Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Optimizing Electron Transfer from CdSe QDs to Hydrogenase for Photocatalytic H_2 Production

Monica L. K. Sanchez,[a] Chang-Hao Wu,[b] Michael W. W. Adams,[b] R. Brian Dyer*[a]

[a]*Department of Chemistry, Emory University, Atlanta, GA 30322 USA.*
[b]*Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602 USA.*

Note: All reagents were purchased from Sigma Aldrich and were used as is except where noted.

Section 1. Synthesis of Mediators

Preparation of 2-Carbon Linker Mediators: DQ52 and DQ42

Procedure as modified from literature.[2] To an air free 3-neck round bottom flask, 5 mL of 1,2-dibromoethane and 0.25g of 4,4’-dimethyl-2,2’-bipyridine (or 5,5’-dimethyl-2,2’-bipyridine for DQ52) was added. The reaction mixture was refluxed at 125 °C and allowed to react for 12-18 hrs at which point a large amount of pale-yellow precipitate had formed within the flask. After the reaction ran to completion, the vessel was cooled down with an ice bath and the solid collected on a frit. Excess reagents were washed from the product with cold acetone and hexane.

The final product was crystallized from methanol and characterized. The crystals collected were off white. Yield: 62%

Preparation of 3-Carbon Linker Mediators: DQ03, DQ43, and DQ53

Procedure as modified from literature.[2] To an air free 3-neck round bottom flask, 5 mL of 1,3-dibromopropane and 0.25g of 4,4’-dimethyl-2,2’-bipyridine (or 5,5’-dimethyl-2,2’-bipyridine for DQ53 or 2,2’-bipyridine for DQ03) was added. The reaction mixture was refluxed at 100-110 °C and allowed to react for 3-5 hrs at which point a large amount of pale-yellow precipitate had formed within the flask. After the reaction ran to completion, the vessel was cooled down with an ice bath and the solid collected on a frit. Excess reagents were washed from the product with cold acetone and hexane.

The final product was crystallized from methanol/ethanol and characterized. The crystals collected were off white or yellow. Yield: 73%

Section 2. Physical Characterization of Mediators++

^{1}H-NMR spectra of oxidized mediators

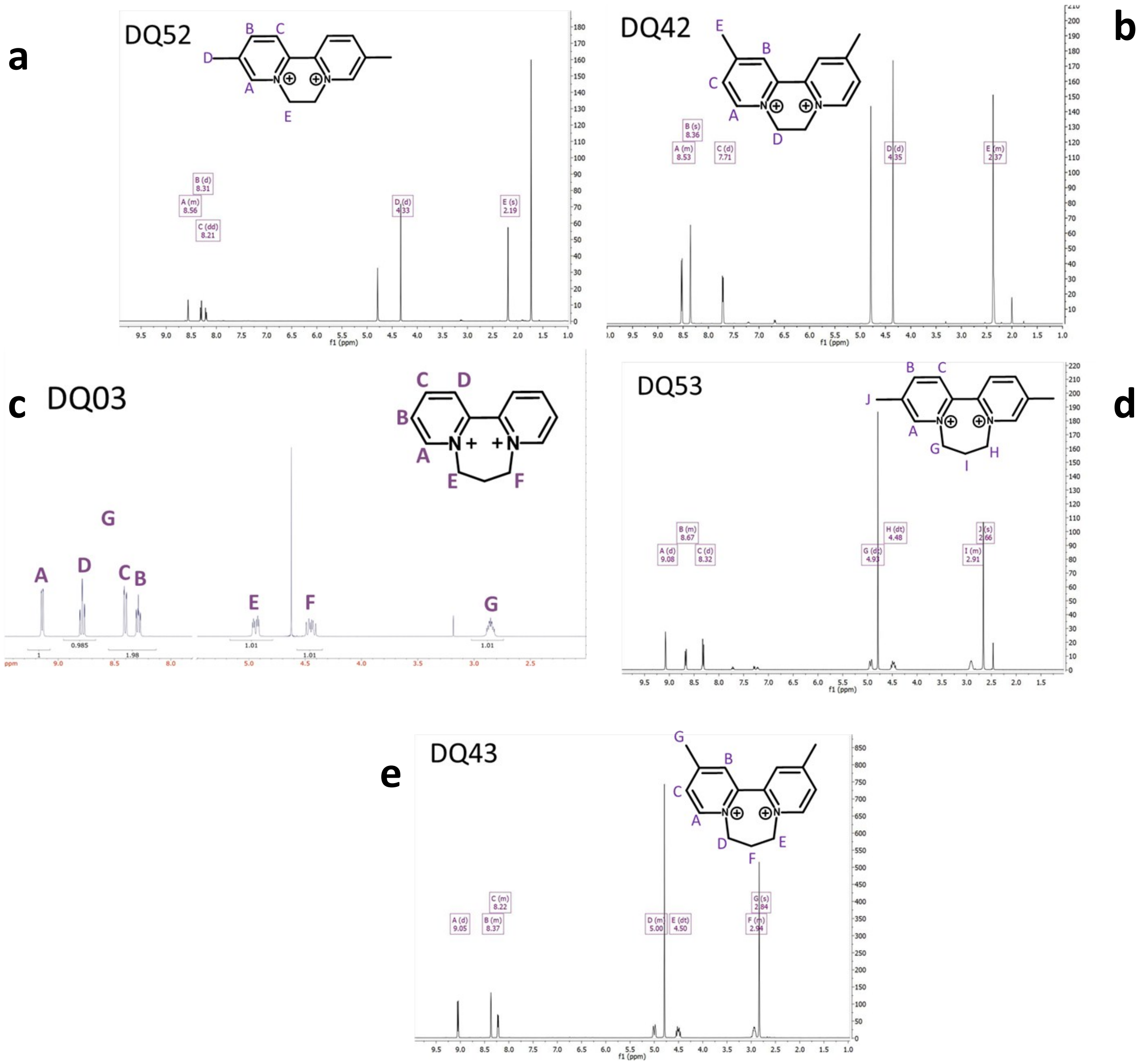


Figure S1. Proton NMR spectra of the oxidized (DQXX^{2+}) mediators in D_2O, collected on a Bruker Inova 400 MHZ instrument. Spectra are the average of 16 scans and are referenced to TMS. a) DQ52, b) DQ42, C) DQ03, d) DQ53, and e) DQ43. The three broad multiplet features that are assigned to the bridging protons are the result of diastereotopic protons resulting from a chiral molecule. A planar molecule would not have this chirality and so broad features at these shifts 2.9, 4.5, and 5.0 would not be expected. This phenomenon has been reported for molecules related to these.[3]

Section 3. UV-Vis Absorbance Spectra of Mediators^{++}

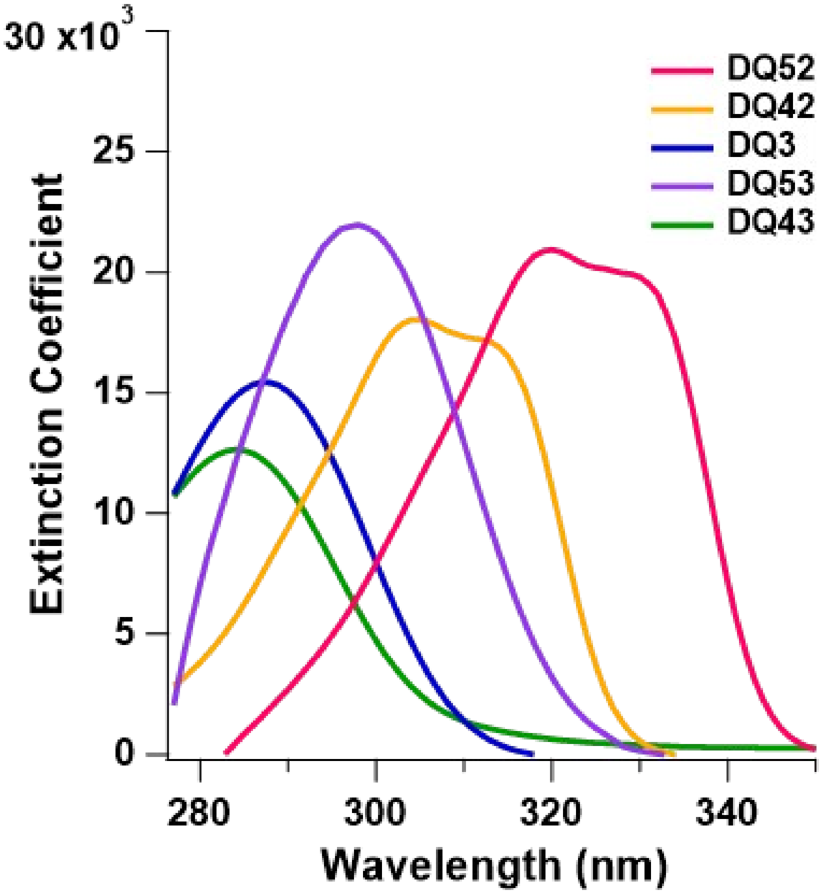


Figure S2. UV-Vis absorbance spectra of the oxidized mediators (DQXX^{2+}) in water. Spectra were collected on a Perkin Elmer Lambda 35 spectrometer.

Section 4. Single Crystal X-ray Crystallography of Mediators

Single crystals of each mediator were grown by vapor diffusion of chloroform into concentrated mediator stock solutions in methanol or ethanol. Representative structures are depicted below.

Table S1: Summary of Structural Properties

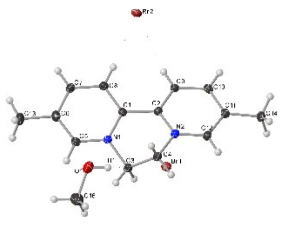
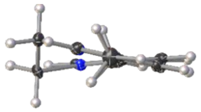
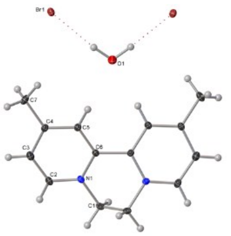
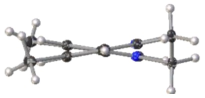
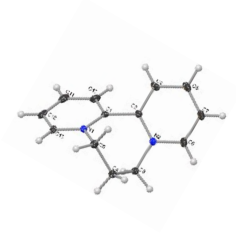
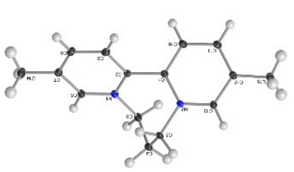
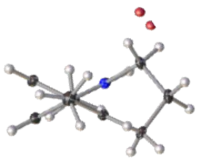
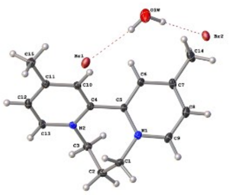
	Front View	Side View	Torsional Twist	Reduction Potential (mV) vs. NHE
DQ52			18°	-470
DQ42			20.8°	-490
DQ03			52.5°	-550
DQ53			55.5°	-635
DQ43			52.3°	-670

Table S2: X-ray Structure Parameters

Compound	**DQ52**	**DQ42**	**DQ03**	**DQ53**	**DQ43**
Formula	$C_{15}H_{20}Br_2N_2O$	$C_{14}H_{18}Br_2N_2O$	$C_{13}H_{15}Br_2N_2O_{0.5}$	$C_{15}H_{26}Br_2N_2O_4$	$C_{15}H_{20}N_2OBr_2$
$D_{calc.}$/ g cm^{-3}	1.628	1.742	1.766	1.606	1.632
μ/mm^{-1}	6.213	5.443	5.857	4.297	4.926
Formula Weight	808.30	390.12	367.07	458.20	404.15
Colour	colourless	colourless	colourless	colourless	colorless
Shape	block	needle	prism	prism	prism
Size/mm^3	0.26×0.22×0.18	0.20×0.12×0.09	0.69×0.47×0.29	0.53×0.39×0.14	0.33
T/K	100.00(10)	100(2)	100(2)	100(2)	0.30
Crystal System	triclinic	monoclinic	monoclinic	monoclinic	0.29
Space Group	P-1	$C2/c$	$C2/c$	$I2/c$	100(2)
a/Å	7.3138(2)	20.6558(11)	44.5365(10)	21.061(2)	monoclinic
b/Å	10.0105(3)	11.4656(3)	14.2236(5)	6.7995(7)	$P2_1/c$
c/Å	12.1578(2)	6.9238(3)	17.4936(4)	26.557(3)	11.1474(10)
α/°	104.745(2)	90	90	90	14.4208(12)
β/°	96.597(2)	114.868(6)	94.743(2)	94.682(12)	11.2139(10)
γ/°	103.047(3)	90	90	90	90
V/Å^3	824.44(4)	1487.73(13)	11043.7(5)	3790.4(7)	114.1500(10)
Z	1	4	32	8	90
Z'	0.5	0.5	4	1	1644.9(2)
Wavelength/Å	1.54184	0.710730	0.710730	0.710730	4
Radiation type	CuK$_\alpha$	MoK$_\alpha$	MoK$_\alpha$	MoK$_\alpha$	1
Θ_{min}/°	3.824	2.082	2.246	1.539	2.002
Θ_{max}/°	77.039	30.500	30.999	30.541	30.614
Measured Refl.	9300	16543	73108	24618	21850
Independent Refl.	3321	2261	17503	5768	5049
Reflections with I > 2(I)	3209	1887	10735	5118	4201
R_{int}	0.0273	0.0658	0.0648	0.0305	0.0431
Parameters	188	91	658	229	190
Restraints	1	27	726	60	3
Largest Peak	0.524	0.915	1.331	0.717	1.251
Deepest Hole	-0.565	-0.592	-0.607	-0.953	-0.606
GooF	1.117	1.083	1.042	1.087	1.062
wR_2 (all data)	0.0801	0.0855	0.1179	0.0772	0.0903
wR_2	0.0787	0.0809	0.1004	0.0735	0.0847
R_1 (all data)	0.0313	0.0496	0.1074	0.0374	0.0466
R_1	0.0286	0.0374	0.0524	0.0310	0.0353

Section 5. Cyclic Voltammetry

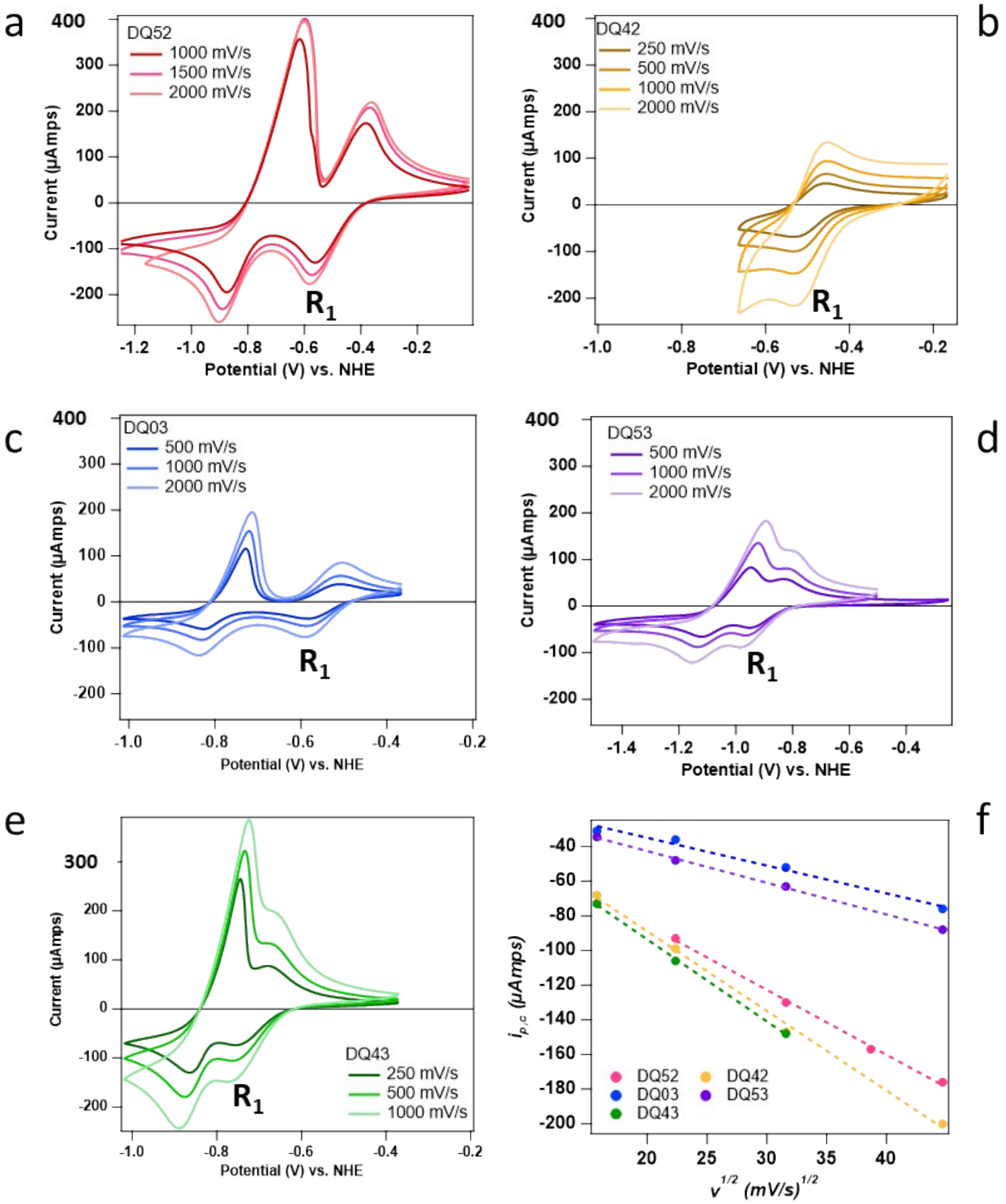
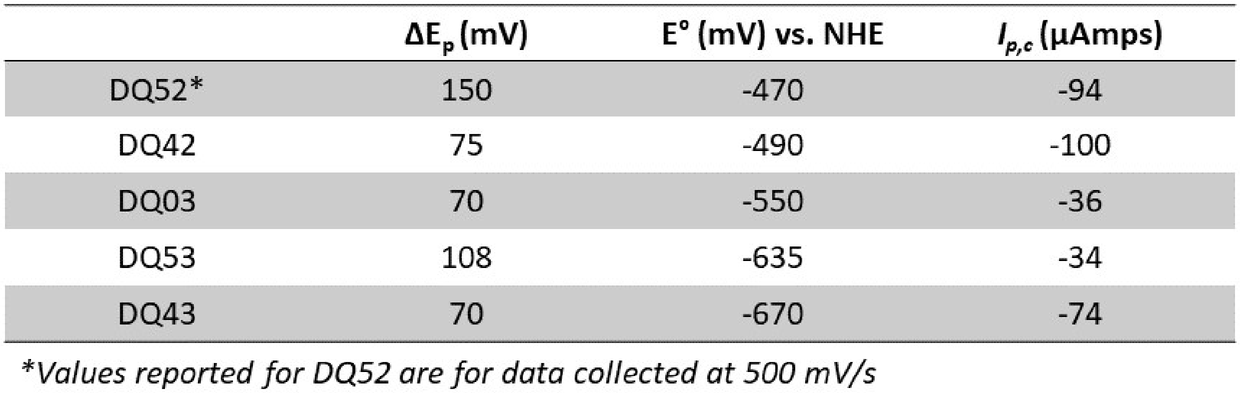


Figure S3. Cyclic voltammograms in water for each of the mediators pictured above: a) DQ42, b) DQ52, c) DQ03, d) DQ53, e) DQ43 and f) a plot of $v^{1/2}$ vs. $i_{p,c}$. Cyclic voltammograms for each of the mediators were collected using a glassy carbon working electrode, Ag/AgCl (.1 M) (vs. NHE .2881 V)[4] as reference and Pt wire counter electrode. Cyclic voltammograms were recorded on a Gamry Instruments Potentiostat, Reference 600. The scan rates are indicated in each voltammogram. The mediator solutions each contained 10 mM mediator, 10 mM borate buffer and 100 mM KCl salt. The solution was purged under N_2 for 10 min prior to data collection.

In the cyclic voltammograms (plots a-e), the peak-to-peak separation observed in the first cathodic wave when scanning at rates from .25 V/s up to 2 V/s for all 5 of the mediators indicates a high degree of reversibility for the first ET event of the first cathodic wave.The linear dependence on the scan rate is observed in the plot f of figure S3 indicates that the mediators remain homogeneous through the redox event.

Table S1. Values from Cyclic Voltammograms



	ΔE_p (mV)	E° (mV) vs. NHE	$I_{p,c}$ (µAmps)
DQ52*	150	-470	-94
DQ42	75	-490	-100
DQ03	70	-550	-36
DQ53	108	-635	-34
DQ43	70	-670	-74

**Values reported for DQ52 are for data collected at 500 mV/s*

Section 6. Preparation of CdSe QDs

CdSe QD synthesis: Procedure was modified from a literature procedure.[5] Briefly CdO (0.06g), trioctylphosphine oxide (TOPO), (3 g), and octadecyl phosphonic acid (ODPA) (0.28g) were combined in a three neck flask and put under vacuum while heating to 150 °C for 1.5 hrs. After which put reaction vessel under N_2 atmosphere. Once under N_2, heat the flask to 300 °C to 350 °C until the reaction mixture goes clear indicating that the Cd has fully dissolved in the solvent. Once the reaction mixture is clear, 1.5 mL of trioctylphosphine (TOP) was injected. Once the desired reaction temperature recovers to ~340 °C inject the Se precursor solution. The reaction was allowed to run for 5 min. For slightly larger or smaller particles, the reaction time was adjusted accordingly. Once, the reaction was completed, the vessel was removed from heat and cool to room temperature. A small addition of hexane or toluene will keep the reaction mixture in solution phase.
*ODPA was purchased from Alfa Aesar

The particles were purified by precipitation via centrifugation with MeOH or Acetone and resuspended in chloroform. This procedure was repeated three times to remove excess ligand and unreacted cadmium before proceeding to the ligand exchange reaction.

The Se precursor solution was prepared by dissolving 0.058 g of Se into 0.45 mL of TOP.

Ligand Exchange Procedure

The nonpolar ligand was exchanged with mercaptopropionic acid (MPA) via a ligand exchange procedure.[5] Briefly, 5 mg of purified quantum dots (QDs) were refluxed for 2 hrs under N_2 in the dark in 15 mL of methanol with 10 mM MPA. The pH of this reaction mixture had been adjusted to pH 10 using tetramethyl ammonium hydroxide (TMAOH) prior to refluxing. After the reaction had gone to completion, the exchanged QDs were then isolated by centrifugation and resuspended in 10 mM MPA, 10mM triscarboxyethyl phosphine (TCEP), 50 mM borate buffer and stored in an air free environment for future use.

Section 7. Physical Characterization of CdSe QDs

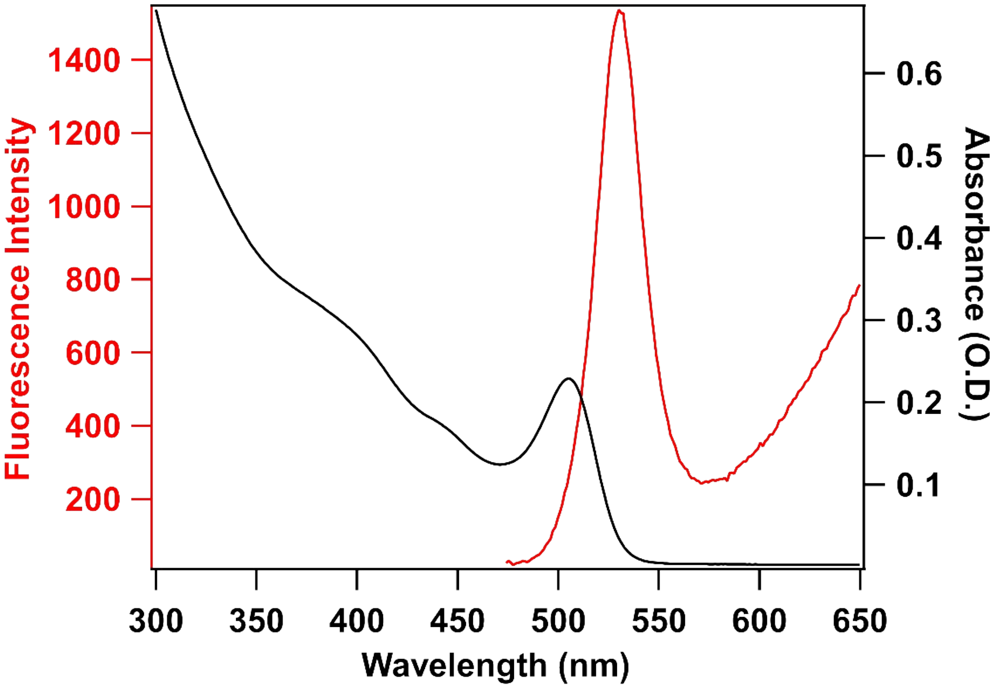


Figure S4. The CdSe QDs were characterized by fluorescence and uv-vis absorbance spectroscopies. The size and extinction coefficient of the QDs were calculated from the band edge absorbance using a procedure previously described. [1] Fluorescence spectra were collected on Horiba Scientific fluorometer and absorbance spectra wer collected on a Perkin Elmer Lambda 35 spectrometer.

Section 8. Quantum Efficiency Methodology and Experimental Setup

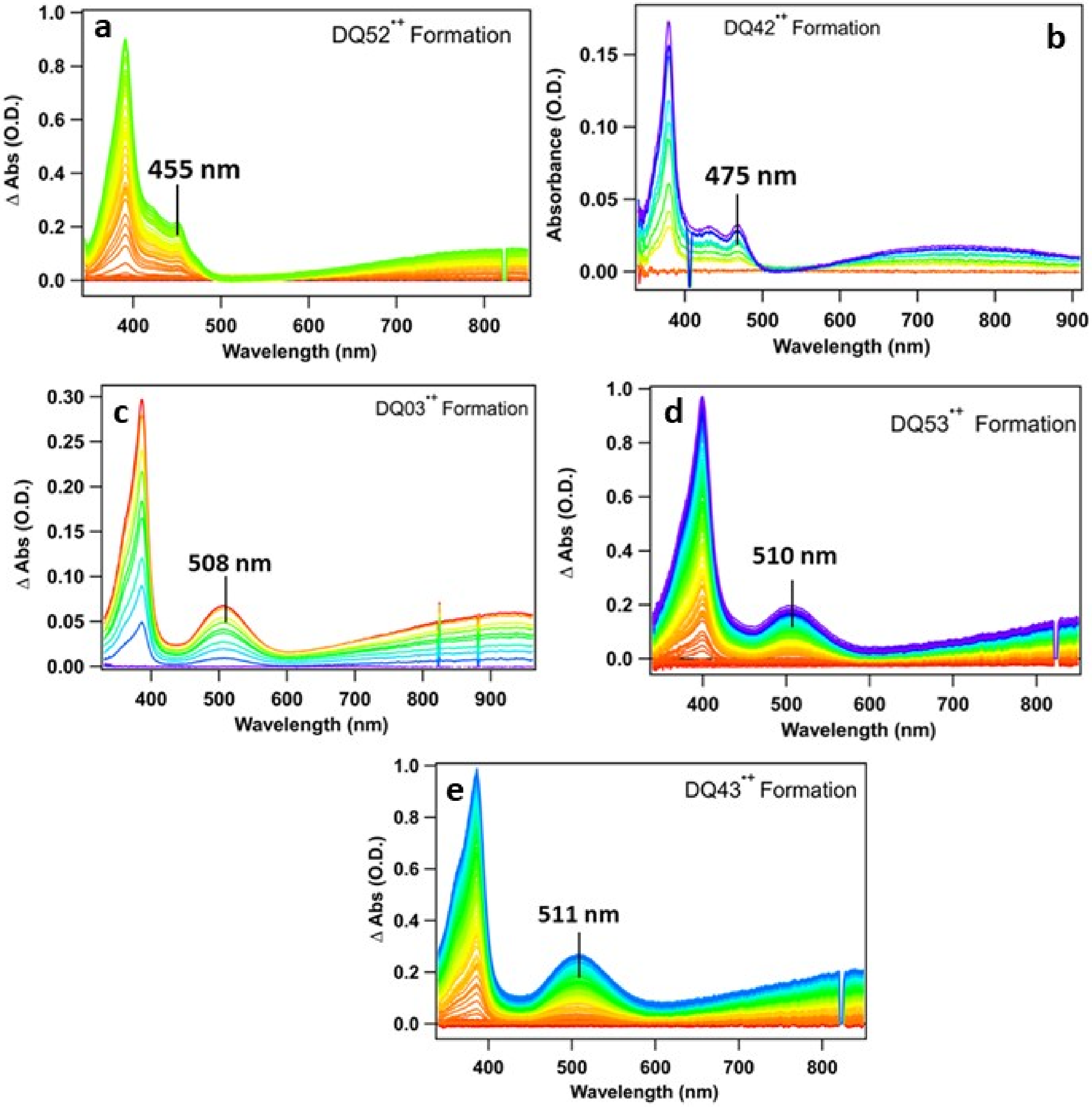


Figure S5. Representative radical absorbance spectra during photoreduction: a) DQ52, b) DQ42, c) DQ03, d) DQ53 and e) DQ43. The complete absorbance spectra were collected during quantum efficiency measurements as a function of illumination time. The wavelength probed for quantification of QE is labeled in black and indicated with a line.

Quantum efficiency measurements were carried out similarly to what has been described previously.[6] The sample contained quantum dots (0.1 - 0.2 O.D.), 50 mM cysteine, and 5 mM mediator, the total volume for each sample was 1.4 mL in 50 mM phosphate buffer, pH 7.4. The sample was prepared inside of a glovebox and buffer exchanged prior to use. The cuvette was sealed with a rubber septum. During the experiment, N_2 flow as introduced 10 min prior to the start of the experiment and continuously run through the headspace of the cuvette to prevent O_2 contamination. The sample was also stirred continuously.

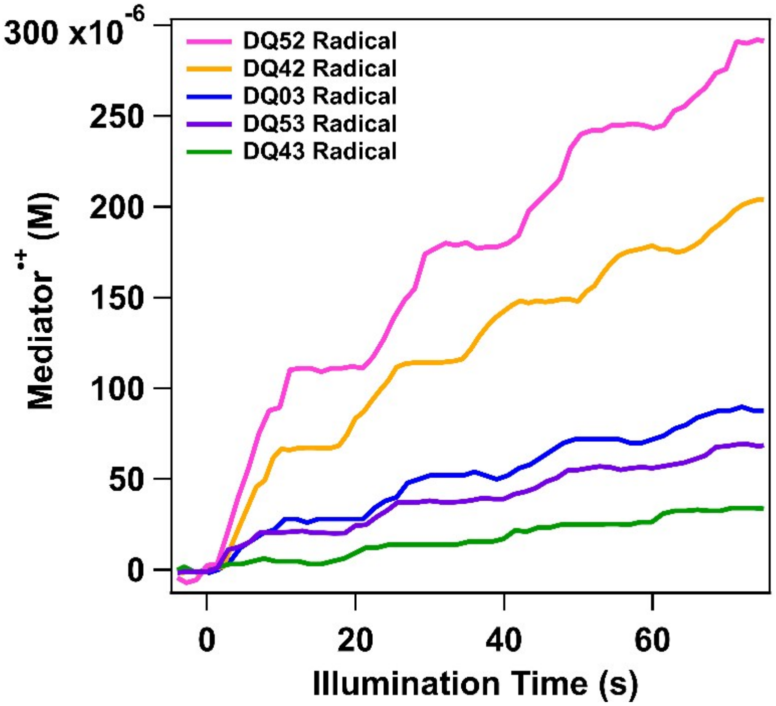


Figure S6. Representative spectra depicting radical population growth vs time collected using an Ocean Optics spectrometer.

The sample was illuminated with a 405 nm diode purchased from Thor labs and the absorbance monitored with a fiber optically coupled ocean optics uv-visible spectrometer. The sample was illuminated in 10 second intervals and allowed to equilibrate with the laser off for 10 sec intervals, producing the steps observed in Fig. S6. This process ensured that the sample was not O_2 contaminated which would result in artificially lower QE. QE was calculated by dividing moles of radical generated by moles of photons absorbed by the sample. Figure S6 has examples of photo-titration experiment for each of the mediators. The absorbance feature at ~450 nm for DQ42 and -52 and the absorbance feature at ~510 nm for DQ03, -43 and -53 were monitored for QE calculations.

QE was calculated using the equation below. Moles of mediator radical generated divided by the moles of photons absorbed. We accounted for the index of refraction by multiplying by 9.15 based on previously reported measurements. For H_2 production assays mediator radical is replaced with 2*moles H_2 produced.

$$\mathrm{QE} = \frac{\text{Mols of mediator radical}}{\text{Mols of photons}}$$

$$= \frac{\text{mols of mediator radical}}{(\text{Photons per sec}) * (1 - (10^{(\text{QD abs at 405 nm})})) * (\text{sec})}$$

Section 9. Hydrogen Production Assays

These measurements were performed analogously as the mediator reduction experiments except for a few minor changes besides the inclusion of hydrogenase.[6] A pressure sensor was inserted into the cap of the cuvette to measure the change in pressure. The change in pressure recorded was used to determine the mols of H_2 generated with this method. The headspace of the cuvette was also purged of any H_2 prior to the start of the experiment. The QE measured are listed in Table S2.

	QE_{rad} at 10 sec		**QE_{hyd} Production (at 5000 sec)**		
	$[\text{Mediator}^{\bullet+}]$	QE (%)	Mols H_2	QE (%)	$QE_{corrected}$ (%)
DQ52	.00011	81.3 ± 4.0	$4.09*10^{-7}$	1.72	4.73
DQ42	$6.65*10^{-5}$	74.3 ± 4	$8.13*10^{-7}$	2.86	7.85
DQ3	$2.79*10^{-5}$	31 ± .5	$2.70*10^{-6}$	11.36	14.8
DQ53	$2.06*10^{-5}$	26 ± 4.2	$3.70*10^{-6}$	16.76	16.78
DQ43	$4.64*10^{-6}$	5 ± 1.2	$2.65*10^{-6}$	11.3	11.5

Table S1: QE_{rad} and QE_{hyd} measured for each of the mediators.

References

1. Yu, W. W.; Qu, L.; Guo, W.; Peng, X., Experimental Determination of the Extinction Coefficient of CdTe, CdSe, and CdS Nanocrystals. *Chemistry of Materials* **2003,** *15* (14), 2854-2860.
2. Xiao, Y.; Chu, L.; Sanakis, Y.; Liu, P., Revisiting the IspH Catalytic System in the Deoxyxylulose Phosphate Pathway: Achieving High Activity. *Journal of the American Chemical Society* **2009,** *131* (29), 9931-9933.
3. Campa, C.; Camps, J.; Font, J.; De March, P., Conformational studies of annulated 2,2'-bipyridinium salts. *The Journal of Organic Chemistry* **1987,** *52* (4), 521-525.
4. Meites, L., *Handbook of Analytical Chemistry*. McGraw-Hill: New York, 1963; p 1788.
5. Zhu, H.; Song, N.; Lv, H.; Hill, C. L.; Lian, T., Near Unity Quantum Yield of Light-Driven Redox Mediator Reduction and Efficient H2 Generation Using Colloidal Nanorod Heterostructures. *Journal of the American Chemical Society* **2012,** *134* (28), 11701-11708.
6. Chica, B.; Wu, C.-H.; Liu, Y.; Adams, M. W. W.; Lian, T.; Dyer, R. B., Balancing electron transfer rate and driving force for efficient photocatalytic hydrogen production in CdSe/CdS nanorod–[NiFe] hydrogenase assemblies. *Energy & Environmental Science* **2017,** *10* (10), 2245-2255.