Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supporting Information for Investigating the Use of Conducting Oligomers and Redox Molecules in CdS-MoFeP Biohybrids

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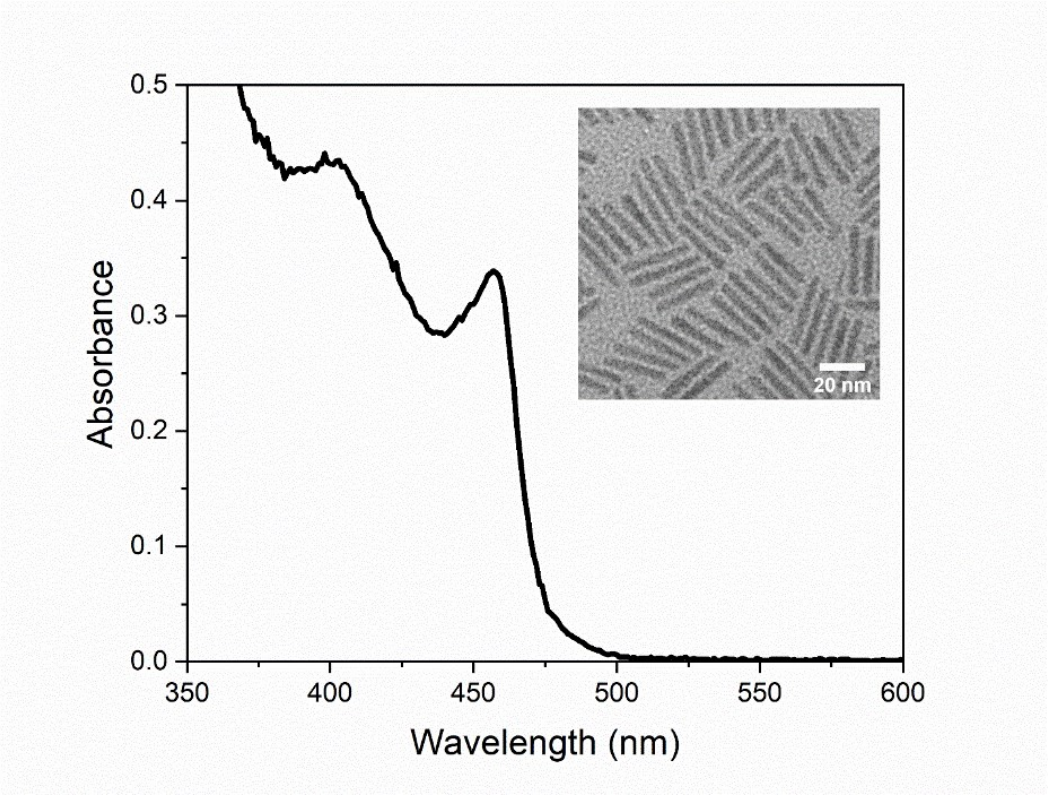

Figure S1. UV-Vis spectrum of as-synthesized CdS nanorods (NRs) with the inset showing the corresponding TEM image.

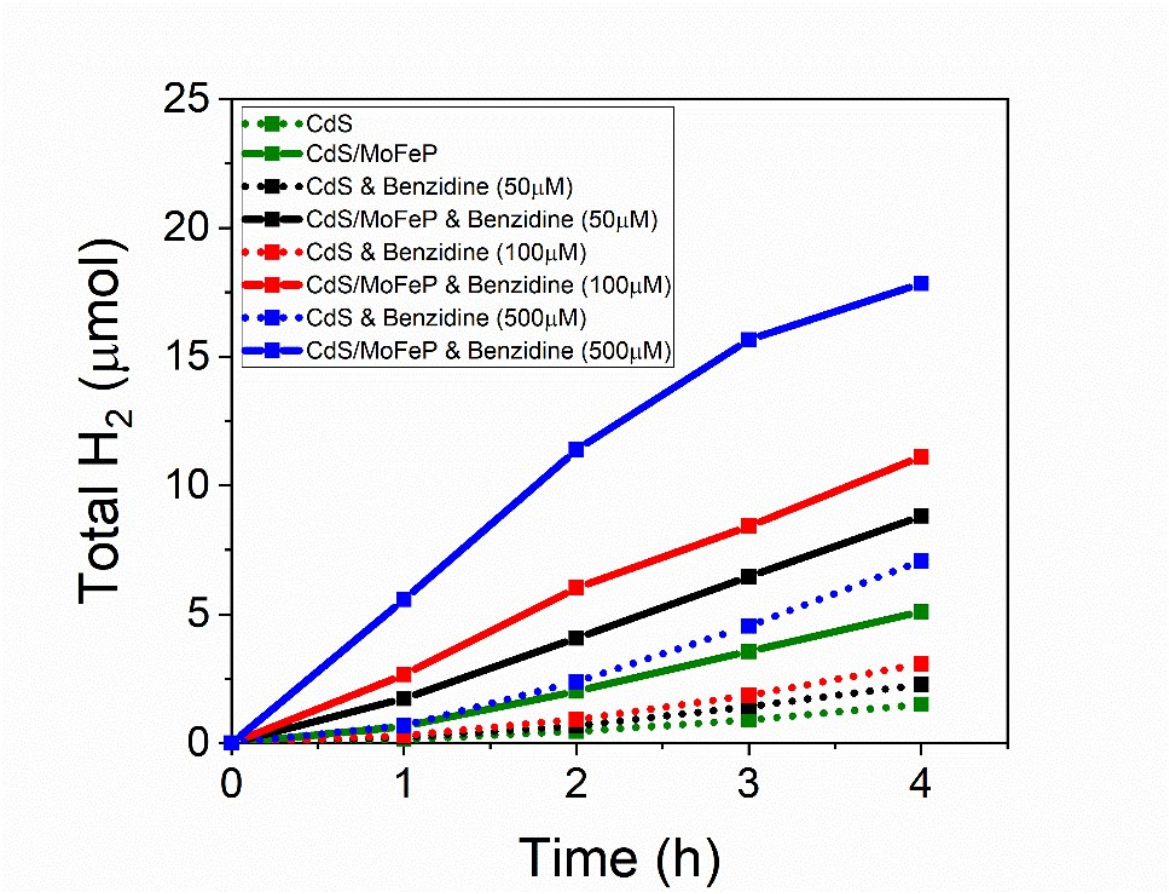

Figure S2. Total H_2 from TGA-CdS/MoFeP biohybrid complexes in the presence of 0 to 500 μM benzidine with the no protein controls. CdS NRs act as the electron supplier under irradiation in an Ar purged vial containing 50 mM PB, 100 mM NaCl, 500 mM ascorbic acid (AA), 500 nM CdS, and 500 nM MoFeP at pH 7.4 over 4 h. Error bars indicate one standard deviation (SD) from duplicate measurements.

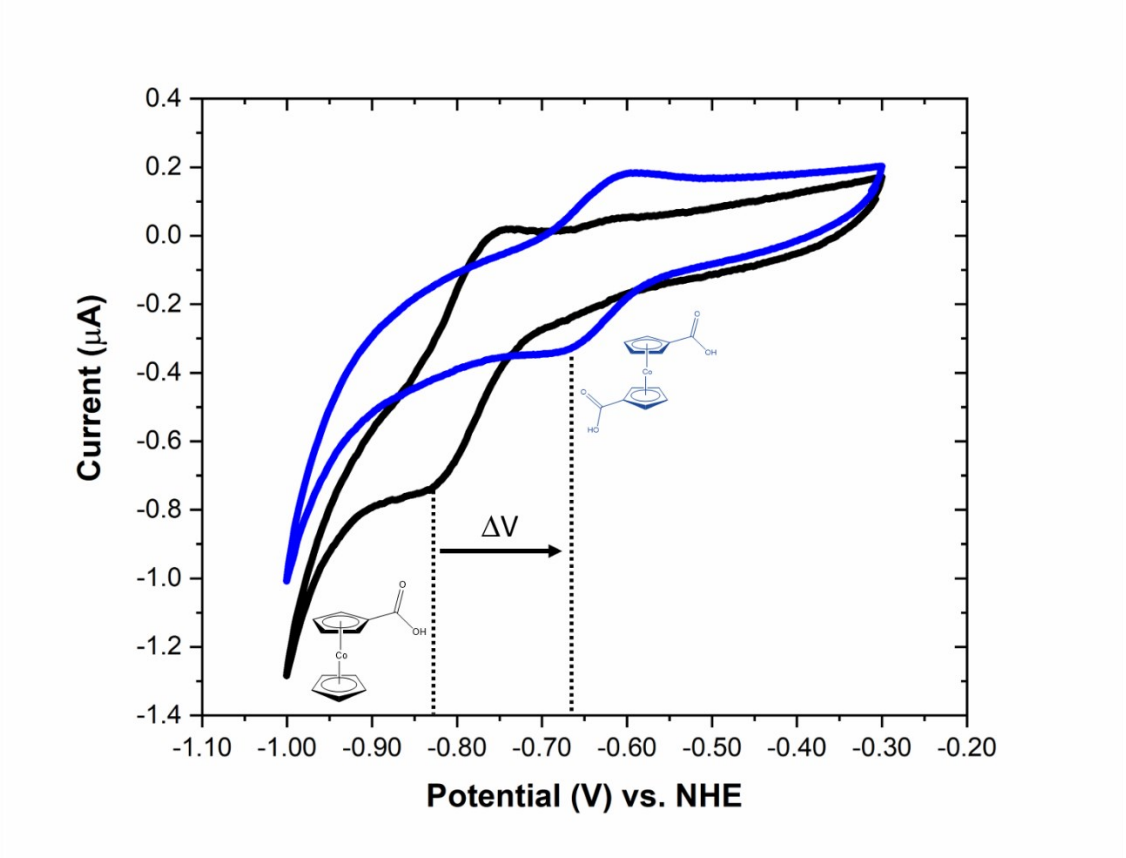

Figure S3. Cyclic voltammogram of COOH-cobaltocene (-840 mV vs. NHE) and di-COOH-cobaltocene (-690 mV vs. NHE) in 50 mM PB and 100 mM NaCl with a 10 mV/s scan rate.

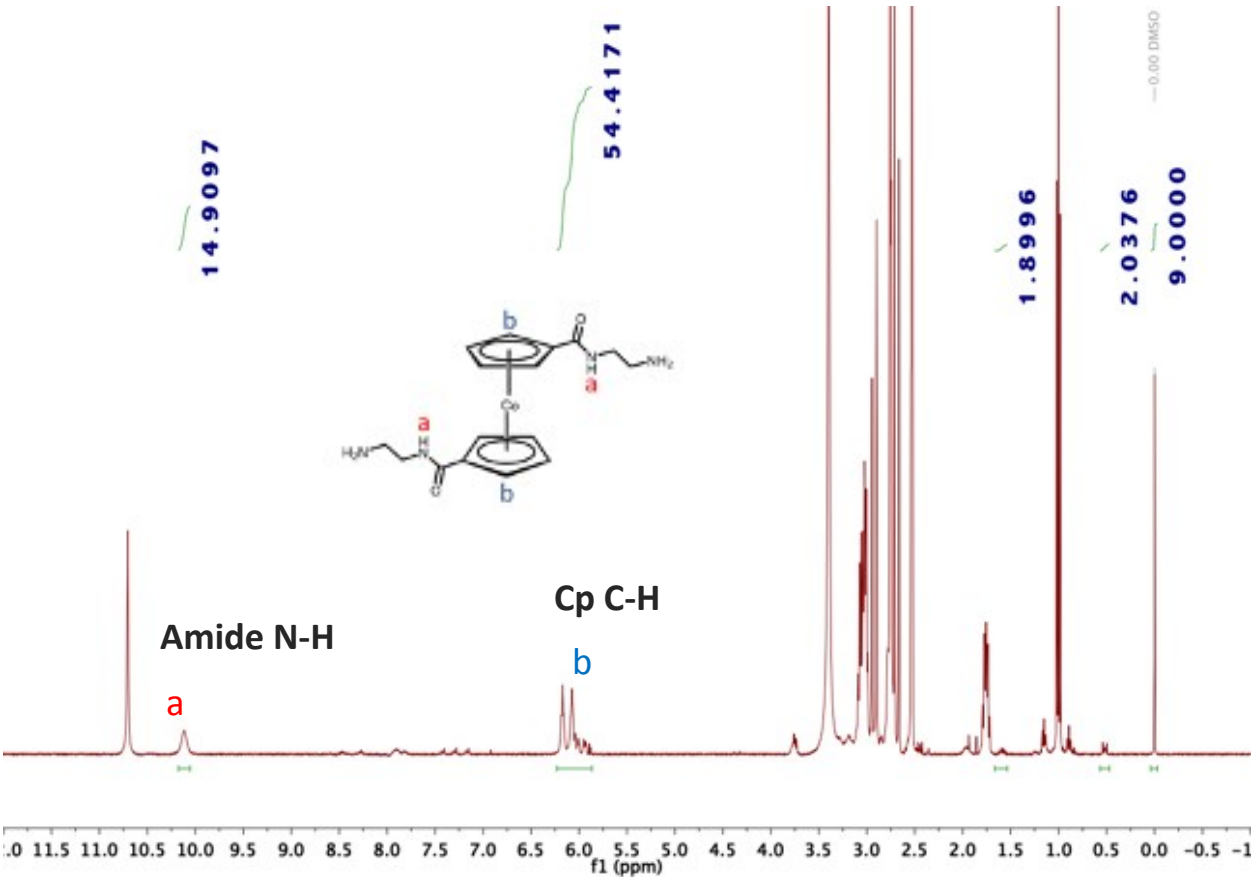

Figure S4. ^{1}H NMR spectra of diamine-cobaltocene in DMSO-d6 with 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) as an internal standard.

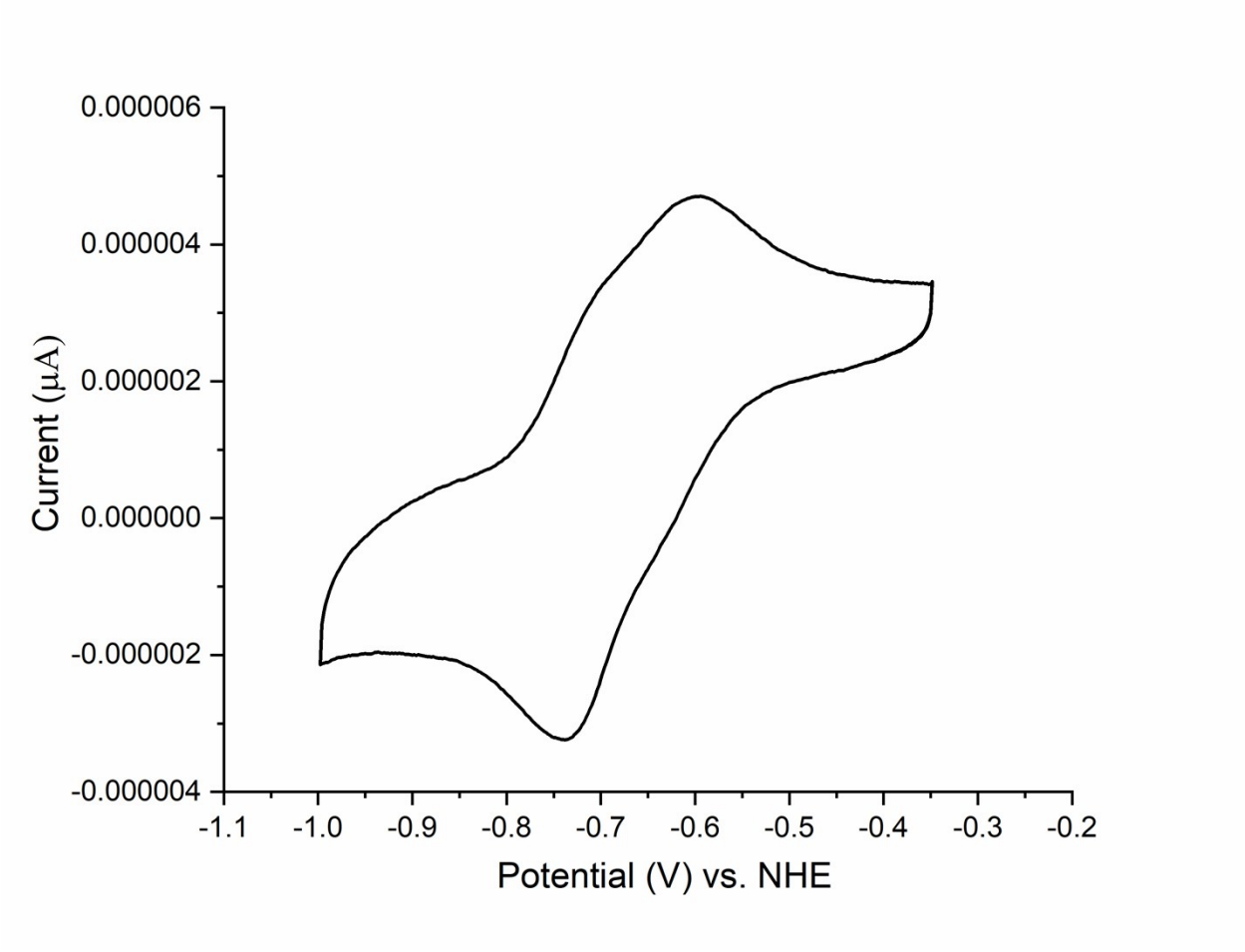

Figure S5. Cyclic voltammogram of diamide-cobaltocene (-740mV vs. NHE) in 50mM PB and 100mM NaCl with 10mV/s scan rate.

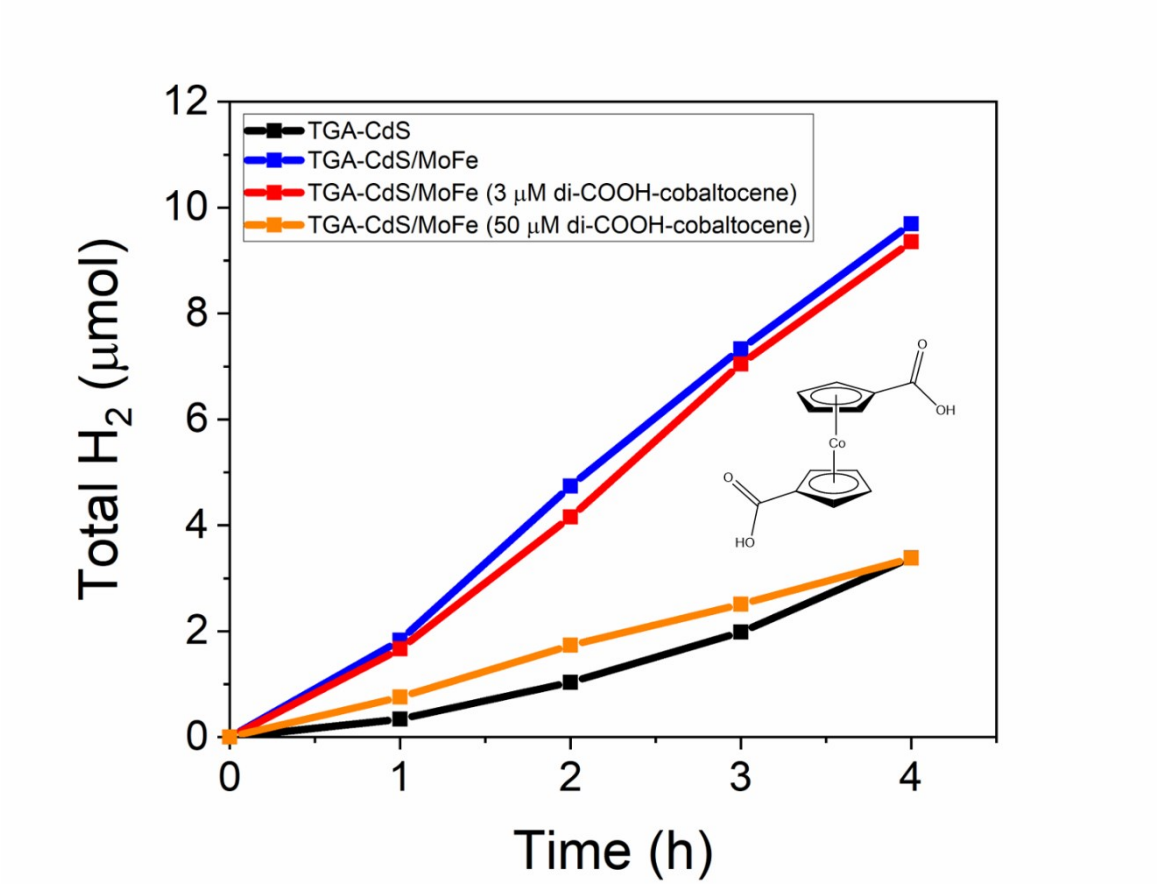

Figure S6. Total H_2 from TGA-CdS/MoFeP biohybrid complexes in the presence of 3 and 50 μM di-COOH-cobaltocene. CdS NRs act as the electron supplier under irradiation in an Ar purged vial containing 50 mM PB, 100 mM NaCl, 500 mM ascorbic acid (AA), 500 nM CdS, and 500 nM MoFeP at pH 7.4 over 4 h.

Experimental Details

***General Information**:* All chemicals and solvents were analytical grade and used as received from commercial sources. Ultraviolet-visible spectra were acquired on a DU 730 spectrophotometer (Beckman Coulter). Anaerobic experiments were performed in an UNIlab glovebox (MBRAUN) under an argon atmosphere.

***CdS Nanocrystal Synthesis**:* The CdS nanorod (NR) synthesis was based on a previously reported procedures from a seeded growth approach.[1,2] First, CdS seeds were prepared by mixing 100 mg cadmium oxide (CdO, 99.95%, Alfa Aesar), 603 mg n-octadecylphosphonic acid (ODPA, 97%, Alfa Aesar), and 3.30 g trioctylphosphine oxide (TOPO, 99%, Strem Chemicals, Inc.) in a 100 mL three-neck round-bottom flask. The mixture was degassed for 10 min under vacuum and then heated to 120°C and held under vacuum for 1 h. After degassing, Ar was gently purged over the solution and the temperature was increased to 300°C for 30 min to dissolve the CdO. The solution should change from dark brown to optically clear and colorless. The solution was next cooled back to 120°C and degassed under vacuum for 30 min. Next, under Ar the temperature is heated to 320°C. Once the temperature is stabilized, a mixture of 179 mg hexamethyldisilathiane ($(TMS)_2S$, synthesis grade, Sigma Aldrich) in 3 g of tributylphosphine (TBP, 97%, Sigma Aldrich) is injected quickly. The heat-controller is set to 250°C and the nanocrystals (NCs) are allowed to grow for 7.5 min. The reaction was stopped by removing the heating mantle. Once cool, toluene was injected and the CdS seeds were precipitated with methanol. The CdS seeds were washed two more times with toluene/methanol in a glovebox and then dissolved in trioctylphosphine (TOP, 97%, Sigma Aldrich). The CdS seeds, shown in Figure S6, had a first exciton peak at 395 nm with an estimated molar absorptivity (ε) of 3.18×10^5 M^{-1} cm^{-1}.[3]

CdS NRs were then synthesized by degassing 86 mg CdO, 3 g TOPO, 290 mg ODPA, and 80 mg hexylphosphonic acid (HPA, 97%, Strem Chemicals, Inc.) for 10 min under vacuum and then heated to 120°C and held for 1 h. The solution was heated to 350°C under Ar and held for 30 min. Then 1.5 mL of TOP was then injected, and the temperature was allowed to stabilize back to 350°C before swiftly injecting the seed-containing solution containing 124 mg sulfur (S, 99.998%, Sigma Aldrich) in 1.5 mL of TOP with 8.0×10^{-8} mol CdS QD seeds. The NCs were allowed to grow for 9 min then the solution was cooled. The CdS NCs were first precipitated with a 1:1:1 mixture of acetone, toluene, and methanol using centrifugation (8,000 rcf, 5 min). The pelleted NCs were than resuspended in toluene, washed with octylamine, and precipitated with methanol (8,000 rcf, 5 min). The NCs were then resuspended in toluene, washed with octanoic acid, and

precipitated with isopropanol for a total of three times (8,000 rcf, 5 min). The CdS NCs were stored in toluene at 4°C. CdS NCs were imaged using a FEI Tecnai 12 Spirit TEM and 100 NCs were measured using ImageJ, Figure S1. A molar absorptivity of 5.80 x 10^6 M^{-1} cm^{-1} was used to determine NR concentrations.

Ligand Exchange*:* CdS NCs were solubilized in water by ligand exchange with thioglycolic acid (TGA, ≥98%, Sigma Aldrich) using a modified procedure.[4,5] CdS NCs were first precipitated from toluene using methanol and centrifugation (8,000 rcf, 10 min). Then 0.3175 mmol of the respective ligand was dissolved in 5 mL of methanol. The pH of this solution was then adjusted to 11 using tetramethylammonium hydroxide pentahydrate salt (97%, Sigma Aldrich). The precipitated CdS NCs were then resuspended in the methanol ligand solution. The yellow solution is initially cloudy but after sonication for a few seconds the solution becomes clear. The now water-soluble NCs are precipitated with toluene and centrifuged (8,000 rcf, 10 min). The supernatant was removed, and the NCs are dried under vacuum before being resuspended in DI water and washed with 600 μL of DI H_2O using a 30 kDa Nanosep centrifugal filter (Pall Corporation).

Nitrogenase Expression and Purification*: Azotobacter vinelandii* (DJ995) with expression of his-tagged wild type MoFe protein (MoFeP) were kindly provided by Dr. Dennis Dean and were grown and expressed using a modified published procedure.[6] Cells were cultured using modified Burk media containing 2% sucrose, 1.7 mM $MgSO_4$, 0.9 mM $CaCl_2$-$2H_2O$, 1 mM Na_2MoO_4-$2H_2O$, 0.04 mM $FeSO_4$-$7H_2O$, 10 mM NH_4Cl, 1.6 mM KH_2PO_4, and 5.2 mM K_2HPO_4.[7,8] A solution of sucrose/Mg/Ca were autoclaved separately to the phosphate buffer solution. Autoclaved solutions were allowed to cool before mixing. The Fe, Mo, and NH_4^+ solutions were added to the sucrose/Mg/Ca solution in order to achieve the final concentrations above, followed by the addition of phosphate buffer. Plates were prepared using an agar concentration of 16 g/L.

Cultures were grown at 30°C in 1 L flasks from starter cultures at ≈250 rpm. First, plates were streaked with cell stocks stored at -80°C and allowed to grow overnight (16-24 h). Once there is sufficient growth on the plate, the plate is pricked and introduced to a 100 mL starter culture in modified Burke's media with 10 mM NH_4Cl and incubated at 30 °C and ≈250 rpm. A separate 100 mL growth, without Fe salts, is also started to ensure there is no contamination. When *A. vinelandii* cells are iron deficient, the cells excrete a fluorescently green siderophore under UV light which is used as an indication of little to no contamination. The starter culture was grown until the optical density at 600 nm (OD_{600}) was about 1.0 (≈16-24 h). Next, 1 L cultures were started with the addition of 5 mL of the starter culture. Once an $OD_{600} \approx 1$ was reached, the cells were spun down (4,000 rcf, 3 min) and resuspended in modified Burk's media without NH_4Cl for 4 h to afford expression of the MoFeP. The cells were then spun down and washed with 20 mM phosphate buffer (PB) containing 300 mM NaCl, (4,000 rcf, 3 min) before the cells were frozen in liquid nitrogen and stored in -80°C until purification.

The his-tagged MoFePs of nitrogenase were purified in an Ar atmosphere glovebox (< 1.0 ppm O_2) using Ar purged buffers. Frozen cells (≈1-2 g) were initially thawed and washed with 15 mL of lysis buffer (20 mM PB, 100 mM NaCl, pH 7.4) containing 5 mM sodium dithionite (DT). The cells were removed via centrifugation (8,000 rcf, 10 min, 4°C) and resuspended in 10 mL of fresh lysis buffer. Cells were lysed at 4°C using a Branson 150W sonifier with a 1/8" probe (70% amplitude, 15 sec ON, 30 sec OFF, 30 min runtime). The cell lysate was first centrifuged (10,000 rcf, 30 min, 4°C) and the supernatant was transferred to a clean centrifugal tube before a second centrifugal step (10,000 rcf, 60 min, 4°C). The transparent reddish black lysate was then adjusted to 500 mM NaCl and 30 mM imidazole.

His-tagged MoFeP were purified using immobilized metal affinity chromatography (IMAC, GE Healthcare, HisTrap High Performance, 1 mL). Due to the use of the reducing agent DT, a blank run without DT was performed based on the manufacturer's recommendation on the column before protein purification. After blanking, the column was washed with 3-5 column volumes of distilled water and then equilibrated with 5 column volumes of binding buffer (20 mM PB, 500 mM NaCl, 30 mM imidazole, 1 mM DT, pH 7.4). The pretreated lysate was then run through the column and washed with 18 column volumes of wash buffer (20 mM PB, 500 mM NaCl, 40 mM imidazole, 1 mM DT, pH 7.4). His-tagged MoFeP was then eluted with 1-2 column volumes of elution buffer (20 mM PB, 500 mM NaCl, 250 mM imidazole, 1 mM DT, pH 7.4). Excess imidazole was removed by dialysis in 50 mM PB, 100 mM NaCl, and 1 mM DT using a 20 kDa filters (Slide-A-Lyzer Mini, ThermoFisher Scientific).

Protein purity was confirmed by SDS-PAGE and concentrations were determined using Fe chelation based on previously published work.[8] Fe chelation was performed in an anaerobic quartz cuvette with 6.4 M guanidine HCl and 6.25 mM 2,2-bipyridine. The reaction was monitored at 522 nm and the concentration was determined using an extinction coefficient of 8,650 M^{-1} cm^{-1}. MoFeP was frozen in 50 μL aliquots at 6 μM (1.38 mg/mL) and stored at -80°C.

Cobaltocene Modification & Characterization: 1,1'-Dicarboxy-cobaltocenium hexafluorophosphate (di-COOH-cobaltocene, 1 molar equivalent, MCAT GmbH) modifications were performed using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 15 molar equivalents, Anaspec Inc.) and sulfo-N-hydroxysuccinimide (sulfo-NHS, 20 molar equivalents, ThermoFisher Scientific) coupling chemistry with ethylenediamine dihydrochloride (2.1 molar equivalents, Sigma Aldrich) under anaerobic conditions. The amide reactions were performed in a mixture DMSO and water for 24 h and then dried using lyophilization prior to characterization followed by ^{1}H NMR in DMSO-d6 with 4,4-dimethyl-4-silapentane-1-sulfonic acid as an internal standard (Figure S4).

Agarose Gel Electrophoresis. To characterize NC-protein interactions, a 0.6 wt% agarose (Sigma Aldrich) gel in 0.1 M sodium borate was prepared. Agarose is first mixed with the buffer and this

hazy solution is then microwaved until boiling, mixed, and then microwaved again to boiling to yield a clear, colorless solution. This mixture is then poured into a gel tray and allowed to solidify. CdS NPs were synthesized using a previously procedure.[9] Prepared solutions of CdS NPs with and without the MoFeP were then pipetted into the wells. Excess NPs were used to improve contrast in the gel prior to staining. A potential of 200 V was applied for 10 minutes. Before staining, the gel was gently mixed in an aqueous solution of 50% ethanol for 15 min before soaking overnight in a Coomassie solution overnight. The stained gel was then washed with water several times to remove excess Coomassie before imaging.

Biohybrid Photoreactions*:* CdS/MoFeP reactions were prepared by mixing a buffered solution containing 50 mM PB, 100 mM NaCl, and 500 mM ascorbic acid (AA, 99%, Sigma Aldrich) at pH 7.4 with 280 pmol of CdS so that the final volume is 550 μL. The solution was then sealed in a 2 mL glass vial and gently purged with Ar for 30 min before being transferred to a glovebox for the protein injection. Protein modifications are described below. In the glovebox, a 50 μL MoFeP aliquot was then injected into the reaction vial yielding a total reaction volume of 600 μL. A CdS only control reaction was run in parallel for each test. For the control reaction, instead of injecting the MoFeP aliquot, a 50 μL aliquot of the dialysis buffer was injected, which was stored at -80°C with the MoFeP. The reactions were then removed from the glovebox, mixed for 1 h, and then irradiated using a 465 $\pm$ 30 nm blue LED light for 4 h. H_2 was measured every hour of irradiation using a HP/Agilent 6890 (G1540A) system equipped with a 5-Å column and a thermal conductivity detector by injecting a 30 μL sample. H_2 was quantified using a calibration curve (Figure S7).

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