Light-driven carbon–carbon bond formation via CO₂ reduction catalyzed by complexes of CdS nanorods and a 2-oxoacid oxidoreductase

Hayden Hamby^{a,1} , Bin Li^{b,2}, Katherine E. Shinopoulos^{a,3}, Helena R. Keller^c, Sean J. Elliott^b, and Gordana Dukovic^{a,4}

^aDepartment of Chemistry, University of Colorado Boulder, Boulder, CO 80309; ^bDepartment of Chemistry, Boston University, Boston, MA 02215; and ^cMaterials Science and Engineering, University of Colorado Boulder, Boulder, CO 80303

Edited by Catherine J. Murphy, University of Illinois at Urbana–Champaign, Urbana, IL, and approved November 18, 2019 (received for review March 18, 2019)

Redox enzymes are capable of catalyzing a vast array of useful reactions, but they require redox partners that donate or accept electrons. Semiconductor nanocrystals provide a mechanism to convert absorbed photon energy into redox equivalents for enzyme catalysis. Here, we describe a system for photochemical carbon–carbon bond formation to make 2-oxoglutarate by coupling CO₂ with a succinyl group. Photoexcited electrons from cadmium sulfide nanorods (CdS NRs) transfer to 2-oxoglutarate:ferredoxin oxidoreductase from *Magnetococcus marinus* MC-1 (MmOGOR), which catalyzes a carbon–carbon bond formation reaction. We thereby decouple MmOGOR from its native role in the reductive tricarboxylic acid cycle and drive it directly with light. We examine the dependence of 2-oxoglutarate formation on a variety of factors and, using ultrafast transient absorption spectroscopy, elucidate the critical role of electron transfer (ET) from CdS NRs to MmOGOR. We find that the efficiency of this ET depends strongly on whether the succinyl CoA (SCoA) cosubstrate is bound at the MmOGOR active site. We hypothesize that the conformational changes due to SCoA binding impact the CdS NR–MmOGOR interaction in a manner that decreases ET efficiency compared to the enzyme with no cosubstrate bound. Our work reveals structural considerations for the nano–bio interfaces involved in light-driven enzyme catalysis and points to the competing factors of enzyme catalysis and ET efficiency that may arise when complex enzyme reactions are driven by artificial light absorbers.

nanocrystal | redox enzyme | electron transfer | photochemistry

In learning how to catalyze complex chemical reactions, chemists often turn to nature for inspiration (1–4). Redox enzymes catalyze a vast variety of reactions in nature by moving multiple electrons and substrates through catalytic cycles with low energy loss and high selectivity (5, 6). As the complexity of chemical transformations increases, so does the utility of chemical pathways that have evolved in nature to target those specific transformations (7–10). However, in nature, redox enzymes operate as parts of complex biochemical cycles in which the electrons involved in redox transformations travel via indirect, tortuous, and often diffusion-limited pathways (2, 10). Recently, there has been a surge in research directed at activating these biological catalysts with photosensitizers so that photon energy is converted into redox equivalents (11, 12). Colloidal semiconductor nanocrystals, in particular, have been shown to be excellent partners for redox enzymes, allowing reduction reactions such as H⁺ to H₂, N₂ to NH₃, and CO₂ to CO to be driven directly by photoexcited electrons (13–17). Due to quantum confinement, nanocrystals have easily tunable electronic structure, enabling control of their band gaps and redox potentials (18–20). They are also strong light absorbers with surfaces that can be chemically adapted to interact with enzymes without inhibiting activity (12, 21).

While several nanocrystal–enzyme systems have demonstrated promising light-driven catalytic activity, we do not yet have enough understanding of how electrons move through these systems to provide rational design principles. Insights so far

come primarily from complexes of cadmium sulfide (CdS) nanocrystals with hydrogenases to drive the relatively simple and fast 2-electron reduction of 2 protons to H₂ (13, 22–26). However, because the utility of enzyme catalysis increases with increasing chemical complexity that is difficult to access by artificial means (7–10), it is important to understand how enzymes that undergo complicated catalytic cycles can be driven by photoexcited nanocrystals.

In this work, we address such catalytic complexity by targeting carbon–carbon (C–C) bond formation via CO₂ reduction. The enzyme we selected, a 2-oxoglutarate:ferredoxin oxidoreductase (OGOR), is one component of the reductive tricarboxylic acid cycle, an alternative system for autotrophic growth found in many microorganisms (27–29). OGOR catalyzes the conversion of CO₂ and succinyl coenzyme A (SCoA) to 2-oxoglutarate and CoA. This reaction occurs with a standard redox potential, E^o, on the order of –500 mV vs. standard hydrogen electrode (SHE) at pH 7 (8, 29, 30) and utilizes low potential electrons donated from the redox partner ferredoxin (Fd) (30). Here we make use of the recently reported OGOR from *Magnetococcus marinus* MC-1

Significance

Nature uses enzymes to catalyze a vast array of complex chemical reactions. Enzymes that catalyze reduction reactions require partners that provide the electrons ultimately used in catalysis. In nature, the associated electron transfer sequences can be complicated, energy-inefficient, and rate determining. Here, we employ artificial electron donors, photoexcited semiconductor nanocrystals, to provide the electrons for catalysis. We couple these nanocrystals with an enzyme that catalyzes the formation of carbon–carbon bonds via CO₂ reduction. With this architecture, we demonstrate light-driven formation of 2-oxoglutarate, storing some of the photon energy in the chemical product. We examine the role of electron transfer from the nanocrystal to the enzyme in the light-driven chemical conversion and learn that the enzyme modulates this process during catalysis.

Author contributions: H.H., B.L., K.E.S., S.J.E., and G.D. designed research; H.H., K.E.S., and H.R.K. performed research; B.L. and S.J.E. contributed new reagents/analytic tools; H.H. and H.R.K. analyzed data; and H.H., B.L., S.J.E., and G.D. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

Published under the PNAS license.

¹Present address: Laboratory Services, Oregon Department of Agriculture, Portland, OR 97209.

²Present address: Department of Chemistry, University of Michigan, Ann Arbor, MI 48109.

³Present address: Dupont Electronics & Imaging, Marlborough, MA 01752.

⁴To whom correspondence may be addressed. Email: gordana.dukovic@colorado.edu.

This article contains supporting information online at <https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1903948116/-DCSupplemental>.

First published December 18, 2019.

(Mm), which uses a native Fd redox partner (MmFd1) to supply electrons for the generation of 2-oxoglutarate (31).

We describe catalytic activity of MmOGOR driven by photoexcited electrons from CdS nanorods (NRs) to photochemically produce 2-oxoglutarate (Fig. 1). The NRs act as an Fd mimic that generates reducing equivalents by light absorption (rather than chemical reduction), injects them into MmOGOR, and supports catalytic turnover. The use of CdS NRs to drive catalysis allows us to 1) demonstrate direct photochemical activation of a complex enzymatic catalytic cycle that involves large substrates, significant conformational changes during catalysis, and eventual formation of C–C bonds and 2) examine the relationship between the electron transfer (ET) from photoexcited CdS NRs to MmOGOR and the catalytic cycle of the enzyme. When coupled with CdS NRs, MmOGOR catalytic activity resembles that of MmFd1-driven MmOGOR, including a similar maximum turnover frequency (TOF_{max}) for 2-oxoglutarate formation. However, the quantum yield (QY) of product formation is only $\sim 1\%$, meaning that only 1% of photoexcited electrons generated in CdS NRs are used for 2-oxoglutarate formation. We use transient absorption (TA) spectroscopy of NRs to probe the kinetics of ET from the NRs to MmOGOR and inform on the origins of this inefficiency. We find that the efficiency of ET from NRs to MmOGOR decreases dramatically when the cosubstrate SCoA is bound to the enzyme active site. Dynamic light scattering (DLS) measurements show that binding of SCoA to MmOGOR weakens the binding of the enzyme to the CdS NRs. We hypothesize that the conformation changes in MmOGOR in the presence of SCoA modify the NR–enzyme interaction in a way that decreases the fraction of photoexcited electrons transferred to the enzyme active site poised for catalysis. Our work reveals previously unappreciated complexities of the nanocrystal-driven enzyme redox catalysis, namely the impact of conformational changes during catalysis on the ability of the enzyme to accept electrons from the nanocrystal. As such, our work illustrates how achieving synergistic and energy-efficient nanocrystal-

driven enzyme catalysis will require an understanding of the interplay between enzyme catalysis and ET as well as its control.

Results and Discussion

Photochemical 2-Oxoglutarate Formation in a Mixture of CdS NRs and MmOGOR. Fig. 1 shows an estimated energy level diagram for the light-driven 2-oxoglutarate formation from the SCoA and CO_2 starting materials using the combination of CdS NRs and MmOGOR. Structural and optical characterization of CdS NRs can be found in *SI Appendix, Fig. S1*. The energy used to form 2-oxoglutarate at the MmOGOR active site is supplied by a continuous wave 405-nm laser that excites electrons above the band gap in CdS NRs. Upon subpicosecond carrier cooling, the electrons are at the lowest conduction band level (32, 33). Its energy corresponds to a potential of ≤ -0.7 V (all potentials are reported vs. SHE at pH 7) (15, 34, 35), which is comparable to the redox potential of [4Fe–4S] clusters in MmFd1, the natural Fd partner for MmOGOR (-0.635 V and -0.485 V for the 2 [4Fe–4S] clusters) (31). The redox potential of the [4Fe–4S] cluster in MmOGOR has been estimated as ~ -0.5 V (31). In addition to their energy level structure, CdS NRs were chosen because of their high molar absorptivity ($\sim 7 \times 10^7 \text{ M}^{-1} \text{ cm}^{-1}$ per particle at 405 nm), which allows for a high excitation rate per particle (~ 50 to 1,000 photons absorbed per NR per second of illumination, depending on the laser power). Photoexcited electrons removed from the NRs are regenerated using a pH 6.8 buffer mixture containing radical scavenging buffers, including one that has previously been used to scavenge holes from CdS nanocrystals (14, 36). We supply CO_2 by adding bicarbonate to the buffered solution, which maintains the concentration of CO_2 at ~ 7.8 mM.

Fig. 1, *Inset* depicts, to scale, a complex of a CdS NR and one MmOGOR homodimer using the recently reported crystal structure of MmOGOR (Protein Data Bank [PDB] ID code 6N2N) (31). CdS NR surfaces are capped with 3-mercaptopropionate (MPA), a ligand that has previously been used to interface CdS NRs with enzymes such as hydrogenase and MoFe protein of nitrogenase (13, 15). At pH > 6 , the carboxylate group of MPA is deprotonated, lending an overall negative charge to the nanocrystal surface. In this regard, the NRs resemble the redox partners of MmOGOR, Fds, which are predominately negatively charged near neutral pH (31, 37, 38). In complexes of MPA-functionalized CdS NRs and a hydrogenase enzyme, the negative charge of the surface-capping ligands on the NRs enables a biomimetic interaction wherein the NRs bind at the positively charged pocket where Fd docks in nature (13). Based on this precedent, in Fig. 1, *Inset*, we depict the NRs as binding near the positively charged area on the MmOGOR surface.

Direct evidence of photochemical 2-oxoglutarate formation using the CdS–MmOGOR system came from high-resolution mass spectrometry (MS) measurements of the filtered reaction mixture (*SI Appendix, Fig. S2*). Furthermore, when bicarbonate isotopically labeled with ^{13}C was used as a starting material, we detected ^{13}C -labeled 2-oxoglutarate (*SI Appendix, Fig. S3*). While MS provides a signature of the photochemical product, it is not well suited for quantitative rate measurements. For that, we turned to in situ assay methods. To monitor light-driven 2-oxoglutarate formation in real time, we adapted an assay that has been previously used for the measurement of oxoacid formation with MmOGOR and similar enzymes (31, 39, 40). This assay employs a second enzyme, glutamate dehydrogenase (GDH), which consumes reduced nicotinamide adenine dinucleotide (NADH) while catalyzing the amination of 2-oxoglutarate (Fig. 2) (39, 40). GDH turnover is $\sim 1,000$ times faster than the turnover of MmOGOR, so that the NADH consumption reports on the 2-oxoglutarate formation rate. We monitor the depletion of NADH in situ under illumination by its absorbance spectrum. The reaction mixture for the assay exhibits a superposition of the

Fig. 1. Energy level diagram with approximate redox potentials for photochemical 2-oxoglutarate formation. VB = valence band; CB = conduction band. Light absorption by CdS NRs is followed by ET to MmOGOR, where SCoA and CO_2 combine to make 2-oxoglutarate and CoA. Photoexcited holes are scavenged by the buffer mixture, D. (*Inset*) Schematic to scale depiction of a CdS NR bound to MmOGOR (PDB ID code 6N2N) showing the surface residue charges as well as the [4Fe–4S] clusters and active sites in MmOGOR and the surface-capping ligands on the NRs. Positively charged residues are shown in blue, neutral are in white, and negative are in red. Atom colors: C (gray), N (blue), O (red), P (orange), S (yellow), Fe (rust), Cd (off-white).

Fig. 2. Reaction scheme and representative absorption spectra over a total of 10 min of illumination during the in situ product detection assay, overlaid with the NADH spectrum. (Inset) Absorbance at 340 nm ($a_{340\text{nm}}$), the maximum of NADH absorbance, as a function of time during the assay.

CdS NR and NADH absorption spectra as shown in Fig. 2. Both components absorb much more strongly than MmOGOR, which does not show detectable signal in the absorption spectra at these concentrations. In the dark, we observe a negligible absorbance change. After light is turned on, we see a decrease in NADH absorbance while the CdS NR absorption remains constant, as seen in the lowest-energy absorption peak at 460 nm (Fig. 2). To monitor 2-oxoglutarate formation, we measure the absorbance at 340 nm during concurrent illumination with 405-nm light (Fig. 2, *Inset*). Control experiments with SCoA, GDH, MmOGOR, and CdS NRs removed (*SI Appendix, Table S1*) show that all 4 components are needed to observe the NADH consumption rate of the complete system. These control experiments account for a small degree of background NADH consumption due to processes other than enzymatic 2-oxoglutarate generation. The photochemical 2-oxoglutarate generation rate of the complete system is measured from the slope of the absorbance change at 340 nm during illumination, with the slope of NADH consumption in the dark and in the absence of SCoA during illumination subtracted. The enzyme TOF, defined as moles of product per mole of OGOR per minute of illumination,

is calculated using ϵ_{340} for NADH ($6,400 \text{ M}^{-1} \text{ cm}^{-1}$) and the enzyme concentration (39).

Characterization of Photochemical 2-Oxoglutarate Formation by the CdS NR–MmOGOR System. To compare the photochemical 2-oxoglutarate production by CdS–MmOGOR complexes with the previously described natural Fd-driven activity of the same enzyme (31), we investigated the dependence of the CdS–MmOGOR reaction on reactant concentrations and excitation frequency. Fig. 3*A* shows the dependence of the light-driven enzyme TOF on the concentration of SCoA. The concentrations of CdS NRs and MmOGOR were 44 nM and 100 nM, respectively. CO_2 concentration was 7.8 mM, and excitation frequency was 890 photons absorbed per NR per second. At lower SCoA concentrations, TOF increases linearly and saturates above 0.1 mM. The functional form of the dependence resembles that seen in Michaelis–Menten kinetics. For the purposes of comparison with Fd-driven OGOR systems in the literature, we analyze our data using the Michaelis–Menten treatment.

TOF_{max} for the data in Fig. 3*A* is 21 ± 0.87 moles of product per mole of MmOGOR per minute of illumination, which is similar to the TOF_{max} obtained for Fd-driven MmOGOR (27.2 moles of product per mole of enzyme per minute) (31). The Michaelis constant K_M describes the concentration of SCoA at which the TOF is at half its maximum and informs on the strength of substrate binding to the enzyme. For our system, K_M for SCoA is $0.050 \pm 0.0086 \text{ mM}$ (Fig. 3*A*), which is similar to that obtained for Fd-driven MmOGOR at $K_M = 0.032 \text{ mM}$ (31). Approximating TOF_{max} as a lower limit to k_{cat} , the ratio of these parameters, $\text{TOF}_{\text{max}}/K_M$, defines the catalytic efficiency, which in our system is $7,000 \text{ s}^{-1} \text{ M}^{-1}$ for SCoA. This is within a factor of 2 of $\text{TOF}_{\text{max}}/K_M$ for SCoA in the natural Fd-driven system, which has a value of $\sim 14,000 \text{ s}^{-1} \text{ M}^{-1}$ at 30°C . Our experiments were carried out at room temperature (22°C) for compatibility with the optical excitation apparatus. The similarity between MmOGOR activity driven by Fd and by NRs suggests that the enzyme function is not adversely impacted by NR binding and, more specifically, that binding of SCoA to the MmOGOR active site is not significantly perturbed by the presence of the CdS NRs.

The kinetics of 2-oxoglutarate production also depend on CO_2 concentration (Fig. 3*B*). The concentrations of CdS NRs and MmOGOR were 44 nM and 100 nM, respectively. The concentration of SCoA was held constant at 100 μM , and the excitation frequency was 890 photons absorbed per NR per second. In this experiment, the MmOGOR TOF reaches a maximum near $39 \pm 3.7 \text{ mol}$ of product per mole of MmOGOR per minute of illumination, obtained using a fit to the Michaelis–Menten model.

Fig. 3. Dependence of MmOGOR TOF on species participating in light-driven 2-oxoglutarate formation using the CdS–MmOGOR system. Solid black lines are fits to the Michaelis–Menten model. (A) MmOGOR TOF dependence on SCoA concentration is similar to that observed in the Fd-driven system. (B) The dependence on concentration of CO_2 suggests that CO_2 binding is weaker than that of SCoA. (C) The dependence of MmOGOR TOF on NR excitation frequency shows both the linear regime where the product formation is limited by the electron flux and the saturation regime where the enzyme limits the turnover.

The measured K_M is larger for CO_2 (3.0 ± 1.0 mM) than for SCoA, implying that CO_2 does not bind as strongly. The weaker binding of CO_2 has also been observed in an OGOR analog, pyruvate:Fd oxidoreductase from *Moorella thermoacetica*, during pyruvate synthesis, suggesting a similarity of the CO_2 binding sites between the 2 enzymes (39). This similarity illustrates the overall biochemical challenge of binding CO_2 , compared to a much larger, more chemically diverse SCoA molecule (or acetyl-CoA in the case of pyruvate synthesis) (39).

Fig. 3C shows the dependence of light-driven MmOGOR TOF on the excitation frequency of the NRs. The concentrations of CdS NRs and MmOGOR were 44 nM and 100 nM, respectively, while SCoA and CO_2 were at 100 μM and 7.8 mM, respectively. At lower excitation frequencies, under 200 photons absorbed per NR per second, the TOF increases linearly, suggesting a regime where the TOF is limited by electron flux from the NRs to OGOR (13). TOF saturates at higher excitation frequencies, where the TOF is limited to 39 ± 3 moles of product per mole of MmOGOR per minute of illumination. In this saturation regime, the product formation is limited by the enzyme turnover. The analogous experiment in the Fd-driven system is the dependence of TOF on Fd concentration, which follows the same Michaelis–Menten functional form, and the TOF_{max} is 30.6 moles of product per mole of enzyme per minute (31). While the saturation due to enzyme turnover at high excitation is expected, the excitation frequency needed to reach that saturation is surprisingly high. The QY of 2-oxoglutarate formation (QY_{OG}), defined as molecules of product formed per 2 photons absorbed (because it takes 2 electrons to make a product molecule), is $\sim 1\%$ in the linear region. This result means that only 1% of photoexcited electrons end up in a 2-oxoglutarate molecule, while the remaining 99% decay by unproductive pathways. This observation is in contrast to QYs of H_2 formation in systems that combine CdS NRs with hydrogenase, where the QYs are on the order of tens of percent (13).

Characterization of ET from CdS NRs to MmOGOR. The upper limit on the value of QY_{OG} is defined by the efficiency of injection of photoexcited electrons from a CdS NR to MmOGOR. Consequently, to shed light on factors that control the value of QY_{OG} , we examine the kinetics of that step using TA spectroscopy. This technique monitors the decay of photoexcited electrons in the NRs on the timescales from 100 fs to ~ 1 μs . The TA spectrum of CdS NRs exhibits a strong bleach feature around 460 nm (Fig. 4, *Inset*), which corresponds to photoexcited electrons at the conduction band edge (32). The bleach signal decays due to relaxation processes such as electron–hole recombination, electron trapping,

and ET (23, 24, 32, 33). Fig. 4A compares the decay of the bleach signal for CdS NRs and a 2.5:1 molar mixture of MmOGOR (1.7 μM) and CdS NRs (670 nM). As has been observed with hydrogenase, bleach decay is faster in the presence of MmOGOR due to ET (22–24). Notably, when SCoA (1.0 mM) is added to the mixture of CdS NRs and MmOGOR (Fig. 4B), the change in the CdS NR bleach decay is much less pronounced, indicating that ET from CdS NRs to MmOGOR is less efficient in the presence of cosubstrate SCoA than when it is absent.

Quantitative modeling of the TA data provides insights into the source of this decreased efficiency. To describe the electron decay kinetics in the mixture of CdS NRs and MmOGOR, we follow a model previously developed for complexes of CdS NRs with hydrogenase (23). The decay of the CdS NR bleach is multiexponential and can be attributed to electron–hole recombination with the rate constant k_0 and electron trapping on the NR surface, which occurs with the rate constant k_{tr} . The trap density is described with a Poisson distribution with the average number of traps $\langle N_{\text{tr}} \rangle$. The additional decay due to ET occurs with a rate constant k_{ET} . A mixture of CdS NRs with MmOGOR leads to a distribution of population of complexes (0, 1, 2, etc., enzymes per NR), which can also be described with a Poisson distribution with the average number of enzymes that can accept electrons per NR abbreviated as $\langle N_{\text{OGOR}} \rangle$ (22–24). The values of these parameters for the TA experiments with and without SCoA are shown in *SI Appendix, Table S2*. As expected, the parameters associated with CdS NR relaxation (k_0 , k_{tr} , $\langle N_{\text{tr}} \rangle$) are similar in both cases (and similar to previously reported values) (23, 24), so we focus on parameters that determine the efficiency of ET, namely $\langle N_{\text{OGOR}} \rangle$ and k_{ET} . For the case of the mixture of CdS NRs with MmOGOR without SCoA added (Fig. 4A), $\langle N_{\text{OGOR}} \rangle$ is 1.4 ± 0.2 and k_{ET} is $2.1 \pm 0.4 \times 10^7 \text{ s}^{-1}$. This value of k_{ET} is of the same order of magnitude as that reported for the CdS NR–hydrogenase system with the same surface-capping ligands (23, 24). The value of k_{ET} is also comparable to k_0 and the rate of electron trapping. In other words, ET from a CdS NR to MmOGOR is in direct kinetic competition with the other electron relaxation processes in the NR. When SCoA is added, $\langle N_{\text{OGOR}} \rangle$ decreases by a factor of 4, to 0.35 ± 0.1 , indicating a factor of 4 decrease in the number of adsorbed enzymes accepting electrons from CdS NRs. The value of k_{ET} is more difficult to compare due to a large error in the fit value ($2.6 \pm 1.3 \times 10^7 \text{ s}^{-1}$) in the presence of SCoA, but it stays within the same order of magnitude in the 2 cases. Overall, the TA data suggest that binding of SCoA to MmOGOR results in a decreased number of enzymes capable of accepting electrons bound per NR.

Characterization of Binding between CdS NRs and MmOGOR. Inspired by the TA data, we characterized the binding between MmOGOR (480 nM) and CdS NRs (250 nM) in the absence and presence of SCoA using DLS, a technique that measures particle hydrodynamic diameter by tracking particle diffusion (Fig. 5 and *SI Appendix, Table S3*). As shown in Fig. 5A, DLS measurements report an average hydrodynamic diameter of CdS NRs of 22.1 ± 6.5 nm, which is comparable to the NR dimensions we measured from transmission electron microscopy (TEM) images (25.0 ± 2.4 nm in length and 4.3 ± 0.4 nm in diameter). Due to the nonisotropic particle shape, the relationship between hydrodynamic diameter measured by DLS and particle dimensions is not straightforward, but DLS is qualitatively consistent with the TEM results. The hydrodynamic diameter of MmOGOR is 10.0 ± 2.0 nm (Fig. 5A), which is consistent with the longest distance of 12.3 nm determined from the crystal structure (31). When MmOGOR and the NRs are mixed at a ratio of 2 enzymes to 1 NR, the average value of hydrodynamic diameter is 61.7 ± 27.0 nm (Fig. 5B). We attribute this larger size to complexation between the CdS NRs and MmOGOR. The large SD is likely due to a distribution of populations of complexes (e.g., 1 enzyme per NR, 2 enzymes per

Fig. 4. Comparison of TA decay kinetics of the CdS NR bleach at 444 nm in the absence and presence of MmOGOR when SCoA is (A) absent and (B) included in the sample mixture. (*Inset*) TA spectrum of CdS NRs at 1-ns time delay. The TA data indicate that ET efficiency decreases when SCoA is added. ΔA = change in probe absorbance due to the pump; λ = wavelength.

Fig. 5. DLS measurements for characterization of CdS NR–MmOGOR binding: (A) CdS NRs and MmOGOR; (B) mixture of CdS NRs and MmOGOR in a 1:2 molar ratio and the same mixture with SCoA added. The increase in average particle hydrodynamic diameter in the CdS–MmOGOR sample is due to complexation between the NRs and MmOGOR. The addition of SCoA leads to dissociation of NR–MmOGOR complexes.

NR, etc.). We caution that the differences in refractive indices between the 2 dissimilar components, strong dependence of scattering intensity on particle size, and the nonisotropic shape of the NRs preclude us from making a quantitative interpretation of the measured hydrodynamic diameter values. Rather, we observe a clear qualitative increase in the average particle size when CdS NRs are mixed with MmOGOR.

When SCoA is present in the CdS NR–MmOGOR mixture, DLS data suggest that less MmOGOR is adsorbed on CdS NRs (Fig. 5B and *SI Appendix, Table S3*). Fig. 5B shows how the hydrodynamic particle diameters change when 50 μ M SCoA is added to a mixture of 250 nM CdS NRs and 480 nM MmOGOR. While the CdS–MmOGOR sample shows an average diameter of 61.7 ± 27 nm, after SCoA addition, 2 populations are detected, both at smaller average diameters (33.2 ± 5.8 and 15.4 ± 2.6 nm). A shift to smaller diameters indicates increased dissociation of CdS–MmOGOR complexes. In combination with the TA data, the DLS data suggest that, while the presence of CdS NRs does not markedly impact the binding of SCoA to the MmOGOR active site (Fig. 3A), the binding of SCoA to the MmOGOR active site strongly impacts the interaction of MmOGOR with CdS NRs. The decrease in the number of bound enzymes accepting electrons, seen in the TA measurements, is consistent with the weaker CdS–MmOGOR interaction seen in the DLS experiments.

Structural Basis for the Substrate-Dependent ET. Our hypothesis for the weaker CdS–MmOGOR interaction once SCoA is bound is based on the structural information from the recent crystallographic study of MmOGOR cocrystallized with 2-oxoglutarate and CoA (31). Once SCoA is bound, the enzyme undergoes a conformational change with domain III (the enzyme domain near the presumed site of CdS NR binding) adopting a “swung-in” conformation (*SI Appendix, Fig. S4*). The swung-in conformation brings residues that interact with the cosubstrate near the active site to be poised for chemical conversion. At the same time, the enzyme surface changes in a way that could impact the interaction with CdS NRs. While the structural changes shown in *SI Appendix, Fig. S4* may appear subtle, the electrostatic interactions at the surface could change substantially enough to impact binding. Furthermore, the distance between the [4Fe–4S] cluster of MmOGOR, which relays the injected electron to the active site, and the CdS NR surface could change enough to impact k_{ET} (which usually depends exponentially on distance) (24, 41, 42). Finally, this conformational change may impact the driving force for electron injection into the [4Fe–4S] cluster of MmOGOR. We therefore propose that the conformational change

in MmOGOR upon SCoA binding impacts the strength of the NR–enzyme interactions and impacts the ET pathway.

MmOGOR, like many other thiamine pyrophosphate-dependent enzymes, consists of 2 active sites which are known to be structurally and functionally asymmetrical during catalysis (43). Based on the K_M value for SCoA (Fig. 3A), at the SCoA concentration of 1 mM, on average, one of the 2 active sites has an SCoA molecule bound in the TA experiment. Because the interaction with the SCoA-bound subunit is weaker, the CdS NR is more likely to be bound to the other subunit, such that ET sends the electron to the active site that is not poised for catalysis. This asymmetry creates a competition for the electron in which the active site with SCoA bound is less likely to receive it. In other words, while the overall ET efficiency decreases when SCoA is added to the CdS–MmOGOR mixture, that decrease is disproportionately borne by the side of the enzyme that is poised for catalysis. The overall result of conformational change upon SCoA binding is decreased efficiency of ET from an NR to the MmOGOR active site with SCoA bound, resulting in a relatively low QY_{OG} .

Thanks to the broad tunability of properties of semiconductor nanocrystals, it may be possible to modify their interaction with MmOGOR to make it more conducive to ET. As a proof of principle, in *SI Appendix, Table S4*, we show that MmOGOR can be driven by CdS and CdSe quantum dots (QDs) with diameters of 4.3 and 3.1 nm, respectively. These particles are closer in size to Fd from *Pseudomonas aeruginosa*, a homolog of MmFd1, which has the approximate dimensions of $4 \times 3 \times 3$ nm (44). Because these particles are ~ 15 times smaller in volume than the NRs used above, they absorb less light and therefore make less 2-oxoglutarate. The relative QY of product formation, which normalizes for photon absorption, is highest for CdSe QDs with 3.1 nm diameter (*SI Appendix, Table S4*). Both the particle composition and size impact the driving force for ET. Particle size also determines how it fits in the Fd-binding pocket of the enzyme. Further work is needed to disentangle the effects of particle composition, size, and shape to identify the optimal configuration for driving MmOGOR catalysis with light. Furthermore, it has been shown that the ligands capping nanocrystal surfaces play a critical role in ET to enzymes by impacting factors such as donor–acceptor distance (and therefore electronic coupling) as well as effective particle size (24). Ligands can also change the driving force for ET (35). We expect that nanocrystal surface functionalization strategies can be developed to control the ET process. Finally, strategies for covalently binding hydrogenases to CdS surfaces of nanocrystals have been reported before (26, 45) and may be adaptable for directing nanocrystal–MmOGOR binding and the subsequent ET. Development of new electrostatic and covalent binding strategies should take into account the enzyme domain movement during catalysis. Beyond the C–C bond formation example reported here, the domain motions that appear critical to timing of ET steps may be a more general phenomenon in complex redox biocatalysts, requiring multiple substrates as well as the control of proton and ET steps. Our work described here highlights the complexities that arise when driving enzyme catalysis of large molecule transformations with photoexcited nanocrystals and provides an initial set of guiding principles for the design of the next generation of nanocrystal–enzyme architectures.

Conclusions

We reported and examined the photochemical formation of 2-oxoglutarate from CO_2 and SCoA in a mixture of CdS NRs and MmOGOR. CdS NRs serve as light absorbers that transfer electrons to MmOGOR, where catalysis occurs. We find that interaction with NRs does not adversely impact the catalytic activity of the enzyme. However, the QY of 2-oxoglutarate production is relatively low, meaning that most photoexcited electrons are wasted rather than converted to the product. Our work reveals that the product formation is limited by conformational

changes in the enzyme that occur when the cosubstrate SCoA is bound. These changes impact the interactions between CdS NRs and MmOGOR in a manner that decreases ET efficiency to the active site poised for catalysis. In other words, the enzyme modifies the electron injection during its catalytic cycle in a manner that reduces the overall efficiency. Our work brings to light challenges in the design of systems where complex enzyme catalysis is driven by photoexcited electrons from semiconductor nanocrystals. In particular, we reveal a tug of war between the processes required for enzyme catalysis and the need for nanocrystal–enzyme interaction that enables electron flux to the catalyst. This interaction is potentially tunable via modification of nanocrystal properties. Our work also raises the question of whether similar modification of ET is also present in the natural system, where the electrons for catalysis come from an Fd partner. As such, the system that uses nanocrystals as electron donors can serve as a tool to learn about the catalytic behavior of this family of enzymes.

Materials and Methods

The CdS NRs synthesis is detailed in *SI Appendix, section I*. The as-synthesized CdS NRs are capped with nonpolar ligands and are soluble in organic

solvents. For coupling with MmOGOR in aqueous solution, we exchange these native ligands to 3-MPA ligands as detailed in *SI Appendix, section I*. Expression and purification of MmOGOR was reported previously (31) and is detailed in *SI Appendix, section I*. MS measurements and the product formation assay using GDH are described in detail in *SI Appendix, section II*. TA spectroscopy and kinetic modeling are described in *SI Appendix, section III*. DLS measurements are detailed in *SI Appendix, section IV*.

Data Availability. All data are included in the manuscript and *SI Appendix*.

ACKNOWLEDGMENTS. Seed funding for this work was provided by a Scialog Collaborative Innovation Award from the Research Corporation for Science Advancement. The work was subsequently supported by the US Department of Energy, Office of Science, Basic Energy Sciences, under Award DE-SC0010334, which funded nanocrystal synthesis, photochemical 2-oxoglutarate formation, and ET studies. Enzyme preparation was supported by the US Department of Energy, Office of Science, Basic Energy Sciences, under Award DE-SC0012598. We thank Dr. James Utterback for help with kinetic modeling of TA data, Dr. Annette Erbse for help with the DLS experiments carried out at the Shared Instruments Core Facility in the Department of Biochemistry, University of Colorado (CU) Boulder, and Dr. Danijel Djukovic for assistance with MS experiments carried out at CU Boulder on an instrument funded by NIH Grant S10-RR026641.

1. A. M. Appel *et al.*, Frontiers, opportunities, and challenges in biochemical and chemical catalysis of CO₂ fixation. *Chem. Rev.* **113**, 6621–6658 (2013).
2. J. A. Cracknell, K. A. Vincent, F. A. Armstrong, Enzymes as working or inspirational electrocatalysts for fuel cells and electrolysis. *Chem. Rev.* **108**, 2439–2461 (2008).
3. S. Sultana, P. C. Sahoo, S. Martha, K. Parida, A review of harvesting clean fuels from enzymatic CO₂ reduction. *RSC Advances* **6**, 44170–44194 (2016).
4. A. Magnuson *et al.*, Biomimetic and microbial approaches to solar fuel generation. *Acc. Chem. Res.* **42**, 1899–1909 (2009).
5. C. Léger, P. Bertrand, Direct electrochemistry of redox enzymes as a tool for mechanistic studies. *Chem. Rev.* **108**, 2379–2438 (2008).
6. D. Umeno, A. V. Tobias, F. H. Arnold, Diversifying carotenoid biosynthetic pathways by directed evolution. *Microbiol. Mol. Biol. Rev.* **69**, 51–78 (2005).
7. L. C. Seefeldt, B. M. Hoffman, D. R. Dean, Mechanism of Mo-dependent nitrogenase. *Annu. Rev. Biochem.* **78**, 701–722 (2009).
8. S. W. Ragsdale, Pyruvate ferredoxin oxidoreductase and its radical intermediate. *Chem. Rev.* **103**, 2333–2346 (2003).
9. A. Bachmeier, B. Sirtanaratkul, F. A. Armstrong, “Enzymes as exploratory catalysts in artificial photosynthesis” in *From Molecules to Materials: Pathways to Artificial Photosynthesis*, E. A. Rozhkova, K. Ariga, Eds. (Springer International, Cham, Switzerland, 2015), pp. 99–123.
10. R. M. Evans *et al.*, The value of enzymes in solar fuels research—Efficient electrocatalysts through evolution. *Chem. Soc. Rev.* **48**, 2039–2052 (2019).
11. S. H. Lee, D. S. Choi, S. K. Kuk, C. B. Park, Photobiocatalysis: Activating redox enzymes by direct or indirect transfer of photoinduced electrons. *Angew. Chem. Int. Ed. Engl.* **57**, 7958–7985 (2018).
12. N. Kornienko, J. Z. Zhang, K. K. Sakimoto, P. Yang, E. Reisner, Interfacing nature's catalytic machinery with synthetic materials for semi-artificial photosynthesis. *Nat. Nanotechnol.* **13**, 890–899 (2018).
13. K. A. Brown, M. B. Wilker, M. Boehm, G. Dukovic, P. W. King, Characterization of photochemical processes for H₂ production by CdS nanorod-[FeFe] hydrogenase complexes. *J. Am. Chem. Soc.* **134**, 5627–5636 (2012).
14. Y. S. Chaudhary *et al.*, Visible light-driven CO₂ reduction by enzyme coupled CdS nanocrystals. *Chem. Commun. (Camb.)* **48**, 58–60 (2012).
15. K. A. Brown *et al.*, Light-driven dinitrogen reduction catalyzed by a CdS:nitrogenase MoFe protein biohybrid. *Science* **352**, 448–450 (2016).
16. K. A. Brown, S. Dayal, X. Ai, G. Rumbles, P. W. King, Controlled assembly of hydrogenase-CdTe nanocrystal hybrids for solar hydrogen production. *J. Am. Chem. Soc.* **132**, 9672–9680 (2010).
17. L. Zhang, M. Can, S. W. Ragsdale, F. A. Armstrong, Fast and selective photoreduction of CO₂ to CO catalyzed by a complex of carbon monoxide dehydrogenase, TiO₂, and Ag nanoclusters. *ACS Catal.* **8**, 2789–2795 (2018).
18. M. G. Bawendi, M. L. Steigerwald, L. E. Brus, The quantum mechanics of larger semiconductor clusters (“quantum dots”). *Annu. Rev. Phys. Chem.* **41**, 477–496 (1990).
19. X. Peng *et al.*, Shape control of CdSe nanocrystals. *Nature* **404**, 59–61 (2000).
20. C. Burda, X. Chen, R. Narayanan, M. A. El-Sayed, Chemistry and properties of nanocrystals of different shapes. *Chem. Rev.* **105**, 1025–1102 (2005).
21. P. W. King, Designing interfaces of hydrogenase-nanomaterial hybrids for efficient solar conversion. *Biochim. Biophys. Acta* **1827**, 949–957 (2013).
22. M. B. Wilker *et al.*, Electron transfer kinetics in CdS nanorod-[FeFe]-hydrogenase complexes and implications for photochemical H₂ generation. *J. Am. Chem. Soc.* **136**, 4316–4324 (2014).
23. J. K. Utterback *et al.*, Competition between electron transfer, trapping, and recombination in CdS nanorod-hydrogenase complexes. *Phys. Chem. Chem. Phys.* **17**, 5538–5542 (2015).
24. M. B. Wilker *et al.*, Role of surface-capping ligands in photoexcited electron transfer between CdS nanorods and [FeFe] hydrogenase and the subsequent H₂ generation. *J. Phys. Chem. C* **122**, 741–750 (2018).
25. B. L. Greene, C. A. Joseph, M. J. Maroney, R. B. Dyer, Direct evidence of active-site reduction and photodriven catalysis in sensitized hydrogenase assemblies. *J. Am. Chem. Soc.* **134**, 11108–11111 (2012).
26. B. Chica *et al.*, Balancing electron transfer rate and driving force for efficient photocatalytic hydrogen production in CdSe/CdS nanorod-[NiFe] hydrogenase assemblies. *Energy Environ. Sci.* **10**, 2245–2255 (2017).
27. D. A. Bazylinski *et al.*, *Magnetococcus marinus* gen. nov., sp. nov., a marine, magnetotactic bacterium that represents a novel lineage (Magnetococcaceae fam. nov., Magnetococcales ord. nov.) at the base of the Alphaproteobacteria. *Int. J. Syst. Evol. Microbiol.* **63**, 801–808 (2013).
28. T. J. Williams, C. L. Zhang, J. H. Scott, D. A. Bazylinski, Evidence for autotrophy via the reverse tricarboxylic acid cycle in the marine magnetotactic coccus strain MC-1. *Appl. Environ. Microbiol.* **72**, 1322–1329 (2006).
29. G. Fuchs, Alternative pathways of carbon dioxide fixation: Insights into the early evolution of life? *Annu. Rev. Microbiol.* **65**, 631–658 (2011).
30. B. Li, S. J. Elliott, The catalytic bias of 2-oxoacid:ferredoxin oxidoreductase in CO₂: Evolution and reduction through a ferredoxin-mediated electrocatalytic assay. *Electrochim. Acta* **199**, 349–356 (2016).
31. P. Y.-T. Chen, B. Li, C. L. Drennan, S. J. Elliott, A reverse TCA cycle 2-oxoacid:ferredoxin oxidoreductase that makes C-C bonds from CO₂. *Joule* **3**, 595–611 (2019).
32. K. Wu, H. Zhu, Z. Liu, W. Rodriguez-Córdoba, T. Lian, Ultrafast charge separation and long-lived charge separated state in photocatalytic CdS-Pt nanorod heterostructures. *J. Am. Chem. Soc.* **134**, 10337–10340 (2012).
33. V. I. Klimov, Spectral and dynamical properties of multiexcitons in semiconductor nanocrystals. *Annu. Rev. Phys. Chem.* **58**, 635–673 (2007).
34. H. W. Tseng, M. B. Wilker, N. H. Damrauer, G. Dukovic, Charge transfer dynamics between photoexcited CdS nanorods and mononuclear Ru water-oxidation catalysts. *J. Am. Chem. Soc.* **135**, 3383–3386 (2013).
35. P. R. Brown *et al.*, Energy level modification in lead sulfide quantum dot thin films through ligand exchange. *ACS Nano* **8**, 5863–5872 (2014).
36. J. K. Grady, N. D. Chasteen, D. C. Harris, Radicals from “Good’s” buffers. *Anal. Biochem.* **173**, 111–115 (1988).
37. L. Pieuille *et al.*, Multiple orientations in a physiological complex: The pyruvate-ferredoxin oxidoreductase-ferredoxin system. *Biochemistry* **43**, 15480–15493 (2004).
38. R. C. Valentine, Bacterial ferredoxin. *Bacteriol. Rev.* **28**, 497–517 (1964).
39. C. Furdul, S. W. Ragsdale, The role of pyruvate ferredoxin oxidoreductase in pyruvate synthesis during autotrophic growth by the Wood-Ljungdahl pathway. *J. Biol. Chem.* **275**, 28494–28499 (2000).
40. M. Yamamoto, T. Ikeda, H. Arai, M. Ishii, Y. Igarashi, Carboxylation reaction catalyzed by 2-oxoglutarate:ferredoxin oxidoreductases from *Hydrogenobacter thermophilus*. *Extremophiles* **14**, 79–85 (2010).
41. H. B. Gray, J. R. Winkler, Electron transfer in proteins. *Annu. Rev. Biochem.* **65**, 537–561 (1996).
42. C. C. Moser, J. M. Keske, K. Warncke, R. S. Farid, P. L. Dutton, Nature of biological electron transfer. *Nature* **355**, 796–802 (1992).
43. E. M. Ciszak, L. G. Korotchkina, P. M. Dominiak, S. Sidhu, M. S. Patel, Structural basis for flip-flop action of thiamin pyrophosphate-dependent enzymes revealed by human pyruvate dehydrogenase. *J. Biol. Chem.* **278**, 21240–21246 (2003).
44. P. Giasas *et al.*, The structure of the 2[4Fe-4S] ferredoxin from *Pseudomonas aeruginosa* at 1.32-Å resolution: Comparison with other high-resolution structures of ferredoxins and contributing structural features to reduction potential values. *J. Biol. Inorg. Chem.* **11**, 445–458 (2006).
45. C. Hamon *et al.*, Synthesis and enzymatic photo-activity of an O₂ tolerant hydrogenase–CdSe@CdS quantum rod bioconjugate. *Chem. Commun. (Camb.)* **50**, 4989–4992 (2014).