2017-04-17

Functional Nanomaterials for Environmental Applications and Bioassemblies

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UNIVERSITY OF MIAMI

FUNCTIONAL NANOMATERIALS FOR ENVIRONMENTAL APPLICATIONS AND BIOASSEMBLIES

By
Michelle Anne Nguyen

A DISSERTATION

Submitted to the Faculty of the University of Miami in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Coral Gables, Florida
May 2017
UNIVERSITY OF MIAMI

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

FUNCTIONAL NANOMATERIALS FOR ENVIRONMENTAL APPLICATIONS AND BIOASSEMBLIES

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The rational design of nanomaterials has yielded new technologies that have revolutionized numerous diverse fields. The work detailed herein first describes the application of photocatalytic nanomaterials towards the environmental remediation of harmful toxins. Specifically, a low-temperature solution-phase synthetic route for size-controlled Cu$_2$O octahedra particles was developed, and these materials were evaluated as catalysts for the photocatalytic degradation of aromatic organic compounds. Moreover, cubic Cu$_2$O/Pd composite structures were fabricated and demonstrated to be effective photocatalysts for the generation of H$_2$ and the reductive dehalogenation of polychlorinated biphenyls, well-known carcinogens present at many contaminated sites around the world. This photocatalytic approach to environmental remediation exemplifies the adaptation of light-driven technologies and sustainable practices to energy-intensive catalytic systems. In addition, this work also investigates the organic/inorganic interface of peptide-mediated Au nanoparticles as a means to identify rational design principles for materials binding peptide sequences for the advancement of stimuli-responsive bionanoassemblies. Factors inherent to peptide sequences that can promote strong materials-binding affinity and/or effective nanoparticle stabilization capability were identified in order to progress biomimetic technologies. These findings were elucidated
using a combinational approach of peptide binding experiments to Au in partnership with molecular dynamics simulations. Overall, this work demonstrates the growing applications of nanomaterials in remediation technologies and aids in the understanding of the origins of peptide material affinity and nanoparticle stabilization.
ACKNOWLEDGEMENTS

I am forever grateful to have Dr. Marc Knecht as my Ph.D. advisor and mentor; he has continuously pushed me to be my best. Because of him, I am the chemist and teacher I am today. I also thank my committee members for taking the time out of their busy schedules to oversee my progress throughout my graduate career: Dr. James Wilson, Dr. Rajeev Prabhakar, and Dr. Kevin Collins. I am especially grateful to Dr. Wilson; if I had not met him as an undergraduate at the University of San Francisco, I would have never changed my major to Chemistry and I would most certainly not be at the University of Miami today. Additionally, I acknowledge and thank our collaborators for contributing to my work: Dr. Elsayed Zahran, Dean Leonidas Bachas, Dr. Nicholas Bedford, Dr. Zak Hughes, Dr. Tiffany Walsh, Dr. Yue Li, Yang Liu, and Dr. Mark Swihart. Furthermore, I acknowledge and thank the undergraduate researchers I have mentored and who have contributed to my work: Azaan Wilbon, Robert Goodin, Alex Besmer, Vincent Cendan, Manuel Portela, Carla Bobé, and Fatima Chagani. I am especially honored to have taught and mentored Azaan; it has been incredibly rewarding to watch him grow, both intellectually and emotionally, into the person he is today. Finally, I thank my labmates, both past and present, for their camaraderie and solidarity. I especially thank Catherine Munro for throwing the most outrageous parties in the lab office and for fixing all the instruments when they break. I thank Randy Lawrence for his unwavering positivity over the past several years.

Words cannot express the deep love and affection I hold for my mom. She has endured painful hardships and made countless sacrifices to give me the life, education, and opportunities she never had growing up. For that, I am eternally grateful for her and
am extremely proud to be her daughter. I would like to thank my grandparents, Ông Bà. They welcomed my mom and I into their family. They love and care for us like we are their blood, and I am forever grateful for them. Lastly, I thank Kim Nguyen from the bottom of my heart for being the linchpin that holds our family together. I consider Kimmy to be the big sister I never had. She is always looking out for me, and she always has my back. I look up to her, and I am in absolute awe of the strength that she exudes.
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Chapter 1. Introduction

1.1 The Rational Design of Functional Nanomaterials
The energy crisis, climate change, and antibiotic resistant superbugs are just some of the issues mankind will face in the coming decades. These challenges and others have brought about a need for the development of innovative tools, methods, and materials in order to help scientists, engineers, and clinicians problem solve. The recent use of nanomaterials in a wide range of industries, such as electronics and medicine, has yielded significant progress toward resolving problems that previous technologies could not achieve. For example, the application of nanomaterials in the field of medicine has revolutionized how diseases are detected, imaged, and treated.1-2 The rational design of functional nanomaterials has led to groundbreaking innovations, including controlled drug delivery systems and technologies that capture, convert, and store renewable energy.3-6 The following examples of nanomaterials and their uses promote the concept of rational design and demonstrate how researchers have creatively problem-solved societal needs. Additionally, these examples highlight contributions nanotechnology has made in the areas of energy, catalysis, environmental remediation, and multicomponent assemblies.

1.2 Photocatalytic Semiconductors
Sustainable energy production at an industrial-scale is needed in order to meet the ever-increasing worldwide demand for energy and to prevent the ecological destruction of the planet. Therefore, accelerating the development of energy technologies is of the utmost importance. Photocatalytic nanomaterials offer an environmentally friendly alternative to traditional thermally-driven catalysts. During a photocatalytic process, a photocatalyst,
such as a metal oxide semiconductor, is irradiated with light and subsequently absorbs the incident photons. As illustrated in Figure 1.1, if the energy of the photon is equal to or greater than the band gap ($E_g$) of the material, an electron ($e^-$) is excited from the valence band to the conduction band, thereby generating a positive hole ($h^+$) in the valence band.\(^7\) The electron–hole charge carriers ($e^-_{CB} + h^+_{VB}$) can either undergo recombination or migrate to the surface of the catalyst to initiate reactions with surface adsorbed substrates.

For example, the photoexcited electrons in the conduction band can react with surface adsorbed oxygen to form superoxide radicals (eqn 1) that can participate in the

**Figure 1.1.** Schematic diagram illustrating semiconductor photocatalysis. Upon irradiation with energy equivalent to or greater than the band gap of the semiconductor photocatalyst, electrons in the valance band are excited to the conduction band, leaving positive holes in the valance band. These photo-generated electrons and holes initiate reduction and oxidation reactions, respectively. Adapted with permission from Banerjee, S.; Pillai, S. C.; Falaras, P.; O’Shea, K. E.; Byrne, J. A.; Dionysiou, D. D. New Insights into the Mechanism of Visible Light Photocatalysis. *J. Phys. Chem. Lett.* 2014, 5, 2543-2554. Copyright 2014 American Chemical Society.
degradation of organic pollutants, whereas positive holes in the valence band can oxidize surface adsorbed water to generate hydroxyl radicals (eqn 2), which in turn can oxidize organic pollutants.

\[
e^{-}_{CB} + O_2 \rightarrow 'O_2^- \quad (1)
\]

\[
h^{+}_{VB} + H_2O \rightarrow 'OH + H^+ \quad (2)
\]

1.2.1 Wide vs. Narrow Band Gap Semiconductors

Various strategies have been developed to improve the absorption capability of semiconductors in order to exploit a wider range of the solar spectrum. One such strategy is to utilize narrow band gap semiconductors, or visible light photocatalysts, over wide band gap semiconductors. Wide band gap semiconductors, such as TiO₂ and ZnO, are typically restricted in their light absorption capabilities. The anatase form of TiO₂ and ZnO have band gap energies of 3.2 eV and 3.0 eV, respectively, which restricts their light absorption to ultraviolet (UV) wavelengths only (wavelengths ≤390 nm). This limits the practical applications of standalone TiO₂- and ZnO-based photocatalysts for solar light harvesting, as over 50% of the sun’s radiation is emitted in the visible and infrared regions of the electromagnetic spectrum.8 Narrow band gap semiconductors, on the other hand, are of particular interest because they can absorb photons from both UV and visible light for photoexcitation and electron/hole pair generation, thus taking advantage of a wider range of the solar spectrum. An example of a visible light photocatalyst is Cu₂O, which has a band gap of 2.17 eV. This small band gap allows Cu₂O to absorb photons from wavelengths ≤570 nm. Because visible light is one of the main components of the solar spectrum, a primary goal in photo-based processes is to use visible light to drive the reaction.
Many metal oxide semiconductors have been found to be highly photocatalytically active for both reduction and oxidation reactions. However, not all semiconductors are capable of initiating the same reactions. As previously discussed, upon irradiation with energy equivalent to or greater than the band gap of the semiconductor photocatalyst, electrons in the valance band are excited to the conduction band, leaving positive holes in the valence band. These photo-generated electrons and holes initiate reduction and oxidation reactions, respectively. To achieve a particular reduction reaction, the conduction band must be more negative than the reduction potential of the reaction. Similarly, to achieve a specific oxidation reaction, the valence band must be more positive than the oxidation potential of the reaction. For example, Cu$_2$O has band edge potentials suitable for the reduction of molecular oxygen to superoxide but not for the oxidation of water to hydroxyl radicals. Figure 1.2 shows the energy positions of conduction and valence band edges for selected metal oxide semiconductors at pH 1.\textsuperscript{9-11} The conduction band for Cu$_2$O is more negative than the reduction potential of O$_2$ to *O$_2^-$ (−0.33 V vs. NHE at pH 1),\textsuperscript{12} therefore Cu$_2$O is capable

![Figure 1.2. Band energy diagram of selected metal oxide semiconductors.](image)
of producing superoxide; however, both TiO$_2$ and ZnO are incapable of producing superoxide because their conduction bands are less negative than the reduction potential of the reaction. In the same manner, Cu$_2$O cannot produce hydroxyl radicals since its valance band is less positive than the oxidation potential of H$_2$O to $^\circ$OH/H$^+$ (+2.72 V vs. NHE at pH 1),$^{12}$ whereas both TiO$_2$ and ZnO can produce hydroxyl radicals because their valance bands are more positive than the oxidation potential of the reaction. Utilizing photocatalysts that exhibit sufficiently small band gap energies for visible light absorption and that have conduction and valence bands at potentials appropriate for the desired reaction are essential to advancing catalyst technologies toward more sustainable practices.

### 1.3 Cu$_2$O Nanomaterials

The synthesis of inorganic materials on the nano and micron scale with simultaneous control over the shape and size has been of great interest for a variety of applications.$^{13-15}$ This is of particular importance for the use of such materials as catalysts for numerous chemical transformations. In this regard, the synthetic procedures used to access precise shape and size control can be exploited to manipulate the atomic display of the material. For instance, changing of the particle shape allows for the ability to selectively display particular facets and vary the number and type of defect atoms (edge and vertex) present within the structure.$^{16-18}$ These two features work in conjunction to mediate the final reactivity of the structure via the development of different active sites at the particle interface responsible for the catalytic reactivity. Unfortunately, the ability to fine tune the size, shape, and overall composition of inorganic materials remains complex where facile and straightforward approaches are needed to enhance the fabrication of structures with optimized catalytic reactivity.
1.3.1 Synthetic Strategies for Shape-Controlled Cu$_2$O Nanoparticles and Their Comparative Photocatalytic Activity

Copper(I) oxide is a p-type semiconductor with a small band gap of 2.17 eV, making it capable of absorbing photons from both UV and visible light. Copper(I) oxide materials are attractive for catalytic applications due to their utilization of renewable solar energy to drive the reaction. Limiting the size of the materials to the nanoscale guarantees optimization of the particle surface-to-volume ratio, which maximizes the exposure of the photocatalytic materials for increased reactivity. Moreover, different particle morphologies can alter the material’s photocatalytic properties. Recently, various synthetic approaches have been reported for the controlled production of differently shaped Cu$_2$O nanoparticles.\textsuperscript{19-20} For instance, Sui et al. has developed a low temperature solution-phase method for the mass synthesis of Cu$_2$O crystals with different morphologies by reducing a copper-citrate complex with glucose in the presence of polyvinylpyrrolidone (PVP, $M_w = 30,000$) at 80 °C.\textsuperscript{21} An interesting shape evolution from cubes, truncated octahedra, (truncated) octahedra, octahedra, and finally to spheres was realized using this procedure. In this system, the morphological evolution was investigated as a function of the PVP concentration. To achieve shape tunability of the Cu$_2$O crystals, the copper-citrate complex was reduced with glucose under five different PVP concentrations: 0.5, 1.5, 2.5, 4.5, and 9 mM.

Field-emission scanning electron microscopy (FESEM) images from the reaction using 0.5 mM PVP displayed well-defined Cu$_2$O cubes with sharp edges, uniform shape, and an average edge length of \~800 nm (Figure 1.3a).\textsuperscript{21} Analysis of the materials prepared with 1.5 mM PVP resulted in Cu$_2$O truncated octahedra with a narrow edge length distribution of \~300–500 nm (Figure 1.3b).\textsuperscript{21} Increasing the PVP concentration to
2.5 mM led to the truncation of the octahedral vertexes, as seen in Figure 1.3c. These structures were termed “(truncated) octahedra” by the authors. The (truncated) octahedra had a side length ranging from 600 to 800 nm and small square facets at the vertices. The materials generated using 4.5 mM PVP yielded fully developed Cu$_2$O octahedra.

![Figure 1.3](image)

**Figure 1.3.** FESEM images of the Cu$_2$O crystals synthesized using different concentrations of PVP: (a) 0.5 mM—cubes (scale bar 100 nm), (b) 1.5 mM—truncated octahedra (scale bar 1 µm), (c) 2.5 mM—(truncated) octahedra (scale bar 1 µm), (d) 4.5 mM—octahedra (scale bar 1 µm), and (e) 9 mM—spheres (scale bar 100 nm). Adapted with permission from Sui, Y.; Fu, W.; Yang, H.; Zeng, Y.; Zhang, Y.; Zhao, Q.; Li, Y.; Zhou, X.; Leng, Y.; Li, M.; Zou, G. Low Temperature Synthesis of Cu$_2$O Crystals: Shape Evolution and Growth Mechanism. *Cryst. Growth Des.* **2010**, *10*, 99-108. Copyright 2010 American Chemical Society.
The structures possessed perfect octahedral morphology with sharp corners and well-defined edges. The octahedra edge lengths ranged from 1 to 1.2 µm. This is slightly increased from the (truncated) octahedra side lengths of 600–800 nm. Finally, when 9 mM PVP was used in the reaction, loose spherical particles with rough surfaces were obtained (Figure 1.3e). The spheres were monodisperse in size with a diameter of 300–400 nm. In this synthetic procedure, PVP acted as the stabilizing polymer to control the shape of the particles. In other methods, however, particle structure is dictated by the amount of reductant, as opposed to the amount of capping agent. One such example is discussed next.

Huang and coworkers have demonstrated a solution-phase approach for tailoring the morphology of Cu$_2$O structures. In this study, Cu$_2$O nanocrystals with systematic shape evolution were synthesized by mixing an aqueous solution of CuCl$_2$, sodium dodecyl sulfate (SDS), NaOH, and NH$_2$OH·HCl reductant at room temperature. Extensive characterization of the reaction under various parameters demonstrated that the NH$_2$OH·HCl amount strictly controlled the structure of the materials, as well as their catalytic functionality. By varying the volume of NH$_2$OH·HCl (0.1 M) added to the reaction, a series of Cu$_2$O nanostructures from nanocubes to rhombic dodecahedra were synthesized. Figure 1.4 gives a summary of the reaction conditions used and shows scanning electron microscopy (SEM) images of each particle shape viewed from two different orientations. By progressively increasing the volume of NH$_2$OH·HCl added, Cu$_2$O cubes (0.40 mL), face-raised cubes (0.60 mL), edge- and corner-truncated octahedra (0.92 mL), all-corner-truncated rhombic dodecahedra (0.98 mL), {100}-truncated rhombic dodecahedra (1.20 mL), and finally rhombic dodecahedra (2.40 mL)
were synthesized. The average particle sizes for the cubes, edge- and corner-truncated octahedra, {100}-truncated rhombic dodecahedra, and rhombic dodecahedra were 202 ± 15 nm, 142 ± 15 nm, 273 ± 25 nm, and 290 ± 24 nm, respectively. This was the first time Cu₂O rhombic dodecahedra with sizes of ~300 nm had been synthesized.

**Figure 1.4.** SEM images and the corresponding schematic drawings of the Cu₂O nanocrystals synthesized with morphology evolution from cubes to rhombic dodecahedra upon increasing the amount of NH₂OH·HCl added to the reaction mixture. Two orientations are shown for each particle shape. Adapted with permission from Huang, W.-C.; Lyu, L.-M.; Yang, Y.-C.; Huang, M. H. Synthesis of Cu₂O Nanocrystals from Cubic to Rhombic Dodecahedral Structures and Their Comparative Photocatalytic Activity. *J. Am. Chem. Soc.* 2011, *134*, 1261-1267. Copyright 2011 American Chemical Society.
As the reaction proceeded during nanocrystal growth, a series of color changes in the reaction solution was observed. Immediately after adding NaOH to the aqueous mixture of CuCl₂ and SDS, the solution turned light blue due to the formation of Cu(OH)₂ and/or Cu(OH)₄²⁻. For the synthesis of Cu₂O nanocubes, the solution color turned from light blue to green within seconds after adding the NH₂OH·HCl reductant, indicating the onset of nanocrystal growth. After 20 s, the solution color turned green-yellow and then deep yellow-orange after 40 s. After 40 s, no further color changes were observed. Since it is known that Cu₂O nanocrystals in the 200–300 nm size range exhibit this color,²³⁻²⁴ the observation implied that the growth rate of the nanocubes was quite fast. Similar color changes were also observed in the synthesis of Cu₂O rhombic dodecahedra, but the evolution proceeded at a much slower rate. The solution was still green 3 min following the addition of NH₂OH·HCl, yellow between 8 and 10 min, and orange from 10 to 60 min. This observation suggested a much slower growth rate in the formation of rhombic dodecahedra particles. As such, an important insight from this observation is that the nanocrystal morphology is highly related to the particle growth rate.

Further work using the synthesized Cu₂O nanocrystals revealed high activity for their use as photocatalysts for the photodegradation of organic pollutants.²² The cubic and rhombic dodecahedral Cu₂O structures were used as catalysts for the photodegradation of methyl orange dye to investigate the facet-dependent photocatalytic activity of Cu₂O nanocrystals. Previously, it was demonstrated that Cu₂O cubes bound exclusively by {100} facets were inactive toward the photodegradation of methyl orange, while Cu₂O octahedra bound exclusively by {111} facets exhibited moderate activity.¹⁹,²⁵ Figure 1.5
presents the reaction analysis for the photodegradation of methyl orange as a function of time using the cubic and rhombic dodecahedral Cu$_2$O nanocrystals as photocatalysts.\textsuperscript{22} As observed before, the cubes were essentially inactive. However, after 90 min of photoirradiation, the methyl orange dye was completely photodecomposed by the Cu$_2$O rhombic dodecahedra nanocrystals. The results showed that Cu$_2$O rhombic dodecahedra bound exclusively by \{110\} facets were far more photocatalytically active for the photodegradation of methyl orange than the Cu$_2$O cubes and octahedra. This indicates that the morphology of the Cu$_2$O nanocrystals has dramatic impacts on the photocatalytic properties and functionality of the materials.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure15.png}
\caption{Reaction analysis for the photodegradation of methyl orange dye as a function of time using Cu$_2$O cubic and rhombic dodecahedral nanocrystals as the photocatalysts. Adapted with permission from Huang, W.-C.; Lyu, L.-M.; Yang, Y.-C.; Huang, M. H. Synthesis of Cu$_2$O Nanocrystals from Cubic to Rhombic Dodecahedral Structures and Their Comparative Photocatalytic Activity. \textit{J. Am. Chem. Soc.} \textbf{2011}, \textit{134}, 1261-1267. Copyright 2011 American Chemical Society.}
\end{figure}
1.3.2 Synthetic Strategies for Size-Controlled Cu$_2$O Nanoparticles

The size and shape of inorganic materials are well known to have great effects on their catalytic properties, thus recent studies have focused on controlling such structural properties. A common method used to adjust the size of nanoparticles is to manipulate the stabilizing polymer concentration. Generally, it is expected that as the concentration of the capping agent increases, the particle size will decrease because the capping agent blocks sites for further nucleation and growth. However, this is not always the case, as is observed in the following example. Gou et al. have prepared highly uniform Cu$_2$O nanocubes by using a simple solution approach. Aqueous CuSO$_4$ was reduced with ascorbic acid in the presence of poly(ethylene) glycol (PEG, $M_w = 600$) as a stabilizer and NaOH. The average edge lengths of the cubes were controlled from 25 to 200 nm by changing the reagent addition order and the PEG concentration. For these reactions, aqueous CuSO$_4$ was co-mixed with various volumes of PEG (0.05 M). Ascorbic acid and NaOH were then added to the Cu(II)-PEG complex in three different manners (A, B, and C). For procedure A, ascorbic acid was added to the reaction first and then NaOH was added 1 min later. In procedure B, ascorbic acid and NaOH were mixed together before being added to the Cu(II)-PEG complex. For procedure C, NaOH was added 1 min before adding ascorbic acid to the reaction.

Transmission electron microscopy (TEM) characterization of the structures fabricated according to procedure A using 1 mL of PEG demonstrated the formation of irregularly shaped Cu$_2$O particles that were less than 30 nm in size (Figure 1.6a). For procedure A, in which ascorbic acid was added before NaOH, PEG concentrations were varied by a factor of 20 with no change in particle size or shape observed. In procedure
B, in which the ascorbic acid and NaOH were simultaneously added, decreasing the PEG concentration led to smaller particle sizes while retaining uniform cubic morphology. Figures 1.6b–d present TEM images and size histograms for Cu$_2$O cubes prepared using 4, 2, and 1 mL of PEG with an average size of 80, 50, and 25 nm, respectively. For procedure C, in which NaOH was added before ascorbic acid, the relationship between
particle size and PEG concentration was difficult to elucidate. Uniform Cu$_2$O cubes with an average size of 150 nm were observed when 2 mL of PEG were employed, while nanocubes with an average size of 80 nm were formed when the volume of PEG increased to 4 mL. However, increasing the PEG volume further to 8 mL produced aggregates that were larger than 200 nm. For low concentrations of PEG (1 mL), a bimodal distribution of large cubes (~200 nm) and smaller cubes (~60 nm) were observed (Figure 1.6e).23 Taken altogether, this work demonstrated that increasing the capping agent concentration can lead to larger structures instead of smaller ones.

Two synthetic procedures have been presented where varying the capping agent concentration resulted in different structural changes. In the first method, the capping agent was used to alter the particle morphology. In the second approach, the capping agent was employed to modify the size of cubic particles. This demonstrates that the synthesis of inorganic nanoparticles does not follow a cookie cutter template. Changing the capping agent parameters in one reaction may have drastically different results in another reaction. Overall, this exemplifies the subtle (size-control) yet complex (shape-control) influence of reagent parameters and synthetic conditions on the final particle morphology and size.

Through a different technique, size-tuning capabilities were achieved using a modified seed-mediated approach. Huang and coworkers have reported the production of monodisperse Cu$_2$O nanocubes with five different sizes ranging from 40 to 420 nm.27 To prepare Cu$_2$O nanocubes of different sizes (Figure 1.7), an aqueous solution of CuSO$_4$ and SDS, which served as the capping surfactant, was transferred to a bottle labeled A.27 The same solution was also added to five other bottles labeled B–F. Next, sodium
ascorbate (SA) reductant was added to bottle A followed by NaOH. An aliquot of 1 mL was transferred to bottle B. After 2 h, the solution in bottle A displayed a brownish-yellow color, indicating the formation of Cu₂O particles. Sodium ascorbate and NaOH were then added to bottle B, which at this point contained only CuSO₄, SDS, and 1 mL of the seed solution. Again, 1 mL of the solution in bottle B was transferred to bottle C. A bright yellow solution was obtained in bottle B after 2 h. The same procedure was followed to prepare the solutions in bottles C–F.

**Figure 1.7.** Schematic illustration of the seed-mediated procedure used to grow Cu₂O nanocubes of different sizes. The procedure used to prepare samples C–F is the same as that for sample B. Adapted from Kuo, C. H.; Chen, C. H.; Huang, M. H. Seed-Mediated Synthesis of Monodispersed Cu₂O Nanocubes with Five Different Size Ranges from 40 to 420 nm. *Adv. Funct. Mater.* **2007**, *17*, 3773-3780 with permission from John Wiley and Sons.
Figure 1.8. SEM (columns 1 and 2) and TEM images (columns 3 and 4) of the Cu$_2$O nanocubes produced for samples A–F. Columns 2 and 4 are the enlarged images of the respective samples. Adapted from Kuo, C. H.; Chen, C. H.; Huang, M. H. Seed-Mediated Synthesis of Monodispersed Cu$_2$O Nanocubes with Five Different Size Ranges from 40 to 420 nm. Adv. Funct. Mater. 2007, 17, 3773-3780 with permission from John Wiley and Sons.
SEM and TEM analysis of sample A revealed the formation of large quasi-spherical structures and seed particles with average diameters of 340 ± 42 nm and 14.1 ± 2.5 nm, respectively (Figure 1.8A). Sample B yielded the first and smallest Cu$_2$O nanocubes of the series with an average diameter of 41.2 ± 3.4 nm (Figure 1.8B). Cu$_2$O nanocubes with progressively larger sizes were synthesized for samples C, D, E, and F; the resulting nanocubes in these samples had diameters of approximately 65, 100, 230, and 420 nm, respectively (Figures 1.8C–F). As seen in Figure 1.8, well-defined nanocubes were the exclusive shape formed. This is attributed to the use of CuSO$_4$ and SDS as the copper ion source and capping surfactant, respectively. When Cu(NO$_3$)$_2$ was employed as the copper ion source, fused nanoparticle aggregates were produced. When cetyltrimethylammonium bromide (CTAB) was used as the capping surfactant with either CuSO$_4$ or Cu(NO$_3$)$_2$ as the copper ion source, extensive aggregation of irregularly shaped particles was observed. These results again illustrate the significant effect reagent parameters can have on the final product morphology and size.

1.4 Stimuli-Responsive Bionanoassemblies

Being able to alter the properties of a material via an external factor, such as light, temperature, or pH, is an attractive consideration when designing advanced functional materials. A stimuli-responsive material has one or more properties that can be significantly changed in a controlled manner by an external force. A multitude of materials that respond to optical, thermal, and electrical stimuli have been reported, and these properties have been used for the development of dynamic assemblies and devices. One area where stimuli-responsive materials are making a significant impact is plasmonic assemblies.
1.4.1 Bionanocombinatorics

Bionanocombinatorics is a newly developed approach for the fabrication of stimuli-responsive assemblies using engineered biomolecules and nanostructures. This strategy exploits the specific non-covalent interactions between peptides and inorganic nanoparticles to produce reconfigurable biological nanoassemblies using switchable molecules that link two different materials binding sequences, thereby allowing a single molecule to bridge two different inorganic nanoparticles. Initially, peptide sequences with desired binding characteristics for material selectivity were identified. Through experimental binding measurements and advanced molecular simulations, an understanding of the binding affinity of peptide sequences for inorganic surfaces at the molecular level was achieved. This knowledge of binding affinities guided the synthesis of peptide-capped nanoparticles in addition to the synthesis of remotely actuated molecular switches. When employed in concert, these components produce responsive bionanocombinatoric assemblies.

1.4.2 PARE-Mediated Multi-Material Nanoparticle Assembly

Peptide assembling responsive elements (PAREs) are a class of biomolecular linkers that are created using materials binding sequences (MBSs) and peptide actuating spacers (PASs), as illustrated in Figure 1.9. Materials binding sequence 1 (MBS1) and materials binding sequence 2 (MBS2), located at opposite ends of the PARE, selectively bind materials 1 (Au) and 2 (Ag), respectively. Connecting MBS1 and MBS2 is a PAS. The PAS is a molecule with at least two distinct conformational states, with switching between these states triggered by an external stimulus.
To identify materials binding sequences best suited for bionanocombinatorics, Tang et al. quantitatively measured the binding kinetics and thermodynamics for 12 peptide sequences to gold via quartz crystal microbalance (QCM) analysis (Table 1.1). In addition to gold binding peptides, sequences that were not specifically isolated for their ability to bind to gold were included in this set as well. These sequences include two silver binding peptides, AgBP1 and AgBP2, a quartz binding peptide, QBP1, and a palladium binding peptide, Pd4. Figure 1.10 presents the QCM analysis for two of the peptides studied, AuBP1 and Pd4. Figure 1.10a specifically presents the observed frequency changes for the AuBP1 peptide at five different concentrations. Note that inverted plots are displayed for more intuitive data interpretation. Higher peptide concentrations produced larger and faster frequency changes, reflecting increased binding rates. Analysis for the Pd4 peptide (Figure 1.10b) showed that it also bound appreciably to the gold surface; however, it ultimately proved to be the weakest binder among the peptides in Table 1.1.
Fitting each QCM-derived binding curve to Langmuir kinetics provided pseudo-first order adsorption rate constants \( (k_{\text{obs}}) \). Plotting the \( k_{\text{obs}} \) values as a function of the AuBP1 peptide concentration, as shown in the right panel of Figure 1.10a, gives the binding constants for adsorption \( (k_a) \) and desorption \( (k_d) \) as the slope and \( y \)-intercept of the best fit line of the plot, respectively.\(^{34}\) The binding equilibrium constant can then be calculated as \( K_{eq} = k_a/k_d \). The binding affinity of a peptide sequence to an inorganic surface is defined as the change in Gibbs free energy between the peptide bound and unbound states, \( \Delta G = \Delta H - T\Delta S \). The value of \( \Delta H \) is associated with the number and strength of contact residues, defined as residues with strong and persistent non-covalent interactions with the surface. Conversely, the value of \( \Delta S \) depends on the number of possible configurations, or in other words the conformational freedom, of the adsorbed peptide on the inorganic surface. Additionally, the value of \( \Delta G \) can also be obtained

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>( \Delta G_{\text{abs}} ) (kJ/mol)</th>
<th>Anchor assignment</th>
<th>Entropy assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuBP1</td>
<td>WACRKLVLRRRE</td>
<td>-37.6 ± 0.9</td>
<td>Strong</td>
<td>Medium</td>
</tr>
<tr>
<td>GBP1</td>
<td>MGKTSATSGTIQG</td>
<td>-37.6 ± 1.0</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>B1</td>
<td>LKAHPPLPSLPS</td>
<td>-36.6 ± 1.2</td>
<td>Weak</td>
<td>Medium</td>
</tr>
<tr>
<td>AuBP2</td>
<td>WALKRSIRQRSV</td>
<td>-36.4 ± 0.3</td>
<td>Strong</td>
<td>Medium</td>
</tr>
<tr>
<td>Midas2</td>
<td>TGTSLDATPMV</td>
<td>-35.7 ± 1.2</td>
<td>Weak</td>
<td>High</td>
</tr>
<tr>
<td>AgBP2</td>
<td>EQLGVKELRGGV</td>
<td>-35.3 ± 1.2</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>Z2</td>
<td>RMRKMKM</td>
<td>-35.0 ± 0.6</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>QBP1</td>
<td>PPPWLPYPPWS</td>
<td>-35.0 ± 1.1</td>
<td>Strong</td>
<td>Medium</td>
</tr>
<tr>
<td>A3</td>
<td>YSSGAPPMPPE</td>
<td>-31.8 ± 0.3</td>
<td>Strong</td>
<td>High</td>
</tr>
<tr>
<td>AgBP1</td>
<td>TGIKAAMPN</td>
<td>-31.6 ± 0.2</td>
<td>Strong</td>
<td>High</td>
</tr>
<tr>
<td>Z1</td>
<td>KHHCWHW</td>
<td>-31.3 ± 0.1</td>
<td>Strong</td>
<td>Low</td>
</tr>
<tr>
<td>Pd4</td>
<td>TSNAVHTPLRL</td>
<td>-30.3 ± 0.2</td>
<td>Weak</td>
<td>High</td>
</tr>
</tbody>
</table>

through the binding equilibrium constant, \( \Delta G = -RT \ln(K_{eq}) \). Using this approach, the \( \Delta G \) of binding for the AuBP1 peptide on gold was determined to be \(-37.6 \pm 0.9 \) kJ/mol. This value reflects a peptide sequence that strongly binds to gold. The right panel of Figure 1.10b presents the same analysis for the Pd4 peptide.\(^{34} \) For Pd4, the \( \Delta G \) value was calculated to be \(-30.3 \pm 0.2 \) kJ/mol. This value indicates that the Pd4 sequence does bind to gold but less strongly than AuBP1. This analysis was carried out for each of the
peptides in Table 1.1, where they are listed in order of decreasing $\Delta G$ values. These ranged from $-30.3$ to $-37.6$ kJ/mol for the weakest (Pd4) and strongest (AuBP1 and GBP1) binders, respectively.

Advanced molecular simulations were carried out on the same set of 12 peptides. Likely adsorbed-peptide conformations for each sequence were identified through this work, and the binding affinities derived from the QCM studies were analyzed in light of the structural predictions to elucidate the molecular basis for the differing degrees of binding. For each peptide, the residues that made the most persistent surface contact were identified. The resulting contact residues are highlighted in green for each sequence in Table 1.1. Moreover, the enthalpic contribution to the experimentally determined $\Delta G$ values was estimated for each sequence by assigning a score to each contact residue based on previous calculations of amino acid binding strengths to gold. These scores are then added up to provide a total enthalpic score for each peptide, which is then used to classify the binding enthalpy as weak, medium, or strong for each sequence. Furthermore, the configurational entropic contribution to $\Delta G$ was estimated for each sequence as well. From this, guidelines for classifying peptide sequences as either enthalpically- or entropically-driven binders were established. For example, strong binding could arise from a large number of strongly binding residues (enthalpically-driven binding) or from fewer strongly binding residues combined with high configurational entropy based on the peptide’s ability to assume a large number of distinct conformations in the surface-adsorbed state (entropically-driven binding). The configurational entropy classification for each peptide is summarized in Table 1.1.
Five contact residues were identified for AuBP1, as indicated in Table 1.1. These contact residues imbue a strong enthalpic contribution to the binding, which is combined with a medium entropic contribution. For this reason, AuBP1 is classified as an enthalpically-driven binder. A typical configuration of the surface-bound state for AuBP1 is depicted in Figure 1.11, revealing the close proximity of the entire peptide chain to the gold surface. This can be attributed to the even spacing of the five contact residues along the peptide chain, ensuring that the peptide backbone cannot form loops that project away from the surface. The weakest binder, Pd4, has only two contact residues (two His), which leads to a classification of the binding enthalpy contribution as weak. On the other hand, the entropic contribution for this peptide is classified as high. As such, the very modest binding enthalpy is outweighed by the entropic contribution, resulting in the classification of Pd4 as an entropically-driven binder. The Pd4 peptide chain can support moderate backbone mobility between the two contact points (positions 6 and 11) and has a weakly bound N-terminal domain. These findings are illustrated in

![Typical structures](image)

**Figure 1.11.** Typical structures (in both plan view and side view) for the surface adsorbed conformations of AuBP1. The anchor residues are highlighted with relatively thicker bonds. Waters are not shown for clarity. Adapted with permission from Tang, Z.; Palafox-Hernandez, J. P.; Law, W.-C.; Hughes, Z. E.; Swihart, M. T.; Prasad, P. N.; Knecht, M. R.; Walsh, T. R. Biomolecular Recognition Principles for Bionanocombinatorics: An Integrated Approach to Elucidate Enthalpic and Entropic Factors. *ACS Nano* 2013, 7, 9632-9646. Copyright 2013 American Chemical Society.
Figure 1.12. Typical structures (in both plan view and side view) for the top two clusters of the adsorbed conformations of Pd4 (top, cluster 1; bottom, cluster 2). In each case, the anchor residues are highlighted with relatively thicker bonds. Waters are not shown for clarity. Adapted with permission from Tang, Z.; Palafox-Hernandez, J. P.; Law, W.-C.; Hughes, Z. E.; Swihart, M. T.; Prasad, P. N.; Knecht, M. R.; Walsh, T. R. Biomolecular Recognition Principles for Bionanocombinatorics: An Integrated Approach to Elucidate Enthalpic and Entropic Factors. *ACS Nano* **2013**, *7*, 9632-9646. Copyright 2013 American Chemical Society.

Figure 1.12, where the top two clusters for the surface-adsorbed Pd4 sequence are shown. All in all, Tang et al. elucidated the important role of configurational entropy in the adsorbed state in determining the overall binding affinity of peptides to inorganic materials and applied this insight to classify materials binding peptides as either enthalpically- or entropically-driven binders.
In later work, Palafox-Hernandez et al. expanded the QCM binding analysis and molecular dynamics simulations to silver surfaces using four peptides: two specific for Ag (AgBP1 and AgBP2) and two specific for Au (AuBP1 and AuBP2). The sequences and ΔG values for binding on Au and Ag for all of the peptides studied are presented in Table 1.2. Note that the ΔG values listed for Au binding are from the work completed by Tang et al. The authors compared the ΔG values of these four peptides on both Au and Ag surfaces to determine if materials selective binding is possible. Gold and silver were chosen as target materials because they both exhibit localized surface plasmon resonance for use in plasmonic assemblies. However, Au and Ag present a significant challenge to achieving material selective peptide binding, as they have similar structures (face centered cubic – fcc) and dielectric properties.


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<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>pI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Material</th>
<th>ΔG&lt;sup&gt;b,c&lt;/sup&gt; (kJ/mol)</th>
<th>Anchor Assignment&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Entropy Assignment&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuBP1</td>
<td>WAGAKRLVLRRRE</td>
<td>11.7</td>
<td>Au</td>
<td>-37.6 ± 0.9</td>
<td>strong</td>
<td>medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ag</td>
<td>-35.3 ± 0.8</td>
<td>weak</td>
<td>high</td>
</tr>
<tr>
<td>AuBP2</td>
<td>WALRRSIRRSQSY</td>
<td>12.0</td>
<td>Au</td>
<td>-36.4 ± 0.3</td>
<td>medium</td>
<td>medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ag</td>
<td>-36.7 ± 0.8</td>
<td>weak</td>
<td>medium</td>
</tr>
<tr>
<td>AgBP1</td>
<td>TGIFKSARAMRN</td>
<td>12.0</td>
<td>Au</td>
<td>-31.6 ± 0.2</td>
<td>medium</td>
<td>high</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ag</td>
<td>-35.9 ± 1.0</td>
<td>weak</td>
<td>high</td>
</tr>
<tr>
<td>AgBP2</td>
<td>EQLGVKELRGV</td>
<td>8.9</td>
<td>Au</td>
<td>-35.3 ± 1.2</td>
<td>medium</td>
<td>medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ag</td>
<td>-36.2 ± 1.0</td>
<td>weak</td>
<td>high</td>
</tr>
</tbody>
</table>

<sup>a</sup>Calculated using [http://web.expasy.org/compute_pil/](http://web.expasy.org/compute_pil/). <sup>b</sup>Top values represent Au binding; bottom values represent Ag binding. <sup>c</sup>Values for Au from *ACS Nano*, 2013, 7, 9632–964.
Figure 1.13 displays the QCM binding analysis of the four selected peptides on the Ag surface. In this analysis, the change in resonance frequency is presented, which directly corresponds to the mass of peptide adsorbed at the metal surface. Interestingly, all four sequences demonstrated equivalent binding affinities for the Ag surface, with $\Delta G$ values ranging from $-35.3 \pm 0.8$ kJ/mol for the weakest binder (AuBP1) to $-36.7 \pm 0.8$ kJ/mol for the strongest binder (AuBP2). In contrast, for binding on the Au surface, the $\Delta G$ values ranged from $-31.6 \pm 0.2$ kJ/mol (AgBP1) to $-37.6 \pm 0.9$ kJ/mol (AuBP1). This suggests that selective binding of one peptide over another on the Ag
surface is unlikely; however, substantial selectivity of AuBP1 binding over AgBP1 binding is likely on the Au surface. These results reveal that compositional selectivity is possible on Au as opposed to the minimal selectivity that can be achieved on Ag.

Figure 1.14. Degree of residue-surface contact, indicated by colored circles, for all four sequences adsorbed on Au (left) and Ag (right) aqueous interfaces. Adapted with permission from Palafox-Hernandez, J. P.; Tang, Z.; Hughes, Z. E.; Li, Y.; Swihart, M. T.; Prasad, P. N.; Walsh, T. R.; Knecht, M. R. Comparative Study of Materials-Binding Peptide Interactions with Gold and Silver Surfaces and Nanostructures: A Thermodynamic Basis for Biological Selectivity of Inorganic Materials. *Chem. Mater.* 2014, 26, 4960-4969. Copyright 2014 American Chemical Society.
Advanced molecular simulations were carried out on the same set of four peptides on the two metal surfaces. Likely binding-conformations for all of the peptides on both Au and Ag were identified. Figure 1.14 presents the degree of surface contact for each residue of the four different peptides on the two metallic surfaces. These data show that all four peptides displayed a greater degree of contact overall with the Au surface compared with the Ag surface. On the Au surface, all four peptide sequences demonstrated a strong degree of residue-surface contact, somewhat uniformly distributed along the length of the chain. Conversely, all four sequences showed fewer contact points overall when adsorbed on Ag. The distribution of degree of contact for Ag was much more narrow compared to Au for all four peptide sequences.

The enthalpic contribution to the experimentally determined $\Delta G$ values was estimated for each peptide sequence. These assignments are summarized in Table 1.2 and indicate a clear difference in the enthalpic character of the peptide binding to Au relative to Ag. The conformational entropy of each adsorbed peptide was also estimated (Table 1.2). These data show that the conformational entropy was higher for adsorption on Ag, which suggests that all of the sequences supported a greater number of distinct adsorbed conformations at the aqueous Ag interface than the Au interface. The residue-surface contact data complements the configurational entropy analysis in that both sets of data indicate that the mode of adsorption onto the two aqueous metal interfaces is very different, with adsorption on Au appearing to be more enthalpically-driven, in contrast to the entropically-driven binding on Ag. Molecular simulations showed flatter configurations on Au and a greater variety of adsorbed conformations on Ag.
Representative snapshots from the molecular dynamics simulations, given in Figure 1.15, illustrate this contrast in the adsorption for Au- and Ag-binding, in this instance for the AgBP2 peptide.\textsuperscript{38}

Taken together, Palafox-Hernandez et al. exposed key principles that determine the ability of peptide sequences to selectively bind to one material over another. Their work revealed different binding modes on the chemically-similar Au and Ag surfaces, specifically enthalpically-driven binding on Au and entropically-driven binding on Ag.
Overall, it was shown that thermodynamically different binding modes at metallic interfaces can enable selective binding on very similar inorganic surfaces.

A significant challenge in designing materials binding sequences is understanding the relationship between peptide sequence and corresponding materials binding selectivity and affinity.\textsuperscript{34,38,41} There is an increasing need for computational approaches that are capable of validating and guiding experimental data and design in addition to predicting peptide-binding behavior. At present, it is painstakingly difficult to design a peptide sequence that can selectively bind to only one material in an environment with multiple similar materials. To that end, a deeper understanding of the fundamental principles that control peptide material selectivity and affinity is required.

1.5 Statement of Research
The purpose of this research is twofold: 1) to exploit photocatalytic nanomaterials for environmental remediation applications and 2) to identify rational design principles for materials binding peptide sequences for the advancement of stimuli-responsive bionanoassemblies. The work that is discussed herein demonstrates the growing applications of nanomaterials in remediation technologies and aids in the understanding of the origins of peptide material affinity and nanoparticle stabilization.

Chapter 2 focuses on the development of a low-temperature, solution-phase synthetic route for size-controlled Cu$_2$O octahedra particles. This chapter examines the impact of changing certain reagent parameters, specifically the Cu$^{2+}$ and reductant concentrations, on the final particle composition and size. Moreover, the Cu$_2$O octahedra
materials are evaluated as catalysts for the photodegradation of aromatic organic compounds. Finally, an analysis is carried out on the post-reacted materials to gain insights regarding the relationship between surface structure and catalytic functionality.

In Chapter 3, a new catalytic approach for sustainable environmental remediation is presented. A semiconductor-metal multicomponent material is exploited to facilitate light-promoted tandem catalysis. Specifically, a synthetic method to form cubic Cu$_2$O/Pd composite structures is reported and their use as photocatalytic materials for the generation of H$_2$ and the reductive dehalogenation of polychlorinated biphenyls (PCBs) is demonstrated. PCBs are well-known carcinogens present at many contaminated sites around the world. This light-driven tandem catalytic system is one of the first to be employed for environmental remediation purposes. Lastly, the translation of photo-based approaches to non-photo-responsive catalytic reactions is exemplified in this chapter.

Chapters 4 and 5 aim to identify factors inherent to peptide sequences that can promote strong materials-binding affinity and/or effective nanoparticle stabilization capability. Specifically, these two chapters investigate the influence of the global and local sequence on Au binding affinity and the colloidal stability of Au nanoparticles. In Chapter 4, Pd4 is employed as a parent peptide to identify the effects of amino acid sequence and the local context of the anchor residues on the binding affinity to Au. To achieve this, a peptide synthetic mutation analysis is carried out, including point mutation of anchor residues with alanine, scrambling of the peptide sequence, and clustering of the anchor residues at the N-terminus. Additionally, peptide-capped Au nanoparticles are prepared using each sequence to elucidate correlations between colloidal stability and peptide-binding behavior. Chapter 5 expands the peptide sequence mutation analysis to
two more peptides, A3 and AuBP1. Both A3 and AuBP1 are well-known Au-binding sequences. These mutation studies will be substantially more complex than the Pd4 studies, as the AuBP1 and A3 peptides each have three significant anchor residues, while Pd4 has only two. Overall, Chapters 4 and 5 analyze how anchor residues work in concert with neighboring residues and how altering the global sequence of the peptide changes materials-binding affinity and nanoparticle stability.
Chapter 2. Direct Synthetic Control over the Size, Composition, and Photocatalytic Activity of Octahedral Copper Oxide Materials: Correlation Between Surface Structure and Catalytic Functionality

2.1 Overview

We report a synthetic approach to form octahedral Cu$_2$O microcrystals with a tunable edge length and demonstrate their use as catalysts for the photodegradation of aromatic organic compounds. In this particular study, the effects of the Cu$^{2+}$ and reductant concentrations and stoichiometric ratios were carefully examined to identify their roles in controlling the final material composition and size under sustainable reaction conditions. Varying the ratio and concentrations of Cu$^{2+}$ and reductant added during the synthesis determined the final morphology and composition of the structures. Octahedral particles were prepared at selected Cu$^{2+}$:glucose ratios that demonstrated a range of photocatalytic reactivity. The results indicate that material composition, surface area, and substrate charge effects play important roles in controlling the overall reaction rate. In addition, analysis of the post-reacted materials revealed photocorrosion was inhibited and that surface etching had preferentially occurred at the particle edges during the reaction, suggesting that the reaction predominately occurred at these interfaces. Such results advance the understanding of how size and composition affect the surface interface and catalytic functionality of materials.

2.2 Background

Because of the impending energy crisis, photocatalytic processes have become particularly attractive based on their reliance on renewable solar light as the main energy source to drive the reaction. Over the past few decades, major advances have occurred in the design and fabrication of highly active and efficient photocatalysts as viable alternatives to conventional catalytic materials.\textsuperscript{42-44} Various photocatalysts, especially metal oxide semiconductors such as TiO$_2$ and ZnO, have been extensively investigated for their use in photo-based processes.\textsuperscript{45-50} Unfortunately, their wide band gaps restrict their light absorption capabilities to ultraviolet wavelengths only. This results in inefficient use of solar energy as most of the solar spectrum is in the visible and infrared regions;\textsuperscript{51} photons within this energy range cannot photoexcite TiO$_2$ and ZnO.\textsuperscript{7,52} Alternatively, the use of narrow band gap semiconductor photocatalysts could effectively exploit visible light absorption for photoexcitation and electron/hole pair generation to take advantage of a significantly greater portion of solar energy that is available.

An exciting material for visible light photocatalysis is Cu$_2$O, which is a $p$-type semiconductor with a small band gap of 2.17 eV.\textsuperscript{19} Cu$_2$O is inexpensive, abundantly available, and has already shown promising activity for solar and energy applications, such as visible light-driven proton reduction for H$_2$ evolution,\textsuperscript{9,53} solar energy photovoltaics,\textsuperscript{54} and electrode material in lithium-ion batteries.\textsuperscript{55-56} Importantly, Cu$_2$O has become an attractive material for the photocatalytic degradation of environmental pollutants,\textsuperscript{44,57-59} where it has demonstrated the ability to break down aromatic dyes that are known to be difficult to degrade.\textsuperscript{60} This reaction typically occurs through the generation of reactive oxygen species at the metal oxide surface, leading to pollutant
degradation. By controlling the facet display of the Cu$_2$O via changes in the particle shape and size, the visible-light-based reactivity of the materials could be enhanced.

To understand the reactivity, Cu$_2$O nanocrystals and microcrystals have been prepared in various morphologies, including cubes, spheres, octahedra, and rhombic dodecahedra. Although various approaches have been followed for the production of shape- and size-controlled Cu$_2$O particles, many of these methods are energy intensive (>100 °C) with long reaction times. As such, the development of sustainable, facile, and low-temperature routes to size- and shape-controlled Cu$_2$O materials has gained increasing interest, but the ability to control both parameters simultaneously remains challenging. For example, Gou et al. reported the synthesis of Cu$_2$O nanocubes with a tunable edge length of 25 – 200 nm by using CuSO$_4$, poly(ethylene) glycol, ascorbic acid, and NaOH in water at room temperature. Zhang and co-workers have reacted CuCl$_2$, NaOH, and sodium ascorbate or hydrazine at room temperature to form Cu$_2$O nanocubes, octahedrons, cuboctahedrons, spheres, and facet-etched cubes. In an additional approach, Liang et al. synthesized polyhedral 50-facet Cu$_2$O microcrystals enclosed with high-index {211}, {522}, or {311} facets by mixing Cu(CH$_3$COO)$_2$ and glucose at 70 °C. Such energy-efficient processes are required for sustainable routes toward important Cu$_2$O structures; however, the effects of the reagents in the reaction mixture remain unclear. For instance, several reductants are employed to partially reduce Cu$^{2+}$ to Cu$^+$ in the final Cu$_2$O materials. The choice of reductant is of critical importance because it must be strong enough to reduce the metal ions under kinetically controlled conditions and to slowly grow the oxide crystal of a specific shape; such effects will undoubtedly play a role in determining the final particle size. As a result, the identity, concentration,
and reaction rate of the reductant are of significant importance to the final structure, where little understanding of such effects is known.

In this chapter, we present a facile synthetic method to generate Cu$_2$O octahedral microcrystals, where the edge length of the structure can be selected for based on reductant effects on the reaction. The structures are prepared in aqueous solution using CuCl$_2$, NaOH, cetyltrimethylammonium bromide (CTAB), and glucose at a relatively low temperature (50 °C), which is notably lower than that observed in many other synthetic approaches. Glucose was specifically selected as the reductant because (i) it is an ecologically friendly alternative to standard reductants and (ii) the rate of reduction can be easily controlled based on glucose isomerization. Such reductant effects play a significant role in controlling the overall material composition, morphology, and dimensions. Once prepared, the materials were extensively characterized to confirm their morphology, size, and composition using scanning electron microscopy (SEM), powder X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), ultraviolet-visible light diffuse reflectance spectroscopy (UV-vis DRS), and Brunauer–Emmett–Teller (BET) analysis. From the synthetic analysis, the effects of the Cu$^{2+}$ and reductant concentrations and stoichiometric ratios were investigated, indicating that the size and composition of the materials were directly related to the Cu$^{2+}$ concentration and rate of reduction. Once the materials were characterized, their photocatalytic activities were investigated by monitoring the degradation of methyl orange and methylene blue. From this study, the effects of the material composition and surface area were observed to play a significant role in the reactivity. Of importance, analysis of the materials after the reaction revealed a substantial change in particle surface structure, where etching at the
oxide edges was noted. This suggests that such regions may serve as the photocatalytic active site for radical generation, thus contributing to the understanding of photocatalytic mechanisms and charge carrier pathways. These results are important as they provide a route to size-controlled Cu$_2$O materials for photocatalysis. They also provide intriguing information concerning the catalytic activity of these structures based on particle composition and size, including potential surface reaction sites.

2.3 Materials and Methods

2.3.1 Chemicals

NaOH was acquired from Acros Organics, while anhydrous CuCl$_2$, methyl orange, and methylene blue were attained from Alfa Aesar. CTAB and glucose were purchased from Sigma-Aldrich. All chemicals were used as received without further purification. Milli-Q water (18 MΩ·cm) was used for all experiments.

2.3.2 Synthesis of Cu$_2$O Octahedra

The following synthesis was adapted from a previous study for the fabrication of Cu-based nanotubes.$^{67}$ The production of the octahedra is described using the Cu$^{2+}$:glucose ratio to differentiate the samples. In a round-bottom flask, 0.8, 1.5, 2.2, or 2.9 mmol of anhydrous CuCl$_2$ were dissolved in 100 mL of a 3.0 M aqueous solution of NaOH by stirring and sonicating. After placing the flask in a 50 °C oil bath, 6 mmol of solid CTAB was added directly to the solution, which was then stirred for 30 min at the elevated temperature. Immediately after removing the flask from the oil bath, 0.8 mmol of glucose was added. Afterward, the solution was stirred for 1 h at room temperature. The precipitate was collected by centrifugation, washed several times with water and ethanol, and dried under vacuum for at least 12 h. Separate reactions were also conducted using
the exact same procedures; however, the glucose amount was increased to be equal to the Cu$^{2+}$ amounts of 1.5, 2.2, or 2.9 mmol.

2.3.3 Photocatalytic Degradation Experiments

For each photocatalytic experiment, sufficient Cu$_2$O materials were added to reach either a surface area of 0.065 m$^2$ (constant-surface area conditions), as determined by BET analysis, or a mass of 10.0 mg (constant-mass conditions). For this, the Cu$_2$O sample was placed into a 100 mm × 50 mm crystalizing dish, to which 20.0 mL of a 30.0 mg/L (91.6 µM) aqueous methyl orange or methylene blue solution was added. Next, the dish was sonicated for 10 s and then capped with a quartz cover. Before light irradiation, the reactions were stirred for 30 min in the dark to allow for dye adsorption to the particle surface. Once complete, the reaction was stirred while being irradiated with an Oriel Sol1A Class ABB solar simulator employing a 1000 W Xe arc lamp operating at ~100 mW/cm$^2$. The sample to light source distance was ~20 cm. Aliquots (150 µL) of the reaction mixture were extracted every 10 min for 3.0 h to analyze the reaction progression via UV-vis analysis.

2.3.4 Characterization

SEM was performed using an FEI XL-30 Field Emission ESEM/SEM operating at 20 kV. To prepare the sample, the Cu$_2$O materials were dispersed in ethanol via sonication, after which 10 µL of the sample was drop-casted onto an aluminum stub. High-energy XRD (HE-XRD) patterns were collected at the Advanced Photon Source at Argonne National Laboratory using beamline 11-ID-C at 115 keV. Powders of Cu$_2$O microcrystals were loaded into 2 mm outer diameter thin-walled quartz capillaries for each sample. A large area detector was employed to collect two-dimensional (2D)
patterns, which were then integrated into one-dimensional (1D) patterns using the Fit2D program.\textsuperscript{68} XPS characterization was performed on a Perkin-Elmer 5100 XPS system. UV-vis DRS analysis was completed on a Shimadzu Model UV-2600 system. BET surface area analysis was carried out on a Quantachrome Model NOVA 1200 system. Finally, UV-vis spectra of the reaction analysis were acquired using an Agilent Model 8453 UV-vis spectrophotometer employing a 2.00 mm path length quartz cuvette.

2.4 Results and Discussion

2.4.1 Materials Synthesis and Characterization

In this contribution, size-controlled Cu$_2$O octahedra are generated using a mixture of CuCl$_2$, NaOH, CTAB, and glucose at a relatively low temperature (Figure 2.1). For this, glucose acts as the reductant at 50 °C, where it isomerizes to the linear form to expose an aldehyde\textsuperscript{69} that can reduce Cu$^{2+}$ to Cu$^+$.\textsuperscript{70} As this reduction process is anticipated to lead to control over the morphology and composition of the final structure, fabrication of Cu$_2$O was processed where the Cu$^{2+}$:glucose ratio was varied, as was the actual concentrations of the two species. This was performed to determine the effects of glucose over the final morphology of the materials, where minimal understanding of the reductant control over particle size, structure, and composition is presently known.

In the initial reaction system, the Cu$^{2+}$ concentration was gradually increased, while the glucose and CTAB concentrations remained constant. In this regard, the materials were synthesized with Cu$^{2+}$:glucose ratios of 0.8:0.8, 1.5:0.8, 2.2:0.8, and 2.9:0.8. Note that the values listed in the ratios represent the actual number of millimoles of the reagent in the reaction, and this notation will be employed herein to differentiate samples. Upon completion, a brick-red precipitate was generated for most of the samples
consistent with previous strategies for the production of Cu$_2$O.\textsuperscript{71-72} For the 2.9:0.8 sample, however, a black precipitate was observed, indicating a change in particle composition and/or morphology. Once the materials were purified and dried, they were imaged using SEM. Figure 2.2 presents the morphology of the particles prepared using the constant glucose concentration. Generally, high yields of well-defined octahedral Cu$_2$O structures were generated with varying edge lengths based on the Cu$^{2+}$:glucose ratio. The average edge lengths for each sample are summarized in Table 2.1. Figure 2.2 specifically presents the materials fabricated at a Cu$^{2+}$:glucose ratio of 0.8:0.8. Here, well-defined octahedrally shaped materials are generated with an average edge length of
2.7 ± 0.8 µm. Shape classification analysis, as shown in Table 6.1 in Chapter 6, indicated that 79% of the materials were octahedra. As the Cu²⁺:glucose ratio changed to 1.5:0.8 (Figure 2.2b), a clear shift in particle size was observed. While it was evident that octahedral materials were again prepared (92% octahedra in the sample), the average particle size increased to 4.4 ± 1.1 µm. Interestingly, for the 2.2:0.8 materials (Figure 2.2c), a bimodal material set was noted with 78% of the materials being octahedra. For this, two populations were observed with edge lengths of 1.7 ± 0.6 and 4.4 ± 1.1 µm. Finally, when the highest Cu²⁺ concentration was employed (2.9:0.8, see Figure 2.2d), the morphology of the materials began to vary. While the structures were generally

**Figure 2.2.** SEM images of the Cu₂O octahedra synthesized in the presence of CTAB at Cu²⁺:glucose mole ratios of (a) 0.8:0.8, (b) 1.5:0.8, (c) 2.2:0.8, and (d) 2.9:0.8. Scale bars = 5 µm.
octahedral (76% were classified as octahedra), the surface became very rough. For these structures, an edge length of 4.5 ± 0.8 µm was determined. For all particle sizing and shape analyses, at least 100 particles were measured over multiple SEM images (see Table 6.1 and Figure 6.1 in Chapter 6).

While interesting materials were prepared using a constant glucose concentration, greater reductant amounts may directly affect the material composition, size, and/or shape. To examine the effect of the reductant, a second set of materials was generated where the Cu\(^{2+}\) concentrations employed were the same as those above; however, the glucose concentrations were increased to match the amount of Cu\(^{2+}\). In this system, as the amount of Cu\(^{2+}\) increased, the edge length of the structures also linearly increased. Figure 2.3a specifically presents the materials prepared at a Cu\(^{2+}\):glucose ratio of 1.5:1.5. Note that a lower Cu\(^{2+}\) and glucose concentration (0.8:0.8) sample was prepared above, which also maintains equal concentrations of Cu\(^{2+}\) and glucose. In this sample, Cu\(_2\)O octahedra are again prepared (86% octahedra) with an edge length of 2.4 ± 0.5 µm. As the reagent

<table>
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<th>Cu(^{2+}):Glucose Mole Ratio</th>
<th>Edge Length (µm)</th>
<th>Surface Cu(^{2+}) Percentage</th>
<th>(E_g) (ev)</th>
<th>Surface Area (m(^2)/g)</th>
<th>(k_{mase}) (10(^{-2}) min(^{-1}))</th>
<th>(k_{SA}) (10(^{-2}) min(^{-1}))</th>
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<td>0.8:0.8</td>
<td>2.7 ± 0.8</td>
<td>80.8%</td>
<td>2.03</td>
<td>0.69</td>
<td>1.18 ± 0.05</td>
<td>5.16 ± 0.66</td>
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<td>1.5:0.8</td>
<td>4.4 ± 1.1</td>
<td>64.0%</td>
<td>2.02</td>
<td>0.82</td>
<td>2.01 ± 0.17</td>
<td>2.80 ± 0.57</td>
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<td>2.2:0.8</td>
<td>1.7 ± 0.6</td>
<td>46.4%</td>
<td>2.00</td>
<td>3.05</td>
<td>0.69 ± 0.07</td>
<td>0.05 ± 0.02</td>
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<tr>
<td>2.9:0.8</td>
<td>4.5 ± 0.8</td>
<td>38.0%</td>
<td>1.98</td>
<td>12.93</td>
<td>0.70 ± 0.05</td>
<td>0.08 ± 0.07</td>
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<tr>
<td>1.5:1.5</td>
<td>2.4 ± 0.5</td>
<td>61.4%</td>
<td>2.02</td>
<td>0.43</td>
<td>1.31 ± 0.53</td>
<td>2.87 ± 0.27</td>
</tr>
<tr>
<td>2.2:2.2</td>
<td>3.6 ± 0.7</td>
<td>71.6%</td>
<td>2.02</td>
<td>0.56</td>
<td>0.51 ± 0.12</td>
<td>4.18 ± 1.42</td>
</tr>
<tr>
<td>2.9:2.9</td>
<td>4.3 ± 1.0</td>
<td>61.9%</td>
<td>2.03</td>
<td>0.24</td>
<td>0.30 ± 0.09</td>
<td>2.75 ± 0.79</td>
</tr>
</tbody>
</table>

*Surface area determined via BET.
concentrations were increased in the 2.2:2.2 (Figure 2.3b) and 2.9:2.9 (Figure 2.3c) samples, the average edge lengths also increased to 3.6 ± 0.7 and 4.3 ± 1.0 µm, respectively. For these structures, only a single population of brick-red materials was noted, where either 75% (2.2:2.2) or 67% (2.9:2.9) of the structures were octahedra.

To understand the effect and fate of the surfactant over the particle morphology, a set of control experiments was conducted. In this initial study, the Cu$_2$O materials were prepared at a Cu$^{2+}$:glucose molar ratio of 1.5:0.8 in the absence of CTAB (Figure 2.3d). In this reaction, the majority of materials presented multiple interfaces with sharp edges, suggesting overgrowth of octahedra. Occasionally octahedra were present in the sample,
but they represented only a minor fraction of the population (24%). Such a population percentage is significantly lower than those achieved for materials fabricated with CTAB present in the reaction mixture. These structures were also polydisperse in size, ranging from 0.8 µm to 6.0 µm in size. This suggested that the surfactant played an important role in controlling the octahedral morphology; however, additional studies were completed to determine the fate of the CTAB. For this, materials fabricated at a ratio of 1.5:0.8 in both the presence and absence of CTAB were also characterized by thermogravimetric analysis (TGA) and IR spectroscopy (see Figure 6.2 in Chapter 6). Generally, both characterization methods indicated that no CTAB was retained by the materials after the extensive washing procedure; no mass loss of CTAB combustion was evident from TGA, and no vibrations of the surfactant were observed in the IR analysis. From this, it is evident that the surfactant plays a role in controlling the morphology and particle size. Furthermore, the TGA and IR analyses indicate that the interactions between the surfactant and oxide are likely to be quite weak, as evidenced by the fact that the purification process completely removes this component. Taken together, this suggests that the surfactant is responsible for controlling the particle morphology during the oxide growth process, but this condition is not required to maintain long-term particle shape stability.

To study the phase purity of the materials, XRD was employed (Figure 2.4). Diffraction patterns of the as-synthesized structures were compared with patterns for bulk Cu$_2$O and CuO, where every peak can be indexed to either composition. The diffraction patterns for the materials fabricated at a Cu$^{2+}$:glucose ratio of 0.8:0.8, 1.5:0.8, or 2.2:0.8 are consistent with the cubic phase of Cu$_2$O, displaying reflections at 2.08, 2.55, 2.95,
Figure 2.4. XRD patterns of the octahedral materials. The gray lines denote diffraction peaks attributed to Cu$_2$O, while the pink lines denote peaks indexed to CuO.
4.17, 4.89, and 5.10 Å⁻¹, corresponding to the (110), (111), (200), (220), (311), and (222) lattice planes of Cu₂O, respectively.⁷³ When the highest Cu²⁺ concentration was used to give a Cu²⁺:glucose ratio of 2.9:0.8, one minor CuO peak was observed at 2.71 Å⁻¹, indexed to the (111) lattice.⁷³ Such results suggest that Cu₂O is the dominant species present in the samples with lower Cu²⁺ concentrations, but that the amount of CuO in the material increases in the 2.9:0.8 structures. In comparison, for the materials generated at equal Cu²⁺ and glucose concentrations (1.5:1.5, 2.2:2.2, and 2.9:2.9 samples), the XRD patterns can be fully ascribed to Cu₂O with no CuO reflections. This indicates that the amount of the reductant in the sample and the reagent concentrations play an important role in controlling particle size and composition; however, additional analysis via XPS was employed to corroborate such effects.

The surface composition of the as-synthesized materials was examined via XPS analysis. Figure 2.5a presents the analysis for the 0.8:0.8 structures, where peak fitting of the data revealed two Cu 2p₃/₂ peaks at 932.5 and 933.7 eV, consistent with Cu⁺ and Cu²⁺, respectively.⁷⁴ From this assessment, the materials were composed of 80.8% Cu⁺ and 19.2% Cu²⁺. Note that this analysis is only able to analyze the surface of the octahedra and not the entire material; the amount of oxidized Cu²⁺ is likely to be higher at the surface because of O₂ exposure. Similar analyses were then conducted for all of the materials prepared at the selected ratios. Generally, higher amounts of Cu²⁺ were observed from those samples with low glucose and high Cu concentrations in the reaction. For instance, when the materials were fabricated at Cu²⁺:glucose ratios of 1.5:0.8, 2.2:0.8, and 2.9:0.8, the amount of surface Cu⁺ in the samples decreased linearly with values of 64.0%, 46.4%, and 38.0%, respectively, with Cu²⁺ making up the balance
Figure 2.5. XPS analysis of the Cu$_2$O octahedral materials prepared at Cu$^{2+}$:glucose mole ratios of (a) 0.8:0.8, (b) 1.5:0.8, (c) 2.2:0.8, (d) 2.9:0.8, (e) 1.5:1.5, (f) 2.2:2.2, and (g) 2.9:2.9. Panel (h) shows a comparison of the Cu$^+$ percentage in the sample as a function of the material.
of Cu. Note the enhancement of the broad peak at ~942 eV as the Cu$^{2+}$:glucose ratio increases. This peak arises from the shakeup structure associated with the presence of CuO.\textsuperscript{75} When the amount of glucose in the reaction was increased and maintained at the same concentration as that of the Cu$^{2+}$ in the reaction, as in the 1.5:1.5, 2.2:2.2, and 2.9:2.9 samples, the amount of Cu$^+$ in the sample remained roughly similar, ranging between 61.4\% and 71.6\%. Figure 2.5h presents a comparison in the amount of Cu$^+$ in the sample, indicating that higher amounts of Cu$^+$ are present in the sample at higher glucose concentrations. While the surface (XPS) and bulk (XRD) measurements corroborate each other to suggest that Cu$_2$O is present in all samples, XPS analysis indicates the presence of surface Cu$^{2+}$ for all the samples. This is attributed to the relatively minor amount, as compared to the total structure as observed \textit{via} XRD, and amorphous nature of CuO as a result of Cu$_2$O surface oxidation.\textsuperscript{76-78}

The UV-vis absorbance properties of the materials were assessed using UV-vis DRS, and the absorption spectra of the Cu$_2$O structures are presented in Figure 2.6a. The particles displayed a strong absorption in the visible region, where the onset of visible light absorption for the 2.9:0.8 sample started at ~750 nm, compared to ~650 nm for all of the other Cu$_2$O octahedra. This suggests that the composition of the 2.9:0.8 sample is different from the other materials. To support this hypothesis, the band gaps of the Cu$_2$O octahedra were determined by using the Kubelka-Munk function, \( F(R_x) \), where the values can be obtained from the tangent line of the Tauc plot.\textsuperscript{79} As presented in Figure 2.6b, the band gap of the 0.8:0.8 sample was calculated to be 2.03 eV, which is lower than that of Cu$_2$O (2.17 eV).\textsuperscript{19} This is likely associated with the small amount of CuO in the sample, which is supported by the observed trends in band gap energies. Generally, the band gaps
Figure 2.6. (a) UV-vis absorption spectra of the Cu₂O octahedral materials. (b–h) Tauc plots of the materials prepared at Cu²⁺:glucose molar ratios of (b) 0.8:0.8, (c) 1.5:0.8, (d) 2.2:0.8, (e) 2.9:0.8, (f) 1.5:1.5, (g) 2.2:2.2, and (h) 2.9:2.9.
correlated well with the amount of oxidized Cu\(^{2+}\) in the sample as observed via XPS. For instance, when the materials were fabricated at Cu\(^{2+}\):glucose ratios of 1.5:0.8, 2.2:0.8, and 2.9:0.8, the band gap decreased to values of 2.02, 2.00, and 1.98 eV, respectively. This shift towards lower band gap values with increasing CuO amounts is not surprising since CuO has a much smaller band gap (1.4 eV) than Cu\(_2\)O and, therefore, absorbs more visible light\(^8\). Furthermore, for the 1.5:1.5, 2.2:2.2, and 2.9:2.9 samples, the band gap ranged between 2.02 eV and 2.03 eV, suggesting a higher fraction of Cu\(_2\)O in the materials.

Taken together, significant structural differences can be noted in these materials based on the effect of the reductant. For this work, the major difference in the preparation process for the two different sets of materials was the amount of glucose introduced during the reaction: either a constant and lower glucose concentration (0.8 mmol) or a glucose concentration equal to the Cu\(^{2+}\) concentration. This generally resulted in greater amounts of CuO in the sample for those materials prepared at the highest Cu\(^{2+}\):glucose ratios; however, these materials still maintained the octahedral structure. This suggests that the CTAB plays an important role in maintaining the shape, as supported by the control reactions studied with no surfactant. Interestingly, the particle size was dependent upon the Cu\(^{2+}\) concentration in the sample for both reaction conditions. This may occur based on manipulation of the nucleation and growth kinetics of the materials. In this regard, should similar numbers of nuclei be developed in each sample, regardless of the Cu\(^{2+}\) concentration, the overall particle edge length would increase based on the amount of Cu\(^{2+}\) in the reaction, as observed herein. Should greater numbers of nuclei be developed as the Cu\(^{2+}\) concentration increases, additional sites for particle growth would
be present in solution, resulting in smaller particles. Since this does not occur, this supports the formation of similar numbers of nuclei in each sample; however, additional studies are required to determine this fine level of detail.

2.4.2 Photocatalytic Reactivity

Once the materials were fully characterized, their photocatalytic reactivity was evaluated. For this, the photocatalytic degradation of aromatic organic compounds was examined using all of the prepared particles irradiated by a solar simulator. The reactivity of the materials was examined using two specific dyes—anionic methyl orange and cationic methylene blue—to ascertain the electrostatic effects on the material reactivity. In the first set of reactions, 10.0 mg of particles for each sample were mixed into an aqueous solution of the dye and allowed to equilibrate in darkness for 30 min, followed by light irradiation for 3.0 h. Figure 2.7a presents the UV-vis spectra of the degradation of methyl orange using the 0.8:0.8 particles. In the spectrum prior to the reaction, a peak at 464 nm was observed, arising from the absorbance of the dye in the mixture. As the reaction ensues, the peak decreases because of the photocatalytic degradation of the dye, until it is completely consumed. By monitoring the degradation of the dye over time, the kinetics of the reaction was determined and compared for each sample. Figure 2.7b presents the photodegradation analysis of methyl orange when a particle mass of 10.0 mg was used for each reaction. In this setup, the dye molecules were allowed to interact with the octahedra for 30 min prior to light irradiation; no changes in the concentration of the dye solution were observed when a catalyst mass of 10.0 mg was used for each reaction. Because of the conduction and valence band positions of the materials, it was anticipated that those structures with the greatest amount of Cu$_2$O would be the most reactive.\textsuperscript{10,12,42}
Figure 2.7. Photocatalytic degradation of methyl orange: (a) the degradation of the dye using the 0.8:0.8 sample at a loading of 10.0 mg in the reaction (spectra were collected every 10 min); (b, c) the overall analysis for the reaction for all of the particles studied when (b) a catalyst mass of 10.0 mg was used in the reaction and (c) a total oxide surface area of 0.065 m² was used in the reaction. Prior to light irradiation, the materials were allowed to interact with the dye molecules for 30 min in darkness.
Interestingly, under these conditions, the Cu$_2$O structures prepared at a Cu$^{2+}$:glucose ratio of 1.5:0.8 were the most efficient for the degradation process. In this regard, 90% of the dye was degraded within 110 min, resulting in a pseudo-first order rate constant ($k_{\text{mass}}$, which indicates that the kinetic analysis was completed using a Cu$_2$O mass of 10.0 mg) of $(2.01 \pm 0.17) \times 10^{-2} \text{ min}^{-1}$. Such a result was quite interesting as this sample did not possess the greatest amount of Cu$_2$O in the material (64.0%), as compared to the octahedra prepared at a ratio of 0.8:0.8 (80.8%). For the latter sample, 170 min of irradiation was required to reach 90% degradation with a $k_{\text{mass}}$ value of $(1.18 \pm 0.05) \times 10^{-2} \text{ min}^{-1}$. Further decreased rates of photodegradation were observed for the rest of the samples prepared (Table 2.1) when a catalyst mass of 10.0 mg was used for each reaction. Additional control studies were also conducted using commercially available bulk Cu$_2$O and CuO, both of which were inactive for the photocatalytic process.

Generally, while the synthesized materials were photocatalytically reactive for the degradation of the anionic methyl orange dye, no evident trends with respect to material composition were observed, suggesting that additional structural properties of the oxides played a critical role in the overall reactivity.

While employing a constant catalyst mass is a standard process, the actual catalytic surface area is likely to differ among the materials prepared at the different Cu$^{2+}$:glucose ratios. This is especially true due to changes in the material composition between the samples. To quantify such effects, BET analysis was conducted to determine the available catalytic surface area of the materials, which is summarized in Table 2.1. From this analysis, it is clear that drastically different surface areas are present for the materials ranging from 0.69 m$^2$/g to 12.93 m$^2$/g for the 0.8:0.8 to 2.9:0.8 samples,
respectively. Note that the surface area observed by BET is larger than the calculated geometric surface area for nearly all of the Cu$_2$O octahedra, suggesting that the materials are porous (see Table 6.2 in Chapter 6). This different surface area is likely to be quite important to the overall reactivity, in combination with the material composition; therefore, modified photocatalytic reactions were carried out where the total oxide surface area (as determined by BET) was set constant to 0.065 m$^2$, and the catalyst mass employed in each reaction was varied. From this analysis, shown in Figure 2.7c, drastically different reactivities were observed for the materials for the photodegradation of methyl orange. Again, the materials were allowed to interact with the dye molecules for 30 min in darkness prior to light irradiation. Under constant-surface-area reaction conditions, dye adsorption was minimal (<10%) compared to the photodegradation activity, indicating that the removal of methyl orange was due to the photocatalytic activity of the Cu$_2$O. Almost complete degradation of the dye was achieved in 80 min when the 0.8:0.8 octahedra were employed as a catalyst. At this point in time, 95% of the dye was degraded, giving rise to a $k_{SA}$ value ($k_{SA}$, which indicates that the kinetic analysis was for the 0.065 m$^2$ catalyst reactions) of $(5.16 \pm 0.66) \times 10^{-2}$ min$^{-1}$. When considering the other Cu$^{2+}$:glucose materials, a trend was clearly evident where the reaction rates were directly proportional to the composition of Cu$_2$O in the sample; when the amount of Cu$_2$O in the octahedral materials increased, the overall reaction rate also increased (Table 2.1). This was anticipated based upon the known conduction and valence band potentials of Cu$_2$O versus CuO, relative to the redox potentials for radical formation; although CuO has a much smaller band gap than Cu$_2$O and absorbs more visible light, the conduction band energy ($E_{CB}$) and the valence band energy ($E_{VB}$) of CuO are insufficient
to catalyze the production of hydroxyl and superoxide radicals, which are primary initiators for the photocatalytic oxidation of aromatic organic compounds.\textsuperscript{10,12,42} Although the presence of CuO is undesirable, CuO contamination is inevitable due to surface oxidation.\textsuperscript{78} Therefore, it is essential to determine at what point the amount of CuO affects the photocatalytic activity. This is especially important for low-temperature synthetic routes in order to maximize Cu\textsubscript{2}O amounts for increased reactivity. Based on the \( k_{SA} \) values, when the amount of surface Cu\textsubscript{2}O in the octahedral materials is <50\%, photocatalytic performance is significantly diminished.

When comparing the mass-constant and surface-area-constant studies, slight variations in the amount of dye adsorbed onto the oxide surface are noted. To investigate whether this process could reach equilibrium, the methyl orange dye was mixed with the 0.8:0.8 octahedra in darkness. Over time, aliquots were extracted, and the amount of dye adsorbed onto the particle surface was quantified for both the mass-constant and surface-area-constant conditions (see Figure 6.3 in Chapter 6). For the surface-area-constant conditions, minimal changes in the dye concentration in the reaction solution are evident, indicating that little dye adsorption occurred over the 6 h study. For the mass-constant conditions, however, a gradual decrease in the solution dye concentration was noted, which was consistent with removal of the dye from solution. In this regard, after 6 h, the dye concentration decreased by 71\%. Interestingly, based on the reagent footprint, more dye would be adsorbed onto the oxide than what would be anticipated based on the measured oxide surface area. This suggests that a slower, secondary dye degradation
process is occurring; however, the rate for this process is substantially slow enough that it would not affect the photocatalytic process being studied, where 90% of the dye was degraded within <3 h, using these materials.

The effects of the surface area were also evident, wherein the catalysts with higher surface areas also had higher reactivities. This was apparent for the materials prepared at a Cu\(^{2+}\):glucose ratio of 2.9:0.8. These samples had the lowest percentage of Cu\(_2\)O in the final structures (38.0%); therefore, they should have been the least reactive. When the reaction was carried out with a catalyst mass of 10.0 mg, they displayed a \(k_{mass}\) value of \((0.70 \pm 0.05) \times 10^{-2} \text{ min}^{-1}\), which was in the middle of the values observed. When considering the available surface area of these structures (12.93 m\(^2\)/g), this value represented the highest surface area per mass for all of the materials studied. As a result, the largest total surface area was presented for this sample when a constant catalyst mass of 10.0 mg was used in the reactions. When the reaction conditions were changed and the total surface area was set constant for each octahedra sample, the \(k_{SA}\) value significantly decreased to \((0.08 \pm 0.07) \times 10^{-2} \text{ min}^{-1}\), which was near the lowest value reported. One final point for the reactions employing a constant oxide surface area of 0.065 m\(^2\), the \(k_{SA}\) reactivity became apparent for the bulk Cu\(_2\)O structures as these materials presented a very low specific surface area (0.40 m\(^2\)/g). As anticipated, no reactivity for the bulk CuO materials was observed, consistent with the band gap positioning. These results support the notion that the metal oxide surface participates in the reaction, either through electron transfer processes for radical formation or through dye adsorption.

Charged inorganic interfaces are typically developed when using oxide-based materials. Thus, the charge on the degrading substrate plays a role in the reactivity. The
Results above employed methyl orange, which is an anionic dye. The same reaction was studied using methylene blue, which is a cationic dye, and 10.0 mg of catalyst mass.

Figure 2.8a presents the UV-vis spectra of the methylene blue degradation reaction using the 0.8:0.8 particles. A peak at 664 nm was observed in the spectrum prior to the reaction, arising from the absorbance of the dye. When any of the octahedral materials were employed as the catalyst, no enhanced methylene blue degradation was noted, compared to the catalyst-free system, suggesting no catalytic effect of the particles toward methylene blue (Figure 2.8b). This behavior is consistent with previous observations for
Cu₂O octahedra and hexapods bound by {111} facets that display positively charged Cu⁺ atoms, suggesting that the surface of the octahedra is positively charged to repel the cationic dye. This would explain the lack of reactivity for the degradation of the cationic methylene blue dye, in contrast to the rapid degradation reactivity for the anionic methyl orange dye that is electrostatically attracted to the positively charged oxide. This also supports the hypothesis that some type of interaction between the substrate and surface is required for photocatalytic reactivity; should the dye not interact with the oxide surface, no charge effects should be evident for the different reactions.

Taken together, these catalytic results suggest that both the composition of the materials, which affects the photogeneration of electron/hole pairs, and the available oxide surface area play a role in the overall reaction. Generally, for all of the reactions, increased reactivity is noted from materials with greater Cu₂O compositions. This is specifically noted in the reactions where the total surface area was set to 0.065 m², but is also generally evident in the reactions where a 10.0 mg catalyst mass was employed. When considering systems with similar Cu₂O compositions, those materials with greater surface areas were generally more reactive. This suggests that the substrate must interact with the oxide surface, in combination with the reactive oxygen species, to drive the dye degradation process. As such, both electron/hole pair formation and available surface area played an important role in the overall reactivity.

For Cu₂O, a key issue is photostability since photocorrosion leads to the reduction of its photocatalytic activity. Therefore, the photostability of the 0.8:0.8 and 1.5:0.8 Cu₂O octahedra particles was investigated; these materials were selected because they exhibited the highest $k_{SA}$ and $k_{mass}$ values, respectively. Figure 2.9 presents SEM images
of the particles after the photocatalytic degradation of methyl orange under both 10.0 mg mass and 0.065 m$^2$ surface area reaction conditions. Additional SEM images of all the materials presented for the photostability analysis can be found in Chapter 6 (Figures 6.7 and 6.8). Imaging of the octahedra post-photodegradation when a particle mass of 10.0 mg was used for each reaction (Figures 2.9a and 2.9b) shows that the particles undergo surface etching at the edges and vertices while retaining their octahedral structure. Similar surface reconstructions were observed when the total surface area was set constant in the reaction (Figures 2.9c and 2.9d); however, the etching is not as drastic since a greater mass of material was employed to reach a surface area of 0.065 m$^2$. XRD
was further used to study each post-reaction material (see Figure 6.4 in Chapter 6). The XRD patterns show that the composition of the structures remained unchanged, with no reflections arising from CuO or Cu under both constant-mass or constant-surface area conditions.

To ensure that the surface etching is due to photocatalysis, a series of control studies were conducted. SEM analysis of the 0.8:0.8 materials after a 6 h dye adsorption equilibration period in darkness under both constant-mass and constant-surface-area conditions (see Figure 6.5 in Chapter 6) showed that no surface reconstruction occurred. In a second control, the 0.8:0.8 particles under both constant-mass and constant-surface-area conditions were photoirradiated in water for 3 h in the absence of dye. Here, surface etching on the edges and vertices of the octahedra was again observed (see Figure 6.6 in Chapter 6); however, pitting of the \{111\} facets became more pronounced. This suggests that radical formation is likely occurring at the edges, where the pitting effect indicates that the particles are being photocorroded when no dye molecules are present.\(^{85-86}\)

Taken together, these post-reaction analyses provide important information concerning the catalytic activity of the Cu\(_2\)O materials. In the reaction system with the dye, etching of the octahedra edges with minimal surface pitting is evident. This suggests that electron migration and the formation of the reactive oxygen species preferentially occurs at the particle edges and vertices, giving rise to the etching effect. Such a mechanistic hypothesis is supported by both the XRD and SEM controls. In this regard, the XRD analysis confirmed that no significant compositional change in the materials was evident, with Cu\(_2\)O forming the bulk of the structure. Adsorption of the dye to the oxide surface also did not result in changes to the octahedral shape; however, irradiation
of the materials in the absence of the dye did give rise to edge etching and pitting of the \{111\} facets. Without any dye present in solution, the Cu$_2$O materials undergo photocorrosion. Based on the electrostatic results observed for the cationic and anionic dyes, it is likely that the methyl orange is adsorbed onto the Cu$_2$O facets; however, additional characterization would be required to confirm this hypothesis. Nevertheless, these post-reaction analyses indicate that the redox reactions for reactive oxygen species preferentially occur at the particle edges and vertices to drive dye degradation.

2.5 Summary and Conclusions
In summary, we have generated size-controlled Cu$_2$O octahedral microcrystals via a facile synthetic approach using aqueous solutions at low temperatures. It has been demonstrated that the size of the octahedra can be tuned by changing the concentrations of the reagents, and that higher amounts of reductant result in materials with a greater Cu$^+$ content. In addition, the octahedral shape of the structures was maintained regardless of material composition, but was dependent on the CTAB in the mixture. Furthermore, the as-synthesized octahedra were highly effective photocatalysts for the decomposition of the anionic methyl orange dye, but demonstrated minimal photocatalytic activity for cationic methylene blue. The results indicate that three specific considerations controlled the overall reaction rate: the charge of the inorganic interface, the composition of the materials, and the surface area of the catalytic structures. Post-photodegradation analysis showed that (i) the Cu$_2$O octahedra were stable and (ii) particle surface etching had occurred, specifically on the edges of the particles, suggesting that the redox reactions preferentially occurred at these regions. These four characteristics worked in concert to mediate the overall reactivity, which could be selectively tuned by the synthetic pathway.
to achieve different sizes and compositions of these materials, providing enhanced control over the catalytic properties. Such results could be important in metal oxide catalyst design, where the size and composition of the structure are key elements in obtaining sustainable and efficient catalytic functionality.
Chapter 3. Converting Light Energy to Chemical Energy: A New Catalytic Approach for Sustainable Environmental Remediation‡

3.1 Overview

We report a synthetic approach to form cubic Cu$_2$O/Pd composite structures and demonstrate their use as photocatalytic materials for tandem catalysis. Pd nanoparticles were deposited onto Cu$_2$O cubes, and their tandem catalytic reactivity was studied via the reductive dehalogenation of polychlorinated biphenyls. The Pd content of the materials was gradually increased to examine its influence on particle morphology and catalytic performance. Materials were prepared at different Pd amounts and demonstrated a range of tandem catalytic reactivity. H$_2$ was generated via photocatalytic proton reduction initiated by the Cu$_2$O, followed by Pd-catalyzed dehalogenation using in situ-generated H$_2$. The results indicate that material morphology and composition and substrate steric effects play important roles in controlling the overall reaction rate. Additionally, analysis of the post-reacted materials revealed that a small number of the cubes had become hollow during the photodechlorination reaction. Such findings offer important insights regarding photocatalytic active sites and mechanisms, providing a pathway toward converting light-based energy to chemical energy for sustainable catalytic reactions not typically driven via light.

3.2 Background

Given the current global energy state, a push for rapid access to sustainable energy, such as solar cells and renewable fuels, has emerged. Accordingly, an expansion in research in the area of photocatalysis has occurred over the past decade. Although significant effort has been exerted to design and fabricate highly active photocatalysts to replace conventional catalytic materials, little progress has been made toward reducing the overall energy, materials, and resources necessary to create and maintain these light-harvesting systems. As such, existing catalytic technologies must evolve toward adopting sustainable synthetic practices that minimize environmental and economic impacts. To this end, the ability to easily synthesize and characterize functional nanomaterials with controlled size, shape, composition, and overall structural morphology will allow us to achieve catalytic technologies with optimized reactivity.

While photocatalysis is appealing, it is traditionally limited to redox chemistries. Thus, the application of photo-based approaches to non-photo-responsive catalytic reactions remains exceedingly rare. One approach to address this limitation is to fabricate multicomponent photocatalytic architectures with multiple catalytic domains. For this approach, semiconductor materials are of particular interest because of their photo-induced charge transfer properties, where fast transfer of charges across the semiconductor interface is critical for high energy conversion efficiency. These charge transfer processes can be greatly influenced by the presence of a noble metal cocatalyst coupled to the semiconductor, which can readily accept photo-induced electrons to efficiently transfer them to surface adsorbed acceptor molecules such as to produce H₂. Additionally, these metal nanoparticle cocatalysts frequently act as
an electron reservoir and consequently promote charge separation within the semiconductor-metal assembly. Solar H$_2$ production from photocatalytic water splitting is one of the most notable applications of photoinduced electron transfer, along with being a promising route to achieving renewable energy. Advancing toward sustainable chemical processes, the photocatalytically generated H$_2$ could be employed in other catalytic reactions, such as hydrogenation or hydrodehalogenation, which could also occur at the metal nanoparticle surface. Use of such a light-promoted tandem catalytic system has been demonstrated recently. For instance, Hirai and co-workers have reported the N-monoalkylation of primary amines in alcohol solvents by tandem photocatalytic and catalytic reactions on TiO$_2$ loaded with Pd particles (Pd/TiO$_2$). In separate work, we reported the use of Cu$_2$O cubes with galvanically deposited Pd nanoparticles on the oxide surface (Cu$_2$O/Pd) as catalysts for the reductive dechlorination of polychlorinated biphenyls (PCBs), a well-known environmental persistent organic toxicant present at many contaminated sites around the world. For this, photoactivation of the oxide component results in H$_2$ production, facilitated by Pd, via proton reduction. This H$_2$ was subsequently activated on the Pd metal surface from which PCB dechlorination was processed in a tandem-like fashion. From this, it is clear that semiconductor-metal multicomponent materials can be tailored to facilitate light-promoted tandem catalysis, catering to reactions not typically driven via light.

In this contribution, we demonstrate that the Cu$_2$O/Pd tandem photocatalytic activity is highly sensitive to both the overall composite material morphology and the substrate structure for nontraditional photocatalytic reactions (Figure 3.1). To examine the structural effect, Cu$_2$O cubes were generated where varying amounts of Pd
Figure 3.1. Reactivity of Cu$_2$O/Pd composite structures for photo-driven tandem catalysis.

nanoparticles were galvanically deposited on the oxide surface (0-15 wt %). The materials were extensively characterized to confirm their morphology, size, composition, and component arrangement using scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDS), inductively coupled plasma optical emission spectrometry (ICP-OES), and powder X-ray diffraction (XRD). Light-promoted tandem catalytic activities of the materials were investigated by monitoring the reductive dechlorination of PCBs. Under light irradiation, H$_2$ was produced via proton reduction by the Cu$_2$O material facilitated by the Pd nanodomains.
Subsequently, the \textit{in situ} generated H\textsubscript{2} was used for Pd-catalyzed dehalogenation of PCBs. For this, PCB structures that positioned the Cl substituent at the \textit{ortho}, \textit{meta}, and \textit{para} position were reductively dechlorinated to generate the final biphenyl product, and the corresponding reaction rate constants were quantified. Comparison of the rate constants demonstrated a general dechlorination reactivity order of \textit{para} > \textit{meta} \geq \textit{ortho}, with maximal catalytic rates obtained using Cu\textsubscript{2}O/Pd materials with 9 wt % Pd deposited at a composite material catalyst loading of 2 mg/mL. These findings provide intriguing information concerning the active sites and composite interface of photocatalytic materials, as well as how such structure-defined properties affect the overall reaction kinetics for photo-initiated tandem systems. They also increase the understanding of material structural effects over catalytic reactivity, opening pathways toward new structures with enhanced reactivity or translation of such photo-based approaches to new catalytic processes, all of which are highly important for environmental remediation of halogenated organic compounds such as PCBs.

3.3 Materials and Methods

3.3.1 Chemicals

CuSO\textsubscript{4} and Na\textsubscript{2}CO\textsubscript{3} were obtained from BDH Chemicals, while Pd(CH\textsubscript{3}COO)\textsubscript{2} was acquired from Strem Chemicals. HR-GC hexanes and 200 proof ethanol were attained from EMD Millipore and Pharmco-AAPER, respectively. Finally, polyvinylpyrrolidone (PVP; MW \approx 29,000 g/mol), sodium citrate, glucose, 2-chlorobiphenyl (PCB 1), 3-chlorobiphenyl (PCB 2), 4-chlorobiphenyl (PCB 3), and biphenyl were purchased from Sigma-Aldrich. All chemicals were used as received without further purification. Milli-Q water (18 M\textOmega\cdot\text{cm}) was used for all experiments.
3.3.2 Synthesis of Cu₂O/Pd Cubes

Cu₂O cubes were prepared according to synthetic methods established by Sui et al.²¹ In detail, 3.0 g of PVP was dissolved in 180 mL of an aqueous 0.038 M CuSO₄ solution with vigorous stirring in a round-bottom flask. Upon complete dissolution of the PVP, the solution was stirred for an additional 10 min. Next, 40 mL of an aqueous 0.37 M sodium citrate and 0.61 M sodium carbonate solution mixture was slowly added dropwise with continuous stirring. This was followed by the addition of 50 mL of an aqueous 1.4 M glucose solution, which was again slowly added dropwise with continuous stirring. After complete glucose incorporation, the reaction was stirred for 10 min. The flask was then placed in a 70 °C water bath for 2 h without stirring. Once complete, the dark orange precipitate was filtered through a 0.2 μm polycarbonate membrane, thoroughly washed with water and ethanol, and dried under vacuum at 60 °C for at least 12 h.

The as-synthesized Cu₂O cubes were subsequently coated with Pd nanoparticles via a galvanic exchange reaction.⁴⁴ The production of the Cu₂O/Pd cubes is described using the deposited Pd mass % to differentiate the samples. The fabrication of 1 wt % Pd Cu₂O/Pd materials is described below; however, changes to the mass of Pd added were employed to reach the appropriate metal loading. In a round-bottom flask, 11.3 mg of Pd(CH₃COO)₂ was dissolved by slowly adding 200 proof ethanol until a total volume of 150 mL was reached with stirring and sonication. Upon complete dissolution of the Pd(CH₃COO)₂, 450 mg of the Cu₂O material was added to the solution and sonicated until the Cu₂O powder was well dispersed. The solution was stirred for 24 h in the dark as the galvanic exchange reaction proceeded. The Cu₂O/Pd 1 wt % cubes were then filtered,
washed with ethanol, dried under vacuum at 60 °C, and then stored in a vacuum desiccator.

3.3.3 Photocatalytic Reductive Dechlorination of PCBs

For each photocatalytic experiment, 100 mg of the Cu$_2$O/Pd materials was added to a 60 mL borosilicate glass vial capped with a Teflon-coated silicone septum closure. To this, 50 mL of a 50/50 ethanol/water (v/v) solution of the specific PCB congener at a concentration of 25 µM was added after being bubbled with N$_2$ for at least 30 min. This resulted in a catalyst loading of 2 mg/mL. Next, the headspace of the vial was purged with N$_2$, and the Cu$_2$O/Pd materials were suspended by sonication. Once the materials were fully dispersed, the reaction was vigorously stirred while being irradiated with a 450 W medium pressure Hg-vapor lamp operating at ~60 mW/cm$^2$, based on the manufacturer's specifications, where the sample to light source distance was ~10 cm. To analyze the reaction progression, 500 µL aliquots of the reaction mixtures were taken at predetermined time intervals in at least triplicate trials. Each aliquot was combined with 500 µL of hexanes in order to extract the unreacted PCB and the biphenyl product for gas chromatography (GC) analysis. Separate reactions were also conducted using the exact same procedures; however, the amount of Cu$_2$O/Pd particles was adjusted to 50 and 150 mg to give 1 and 3 mg/mL catalyst loadings, respectively. All experiments were conducted under ambient conditions and at room temperature.

3.3.4 Characterization

SEM was performed using a FEI/Philips XL-30 Field Emission SEM equipped with an Advanced Detector Technologies EDS detector, while TEM studies were completed using a JEOL JEM-1400 TEM. ICP-OES analysis was performed using a Varian Vista-
Pro CCD Simultaneous ICP-OES spectrophotometer. Powder XRD analysis was completed on a Philips MRD X'Pert diffractometer using Cu Kα radiation. Finally, reaction progression analysis was conducted on an Agilent 7820A GC equipped with a flame ionization detector and on an Agilent 5975C GC-MS. All chromatograms, unless otherwise noted, showed only the presence of PCB, biphenyl, and hexane.

3.4 Results and Discussion

3.4.1 Materials Synthesis and Characterization

As illustrated in Figure 3.1, to achieve light-driven PCB degradation, the photocatalytic capability of Cu₂O was combined with the hydrodehalogenation activity of Pd to produce a composite structure for photo-driven tandem catalysis. For this, H₂ generation is processed via proton reduction by Cu₂O photoexcitation facilitated by the Pd, followed by reductive dehalogenation using the in situ-generated H₂ at the Pd component. As previously reported, the Cu₂O/Pd materials have shown significant tandem catalytic activity for the reductive dehalogenation of PCBs. To understand both the fabrication of the composite structure and how this structure affects the reaction kinetics, the Pd content of the composite material was varied between 0 and 15 wt % Pd. In this regard, the Cu₂O/Pd materials were synthesized to have a final deposited Pd mass of 1, 3, 6, 9, or 15% where the percent value listed represents the actual Pd mass % (relative to the Cu₂O mass) deposited onto the oxide surface. Prior to Pd deposition, Cu₂O cubes were generated with an edge length of 665 ± 55 nm (Figure 3.2a). Figure 3.2 also presents SEM images of the Cu₂O/Pd materials after the galvanic deposition process. In general, high yields of well-defined cubic composite structures were generated for all of the samples. Figure 3.2b specifically presents the Cu₂O/Pd 1% materials. Here, distinct cubes
were generated with an average edge length of $663 \pm 69$ nm. Such dimensions indicate that the addition of 1% Pd by mass does not substantially alter the size of the cubes as compared to that of the initial Cu$_2$O structures. As the Pd mass increased to 3, 6, 9, and 15% (Figure 3.2c–f), a shift in particle size was observed; although it was evident that

**Figure 3.2.** SEM images of the Cu$_2$O/Pd composite structures with a Pd mass percent deposited of (a) 0% (bare Cu$_2$O cubes), (b) 1%, (c) 3%, (d) 6%, (e) 9%, and (f) 15%.
cubic materials were again prepared, the average particle size increased to 689 ± 32, 738 ± 67, 752 ± 63, and 814 ± 91 nm, respectively. This increase in particle size likely arises from the deposition of the Pd materials, as confirmed by TEM analysis (discussed below). For all particle sizing, at least 100 cubes were measured over multiple SEM images (Figure 6.9 in Chapter 6). Interestingly, an ombré color effect from light to dark was observed for the color of the materials as the Pd content increased. To this end, a dark amber color was noted for the Cu$_2$O/Pd 1% sample, whereas the Cu$_2$O/Pd 3% particles were light brown in color. As the Pd mass increased to 6, 9, and 15%, dark brown to black colors were observed for these materials, respectively, indicative of the significantly higher amounts of Pd deposited.

To thoroughly examine the structural effects of the various Pd amounts on the composite materials, TEM analysis was conducted (Figure 3.3). Figure 3.3a presents the bare Cu$_2$O cubes prior to Pd deposition, where the left image shows the overall cube structure and the right image displays a high-magnification analysis of the oxide edge. Unfortunately, due to the material thickness, imaging of the cube facet is not possible. In this sample, it is clear that the surface of the metal oxide cube is smooth, as anticipated. Figure 3.3b presents the analysis for the Cu$_2$O/Pd 1% structures, where it is evident that the Pd nanoparticles were directly deposited on the cube surface. To this end, the metallic nanomaterials were imaged on the Cu$_2$O surface as the light gray rough region at the oxide edge as compared to the Pd-free materials. As Figure 3.3b depicts, the size and spatial distribution of the noble metal components were disperse, likely arising from the galvanic deposition process, and prior elemental mapping studies confirmed that the Pd remains on the oxide surface. As the Pd content increased in the Cu$_2$O/Pd 3, 6, 9, and
Figure 3.3. TEM images of (a) bare Cu$_2$O cubes and (b–f) Cu$_2$O/Pd materials with a deposited Pd mass of (b) 1%, (c) 3%, (d) 6%, (e) 9%, and (f) 15%. The left panel displays a single cube and the right panel shows the zoomed-in area indicated in the left image.
15% (Figure 3.3c–f) samples, the deposited Pd layer topology of the surface became progressively rougher and flake-like, suggesting that multiple layers of Pd materials were incorporated.

Additional characterization to confirm the composite material morphology was conducted (Figure 3.4). To quantify the Pd content in the composite structures, EDS was employed. From the EDS analysis of the Cu$_2$O/Pd 9% materials (Figure 3.4a), Pd was successfully deposited as evidenced by a strong peak at ~2.8 keV corresponding to the Pd L$_\alpha$ X-ray line. As anticipated, Cu was also present in the sample arising from the Cu$_2$O core component. Similar analyses were conducted for the Cu$_2$O/Pd 1, 3, 6, and 15% materials. The EDS assessment indicated that the deposited Pd mass percent depended on the added Pd mass percent (relative to the Cu$_2$O sample mass) in the galvanic exchange reaction. The plot presented in Figure 3.4b shows the linear relationship between the Pd mass percent added into the galvanic exchange reaction and the Pd mass percent deposited on the Cu$_2$O surface. For instance, when 153 mg of Pd(CH$_3$COO)$_2$ (17% Pd mass) was added in the reaction with 450 mg of Cu$_2$O, 9.1 ± 0.1% Pd mass was deposited as measured by EDS. In general, diminished deposition amounts were observed as compared to the reaction stoichiometry, most likely due to incomplete Pd$^{2+}$ reduction at the Cu$_2$O interface as the filtrate was pale yellow in color for the higher Pd loadings. Pd wt % amounts for the Cu$_2$O/Pd materials were also confirmed with ICP-OES and are presented in Table 6.3 in Chapter 6. Finally, the crystallinity of the materials was studied via powder XRD (Figure 3.4c). Diffraction patterns of the as-synthesized structures were compared with the pattern for bulk Cu$_2$O. The diffraction patterns for all of the Cu$_2$O/Pd materials are consistent with the cubic phase of Cu$_2$O, displaying reflections at 29.4, 36.3,
42.2, 61.4, 73.6, and 77.4° 2θ, corresponding to the (110), (111), (200), (220), (311), and (222) lattice planes of Cu$_2$O, respectively. Furthermore, no peaks arising from Cu$^0$ metal, CuO, or Pd were observed. Such results suggest that Cu$_2$O is the dominant species present in the composite materials, consistent with the SEM, TEM, and EDS results. Additionally, the Pd layer on the Cu$_2$O surface is quite thin, especially when considering the thickness of the oxide material, and beyond the detection limit of the technique.
Taken together, significant structural differences can be noted in these materials based upon the effect of the Pd content. For this study, the major difference in the preparation process for the different materials was the amount of Pd introduced during the galvanic exchange reaction, where the amount of Pd deposited on the Cu$_2$O cubes was directly proportional to the amount of Pd$^{2+}$ added into the galvanic exchange reaction. This generally resulted in an increasingly rough and flakey material surface as greater amounts of Pd were deposited; however, all the different final composite structures maintained a cubic morphology, where the size increased proportional to the amount of Pd deposited on the oxide surface. While the XRD measurements indicate that only Cu$_2$O is present in the samples, EDS and ICP-OES confirmed the presence of surface Pd for all the samples, which was visually observed by TEM. This effect is attributed to the thinness of the Pd layer on the surface of the oxide cube.\textsuperscript{105}

3.4.2 Photocatalytic Reactivity

The photocatalytic reductive dechlorination of PCBs was examined using the Cu$_2$O/Pd 1, 3, 6, 9, and 15\% particles irradiated with a Hg-vapor lamp in a photochemical cabinet. The reactivity of the materials was evaluated using three monochlorinated PCB congeners to ascertain steric effects on the material reactivity: PCB 1, PCB 2, and PCB 3. These PCB substrates were specifically selected due to their positioning of the Cl group at the ortho, meta, and para positions, respectively. Such structural features are known to
Figure 3.5. Overall reaction analysis for the photocatalytic reductive dechlorination of PCBs 1, 2, and 3 using the Cu$_2$O/Pd materials at a catalyst loading of 2 mg/mL in the reaction: (a) Cu$_2$O/Pd 1%, (b) Cu$_2$O/Pd 3%, (c) Cu$_2$O/Pd 6%, (d) Cu$_2$O/Pd 9%, and (e) Cu$_2$O/Pd 15%. Note that lines are added to guide the eye.
affect the catalytic reactivity based on steric constraints. For these reactions, 100 mg of particles were mixed into 50 mL of the 25 µM PCB solution, resulting in a 2 mg/mL catalyst loading, followed by light irradiation for 250 h. Figure 3.5a presents the dechlorination analysis of PCBs 1, 2, and 3 using the Cu$_2$O/Pd 1% materials. By monitoring the dechlorination of the PCBs over time, the kinetics of the reactions were determined and compared. For this system, PCB 3 (chlorine in the para position) was dechlorinated the most efficiently. In this regard, 89% of PCB 3 was dechlorinated after 250 h, resulting in a pseudo-first-order rate constant $k_{PCB3}$ of $(7.7 \pm 0.7) \times 10^{-3}$ h$^{-1}$. Such a result was anticipated because PCB 3 is the least sterically hindered congener, as compared to the other substrates. When PCBs 2 and 1 were employed in the reaction system using the Cu$_2$O/Pd 1% tandem catalysts, $k_{PCB2}$ and $k_{PCB1}$ values of $(2.3 \pm 0.5) \times 10^{-3}$ h$^{-1}$.
Identical analyses were conducted for all of the composite structures prepared at the selected Pd loadings (Figure 3.5). For all of the particles prepared, the rate constants for PCB photodechlorination generally maintained the \( \text{para} (k_{\text{PCB3}}) > \text{meta} (k_{\text{PCB2}}) \geq \text{ortho} (k_{\text{PCB1}}) \) trend (Figure 3.6). In this regard, when the Cu$_2$O/Pd 3% sample catalyzed the reaction, rate constants of \((3.4 \pm 0.5) \times 10^{-3}\), \((2.7 \pm 0.1) \times 10^{-3}\), and \((6.3 \pm 0.3) \times 10^{-3}\) h$^{-1}$ were observed for the PCB 1, 2, and 3 substrates, respectively. Note that this is the only sample where \(k_{\text{PCB2}} < k_{\text{PCB1}}\). The \(k\) values determined for the Cu$_2$O/Pd 6% catalyzed reaction were \((2.5 \pm 0.2) \times 10^{-3}\) (PCB 1), \((4.0 \pm 0.2) \times 10^{-3}\) (PCB 2), and \((7.7 \pm 0.3) \times 10^{-3}\) h$^{-1}$ (PCB 3), which were slightly higher than those noted for the Cu$_2$O/Pd 3% system.

Interestingly, the material with 9 wt % Pd content (Cu$_2$O/Pd 9%; Figure 3.5d) was the most efficient dechlorination system. Complete dechlorination of PCB 3 was practically achieved in 75 h. At this time point, 98% of the substrate was dechlorinated, giving rise to a \(k_{\text{PCB3}}\) value of \((50.8 \pm 2.9) \times 10^{-3}\) h$^{-1}$. Almost complete dechlorination (95%) of PCB 2 was reached after 250 h of irradiation, with a corresponding \(k_{\text{PCB2}}\) value of \((11.0 \pm 0.7) \times 10^{-3}\) h$^{-1}$. For the PCB 1 substrate, diminished reactivity was noted, as anticipated, where 39% of this reagent was dechlorinated in 250 h, with a \(k_{\text{PCB1}}\) value of \((1.9 \pm 0.1) \times 10^{-3}\) h$^{-1}$. When using the material with the highest Pd loading studied (Cu$_2$O/Pd 15%; Figure 3.5e), reduced reactivity was observed as compared to the Cu$_2$O/Pd 9% sample. In this regard, when the Cu$_2$O/Pd 15% sample catalyzed the reaction, rate constants of \((2.4 \pm 0.2) \times 10^{-3}\), \((6.3 \pm 0.3) \times 10^{-3}\), and \((36.5 \pm 1.3) \times 10^{-3}\) h$^{-1}$ were observed for the PCB 1, 2, and 3 congeners, respectively. These lower \(k\) values, as
compared to Cu$_2$O/Pd 9%, may be due to the extensive Pd coverage of the oxide core, therefore obstructing light absorption. Nevertheless, these results indicate that the reactivity of the Cu$_2$O/Pd materials for PCB photodechlorination is maximized for 9 wt % Pd on the Cu$_2$O core.

When considering all of the Cu$_2$O/Pd materials studied, a trend was evident where the rate of reductive dehalogenation was correlated to the location of the Cl substituent in the PCB molecule; catalytic removal of chlorine is typically favored in the order of \textit{para} > \textit{meta} ≥ \textit{ortho} positions. In general, while all of the synthesized structures were photocatalytically reactive for the reductive dechlorination of all of the PCB congeners studied, the most significant dechlorination was observed for PCB 3, regardless of which Cu$_2$O/Pd sample was used as a catalyst. From this, it is evident that substrate steric effects play an extremely important role in the material reactivity. In particular, the rate constants for PCB 1 were the lowest among all of the Cu$_2$O/Pd materials, ranging over a small window of $(1.9–3.4) \times 10^{-3} \text{ h}^{-1}$. This suggests that the amount of Pd deposited on the Cu$_2$O has little effect on the dechlorination of PCB 1, most likely due to its steric hindrance. Furthermore, dechlorination of PCBs 2 and 3 was observed to be enhanced as the mass of Pd increased in the catalytic materials. This likely arises from the optimized inorganic morphology that displays increased Pd materials for reductive dechlorination, as well as the accessibility of the Cl substituent in the substrate structure. Taken together, these catalytic results suggest that the Pd content, composite structures, and substrate steric effects work synergistically to influence the overall reactivity.
On the basis of the changes in particle structure, such information can be correlated to the observed reactivity to determine structure/function relationships. In general, the greatest reactivity for the photodechlorination reaction was observed from the Cu$_2$O/Pd 9% sample. Such results were quite interesting as this material did not possess the greatest amount of surface-deposited Pd. To elucidate the structure/function relationships of the composite material, a set of control experiments was carried out. To probe the photocatalytic effect of the Cu$_2$O materials, a solution of PCB 3 was bubbled with H$_2$ for 5 h in the presence of Cu$_2$O/Pd 9% particles at a catalyst loading of 2 mg/mL while in the dark (Figure 6.10a in Chapter 6). After 3 h, complete dechlorination of PCB 3 was observed with only a modest amount of biphenyl being detected. It is likely that biphenyl was the dominant product generated, as indicated by GC analysis; however, due to the catalytic setup, aerosolization of the biphenyl occurred, resulting in diminished amounts detected. To confirm this aerosolization effect, a solution of biphenyl was bubbled with H$_2$ for 5 h in the absence of a photocatalyst while in the dark (Figure 6.10b in Chapter 6). Within 2.5 h, complete aerosolization of biphenyl was observed. Taken together, these results suggest that the production of H$_2$ is the rate-determining step for the tandem catalytic process. In this regard, the high degree of Pd coverage for this sample is anticipated to facilitate H$_2$ generation to greater degrees than those materials with lesser amounts of Pd, as observed herein, due to diminished charge recombination effects; however, when more Pd is deposited on the surface, as in the Cu$_2$O/Pd 15% sample, lower reactivity is observed due to diminished light absorptivity by the Cu$_2$O core. In addition to activating the reductive dehalogenation step of the tandem catalytic process, the Pd metal surface likely increases the overall surface area of the composite
structure, providing additional adsorption sites for H\(^+\) ions. Pd could then reduce adsorbed H\(^+\) ions through the electrons transferred from the Cu\(_2\)O conduction band to form H\(_2\).\(^{111}\) Previous BET surface analysis has confirmed that surface-deposited Pd increases the overall surface area of the composite material.\(^{44}\) Therefore, it is possible for H\(_2\) generation to occur at both the Cu\(_2\)O and Pd sites. Altogether, these results provide important insights into the catalytic functions of the individual components of the composite structure and their overall roles in the tandem catalytic process.

To confirm that the observed reactivity arose from the particles and not light-based degradation of PCBs, a series of additional control studies was conducted. For this, solutions of PCBs 1, 2, and 3 were irradiated for 250 h in the absence of the photocatalyst (Figure 6.11 in Chapter 6). In general, while all of the PCB congeners demonstrated varying degrees of photo-based degradation, biphenyl formation was negligible. For instance, PCB 1 was quickly converted into other oxidative, and non-reductive, dechlorination products, such as hydroxybiphenyl as detected via GC-MS, with minor generation of biphenyl. Furthermore, in agreement with previous studies,\(^{109-110}\) it was observed that PCB photodegradation was favored in the order of Cl substitution at the ortho > meta > para positions under light irradiation in the absence of a photocatalyst. Such a trend is in direct opposition to the current results, where photocatalytic reductive dehalogenation of PCBs favored a Cl substitution order of para > meta ≥ ortho positions, with biphenyl as the only product formed. Taken together, this suggests that other photon-driven chemical processes that do not follow reductive dechlorination pathways are occurring in the absence of the catalyst to generate such products as hydroxylated PCBs. It should be noted that hydroxylated PCBs have enhanced toxicity compared to that of the
parent substrate\textsuperscript{112-114} thus, reductive pathways of degradation, as provided by the Cu\textsubscript{2}O/Pd material, are preferred.

While the above control reactions confirmed the Cu\textsubscript{2}O/Pd reductive dechlorination reactivity, it is essential to determine at what concentration the catalyst loading affects the tandem photocatalytic performance. This is especially important for transitioning energy- and material-intensive processes to photocatalytic routes in order to maximize reactivity while minimizing the consumption of resources. In this regard, modified photocatalytic reactions using the Cu\textsubscript{2}O/Pd 9% materials were carried out where the catalyst mass employed was varied to provide loadings of 1 and 3 mg/mL. Note that all of the reactions discussed above have a catalyst loading of 2 mg/mL. From this analysis, shown in Figure 3.7a, different reactivities were observed for the various catalyst loadings for the dechlorination of PCB 3. Interestingly, under these conditions, 2 mg/mL was the optimal catalyst loading for the dechlorination process. As mentioned above, under these conditions 98% of PCB 3 was dechlorinated in 75 h, with a $k_{PCB3}$ value of $(50.8 \pm 2.9) \times 10^{-3}$ h\textsuperscript{-1} (Figure 3.7b and Table 6.5 in Chapter 6). The 1 mg/mL catalyst loading gave a decreased $k_{PCB3}$ value of $(17.6 \pm 0.7) \times 10^{-3}$ h\textsuperscript{-1}. A further diminished $k_{PCB3}$ value of $(9.9 \pm 0.9) \times 10^{-3}$ h\textsuperscript{-1} was observed for the 3 mg/mL catalyst loading. These results indicate that 2 mg/mL is the most efficient catalyst loading for the dechlorination process. At a lower catalyst loading, the decreased amount of catalytic materials presented led to lower reactivity, whereas for the 3 mg/mL catalyst loading, the high material concentration resulted in inefficient light absorption by the materials, causing diminished reactivity.
From the above studies, it is clear that the Cu₂O/Pd materials control the overall photo-driven reductive dechlorination process and that the reaction rates are highly sensitive to the composite structure and catalyst loading. To probe whether the photocatalytic reductive dechlorination reaction affects the material structure, SEM and TEM analyses were conducted on the Cu₂O/Pd 9% materials after being used for the dechlorination of PCB 3 at a catalyst loading of 2 mg/mL, as shown in Figure 3.8. Additional SEM and TEM images of the materials presented for the postreaction analysis can be found in Chapter 6, Figure 6.12. Imaging of the Cu₂O/Pd 9% cubes post-dechlorination showed that while the majority of the structures remained intact, some of the particles became hollow (~29%). In this regard, Figure 3.8a shows a large area SEM image of the materials where fully intact materials are present, although hollow structures are also evident. Figure 3.8b shows a zoomed-in SEM image of the cubic materials in the red box of Figure 3.8a. In this image, it is apparent that significant internal structural changes have occurred for this set of materials. TEM analysis of these materials further exposes the dramatic structural changes to the particles that become hollow during the
reaction (Figure 3.8c). In this image, it is clear that the Cu$_2$O/Pd materials have been internally changed, whereby the oxide component was potentially partially etched while the Pd layer remained intact. To examine the material composition of the post-dechlorinated particles, EDS analysis was performed. These assessments indicated that the Pd content (relative to the Cu$_2$O mass) of the composite structures increased from 9.1 ± 0.1% Pd mass before the reaction to 9.6 ± 0.6% Pd mass after the reaction occurred, suggesting that the morphological changes arose predominantly from partial etching of the oxide component.

To identify and understand the structural modifications to the particle morphology due to photocatalysis as opposed to photocorrosion, a set of control experiments was conducted. In this study, at a catalyst loading of 2 mg/mL, the Cu$_2$O/Pd 9% materials were photoirradiated in the reaction solvent for 250 h in the absence of PCBs. SEM analysis showed that the majority of the cubes were still whole (71% of the sample); however, 29% of the materials appeared to be pitted, suggesting that photocorrosion had occurred (Figure 6.13a in Chapter 6). This pitted morphology was significantly different than that observed after the photodechlorination reaction, which generated hollow structures, suggesting that the hollowing effect was not due to photocorrosion. To

Figure 3.8. SEM and TEM images of the Cu$_2$O/Pd 9% structures after the photocatalytic reductive dechlorination of PCB 3 using a catalyst loading of 2 mg/mL. (a, b) SEM images and (c) TEM images. Panel (b) shows a zoomed-in image of the red box in panel (a).
reaffirm this hypothesis, a second control study was completed where bare Cu$_2$O cubes at a catalyst loading of 2 mg/mL were photoirradiated in the solvent for 250 h. In this analysis, pronounced surface reconstruction was observed (Figure 6.13b in Chapter 6), displaying jagged overgrowths on the oxide. Although no pitting was evident in the bare Cu$_2$O sample, hollow cubes were also not observed, supporting the hypothesis that photocorrosion does not drive Cu$_2$O/Pd material etching.

These postreaction analyses provide important insights concerning the photocatalytic mechanism of the Cu$_2$O/Pd composite structures. In the reaction system with PCB 3, hollowing of the cubes is evident. This control study suggests that the hollow cubes are a result of photodechlorination, which may affect the recyclability of the materials *vide infra*; pitting is evident from the Cu$_2$O/Pd 9% materials and particle surface reconstruction is observed in the bare Cu$_2$O cubes after being photoirradiated in ethanol/water. Additionally, the post-dechlorination wide-area EDS analysis shows that the Pd mass percent is higher after the reaction. Taken together, these postreaction analyses suggest that photodechlorination is responsible for the hollowing of the cubes. Although speculative, it is possible that the shell of the hollow cubes is composed of mainly Pd; however, additional studies are required to determine this fine level of detail.

Because of the observed structural changes of the Cu$_2$O/Pd after the first catalytic cycle, a reduction in the photocatalytic activity for subsequent catalytic cycles could be observed. As such, recyclability studies of the composite materials as catalysts for PCB photodechlorination were conducted using Cu$_2$O/Pd 7.5% materials with PCB 3 at a catalyst loading of 2 mg/mL. Figure 6.14 in Chapter 6 presents the dechlorination analysis and the resulting rate constants for the recyclability studies. For this process,
when the 75 h reaction period of the first cycle was completed, the Cu$_2$O/Pd 7.5% material was filtered, washed, and dried. Once the material was completely dry, it was weighed, and the appropriate volume of PCB 3 was added in order to maintain a catalyst loading of 2 mg/mL. For the first cycle, 88% of PCB 3 was dechlorinated after 75 h, resulting in a $k_{PCB3}$ value of $(29.8 \pm 2.0) \times 10^{-3} \text{ h}^{-1}$. For the second cycle, 60% of PCB 3 was dechlorinated after 75 h, giving a $k_{PCB3}$ value of $(10.6 \pm 1.2) \times 10^{-3} \text{ h}^{-1}$. As is evident, diminished reactivity was noted for the second reaction cycle, likely arising from the catalytic material degradation. It is evident that the redox potential of Cu$_2$O intrinsically exists within its band gap energy, which might lead to self-oxidation or self-reduction and depletion of copper species into the solution.$^{115}$ Additionally, Cu$_2$O oxidation to CuO at the composite material surface could be occurring resulting in diminished reactivity; however, partial reduction back to Cu$_2$O should restore the reactivity. Additional studies are required to determine the actual basis of the reactivity changes.

Optimization of this tandem catalytic system is still in progress; however, in comparison to other catalysts used for the dechlorination of PCBs, the Cu$_2$O/Pd composite materials are highly unique in that light is used as the energy source to drive the reaction. For this, the most established class of materials for PCB dechlorination is based on Pd/Fe nanostructures. For example, Wang et al. reported the synthesis of nanoscale Pd/Fe particles for the dechlorination of PCBs.$^{116}$ Within 17 h, complete dechlorination of the PCB congeners of Aroclor 1254 by Pd/Fe nanoparticles was observed. The initial PCB solution mixture concentration was 5 mg/L, and the catalyst loading was 50 mg/mL. Although complete dechlorination was achieved within 17 h, a less concentrated PCB solution and a significantly higher catalyst loading than those
employed herein were used. In an additional approach, Zahran et al. synthesized Pd/Fe bimetallic nanotubes that demonstrated high reactivity.\textsuperscript{117} Dechlorination of 25 µM 3,3’4,4’-tetrachlorobiphenyl using Pd/Fe bimetallic nanotubes with 0.9 wt % Pd was achieved in 25 h at a catalyst loading of 0.25 mg/mL. This high dechlorination efficiency was attributed to the high surface area to volume ratio of the hollow nanotubes structure. For these systems, the production of H\textsubscript{2} by Fe\textsuperscript{0} in aqueous solution leads to iron corrosion. As such, H\textsubscript{2} production and iron reactivity decrease over time; therefore, the Pd/Fe materials are not sustainable as a result of surface oxidation and precipitation of iron oxides/hydroxides on the surface of the iron.\textsuperscript{118-119} While these materials are the most reactive for PCB dechlorination, they are not directly comparable to the Cu\textsubscript{2}O/Pd systems due to the significant differences in H\textsubscript{2} generation (Fe oxidation vs. photocatalysis). While the Cu\textsubscript{2}O/Pd materials demonstrate lower reactivity, they represent a new avenue to materials with potential long-term reactivity for in-field photodechlorination using sunlight as the energy source. Further studies are underway to enhance the reactivity of the tandem catalysts, including pathways to enhance their recyclability.

3.5 Summary and Conclusions

In summary, we have generated cubic Cu\textsubscript{2}O/Pd composite structures with tandem photocatalytic reactivity. It was demonstrated that the Pd content in the material affects the particle surface morphology, where higher amounts of Pd result in structures with a flakey topology. Additionally, the cubic shape of the particles was maintained regardless of the material composition. Although simple, this architecture is strategic for transitioning energy- and material-intensive reactions that require H\textsubscript{2} as a reagent to more sustainable, photocatalytic methods. The as-synthesized Cu\textsubscript{2}O/Pd particles demonstrated
high tandem catalytic performance for the reductive dehalogenation of PCBs 3 and 2, but showed slow dechlorination rates for PCB 1. The data indicate that the overall dechlorination reaction rate results from a synergistic effect of several key factors, including the surface morphology and composition of the materials and the steric effects of the substrate. Postreaction analysis showed that some of the Cu$_2$O/Pd cubes had become hollow following dechlorination. The light-driven tandem catalytic system demonstrated herein exemplifies how current photocatalyst materials for H$_2$ production can be applied to reactions not typically fueled by light. The fundamental understanding of material structural effects over catalytic function and reactivity is important in the design of photocatalytic systems for sustainable reactivity.
Chapter 4. Elucidating the Influence of Materials-Binding Peptide Sequence on Au Surface Interactions and Colloidal Stability of Au Nanoparticles

4.1 Overview

Peptide-mediated synthesis and assembly of nanostructures opens new routes to functional inorganic/organic hybrid materials. However, understanding of the many factors that influence the interaction of biomolecules, specifically peptides, with metal surfaces remains limited. Understanding of the relationship between peptide sequence and resulting binding affinity and configurations would allow predictive design of peptides to achieve desired peptide/metal interface characteristics. Here, we measured the kinetics and thermodynamics of binding on a Au surface for a series of peptide sequences designed to probe specific sequence and context effects. For example, context effects were explored by making the same mutation at different positions in the peptide and by rearranging the peptide sequence without changing the amino acid content. The degree of peptide-surface contact, predicted from advanced molecular simulations of the surface-adsorbed structures, was consistent with the measured binding constants. In simulations, the ensemble of peptide backbone conformations showed little change with point mutations of the anchor residues that dominate interaction with the surface. Peptide-capped Au nanoparticles were produced using each sequence. Comparison of simulations with nanoparticle synthesis results revealed a correlation between the colloidal stability of the Au nanoparticles and the degree of structural disorder in the surface-adsorbed peptide structures for this family of sequences. These findings suggest new directions in

the optimization and design of biomolecules for \textit{in situ} peptide-based nanoparticle growth, binding, and dispersion in aqueous media.

4.2 Background

Growing interest in biomimetic materials has led to the use of both traditional (\textit{e.g.} proteins and peptides)\textsuperscript{120-121} and unconventional (\textit{e.g.} peptoids and biopolymers)\textsuperscript{122-123} biomolecules to synthesize and assemble inorganic materials. Exploiting materials recognition properties of biomolecules could allow the creation of functional nanoassemblies that incorporate both inorganic and organic components.\textsuperscript{124-126} In particular, bionanocombinatorics is an emerging field that aims to control the assembly of nanomaterials using biological interactions. Peptides provide a unique pathway to achieving this objective. For peptides that bind to inorganic surfaces through multiple noncovalent interactions, both enthalpic and entropic components of binding affinity must be considered when predicting and interpreting binding behavior.\textsuperscript{34} Biocombinatorial selection techniques used to identify material-binding peptides generally select for overall binding affinity and do not provide information on the components of binding affinity separately. Based on their binding affinity, such peptides have been used to cap and direct the growth of various inorganic nanoparticles under mild conditions.\textsuperscript{127-129} Specifically, Au nanoparticles are of high interest due to their potential applications in catalysis,\textsuperscript{130-132} energy harvesting and storage,\textsuperscript{133-134} sensors,\textsuperscript{135-136} optics/plasmonics,\textsuperscript{137-138} and as components in nanoassemblies.\textsuperscript{38,139-140} Additionally, Au nanoparticles exhibit a localized surface plasmon resonance (LSPR), which provides a simple means of detecting changes in the size, shape, and aggregation state of the nanostructures \textit{via} changes in the LSPR optical extinction spectrum.
While experimental measurements of peptide binding affinity for a target surface are possible, experimentally characterizing the peptide-metal interface at the molecular level is difficult, if not impossible. As a result, molecular simulation approaches, along with complementary experimental data, are essential for separating out enthalpic and entropic contributions of peptide binding, elucidating configurations of the peptide-metal interface, and linking these to the peptide-surface binding affinity. We previously demonstrated that material-binding peptides can be categorized as either enthalpically- or entropically-driven binders. Enthalpically driven material-binding peptides tend to have several anchor residues (those residues with strong and persistent non-covalent interactions with the surface) spaced throughout the sequence. These anchor the biomolecule to the surface, resulting in a limited number of surface-binding configurations. However, strong materials-binding affinity can also arise from the presence of a large number of relatively-weakly-bound adsorbed peptide configurations. Such entropically-driven binding peptides have fewer anchor residues, allowing them to adopt many different binding conformations on the inorganic surface. The conformational entropic contribution to binding associated with the large number of possible configurations contributes to higher binding affinity with fewer strong anchor residues compared to enthalpically driven binders.

We previously classified the Pd4 peptide (TSNAVHPTLRHL), originally isolated based on its affinity for Pd, as an entropically driven binder on Au. This sequence contains only two anchor residues (His6 and His11), resulting in many distinct surface-adsorbed conformations. In comparison, other sequences such as AuBP1 (WAGAKRLVLRRE) and AuBP2 (WALRRSIRRQSY), originally isolated as
Au-binding peptides\textsuperscript{141} were classified as enthalpic binders. They have five and four anchor residues, respectively, with a significantly smaller number of thermally-accessible conformations on Au compared to Pd\textsubscript{4}\textsuperscript{141}. While these broad classifications advanced the understanding of how peptides bind to inorganic surfaces, the effects of the global and local sequence on binding remain poorly understood. In this regard, the importance of sequence, and not just overall residue content, is clearly important; however, how the anchor residues work in concert with neighboring residues and how altering the global sequence of the peptide changes materials binding affinity remain unknown.

Here, Pd\textsubscript{4} was employed as a parent peptide to identify the effects of amino acid sequence and the local context of the anchor residues, and to explore how these effects act synergistically to mediate binding affinity for the Au surface. Our modifications included point mutation of histidine anchor residues with alanine (with the aim to diminish localized binding affinity), scrambling of the peptide sequence, and clustering of the anchor residues at the N-terminus (Table 4.1). The Pd\textsubscript{4} peptide was initially studied due to the limited number of anchor residues (two in total)\textsuperscript{34} and its known affinity for Au. Peptide binding affinities on Au were quantified using Quartz Crystal Microbalance (QCM) measurements, which revealed significant changes in binding affinity between sequences. This information was combined with findings from advanced molecular simulation studies, in which the Boltzmann-weighted ensemble of adsorbed conformations was predicted for each of the sequences. Taken together, the experimental and computational results reveal underlying causes for the observed variation in peptide-surface binding. We also prepared peptide-capped Au nanoparticles using each sequence and characterized them by UV-vis spectroscopy and transmission electron microscopy.
(TEM). These results demonstrated that the local context of the anchor residues, as well as the global peptide sequence, plays a key role in controlling material affinity. Understanding of these sequence effects can also be exploited to enhance the quality and colloidal stability of peptide-capped Au nanoparticles. Thus, these studies advance efforts to identify heuristics for the design of peptides with affinity and potential selectivity for inorganic surfaces. The ability to rationally tune the binding strength and binding mode on specific surfaces would be extremely valuable for creating peptide-organized structures for optical, catalytic, and nanoparticle self-assembly applications.

Table 4.1. Adsorption Analysis for the Parent Pd4 Peptide and Mutants on Au. Anchor residues are highlighted in red, mutated residues in blue, and scrambled residues in green.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>( \Delta G ) ((\text{kJ mol}^{-1}))</th>
<th>( \theta ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd4</td>
<td>TSNAVHPTLRHL</td>
<td>-31.4 ± 0.1</td>
<td>79.8 ± 0.5</td>
</tr>
<tr>
<td>Pd4-1</td>
<td>TSNAVAPTLRHL</td>
<td>-27.2 ± 0.2</td>
<td>41.2 ± 1.6</td>
</tr>
<tr>
<td>Pd4-2</td>
<td>TSNAVHPTLRAL</td>
<td>-30.7 ± 0.1</td>
<td>75.0 ± 0.9</td>
</tr>
<tr>
<td>Pd4-3</td>
<td>TSNAVAPTLRAL</td>
<td>-32.0 ± 0.8</td>
<td>82.8 ± 5.0</td>
</tr>
<tr>
<td>Pd4-4</td>
<td>SPHTTNHRALLV</td>
<td>-33.1 ± 0.2</td>
<td>88.8 ± 0.8</td>
</tr>
<tr>
<td>Pd4-5</td>
<td>HHTSNAVPTLRL</td>
<td>-35.5 ± 0.2</td>
<td>95.3 ± 0.4</td>
</tr>
</tbody>
</table>

\(^a\)From QCM experiments: adsorption Gibbs free energy \((\Delta G)\) values, as well as surface coverage at the highest peptide concentration studied \((15 \mu\text{g/mL peptide})\). Values are given as mean ± one standard deviation from at least three independent experiments.
4.3 Materials and Methods

4.3.1 Chemicals

All Fmoc-protected Wang resins, Fmoc-protected amino acids, N,N'-diisopropylethylamine (DIPEA), piperidine, 1-hydroxybenzotriazole hydrate (HOBT hydrate), and N-[(1H-benzotriazol-1-yl)(dimethylamino)methylene]-N-methylmethanaminium hexafluoro-phosphate N-oxide (HBTU) were purchased from Advanced ChemTech. N,N'-dimethylformamide, methanol, diethyl ether, ammonium hydroxide (20%), and hydrogen peroxide (30%) were obtained from BDH Chemicals, while trifluoroacetic acid (TFA) and triisopropylsilane (TIS) were acquired from Alfa Aesar. HAuCl₄ and NaBH₄ were purchased from Sigma-Aldrich and Acros Organics, respectively. Finally, acetonitrile was purchased from EMD Millipore. All chemicals were used as received without further purification. Milli-Q water (18 MΩ·cm) was used for all experiments.

4.3.2 Peptide Synthesis

Peptides were generated using a TETRAS peptide synthesizer (CreoSalus) following standard solid-phase Fmoc protocols.¹⁴² A cleavage cocktail of 95% TFA, 2.5% TIS, and 2.5% water was employed to cleave the peptides from the resins. The crude peptides were purified via reversed-phase high performance liquid chromatography (HPLC) with a 0.1% TFA aqueous solution and acetonitrile gradient. Finally, the purified peptides were confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. All peptide solutions were lyophilized and stored at −80 °C prior to use.
4.3.3 QCM Analysis

All QCM measurements, including those for dissipation energy, were conducted on a Q-Sense E4 instrument (Biolin Scientific) following previously reported procedures. Standard polycrystalline gold crystal sensors were employed and cleaned according to manufacturer protocols prior to use. In brief, the gold QCM sensors were first subjected to UV-ozone exposure, followed by immersion in a 5:1:1 (v/v/v) water/ammonium hydroxide/hydrogen peroxide solution and an additional UV/ozone treatment. Afterward, an aqueous peptide solution at a concentration between 2.5 to 15.0 µg/mL was flowed over the sensor surface at a rate of 150 µL/min. The frequency change and dissipation energy were recorded for 30 min to ensure saturation. This frequency change is directly related, via the Sauerbrey equation, to the mass of peptide adsorbed, from which binding kinetic parameters can be determined. All measurements were performed at 22.5 °C, and the pH of the peptide solutions ranged from 3.9 – 4.2.

4.3.4 Molecular Simulations

To investigate the effect of the variations of the parent Pd4 peptide sequence on the peptide-surface adsorption, the conformational ensemble of the adsorbed peptides was predicted using Replica Exchange with Solute Tempering (REST) molecular dynamics (MD) simulations. Each simulation comprised a single peptide chain adsorbed at the Au(111) interface (represented by a five-layer Au slab) in the presence of liquid water. Three-dimensional periodic boundary conditions were applied throughout. The polarizable GolP-CHARMM force-field, the CHARMM22* force-field, and the modified TIP3P potential were used to describe the interactions involving the Au surface, the peptide, and water, respectively. All REST simulations were performed at
a thermal temperature of 300 K. Additional details about the simulation procedures and analysis of the simulation trajectories are provided in the ‘Simulation Details’ Section in Chapter 6.3. Note that previous studies\textsuperscript{153} have shown that the binding modes of peptide sequences at the described planar surface are very similar to the binding modes on Au NPs dispersed in solution. Thus REST simulations of peptides at the aqueous Au (111) interface are able to provide useful insight into the principal contact modes and conformational entropic contributions of the same peptide adsorbed onto the surface of Au NPs, which is in turn related to colloidal stability of the Au NPs.

At present, capturing the true complexity of the polycrystalline Au substrate used in the QCM experiments using atomistic MD simulation is not possible. A viable means of addressing this challenge is to focus on the dominant Au crystal orientations present on the polycrystalline Au surface. Based on previously-published analyses,\textsuperscript{154} the Au(111) surface is expected to be the dominant facet at the aqueous interface of polycrystalline Au. In particular, because our study encompasses seventeen different peptide sequences (see below), for pragmatic reasons we focussed our REST MD simulations solely on the aqueous Au(111) interface. Probing the binding of these peptides at other Au surfaces, such as the Au(100) interface that is predicted to be the next most dominant facet on the polycrystalline Au surface, would be interesting. However, for flat planar Au surfaces (i.e. not those present on nanoparticle surfaces), previous studies suggest that the Au(100) native surface relaxes to the Au(100) (5\times1) reconstruction,\textsuperscript{154} which features a lateral hexagonal arrangement of Au atoms in the surface plane that is very similar to that of Au(111). Due to this lateral hexagonal arrangement, our previous studies indicate that peptide adsorption to the aqueous Au(100) (5\times1) surface is similar to that corresponding
to Au(111). In particular, the anchor residues identified on Au(111) were generally found to be strong-binding residues on Au(100) (5\times1).\textsuperscript{154} We therefore suggest that the Au(111) surface is an appropriate structural model for comparison with experimental data obtained from polycrystalline Au QCM sensor surfaces.

Special attention was paid to the protonation state of the histidine residues (see Results and Discussion). For the original sequence and each mutated sequence (see Table 4.1), we separately considered the deprotonated (charge-neutral) and protonated (positively-charged) states of each histidine present in that sequence. For each sequence that contained only one histidine, this resulted in two different REST simulations: one with the histidine modeled as protonated (denoted PS1) and one with the histidine deprotonated (denoted PS0). For sequences with two histidines, this resulted in four different REST simulations: 1) no histidines protonated (denoted PS0), 2) only the first histidine in the sequence protonated (denoted PS1A), 3) only the second histidine in the sequence protonated (denoted PS1B), and 4) both histidines protonated (denoted PS2). In summary, we performed a total of seventeen REST simulations to cover all possibilities for the mutant sequences.

4.3.5 Au Nanoparticle Synthesis

Peptide-capped Au nanoparticles were prepared according to synthetic methods established by Li et al.\textsuperscript{130} The fabrication of peptide-capped Au nanoparticles with a Au:peptide ratio of 2 is described; however, changes to the reagent volumes (to equal a total volume of 5.0 mL) were employed to reach the appropriate metal loading. In detail, 500 µL of an aqueous 1.0 mM peptide solution was added to 4.460 mL of water in a vial. Next, 10 µL of an aqueous 100 mM H\textsubscript{Au}Cl\textsubscript{4} solution was added. The solution was briefly
agitated and allowed to stand for 10 min. This was followed by the addition of 30 µL of a freshly prepared aqueous 100 mM NaBH₄ solution. A NaBH₄:Au ratio of 3 was used for all syntheses. The solution was briefly agitated, and the reaction was allowed to proceed undisturbed at room temperature for 1.0 h to ensure complete reduction. Identical synthetic protocols were used to prepare Au nanoparticles at a Au:peptide ratio of 1:1; however, the amount of the stock HAuCl₄ solution was decreased to 5 µL and the water volume was increased to reach a final reaction volume of 5.0 mL.

4.3.6 Nanoparticle Characterization

TEM studies were performed using a JEOL JEM-2010 microscope operating at 200 kV. The samples were prepared by drop casting 5 µL of the colloidal nanoparticle dispersion onto a carbon-coated 200 mesh Cu grid, which was allowed to dry overnight. UV-vis monitoring of particle formation and aggregation employed an Agilent 8453 photodiode array spectrophotometer. All samples were analyzed using a quartz cuvette with a 1 cm optical path length.

4.4 Results and Discussion

4.4.1 QCM Analysis of Peptide Binding to Au

Pd4, originally isolated as a Pd-binding peptide, also binds to Au. Our previously-published molecular simulations predicted that the native Pd4 peptide has two anchor residues for Au binding, specifically two histidines at the 6 and 11 positions. To elucidate effects of sequence and anchor residue context on peptide binding, a set of six different Pd4 variant peptide sequences was prepared (Table 1). More specifically, to understand how the binding thermodynamics change when one or both of the anchor residues is removed, two peptides, Pd4-1 and Pd4-2, were prepared in which a single
histidine residue was replaced with alanine at the 6 or 11 position, respectively. A doubly mutated peptide, Pd4-3, was generated with both histidines replaced by alanines. Previous predictions of amino acid binding free energies on aqueous Au(111) suggested that alanine is not a strong binder. Thus, minimal Au surface binding at the alanine sites was anticipated. However, these sequences were designed to probe how the local context of anchor residues affected the surface conformation and Au-binding affinity of the peptide. To probe the effects of the global sequence for Au binding behavior (i.e. to test the possibility that overall binding affinity is just a summation of the individual residues’ affinities, in which details of the sequence play no role in binding), two additional peptides were designed: the Pd4-4 sequence (a random rearrangement of the residues present in the native Pd4 peptide) and the Pd4-5 peptide (which has the two histidine anchors grouped together at the N-terminus).

QCM analysis was used to determine peptide binding kinetics and thermodynamics for each sequence. Figure 4.1a presents the observed frequency changes for the Pd4-5 peptide at five different concentrations as an example. The full QCM analysis for all of the peptides is presented in Chapter 6, Figure 6.15. Higher peptide concentrations produced faster frequency changes, reflecting increased binding rates. The dissipation energy, which is an indicator of the viscoelasticity of the bound peptide layer, is shown for the highest peptide concentration (15 µg/mL). For all of the peptides studied here, the dissipation energy was <5% of the total frequency change. This indicates that the adsorbed peptide layer was rigid, and that multiple layers were not formed. Note that for all QCM data, inverted plots are displayed for more intuitive data interpretation.
Fitting each QCM-derived binding curve to Langmuir kinetics provided pseudo-first order adsorption rate constants ($k_{obs}$) as in prior studies.\textsuperscript{34,143-144} Plotting the $k_{obs}$ values as a function of the peptide concentration gives the binding constants for adsorption ($k_a$) and desorption ($k_d$) as the slope and $y$-intercept of the best fit line of the plot, respectively (Figure 4.1b). Using these values, the binding equilibrium constant, $K_{eq}$, can then be calculated as $k_a/k_d$. The binding affinity, defined as the Gibbs free energy change ($\Delta G$) for peptide binding, is then obtained as $\Delta G = -RT \ln(K_{eq})$. Using this approach, the $\Delta G$ of binding for the Pd4-5 peptide on Au was determined to be $-35.5 \pm 0.2$ kJ/mol. This analysis was carried out for each of the peptides in the designed library to yield the $\Delta G$ values presented in Table 4.1. These ranged from $-27.2$ to $-35.5$ kJ/mol for the weakest (Pd4-1) and strongest (Pd4-5) binders, respectively. Note that the pH of the peptide solutions for the QCM analysis ranged from 3.9 to 4.2. A full summary of the $k_a$, $k_d$, and $K_{eq}$ values for each peptide is provided in Table 6.6 in

**Figure 4.1.** QCM analysis of the Pd4-5 peptide to obtain $k_a$ and $k_d$ values. Part (a) shows the inverted frequency change vs. time for five concentrations, as well as the dissipation energy plot for the highest concentration studied. Part (b) shows $k_{obs}$ values vs. peptide concentration, obtained from the data in part (a) by fitting using Langmuir kinetics. Note that no error bars are presented as they are smaller than the points in the graph. The slope and $y$-intercept in part (b) provide the rate constants for adsorption and desorption, respectively.
Chapter 6. The native Pd4 peptide has a $\Delta G$ value of $-31.4 \pm 0.1$ kJ/mol. This value is similar to values reported in previous studies of Pd4 binding on Au.\textsuperscript{4} When a single histidine was replaced by alanine, as in Pd4-1 and Pd4-2, lower $\Delta G$ values of $-27.2 \pm 0.2$ kJ/mol and $-30.7 \pm 0.1$ kJ/mol, respectively, were measured. This indicates that mutation of the individual histidine anchor residues does substantially affect the Au affinity; however, the effect was larger for the residue at the 6 position. When histidines at both the 6 and 11 positions were replaced with alanines (Pd4-3), we observed an unexpected increase in the Au binding affinity ($\Delta G = -32.0 \pm 0.8$ kJ/mol). Note that while the average $\Delta G$ values for Pd4 and Pd4-3 are different, the error limits for the measured values overlap. The relatively strong affinity of Pd4-3 was surprising; mutation of a second anchor residue (histidine) would be expected to further decrease the binding affinity, given the decreases observed upon replacement of either of the anchor residues individually. Finally, an increase in binding affinity was noted for the remaining two peptide variants, Pd4-4 and Pd4-5. A $\Delta G$ value of $-33.1 \pm 0.2$ kJ/mol was determined for the Pd4-4 peptide, while the strongest binding of the set was observed from the Pd4-5 peptide. The weakest binding was observed from the Pd4-1 peptide.

From Langmuir fitting of the QCM data, the steady-state fractional surface coverage, $\theta$, of the sensor by the peptide can also be directly determined.\textsuperscript{34} For the Pd4-5 peptide at the highest concentration studied (15 $\mu$g/mL peptide), the surface coverage reached a maximum value of $95.3 \pm 0.4\%$, consistent with its strong binding affinity. At the other extreme of binding affinity, Pd4-1 at the same concentration had a maximum surface coverage of $41.2 \pm 1.6\%$. 
4.4.2 Molecular Dynamics Simulations of Peptide Binding to Au

The conformational ensemble of adsorbed peptide states provided by our molecular simulation data allows for conceptual links to be established between the primary sequence of the peptide, the observed experimental binding data, and the predicted structure(s) of the peptide in the adsorbed state. One additional challenge in predicting the conformational ensemble of the Pd4 sequence and its variants is associated with the presence of His residues. In the amino acid form, the side-chain in histidine is likely to be protonated (i.e. carry an overall +1 charge) in aqueous solution at the pH values investigated experimentally (pH 3.9 – 4.2); the relevant pK\textsubscript{a} value is ~6.2 for the imidazole side-chain. However, the pK\textsubscript{a} corresponding to protonation of the side-chain of a His residue in a peptide sequence in solution may differ substantially from that of the corresponding amino acid.\textsuperscript{155} This is due to the modification in the immediate dielectric environment of the relevant His in a protein/peptide, which can depend on the conformational state (or ensemble of states) of the protein/peptide. For example, the degree to which the residue is shielded from water, based upon the biomolecular structure, can substantially affect the localized dielectric environment around the histidine, thus potentially altering the protonation state of the imidazole side chain.\textsuperscript{156-157} Moreover, the relevant pK\textsubscript{a} of a His residue in the surface-adsorbed state at the aqueous Au interface might also differ from that of a free (i.e. un-adsorbed) peptide in solution.

At present, the prediction of pK\textsubscript{a} values for residues such as His for peptides/proteins in aqueous solution, which can account for environment/conformation effects, is extremely challenging.\textsuperscript{158} Going further, to the authors’ knowledge, information regarding the influence exerted by the presence of the aqueous Au interface
on the $pK_a$ of His residues in surface-bound peptide is also scarce. Therefore, the protonation state of each His residue in the surface-adsorbed state of the peptide may not match expectations based on the known $pK_a$ values generated for the His amino acid.

These questions discussed above represent a grand challenge in molecular simulation and are not the focus of the present study. Herein, we only consider how the conformational ensemble of the surface-adsorbed peptide sequences can be modulated by the protonation state of each His residue. As will be demonstrated, the position and local environment of each His residue, in addition to the protonation state of the His side-chain, can all influence this conformational ensemble. Therefore, in the REST simulations, each protonation state of each His residue for every peptide sequence that contained any His residues (i.e. Pd4, Pd4-1, Pd4-2, Pd4-4 and Pd4-5) was considered. These sequences comprise a special case in the simulation dataset; however, unless specified otherwise, the results discussed below will refer to the case where all the His in the sequence were protonated. Snapshots of representative structures of the Pd4-1 and Pd4-5 peptides, which are the two peptides that feature the greatest difference in terms of the QCM-measured binding affinity, are shown in Figure 4.2. Corresponding snapshots of the other peptides are shown in Figures 6.16–6.19 in Chapter 6.

As mentioned above, our previously-published simulation studies on the adsorption of Pd4 at the aqueous Au interface have identified Pd4 as an entropically-driven binder,\textsuperscript{34} i.e. that Pd4 supports a large number of distinct conformations in the surface-adsorbed state. The number of distinct surface-adsorbed conformations of a peptide can be determined from a clustering analysis,\textsuperscript{159} where like structures are grouped together on the basis of peptide backbone conformational similarity (denoted herein as
From this cluster analysis, both the total number of distinct adsorbed conformations, \( n \), and their relative population, \( p_i \), can be determined. As introduced in earlier studies, the conformational entropic contribution, \( S_{\text{conf}} \), associated with the surface-adsorbed peptide can then be estimated from:

\[
S_{\text{conf}} = \sum_{i=1}^{n} -p_i \ln(p_i)
\]
These $S_{\text{conf}}$ values are summarized in Table 6.7 in Chapter 6; the actual values of total number of distinct conformations (referred to as clusters), $n$, and the populations, $p_i$, for the top-ten most populated clusters, are also provided in Table 6.8 in Chapter 6. In terms of a relative $S_{\text{conf}}$ score, the native Pd4 sequence was previously ranked as high.$^{34}$ The current data show that almost all of the variants of the Pd4 sequence could also be classified as high in terms of $S_{\text{conf}}$ score. In the case of three of the variants, Pd4-1, Pd4-2, and Pd4-4, the conformational entropy contribution was substantially enhanced relative to the parent sequence (see Table 6.7 in Chapter 6).

In addition, the adsorbed configurations of each variant sequence were compared in terms of structural similarity against those obtained for the parent peptide sequence. If the root mean-squared deviation (RMSD) between the relative positions of the peptide backbone atoms inherent to the representative structures of the two clusters was less than or equal to 2 Å (the same spatial cut-off used for the clustering analysis, see the ‘Simulation Details’ Section in Chapter 6.3) then the two conformations were classified as a match, i.e. the peptide backbone conformations were similar. The top-ten most populated clusters of the parent peptide have thus been compared against the ten most populated clusters of each of the variants (see Table 6.9 in Chapter 6). Both the Pd4-1 and Pd4-3 sequences shared reasonable structural similarity with the original Pd4 sequence (i.e. both Pd4-1 and Pd4-3 possess several matched structures with Pd4). In particular, the most populated cluster of Pd4-1 and Pd4-3 both have a structural match with the most populated cluster of Pd4. Pd4-2 had fewer matching conformations with Pd4 but still featured one match between clusters with a non-negligible population. These findings suggest that the Pd4 sequence possesses conformational stability. In other words,
Pd4 shows a lack of conformational susceptibility with regards to the mutation of individual residues, even if those residues are anchors. These results do not necessarily suggest a general finding; for example, this may only hold for entropically-driven materials-binding peptides. Further comprehensive and systematic investigations are needed to fully elucidate the rules governing this phenomenon. The more substantial changes to peptide sequence inherent to the other two mutants (Pd4-4 and Pd4-5) resulted in few or no matches with Pd4, respectively. Overall, the extensive conformational ensemble identified for all six peptide sequences, combined with the conformational recalcitrance of the sequences corresponding with point mutations, indicates that the underlying explanation for the differences in the experimentally-measured binding affinities is related to the anchor residues, i.e. the histidines.

Figure 4.3 shows the predicted degree of direct Au surface contact calculated for each residue in each of the six peptides, in the instance where all His residues are protonated. Figure 6.20 in Chapter 6 shows the residue-surface contact averaged over different configurations of the protonation states, while the numerical contact data (as percentages) are provided in Table 6.10 in Chapter 6 for all possible combinations of histidine protonation state for every sequence. From these data, the anchor residues can be identified and the degree to which the mutations of the peptide chain alter the binding of residues within the peptide can be assessed. For the parent Pd4 sequence, H6 and H11 are anchor residues in all protonation state scenarios (Table 6.10 in Chapter 6). At pH 4, R10 is also an anchor residue. Overall, the peptide is predicted to be adsorbed to the Au surface at two locations in the sequence: in the middle of the chain and close to the C-terminus of the chain. In Pd4-1 the H6A mutation generates a change in the residue-
surface contact of the peptide (Figure 4.3 and Table 6.10 in Chapter 6). In this case, the A6 residue showed only weak contact with the Au(111) surface, while the A4 residue became an anchor. Interestingly, this is the only non-His/Arg residue that acted as an anchor in all the systems considered. At the C-terminal end of Pd4-1, H11 remained an anchor residue but R10 did not. However, the enthalpic contribution of A4 to the overall peptide binding strength is predicted to be considerably less than that of a His or Arg residue.\textsuperscript{38,147-148,161-162} In summary, when compared to Pd4, Pd4-1 suffered a reduction
from two strong anchor residues (in Pd4) to one strong anchor (H11) and one weak anchor (A4), which may explain why the measured binding affinity of Pd4-1 is weaker than Pd4.

In the case of Pd4-2, the H11A mutation is seen to exert less effect on the surface contact points along the peptide chain. H6 remained an anchor (regardless of protonation state, see Table 6.10 in Chapter 6); in addition, despite the (expectedly) weak binding of A11, R10 assumed the role of the sole strong surface contact point in the C-terminal region of the chain. Both His and Arg are thought to be strong binding residues at the aqueous Au interface.\textsuperscript{34} We propose that for a dyad sequence motif where His and Arg are adjacent in the sequence (\textit{i.e.} either HR or RH), this can lead to a competition between the two residues in determining which residue acts as the surface contact point. However, this competition to act as an anchor appears to also be influenced by the His protonation states (see data for Pd4 and Pd4-4 in Table 6.10 in Chapter 6).

The mutation of both His residues in Pd4-3 removed two anchor points. Intuitively, this would suggest that Pd4-3 should bind more weakly than Pd4; however, as seen for Pd4-2, R10 again assumed the role as anchor residue in the absence of H11. Moreover, while Pd4-3 did not feature any additional anchor residues, this sequence did however exhibit a substantial contiguous segment (N3-P7) that featured moderate binding. For the Pd4 parent, the surface contact for residues in the N3-P7 segment varies with the protonation state of the His residues (Table 6.10 in Chapter 6); if the contact is averaged over all the possible protonation states (Figure 6.20 in Chapter 6), the contact of the N3-P7 segment in Pd4-3 is comparable to that of Pd4. In particular, the contact of the N3-A4-V5 triad is higher in Pd4-3 than the parent peptide. The probability of at least two
of these three residues being adsorbed simultaneously is 51% and 35% for Pd4-3 and Pd4, respectively. This, in partnership with the anchor at R10, could in part account for the similarity in binding strength of Pd4-3 compared with Pd4. Despite the scrambling of the sequence in Pd4-4, both of the His residues and the Arg residue all remained as anchors for three out of the four His protonation configurations considered here. While we cannot determine the relative weighting of these four protonation configurations in the overall ensemble of Pd4-4, a naïve averaging of the number of anchors over the four states suggests a greater number of anchor points compared with the corresponding analysis for Pd4. This assumption is reasonable given the lack of certainty regarding the pK\textsubscript{a} of each His residue when the peptide is adsorbed at the aqueous Au interface. These results are consistent with the experimentally-observed binding constants.

The data from the QCM experiments indicate that Pd4-5 was the strongest binder to Au. For the doubly-protonated state (where both His are protonated), at first glance it may seem counter-intuitive that Pd4-5 featured only one single strong anchor residue, R11. However, further analysis (see a summary of conditional residue-surface binding probabilities in Table 6.11 in Chapter 6) demonstrated that the majority (92%) of the simulation trajectory had at least one of the His residues also in contact. In other words, a competitive binding scenario, similar to that seen for RH/HR, was also noted for the HH dyad motif, where the binding of the two His residues was seen to toggle. Our analysis confirmed that only 8% of the trajectory featured neither of the His residues in a surface-bound state. Therefore, any given adsorbed conformation of doubly-protonated Pd4-5 was likely to have at least two strong surface contact points (R11 and either H1 or H2, or all three residues), as indicated in Figure 4.2b. As with Pd4-4, the relative proportion of
each protonation configuration is unknown, but again, an unweighted average over states suggests that Pd4-5 had a greater average number of anchors than did Pd4. This could in part explain the stronger binding affinity observed for Pd4-5.

4.4.3 Au Nanoparticle Synthesis and Characterization

With a clearer understanding of the binding between the Au surface and the peptides of Table 4.1, Au nanoparticles were fabricated using the biomolecules as capping ligands. For nanoparticle synthesis, each peptide was mixed with Au$^{3+}$ ions at Au:peptide molar ratios of 1:1 and 2:1. After allowing time for complexation of the metal ions to the peptide, each solution was reduced with a 3-fold molar excess of NaBH$_4$ relative to Au$^{3+}$. In all cases, a dramatic color change was observed upon reduction. The absorbance of the nanoparticle dispersions was characterized using UV-vis spectroscopy (Figure 4.4). Figure 4.4a specifically presents the parent Pd4 system. The spectrum of the Pd4 peptide solution was featureless, while those of the Au$^{3+}$/peptide complex showed a weak absorbance peak at 300 nm, at both Au:peptide ratios. After reduction, an absorbance peak associated with the Au nanoparticle LSPR was observed. For a Au:peptide ratio of 2, the LSPR absorbance was quite broad and red-shifted relative to the typical position for the LSPR of Au particles. However, when a Au:peptide ratio of 1 was employed, a distinct LSPR band was evident at 525 nm. This suggests that the Au nanoparticles prepared at a ratio of 2 are larger, more polydisperse, and/or more aggregated in solution compared to particles prepared at the lower ratio.$^{163}$ Identical analyses were conducted for each of the peptides at both ratios. Overall, for all of the peptide-capped Au
nanoparticles produced at a Au:peptide ratio of 2, the LSPR absorbance was broad and red-shifted, beginning near 580 nm, while at a ratio of 1, the materials typically had a distinct LSPR band that was positioned between 525 and 550 nm.

**Figure 4.4.** UV-vis analysis of Au nanoparticles capped with (a) Pd4, (b) Pd4-1, (c) Pd4-2, (d) Pd4-3, (e) Pd4-4, and (f) Pd4-5 peptide. Ratios represent molar ratio of Au:peptide.
TEM analysis was conducted to examine the effect of peptide sequence/mutations and the Au:peptide ratio on particle size, shape, and stability. Figure 4.5 presents TEM images of the Au nanoparticles capped with each of the peptides after 1 h of reduction. The top and bottom rows display the materials prepared at ratios of 2 and 1, respectively. For the Pd4-capped materials fabricated at a ratio of 2, polydisperse particles with an average size of $7.7 \pm 3.6$ nm were observed, consistent with previous studies;\textsuperscript{130,163} however, the particles generated with the alanine-substituted peptides decreased in size to $5.2 \pm 2.0$ nm (Pd4-1), $3.9 \pm 1.1$ nm (Pd4-2), and $5.3 \pm 1.7$ nm (Pd4-3). For the materials capped with the Pd4-4 peptide that randomized the peptide sequence and the Pd4-5 sequence that grouped the histidine anchor residues at the N-terminus, particles of $5.3 \pm 2.8$ nm and $4.1 \pm 1.6$ nm were noted, respectively. Overall, all of the modified or mutated peptides generated smaller Au nanoparticles than the parent Pd4 sequence; however, no clear trends with respect to biomolecule binding strength ($\Delta G$) and average particle size were evident. Furthermore, all of the particles were polydisperse in size and overall morphology.

**Figure 4.5.** TEM analysis of the Au nanoparticles capped with the indicated mutant peptides. TEM grids were prepared 1 h after reduction. The top row displays the materials fabricated at a Au:peptide ratio of 2, while the bottom row shows the structures generated at a ratio of 1. All scale bars are 50 nm.
Different results were obtained when the synthesis reactions were carried out at a Au:peptide ratio of 1. Spherical particles with an average diameter of $3.0 \pm 0.6$ nm (Pd4), $3.6 \pm 0.9$ nm (Pd4-1), $2.6 \pm 0.5$ nm (Pd4-2), $3.5 \pm 1.1$ nm (Pd4-3), $3.2 \pm 1.1$ nm (Pd4-4), and $2.7 \pm 0.5$ nm (Pd4-5) were formed at this lower Au:peptide ratio. For all particle sizing, at least 100 particles were measured over multiple TEM images. Size distribution histograms are available in Chapter 6, Figures 6.21 and 6.22. The effect of decreasing the Au:peptide ratio on particle size and shape is illustrated in these samples. In general, after 1 h of reduction, using a 2:1 ratio to prepare the materials gave larger, more polydisperse, and somewhat aggregated particles, while employing a 1:1 ratio produced smaller, more spherical particles. Such effects likely arise from insufficient peptide in solution at the higher ratio (2:1) to passivate the Au nanoparticle surfaces at smaller particle sizes, leading to coalescence of the materials to generate the observed sample. At the smaller ratio (1:1), enough peptide is present in the reaction to cap the growing nanoparticles at the smaller sizes. These results are consistent with the UV-vis spectra of the materials, in which all the structures generated at a ratio of 2, independent of which peptide was used to cap them, displayed a broad and red shifted Au LSPR absorbance relative to the distinct LSPR peak at 525-550 nm for the particles prepared at a ratio of 1.

Figure 4.6 presents UV-vis and TEM analyses of samples 24 h after reduction. The aggregation process was monitored by measuring the absorbance at the LSPR peak of the Au nanoparticles over 24 h, taking one spectrum every 10 min for 24 h. For the reactions using a Au:peptide ratio of 2, as presented in Figure 4.6a, aggregation of the particles prepared with Pd4-1, Pd4-2, and Pd4-4 was observed in less than 24 h. Although a significant increase in absorbance was evident initially for the Pd4-3 capped materials,
arising from the nucleation and initial growth of the particles, a sharp decrease in the absorbance was noted beginning at 16 h, indicating precipitation was occurring. The Pd4-5 reaction products were stable for the duration of the experiment as seen in the plateau of its LSPR absorbance. Interestingly, the absorbance for the Pd4 particles slowly decreased over 24 h and ended at the same absorbance as the Pd4-3 reaction when it had precipitated; however, the Pd4-capped materials remained stable and dispersed in water. This suggests that the Pd4-capped particles were somewhat aggregated in solution, even though they do not aggregate to a sufficient extent to show visible precipitation. TEM
imaging of the Pd4 and Pd4-5 capped materials 24 h post reduction revealed particle sizes of 5.5 ± 2.4 nm and 3.7 ± 2.1 nm, respectively, which are relatively similar to the particle sizes after 1 h of reduction. When a Au:peptide ratio of 1 was employed, a similar trend was also found in that materials produced using Pd4 and Pd4-5 remained stable over 24 h; however, bulk precipitation was evident in the particles capped with Pd4-1, Pd4-2, Pd4-3, and Pd4-4 peptides (Figure 4.6b). TEM imaging 24 h after reduction gave identical particle sizes of 2.7 ± 0.5 nm (Pd4) and 2.7 ± 0.5 nm (Pd4-5), which are again similar to the particle sizes after 1 h of reduction.

Overall, no clear trend was evident between the binding strengths of the peptides and the size of the particles produced. For example, Pd4-4 and Pd4-5 have the strongest affinity for Au based upon their ∆G values; however, these sequences generated Au nanoparticles with neither the largest or smallest sizes among all the peptides studied. This suggests that additional binding parameters beyond surface affinity govern particle formation and growth using peptides. Interestingly, the Pd4 and Pd4-5 peptides generated nanoparticles that remained stable for more than 24 h at both ratios of 2 and 1, even though their ∆G values were very different. This indicates that particle stability depends upon other factors such as the conformation in which the peptide binds to the metal surface, rather than simply the affinity of the peptide for the NP material.

To date, there has been no systematic rationale to understand or predict why a given peptide sequence may or may not produce a stable dispersion of peptide-capped Au nanoparticles in aqueous solution. Aggregation is likely if patches of exposed Au surface are present on two neighboring nanoparticles in solution. This condition is more probable for a peptide/nanoparticle interface that features a relatively high degree of
conformational entropic contribution to the binding ($S_{\text{conf}}$), i.e., a large number of distinct adsorbed structures and few strong anchor points. In terms of the underlying potential energy landscape that corresponds to a high value of $S_{\text{conf}}$, two scenarios are plausible. In the first, the large number of distinct surface-adsorbed conformations could be separated by very low (less than or equal to $k_B T$) potential energy barriers. This would result in a highly dynamic, disordered system where the adsorbed peptide conformations could readily interconvert at room temperature, producing a high degree of mobility of the surface-adsorbed peptides. The second scenario would correspond to the presence of high (much greater than $k_B T$) potential energy barriers with respect to interconversion between the large number of distinct conformations. In this case, the peptide conformations would be frozen into a wide range of adsorbed structures, and would be essentially immobile on the surface, presumably from their first instance of contact on the Au nanoparticle surface. In the former scenario, a high $S_{\text{conf}}$ value, corresponding to high mobility of surface-adsorbed peptides, could be conducive to the thermally-activated exposure of patches on the Au nanoparticle surface. Our predicted averaged $S_{\text{conf}}$ values, provided in Figure 4.7 (see Table 6.7 in Chapter 6 for the numerical values), indicate that the sequences Pd4-1, Pd4-2, Pd4-3, and Pd4-4, which correspond to unstable nanoparticle dispersions, all have very high $S_{\text{conf}}$ values. This contrasts with Pd4 and Pd4-5, which have the lowest $S_{\text{conf}}$ values in the set and also yield the only nanoparticle dispersions that remained stable after 24 h. These data suggest a connection between the $S_{\text{conf}}$ value and nanoparticle stability in solution. However, because multiple factors are likely to influence nanoparticle stability, extension of this hypothesis to other peptide systems requires further investigation.
In a previous study of peptide-mediated Au NP formation using a diverse set of sequences,\textsuperscript{130} we also found that there was no direct correlation between binding affinity and the size of the resulting NPs. Consistent with the present study, this shows that simply selecting sequences for their overall binding affinity for a particular inorganic material may not guarantee identification of sequences that will produce stable peptide-capped NPs. This earlier study also showed that the catalytic activity of the NPs correlated with the number of strong anchor residues. Peptides characterized as enthalpic binders, with a relatively large number of anchor residues exhibited high activation energies for a prototypical surface catalysed reaction. This picture was refined in the study by Bedford \textit{et al.}\textsuperscript{153} With the present study, a coherent picture begins to emerge. Synthesis of small, colloidally stable peptide-capped NPs seem to be favored by use of enthalpically-driven binders with relatively low conformational entropy. Sequences with

\textbf{Figure 4.7.} Values of the conformation contribution to the entropy of peptide-surface binding ($S_{\text{conf}}$) for the parent Pd4 peptide and all variants on this sequence. Numerical values are provided in Table 6.7 in Chapter 6.
fewer anchor residues and high conformational entropy provide greater exposure of the inorganic surface, which may be desirable, e.g. for improved catalytic activity, but may also promote aggregation.

4.5 Summary and Conclusions

In conclusion, we have presented a peptide synthetic mutation analysis that advances the understanding of the role of peptide sequence in controlling and modulating the affinity of the biomolecule for its target inorganic surface. In this regard, mutation of anchor points demonstrated that the local affinity can be modulated based upon the neighboring residues in the sequence. Thus, sequence context effects can influence the materials-binding ability of peptides. This observation was corroborated by both experimental and computational findings that pointed to important thermodynamic considerations for isolating the underlying basis of binding affinity. Further Au nanoparticle synthesis results demonstrated that the overall particle morphology was not directly associated with the $\Delta G$ of binding to the material surface, suggesting that other biomolecular factors govern the size, shape, and stability of peptide-capped materials. A feature that is suggested to govern the stability of the peptide-capped nanoparticle dispersion was identified, namely the number of distinct conformations of the peptide in the surface-adsorbed state. Such results are important for the creation of rules for the $a$ priori design of new biomolecules that can tune peptide affinity and specificity, as well as nanoparticle capping and stabilization ability for target inorganic structures. Such capabilities would be of significant use in the one-pot, aqueous synthetic design of functional materials, for applications wherein the ligand overlayer structure/morphology is critical to the intended functionality. For instance, controlling the bioligand surface display could be important
for applications in biosensing, especially for Au nanorings\textsuperscript{164-165} that are sensitive to the biomolecule orientation, and catalysis where selective peptide surface structures could be designed to enhance the particle functionality.
Chapter 5. Influence of Peptide Sequence Manipulation on Au Binding and Nanoparticle Stabilization*

5.1 Overview

To further advance peptide-based material fabrication and assembly, a comprehensive understanding of the many factors that influence materials-binding affinity and nanoparticle stability is required. Here, quartz crystal microbalance studies and molecular dynamics simulations were employed to characterize the binding affinity and binding properties to Au of variants of the Au-binding A3 and AuBP1 peptides. By studying the binding behavior of the mutant sequences, we were able to classify the binding properties of the parent peptides as either conformationally-recalcitrant or conformationally-susceptible to sequence manipulation. Au nanoparticle synthesis with the parent and mutant peptides revealed that conformational entropy along with other biomolecular factors govern nanoparticle stability. These results symbolize an important step toward predicting and realizing materials-binding affinity and nanoparticle stabilization capability from peptide sequence information alone.

5.2 Background

Bio-inspired peptide-mediated strategies have been previously demonstrated as an effective approach to nucleate, grow, stabilize, activate, and organize inorganic nanoparticles in aqueous media.\textsuperscript{121,130,163} Despite these successes, broader applicability of such strategies is currently impeded due to a lack of fundamental understanding of how to manipulate the surface-adsorbed structure, and consequently the binding strength, of

these materials-binding peptides. Previous studies have clearly demonstrated that a peptide sequence that shows strong materials-binding affinity does not necessarily also serve as an effective agent for stabilizing peptide-decorated nanoparticles dispersed in solution.\textsuperscript{166-167} Therefore, the identification of factors inherent to peptide sequences that can promote either strong materials-binding affinity or effective nanoparticle stabilization capability, or both, remains as a key unresolved question.

Earlier studies have reported investigations of the materials-binding and nanoparticle stabilization capabilities of peptide sequences that have been altered, \textit{e.g.} via point mutations or by shuffling the sequence.\textsuperscript{168} However, the majority of these studies lacked accompanying detailed molecular-level structural data to enable clear conceptual links between peptide sequence, conformation(s), and properties. Moreover, the systematic alteration of known materials-binding sequences that have a wealth of pre-established binding and structural data has not been a focus of this previous work, but is precisely what is required as first steps to elucidate these structure/property relationships. In terms of linking the peptide structure to its properties, the degree of localization of the structural effect of point mutations, chiefly their influence on the corresponding properties of a peptide such as surface binding affinity, is a poorly understood phenomenon in a general sense. This lack of a predictable response to mutations severely hinders clear interpretation of experimental procedures that seek to identify which residues are the most critical for mediating surface binding as generated by approaches such as alanine scan experiments.

While our work summarized herein does not claim to definitively address this general problem, herein we summarize our progress to continue to systematically build a
structure/property knowledge-base for peptide-mediated nanomaterials that will ultimately provide the foundation for reliable data mining of these properties in the future. Our previous work has provided a comprehensive survey and quantification of the Au-surface binding strengths and surface-adsorbed conformations of 12 known materials-binding peptide sequences. These data provide a solid platform for such systematic mutation/alteration studies, particularly because many of these binding sequences were derived from very different sources (e.g. biocombinatorial screening or isolated from natural sources). Consequently, this set of peptide sequences is very diverse in terms of composition and sequence motifs and have been identified previously to act as either enthalpic or entropic binders. The sequence diversity in this set enables us to compare and contrast the properties and related conformational response of different Au-binding sequences when altered in systematic ways, with particular focus on materials-binding affinity and nanoparticle stabilization.

Here, we quantify and elucidate how the mutations of two peptides with very different conformational susceptibilities can influence both the non-covalent binding affinity at aqueous Au interfaces and the ability of these biomolecules to nucleate, grow, and stabilize Au nanoparticles in aqueous media. Specifically, we provide an in-depth analysis and characterization of the Au-binding behavior of mutants of two well-known Au-binding sequences: A3 (AYSSGAPMPPF) and AuBP1 (WAGAKRVLVRRE). Our mutation studies provide valuable insights into sequence characteristics that control peptide-materials binding affinity and can explain how and why particular mutants of these sequences can stabilize Au nanoparticles in solution. Following our work on the Pd4 peptide sequence, we considered five sequence variations for both A3 and AuBP1,
Table 5.1. Adsorption Analysis for Parent A3 and AuBP1 Peptides and Mutants on Au\textsuperscript{a}. Anchor residues are highlighted in red, mutated residues in blue, and scrambled residues in green.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>ΔG (kJ mol\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3</td>
<td>AYSSGAPPMPPF</td>
<td>-31.6 ± 0.5</td>
</tr>
<tr>
<td>A3-1</td>
<td>AASSGAPPMPPF</td>
<td>-36.6 ± 1.1</td>
</tr>
<tr>
<td>A3-2</td>
<td>AYSSGAPPAPPF</td>
<td>-28.8 ± 0.9</td>
</tr>
<tr>
<td>A3-3</td>
<td>AYSSGAPPMPPA</td>
<td>-30.5 ± 0.2</td>
</tr>
<tr>
<td>A3-4</td>
<td>YPPAPSAPSFMG</td>
<td>-30.6 ± 0.1</td>
</tr>
<tr>
<td>A3-5</td>
<td>YMFASSGAPPPP</td>
<td>-28.4 ± 0.2</td>
</tr>
<tr>
<td>AuBP1</td>
<td>WAGAKRLVLRRE</td>
<td>-40.7 ± 2.1</td>
</tr>
<tr>
<td>AuBP1-1</td>
<td>AAGAKRLVLRRE</td>
<td>-32.2 ± 0.5</td>
</tr>
<tr>
<td>AuBP1-2</td>
<td>WAGAKALVLRRE</td>
<td>-35.9 ± 0.7</td>
</tr>
<tr>
<td>AuBP1-3</td>
<td>WAGAKRLVLRAE</td>
<td>-33.3 ± 0.7</td>
</tr>
<tr>
<td>AuBP1-4</td>
<td>ALRWVGRRAELK</td>
<td>-32.6 ± 0.0</td>
</tr>
<tr>
<td>AuBP1-5</td>
<td>WRRAGAKLVLRE</td>
<td>-34.2 ± 0.3</td>
</tr>
</tbody>
</table>

\textsuperscript{a}From QCM experiments: adsorption Gibbs free energy (ΔG) values are given as mean ± one standard deviation from at least three independent experiments.

as summarized in Table 5.1. For each parent peptide, we replaced each known anchor residue, identified from our previous work,\textsuperscript{34} with alanine (sequences A3-1 to A3-3 and AuBP1-1 to AuBP1-3). We also considered a randomized version of each peptide sequence (A3-4 and AuBP1-4) and a variation of the peptide sequence where all anchors were grouped together at the N-terminus (A3-5 and AuBP1-5). In comparing these with the original parent biomolecules, this results in a total of 12 unique peptide sequences that we considered in the present work. Using a combination of quartz crystal microbalance (QCM) measurements, nanoparticle synthesis and characterization, and
advanced molecular dynamics (MD) simulations, we have quantified and evaluated how each of these 12 sequences adsorbs to Au surfaces and explored the connections between the resulting binding properties and the ability of these peptides to support the nucleation, growth, and stabilization of Au nanoparticles in aqueous media. Furthermore, these results provide key insights into the context effects of the localized residue mutations for the binding of peptides to their target inorganic surface, providing a pathway to the \textit{de novo} design of new sequences with enhanced affinity/properties and potentially inorganic material selectivity.

5.3 Materials and Methods

5.3.1 Chemicals

Ammonium hydroxide (20\%) and hydrogen peroxide (30\%) were purchased from BDH Chemicals. HAuCl$_4$ and NaBH$_4$ were obtained from Sigma Aldrich. All peptides used were acquired from Genscript at >90\% purity. Finally, all chemicals that were used in this study were used as received without further purification. Milli-Q water (18 M\(\Omega\) cm) was employed for all experiments.

5.3.2 QCM Binding Experiments

All QCM measurements, including those for dissipation energy, were conducted on a Q-Sense E4 instrument (Biolin Scientific) following established methods.$^{34,145}$ Standard polycrystalline Au crystal sensors were cleaned according to manufacturer protocols before use. Briefly, the Au QCM sensors were subjected to UV-ozone exposure, followed by immersion in a 5:1:1 (v/v/v) water/ammonium hydroxide/hydrogen peroxide solution and an additional UV/ozone treatment. An aqueous peptide solution at a concentration between 2.5 to 15 \(\mu\)g/mL was flowed over the sensor surface at a rate of 150 \(\mu\)L/min. The
frequency change and dissipation energy were recorded for 30 min to ensure saturation. The frequency change is directly related, via the Sauerbrey equation, to the mass of peptide adsorbed, from which binding kinetic parameters can be determined. All QCM experiments were performed at 22.5 °C, and the pH of the peptide solutions ranged from 4.1 – 6.0 depending on the sequence. Peptide solutions were made by dissolving the peptide in deionized water only to the appropriate concentration.

5.3.3 Synthesis and Characterization of Peptide-Capped Au Nanoparticles

Peptide-capped Au nanoparticles were prepared following previously established synthetic methods. The peptide-capped Au nanoparticles were produced with a Au:peptide ratio of 2. Briefly, 500 µL of an aqueous 1.0 mM peptide solution was added to 4.460 mL of water. Next, 10 µL of an aqueous 100 mM HAuCl₄ solution was added. The solution was gently agitated and after a 10 min complexation period, 30 µL of a freshly prepared aqueous 100 mM NaBH₄ solution was added. A NaBH₄:Au ratio of 3 was used for all syntheses. The solution was gently agitated again, and the reaction was allowed to proceed undisturbed at room temperature for 1.0 h to ensure complete reduction.

Transmission electron microscopy (TEM) studies were conducted using a JEOL JEM-2010 microscope operating at a working voltage of 200 kV. The samples were prepared by drop-casting 30 µL of the colloidal nanoparticle dispersion onto a carbon-coated 400 mesh Cu grid, which was allowed to dry under ambient conditions. UV-vis optical absorbance of the Au nanoparticles was measured using a Shimadzu 3600 UV-visible-NIR scanning spectrophotometer. All samples were analyzed using a quartz cuvette with a 1 cm optical path length.
5.3.4 Molecular Simulations

We used a polarizable force-field for Au, namely GolP-CHARMM. Additional details, including simulation analyses and evidence of REST sampling efficacy (replica mobilities) and sampling equilibration, can be found in the ‘Simulation Details’ section of Chapter 6.4. The capabilities of the GolP-CHARMM force-field have been recently demonstrated in the near-reproduction of the experimentally-determined binding free energy of the AuBP1 peptide at the aqueous Au interface.

5.4 Results and Discussion

5.4.1 QCM Analysis of Peptide Binding to Au

Although the A3 and AuBP1 parent sequences are both Au-binding peptides and have three anchor residues for Au-binding each, they significantly differ to the degree in which they bind to Au. To this end, the AuBP1 is a substantially stronger binder as compared to the A3. To analyze the sequence characteristics that affect the kinetic and thermodynamic properties that control the Au-binding behavior of A3 and AuBP1, a set of five different mutant peptide sequences was prepared for each parent peptide. QCM measurements were employed to quantify the free energy of binding ($\Delta G$) of each peptide of the library for Au surfaces, with values provided in Table 5.1. As an example of the QCM analysis, Figure 5.1a presents the observed frequency changes for the A3-1 peptide at five different concentrations. Higher peptide concentrations yielded faster and larger frequency changes, reflecting increased binding rates. The dissipation energy, which is indicative of the viscoelasticity of the bound peptide layer, is shown for the highest peptide concentration (15 µg/mL). For all of the peptides studied here, the dissipation energy was observed to be <5% of the total frequency change. This indicates that the
An adsorbed peptide layer was rigidly bound to the Au surface, strongly suggesting that only a single peptide layer was formed. Note that for all QCM data, inverted plots are displayed for more intuitive data interpretation. The QCM analysis for all of the peptides is presented in the Supporting Information, Figures 6.23–6.24.

Psuedo-first-order adsorption rate constants ($k_{\text{obs}}$) were obtained by fitting each QCM binding curve to Langmuir kinetics. The binding constants for adsorption ($k_a$) and desorption ($k_d$) are given by the slope and $y$-intercept, respectively, of the best-fit line when the $k_{\text{obs}}$ values are plotted as a function of the peptide concentration (Figure 5.1b). The binding equilibrium constant, $K_{\text{eq}}$, is then calculated as $k_a/k_d$, and finally, the binding affinity for peptide binding, defined as the change in Gibbs free energy ($\Delta G$) between the peptide bound and unbound states, is determined by $\Delta G = -RT \ln(K_{\text{eq}})$. A list of the $k_a$, $k_d$, and $K_{\text{eq}}$ values for each peptide in Table 5.1 can be found in the Supporting Information, Table 6.12.
For the A3 mutant peptides, the $\Delta G$ values ranged from $-28.4$ to $-36.6$ kJ/mol for the weakest (A3-5) and strongest (A3-1) binders, respectively. For the parent A3 peptide, a $\Delta G$ value of $-31.6 \pm 0.5$ kJ/mol was measured, equivalent to previously reported values.\textsuperscript{34} Modification of the tyrosine at the second position to alanine (i.e. the Y2A mutation) in A3-1 resulted in a substantial increase in binding affinity to $-36.6 \pm 1.1$ kJ/mol, which was quite surprising in light of the removal of a strong anchor residue. Interestingly, when the second or third anchor residue was replaced by alanine, as in A3-2 (M9A mutation) and A3-3 (F12A mutation), lower $\Delta G$ values of $-28.8 \pm 0.9$ kJ/mol and $-30.5 \pm 0.2$ kJ/mol were measured, respectively. This suggests that the local context of anchor residues is an important factor that may affect the Au-binding affinity of the peptide. Finally, all further changes, including sequence scrambling or clustering of anchor residues at the N-terminus resulted in diminished Au affinity as compared to the parent A3 sequence; a $\Delta G$ value of $-30.6 \pm 0.1$ kJ/mol was determined for the A3-4 peptide, while the weakest binding of the set was observed from the A3-5 peptide with a $\Delta G$ value of $-28.4 \pm 0.2$ kJ/mol.

Modification of the AuBP1 peptide provided interesting results. QCM measurements on the parent sequence gave a $\Delta G$ value of $-40.7 \pm 2.1$ kJ/mol, which is similar to previously reported values.\textsuperscript{34} Note that this peptide sequence represents one of the strongest known binding sequences for Au. Each modification to this sequence resulted in a significant reduction in Au affinity. For example, in each instance when a single anchor residue was replaced by alanine, as in the AuBP1-1 (W1A mutation), AuBP1-2 (R6A mutation), and AuBP1-3 (R11A mutation) peptides, the modifications resulted in substantially lower affinity with $\Delta G$ values of $-32.2 \pm 0.5$ kJ/mol, $-35.9 \pm 0.7$ kJ/mol, respectively.
kJ/mol, and $-33.3 \pm 0.7$ kJ/mol, respectively. This indicates that mutation of the individual anchor residues does greatly affect the Au affinity; however, the effect was largest for the AuBP1-1 and AuBP1-3 sequences, in which the alanine mutation occurs at the N-terminus or the C-terminus, respectively. A dramatic decrease in binding affinity was observed for the scrambled sequence; a $\Delta G$ value of $-32.6 \pm 0.9$ kJ/mol was determined for AuBP1-4. The AuBP1-5 peptide (anchors clustered at the N-terminus) exhibited a smaller decrease in binding affinity with a $\Delta G$ value of $-34.2 \pm 0.4$ kJ/mol.

5.4.2 Molecular Dynamics Simulations of Peptide Binding to Au

Replica-Exchange with Solute Tempering (REST) MD simulations were used to predict the conformational ensemble of each of the twelve peptide sequences in the surface-adsorbed state on the aqueous Au(111) interface. We start with the six A3-based sequences, where the degree of residue-surface contact with the Au surface was determined from our simulations, as summarized in Table 5.2. When adsorbed at the aqueous Au interface, the parent A3 peptide is predicted to have three anchor residues, Y2, M9, and F12 (see red-highlighted entries in Table 5.2), which is why these residues were selected for point mutations with alanine. Naïvely, it might be expected that mutation of Y2 for alanine, as was done to create the A3-1 sequence, would result in a reduction of Au-binding affinity. However, in agreement with the unexpected results from the QCM observations, the enthalpic contribution to the overall peptide binding, as determined by the number and strength of the resulting anchor residues, appeared to increase, while the surface contact of several non-anchor residues is also seen to increase (Table 5.2). The impact of the Y2 mutation appears to be remarkably non-local, causing a predicted increase in surface contact for the central segment of the peptide chain, while
the remaining M9 and F12 sites also remained as anchor residues. In contrast with the results for A3-1, the A3-2 mutation suggests a very localized impact on binding compared with the parent peptide, with an accompanying predicted drop in binding strength due to the deletion of the strong adsorption arising from the methionine (M9). The surface contact of all other residues in A3-2 remained largely invariant to this mutation.

The sequence arising from the mutation of phenylalanine (F12), namely A3-3, resulted in surface-peptide binding that was intermediate between A3-1 and A3-2 (see Table 5.2), corresponding with a concomitant change in measured adsorption free energy that was also in between that of A3-1 and A3-2 (Table 5.1). The randomized sequence, A3-4, also showed a drop in adsorption free energy, which our simulations suggest is due
to the reduction in binding of F10, while the tyrosine and methionine residues remained as anchors. The residue-surface contact of the final variant, A3-5, reflects the greatest reduction in binding strength compared with the parent sequence. In this case, both methionine and phenylalanine exhibited substantially reduced binding.

![Image of peptide conformations](image_url)

**Figure 5.2.** Most likely structures of the six A3-based peptides adsorbed at the aqueous Au interface, predicted from the REST simulations. Water not shown for clarity.

We attribute these trends in binding properties in part to the conformational response of A3 to mutation and sequence rearrangement. Specifically, we quantified and compared the degree of structural similarity of the peptide backbone conformations for each of the six peptides adsorbed at the Au interface (see Simulation Details and Table 6.13 in Chapter 6) and found that A3-1, A3-2, and A3-3 (corresponding with the three alanine point mutations) showed remarkable similarity in backbone conformation
compared with the parent peptide in the adsorbed state.\textsuperscript{34} On the other hand, A3-4 and A3-5, which corresponded to global rearrangements of the peptide sequence, shared very little conformational similarity. To illustrate this point, we provide the most likely Au-adsorbed structures, as determined by clustering our reference replica REST trajectories (see Methodology), in each of the six cases in Figure 5.2. This conformational recalcitrance to point mutations was also noted for the Pd4 sequence in our previous work.\textsuperscript{167}

**Table 5.3. Degree of residue-surface contact (expressed as a percentage) for each of the six AuBP1-based peptide sequences, adsorbed at the aqueous Au interface, predicted from the REST simulations.** White, blue, green and red colors indicate negligible, low, medium and strong binding, respectively.

<table>
<thead>
<tr>
<th>AuBP1</th>
<th>AuBP1-1</th>
<th>AuBP1-2</th>
<th>AuBP1-3</th>
<th>AuBP1-4</th>
<th>AuBP1-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>W 88</td>
<td>A 12</td>
<td>W 91</td>
<td>W 93</td>
<td>A 11</td>
<td>W 87</td>
</tr>
<tr>
<td>A 45</td>
<td>A 37</td>
<td>A 12</td>
<td>A 32</td>
<td>L 35</td>
<td>R 53</td>
</tr>
<tr>
<td>G 64</td>
<td>G 50</td>
<td>G 39</td>
<td>G 43</td>
<td>R 70</td>
<td>R 56</td>
</tr>
<tr>
<td>A 53</td>
<td>A 48</td>
<td>A 17</td>
<td>A 54</td>
<td>W 90</td>
<td>R 67</td>
</tr>
<tr>
<td>K 23</td>
<td>K 19</td>
<td>K 27</td>
<td>K 16</td>
<td>V 23</td>
<td>G 52</td>
</tr>
<tr>
<td>R 92</td>
<td>R 85</td>
<td>A 56</td>
<td>R 71</td>
<td>G 70</td>
<td>A 28</td>
</tr>
<tr>
<td>L 44</td>
<td>L 33</td>
<td>L 38</td>
<td>L 58</td>
<td>R 65</td>
<td>K 11</td>
</tr>
<tr>
<td>V 42</td>
<td>V 63</td>
<td>V 23</td>
<td>V 45</td>
<td>R 66</td>
<td>L 35</td>
</tr>
<tr>
<td>L 50</td>
<td>L 40</td>
<td>L 45</td>
<td>L 33</td>
<td>A 17</td>
<td>V 19</td>
</tr>
<tr>
<td>R 76</td>
<td>R 74</td>
<td>R 77</td>
<td>R 71</td>
<td>E 33</td>
<td>L 30</td>
</tr>
<tr>
<td>R 85</td>
<td>R 53</td>
<td>R 84</td>
<td>A 42</td>
<td>L 16</td>
<td>A 17</td>
</tr>
<tr>
<td>E 50</td>
<td>E 25</td>
<td>E 51</td>
<td>E 21</td>
<td>K 6</td>
<td>E 22</td>
</tr>
</tbody>
</table>

In complete contrast to the predicted change in binding for the variants of A3, and for Pd4,\textsuperscript{34} the variations of the AuBP1 sequence resulted in very different binding outcomes. In terms of the predicted residue-surface contact, our results indicate a reduction in binding for all sequences compared with the parent AuBP1 peptide (see
Table 5.3). Moreover, the QCM experimental data suggest that AuBP1-1 and AuBP1-4 suffered the greatest reduction in adsorption free energy on the Au surface. As our residue-surface contact data suggest (Table 5.3), these two sequences showed the greatest loss of contact, particularly for the remaining anchors of the parent sequence. For example, in AuBP1-1, the W1A mutation not only resulted in reduced binding at the A1 position, but also diminished surface contact for the anchors R10 and R11, both of which are distant from the site of the point mutation. The AuBP1-based sequence that showed the least reduction in binding at the Au surface, AuBP1-2, also showed the least reduction in predicted residue-surface contact (Table 5.3).

In Figure 5.3, we show the most likely structures of the six AuBP1-based peptides adsorbed at the aqueous Au interface. From these data, in partnership with a mathematical comparison of the structural similarity of the peptide conformations (see Table 6.14 in Chapter 6), we found that the AuBP1 mutants had very little commonality in terms of backbone structure. This finding indicates that, unlike Pd4 and A3, AuBP1 is a conformationally-susceptible sequence that in some instances supported a dramatic conformational response to point alanine mutations. Overall, our predictions from the REST-MD simulations corroborate the QCM measurements of peptide binding strength. From our current work and comparisons with past studies, we have identified two classes of peptides: conformationally-recalcitrant peptides, where the ensemble of adsorbed structures does not change appreciably for point mutations, and conformationally-susceptible peptides, which can result in very different structures (in a non-localized sense) upon point mutation. The presence of proline in a sequence is likely to be instrumental in conferring conformationally-recalcitrant behaviors, as each of the Pd4- and A3-based sequences contain one and four proline residues, respectively.
All in all, the MD simulations of the A3 variants demonstrated that alanine mutations near the termini ends of the sequence, as in the case of A3-1 (Y2A) and A3-3 (F12A), can slightly enhance residue-surface contact in the center of the peptide. Additionally, binding of tyrosine is less susceptible to sequence context effects compared with other anchors. Tyrosine remains an anchor residue independent of point mutation or rearrangement of the A3 sequence, as seen for each of the A3 mutant peptides except for A3-1, which does not possess a tyrosine residue. In contrast with AuBP1, alanine point mutations to the A3 sequence have a very localized effect; peptide binding distant from the mutation site is largely unaffected. Lastly, it was shown that the A3 mutant peptides with alanine point mutations (A3-1, A3-2, and A3-3) all have very similar structures compared with the parent sequence and each other, which can explain the localized

Figure 5.3. Most likely structures of the six AuBP1-based peptides adsorbed at the aqueous Au interface, predicted from the REST simulations. Water not shown for clarity.
effects on residue-surface contact. To this end, we classify the binding properties of A3 as conformationally-recalcitrant to sequence manipulation. This conformationally-recalcitrant behavior is attributed to proline-rich sequences that confer backbone rigidity.

The MD simulations of the AuBP1 variants indicated that binding of tryptophan is less susceptible to sequence context effects compared with other anchors. Similar to tyrosine in the A3-based sequences, tryptophan remains an anchor residue independent of point mutation or rearrangement of the AuBP1 sequence, as seen for each of the AuBP1 mutant peptides except for AuBP1-1, which does not possess a tryptophan residue. In contrast to A3, alanine point mutations to the AuBP1 sequence, as in the case of AuBP1-1, AuBP1-2, and AuBP1-3, generally weaken residue-surface contact throughout the sequence, as this effect is not localized to the mutation site. Scrambling (AuBP1-4) and anchor-grouping (AuBP1-5) both weaken residue-surface contact throughout the sequence, in addition to resulting in loose binding at the C-terminus in each case. Finally, it was shown that sequence manipulation drastically alters the conformational ensemble of the AuBP1 mutant peptides compared with the parent sequence and each other. Mathematical analysis of the structural similarity indicates very few mutant structures in common, which can explain the non-localized global weakening of residue-surface contact. To this end, we classify the binding properties of AuBP1 as conformationally-susceptible to sequence manipulation.
5.4.3 Au Nanoparticle Synthesis and Characterization

Figure 5.4 presents TEM images and UV-vis spectroscopy analyses of Au nanoparticles synthesized using the A3 mutant peptides, approximately 1 hour after reduction. The A3 parent and four of the five mutant peptides (A3-1, A3-3, A3-4, and A3-5) produced small and spherical Au nanoparticles with diameters of ~2–3 nm that exhibited well-defined LSPR peaks at ~520 nm, while the A3-2 mutant peptide produced larger, somewhat irregular nanoparticles and clusters that exhibited a broadened and red-shifted LSPR peak (see Figure 5.4 for the actual size values). For all particle sizing, at least 100 particles were measured over multiple TEM images. Size distribution histograms are available in Chapter 6, Figures 6.25 and 6.26. Although A3-2 has a reduced binding affinity compared to A3, it does not have the lowest binding affinity in the set. The A3-5 peptide, which has even lower binding affinity, also has notably slower binding kinetics than A3-2 (Table 6.12 in Chapter 6) and a much different binding conformation on the Au surface (Figure 5.2) compared to A3-2. Both A3-2 and A3-5 have two strong anchor residues, but in A3-2 they are spaced at opposite ends of the sequence, while in A3-5 they are closer together. This may limit the ability of A3-2 to cap very small nanoparticles during growth.

Long-term stability testing showed that the Au nanoparticle dispersions remained stable for at least 1 week, except for the A3-4 capped nanoparticles. Au nanoparticles capped with the A3-4 peptide showed signs of precipitation within hours after the synthesis. This is consistent with the minor aggregation of the A3-4 capped nanoparticles visible in the TEM image of Figure 5.4. While the three strong anchor residues were preserved to passivate and stabilize the Au nanoparticles during synthesis, the reduced
binding of F10 that arises from sequence randomization may have significantly impaired its conformational advantage in mediating nanoparticle stability. This presents an interesting contrast with the A3-2 capped particles, which were larger and more irregular than the other materials, but which remained stably dispersed. Thus, the A3-2 peptide was less able to cap the particles and limit their growth during the rapid synthesis process compared to the other sequences, but was nonetheless able to stabilize the nanoparticle dispersion. The A3-4 peptide was able to limit the nanoparticle growth during synthesis, but was not able to stabilize the nanoparticle dispersion as well as the other sequences. Aggregation of Au nanoparticles with one another at points where the Au surface is exposed (due to peptide desorption or low coverage) is more probable for a

Figure 5.4. TEM and UV-vis analyses of the Au nanoparticles capped with the indicated A3 mutant peptides. All scale bars are 20 nm.
peptide/inorganic interface that features a relatively high degree of conformational entropy ($S_{\text{conf}}$). As demonstrated in our prior work on the Pd4 mutant peptides,$^{167}$ $S_{\text{conf}}$ values from MD simulations correlate with this outcome. The predicted averaged $S_{\text{conf}}$ values, provided in Table 6.15 in Chapter 6, indicate that the A3-4 sequence, which produces an unstable nanoparticle dispersion, has the highest $S_{\text{conf}}$ value among the A3 parent and mutant peptides. This contrasts with A3, A3-1, A3-2, A3-3, and A3-5, which have relatively lower $S_{\text{conf}}$ values and also yield nanoparticle dispersions that remain stable after 1 week.

Similarly, AuBP1 and its mutant peptides were used to synthesize Au nanoparticles, as presented in the TEM images and UV-vis studies in Figure 5.5. The AuBP1 parent and three of the five mutant peptides (AuBP1-2, AuBP1-4, and AuBP1-5) produced well-defined spherical Au nanoparticles with diameters of ~3 nm that exhibited distinct LSPR peaks at ~520 nm. In contrast, the AuBP1-1 peptide showed a reduced ability to cap the Au nanoparticles during nucleation and growth, resulting in larger and irregularly shaped particles aggregated together that exhibited an extremely broad and red-shifted LSPR peak. Interestingly, the AuBP1-3 peptide produced nanoparticles that appear aggregated in the TEM image; however, a well-defined LSPR peak was exhibited for this sample, indicating the presence of small and stable nanoparticles.

Long-term stability testing showed that the Au nanoparticles capped with the AuBP1-1, AuBP1-2, and AuBP1-3 peptides precipitated within hours after the synthesis, while all other sequences supported stable dispersions for at least 1 week. However, a high degree of conformational entropy cannot explain this lack of nanoparticle stability;
the predicted $S_{\text{conf}}$ values do not correlate with this outcome (see Table 6.16 in Chapter 6). As such, multiple factors are likely to influence nanoparticle stability. One possible factor is peptide-peptide interactions. The conformational entropy predicted from MD simulations does not account for inter-peptide interactions. The positive and negative charges of AuBP1 promote strong electrostatic inter-peptide interactions, which are not featured in A3 or Pd4. Sequences with positive and negative charges could be more complex to predict in terms of nanoparticle stability. Another possible factor that may influence nanoparticle stability is peptide-binding properties. The AuBP1 sequence is conformationally-susceptible to sequence manipulation, while the A3 and Pd4

**Figure 5.5.** TEM and UV-vis analyses of the Au nanoparticles capped with the indicated AuBP1 mutant peptides. All scale bars are 50 nm.
sequences are conformationally-recalcitrant to sequence manipulation. However, additional studies are required to test these hypotheses, such as by exploring point mutations of other Au-binding peptides with multiple charges.

5.5 Summary and Conclusions

In summary, this combination of experimental and computational analyses demonstrated the effects of peptide sequence manipulation on the binding of the AuBP1 and A3 peptides to Au. Clear differences in binding affinity resulted from the selected mutations and modifications to the sequences. Computational modeling showed how the structures of the parent peptides on the aqueous Au surface changed upon altering the sequences, which correlated well to the QCM binding studies. Through this work, two classes of peptides were identified: conformationally-recalcitrant peptides, where the ensemble of adsorbed structures does not change appreciably for point mutations, and conformationally-susceptible peptides, which can result in very different structures upon point mutation. Peptide-mediated Au nanoparticle stability studies showed that conformational entropy can explain the nanoparticle stability outcomes for the A3 peptides, but not for the AuBP1 peptides. This indicates that multiple factors are likely to influence nanoparticle stability. Such results are important as they enable the future rational design of peptide sequences with specific materials-binding and nanoparticle stabilization capabilities.
Chapter 6. Additional Information

This chapter includes additional data for each of the presented chapters in this dissertation. The reference in the figure/table caption indicates the page of citation from the main text.

6.1 Additional Information for Chapter 2

Table 6.1. Shape Control Analysis for the Cu₂O Octahedra (ref. pg. 40 & 42)

<table>
<thead>
<tr>
<th>Cu²⁺:Glucose Mole Ratio</th>
<th>Octahedral</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8:0.8</td>
<td>79%</td>
<td>21%</td>
</tr>
<tr>
<td>1.5:0.8</td>
<td>92%</td>
<td>8%</td>
</tr>
<tr>
<td>2.2:0.8</td>
<td>78%</td>
<td>22%</td>
</tr>
<tr>
<td>2.9:0.8</td>
<td>76%</td>
<td>24%</td>
</tr>
<tr>
<td>1.5:1.5</td>
<td>86%</td>
<td>14%</td>
</tr>
<tr>
<td>2.2:2.2</td>
<td>75%</td>
<td>25%</td>
</tr>
<tr>
<td>2.9:2.9</td>
<td>67%</td>
<td>33%</td>
</tr>
<tr>
<td>1.5:0.8</td>
<td>24%</td>
<td>76%</td>
</tr>
<tr>
<td>No CTAB</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 6.1. Size analysis of the Cu₂O octahedra synthesized in the presence of CTAB at Cu²⁺:glucose mole ratios of (a) 0.8:0.8, (b) 1.5:0.8, (c) 2.2:0.8, (d) 2.9:0.8, (e) 1.5:1.5, (f) 2.2:2.2, and (g) 2.9:2.9. Panel (h) presents the materials prepared at a Cu²⁺:glucose mole ratio of 1.5:0.8, but in the absence of CTAB. (ref. pg. 42)
Figure 6.2. Analysis of the fate of the CTAB during Cu$_2$O synthesis. Panel (a) presents the TGA analysis of the materials fabricated at a Cu$^{2+}$:glucose ratio of 1.5:0.8 in the presence (green plot) and absence (blue plot) of CTAB. The red plot displays the analysis of just CTAB where complete decomposition of the surfactant is evident at 225 °C. No mass loss for the decomposition of CTAB was observed from the oxide materials, indicating that no surfactant was associated with the structures. Note that the increase in mass at temperatures >400 °C is associated with the oxidation of Cu$_2$O to CuO, with the gain in mass arising from incorporation of additional oxygen into the materials. Panel (b) presents the IR analysis where no vibrations associated with the surfactant are observed in the materials fabricated in the presence of CTAB. In fact, the spectrum of the Cu$_2$O structures prepared in the presence of CTAB was nearly identical to that of the materials generated without CTAB. Together, these two studies indicate that no surfactant was associated with the oxide structures after the materials were washed. (ref. pg. 44)
Table 6.2. Calculated Geometric and BET Surface Areas for the Cu$_2$O Octahedra (ref. pg. 54)

<table>
<thead>
<tr>
<th>Cu$^{2+}$:Glucose Mole Ratio</th>
<th>Edge Length ($\mu$m)</th>
<th>$S_{\text{BET}}$ (m$^2$ g$^{-1}$)</th>
<th>$S_{\text{BET}}$ (10$^{-11}$ m$^2$ particle$^{-1}$)</th>
<th>$S_{\text{geometric}}$ (10$^{-11}$ m$^2$ particle$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8:0.8</td>
<td>2.7 ± 0.8</td>
<td>0.69</td>
<td>3.84</td>
<td>2.53</td>
</tr>
<tr>
<td>1.5:0.8</td>
<td>4.4 ± 1.1</td>
<td>0.82</td>
<td>19.8</td>
<td>6.71</td>
</tr>
<tr>
<td>2.2:0.8</td>
<td>1.7 ± 0.6</td>
<td>3.05</td>
<td>4.25</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>4.4 ± 1.1</td>
<td></td>
<td>73.6</td>
<td>6.71</td>
</tr>
<tr>
<td>2.9:0.8</td>
<td>4.5 ± 0.8</td>
<td>12.93</td>
<td>334</td>
<td>7.01</td>
</tr>
<tr>
<td>1.5:1.5</td>
<td>2.4 ± 0.5</td>
<td>0.43</td>
<td>1.68</td>
<td>2.00</td>
</tr>
<tr>
<td>2.2:2.2</td>
<td>3.6 ± 0.7</td>
<td>0.56</td>
<td>7.39</td>
<td>4.49</td>
</tr>
<tr>
<td>2.9:2.9</td>
<td>4.3 ± 1.0</td>
<td>0.24</td>
<td>5.40</td>
<td>6.41</td>
</tr>
</tbody>
</table>
Figure 6.3: Adsorption of the methyl orange dye onto the 0.8:0.8 Cu$_2$O octahedra under mass-constant (red plot) and surface-area-constant (green plot) conditions in darkness. As is evident, negligible dye adsorption is observed under the surface-area-constant conditions; however, a significant dye concentration decrease is noted for the mass-constant system. More dye is lost than would be anticipated by simple surface adsorption of the dye onto the oxide, suggesting that a slow, secondary degradation process is occurring. The rate of this process is sufficiently slow enough that it does not affect the photocatalytic degradation of the dye. (ref. pg. 55)
Figure 6.4. XRD patterns of the 0.8:0.8 and 1.5:0.8 octahedral Cu$_2$O materials after the photocatalytic degradation of methyl orange under both mass and surface area normalized reaction conditions. The gray lines denote diffraction peaks attributed to Cu$_2$O, while the pink lines denote peaks indexed to CuO. Note that no diffraction peaks associated with CuO were evident in the samples. (ref. pg. 60)
Figure 6.5. SEM images of the 0.8:0.8 materials after a 6 h dye adsorption equilibration period under (a) mass and (b) surface area normalized conditions. (ref. pg. 60)

Figure 6.6. SEM images of the 0.8:0.8 materials after being photoirradiated in DI water for 3 h in the absence of dye under (a and c) mass and (b and d) surface area normalized conditions. (ref. pg. 60)
Figure 6.7. Additional SEM images of the 0.8:0.8 materials presented for the photostability analysis. Panels (a and e) present the materials before any reaction analysis. Panels (b and f) and (i and l) present the materials after the photodegradation of methyl orange under (b and f) mass and (i and l) surface area normalized conditions. Panels (c and g) and (j and m) present the materials after a 6 h dye adsorption equilibration period under (c and g) mass and (j and m) surface area normalized conditions. Panels (d and h) and (k and n) present the materials after being photoirradiated in water for 3 h in the absence of dye under (d and h) mass and (k and n) surface area normalized conditions. (ref. pg. 59)
Figure 6.8. Additional SEM images of the 1.5:0.8 materials presented for the photostability analysis. Panels (a and d) present the materials before any reaction analysis. Panels (b and e) and (c and f) present the materials after the photodegradation of methyl orange under (b and e) mass and (c and f) surface area normalized conditions. (ref. pg. 59)
6.2 Additional Information for Chapter 3

Figure 6.9. Size analysis of the Cu$_2$O/Pd materials with a Pd mass of (a) 1%, (b) 3%, (c) 6%, (d) 9%, and (e) 15%. Panel (f) presents the size analysis of the bare Cu$_2$O cubes. (ref. pg. 72)
Table 6.3. ICP-OES Analysis for the Cu₂O/Pd Materials (ref. pg. 74)

<table>
<thead>
<tr>
<th>Cu₂O/Pd</th>
<th>Pd wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>0.92</td>
</tr>
<tr>
<td>3%</td>
<td>2.35</td>
</tr>
<tr>
<td>6%</td>
<td>5.65</td>
</tr>
<tr>
<td>9%</td>
<td>7.62</td>
</tr>
<tr>
<td>15%</td>
<td>14.99</td>
</tr>
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</table>

Table 6.4. Pseudo-First-Order Rate Constants for the PCB Photodechlorination Reactions for Each of the Cu₂O/Pd Materials Indicated at a Catalyst Loading of 2 mg/mL (ref. pg. 78)

<table>
<thead>
<tr>
<th>Cu₂O/Pd</th>
<th>$k_{PCB_1}$ (10⁻³ h⁻¹)</th>
<th>$k_{PCB_2}$ (10⁻³ h⁻¹)</th>
<th>$k_{PCB_3}$ (10⁻³ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>2.4 ± 0.7</td>
<td>2.3 ± 0.5</td>
<td>7.7 ± 0.7</td>
</tr>
<tr>
<td>3%</td>
<td>3.4 ± 0.5</td>
<td>2.7 ± 0.1</td>
<td>6.3 ± 0.3</td>
</tr>
<tr>
<td>6%</td>
<td>2.5 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>7.7 ± 0.3</td>
</tr>
<tr>
<td>9%</td>
<td>1.9 ± 0.1</td>
<td>11.0 ± 0.7</td>
<td>50.8 ± 2.9</td>
</tr>
<tr>
<td>15%</td>
<td>2.4 ± 0.2</td>
<td>6.3 ± 0.3</td>
<td>36.5 ± 1.3</td>
</tr>
</tbody>
</table>
Figure 6.10. The overall reaction analysis for (a) the dechlorination of PCB 3 following H₂ being bubbled into the reaction for 5 h in the presence of Cu₂O/Pd 9% materials at a catalyst loading of 2 mg/mL while in the dark and (b) the aerosolization of biphenyl following H₂ being bubbled into the reaction for 5 h in the absence of a photocatalyst while in the dark. Note that lines are added to guide the eye. (ref. pg. 80–81)

Figure 6.11. The overall reaction analysis for the photodegradation of PCBs 1, 2, and 3 following light irradiation for 250 h in the absence of a photocatalyst. Note that lines are added to guide the eye. (ref. pg. 82)
Table 6.5. Pseudo-First-Order Rate Constants for the PCB 3 Photodechlorination Reaction Using the Cu\textsubscript{2}O/Pd 9% Materials at the Indicated Catalyst Loadings (ref. pg. 83)

<table>
<thead>
<tr>
<th>Cu\textsubscript{2}O/Pd 9%</th>
<th>1 mg/mL</th>
<th>2 mg/mL</th>
<th>3 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{\text{PCB 3}}) (10\textsuperscript{-3} h\textsuperscript{-1})</td>
<td>17.6 ± 0.7</td>
<td>50.8 ± 2.9</td>
<td>9.9 ± 0.9</td>
</tr>
</tbody>
</table>

Figure 6.12. Additional SEM and TEM images of the Cu\textsubscript{2}O/Pd 9% cubes presented for the post-dechlorination analysis. Panels (a–b) present SEM images and panel (c) displays a TEM image when a catalyst loading of 2 mg/mL was used in the reaction. (ref. pg. 84)
Figure 6.13. SEM images of (a) the Cu$_2$O/Pd 9% materials and (b) the bare Cu$_2$O cubes after being photoirradiated in ethanol/water for 250 h in the absence of PCB. The right panel shows the yellow box zoomed-in. (ref. pg. 85)

Figure 6.14. The reaction analysis for the recyclability study of the Cu$_2$O/Pd 7.5% materials for the photodegradation of PCB 3 at a catalyst loading of 2 mg/mL. Note that lines are added to guide the eye. (ref. pg. 86)
6.3 Additional Information for Chapter 4

Simulation Details (ref. pg. 97 & 106)

System Setup: Each system simulated consisted of a single peptide chain, a Au(111) slab, 6605 water molecules and a number of Cl$^-$ counter-ions such as to ensure the overall charge neutrality of the system. The Au(111) slab was based on a $p(20 \times 24)$ supercell and was five atomic layers thick. The overall cell dimensions were $58.6 \times 60.9 \times 67.6$ Å$^3$, resulting in an inter-slab distance of greater than 55 Å along the cell direction perpendicular to the slab surface. The density of water in the center of the system was equivalent to the density of liquid water at 300 K and 1 atm.

All peptide chains were modeled in the zwitterionic form, i.e. the N- and C-termini of the peptide were uncapped (i.e. modeled as NH$_3^+$ and COO$^-$ respectively), consistent with the peptides used experimentally. At neutral pH the arginine residue will be protonated, however, the protonation state of the histidine residues is more complex, with both the protonated and non-protonated forms likely present in solution. Experimentally the pH of the solution during the QCM experiments was measured as pH 3.9-4.2. In addition, it is possible that the probability distribution of protonation states of the His residues in the sequence when the peptide is free in solution may differ from those when the peptide is in the surface-adsorbed state. To ensure the effect of protonation state was accounted for in the simulations, all possible protonation states of the histidine residues were considered, a total of seventeen systems. The naming convention of the different protonation states is as follows; PS0 – no His protonated; PS1 – one His protonated; PS2 – 2 His protonated. For those peptides containing two His
residues (Pd4, Pd4-4 and Pd4-5) there are two PS1 states; PS1A – 1\textsuperscript{st} His protonated, 2\textsuperscript{nd} His unprotonated and PS1B – 1\textsuperscript{st} His unprotonated, 2\textsuperscript{nd} His protonated. In all simulations the side-chain of Arg was positively-charged.

**REST Simulation Details:** All simulations were performed using Gromacs version 5.0\textsuperscript{170} The GolP-CHARMM FF\textsuperscript{147-148} was used to model the interactions with the Au(111) surface, the peptide was modeled using the CHARMM22* FF\textsuperscript{149-150} and the modified version of TIP3P water\textsuperscript{151-152} (compatible with the CHARMM FFs) was employed. All simulations were performed in the canonical (\textit{NVT}) ensemble, at 300 K with temperature regulated by a Nose-Hoover\textsuperscript{171-172} thermostat. Note that all replicas were run at the same thermal temperature of 300 K. The LJ non-bonded interactions were tapered to zero between 10 and 11 Å, while the electrostatic interactions were treated using a particle mesh Ewald (PME)\textsuperscript{173} summation with a real-space cutoff of 11 Å. A timestep of 1 fs was used, each system simulated for $20\times10^6$ MD steps and exchanges between adjacent replicas attempted every 1000 steps ($\equiv$ 1ps). Coordinates were saved every 1000 steps.

The REST approach is a version of Hamiltonian replica exchange molecular dynamics (H_REMD) that provides advanced sampling of the conformational space of a system.\textsuperscript{34,145-146} For fuller details of the technique, its implementation in GROMACS and investigation of the improvements sampling efficiency we refer readers to Terakawa \textit{et al.}, Wright \textit{et al.} and Tang \textit{et al.}\textsuperscript{34,145-146}

In the present study the ‘effective temperature’ window spanned 300-430 K across sixteen replicas. The $\lambda$ (scaling) values used for the replicas were 0.000, 0.057, 0.114, 0.177, 0.240, 0.310, 0.382, 0.458, 0.528, 0.597, 0.692, 0.750, 0.803, 0.855, 0.930,
and 1.000. The initial configuration of the peptide in each replica differed, covering a range of different secondary structure motifs, e.g. α-helix, β-turn, PPII helix and random coil.

**Simulation Analysis:** The degree of residue-surface contact was determined by calculating the fraction of the total REST-MD reference trajectory (i.e. the trajectory that corresponds to the unscaled Hamiltonian, \( \lambda=0.000 \)) that a reference site on a residue was less than or equal to a cutoff distance of the Au(111) surface. The reference sites and cutoff-distance are the same as used in previous work. An anchor residue is defined as a residue that was in direct contact with the Au(111) surface for 60% or more of the trajectory.

To determine the Boltzmann-weighted ensemble of peptide conformations the Daura clustering algorithm, with a 2 Å cutoff for the atoms in the peptide backbone was employed. The clustering analysis was performed over the full 20,000 frames of the reference trajectory. The percentage population of each cluster was determined from the fraction of the total 20,000 frames that were assigned to that cluster.
Figure 6.15. QCM analysis of the peptides to obtain $k_a$ and $k_d$ values: (a) Pd4, (b) Pd4-1, (c) Pd4-2, (d) Pd4-3, and (e) Pd4-4. The left panel shows the inverted frequency change vs. time, as well as the dissipation energy plot for the highest concentration studied. The right panel shows the plot of $k_{obs}$ values vs. peptide concentration, obtained from the data in the left panel by fitting with Langmuir kinetics.
Table 6.6. Adsorption and Desorption Analysis for Au Surface Binding Including $k_a$, $k_d$, $K_{eq}$, $\Delta G$, and $\theta$ Values for All Peptides (ref. pg. 101–102)

<table>
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<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>$p_I$</th>
<th>$k_a$ (M$^{-1}$ s$^{-1}$)</th>
<th>$k_d$ (10$^{-3}$ s$^{-1}$)</th>
<th>$K_{eq}$ (10$^4$ M$^{-1}$)</th>
<th>$\Delta G$ (kJ mol$^{-1}$)</th>
<th>$\theta$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd4</td>
<td>TSNAVHPTLRHL</td>
<td>9.47</td>
<td>3491 ± 22</td>
<td>9.86 ± 0.31</td>
<td>35.5 ± 1.2</td>
<td>-31.4 ± 0.1</td>
<td>79.8 ± 0.5</td>
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<td>TSNAVA PTLRHL</td>
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<td>741 ± 36</td>
<td>11.80 ± 0.19</td>
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<td>-27.2 ± 0.2</td>
<td>41.2 ± 1.6</td>
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<tr>
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<td>TSNAVHPTLRAL</td>
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<td>1609 ± 28</td>
<td>5.97 ± 0.18</td>
<td>27.0 ± 1.3</td>
<td>-30.7 ± 0.1</td>
<td>75.0 ± 0.9</td>
</tr>
<tr>
<td>Pd4-3</td>
<td>TSNAVA PTLRAL</td>
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<td>-32.0 ± 0.8</td>
<td>82.8 ± 5.0</td>
</tr>
<tr>
<td>Pd4-4</td>
<td>SPHTTNHRALLV</td>
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<td>4214 ± 56</td>
<td>5.93 ± 0.42</td>
<td>71.4 ± 5.5</td>
<td>-33.1 ± 0.2</td>
<td>88.8 ± 0.8</td>
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<tr>
<td>Pd4-5</td>
<td>HHTSNAVPTLRL</td>
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<td>4204 ± 36</td>
<td>2.29 ± 0.19</td>
<td>184.4 ± 16.7</td>
<td>-35.5 ± 0.2</td>
<td>95.3 ± 0.4</td>
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</table>

Figure 6.16. Representative snapshots of Pd4 adsorbed on the Au(111) interface. The anchor residues (H6, R10 and H11) are colored green and the peptide backbone is colored purple. Water molecules are not shown for clarity. (ref. pg. 104)
Figure 6.17. Representative snapshots of Pd4-2 adsorbed on the Au(111) interface. The anchor residues (H6 and R10) are colored green and the peptide backbone is colored purple. Water molecules are not shown for clarity. (ref. pg. 104)
Figure 6.18. Representative snapshots of Pd4-3 adsorbed on the Au(111) interface. The anchor residue (R10) is colored green and the peptide backbone is colored purple. Water molecules are not shown for clarity. (ref. pg. 104)
Figure 6.19. Representative snapshots of Pd4-4 adsorbed on the Au(111) interface. The anchor residues (H3, H7 and R8) are colored green and the peptide backbone is colored purple. Water molecules are not shown for clarity. (ref. pg. 104)
Table 6.7. Conformational Entropic Contribution Scores, $S_{\text{conf}}$, for the Different Peptides Systems Simulated (ref. pg. 106 & 117)

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Table 6.8. Percentage population of the top ten most populated clusters of the Pd4 family of peptides adsorbed at the aqueous Au(111) interface. Data for all the different protonation states of the peptides are shown; PS0 (no His protonated), PS1 (one His protonated) and PS2 (two His protonated). In the case where there are two His present in the sequence, PS1A denotes that only the first His in the sequence is protonated, while PS1B denotes that only the second His in the sequence is protonated. The total number of clusters for each case is given in parentheses in the table header. (ref. pg. 106)

<table>
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<tr>
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Table 6.9. Comparison of cluster centroids of the top ten most populated clusters, comparing the Pd4 peptide backbone structure to each of the mutants. A matched peptide configuration has a RMSD less than 0.2 nm. Pairs where both structures come from one of the top five most populated clusters are highlighted in yellow. (ref. pg. 106)

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Figure 6.20. Degree of residue-surface contact for each of the 6 peptides, data is averaged over all three protonation states simulated for Pd4, Pd4-4 and Pd4-5. (ref. pg. 107 & 109)
Table 6.10. Residue-surface contact data (percentages) determined from the REST MD simulations. Data for all the different protonation states of the peptides are shown; PS0 (no His are protonated), PS1 (only one His is protonated; for those sequences with two histidines, in PS1A the first histidine in the sequence is protonated, in PS1B the second histidine in the sequence is protonated) and PS2 (both His are protonated). Data for protonated His residues are underlined. Anchor residues (defined as greater than 60% degree of contact) are highlighted in yellow. (ref. pg. 107–109)

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</table>
Table 6.11. Surface contact data (percentages) showing the breakdown of conditional probabilities of contact states for the two histidine residues in peptides Pd4, Pd4-4 and Pd4-5, determined from the REST MD simulations. Data for all the different protonation states of the peptides are shown; PS0 (no His protonated), PS1 (one His protonated) and PS2 (two His protonated). His A and His B are the first and second histidines as they appeared in the sequence, respectively. (ref. pg. 110)

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<th>His B only</th>
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<th>Both His and Arg</th>
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Figure 6.21. Size analysis of the Au nanoparticles prepared at a Au:peptide ratio of 2 capped with the indicated peptide. (ref. pg. 114)
Figure 6.22. Size analysis of the Au nanoparticles prepared at a Au:peptide ratio of 1 capped with the indicated peptide. (ref. pg. 114)
6.4 Additional Information for Chapter 5

Simulation Details (ref. pg. 127)

System Setup: Each system simulated consisted of a single peptide chain, a Au(111) slab, 6605 water molecules, and a number of Cl\(^-\) counter-ions such as to ensure the overall charge neutrality of the system. The Au(111) slab was based on a \(p(20\times24)\) supercell and was five atomic layers thick. The overall cell dimensions were 58.6\(\times\)60.9\(\times\)67.6 Å\(^3\), resulting in an inter-slab distance of greater than 55 Å along the cell direction perpendicular to the slab surface. The density of water in the center of the system was equivalent to the density of liquid water at 300 K and 1 atm.

All peptide chains were modeled in the zwitterionic form, i.e. the N- and C-termini of the peptide were uncapped (i.e. modeled as \(\text{NH}_3^+\) and \(\text{COO}^-\) respectively), consistent with the peptides used experimentally. At neutral pH, the arginine residue will be protonated. In all simulations, the side-chain of Arg was positively-charged. Experimentally the pH of the solution during the QCM experiments was measured as pH 4.1-6.0.

REST Simulation Details: All simulations were performed using Gromacs version 5.0.\(^{170}\) The GolP-CHARMM FF\(^{147-148}\) was used to model the interactions with the Au(111) surface, the peptide was modeled using the CHARMM22* FF\(^{149-150}\) and the modified version of TIP3P water\(^{151-152}\) (compatible with the CHARMM FFs) was employed. All simulations were performed in the canonical (\(NVT\)) ensemble, at 300 K with temperature regulated by a Nose-Hoover\(^{171-172}\) thermostat. Note that all replicas were run at the same thermal temperature of 300 K. The LJ non-bonded interactions were tapered to zero between 10 and 11 Å, while the electrostatic interactions were treated...
using a particle mesh Ewald (PME)\textsuperscript{173} summation with a real-space cutoff of 11 Å. A timestep of 1 fs was used, each system simulated for $20 \times 10^6$ MD steps and exchanges between adjacent replicas attempted every 1000 steps (\(\equiv 1\)ps). Coordinates were saved every 1000 steps.

The REST approach is a version of Hamiltonian replica exchange molecular dynamics (H_REMD) that provides advanced sampling of the conformational space of a system.\textsuperscript{34,145-146} For fuller details of the technique, its implementation in GROMACS and investigation of the improvements sampling efficiency we refer readers to Terakawa et al., Wright et al. and Tang et al.\textsuperscript{34,145-146}

In the present study the ‘effective temperature’ window spanned 300-430 K across sixteen replicas. The \(\lambda\) (scaling) values used for the replicas were 0.000, 0.057, 0.114, 0.177, 0.240, 0.310, 0.382, 0.458, 0.528, 0.597, 0.692, 0.750, 0.803, 0.855, 0.930, and 1.000. The initial configuration of the peptide in each replica differed, covering a range of different secondary structure motifs, e.g. \(\alpha\)-helix, \(\beta\)-turn, PPII helix and random coil.

**Simulation Analysis:** The degree of residue-surface contact was determined by calculating the fraction of the total REST-MD reference trajectory (i.e. the trajectory that corresponds to the unscaled Hamiltonian, \(\lambda=0.000\)) that a reference site on a residue was less than or equal to a cutoff distance of the Au(111) surface. The reference sites and cutoff-distance are the same as used in previous work.\textsuperscript{34} An anchor residue is defined as a residue that was in direct contact with the Au(111) surface for 60% or more of the trajectory.
To determine the Boltzmann-weighted ensemble of peptide conformations the Daura clustering algorithm,\textsuperscript{159} with a 2 Å cutoff for the atoms in the peptide backbone was employed. The clustering analysis was performed over the full 20,000 frames of the reference trajectory. The percentage population of each cluster was determined from the fraction of the total 20,000 frames that were assigned to that cluster.
Figure 6.23. QCM analysis of the peptides to obtain $k_a$ and $k_d$ values: (a) A3, (b) A3-2, (c) A3-3, (d) A3-4, and (e) A3-5. The left panel shows the inverted frequency change vs. time, as well as the dissipation energy plot for the highest concentration studied. The right panel shows the plot of $k_{obs}$ values vs. peptide concentration, obtained from the data in the left panel by fitting with Langmuir kinetics. (ref. pg. 128)
Figure 6.24. QCM analysis of the peptides to obtain $k_a$ and $k_d$ values: (a) AuBP1, (b) AuBP1-1, (c) AuBP1-2, (d), AuBP1-3, (e) AuBP1-4, and (f) AuBP1-5. The left panel shows the inverted frequency change vs. time, as well as the dissipation energy plot for the highest concentration studied. The right panel shows the plot of $k_{obs}$ values vs. peptide concentration, obtained from the data in the left panel by fitting with Langmuir kinetics. (ref. pg. 128)
Table 6.12. Adsorption and Desorption Analysis for Au Surface Binding Including $k_a$, $k_d$, $K_{eq}$, and $\Delta G$ Values for All Peptides (ref. pg. 128 & 137)

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<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>$pI$</th>
<th>$k_a$ ($10^3$ M$^{-1}$ s$^{-1}$)</th>
<th>$k_d$ ($10^3$ s$^{-1}$)</th>
<th>$K_{eq}$ ($10^4$ M$^{-1}$)</th>
<th>$\Delta G$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3</td>
<td>AYSSGAPPMPPF</td>
<td>5.57</td>
<td>4.23 ± 0.33</td>
<td>11.07 ± 1.61</td>
<td>38.9 ± 7.6</td>
<td>-31.6 ± 0.5</td>
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<td>A3-1</td>
<td>AASSGAPPMPPF</td>
<td>5.57</td>
<td>3.78 ± 0.07</td>
<td>1.36 ± 0.55</td>
<td>318.0 ± 135.2</td>
<td>-36.6 ± 1.1</td>
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<td>A3-2</td>
<td>AYSSGAPPAPPF</td>
<td>5.57</td>
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<td>A3-3</td>
<td>AYSSGAPPMPPA</td>
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<td>YPPAPSAPSFGM</td>
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<td>0.76 ± 0.04</td>
<td>2.99 ± 0.10</td>
<td>25.6 ± 1.0</td>
<td>-30.6 ± 0.1</td>
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<tr>
<td>A3-5</td>
<td>YMFASSGAPPPP</td>
<td>5.52</td>
<td>0.85 ± 0.05</td>
<td>8.08 ± 0.41</td>
<td>10.5 ± 1.0</td>
<td>-28.4 ± 0.2</td>
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<tr>
<td>AuBP1</td>
<td>WAGAKRLVLRRE</td>
<td>11.71</td>
<td>4.69 ± 0.10</td>
<td>0.38 ± 0.25</td>
<td>1973.9 ± 1745.3</td>
<td>-40.7 ± 2.1</td>
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<td>8.71 ± 1.21</td>
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<td>-32.2 ± 0.5</td>
</tr>
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<td>WAGAKALVLRRE</td>
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<td>228.2 ± 69.3</td>
<td>-35.9 ± 0.7</td>
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<td>WAGAKRLVLRAE</td>
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<td>57.8 ± 1.0</td>
<td>-32.6 ± 0.0</td>
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<td>3.49 ± 0.40</td>
<td>109.6 ± 13.6</td>
<td>-34.2 ± 0.3</td>
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Table 6.13. Comparison of cluster centroids of the top ten most populated clusters, comparing the A3 peptide backbone structure to each of the mutants. Pairs where both structures come from one of the top five most populated clusters are highlighted in orange. (ref. pg. 132)
Table 6.14. Comparison of cluster centroids of the top ten most populated clusters, comparing the AuBP1 peptide backbone structure to each of the mutants. Pairs where both structures come from one of the top five most populated clusters are highlighted in orange. (ref. pg. 134)

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Figure 6.25. Size analysis of the Au nanoparticles capped with the indicated A3-based peptide. (ref. pg. 137)
Figure 6.26. Size analysis of the Au nanoparticles capped with the indicated AuBP1-based peptide. (ref. pg. 137)
Table 6.15. Percentage population of the top ten most populated clusters of the A3 family of peptides adsorbed at the aqueous Au(111) interface and conformational entropic contribution scores, $S_{\text{conf}}$, for the different peptides simulated. (ref. pg. 139)

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<td>2.98</td>
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Table 6.16. Percentage population of the top ten most populated clusters of the AuBP1 family of peptides adsorbed at the aqueous Au(111) interface and conformational entropic contribution scores, $S_{\text{conf}}$, for the different peptides simulated. (ref. pg. 140)

<table>
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