Photocatalytic Bionanomaterial for Renewable Energy

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PHOTOCATALYTIC BIONANOMATERIALS FOR RENEWABLE ENERGY

by

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B.A., Zhejiang University, 2012

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has been approved for the Department of Chemical and Biological Engineering

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The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
Photocatalytic Bionanomaterial for Renewable Energy

Thesis directed by Professor Jennifer N. Cha

Photocatalytic materials and systems have been studied in recent years for generating fuels from renewable energy resources. My doctoral research has focused on studying the use of biological mediated self-assembly to fabricate well-organized Z-scheme nanoparticle systems for both enhancing water splitting as well as CO$_2$ reduction. Using DNA as a template in nanomaterial assembly allows the ability to guide the positioning of each nanomaterial to build an effective photocatalytic Z-scheme. In this dissertation, I discuss the use of DNA as a structure directing agent to assemble different nanocrystals for water splitting to improve H$_2$ production upon solar irradiation. In addition, I report the utilization of such nanocrystal Z-scheme system for showing enhanced reduction of CO$_2$, and also the application of alcohol dehydrogenase in further reduction of formaldehyde to methanol. The physical and chemical characterization methods were used to analyze these systems, and the results of their performance on different reactions will be presented in this assay.
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1 Introduction

1.1 Nanomaterial for Renewable Energy Generation

Energy is extremely crucial for human development, and it’s also the essential element in our everyday life. As the population, urbanization and modernization increased in human development, the demand for energy increased rapidly. The main global primary energy resources are composed of traditional fossil fuels, nuclear power and renewable energy, in which the fossil fuels still provide the majority of the global energy demands nowadays.\(^1\) However, with the depletion of the fossil fuel reserves, the present energy systems are paying attention more and more to the other energy sources. Regardless to the limited fossil fuel reserves, the enormous impacts on the environment and the climate from the fossil fuels is another issue. Especially in China, the high-speed economic development and tremendous energy consumption brings not only the challenges of energy supply, but also the serious environment issues. China is the largest energy consumer and CO\(_2\) emitting country in the world now, and set the target of decreasing the CO\(_2\) emissions per unit of GDP by 40-45% of 2005 levels by 2020.\(^2\) That requires abundant development and research on the renewable energy to help changing the inappropriate energy consumption structure.

Many methods have been used to tackle the challenges of the renewable energy generation. Most popular methods are harvesting solar light, and converting it to electricity or fuels, especially fuels that can be stored and transported. But the efficiency of current renewable energy technologies is not competitive with the traditional fossil energy technologies.
Semiconductor, as a material can absorb light and generate electron and hole pairs, can be used in converting solar energy to either electricity or fuels. The photogenerated electrons and holes will be separated in the semiconductor, and move out of the material to either go into an external circuit in photovoltaic devices, or start a reduction/oxidation reaction on the surface of the semiconductor to generate useable fuels. Recently, with the rising interests in nanomaterial, the development of semiconductor nanomaterial also opens a new door to the development of renewable energy technology. Nanomaterial is the materials with at least one dimension within 1-100nm in physical size. It could be in different shapes, from nanoparticle, to nanorods and nanowires. As the size of the material decreases, the surface-to-volume ratio of the material will increase, which brings some unique properties of the materials. The atoms on the surface are usually very active and can bond with adjacent molecules and lower the total energy. This property can cause an increased chemical activity, thus start a catalysis reaction. This property of semiconductor nanomaterial brings a possibility of using small amount of materials to achieve high activities in reaction which is particularly attracting for economic and environmental consideration. The other advantage of nanomaterial is its intrinsic properties are affected by their size. For semiconductor nanomaterial, it means the electronic band gap will increase as the size of the nanomaterial decreases. As the electronic transitions between the valence band and conduction band is the basis of the optical absorption and emission of a semiconductor, the control of the optical property of the semiconductor can be achieved through tuning the size of the nanomaterial. In addition, the transport of electrons and holes to the surface of the semiconductor for charge separation and surface reaction will be easier in nanomaterial, as the diffusion length gets shorter than that in the bulk material.
Because of all the advantages of semiconductor nanomaterial we’ve been discussed above, a lot of studies have been done on it for renewable generation. TiO$_2$ nanoparticle is one of the most commonly used materials in photocatalysis system. TiO$_2$ powder was discovered in the early 20$^{th}$ century to show photoactivity, and was used for photodegradation of various pollutants. Then in the 21$^{st}$ century, TiO$_2$ nanoparticles were widely used in photocatalysis systems.\textsuperscript{4,5} TiO$_2$ nanoparticles have a band gap 3.0-3.4eV which will make it absorb mainly the UV light. But the wide band gap also enables it to suit for more photocatalysis reactions, as the conduction band/valance band has enough energy to reduce/oxidize more molecules. In addition, the TiO$_2$ nanoparticles show the advantage of cost-effective, chemical stable and biocompatible over the other semiconductor nanomaterial. Researchers started using TiO$_2$ nanoparticles to split water to generate oxygen and hydrogen as fuel, and it’s also been shown to use for CO$_2$ reduction.\textsuperscript{4,6} Metal-doped and non-metal doped, such as Nd, N and C, TiO$_2$ nanomaterials have been developed later on to enhance its photocatalytic performance.\textsuperscript{5} These doped TiO$_2$ can be considered as second and third generation of TiO$_2$ used in photocatalysis. The photoactivity of doped TiO$_2$ depends on several factors including dopant concentration, the energy level of the dopants and the distribution of the dopants, which have gained a lot of interests. Metal doping on TiO$_2$ has been studied to show that it creates mid-gap states or even change the valence band, thus narrowing the bandgap. For example, Li an co-workers found that Nd$^{3+}$-doped TiO$_2$ nanoparticles can narrow the bandgap by 0.55eV because of the new electron states introduced into the bandgap of TiO$_2$ by Nd$^{3+}$ ions.\textsuperscript{7} Bandgap narrowing of TiO$_2$ nanoparticles can also be achieved through non-metal doping. The narrow bandgap of TiO$_2$ nanoparticles shifts the absorption spectrum to the lower energy
region, or the visible light region, which provide more possibilities of using TiO$_2$ nanoparticles as light harvesting material and photocatalytic material.

Despite all the studies about TiO$_2$ nanomaterial, the overall efficiency of it in renewable energy generation still has a lot to improve. Tremendous new nanomaterial has been developed to improve the photocatalytic performance. From the solar energy utilization point of view, the bandgap of the new photocatalysts needs to absorb mainly the visible light. A few chalcogenides and metal oxides were developed initially, including CdS and WO$_3$. Metal chalcogenides such as CdS and CdSe show the ability of splitting water to H$_2$ and O$_2$ under visible light. But because the S$^{2-}$ and Se$^{2-}$ anions are more likely to be oxidized than water, both the photocatalysts are not stable in water solution due to the degradation. While at the same time, the WO$_3$ don’t have the ability to reduce H$^+$ to H$_2$. To date, numerous efforts have been made to develop the photocatalysts that can work under visible light. Maeda and Domen’s group has shown many attempts on developing such materials, especially (oxy)nitrides and oxysulfides, as photocatalysts for overall water splitting. In their studies, (Ga$_{1-x}$Zn$_x$)(N$_{1-x}$O$_x$) and (Zn$_{1+x}$Ge)(N$_2$O$_x$) solid solutions have shown the overall water splitting ability under visible light without obvious degradation.

To achieve a better photocatalytic performance with the semiconductor nanomaterials, the reduction and oxidation reaction on the active site is also very important other than the photogenerated electrons and holes separation. Co-catalysts have also been studied to further improve the catalytic activity and selectivity of the system. The co-catalysts are usually noble metal (Pt, Rh et al) or transition-metal oxide (NiO$_2$, RuO$_2$ et al), and are usually loaded onto the light harvesting material as nanoparticles. The co-catalysts can reduce the activation energy for surface chemical reactions and provide active sites, thus promote the photocatalytic reaction on the
Semiconductor nanomaterials. Beside to offer active site for redox reaction, co-catalysts loaded on a photocatalysts can also extract charge carriers from the photocatalysts and offer an electron sink to improve multi-electron reduction reactions. It has been shown that SrTiO$_3$ nanoparticles can be modified with Ru nanoparticles on the surface as co-catalysts and enhance the proton reduction ability.$^{10}$ TaON has also been reported to have RuO$_2$ loaded and promote water oxidation reaction, and have Pt loaded to promote water reduction reaction.$^{11}$ The selection of co-catalysts on photocatalysts need to take in consideration of different aspects. As both Pt and Ru can be a co-catalyst for H$_2$ evolution, Ru loaded ZrO$_2$/TaON shows less activity in H$_2$ evolution in the presence of IO$_3^-$/I$^-$ aqueous system. And the size of Pt nanoparticles affects the activity, as the smaller size in this case contributes more in the enhancement of H$_2$ evolution. In some cases, such as the CO$_2$ reduction system with C$_3$N$_4$ as light harvesting material, the metal loading onto the photocatalysts need to be an electron sink, but not an efficient co-catalysts for H$_2$ evolution.$^{12}$ In this case, metal nanoparticles such as Ag, Cu, Au and Pt can all be loaded on C$_3$N$_4$ as an electron sink, but because of Pt acting as a well-known co-catalysts for H$_2$ evolution which is a competing reaction with CO$_2$ reduction, it is eliminated from the list.

The last to mention in the renewable energy conversion with nanomaterial is the use of biomaterial, especially enzyme. The advantage of using enzyme instead of photocatalysts is the reaction selection of enzyme. As a complicated biomaterial, enzyme can only catalyze certain reaction, which is an effective way inhibit strong competing reaction in photocatalysis reaction. For example, in CO$_2$ reduction, the strong competing reaction is H$_2$ generation, as they have the similar redox potential, while most photocatalyst has challenges in the product selection. But enzyme can tackle this problem. Also taking the interesting topic of CO$_2$ reduction as an example,
many studies have been done with enzyme, and people are trying to develop more efficient system with enzyme to reduce CO\textsubscript{2}, including combining photocatalysts with enzyme\textsuperscript{13-17}. But the efficiency of enzyme in those most desired reactions in renewable energy conversion still has a lot to improve. Also the stability and mild reaction condition limit the application of enzyme.

### 1.2 Z-Scheme Photocatalytic Reaction

Despite all the modification and development of the semiconductor nanomaterial, there’s still some intrinsic property of semiconductor nanomaterial that becomes obstacle in the development of a photocatalyst driven by one-step photoexcitation. It is difficult for a single-component photocatalyst to possess a wide light absorption range to maximize the utilize of solar light which requires narrow bandgap, and also has strong redox ability which requires wide bandgap.

What we can learn from nature is not only the small systems as enzyme. In nature, the green plants also use solar energy to convert H\textsubscript{2}O and CO\textsubscript{2} into O\textsubscript{2} and carbohydrate as fuel, which is known as photosynthesis, also known as the Z-scheme photosynthesis. In the Z-scheme photosynthesis, there’re two steps of photoexcitation included. Photosystem I (PS I) and photosystem II (PS II) harvest solar energy and excite electrons to the excitation state. The two photosystems are connected with a series of electron mediators. Water oxidation takes places in PS II. The electrons excited in PS II flowed through the electron mediators to the ground state in PS I, then PS I pump electrons from ground state to excited state which has higher energy than the excite state in PS II, and transport them through another series of electron mediators to reduce co-enzyme NADP\textsuperscript{+} to NADP which is used to convert CO\textsubscript{2} into carbohydrate in the dark reaction. The efficiency of charge separation in PS I is close
to 100% in this system, which is the ultimate goal that researchers desired to achieve in photocatalysts.$^{18,19}$

To mimic the nature photosynthesis system, researchers replace the PS II and PS I in the nature photosynthesis with two types of material structures which play the same role to generate excited electrons. A dye molecule or semiconductor is usually employed as excitation site, and one behaves as electron donor and the other behaves as electron acceptor. Same as the nature photosynthesis system, this system enhances the charge separation efficiency, lower down the possibility of back electron transfer and electron-hole recombination. Besides, the two materials can have narrow bandgap or the gap between the highest occupied molecular orbital and the lowest unoccupied molecular orbital, as long as two gaps have overlapping section, and conduction band of electron acceptor material has enough energy to do the reduction work, while the valence band of electron donor material is enough to start the oxidation reaction. The collaboration of two semiconductors solves the problem of co-exist of efficient light utilization and the high redox activity in one system. In addition to the energy level, the stability of the artificial photosynthesis system is improved, and both photocatalyst can be more carefully chosen to fit into reduction and oxidation reaction. Taken water splitting as an example, the two photocatalyst materials don’t need to have a bandgap larger than 1.83eV while also fit with both water oxidation and reduction redox potential, which is required in single-component water splitting system. The requirements of the system lowered down to 1) the valence band of electron donor material is more positive than water oxidation potential (+0.82V); 2) the conduction band of electron acceptor material is more negative than water reduction potential (-0.41V). This system lowers down the requirement for choosing
the photocatalytical materials, and enables the use of photocatalysts with better catalytic activity.

A lot of materials have been developed in Z-scheme photosynthesis, especially water splitting as the most interested reaction. Those include Pt/SrTiO$_3$:Rh, TaON, BiVO$_4$, and many more doped or seeded semiconductors. The TiO$_2$ and WO$_3$ as mentioned before, are used as water oxidation material at the beginning. Even TiO$_2$ nanoparticle is combined with Pt loaded TiO$_2$ nanoparticle to form the full water splitting system, in which the Pt/TiO$_2$ serves as H$_2$ evolution photocatalyst. Both TiO$_2$ and WO$_3$ have selectivity of water oxidation even in the presence of electron donors such as Fe$^{2+}$ or I$^-$, which are thermodynamically more likely to be oxidized than water, because of the intrinsic surface properties of the two materials. In another case, the Z-scheme system can be construct with the same SrTiO$_3$ semiconductor with different doping materials. The Na-and V-codoped Ru/SrTiO$_3$ functions as O$_2$ evolution photocatalysts, while the Rh doped Ru/SrTiO$_3$ functions as H$_2$ evolution photocatalysts.

But beside the selection of the two photocatalysts, the electron transfer between the two photocatalysts is the major issue in the Z-scheme system. People have done a lot of researches on improving the charge transport between the two particles. One method is to bring the two particles close to enhance the interparticle electron transfer. By changing the system condition, like pH value, the particles can get aggregate thus shorten the interparticle distance. Or using the direct way of growing one semiconductor on to the other one to form Z-scheme system without distance between the two particles. But both of them lowered the surface area of nanoparticles, and may also bring in the complexity of finding new synthetic method.
Using redox mediator to shuttle between the two particles is another method, bring electrons from one material to the other.\textsuperscript{23,10} The shuttle redox mediator now is the most common way of achieving the electron transfer between the two semiconductors nowadays. The most applied redox mediators are $\text{Fe}^{3+}/\text{Fe}^{2+}$ and $\text{IO}_3^-/\text{I}^-$, while the other mediators such as $\text{NO}_3^-/\text{NO}_2^-$ and $\text{Co}^{3+}/\text{Co}^{2+}$ have also been employed. But this method highly depends on the diffusion of the mediator and might also compete with the desire reduction and oxidation reaction. Thus the system activity has a volcano-type of relationship with the concentration of the redox mediators.\textsuperscript{9} At low concentration, the increasing of mediator concentration improves the photocatalytic reaction, while at high concentration, the increasing of mediator concentration inhibit the photocatalytic reaction due to the competitive oxidation or reduction reaction with the desired reactions. Such undesired back reaction is the main drawback of the ion redox mediators.

Later on, more and more researches start to focus on using solid metal as electron transfer material between two nanoparticles.\textsuperscript{24,25} The metal in between the two semiconductors forms the Ohmic contact with low contact resistance, then provide a place for the electrons from PSII and holes form PSI to combine, thus separate the electrons and holes in the whole system. With this redox mediator free system, the back reaction caused by the redox mediator can be eliminated. The first all-solid-state Z-scheme system is developed with $\text{TiO}_2$ and $\text{CdS}$ as two photocatalysts, and Au in between them as the electron transfer route.\textsuperscript{25} It’s achieved through depositing Au nanoparticles on the $\text{TiO}_2$ surface, and growing CdS shells on AuNPs. The $\text{TiO}_2$-Au-CdS system shows high photocatalytic activity in water splitting, and proved that the AuNPs promote charge separation in the system. Also to improve the light absorption of $\text{TiO}_2$, C-doped TiO$_2$ is employed to enhance the
visible light absorption. Later on, more and more all-solid-state Z-scheme system has been reported. The conductor between the two semiconductors have more and more choices, such as Ag, Cd, W, Pt and Cu. They are either using the different metal conductor, or using the same metallic element with donor semiconductor or acceptor semiconductor which might help the electron transfer through the solid-solid interface. Beside the electron and hole combination place, AuNP has also been reported to have surface plasma resonance and can respond to the visible light. Thus the metal mediator might also serves as a photosensitizer when the semiconductor photocatalysts cannot absorb visible light well. In addition to synthesize metal nanoparticles between the two catalysts, low cost metal oxide and nonmetal materials with good conductivity can also be used in the all-solid-state system. Reduced graphene oxide is one of the materials, and can absorb the semiconductor photocatalysts on it which simplified the synthesis process. It’s been used in water splitting system, and also been used in the CO\textsubscript{2} reduction system. But as the graphene oxide nowadays is still under developing that it’s hard to avoid aggregation in the solution, this method is still not well-defined.

Other than the traditional semiconductor method, molecule is also frequently used in Z-scheme system. Many dye molecules are photosensitive and can generate electrons and holes under illumination. They are repeatedly used in photovoltaic devices, but are also able to be used in the artificial photosynthetic system. They have the advantage of easily diffusing in the solution and allow a homogeneous system to be built, but the stability of the system and the products separation still remains to be enhanced. Last, taking the construction form of artificial photosynthetic system into consideration, there’re mainly two types of Z-scheme system, on electrode or in
solution. The system in solution have a better diffusion ability, while the system on electrode has the advantage of collecting products and separation of products.\textsuperscript{19}

Zscheme artificial photosynthetic system has been studied for decades, and a lot of knowledge has been gain from it. Now with more technology has been risen up in nanomaterials field, it brings us more possibility of delicate study of the Zscheme system and the affecting factors.

1.3 Biomaterial in Nanotechnology

DNA, the nucleic acid, is used as genetic material in nature, but it has been found a significant role now as structure material. Although each base in DNA can pair with every other base, the Watson-Crick base pairing is more preferred. The selfassociation of complementary nucleic acid molecules is the fundamental of all the DNA structure nanotechnology. The hybridization between two single strand DNA can forms into double strand DNA, which has a persistent length as 30nm. These features motivated many researchers in nanotechnology filed to take advantages of it and manipulate them for construction purpose, especially after the synthesis of DNA becomes more commercially available. By controlling the distance and orientation among nanomaterials to build complicated structure on the nanometer scale, new optical, electrical or magnetic characteristic will be created in the materials which will change the way people building devices. Nature is providing people a tool box with plenty of choices.

DNA can be used for the construction of 1-D, 2-D or even 3-D structure. The simplest DNA assembly includes just two nanostructures. Xu et al has reported using single hairpin strand DNA to connect two gold-silver core-shell nanoparticles to form a switchable nanodumbell structure.\textsuperscript{30} In the absence of complementary DNA strand, the single strand DNA forms hairpin and shorten the distance between the two
nanoparticles, while in the presence of the complementary DNA, the single strand DNA hybridizes with the complementary DNA to form linear DNA and increase the interparticle distance. The differences of interparticle distance changes the surface-enhanced Raman signal and can be used bio-sensing devices. Tel-Vered et al has reported using DNA as scaffolds to structural ordering rely units and photoactive semiconductor nanoparticles or molecular photosensitizers on electrode surfaces. They proved that with DNA templates, they can sequentially order the different materials, and further control the charge separation and the resulting of photocurrent, and the current direction. In addition, they demonstrate that it provides a “click-type” method, to combine the components, switch the position, and cleave off components on DNA with endonuclease-induced scission. These result shows a powerful application of DNA with all its binding specific properties. DNA has also been reported as a template to promote chemical reaction. The aniline-terminated DNA strand can form template with hydrazine-terminated DNA, and increase the rate of hydrazone formation as a catalyst, and increase the rate to at most more than 85-fold above that of the uncatalyzed reaction.

To expend the utilization of DNA as a structural material, and also take full advantages of the specific bonding among DNA, scaffolded DNA origami is built up. DNA origami is using numerous short strand DNA to fold onto single long strand DNA, to form a desired shape, either in 2-D or in 3-D. These shapes are always around 100nm, and obtain much more complexity we can get from other templating materials but synthesized with simply one-pot method. This is a great example shows DNA is the material that can be programed and delicate designed base on its specific bonding. DNA origami shows a new door to self-assembly, which can be applied to create complex nanosystem, and examining the effects of spatial organization which
might change the electronic or plasmonic properties of the materials. People have shown that it’s able to organize AuNP chain structure by triangle DNA origami with well-controlled orientation and sub-10nm spacing. This structure may be used to enhance local-field.34 Beside assemble nanoparticles on just one DNA origami within 100nm, people also showed the possibility of creating large areas of patterned nanoparticles by combing the DNA origami templating method with the top-down lithography method.35 By modifying the substrate surface with lithography method, 100nm sized DNA origami can be absorbed on to the surface in pattern. As the nanoparticles can be organized on DNA origami with sub-10nm dimensions, it’s able to achieve sub-10nm dimension organization of nanomaterials in large areas with this method. This method provides the possibility of using DNA origami to help build real devices in electronic, photonic or biology. DNA origami has also been reported as template to direct the gold nanorods (AuNRs) orientation. The AuNRs with a size of 12 x 42 nm$^2$ can be immobilized on triangle DNA origami scaffold, and guided in different orientation.36 With this method, several different AuNR dimer structures with designed angles between metallic components can be assembled. Also AuNPs can be immobilized together with AuNRs and have different relative position with AuNRs. This method allows site-specific placement of a nanoparticle close to a AuNR, and supports the study of the dependence of photonic interactions between AuNRs and AuNPs on the interparticle distance and geometrical position.

The development of DNA template is not limited in the discrete cluster field, it was applied even more to building nanoparticles into a specific crystal lattice in 3-D. By control the DNA sequence on the AuNPs, AuNPs can be guided to the self-assembly of different crystalline state, from micrometer-sized face-centred-cubic to body-centred-cubic crystal structures.37,38 With different AuNPs in different crystal
structures, the plasmonic properties of the macro-structure can be easily tuned and suit for different desire.

Beside DNA, other biomolecules can also be used in nano-assembly. For example, the common used biotin-straotavidin pairs can be used to connect AuNRs end-to-end due to the lack of ligand on the end of AuNRs which allows the biotin modification only on the end of AuNRs. The end-to-end or side-by-side AuNR assemblies can also be achieved with complementary antibody and antigen, and realized the detection of microcystin-LR.

Even with all the other biomolecules be able to act as a template for nano-self-assembly, DNA still has its own unique properties. One of the special characteristic is the conductive property. The double strand DNA is formed through π-stacked base pairs, while the π-stacked materials usually have conductive characteristic, which brings in the possibility of DNA also obtains conductive property. Gill et al has shown by intercalating doxorubicin or reduced methylene blue into the DNA base pairs, the CdS nanoparticle/dsDNA assemblies on electrode can switch the directions of the photocurrents. The intercalator increased the photocurrent through DNA. But without the intercalators, a number of reports also showed the observation of charge transport through DNA. Reports have shown that electron can be transferred through 34nm length of dsDNA by the current-voltage curve measurement. The dsDNA has one end attached on the electrode while has the other end attached on the electron accepting molecules. The study showed that the mismatch or single strand DNA affect the charge transport significantly. The other study using molecules as donor and acceptor to form the donor-DNA-acceptor system, and applying femtosecond laser flash photolysis to study the charge transport dynamics, and proved the hopping mechanism of DNA charge transport. The charge transport through DNA has been
reported in different rate, covering several orders of magnitude. And it’s been reported both dependent and independent on the sequence, hydration, length, and hybridization. The vast differences in the results can be related with the sample preparation methods, especially the DNA conjugation method, and the measurement methods. It’s hard to compare all the different results and give a conclusion of the charge transport mechanism and charge transport rate through DNA. But it’s clear that DNA can support change transport over long distance when it’s properly couple between a donor and an acceptor.

Most of the exciting works from DNA templating comes from the last 20 years’ study. It’s still a new field with a lot more to be discovered. But what it brought to us is already astonishing. DNA templating method allows us to precisely control the nanomaterial organization to nanometer precision and create incredible complicate system. The application of DNA to more and more fields will bring us to a new level of nanomaterial study.
2 Water splitting study with Z-scheme system

2.1 Introduction

In recent years, a wide range of inorganic nanostructures with high surface areas and tunable band gaps have been synthesized and used as photocatalysts for splitting water into hydrogen and oxygen.\textsuperscript{21,23,10,25,44,45} To increase their activity, “Z-scheme” photocatalytic systems have been implemented in which multiple types of photoactive materials simultaneously oxidize water and reduce molecules upon photoillumination.\textsuperscript{21,23,10,25,44} In some cases, redox molecules or electron mediators have also been used to aid in electron shuttling between the different catalysts, facilitate charge separation, and inhibit recombination events. For example, Sasaki, \textit{et al.} recently showed that by mixing Ru/SrTiO\textsubscript{3}:Rh with BiVO\textsubscript{4} and inducing aggregation through pH changes that higher amounts of H\textsubscript{2} and O\textsubscript{2} could be produced than either material alone.\textsuperscript{21} In later work, they showed that adding [Co(bpy)\textsubscript{3}]\textsuperscript{2+}/[Co(bpy)\textsubscript{3}]\textsuperscript{3+} as redox mediators further enhanced water splitting performance.\textsuperscript{10} Abe \textit{et al} reported similar work in which IO\textsubscript{3}⁻/I\textsuperscript{⁻} was utilized to shuttle electrons between TiO\textsubscript{2}-rutile and Pt-loaded TiO\textsubscript{2}-anatase to produce H\textsubscript{2} under UV light.\textsuperscript{23} In order to achieve catalysis under visible light, Kato and coworkers used a Pt/SrTiO\textsubscript{3}:Rh-BiVO\textsubscript{4} system with Fe\textsuperscript{3+}/Fe\textsuperscript{2+} as redox mediators.\textsuperscript{44} Recently, solid-state Z-scheme systems have also been synthesized in which control over the interface between the different materials was found to be critical for higher fuel production.\textsuperscript{24,25,27,46–48,26} For example, Tada \textit{et al.} developed an anisotropic CdS/Au/TiO\textsubscript{2} system in which all the components were spatially fixed with Au
facilitating electron transfer.\textsuperscript{26} Metals other than gold have also been tested, including Ag,\textsuperscript{47} Cd,\textsuperscript{24} Cu,\textsuperscript{48} ITO,\textsuperscript{47} and reduced graphene oxide.\textsuperscript{27}

In almost all of these cases, optimal catalysis is typically obtained by interfacing different materials through aggregation (e.g. electrostatic interactions\textsuperscript{21}) or epitaxial nucleation of one material on top of another. These methods, however, tend to decrease the overall accessible catalytic surface area because of the limited control over spatial organization of the separate components. In addition, the scope of different materials that can be produced by direct chemical synthesis is limited. Finally, since electron transfer largely depends on diffusion, simply mixing electron mediators with different catalysts in solution requires a high concentration of redox molecules to avoid rate limitations but which can lead to undesired side oxidation/reduction reactions. To address these challenges, we utilize DNA in this work as a structure-directing agent\textsuperscript{49-52} to spatially organize well-defined TiO\textsubscript{2} and Pt

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Depiction of DNA linked TiO\textsubscript{2}-benzoquinone-Pt@CdS forms well-defined Z-scheme system and enhance water splitting reaction under illumination. Benzoquinone works as an electron mediator.}
\end{figure}
decorated CdS (Pt@CdS) nanocrystals. By using DNA as an assembler a significant increase in H\textsubscript{2} production was observed upon photoillumination as compared to Pt@CdS or TiO\textsubscript{2} alone directly in solution. Potential limitations in H\textsubscript{2} production caused by negatively charged DNA on the Pt@CdS nanoparticles was simply fixed by controlling the amount of DNA per CdS nanorod. DNA also allowed positioning of a single or series of electron mediators site-specifically in between the two catalysts.

2.2 Method

Materials: titanium(IV) butoxide, oleic acid, and oleylamine, Cadmium oxide, sulfur, octanoic acid, octadecylphosphonic acid, Trioctylphosphine oxide, Trioctylphosphine, (1,5-cyclooctadiene)dimethylplatinum(II), triethanolamine, mercaptoethanol, O-(2-Aminoethyl)-O’-(2-azidoethyl)pentaethylene glycol (amine-(PEG)$_6$-azide), copper(II) sulphate, Tripotassium 5,5′,5′′-[2,2′,2′′-nitrilotris(methylene)tris(1H-benzimidazole-2,1-diyl)]tripentanoate hydrate, sodium ascorbate, pH 7 phosphate buffer, benzoquinone, 2,3-Dichloro-1,4-napthoquinone (DCNQ), ethanol, toluene, isopropanol. All DNA were purchased from Integrated DNA Technologies.

Synthesis of nanocrystals: The TiO\textsubscript{2} nanocrystals were produced following Dinh’s method, using a solvothermal approach with both oleic acid and oleylamine as capping ligands. The CdS NRs were synthesized using previously reported procedures (following sample A synthesis in Robinson et al method). The CdS NRs was stored in toluene. To decorate CdS NR with Pt nanoparticles, Dukovic’s photodeposition method was used. For this, 400 \(\mu\)L 20 mM (1,5-cyclooctadiene)dimethylplatinum(II) in toluene was mixed with 200 \(\mu\)L 1.8 \(\mu\)M CdS NRs solution, and 56 \(\mu\)L triethanolamine was added as an electron scavenger. The mixture was photoirradiated
at 460 nm for 20 min. The product was purified with 220nm cutoff filter, following by ligand exchange with mercaptoethanol for 10min, then the water solution was purified with 30K microcentrifuge filter. For ligand exchange of TiO$_2$ nanoparticles, 10 µL of amine-(PEG)$_n$-azide was added to 1.5 mL TiO$_2$ NPs in toluene. After stirring for 1 h, water was added to form a biphasic solution, which was then stirred overnight to enable complete phase transfer of the TiO$_2$ from toluene to water.

**DNA conjugation to nanocrystals**: To attach DNA to the CdS NRs, thiolated DNA (DNA-1 or DNA-4) were mixed with the mercaptoethanol conjugated CdS NRs overnight, followed by microcentrifuge filtration to remove unbound DNA. UV-Vis measurements (Beckman Coulter DU 730) of the filtrate was used to determine the amount of oligonucleotides attached to each Pt@CdS nanorod. DNA conjugation to TiO$_2$ NP was achieved through click chemistry with some modifications published methods. The azide groups on the TiO$_2$ NPs were reacted with 2 equivalents of alkyne-DNA (DNA-2 or DNA-5), 250 µM copper(II) sulfate, 250 µM Tripotassium 5,5′,5″-[2,2′,2″-nitrilotris(methylene)tris(1H-benzimidazole-2,1-diyl)]tripentanoate hydrate and 1 mM sodium ascorbate in 0.1M pH7 phosphate buffer for 5 hrs, and purified through microcentrifuge filtration.

The DNA-conjugated TiO$_2$ NPs were hybridized to the DNA conjugated Pt@CdS NRs by annealing equimolar amounts of each photocatalyst, and DNA-3 in 100 mM NaCl at 57°C for 30min, then cooled down to 4°C. To functionalize DNA-3 with benzoquinone, a slight molar excess of BQ was incubated with the amine-terminated DNA-3 at 60°C for 1 h and the product was purified via G-25 column (GE Healthcare Life Science). To modify DNA-4 with DCNQ, the thiol group of DNA-4 was first conjugated to the Pt@CdS NR and the exposed amine group was then reacted with DCNQ via Michael addition with excess DCNQ removed by
microcentrifuge filtration. In order to attach DCNQ to DNA-5, click chemistry was first run to couple the DNA-5 to TiO$_2$ as there was concern about the deleterious effects the copper catalysts might exert on DCNQ. Next, we reacted 35 μM DNCQ with the TiO$_2$-DNA-5 at 50°C for 12 hrs and removed excess quinone by microcentrifuge filtration.

**Measurement:** For measuring H$_2$ and O$_2$ production, in all cases, the photocatalyst solutions in cuvette (Starna Cells) were first degassed with argon for 30 minutes followed by photoirradiation under 1 sun for 2 hours during which time samples were periodically extracted from the vapor phase for gas chromatography (GC) equipped with 5-A° column and TCD detector measurements. The amount of O$_2$ generated from the photocatalysts was calculated by subtracting the initial O$_2$ present from the total O$_2$ measured after the photoreactions.

**Solar-to-hydrogen energy conversion efficiency calculation:** The energy conversion efficiency calculation was based on the following equation

\[
\text{solar energy conversion efficiency (\%)} = \frac{[\text{standard Gibbs free energy of water, } \Delta G] \times [\text{rate of } H_2 \text{ evolution}]}{[\text{energy density of incident light}] \times [\text{irradiated area}]}
\]

In the experiment, the power output of 1 sun (AM1.5) simulator is 97mW/cm$^2$ (measured with OPHIR Photonics power sensor), while the irradiated area is 0.6cm$^2$. The rate of H$_2$ evolution was calculated as the slope of H$_2$ production vs time plot, and the standard Gibbs free energy of water is 237kJ/mol.

### 2.3 Result and discussion

Well-defined Pt decorated CdS nanorods (NRs) and TiO$_2$ nanoparticles (NPs) were implemented as photocatalysts for the DNA-linked Z-scheme. Based on their
respective energy levels, CdS or Pt@CdS should be able to efficiently reduce $\text{H}^+$ to $\text{H}_2$ but TiO$_2$ cannot. TiO$_2$ NPs and CdS NRs were synthesized according to previous reports.$^{53,54}$ As shown in Figure 2.3.2a, the as-synthesized TiO$_2$ NPs possessed truncated rhombic shapes that were approximately 25 nm in length and 15 nm in diameter. The X-ray diffraction (XRD) analysis of the TiO$_2$ particles (Figure 2.3.1a) proved them to have an anatase structure and the UV-Vis measurements showed a strong absorption at ~350nm (Figure 2.3.2c). As shown in Figure 2.3.2b, the CdS nanorods were ~6 nm in width and average 60 nm in length, which corresponded to an absorption onset of ~460 nm. Since the bandgap of the synthesized CdS NRs is

![Figure 2.3.1](image_url)

**Figure 2.3.1** (a) XRD spectrum of TiO$_2$ (anatase); (b) Cyclic voltammetry (CV) of TiO$_2$ in solution.
\( \sim 2.7 \text{eV} \), the rods should absorb all photon wavelengths less than 490nm, while the TiO\(_2\) bandgap of \( 3.83 \text{eV} \) causes it to absorb primarily in the UV range. The valence band and conduction band of the TiO\(_2\) NPs were investigated by cyclic voltammetry (CV) (Figure 2.3.1b).\(^{55}\) As shown in Figure 2.3.2d, the conduction band of the TiO\(_2\) NPs was determined to be \(-0.48\text{V vs. NHE}\), while the conduction band of the CdS NRs is \(-0.9\text{V vs. NHE}\).\(^{42}\) At pH 7, the \( \text{H}_2 \) redox potential is \(-0.42\text{V vs NHE}\). Because the conduction band of the TiO\(_2\) NPs is similar to the redox potential of \( \text{H}_2 \), it is difficult for TiO\(_2\) to photoreduce protons, thereby making the TiO\(_2\)-CdS system an ideal platform for studying Z-scheme photocatalysis. Next, in order to generate \( \text{H}_2 \)
from the CdS NRs, Pt seeds were nucleated on the CdS through photodeposition. TEM analysis showed that before and after platinum nucleation the CdS nanocrystals retained their shape and size and that on average one Pt NP was bound per nanorod (Figure 2.3.2b). Next, ligand exchange methods were developed to transfer the as-synthesized TiO$_2$ and CdS NPs into aqueous environments. After trying various small molecules, we found that the most successful way to transfer the TiO$_2$ NPs from toluene to water was to add a large excess of amine-(PEG)$_6$-azide. For the Pt@CdS NRs, we used our previously developed method of small molecule ligand exchange via mercaptoethanol or thioglycolic acid.$^{56}$

Once the TiO$_2$ and Pt@CdS NPs were transferred to the aqueous phase, DNA

![Figure 2.3.3](image)

**Figure 2.3.3** (a) Chemical methods used to attach DNA to the TiO$_2$ NPs and CdS NRs; (b) DNA-TiO$_2$ reacted with gold nanoparticles bound with complementary DNA; (c) TEM and UV-vis spectra of DNA modified TiO$_2$ NCs.
strands were conjugated to the NPs. Previously developed methods were used to conjugate ~200 DNA strands to each Pt@CdS NR. For the TiO$_2$ NPs, click chemistry was implemented to form covalent bonds between the azide groups on the TiO$_2$ and alkyne-terminated DNA (Figure 2.3.3a). To qualitatively characterize DNA attachment, we reacted the DNA-TiO$_2$ with gold nanoparticles bound with complementary DNA. The AuNPs clearly associated with the TiO$_2$ NPs through DNA hybridization (Figure 2.3.3b). The use of copper catalysts to conjugate the DNA to the TiO$_2$ did not appear to affect the morphology or the absorption of the TiO$_2$ NPs (Figure 2.3.3c).

Once the DNA strands were conjugated to both catalysts, we probed their ability to produce H$_2$ at specific catalyst concentrations and compared these results to the azide-conjugated TiO$_2$ and ME-Pt@CdS. First, the azide-terminated TiO$_2$ NPs as expected showed almost no H$_2$ production (Figure 2.3.4a). In contrast, 43 nM mercaptoethanol coated Pt@CdS NRs (ME-Pt@CdS) produced ~230 nmoles of H$_2$. Next, we measured H$_2$ production from the Pt@CdS NRs conjugated with ~200 DNA strands per particle. As shown in Figure 2.3.4a, having a large number of DNA

![Figure 2.3.4](image)

**Figure 2.3.4** (a) H$_2$ production from Pt@CdS NRs conjugated with different amounts of DNA (molar ratio of CdS:DNA=1:2, 1:10, 1:200), and amine-(PEG)$_4$-azide coated TiO$_2$. Using molar ratios of CdS:DNA of 1:2 and 1:200 produced NRs attached with ~1.92 and ~189 DNA oligonucleotides respectively as determined by UV Vis measurements. (b) H$_2$ production from Pt@CdS NRs with mercaptoethanol (ME) (black squares) and thioglycolic acid (TGA) (red circles).
strands attached caused a significant decreased in H₂ production as compared to the ME-Pt@CdS NRs. To determine if this effect was due to the presence of negatively charged groups at the photocatalyst surface, we also measured the amount of H₂ produced from Pt@CdS NRs conjugated with thioglycolic acid (TGA). As shown in Figure 2.3.4b, a substantial decrease in H₂ production was also observed from the negatively charged TGA-Pt@CdS as compared to the ME-Pt@CdS, indicating that the decrease in H₂ production could be caused by the high negative charge of TGA or DNA. It is also possible in the case of DNA that a high coverage of oligonucleotides covering a particle surface could also hinder proton adsorption. However, decreasing the amount of DNA attached to the Pt@CdS NRs to ~2-5 oligonucleotides per particle led to an improvement in H₂ production (Figure 2.3.4a) that gave results comparable to the ME-Pt@CdS NRs.

In order to assemble the TiO₂-Pt@CdS clusters through DNA interactions, the thiolated DNA (DNA-1) on the Pt@CdS was designed to contain two domains, one of which is complementary to the DNA on the TiO₂ (DNA-2), with the other complementary to a third amine terminated strand (DNA-3) (Table 2.1). DNA-3 was

<table>
<thead>
<tr>
<th>DNA name</th>
<th>DNA usage</th>
<th>DNA sequence</th>
</tr>
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<tbody>
<tr>
<td>DNA 1</td>
<td></td>
<td>thiol-CATTACGCTTCCCTATTA</td>
</tr>
<tr>
<td>DNA 2</td>
<td></td>
<td>alkylne-TAATAGGAA</td>
</tr>
<tr>
<td>DNA 3</td>
<td></td>
<td>amine-TTTTTTAGCCGTAATG</td>
</tr>
<tr>
<td>DNA 4</td>
<td></td>
<td>thiol-CATTACGCTTCCCTATTA-amine</td>
</tr>
<tr>
<td>DNA 5</td>
<td></td>
<td>alkylne-TAATAGGAA-amine</td>
</tr>
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**Table 2.3.1 DNA design and sequence.**
used later to place electron mediators such as benzoquinone site specifically in between the TiO$_2$ and Pt@CdS. The DNA strands bridging the two photocatalysts were also designed to be 20 bases in length with a ~40% G/C composition as this would yield good thermal stability in 100mM NaCl and separate all the different components by 3-6 nm. TEM analysis of the DNA assembled catalysts showed the

![Representative TEM images of the DNA linked Pt@CdS-TiO$_2$ assemblies. Scale bar: 20 nm](image1)

![Dynamic light scattering (DLS) data of DNA linked Pt@CdS-TiO$_2$ assemblies.](image2)

**Figure 2.3.5** (a) Representative TEM images of the DNA linked Pt@CdS-TiO$_2$ assemblies. Scale bar: 20 nm; (b) Dynamic light scattering (DLS) data of DNA linked Pt@CdS-TiO$_2$ assemblies.

TiO$_2$ and Pt@CdS nanoparticles to be well associated with each other with no large aggregates forming (Figure 2.3.5a), and it’s also prove by the dynamic light scattering measurements which showed an average cluster size of ~247nm (Figure 2.3.5b). Next, the TiO$_2$-Pt@CdS clusters were tested for H$_2$ production. After photoirradiation under 1 sun for 2 hours, the DNA assembled TiO$_2$-Pt@CdS nanoparticle clusters (Figure 2.3.6a) produced a significantly higher amount of H$_2$ than the TiO$_2$ or Pt@CdS NRs
alone, with an overall gain of about 2.8 over Pt@CdS NR alone. Oxygen generated from the DNA linked Pt@CdS-TiO₂ was also determined and showed approximately a 2:1 H₂:O₂ ratio as determined by GC (Figure 2.3.7). It should also be noted that the catalyst amount used in this work (~0.1mg) was significantly less than that used in other reports. To test the importance of DNA assembly, H₂ production was measured from equimolar mixtures of mercaptoethanol conjugated Pt@CdS NRs and amine-PEG-azide-coated TiO₂ NPs. In contrast to the DNA assembled TiO₂-Pt@CdS catalysts, a dispersed solution of the two photocatalysts showed no improvement over Pt@CdS itself (Figure 2.3.6a), illustrating that any H₂ production enhancement was due solely to the DNA interaction. Furthermore, TEM analysis showed the DNA linked TiO₂-Pt@CdS assemblies also proved to be stable during photoillumination.

Figure 2.3.6 (a) H₂ production from, DNA conjugated Pt@CdS (red circles) DNA linked TiO₂-Pt@CdS (black squares) and simple mixtures of unlinked Pt@CdS and TiO₂ (pink triangles). (b) H₂ production from DNA conjugated Pt@CdS (red circles) and from DNA linked Pt@CdS-benzoquinone-TiO₂ system (blue triangles).
The next hypothesis tested was that placement of electron mediators specifically between the two photocatalysts would enhance H₂ production further by inhibiting back reactivity to facilitate charge separation. Benzoquinone (BQ) was chosen as a candidate because its reduction potential of 0.283V vs. NHE at pH 7 lies between the conduction band (CB) of TiO₂ NP and the valence band (VB) of CdS NR. BQ was attached to DNA-3 strands through a Michael addition. Mass spec analysis confirmed the successful conjugation of BQ to the DNA-3 strand (Figure 2.3.8). Cyclic voltammetry (CV) studies revealed that amine functionalization of BQ altered its reduction potential to ~0.073V vs NHE at pH 7 (Figure 2.3.9a), which is still in between the CB and VB of TiO₂ and CdS, respectively. We next hybridized equimolar amounts of the DNA-1-Pt@CdS, DNA-2-TiO₂, and the DNA-3-BQ to make 43nM concentrations of the Pt@CdS-BQ-TiO₂ and H₂ production was measured. As shown in Figure 2.3.6b, intercalating benzoquinone between the Pt@CdS and TiO₂ caused a further increase in H₂ production, with an overall 3.4-fold gain as

**Figure 2.3.7** Hydrogen and oxygen generation from the DNA linked Pt@CdS-TiO₂ clusters.
compared to Pt@CdS itself and a 1.2-fold gain over the TiO$_2$-Pt@CdS assemblies without BQ. It should also be noted here that this process required a very low concentration (nM) of electron mediator. As a comparison, previously reported examples of non-assembled catalysts in solution required millimolar concentrations of redox mediators,\textsuperscript{10} which can lead to deleterious effects such as competing oxidation-reduction reactions. Furthermore, when nanomolar concentrations of BQ were simply mixed with either the DNA assembled TiO$_2$-Pt@CdS clusters or the Pt@CdS alone, no H$_2$ was produced due to BQ being competitively photoreduced in place of protons (Figure 2.3.9b). The solar-to-hydrogen (STH) energy conversion efficiency was also calculated. With 0.1mg of Pt@CdS in solution, the STH values increased from 0.011% with Pt@CdS to 0.031% with TiO$_2$-Pt@CdS to 0.037% with the TiO$_2$-BQ-Pt@CdS under one sun illumination at 97mW/cm$^2$. The small STH values are due to the low catalyst concentration in solution.

Figure 2.3.8 Mass spectra of amine modified DNA and benzoquinone modified DNA.
In addition to utilizing a single electron mediator, the incorporation of a second mediator was tested. For this, 2,3-Dichloro-1,4-napthoquinone (DCNQ) was used as its reduction potential of -0.077 V vs NHE is more positive than the CB of TiO$_2$ but more negative than the redox potential of BQ. In this case, the excited electrons are expected to travel from TiO$_2$ to DCNQ to BQ to Pt@CdS (Figure 2.3.10a). To achieve this, the DNA-1 strand was modified to include a thiol group at its 5’ end and an amine at the 3’ end (DNA-4), to place the DCNQ in close proximity to the TiO$_2$ NP (Figure 2.3.10b). In testing the TiO$_2$-DCNQ-BQ-Pt@CdS clusters however for catalysis, we found that less H$_2$ was produced than the Pt@CdS-BQ-TiO$_2$ system (Figure 2.3.10c). Although an additional redox mediator may facilitate the electron transfer, it is also possible that two intermediates may slow down the kinetics of the process due to the additional redox step. Because chronoamperometry studies however showed that the electron transfer rates of BQ and DCNQ do not differ significantly from each other (Figure 2.3.11), another more likely scenario is that the close proximity of the DCNQ to the TiO$_2$ leads to back reactivity, preventing electron transfer.
mobility from DCNQ to BQ. In order to investigate this further, we modified DNA-2 to possess an alkyne at its 3’ end and an amine at its 5’ end (DNA-5) (Table 1). This design would cause the DCNQ to be separated from the TiO$_2$ surface by ~3nm but be immediately next to the BQ (Figure 2.3.10d). The TiO$_2$-DNA-5-DCNQ were next hybridized with the DNA-1-Pt@CdS and DNA-3-BQ and tested for H$_2$ production. As shown in Figure 2.3.10c, while increasing the distance between the DCNQ and TiO$_2$ did increase the H$_2$ production from the first design of TiO$_2$-DCNQ-BQ-Pt@CdS system (with DNA-4), the overall H$_2$ yield was comparable to the TiO$_2$-BQ-Pt@CdS but not higher. Because the two quinones have similar energy levels and similar electron transfer rate, the electron transfer might not significantly change from

**Figure 2.3.10** (a) Energy band diagram scheme of the Pt@CdS-BQ-DCNQ-TiO$_2$ system. (b) Scheme of H$_2$ production from Pt@CdS-BQ-DCNQ-TiO$_2$ design 1 system. (c) H$_2$ production from Pt@CdS-BQ-DCNQ-TiO$_2$ assemblies versus Pt@CdS-BQ-TiO$_2$ (red circles) or Pt@CdS-DNA alone (black squares) Design 1 (blue triangles) Design 2 (Pink triangles). (d) Scheme of H$_2$ production from CdS-BQ-DCNQ-TiO$_2$ design 2 system.
the Pt@CdS-BQ-TiO₂ system, or the electron may even be prevented from transferring to the Pt@CdS by undergoing cyclic oxidation/reduction processes between the DCNQ and BQ. In order to cause an improvement in photocatalysis and H₂ production then, alternate quinones, increasing the distance between the mediators or using dynamic hybridization events need to be investigated in future studies.

2.4 Conclusion

In conclusion, a method was demonstrated for self-assembling different photocatalysts and electron mediators using DNA interactions for enhanced H₂ production. DNA was used to construct a well-organized Z-scheme photocatalytic system that could produce H₂ directly in water under solar illumination. The DNA assembled Pt@CdS and TiO₂ clusters showed a significant improvement in H₂ production as compared to either single photocatalysts or unassembled, dispersed catalyst mixtures. Using DNA also enabled us to site specifically place electron mediators in between the TiO₂ and CdS and we found that while the use of

![Figure 2.3.11](image_url) Chronoamperometry results which show that the BQ and DCNQ have similar oxidation/reduction rate.
benzoquinone alone improved H\textsubscript{2} production, utilizing an additional mediator did not improve catalysis. These results demonstrate that to increase the production of H\textsubscript{2} even more, both the redox potentials and spatial positioning of all the different Z-scheme components need to be further optimized and this will be studied in future work.
3 Methanol generation with enzyme coupled photoelectrochemical cells

3.1 Introduction

In Chapter 2, we’ve discussed the extensive research about developing renewable energy such as hydrogen, and demonstrated a new method of splitting water to generate hydrogen as renewable energy. However, with decreasing fossil fuel reserves and rising levels of CO₂, the search for renewable energy while at the same time converting CO₂ has grown in importance. While splitting water to oxygen and hydrogen has been showed successfully, the reduction of CO₂ to a liquid fuel such as methanol has remained significantly more challenging due to required six-electron and proton transfer to CO₂. Previously, photoelectrochemical cells (PECs) or aqueous suspensions of semiconductor powders were used to reduce CO₂ into useful fuels. In early work by Halmann et al., PECs were constructed by using p-GaP cathodes and silicon anodes to reduce CO₂ into formate, formaldehyde, and methanol under applied bias. The approach was further extended by Honda and coworkers who used wide bandgap semiconductors such as TiO₂ in aqueous suspensions to generate formaldehyde and methanol. In later work, semiconductors including TiO₂, p-GaP, TaON, p-type N-doped Ta₂O₅, p-type InP, CdS, Bi₂WO₆ hollow microspheres, and BiVO₄ have been used to photoreduce CO₂ to primarily formic acid with low micromolar amounts of methanol as detected by GC-MS. Recently, Bocarsly and coworkers utilized the organohydride pyridine in conjunction with electrochemical and photoelectrochemical cells to under bias, promote the reduction of CO₂ to methanol. Furthermore, Boston et al. showed that
combining the photoactive ruthenium(II) trisphenanthroline with pyridine could also reduce CO$_2$ in the presence of the sacrificial electron donors to form millimolar and micromolar amounts of formate and methanol respectively.

Lastly, in addition to using purely synthetic systems, research has explored the use of multiple enzymes to reduce CO$_2$ which will be discussed more later. To improve enzyme activity, Weaver and co-workers also coupled formate dehydrogenase with p-type InP electrodes to increase CO$_2$ reduction to formate.

Despite significant effort in recent years, reducing CO$_2$ to methanol has remained a difficult challenge because it requires a 6-electron, 6-proton transfer process. Instead, most catalytic systems produce 2-electron 2-proton products such as formic acid or CO. Furthermore, even if formate could be further reduced to formaldehyde, converting formaldehyde to methanol in water is further hindered by the fast equilibrium of formaldehyde to the hydrate form methanediol, adding a rate-limiting step of dehydration back to formaldehyde to allow further reduction.

To mitigate these challenges, we demonstrate here that a combination of the enzyme alcohol dehydrogenase (ADH) with synthetic photovoltaic cells (PEC) significantly increased the production of methanol from CO$_2$ beyond what is typically possible with either inorganic catalysts or enzymes alone. Combining photocatalysts and biocatalysts has received increased attention recently, with much of the research focused on the use of coupled electron transfer. In this work, we first show that ADH isolated from *Saccharomyces cerevisiae* easily reduces formaldehyde in the presence of NADH starting from very low micromolar amounts of substrate, which is critical because electrochemical yields of formaldehyde from CO$_2$ are expected to be very low. Furthermore, we demonstrate that the ADH is unique in that while the enzyme can dehydrogenate ethanol and butanol to their
respective aldehydes, it cannot dehydrogenate methanol back to formaldehyde. Thus, this enzyme is particularly useful for increasing methanol production from PEC driven CO$_2$ reduction with no possible back reactivity.

![Diagram](Image)

**Figure 3.1.1** Depiction of a combination of the enzyme alcohol dehydrogenase with synthetic photoelectrochemical cells significantly increased the production of methanol from CO$_2$.

### 3.2 Method

**Materials:** Alcohol Dehydrogenase from *Saccharomyces cerevisiae*, Formaldehyde Dehydrogenase from *Pseudomonas sp.*, and Formate Dehydrogenase from *Candida boidinii* were all purchased from Sigma-Aldrich. $^1$H NMR spectroscopic measurements were performed using a Bruker Advance-III 400 NMR system. The UV-Vis measurements were performed with a Beckman Coulter DU 730 UV/Vis Spectrophotometer.

*Synthesis of $\{\text{Cp}^*\text{Rh(bpy)H}_2\text{O}\}^{2+}$:* $\{\text{Cp}^*\text{Rh(bpy)H}_2\text{O}\}^{2+}$ was synthesized as follows: 50mg RhCl$_3$$\cdot$H$_2$O was refluxed under N$_2$ in 10mL of methanol with 37.4 µL
of 1,2,3,4,5-pentamethylcyclopentadiene for 24 h. The solution was put in ice for 3 h, and the resulting red precipitate was collected and resuspended in methanol. With the addition of 74.7 mg 2,2-bipyridine, the suspension cleared up immediately and a yellow solution was formed. [Cp*Rh(bpy)Cl]Cl was precipitated by the addition of diethyl ether into the yellow solution. Stock solutions were prepared in water, and [Cp*Rh(bpy)Cl]Cl readily hydrolyzes to [Cp*Rh(bpy)(H$_2$O)]$^{2+}$. Lyophilization was used to remove methanol and diethyl ether from the sample.

**NiO/CdTe electrode preparation:** The CdTe nanoparticles (NPs) were synthesized using a hydrothermal method. 0.71 g of CdCl$_2$ and 580 ml of thioglycolic acid (TGA) were added to 20 ml of H$_2$O in a pressure vessel. The pH was adjusted to 9 and the solution was kept under Ar atmosphere (solution A). Te (100mg) and NaBH$_4$ (110mg) were stirred in 10ml of H$_2$O under vacuum for 2 h to form reduced Te (solution B, purple pink color). Solution B was then added to solution A and the vessel was quickly sealed. The temperature was raised to 100 C and run for 24 h. The NPs were cleaned by successive washes and centrifugation with water and acetone. A CdTe paste was prepared by adding the CdTe NP stock solution to ethylene glycol in equal volume. The solution was mixed and then the particles were collected by centrifugation to remove excess ligands. NiO nanoparticles were commercially purchased (Solaronix). A 0.9 x 2.2 cm ITO electrode was used to brush a thin layer of NiO paste on. The brushed area was 0.9 x 1.5 cm$^2$. The NiO coated electrode was annealed at 200 C for 18 h. Next, the CdTe paste was brushed on the annealed NiO substrates to form a black layer after calcination at 200 C for 18 h.

**PEC reaction setup and condition:** Biased photoelectrochemical reactions were performed in a three-compartment cell where Pt was used as the counter electrode and Ag/AgCl was used as the reference. A blue LED source that showed a
maximum emission at 465 nm was utilized. 1 cm² of the working electrode was immersed into the buffer solution (0.1M phosphate buffer, pH 6.8). The reaction solution with the organocatalyst was purged in separate vials with CO₂ for 1h. The purged solution was then injected into the photoelectrochemical cell. In the biased experiments, total volume of reaction solution was 15 mL and CdTe/NiO electrode was biased at -0.3mM, 17C.

**ADH reaction condition:** In all the ADH reactions with the PEC product solutions, 10 units of ADH was used. 374 µL of PEC solution was mixed with 1.8mM of NADH and ADH. ¹H NMR spectroscopy was used to follow the reactions.

**Control experiment of ADH, FaldDH and FDH:** In all the ADH reactions, 10 units of ADH were used. The reactions were performed in 0.1M of buffer solution (PB buffer for pH 7, or citrate buffer for pH 5). NADH or NAD concentration was 1.8mM, if not specially noted otherwise. In the FaldDH and FDH reaction, 1 unit of either FaldDH or FDH was used. Reaction products were measured by either UV-Vis or ¹H NMR analysis.

### 3.3 Result and discussion

Experimentally, the process of CO₂ reduction typically results in decreasing amounts of the products formic acid (formate), formaldehyde, and methanol. The reduction potentials for producing formaldehyde and methanol can be significantly lowered in energy if multiple electrons and protons can be transferred simultaneously to CO₂. However, since a simultaneous 6e⁻, 6H⁺ transfer to CO₂ to produce methanol is highly difficult to achieve, it is expected that a natural progression of reducing CO₂ to formate, formate to formaldehyde and formaldehyde to methanol might ensue. While reducing an aldehyde should be relatively facile, the reduction of
formaldehyde in water is particularly challenging due to the equilibrium being greatly favored by $\sim 10^3$ toward the hydrate form methanediol with fast reaction rates.$^{92}$ This rapid conversion to methanediol also therefore greatly lowers the amount of formaldehyde in solution available for reduction to methanol. Since at best, electrochemical or photoelectrochemical systems produce low millimolar amounts of formate, the amount of formaldehyde is expected to be in the mid to low micromolar range which is a very low reactant concentration for synthetic reductases. However, biological catalysts can perform well at sub-millimolar substrate concentrations due to the low $K_D$ values (dissociation constant) between enzymes and reactants.$^{96,104,105}$ Furthermore, if an enzyme could reduce the low amounts of formaldehyde, the equilibrium between methanediol and formaldehyde would shift to producing more formaldehyde and therefore more methanol.

In order to test enzyme activity, we first reacted an alcohol dehydrogenase (ADH) from *Saccharomyces cerevisiae* with varying concentrations of formaldehyde in the presence of NADH in phosphate buffer. The formation of methanol was tracked by $^1$H NMR spectroscopy and NADH consumption by UV-Vis spectroscopy. Reacting 0.1 unit (3.1 nM) ADH with 50, 100, 250, and 450 μM amounts of formaldehyde caused rapid methanol generation in which the methanol yield matched the conversion of NADH to NAD$^+$ (Figure 3.3.1 and 3.3.2). Furthermore, even at 50 μM formaldehyde, ~33 μM methanol was formed (~66% yield). If we consider that 50 μM formaldehyde should equilibrate to produce about 80% methanediol (40 μM) and 20% formaldehyde (10 μM), the enzyme not only is efficient at converting low micromolar concentrations of formaldehyde but can also produce amounts of methanol that are greater than the starting amounts of formaldehyde available for reduction. Lastly, as control experiments, ADH was also reacted with CO$_2$ and formic
acid which showed the production of neither formic acid nor methanol by NMR (Figure 3.3.3).

Because most alcohol dehydrogenases are known to undergo reversible oxidation-reduction reactions, the ADH was next tested for its rate of methanol oxidation, which would lower or limit methanol yields. To study this, ADH was reacted with 500 µM methanol and NAD\(^+\), and UV-Vis and \(^1\)H NMR spectroscopy were used to measure NADH production and methanol consumption, respectively. As shown in Figure 3.3.3, combining ADH and methanol led to no detectable amounts of NADH by UV-Vis, and, correspondingly, no decrease in the proton peak for methanol.

**Figure 3.3.1** UV-Vis and \(^1\)H NMR spectra of ADH combined with different concentrations of formaldehyde in the presence of NADH. UV-Vis measurements showed the consumption of NADH from reacting ADH with (a) 50 µM and (b) 100 µM formaldehyde. \(^1\)H NMR spectroscopic measurements showing increase in methanol production from reacting ADH with NADH and (c) 50 µM and (d) 100 µM formaldehyde. (The methanol peak shown before the reaction comes from the commercially purchased formaldehyde)
was observed. No detectable increase in NADH or decrease in methanol was observed.

**Figure 3.3.2** NMR and UV-vis result of reaction of ADH with different concentration of formaldehyde (in the presence of NADH and PB buffer). UV-vis measurement of NADH decreasing with 250uM (a) and 450uM (b) formaldehyde. NMR measurement of methanol increasing with 250uM (c) and 450uM (d) formaldehyde.

**Figure 3.3.3** $^1$H NMR spectra showing combined ADH and methanol (insert: UV Vis measurement of NADH).
even after 12 h. However when ethanol or butanol was used instead as the substrate, the ADH performed as expected and quickly converted NAD$^+$ to NADH (Figure 3.3.4). In order to explain this phenomena, the Michaelis constant ($K_M$) of ADH with methanol which is defined as the concentration at which the rate of the enzyme reaction is half the maximum reaction rate was measured to be 148 mM (Figure 3.3.5). Based on this result, we determined that while ADH can in fact oxidize

![Figure 3.3.4](image1.png) **Figure 3.3.4** UV-Vis results of reacting ADH with ethanol (a) and butanol (b) in the presence of NAD$^+$.

![Figure 3.3.5](image2.png) **Figure 3.3.5** (a) The NADH increasing in the reaction of ADH and NAD, with varies starting concentration of methanol; (b) The $K_M$ value calculation for methanol in the reaction of ADH with methanol, based on the equation $\frac{1}{V_0} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$. The $K_M$ value is 148 mM.
methanol back to formaldehyde, the substrate concentration needed was substantially higher than what could be produced from CO₂ reduction, enabling the use of the enzyme for increasing methanol production from CO₂.

To study this, we next utilized a photoelectrochemical cell (PEC) to reduce CO₂ prior to adding the enzyme. This was done because many hours of photoirradiation are typically needed to produce low micromolar or millimolar amounts of products from CO₂. The PEC was built from a NiO/CdTe photocathode and a Pt counter electrode. CdTe nanoparticles (NPs) were first synthesized using published procedures. The bandgap of the CdTe was determined previously to be ~2 eV and the conduction band of the CdTe was measured to be -1.53 V vs. Ag/AgCl through electrochemical studies, which is sufficient enough to reduce CO₂. To produce the photocathode, NiO was first deposited on ITO to act as a hole collector, then a CdTe paste was brushed onto the NiO layer and the substrates were calcined. In order to increase CO₂ reduction, a diffusional catalyst, CpRh(bpy)Cl₂ was utilized in conjunction with the PECs to improve product yields by ~85 fold. This particular metal complex was also chosen because it had been shown in previous reports to be an effective hydride donor at neutral pH and while not interfering with enzyme activity. We next coupled the NiO/CdTe cathode to a Pt anode, and CO₂ was then bubbled into the electrochemical cell containing 0.4 mM organocatalyst CpRh(bpy)Cl₂ in 0.1 M phosphate buffer (pH 6.8). After 17 h photoirradiation, ~2 mM of formate and ~10 µM of methanol were detected by ¹H NMR spectroscopy (Figure 3.3.6). Formaldehyde was difficult to detect because the expected methanediol peak was obscured by the large water peak. Nevertheless, a small amount of methanol would indicate that at least small amounts of formaldehyde or methanediol were present in the product mixture.
After photoirradiation, 10 units (0.4 µM) of ADH and 1.8 mM NADH were next added to 374 µL of the PEC product solution in the dark, and methanol formation was monitored by $^1$H NMR spectroscopy. While the PEC product solution initially showed 10 µM methanol, addition of the enzyme increased the methanol concentration by ~5 fold to 47 µM methanol within 30 min (Figure 3.3.6). This not only showed that formaldehyde and/or methanediol was present in the PEC product mixture, but also that the ADH was able to convert very low concentrations of formaldehyde to methanol. To determine the amount of methanol generated in real time a calibration curve of peak area versus methanol concentration was built (Figure 3.3.7). Enzyme reactions over 30 min ~ 1 h showed a gradual 4-5 fold increase in methanol production (Figure 3.3.8). Although the total amount of methanol from CO$_2$ reduction clearly depends on the PEC performance, over multiple runs with different
PEC reactions, addition of ADH generally showed an average gain of 3.22 fold in methanol concentration within 30 min (Figure 3.3.9a). Since each PEC reaction can generate varying amounts of product (formate, formaldehyde, methanol) from one run

![Figure 3.3.7](image.png)

**Figure 3.3.7** Standard curve of methanol peak in NMR

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![Figure 3.3.8](image.png)

**Figure 3.3.8** $^1$H NMR spectra of measuring methanol formation over time upon adding (a) ADH to the PEC product solution and (b) FalDH and ADH to a different aliquot of the exact same PEC production solution shown in (a). At time zero, the PEC product solutions showed 24 µM methanol. The addition of (b) FalDH and ADH yielded 103 µM methanol and (a) ADH alone gave 96 µM methanol.

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![Figure 3.3.8](image.png)

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to the next, the observed deviations in methanol yields before and after adding the enzyme are not unexpected (Figure 3.3.9a and Table 3.3.1). As described earlier,
because the ADH cannot oxidize methanol, the methanol yield remained constant with no later decreases due to back reactions.

In addition to studying the ADH enzyme with the PEC driven CO$_2$ reactions, we also tested other enzymes such as formate dehydrogenase (FDH) and

Figure 3.3.9 (a) Comparison of methanol production from PEC reaction before and after adding ADH. Error bars indicate 1 standard error from 5 replicate experiments. Additional $^1$H NMR results showing an increase in methanol upon adding ADH to a PEC product solution. (b) Methanol increasing along with time.

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Table 3.3.1 Table showing amounts of methanol production before and after ADH addition to the PEC reactions in five separate experiments. Standard errors and deviations are shown for all of the five experiments run.
formaldehyde dehydrogenase (FalDH), since previous reports stated that using a tandem series of enzymes could produce measurable amounts of methanol from CO$_2$.\textsuperscript{83–85} It must be noted that these studies however primarily used gas chromatography to detect methanol directly from buffered solutions that in some cases also contained the different enzymes which can often be difficult to do. For our studies, we first combined 1 unit of FDH with a saturated CO$_2$ solution in buffer and measured formate production by $^1$H NMR spectroscopy. However, only 80 µM formate was produced after 12 h. This result matched the NAD$^+$ conversion by UV Vis spectroscopy (Figure 3.3.10), which showed extremely slow enzyme kinetics. We next reacted 1 or 3 units of FalDH with 500 µM to 20mM formate and NADH and

![Figure 3.3.10 NMR result of FDH reaction with CO$_2$]
monitored their conversion by $^1$H NMR and UV Vis spectroscopy respectively. As shown in Figure 3.3.11c, little NADH conversion to NAD$^+$ was observed even after 5 h. These results were also confirmed by analysis of NMR, which showed no decrease in the formate peak (Figure 3.3.11a). While lowering the pH to 5 might be expected to

**Figure 3.3.11** 1 unit of FalddH reaction with formate (a, pH 7; b, pH 5); UV-vis result of FalddH reaction with formate (c) and reaction with formaldehyde at pH 7 (d); 3 unit of FalddH overnight reaction with 2mM of formic acid at pH 5 (e); 1 unit of FalddH overnight reaction with 20mM of formic acid at pH 7 (f)
push the formate reduction by FalDH by protonating formate, FalDH activity did not improve significantly at pH 5 (Figure 3.3.11b). In contrast, when the FalDH was mixed with NAD\(^+\) and formaldehyde, the NADH peak clearly increased in 1 h, indicating that formaldehyde is easily oxidized to formic acid (Figure 3.3.11d).

Although both FDH and FalDH demonstrated low conversion efficiencies as hydrogenases, we next also tested all three enzymes, FDH, FalDH and ADH in the presence of CO\(_2\) and a 1.8mM of NADH. However, as shown in Figure 3.3.12 using all three enzymes simultaneously only produced sub-100 \(\mu\)M amounts of formate and no methanol. Finally, since the PEC reactions could generate measurable millimolar amounts of formate from CO\(_2\), we also tested the addition of FaldH and ADH to the PEC product mixture in order to see if this could increase the production of methanol. However, due to the low reactivity of FalDH with formate, as shown in Figure 3.3.9, using both FalDH and ADH showed no difference in methanol production as compared to when ADH was used alone.

Figure 3.3.12 FDH, FaldDH and ADH reaction with CO\(_2\)
3.4 Conclusion

We have demonstrated in this work the novel use of biological catalysts with photoelectrochemical cells to increase the production of methanol by CO$_2$ reduction in water. While many synthetic catalysts have been studied to push CO$_2$ reduction, these processes produce mainly formic acid or formate, with very low levels of methanol. Furthermore, although formaldehyde should be a product of CO$_2$ reduction, the equilibrium causes it to primarily exist in its hydrate form, methanediol, decreasing the amount of available aldehyde for reduction. Because biological catalysts are known to work highly effectively with low substrate concentrations, we first showed that an alcohol dehydrogenase could reduce formaldehyde down to 50 $\mu$M formaldehyde. Furthermore, we demonstrated that the ADH is an ideal catalyst for increasing methanol production from CO$_2$ as it could not undergo back reaction to oxidize methanol back to formaldehyde. By combining ADH with products generated from photoelectrochemical cells, we were able to induce 3-4 fold gains in methanol production from CO$_2$ and water. Other enzymes such as FDH and FalDH showed poor activity in reducing CO$_2$ or formate, respectively, and that combinations of FDH, FalDH and ADH could therefore only yield ~80 $\mu$M formate with no methanol. Due to this low activity of FalDH with formate, using both FalDH and ADH with the PEC generated reduced CO$_2$ products showed no improvement over ADH alone. These results also are cost advantageous since out of the three enzymes, ADH is markedly lower in price (76 USD/30 kUN) as compared to the FDH (176 USD/50 UN) or FalDH (138 USD/5 UN). This work thus demonstrates the power of combinations of biological catalysts with synthetic photosystems to ultimately increase the generation of liquid fuel from CO$_2$ and water.
4 DNA for assembly and charge transport for photocatalytic reduction of CO₂

4.1 Introduction

As stated in the previous chapters, the development of renewable energy while also reduce CO₂ generated by fossil fuel has become important. Scientists in this field have been inspired by the natural photosynthetic process, in which photosystems and solar energy are used to drive oxidation/reduction process to produce ATP, NADPH and carbohydrates. However, it was showed that mimicking the system with three enzymes has low efficiency. So recent efforts have studied photoelectrochemistry or solution based photocatalysis, as well as bio-hybrid materials to convert solar energy, CO₂ to usable fuels, as described in Chapter 3.

Z-scheme system, as described in Chapter 2, can both improve the overall stability of the photoactive elements and enlarge the overall band gap of the system to yield electrons and holes of sufficient energy for CO₂ reduction. In some cases, redox molecules or electron mediators have also been used to aid electron shuttling between the different catalysts, facilitate charge separation, and inhibit recombination events. For example, Kuriki and coworkers recently utilized both mesoporous graphitic carbon nitride and a Ru(II) binuclear complex as two photocatalysts for selectively reducing CO₂ to formic acid under visible light. In other work, Kang et al. converted CO₂ to formate with ~1% efficiency using a combination of synthetic CuFeO₂/CuO photocathodes and Pt anodes. Despite these successes, the photocatalytic output of nanoparticle-based Z-scheme systems must be
significantly improved. For example, simply mixing different photoactive nanocrystals in solution does not provide specific donor-acceptor nanocrystal interactions, which allows the electrons to traverse many different pathways, including back transfer. Instead, controlling the organization and spatial distribution of each donor-acceptor nanocrystal pair would drive the photogenerated electrons from one nanoparticle to the next in the desired direction. As one example of this, we demonstrated in Chapter 2 that coupling TiO$_2$ nanoparticles and Pt@CdS with DNA led to full splitting of water to H$_2$ and O$_2$ upon solar irradiation.$^{134}$

Because of Nature's inherent ability for self-recognition, biomolecules have been extensively studied as tools to organize nanoscale systems into hierarchical arrays with high precision.$^{30,56,134–136,36}$ For example, Belcher and coworkers have utilized virus scaffolds to nucleate a wide variety of inorganic nanomaterials for use as batteries and solar harvesting agents.$^{137–139}$ In recent years, Mirkin and coworkers have pioneered the use of DNA to fabricate a myriad of highly ordered 3-dimensional crystals from metal, semiconductor and organic building blocks.$^{37,38,140–143}$ Furthermore, 2- and 3-D DNA scaffolds (origami) have been used to place nanoscale objects into periodic arrays.$^{33,144–148}$ Beyond templating nanocrystal assembly, DNA has also been shown to facilitate charge transfer over varying nucleobase lengths.$^{42,41,43,149,150}$ Thus, this technique is also expected to be applied in the CO$_2$ reduction system, and improve the CO$_2$ reduction efficiency.

In this work, well-defined Z-scheme architectures for CO$_2$ reduction were produced through DNA self-assembly. Sufficient electron transfer was achieved to reduce CO$_2$ to usable fuels with a significant, 5-6 fold increase over simply mixing the two photocatalysts. Beside using DNA to shorten the distance of the two photocatalysts and enhance the CO$_2$ reduction efficiency, the effect of different DNA
lengths was also studied. It was observed that the Z-scheme effect still exists while using 80mer DNA (~24nm in length) as a linker, which is believed due to the electron transfer through DNA. Furthermore, different from the results showed in Chapter 2, a strong dependence between the lengths of the DNA strands used to link the donor-acceptor nanocrystals and CO₂ reduction was observed. Optimal interparticle spacing was found for DNA lengths of 9-10 nm, which has 5 folds higher CO₂ reduction efficiency than linking the particles by the synthetic polymer polyethylene glycol (PEG), indicating a possible need to balance between facilitating electron transfer and reducing back reaction rates. These results demonstrate both the unique role of DNA as a tool for facilitating nanoparticle organization as well as electron transfer between donor-acceptor nanocrystals to investigate and optimize Z-scheme based photocatalysis.

Figure 4.1.1 Depict of CO₂ reduction Z-scheme system constructed with DNA as template
4.2 Method

**TiO$_2$ Nanoparticle and CdS nanorod Synthesis:** Refer to Section 2.2.

**AuNP seeds growth on CdS NR:** To synthesize the Au seeded CdS nanorods (Au@CdS), the method developed by Ben-Shahar and coworkers was used. A precursor solution containing octadecylamine (ODA, 42 mg), di-dodecylammoniumbromide (DDAB, 21 mg) and AuCl$_3$ (7.8 mg) in toluene (10 mL) was sonicated for 10 min to dissolve the AuCl$_3$. Then 1.68 mL of this precursor solution was injected into CdS NR solution (0.34 uM, 1.5 mL) under argon. The solution was next stirred for 1 h in the dark, and then washed with ethanol and acetone three times. The as-synthesized Au@CdS was dissolved in toluene.

**[Cp*Rh(bpy)H$_2$O]$^{2+}$ Synthesis:** Refer to Section 3.2.

**DNA Conjugation to TiO$_2$ and Au@CdS:** First, the as-synthesized TiO$_2$ and Au@CdS were transferred from toluene to water through ligand exchance as described in Section 2.2. To attach DNA to the Au@CdS NRs, thiolated DNA were mixed with the mercaptoethanol conjugated Au@CdS NRs at pH 9 overnight, followed by microcentrifuge filtration to remove unbound DNA. UV-Vis of the filtrate was measured to determine the amount of oligonucleotides attached to each Au@CdS nanorod. DNA conjugation to TiO$_2$ NP was achieved through click chemistry with some modifications published methods. The amount of DNA conjugated on TiO$_2$ was measured with $^1$H NMR for DNA peaks, and compare with the DNA peaks on Au@CdS-DNA spectra. The DNA-conjugated TiO$_2$ NPs (8.8 pmole) were hybridized to the DNA conjugated Au@CdS NRs (22 pmole) by thermal annealing in 100 mM NaCl (at 10°C higher than the melting temperature of the DNA strands used) for 20 min, then cooled down slowly to 4°C.
Polyethyleneglycol (PEG) Conjugation to TiO$_2$ and Au@CdS: PEG chains were conjugated to TiO$_2$ and Au@CdS nanocrystals by using thiol-(PEG)$_{5k}$-DBCO. The thiol-(PEG)$_{5k}$-DBCO was reacted with amine-(PEG)$_{6}$-azide coated TiO$_2$ NPs (molar ratio of 50:1 thiol-(PEG)$_{5k}$-DBCO:TiO$_2$) in phosphate buffer overnight, through a copper free click reaction between DBCO and azide. Then the disulfide bonds formed during the reaction was reduced with TCEP for 2 h, followed with purifying through microcentrifuge filtration. The PEG conjugated TiO$_2$ was then reacted with Au@CdS at pH 9. The TiO$_2$ and Au@CdS amount used in PEG linker experiments were the same with the one used in the DNA linker experiments.

CO$_2$ Reduction: For CO$_2$ reduction, NPs were dispersed in 1 mL water containing 0.09 M ascorbic acid, 0.3 M phosphate buffer, 10 mM NaCl and 0.32 mM [Cp*Rh(bpy)(H$_2$O)]$^{2+}$. The solution was degassed with argon for 1 h, then followed with 1 h CO$_2$ purging to achieve saturated CO$_2$ amount in water solution. The reaction was done in sealed cuvette and photoilluminated under 1 sun for 11 h. 400 uL of the reaction solution was used for $^1$H NMR measurements, and 413.8 uL of the solution was used to react with 20 units of ADH and 1.8 mM of NADH for 1 h, followed with $^1$H NMR measurements. For $^1$H NMR measurement, 1 mM of DSS was used as the reference. As the formate peak is overlapping with the [Cp*Rh(bpy)(H$_2$O)]$^{2+}$ peak, the [Cp*Rh(bpy)(H$_2$O)]$^{2+}$ peak at 8.97ppm was used as reference peak to calculate the [Cp*Rh(bpy)(H$_2$O)]$^{2+}$ peak at 8.44 ppm. Then the integration of [Cp*Rh(bpy)(H$_2$O)]$^{2+}$ peak at 8.44 ppm was subtracted from the integration of both formate and [Cp*Rh(bpy)(H$_2$O)]$^{2+}$ peaks at 8.44 ppm to get the formate peak integration.

H$_2$ Generation: For H$_2$ generation, in all cases, the photocatalyst solutions were first degassed with argon for 1 h followed by photoirradiation under 1 sun for 2
hours during which time samples were periodically extracted from the vapor phase for gas chromatography (GC) measurements.

4.3 Result and discussion

The large persistence length of linear double stranded DNA (100 bases, or 30 nm) allowed us in this study to systematically control the distance between two semiconductor nanocrystals. TiO$_2$ and CdS nanocrystals were chosen as the photocatalysts since the valence and conduction bands of TiO$_2$ and CdS, respectively, are of ample energy to oxidize electron donors such as ascorbic acid or water and reduce CO$_2$ to formate, formaldehyde, or methanol (Figure 4.3.1A). For an efficient Z-scheme system to function, the photogenerated electrons in the donor nanocrystals

![Figure 4.3.1](image-url)  
(A) CO$_2$ photoreduction mediated by DNA linked TiO$_2$ and Au@CdS
(B) Energy diagram of the TiO$_2$-CdS Z-scheme system.
(e.g. TiO$_2$) must be able to travel to the valence band of the acceptor nanocrystals (e.g. CdS) at a sufficient rate. To study the possibility of using DNA to enable this, TiO$_2$ nanoparticles (NPs) were first synthesized using methods developed by Dinh et al.\textsuperscript{53} Transmission electron microscopy (TEM) analysis showed the TiO$_2$ nanoparticles to possess truncated rhombic shapes that were $\sim$23.3 $\pm$ 1.9 nm in length and $\sim$14 $\pm$ 1.6 nm in diameter (Figure 4.3.2A). The TiO$_2$ nanoparticles were anatase in crystal structure as determined by XRD and cyclic voltammetry (CV) measurements showed a conduction band at -0.48 vs NHE and a valence band 3.35 V vs NHE.\textsuperscript{134} These energy levels confirm that the TiO$_2$ nanocrystals can oxidize electron donors such as ascorbic acid (AA) or water but be highly inefficient in reducing CO$_2$ (Figure 4.3.1B).

CdS nanorods (NRs) were synthesized next using procedures developed by

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Figure 4.3.2 TEM images of (A) TiO$_2$ nanoparticles and (B) Au@CdS nanorods; (C) UV-Vis absorption spectra of TiO$_2$ and Au@CdS at concentrations used to self-assemble the photocatalytic clusters; (D) Chemistry used to couple DNA strands to the TiO$_2$ and Au@CdS nanocrystals. Scale bar 100 nm.
Robinson et al and further modified with Au metal seeds as shown by Ben-Shahar and coworkers. The main reason to synthesize gold decorated CdS (Au@CdS) was to improve the charge separation in CdS, and potentially aid in electron transfer from the CdS to an acceptor such as CO\textsubscript{2} or an organohydride\textsuperscript{152-155}. While Pt could also have been used, as Pt is a strong catalyst for H\textsubscript{2} formation\textsuperscript{23,12,134} as stated in Chapter 2, and thereby would compete with CO\textsubscript{2} reduction, Au was chosen instead. TEM analysis showed the CdS NRs to be ~61 nm ± 11 nm in length and 6 ± 0.9 nm in diameter. The CdS nanorods have a conduction band at -0.9V vs NHE which is negative enough to reduce CO\textsubscript{2}, and have a valence band at 1.8V vs NHE. After seeding Au growth, TEM analysis showed each CdS NR to possess ~15 Au seeds sized ~1-2nm per particle (Figure 4.3.2B).

Next, to attach DNA strands to the as-synthesized TiO\textsubscript{2} and Au@CdS, ligand exchange was performed to transfer the particles from toluene to water. For this, the TiO\textsubscript{2} and Au@CdS nanocrystals were reacted with amine-(PEG)\textsubscript{6}-azide and mercaptoethanol. After phase transfer, the azide-modified TiO\textsubscript{2} were next reacted with alkyne-terminated DNA strands through click chemistry and a 500:1 molar ratio of DNA:TiO\textsubscript{2}. For the Au@CdS, thiolated DNA strands were reacted with the mercaptoethanol modified NRs overnight at pH 9 using 250:1 molar ratios of DNA:Au@CdS (Figure 4.3.2D). To determine the amount of DNA attached per Au@CdS, unbound DNA was removed by microcentrifuge filtration and measured by UV-Vis and was determined to be ~100 strands per Au@CdS. With the DNA conjugated TiO\textsubscript{2}, since the presence of the copper catalyst interfered with UV-Vis measurements, the \textsuperscript{1}H NMR spectrum of the DNA conjugated Au@CdS was obtained and used to calibrate the amount of DNA bound to TiO\textsubscript{2} (Figure 4.3.3A). From this,
we were able to detect ~ 100 strands of DNA attached to each TiO$_2$ nanoparticle as well.

The TiO$_2$, CdS and Au@CdS nanocrystals were next tested for CO$_2$ reduction in both the absence and presence of DNA strands attached. To start, while both TiO$_2$ and CdS should thermodynamically be of sufficient energy to oxidize water, due to the inherent difficulties of photoreducing CO$_2$ to any detectable level using water

Figure 4.3.3 A. NMR spectra of DNA on Au@CdS compared with NMR spectra of mercaptoethanol coated Au@CdS. (without AA in the solution). B. CO$_2$ reduction result with mercaptoethanol coated CdS, mercaptoethanol coated Au@CdS and DNA conjugated Au@CdS.
alone as an electron source, sacrificial electron donors were utilized. In addition, because previous studies showed that using a diffusional molecular catalyst such as pyridine or the metal complex Rh*Cp(bpy)_2Cl_2 can significantly increase CO_2 reduction by acting as hydride transfer agents,^{152,155} Rh*Cp(bpy)_2Cl_2 was added to the photocatalysts in solution. For testing the activity of each semiconductor nanocrystal, nanomolar solutions of either TiO_2, CdS or Au@CdS were mixed with an excess of ascorbic acid (90 mM) and Rh*Cp(bpy)_2(H_2O)_2 (0.32 mM) in phosphate buffer (pH 7) and degassed first for 1 h with Ar followed by a 1 h purge with CO_2. The sealed solutions were next photoirradiated using a solar simulator (1 sun) for 11 h after which the products formed were characterized by ^1H NMR. As shown in Figure 4.3.5A, TiO_2 nanoparticles conjugated either with amine-(PEG)_n-azide or DNA showed as expected, little to no ability to reduce CO_2 to any measurable degree. Using 22 nM CdS NRs however showed the production of 38 ± 5.3 uM formate from CO_2 and by modifying the CdS NRs further with Au seeds, a ~ 3 fold increase in CO_2 reduction to 91±12 uM formate was detected. These CdS or Au@CdS results showed similar yields whether bound with either mercapethanol alone or single stranded or double stranded DNA (Figure 4.3.3B).

For CO_2 reduction, formaldehyde may be produced in addition to formate and methanol include (Figure 4.3.1A). While formate and methanol can be directly observed in ^1H NMR spectra, the rapid equilibrium of formaldehyde with its hydrate form methanediol complicates formaldehyde quantification. To overcome this, we showed in Chapter 3 that the enzyme alcohol dehydrogenase (ADH) can reduce ultra-low levels of formaldehyde to methanol, despite the large equilibrium shift toward methanediol, without any back reactivity.^{156} Therefore, after running a CO_2 photoreduction overnight, ADH can be added next in the dark to reduce any
formaldehyde present to methanol. This led to an increase in the methanol peak in NMR which then correlates to how much formaldehyde was initially present ($[\text{MeOH}]_{\text{after ADH}} - [\text{MeOH}]_{\text{before ADH}}$). In adding ADH after the photoreactions with non-

![Figure 4.3.4](image)

**Figure 4.3.4** CO$_2$ reduction result from Au@CdS, TiO$_2$, mixed Au@CdS and TiO$_2$, and different length of DNA linker linked TiO$_2$ and Au@CdS. (A) NMR spectra of the product after CO$_2$ reduction under illumination. (B) Change of methanol peak in NMR spectra after adding ADH to the CO$_2$ reduction solution.
DNA and DNA conjugated TiO\textsubscript{2}, CdS and Au@CdS, only the Au@CdS NRs showed a production of formaldehyde with an average yield of \(~8.1 \pm 0.9\) uM (Figure 4.3.4).

Next, we tested the CO\textsubscript{2} photoreduction from simple binary mixtures of TiO\textsubscript{2} and Au@CdS nanocrystals dispersed randomly in solution. To do this, we first optimized the molar ratio of TiO\textsubscript{2} to Au@CdS to balance the amount of solar energy absorbed by each semiconductor. Since TiO\textsubscript{2} primarily absorbs \(< ~400\) nm while CdS shows an absorption onset at \(~460\) nm which continues into the UV, the concentrations of each were tuned to maximize the absorption profiles at the respective wavelengths (Figure 4.3.2C). Based on these studies, the optimal molar ratio of TiO\textsubscript{2}:Au@CdS was determined to be 1:2.5. Using this value, a 8.8 nM solution of TiO\textsubscript{2} conjugated with the DNA 7 (Table 4.3.1) was mixed with a 22 nM solution of Au@CdS modified with DNA 1 (Table 4.3.1) to prevent or greatly

<table>
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<tr>
<th>DNA name</th>
<th>DNA sequence</th>
<th>T\textsubscript{m} /\degree C</th>
<th>Note</th>
</tr>
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<tr>
<td>DNA 1</td>
<td>5'-thiol-TTT TTT TTT TTT TTT-3'</td>
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<td>Control on Au@CdS</td>
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<tr>
<td>DNA 2</td>
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<td>10mer on Au@CdS</td>
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<tr>
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<td>30mer on Au@CdS</td>
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<tr>
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<td>48.3</td>
<td>40mer on Au@CdS</td>
</tr>
<tr>
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<td>53</td>
<td>80mer on Au@CdS</td>
</tr>
<tr>
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<td>22.3</td>
<td>10mer on TiO\textsubscript{2}</td>
</tr>
<tr>
<td>DNA 8</td>
<td>5'-hexynyl-TAA TAG GGA AAG CCG TAA TG-3'</td>
<td>37.2</td>
<td>20mer on TiO\textsubscript{2}</td>
</tr>
<tr>
<td>DNA 9</td>
<td>5'-hexynyl-TAA TAG GGA AAG CCG TAA TGT CAC TCA CTC-3'</td>
<td>46.3</td>
<td>30mer on TiO\textsubscript{2}</td>
</tr>
<tr>
<td>DNA 10</td>
<td>5'-hexynyl-TAT TAG GGT ATT G-3'</td>
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<td>40mer part on TiO\textsubscript{2}</td>
</tr>
<tr>
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<td>5'-hexynyl-AAA CTC ACT CAC TCA CTG TTA GGA GTC GTG GGA TTA TTA TTA GGG TAT TGC TGA GTG TGA CCT ACT CAC TCA AA-3'</td>
<td>80mer on TiO\textsubscript{2}</td>
<td></td>
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<tr>
<td>DNA 12</td>
<td>5'-CT GAG GTT CAC TCA-3'</td>
<td></td>
<td>Hairpin part on 40mer DNA</td>
</tr>
<tr>
<td>DNA 13</td>
<td>5'-CTC ACA TCT CAA A-3'</td>
<td></td>
<td>Part of 40mer DNA</td>
</tr>
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</table>

**Table 4.3.1** DNA name and sequence used in this study.
minimize any DNA hybridization between the two. At these concentrations, the average distance between each particle (TiO$_2$/TiO$_2$, TiO$_2$/Au@CdS or Au@CdS/Au@CdS) was calculated to be ~350 nm. As with the single particle composition studies, the TiO$_2$/Au@CdS mixtures were mixed with the donor AA and Rh*Cp(bpy)$_2$Cl$_2$, degassed with Ar followed by CO$_2$ and photoirradiated for 11 h. As shown in Figure 4.3.5A, the photoreactions showed little to no enhancement in CO$_2$ reduction as compared to Au@CdS alone, demonstrating little Z-scheme mediated catalysis.

Next, DNA hybridization was used to control the distance between the donor (TiO$_2$) and acceptor (CdS) nanocrystal and study its effect on CO$_2$ photoreduction.

**Figure 4.3.5** (A) Bar graph showing the overall yields of CO$_2$ photoreduction products from Au@CdS alone, a mix of Au@CdS and TiO$_2$ and DNA assembled TiO$_2$-Au@CdS clusters as a function of DNA length in between the photocatalysts. TEM images showing the interparticle distances mediated by the lengths of DNA strands used: (B) 10 bases; (C) 20 bases; (D) 30 bases; (E) 40 bases; (F) 80 bases. Scale bar 20 nm.
For this, we chose to use double stranded DNA (dsDNA) whose lengths varied from 10 to 80 bases (Figure 4.3.5B-F). As dsDNA has a persistence length of ~100 bases or ~30 nm, DNA bases of 10, 20, 30, 40 and 80 base would correlate to interparticle distances of ~3, ~6, ~9, ~12 and ~24 nm successively. In order to verify that dsDNA can be used to control the spacing between the photocatalysts prior to any studies with CO₂, dimers of TiO₂ and Au@CdS nanoparticles were first fabricated by DNA annealing and characterized by TEM. As shown in Figure 4.3.5B-F, TEM analysis of the TiO₂-Au@CdS clusters linked by 10, 20, 30, 40 and 80 mer dsDNA showed interparticle spacings of 2.3, 5.4, 9.1, 12.3 and 25.4 nm respectively which were close to the expected distances. It should however be mentioned that these samples were measured in a dry state and therefore may be slightly different from what is the actual spacing between TiO₂ and Au@CdS when hydrated.

Once we determined that DNA could control the distance between TiO₂ and Au@CdS, the photoreduction of CO₂ was tested as a function of DNA length. For this, the DNA strands were first conjugated to the TiO₂ and Au@CdS nanocrystals using 100:1 DNA:NP molar ratios. In each case, the amount of DNA loaded per nanocrystal was kept constant as determined by UV-Vis spectroscopy of unbound DNA as well as ¹H NMR of the DNA conjugated nanoparticles. The DNA conjugated TiO₂ nanocrystals were then annealed with the Au@CdS NRs using TiO₂:Au@CdS molar ratios of 1:2.5 and tested for CO₂ reduction in the presence of ascorbic acid and Rh*Cp(bpy)₂Cl₂. First, as opposed to either TiO₂, Au@CdS alone or just mixing the two, the DNA assembled TiO₂-Au@CdS showed a significant 2.94-5.28 fold increase in CO₂ photoreduction as evidenced by the amounts of formate, formaldehyde and methanol generated (Figure 4.3.5A and Figure 4.3.4). In testing TiO₂-Au@CdS clusters assembled using different lengths of DNA ranging from 10 to 80 bases, we
found that overall, the highest yields of CO₂ photoreduction was measured from TiO₂-Au@CdS assemblies separated by DNA 30 bases in length or ~ 9 nm in distance. As shown in Figure 4.3.5A, an average production of 484 ± 14.3 uM formate, 14 ± 2.4 uM formaldehyde and 26 ± 2.3 uM of methanol was detected which correlates to ~1.55% (33.8mM of saturated CO₂ in solution as initial concentration\textsuperscript{157,158}) CO₂ conversion at pH 7. As we decreased the DNA length to 10 and 20 bases, a measurable decrease in CO₂ reduction was observed with the 10 and 20mer DNA strands yielding 0.87% and 1.05% CO₂ to fuel conversion respectively. While the amount of CO₂ reduction with the 10 and 20mer DNA linked TiO₂-Au@CdS is ~3.05 and ~3.65 folds higher as compared with simply mixing the two nanocrystals in solution, as compared to the 30mer dsDNA, a ~33% to 45% decrease in CO₂ conversion was seen. These results were somewhat unexpected, as we hypothesized that the electron transfer in the Z-scheme system would function most efficiently when the spacing between the TiO₂ and Au@CdS was as small as possible. Thus, we theorize that the increased distance may also reduce back electron transfer.

When the dsDNA lengths were increased further to 40 and 80 bases, while a drop in CO₂ reduction as compared to the 30mer DNA was seen by ~25% and ~27% respectively, the photocatalytic performance of the TiO₂-Au@CdS nanoparticle clusters did not diminish further and was still 4.07 and 3.96 folds respectively over mixing the two particle types in solution. These results demonstrate that electron transfer from TiO₂ to Au@CdS can be achieved over DNA lengths that correspond to distances as large as 24 nm. In order to study the effect of using another organic linker in place of DNA, we next coupled the TiO₂ nanoparticles to the Au@CdS NRs by using a 5K polyethylene glycol (PEG) spacer. For this, dibenzocyclooctyne (DBCO)-PEG5K-thiol was reacted first with the azide modified TiO₂ using 50:1 molar ratios of
PEG:TiO$_2$. It was critical to control the amount of PEG attached per nanocrystal to prevent the PEG assembled nanoparticle clusters from aggregating out of solution due to lack of charge on the polymer. Next, the thiol terminated PEG modified TiO$_2$ were reacted with the Au@CdS to build the PEG linked TiO$_2$-Au@CdS clusters (Figure 4.3.6). After cleaning, the PEG assembled nanocrystals were tested for CO$_2$ reduction in the presence of ascorbic acid and Rh*Cp(bpy)$_2$Cl$_2$ and as shown in Figure 4.3.7A, low amounts (0.45%) of CO$_2$ to fuel conversion was detected after 11 h photoirradiation. As a PEG length of 5K would lead to an interparticle spacing of ~11 nm, these results can be compared to the TiO$_2$-Au@CdS clusters assembled with 30 and 40 bases showing that the DNA assembled clusters to be 2.6-3.4 fold more photocatalytically active than the TiO$_2$-Au@CdS particles linked by PEG.

In addition to using linear dsDNA for the assembly, we tested the effect of using DNA that also contained a hairpin domain. This was done primarily to determine if the hairpin affected CO$_2$ reduction from the TiO$_2$-Au@CdS photocatalysts and how it compared to using completely linear DNA. For this, since the TiO$_2$-Au@CdS clusters assembled with 40mer DNA was initially formed by having the 40 base strand attached to the Au@CdS be hybridized to 3 shorter
complementary strands (Figure 4.3.7E), to put a hairpin at the center of the 40mer DNA, the middle complementary strand (DNA 12) was simply removed for the assembly (Figure 4.3.7D). In testing this linear-hairpin 40mer DNA for CO₂ reduction,

![Graph showing CO₂ photoreduction products](image)

**Figure 4.3.7** (A) Bar graph showing the overall yields of CO₂ photoreduction products from TiO₂-Au@CdS clusters assembled using 5K PEG, 30mer DNA (100:1 DNA:NP), 40mer DNA composed of both a hairpin and a linear domain, 40mer linear DNA and 30mer DNA (30:1 DNA:NP). Schematic image showing expected distances between TiO₂ and Au@CdS using (B) 5K PEG, (C) 30mer DNA (D) 40mer linear DNA (E) 40mer composed of both a hairpin and a linear domain.

we discovered that as opposed to pure linear 40mer dsDNA, significantly higher CO₂ to fuel conversion was detected and more importantly, the value obtained was close to what was seen for pure 30mer linear dsDNA (Figure 4.3.7A and Figure 4.3.8). In looking carefully at the distance between TiO₂ and Au@CdS with the linear-hairpin 40mer DNA, we found that if we take into account each of the 13 bases of linear dsDNA to equal 3.9 nm and the width of the hairpin to equal ~2 nm, the interparticle spacing between TiO₂ and Au@CdS is found to be ~9.8 nm which is quite close to the expected ~9 nm of the 30mer DNA. These results highlight that an optimal
interparticle distance of 9-10 nm exists with the TiO$_2$-Au@CdS system if DNA is used as the linker. These combined results demonstrate that there is a distinct correlation between interparticle spacing and Z-scheme assisted photocatalysis and that DNA can indeed aid in the charge transfer from one nanocrystal to the next.

Finally, we examined the effect of DNA density per particle on photocatalytic efficiency. For this, the TiO$_2$ and Au@CdS nanocrystals were first conjugated with ~30 30mer DNA oligonucleotides, as compared to 100 strands per nanocrystal in the aforementioned studies. As shown in Figure 4.3.7A and Figure 4.3.9 (30mer, 30:1 DNA:NP), the decrease in DNA density between the TiO$_2$ and Au@CdS led to a ~30%

**Figure 4.3.8** CO$_2$ reduction result from hairpin 40mer DNA linked TiO$_2$-Au@CdS, and compared with the result from linear 40mer and 30mer DNA linked TiO$_2$-Au@CdS.

**Figure 4.3.9** CO$_2$ reduction result from 30mer DNA linked TiO$_2$-Au@CdS with different loading density of DNA on NPs.
decrease in product formation. To further explore the role of DNA in aiding electron transfer between semiconductor nanocrystals, we applied the DNA assembled photocatalytic clusters for H₂ generation as in Chapter 2, but with ascorbic acid as electron donor. For this, Pt decorated CdS (Pt@CdS) nanorods were used in place of Au@CdS because Pt is a strong catalyst for H₂ production. However, in earlier work we showed that having negatively charged DNA on the photocatalysts can significantly decrease H₂ generation. Thus, the amount of DNA loaded per particle was drastically reduced from 100:1 DNA:NP (used for CO₂ reduction) to ~2:1 for these studies using ascorbic acid as a donor. After degassing with Ar, the amount of H₂ formed over 2 h under solar illumination was measured by sampling the vapor phase and running GC. As shown in Figure 4.3.10, in distinct contrast to that observed for CO₂, an increase in H₂ was observed as the DNA length shortened from 30 to 10 bases. An explanation for this is that because few DNA strands are present between the TiO₂ and Pt@CdS nanocrystals, fewer avenues for electron transfer exist, thereby causing particles in closer proximity to show improved Z-scheme performance.

![Figure 4.3.10 Results of H₂ production from TiO₂-Pt@CdS clusters with varying lengths of DNA. In this instance, the amount of DNA linking the two particles was ~2:1 DNA:nanoparticle.](image)
4.4 Conclusion

To summarize, we demonstrate here the power of DNA for assembling donor-acceptor nanocrystals to facilitate Z-schemes for photocatalysis. In this work, we show that TiO$_2$ and Au@CdS nanoparticles spaced with DNA lengths ranging from 10-80 bases can yield $\sim$2.94 to 5.25 fold improvement in CO$_2$ reduction over either simply mixing the photocatalysts in solution or using a 5K PEG linker. These results not only show that DNA hybridization can couple donor-acceptor nanocrystals for Z-scheme photocatalysis, but also that charge transport can occur over distances as long as 24 nm when DNA is used as the linker. In addition, we show that a linear 30mer dsDNA or a 40mer DNA composed of both linear and hairpin DNA leads to the highest amount of CO$_2$ conversion, showing that with TiO$_2$ and Au@CdS as the donor-acceptor photocatalysts, the optimal interparticle spacing is $\sim$9-10 nm. We hypothesize that, at this distance, a balance is reached between the rates of forward electron transfer and electron donor oxidation. Lastly, we show that lowering the amount of DNA linking the photocatalysts can clearly influence the yields of CO$_2$ reduction or water splitting, showcasing the role of DNA in mediating electron transfer. In future studies, time-resolved spectroscopic studies will be run to directly measure the electron transfer kinetics between the different photocatalysts assembled by the DNA strands. It is envisioned that these future studies, combined with the results shown here, will open up new avenues in bio-nanoelectronics to biohybrid materials for energy harvesting and renewable energy.
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