January 2007

PROGRESS TOWARDS A MODEL FLAVOENZYME SYSTEM

Kevin M. Bardon
University of Massachusetts Amherst, kbardon3@yahoo.com

PROGRESS TOWARDS A MODEL FLAVOENZYME SYSTEM

A Thesis Presented

by

KEVIN MATTHEW BARDON

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

Master of Science

September 2007

Biochemistry and Molecular Biology

PROGRESS TOWARDS A MODEL FLAVOENZYME SYSTEM

A Thesis Presented

by

KEVIN MATTHEW BARDON

Approved as to style and content by:

_____
Vincent M. Rotello, Chair

_____
Maurille J. Fournier, Member

_____
Daniel J. Schnell, Department Head
Biochemistry and Molecular Biology

ABSTRACT

PROGRESS TOWARDS A MODEL FLAVOENZYME SYSTEM

SEPTEMBER 2007

KEVIN MATTHEW BARDON, B.S., UNIVERSITY OF MASSACHUSETTS AMHERST

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Vincent M. Rotello

The foundation for supramolecular chemistry is in nature; by studying these archetypes, chemists have devised methods of recreating these complex interactions in the laboratory. Of particular interest is the interplay between enzyme- more specifically, its active site- and the target substrate. Utilizing recent advancements in self-assembled monolayers, progress towards a more-accurate flavoenzyme model has been demonstrated.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

CHAPTER 1

MOLECULAR RECOGNITION: ITS HISTORY AND APPLICATIONS

1.1 Introduction

Traditionally,[1] synthetic chemists have pursued the formation of covalent bonds in order to construct desirable molecules. This methodology has afforded innumerable synthetic triumphs, most notably in the areas of pharmaceuticals and polymer science.[2] However, the efficacy of this piece-wise assembly of small molecules has plateaued.[3] At a small size and limited complexity, good control over the activity of the compound may be maintained; as the size and complexity are increased, control is lost.[4] Molecular recognition provides the platform by which these disparate goals may be concurrently sought.[5]

The seeds of molecular recognition as an individual discipline were sown nearly forty years ago. Carl Pedersen's discovery of crown ethers,[6] Jean-Marie Lehn's use of cryptands[7] and Dennis Cram's exploitation of the crown ethers and spherands in a host-guest sense[8] established molecular recognition as a useful tool available to the traditional synthetic chemist. The pioneering work of this remarkable trio, duly acknowledged by their reception of the Nobel Prize in Chemistry in 1987,[9] lent credence to the importance of this new field and paved the way for the discoveries which are still occurring today.

Molecular recognition may be defined as the control, both intra- and inter-molecularly, of noncovalent interactions. Included in these noncovalent interactions are

hydrogen bonding, solvophobic effects and π-π interactions, weak forces when quantified individually but, when concerted, strong enough to form hierarchical structures and supramolecular arrays.[10] As such, the buzzwords of "supramolecular chemistry", "self-assembly" and "host-guest chemistry" may all be shepherded under the aegis of molecular recognition to produce this highly interdisciplinary field of chemical research. Using complementarity of size, shape and chemical functionalities, along with the above-mentioned noncovalent interactions, structural complexity is enhanced beyond the primary composition of the individual molecule(s).[5] Drawing inspiration from the countless examples of molecular recognition found in Nature, the ultimate aim is to create functioning, controlled and organized nanoscale devices.[11]

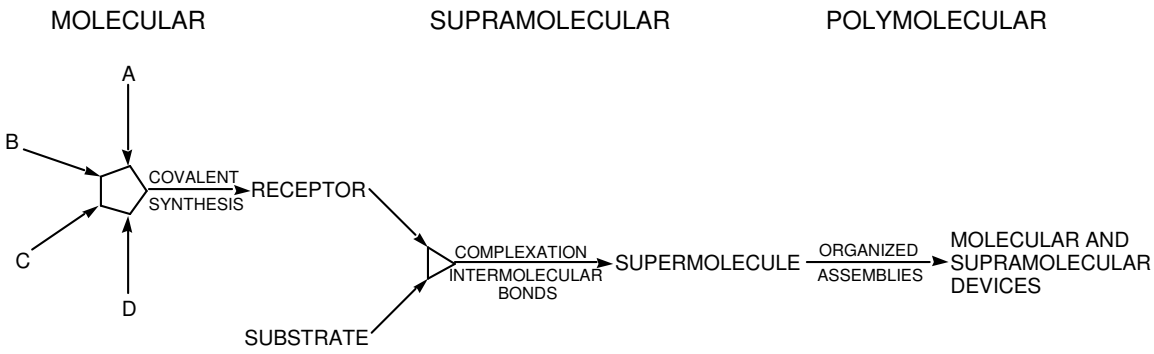

Figure 1.1: Progression from molecular to supramolecular chemistry

1.2 Major Themes and Challenges of Molecular Recognition

Several major challenges confront the chemist concerned with molecular recognition. While advances in covalent chemistry continue to be made, the vast majority have already been discovered. Thus, in pursuit of the advanced arrays

available through noncovalent chemistry, the tools of the trade- noncovalent interactions- must be mastered.

Current understanding of noncovalently controlled phenomena is lacking. The collection of examples from nature of complex aggregates and arrays formed largely through noncovalent interactions is overwhelming, yet no one can put to paper the recipe for even a small molecule to bind tightly and efficiently to the active site of a known enzyme. The "rational drug design" of the pharmaceutical industry provides only a jumping point, from which trial and error and serendipity eventually lead to a successful compound. Even when armed with the exact primary sequence of amino acids and high-resolution crystal structure, it remains impossible to foretell the folding pattern of a protein. A possible explanation for this is that the full collection and the importance of each of these noncovalent interactions are not yet fully defined. Hydrogen bonds between uncharged groups are typically favored by <5 kcal/mol in nonpolar environments, such as the interior, hydrophobic domain of a protein.[12] In theory, these weak interactions could easily be undone by the dynamic nature of the covalent bonds of the protein. Yet the protein maintains its structure and the scientist is left to determine how much a given hydrogen bond and these disconcerting forces contribute to the stability of the native structure.[11]

Understanding noncovalent interactions enough to install them in the design and synthesis of novel structures equal in structural and functional complexity as their biological prototypes demands a completely different intellectual and technical approach. Covalent bonds, largely reliant on the individual enthalpy of formation, are kinetically stable whence formed. Factors such as bond energy, strain and

stereoelectronic interactions influence formation but are mostly too weak to disrupt covalent bonds that are already formed. Molecular recognition, however, relies upon noncovalent interactions in which the products are equilibrating structures. These products reflect the balance reached between enthalpy and entropy.[13] Therefore, in envisioning these syntheses, potential products must be measured in terms of thermodynamic minima in equilibrating mixtures. Thus, one must first predict these possible outcomes and then skew the equilibrium in the direction of the desired product.

In order to address these challenges, three basic goals have been set.[5] The first is to define strategies for the assembly of supramolecular aggregates. The basic strategies for the use of molecular recognition are preorganization[9,15] and peripheral crowding.[16] Preorganization describes the use of covalent bonds that set a compound in a certain way to minimize the unfavorable entropy of forming an aggregate. As was previously mentioned, singular noncovalent interactions account for very little enthalpy. Even many concerted noncovalent interactions may not be able to overcome the number of unfavorable entropic terms incurred by losses in freedom in translation and rotation of components on formation of the aggregate. Preorganizing components of the aggregate to a common hub couples the entropic cost of formation, resulting in a lessened entropic loss and a mitigated formation of the aggregate. Peripheral crowding describes the use of bulky groups that coerce formation of the desired aggregate by making the formation of side products energetically unfavorable. This manipulation of conformational enthalpy works particularly well when a strong 1:1 complementary binding may occur.

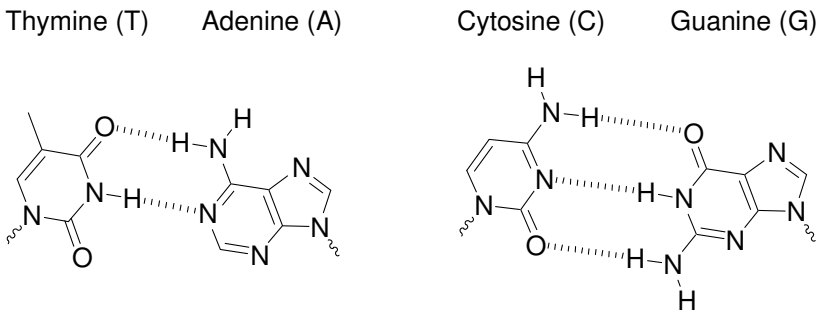

Figure 1.2: Demonstration of preorganization in DNA base pairs and 1:1 complementary binding

The second goal for the study of molecular recognition is to develop techniques for the complete characterization and study of these entities. While seemingly trivial, the characterization of these supramolecular aggregates has presented a significant challenge. Therein lies the cause for the lack of knowledge and understanding of the effects and contributions of the varied noncovalent interactions. Traditional methods of characterization, such as mass spectroscopy and X-ray crystallography, are not applicable to these studies.[17] Instead, characterization of these aggregates may be achieved by combining data from any of several methods. Individually, these methods are insufficient; collectively, a clear picture of the recognition event is presented.

Nuclear magnetic resonance (NMR) spectroscopy titrations can be valuable in two ways. The first is used to assess the stoichiometry of complexation. Supramolecular aggregates are often much more soluble in chloroform than one or both of the starting materials. At varying concentrations of the starting materials, hydrogen bond donors and acceptors interact. Complete dissolution is achieved as the more hydrophilic groups are bound and the aggregate is able to dissolve into the hydrophobic solvent, providing a qualitative observation of the correct stoichiometry. Titration

experiments monitored by NMR corroborate this finding. The spectrum of one of the insoluble starting materials alone results in a broadened, nondescript spectrum in chloroform. As the second starting material is added, the spectrum becomes increasingly well defined until the correct stoichiometry is achieved; further addition of the second reactant does not affect the intensity or sharpness of the spectrum since this added amount does not dissolve. The resultant titration curve delineates the point at which the proper ratio of the starting materials is reached.[11]

A second NMR experiment may be employed to determine the steady-state association constant for the aggregate. This experiment is most useful as a means to interpret the extent of hydrogen bonding. Using a steady host concentration, aliquots of guest are added and the migration of the hydrogen-bonded proton(s) is observed. The plot of the chemical shifts of H(3) as a function of receptor concentration yield a titration curve. Association constants may then be determined through nonlinear least-squares fitting, assuming a proper fit to the expected 1:1 binding isotherm.

Gel permeation chromatography is useful for two reasons. First, it can establish that the aggregate exists as a single entity by the elution of only a single product peak from the column. Second, it allows for a qualitative estimate of the stability of the species. Based on retention time, the stoichiometry found by NMR titration may be affirmed when the single peak emerges at a predicted time. The shape of the peak is also of interest. If dissociation of the aggregates occurs, then the peak will display tails. The extent of tailing correlates to the order of stability of the aggregate.[18]

Progress in the field of molecular recognition continues. As the many disciplines that comprise the field make headway in the breadth and depth of

understanding of these phenomena- their origin in nature, application to synthetic goals and evolution to molecular engineering- these challenges will be more successfully met.

1.3 Examples of Molecular Recognition in Nature

A prototypical example of molecular recognition in a biological system is the self-assembly of the deoxyribonucleic acid (DNA) double helix. DNA polymerase enzymes covalently link nucleotides along a phosphate backbone. A simplistic view of the recognition event would describe these oligonucleotide strands as contacting each other and further propagating the growing helix by the matching of complementary base pairs. Dynamic assembly and disassembly allow for correction of mismatched base pairs, leading to the appropriate alignment between the two strands and, hence, the most stable structure, at thermodynamic equilibrium. The reality is more complicated, with the formation of stemloops and hairpins, by hydrodynamic effects and assistance from proteins and by interstrand penetration.[19] Helix formation therefore proceeds via a two step process. In the first, nucleation, step, three to four base pairs form the helix nucleus.[20] This alignment of so few base pairs is energetically disfavored.[21] The second step-propagation of the helix-introduces additional base pairs to the helix. This addition leads to a negative overall free energy of formation for the double helix, and formation continues until the helix is complete.

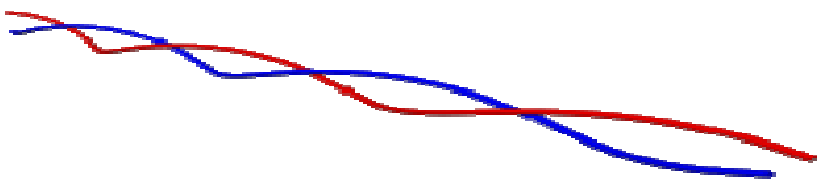

Figure 1.3: Illustration of a DNA double helix

The DNA helix assembly illustrates several key concepts in molecular recognition. First, the many favorable noncovalent interactions that occur during the molecular recognition event can easily overcome the initial unfavorable equilibrium. The entropic price for associating the two oligonucleotide strands in solution is settled by the numerous hydrogen bonds that are formed and the strong overall hydrophobic effect.[22] DNA helix formation also reinforces the importance of preorganization. In doing thus, the assembly process quickly changes from thermodynamically unfavorable to favorable and the complete double helix is formed. Of course, *in vivo* DNA helix formation is much more complex and involves a multitude of additional factors.

1.4 Synthetic Examples of Molecular Recognition

The use of cyclodextrins displays a clear example of translation of biological principles to synthetic achievement in the field of molecular recognition. Cyclodextrins (CDs) are a series of cyclic oligosaccharides consisting of six or more α-1,4-linked D-glucopyranose rings.[23] The cyclodextrins are labeled according to the number of glucopyranose rings present: α-CD has six, β-CD has seven, and γ-CD has eight. The defining structural feature of these smaller CDs is the rigid cavity that results from the overall morphology of the molecules. Hydroxyl groups are exposed on the top and bottom faces of the bucket, while a reasonably hydrophobic interior of the bucket is maintained. This hydrophobic cavity has a strong affinity for molecular guests of all types. In all cases at least some part of the guest is noncovalently bound within the cavity of the cyclodextrin molecule, the result of several noncovalent phenomena, namely van der Waals interactions, hydrophobic binding and solvophobic effect.[24]

Although the individual contribution of these forces is dependent of the particular cyclodextrin, guest and solvent, the overall effect is the same.

First observed and isolated in the early 1970s,[25] early applications for cyclodextrins were in peptidomimetic systems.[26] More recently, however, the focus of cyclodextrin research has turned towards their use as ready-made components for the construction of nanoscale entities through self-assembly. Many examples of synthetic advances have been derived from the ease with which cyclodextrins form strong complexes in aqueous solution with many different types of guests.[22]

1.5 References

(1) Corey, E. J.; Cheng, X.-M. *The Logic of Chemical Synthesis*; Wiley: New York, **1989**. (b) Nicolaou, K. C.; Sorensen, E. J. *Classics in Total Synthesis*; VCH: Weinheim, Germany, **1995**.

(2) Nicolaou, K. C. "The Total Synthesis of Brevetoxin B: A Twelve-year Odyssey in Organic Synthesis" *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 589-607.

(3) Whitesides, G. M. "What Will Chemistry Do in the Next Twenty Years?" *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1209-1218. (b) Seebach, D. "Organic Synthesis-Where Now?" *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1320-1367.

(4) *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Pergamon: Oxford, U.K., **1996**.

(5) Lehn, J.-M. *Supramolecular Chemistry*; VCH: Weinheim, Germany, **1995**.

(6) Pedersen, C. J. "Cyclic Polyethers and Their Complexes with Metal Salts" *J. Am. Chem. Soc.* **1967**, *89*, 7017-7036.

(7) Lehn, J.-M. *Struct. Bonding*; Berlin, Germany, **1973**.

(8) Cram, D. J.; Cram, J. M. *Science* **1974**, *183*, 803-809.

(9) For Nobel lectures see: (a) Pedersen, C. J. "The Discovery of Crown Ethers" *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1021-1027. (b) Lehn, J.-M. "Supramolecular Chemistry-Scope and Perspectives: Molecules, Supermolecules

and Molecular Devices" *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 89-112. (c) Cram, D. J. "The Design of Molecular Hosts, Guests, and Their Complexes" *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1009-1020.

(10) Whitesides, G. M. "Self-Assembling Materials" *Sci. Am.* **1995**, *273* (3), 146-149.

(11) Whitesides, G. M.; Simanek, E. E.; Mathias, J. P.; Seto, C. T.; Chin, D. N.; Mammen, M.; Gordon, D. M. "Noncovalent Synthesis: Using Physical-Organic Chemistry to Make Aggregates" *Acc. Chem. Res.* **1995**, *28*, 37-44.

(12) Janin, J.; Miller, S.; Chothia, C. "Surface, Subunit Interfaces and Interior of Oligomeric Proteins" *J. Mol. Biol.* **1988**, *204*, 155-164.

(13) Conn, M. M.; Rebek, J., Jr. "Self-Assembling Capsules" *Chem. Rev.* **1997**, *97*, 1647-1668.

(14) Seto, C. T.; Whitesides, G. M. "Molecular Self-Assembly through Hydrogen-Bonding Supramolecular Aggregates Based on the Cyanuric Acid:Melamine Lattice" *J. Am. Chem. Soc.* **1993**, *115*, 905-916.

(15) Mathias, J. P.; Simanek, E. E.; Whitesides, G. M. "Self-Assembly through Hydrogen-Bonding-Peripheral Crowding-A New Strategy for the Preparation of Stable Supramolecular Aggregates Based on Parallel, Connected CA(3).M(3) Rosettes" *J. Am. Chem. Soc.* **1994**, *116*, 4326.

(16) Zerkowski, J. A.; MacDonald, J. C.; Seto, C. T.; Wierda, D. A.; Whitesides, G. M. "Design of Organic Structures in the Solid-State-Molecular Tapes Based on the Network of Hydrogen-Bonds Present in the Cyanuric Acid:Melamine Complex" *J. Am. Chem. Soc.* **1994**, *116*, 2382.

(17) Mathias, J. P.; Seto, C. T.; Simanek, E. E.; Whitesides, G. M. "Self-Assembly through Hydrogen-Bonding-Preparation and Characterization of Three New Types of Supramolecular Aggregates Based on Parallel Cyclic CA(3).M(3) Rosettes" *J. Am. Chem. Soc.* **1994**, *116*, 1725.

(18) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry, Part III*; W. H. Freeman: San Francisco, **1980**.

(19) Pörschke, D.; Eigen, M. *J. Mol. Biol.* **1971**, *62*, 361.

(20) Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, **1984**.

(21) Philp, D.; Stoddart, J. F. "Self-Assembly in Natural and Unnatural Systems" *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1154-1196.

(22) Saenger, W. "Cyclodextrin Inclusion Compounds in Research and Industry" *Angew. Chem. Int. Ed. Engl.* **1980**, *82*, 343.

(23) Müller, N. "Search for a Realistic View of Hydrophobic Effects" *Acc. Chem. Res.* **1990**, *23*, 23.

(24) Schardinger, F. Z. Unters. Nahr. Genussm. Gebrauchsgegenstaende **1903**, 6, 865.

(25) Griffiths, D. W.; Bender, M. L. *Adv. Catal.* **1973**, *23*, 209.

(26) Stoddart, J. F. "Cyclodextrins, Off-the-Shelf Components for the Construction of Mechanically Interlocked Molecular-Systems" *Angew. Chem. Int. Ed. Engl.* **1992**, *31***,** 846.

CHAPTER 2

FLAVOENZYME MODELS

2.1 Protein Background

Proteins are ubiquitous biological macromolecules and wonderful motifs for the study of supramolecular organization. The structures of these many proteins are, by convention, hierarchically organized in terms of structural complexity. The primary structure is the linear monomer (amino acid) sequence along the polypeptide chain. The secondary structure denotes the local interaction of the amino acids, usually taking the shape of α-helices or β-sheets. Formation of these secondary structures is in part determined by the primary sequence, as in the case of glycine or proline residues allowing for the tight turns necessary in β-sheets. The tertiary structure is the way a single polypeptide chain folds into one or more domains of secondary structure. Lastly, the quarternary structure is the association of several polypeptide chains; these associations can function independently or cooperatively.[1]

The fundamental unit of tertiary structure is the domain. However, domains are also units of function. Often times the structural domain of the polypeptide coincides with a functional domain in the same position. An example of this is the λ–repressor protein. In this protein the structural domain found at the N-terminus functions to bind DNA while the structural domain found at the C-terminus functions to hold together two polypeptide chains into a dimeric repressor molecule.[1]

A protein in its native, tertiary conformation folds such that the entropic price of the solvent/protein interaction is minimized. This is accomplished by exposing the

hydrophilic residues to the solvent while sequestering the hydrophobic residues in the interior. An additional feature of this adaptation is ability to form pockets, less polar than would normally be allowed in aqueous media. These characteristics are especially pertinent to enzymes, a class of proteins that catalyze various biological reactions. The site of catalysis for the enzyme- the active site- is most often found in the interior, less polar region. There, small molecules selectively associate with the enzyme active site based upon specific interactions of complementarity of functionality and/or structure.[2]

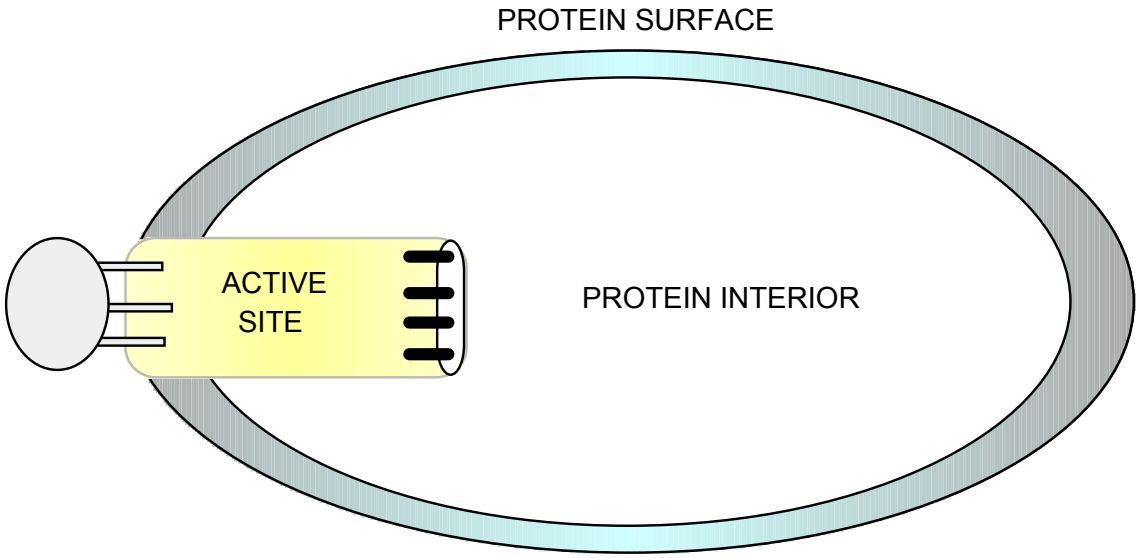

Figure 2.1: Schematic representation of an enzyme, with hydrophilic surface, hydrophobic interior and selective active site. For illustration purposes, a substrate molecule with specific recognition elements is shown entering the active site.

2.2 Flavoenzymes

Flavoenzymes comprise an incredibly diverse family of proteins.[3] Flavoenzymes are responsible for catalyzing the dehydrogenation of many different types of compounds, including dithiols, reduced nicotinamide nucleotides, alcohols and

α-hydroxy acids, amines and α-amino acids, and even saturated C-C bonds, under suitably activated conditions.[4] Owing to this vast array of redox processes and their biological necessity, flavoenzymes are omnipresent in organisms; this has allowed the flavoenzymes to be one of the most studied and understood protein families.

Although the structures and functions of these proteins vary widely, the commonality shared by the flavoenzymes is the flavin coenzyme. Through specific, non-covalent interactions, the flavin molecule binds to the active site of the flavoenzyme and functions in a wide variety of enzymatic reactions involving either two-electron or one-electron transfers. In the process of catalyzing these dehydrogenation reactions, the flavin is itself reduced, and, in order to function catalytically, the oxidized flavin must be regenerated.[5]

The cycling of flavin is a rigidly controlled process. Control of the oxidation state of the flavin molecule by the flavoenzyme is achieved through the specific interactions formed in the binding site of the enzyme. Thus, through hydrogen bonding,[6] π-stacking[7] and other electrostatic interactions,[8] flavoenzymes selectively stabilize specific oxidation and protonation states of the coenzyme.

The importance of this control is evidenced by the stabilization of the flavin radical.[4] In free solution, the flavin radical exists in very rapid equilibrium with its oxidized and fully reduced forms, resulting in the thermodynamic destabilization of the radical. In free solution the flavin radical also ionizes, so that under physiological conditions either the neutral or anion radical could be expected as significant forms. These two factors limit the appearance of the neutral radical flavin in free solution. When bound to a protein, however, the stability of the flavin radical is generally

enhanced, and either the neutral or the anion form is stabilized. Flavoenzymes can thereby be regarded as complex molecular devices that, through the use of specific noncovalent interactions, control the redox events of the flavin coenzyme and extend its range of biological reactivity.[4]

2.3 Solution-based Receptors

Solution-based receptors play an important role in both natural and synthetic systems. Archetypes of this specific binding in nature include enzyme-substrate, antigen-antibody, and complementary DNA annealing. Landmarks in the development of analogous synthetic receptors include cryptand-ion,[9] cyclophane-aromatic,[10] boronic acid-sugar,[11] and guanidinium-carboxylate[12] interactions. These synthetic hosts have been studied to determine their selectivity for different target guests over similar guest molecules. In order to engineer these receptors, the identity of the proper recognition entities to be bound to the receptor must be known. The tools of molecular recognition- noncovalent forces, such as hydrogen bonding and aromatic stacking- are used in this process. Through rational design and computer modeling a hypothetical system may be conceived, after which trial and error testing seeks to optimize the ability of the receptor to recognize the guest.[13]

2.4 Solution-based Flavin Receptors

The flavoenzyme family of proteins is an extensively studied one, with the crystal structures of no less than ten constituent proteins already solved.[4] Despite this, the complex structural and mechanistic properties of the native enzymes allow for

considerable debate about the reaction pathways and mechanisms utilized by flavoenzymes.[7,14] Solution-based flavin receptors offer insight into these questions. The comparative simplicity of these synthetic models, as compared to the native proteins, enables greater focus on individual interactions and pathways because only the active site and its direct neighbors are involved in the recognition event.[15] Thus, simple synthetic models increase the understanding of large and complex flavoenzyme systems at the molecular level.

Flavoenzymes modulate the redox behavior of the flavin cofactor through stabilization of reactive intermediates.[4] Hydrogen bonding between the enzyme active site and its cofactor plays a large part in this stabilization. To this end, synthetic receptors were designed to reproduce the specific hydrogen bond patterns found in flavoenzymes and to then isolate, observe and quantify these interactions.

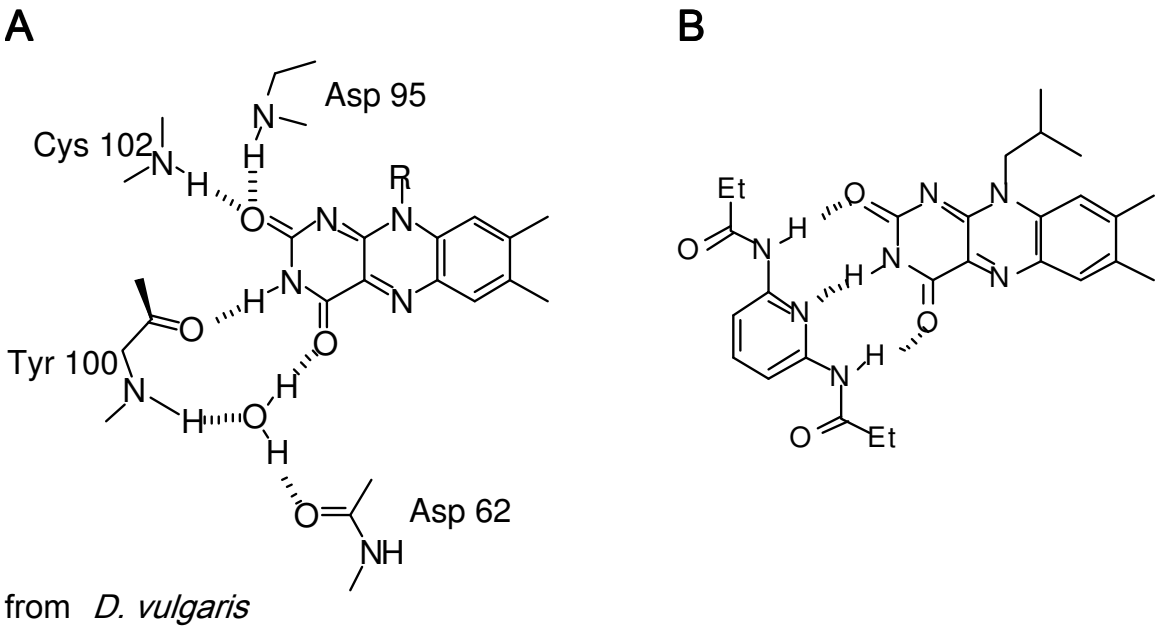

Figure 2.2: Illustration of a) native flavoenzyme active site/flavin cofactor interaction and b) synthetic flavoenzyme active site mimic (DAP)/flavin interaction.

In order to quantify the energetic effects of hydrogen bonding on the flavin-stabilized redox processes, a family of receptors based on 2,6-diaminopyridine were synthesized.[16] These receptors faithfully reproduce the enzyme-cofactor interactions, occurring at O(2), N(3)H and O(4) of the flavin cofactor, found in a number of systems for which the enzyme structure is known.[17] Here again the importance of these synthetic models is shown, as the use of mutation studies on actual enzymes are significantly hindered by the positioning of these hydrogen bonds on main chain amides.

The results from this simple, solution-based model system show that complexation of flavin with the receptor lowered the reduction potential of the flavin.[16] This behavior directly mimics that of the native flavoenzyme-cofactor complex.

2.4.1 Xanthene-scaffold Flavin Receptors

Enzyme-cofactor π-stacking interactions have been shown to aid in the modulation of flavin reactivities. An example of this is found in a class of flavoenzymes called the flavodoxins.[4] These proteins use a molecule of flavin mononucleotide (FMN) as the cofactor in a highly conserved binding site containing the aromatic amino acids tryptophan and tyrosine.[17] Binding of FMN within the active site lowers the potential necessary for semiquinone formation. Once again, the structural complexity of the native enzyme precludes the employment of mutation studies involving the true enzyme system. Hence, a synthetic model is necessary in order to observe the effects of these interactions.

Utilizing the molecular recognition precept of preorganization, a novel solution-based receptor model was synthesized.[18] The xanthene scaffold organizes both the

hydrogen-bonding event and the π-interaction, such that binding of the cofactor through hydrogen-bonding correctly orients the flavin over the aromatic surface. The modularity of the scaffold then allows for the aromatic surface to be varied while keeping other interactions constant.

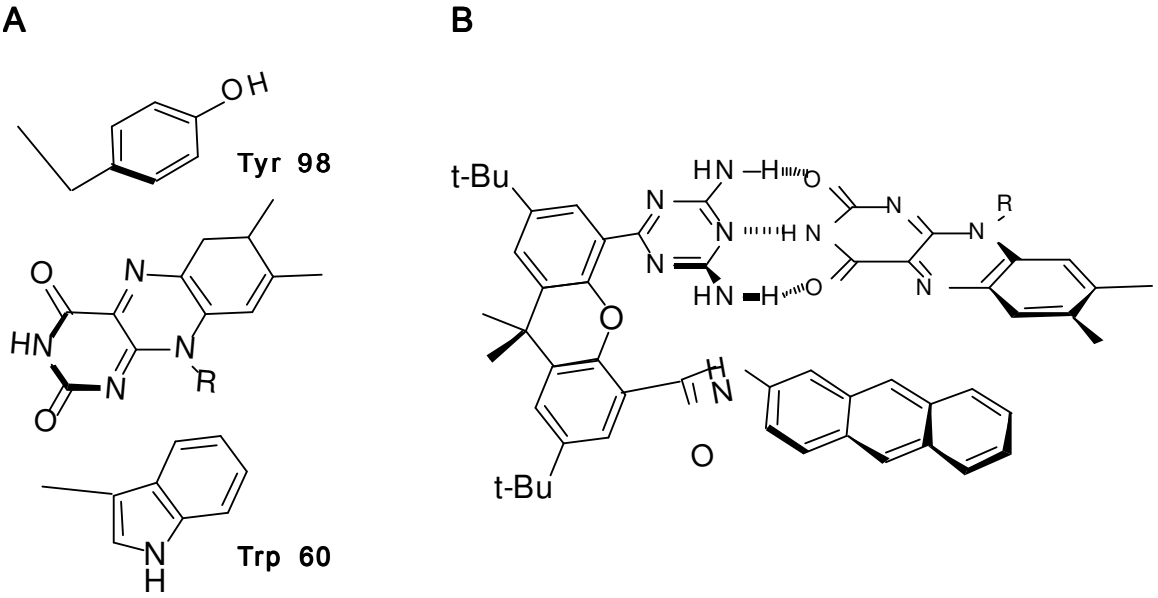

Figure 2.3: Illustration of aromatic stacking in a) native flavoenzyme and b) xanthene system

Stacking interactions between the xanthene receptor and flavin were observed[18] by both fluorescence quenching and NMR titration. Through the use of a control flavin molecule, incapable of hydrogen bonding by the substitution of a methyl group for the N(3)H, hydrogen bonding between receptor and flavin resulted in fluorescence quenching. Greater quenching of the flavin fluorescence was achieved with larger π-surfaces, indicating the extent of π-overlap achieved. In like manner, association constants obtained by NMR constant-host titration show stronger binding between

receptor and flavin as the π-surface is extended; the tightest binding occurred when an anthryl group was used.[18]

This original xanthene-scaffold receptor used only diaminotriazine[19] as the hydrogen-bond complement to flavin. The synthesis of 4-bromo DAP, followed by Stille coupling[20] reaction to the xanthene scaffold enabled the direct linkage of this hydrogen bonding molecule. Apart from the first step, which involved a high temperature melt with only phosphorous pentabromide and chelidamic acid followed by esterification at the 2- and 6-positions with a methanol wash, the synthetic route is the same as that used for the synthesis of 4-hydroxy DAP. An additional characteristic found in this route was the ability of the reaction intermediate 4-bromo-2,6-diaminopyridine to be easily sublimed; this increased both the yield and purity of the Hofmann rearrangement. The Stille reagent was synthesized using 4-bromo-2,6-diamidopyridine, bis(tributyltin) and a palladium catalyst ($(Ph_3P)_2PdCl_2$). Addition of the Stille reagent to the xanthene scaffold utilized the same palladium catalyst.

The interchanging of the hydrogen-bonding molecules DAT and DAP, along with the altering of π-surfaces and electron-donating groups, enhanced understanding of the multiple noncovalent effects which transpire to tightly bind the flavin cofactor to the synthetic receptor. The results from this experiment indicate that, under neutral conditions, the polarizability of the DAP receptor allows for tighter binding of the flavin molecule, thereby more closely replicating the interaction between a flavoenzyme and its cofactor.

2.5 References

(1) Branden, C.; Tooze, J. *Introduction to Protein Structure*; Garland: New York, **1991**.

(2) Peczuh, M. W.; Hamilton, A. D. "Peptide and Protein Recognition by Designed Molecules" *Chem. Rev.* **2000**, *100*, 2479-2494.

(3) *Chemistry and Biochemistry of Flavoenzymes*; Müller, F., Ed.; CRC: Boca Raton, **1991**.

(4) Massey, V.; Hemmerich, P. "Active-site Probes of Flavoproteins" *Biochem. Soc. Trans.* **1980**, *8*, 246-257.

(5) Cuello, A. O.; McIntosh, C. M.; Rotello, V. M. "Model Systems for Flavoenzyme Activity. The Role of N(3)-H Hydrogen Bonding in Flavin Redox Processes" *J. Am. Chem. Soc.* **2000**, *122*, 3517-3521.

(6) Sanner, C.; Macheroux, P.; Ruterjans, H.; Müller, F.; Bacher, A. *Eur. J. Chem.* **1985**, *151*, 49-65. Stockman, B.; Krezel, A.; Markley, J.; Leonhardt, K.; Straus, H. "^{1}H, ^{13}C, and ^{15}N NMR Spectroscopy of Anabaena-7120 Flavodoxin-Assignment of β-Sheet and Flavin Binding-Site Resonances and Analysis of Protein-Flavin Interactions" *Biochemistry* **1990**, *29*, 9600-9609. Fox, K.; Karplus, P. "The Flavin Environment in Old Yellow Enzyme-An Evaluation of Insights from Spectroscopic and Artificial Flavin Studies" *J. Biol. Chem.* **1999**, *274*, 9357-9362.

(7) Swenson, R.; Krey, G. "Site-Directed Mutagenesis of Tyrosine-98 in the Flavodoxin from *Desulfovibrio vulgaris*-Regulation of Oxidation-Reduction Properties of the Bound FMN Cofactor by Aromatic, Solvent, and Electrostatic Interactions" *Biochemistry* **1994**, *33*, 8505-8514. Watt, W.; Tulinsky, A.; Swenson, R.; Wattenpugh, K. "Comparison of the Crystal-Structures of a Flavodoxin in its Three Oxidation States at Cryogenic Temperatures" *J. Mol. Biol.* **1991**, *218*, 195-208. Schreuder, H.; van der Laan, J.; Hol, W.; Drenth, J. "Crystal-Structure of Para-Hydroxybenzoate Hydroxylase Complexed with its Reaction Product 3,4-Dihydroxybenzoate" *J. Mol. Biol.* **1988**, *199*, 637-648. Palfey, B.; Moran, G.; Entsch, B.; Ballou, D.; Massey, V. "Substrate Recognition by 'Password' in p-Hydroxybenzoate Hydroxylase" *Biochemistry* **1999**, *38*, 1153-1158.

(8) Zhou, Z.; Swenson, R. "Electrostatic Effects of Surface Acidic Amino-Acid-Residues on the Oxidation-Reduction Potentials of the Flavodoxin from *Desulfovibrio vulgaris*" *Biochemistry* **1995**, *34*, 3183-3192. Zhou, Z.; Swenson, R. "Evaluation of the Electrostatic Effect of the 5'-phosphate of the Flavin Mononucleotide Cofactor on the Oxidation-Reduction Potentials of the

Flavodoxin from *Desulfovibrio vulgaris*" *Biochemistry* **1996**, *35*, 12443-12454. Aliverti, A.; Deng, Z.; Ravasi, D.; Piubelli, L.; Karplus, P.; Zanetti, G. "Probing the Function of the Invariant Glutamyl Residue 312 in spinach Ferredoxin-NADP(+) Reductase" *J. Biol. Chem.* **1998**, *273*, 34008-34015.

(9) Grell, E.; Warmuth, R. "Caged Cations" *Pure Appl. Chem.* **1993**, *65*, 373-379. Lehn, J.-M. *Supramolecular Chemistry: Concepts and Perspectives*; VCH: New York, **1995**.

(10) Diederich, F. "Complexation of Neutral Molecules by Cyclophane Hosts" *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 362-386.

(11) James, T. D.; Sandanayake K. R. A. S.; Shinkai, S. "Saccharide Sensing with Molecular Receptors Based on Boronic Acid" *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1910-1922.

(12) Schmidtchen, F. P.; Berger, M. "Artificial Organic Host Molecules for Anions" *Chem. Rev.* **1997**, *97*, 1609-1646; Linton, B.; Hamilton, A. D. "Calorimetric Investigation of Guanidinium-Carboxylate Interactions" *Tetrahedron* **1999**, *55*, 6027-6038.

(13) Lavigne, J. J.; Anslyn, E. V. "Sensing a Paradigm Shift in the Field of Molecular Recognition: From Selective to Differential Receptors" *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 3118-3130.

(14) For studies on flavoenzyme mechanisms, see: (a) Kim, J. M.; Bogdan, M. A.; Mariano, P. S. *J. Am. Chem. Soc.* **1993**, *115*, 10591. (b) Hemmerich, P. *The Enzymes, Vol. XII*; Boyer, P. D., Ed.; Academic Press: New York, **1976**. (c) Bach, R.; Su, M.-D. *J. Am. Chem. Soc.* **1994**, *116*, 5392.

(15) For previously published model systems for flavoenzymes, see: (a) Levine, H. L.; Kaiser, E. T. "Oxidation of Dihydronicotinamides by Flavopapain" *J. Am. Chem. Soc.* **1978**, *100*, 7670. (b) Schultz, P. G. "Catalytic Antibodies" *Acc. Chem. Res.* **1989**, *22*, 287. (c) Tabushi, I.; Kodera, M. "Flavocyclodextrin as a Promising Flavoprotein Model-Efficient Electron-Transfer Catalysis by Flavocyclodextrin" *J. Am. Chem. Soc.* **1987**, *109*, 4734. (d) Akiyama, T.; Simeno, F.; Murakami, M.; Yoneda, F. "Flavin-6-Carboxylic Acid as Novel and simple Flavoenzyme Models-Nonenzymatic Stabilization of the Flavin Semiquinone Radical and the 4A-Hydroperoxyflavin by Intramolecular Hydrogen-Bonding" *J. Am. Chem. Soc.* **1992**, *114*, 6613. (e) Takeda, J.; Ohta, S.; Hirobe, M. "Synthesis and Characterization of Novel Flavin-Linked Porphyrins-Mechanism for Flavin-Catalyzed Intermolecular and Intramolecular-2e/1e Electron-Transfer Reactions" *J. Am. Chem. Soc.* **1987**, *109*, 7677.

(16) Breinlinger, E.; Niemz, A.; Rotello, V. M. “Model Systems for Flavoenzyme Activity. Stabilization of the Flavin Radical Anion through Specific Hydrogen Bond Interactions” *J. Am. Chem. Soc.* **1995**, *117*, 5379-5380.

(17) Stockman, B. J.; Richardson, T. E.; Swenson, R. P. "Structural Changes Cause by Site-Directed Mutagenesis of Tyrosine-98 in *Desulfovibrio vulgaris* Flavodoxin Delineated by ^{1}H and ^{15}N NMR Spectroscopy-Implications for Redox Potential Modulation" *Biochemistry* **1994**, *33*, 15298-15308.

(18) Breinlinger, E.; Rotello, V. M. “Model Systems for Flavoenzyme Activity. Modulation of Flavin Redox Potentials through π-Stacking Interactions” *J. Am. Chem. Soc.* **1997**, *119*, 1165-1166.

(19) Nowick, J. S.; Ballester, P.; Ebmeyer, F.; Rebek, J. "Convergent Functional Groups. 9. Complexation in New Molecular Clefts" *J. Am. Chem. Soc.* **1990**, *112*, 8902-8906. Park, T.; Schroeder, J.; Rebek, J. "New Molecular Complements to Imides-Complexation of Thymine Derivatives" *J. Am. Chem. Soc.* **1991**, *113*, 5125-5127.

CHAPTER 3

SELF-ASSEMBLED MONOLAYERS AND MONOLAYER PROTECTED GOLD CLUSTERS

3.1 Introduction

The introduction to monolayers is sequentially presented, from the general topic of self-assembled monolayers on any substrate to the more-specific one concerning clusters on a gold substrate.

3.1.1 Introduction to Self-Assembled Monolayers

The importance and applicability of self-assembled monolayers (SAMs) has burgeoned in recent years.[1] This area of study humbly began in 1946 when the Zisman laboratory published the preparation of a monomolecular layer by adsorption of a surfactant onto a clean metal surface.[2] Because the potential of self-assembly was not recognized, Zisman, et al's work went largely unnoticed. Early work initiated in the laboratory of Kuhn at Göttingen, was followed more recently by Nuzzo and Allara,[3] who demonstated that SAMs of alkanethiolates on gold can be prepared by the adsorption of di-n-alkyl disulfides from dilute solutions. The progression from moisture-sensitive silanes to stable disulfides, along with the ease of using crystalline gold surfaces, led to the success of this work. Although other metal surfaces and adsorbing ligands may be used in the production of SAMs, monolayers of alkane-thiolates on gold remain the most studied and best understood.

In nature, self-assembly of simple components results in supermolecular hierarchical organizations, providing a path to very complex systems.[4] The formation of monolayers by adsorption of ligands to surfaces is one example of this general phenomenon of self-assembly. SAMs provide unique opportunities to better study and understand fundamental concepts such as self-organization, structure-property relationships, and interfacial phenomena. The ability to engineer both head and tail groups of the constituent molecules makes SAMs model systems for a more complete understanding of phenomena affected by intermolecular, molecular-substrates and molecule-solvent interactions such as ordering and growth, wetting, adhesion, lubrication, and corrosion. The reproducibility and accessibility of SAMs lends them to the study of physical chemistry and statistical physics in two dimensions. Applications and opportunities in chemical and biochemical sensing arise from the biomimetic and biocompatible nature of SAMs. In general, self-assembled monolayers on gold surface provide a versatile tool invaluable in many areas of study.[5]

3.1.2 Introduction to Monolayer-Protected Clusters

Although the self-assembly of thiolate ligands on gold clusters was first recognized more than a decade ago,[6] its practical application came about more recently. Schiffrin and co-workers coupled the classical Faraday[6b] two-phase colloid preparation with modern phase-transfer catalysts and alkanethiolate/Au chemistry; the result was the formation of very small clusters of gold atoms (<5 nm average core diameter) coated with alkanethiolate monolayers (AuMPCs).[7]

Several characteristics of AuMPCs differentiate them from other methods of colloid and nanoparticle preparation.[6b] AuMPCs can be repeatedly isolated from and redissolved in common organic solvents without irreversible aggregation or decomposition. This trait and the air stability of AuMPCs are significant in that they are conducive to handling and functionalization via methods already familiar to molecular chemists. Additionally, AuMPCs provide considerable flexibility in characterization, as compared to earlier nanoparticle entities.[8]

The physical attributes of MPCs are very appealing as well. The size limit for current 'engineering down' technology, utilizing such methods as lithography and etching, is roughly 1 μm. In order to continue making rapid advancements in technology, a paradigm shift toward functional nanoscale devices is necessary. MPCs carry the potential to fill this void. By 'engineering up', from molecular size to a controlled supramolecular assembly, it is possible to fabricate controlled nanoscale entities with remarkable applications.[9]

3.2 Synthesis and Characterization of Monolayer Protected Gold Clusters

The pioneering Schiffrin reaction[7] involved the equimolar addition of alkanethiol to organic-phase $AuCl_4^-$. This was accomplished through the use of a phase transfer catalyst. Subsequent reduction by BH_4^- yields alkanethiolate-protected gold clusters possessing a core diameter of 1-3 nm. Although it has not been precisely determined, the reaction appears to adhere to a nucleation-growth-passivation process. The evidence for this argument is thus: first, a greater thiol:gold mole ratio produces smaller average MPC core sizes than does an equimolar ratio;[8,10] second, rapid addition

of the borohydride reductant and reaction in an ice bath yields smaller, more monodisperse MPCs;[8,11] and third, immediate quenching of the reaction upon reduction gives higher abundances of very small core sizes (<2 nm).[12] These results attest to the tunability of the reaction and offer insight toward its behavior.

Schiffrin and co-workers were able to significantly modify the structure and properties of the protecting ligand structures.[13] Simple alkanethiolate MPCs are nonpolar, while highly non-polar head groups can be added to thiols to yield water-soluble MPCs.[13] Functionality may also be added to MPCs, by both reaction and displacement. Schiffrin reported the esterification of p-mercaptoethanol-protected MPCs using propionic anhydride,[14] and since this early example several more reactions using MPCs have been examined.[15] The use of ligand place-exchange as a tool for MPC functionalization was championed by Murray and co-workers.[16] It was reported that MPCs with alkanethiolate monolayers could be functionalized with some other thiolate according to the reaction, where *x* and *m* are the numbers of new and original ligands, respectively.[16] Several factors influence this place-exchange, including the mole ratio of incoming to outgoing ligand, the relative steric bulk of the ligands, as well as the difference in chainlength of the two ligands. The dynamics and mechanism of the place-exchange reaction have been investigated and the results indicate exchange to have a 1:1 stoichiometry, to be an associative process, and to yield the exchanged ligand in solution as a thiol, as opposed to disulfides or other oxidized sulfur species. The time-dependence of place-exchange owes to the accessibility of the various core surface binding sites, with vertexes and edges accordingly more available than are terrace sites.[16c]

$$x(R'SH) + (RS)_mMPC \longrightarrow x(RSH) + (R'S)_x(RS)_{m-x}MPC$$

Figure 3.1: Stoichiometry of the Murray place exchange reaction

The above-mentioned properties of gold MPCs enable their characterization by a number of analytical methods. The content provided by these analytical methods can be organized as pertaining to either the gold core or the surrounding monolayer. The size characteristics of the core may be measured using any or all of the following: scanning tunneling microscopy (STM);[17] atomic force microscopy (AFM);[17] transmission electron microscopy (TEM);[8] small-angle X-ray scattering (SAXS);[17] laser desorption-ionization mass spectrometry (LDI-MS);[11] and X-ray diffraction (XRD).[10] TEM allows for simultaneous observation of the aggregation and core-core spacing,[18] as well. Each of these methods contributes information as to the core dimensions of MPCs, generally in good agreement with each other.

Multiple techniques have also been used to study the monolayers of gold MPCs.[8,17,19] Because of the difficulty involved in obtaining MPCs with low polydispersity in size, most studies have based the gathered information on averages. Elemental analysis[17] is used to confirm the nature of intact ligands. X-ray photoelectron spectroscopy (XPS),[8] thermogravimetric analysis (TGA)[8,17] and/or core size analysis is used to determine the average number of ligands per core. Core size analysis is used due to the tendency of the core to form closed shell structures ("magic numbers"); by knowing the core magic number, the number of ligands may then be calculated. NMR spectroscopy proves invaluable in elucidating the structure and content of MPC monolayers.[20] The NMR resonances of both ^{1}H and ^{13}C spectra are characteristically

broadened as compare to the spectra of free alkanethiols. This broadening is the result of several factors,[8,20] including a distribution of chemical shifts dependent on where the ligand is bound on the gold (vertex, terrace, etc), spin-spin relaxational (T_2) broadening, and a loosening of the monolayer packing density from near-core towards the chain terminus.

Additionally, the amount of disorder and the transition from order to disorder may be determined. Vibrational spectroscopy has shown[5] that, although the alkanethiolate chains of MPCs mostly lie in an all-trans, zigzag conformation, gauche defects are detected. These defects are most likely present in longer chainlengths and when the MPC is above the chain melting temperature. Vibrational spectroscopy has also proved useful in showing intramolecular hydrogen bonding[13] and intermolecular interdigitation[19] of chain domains. The order-disorder transition in solid-state alkanethiolate MPCs may be detected by differential scanning calorimetry (DSC).[19] DSC studies have shown that transition temperatures increase with chainlength, as longer-chain alkanethiolates typically have a larger crystalline domain.

3.3 Diverse, Polyfunctional Monolayer-Protected Gold Clusters

Polyhomo- and heterofunctionalization is a common feature of proteins and protein complexes. Previous synthetic examples of polyfunctionalization include polymeric and dendrimeric materials.[21] The advantages of gold MPCs, including air stability, relatively low cost and tunable solubility, make them very attractive for use as chemical reagents.[8] If MPCs could be polyfunctionalized, then their application to

multistep, mutually supporting catalytic and electron donor/acceptor reactions is readily foreseen.

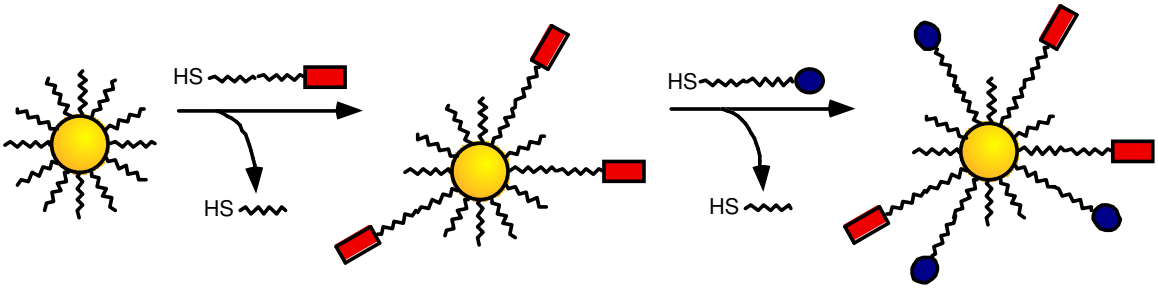

Figure 3.2: Schematic of the Murray place exchange, used in this case to make poly-heterofunctionalized MPC

The fundamentals for polyfunctionalization are based upon place-exchange reactions in solution mixtures of ω-functionalized alkanethiols with alkanethiolate/Au MPCs.[16] The use of place exchange reactions to introduce functionality foregoes the possible alterations in the Au core size that could occur in *de novo* synthesis using a mixture of thiols; this statement has been confirmed by SAXS experiments on the core.[22]

It is evident that two routes to poly-hetero-ω-functionalized are possible: simultaneous exchange of a mixture of free thiols onto an MPC and stepwise exchange.[22] Results from simultaneous exchange show that ligand interaction and chain length largely determine which thiols will adsorb to the gold and which will remain in solution. Ligands with a lot of steric bulk, such as ferrocene-tagged thiols, are preferentially excluded while similar thiols with less steric bulk are adsorbed.[15] Likewise, thiols of longer chainlengths are preferentially adsorbed, and, once adsorbed, preferentially exclude the subsequent adsorption of shorter thiols.

Stepwise assembly of polyfunctionalized gold MPCs was accomplished using ω-functionalized ligands with progressively longer chain lengths.[22] This method also revealed the extent to which an exchange reaction can displace both functionalized and non-functionalized ligands from the gold cluster. Again, the results show that ligands of short chain length and bulky functionality are the least thermodynamically stable. The lesson learned is that it is thermodynamically unfavorable to bury bulky functionality within the monolayer.[22]

Another method of adding functionality to the MPC is to undergo reactions with the ω-functionality already present. It has been shown that ω-Br-alkanethiolate/Au MPCs readily perform S_N2 displacements with alkylamines. The potential for MPCs to be used as "nanofactory" cluster molecules is apparent by the high reactivity of this prototype, with over 20 S_N2 reactions per cluster molecule. The use of this strategy in lieu of the place-exchange method eases the need for purification and requires less time for reaction to occur; thus, coupling reactions on the clusters presents a simple route to obtain polyfunctionalized MPCs with a wide variety of structural groups. Using simple amide and esterification reactions, biologically and technologically relevant entities may be coupled to the MPC, presented on the MPC surface and utilized in a number of critical roles.

3.4 References

(1) Ulman, A. *An Introduction to Ultrathin Organic Films*; Academic Press: Boston, 1991.

(2) Bigelow, W. C.; Pickett, D. L.; Zisman, W. A. *J. Colloid Interface Sci.* **1946**, *1*, 513.

(3) Nuzzo, R. G.; Allara, D. L. “Adsorption of Bifunctional Organic Disulfides on Gold Surfaces” *J. Am. Chem. Soc.* **1983**, *105*, 4481-4483.

(4) Kuhn, H.; Ulman, A. In *Thin Films*; Ulman, A., Ed.; Academic Press: New York, 1995; Vol. 20.

(5) Ulman, A. “Formation and Structure of Self-Assembled Monolayers” *Chem. Rev.* **1996**, *96*, 1533-1554.

(6) Schmid, G., Ed. *Clusters and Colloids*; VCH: Weinheim, **1994**. (b) Hayat, M. A., Ed. *Colloidal Gold: Principles, Methods, and Applications*; Academic Press: New York, **1989**; Vols. 1-2.

(7) Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R. “Synthesis of Thiol-derivatised Gold Nanoparticles in a Two-Phase Liquid-Liquid System” *J. Chem. Soc., Chem. Commun.* **1994**, 801-802.

(8) Hostetler, M. J.; Wingate, J. E.; Zhong, C.-Z.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. "Alkanethiolate Gold Cluster Molecules with Core Diameters from 1.5 to 5.2 nm: Core and Monolayer Properties as a Function of Core Size" *Langmuir* **1998**, *14*, 17-30.

(9) Philp, D.; Stoddart, J. F. "Self-Assembly in Natural and Unnatural Systems" *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1154-1196.

(10) Leff, D. V.; O'Hara, P. C.; Heath, J. R.; Gelbart, W. M. "Thermodynamic Control of Gold Nanocrystal Size: Experiment and Theory" *J. Phys. Chem.* **1995**, *99*, 7036-7041.

(11) Whetten, R. L.; Khoury, J. T.; Alvarez, M. M.; Murthy, S.; Vezmar, I.; Wang, Z. L.; Stephen, P. W.; Cleveland, C. L.; Luedtke, W. D.; Landman, U. "Nanocrystal Gold Molecules" *Adv. Mater.* **1996**, *5*, 428-433.

(12) Schaaf, T. G.; Shafigullin, M. N.; Khoury, J. T.; Vezmar, I.; Whetten, R. L.; Cullen, W.; First, P. N.; Gutierrez-Wing, C.; Ascensio, J.; Jose-Yacaman, M. J. "Isolation of Smaller Nanocrystal Au Molecules: Robust Quantum Effects in Optical Spectra" *J. Phys. Chem. B* **1997**, *101*, 7885-7891. (b) Alvarez, M. M.; Khoury, J. T.; Schaaf, T. G.; Shafigulin, M.; Vezmar, I.; Whetten, R. L. "Critical States in the Growth of Au Clusters" *Chem. Phys. Lett.* **1997**, *266*, 91-98.

(13) Schaaf, T. G.; Knight, G.; Shafigullin, M. N.; Borkman, R. F.; Whetten, R. L. "Isolation and Selected Properties of a 10.4 kDa Gold:Glutathione Cluster Compound" *J. Phys. Chem. B* **1998**, *102*, 10643-10646. (b) Templeton, A. C.; Chen, S.; Gross, S. M.; Murray, R. W. "Water-Soluble, Isolable Gold Clusters

Protected by Tiopronin and Coenzyme A Monolayers" *Langmuir* **1999**, *15*, 66-76. (c) Wuelfing, W. P.; Gross, S. M.; Miles, D. T.; Murray, R. W. "Nanometer Gold Clusters Protected by Surface-Bound Monolayers of Thiolated Poly(ethylene glycol) Polymer Electrolyte" *J. Am. Chem. Soc.* **1998**, *120*, 12696-12697. (d) Chen, S.; Kimura, K. "Synthesis and Characterization of Carboxylate-Modified Gold Nanoparticle Powders Dispersible in Water" *Langmuir* **1999**, *15*, 1075-1082. (e) Chen, S.; Murray, R. W. "Arenethiolate Monolayer-Protected Gold Clusters" *Langmuir* **1999**, *15*, 682-689. (f) Johnson, S. R.; Evans, S. D.; Mahon, S. W.; Ulman, A. "Alkanethiol Molecules Containing an Aromatic Moiety Self-Assembled onto Gold Clusters" *Langmuir* **1997**, *13*, 51-57. (g) Brust, M.; Fink, J.; Bethell, D.; Schiffrin, D. J.; Kiely, C. "Synthesis and Reactions of Functionalised Gold Nanoparticles" *J. Chem. Soc., Chem. Commun.* **1995**, 1655-1656. (h) Buining, P. A.; Humbel, B. M.; Philipse, A. P. Verkleij, A. J. "Preparation of Functional Silane-Stabilized Gold colloids in the (Sub)nanometer Size Range" *Langmuir* **1997**, *13*, 3921-3926.

(14) Bethell, D.; Schiffrin, M.; Schiffrin, D. J.; Kiely, C. "From Monolayers to Nanostructured Materials: An Organic Chemist's View of Self-Assembly" *J. Electroanal. Chem.* **1996**, *409*, 137-143.

(15) Templeton, A. C.; Hostetler, M. J.; Kraft, C. T.; Murray, R. W. "Reactivity of Monolayer-Protected Gold Cluster Molecules: Steric Effects" *J. Am. Chem. Soc.* **1998**, *120*, 1906-1911.

(16) Hostetler, M. J.; Green, S. J.; Stokes, J. J.; Murray, R. W. "Monolayers in Three Dimensions: Synthesis and Electrochemistry of ω-Functionalized Alkanethiolate-Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1996**, *118*, 4212-4213. (b) Ingram, R. S.; Hostetler, M. J.; Murray, R. W. "Poly-hetero-ω-functionalized Alkanethiolate-Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1997**, *119*, 9175. (c) Hostetler, M. J.; Templeton, A. C.; Murray, R. W. "Dynamics of Place-Exchange Reactions on Monolayer-Protected Gold Cluster Molecules" *Langmuir* **1999**, *15*, 3782-3789.

(17) Terrill, R. H.; Postlethwaite, T. A.; Chen, C.-H.; Poon, C.-D.; Terzis, A.; Chen,A.; Hutchison, J. E.; Clark, M. R.; Wignall, G.; Londono, J. D.; Superfine, R.; Falvo, M.; Johnson, C. S., Jr.; Samulski, E. T.; Murray, R. W. "Monolayers in Three Dimensions: NMR, SAXS, Thermal, and Electron Hopping Studies of Alkanethiol Stabilized Gold Clusters" *J. Am. Chem. Soc.* **1995**, *117*, 12537-12547.

(18) Fink, J.; Kiely, C. J.; Bethell, D.; Schiffrin, D. J. "Self-Organization of Nanosized Gold Particles" *Chem. Mater.* **1998**, *10*, 922-926.

(19) Badia, A.; Cuccia, L.; Demers, L.; Morin, F.; Lennox, R. B. "Structure and Dynamics in Alkanethiolate Monolayers Self-Assembled on Gold Nanoparticles:

A DSC, FT-IR, and Deuterium NMR Study" *J. Am. Chem. Soc.* **1997**, *119*, 2682-2692.

(20) Badia, A.; Singh, S.; Demers, L.; Cuccia, L.; Brown, G. B.; Lennox, R. B. "Self-Assembled Monolayers on Gold Nanoparticles" *Chem. Eur. J.* **1996**, *2*, 359-363. (b) Badia, A.; Gao, W.; Singh, S.; Demers, L.; Cuccia, L.; Reven, L. "Structure and Chain Dynamics of Alkanethiol-Capped Gold Colloids" *Langmuir* **1996**, *12*, 1262-1269. (c) Badia, A.; Demers, L.; Dickinson, L.; Morin, F. G.; Lennox, R. B.; Reven, L. "Gold-Sulfur Interactions in Alkylthiol Self-Assembled Monolayers Formed on Gold Nanoparticles Studied by Solid-State NMR" *J. Am. Chem. Soc.* **1997**, *119*, 11104-11105.

(21) Bar, G.; Rubin, S.; Cutts, R. W.; Taylor, T. N.; Zawodzinski, T. A. "Dendrimer-Modified Silicon Oxide surfaces as Platforms for the Deposition of Gold and Silver Colloid Monolayers: Preparation Method, Characterization, and Correlation Between Microstructure and Optical Properties" *Langmuir* **1996**, *12*, 1172-1179.

(22) Ingram, R. S.; Hostetler, M. J.; Murray, R. W. "Poly-hetero-ω-functionalized Alkanethiolate-Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1997**, *119*, 9175-9178.

CHAPTER 4

THE APPLICATION OF MONOLAYER PROTECTED GOLD CLUSTERS TO SYNTHETIC FLAVOENZYME MODELS

4.1 Introduction

With the development of the Brust-Schiffrin[1] procedure and the Murray place-exchange reaction,[2] monolayer protected gold clusters began to receive the attention due to such a versatile system. Chemists favor MPCs for their remarkable ease of handling, ability to control reaction product and many methods of characterization.[3] The applicability of MPCs to a diverse range of functions also beholden them to industry. Already, MPCs have been put to use as catalysts,[4] chemical and biological sensors,[5] nanoreactors,[6] polymer additives[7] and as building blocks for the self-assembly of hierarchical structures;[8] future areas of application include drug delivery, gene therapy, optical switches[9] and electronic inks.[10]

The presentation of a variety of functional groups on the monolayer surface is made possible by the Murray place-exchange reaction.[2] Regardless of whether functional groups are added in a stepwise or simultaneous reaction, adsorption of incoming ligands is largely dependent on chainlength and steric bulk. Ligands with short chain length and bulky functional groups adsorb at a very low rate and are easily exchanged out of the monolayer; thiols of longer chain length and with less bulky groups are more easily exchanged into the monolayer and are more likely to remain adsorbed to the core. It has been demonstrated that these characteristics may be tuned

so as to provide gold clusters with poly-homo- or heterofunctionalization and high reactivity with proper substrates.[11]

The necessary components of an enzymatic model include proper substrate selectivity and sequestration.[12] Native enzymes retain their solubility while simultaneously providing a region of tuned polarity to satisfy the requirements of the substrate.[13] From the many advantages of MPCs and the previous studies conducted on them, it follows that monolayer protected gold clusters ought to provide a useful application to a more exact biomimetic enzyme model.

4.2 AuMPC-based Enzyme Models

Self-assembled monolayer (SAM) enzyme model systems have displayed the ability to sequester a substrate molecule in a biomimetic fashion. Yet these systems still carry the inherent limitations associated with SAMs, such as difficulty in scale-up production, characterization and proper distribution to the substrate in solution.[12] These drawbacks may be overcome by the use of AuMPCs as enzyme models.

Redox-modulated recognition of flavin by functionalized gold nanoparticles was accomplished by Boal and Rotello.[14] This work built on prior research by Rotello and co-workers, which established that the recognition of the flavin–diaminopyridine dyad can be enhanced via reduction of the oxidized flavin molecule to the radical flavin anion.[15] Diamidopyridine-functionalized MPCs (DAP-Au) were prepared via Murray place-exchange reaction of octanethiol MPC and purified via sequential precipitation. Recognition of flavin was demonstrated via NMR titration. The downfield shift of the flavin N(3) proton, upon addition of DAP-Au, fits a 1:1 binding isotherm. The resulting

association constant (K_a) is in accordance with values previously observed for DAP recognition by the oxidized flavin molecule.

After flavin recognition by the gold nanoparticles was demonstrated, electrochemistry was used to illustrate the redox-induced modulation of the host-guest interactions. The results of this voltammetry titration correspond to a stabilization of the radical flavin anion by nearly 2 kcal/mol. Thus, a model system of variable surface functionality with electrochemically controlled host-guest interactions was produced.

More recently, Boal and Rotello have demonstrated an enhanced MPC-based flavoenzyme model. The benefits of preorganization, a central tenet in molecular recognition and demonstrated by the xanthene-based system, were combined with the benefits of MPCs. The specific interactions utilized by flavoproteins to sequester the flavin cofactor, namely hydrogen bonding and aromatic stacking, were mimicked through the use of diamidopyridine as hydrogen bond receptor and pyrene as a large, electron-rich aromatic surface.[16]

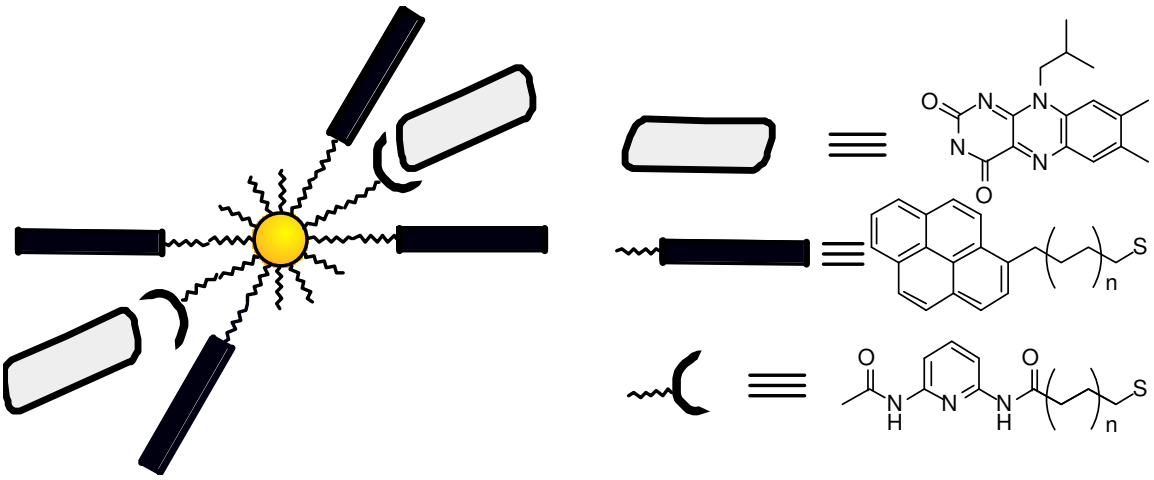

Figure 4.1: Representation of the multivalent recognition and binding of flavin by MMPC of Boal and Rotello

With the goals of adding complexity to the system and recreating the active site of a native enzyme, a series of these mixed monolayer-protected gold clusters (MMPCs) was prepared. Recognition of the oxidized flavin molecule followed previous protocol, with the shifts of the N(3) proton on flavin, as seen by constant-host NMR titration, fit to a 1:1 binding isotherm. A large radial effect was observed for those MMPCs with ditopic receptors, with flavin binding doubled as compared to binding by the monotopic receptors. Ditopic MMPCs with shorter chainlengths produce even stronger binding to flavin (~5 times as compared to monotopic MMPCs). This effect is a result of the increased order, and therefore greater preorganization, of the monolayer as it nears the core.[16] This work demonstrates a biomimetic system with the means of controlling guest redox potential while simultaneously regulating redox-state specific host-guest interaction. Functionalized MMPCs have unique characteristics that may be exploited in the creation of better enzyme models and molecule-based materials.

4.3 2,6-diamido-4-hydroxypyridine-based MPC Receptors

In an effort to more precisely mimic the hydrogen bonding geometry between the active site of flavoenzymes and the flavin cofactor, 2,6-diamido-4-hydroxypyridine (HO-DAP) functionalized thiols were synthesized. Two synthetic routes were employed to obtain the thioacetate. The first involved the anti-Markovnikov addition of thiolacetic acid to the terminal double bond of an ω-bromoalkene, while the second involved the statistical halo-substitution of one bromide using potassium thioacetate. The result for both routes was the ω-bromothioacetate. Halo-substitution of the

bromide with HO-DAP and subsequent basic cleavage of the protecting group yielded the desired thiol.

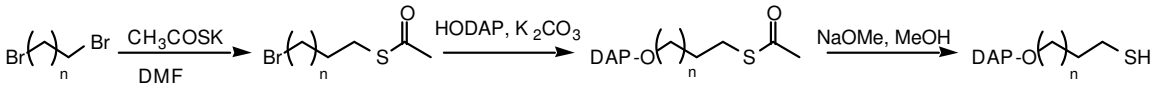

Figure 4.2: General synthesis of 2,6-diamido-4-oxypyridine-functionalized monothiols

These thiols were then added to the gold cluster via the Murray place exchange reaction. The extent of functionality added to the MMPC was monitored by ^{1}H NMR. The advantage of linking diamidopyridine at the 4- position was the display of hydrogen bond sites facing away from the gold core, whereas attachment at the 2- position resulted in a geometry perpendicular to the core. Crystallographic data from native active sites[17] shows that the flavoenzyme active site resides roughly perpendicular to the extended hydrophobic area surrounding it; the display of the DAP receptor afforded via its attachment at the 4- position most closely resembles this.

Attempts to bury the DAP functionality within the monolayer, in an attempt to fashion a model 'active site' failed. The idea was that if the hydrocarbon monolayer could be extended beyond the DAP receptor via place exchange with long chain alkanethiols then the DAP receptor would serve as an interior pocket of increased polarity and binding specificity with its substrate, and the extended hydrocarbon monolayer would retain its hydrophobicity; thus, the topology of a native enzyme would be closely modeled. Place exchange reactions using alkanethiols long enough to effectively bury the receptor within the monolayer resulted in complete exchange of the functionalized thiols. The steric bulkiness of the HO-DAP molecule and the shorter

chain length of the functionalized thiol, as compared to the incoming alkanethiol, greatly favored the desorption of the functionalized thiol.

These functionalized thiols were retained so long as the length of this adsorbed thiol was not exceeded- by more than several carbons- by the length of the incoming thiol; however, the product of this exchange reaction less closely modeled native flavoenzyme architecture. ^{1}H NMR titrations, with flavin cofactor as constant host and HO-DAP-Au as guest, showed less binding than was achieved using DAP-functionalized thiols attached at the 2- position. Clearly, the resistance to desorption of the functionalized thiol must be greatly improved if a true biomimetic 'active site' is to be achieved.

4.4 Self-Assembled Alkanedithiols Monolayers on Gold

The adsorption of alkanethiols onto gold surfaces leads to the formation of self-assembled monolayers (SAMs).[12] Although research has shown that SAMs exhibit moderate stabilities at room temperature,[18] research has also shown that exposure to oxygen, elevated temperature or free thiol in solution will desorb the initial monolayer.[19] This moderate stability of normal SAMs limits their application to a number of long-term applications which require more robust systems to be effective.

Several strategies toward the production of a more stable system have been undertaken. These include the use of underpotential-deposited (UPD) metal substrates,[20] the incorporation of "cross-linking" groups within the alkyl chains[21] and the use of adsorbates capable of multiple sulfur-gold interactions.[22] This latter strategy was the focus of the work by Shon and Lee, based on the hypothesis that the entropy-

driven 'chelate effect' ought to provide greater stability to SAM-based materials.[23] In addition, as 1,3-dithiols were the choice of chelating ligand, it was determined that the intramolecular formation of disulfides was greatly disfavored.[24]

Three types of ligands were synthesized in order to determine the differences in stability between mono- and dithiols. Dithiols were synthesized using standard malonic ester manipulations.[25] The advantages to this route include the low cost of starting materials and the facile opportunity to generate a wide variety of dithiols. Included in this variety are mono- and dialkylated species, as well as asymmetric substrates. The monothiols that were used in this study were commercially available.

Thermal desorption studies at increasing temperatures were undertaken to determine the stability of the various thiols. In each case and irrespective of temperature, the order of stability of the thiols, as measured by the fraction of SAM remaining as a function of time, was dialkylated dithiol > monoalkylated dithiol > normal monothiol.[26] Four explanations were given in an attempt to rationalize the enhanced stability of the dithiol-based SAMs. First, desorption of a dithiol molecule requires the nearly simultaneous breaking of two sulfur-gold interactions.[22] This entropy-driven effect contributes to the enhanced stability of the system. Second, decomposition of a monolayer of alkanethiols results in disulfide formation.[24] The use of 1,3-dithiols drastically inhibits this *intramolecular* process, due to the ring strain that is generated in the formation of such dithiolanes. Third, although the *intermolecular* desorption of dithiols is allowed energetically, its requirement of the concurrent desorption of four tethered sulfur atoms is highly improbable and entropically disfavored. Fourth, diffusion of some monothiols on the gold surface from high-energy-

barrier sites to low-energy ones allows for more complete desorption.[27] A monolayer composed of dithiols, and, hence, with two distinct sulfur-gold interactions, would not bear this same diffusion; desorption is less complete.

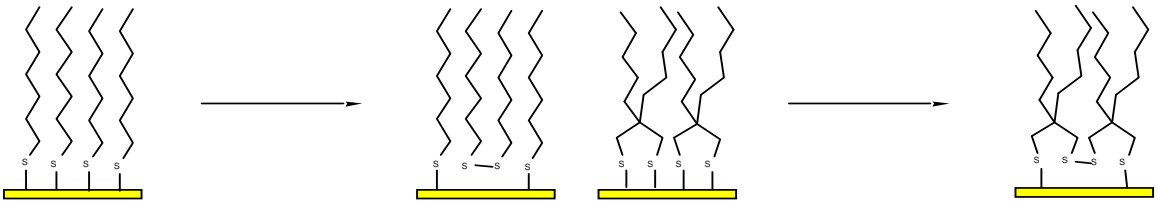

Figure 4.3: Representation of the enhanced stability afforded a dithiol system via the "chelate effect", as reported by Shon and Lee

Displacement studies on preformed SAMs confirm the stability of the dithiol-based monolayer. At room temperature it was shown that the SAM composed of normal alkanethiol was more readily displaced that the dithiol SAM of corresponding chain length. Thermodynamic displacement experiments reinforced the previous results. Although kinetics data showed that adsorption of the monothiol proceeded more rapidly than that of the dithiol, these studies demonstrated a clear preference for the adsorption of the dithiol.[26]

4.5 Synthesis of Dithiol Monolayer Protected Gold Clusters

The self-assembled dithiol monolayer on gold surface, as described by Shon and Lee,[26] exhibited a significant increase in stability when compared to the standard n-alkanethiol monolayer. It follows, then, that this system would afford greater stability in an MPC system, as well. Dithiols were synthesized following the same route as Shon and Lee[25] with one exception; HMPA (hexamethylphosphoramide) was replaced by

DMF (dimethylformamide) as the solvent for the nucleophilic addition of thioacetate for reasons of safety.

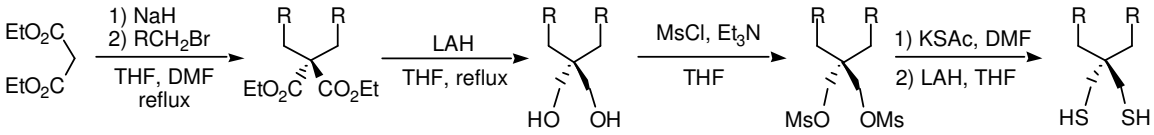

Figure 4.4: Synthesis of dialkyl dithiol ligand

Once the dithiol had been synthesized in sufficient quantity, its reduction in the presence of organic-phase gold salt followed standard protocol.[1] The structure of dithiol MPC very closely resembles that of n-alkanethiol MPC, as determined by ^{1}H NMR.

4.6 Investigation of Dithiol MPC Stability Using NaCN-Induced Decomposition

Using 2D alkanethiolate/gold SAMs, it has previously been demonstrated[28] that cyanide causes decomposition of the monolayer to the corresponding disulfide. The rate of decomposition can be correlated to the extent to which an individual alkanethiolate monolayer provides a protective barrier for the gold surface.[29] This rate of decomposition may be monitored via the change in absorbance at 340nm, as measured by UV-vis spectroscopy.

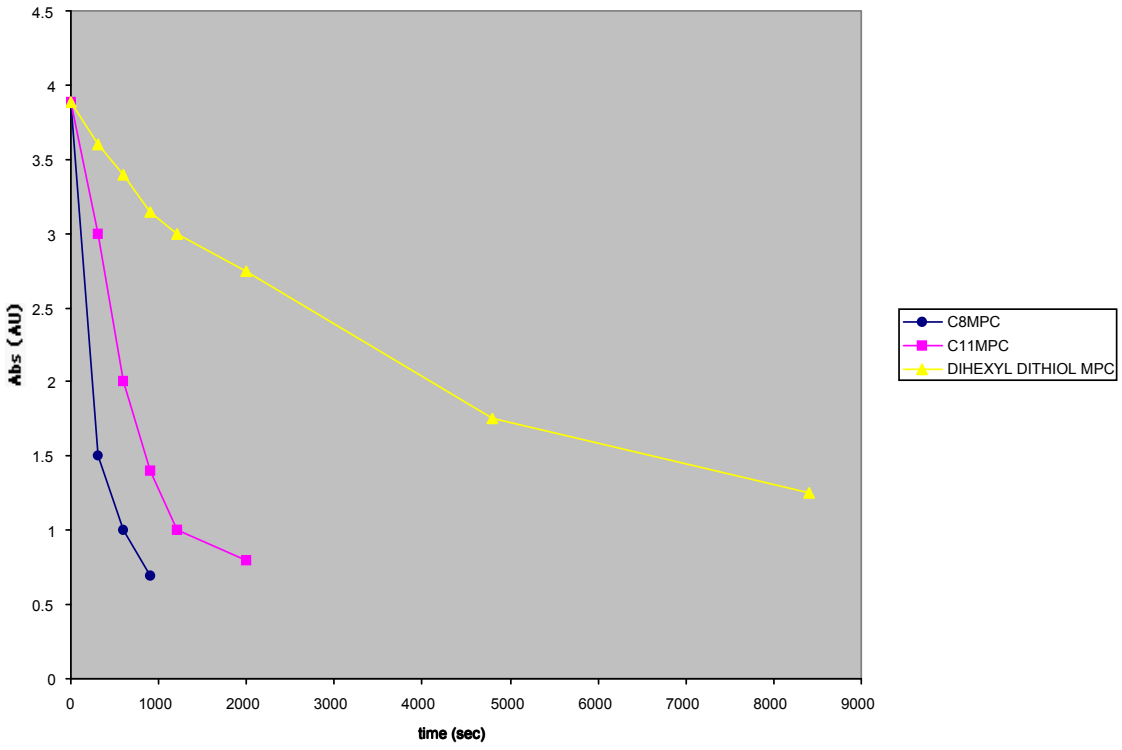

Figure 4.5: NaCN-induced decomposition of MPCs, measured at 340 nm

Murray and co-workers observed the absorbance-decay rate of cyanide-induced decomposition for several different MPCs of varying alkanethiolate chain length. As expected, the results show that decomposition is most rapid for clusters protected by short ligands, and slows for longer chain lengths. For example, dodecanethiolate-protected MPC decomposes two times more slowly than the corresponding octanethiolate MPC. This allows for the assumption that the longer dodecanethiolate ligands are more effective at preventing the cyanide ion from approaching the gold cluster. Reasons for this include the increased size of steric barrier and hydrophobic shell imposed by the larger thiolates. Murray also determined that extension of the

monolayer at or beyond a length of ten carbon atoms resulted in no significant increase in protection from decomposition.[29]

The results obtained from a repeat of the above-mentioned work, using only monolayers composed of octanethiolate or undecenethiolate ligands, were in excellent agreement. As was expected, the undecenethiolate MPC decomposed roughly two times more slowly than the octanethiolate MPC.

Decomposition of dihexyl-1,3-dithiolate MPC gave interesting results. The rate of decomposition of this system, with a carbon chain length roughly equivalent to that of octanethiol, was nearly five times slower than that of undecenethiolate. The main reason for this remarkable stability is believed to be the 'chelate effect', afforded by the use of the 1,3-dithiol.[26] These results indicate the significantly increased robustness of the dithiol-gold interaction, a highly desirable trait in the quest of adding more securely anchored functionality to the MPC system.

Thermogravimetric analysis (TGA) is another method used to assess the stability of the MPC monolayer. TGA measures the fractional weight loss that occurs as a function of increasing temperature. This decline in weight is attributed to the thermally-labile portion of the sample; in the case of a Au-MPC, it refers to the organic thiolate monolayer. For octanethiolate MPC, experiments show that the maximal thermal degradation occurs at roughly 190^{o}C. Experiments with dihexyl-1,3-dithiolate MPC, using the same TGA acquisition parameters as the octanethiolate MPC experiment, show that maximal thermal degradation occurs at roughly 220^{o}C. These results illustrate the enhanced stability achieved through the use of dithiolate ligand as the

monolayer constituent, and further posit the dithiol system as a sound choice of adsorbed ligand in the generation of immobilized functionality on a Au-MPC.

4.7 Experimental Method

General. All starting materials were purchased from Aldrich Chemical Co. and used as received. All solvents were reagent grade and used as received. ^{1}H NMR spectra were recorded at 200 MHz in $CDCl_3$ or DMSO (purchased from Cambridge Isotope Labs, Inc.) and referenced internally to TMS at 0.0ppm.

4.7.1 Synthesis

4.7.1.1 2,6-diamido-4-hydroxypyridine

In a flame-dried 1000mL round-bottom flask, chelidamic acid (1 eq) and potassium carbonate (large excess) were stirred in roughly 300mL acetone. This solution was allowed to stir for twenty minutes, after which benzyl bromide (3.5 eq) in one portion. Under dry tube, the reaction mixture was refluxed at 80^{o}C for 72h. Upon completion and return to room temperature, acetone was removed under reduced pressure. The dry material was suspended in EtOAc and stirred for a short period of time. 2M HCl was used to neutralize the excess base; resultant pH was ~4. Product was extracted from the aqueous portion three times, after which it was dried over $MgSO_4$. Simple filtration afforded the product as a slightly off-white solid. This benzyl ester compound was then used without further purification.

Upon dissolution in methanol at 65^{o}C, ammonia gas was bubbled through the solution until precipitation of the next intermediate occurred. This compound, a

diamide, was refrigerated overnight and then cold filtered with methanol to afford pure white product. To ensure its dryness, it was left for 72h under high vacuum in a water pistil.

Hofmann rearrangement[30] of the diamide to the diamine followed. At O°C, a 5M KOH/H_2O solution was made using 100mL water in a 250mL round-bottom flask. Br_2 (l) (2 eq) was added in one portion. This mixture was allowed to stir until nearly all of the bromine had been changed to HOBr, as evidenced in the solution changing color from yellow to white. To this mixture was added the diamide. After approximately 30 minutes of stirring following complete addition of the diamide, the reaction mixture was immediately raised to 90°C in a pre-heated oil bath. This reaction mixture was then stirred open to atmosphere at 90°C for a period of 5h. Upon completion, the reaction mixture was acidified (pH ~4) with 2M HCl. This mixture was then saturated with NaCl in order to precipitate product. This mixture was vacuum filtered through a frit, fitted with filter paper. The solid obtained was then free-based with K_2CO_3 at low heat in water. Extraction three times with EtOAc afforded clean 4-benzyloxy-2,6-diaminopyridine (65 % yield). ^{1}H NMR (DMSO, 200 MHz): δ (ppm) 7.35 (m,5H), 5.30 (ds, 6H, J = 6 Hz), 4.96 (s, 2H).

Acylation of the diamine is the next step. In a dry, 3-neck flask under inert conditions, the diamine (1 eq) is dissolved in dried CH_2Cl_2 (~100mL). Et_3N (2.2 eq) is then added. A flame-dried addition funnel with volume of inert gas is then fitted onto the 3-neck flask. ~60mL dried CH_2Cl_2 added. Propionyl chloride (2.2 eq) was then dissolved into the CH_2Cl_2. Dropwise addition of this solution occurred for several hours. Upon complete addition, the organic portion was extracted and evaporated onto

silica powder. Flash column chromatography of this crude material afforded the desired diacylated compound as the major product (70 % yield). ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.14 (bs, 2H), 7.65 (bs, 2H), 7.37 (m, 5H), 5.08 (s, 2H), 2.30 (q, 4H, J = 8 Hz), 1.16 (t, 6H, J = 7 Hz).

Deprotection of the benzyl group used 5 % Pd on activated carbon in methanol under positive H_2 atmosphere. Reaction mixture was stirred overnight to ensure completion. Hot filtration with methanol used in an attempt to release product adsorbed to carbon. Methanol removed from filtrate *in vacuo* to afford desired 2,6-diamido-4-hydroxypyridine compound (95 % yield). ^{1}H NMR (DMSO, 200 MHz): δ (ppm) 10.53 (bs, 1H), 9.76 (s, 2H), 7.26 (s, 2H), 2.39 (q, 4H, J = 8 Hz), 1.04 (t, 6H, J = 7 Hz).

4.7.1.2 General Ether-linked diamidopyridine-functionalized thiol synthesis

In a 100mL round-bottom flask, α,ω-dibromoalkane (3 eq) dissolved in DMF (~10mL DMF/g alkane). After several minutes of stirring, potassium thioacetate (1 eq) added and reaction mixture allowed to stir overnight at room temperature. H_2O added to reaction mixture. Organic material extracted from DMF/H_2O using 1:1 Hexane/EtOAc mixture. This was then dried over $MgSO_4$. Filtration and removal of solvent under reduced pressure yielded crude oil. Flash column chromatography of this crude material gave desired ω-bromoalkylthioacetate in moderate yield (45%). ^{1}H NMR (for prototypical bromopentylthioacetate) ($CDCl_3$, 200 MHz): δ (ppm) 3.40 (t, 2H, J = 7 Hz), 2.86 (t, 2H, J = 7 Hz), 2.32 (s, 3H), 1.85 (bm, 2H), 1.53 (bm, 2H), 1.33 (m, 2H).

Bromoalkylthioacetate (1.3 eq) then added by pipet to a large, dry vial. Potassium carbonate (3 eq) was then added. These reagents were dissolved in ~12mL

DMF and thoroughly sparged with argon. NaI added in catalytic amount and allowed to stir for several minutes, after which hydroxydiamidopyridine was added. Reaction mixture temperature raised to 65^{o}C and stirred under dry tube for 72h. Extraction of organic material from DMF/H_2O using Hexane/EtOAc mixture gave crude oil. Purification using flash column chromatography gave desired functionalized thioacetate (75% yield). ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.55 (bs, 2H), 7.52 (bs, 2H), 4.03 (t, 2H, J = 6 Hz), 2.89 (t, 2H, J = 7 Hz), 2.42 (q, 4H, J = 3 Hz), 2.32 (s, 2H), 1.78 (bm, 2H), 1.66 (bm, 2H), 1.56 (m, 2H), 1.23 (t, 6H, J = 7 Hz).

Basic cleavage of the thioacetate group followed. The diamidopyridine-functionalized thioacetate (1 eq), added to round-bottom flask via pipet, was dissoved in methanol (5 mL). Reaction mixture thoroughly sparged with argon. Solution of NaOMe (25% wt in MeOH, 10 eq) was also thoroughly sparged. After addition of NaOMe solution to reaction mixture, resultant reaction mixture was degassed for an additional 20 min. This reaction mixture was then stirred overnight under inert conditions. Work-up involved quenching of excess base with ammonium chloride/aqueous solution, evaporation of methanol under reduced pressure and subsequent extraction of organic content. After drying over $MgSO_4$, crude oil recovered. This oil was eluted through a silica plug and desired thiol obtained (80% yield). ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.53 (bs, 2H), 7.52 (bs, 2H), 4.04 (t, 2H, J = 6 Hz), 2.51 (q, 2H, J = 8 Hz), 2.41 (q, 4H, J = 7 Hz), 1.78 (bm, 2H), 1.61 (bm, 6H), 1.27 (t, 6H, J = 7 Hz).

4.7.1.3 Dithiol Synthesis

A solution of NaH in THF and DMF was prepared at 0°C under argon. To this solution was added slowly diethyl malonate. The mixture was stirred at room temperature for 15 min, after which bromoalkane was added. The mixture was then refluxed for 6h. Extraction of organic portion and flash column chromatography gave product. This compound was then reduced by LAH in THF at room temperature, and the reaction mixture refluxed for 2h. 1M HCl was used to quench excess LAH after which reaction mixture was allowed to stir an additional 30 min. Extraction of organic portion with subsequent drying over $MgSO_4$ yielded desired intermediate diol. This diol was then dissolved in THF. Triethylamine was added. To this stirring mixture was added methanesulfonyl chloride, dropwise over a period of 5 min. The organic portion was extracted with ether and then dried over $MgSO_4$ to afford mesylated intermediate. This dimesylated compound was then placed in DMF, along with potassium thioacetate. The reaction mixture was heated to 65°C for 72h. Upon cooling, the organic portion was extracted from the DMF. Crude oil was purified by flash column chromatography. The thioacetate groups were removed using LAH in THF. This crude oil was then purified by flash column chromatography. NMR data is for 2,2-dihexyl-1,3-propanedithiol. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 2.52 (ds, 4H, J = 9 Hz), 1.33-1.10 (bm, 16 H), 1.11 (t, 4 H, J = 8 Hz), 0.91 (t, 6 H).

4.7.1.4 MPC Synthesis

In a 100mL round-bottom flask, $HAuCl_4 \cdot 3H_2O$ (1 eq, 0.5 mmol) was dissolved in distilled H_2O (20mL). A solution of tetraoctylammonium bromide (2.5 eq, 2 mmol)

in toluene (40mL) was added under stirring. To this biphasic mixture was added the desired thiol ligand (3 eq). Reaction mixture cooled to 0°C in an ice bath. $NaBH_4$ (20 eq) dissolved in distilled H_2O (10 mL) in a 20mL vial. Upon dissolution, reductant added in one portion to reaction mixture. After generation of gas had subsided, the reaction mixture was allowed to warm to room temperature and stirred for an additional 3h. After extraction of the organic layer and *in vacuo* removal of toluene, the resultant thick brown solid was precipitated from ethanol and/or acetonitrile overnight in the refrigerator. Collection on a filter paper via vacuum filtration, and additional washes with cold ethanol afforded the desired monolayer protected gold clusters. Sample MPC data (unfunctionalized alkanethiolate MPCs, whether composed of mono- or dithiols, have very similar NMR data). ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 1.57 (bs) 1.28 (bm) 0.88 (bm).

4.7.2 NaCN-Induced MPC Decomposition

To a 3mL solution of desired MPC (8.28μM in THF, final concentration ~ 7μM) was added 0.5mL of an aqueous NaCN solution (final concentration 8.7mM), followed by briefly agitating the mixture. The decay in absorbance at 340nm was monitored using a UV-vis spectrophotometer beginning ~8s after addition of NaCN solution. The decomposition rates were analyzed by comparing the maximal rate of decomposition as evidenced by the fastest decay in absorption between two time points of the decay curve.

4.8 References

(1) Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R. "Synthesis of Thiol-derivatised Gold Nanoparticles in a Two-Phase Liquid-Liquid System" *J. Chem. Soc., Chem. Commun.* **1994**, 801-802.

(2) Hostetler, M. J.; Green, S. J.; Stokes, J. J.; Murray, R. W. "Monolayers in Three Dimensions: Synthesis and Electrochemistry of ω-Functionalized Alkanethiolate-Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1996**, *118*, 4212-4213. (b) Ingram, R. S.; Hostetler, M. J.; Murray, R. W. "Poly-hetero-ω-functionalized Alkanethiolate-Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1997**, *119*, 9175. (c) Hostetler, M. J.; Templeton, A. C.; Murray, R. W. "Dynamics of Place-Exchange Reactions on Monolayer-Protected Gold Cluster Molecules" *Langmuir* **1999**, *15*, 3782-3789.

(3) Hostetler, M. J.; Wingate, J. E.; Zhong, C.-Z.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. "Alkanethiolate Gold Cluster Molecules with Core Diameters from 1.5 to 5.2 nm: Core and Monolayer Properties as a Function of Core Size" *Langmuir* **1998**, *14*, 17-30.

(4) Li, H.; Luk, Y.-Y.; Mrksich, M. "Catalytic Asymmetric Dihydroxylation by Gold Colloids Functionalized with Self-Assembled Monolayers" *Langmuir* **1999**, *15*, 4957-4959.

(5) Bruchez, M., Jr.; Moronne, M.; Gin, P.; Weiss, S.; Alivasatos, A. P. "Semiconductor Nanocrystals as Fluorescent Biological Labels" *Science* **1998**, *281*, 2013-2016. (b) Chan, W. C. W.; Nie, S. "Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection" *Science* **1998**, *281*, 2016-2018. (c) Sampath, S.; Lev, O. "3D Organized Self-Assembled Monolayer Electrodes: A Novel Biosensor Configuration" *Adv. Mater.* **1997**, *9*, 410-412.

(6) Kaifer, A. E. "Interplay Between Molecular Recognition and Redox Chemistry" *Acc. Chem. Res.* **1999**, *32*, 62-71. (b) Liu, J.; Xu, R. L.; Kaifer, A. E. "In Situ Modification of the Surface of Gold Colloidal Particles: Preparation of Cyclodextrin-Based Rotaxanes Supported on Gold Nanospheres" *Langmuir* **1998**, *14*, 7337-7339.

(7) Watson, K. J.; Zhu, J.; Nguyen, S. T.; Mirkin, C. A. "Hybrid Nanoparticles with Block Copolymer Shell Structures" *J. Am. Chem. Soc.* **1999**, *121*, 402-403.

(8) Mucic, R. C.; Storhoff, J. J.; Mirkin, C. A.; Letsinger, R. L. "DNA-Directed Synthesis of Binary Nanoparticle Network Materials" *J. Am. Chem. Soc.* **1998**,*120*, 12674-12675. (b) Niemeyer, C. M.; Bürger, W.; Peplies, J. "Covalent DNA-Streptavidin Conjugates as Building Blocks for Novel Biometallic

Nanostructures" *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2265-2268. (c) Brousseau, L. C.; Novak, J. P.; Marinakos, S. M.; Feldheim, D. L. "Assembly of Phenylacetylene-Bridged Gold Nanocluster Dimers and Trimers" *Adv. Mater.* **1999**, *11*, 447-449. (d) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storrhoff, J. J. "A DNA-Based Method for Rationally Assembling Nanoparticles into Macroscopic Materials" *Nature* **1996**, *382*, 607-609. (e) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Lowteh, C. J.; Bruchez, M. P., Jr.; Schultz, P. G. "Organization of 'Nanocrystal Molecules' Using DNA" *Nature* **1996**, *382*, 609-622. (f) Boal, A. K.; Ilhan, F.; DeRouchey, J. E.; Thurn-Albrecht, T.; Russell, T. P.; Rotello, V. M. "Self-Assembly of Nanoparticles into Structured Spherical and Network Aggregates" *Nature* **2000**, *404*, 746-748. McIntosh, C. M.; Esposito, E. A.; Boal, A. K.; Simard, J. M.; Martin, C. T.; Rotello, V. M. "Inhibition of DNA Transcription Using Cationic Mixed Monolayer Protected Gold Clusters" *J. Am. Chem. Soc.* **2001**, *123*, 7626-7629.

(9) Schon, G.; Simon, U. "A Fascinating New Field in Colloidal Science: Small Ligand-Stabilized Metal-Clusters as Possible Application in Microelectronics. 1. State-of-the-Art" *Polym. Sci.* **1995**, *273*, 101-117. (b) Schon, G.; Simon, U. "A Fascinating New Field in Colloidal Science: Small Ligand-Stabilized Metal-Clusters as Possible Application in Microelectronics. 2. Future Directions" *Polym. Sci.* **1995**, *273*, 202-218.

(10) Murray, C. B.; Kagan, C. R.; Bawendi, M. G. "Synthesis and Characterization of Monodisperse Nanocrystals and Close-Packed Nanocrystal Assemblies" *Annu. Rev. Mater. Sci.* **2000**, *30*, 545-610. (b) Sun, S.; Murray, C. B.; Weller, D.; Folks, L.; Moser, A. "Monodisperse FePt Nanoparticles and Ferromagnetic FePt Nanocrystal Superlattices" *Science* **2000**, *287*, 1989-1992.

(11) Ingram, R. S.; Hostetler, M. J.; Murray, R. W. "Poly-hetero-ω-functionalized Alkanethiolate-Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1997**, *119*, 9175-9178.

(12) Ulman, A. “Formation and Structure of Self-Assembled Monolayers” *Chem. Rev.* **1996**, *96*, 1533-1554.

(13) Peczuh, M. W.; Hamilton, A. D. "Peptide and Protein Recognition by Designed Molecules" *Chem. Rev.* **2000**, *100*, 2479-2494.

(14) Boal, A. K.; Rotello, V. M. “Redox-Modulated Recognition of Flavin by Functionalized Gold Nanoparticles” *J. Am. Chem. Soc.* **1999**, *121*, 4914-4915.

(15) For examples, see: Breinlinger, E.; Rotello, V. M. “Model Systems for Flavoenzyme Activity. Modulation of Flavin Redox Potentials through π-Stacking Interactions” *J. Am. Chem. Soc.* **1997**, *119*, 1165-1166. Breinlinger, E.; Niemz, A.; Rotello, V. M. “Model Systems for Flavoenzyme Activity. Stabilization of the

Flavin Radical Anion through Specific Hydrogen Bond Interactions" *J. Am. Chem. Soc.* **1995**, *117*, 5379-5380.

(16) Boal, A. K.; Rotello, V. M. "Radial Control of Recognition and Redox Processes with Multivalent Hosts" *J. Am. Chem. Soc.* **2001**, *124*, 5019-5024.

(17) Fox, K.; Karplus, P. "The Flavin Environment in Old Yellow Enzyme-An Evaluation of Insights from Spectroscopic and Artificial Flavin Studies" *J. Biol. Chem.* **1999**, *274*, 9357-9362.

(18) Laibinis, P. E.; Whitesides, G. M. "Self-Assembled Monolayers of n-Alkanethiolates on Copper are Barrier Films that Protect the Metal against Oxidation by Air" *J. Am. Chem. Soc.* **1992**, *114*, 9022-9028. Kumar, A. K.; Biebuyck, H. A.; Whitesides, G. M. "Patterning Self-Assembled Monolayers-Applications in Materials Science" *Langmuir* **1994**, *10*, 1498-1511.

(19) Schoenfisch, M. H.; Pemberton, J. E. "Air Stability of Alkanethiol Self-Assembled Monolayers on Silver and Gold Surfaces" *J. Am. Chem. Soc.* **1998**, *120*, 4502-4513. Lee, M.-T.; Hsueh, C.-C.; Freund, M. S.; Ferguson, G. S. "Air Oxidation of Self-Assembled Monolayers on Polycrystalline Gold: The Role of the Gold Substrate" *Langmuir* **1998**, *14*, 6419-6423. Scott, J. R.; Baker, L. S.; Everett, W. R.; Wilkins, C. L.; Fritsch, I. "Laser Desorptio Fourier Transform Mass Spectrometry Exchange Studies of Air-Oxidized Alkanethiol Self-Assembled Monolayers on Gold" *Anal. Chem.* **1997**, *69*, 2636-2639.

(20) Jennings, G. K.; Laibinis, P. E. "Underpotentially Deposited Metal Layers of Silver Provide Enhanced Stability to Self-Assembled Alkanethiol Monolayers on Gold" *Langmuir* **1996**, *12*, 6173-6175. Jennings, G. K.; Laibinis, P. E. "Self-Assembled n-Alkanethiolate Monolayers on Underpotentially Deposited Adlayers of Silver and Copper on Gold" *J. Am. Chem. Soc.* **1997**, *119*, 5208-5214.

(21) Clegg, R. S.; Hutchison, J. E. "Hydrogen-Bonding, Self-Assembled Monolayers: Ordered Molecular Films for Study of Through-Peptide Electron Transfer" *Langmuir* **1996**, *12*, 5239-5243. Clegg, R. S.; Reed, S. M.; Hutchison, J. E. "Self-Assembled Monolayers Stabilized by Three-Dimensional Networks of Hydrogen Bonds" *J. Am. Chem. Soc.* **1998**, *120*, 2486-2487.

(22) Garg, N.; Lee, T. R. "Self-Assembled Monolayers Based on Chelating Aromatic Dithiols on Gold" *Langmuir* **1998**, *14*, 3815-3819. Whitesell, J. K.; Chang, H. K. "Directionally Aligned Helical Peptides on Surfaces" *Science* **1993**, *261*, 73-76. Wooster, T. T.; Gamm, P. R.; Geiger, W. E.; Oliver, A. M.; Black, A. J.; Graig, D. C.; Paddon-Row, M. N. "Synthesis and Surface-Confined Electrochemistry of Dimethoxynaphthalene Fused through Rigid Norbornylogous Spacers to Thiolate, Dithiolate, and Disulfide Groups" *Langmuir* **1996**, *12*, 6616-6626.

(23) Purcell, K. F.; Kotz, J. C. *Inorganic Chemistry*; W. B. Saunders: Philadelphia, **1977**. Huheey, J. E. *Inorganic Chemistry*; Harper Collins: Singapore, **1983**.

(24) Burns, J. A.; Whitesides, G. M. "Predicting the Stability of Cyclic Disulfides by Molecular Modeling-Effective Concentrations in Thiol-Disulfide Interchange and the Design of Strongly Reducing Thiols" *J. Am. Chem. Soc.* **1990**, *112*, 6296-6303. Biebuyck, H. A.; Whitesides, G. M. "Interchange between Monolayers on Gold Formed from Unsymmetrical Disulfides and Solutions of Thiols-Evidence for Sulfur Sulfur Bond-Cleavage by Gold Metal" *Langmuir* **1993**, *9*, 1766-1770. Kolega, R. R.; Schlenoff, J. B. "Self-Assembled Monolayers of an Aryl Thiol: Formation, Stability, and Exchange of Adsorbed 2-Naphthalenethiol and bis(2-naphthy) Disulfide on Gold" *Langmuir* **1998**, *14*, 5469-5478.

(25) Shon, Y.-S; Lee, T. R. "Chelating Self-Assembled Monolayers on Gold Generated from Sprioalkanedithiols" *Langmuir* **1999**, *15*, 1136-1140.

(26) Shon, Y.-S; Lee, T. R. "Desorption and Exchange of Self-Assembled Monolayers (SAMs) on Gold Generated from Chelating Alkanedithiols" *J. Phys. Chem. B* **2000**, *104*, 8192-8200.

(27) Walczak, M. M.; Alves, C. A.; Lamp, B. D.; Porter, M. D. "Electrochemical and X-Ray Photoelectron Spectroscopic Evidence for Differences in the Binding-Sites of Alkanethiolate Monolayers Chemisorbed at Gold" *J. Electroanal. Chem.* **1995**, *396*, 103-114.

(28) Kumar, A.; Whitesides, G. M. "Features of Gold Having Micrometer to Centimeter Dimensions Can Be Formed through a Combination of Stamping with an Elastomeric Stamp and an Alkanethiol "Ink" Followed by Chemical Etching" *Appl. Phys. Lett.* **1993**, *63*, 2002-2004. Gorman, C. B.; Biebuyck, H. A.; Whitesides, G. M. "Use of a Patterned Self-Assembled Monolayer to Control the Formation of a Liquid Resist Pattern on a Gold Surface" *Chem. Mater.* **1995**, *7*, 252-254.

(29) Templeton, A. C.; Hostetler, M. J.; Kraft, C. T.; Murray, R. W. "Reactivity of Monolayer-Protected Gold Cluster Molecules: Steric Effects" *J. Am. Chem. Soc.* **1998**, *120*, 1906-1911.

BIBLIOGRAPHY

- Corey, E. J.; Cheng, X.-M. *The Logic of Chemical Synthesis*; Wiley: New York, **1989**. (b) Nicolaou, K. C.; Sorensen, E. J. *Classics in Total Synthesis*; VCH: Weinheim, Germany, **1995**.

- Nicolaou, K. C. "The Total Synthesis of Brevetoxin B: A Twelve-year Odyssey in Organic Synthesis" *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 589-607.

- Whitesides, G. M. "What Will Chemistry Do in the Next Twenty Years?" *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1209-1218. (b) Seebach, D. "Organic Synthesis-Where Now?" *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1320-1367.

- *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Pergamon: Oxford, U.K., **1996**.

- Lehn, J.-M. *Supramolecular Chemistry*; VCH: Weinheim, Germany, **1995**.

- Pedersen, C. J. "Cyclic Polyethers and Their Complexes with Metal Salts" *J. Am. Chem. Soc.* **1967**, *89*, 7017-7036.

- Lehn, J.-M. *Struct. Bonding*; Berlin, Germany, **1973**.

- Cram, D. J.; Cram, J. M. *Science* **1974**, *183*, 803-809.

- For Nobel lectures see: (a) Pedersen, C. J. "The Discovery of Crown Ethers" *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1021-1027. (b) Lehn, J.-M. "Supramolecular Chemistry-Scope and Perspectives: Molecules, Supermolecules and Molecular Devices" *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 89-112. (c) Cram, D. J. "The Design of Molecular Hosts, Guests, and Their Complexes" *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1009-1020.

- Whitesides, G. M. "Self-Assembling Materials" *Sci. Am.* **1995**, *273* (3), 146-149.

- Whitesides, G. M.; Simanek, E. E.; Mathias, J. P.; Seto, C. T.; Chin, D. N.; Mammen, M.; Gordon, D. M. "Noncovalent Synthesis: Using Physical-Organic Chemistry to Make Aggregates" *Acc. Chem. Res.* **1995**, *28*, 37-44.

- Janin, J.; Miller, S.; Chothia, C. "Surface, Subunit Interfaces and Interior of Oligomeric Proteins" *J. Mol. Biol.* **1988**, *204*, 155-164.

- Conn, M. M.; Rebek, J., Jr. "Self-Assembling Capsules" *Chem. Rev.* **1997**, *97*, 1647-1668.

- Seto, C. T.; Whitesides, G. M. "Molecular Self-Assembly through Hydrogen-Bonding Supramolecular Aggregates Based on the Cyanuric Acid:Melamine Lattice" *J. Am. Chem. Soc.* **1993**, *115*, 905-916.

- Mathias, J. P.; Simanek, E. E.; Whitesides, G. M. "Self-Assembly through Hydrogen-Bonding-Peripheral Crowding-A New Strategy for the Preparation of Stable Supramolecular Aggregates Based on Parallel, Connected CA(3).M(3) Rosettes" *J. Am. Chem. Soc.* **1994**, *116*, 4326.

- Zerkowski, J. A.; MacDonald, J. C.; Seto, C. T.; Wierda, D. A.; Whitesides, G. M. "Design of Organic Structures in the Solid-State-Molecular Tapes Based on the Network of Hydrogen-Bonds Present in the Cyanuric Acid:Melamine Complex" *J. Am. Chem. Soc.* **1994**, *116*, 2382.

- Mathias, J. P.; Seto, C. T.; Simanek, E. E.; Whitesides, G. M. "Self-Assembly through Hydrogen-Bonding-Preparation and Characterization of Three New Types of Supramolecular Aggregates Based on Parallel Cyclic CA(3).M(3) Rosettes" *J. Am. Chem. Soc.* **1994**, *116*, 1725.

- Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry, Part III*; W. H. Freeman: San Francisco, **1980**.

- Pörschke, D.; Eigen, M. *J. Mol. Biol.* **1971**, *62*, 361.

- Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, **1984**.

- Philp, D.; Stoddart, J. F. "Self-Assembly in Natural and Unnatural Systems" *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1154-1196.

- Saenger, W. "Cyclodextrin Inclusion Compounds in Research and Industry" *Angew. Chem. Int. Ed. Engl.* **1980**, *82*, 343.

- Müller, N. "Search for a Realistic View of Hydrophobic Effects" *Acc. Chem. Res.* **1990**, *23*, 23.

- Schardinger, F. Z. Unters. Nahr. Genussm. Gebrauchsgegenstaende **1903**, 6, 865.

- Griffiths, D. W.; Bender, M. L. *Adv. Catal.* **1973**, *23*, 209.

- Stoddart, J. F. "Cyclodextrins, Off-the-Shelf Components for the Construction of Mechanically Interlocked Molecular-Systems" *Angew. Chem. Int. Ed. Engl.* **1992**, *31***,** 846.

- Branden, C.; Tooze, J. *Introduction to Protein Structure*; Garland: New York, **1991**.

- Peczuh, M. W.; Hamilton, A. D. "Peptide and Protein Recognition by Designed Molecules" *Chem. Rev.* **2000**, *100*, 2479-2494.

- Chemistry and Biochemistry of Flavoenzymes; Müller, F., Ed.; CRC: Boca Raton, **1991**.

- Massey, V.; Hemmerich, P. "Active-site Probes of Flavoproteins" *Biochem. Soc. Trans.* **1980**, *8*, 246-257.

- Cuello, A. O.; McIntosh, C. M.; Rotello, V. M. "Model Systems for Flavoenzyme Activity. The Role of N(3)-H Hydrogen Bonding in Flavin Redox Processes" *J. Am. Chem. Soc.* **2000**, *122*, 3517-3521.

- Sanner, C.; Macheroux, P.; Ruterjans, H.; Müller, F.; Bacher, A. *Eur. J. Chem.* **1985**, *151*, 49-65. Stockman, B.; Krezel, A.; Markley, J.; Leonhardt, K.; Straus, H. "^{1}H, ^{13}C, and ^{15}N NMR Spectroscopy of Anabaena-7120 Flavodoxin-Assignment of β-Sheet and Flavin Binding-Site Resonances and Analysis of Protein-Flavin Interactions" *Biochemistry* **1990**, *29*, 9600-9609. Fox, K.; Karplus, P. "The Flavin Environment in Old Yellow Enzyme-An Evaluation of Insights from Spectroscopic and Artificial Flavin Studies" *J. Biol. Chem.* **1999**, *274*, 9357-9362.

- Swenson, R.; Krey, G. "Site-Directed Mutagenesis of Tyrosine-98 in the Flavodoxin from *Desulfovibrio vulgaris*-Regulation of Oxidation-Reduction Properties of the Bound FMN Cofactor by Aromatic, Solvent, and Electrostatic Interactions" *Biochemistry* **1994**, *33*, 8505-8514. Watt, W.; Tulinsky, A.; Swenson, R.; Wattenpugh, K. "Comparison of the Crystal-Structures of a Flavodoxin in its Three Oxidation States at Cryogenic Temperatures" *J. Mol. Biol.* **1991**, *218*, 195-208. Schreuder, H.; van der Laan, J.; Hol, W.; Drenth, J. "Crystal-Structure of Para-Hydroxybenzoate Hydroxylase Complexed with its Reaction Product 3,4-Dihydroxybenzoate" *J. Mol. Biol.* **1988**, *199*, 637-648. Palfey, B.; Moran, G.; Entsch, B.; Ballou, D.; Massey, V. "Substrate Recognition by 'Password' in p-Hydroxybenzoate Hydroxylase" *Biochemistry* **1999**, *38*, 1153-1158.

- Zhou, Z.; Swenson, R. "Electrostatic Effects of Surface Acidic Amino-Acid-Residues on the Oxidation-Reduction Potentials of the Flavodoxin from *Desulfovibrio vulgaris*" *Biochemistry* **1995**, *34*, 3183-3192. Zhou, Z.; Swenson, R. "Evaluation of the Electrostatic Effect of the 5'-phosphate of the Flavin Mononucleotide Cofactor on the Oxidation-Reduction Potentials of the Flavodoxin from *Desulfovibrio vulgaris*" *Biochemistry* **1996**, *35*, 12443-12454. Aliverti, A.; Deng, Z.; Ravasi, D.; Piubelli, L.; Karplus, P.; Zanetti, G. "Probing the Function of the Invariant Glutamyl Residue 312 in spinach Ferredoxin-NADP(+) Reductase" *J. Biol. Chem.* **1998**, *273*, 34008-34015.

- Grell, E.; Warmuth, R. "Caged Cations" *Pure Appl. Chem.* **1993**, *65*, 373-379. Lehn, J.-M. *Supramolecular Chemistry: Concepts and Perspectives*; VCH: New York, **1995**.

- Diederich, F. "Complexation of Neutral Molecules by Cyclophane Hosts" *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 362-386.

- James, T. D.; Sandanayake K. R. A. S.; Shinkai, S. "Saccharide Sensing with Molecular Receptors Based on Boronic Acid" *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1910-1922.

- Schmidtchen, F. P.; Berger, M. "Artificial Organic Host Molecules for Anions" *Chem. Rev.* **1997**, *97*, 1609-1646; Linton, B.; Hamilton, A. D. "Calorimetric Investigation of Guanidinium-Carboxylate Interactions" *Tetrahedron* **1999**, *55*, 6027-6038.

- Lavigne, J. J.; Anslyn, E. V. "Sensing a Paradigm Shift in the Field of Molecular Recognition: From Selective to Differential Receptors" *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 3118-3130.

- For studies on flavoenzyme mechanisms, see: (a) Kim, J. M.; Bogdan, M. A.; Mariano, P. S. *J. Am. Chem. Soc.* **1993**, *115*, 10591. (b) Hemmerich, P. *The Enzymes, Vol. XII*; Boyer, P. D., Ed.; Academic Press: New York, **1976**. (c) Bach, R.; Su, M.-D. *J. Am. Chem. Soc.* **1994**, *116*, 5392.

- For previously published model systems for flavoenzymes, see: (a) Levine, H. L.; Kaiser, E. T. "Oxidation of Dihydronicotinamides by Flavopapain" *J. Am. Chem. Soc.* **1978**, *100*, 7670. (b) Schultz, P. G. "Catalytic Antibodies" *Acc. Chem. Res.* **1989**, *22*, 287. (c) Tabushi, I.; Kodera, M. "Flavocyclodextrin as a Promising Flavoprotein Model-Efficient Electron-Transfer Catalysis by Flavocyclodextrin" *J. Am. Chem. Soc.* **1987**, *109*, 4734. (d) Akiyama, T.; Simeno, F.; Murakami, M.; Yoneda, F. "Flavin-6-Carboxylic Acid as Novel and simple Flavoenzyme Models-Nonenzymatic Stabilization of the Flavin Semiquinone Radical and the 4A-Hydroperoxyflavin by Intramolecular Hydrogen-Bonding" *J. Am. Chem. Soc.* **1992**, *114*, 6613. (e) Takeda, J.; Ohta, S.; Hirobe, M. "Synthesis and Characterization of Novel Flavin-Linked Porphyrins-Mechanism for Flavin-Catalyzed Intermolecular and Intramolecular-2e/1e Electron-Transfer Reactions" *J. Am. Chem. Soc.* **1987**, *109*, 7677.

- Breinlinger, E.; Niemz, A.; Rotello, V. M. "Model Systems for Flavoenzyme Activity. Stabilization of the Flavin Radical Anion through Specific Hydrogen Bond Interactions" *J. Am. Chem. Soc.* **1995**, *117*, 5379-5380.

- Stockman, B. J.; Richardson, T. E.; Swenson, R. P. "Structural Changes Cause by Site-Directed Mutagenesis of Tyrosine-98 in *Desulfovibrio vulgaris* Flavodoxin Delineated by ^{1}H and ^{15}N NMR Spectroscopy-Implications for Redox Potential Modulation" *Biochemistry* **1994**, *33*, 15298-15308.

- Breinlinger, E.; Rotello, V. M. "Model Systems for Flavoenzyme Activity. Modulation of Flavin Redox Potentials through π-Stacking Interactions" *J. Am. Chem. Soc.* **1997**, *119*, 1165-1166.

- Nowick, J. S.; Ballester, P.; Ebmeyer, F.; Rebek, J. "Convergent Functional Groups. 9. Complexation in New Molecular Clefts" *J. Am. Chem. Soc.* **1990**, *112*, 8902-8906. Park, T.; Schroeder, J.; Rebek, J. "New Molecular Complements to Imides-Complexation of Thymine Derivatives" *J. Am. Chem. Soc.* **1991**, *113*, 5125-5127.

- Ulman, A. An Introduction to Ultrathin Organic Films; Academic Press: Boston, 1991.

- Bigelow, W. C.; Pickett, D. L.; Zisman, W. A. *J. Colloid Interface Sci.* **1946**, *1*, 513.

- Nuzzo, R. G.; Allara, D. L. "Adsorption of Bifunctional Organic Disulfides on Gold Surfaces" *J. Am. Chem. Soc.* **1983**, *105*, 4481-4483.

- Kuhn, H.; Ulman, A. In *Thin Films*; Ulman, A., Ed.; Academic Press: New York, 1995; Vol. 20.

- Ulman, A. "Formation and Structure of Self-Assembled Monolayers" *Chem. Rev.* **1996**, *96*, 1533-1554.

- Schmid, G., Ed. *Clusters and Colloids*; VCH: Weinheim, **1994**. (b) Hayat, M. A., Ed. *Colloidal Gold: Principles, Methods, and Applications*; Academic Press: New York, **1989**; Vols. 1-2.

- Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R. "Synthesis of Thiol-derivatised Gold Nanoparticles in a Two-Phase Liquid-Liquid System" *J. Chem. Soc., Chem. Commun.* **1994**, 801-802.

- Hostetler, M. J.; Wingate, J. E.; Zhong, C.-Z.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. "Alkanethiolate Gold Cluster Molecules with Core Diameters from 1.5 to 5.2 nm: Core and Monolayer Properties as a Function of Core Size" *Langmuir* **1998**, *14*, 17-30.

- Philp, D.; Stoddart, J. F. "Self-Assembly in Natural and Unnatural Systems" *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1154-1196.

- Leff, D. V.; O'Hara, P. C.; Heath, J. R.; Gelbart, W. M. "Thermodynamic Control of Gold Nanocrystal Size: Experiment and Theory" *J. Phys. Chem.* **1995**, *99*, 7036-7041.

- Whetten, R. L.; Khoury, J. T.; Alvarez, M. M.; Murthy, S.; Vezmar, I.; Wang, Z. L.; Stephen, P. W.; Cleveland, C. L.; Luedtke, W. D.; Landman, U. "Nanocrystal Gold Molecules" *Adv. Mater.* **1996**, *5*, 428-433.

- Schaaf, T. G.; Shafigullin, M. N.; Khoury, J. T.; Vezmar, I.; Whetten, R. L.; Cullen, W.; First, P. N.; Gutierrez-Wing, C.; Ascensio, J.; Jose-Yacaman, M. J. "Isolation of Smaller Nanocrystal Au Molecules: Robust Quantum Effects in Optical Spectra" *J. Phys. Chem. B* **1997**, *101*, 7885-7891. (b) Alvarez, M. M.; Khoury, J. T.; Schaaf, T. G.; Shafigulin, M.; Vezmar, I.; Whetten, R. L. "Critical States in the Growth of Au Clusters" *Chem. Phys. Lett.* **1997**, *266*, 91-98.

- Schaaf, T. G.; Knight, G.; Shafigullin, M. N.; Borkman, R. F.; Whetten, R. L. "Isolation and Selected Properties of a 10.4 kDa Gold:Glutathione Cluster Compound" *J. Phys. Chem. B* **1998**, *102*, 10643-10646. (b) Templeton, A. C.; Chen, S.; Gross, S. M.; Murray, R. W. "Water-Soluble, Isolable Gold Clusters Protected by Tiopronin and Coenzyme A Monolayers" *Langmuir* **1999**, *15*, 66-76. (c) Wuelfing, W. P.; Gross, S. M.; Miles, D. T.; Murray, R. W. "Nanometer Gold Clusters Protected by Surface-Bound Monolayers of Thiolated Poly(ethylene glycol) Polymer Electrolyte" *J. Am. Chem. Soc.* **1998**, *120*, 12696-12697. (d) Chen, S.; Kimura, K. "Synthesis and Characterization of Carboxylate-Modified Gold Nanoparticle Powders Dispersible in Water" *Langmuir* **1999**, *15*, 1075-1082. (e) Chen, S.; Murray, R. W. "Arenethiolate Monolayer-Protected Gold Clusters" *Langmuir* **1999**, *15*, 682-689. (f) Johnson, S. R.; Evans, S. D.; Mahon, S. W.; Ulman, A. "Alkanethiol Molecules Containing an Aromatic Moiety Self-Assembled onto Gold Clusters" *Langmuir* **1997**, *13*, 51-57. (g) Brust, M.; Fink, J.; Bethell, D.; Schiffrin, D. J.; Kiely, C. "Synthesis and Reactions of Functionalised Gold Nanoparticles" *J. Chem. Soc., Chem. Commun.* **1995**, 1655-1656. (h) Buining, P. A.; Humbel, B. M.; Philipse, A. P. Verkleij, A. J. "Preparation of Functional Silane-Stabilized Gold colloids in the (Sub)nanometer Size Range" *Langmuir* **1997**, *13*, 3921-3926.

- Bethell, D.; Schiffrin, M.; Schiffrin, D. J.; Kiely, C. "From Monolayers to Nanostructured Materials: An Organic Chemist's View of Self-Assembly" *J. Electroanal. Chem.* **1996**, *409*, 137-143.

- Templeton, A. C.; Hostetler, M. J.; Kraft, C. T.; Murray, R. W. "Reactivity of Monolayer-Protected Gold Cluster Molecules: Steric Effects" *J. Am. Chem. Soc.* **1998**, *120*, 1906-1911.

- Hostetler, M. J.; Green, S. J.; Stokes, J. J.; Murray, R. W. "Monolayers in Three Dimensions: Synthesis and Electrochemistry of ω-Functionalized Alkanethiolate- Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1996**, *118*, 4212-4213. (b) Ingram, R. S.; Hostetler, M. J.; Murray, R. W. "Poly-hetero-ω-functionalized Alkanethiolate-Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1997**, *119*, 9175. (c) Hostetler, M. J.; Templeton, A. C.; Murray, R. W. "Dynamics of Place-Exchange Reactions on Monolayer-Protected Gold Cluster Molecules" *Langmuir* **1999**, *15*, 3782-3789.

- Terrill, R. H.; Postlethwaite, T. A.; Chen, C.-H.; Poon, C.-D.; Terzis, A.; Chen,A.; Hutchison, J. E.; Clark, M. R.; Wignall, G.; Londono, J. D.; Superfine, R.; Falvo, M.; Johnson, C. S., Jr.; Samulski, E. T.; Murray, R. W. "Monolayers in Three Dimensions: NMR, SAXS, Thermal, and Electron Hopping Studies of Alkanethiol Stabilized Gold Clusters" *J. Am. Chem. Soc.* **1995**, *117*, 12537-12547.

- Fink, J.; Kiely, C. J.; Bethell, D.; Schiffrin, D. J. "Self-Organization of Nanosized Gold Particles" *Chem. Mater.* **1998**, *10*, 922-926.

- Badia, A.; Cuccia, L.; Demers, L.; Morin, F.; Lennox, R. B. "Structure and Dynamics in Alkanethiolate Monolayers Self-Assembled on Gold Nanoparticles: A DSC, FT-IR, and Deuterium NMR Study" *J. Am. Chem. Soc.* **1997**, *119*, 2682-2692.

- Badia, A.; Singh, S.; Demers, L.; Cuccia, L.; Brown, G. B.; Lennox, R. B. "Self-Assembled Monolayers on Gold Nanoparticles" *Chem. Eur. J.* **1996**, *2*, 359-363. (b) Badia, A.; Gao, W.; Singh, S.; Demers, L.; Cuccia, L.; Reven, L. "Structure and Chain Dynamics of Alkanethiol-Capped Gold Colloids" *Langmuir* **1996**, *12*, 1262-1269. (c) Badia, A.; Demers, L.; Dickinson, L.; Morin, F. G.; Lennox, R. B.; Reven, L. "Gold-Sulfur Interactions in Alkylthiol Self-Assembled Monolayers Formed on Gold Nanoparticles Studied by Solid-State NMR" *J. Am. Chem. Soc.* **1997**, *119*, 11104-11105.

- Bar, G.; Rubin, S.; Cutts, R. W.; Taylor, T. N.; Zawodzinski, T. A. "Dendrimer-Modified Silicon Oxide surfaces as Platforms for the Deposition of Gold and Silver Colloid Monolayers: Preparation Method, Characterization, and Correlation Between Microstructure and Optical Properties" *Langmuir* **1996**, *12*, 1172−1179.

- Ingram, R. S.; Hostetler, M. J.; Murray, R. W. "Poly-hetero−ω-functionalized Alkanethiolate-Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1997**, *119*, 9175-9178.

- Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R. “Synthesis of Thiol-derivatised Gold Nanoparticles in a Two-Phase Liquid-Liquid System” *J. Chem. Soc., Chem. Commun.* **1994**, 801-802.

- Hostetler, M. J.; Green, S. J.; Stokes, J. J.; Murray, R. W. "Monolayers in Three Dimensions: Synthesis and Electrochemistry of ω-Functionalized Alkanethiolate-Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1996**, *118*, 4212-4213. (b) Ingram, R. S.; Hostetler, M. J.; Murray, R. W. "Poly-hetero-ω-functionalized Alkanethiolate-Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1997**, *119*, 9175. (c) Hostetler, M. J.; Templeton, A. C.; Murray, R. W. "Dynamics of Place-Exchange Reactions on Monolayer-Protected Gold Cluster Molecules" *Langmuir* **1999**, *15*, 3782-3789.

- Hostetler, M. J.; Wingate, J. E.; Zhong, C.-Z.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. "Alkanethiolate Gold Cluster Molecules with Core Diameters from 1.5 to 5.2 nm: Core and Monolayer Properties as a Function of Core Size" *Langmuir* **1998**, *14*, 17-30.

- Li, H.; Luk, Y.-Y.; Mrksich, M. "Catalytic Asymmetric Dihydroxylation by Gold Colloids Functionalized with Self-Assembled Monolayers" *Langmuir* **1999**, *15*, 4957-4959.

- Bruchez, M., Jr.; Moronne, M.; Gin, P.; Weiss, S.; Alivasatos, A. P. "Semiconductor Nanocrystals as Fluorescent Biological Labels" *Science* **1998**, *281*, 2013-2016. (b) Chan, W. C. W.; Nie, S. "Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection" *Science* **1998**, *281*, 2016-2018. (c) Sampath, S.; Lev, O. "3D Organized Self-Assembled Monolayer Electrodes: A Novel Biosensor Configuration" *Adv. Mater.* **1997**, *9*, 410-412.

- Kaifer, A. E. "Interplay Between Molecular Recognition and Redox Chemistry" *Acc. Chem. Res.* **1999**, *32*, 62-71. (b) Liu, J.; Xu, R. L.; Kaifer, A. E. "In Situ Modification of the Surface of Gold Colloidal Particles: Preparation of Cyclodextrin-Based Rotaxanes Supported on Gold Nanospheres" *Langmuir* **1998**, *14*, 7337-7339.

- Watson, K. J.; Zhu, J.; Nguyen, S. T.; Mirkin, C. A. "Hybrid Nanoparticles with Block Copolymer Shell Structures" *J. Am. Chem. Soc.* **1999**, *121*, 402-403.

- Mucic, R. C.; Storhoff, J. J.; Mirkin, C. A.; Letsinger, R. L. "DNA-Directed Synthesis of Binary Nanoparticle Network Materials" *J. Am. Chem. Soc.* **1998**,*120*, 12674-12675. (b) Niemeyer, C. M.; Bürger, W.; Peplies, J. "Covalent DNA-Streptavidin Conjugates as Building Blocks for Novel Biometallic Nanostructures" *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2265-2268. (c) Brousseau, L. C.; Novak, J. P.; Marinakos, S. M.; Feldheim, D. L. "Assembly of Phenylacetylene-Bridged Gold Nanocluster Dimers and Trimers" *Adv. Mater.* **1999**, *11*, 447-449. (d) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storrhoff, J. J. "A DNA-Based Method for Rationally Assembling Nanoparticles into Macroscopic Materials" *Nature* **1996**, *382*, 607-609. (e) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Lowteh, C. J.; Bruchez, M. P., Jr.; Schultz, P. G. "Organization of 'Nanocrystal Molecules' Using DNA" *Nature* **1996**, *382*, 609-622. (f) Boal, A. K.; Ilhan, F.; DeRouchey, J. E.; Thurn-Albrecht, T.; Russell, T. P.; Rotello, V. M. "Self-Assembly of Nanoparticles into Structured Spherical and Network Aggregates" *Nature* **2000**, *404*, 746-748. McIntosh, C. M.; Esposito, E. A.; Boal, A. K.; Simard, J. M.; Martin, C. T.; Rotello, V. M. "Inhibition of DNA Transcription Using Cationic Mixed Monolayer Protected Gold Clusters" *J. Am. Chem. Soc.* **2001**, *123*, 7626-7629.

- Schon, G.; Simon, U. "A Fascinating New Field in Colloidal Science: Small Ligand-Stabilized Metal-Clusters as Possible Application in Microelectronics. 1. State-of-the-Art" *Polym. Sci.* **1995**, *273*, 101-117. (b) Schon, G.; Simon, U. "A Fascinating New Field in Colloidal Science: Small Ligand-Stabilized Metal-Clusters as Possible Application in Microelectronics. 2. Future Directions" *Polym. Sci.* **1995**, *273*, 202-218.

- Murray, C. B.; Kagan, C. R.; Bawendi, M. G. "Synthesis and Characterization of Monodisperse Nanocrystals and Close-Packed Nanocrystal Assemblies" *Annu. Rev. Mater. Sci.* **2000**, *30*, 545-610. (b) Sun, S.; Murray, C. B.; Weller, D.; Folks, L.; Moser, A. "Monodisperse FePt Nanoparticles and Ferromagnetic FePt Nanocrystal Superlattices" *Science* **2000**, *287*, 1989-1992.

- Ingram, R. S.; Hostetler, M. J.; Murray, R. W. "Poly-hetero-ω-functionalized Alkanethiolate-Stabilized Gold Cluster Compounds" *J. Am. Chem. Soc.* **1997**, *119*, 9175-9178.

- Ulman, A. “Formation and Structure of Self-Assembled Monolayers” *Chem. Rev.* **1996**, *96*, 1533-1554.

- Peczuh, M. W.; Hamilton, A. D. "Peptide and Protein Recognition by Designed Molecules" *Chem. Rev.* **2000**, *100*, 2479-2494.

- Boal, A. K.; Rotello, V. M. “Redox-Modulated Recognition of Flavin by Functionalized Gold Nanoparticles” *J. Am. Chem. Soc.* **1999**, *121*, 4914-4915.

- For examples, see: Breinlinger, E.; Rotello, V. M. "Model Systems for Flavoenzyme Activity. Modulation of Flavin Redox Potentials through π-Stacking Interactions" *J. Am. Chem. Soc.* **1997**, *119*, 1165-1166. Breinlinger, E.; Niemz, A.; Rotello, V. M. "Model Systems for Flavoenzyme Activity. Stabilization of the Flavin Radical Anion through Specific Hydrogen Bond Interactions" *J. Am. Chem. Soc.* **1995**, *117*, 5379-5380.

- Boal, A. K.; Rotello, V. M. "Radial Control of Recognition and Redox Processes with Multivalent Hosts" *J. Am. Chem. Soc.* **2001**, *124*, 5019-5024.

- Fox, K.; Karplus, P. "The Flavin Environment in Old Yellow Enzyme-An Evaluation of Insights from Spectroscopic and Artificial Flavin Studies" *J. Biol. Chem.* **1999**, *274*, 9357-9362.

- Laibinis, P. E.; Whitesides, G. M. "Self-Assembled Monolayers of n-Alkanethiolates on Copper are Barrier Films that Protect the Metal against Oxidation by Air" *J. Am. Chem. Soc.* **1992**, *114*, 9022-9028. Kumar, A. K.; Biebuyck, H. A.; Whitesides, G. M. "Patterning Self-Assembled Monolayers-Applications in Materials Science" *Langmuir* **1994**, *10*, 1498-1511.

- Schoenfisch, M. H.; Pemberton, J. E. "Air Stability of Alkanethiol Self-Assembled Monolayers on Silver and Gold Surfaces" *J. Am. Chem. Soc.* **1998**, *120*, 4502-4513. Lee, M.-T.; Hsueh, C.-C.; Freund, M. S.; Ferguson, G. S. "Air Oxidation of Self-Assembled Monolayers on Polycrystalline Gold: The Role of the Gold Substrate" *Langmuir* **1998**, *14*, 6419-6423. Scott, J. R.; Baker, L. S.; Everett, W. R.; Wilkins, C. L.; Fritsch, I. "Laser Desorptio Fourier Transform Mass Spectrometry Exchange Studies of Air-Oxidized Alkanethiol Self-Assembled Monolayers on Gold" *Anal. Chem.* **1997**, *69*, 2636-2639.

- Jennings, G. K.; Laibinis, P. E. "Underpotentially Deposited Metal Layers of Silver Provide Enhanced Stability to Self-Assembled Alkanethiol Monolayers on Gold" *Langmuir* **1996**, *12*, 6173-6175. Jennings, G. K.; Laibinis, P. E. "Self-Assembled n-Alkanethiolate Monolayers on Underpotentially Deposited Adlayers of Silver and Copper on Gold" *J. Am. Chem. Soc.* **1997**, *119*, 5208-5214.

- Clegg, R. S.; Hutchison, J. E. "Hydrogen-Bonding, Self-Assembled Monolayers: Ordered Molecular Films for Study of Through-Peptide Electron Transfer" *Langmuir* **1996**, *12*, 5239-5243. Clegg, R. S.; Reed, S. M.; Hutchison, J. E. "Self-Assembled Monolayers Stabilized by Three-Dimensional Networks of Hydrogen Bonds" *J. Am. Chem. Soc.* **1998**, *120*, 2486-2487.

- Garg, N.; Lee, T. R. "Self-Assembled Monolayers Based on Chelating Aromatic Dithiols on Gold" *Langmuir* **1998**, *14*, 3815-3819. Whitesell, J. K.; Chang, H. K. "Directionally Aligned Helical Peptides on Surfaces" *Science* **1993**, *261*, 73-76. Wooster, T. T.; Gamm, P. R.; Geiger, W. E.; Oliver, A. M.; Black, A. J.; Graig, D. C.; Paddon-Row, M. N. "Synthesis and Surface-Confined Electrochemistry of Dimethoxynaphthalene Fused through Rigid Norbornylogous Spacers to Thiolate, Dithiolate, and Disulfide Groups" *Langmuir* **1996**, *12*, 6616-6626.

- Purcell, K. F.; Kotz, J. C. *Inorganic Chemistry*; W. B. Saunders: Philadelphia, **1977**. Huheey, J. E. *Inorganic Chemistry*; Harper Collins: Singapore, **1983**.

- Burns, J. A.; Whitesides, G. M. "Predicting the Stability of Cyclic Disulfides by Molecular Modeling-Effective Concentrations in Thiol-Disulfide Interchange and the Design of Strongly Reducing Thiols" *J. Am. Chem. Soc.* **1990**, *112*, 6296-6303. Biebuyck, H. A.; Whitesides, G. M. "Interchange between Monolayers on Gold Formed from Unsymmetrical Disulfides and Solutions of Thiols-Evidence for Sulfur Sulfur Bond-Cleavage by Gold Metal" *Langmuir* **1993**, *9*, 1766-1770. Kolega, R. R.; Schlenoff, J. B. "Self-Assembled Monolayers of an Aryl Thiol: Formation, Stability, and Exchange of Adsorbed 2-Naphthalenethiol and bis(2-naphthy) Disulfide on Gold" *Langmuir* **1998**, *14*, 5469-5478.

- Shon, Y.-S; Lee, T. R. "Chelating Self-Assembled Monolayers on Gold Generated from Sprioalkanedithiols" *Langmuir* **1999**, *15*, 1136-1140.

- Shon, Y.-S; Lee, T. R. "Desorption and Exchange of Self-Assembled Monolayers (SAMs) on Gold Generated from Chelating Alkanedithiols" *J. Phys. Chem. B* **2000**, *104*, 8192-8200.

- Walczak, M. M.; Alves, C. A.; Lamp, B. D.; Porter, M. D. "Electrochemical and X-Ray Photoelectron Spectroscopic Evidence for Differences in the Binding-Sites of Alkanethiolate Monolayers Chemisorbed at Gold" *J. Electroanal. Chem.* **1995**, *396*, 103-114.

- Kumar, A.; Whitesides, G. M. "Features of Gold Having Micrometer to Centimeter Dimensions Can Be Formed through a Combination of Stamping with an Elastomeric Stamp and an Alkanethiol "Ink" Followed by Chemical Etching" *Appl. Phys. Lett.* **1993**, *63*, 2002-2004. Gorman, C. B.; Biebuyck, H. A.; Whitesides, G. M. "Use of a Patterned Self-Assembled Monolayer to Control the Formation of a Liquid Resist Pattern on a Gold Surface" *Chem. Mater.* **1995**, *7*, 252-254.

- Templeton, A. C.; Hostetler, M. J.; Kraft, C. T.; Murray, R. W. “Reactivity of Monolayer-Protected Gold Cluster Molecules: Steric Effects” *J. Am. Chem. Soc.* **1998**, *120*, 1906-1911.