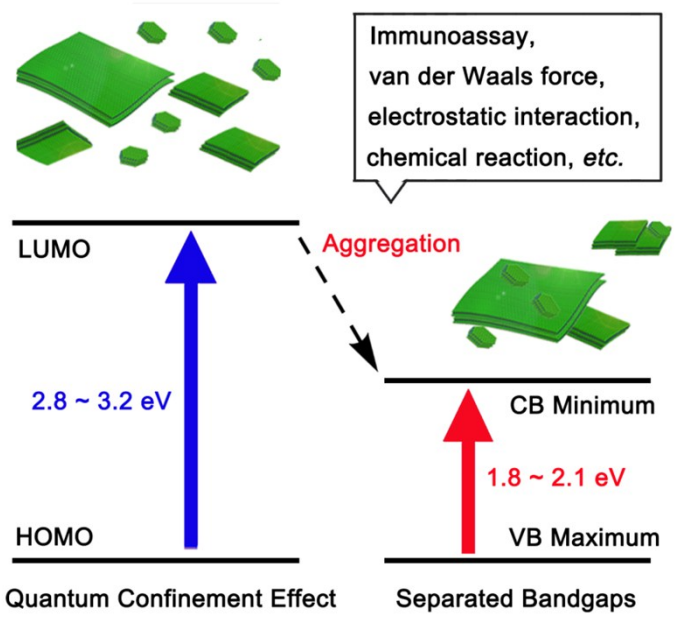

Fig. S6 Scheme illustration indicating that aggregation of LE MoS_2 induced alternation of photonic absorption performances, derived from synergistic mechanism of separated electronic bandgaps and quantum confinement effect of MoS_2 nanodots.