Supporting Information

Tailoring Electron Transfer Pathway for Photocatalytic N_2-to-NH_3 Reduction in a CdS Quantum Dots-Nitrogenase System

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1. Photoreduction of $(SPr)_2V$ by CdS QDs-ME

To study the photoreduction of $(SPr)_2V$, the reaction solution after 10 minutes irradiation was transferred to a quartz cuvette, and the amount of $[(SPr)_2V^{\cdot}]^-$ was determined. The corresponding UV-vis spectra are shown in Figures S1 and S2.

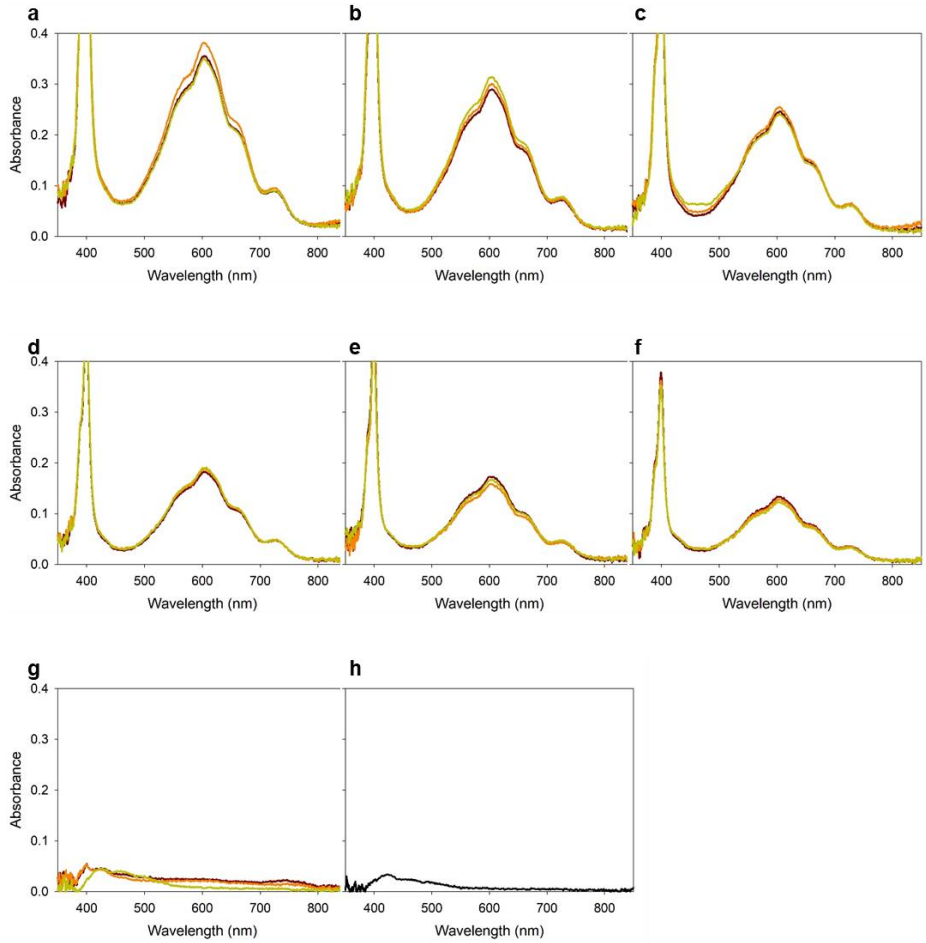

Figure S1. Photoreduction of $(SPr)_2V$ by CdS QDs-ME as a function of $[SPr_2V]$. The photoreduction of $(SPr)_2V$ (4 mM - **a**, 2 mM - **b**, 1 mM - **c**, 0.5 mM - **d**, 0.25 mM - **e**, 0.125 mM - **f**, 0 mM – **g**) was studied in the presence of 0.1 µM CdS QDs-ME. **h**, The spectrum of the reaction mixture containing 0.1 µM CdS QDs-ME before illumination. All experiments were performed in nitrogenase activity buffer, 2 mM ME, excitation wavelength 405 nm, 10 minutes, 0.5 mL.

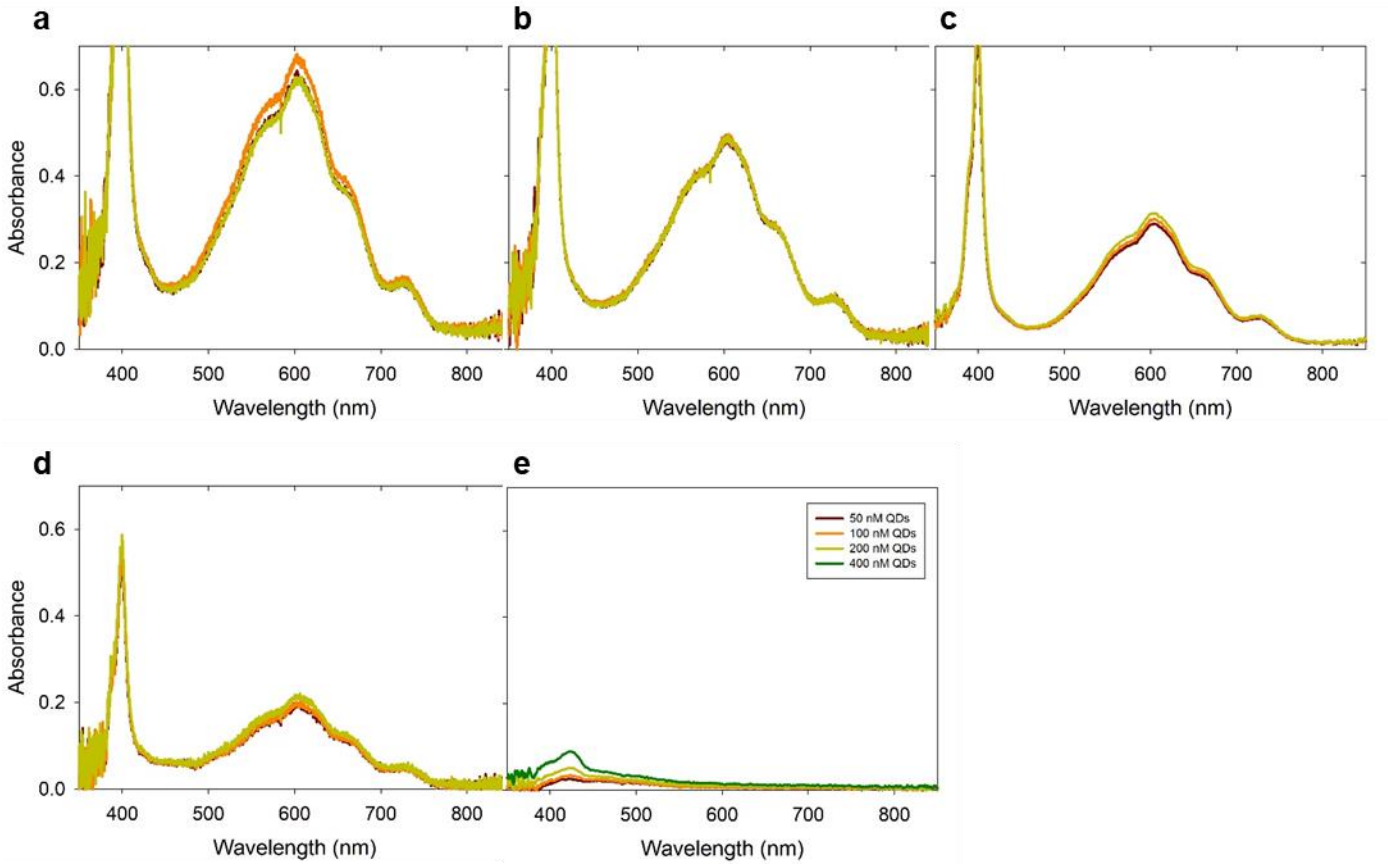

Figure S2. Photoreduction of $(SPr)_2V$ as a function of [CdS QDs-ME]. The photoreduction of 2 mM $(SPr)_2V$ in the presence of 400 nM (**a**), 200 nM (**b**), 100 nM (**c**), 50 nM (**d**) CdS QDs-ME. **e**, The spectra of reaction mixture containing 2 mM $(SPr)_2V$, 2 mM ME with various concentrations of QDs-ME before illumination. All experiments were performed in nitrogenase activity buffer, 2 mM ME, excitation wavelength 405 nm, 10 minutes, 0.5 mL.

2. ME and Photon Flux Effects on the Photoreduction of $(SPr)_2V$ by CdS QDs-ME

ME effect on photoreduction $(SPr)_2V$ by CdS QDs-ME. To examine the effect of ME, the photoreduction of $(SPr)_2V$ was performed in the presence of different concentrations of ME. After 10 minutes irradiation, the reaction solution was transferred in a quartz cuvette, and the amount of $[(SPr)_2V^{\cdot}]^-$ was determined. Above 10 mM ME, a decrease in the formation of $[(SPr)_2V^{\cdot}]^-$ was observed (Figure S3a).

Photon flux effect on photoreduction $(SPr)_2V$ by CdS QDs-ME. Similar studies were performed to examine the light intensity effect. Light intensity was determined using a MQ-500 Quantum Meter, Apogee Instruments, USA. Figure S3b shows an increase of the reduced mediator with increasing of photon flux.

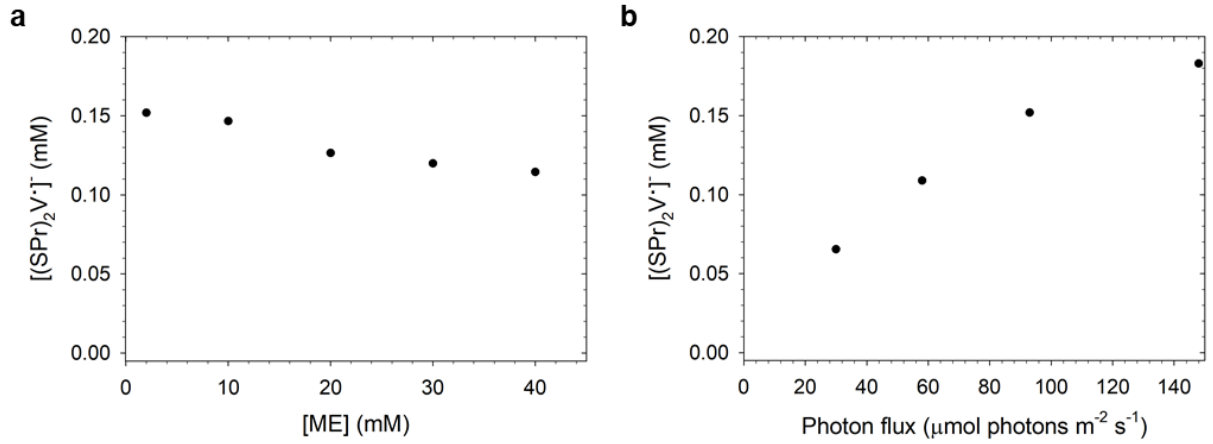

Figure S3. $[(SPr)_2V^{\cdot}]^-$ versus [ME] (a) and photon flux (b). All experiments were performed in the nitrogenase activity buffer, 2 mM $(SPr)_2V$, 100 nM CdS QDs-ME, n=1, 0.5 mL, under argon.

3. The CdS QDs Ligand Test

The effect of the CdS QDs ligands was studied. The ligands were 3-mercaptopropionic acid, 2-mercaptoethylamine, 2-(dimethylamino)ethanethiol, 2-mercaptoethanol, cysteine. The experiments were performed also in 100 mM MOPS pH 7.0 without additives. In the activity buffer, the amount of reduced mediator was lower than in 100 mM MOPS, most probably due to the additives interacting with CdS QDs and hindering the electron transfer to the mediator (Figure S4).

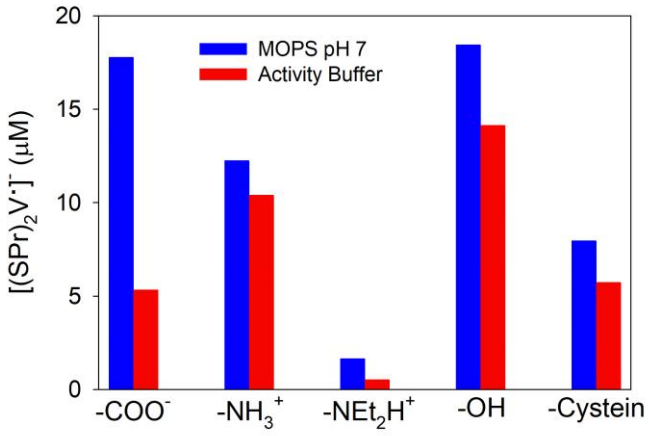

Figure S4. Photoreduction of [(SPr)$_2$V] by CdS QDs-Ligand. The study was performed in the presence of 2 mM (SPr)$_2$V, 50 nM CdS QDs-Ligand, in 100 mM MOPS pH 7.0 (in blue) and in nitrogenase activity buffer (in red). All experiments featured 50 nM CdS QDs-Ligand and 2 mM (SPr)$_2$V, excitation wavelength 405 nm (photon flux 40 μmol m^{-2} s^{-1}), 3 min, 1 mL, n=1, under argon.

4. CdS QDs-ME and ME Effects on Nitrogenase Activity

CdS QDs-ME and nitrogenase activity. To examine a potential inhibitory effect of CdS QDs on nitrogenase activity, the previously developed spectrophotometric assay[1] was performed varying CdS QDs-ME concentration (Figure S5a). No significant effects of CdS QDs-ME on nitrogenase activity was observed.

ME and nitrogenase activity. To examine a potential inhibitory effect of the ligand ME on nitrogenase activity, the previously developed spectrophotometric activity assay was performed varying ME concentration (Figure S5b and c). Some inhibitory effects were observed above 40 mM ME.

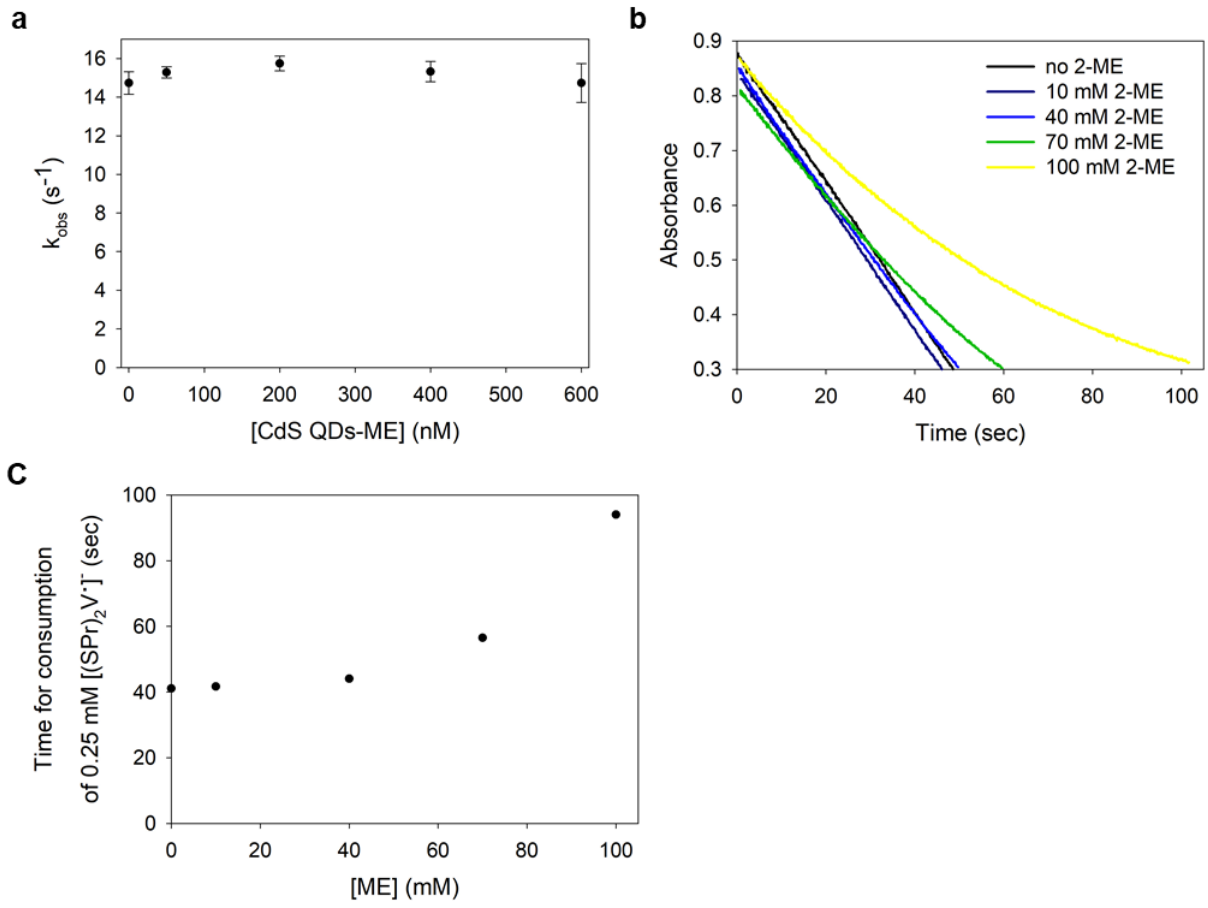

Figure S5. Spectrophotometric studies of CdS QDs-ME and ME effects on nitrogenase activity. a, k_{obs} (electrons consumed by nitrogenase per second) versus [CdS QDs-ME]. **b**, Absorbance versus time in the presence of different concentrations of ME. **c**, Time for consumption of 0.25 mM $[(SPr)_2V^{\cdot}]^-$ by nitrogenase versus different concentrations of ME. All experiments were performed in nitrogenase activity buffer, 0.4 µM MoFeP, 6 µM FeP, 0.5 mM $[(SPr)_2V^{\cdot}]^-$, 100 nM CdS QDs-ME, cuvette pathlength 2 mm, n=3, 0.5 mL, under argon, the wavelength 600 nm, if not otherwise stated.

5. UV-vis Studies of $(SPr)_2V$ and TQ photochemical reduction.

$(SPr)_2V$ and TQ were reduced in activity assay buffer containing 0.1 µM CdS QDs-ME and 2 mM corresponding mediator. After 10 minutes of illumination, the solutions were transferred in spectroscopic cuvettes, and the amount reduced mediator was quantified using extinction coefficients ε $((SPr)_2V_{red})^1$ of 9925 M^{-1} cm^{-1} at 600 nm and ε $(TQ_{red})^2$ of 2000 M^{-1} cm^{-1} at 493 nm (Figure S6). This resulted in 75 µM $[(SPr)_2V^{\cdot}]^-$ and 92 µM TQ_{red}.

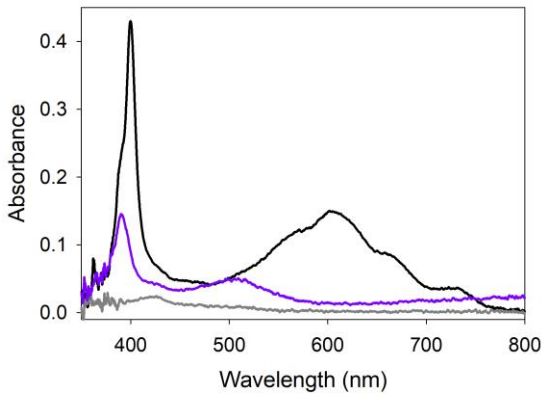

Figure S6. Spectroscopic study of photochemically reduced $(SPr)_2V$ (in black) and TQ (in purple). The spectrum of a mixture of 2 mM TQ, 100 nM CdS QDs-ME, 2 mM ME, in activity assay buffer is shown in gray. All experiments were performed in nitrogenase activity buffer, 2 mM mediator, 0.1 µM CdS QDs-ME, 2 mM ME, n=1, 0.5 mL, under argon.

6. Estimated quantum yields

The photon flux was measured using a MQ-500 Quantum Meter, Apogee Instruments, USA. Total incident photons were calculated taking into account illumination time (600 s) and area (7×10^{-5} m^{-2}). The number of absorbed photons was calculated accounting for the optical absorbance of QDs at 405 nm (0.17 A.U. or 32 % of absorbed light) and the circular shape of the vial (0.78).

Parameter	Value
Photon flux at the sample	93 µmol m^{-2} s^{-1}
Area	7×10^{-5} m^{-2}
Illumination time	600 s
Total incident photon	3.9 µmol
Photons absorbed	1.26 µmol

The quantum yields (QYs) were estimated for the conversion of absorbed photons to NH_3 ($3e^-$ per molecule) and both NH_3 and H_2 ($2e^-$ per molecule):

Quantum Yield = (mol e– used in product formation) ÷ (mol of absorbed photons) x 100%.

8. References.

(1) Badalyan, A.; Yang, Z.-Y.; Hu, B.; Luo, J.; Hu, M.; Liu, T. L.; Seefeldt, L. C. An Efficient Viologen-Based Electron Donor to Nitrogenase. *Biochemistry* **2019**, *58*, 4590–4595

(2) Tsukahara, K., Wilkins, R.G. Kinetics of Reduction of Eight Viologens by Dithionite Ion. *J. Am. Chem. Soc.* **1985**, *107*, 2632-2635.