

©Copyright 2023

Kacper J. Lachowski

Bottom-Up Synthesis of Colloidal Systems Using Sequence Defined Molecules

Kacper J. Lachowski

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

Doctor of Philosophy

University of Washington

2023

Reading Committee:

Lilo D. Pozzo, Chair

James J. De Yoreo

Suzie H. Pun

Program Authorized to Offer Degree:

Molecular Engineering

University of Washington

Abstract

Bottom-Up Synthesis of Colloidal Systems Using Sequence Defined Molecules

Kacper J. Lachowski

Chair of the Supervisory Committee:
Boeing-Roundhill Professor for Excellence in Engineering Lilo D. Pozzo
Chemical Engineering

Self-assembled colloidal nanoparticles can be used to deliver vaccines, sense pathogenic materials, and mark tumors. In response to light, they catalyze the formation of clean fuels or help transform it directly into usable energy. Quantum dots are used in commercial displays and will be a key step in new forms of computing and information storage. The performance of nanoparticles is dictated by their structure and composition and can be controlled using sequence defined molecules. The latter include any polymeric or oligomeric materials in which the exact sequence is precisely controlled using enzymatic processes or synthetic chemistry. Their physicochemical diversity and modularity are used to intervene in chemical processes occurring during synthesis, stabilize specific crystal facets, or form templates that guide nanomaterial growth. Yet the multivariate relationship between experimental parameters and intermolecular reactions that govern nanomaterial self-assembly is difficult to study using traditional experimental methods.

In this work, gold nanoparticle synthesis in the presence of peptides is used as a model system for developing and integrating experimental automation with computational approaches to extract information for guiding sequence design. Several peptide variants were selected through systematic variations of a gold binding peptide, and nanoparticles were synthesized using a liquid handling robot in a large design space of reagent concentrations. The plasmonic response of nanoparticles was used as a fast proxy for changes in structures and

was analyzed using functional data analysis methods. The analysis resulted in a metric for quantifying how changes in peptide design affect nanoparticle synthesis outcomes, and the conclusions were corroborated with small-angle X-ray scattering and electron microscopy. Next, the relationship between substitution of methionine in a peptide sequence and an increase in particle anisotropy was assessed. A programmed liquid handling robot was used to dynamically intervene in nanoparticle synthesis to control the resulting structure and stability of anisotropic nanoparticles. Finally, highlights of how small-angle X-ray scattering can work in parallel with computational methods to study colloidal self-assembly mechanisms are presented.

TABLE OF CONTENTS

	Page
List of Figures	v
List of Tables	xi
Chapter 1: Introduction and Motivation	1
1.1 Objectives	1
1.2 Inorganic Nanomaterials	2
1.2.1 Properties and Applications	2
1.2.2 Overview of Synthesis Methods	5
1.3 Sequence Defined Molecules for Bioinspired Nanomaterial Synthesis	11
1.3.1 Biomineralization	11
1.3.2 Bioinspired Nanomaterial Synthesis	12
1.3.3 Design and Use of Sequence Defined Molecules	14
Chapter 2: Theoretical Background	17
2.1 Intermolecular Forces and Colloidal Stability	17
2.1.1 Intermolecular Forces	17
2.1.2 Colloidal Interactions and Stability	20
2.1.3 Interparticle Interactions and DLVO Theory	21
2.2 Nucleation and Particle Growth	26
2.2.1 Classical Nucleation Theory	26
2.2.2 Particle Growth	28
2.2.3 Growth of Metal Nanoparticles	31
2.3 Surface Plasmon Resonance	33
2.3.1 Fundamentals and Origin	33
2.3.2 Mie Scattering	34
2.3.3 UV-Vis Spectroscopy as a Proxy for Structural Changes	35

Chapter 3:	Experimental Methods	38
3.1	Small-Angle Scattering	38
3.1.1	General Theory	39
3.1.2	Instrumentation	41
3.2	Optical Spectroscopy	43
3.2.1	Ultraviolet and Visible Light Spectroscopy	43
3.2.2	Fluorescence Spectroscopy	44
3.2.3	Circular Dichroism Spectroscopy	45
3.3	Transmission Electron Microscopy	46
3.4	Scanning Electron Microscopy	47
3.5	Dynamic Light Scattering	48
Chapter 4:	Multivariate Analysis of Peptide-Driven Nucleation and Growth of Au Nanoparticles	52
4.1	Introduction	53
4.2	Materials and Methods	57
4.2.1	Materials	57
4.2.2	Sample Preparation	58
4.2.3	UV-Vis Sample Characterization	58
4.2.4	Electron Microscopy	58
4.2.5	USAXS Measurements and Data Analysis	59
4.3	Results and Discussion	59
4.3.1	Effects of Peptide on Au Nanoparticle Synthesis	59
4.3.2	Comparison of Peptide Mediated Nanostructure Formation Using the Full Parameter Space	67
4.3.3	Application of FPCA to UV-Vis Spectroscopy	68
4.3.4	Application of GFT to High-Throughput Experimentation Data	70
4.3.5	Structural Characterization Using Electron Microscopy and USAXS .	72
4.4	Conclusion	80
Chapter 5:	Anisotropic Gold Nanomaterial Synthesis Using Peptide Facet Specificity and Timed Intervention	82
5.1	Introduction	83
5.2	Materials and Methods	86

5.2.1	Materials	86
5.2.2	Sample Preparation and Dynamic Intervention	86
5.2.3	UV-Vis Characterization	87
5.2.4	In-Situ SAXS Experiments and Data Fitting	87
5.2.5	Electron Microscopy	88
5.2.6	Atomic Force Microscopy	88
5.2.7	Dynamic Light Scattering	88
5.3	Results and Discussion	89
5.3.1	Morphogenesis of Interconnected Anisotropic Particles	89
5.3.2	Dynamic Intervention Using Z2	94
5.3.3	Effect of Z2 Addition on Particle Growth Pathways	101
5.4	Conclusion	105
Chapter 6:	Applications of Small-Angle Scattering in Hierarchical Self-Assembly	106
6.1	Hierarchical Self-Assembly Pathways of Peptoid Helices and Sheets	106
6.1.1	Introduction	107
6.1.2	Methods	108
6.1.3	Results and Discussion	109
6.2	Predictive Theoretical Framework for Dynamic Control of Bioinspired Hybrid Nanoparticle Self-Assembly	111
6.2.1	Introduction	112
6.2.2	Methods	113
6.2.3	Results and Discussion	115
Chapter 7:	Conclusion and Outlook	121
7.1	Summary	121
7.2	Key Results and Perspectives	122
Bibliography		126
Appendix A:	Multivariate Analysis of Peptide-Driven Nucleation and Growth of Au Nanoparticles	147
A.0.1	Materials	147
A.0.2	UV-Vis Spectra	147

A.0.3	Determination of Lipidated Peptide Critical Micelle Concentration . .	151
A.0.4	Au Reduction Kinetics Assay	152
A.1	USAXS Scattering Profiles and AUTORG Results	154
A.1.1	SAXS	157
A.1.2	USAXS Sample Holder Assembly	159
A.1.3	Opentrons Protocol Details	160
Appendix B:	Anisotropic Gold Nanomaterial Synthesis Using Peptide Facet Spec-	
	ificity and Timed Intervention	162
B.1	Liquid Handling Robot Program	162
B.2	SAXS Sample Holder	162
B.3	Atomic Force Microscopy Analysis	163
B.4	Cuvette Based Colloidal Stability Assay	170

LIST OF FIGURES

Figure Number	Page
2.1	Equilibrium number density profile when equilibrium between sedimentation and diffusion is reached for spherical gold nanoparticles of different diameters in water at 298 K as a function of sedimentation distance (mm).
	21
2.2	Electrostatic potential ψ as a function of distance from a surface in aqueous solution containing different concentrations of NaCl at 298K with a surface potential $\psi_0 = 28$ mV.
	24
2.3	A shows the contributions from van der Waals and electrostatic forces to the combined DLVO interaction potential for an arbitrary 200 nm diameter pair of particles in 0.1 M NaCl at 298.5K, and a $\psi_\delta=30$ mV. B is the same system but shows the effect of changing ion concentration.
	25
2.4	A is an example of eq. (2.14) which demonstrates the contributions of the bulk and surface free energy terms to the thermodynamic stability of a nucleus as a function of the radius of the nucleus. At the critical value of free energy ΔG_{crit} , the nucleus reaches a thermodynamically stable size at which point association of new monomers happens at a much greater rate relative to dissociation. The region below this critical point is highlighted in red in A to indicate that dissociation is favored. A schematic of homogeneous nucleation is shown in B where the black circles represent monomers that may associate or dissociate to form a nucleus that is approximated to be a sphere. Clusters of monomers which are at least the size of the critical nucleation radius are thermodynamically stable and can continue to grow. C is a schematic of heterogeneous nucleation in which the nucleus N is represented by a hemisphere on a substrate S in a liquid L . The red, blue, and purple arrows correspond to the interfacial energies γ_{S-N} , γ_{S-L} , and γ_{N-L} , respectively
	29
2.5	Localized surface plasmon resonance schematic
	33
2.6	A Quasistatic approximation of a 20 nm diameter gold nanoparticle's extinction, scattering, and absorption cross-sections in water. B and C show ϵ_1 and ϵ_2 , the real and imaginary components of gold's dielectric function, respectively[1].
	36

3.1	Experimental setup for a typical small-angle scattering experiments. A collimated X-ray or neutron beam passes through a sample and scatters radiation with an angular distribution of intensity captured by a detector. The 2D intensity is then integrated to obtain a 1D scattering profile. The black vectors represent the incident and transmitted beam, the blue vector represents the scattered beam, and the red vector is the scattering vector q .	39
3.2	Schematic of a typical circular dichroism spectroscopy experiment. A monochromatic light source is circularly-polarized before passing through a sample. Depending on the optical activity of the sample, the difference in right and left circularly-polarized light is detected.	46
3.3	Experimental setup of dynamic light scattering. A detector measures the scattered light intensity at some angle θ . The scattering vector q depends on θ and the wavelength of incident light and is related to the size of scatterers. The measured intensity over time is analyzed using an autocorrelation function.	48
3.4	Major diameter of hexagonal nanoplatelets with a thickness of 10 nm obtained using equations and parameters obtained in the literature[2].	51
4.1	Project Overview	57
4.2	UV-Vis spectra of Au Nanoparticles prepared in the presence of different peptides.	60
4.3	Principal component representations of spectra	70
4.4	Distances between peptide synthesis spaces obtained after applying a graph Fourier transform to FPCA representation of each synthesis space	71
4.5	USAXS profiles of gold nanoparticles formed in the presence of peptides.	72
4.6	Radius of gyration results obtained from AUTORG plotted as a function of peptide.	73
4.7	Electron microscopy results.	75
4.8	Schematic showing the effects of Z2 and its variants on Au nanoparticle synthesis outcomes.	79
5.1	UV-Vis spectra of Au nanoparticles formed using 0.2 mM HAuCl_4 , 0.1 mM HEPES, and 0.04 mM of peptide. Increasing the amount of methionines substituted for isoleucine in the Z2 sequence leads to a red shift in the plasmon resonance peak position at higher wavelengths indicating that larger plates are being formed. Z2, Z2M6I, Z2M46I, and Z2M246I correspond to sequence RMRMKMK, RMRMKIK, RMKIKIK, and RIRIKIK, respectively.	90

5.2	A SEM of an aggregate of irregularly shaped plate-like and spherical particles. B TEM of samples prepared in presence of Z2M246I. Presence of wrinkle-like defects suggests that the structures are thin. C SAED of the basal facet of an isolated plate-like particle which was indexed as the [111] direction. Points in the circle and square are from $\{220\}$ and $1/3\{422\}$ reflections, respectively. D and the inset figure correspond to an HRTEM image and its FFT of a plate-like structure, respectively. The inner ring of points corresponds to 0.25 nm interplanar spacing which is consistent with reflections from $\frac{1}{3}\{422\}$	92
5.3	A AFM image showing plate-like particles with an approximate thickness of 10 nm, as indicated by the annotations and the corresponding height profile in B.	93
5.4	A Time-resolved SAXS profiles characterizing the formation of Au structures formed using 0.04 mM Z2M246I, 0.2 mM HAuCl ₄ , and 1 mM HEPES. Once the scattering intensity was sufficiently high to fit (approximately 10 minutes), the scattering profile at each point was fitted using a Guinier-Porod model, and the fit parameters were plotted as a function of time in B and C. The dimension variable increased to and stayed near a value of 2, and is consistent with plate-like structures. The radius of gyration of time points which had a dimension variable near 2 were converted to thickness, and demonstrated that the thickness plateaus to a value of approximately 10 nm. The intensity of scattering at low-q ($q=0.007 \text{ \AA}^{-1}$) is proportional to the concentration of particles, and is stable after approximately 30 minutes.	95
5.5	Overview of the synthesis and microplate sedimentation assays used in this section. Samples were synthesized using a liquid handling robot and custom script as described in the methods section. The sedimentation assay consists of two transfer steps in which 200 μL aliquots are transferred from 600 μL of sample in the 48-well reaction plate to wells in the 96-well plate used for UV-Vis characterization. Comparison of spectra from samples which were transferred immediately, or 12-15 hours after all synthesis steps were completed, allows for differentiation between extents of sedimentation between samples. For example, a sample that sediments significantly in the reaction plate during the 12-15 hour period will have much lower optical extinction in the second aliquot transferred to the microplate used in UV-Vis characterization relative to the first aliquot.	96
5.6	A and B correspond to spectra taken of samples transferred from the reaction plate immediately after all pipetting commands were finished, and after a delay period of 12-15 hours.	97

5.7	A and B correspond to changes in optical extinction at $\lambda=400$ nm and 1000 nm for samples prepared with Z2 being added after a different number of delay periods, or without Z2. Optical extinction at 400 nm is associated with overall Au(0) concentration, and optical extinction at 1000 nm suggests presence of plate-like particles.	98
5.8	SEM images of Au nanoparticles obtained by mixing in Z2 after a different amount of delay periods. A, B, C, and D correspond to delay periods of 0, 6, 8, and 10, respectively. The particles in A are primarily spherical particles ranging from ca. 10 to 30 nm in diameter, and some nanotriangles are present. Particles obtained after 6 or 8 delay periods also feature some spherical particles, but have a larger proportion of plate-like nanoparticles. Z2 addition after 10 delay periods forms primarily plate-like nanoparticles which appear less aggregated than in the control sample with no Z2.	100
5.9	A Intensity weighted distribution of diffusion coefficients for samples in which Z2 was added after a different number of delay periods, and for the control sample in which no Z2 is added. B The diffusion coefficients of peaks with the largest intensity weighted area were converted to effective diameters as described in the methods section, and plotted as a function of the delay time.	101
5.10	In-situ UV-Vis spectra of gold nanoparticle formation over time in the presence of Z2 A, or Z2M246I B. The asterisk in B marks the 9 minute time point at which the prominence of a peak at higher wavelengths that is attributed to the longitudinal plasmon resonance mode of plate-like gold particles.	102
5.11	In-situ UV-Vis spectra of gold nanoparticle formation over time in which only Z2M246I is present initially, and Z2 is added at different time points: A 1 minute, B 5 minutes, C 9 minutes, D 13 minutes.	103
6.1	Proposed hierarchical self-assembly pathway of NBrpe6Nc6	108
6.2	SAXS and CD measurements of NBrpe6Nc6 aggregates in pure methanol solvent where the peptoids are dispersed, and a 75:25 v/v methanol/water solvent where we see evidence of peptoid assembly.	111
6.3	Core-shell fractal model and schematic of protein induced SiNP assembly	114
6.4	Smeared USAXS data of all sfGFP:SiNP molar fractions and pH conditions that were characterized. A,B, and C correspond to sfGFP:SiNP molar fractions of 1:1, 3:1, and 5:1, respectively. Scattering from SiNP measured at a pH of 7.5 at the same particle concentration with no protein present is plotted for reference. Solid lines correspond to the fitted model.	119

A.1	UV-Vis spectra of Au Nanoparticles prepared in the presence of different peptides as indicated by the color labeling. HEPES concentration is 0 mM, HAuCl ₄ concentration decreases from top to bottom (0.2, 0.117, 0.0684, 0.04 mM), and peptide concentration increases from left to right (0, 0.04, 0.0894, 0.2 mM).	148
A.2	UV-Vis spectra of Au Nanoparticles prepared in the presence of different peptides as indicated by the color labeling. HEPES concentration is 0.01 mM, HAuCl ₄ concentration decreases from top to bottom (0.2, 0.117, 0.0684, 0.04 mM), and peptide concentration increases from left to right (0, 0.04, 0.0894, 0.2 mM).	149
A.3	UV-Vis spectra of Au Nanoparticles prepared in the presence of different peptides as indicated by the color labeling. HEPES concentration is 0.1 mM, HAuCl ₄ concentration decreases from top to bottom (0.2, 0.117, 0.0684, 0.04 mM), and peptide concentration increases from left to right (0, 0.04, 0.0894, 0.2 mM).	150
A.4	Ratio of fluorescence intensity at 384 to 372 nm resulting from excitation at 334 nm as a function of peptide concentration. Adsorption of pyrene to micelles leads to a change in the fluorescence spectra which is used to estimate the CMC by finding intersection of two lines fitted to the data. The CMC of MZ2, MZ2R, and PZ2 were estimated to be 0.47 mM, 0.33 mM, and 0.24 mM, respectively.	152
A.5	Absorbance at 400 nm as a function of time (minutes) for Z2, Z2M246I, and MZ2.	153
A.6	Smeared SAXS data. Sample compositions: 0 mM HEPES; peptide left to right 0.040, 0.060, 0.089, 0.134, and 0.200 mM; HAuCl ₄ top to bottom 0.200, 0.117, 0.068, and 0.040 mM.	154
A.7	Smeared SAXS data. Sample compositions: 1 mM HEPES; peptide left to right 0.040, 0.060, 0.089, 0.134, and 0.200 mM; HAuCl ₄ top to bottom 0.200, 0.117, 0.068, and 0.040 mM.	155
A.8	Radius of gyration results obtained from AUTORG plotted as a function of peptide:HAuCl ₄ molar ratio for all lipidated peptides and a HEPES concentration of 0 mM. Error bars indicate standard deviation. Profiles which did not contain a Guinier region that could be fitted were marked using black and plotted at a fixed R_g of 10 Å.	156

A.9	Radius of gyration results obtained from AUTORG plotted as a function of peptide:HAuCl ₄ molar ratio for non-lipidated peptides (except AG3) and a HEPES concentration of 0 mM. Error bars indicate standard deviation. Profiles which did not contain a Guinier region that could be fitted were marked using black and plotted at a fixed R_g of 10 Å.	157
A.10	Smeared small-angle scattering of MZ2 dissolved in water (4.2 mM) fit with a cylinder model (radius = 5.2 nm).	158
A.11	Process of assembling USXAXS sample holder, and the frame which holds up to two plates during experiments. A 3D printed mock cartridge is used to hold silver behenate for USAXS calibration.	159
A.12	Opentrons OT-2 deck layout: 1, Agilent 6 column reservoir, water; 2, stocks in 20 mL scintillation vials seated in 3D printed vial holder; 4 and 5, 48 well microplates; 7 and 10, 300 μ L pipette tips. Stock distribution in slot 2: A1, peptide; A3 and A4, HEPES; B1, HAuCl ₄	161
B.1	AFM image of sample in which Z2 was added after 0 delay periods	164
B.2	Line profiles of measurements annotated in fig. B.1. A, B, C, D, E, and F correspond to measurements 1, 2, 3, 4, 5, and 6.	165
B.3	AFM image of sample in which Z2 was added after 6 delay periods	166
B.4	Line profiles of measurements annotated in fig. B.3. A, B, C, D, E, and F correspond to measurements 1, 2, 3, and 4.	167
B.5	Line profiles of measurements annotated in fig. B.3. A, B, C, and D correspond to measurements 5, 6, 7, and 8.	168
B.6	AFM image of sample in which Z2 was added after 10 delay periods	169
B.7	Line profiles of measurements annotated in fig. B.6. A and B correspond to measurements 1 and 2.	169
B.8	UV-Vis spectra of samples prepared with Z2 added at different times, and a control with no Z2 added. A, B, C, D, E, F, and G correspond to samples with delay periods of 0, 2, 4, 6, 8, 10, and 12. H is the control sample with no Z2 added.	170
B.9	Images of samples prepared with Z2 added at different times, and a control with no Z2 added.	171

LIST OF TABLES

Table Number	Page
4.1 Peptides used for Au nanoparticle synthesis	61
6.1 Results of fitting a fractal aggregate model composed of core-shell subunits, or a simple spherical model to all samples.	120
A.1 List of items used per plate	160

ACKNOWLEDGMENTS

I have a long list of mentors that have genuinely helped and inspired me, coworkers that I could count on and confide in, and staff that went above and beyond their duties to help me progress. At the top of this list, but at the foundation of my career in science, is Professor Lilo Pozzo. Lilo helped me find and then break past any limits I thought existed in myself or in my work. Likewise, when I ventured too far into a rabbit hole, she helped me get back on track. Our shared passion for cooking and science, and Lilo's leadership through pandemics, hurricanes, and scattering trips, has been a privilege to experience and learn from, thank you Lilo. I would also like to thank my committee members, Professors Suzie Pun, James De Yoreo, and Sarah Keller. Suzie, thank you for opening the door to collaborations early on in my time as a PhD student, and for remaining on my committee. Jim, thank you for the work and time that you put into making collaborative work possible, and for helping me engage more deeply in my work. Sarah, thank you for your support during exams and reminding me to take ownership of my work. Others that have inspired, supported, or collaborated with me include Professors David Beck, François Baneyx, Andrew Ferguson, and Jim Pfaendtner. Senior colleagues in the Pozzo lab have been both mentors, and friends that mutually supported one another. Thank you Dr. David Li, Dr. Yi-Ting Lee, Dr. Caitlyn Wolf, Dr. Ryan Kastilani, and Dr. Jaime Rodriguez. I am also thankful to have worked alongside other Pozzo lab members: Sage Scheiwiller, Maria Politi, Huat Chiang, Brenden Pelkie, Beth Harmon, Cameron Tavakoli, Karen Li, Claire Benstead, Abdul Moez, Dr. Kiran Vaddi, Dr. Shayna Hilburg, and Dr. Fabio Baum. We have learned so much both through and about one another, and I am looking forward to watching each of you achieve your goals in the future. My fortune in meeting great colleagues, mentors, and collaborators

has extended to people working in the center for the science of synthesis across scales, thank you Dr. Shuai Zhang, Dr. Sakshi Yadav, Dr. Mingfei Zhao, Dr. Xin Qi, Yifeng Cai, Nada Naser, Kaylyn Torkelson, Professor Chun-Long Chen, Renyu Zheng, Wenhao Zhou, Dr. Biao Jin, Fatima Davila, Harley Pyles, and Zhixing Lin. I am also grateful for Ellen Lavoie, Dr. Yun Liu, Dr. Ivan Kuzmenko, and Dr. Jan Ilavsky, for helping me make the most of my time on their instruments by being patient, thorough, and fun to work with.

Outside of work, I had a net of friends and roommates that made me feel welcome and helped me to break out of my comfort zone and enrich my life. Our times spent on the Burke-Gilman, playing board games, or just having heart to heart conversations kept me going no matter how dark everything appeared, I am thankful for Alex, Kendrick, Blake, Kiel, Serena, Stephen, Andres, and Jiacheng. In the past few years I have also had the pleasure of calling Marielle my partner, and in the past few months her support has been especially indispensable. Finally, I am thankful to my mom and dad, who have been with me from the very beginning. Their decisions and sacrifices allowed me to find a fulfilling and beautiful path in life. I am eternally grateful to them, and I strive to match them in the love they have shown me.

DEDICATION

To my parents, Dariusz Lachowski and Dorota Lachowska.

Chapter 1

INTRODUCTION AND MOTIVATION

1.1 *Objectives*

The primary objective of this thesis is to advance molecular design for bottom-up nanomaterial synthesis. This chapter will provide a review of how the structure and composition of various nanoscale inorganic materials give rise to unique properties that distinguish them from their bulk phase counterparts. Notable synthetic approaches for each category of materials are described next to introduce common approaches for controlling and studying nucleation and growth mechanisms. Finally, the chapter concludes by describing bioinspired approaches to nanomaterial synthesis, and delineates three distinct but synergistic approaches used in sequence defined molecule design for applications in nanomaterial synthesis. Relevant theoretical background and experimental techniques are described in chapter 2 and chapter 3. The unifying theme in the thesis is that understanding the context in which intermolecular interactions and chemical reactions take place during nanoparticle synthesis is necessary to guide molecular design. This theme is introduced as peptide presentation in chapter 4, which encompasses the different ways that a peptide interacts with precursors and particles during synthesis. These interactions were studied by making rationally guided changes to a gold binding peptide, and by evaluating the effects of these changes on gold nanoparticle synthesis outcomes in large and multivariate experimental design space. Gold nanoparticle synthesis is chosen as a model system because it is well understood, and because plasmon resonance allows for UV-Vis spectroscopy to serve as a rapid proxy for structural changes. Functional data analysis of the spectra yielded a quantitative metric for comparing effects of different peptide modifications. Following up on this work, chapter 5 takes a closer look at the origin of and methods for manipulating anisotropic particle growth in the presence

of gold binding peptides studied in the preceding chapter. Here, the context in which peptides interact with precursors and particle surfaces is studied by changing the time at which a peptide is added to a reaction mixture in which a peptide with facet specific binding is already present. We demonstrate that the time of addition changes the colloidal stability, proportion, and size of anisotropic particles that are formed. Next, chapter 6 summarizes experimental contributions to two projects that fit into the theme of this thesis. In the first project, the stages of peptoid self-assembly as a result of changing solvent composition are described by a combined effort of molecular dynamics simulations validated by experimental methods. My primary contribution was the identification of an intermediate extended peptoid structure using small-angle scattering. In the next project, I used ultra-small-angle X-ray scattering to characterize the structure of silica nanoparticles which aggregated in the presence of a pH responsive homobifunctional protein construct. The scattering data were used to validate a coarse grained simulation of nanoparticle aggregation. A summary of the major findings from each chapter and a perspective on future directions for research that builds on this thesis is provided in chapter 7.

1.2 Inorganic Nanomaterials

1.2.1 Properties and Applications

Metal nanomaterials are the primary focus of this thesis, but other inorganic systems will also be discussed to motivate the use of sequence defined molecules towards control of structure at the nanoscale. The electric field of light passing through metal nanoparticles polarizes and displaces the electrons in the conduction band, which are then attracted back to the positively charged lattice [3]. This optical response, known as localized surface plasmon resonance (LSPR), requires that the size of the particle or one of its structural features be smaller than the wavelength of light. LSPR enables several realized and envisioned uses of metal nanoparticles in the areas of biosensors [4], photocatalysis [5], bioimaging [6], photodynamic therapy [7], displays [8], and other applications. Noble metals, and Au in

particular, have received the most attention due to their chemical stability, relatively simple synthesis, and the breadth of possible applications that LSPR affords. However, non-noble metals and hybrids (e.g. nonnoble and noble) also receive attention motivated by the need to balance cost, material availability, stability, and performance [9].

The modification of composition is highly developed and widely used in the field of semiconductors. Electronic properties of semiconductors, whose conductivity is between that of a conductor and insulator, can be tuned through doping. Doping is the incorporation of small quantities of impurities in the form of atoms different from the bulk of the semiconductor material. The amount of devices and applications that have been created or transformed through the use of semiconductor components has grown exponentially since the invention of the transistor. Colloidal semiconductors whose dimensions approach that of the Bohr radius have a band gap structure which is more similar to individual atoms than the bulk material due to quantum confinement [10], and are typically referred to as quantum dots (QDs). The accessible energy levels of electrons in QDs are discretely spaced, and the energy required to transition from the valence band to the conduction band (band gap energy) depends on the size of the QD. Hence, the photoluminescent properties (e.g. emission wavelength, emission line width, quantum yield) of QDs are modified by adjusting the composition and dimensions of the core and any additional shell. The properties of QDs have been leveraged in displays, luminescent solar concentrators, bioimaging, photocatalysis, biosensors, photovoltaics, and various optoelectronic devices (i.e. phototransistors, photodetectors) [10]. In 2015 Protesescu et al. [11] demonstrated a new method for synthesizing QDs, which were hitherto primarily composed of metal chalcogenides (e.g. Cd, Pb, In and S, Se, Te, As), from metal halide QDs (CsPbX_3 , $\text{X}=\text{Cl, Br, and I}$) with a Perovskite crystal structure. Perovskites have received significant attention in recent years due to high performance and potential in a variety of optoelectronic applications [12, 13].

While the classes of materials introduced so far have clearly desirable properties, metal oxides such as rust (Fe_2O_3) are often associated with the detrimental effects that oxidation has on structural integrity or electric conductivity. On the contrary, metal oxides also have

useful properties that make them widely used in both commodity and specialty applications. Metal oxides are a diverse class of materials which may be insulators (e.g. CuO), semiconductors (e.g. ZnO) or conductors (e.g. Indium tin oxide) [14]. The use of naturally occurring colorful oxides (e.g. ochre, a mixture of ferric oxide and minerals) as pigments or even medicine dates back millennia [15]. Metal oxides such as ZnO and TiO₂ are used in modern pigments or cosmetic formulations due to their high refractive index and high absorption of ultraviolet light [16]. The latter property is useful to limit the degradation of organic materials such as plastics, and in sunscreen formulations to prevent skin damage [16, 17, 18]. In addition to the properties and applications of semiconducting materials already discussed, the application of semiconducting metal oxides towards water photocatalytic water splitting has received significant attention since it was first reported in 1972 [19, 20].

Due to its abundance and ubiquitous use, a discussion of inorganic nanomaterials is incomplete without silica (SiO₂), an oxide of the metalloid silicon. The structure of silica is a network of tetrahedral Si–O bonds that can be crystalline or amorphous. Colloidal silica is typically amorphous, but nanocrystals have also been reported in the literature [21, 22]. The surface chemistry and particle structure (e.g. porosity, shape, size) of silica determine its hydrophobicity, charge, adsorption, and colloidal stability. The surface of silica may include silanols (Si–OH), vicinal silanols (Si–OH which features hydrogen bonds between neighboring hydroxyls), and siloxanes (Si–O–Si). The proportion of silanols and siloxanes affects the hydrophobicity of colloidal silica[21]. Other properties such as surface charge can be modified by substitution of silicon (e.g. by aluminum), and a wide range of chemical functionalities can be introduced via ester or silane linkages [23]. While the primary application of silica by volume is construction (e.g. cement) [21], colloidal forms of silica have an immense number of other applications. Some of the latter include clarification of beverages through flocculation, increasing friction of paper products, scavenging reaction products during polishing of silicon wafers and many others [21]. Silica is also used in hybrid inorganic nanomaterials, such as silica coated plasmonic nanoparticles [24]. The high degree of control over silica growth, etching, porosity, and chemical functionality allows researchers

to probe the effects of separation distance in optically active plasmonic materials.

1.2.2 Overview of Synthesis Methods

Improving synthetic control over structure and composition of inorganic materials is beneficial because the two are directly linked to material performance. Structure encompasses crystal structure, particle shape, size of various features, polydispersity, and hierarchical order (e.g. fractal networks, colloidal crystals, ordered hybrid inorganic-organic structures). Whereas composition refers to atomic identity and its distribution within the inorganic structure. Beyond control over these two aspects, synthetic methods may also be evaluated on multiple criteria such as their yield, purity, environmental impact, safety, complexity, and scalability. Underlying the motivation for improving any of these aspects of synthetic methods is the need for predictable and reproducible results at the very least, and full mechanistic understanding at best. The following survey of synthesis methods will introduce common approaches, important connections between assembly mechanisms and synthetic control, and ongoing challenges.

Synthesis of Metal Nanoparticles through Chemical Reduction

Reduction, the process by which an atom gains electrons, decreases the solubility of metal nanoparticle precursors, and can be controlled to yield metal nanoparticles. Metal salts in an aqueous medium are the most well studied precursors of metal nanoparticles [25], but complexes of metals and organic molecules may also be used as precursors [26]. Reduction is most commonly initiated by chemical reducing agents, but is also carried out using methods such as radiolysis [25, 27] or sonolysis [28]. The pathways through which nucleation and growth occurs depend on the rate of reduction and other experimental conditions, and continue to be intensely studied [25, 29]. The primary tools used to capture the dynamics of nanoparticle growth are UV-Vis spectroscopy, electron microscopy, SAXS, and X-ray absorption spectroscopy.

The Turkevich method, a well known approach for the synthesis of Au nanoparticles[30], consists of adding sodium citrate solution to boiling chloroauric acid. The authors used transmission electron microscopy and UV-Vis spectroscopy to characterize the Au colloids, and identified spherical nanoparticles with a mean diameter of 20 nm. The temperature dependence of the apparent nucleation rate prompted Turkevich et al. to propose an alternative mechanism to the explanation given by LaMer et al. for the formation of sulfur hydrosols[31]. Several competing explanations were proposed in the following decades, but a comprehensive mechanistic understanding has emerged recently thanks to experiments employing simultaneous SAXS and X-ray absorption spectroscopy[32, 33, 34]. X-ray absorption near edge structure spectroscopy was used to track the proportion of Au(III) reduced to Au(0) while SAXS data were fitted to extract particle radii and volume fractions. The four step model proposed by the authors begins with reduction of HAuCl_4 and formation of clusters. In the second step, coalescence of clusters leads to the formation of seed particles. The term seed particle indicates that future growth of particles occurs primarily on seed particle surfaces, and that formation of new particles decreases significantly. In the third step, which begins around the time that particles reach an average diameter of 5 nm, reduced Au diffuses to the surface of existing particles, and Au ions accumulate at the electrical double layer of particles. Finally, once an average diameter of 8-10 nm is reached, the remaining 70-80% of Au is reduced rapidly. In addition to being a reducing agent, citrate and its oxidized byproducts provide electrostatic stabilization of seeds and particles against further coalescence, and provides a pH buffer effect that increases the concentration of less reactive Au complexes responsible for the seed-mediated growth mechanism.

Seed mediated growth has also been applied in the synthesis of non-spherical particles such as nanorods [35, 36], nanostars [36], and nanotriangles [37]. Control over nanoparticle shape is key to obtaining a desired optical response from plasmonic nanostructures. In the case of nanorods, the position of the second LSPR peak can be controlled by changing the length of the rod, while maintaining a narrow diameter. Nanorods with an LSPR peak in the near-infrared region are desirable for bioimaging applications because they coincide with the

spectral window in which tissues have low optical extinction [7]. The key to many syntheses of Au nanorods are cetrimonium bromide (CTAB) and AgNO_3 [38]. CTAB is a surfactant which has an alkyl tail (16 carbons) and a positively charged quaternary ammonium head group. Like citrate, CTAB performs several roles in nanoparticle synthesis: complexation with the metal precursor leading to a change in the redox potential of Au, colloidal stabilization of particles, and complexation with $[\text{Br}-\text{Ag}-\text{Br}]^{-1}$ leading to facet specific adsorption and a symmetry breaking event [38, 39]. Growth of metal nanoparticles through chemical reduction is a complex phenomenon, and it is logical that the Turkevich method, and other syntheses, take a long time and combined effort of the research community to understand. However, due to the progress made in instrumentation, data analysis techniques, and theoretical understanding of colloidal metal nanoparticles [40], coupled with the large design space of possible precursors, reducing agents, capping agents, and surfactants, the field of metal nanoparticle synthesis will continue to thrive.

Synthesis of Quantum Dots Using Solvothermal Methods

High temperatures are used in a variety of colloidal syntheses involving thermal decomposition, nonhydrolytic sol-gel reactions, and chemical reduction[41]. This section will focus on syntheses in which thermal decomposition initiates the formation of QDs in two different solvothermal methods, hot-injection and the heat-up method. Aside from thermal decomposition, temperature is also used to control nucleation and particle growth rates. Minimizing polydispersity and control over the exact size of QDs are primary objectives in QD synthesis, because of the sensitivity of the optical response to QD size. The use of a secondary layer or shell on the QD core is a more recent development which has been highly effective at improving QD photoluminescence stability[42].

The hot-injection method was first described by Murray et al. in 1993 [43]. Murray et al. reacted dimethylcadmium with different silylchalconides and phosphine chalconides to yield colloidal crystals of CdE ($\text{E}=\text{S}, \text{Se}, \text{Te}$). The significance of this publication is that while it was already known that QDs had size dependent optical properties, the described

method was the first to demonstrate control over the size of QDs of multiple compositions over a range of diameters. Hot-injection begins with a quick injection of reagents into a heated solvent (e.g. 300°C) followed by controlled heating. The quick injection is used to achieve rapid homogeneous nucleation through supersaturation, and is similar to the method of hydrosol generation described by LaMer[31]. Forming the majority of nuclei in one step is beneficial to controlling size polydispersity because nuclei will have a similar size initially, and because the growth history of each particle will be similar. The purpose of rapid injection of room-temperature reagents is that it limits the formation of new nuclei by depleting available reagents and decreasing the temperature of the reaction. Additional heating is then applied to reach a particular temperature, depending on the reagents being used, and the desired size[43]. Peng et al. advanced the hot-injection method by providing a deeper understanding of the underlying mechanisms by following QD growth over time using ex situ spectroscopic measurements[44]. By following changes in peak position and width of photoluminescence emission, the authors followed the changes in average particle size over time. Size focusing and Ostwald ripening, which will be described in greater detail in chapter 2, were identified as likely mechanisms associated with size control in the hot-injection method. Size focusing is the decrease in size polydispersity due to the inversely proportional relationship between growth rate and particle size in diffusion limited growth. That is, for any particle above the critical nucleation radius, growth rate will decrease with increasing size. The effect of this is that after sufficient time, particle radii converge to similar values. Ostwald ripening describes the process by which particles whose radius is smaller than the critical nucleation radius dissolve over time, and the dissolved material then adsorbs to larger, more stable particles. Ostwald ripening increases the polydispersity of particle size because particles below the critical radius of nucleation begin to disappear, while larger ones grow. The trends in particle size over time were shown to be consistent with size focusing early on in the synthesis, and resembled Ostwald ripening once the monomer concentration decreased[44]. Both of these landmark papers contained ex situ spectroscopic measurement of aliquots in order to study and optimize growth conditions for a desired size[44, 43]. The

hot-injection method is conceptually simple and is effective at synthesizing monodisperse QDs with a variety of compositions and diameters.

However, the hot-injection method also has certain disadvantages with respect to the throughput of optimizing reaction conditions, a multitude of difficulties with scale up, and the difficulty of studying reactions in situ[45]. In contrast to the separate steps of injection followed by a controlled heating step, the heat-up method is a one-pot synthesis in which reagents are heated to high temperatures that result in precursor decomposition and formation of nanocrystals[41]. The early stages of nucleation and growth are easier to study using in situ techniques in the case of the heat-up method, because rapid mixing at high temperatures is not necessary. In contrast to the ex situ spectroscopic measurements discussed in the context of hot-injection, in situ approaches are able to provide more mechanistic details about structural changes and intermediates in a system which would otherwise be missed by spectroscopic measurements. The heat-up method was used by Castro et al. to study the synthesis of CdSe nanoplatelets (NPLs) using in situ X-ray scattering techniques[46]. Unlike QDs, NPLs are 2D structures in which quantum confinement only occurs along the thickness of the material. Their results indicated that the formation of CdSe NPLs was most consistent with the increase in lateral extension of seed particles, rather than a templating mechanism. Overall, the fundamental and practical applications of QDs continue to motivate research into new syntheses that improve on existing methods.

Sol-Gel Methods

The sol-gel method is named after two distinct stages of synthesis. First, a suspension of particles is formed in a liquid (sol) through hydrolysis and condensation reactions. The sol may then be processed in different ways to yield a film, fibers, a gel, or particles[47]. Next, the sol is either precipitated to yield dispersed particles, or condensation is allowed to continue to form a gel of interconnected particles. The kinetics and extent of hydrolysis and condensation reactions are both highly dependent on the pH. Hydrolysis is faster than condensation in acidic conditions, and condensation is faster than hydrolysis in basic conditions[47]. In turn,

the rates of these reactions determine whether the degree of branching of structures is low or high, respectively. Some of the materials which are synthesized using the sol-gel method include SiO_2 , TiO_2 and ZnO [48, 49, 50, 51].

The Stöber process, named after the method described by Stöber, Fink, and Bohn in 1968[48], is now one of the most well known approaches to forming monodisperse silica spheres. The reaction was carried out in different low molecular weight alcohols and low concentrations of water. Tetraalkyl silicates with different lengths were used as precursors, and ammonium hydroxide was used to catalyze the reaction. The concentration of the latter is a critical parameter of the synthesis because it is a source of highly nucleophilic OH^- ions. In later years researchers sought to clarify the mechanisms of nucleation and growth in the Stöber process using in situ methods to study changes in structure and concentrations of chemical species. Han et al. published a study on the growth mechanisms in the Stöber process that bridged the differences in existing models that described growth at high and low ammonia concentrations through monomer-addition and aggregation-only, respectively[52]. The authors used conductivity to probe changes in the concentration of hydrolyzed silicate species, and dynamic light scattering to follow the hydrodynamic diameter of structures. The authors confirmed that there are two pathways of growth, and that these vary with the concentration of ammonia. In the first stage, hydrolysis and subsequent condensation of a portion of the silica precursor leads to the formation of seed particles. Next, in the second stage, hydrolyzed silica monomers primarily undergo condensation reactions that attach them to existing seed particles. The two stages were temporally separate when high concentrations of ammonia were used, and the second stage began after an induction time. Samples with low concentrations of ammonia also had an induction time during which silica precursor primarily formed new seeds, but both modes of growth followed the induction time. Given this understanding, the authors used LiOH , a stronger nucleophile, in the presence of low concentrations of ammonia to achieve greater control over the size and polydispersity of the resulting colloids. Specifically, use of both ammonia and LiOH allowed for synthesis of silica with diameters less than 250 nm, but which were less polydisperse than those formed

in the presence of ammonia alone.

1.3 Sequence Defined Molecules for Bioinspired Nanomaterial Synthesis

1.3.1 Biomineralization

Unlike the previously discussed examples of laboratory synthesized materials, there are many naturally occurring examples of inorganic materials with complex structure and composition. Many of these materials, such as diatomaceous earth, are used for a variety of applications made possible by their structure. Diatomaceous earth is a sedimentary rock made up largely of the fossilized remains of diatoms, a type of microscopic water dwelling algae[53, 21]. Fossilized diatoms are primarily composed of silica, have nano and micro scale features, a high surface area, and high porosity. These properties lend themselves to many practical applications including filtration, pest control, and serving as a support for catalysts[21]. Before becoming a fossil however, the silicified membrane of the diatom fulfilled a more critical purpose, protecting the diatom from its environment. The synthesis of inorganic materials by living organisms such as diatoms is known as biomineralization. Biomineralization has multiple purposes and is found in all domains of life: plants form calcium oxalate to regulate Ca levels [54], hydroxyapatite provides protection, support, and enables the breakdown of food in animals [55], and certain bacteria use magnetite and greigite nanoparticles to aid with navigation [53]. The nanoparticles (diameter ca. 35-120 nm) found in the latter type of bacteria are relatively monodisperse, magnetically aligned, and feature broken symmetry in the form of elongated particles of cubic crystal lattices[56]. Other notable properties of biomineralized materials are integration with organic components and hierarchical order such as in the case of bone or mollusk shells [56, 55].

Nature employs several mechanisms to control mineralization. Decoration of biological surfaces with functional groups that bind inorganic species from the environment to serve as nucleation sites is a relatively simple example of the latter. Further nucleation and growth is controlled by solution conditions through the organism's metabolism[56]. Organisms can

achieve even finer control by creating confined environments, templates that stabilize specific facets, modulating inorganic precursor concentrations, and stopping crystal growth[56]. Biomolecules, such as proteins and lipids, presented at a specific concentration, conformation, time, and location, are key to achieving each of these tasks. Expression and presentation of biomolecules is ultimately controlled by an organism’s genome and epigenome. The genome is a nucleic acid based set of instructions that, through transcription and translation, produces proteins. The epigenome refers to the different ways that gene expression can be controlled through chemical modifications or interactions with proteins like histones. Proteins may contribute to biomineralization by catalyzing the formation of lipids necessary for compartmentalization and control over local concentrations of chemical species, or by taking on a shape which stabilizes specific facets of a particular mineral. Genetic information passed on to offspring varies in the degree of conservation, and mutations may affect an organism’s fitness - which is the ability to survive and reproduce. Mutations that improve an organism’s fitness are more likely to be passed on in the evolutionary process. Biomineralization can have a profound impact on an organism’s fitness, and is therefore also subject to evolutionary change.

1.3.2 Bioinspired Nanomaterial Synthesis

Nature’s ability to biomineralize structures with high complexity in mild synthesis conditions is a source of inspiration for improving the synthesis of nanomaterials. Many of the methods used by nature in biomineralization have been mimicked in syntheses of inorganic nanomaterials[24, 56, 55]. The use of a structured organic phase, such as a lipid vesicle or collagen network, is fundamental to biomineralization[56]. Liebig et al. studied the growth of gold nano-triangles confined in vesicles and in the presence of a surfactant and polyampholyte, emulating the use of templates in biomineralization. One-step syntheses like these have the potential to save time and resources, but are challenging to control. Droplet reactors may also be viewed as an approach inspired by nature because of their resemblance to confined growth in biological systems. One type of droplet reactor is a flow-through sys-

tem where aqueous droplets containing different reagent concentrations are suspended in an inert carrier oil. This allows for exploration of a large design space by carrying out different reactions in each droplet[57]. In addition to mimicking the physical mechanisms used in biomineralization, researchers have used evolutionary algorithms to optimize synthetic conditions[57, 58]. In this process, experimental information is encoded and attributed to each sample. Samples are then characterized and compared with a target that is either categorical (e.g. presence of a crystalline phase in a sample), or quantitative (e.g. crystal size distribution)[58]. Samples with attributes close to the target are selected for the next round of experiments, and are conserved or mutated until a desired outcome is achieved or other stopping criteria are met. Taking an algorithmic approach to synthesis in which experimental parameters are iteratively explored to minimize a cost function is part of a broader focus of the materials research community to develop new methods for planning and executing experiments[59].

Researchers have also used proteins, and peptides designed from sequences in functional motifs of proteins, to synthesize inorganic materials. Proteins like Mms6 have been purified from magnetotactic bacteria and used to synthesize cuboidal crystals with 20-30 nm dimensions in vitro[55]. Silica forming organisms such as diatoms, produce a variety of amine and phosphate rich proteins such as silaffins, long-chain polyamines, and silacidins that promote and control the growth of silica with higher order morphologies[56, 55]. By transfecting organisms such as *Escherichia Coli* with genes that produce proteins or fragments of proteins associated with biomineralization, researchers can produce these proteins in high yields[53]. Nevertheless, the complexity of structures formed by diatoms has not been replicated in vitro. Moreover, there are many inorganic nanomaterials that organisms have not evolved to synthesize, but which have important practical applications. To this end, researchers have synthesized new sequences of proteins and other sequence defined molecules to control nanomaterial synthesis.

1.3.3 *Design and Use of Sequence Defined Molecules*

The properties and function of sequence defined molecules are determined by their constituent monomers and their order. In addition to naturally occurring biomolecules such as peptides, proteins, and nucleic acids, chemists have also constructed synthetic sequence defined molecules. Peptoids, or N-substituted polyglycines, are a notable example of the latter[60]. Sequence defined molecules can be used in nanomaterial synthesis to bind to specific materials or crystal facets, or to create templates that order the nucleation, growth, and assembly of crystalline materials. The design of sequence defined molecules for these purposes can be classified into three, sometimes overlapping approaches: **1.** directed evolution, **2.** computational methods, and **3.** rational design. Directed evolution is the use of selective evolution in microorganisms to identify a peptide or protein sequence that has desirable properties. The process begins with construction of a library of organisms that display a variety of sequences. These are then exposed to a cycle of selection, amplification, and sequencing. Selection involves incubation on a specific substrate (e.g. an inorganic surface), washing away weak binders, and elution. Thus, the sequences displayed by the next generation are more likely to display an affinity towards the target of interest. Prasad et al. used directed evolution to identify proteins that bind to the (111) facet of gold[61]. These proteins were used as capping agents in the synthesis of polyhedral and plate-like gold nanoparticles. The resulting nanoparticles had limited growth along the (111) direction in the presence of the facet-specific proteins, leading the authors to conclude that the facet specificity of the proteins played a role in regulating the shape of the synthesized nanoparticles.

Despite the efficiency of directed evolution in identifying sequences that meet specific targets, its stochastic nature obscures potential insights about molecular design. The latter can be obtained by computational approaches that simulate interactions between molecules and inorganic surfaces in high detail[62] Computational methods can also be used to study the self-assembly of molecules into structures that template particle assembly, or to simulate the hybrid assembly of sequence defined molecules and inorganic structures[63]. Yan et al.

used molecular dynamics to inform the design of peptoids for gold nanoparticle synthesis[64]. The authors showed that slight differences in side chain chemistry led to significant differences in binding to gold surfaces. Another important factor that affected binding affinity of peptoids towards gold surfaces was the conformational entropy, which is related to the number of thermally accessible conformations that a molecule can take on in contact with a surface. Sequences with higher conformational entropy were more likely to have favorable binding towards a surface. Simulating larger systems like macromolecules and supramolecular assemblies requires computationally efficient methods. *De Novo* protein design is an area in which significant progress has been made towards this end. The Rosetta software suite for protein design includes a tool that estimates the free energy of a given conformation by using the weighted linear sum of several terms related to intermolecular interactions. The Rosetta suite was used by Thomas et al. to redesign a homohexameric protein, STM4215[65]. The modified protein self-assembled into hexagonal 2D arrays that grew to micrometers in scale and retained long-range order. Chemical modification of the solvent accessible terminus of the protein allowed integration of a gold binding peptide sequence. Gold nanoparticles incubated with this construct were observed to decorate the protein array. Nevertheless, computational methods have limitations such as force field inaccuracy, a need to balance the length of simulations based on resources and sampling, and the high cost of modelling events over long timescales[66].

Rational design on the other hand, is the use of prior knowledge about properties and behavior expected from a sequence, subunit, or other chemical modification to inform design choices. Peelle et al. and Tan et al. designed experiments to rank the contribution of each of the 20 naturally occurring amino acids based on their affinity towards inorganic surfaces[67, 68]. Both works used peptides in which an amino acid was interdigitated with a different residue (i.e. XHXHXHX where H is histidine and X is a different amino acid). These results help contextualize the role that individual amino acids have on inorganic nanomaterial synthesis, and may be used to design short sequences with desired properties. Pelle et al. used the ranking of each amino acid’s binding affinity to three different inorganic surfaces

to design sequences which were either specific to one of the inorganic surfaces, or which had an affinity for multiple inorganic surfaces[67].

Overall, the behavior of sequence defined molecules is complex and cannot be fully understood through the summation of contributions of individual subunits. The complexity is related to the length of the sequence, as demonstrated by the intricate structure and function of proteins. The use of all three of the discussed methods is more effective at addressing this complexity than any of the approaches alone. A publication by Mokashi-Punekar et al. took this multi-faceted approach[69]. The authors used a gold-binding sequence identified through directed evolution and employed simulations to identify the importance of methionine in the sequence’s affinity towards gold surfaces. An alkyl tail was conjugated to the sequence to induce peptide self-assembly and control the organization of spherical nanoparticles. Finally, they also showed that changing the oxidation state and position of methionine led to different nanoparticle structures and organization along the peptide ribbon. Their study demonstrates the power of combining the three three discussed methods to design sequence defined molecules.

Chapter 2

THEORETICAL BACKGROUND

2.1 *Intermolecular Forces and Colloidal Stability*

2.1.1 *Intermolecular Forces*

Electrons shared between atoms form covalent bonds that have high directionality and strength. The properties of chemical bonds vary based on other atoms and bonds in a molecule, but are otherwise stable. Significant changes to chemical bonding, such as decomposition or chemical reactions, require breaking of bonds. In contrast, bonds which arise due to physical forces are generally weaker, non-specific, and less directional. Physical forces are sensitive to a variety of molecular properties such as the polarity of the medium, charge of the molecule, temperature, and distance. The interaction potential is the work that a force can perform across a given separation distance, and each type of interaction obeys a scaling property as a function of the separation distance. However, not only can the properties of a molecule vary due to the medium it is in, determining the interaction potential between a pair of molecules requires accounting for contributions of the medium, and is therefore a many-body interaction[70]. The magnitude of intermolecular energy is conventionally gauged by comparison to $k_B T$ (or kT), the product of the Boltzmann constant and the absolute temperature. This is because kT is a threshold that must be overcome for an interaction to result in molecular motion that is more significant than those due to thermal energy.

The strength of a covalent bond is put into perspective when expressed as being on the order of 100 kT . Charge-charge interactions, responsible for ionic bonding, are the only interaction that have a similar or greater magnitude compared with covalent bonds. A single pair of monovalent point charges in vacuum experiences an interaction with a magnitude

that is similar to covalent interactions, and this value increases inside an ionic crystal due to the interaction with other atoms. The equation for the interaction potential between two point charges in a medium is:

$$w_{charge-charge}(r) = \frac{Q_1 Q_2 e^2}{4\pi\epsilon_0\epsilon r} \quad (2.1)$$

Where Q is charge, ϵ_0 is the dielectric permittivity of vacuum, ϵ is the dielectric permittivity of the medium, and r is the separation distance. For molecules in a liquid, the contribution of the liquid is represented using its bulk dielectric constant, which is a continuum approach that treats the liquid as a featureless medium[70]. After accounting for the contribution of interactions with other atoms in a crystal, the lattice energy, this approach is sufficient for predicting the solubility of NaCl in water. This approach also highlights how important the medium is when considering intermolecular potentials, which in this case decreases by a factor of 78 (at 298 K) for a pair of ions due to water's high dielectric constant[70].

In addition to point charges and ions, electrostatic interactions also occur between permanent dipoles, dipoles induced by polarization effects, and any combination of these units[70]. Interestingly, it is the directionality of covalent bonding that gives rise to permanent dipoles. An electric dipole is the phenomenon that describes a pair of opposite charges q separated by the dipole length l . In the simple case of a polar molecule that has unequal sharing of electrons, the electric dipole moment u is equal to the product ql . When a charge Q , such as an ion, and a polar molecule with a dipole moment u are separated by a distance more significant than the dipole length, the interaction potential is expressed as:

$$w_{charge-dipole}(r, \theta) = -\frac{Qu \cos \theta}{4\pi\epsilon_0\epsilon r^2} \quad (2.2)$$

Where θ is the orientation of the dipole relative to the charge. The interaction between an anion and a dipole will be maximum when $\theta = 180^\circ$, which corresponds to the orientation in which the negative component of the dipole is furthest from the anion by convention. Dipole-dipole interactions also depend on the relative orientations of the dipoles, but a special case arises when this angular dependence is less than kT . This occurs when

dipoles are separated by a sufficiently large distance or are in a medium with a high ϵ like that of water[70]. In this scenario, the differences in energy between dipole orientations determine the weight that each has when considering the angle-averaged free energy $w(r, \Omega)$, and the resulting expression for the interaction is:

$$w_{dipole-dipole}(r) = -\frac{u_1^2 u_2^2}{3(4\pi\epsilon_0\epsilon)^2 kT r^6} \quad (2.3)$$

Unlike the charge-charge interaction in eq. (2.1), this interaction scales with r^{-6} , and is inherently attractive. It is also known as the Keesom interaction, and is one of three interactions that make up the van der Waals interactions which are important to understanding the attraction between colloidal particles.

The other two types of interactions both occur as a result of polarization, the formation of an induced dipole u_{ind} due to an electric field E :

$$u_{ind} = \alpha E \quad (2.4)$$

Where α is the polarizability of a molecule. Molecules have an electronic polarizability α_0 associated with them that is associated with the displacement of electrons away from an atom. Polar molecules also have a component associated with the polarizability of the orientations of a dipole. The electric field of an ion, or the permanent dipole in a polar molecule, may induce a dipole in a nonpolar or polar molecule. The interaction between a permanent dipole of a polar molecule and the induced dipole of a nonpolar molecule is the Debye energy shown below:

$$w_{dipole-induced/dipole}(r) = -\frac{u^2 \alpha_0}{(4\pi\epsilon_0\epsilon)^2 r^6} \quad (2.5)$$

Finally, the third interaction occurs between a pair of induced dipoles due to the London dispersion forces[70]. This interaction is more universal because it can take place between any atom or molecule. The origin of the polarization effect is quantum mechanical in nature, but may be understood by the instantaneous formation of charge imbalances which exist at various points of an electron's orbit[70]. The dispersion force between a pair of identical

molecules due to the formation of instantaneous dipoles is given by the London equation to be:

$$w_{\text{instantaneousdipole-induceddipole}}(r) = -\frac{3\alpha_0^2 h\nu}{4(4\pi\epsilon_0)^2 r^6} \quad (2.6)$$

Where h and ν are the Planck constant and frequency, respectively. The van der Waals interaction can then be derived by combining all three contributions from orientation, induction, and dispersion. An improvement to this theory was made by McLachlan[70] to account for the frequency dependence of the polarizability.

2.1.2 Colloidal Interactions and Stability

Colloidal Stability

This thesis is primarily focused on lyophobic colloids that have unfavorable interactions with the solvent they are in. Many of the applications of inorganic nanomaterials require them to remain dispersed and not undergo changes in their size distribution. The state of dispersed lyophobic colloids is not considered thermodynamically stable, but is kinetically stabilized against several processes, i.e. metastable[71]. In terms of free energy, the colloidal stability of a dispersion is a local rather than a global minimum. The depth of this barrier is related to how easily a given process can take the system out of this state. Particle phoresis, net movement of particles in one direction, can lead to an increase in particle concentration and an increase in particle interactions due to a decrease in interparticle distance. Sedimentation or creaming of particles due to gravity is one example of the latter. The below expression gives the particle concentration when motion due to diffusion and sedimentation are at steady state as a function of the direction of sedimentation z [72]:

$$n(z) = n_0 \exp -\frac{\pi d^3 (\rho_P - \rho_M) g}{6kT} z \quad (2.7)$$

Where n_0 is the initial number concentration of particles, d is the diameter of a spherical particle, and ρ_P and ρ_M are the density of the particle and medium respectively. Figure 2.1 presents the resulting number density profile for bare spherical gold nanoparticles of different

diameters in water. This example illustrates that the degree to which a colloid sediments highly depends on its size. Aggregation and coalescence are two mechanisms that result in attachment of colloidal subunits to one another, and are important to both particle growth and stability[34, 72]. Aggregation is the joining of two or more particles by attractive interparticle interactions that may or may not be easily reversible depending on the strength of the interaction. Coalescence also features joining of colloidal units, but results in restructuring of the attached subunits in a way that eliminates interfacial area. The theoretical description of attractive and repulsive interparticle interactions will be described next.

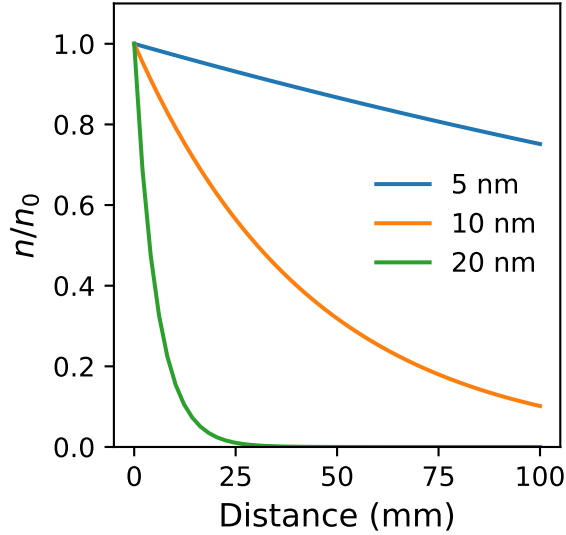


Figure 2.1: Equilibrium number density profile when equilibrium between sedimentation and diffusion is reached for spherical gold nanoparticles of different diameters in water at 298 K as a function of sedimentation distance (mm).

2.1.3 Interparticle Interactions and DLVO Theory

Interparticle interactions are often largely defined by van der Waals and electrostatic forces. The Van der Waals forces introduced in section 2.1.1, dipole-dipole, dipole-induced dipole, and instantaneous dipole-induced dipole, all scale with r^{-6} . Collective Van der Waals inter-

actions, such as between a molecule and a surface, or two spherical particles, can be treated by assuming that the interactions are additive. Summation of interaction potentials is accomplished by integrating the interaction between many atoms or molecules for different geometries such as surfaces, rods, or sheets. For two spheres of radius R separated by a distance $D \ll R$ the interaction potential is[70]:

$$w_{vdW,sphere-sphere}(D) = -\frac{\pi^2 \rho_1 \rho_2 C R}{12D} = -\frac{AR}{12D} \quad (2.8)$$

where ρ is the number density of atoms or molecules in each sphere, and A is the Hamaker constant ($\pi^2 \rho_1 \rho_2 C$). One important takeaway from this equation is that once particles are close enough, their attractive potential drops off with the power of -1 instead of -6. Changes in distance scaling laws be conceptually understood by considering that relative to a pairwise intermolecular interaction, the number of interactions between a molecule and a collection of molecules at some distance away will scale proportionally to the number of molecules in the latter. Without sufficient repulsion, colloids will have an attraction to one another and can aggregate irreversibly if the net strength of their interaction potential $w \gg kT$.

The electrostatic potential between two particles in a medium depends on the concentration of dissolved ionic species. The electrical potential $\psi(x)$ at a distance x away from the surface can be described using an expression for the distribution of ionic species using Stern-Gouy-Chapman theory[72, 34]. Ionic species which have the same or different charge relative to a surface are referred to as co- and counter-ions, respectively. The concentration of counter-ions is higher near the surface than in the bulk solution, and the opposite is true for co-ions. Moving from the surface to the medium, the region with a high concentration of counter ions near the surface is the Stern layer, and is followed by a more diffuse electrical double layer with thickness described by the Debye length $\lambda(\kappa^{-1})$, and the concentration then gradually drops off to the concentration of the bulk suspension. To obtain the functional form of $\psi(x)$, ions are treated as point charges with contributions to the electric potential described by Poisson's equation:

$$\nabla^2 \psi = -\frac{1}{\epsilon \epsilon_0} \rho_e \quad (2.9)$$

Where ϵ and ϵ_0 are the dielectric constants of a medium and the vacuum, respectively, and ρ_e is the spatial charge distribution. The diffusive nature of ions in the double layer is described as a Boltzmann distribution to arrive at the Poisson-Boltzmann equation in which ρ_e is replaced by the sum of the product of n_i concentrations of i ions each with a valence z_i and the elementary charge e :

$$\frac{d^2\psi}{dx^2} = -\frac{1}{\epsilon\epsilon_0} \sum_i z_i e n_{i,\infty} e^{-\frac{z_i e \psi(x)}{kT}} \quad (2.10)$$

By considering the case of a single symmetrical 1:1 electrolyte (e.g. NaCl), and by assuming a low surface potential (i.e. Debye-Hückel approximation), one obtains the linearized Poisson-Boltzmann equation:

$$\frac{d^2\psi}{dx^2} = \kappa^2 \psi(x) \quad (2.11)$$

The electrical potential near a particle surface is described by the boundary condition $\psi(0) = \psi_0, \psi(x) = \psi_0 e^{-\kappa x}$ where $\kappa = \sqrt{\frac{2z^2 e^2 n_\infty}{\epsilon\epsilon_0 kT}}$ is the decay constant. The effect of ionic concentration on the double-layer thickness is shown in fig. 2.2. Higher ionic concentrations result in a thinner double-layer that leads to a more rapid decrease in the electrostatic potential away from the particle surface.

The Poisson-Boltzmann equation can be used to obtain the interparticle potential energy due to electrostatic forces between surfaces or particles in which the electric potential distribution and ion concentration overlap[34]. The Derjaguin approximation is a method for estimating the interaction between two spherical particles of radius R separated by a distance $D \ll R$ by approximating the spheres as parallel-facing annular disks[72], and is valid when the surface potential is a constant value and $\kappa R > 10$:

$$w(D) \approx 2\pi\epsilon\epsilon_0 R \psi_\delta^2 e^{-\kappa D} \quad (2.12)$$

Where ψ_δ is the potential at the center of the Stern layer. Taken together, the attractive and repulsive potential in a colloidal suspension due to van der Waals forces and the electrical double layer, respectively, can be added together to obtain a net interaction potential acting on a particle, i.e. $w_{total}(D) = w_{attractive}(D) + w_{repulsive}(D)$. The resulting equation for

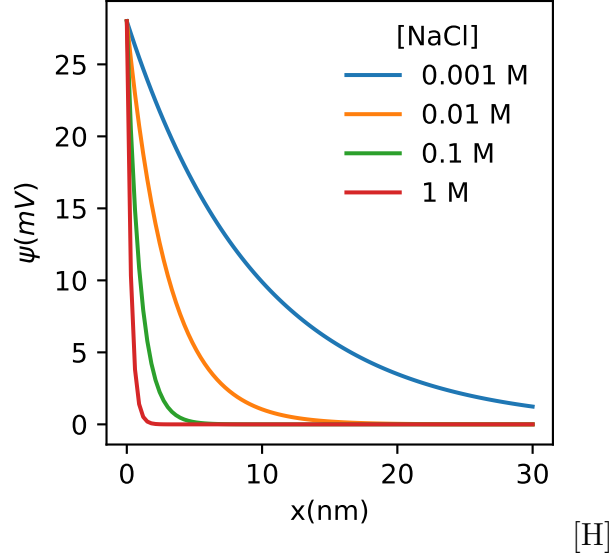


Figure 2.2: Electrostatic potential ψ as a function of distance from a surface in aqueous solution containing different concentrations of NaCl at 298K with a surface potential $\psi_0 = 28$ mV.

the interaction potential between two spheres, given the assumptions for the electrostatic potential and using the Derjaguin approximation, is shown below:

$$w_{total}(D) = -\frac{AR}{12D} + 2\pi\epsilon\epsilon_0 R\psi_\delta^2 e^{-\kappa D} \quad (2.13)$$

This theory is named after the scientists that pioneered it: Derjaguin-Landau-Verwey-Overbeek (DLVO). The attractive and repulsive contributions along with the total pair interaction potential are shown for a hypothetical pair of particles in fig. 2.3 A. This figure illustrates that a suspension of particles can be electrostatically stabilized against aggregation when the repulsive potential due to electrical double layers is greater in magnitude than that of the attractive potential. Generally, as the distance between two particles decreases, the magnitudes of repulsive and attractive forces increase from a value of zero at infinite separation. However, at a close enough distance, the attractive potential will be greater than the repulsive potential, and particles can aggregate. For the example in fig. 2.3 A, as two

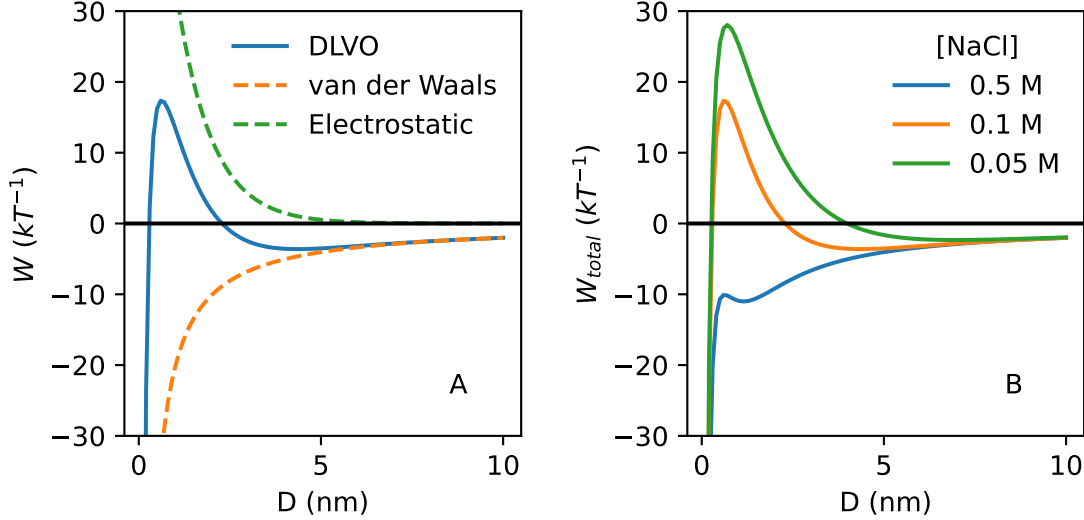


Figure 2.3: A shows the contributions from van der Waals and electrostatic forces to the combined DLVO interaction potential for an arbitrary 200 nm diameter pair of particles in 0.1 M NaCl at 298.5K, and a $\psi_\delta=30$ mV. B is the same system but shows the effect of changing ion concentration.

particles approach there is a local minimum of approximately $3.5 kT$ at a separation distance of 4 nm. While some interparticle association may occur, this attraction is still weak relative to kT . Moreover, the particles are unlikely to spontaneously overcome the higher barrier due to electrostatic repulsion at a separation distance of 0.5 nm that has a value of ca. $18 kT$. On the other hand in fig. 2.3 B, increasing the ionic concentration by a factor of five results in a significant loss of electrostatic repulsion, and is expected to result in irreversible aggregation.

The DLVO theory has been extended to account for many other contributions to interparticle interactions[73, 34, 74]. Colloidal stabilizers, such as polymers, surfactants, or sequence defined molecules can be added to a suspension to provide steric stabilization. Depending on the nature of the system, the added molecules or macromolecules can spontaneously adsorb to the particle interface. Additives that have ionizable functional groups can also provide an electrostatic barrier to aggregation by increasing the charge density of the particle surface.

Therefore, the assumption used in formulating the Poisson-Boltzmann equation that ion adsorption is non-specific is not always valid. In the case of plasmonic metal nanoparticles, the variation of the Hamaker constant with respect to particle size and temperature must be taken into account[75, 73].

2.2 Nucleation and Particle Growth

Theoretical explanations for nanoparticle growth may be broadly be divided into classical and nonclassical mechanisms. The former is a thermodynamic description of how monomers are added to a particle which begins as a nucleus, and has continued to evolve to include new factors[76]. Nonclassical growth on the other hand is a broader definition of growth pathways in which the subunits being added to a particle or crystal are not just individual monomers and instead have higher-order structure[76, 77]. This section will introduce the fundamentals of particle nucleation and growth. Additional phenomena that affect changes in particle structure such as Ostwald ripening, size focusing, and kinetic control over particle shape will be introduced as well.

2.2.1 Classical Nucleation Theory

The classical description of how monomers in a solution begin to spontaneously attach to one another is called nucleation. Nucleation is divided into homogeneous or heterogeneous nucleation depending on whether the nucleus forms in solution or at an interface. The classical description of a homogeneous nucleus is a smooth sphere while a sphere-like cap is considered in heterogeneous nucleation. The essential idea of classical nucleation theory is that monomer association and dissociation both take place until a critical nucleus size is reached, at which point association is more favorable than dissociation. Homogeneous nucleation is described by the Gibbs free energy ΔG of a cluster with spherical symmetry:

$$\Delta G = -\frac{4}{3}\pi r^3 \Delta G_v + 4\pi r^2 \gamma \quad (2.14)$$

Where r is the radius of the nucleus, and γ is the surface energy per unit area. The bulk free energy $\Delta G_v = \frac{kT \ln(s)}{v}$ where S is the supersaturation of the system, and v is the molecular volume. The γ of the nucleus is typically assumed to be the same as for a bulk surface, but is not a good approximation for small nuclei[78]. The expression in eq. (2.14) is plotted in fig. 2.4A for homogeneous gold nucleation in toluene using parameters obtained from Chen et al.[26]. The critical radius of nucleation r_{crit} is defined by the point at which $\frac{\Delta G}{dr} = 0$. As shown in fig. 2.4 B, monomer association is highly favored over dissociation in nuclei with $r > r_{crit}$.

Heterogeneous nucleation occurs at sites such as phase boundaries or impurities including bubbles, other particles, or vessel walls. Adsorption to these sites lowers the critical free energy of nucleation because there is a decrease in the surface area of the interface between the nucleus and the medium it is in. The extent to which this surface area is minimized depends on the contact angle θ of the nucleus on the substrate. For a flat substrate S and a nucleating phase N in a liquid medium L , the Young equation relates the contact angle to the surface energy γ of each interface:

$$\theta = \cos^{-1} \frac{\gamma_{S-L} - \gamma_{S-N}}{\gamma_{N-L}} \quad (2.15)$$

A nucleating phase that has favorable interactions with the substrate (i.e. low γ_{S-N}) relative to the medium will form a nucleus that has a lower contact angle. The critical free energy for heterogeneous nucleation can be obtained using the relation $\Delta G_{crit}^{hetero} = \phi(\theta) \Delta G_{crit}^{homo}$, where $\phi \leq 1$ based on the following equation:

$$\phi = \frac{(2 + \cos \theta)(1 - \cos \theta)^2}{4} \quad (2.16)$$

The value of critical free energy of nucleation is an energetic barrier to formation of nuclei that can be used in an Arrhenius equation to predict the rate of formation of nuclei J after solving for ΔG_{crit} [25]:

$$J = A \exp -\frac{\Delta G_{crit}}{kT} = A \exp -\frac{16\pi\gamma^3 v^2}{3k^3 T^3 \ln S^2} \quad (2.17)$$

This expression demonstrates that for a given nucleating species, the rate of nucleation can be altered by changes in temperature, supersaturation, and surface energy. LaMer et al. used CNT to describe the formation of sulfur hydrosols in 1950[31]. The proposed mechanism consisted of three stages: (I) monomer build up to a critical concentration that is supersaturated, (II) rapid homogeneous nucleation of nuclei followed by a gradual decrease of super saturation, (III) heterogeneous nucleation and growth of particles as a result of monomers diffusing to and reacting at the particle surface. The separation of particle formation into distinct stages was a strength of this work and most likely influenced how researchers conceptualized nanoparticle synthesis following this publication.

2.2.2 Particle Growth

The classical description of particle growth by association of monomers divides the process into two steps: diffusion to the particle surface from solution, and the reaction at the surface, and originates in the work of Lifshitz, Slyozov, and Wagner (LSW theory)[25, 79]. An expression for the growth in particle radius over time $\frac{dr}{dt}$ is obtained by solving for steady state when the rate of monomers reacting at and diffusing to the surface are equal:

$$\frac{dr}{dt} = \frac{DV_m(C_b - C_r)}{r + \frac{D}{k_d}} \quad (2.18)$$

Where D is the diffusion coefficient of monomers, V_m is the molar volume of a bulk crystal, C_b is the bulk concentration of monomers in solution, C_r is the solubility of the particle, and k_d is a first order rate constant of monomer deposition. The dependence of a particle's solubility is a consequence of the increase in pressure across an interface due to its curvature, and is known as the Ostwald-Freundlich equation:

$$C_r = C_\infty \frac{2\gamma V_m}{rRT} \quad (2.19)$$

Where C_∞ is the concentration of a monomer which enters the solution at the interface of two bulk components (i.e. $K_{eq} = a_{solute}/a_{solid} \approx C_\infty$) where is the activity of a components[80]. This relationship can also be derived from the Kelvin equation and is applicable to other

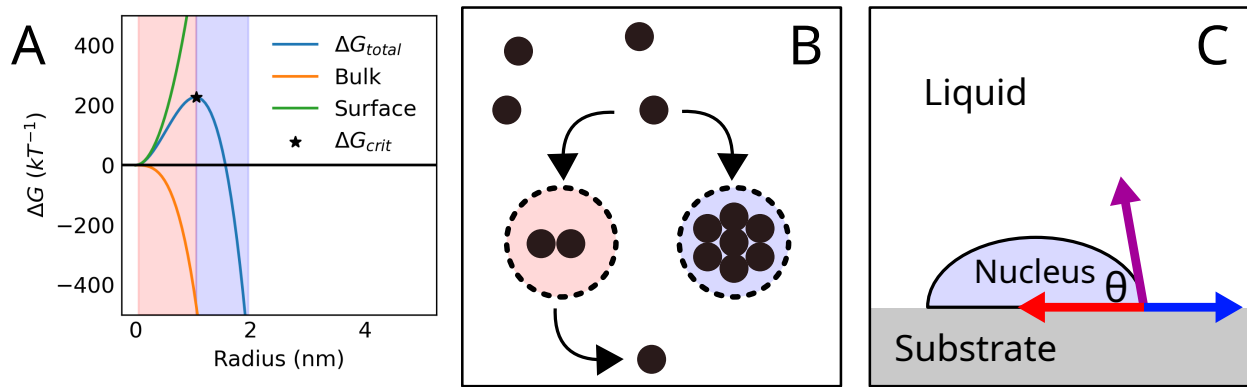


Figure 2.4: A is an example of eq. (2.14) which demonstrates the contributions of the bulk and surface free energy terms to the thermodynamic stability of a nucleus as a function of the radius of the nucleus. At the critical value of free energy ΔG_{crit} , the nucleus reaches a thermodynamically stable size at which point association of new monomers happens at a much greater rate relative to dissociation. The region below this critical point is highlighted in red in A to indicate that dissociation is favored. A schematic of homogeneous nucleation is shown in B where the black circles represent monomers that may associate or dissociate to form a nucleus that is approximated to be a sphere. Clusters of monomers which are at least the size of the critical nucleation radius are thermodynamically stable and can continue to grow. C is a schematic of heterogeneous nucleation in which the nucleus N is represented by a hemisphere on a substrate S in a liquid L . The red, blue, and purple arrows correspond to the interfacial energies γ_{S-N} , γ_{S-L} , and γ_{N-L} , respectively

two phase systems such as bubbles or droplets inside a liquid. Equation (2.19) can also be used to obtain the concentration of monomers in a solution by considering a critical radius r_b which has an equal rate of association and dissociation[79], and therefore does not change in size. The remaining assumptions made in the LSW description of particle growth are that eq. (2.19) can be expanded using a Taylor series given that the term inside the exponential is small, and that the diffusion layer thickness is much greater than the particle radius. LSW theory is therefore meant to describe the process of changes in particle size after nucleation due to Ostwald ripening, the process in which monomers dissociate from particles with $r < r_b$ and associate with particles that have radius $r > r_b$. The final expression is then:

$$\frac{dr}{dt} = \frac{2\gamma V_m^2 C_\infty}{RT(\frac{1}{D} + \frac{1}{k_d r})} \frac{\frac{1}{r_b} - \frac{1}{r}}{r} \quad (2.20)$$

If D or $k_d r$ are much smaller than the other, then particle growth may be considered to be diffusion or reaction rate limited. In LSW theory, $r_b = r_{crit}$, i.e. the critical nucleation radius from CNT is the particle size which will no longer change as a result of unequal rates of monomer association and dissociation. The two limiting conditions are predicted to lead to a distribution of particle sizes $\langle r \rangle = r_{crit}$ for diffusion limited growth, and $\langle r \rangle = \frac{8}{9}r_{crit}$ for reaction limited growth[79].

The applicability of this simplification and the validity of the approximation of eq. (2.19) by using an expansion were examined in more detail using Monte Carlo simulations by Talapin et al.[80]. This work demonstrated that LSW theory is correct in the sense that the final size distribution depends on the initial condition of nucleation as long as the nucleation rate is much higher than particle growth rate. However, when reactions are carried out in finite volumes, the consumption of monomers decreases the level of supersaturation, and the critical radius increases over time[80, 79]. The simulations carried out by the authors demonstrate that the consequence of this change in the critical radius is that the final size distribution also depends on the initial monomer concentration. The authors demonstrated that Ostwald ripening can in fact be used to achieve narrower size distributions of nanoparticles. This conclusion is in accord with the experimental results of hot-injection experiments

by Peng et al. described in chapter 1, in which the authors show that injection of additional monomers during the growth regime focused, or narrowed, the particle size[44].

2.2.3 Growth of Metal Nanoparticles

The validity of using classical descriptions of growth to analyze gold nanoparticle growth was tested by Chen et al.[26] using simultaneous SAXS/WAXS/UV-Vis. Unlike aqueous approaches which use gold salts with an oxidation state of +3, the authors used gold complexes which have an oxidation state of +1 and toluene as a solvent. Additionally, a high concentration of dodecanethiol was used as a surfactant to sterically stabilize the synthesized particles. The authors demonstrate that despite formation of nanoparticle clusters, nanoparticle growth was consistent with nucleation and monomer addition, and is generally in accord with LSW theory. In stark contrast, authors such as Jörg Polte claim that many syntheses of metal nanoparticles feature particle coalescence which is inconsistent with LSW theory[33, 34]. Moreover, Polte argues that Ostwald ripening is unlikely to take place in metal nanoparticle syntheses at low temperatures, and in the absence of reagents that can etch metals. Coalescence of particles is difficult to distinguish from Ostwald ripening using SAXS because both phenomena feature a decrease in the number of particles and an increase in the mean particle radius. Thanks to advancements in cryo TEM, and in-situ TEM and AFM, the existence of nonclassical phenomena such as particle coalescence and oriented attachment have been validated in certain examples of gold nanoparticle growth[81, 82, 83]. Therefore, the description of nanoparticle syntheses in which nonclassical processes take place need to incorporate theories of interparticle interactions. However, as discussed in the preceding section, (extended) DLVO theory has limitations with respect to explaining nanoparticle growth. One of the limitations of DLVO theory is that nanoparticles are considered to be spherical, when in fact they are faceted structures, and can even be highly anisotropic. Zhu et al. used in-situ TEM to study the oriented attachment of citrate stabilized gold nanoparticles[82]. The authors demonstrated that particles had a preference for attachment occurring between 111 facets rather than 100. This was somewhat unexpected in light of the fact that DFT

calculations predict that the surface energy of bare 100 facets is higher than 111. However, because citrate was predicted to have a preference for 100 facets, there was a greater energetic penalty associated with attachment of the 100 facets. Control over the shape of faceted and anisotropic particles is another area in which differences in crystal surface energies play a significant role. The shape of a crystal in thermodynamic equilibrium is predicted using the Gibbs-Wulff theorem by taking into account the differences in surface free energies of different crystallographic facets[40]. Selective binding of species such as ions, precursor complexes, small molecules, or macromolecules towards different facets can decrease their surface energy, and thereby maximize the proportion of a particular facet[39, 40]. In addition, the presence adsorbed species at a surface may decrease deposition of monomers at that facet, and thereby decrease growth in the direction perpendicular to the facet.

Therefore, the design of sequence defined molecules which specifically adsorb to particle surfaces or facets would be a powerful tool for controlling colloidal stabilization of particles against coalescence and biasing facet expression. However, experimental techniques for directly validating that molecules have selectivity for a certain facet are limited[40]. Existing techniques such as surface plasmon resonance or differential capacity rely on the ability to synthesize bulk metal crystals with a high proportion of a specific facet[61, 84]. Insights into molecular design for facet specific binding can also be obtained using infrared spectroscopy by characterizing the molecular conformation of molecules adsorbed to nanoparticles that have a high proportion of a certain facet[85]. Synthesis of high index and high surface energy facets is desirable for catalysis applications[86], and therefore motivates the need for molecular design insights into stabilization of high surface energy facets. Therefore, computational approaches for studying interactions between molecules and inorganic surfaces will continue to be essential to the design of sequence defined molecules for nanoparticle synthesis, and will need to be highly integrated with experimental methods that can validate computational predictions.

2.3 Surface Plasmon Resonance

2.3.1 Fundamentals and Origin

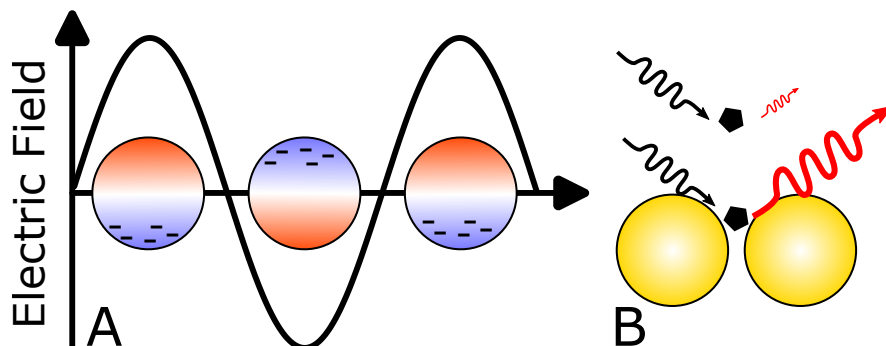


Figure 2.5: A The electric field of a propagating light wave displaces electrons from their equilibrium position resulting in a charge imbalance across an irradiated spherical nanoparticle. B Inelastic scattering of light from an irradiated analyte results in Raman scattering which can be used for sensing applications. Significant signal enhancement is achieved due to the local electric field enhancement as a result of plasmon coupling between two nanoparticles near one another.

Plasmons are produced when an electric field coherently displaces electrons away from their equilibrium with metal atoms, and thus results in a charge imbalance[3, 87]. Unlike in bulk metals, electromagnetic radiation can displace electrons near the surface of a metal to form propagating surface plasmon polaritons. This process is not limited to the surface in nanoparticles with dimensions smaller than the wavelength of light. Light induces polarization of electrons in the conduction band of the whole particle and thereby forms localized surface plasmons. As seen in fig. 2.5 A, the restoring force due to charge displacement is conceptually similar to a harmonic oscillator in which the resonance depends on the wavelength of light, the dielectric function of the medium, the structure of the particle (size and shape), and proximity to other metal nanoparticles. Electric fields near the particle surface are greatly

enhanced as a result of localized surface plasmons. Amplification of electromagnetic fields near a particle surface has been used to increase the signal from phenomena such as Raman scattering and fluorescence[3]. The former is illustrated in fig. 2.5 B which demonstrates that the Raman signal of an analyte excited by light is greater when it is near the intersection of plasmonic nanoparticles due to hotspots generated by coupling between the nanoparticles.

2.3.2 Mie Scattering

By the early 20th century, the strong wavelength dependence in Rayleigh's description of scattering was understood to be responsible for the blue tint of various colloidal suspension[88]. However, the color of gold sols, which have been used in the preparation of red colored glass since at least the 4th century (Lycurgus cup)[3], could not be explained by this same framework. The work of Gustav Mie stood out from his contemporaries in the completeness of comparisons between experiments and his theoretical explanation[88]. The model developed by Mie was able to explain light scattering and absorption by spherical particles larger than the wavelength of light, and was consistent with Rayleigh scattering for particles with sufficiently small dimensions. The approach considers homogeneous spherical particles in a non-absorbing medium, and uses Maxwell equations and multipole expansion of electromagnetic fields to describe scattering, extinction, and absorption cross-sections[87]:

$$\sigma_{scattering} = \frac{2\pi}{|k|^2} \sum_{L=1}^{\infty} (2L+1) (|a_L|^2 + |b_L|^2) \quad (2.21)$$

$$\sigma_{extinction} = \frac{2\pi}{|k|^2} \sum_{L=1}^{\infty} (2L+1) (Re(a_L + b_L)) \quad (2.22)$$

$$\sigma_{absorption} = \sigma_{extinction} - \sigma_{scattering} \quad (2.23)$$

k is the wavevector, and L are integers representing each scattering pole (i.e. dipole, quadrupole, etc.). a_L and b_L are parameters composed of Riccati-Bessel functions which depend on the sphere radius, the wavenumber of light in the medium, the complex refractive index of the metal, and the refractive index of the medium. By approximating the

Bessel functions using a truncated power series[89] limiting L in eq. (2.21) and eq. (2.22) to the dipole term, and only considering nanoparticles with radius r such that $\frac{2\pi r}{\lambda_{medium}} \ll 1$ one obtains a simpler expression for the extinction cross-section[87]:

$$\sigma_{extinction} = \frac{18\pi\epsilon_m^{3/2}V}{\lambda} \frac{\epsilon_2(\lambda)}{[\epsilon_1(\lambda) + 2\epsilon_m]^2 + \epsilon_2(\lambda)^2} \quad (2.24)$$

Where ϵ_m is the dielectric function of the medium, ϵ_1 and ϵ_2 are the wavelength dependent real and imaginary components of the metal's dielectric function, and V is the particle volume. This approximation is known as the quasistatic approximation and holds for particles with a diameter below 20 nm[3]. The important takeaways of this expression are that the magnitude of extinction scales with particle size, the plasmonic properties depend on the frequency dependent dielectric function of the metal as well as its medium, and that a maximum value exists for $\epsilon_1(\lambda) = -2\epsilon_m$. The expression for the scattering cross-section on the other hand is:

$$\sigma_{scattering} = \frac{34\pi^4\epsilon_m^2V^2}{\lambda^4} \frac{(\epsilon_1 - \epsilon_m)^2 + \epsilon_2^2}{(\epsilon_1 + 2\epsilon_m)^2 + \epsilon_2^2} \quad (2.25)$$

And $\sigma_{absorption} = \sigma_{extinction} - \sigma_{scattering}$. The result for extinction, scattering, and absorption cross-sections for a 20 nm diameter gold nanoparticle in water are presented in fig. 2.6. While these results are limited to small spherical nanoparticles in which absorption is apparently the dominant contributor to optical extinction, differences in particle shape and size can result in differences in contributions from absorption and scattering to extinction.

2.3.3 UV-Vis Spectroscopy as a Proxy for Structural Changes

In this work, plasmon resonance is used as a proxy for gold nanoparticle structure. The influence of spherical particle size, effect of two-dimensional symmetry (i.e. nanoplates, nanotriangles), and aggregation are most pertinent to this thesis. By using more terms in the power series expansion of the Bessel functions and including contributions from quadrupoles ($L = 2$), Mie theory is also able to describe optical extinction of larger spherical particles not described by quasistatic approximation[3, 90]. Therefore, it can predict that the position of

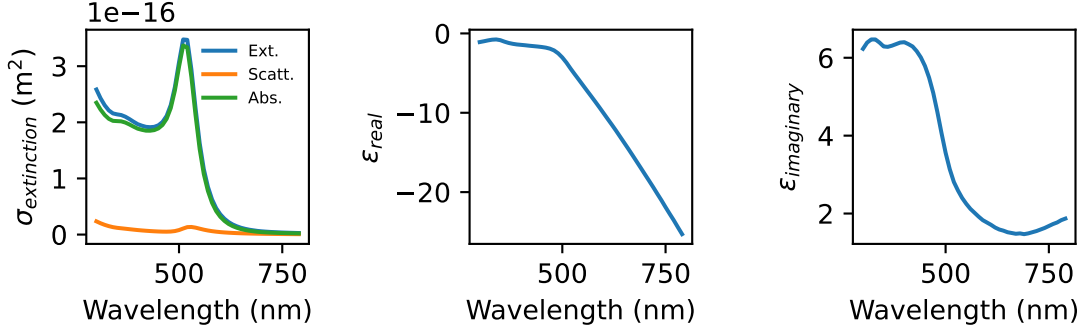


Figure 2.6: A Quasistatic approximation of a 20 nm diameter gold nanoparticle's extinction, scattering, and absorption cross-sections in water. B and C show ϵ_1 and ϵ_2 , the real and imaginary components of gold's dielectric function, respectively[1].

the surface plasmon resonance peak is red-shifted for larger nanoparticle sizes. However, Mie theory is limited to spherical and spheroidal particles, and numerical approaches are commonly used to study surface plasmon resonance. One commonly used numerical approach is the discrete dipole approximation (DDA). Anisotropic particles such as nanotriangles and nanorods have a second plasmon resonance peak due to the appearance of a second, longitudinal plasmon resonance mode. Using the analogy of the harmonic oscillator, the energy of this mode is lower because more electrons dampen the oscillation. O'Brien et al. used DDA to model the optical extinction of thin circular metal disks. The authors found that the transverse mode of resonance, associated with the thickness of metal disks, becomes negligible for disc-like particles thinner than 10 nm. In certain applications of plasmonic nanoparticles, the ratio of absorbance and scattering to optical extinction is also an important parameter that needs to be controlled. Meyer et al. point out that while it is true that the ratio of absorbance to scattering contributions to a plasmon resonance peak decreases with increasing particle size, absorption dependent phenomena such as photothermal heating are not maximized by reducing nanorod length because of differences in absorption efficiency[91]. Numerical simulations were employed by the authors to distinguish contributions to extinc-

tion from absorption and scattering, but this can also be measured experimentally by using an integrating sphere detector. The latter was used by Langhammer et al. to measure absorption and scattering of disk like metal particles on a silica substrate[92]. The authors found that while the ratio of absorption to scattering cross-sections decreased with increasing disk diameter, the ratio only decreased below 1 in the case of Ag and Au but not Pd or Pt. Changes in plasmon resonance occur as a result of coupling between dipolar plasmon modes in assemblies of metal nanoparticles. Depending on the number of particles in an aggregate, a second peak may appear due to coupling. Additionally, due to the convolution of several supported plasmon modes within an aggregate, both peaks will appear broad[3].

Chapter 3

EXPERIMENTAL METHODS

3.1 *Small-Angle Scattering*

Small-angle scattering (SAS) is a versatile technique for characterizing structures with length scales ranging from 0.1 to 1000's of nm. The versatility of SAS is illustrated by its ability to characterize the structure of a wide variety of colloidal systems in the native state[93] or during an application of some external input[94, 95], and with time-resolved changes on the order of milliseconds[96]. SAS encompasses a wide range of instrumental techniques, which may use either collimated X-rays or neutrons, and are designed to optimize experiments depending on the materials being studied as well as temporal and spatial resolution. Finally, SAS is also highly synergistic with computational techniques. SAS profiles can be calculated from simulation results and compared to experiments to refine simulation parameters, or validate hypotheses generated from experiments[97].

3.1.1 General Theory

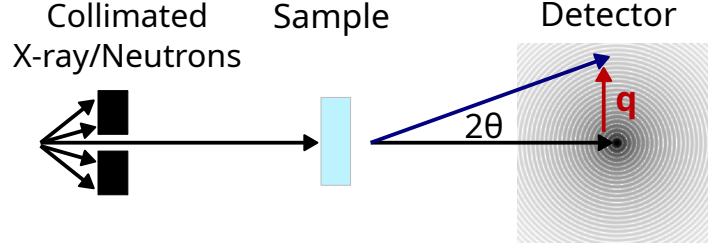


Figure 3.1: Experimental setup for a typical small-angle scattering experiments. A collimated X-ray or neutron beam passes through a sample and scatters radiation with an angular distribution of intensity captured by a detector. The 2D intensity is then integrated to obtain a 1D scattering profile. The black vectors represent the incident and transmitted beam, the blue vector represents the scattered beam, and the red vector is the scattering vector q .

A collimated beam of X-rays or neutrons, represented by the black vector in fig. 3.1, can be transmitted, absorbed, or scattered by structures in a sample. The scattering vector q is the difference between the scattered and incident beams. The magnitude of the scattering vector is determined by both the wavelength of incident radiations λ , as well as the angle between the incident and scattered beam 2θ :

$$q \equiv |\mathbf{q}| = \frac{4\pi}{\lambda} \sin \frac{\theta}{2} \quad (3.1)$$

Scaling by λ allows for interoperability between different radiation sources. The scattered radiation measured at a given q value is the result of interference between scattered waves originating from inhomogeneities in the irradiated sample volume. The relative phase shift between two scattered rays is qr , where r is the vector separating the two origins of the scattered wave. The amplitude of radiation from a sample is the sum of the amplitudes from all scatterers in the path of the beam, where each amplitude is the product of the phase shift and the scattering length. In experiments however, detectors measure the intensity

of scattering. Analysis of experimental scattering intensity as a function of q enables one to extract the spatial distribution of electron density $\rho(r)$ (for X-ray scattering) inside a sample. The scattering intensity is the squared modulus of the Fourier transform of a given material's electron density. However, a continuum approximation of the electron density, the scattering length density ρ , is more tractable for describing small-angle scattering. For a given molecule, ρ is the sum of scattering lengths of all atoms divided by the molecular volume. The scattering length represents the change in amplitude of a scattered wave relative to the incident wave. For SAXS, Thomson scattering is the primary mode of scattering, and the scattering length b at small angles is:

$$b = r_{el}Z \quad (3.2)$$

Where r_{el} is the radius of the electron, and Z is the atomic number. For a two phase system the scattering intensity is:

$$I(q) = \frac{1}{V}(\Delta\rho)^2 \left| \int_{V_1} e^{-iqr} dr \right|^2 \quad (3.3)$$

Here, $\Delta\rho$ is the difference in scattering length density between the particle and its medium and is also referred to as contrast. V is the scattering volume, V_1 is the volume of one of the phases. The spatial distribution of material inside the system is given by $\left| \int_{V_1} e^{-iqr} dr \right|^2$. For a system consisting of discrete inhomogeneities inside a medium, such as particles in a solvent, eq. (3.3) can be rewritten as:

$$I(q) = \frac{N}{V}(\Delta\rho V_P)^2 P(q)S(q) + background \quad (3.4)$$

Here $\frac{N}{V}$ is the number density of particles, and V_P is the volume of a particle. $P(q)$ is the form factor and describes the contributions from particle shape to scattering. $S(q)$ is the structure factor and accounts for correlations between particles, and has a value of 1 in dilute conditions. Analytical solutions for form factors have been obtained for a variety of geometric shapes (e.g. spheres, rods, discs), and parametrizations of simulations of structures (e.g. flexible cylinder). Systems in which correlations between particles or other structures are high, such as in fractal particle aggregates or highly concentrated particle suspensions

require use of a structure factor. However, model fitting is an ill-posed problem which gives non-unique results, and often requires additional knowledge to assign model parameter values.

Structural information can also be obtained using approaches that require no assumptions about particle shape or other model parameters. The form factor of structures in a system with negligible interparticle correlations can be approximated in the low- q limit by the Guinier approximation[98, 72]. This results in the following expression for scattering intensity:

$$I(q) = \phi \exp \frac{-Q^2 R_g^2}{3} \quad (3.5)$$

Where ϕ is the volume fraction when the absolute scattering intensity is known, and R_g is the radius of gyration. The radius of gyration describes a particle's spatial distribution of scattering length density. Higher q values on the other hand probe increasingly smaller structural length scales, and lead to different power laws indicative of the particle's symmetry, and surface roughness[99]. A model which yields continuity between scattering at low- q (Guinier region) and high- q (Porod region) was published by Hammouda[100]. This model allows one to distinguish between particles that are spherical, rod-like, or plate-like. Radii of gyration may then be related to spherical, cylindrical rod-like, and plate-like particles by:

$$R_{g,sphere} = R \sqrt{\frac{3}{5}} \quad (3.6)$$

$$R_{g,cylinder} = \sqrt{\frac{R^2}{2} + \frac{L^2}{12}} \quad (3.7)$$

$$R_{g,plate} = \sqrt{\frac{W^2}{12} + \frac{T^2}{12}} \quad (3.8)$$

Where R is radius, L is length, W is width, and T is thickness[100].

3.1.2 Instrumentation

A schematic of a typical experimental configuration is presented in fig. 3.1. The flight path of radiation from the source to the detector is typically under vacuum to decrease noise, but it

is also possible to measure samples in ambient or other conditions as necessary. A detection system located at a known distance from the sample is used to measure the intensity of the elastically scattered X-rays or neutrons as represented by the red scattering vector in fig. 3.1. Instruments in which the position of the detector can be changed are able to optimize the measurement of a portion of q-space by maximizing the flux of scattered radiation while maintaining sufficient angular resolution. Specifically, shorter sample to detector distances are used to capture wide-angle scattering, and large distances are used to capture scattering at smaller angles. However, extending the sample to detector distance to resolve smaller angles is impractical beyond a certain point because of instrument footprint considerations and the decrease in flux due to a need to reduce the beam size. Above all, there are also issues related to beamstop shadowing effects, slit fluorescence, and parasitic reflections from slits[101]. Ultra-small angle scattering overcomes these challenges and enables measurements at much smaller angles (e.g. less than 1 degree) by using Bonse-Hart crystal optics[101, 102]. The latter rely on a set of channel cut crystals that use Bragg diffraction to select a precise wavelength or direction of a beam. In a USAS instrument, the first set of crystals acts as a monochromator to select a specific wavelength that will pass through a sample. Radiation scattered from the sample then passes through a second set of crystals that are rotated to probe different scattering vectors without the need for large sample to detector distances. The drawback of USAS instruments is that the geometry of the optics only allow high resolution of the scattering vector in the direction perpendicular to the rectangular slit of the channel cut crystal. Scattering parallel to the length of the slit is a source of significant instrumental smearing that can be accounted for, but makes the technique inappropriate for anisotropic samples in which scattering varies with respect to the azimuthal angle.

In addition to instrumental configuration smearing effects, smearing also arises due to gravity in the case of neutrons, detector resolution, and due to polychromaticity of radiation[103]. Smearing changes the observed $I_{observed}(q)$, and needs to be accounted for by either applying instrumental smearing to a model that is being used to fit data, or by applying a desmearing algorithm to the experimental data[103]. The effects of different con-

tributions are well-approximated by using a combination of Gaussian functions which relate the distribution of radiation from a given q vector to an apparent or measured q' vector as a point spread function $R(q, q')$ [103]. Applying instrumental smearing takes as input the geometry of an instrumental configuration (e.g. slit height, width, shape), and computes a weighting vector for each q -value. Desmearing procedures such as the Lake algorithm[104], rely on the assumption that:

$$\frac{I_{observed}(q)}{\int_{q'} I_{observed}(q'), R(q, q') dq'} = \frac{d\sigma/d\Omega(q)}{\int_{q'} d\sigma/d\Omega(q'), R(q, q') dq'} \quad (3.9)$$

In which $d\sigma/d\Omega$ is the scattering cross-section. The meaning of this relation is that the ratio of the experimental intensity with and without smearing is effectively the same as the ratio of the scattering cross section with and without smearing effects. The true scattering cross-section is then estimated by $u(q)$:

$$u_1(q) = \frac{I_{obs}^2(q)}{\int_{q'} I_{observed}(q'), R(q, q') dq'} \quad (3.10)$$

This process is repeated iteratively based on the choice of the user, or since excessive iterations lead to divergence, a stopping criteria like the one proposed by Vad and Sager is reached[103].

3.2 Optical Spectroscopy

Optical spectroscopy uses light to probe chemical signatures in a sample as a function of wavelength. Specific wavelengths of radiation elevate electrons in a molecule from a ground state to an excited state. Spectroscopic measurements may be taken of light emission, absorption, or scattering as a result of these interactions between molecules and light.

3.2.1 Ultraviolet and Visible Light Spectroscopy

Ultraviolet and visible light (UV-Vis) spectroscopy is a simple method for characterizing the presence and concentration of chemical species. The typical wavelength range for UV-Vis experiments ranges from 200 nm to 800 nm. In a common instrument configuration, a white

light source first passes through a monochromator before reaching a sample. The light which is not absorbed or scattered by the sample is then detected, and the absorbance, A , at a particular wavelength is calculated as shown in section 3.2.1. I_0 and I_T are the incident light intensity and transmitted light intensity, ϵ is the molar extinction coefficient, l is the path length of the sample, and c is the concentration of the species which is absorbing light. Other instrument configurations may allow for simultaneous detection of a full spectrum of light by using gratings and a CCD detector.

$$A = \log \frac{I_0}{I_T} \epsilon l C \quad (3.11)$$

3.2.2 Fluorescence Spectroscopy

Molecules which have fused aromatic groups have delocalized electrons that can be excited by absorbing light at a particular wavelength, and then emitting light at a higher wavelength. This phenomenon is known as fluorescence. Conventional fluorescence spectroscopy involves measuring the emission intensity at a specific wavelength or spectrum of wavelengths due to excitation by a single wavelength. Recently, non-aromatic fluorophores have received attention, and molecular aggregation may be an important factor in several systems[105]. Fluorophores may also be used to study the self-assembly of other molecules through direct conjugation or site-specific adsorption. The fluorescence of fluorophores depends on interactions such as dipole-dipole coupling and π -orbital interactions between the fluorophore and its medium. Pyrene was used to characterize the critical micelle concentration of amphiphilic peptides in chapter 4 using a method developed by Kalyanasundaram and Thomas[106]. Trace amounts of pyrene were dissolved in the presence of a range of peptide concentrations, and the ratio of two emission peaks was measured as a function of peptide concentration. Once the peptide concentration is high enough to form micelles, pyrene is adsorbed in the apolar environment formed by the alkyl tails at the center of the micelle, and its emission

spectra are altered.

3.2.3 Circular Dichroism Spectroscopy

The difference between absorption coefficients of left and right circularly polarized light, $\Delta\epsilon = \epsilon_L - \epsilon_R$, is circular dichroism (CD)[107]. Circular dichroism at wavelengths in the ultraviolet to visible range is also referred to as electronic circular dichroism (ECD) to distinguish it from vibrational circular dichroism which occurs at infrared wavelengths. The origin of CD is described by the Rosenfeld for the rotation strength R [107]:

$$R = \text{Im}[\mu^{if} \dot{m}^{if}] \quad (3.12)$$

which is the imaginary component of the dot product of the scalar product of the electric and magnetic dipole transition moments μ and m during a transition from state i to f . An experiment schematic is presented in fig. 3.2. Combined with theoretical calculations, the technique may be used to confirm the absolute configuration and conformation of chiral molecules[107]. Additionally, the technique is sensitive to changes in molecular conformation which occur as a result of self-assembly of both chiral and achiral molecules. Peptide and protein conformations can be experimentally characterized in-situ by comparing CD spectra to models of secondary structure components such as α -helices and β -sheets[107]. CD in supramolecular assemblies of achiral molecules is induced through strong, asymmetric non-covalent intermolecular interactions[108]. Symmetry breaking can be induced when achiral molecules are self-assembled in the presence of chiral molecules or solvents. Interestingly, systems composed of only achiral molecules may form chiral structures such as helical tapes and ribbons. Moreover, the statistical average of structures with opposite supramolecular chirality (e.g. left or right handed helices) may or may not be equal. Self-assembled achiral molecules will only have a CD signal if there is a bias towards structures with one handedness.

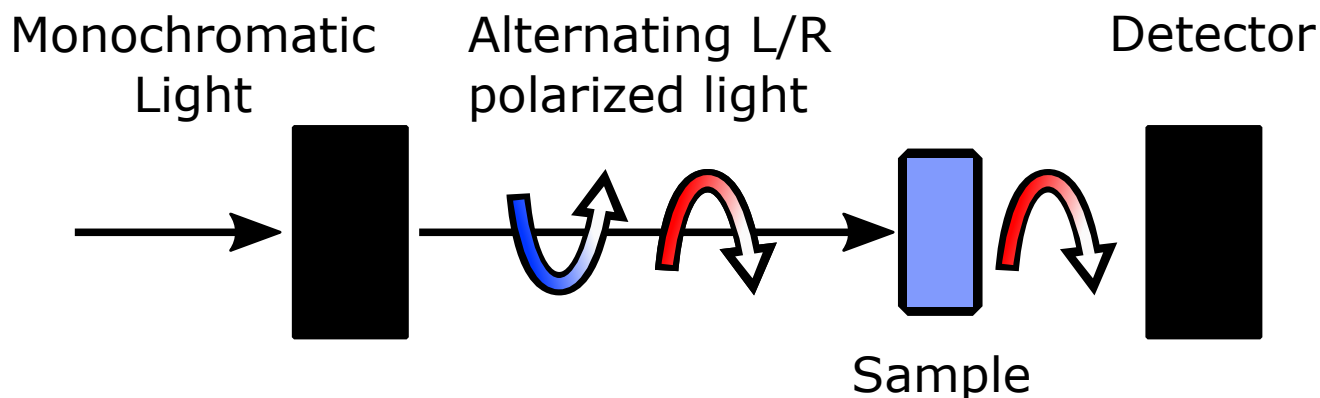


Figure 3.2: Schematic of a typical circular dichroism spectroscopy experiment. A monochromatic light source is circularly-polarized before passing through a sample. Depending on the optical activity of the sample, the difference in right and left circularly-polarized light is detected.

3.3 *Transmission Electron Microscopy*

The basic components of a transmission electron microscope (TEM) are an electron gun, illumination system, objective lens, specimen stage, image-forming system, and an image viewing and recording chamber[109]. Electrons are guided using electromagnetic fields onto thin samples that can transmit, absorb, scatter, or diffract the electrons. Like in other techniques, the resolution limit is related to the wavelength of the probe being used. The wavelength of an electron beam depends on its acceleration voltage, but is many orders of magnitude lower than that of visible light. Electrons accelerated with a voltage of 10-1000 kV have wavelengths between 12.2 and 0.87 pm[109]. Due to instrumental aberrations, the practical resolution limit of TEM instruments in ideal operating conditions is approximately 0.2 nm. TEM is well suited for characterizing inorganic nanoparticles and other materials with nanoscale features.

Important disadvantages of TEM include the need to dry colloidal suspensions on a sur-

face and image under high vacuum, the general difficulty of loading and imaging multiple samples in the same session, and difficulties associated with data analysis. Sample drying may lead to aggregation that is not representative of the sample *in situ*. To some extent, this has been addressed in by use of liquid phase TEM systems[110]. Besides overcoming drying effects, liquid phase TEM has also enabled experimenters to study the dynamics of processes such as particle ripening *in situ*[111]. Advances in throughput of analyzing colloidal suspensions have taken place in the form of microarray deposition instruments as demonstrated by Castro-Hartmann et al.[112]. Regardless of whether imaging is done *in situ* or on a large number of samples, analyzing data and making statistically significant conclusions about particle structure is difficult. Automating image acquisition and analysis using machine learning methods will augment TEM and lead to progress in a variety of research areas such as analysis of biological tissues and particle dynamics[113, 114]. Finally, TEM instruments may also be operated in modes that allow for mapping elemental composition onto structures (energy dispersive X-ray elemental analysis, EDX), or minimize drying effects and resemble structures found *in situ* (cryo electron microscopy).

3.4 Scanning Electron Microscopy

Unlike TEM, scanning electron microscopy (SEM) principles are less analogous to traditional microscopy techniques. While an electron beam passes through samples in TEM, SEM involves scanning or rastering over a sample to create an image. A typical SEM instrument is also operated under high vacuum, and consists of a column that includes an electron source and a lens system, a sample chamber, and several detectors. The electrons generated in order to probe the sample are called primary electrons. The use of detectors in multiple position is related to the ways in which electrons interact with matter and generate signals that can be used to form images. These interactions can be categorized as inelastic or elastic depending on whether the electron's energy changes significantly or not, respectively[109]. Electrons interact with nuclei of atoms that have a large atomic number have a high likelihood of being elastically scattered due to Rutherford scattering. This can lead to a weak change

in trajectory, or a significant change that causes these electrons to become back-scattered. Interactions between primary electrons and electrons in the sample are typically inelastic. When sufficient energy is transferred from the primary electron, secondary electrons are released from the sample and can also be detected to create an image. Electrons which have a higher energy state may relax to lower energy state and emit radiation that is characteristic to the atom. This is the principle behind EDX which can be used in both SEM and TEM. Depending on the imaging mode used, an SEM can achieve a lateral resolution on the order of 1 nm, and focal depths on the order of mm depending on the magnification used[109]. While SEM is also an *ex situ* technique which provides structural information that has lower resolution than TEM, SEM has practical advantages in the analysis of nanoparticles. The latter include the comparable ease with which multiple samples can be loaded in an instrument, and the ability to image larger numbers of particles.

3.5 Dynamic Light Scattering

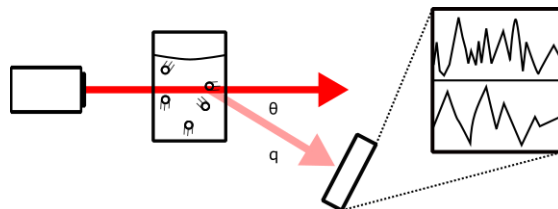


Figure 3.3: Experimental setup of dynamic light scattering. A detector measures the scattered light intensity at some angle θ . The scattering vector q depends on θ and the wavelength of incident light and is related to the size of scatterers. The measured intensity over time is analyzed using an autocorrelation function.

Dynamic light scattering (DLS) is used to characterize the size distribution of colloidal suspensions in liquids by monitoring light scattering over time. As seen in fig. 3.3, the experimental setup consists of a collimated monochromatic light source which passes through

a suspension of colloids which scatter light that is measured by a detector. Light scattering occurs due to differences in the refractive index between a scatterer and its medium. The scattering angle and magnitude (q vector) depend on the size and shape of the scatterer, the refractive index of the solvent and scatterer, and the wavelength of light. When thermal fluctuations are more significant than any other force acting on the colloid (e.g. gravitational), particle dynamics are governed by Brownian motion. Measurement of the scattering intensity I over time t can be analyzed using an autocorrelation function[72]:

$$G(\tau) = \lim_{t \rightarrow \infty} \frac{1}{T} \int_0^T I(t)I(t + \tau)dt \quad (3.13)$$

For an instantaneous interval, $\tau = 0$, the intensity does not change significantly and is therefore correlated. As $\tau \rightarrow \infty$, the signal is not correlated because it does not capture the rate of change in the signal. Therefore, the time averaged product of $I(t)I(t + \tau)$ at $\tau = 0$ and as $\tau \rightarrow \infty$ yields $\langle I^2(t) \rangle$ and $\langle I(t) \rangle^2$, respectively. For a monodisperse population of particles, the autocorrelation function may be fit using an exponential decay model to extract a decay constant Γ , which is related to the diffusion constant D and the scattering vector q by the relation:

$$\Gamma = q^2 D \quad (3.14)$$

The hydrodynamic radius R_h of colloids that can be approximated as spheres in a medium of viscosity μ at a temperature T can be determined using the Stokes-Einstein equation:

$$D = \frac{k_b T}{6\pi\mu R_h} \quad (3.15)$$

A more general relation between the translational diffusion coefficient, D_T , of particles with other geometries is:

$$D_T = \frac{k_b T}{f} \quad (3.16)$$

Where f is a friction coefficient that can be determined or approximated using analytical and numeric methods[115]. In chapter 6, DLS was used to measure the diameter of plate-like gold nanoparticles. The Rayleigh approximation described by Mansfield et al. is intended

for use with thin circular disks, and relates R_h to R_{disk} as follows:

$$R_h = R_{Rayleigh} = \frac{2R_{disk}}{\pi} \quad (3.17)$$

This approximation was found to underestimate the volume weighted diameter of Gibbsite platelets determined using electron microscopy by 37%. A more recently published result by Hernández-Cifre et al. calculated friction coefficients of nanoscale plate-like particles with arbitrary shapes[2]. Friction coefficients can be estimated for a variety of shapes analyzed by the authors by using different values for shape parameters obtained from simulations in the equation:

$$f = 12\eta_0 \left(\sqrt{\frac{A}{\pi}} C + Qh \right) \quad (3.18)$$

Where A is the area of a plate-like particle, C and Q are shape specific fitting parameters[2], and h is the thickness. Provided that h is known, solving for A by using eq. (3.18) in eq. (3.16) can be used to obtain a lateral dimension by relating the area to dimensions of different geometric shapes (e.g. major diameter of hexagon, side length of triangle). For a range of experimentally determined diffusion coefficients D_T of hexagonal nanoplatelets in water at a temperature of 298.5K, the major diameter is shown in fig. 3.4. For a monomodal distribution with moderate polydispersity, the autocorrelation function can be expressed as the overlap of several exponential functions with a distribution of decay constants. Overall, DLS is a simple but useful technique for characterizing the size of dilute dispersions between about 10 nm and 1000 nm in diameter.

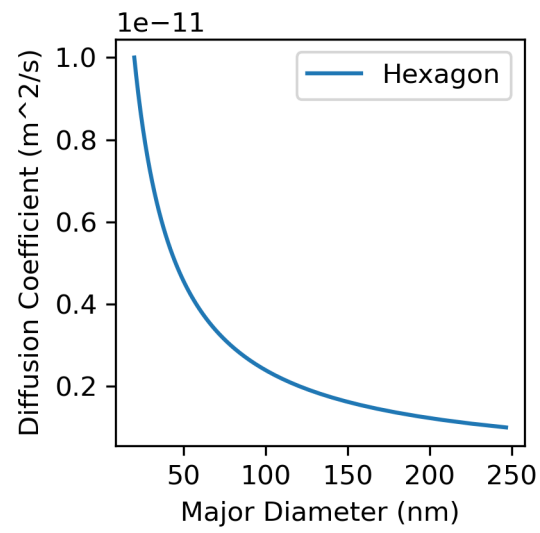


Figure 3.4: Major diameter of hexagonal nanoplatelets with a thickness of 10 nm obtained using equations and parameters obtained in the literature^[2].

Chapter 4

MULTIVARIATE ANALYSIS OF PEPTIDE-DRIVEN NUCLEATION AND GROWTH OF AU NANOPARTICLES

The work presented in this chapter is from: Lachowski, K. J., Vaddi, K., Naser, N. Y., Baneyx, F. & Pozzo, L. D., *Multivariate analysis of peptide-driven nucleation and growth of Au nanoparticles*, Digit. Discov. 1, 427–439 (2022)[116]. Contributions from co-authors include functional data analysis and figures created by Kiran Vaddi. Scanning electron microscopy imaging was performed by Nada Naser.

Abstract

In this work we synthesize gold nanostructures using a liquid-handling robot and present new data analysis methods to quantitatively compare the effect of peptides and peptide modifications on gold nanoparticle synthesis outcomes. The peptides used were gold binding peptides Z2 and AG3, as well as five Z2 variants obtained by sequence modification or conjugation of a lipid tail. Four different concentrations of peptide (0, 0.04, 0.0894, and 0.2 mM), reducing agent (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, HEPES; 0, 0.01, 0.1, and 1 mM), and precursor (HAuCl_4 ; 0.04, 0.0684, 0.117, and 0.2 mM) were used to synthesize 64 different reagent combinations for each of the seven peptides. Each sample was characterized using UV-Vis spectroscopy, which serves as a proxy for changes in nanoparticle structure, and enabled comparisons of how peptide modifications and reagent conditions affect synthesis outcomes. We then used functional data analysis to extract a pairwise signal correlation distance between each of the peptides. The signal correlation distance quantified how different the set of 64 spectra, and therefore the synthesis outcomes, was from one peptide to the other. Spherical nanoparticles synthesized in the presence of peptides were typically 10 to

50 nm in diameter, but the size and shape of nanoparticles varied with peptide properties and reagent conditions. We show that substitution of methionine for isoleucine in Z2 has a profound impact on synthesis outcomes when compared with conjugation of a lipid tail. Electron microscopy and ultra-small angle X-ray scattering were used to corroborate our conclusions from spectroscopy experiments by directly characterizing the structure of a smaller set of samples. Scanning electron microscopy revealed interconnected plate-like structures in samples prepared with a Z2 variant with all methionines substituted by isoleucine. Z2 peptides modified with a lipid tail on the other hand, formed nanoparticles which were more colloiddally stable than those prepared with non-lipidated peptides, contained no plate-like particles, and whose size depended on the ratio of peptide to precursor.

4.1 Introduction

The structure and composition of inorganic colloidal materials enable several unique properties and functions. Structure encompasses the morphology, characteristic dimensions, polydispersity, and crystal structure of nanoscale materials. Control of thermodynamic and kinetic processes during synthesis is required to achieve desired structural features. Particles which are composed of metals (e.g. Au, Ag, Pt, Cu) and have nanoscale structures exhibit localized surface plasmon resonance (LSPR) in response to light [3]. LSPR has been used in chemical sensing, enhanced Raman spectroscopy, light harvesting, catalysis, medical imaging, and other applications [6]. The particle's structure is directly related to its LSPR response. Therefore, simple optical extinction measurements can provide information about particle structure within a sample. In the simplest case of a spherical nanoparticle, the magnitude and wavelength of the LSPR band is related to the diameter of the particle [3]. Different particle morphologies such as nanorods or nanotriangles allow for an additional mode of plasmonic resonance. Proximity to other particles can also modify the optical response through plasmonic coupling. As the size of features directly influences the optical properties of plasmonic particles, monodispersity is a desirable trait to obtain maximum control over the optical response. The crystal structure of metallic nanoparticles may be

generally described as face-centered cubic (fcc), but the exact structural order at the surface can vary depending on the method of synthesis. This is important because the order at the nanoparticle surface is related to its catalytic properties [117].

Efforts at improving control over nanoparticle structure and spatial organization have often taken inspiration from biomineralization. Specifically, researchers seek to emulate the mild synthesis conditions, use of molecules with molecular recognition, and structural complexity that nature has achieved. Sequence-defined molecules such as DNA, peptides, and peptoids are attractive choices for bio-inspired synthesis approaches and have been used to control the nucleation, growth, and self-assembly of inorganic materials [24, 118, 53]. Modifying the sequence of a molecule often has a significant impact on how the molecule interacts with inorganic precursors and particle surfaces [58, 119]. In turn, these modifications can lead to changes in nanoparticle formation mechanisms, colloidal stability, reaction kinetics, and ultimately the function of the synthesized material [117].

Research into understanding molecular design rules for peptide mediated Au nanoparticle research has continued to receive significant attention due to extensive potential applications for plasmonic nanoparticles. Au nanoparticle synthesis also serves as a good model system for method development. Following several discoveries of peptides with strong binding affinities to Au surfaces using biopanning methods, Tan et al.[68] performed a systematic study of each naturally occurring amino acid's contribution to Au binding and reduction. The authors synthesized peptides with each of the amino acids interdigitated with histidine. The interdigitated peptides were then used in experiments to assign scores for each amino acid's Au binding and reducing ability. However, one of the limitations of this work is related to one of the motivations for undertaking it in the first place - the order and identity of amino acids in the sequence can lead to surprising changes in peptide interaction with particle surfaces and precursors. Moreover, as was shown by Munro et al. [120], varying solution and reagent conditions will modify how a peptide performs in nanoparticle synthesis. Therefore, design rules obtained using a particular and narrow set of experimental conditions may be limited in their applicability to other experimental conditions. Munro et al. studied the effects of pH,

ionic strength, and ionic composition on the formation of Au nanoparticles using the peptide AuBP1. However, unlike AuBP1, not all peptides which have a strong binding affinity towards gold surfaces also have an appreciable capability of reducing Au(III), and therefore require the use of a reducing agent. Insights into molecular design rules can be generated by studying sequence rearrangement, amino acid substitutions or additions, and by comparison of known peptides to one another. One example of the latter is the work of Li et al., who compared the use of several Au binding peptides on the formation of Au nanoparticles at fixed molar concentrations of peptide, HAuCl_4 , and a reducing agent [121]. The researchers noted that each peptide’s theoretical isoelectric point was a good predictor of zeta potential sign, but no peptide feature was identified as a good predictor of gold nanoparticle size or polydispersity. Finally, there are also several examples in which conjugation of a lipid tail to the peptide N-terminus resulted in the peptide modifying the synthesis mechanism by acting as a template for nanoparticle growth and assembly [122, 69, 123, 124, 125, 126].

In this paper, we seek to address the lack of systematic experimental and data analysis methods for studying connections between peptide modifications (amino acid content, sequence order, lipid tail conjugation etc.) and synthesis outcomes within a large scope of experimental conditions. There has been a surge of interest and breakthroughs in applying high-throughput experimentation, design of experiments, and new data analysis methods to accelerate colloidal synthesis research. Often, the pursued objective is an optimization of a particular nanoparticle structure or characterization of phases in the experimental design space [127, 128]. In contrast, our work is concerned with using high-throughput experimentation and data analysis to understand the role of peptide modifications on Au nanoparticle synthesis. We demonstrated our approach by using the Au binding peptide Z2 (RMRMKMK) [67] and the mild reducing and buffering agent HEPES. Five additional Z2 variants were obtained to study the influence of different modifications to the peptide. The modifications chosen included substitution of methionine residues for isoleucine, the addition of lipid tails with two different lengths, and the addition of a lipid tail accompanied by a reversal of the sequence. A different Au binding peptide, AG3 (AYSSGAPPMPF), was also included for

comparison. Each of the peptides was used to synthesize a set of 64 samples with varying concentrations of peptide, HAuCl_4 , and HEPES. Each sample was then characterized using UV-Vis spectroscopy. The spectra serve as a fast proxy method for characterizing the nanoparticle structure. That is, although a given spectrum is not a unique representation of a particular nanoparticle structure, we can identify how spectra and therefore structures change as a function of reagent concentrations and peptide identity. We propose an application of functional data analysis methods to the spectral characterizations of plasmonic structures to analyze the high-throughput experimentation data obtained. Finally, we corroborate these differences in nanoparticle synthesis outcomes for each Z2 variant by using a combination of electron microscopy and small-angle scattering, and discuss the significance of changes to peptide chemistry in the context of a large experimental design space.

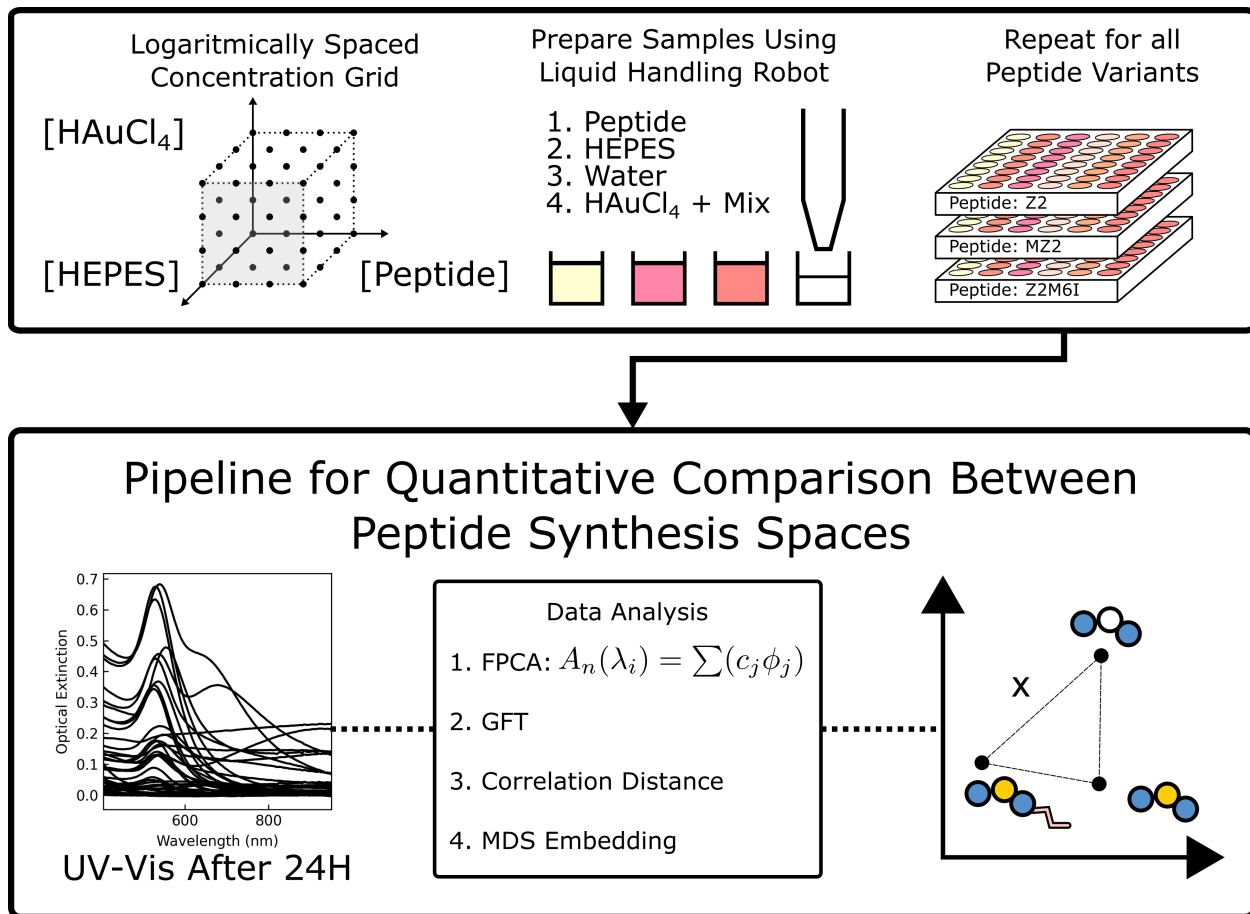


Figure 4.1: A logarithmically spaced concentration grid of reagents is sampled using a liquid-handling robot. UV-Vis spectra are obtained for each sample and the process is repeated for all 7 peptides. Spectra are then analyzed using the data analysis pipeline to recover a quantitative metric for comparing each peptide’s synthesis space.

4.2 Materials and Methods

4.2.1 Materials

Gold (III) chloride trihydrate (HAuCl_4) ($\geq 99.9\%$) was obtained from Sigma-Aldrich (St. Louis, MO, USA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was obtained

from EMD Chemicals (Gibbstown, NJ, USA), and all peptides were acquired from Biomatik Corporation (Cambridge, ON, Canada). Peptides were purified using HPLC to >95% purity by the manufacturer and the lyophilized trifluoroacetate salt was used for experiments without further purification.

4.2.2 Sample Preparation

Stocks of HEPES, HAuCl₄, and peptide were prepared by diluting the reagents with ultra pure water obtained from a Direct-Q 3 UV water purification system with a resistivity of 18.2 MΩ (Millipore Corporation, Bedford, MA, USA). The concentrations of peptide, HEPES, and HAuCl₄ stock used were: 5×10^{-2} wt.% (*ca.* 0.5 mM), 1×10^{-1} wt.% or 1×10^{-2} wt.% (*ca.* 4.18 or 0.418 mM), and 1×10^{-1} wt.% (*ca.* 2.93 mM), respectively. Synthesis of gold nanoparticles was carried out by pipetting the stocks of peptide, HEPES, additional water, and finally chloroauric acid into 48-well Falcon microplates (Corning, Corning, NY, USA) using an Opentrons (Brooklyn, NY, USA) OT-2 liquid handling robot. Each solution was pipetted up and down, the microplates were covered, and the samples were left to react at room temperature. The Python script used to control the liquid handling robot has been shared online [129].

4.2.3 UV-Vis Sample Characterization

Samples were measured 24 hours after synthesis using an Epoch 2 microplate spectrophotometer (BioTek, Winooski, VT, USA). Background correction was performed by subtracting the absorbance of water. Details of functional data analysis are available in the supporting information.

4.2.4 Electron Microscopy

For imaging on the scanning electron microscope (SEM), 5 μL aliquots of samples were deposited on a silicon wafer and air dried overnight. Images were acquired on a FEI (Hillsboro,

OR, USA) Sirion XL30 SEM which was typically operated at 5 kV and with a spot size of 3 using through-lens detection and ultra-high resolution mode. Transmission electron microscopy (TEM) samples were prepared by diluting the sample ten-fold and then casting 7 μL on a lacey carbon coated film, 200 mesh, copper grids purchased from Electron Microscopy Sciences. Grids were dried in air and imaged on a FEI Tecnai F20 at 200 kV.

4.2.5 USAXS Measurements and Data Analysis

Samples were loaded in a custom-made 48-well plate assembly between two Kapton windows for a total sample thickness of 10 mm [130]. USAXS data were collected on the 9-ID-C beamline at the Advanced Photon Source at Argonne National Laboratory using 21 keV X-rays [101]. The USAXS data collection time was 90 seconds, beam size was 0.8 mm by 0.8 mm, and the flux density was approximately 5×10^{12} *photons/second/mm*². Data were reduced using the standard data reduction protocols and software provided by the beamline (Indra).

Mathematical desmearing based on the Lake method [104] was performed using the provided Igor macros [131] to remove instrumental slit smearing effects in preparation for data fitting. The flat background at high- q was then averaged and subtracted from the data before performing automated Guinier analysis. The latter is an estimate of the radius of gyration R_g using the Guinier approximation $I(q) = I(0)\exp(\frac{q^2 R_g^2}{-3})$. Automated Guinier analysis was performed using AUTORG [132].

4.3 Results and Discussion

4.3.1 Effects of Peptide on Au Nanoparticle Synthesis

Au nanoparticles were synthesized in the presence of one of the 7 peptides listed in table 4.1 at 64 different reaction conditions represented by the grid of points in fig. 4.1. The concentrations of peptide, HAuCl_4 , and HEPES were sampled at logarithmically spaced intervals. Sample conditions with no peptide or HEPES were also included in this space. We present a

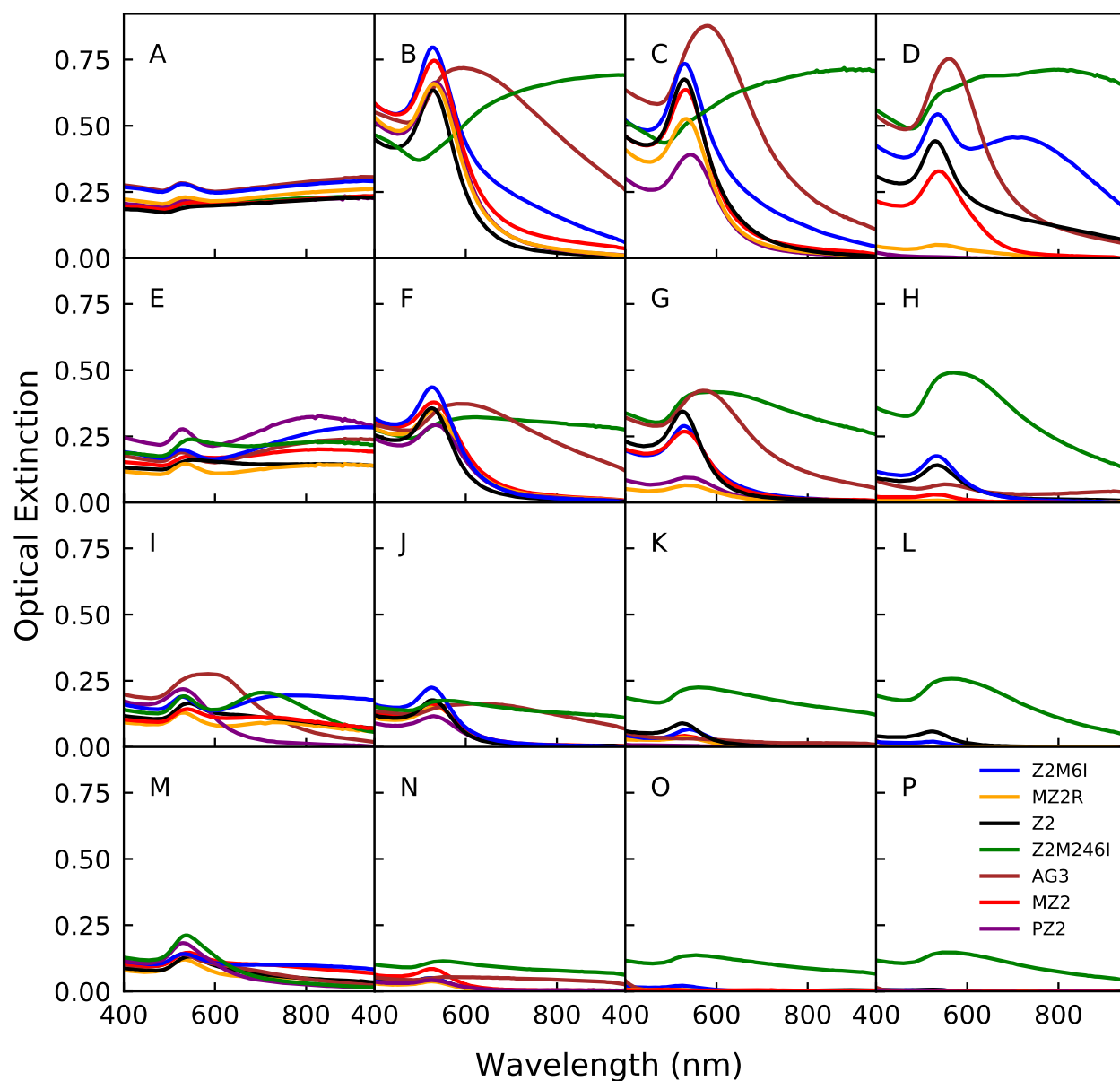


Figure 4.2: UV-Vis spectra of Au Nanoparticles prepared in the presence of different peptides as indicated by the color labeling. HEPES concentration is 1 mM, HAuCl_4 concentration decreases from top to bottom (0.2, 0.117, 0.0684, 0.04 mM), and peptide concentration increases from left to right (0, 0.04, 0.0894, 0.2 mM).

Table 4.1: Peptides used for Au nanoparticle synthesis

Name	Sequence	Lipid Tail	Reference
Z2	RMRMKMK		[67]
MZ2	RMRMKMK	Myristoyl	
MZ2R	KMKRMRM	Myristoyl	
PZ2	RMRMKMK	Palmitoyl	
Z2M6I	RMRMKIK		
Z2M246I	RIRIKIK		
AG3	AYSSGAPPMPPF		[133]

portion of the UV-Vis spectra in fig. 4.2 in which each row from top to bottom corresponds to a decrease in the HAuCl_4 concentration level, each column from left to right represents an increasing level of peptide concentration, and the HEPES concentration is fixed at 1 mM (see appendix A). These spectra can be qualitatively analyzed as a proxy for nanoparticle structure as a function of reagent conditions and peptide identity. Additionally, absorption at 400 nm can be used to determine the concentration of $\text{Au}(0)$ *in-situ* [134]. However, as the cited work points out, the extinction coefficient of $\text{Au}(0)$ at 400 nm depends on particle shape, size distribution, and the particle’s surface chemistry (e.g. peptide or HEPES). Other contributions to optical extinction at this wavelength may include Au clusters and turbidity. Nevertheless, optical extinction at 400 nm is useful for identifying major differences in Au reduction when analyzing UV-Vis spectra. The leftmost column in fig. 4.2 corresponds to the zero peptide condition and is color coded to denote the peptide that was used in the same batch of reactions. HEPES is a mild reducing and buffering agent which forms nanoplates, spherical nanoparticles, or nanostars in the presence of HAuCl_4 [135, 136, 137]. HEPES stabilizes nanoparticles against aggregation through its adsorption to the particle surface [138, 139]. As the HAuCl_4 concentration increases from 40 μM to 200 μM in fig. 4.2 (M,A), the ratio of HEPES: HAuCl_4 decreases. This results in the appearance of a second peak and

broad extinction at higher wavelengths. This is consistent with formation of nanoplates and particle aggregates as seen in fig. 4.7 D.

The addition of peptide causes significant changes in the spectra for all HAuCl_4 levels relative to the no peptide condition. However, the exact changes in spectra vary depending on the peptide identity and concentration. In order to discuss the potential effects of peptides on Au nanoparticle synthesis outcomes, we conceptualized the different ways that peptides can interact with Au precursors and nanoparticles as peptide presentation. Peptide presentation includes, but is not limited to, peptide sequence order, amino acid identity, lipid tail conjugation, charge of residues, solution conditions (pH, ionic strength and identity), proximity of peptide to other peptides, and peptide self-assembly. Peptide presentation in the context of peptide mediated Au nanoparticle synthesis has often been studied by modifying the peptide chemistry - i.e. sequence order, identity, chemical modification, or conjugation of lipid tails to the N-terminus [119, 69, 122]. However, we believe that additional insights and molecular design rules for peptide mediated inorganic nanoparticle synthesis may be identified by sampling the effects of modifications to peptides in a large set of reaction conditions with the aid of high-throughput experimentation and new data analysis approaches. We identified Z2 (RMRMKMK) as a good candidate peptide for developing methods to study how peptide presentation affects nanoparticle synthesis. We selected Z2 because it has a small number of unique amino acids and lacks secondary structure. Unlike many Au binding peptides in the literature which are identified through biopanning methods, Z2 was rationally designed by combining residues with high binding affinities to Au surfaces [67]. Later molecular dynamics simulations concluded that the methionine residues in Z2 act as anchor residues, which the authors define as having a strong and persistent affinity for the simulated Au(111) surface [140]. Additionally, methionine is known to complex with AuCl_4^- which would decrease the reduction rate of Au(III) [68, 141]. We also chose to include AG3 (AYSSGAPPMPPF) in our study in order investigate whether our approach can yield new insights into the similarities and differences of Z2 and AG3 in the context of Au nanoparticle synthesis and stabilization beyond those that have been discussed [121].

We observed that at a fixed concentration of HAuCl_4 and HEPES, high concentrations of Z2 and lipidated Z2 variants led to a decrease in optical extinction at the LSPR peak and at 400 nm. Both of these observations suggest that the concentration of $\text{Au}(0)$ decreases as the concentration of Z2 and lipidated Z2 variants is increased [134]. We hypothesized that the complexation of methionine with HAuCl_4 is responsible for the decrease in formation of Au nanoparticles after 24 hours. Therefore, we synthesized Z2M6I (RMRMKIK) and Z2M246I (RIRIKIK) to study the effect of partially or completely substituting methionine for isoleucine on peptide-mediated growth of Au nanoparticles. The rationale for choosing isoleucine is that it has similar physicochemical properties to methionine [142], but is not expected to bind to Au surfaces [67, 68] nor complex with HAuCl_4 . With the exception of Z2M246I, a low concentration of any Z2 variant generally led to more prominent LSPR peaks and lower optical extinction at higher wavelengths when compared to the no peptide condition at the same concentration of HEPES and HAuCl_4 . An increase in prominence of the the LSPR peak (*ca.* 540 nm) is associated with increased formation of spherical nanoparticles and a decrease in extinction at higher wavelengths is consistent with a decrease in aggregation and formation of nanoplates. All samples synthesized in the presence of Z2M246I on the other hand, had significant and broad optical extinction at wavelengths higher than the typical peak position of an LSPR peak associated with spherical Au nanoparticles. Additionally, samples prepared with Z2M246I were visually distinct from samples prepared with the other Z2 variants in this study. The former sedimented and had a green or black hue, which indicated the formation of large particles or aggregates. Whereas the latter were typically pink, purple, or red and remained dispersed. We also observed that increasing the concentration of Z2M6I or Z2M246I did not lead to a significant decrease in optical extinction at 400 nm. Therefore we concluded that the structures formed in the presence of Z2M246I should be most distinct from others in the study, and that complexation of HAuCl_4 with methionine was an important factor in controlling the proportion of Au(III) that was reduced to $\text{Au}(0)$ after 24 hours.

Several previous works have investigated the use of a lipid tail conjugated to AG3 to

control the *in-situ* formation of Au nanoparticle decorated peptide nanostructures (e.g. micelles, fibrils) [122, 69, 123, 124, 125, 126]. The addition of an alkyl tail to a water soluble peptide makes it amphiphilic, and therefore capable of self-assembling in water. Once self-assembled, peptide nanostructures may act as a template for nucleation or adsorption of Au nanoparticles. Most uses of amphiphilic peptides in the synthesis of Au nanoparticles use just one or a limited set of reaction conditions to draw conclusions about the effect of peptide modifications to Au nanoparticle synthesis outcomes. We were interested in gaining a deeper understanding of the role of peptide lipidation on Au nanoparticle synthesis by using amphiphilic peptides in a large range of reaction conditions. Increasing the length of lipidated peptide tails is one method for decreasing the critical micelle concentration. However, peptide self-assembly can also be affected by the ratio of precursor to peptide due to interactions between peptide residues and the precursor. Ionic interactions between AuCl_4^- and positively charged residues and complexation may lead to changes in the critical micelle concentration and the structure of the peptide-precursor assembly. Therefore, in addition to making substitutions to the Z2 sequence, we also obtained three variants which were modified at the N-terminus with either a palmitic acid tail (PZ2), myristic acid tail (MZ2), or a myristic acid tail with a reversed sequence (MZ2R). SAXS studies of MZ2 dissolved at a concentration of 4.2 mM revealed extended structures which could be fit with a cylindrical model, and are therefore likely to be cylindrical micelles. However, this concentration is much larger than 0.2 mM, the highest concentration used in Au nanoparticle synthesis experiments. We used pyrene fluorescence to estimate the critical micelle concentration of MZ2, MZ2R, and PZ2 to be 0.47 mM, 0.33 mM, and 0.24 mM, respectively (appendix A) [106]. Note that the conditions used in this work are all below the critical micelle concentration. Therefore, the study is limited to the interaction of dissolved peptides with particle surfaces, and does not include the growth of Au over self-assembled peptides. In the case of Z2, reversing the sequence maintains the position of the methionines but swaps the positions of lysines and arginines with one another. The physicochemical similarity of arginine and lysine may suggest that swapping their positions in a sequence should lead to negligible

changes in the peptide’s affinity to Au surfaces and Au nanoparticle synthesis outcomes. However, unlike lysine, arginine has been classified as an anchor residue in sequences other than Z2, and the first arginine in the Z2 sequence was close to being classified as a contact residue by Tang et al. [140]. Therefore, we were interested in whether changing the position of lysine and arginine relative to the lipid tail would lead to an appreciable change in Au nanoparticle synthesis outcomes.

The spectra of samples prepared in the presence of lipidated peptides were generally similar to that of the unmodified Z2. However, unlike Z2, the lipidated variants led to a more significant decrease in overall optical extinction as the peptide concentration increased. Additionally, unlike the non-lipidated peptides in this study, an increase in optical extinction at higher wavelengths for high peptide concentrations was not observed for the lipidated Z2 variants. Had the lipidated peptides led to structural organization of nanoparticles, a red-shift and peak broadening should be observed due to plasmonic coupling [3]. Therefore, we interpreted the lack of optical extinction at higher wavelengths as an indication that the lipidated Z2 variants were unlikely to be organizing Au nanoparticles. However, we expected that the presence of HAuCl_4 should reduce the concentration of lipidated peptide required for its self assembly. As will be discussed in a later section, peptide-precursor structures do exist in the sample, but these do not lead to organized peptide nanostructures decorated by nanoparticles. The decrease in optical extinction for lipidated peptides relative to non-lipidated peptides is likely due to a decrease in the Au reduction rate. A decrease in Au reduction rate could be caused by the peptide-precursor interactions forming structures that inhibit access of HEPES to Au species. This explanation is supported by the fact that samples prepared with PZ2 had lower optical extinction than those prepared with MZ2. The longer length of the palmitic acid tail should lead to stronger association between peptides, and therefore more strongly inhibit reactions of HEPES with Au species. The spectra of Au nanoparticles formed in the presence of MZ2R were comparable to those formed with either MZ2 or PZ2, with the distinction that MZ2R inhibited Au nanoparticle formation to a greater extent than MZ2, and less than PZ2. This observation is in line with the order

of critical micelle concentrations estimated for the three peptides. Overall, changing the positions of arginine and lysine in the sequence did not lead to a significant change in the peptide’s function in modulating Au nanoparticle synthesis. Therefore, the weak impact of this modification should be reflected in later analyses which use the full synthesis space to make pairwise comparisons between peptides.

The spectra from samples synthesized in the presence of AG3 show clear LSPR peaks in most conditions, but these are red-shifted and broadened relative to those synthesized in the presence of Z2. These spectral changes are consistent with formation of larger particles and aggregation. Colloidal instability of AG3 capped particles has been correlated with acidic conditions, the ratio of reducing agent to precursor, and the ratio of peptide to precursor [119]. In contrast to the conditions used in this work, other publications which use HEPES, the AG3 sequence, and modified AG3 variants, utilize higher HEPES concentrations such as 0.1 M HEPES [124]. The differences between synthesis conditions in which Z2 and AG3 form colloiddally stable nanoparticles reveal high dissimilarity between the two sequences.

The present work is focused on measurement of spectra at just one time point (24H) in order to connect peptide properties and reaction conditions with nanoparticle structure. Nevertheless, we also include measurements of the optical extinction at 400 nm over time in nanoparticle syntheses carried out in the presence of Z2, Z2M246I, or MZ2. These measurements demonstrate how peptide modifications affected the Au reduction rate, see appendix A. The slower reduction rate in the presence of MZ2 and the decrease in LSPR features in spectra prepared with high peptide concentrations after 24 hours (e.g. fig. 4.2 D) both demonstrate that peptide lipidation has a significant impact on nanoparticle growth dynamics. These differences in reduction rates are interesting given the similarity of spectra obtained with these reagent concentrations after 24 hours in the case of Z2 and MZ2 (fig. 4.2 B). The similarity in reduction rates between Z2 and Z2M246I on the other hand show that, at least in these conditions, the presence of methionine in the peptide does not appear to have an effect on the Au reduction rate. However, the connection between peptide properties, reaction conditions, and nanoparticle growth dynamics will require extensive in-situ

UV-Vis spectroscopy and SAXS studies in the future.

4.3.2 *Comparison of Peptide Mediated Nanostructure Formation Using the Full Parameter Space*

Each peptide’s synthesis space, consisting of the 4x4x4 set of reagent concentrations and their respective spectra, is a rich dataset of nanoparticle formation outcomes. We posit that using the full dataset would strengthen our analysis of each peptide’s effect on nanoparticle formation. To accomplish this, we use each peptide’s synthesis space in an unsupervised learning approach to study correlations between each peptide. Comparing the synthesis space of peptides would then require comparing the set of spectra along with their sample concentrations. One crucial aspect of this comparison is the way one represents the synthesis space for data analysis purposes. To correlate different synthesis spaces of peptide sequences, we first parameterize the sampled synthesis space by a graph (i.e. a network of first-order connections) using the different component concentrations as nodes and edges obtained using a k -nearest neighbors (k -NN) approach. The above parameterization results in a natural data representation for the synthesis space via signals on the graphs where the signal corresponds to the UV-Vis characterization of the structure of Au nanoparticles. The graph signal approach also allows us to further compress the signals and delineate regions of synthesis spaces for comparison using a Fourier transform [143, 144] that has been well developed for graph signals with readily available open-source code [145]. For example, for a synthesis space comprising of concentration of peptide, buffer, and HAuCl_4 , each sample (a node in the graph) in the synthesis space can be considered as a point in a three-dimensional space. Euclidean distance between the sample concentrations can then be used to obtain edges in the graph using a k -nearest neighbors construction. For each sample in the synthesis space, its characteristic UV-Vis response is collected between the wavelength region of 400-900 nm with 5 nm increments. Given that response of each sample (i.e. the signal at each node of the graph) is a function $x(\lambda)$ (mapping a given wavelength to an absorption intensity) rather than an array of values typically considered in data analysis, we use a principal

component analysis (PCA) adapted for functional data to obtain vector-like descriptors for each sample [146, 147]. This choice of representation allows us to: a) encode the dependency between responses at different wavelengths via an inner product using integrals; b) learn underlying basis functions to represent the functional variations such as peak position and broadening. The approach referred to as FPCA and the graph based signal processing used in this paper are described by the co-author, Kiran Vaddi, in the manuscript and supporting information of Lachowski et al.[116] In the next two subsections, we limit the description of these methods to their basic principles and present the key takeaways.

4.3.3 Application of FPCA to UV-Vis Spectroscopy

Functional data approaches are used to analyze the shape of curves and data that have a defined input and output. UV-Vis optical extinction at a wavelength λ is represented as $x(\lambda)$. We can represent our dataset of spectra as a matrix x_{ij} , where i and j are the sample number and wavelength, respectively. The FPCA of this matrix demonstrates that we observe $\approx 95\%$ of variance using 8 components, and can be qualitatively analyzed through generation of spectra described in the manuscript.

In fig. 4.3, we plot spectra generated from each one of four components where we sample one standard deviation away from the mean (dotted black curve) in positive (blue curve) and negative direction (red curve). We observe that the obtained FPCA components are informative in the following sense: i) moving one standard deviation away from the mean along PC 1 direction changes the peak from being non-existent to a sharp peak thus signifying the primary signal regarding formation of gold nanoparticles. ii) Similarly, PC 2 can be associated with both a peak shift and a change in broad optical extinction at higher wavelengths, which could indicate changes in particle aggregation. iii) PC 3 corresponds to a shift along the intensity axis which we associate with changes in HAuCl_4 concentration under the conditions that forms nanoparticles. iv) We also observe that rest of components are redundant in terms of the information they encode at the spectra level. For example, PC 4 can be considered as mirroring the features encoded in PC 2 while PC 5 is a scaled copy

of PC 3. Further more, PC 6 can be associated with a combination of PC 2 and PC 3; PC 7 and 8 provide minimal changes to the spectra when generated from the model. The above qualitative method is used to further decrease the components to just four given by the top four eigenvalues. For the rest of this paper, we represent each UV-Vis spectra $x(\lambda)$ using its first four principal component scores $f_i, i = [1, 2, 3, 4]$ resulting in a vector representation of a signal on graph parameterization of grid-like synthesis space. Further details on how and why a graph Fourier transform (GFT) was used to compare these synthesis spaces is available in the published work[116].

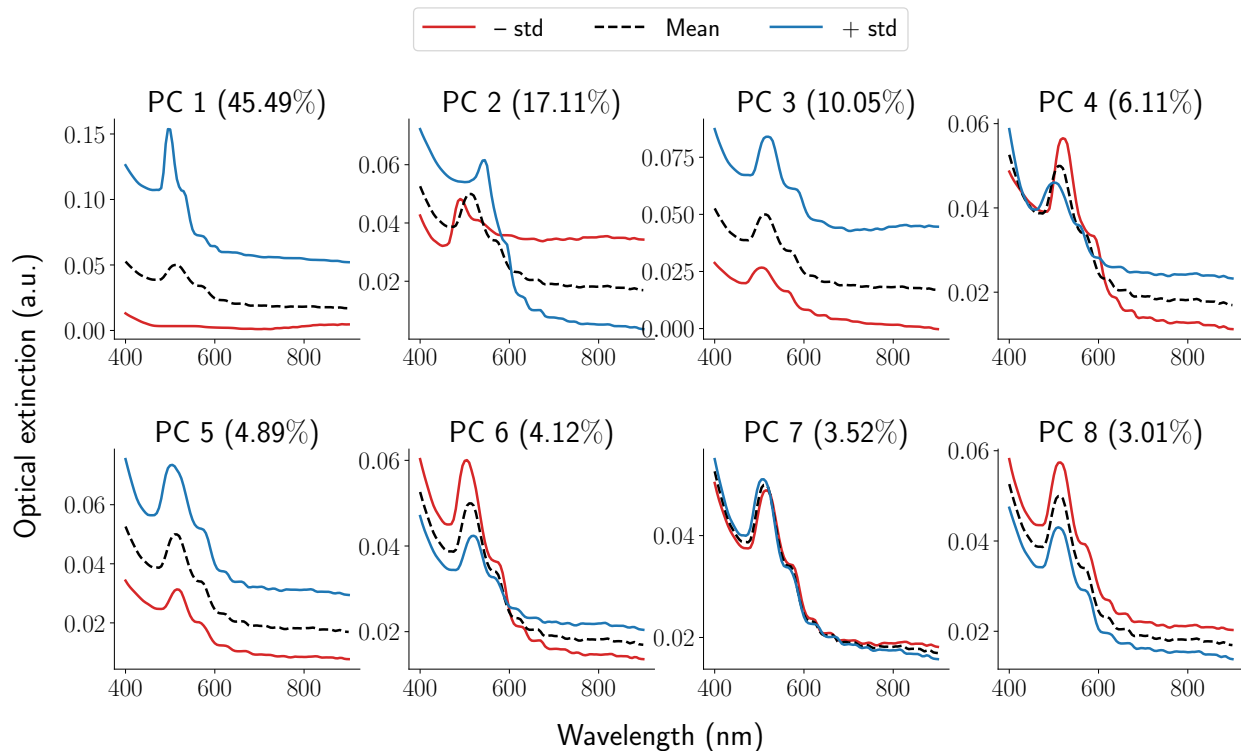


Figure 4.3: Generated samples from the FPCA model of data. Each panel corresponds to a principal component annotated on the top along with corresponding explained variance. For each component, the mean of the functions is denoted using a black-dotted curve while positive and negative standard deviations along the component are denoted using blue and red curves respectively.

4.3.4 Application of GFT to High-Throughput Experimentation Data

In this section, we summarize the results of using GFT to represent the data from each peptide's synthesis space. A correlation distance was used to quantify the similarity between each synthesis space, i.e. the types of Au formed as a result of varying concentrations, and is presented in fig. 4.4.

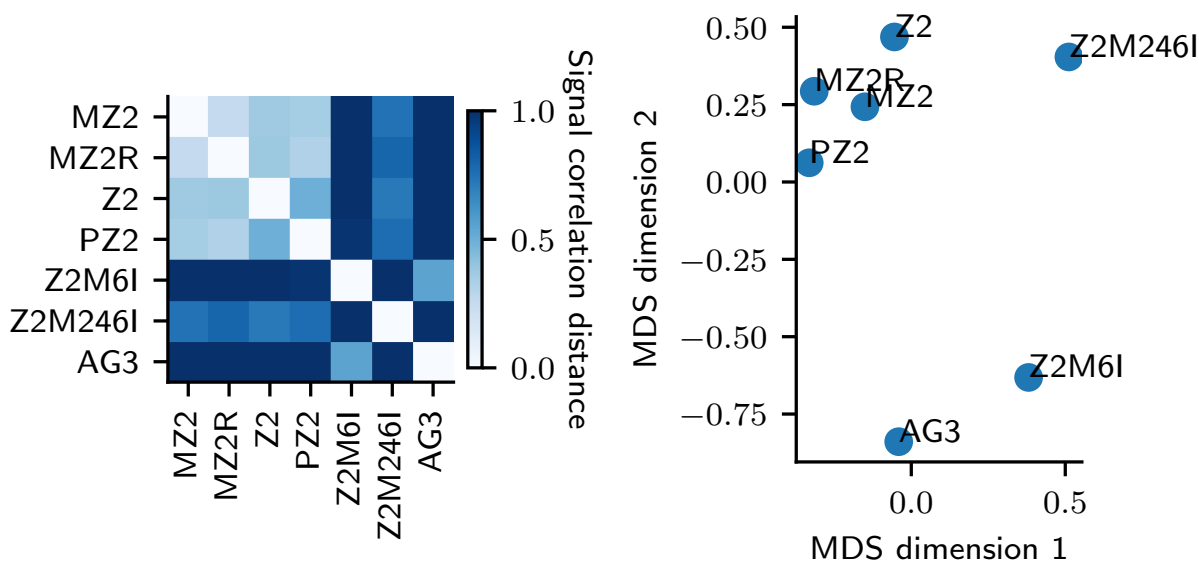


Figure 4.4: Application of graph Fourier transform to high-throughput experimentation data with an FPCA representation of UV-Vis spectra. (left) a correlation distance between graph signal representation in Fourier domain i.e. $\hat{S}$ of different peptides; (right) a metric embedding of the similarity measure shown on the left

On the left panel of fig. 4.4, we used a correlation distance between compressed graph signal of pairs of peptides and represented the similarities using a heatmap plot. The similarity between synthesis spaces is depicted using a continuous color gradient light blue (highly similar) and dark blue (highly dissimilar). A metric embedding using multi-dimensional scaling [148, 149] of the correlation distance is shown on right. We observe that changes to our *base* peptide Z2 result in two distinct modes of synthesis space. Namely, changes to the type of alkyl chain (MZ2, PZ2) and reversing the sequencing (MZ2R) result in very similar synthesis space while changes to actual peptide sequence (AG3, Z2M6I, Z2M246I) result in a diverse synthesis space as quantified by distances in the metric embedding shown in the right panel of fig. 4.4 where the two groups are clearly separated.

4.3.5 Structural Characterization Using Electron Microscopy and USAXS

The correlation distances describe how each pair of peptides differs in the effect it has on Au nanoparticle synthesis across the whole synthesis space. However, it is important to validate the conclusions we drew from UV-Vis spectra by more direct structural characterization methods.

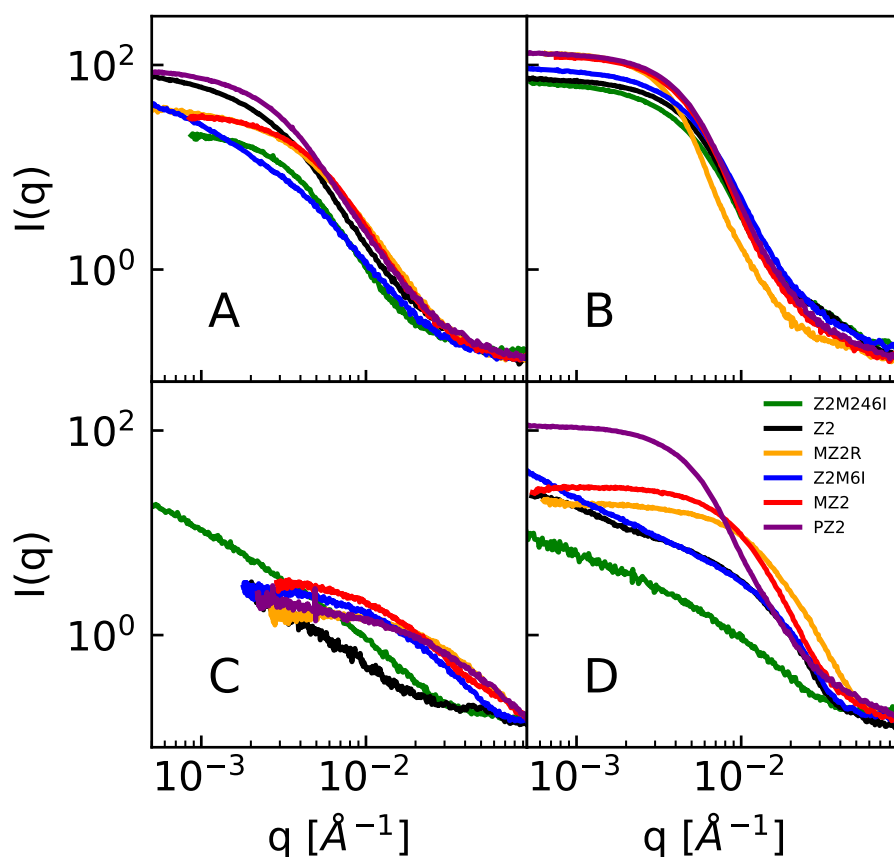


Figure 4.5: A subset of smeared USAXS data. Remaining data is available in appendix A. Concentrations of peptide, HAuCl_4 , and HEPES: A 0.04, 0.2, 0 mM, B 0.2, 0.2, 0 mM, C 0.04, 0.2, 1 mM, D 0.2, 0.2, 1 mM. Note that while most samples show a Guinier region in the absence of HEPES, non-lipidated peptides in the presence of HEPES and HAuCl_4 do not have a Guinier region and may be colloiddally unstable.

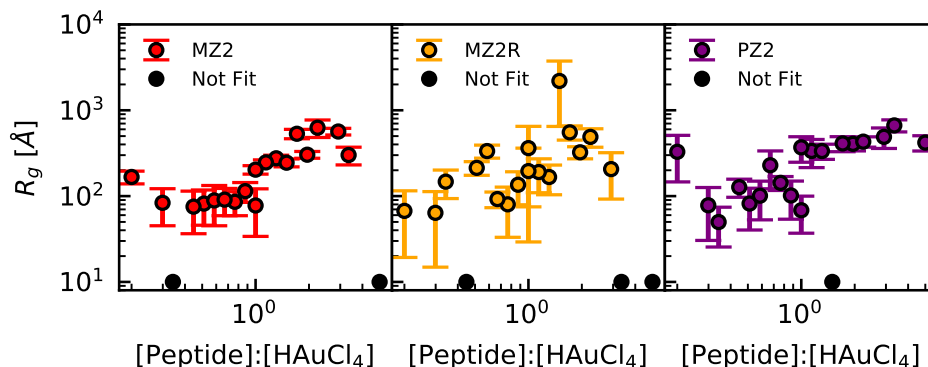


Figure 4.6: Radius of gyration results obtained from AUTORG plotted as a function of peptide:HAuCl₄ molar ratio for all lipidated peptides and a HEPES concentration of 1 mM. Error bars indicate standard deviation. Profiles which did not contain a Guinier region that could be fitted were marked using black and plotted at a fixed R_g of 10 Å. R_g fit results for all peptides in the absence of HEPES are available in appendix A. Data of non-lipidated peptides in the presence of 1 mM HEPES is not presented because the majority of samples did not have a Guinier region.

We repeated the synthesis of Au nanoparticles in the presence of each of the Z2 variants in a slightly modified synthesis space (6x4x2, peptide x HAuCl₄ x HEPES). The bounds of this synthesis space were the same as those used for UV-Vis characterization, but the number of peptide concentrations studied was increased to more finely assess the impact of peptides on synthesis. The HEPES concentrations was 0 or 1 mM to study peptide-precursor structure on its own and because this concentration of HEPES led to more colloiddally stable samples than those formed at lower concentrations, respectively. The USAXS scattering profiles presented in fig. 4.5 show the results for all peptide Z2 variants. Even in the absence of HEPES, most of the scattering profiles show a Guinier region. This is consistent with the formation of hypothesized co-assembled peptide-precursor structures.

The scattering profiles of all Z2 variants in the presence of HAuCl₄ and no HEPES are presented in fig. 4.5 A and B. The scattering profiles of Z2M246I were similar to that of other

Z2 variants which had methionine. Therefore, we concluded that the studied peptide variants co-assemble with the precursor due to ionic interactions between the positively charged residues (arginine and lysine) and AuCl_4^- , and not due to methionine- AuCl_4^- complexation.

In the presence of 1 mM HEPES the scattering profiles of lipidated peptides more closely resembled one another than any of the non-lipidated variants. This can be seen in fig. 4.5 C and D, in which samples which were lipidated show a Guinier region and do not have signs of colloidal instability, unlike samples prepared with non-lipidated peptides. The scattering profiles of non-lipidated peptides in fig. 4.5 D indicate aggregation or the presence of large particles due to the upturn in scattering at low- q . The Guinier region of samples prepared with lipidated peptides in 1 mM HEPES was fit using AUTORG [132]. The results are presented using a log-log plot of R_g as a function of the peptide: HAuCl_4 molar ratio in fig. 4.6. Samples which could not be fit with a Guinier region are shown as black markers with a R_g of 10 Å. All three lipidated Z2 variants show an increase in R_g as a function of peptide: HAuCl_4 ratio. The majority of samples prepared with non-lipidated peptides and 1 mM HEPES did not contain a Guinier region due to aggregation or sedimentation of larger particles and could therefore not be analyzed using AUTORG. The similarity of the scattering profiles of samples prepared with lipidated peptides is in line with the analysis of the signal correlation distance of peptide synthesis spaces. Drawing conclusions from how non-lipidated peptides affected the formation of Au nanoparticles across the synthesis space is precluded by colloidal instability. Nevertheless, future high-throughput experimentation studies of nanoparticle synthesis may utilize small-angle scattering for systems which lack an easy to measure proxy of structure (i.e. LSPR). Fitting of scattering profiles provides a low-dimensional descriptor of the structure (the fit parameters) which can be used to optimize a particular structure as a function of experimental conditions [150]. Alternatively, these model fitting results could be used to compare how sequence-defined molecules or other additives impact nanoparticle synthesis. However, small-angle scattering data fitting is an ill-posed problem and challenges with automated data analysis are continuing to be studied. Challenges that preclude us from applying more complex and automated data fitting to our

dataset are the presence of mixed particle morphologies and sample sedimentation during longer than normal measurement times when queuing large numbers of samples.

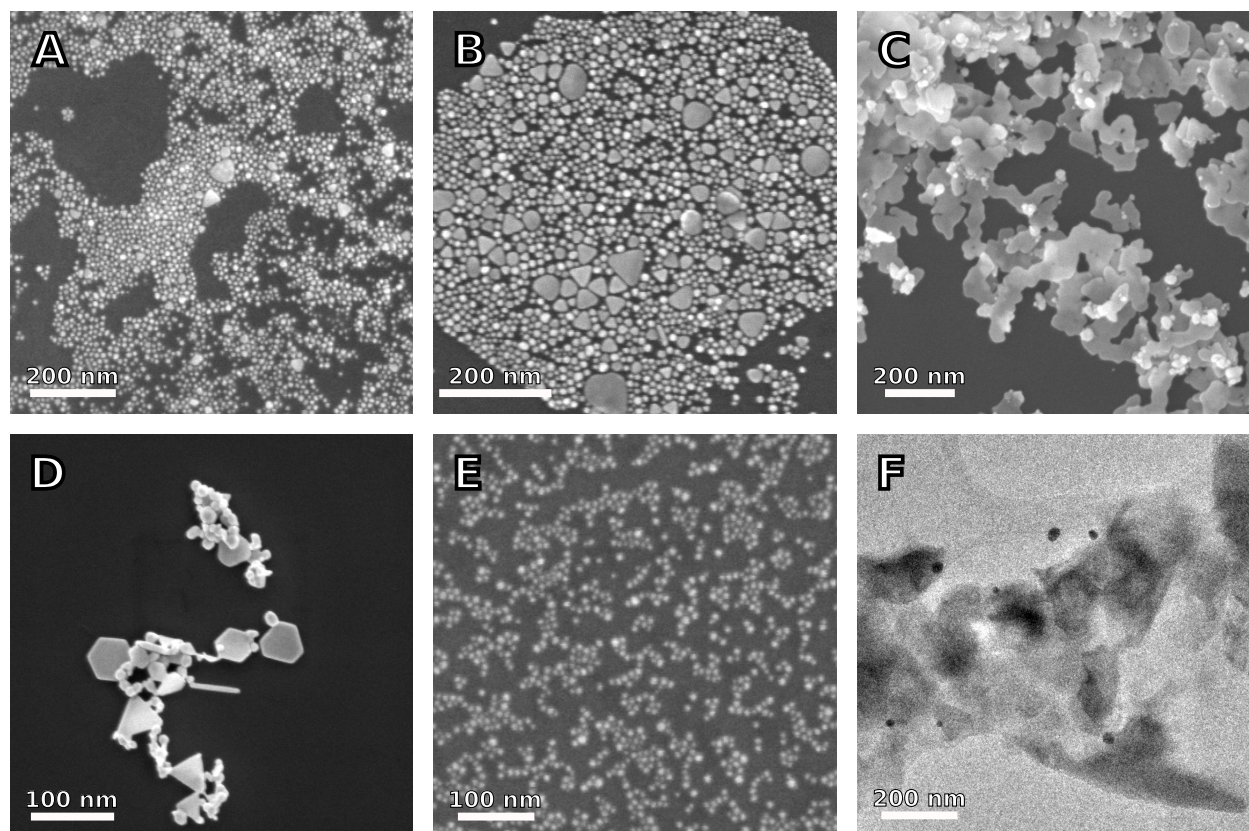


Figure 4.7: Electron microscopy results. A, B, and C: SEM micrographs of Au nanoparticles prepared in the presence of Z2, Z2M6I, and Z2M246I, respectively. The reagent concentrations for these samples were 0.04 mM peptide, 1 mM HEPES, and 0.2 mM HAuCl₄. D: aggregated Au nanoparticles and nanoplates synthesized in 1 mM HEPES and 0.2 mM HAuCl₄. E and F: SEM and TEM micrographs of samples prepared in 1 mM HEPES, 0.2 mM HAuCl₄ and 0.04 or 0.2 mM MZ2R, respectively.

Next, we used SEM and TEM to study the structure of a small subset of samples formed in the presence of different Z2 variants. The SEM image in fig. 4.7 A shows that the sam-

ple synthesized in the presence of Z2 contains both spherical nanoparticles and nanoplates. However, the sample contains fewer nanoplates and is less aggregated than the sample in fig. 4.7 D, which corresponds to the same reagent concentration but with no peptide. Samples prepared with lipidated peptides such as MZ2R contained no plate like particles as seen in fig. 4.7 E and F. Additionally, the particles formed in the presence of MZ2R appear to be more monodisperse relative to the particles formed in the presence of Z2. The spectra which correspond to the reagent concentrations with and without peptides in these micrographs are shown in fig. 4.2 A and B, respectively. These SEM images support our previous interpretation of the spectra that the addition of Z2 leads to less aggregation and fewer nanoplates. Although the presence of nanoplates in the sample prepared with Z2 is not apparent from fig. 4.2 B, higher concentrations of Z2 and Z2M6I did show an increase in optical extinction at higher wavelengths and are likely to contain more plate like structures.

Samples prepared with the lipidated peptide MZ2R in fig. 4.7 E, on the other hand, contain no plate like particles. However, as was previously discussed, lipidation of Z2 was not sufficient to lead to formation of nanoparticle decorated peptide nanostructures. This is due to the fact the peptide concentrations in this study are below the critical micelle concentration. Additionally, Z2 has several charged amino acid groups and is therefore more likely to assemble into less extended or even spherical micelles [151]. Future work into using Z2 to control the assembly of Au nanoparticles could focus on the use of higher peptide concentrations, a lipid tail that is longer than palmitic acid, or the addition of hydrophobic amino acids to the sequence [122]. Using a higher peptide concentration of 0.2 mM caused sample charging in SEM. Therefore, TEM was used to characterize samples formed in the presence of MZ2R at this higher concentration. The image in fig. 4.7 F shows what appears to be a large amorphous aggregate with Au nanoparticles adsorbed on its surface. We interpret this as further evidence of peptide-precursor aggregation. However, the size of these amorphous aggregates is larger than what is to be expected from the fits in fig. 4.6, and the structures are therefore likely to be affected by drying.

The sample prepared in the same conditions as fig. 4.7 A, but with the Z2M6I and

Z2M246I peptides instead of Z2, are shown in fig. 4.7 B and C, respectively. The sample prepared with Z2M6I is similar to that prepared with Z2, with the exception that it contains a higher fraction of plate-like particles. This increase in plate-like particles is consistent with Z2M6I having higher extinction than Z2 at wavelengths above the main LSPR peak, as can be seen in fig. 4.2 B. The SEM image of the sample prepared with Z2M246I primarily contains flat, and interconnected structures. These interconnected structures also have smaller particles deposited on top, but the shape of these smaller particles is not clear. The large structures are similar to other reported flat and branching structures whose formation mechanism was attributed to molecular confinement [152]. However, the reason for why methionine substitution for isoleucine in Z2 leads to promotion of anisotropic structures requires further study. We expected that removal of methionine would decrease the affinity of the peptide towards Au surfaces, and that this could result in structures that more closely resembled those obtained in the absence of peptide. Whereas the rigid aromatic structure of a molecule was implicated as important to achieving anisotropic particle growth [152], we do not expect the Z2M246I variant to be rigid. However, the piperazine ring in HEPES does possess this rigidity and HEPES also leads to formation of plate like structures (fig. 4.7 D). Therefore, we hypothesize that the observed anisotropic growth in the presence of Z2M246I is related to a modification in how HEPES interacts with Au structures, and that a different reducing agent (e.g. citrate) would not result in similar structures. The differences in particle morphology observed in samples containing Z2M6I and Z2M246I support the high signal correlation distances between these peptides and the other Z2 variants presented in fig. 4.4.

We summarize our main findings in fig. 4.8, where we show that each of the Z2 peptide variants formed peptide-precursor aggregates in the absence of HEPES. Given that HAuCl_4 is added last to the solution, we infer that peptide-precursor aggregation is a process that happens in conjunction with Au reduction. High concentrations of peptide variants which contained methionine showed a significant decrease in the amount of $\text{Au}(0)$ formation after 24 hours based on comparison of optical extinction at 400 nm relative to lower peptide concentrations. We note that this change cannot be explained by an increase in the amount of

peptide adsorbed to the surface given that adsorption of organic molecules to citrate capped nanoparticles resulted in an increase in optical extinction [134]. In the case of the lipidated peptides in this study, the difference in to what extent Au(0) formation was suppressed was inversely proportional to the values of critical micelle concentrations. Therefore, the differences in Au(0) formation in the presence of lipidated peptides are likely due to differences in modulation of H_{Au}Cl₄-methionine complexation and availability of H_{Au}Cl₄ to HEPES. These differences arise due to variation in the strength of lipidated peptide-precursor interactions between MZ2, MZ2R, and PZ2. Z2M6I and Z2M246I have one or all three methionine residues substituted with isoleucine, respectively. In Z2M6I, the relative proportion of plate-like particles to spherical particles appears to increase relative to Z2. Whereas for Z2M246I the structures that formed are interconnected and branching plate-like structures. Finally, the optical extinction at 400 nm for Z2M246I is least sensitive to peptide concentration out of all of the peptides studied and therefore confirms that methionine is an important modulator of H_{Au}Cl₄ reduction.

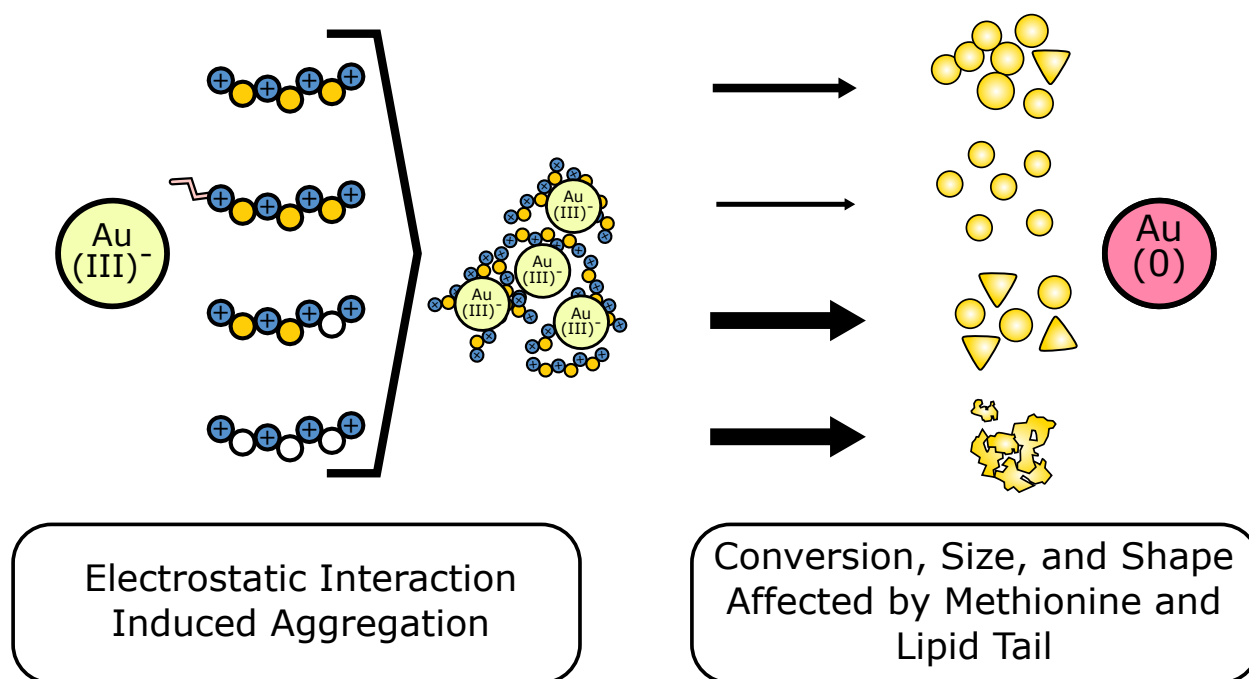


Figure 4.8: Schematic showing the effects of Z2 and its variants on Au nanoparticle synthesis outcomes. All Z2 variants were shown to form peptide-precursor aggregates based on the USAXS data in the absence of HEPES. We inferred that this aggregation occurs due to attractive electrostatic interactions between AuCl_4^{-1} and the positively charged residues present on all Z2 variants, shown above as blue circles. Next, we use thinner and thicker arrow to indicate lower and higher conversion of Au(III) to Au(0) in the Z2 variants, respectively. Conversion was least affected by presence of peptide variants which had methionine (yellow circles) substituted for isoleucine (white circles) (Z2M6I, Z2M246I). Conversion was significantly decreased in the presence of lipidated peptide variants (MZ2, MZ2R, PZ2). Finally, we show how these modifications of Z2 led to differences in nanoparticle size and shape. In short, Z2 lipidation led to more dispersed spherical nanoparticles, and substitution of methionine increased anisotropic particle growth relative to the base Z2 sequence.

4.4 Conclusion

In this paper, we presented a data analysis pipeline for studying large synthesis spaces using high-throughput experimentation. We characterize the formation of Au nanoparticles in the presence of peptides using our high-throughput experimentation framework to sample large synthesis spaces and collect UV-Vis spectra for each sample as a proxy signal for the nanoparticle structure.

We applied our framework to study the differences in nanoparticle synthesis outcomes between six variants of the Au binding peptide Z2 and a different Au binding peptide AG3. We first focus on a portion of the synthesis space (one HEPES concentration) by analyzing the differences between spectra of peptides at specific reagent concentrations, and as these concentrations are varied. This analysis demonstrated the utility of studying modifications to sequence defined molecules while systematically varying experimental conditions. However, in order to fully take advantage of this dataset we described a data analysis framework to discern the correlations between peptide synthesis spaces. Using metrics designed to quantify higher-order correlations and variations between features, we obtain a two-dimensional embedding that provides insights into the similarity between the effect that peptides have on Au nanoparticle synthesis. The framework presented here is generic and only assumes the synthesis space being sampled is continuous. We obtained a Euclidean embedding of the correlations revealing that the synthesis spaces are more affected by the peptide sequence than conjugation of a lipid tail. The conclusions of this analysis were supported by use of USAXS and electron microscopy, which are lower-throughput but more direct methods of structural characterization. We anticipate that the proposed approach will initiate further research aiming at analyzing high-throughput experimentation data with spectral characterization to explore and understand complex synthesis spaces. The hardware and software introduced in this work can also be used to accelerate the identification of phase or state diagrams, or to perform closed-loop experimentation for material retrosynthesis [153]. The latter may be used to either tune experimental conditions or molecular design to achieve

desired nanomaterial structures. There are two notable limitations of the current method:

- i) Although the principal components capture interpretable variations in Au nanoparticle spectra, the method cannot attribute these variations to physically meaningful variables (i.e. reagent concentrations or physicochemical peptide attributes). This can, for example, be improved by using the PCA features in causal models [154, 155].
- ii) Lower dimensional representations obtained from FPCA via principal coefficients are not disentangled. Alternatively, data representations that characterize spectra using disentangled low dimensional representations would provide a direct way of visualizing variations in the synthesis space and their similarities across.

Chapter 5

**ANISOTROPIC GOLD NANOMATERIAL SYNTHESIS USING
PEPTIDE FACET SPECIFICITY AND TIMED
INTERVENTION**

The author would like to acknowledge contributions to this chapter from: Huat Chiang for creating the Python script that was used to control the liquid handling robot, Wenhao Zhou for obtaining atomic force microscopy images, and Ellen Lavoie for helping obtain selected area electron diffraction images.

Abstract

Thin metal particles with two-dimensional symmetry are difficult to synthesize efficiently in a reproducible manner, and are attractive for applications in diverse fields such as medicine and energy. It has long been understood that selective adsorption of molecules to particle surfaces enables control over nanoparticle assembly pathways. However, controlling the time at which a molecule is added after a reaction is already underway to alter synthesis has limited examples in the literature. Moreover, this approach, and the growth of thin particles with two-dimensional symmetry in general, seldom feature examples of in-situ characterization methods that provide mechanistic details. In this work, we study the origin of gold nanoparticle anisotropy as a result of methionine substitutions in a gold binding peptide (Z2, RMRMKMK). The growth of plate-like particles in the presence of Z2M246I (RIRIKIK) is reported using in-situ small-angle X-ray scattering (SAXS) and UV-Vis spectroscopy. 10 nm thick particles with two-dimensional symmetry are identified within the first few minutes of the reaction. We infer that substitution of methionine in Z2 increases the (111) facet selectivity of the peptide and that this is key to plate-like particle growth.

However, the substituted peptide appears to have limited control over the extent of lateral growth and aggregation of nanoplatelets. These limitations result in sedimentation and irreversible aggregation, and apparently limit the potential of Z2M246I. However, we were able to demonstrate improvements in synthetic control by dynamically intervening through the addition of Z2 to nanoplatelets growing in the presence of Z2M246I. Synthesis and dynamic intervention are performed using a liquid handling robot with a custom script that controls the time of reagent addition, and can be adapted for future approaches in which the time of intervention is a key experimental parameter. We use spectroscopic assays to demonstrate that plate-like particles formed as a result of intervention are more stable than in the absence of Z2. Dynamic light scattering and imaging studies demonstrate that the time of addition influences the mean size of plate-like particles, and the proportion of spherical to plate-like particles. Finally, in-situ UV-Vis characterization of plate-like particle growth at different times of intervention is used to infer additional mechanistic insights.

5.1 Introduction

Anisotropically shaped plasmonic nanoparticles such as nanoplates, nanorods, and various morphologies with protruding morphologies exhibit a longitudinal plasmon resonance mode that is not present in spherical nanoparticles[84, 156, 157, 158, 159, 160]. A unique feature of particles with two dimensional symmetry is that the ratio of extinction from transverse and longitudinal modes decreases with decreasing thickness[157]. The position of the longitudinal surface plasmon resonance (SPR) peak can be tuned to near-infrared wavelengths by changing the associated characteristic dimension (e.g. nanorod length, nanotriangle edge length). This is desirable for biomedical applications that take advantage of the low optical extinction of tissues in the near-infrared range[6]. However, particles with two dimensional symmetry need to be thin in order to exhibit optical extinction in the near-infrared and be synthetically accessible and colloidally stable[157]. An added benefit of thin plasmonic structures is that they have a high specific surface area, which makes them advantageous in catalysis applications[152]. Surface enhanced Raman spectroscopy (SERS) is another

application in which anisotropic particles outperform spherical particles[161, 156, 158, 162]. Besides particle anisotropy, the presence of nanoscale tips or gaps leads to an enhancement of the electromagnetic field due to local hotspots that is beneficial to SERS and photocatalysis applications of colloidal nanoparticles[163, 162]. Methods for synthesizing colloidally stable metal nanoparticles that are thin, have two dimensional symmetry, and irregularly shaped edges often require etching of existing plate-like particles which adds additional synthesis steps[156, 158]. This is not optimal given that many syntheses of two-dimensional metal nanomaterials are based on growth of seed particles, which is already a two-step process. Alternative methods that do not require multiple processing steps have been identified in the literature and we point the readers to a recently published review on the subject[164]. On the other hand, as the authors point out, there are limited examples of in-situ characterization methods being applied to the formation process of two-dimensional metal nanomaterials[164].

An under-appreciated experimental parameter in two-dimensional nanomaterial synthesis, which has been applied to anisotropic nanoparticle synthesis in the past, is the time at which a certain reagent is added to the synthesis. A potential reason for the limited exploration of this approach in addition to its complexity, is that it is time consuming and inaccurate to temporally intervene in a synthesis by an experimenter. This concept is illustrated by the addition of metal ion scavenging molecules at different times to stabilize and control the structure of gold nanorods[160, 159]. In another work, a small molecule that had facet selective binding was used to partially reduce HAuCl_4 before addition of the strong reducing agent sodium borohydride[84]. By changing the length of the incubation step, the authors were able to control the characteristic dimensions of highly anisotropic gold nanoparticles such as nanopods. A more sophisticated approach to growing Au–Ag nanostructures was also recently reported by Wang et al.[165]. The authors observed that different particle morphologies were formed in the presence of two different DNA sequences. Particles were grown in the presence of one sequence for different amounts of time, ligand exchange was performed to replace the first sequence for the other, and particle growth was resumed by addition of new precursor. Again, the time at which the synthetic intervention

took place allowed for predictive control over particle structure.

In our previous work, we identified that substitution of methionine with the physicochemically similar, but chemically dissimilar isoleucine, led to dramatically different nanoparticle synthesis outcomes. Specifically, we identified that anisotropic, interconnected, and plate-like gold nanomaterials were formed in the presence of Z2M246I[116]. However, the plate-like particles were not colloiddally stable, and varying the reagent concentration was not effective at limiting the lateral dimension of the plate-like particles. In this work we set out to understand the formation mechanism and origin of the plate-like particles, and to control the colloidal stability and lateral dimension of the nanomaterials. In addition to electron microscopy and AFM imaging studies, in-situ UV-Vis and small-angle X-ray scattering were used to study the formation of anisotropic structures in the presence of Z2M246I. Next, we programmed a liquid-handling robot to dynamically intervene in the synthesis by adding a different peptide at different times. The peptide chosen for this step was Z2, which we previously observed to form highly stable spherical nanoparticles in a wide range of conditions. We hypothesized that addition of Z2 at different times would cap the lateral growth of anisotropic particles being formed in the presence of Z2M246I, and thereby achieve control over the lateral dimension and colloidal stability of plate-like nanoparticles. A microplate based sedimentation assay inspired by another publication[166] was used to screen for synthesis conditions that met our design objectives: formation of plate-like particles and an improvement in colloidal stability relative to the control in which no intervention had taken place. We used UV-Vis spectra of the plasmonic nanoparticles as a proxy for changes in structure and sedimentation. Once the desired synthesis conditions were identified, and the stability of the samples was corroborated using a cuvette based sedimentation assay, we used electron microscopy, AFM, and dynamic light scattering to identify how the time of addition of Z2 changes the resulting structures. Finally, we obtain in-situ UV-Vis spectroscopy to identify changes in the assembly mechanism when intervention takes place at different times, and comment on the importance of methionine in this system.

5.2 Materials and Methods

5.2.1 Materials

All peptides were synthesized by Biomatik Corporation (Cambridge, ON, Canada), purified using HPLC to $> 95\%$ purity by the manufacturer, and the lyophilized trifluoroacetate salt was used without further modification. Gold chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ $\geq 99.9\%$) was obtained from Sigma-Aldrich (St. Louis, MO, USA). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was obtained from EMD Chemicals (Gibbstown, NJ, USA). Ultra-pure water used in the study was obtained from a Direct-Q 3 UV water purification system with a resistivity of $18.2\text{ M}\Omega$ (Millipore Corporation, Bedford, MA, USA).

5.2.2 Sample Preparation and Dynamic Intervention

Aqueous stock solutions of HAuCl_4 (10 mM), HEPES (3 mM), and peptide (0.3 mM) were prepared before each synthesis. Samples used in UV-Vis, imaging, and DLS studies were all prepared using an OT-2 liquid handling robot (Opentrons, Brooklyn, NY, USA), and samples were prepared manually for in-situ SAXS and UV-Vis studies. Except where indicated otherwise, final sample concentrations were 0.4 mM HAuCl_4 , 1.0 mM HEPES, 0.08 mM of Z2M246I (RIRIKIK), and 0.08 mM of Z2 (RMRMKMK). The order in which stocks were added was water, HEPES, Z2M246I, HAuCl_4 , and Z2. A custom Python script (OTTO, <https://github.com/pozzo-research-group/otto>) was used to control the time at which the Z2 peptide was added relative to HAuCl_4 . Samples were prepared in 48-well Falcon microplates (Corning, Corning, NY, USA). For the microplate based sedimentation assay, 200 μL of the sample was automatically transferred to a 96-well plate (Corning, Corning, NY, USA) immediately after all pipetting steps were completed, and then again after a delay period of 12-15 hours to allow for sedimentation to take place. For the cuvette based sedimentation assay, samples were transferred to polystyrene cuvettes after all pipetting steps were completed.

5.2.3 UV-Vis Characterization

UV-Vis measurements of samples in microplates were obtained using an Epoch 2 microplate spectrophotometer (BioTek, Winooski, VT, USA), and a Thermo Scientific Evolution 300 (Waltham, MA, USA) was used to characterize samples in cuvettes. In-situ UV-Vis measurements were carried out in a 48-well microplate format. In all cases data normalization was performed by subtracting absorbance of water measured in the same format.

5.2.4 In-Situ SAXS Experiments and Data Fitting

SAXS measurements were obtained using a Xeuss 3.0 (Xenocs, Grenoble, France) under vacuum and characterized using Cu K_α radiation. The reagent concentrations used were 0.04 mM Z2M246I, 0.2 mM HAuCl₄, and 1 mM HEPES. All reagents were mixed in a centrifuge tube except for HAuCl₄. Next, a cycle of image collection at a 90 cm sample to detector distance with 2 minute exposures was initiated, the precursor was rapidly added to the tube and agitated, and the start time was recorded. The sample was then injected using a syringe into a custom built sample holder located inside the instrument as described in appendix B. Data reduction was performed in XSACT software (Xenocs, Grenoble, France) by azimuthally integrating 2D images to obtain 1D scattering profiles. A water background measurement collected in the same sample holder was subtracted.

Reduced data at each time point were fit using a Guinier-Porod model[100]. This model enables fitting of arbitrary particle shapes by applying a continuity between the Guinier and Porod regions of scattering that correspond to scattering at low-q and high-q, respectively. The combined Guinier-Porod model includes a dimension parameter to account for power-law scattering of non-spherical particles in the intermediate Guinier region. Scattering in the intermediate Guinier region of particles with two-dimensional symmetry will lead to a dimension parameter of 2, and the corresponding radius of gyration is related to the thickness of the particles by $T = R_g\sqrt{12}$. Model fitting was performed using the Sasview/sasmodels package for Python[167].

5.2.5 *Electron Microscopy*

Scanning electron microscopy (SEM) was performed using an Apreo-S (Thermo Fisher, Waltham, MA, USA). Samples in microplates were re-suspended by pipetting up and down, 5 μL aliquots were deposited on clean silicon wafers, and dried overnight. The instrument was operated at 1 kV and 3.1 pA, and images were obtained from in-lens detectors.

For transmission electron microscopy (TEM), samples were also re-suspended, 5 μL was cast on square carbon coated, 200 mesh, copper grids obtained from Electron Microscopy Sciences (Hatfield, PA, USA), and dried overnight. Imaging and Selected area electron diffraction (SAED) were performed using a Tecnai G2 F20 SuperTwin TEM instrument operated at 200 kV.

5.2.6 *Atomic Force Microscopy*

Atomic force microscopy (AFM) imaging was carried out on samples prepared in the same way as for SEM. Samples were characterized using a Dimension Icon AFM (Bruker, CA, USA) in air using PeakForce QNM mode and ScanAsyst-Air probes ($k=0.4\frac{\text{N}}{\text{m}}$, Bruker, CA, USA). Image processing and particle dimension analysis was performed using Gwyddion[168]. Data processing steps included mean plane subtraction, row alignment, correction of horizontal scars, and finally the minimum height value was shifted to zero. Line profile measurements for samples in which Z2 was added are shown in appendix B

5.2.7 *Dynamic Light Scattering*

Dynamic light scattering (DLS) measurements were taken on a Malvern Panalytical (Malvern, United Kingdom) Zetasizer Nano ZS. Samples used were prepared using the liquid-handling robot as previously described, and were transferred to polystyrene cuvettes without dilution. The intensity weighted peak distribution of diffusion coefficients is presented rather than the hydrodynamic diameter, because the latter requires the assumption that the particle shape is spherical. The highest intensity peak obtained from each synthesis condition

is also converted to an equivalent diameter depending on whether it can be attributed to either spherical nanoparticles or nanoplatelets. The equivalent diameter of the latter is obtained by solving for the area A in the expression for the friction coefficient f obtained from Hernández-Cifre et al.[2]:

$$f = 12\eta_0\left(\sqrt{\frac{A}{\pi}}C + Qh\right) \quad (5.1)$$

Where η_0 is the viscosity of the medium, h is nanoplatelet thickness (estimated to be 10 nm based on experimental results), and C and Q are fitting parameters for hexagonal nanoplatelets obtained from numerical simulations[2]. The major diameter of a hexagon with the obtained area is then used to estimate changes in nanoplatelet diameter.

5.3 Results and Discussion

5.3.1 Morphogenesis of Interconnected Anisotropic Particles

Studies of methionine containing peptides identify that the residue contributes significantly to the binding of peptides to gold surfaces[140, 68]. All three methionines in the rationally designed Z2 sequence (RMRMKMK)[67] were predicted to make significant contact with gold surfaces by molecular dynamics studies[140]. Additionally, methionine plays a role in chemical reduction of gold because it can be oxidized and form intermediate Au(I)-methionine species[169]. It has been proposed that Au(I)-methionine complexation may reduce the rate of reduction[68], and impede secondary nucleation on or near the particle surface[170]. The former is evident in our previous work which explored the effect of substituting methionine with isoleucine in the Z2 sequence[116]. We observed that increasing the concentration of methionine containing Z2 variants decreased the concentration of Au(0) 1 day after synthesis based on absorbance at 400 nm, which is proportional to the concentration of Au(0)[134]. In contrast, increasing the concentration of the Z2M246I sequence, with all three methionines substituted for isoleucine, did not decrease the apparent concentration of Au(0) after 24 hours. Moreover, we observed that decreasing the number of methionines led to an incremental increase in particle anisotropy. This can be seen in fig. 5.1, which shows spectra of

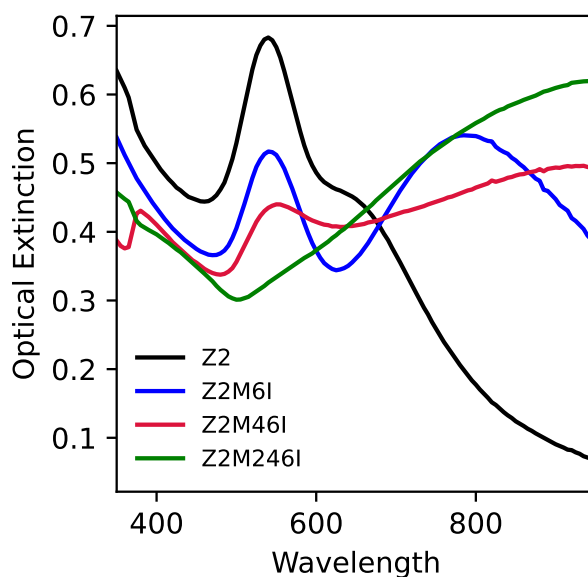


Figure 5.1: UV-Vis spectra of Au nanoparticles formed using 0.2 mM HAuCl_4 , 0.1 mM HEPES, and 0.04 mM of peptide. Increasing the amount of methionines substituted for isoleucine in the Z2 sequence leads to a red shift in the plasmon resonance peak position at higher wavelengths indicating that larger plates are being formed. Z2, Z2M6I, Z2M46I, and Z2M246I correspond to sequence RMRMKMK, RMRMKIK, RMKIKIK, and RIRIKIK, respectively.

gold nanomaterials formed in the presence of variants with increasing numbers of methionine substitutions. While Z2 shows one LSPR peak corresponding to spherical nanoparticles, the remaining variants show contributions from LSPR at higher wavelengths, which is consistent with the longitudinal plasmon resonance mode of plate-like particles identified in the samples using electron microscopy as demonstrated in our previous work and in fig. 5.2 A. In the presence of Z2M246I, no LSPR peak related to spherical particles could be observed in a wide range of synthesis conditions[116]. The SEM image in fig. 5.2 A of a sample formed in the presence of Z2M246I shows aggregates of anisotropic plate-like particles with irregular edges. A TEM image of a similar group of particles with irregular edges and somewhat triangular shape is shown in fig. 5.2 B. The apparent wrinkle-like defects and translucence of the plate-like particles suggests that they are relatively thin. Plate-like particles may exhibit a transverse and lateral mode of plasmon resonance which are affected by the thickness and lateral dimension of the particle. However, the surface plasmon resonance of thin disc-like gold particles is predicted[157] to have minor contributions from the transverse mode, leading to only one LSPR peak as observed for Z2M246I in fig. 5.1. The particles formed in the presence of Z2M246I were identified to be 10 nm thick using AFM as shown in fig. 5.3. Selected area electron diffraction and FFT analysis of HR-TEM images are consistent with (111) basal facets based on the presence of $\{220\}$ and $\frac{1}{3}\{422\}$ reflections[171, 172]. The latter have been attributed to a different types of stacking faults in metal nanoplates[172] and have a 2.5 Å interplanar spacing that was also identified in the FFT of plate-like particle surfaces in the sample. Each of these stacking faults may contribute to anisotropic growth in the [100] direction, and may form as a result of a molecule selectively binding to the (111) face[172].

Time-resolved SAXS curves of nanoparticle growth in the presence of Z2M246I are shown in fig. 5.4. The low-q limit of the instrumental configuration is limited to *c.a.* $0.007 \text{ \AA}^{-1}$ which restricts the measurement to structures with dimensions smaller than approximately 90 nm. The scattering profile at each time point was fit using a Guinier-Porod model[100] and is described in the methods section. As seen in fig. 5.4 B, the fitted dimension parameter reaches

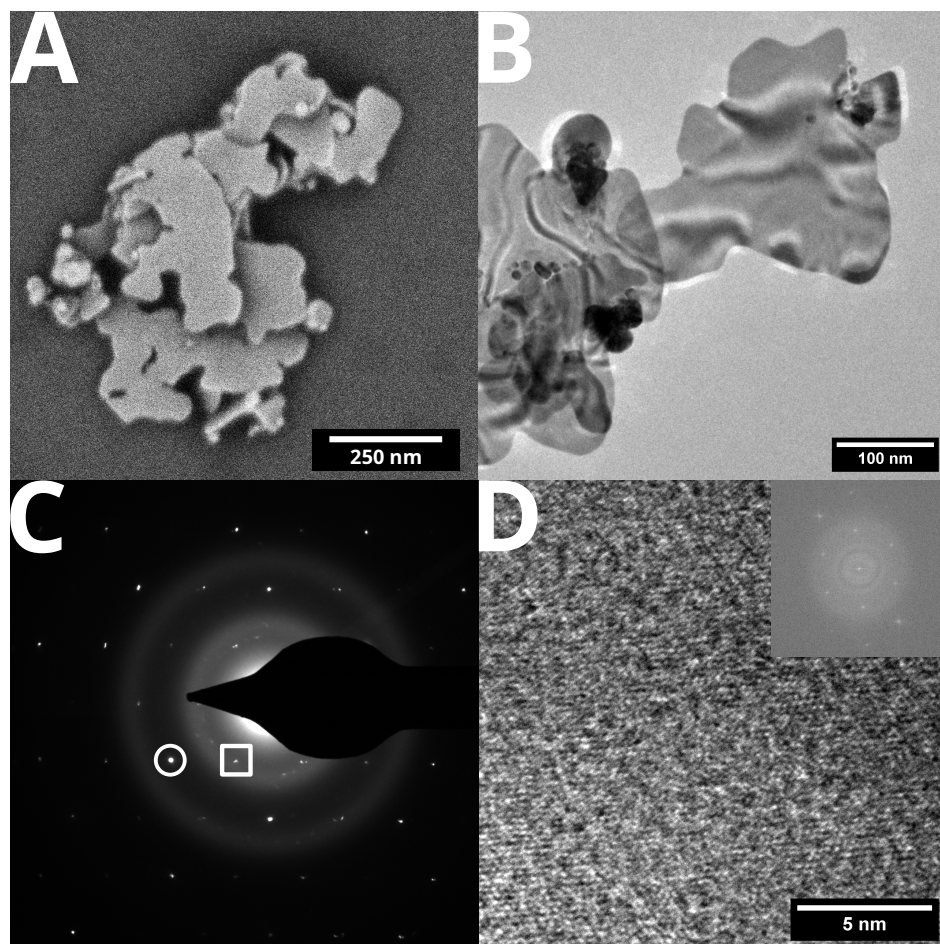


Figure 5.2: A SEM of an aggregate of irregularly shaped plate-like and spherical particles. B TEM of samples prepared in presence of Z2M246I. Presence of wrinkle-like defects suggests that the structures are thin. C SAED of the basal facet of an isolated plate-like particle which was indexed as the $[111]$ direction. Points in the circle and square are from $\{220\}$ and $1/3\{422\}$ reflections, respectively. D and the inset figure correspond to an HRTEM image and its FFT of a plate-like structure, respectively. The inner ring of points corresponds to 0.25 nm interplanar spacing which is consistent with reflections from $\frac{1}{3}\{422\}$.

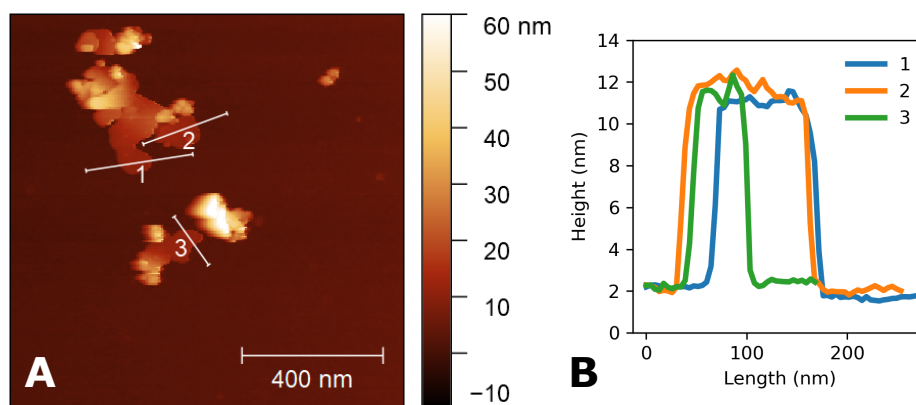


Figure 5.3: A AFM image showing plate-like particles with an approximate thickness of 10 nm, as indicated by the annotations and the corresponding height profile in B.

a value of 2 after ca. 10 minutes, indicating that a symmetry breaking event occurs before this time, and results in plate-like particle growth. The corresponding radius of gyration in each fit is converted to thickness and plotted in fig. 5.4 C, and is approximately 10 nm. The thickness is consistent with the AFM results of particles that were cast several hours after the start of synthesis. Given that the (111) facet is exposed on the plate-like particles observed using TEM, we concluded that there must be a mechanism that limits the thickness of particles by preventing growth in the [111] direction. Furthermore, we believe that this is related to the difference between how Z2 (RMRMKMK) and Z2M246I (RIRIKIK) interact with gold precursors and the surface of particles. Amino acids containing aromatic or sp^2 hybridized atoms, such as arginine, were identified to have a higher preference for binding to (111) vs (100) facets in MD simulations[173]. Similarly, proteins selected on the basis of preferential binding to gold (111) facets using directed evolution displayed a high frequency of lysine and arginine[61]. While methionine is also known to have a strong binding energy to gold surfaces, we have found no evidence of methionine selectivity for (111) surfaces. Therefore, we hypothesized that in the absence of methionine, the remaining arginine and lysine residues in Z2M246I result in a higher preference for binding to the (111) facet of gold

nanomaterials during synthesis. As a result, the (111) facet is stabilized, stacking faults are created, and growth occurs preferentially in the [100] direction.

5.3.2 *Dynamic Intervention Using Z2*

Given the plausibility of Z2M246I having a preference for (111) facets, and the increase in anisotropic particles terminated by (111) facets, it is likely that Z2M246I is responsible for limiting growth in the [111] direction. However, the resulting nanoparticles are colloidally unstable and aggregate irreversibly after several days. We hypothesized that addition of Z2 during synthesis in the presence of Z2M246I would lead to more colloidally stable plate-like particles. The observation that isotropic nanoparticles are formed when using Z2 led us to imply that the sequence has less selective binding to gold facets. Z2 added after the reaction has started will therefore bind to exposed facets with low Z2M246I coverage, such as (100), and limit lateral growth of plate-like structures. A liquid handling robot was used to prepare samples and add Z2 after a specified amount of time as described in the methods section and illustrated in fig. 5.5. By adding Z2 after different delay periods, we expected to gain control over the extent and structure of plate-like particles formed. Each pipetting step was allocated a one minute period, and an additional delay could be added before the next pipetting step to control the time at which Z2 was added after the start of the reaction. The delays were programmed to vary between 0 and 12 delay periods, corresponding to addition of Z2 after approximately 1 and 13 minutes, respectively. Samples were created in one microplate (reaction plate) and then transferred to another microplate (characterization plate). Transfer of samples from the reaction plate to the characterization plate was repeated the following day (12-15 hours). This proved to be a fast method of determining which sample conditions resulted in colloidally unstable gold nanoparticles by comparing the decanted sample taken right after the reaction was started, and after samples were allowed to sediment (see fig. 5.5 for details).

The UV-Vis spectra in fig. 5.6 A and B show the samples transferred before and after sedimentation had time to occur, respectively. The spectra of the control sample prepared

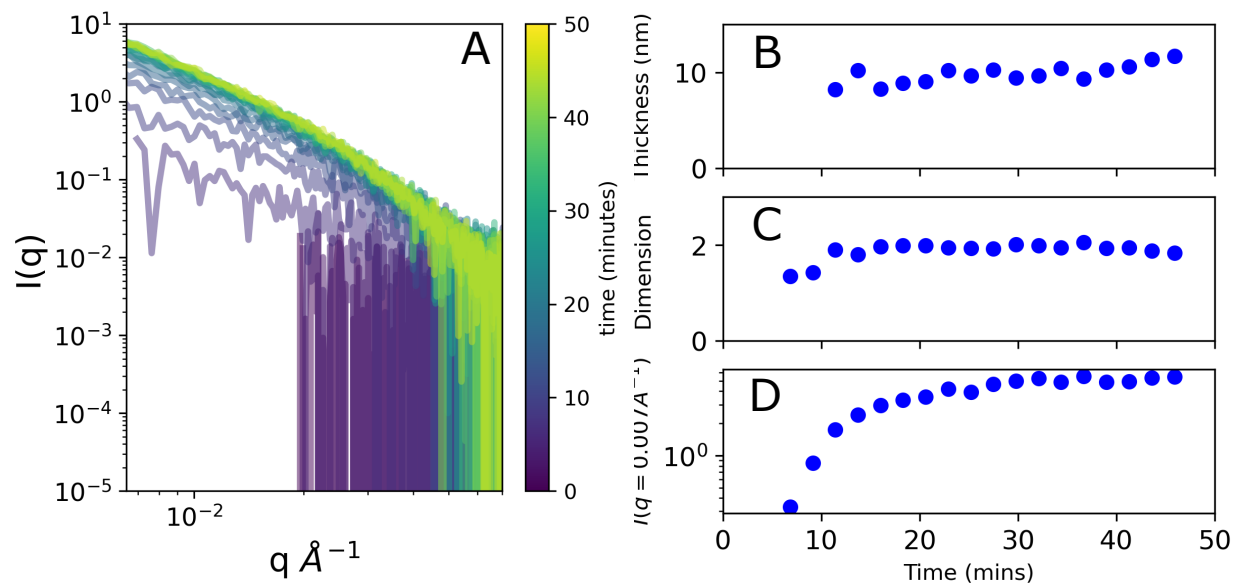


Figure 5.4: A Time-resolved SAXS profiles characterizing the formation of Au structures formed using 0.04 mM Z2M246I, 0.2 mM HAuCl₄, and 1 mM HEPES. Once the scattering intensity was sufficiently high to fit (approximately 10 minutes), the scattering profile at each point was fitted using a Guinier-Porod model, and the fit parameters were plotted as a function of time in B and C. The dimension variable increased to and stayed near a value of 2, and is consistent with plate-like structures. The radius of gyration of time points which had a dimension variable near 2 were converted to thickness, and demonstrated that the thickness plateaus to a value of approximately 10 nm. The intensity of scattering at low- q ($q=0.007 \text{\AA}^{-1}$) is proportional to the concentration of particles, and is stable after approximately 30 minutes.

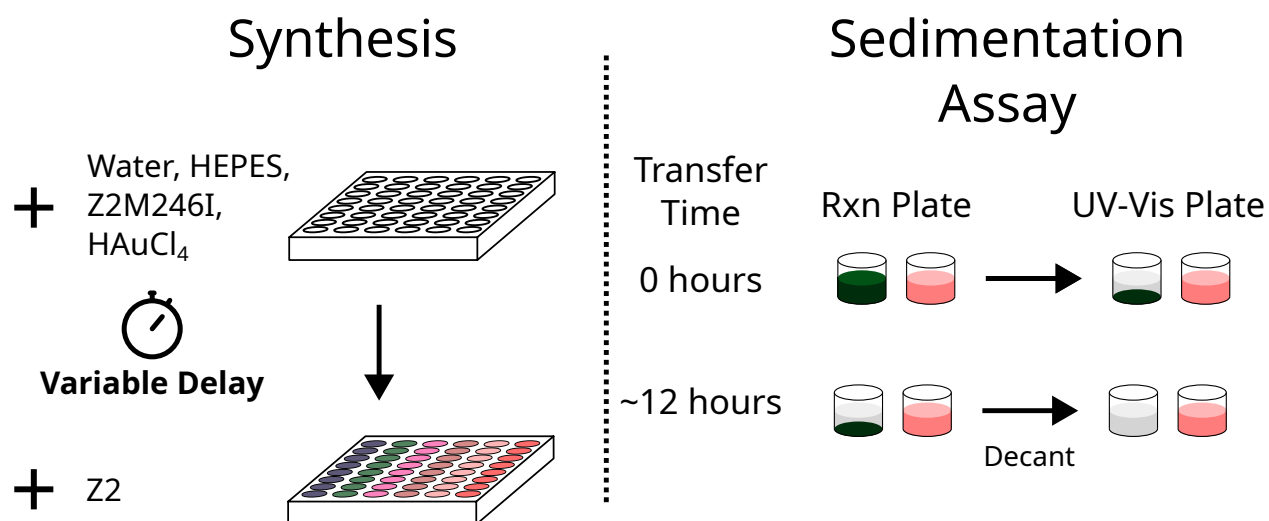


Figure 5.5: Overview of the synthesis and microplate sedimentation assays used in this section. Samples were synthesized using a liquid handling robot and custom script as described in the methods section. The sedimentation assay consists of two transfer steps in which 200 μL aliquots are transferred from 600 μL of sample in the 48-well reaction plate to wells in the 96-well plate used for UV-Vis characterization. Comparison of spectra from samples which were transferred immediately, or 12-15 hours after all synthesis steps were completed, allows for differentiation between extents of sedimentation between samples. For example, a sample that sediments significantly in the reaction plate during the 12-15 hour period will have much lower optical extinction in the second aliquot transferred to the microplate used in UV-Vis characterization relative to the first aliquot.

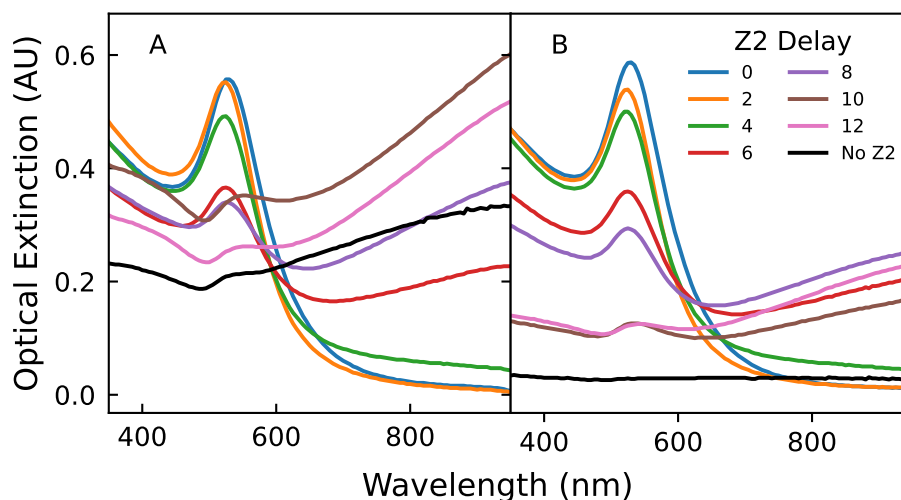


Figure 5.6: A and B correspond to spectra taken of samples transferred from the reaction plate immediately after all pipetting commands were finished, and after a delay period of 12-15 hours.

in the absence of Z2 show minimal optical extinction after decanting and demonstrate that the particles sedimented in the reaction plate. Samples in which Z2 was added early on in the reaction, such as those with a delay period of 0, 2 or 4, feature only one LSPR peak, and are therefore consistent with spherical particles. The spectra of these three samples were similar independent of the time that they were transferred, and demonstrate that the particles formed were more colloidally stable. Addition of Z2 after longer than 4 delay periods resulted in an increase in optical extinction at higher wavelengths, and a decrease in the optical extinction of the first LSPR peak. Optical extinction at higher wavelengths indicates that plate-like particles were present in addition to spherical particles. However, the broadness of extinction at wavelengths greater than ca. 650 nm, such as when Z2 was added after 6 delay periods, suggests that there are multiple in-plane dipolar modes. This can be explained by aggregation, a broad distribution of plate-like particle lateral dimensions, and distinct modes made possible by irregularly shaped particle edges as seen in [158]. Optical extinction

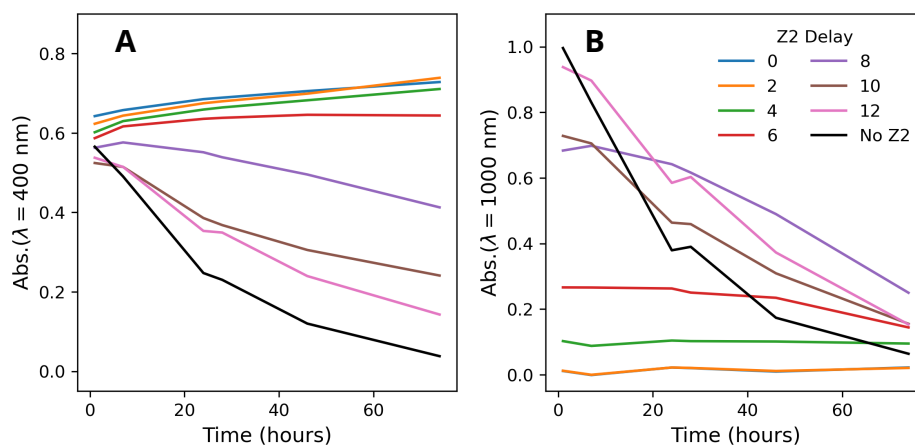


Figure 5.7: A and B correspond to changes in optical extinction at $\lambda=400$ nm and 1000 nm for samples prepared with Z2 being added after a different number of delay periods, or without Z2. Optical extinction at 400 nm is associated with overall Au(0) concentration, and optical extinction at 1000 nm suggests presence of plate-like particles.

in this region of the spectra decreased less significantly than in the control sample, and therefore demonstrates that existing plate-like particles are more colloidally stable. Finally the decrease in optical extinction of the first LSPR peak as the delay time is increased indicates that a smaller proportion of spherical particles is formed when Z2 is added after longer periods of delay.

To corroborate these results, a new batch of samples was synthesized and transferred to cuvettes to perform a colloidal stability assay by measuring changes in UV-Vis spectra over the course of 3 days. Changes in absorption at 400 nm and 1000 nm for each sample are shown in fig. 5.7 A and B to demonstrate changes in overall Au(0) concentration and presence of plate-like particles, respectively. Images of each sample and spectra are included in appendix B. Optical extinction at 400 nm increased slightly or remained nearly constant for samples in which Z2 was added after 6 delay periods or less. This indicates that the majority of the particles in these conditions remained suspended over the time period of

the assay, and are therefore primarily particles small enough for the gravitational force to be negligible relative to random motion. The slight increase over time may be attributed to the slower growth as a result of Au(I)-methionine complexation. The optical extinction at 400 nm decreased most rapidly for the control sample in which no Z2 was added, and is in agreement with the microplate based sedimentation assay. Addition of Z2 at 6 delay periods or later produced mixtures of spherical and plate-like particles in which the latter also appeared to sediment. Nevertheless, the optical extinction at 1000 nm shown in fig. 5.7B for this set of samples was maintained for a longer time, and was ultimately higher than in the control sample. This shows that the intervention with Z2 was successful at improving the colloidal stability of plate-like particles.

Electron microscopy and AFM were used to directly characterize particle structures formed as a result of intervention. SEM images obtained for samples in which Z2 was added after 0, 6, 8, and 10 delay periods are shown in fig. 5.8. The addition of Z2 after no delay periods resulted in spherical particles with a diameter of ca. 10 to 30 nm and some nanotriangles. Samples in which Z2 was added at later times feature fewer spherical particles, and more plate-like particles. The thickness of plate-like particles formed after 6 and 10 delay periods was characterized using AFM as shown in the appendix B. Several smaller nanoplatelets were identified with a thickness of 10 nm in the former sample, and a larger plate-like structure was found to have a thickness of ca. 12 nm in the latter. These observations confirm that addition of Z2 successfully stabilizes the intermediate plate-like structures from further lateral growth and aggregation.

DLS experiments were performed to identify changes in the average size of particles as a result of intervention. The DLS data are presented as an intensity weighted distribution of diffusion coefficients in fig. 5.9 A. The data is presented in terms of diffusion coefficients, which scale inversely with particle size, because the coexisting spherical and plate-like particle morphologies have different friction coefficients. These data support that a population of more slowly diffusing particles forms when Z2 is added at or after 4 delay periods, and that the mean size of this particle population increases when Z2 is added later. The trend in

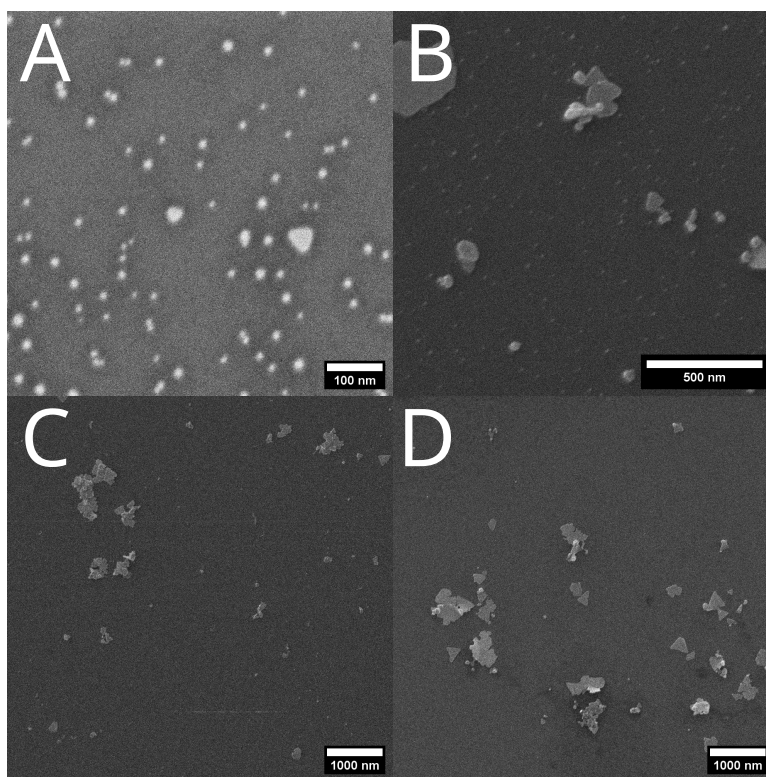


Figure 5.8: SEM images of Au nanoparticles obtained by mixing in Z2 after a different amount of delay periods. A, B, C, and D correspond to delay periods of 0, 6, 8, and 10, respectively. The particles in A are primarily spherical particles ranging from ca. 10 to 30 nm in diameter, and some nanotriangles are present. Particles obtained after 6 or 8 delay periods also feature some spherical particles, but have a larger proportion of plate-like nanoparticles. Z2 addition after 10 delay periods forms primarily plate-like nanoparticles which appear less aggregated than in the control sample with no Z2.

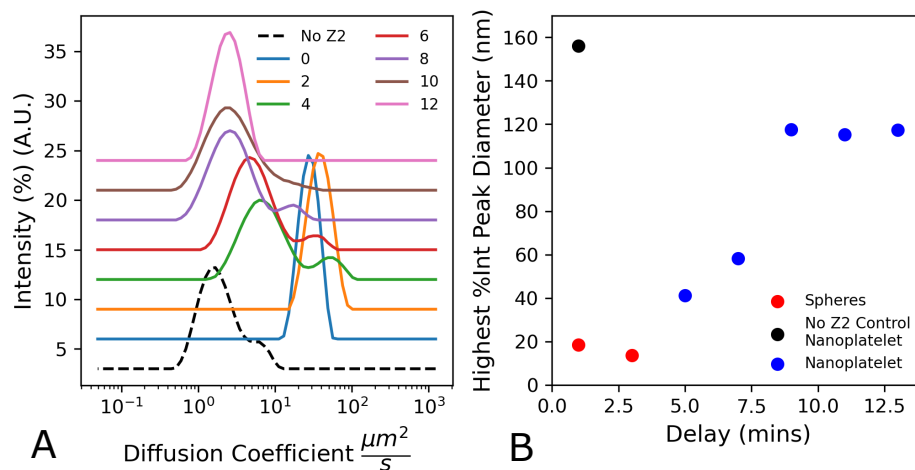


Figure 5.9: A Intensity weighted distribution of diffusion coefficients for samples in which Z2 was added after a different number of delay periods, and for the control sample in which no Z2 is added. B The diffusion coefficients of peaks with the largest intensity weighted area were converted to effective diameters as described in the methods section, and plotted as a function of the delay time.

particle size can be estimated as shown in fig. 5.9 B, in which the diffusion coefficient of the peak with the largest intensity weighted area for each time of Z2 addition is converted to an equivalent diameter as described in the methods section. Based on the microscopy results shown in fig. 5.8, we convert the diffusion coefficients by either assuming a spherical or hexagonal nanoplatelet morphology.

5.3.3 Effect of Z2 Addition on Particle Growth Pathways

In-situ UV-Vis measurements were carried out to study the dynamics of particle growth as a result of the intervention using Z2. First, the control experiments of particle growth in the presence of just Z2 or Z2M246I are presented in fig. 5.10. The single LSPR peak and negligible absorption at wavelengths higher than this peak indicate that dispersed spherical particles were formed without formation of larger clusters, or anisotropic structures.

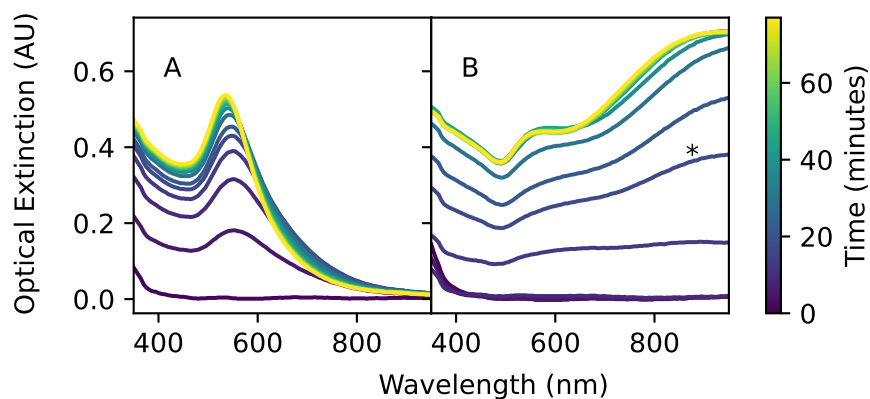


Figure 5.10: In-situ UV-Vis spectra of gold nanoparticle formation over time in the presence of Z2 A, or Z2M246I B. The asterisk in B marks the 9 minute time point at which the prominence of a peak at higher wavelengths that is attributed to the longitudinal plasmon resonance mode of plate-like gold particles.

Increased optical absorption at higher wavelengths which then decrease in intensity have been linked to particle clusters and formation of nuclei near the surface of existing particles, i.e. secondary nucleation[174, 81]. In the case of the Z2M246I sequence, in the absence of methionine, we observe broad optical absorption and lack of a single LSPR peak. A more defined peak appears after approximately 13 minutes as indicated by the asterisk in fig. 5.10 B. Broad optical extinction is consistent with plasmonic particles in close proximity to one another, and the appearance of a more distinct peak later on is likely the longitudinal plasmon resonance mode of anisotropic particles.

Next, we present the in-situ spectra of Au nanoparticle growth occurring after intervention by addition of Z2 at four different intervals in fig. 5.11. In the earliest and latest time points, addition of Z2 results in spectra that resemble growth in the presence of Z2 or Z2M246I. This was expected based on previously discussed imaging and spectroscopy results which showed that the shortest and longest delay times of Z2 addition tended to form structures similar to those found in the presence of either Z2 or Z2M246I on their own. Ad-

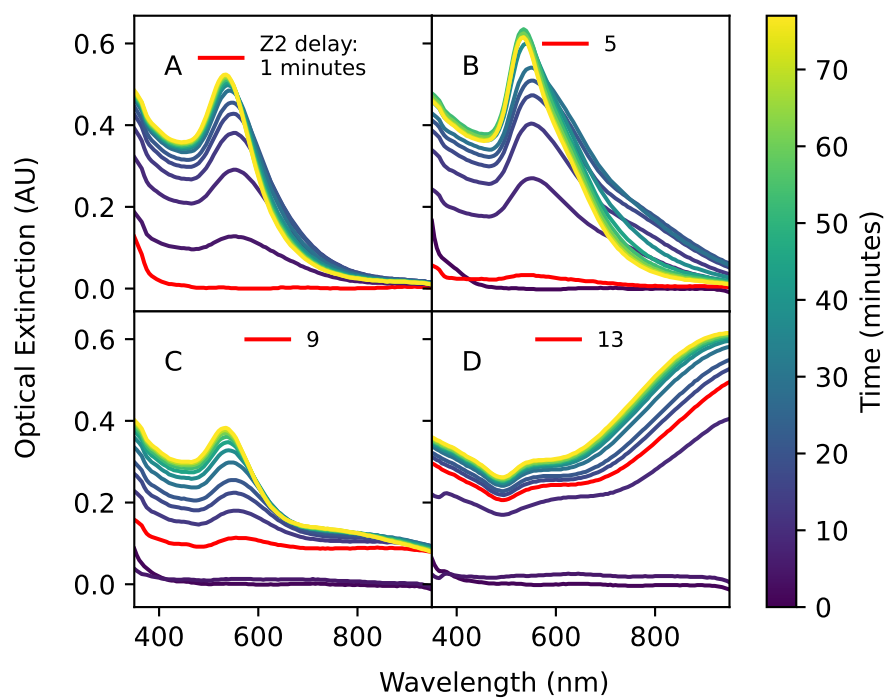


Figure 5.11: In-situ UV-Vis spectra of gold nanoparticle formation over time in which only Z2M246I is present initially, and Z2 is added at different time points: A 1 minute, B 5 minutes, C 9 minutes, D 13 minutes.

ditionally, we believe that these observations are consistent with Z2 having a higher binding free energy to particle surfaces than Z2M246I. The similarity of the structures formed when Z2 is used on its own to when it is added early on to a synthesis in the presence of Z2M246I resembles the result obtained by Wang et al.[165]. The authors demonstrated that replacing a shape-directing molecule with another resulted in unique combinatorial growth pathways only after completing an initial growth stage for a minimum period of time. In our work, growth pathways that were unique from either the control experiment featuring only Z2 or Z2M246I were only observed when Z2 was added at intermediate time points. Addition of Z2 after 5 minutes resulted in spectra with an LSPR peak consistent with spheres, and optical extinction at wavelengths higher than this peak that increased and then decreased in intensity. This suggests that particles were in close proximity to one another at intermediate time points of the reaction and then dispersed. Because HEPES is a weak reducing agent, gold reduction is not yet complete at this time based on the increase in extinction at 400 nm that follows. Therefore, we believe that addition of Z2 at this intermediate time results in complexation of methionine with Au(I), and thereby inhibits secondary nucleation near the surface[170]. Optical extinction at high wavelengths remained relatively constant when Z2 was added 9 minutes after starting the reaction, and the LSPR band from spherical particles continued to grow in intensity. The lack of change in extinction at higher wavelengths suggests that by this point anisotropic particles have already formed, and that there is less Au(I) species for methionine to complex with. While it is difficult to prove mechanistic details from these UV-Vis in-situ experiments, they illustrate that intervention with a peptide that has a different selectivity for particle surfaces can change the dominant mode of growth in a reaction, i.e. from favoring anisotropic to isotropic growth. Moreover, the differences between addition of Z2 at the two intermediate time points lead us to infer that secondary nucleation is likely important in explaining the growth of plate-like particles. This process may be associated with the interconnected appearance of plate-like particles as opposed to individual nanotriangles, and that Z2M246I adsorption at the (111) facet prevents growth of particles in that direction.

5.4 Conclusion

This work clarifies questions about the origin of particle anisotropy as a result of methionine substitution in the Z2 sequence, and uses experiment automation tools to control the dynamics of particle growth through timed intervention. Temporal control over the addition of reagents is used in biomineralization to yield complex structures, but remains an underappreciated experimental approach in nanoparticle synthesis. While methionine substitution likely decreases the overall binding affinity of the peptide towards gold surfaces, it also results in appreciable changes in facet selectivity. We provide in-situ UV-Vis characterization of plate-like gold nanoparticles that form in the presence of a facet selective peptide variant, and as a result of addition of a second peptide which we believe does not have facet selective binding at different times. Together with our in-situ SAXS results we conclude that anisotropic growth likely occurs in clusters where secondary nucleation events extend growth preferentially along the lateral direction of plate-like particles. However, identifying exact mechanistic details as a result of intervention will require in-situ characterization such as SAXS or liquid phase TEM combined with a means of mixing reagents at different times, such as with a microfluidic chip. Addition of methionine containing Z2 at intermediate time points results in pathways in which optical extinction associated with particle aggregation or anisotropy is either reversed, or maintained. The time at which Z2 is introduced controls the proportion of spherical to plate-like nanoparticles, improves the colloidal stability of plate-like particle, and can be used to control the average lateral dimension of plate-like particles. We envision that our experimental automation approach for temporal manipulation of growth pathways can be used by future researchers to achieve control over anisotropic nanoparticle synthesis and other systems.

Chapter 6

APPLICATIONS OF SMALL-ANGLE SCATTERING IN HIERARCHICAL SELF-ASSEMBLY

Abstract

The ability to obtain in-situ structural information using small-angle scattering makes the experimental technique highly synergistic with computational approaches such as molecular dynamics. SAS has been used to optimize parameters in force fields for conjugated polymers and proteins[175, 97]. Additionally, the ability to calculate small-angle scattering profiles from simulations allows for a more direct comparison, as demonstrated by the work of Shimizu et al. in which 1 μ s of coarse grained molecular dynamics simulations were analyzed to extract possible structural ensembles[176]. The purpose of this chapter is to highlight contributions of SAS experiments and model fitting results to collaborative works focused on the hierarchical self-assembly of nanomaterials. These works included themes pertinent to this thesis such as the use of sequence defined molecules to control self-assembly, how different experimental conditions (pH, solvent identity and concentration) alter intermolecular interactions and thereby colloidal self-assembly, and the synergy of SAS and computational methods to address issues related to the design of sequence defined molecules.

6.1 Hierarchical Self-Assembly Pathways of Peptoid Helices and Sheets

The work presented in this section is based on: Zhao, M., Lachowski, K. J., Zhang, S., Alamdari, S., Sampath, J., Mu, P., Mundy, C. J., Pfaendtner, J., De Yoreo, J. J., Chen, C. L., Pozzo, L. D., & Ferguson, A. L., *Hierarchical Self-Assembly Pathways of Peptoid Helices and Sheets*, Biomacromolecules 23, 992–1008 (2022)[177]. All simulations, chemical synthesis, and experiments other than small-angle scattering and circular dichroism were

designed, performed, analyzed, and described by the co-authors in the publication.

6.1.1 Introduction

Peptoids, or N-substituted polyglycines, are peptidomimetic polymers or oligomers in which the side-chain is appended to the nitrogen atom in the amide backbone. The submonomer synthesis method developed for peptoids has advantages over methods used to synthesize peptides in that it eliminates the need for certain protecting groups, and has access to a wider variety of readily accessible building blocks[64, 178]. The three key molecular differences that distinguish peptoids from peptides are the absence of a hydrogen bond donor, absence of a chiral center in the backbone, and the ability to more easily transition to *cis* and *trans* conformations[178]. In principle, the effect of sequence modifications on intermolecular interactions should be more predictable relative to peptides given the lack of backbone hydrogen bonding and chiral centers[118]. This predictability has allowed rational design of peptoids that self-assemble into a variety of shapes such as sheets, fibers, micelles, tubes, ribbons, helices, and vesicles[118]. These structures can be used as templates for the growth and assembly of inorganic nanomaterials by exposing specific functional groups present in the peptoid, or biomolecules such as gold binding peptides, on the surface[118, 179, 180]. In reality, the connection between a peptoid sequence and its self-assembly and interactions with particle surfaces is still complex[64].

One approach for forming self-assembled structures begins by dissolving peptoids in a binary solvent mixture consisting of a good and bad solvent, and then slowly allowing the solvent to evaporate. By using a good solvent which is more volatile than the other, the supersaturation of the peptoid increases due to both the decrease in solvent quality and increase in solute concentration. In this work, the specific peptoid used was NBrpe6Nc6 - a hexamer of N-((4-bromophenyl)ethyl)glycine capped on one end with an amine group, and conjugated to 6-aminohexanoic acid. NBrpe6Nc6 dissolves in mixtures of volatile organic solvents with water, and self-assembles into crystalline nanosheets after solvent evaporation as shown in ex-situ imaging studies. We set out to characterize the pathway taken by pep-

toid monomers to crystalline nanosheets to better understand how side chain chemistry and assembly conditions can be used to control self-assembly. The pathway was determined by characterizing changes in peptoid structure in different solvent compositions. Specifically, all-atom molecular dynamics were used to characterize the free energy of different stages of the pathway, and were validated where possible by small-angle X-ray scattering, circular dichroism, atomic force microscopy, and X-ray diffraction. Based on our results, we proposed that NBrpe6Nc6 follows a hierarchical self-assembly pathway shown in fig. 6.1 which resembles one found in the literature for another peptoid system[181].

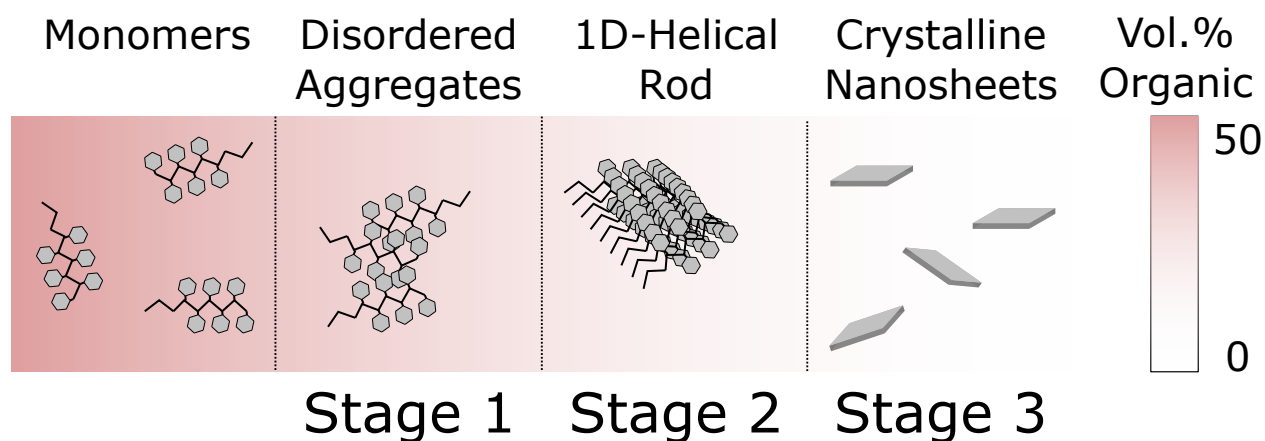


Figure 6.1: Proposed hierarchical self-assembly pathway of NBrpe6Nc6 as a result of changes in organic solvent concentration as indicated by the gradient. Assembly consisted of three stages: formation of disordered aggregates from monomers, organization into 1D-helical rods, and finally packing of rods into nanosheets.

6.1.2 Methods

Trifluoroacetic acid was added to 1 μmol of NBrpe6Nc6 and dried using a stream of nitrogen to obtain a clear film, and finally methanol was added. The stock was used to prepare 6 g/L peptoid solutions in pure methanol and a 75:25 v/v mixture of methanol and water.

The latter turned turbid immediately after addition of water, and was injected into a flow-through quartz capillary with 1 mm outer diameter. Both samples were measured soon after preparation in a Kratky-type instrument (SAXSess, Anton Paar, Graz, Austria) with a Cu K_α source ($\lambda = 1.54\text{\AA}$), and a temperature controlled capillary holder set to 20°C . The fully reduced, slit-smeared SAXS data of the 75:25 v/v methanol/water mixture were analyzed in SASview by fitting with a flexible cylinder model (SASview v.5.0.1, www.sasview.org). The model corresponds to a semiflexible chain with a contour length l_c composed of cylindrical elements of length l_p , the persistence length, and with a radius r [182, 183].

CD measurements were carried out on a Jasco (Oklahoma City, OK, USA) J-1500 CD spectrometer, and were baseline corrected by using the corresponding solvent or solvent mixture. The final CD was then converted to molar ellipticity by normalizing to the concentration of total peptoid. First, a stock of NBrpe6Nc6 in methanol was prepared as described in the preceding section, and measured in a quartz cuvette with 1 mm path length (Hellma, Plainview, NY, USA). Next, the sample was diluted with water to reach a peptoid concentration of 6 g/L and the same 75:25 v/v methanol/water mixture used in SAXS experiments.

6.1.3 Results and Discussion

The suggested pathway shown in fig. 6.1 consisted of three stages over the course of solvent evaporation: monomers forming disordered aggregates, formation of 1D helical rods, and finally 2D crystalline nanosheets. This hypothesis was tested using MD simulations in 50:50, 20:80, and 10:90 v/v acetonitrile:water, as well as pure water and 75:25 v/v methanol/water. Specifically, the association free energy between a monomer and each of the different self-assembled structures was obtained for each of the five solvent conditions, and was in general agreement with the proposed assembly mechanism.

While acetonitrile/water mixtures were reliably used to form nanosheets observed using AFM imaging, these mixtures are challenging to characterize using SAXS due to the formation of self-associating acetonitrile clusters that result in significant microscopic heterogeneity for mixtures ranging from 20-60% acetonitrile[184]. Therefore, methanol was substituted for

acetonitrile in SAXS experiments, and the results for peptoid in pure methanol and 75:25 v/v methanol:water are presented in fig. 6.2 A. The scattering of the sample in pure methanol was found to be consistent with dissolved peptoid monomers. On the other hand, the data obtained for 75:25 v/v methanol/water was fit well using a form factor of randomly oriented, monodisperse flexible cylinders. The diameter was 28 Å, the contour length was greater than the low-q limit of the instrument (>500 Å), and the Kuhn length of 108 Å. This was a surprising result and revealed that the self-assembly of nanosheets may be hierarchical, i.e. that 1D rod structures can assemble into nanosheets. Additionally, the fitted diameter was consistent with the length of a single extended NBrpe6Nc6 molecule, and indicated that the rods are probably stacked peptoid monomers. MD simulations confirmed that this rod-like structure was thermodynamically favorable for both the 75:25 v/v methanol/water and 10:90 v/v acetonitrile/water mixtures. Evidence for rod-like structures, and confirmation that NBrpe6Nc6 ultimately also assembles into nanosheets were obtained using AFM. MD simulations also revealed that peptoid monomer stacking may have either right or left-handed chirality, but that there should not be a net bias towards one handedness over the other. The absence of a CD signal in fig. 6.2 supported this hypothesis.

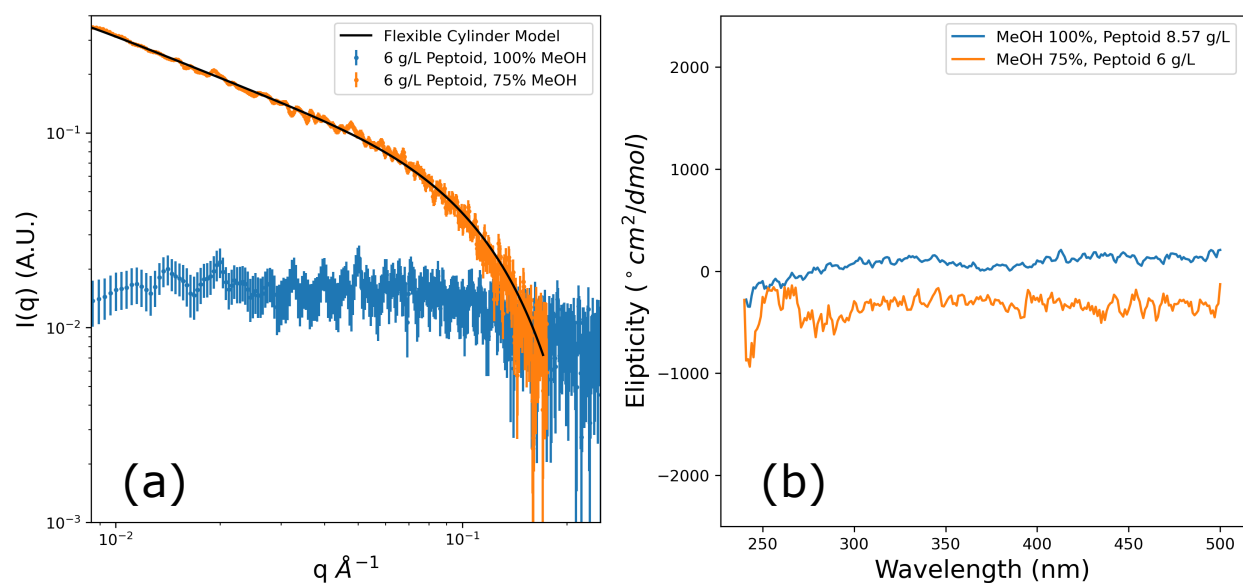


Figure 6.2: SAXS and CD measurements of NBrpe6Nc6 aggregates in pure methanol solvent where the peptoids are dispersed, and a 75:25 v/v methanol/water solvent where we see evidence of peptoid assembly. A The experimental SAXS data at 75:25 v/v methanol/water is well fitted by assuming a flexible cylinder form factor for 1D rods of diameter 28 Å, length greater than 500 Å, and a Kuhn length of 108 Å. B CD measurements of peptoid in both pure methanol and a 75:25 v/v methanol/water solvent show no net chirality as evidenced by the weak ellipticity.

6.2 Predictive Theoretical Framework for Dynamic Control of Bioinspired Hybrid Nanoparticle Self-Assembly

The work in this section is based on: Qi, X., Zhao, Y., Lachowski, K. J., Boese, J., Cai, Y., Dollar, O., Hellner, B., Pozzo, L. D., Pfaendtner, J., Chun, J., Baneyx, F., Mundy, C.J., *Predictive Theoretical Framework for Dynamic Control of Bioinspired Hybrid Nanoparticle Self-Assembly*. ACS Nano 16, 1919–1928 (2022)[63]. All simulations, chemical synthesis, and experiments other than ultra-small-angle scattering were designed, performed, analyzed, and

described by the co-authors in the publication.

6.2.1 *Introduction*

The structure of individual nanoparticles enables properties not observed in the bulk state, such as quantum confinement in semiconductor nanocrystals, localized surface plasmon resonance in metal nanoparticles, and multiple changes in physical properties as a result of higher surface area to volume ratios[79]. Maintaining physical separation between nanoparticles is typically desirable to maintain colloidal stability, and retain the functionality of the system. However, monodisperse colloidal particles may be directed to self-assemble into structures with symmetry and regular nanoscale separation distances. Unlike the color which results from light absorption by specific bonds in chromophores, structural color arises from interference effects in structures with repeating distances similar to the wavelength of light. Structural color is responsible for the iridescence of minerals such as opals which are composed of ordered silica spheres, or insect wings such as in the morpho butterfly[56]. Interference effects due to scattering can be also be tuned to transmit a specific wavelength of light by using the Christiansen effect. The latter is the decrease in scattering from multiphase domains which have the same refractive index at a given wavelength. Xi et al. demonstrated that this effect was present in bicontinuous gels composed of silica nanoparticles, water, and an organic molecule[185]. The authors used temperature to change the proportion of water and the organic molecule in the two domains, which changed their relative refractive indices. In turn, small variations in temperature gave sensitive control over the color of light which could be transmitted through the gel. The authors used small-angle scattering techniques to support that the observed changes were not a result of changes in repeating distance related to silica packing or the bicontinuous domains. Control over the interparticle distance in ordered assemblies such as colloidal crystals is a more commonly used method for engineering structural color[186].

To achieve regular and symmetric packing of particles, directionality in interparticle interactions must be introduced by either manipulating particle shape or by forming patchy

particles with anisotropically patterned surfaces. Both of these fields are highly mature and can often predict the packing of nanoparticles. However, there is poor understanding over how changes in an individual experimental parameter can be used to control the dynamics or reversibility of colloidal assembly. The selective binding of sequence defined molecules to inorganic interfaces, combined with knowledge of how this interaction can be manipulated using temperature, pH, and ionic concentration, can be used to improve the predictability of colloidal self-assembly.

In this work we used Car9[187], a silica binding peptide, to control the dynamic self-assembly of silica nanoparticles. The peptide was expressed in a green fluorescent protein construct which featured two Car9 sequences in solvent accessible positions (sfGFP::Car9). By taking advantage of the high sensitivity of Car9-silica interactions to pH changes, we were able to manipulate the reversible assembly of silica nanoparticles (SiNPs). Specifically, micrometer-scale SiNP aggregates were formed at a pH of 7.5, and dispersed particles were obtained at a pH of 8.5. The theoretical framework used to explain these changes was a coarse grained model which separates the interactions of long range SiNP-SiNP and short range sfGFP::Car9-SiNP interactions. These interactions were modelled using DLVO theory and atomistic molecular dynamics simulations, respectively. The coarse-grained model was consolidated into a rigid-body model which represents SiNP and protein as spheres, as shown in the fig. 6.3. Ultra-small-angle X-ray scattering (USAXS) was used to validate assumptions and tune parameters used in the model.

6.2.2 Methods

Rhodamine-conjugated silica nanoparticles (RhSiNP) terminated with surface silanols were purchased from Micromod Partikeltechnologie GmbH (Rostock, Germany). sfGFP::Car9-Car9 (sfGFP) was mixed in centrifuge tubes with RhSiNP at a 5:1, 3:1, or 1:1 molar ratio in 20 mM Tris-HCl titrated to a pH of 7.5 or 8.5. The mixture was then covered with aluminum foil and rotated using a tube rotator for 30 minutes at room temperature.

USAXS data were collected on the 9-ID-C beamline at the Advanced Photon Source

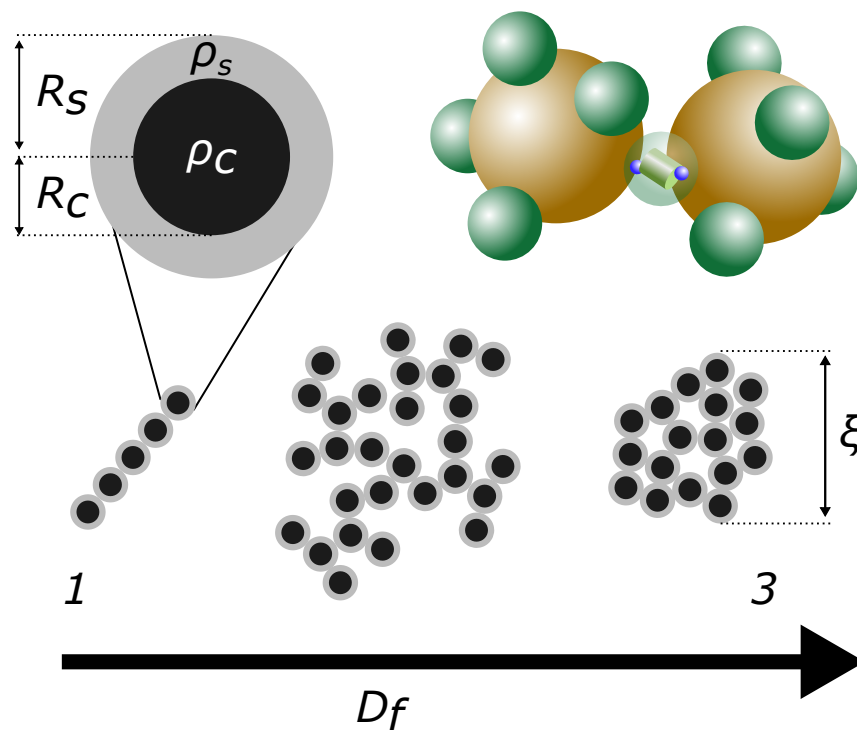


Figure 6.3: Schematic showing the model of the core-shell sphere building block in the top left, and the fractal aggregate on the bottom. Note that in the software implementation of the core-shell sphere form factor, the thickness is the difference between R_s and R_c . In the upper right, the schematic for the coarse-grained model representing SiNP bridging through the homobifunctional sfGFP::Car9 construct.

at Argonne National Laboratory using 21 keV X-rays[101]. USAXS data collection time was 90 seconds. Beam size was 0.8 x 0.8 mm. Flux density was approximately $5 \times 10^{12} \text{photons/second/mm}^2$. Samples were loaded in a custom-made 48-well plate assembly with two Kapton windows and a sample thickness of 10 mm[130]. USAXS data were reduced using standard data reduction protocols and software (Indra) that were provided by the beamline. Data were corrected for instrumental background and for the scattering from the sample container and windows. Mathematical desmearing of the USAXS data was performed to remove instrumental slit smearing effects for direct comparison with SAXS profiles obtained from rigid-body simulations[131]. Smearred USAXS data were also analyzed using SASview/SASmodels[167] and bumps[188] packages for Python.

6.2.3 Results and Discussion

Fitting of surface plasmon resonance measurements demonstrated that the two Car9 sequences in the construct have notably different binding affinities for silica surfaces. Specifically, the Car9 tag located in the tail had a much higher binding energy to silica surfaces, and was assumed to always be bound to dispersed SiNP. In the dispersed state, the statistical distribution of proteins per SiNP was assumed to be equivalent to the molar ratio, i.e. for a 5:1 molar ratio of protein:SiNP, on average, each SiNP had 5 proteins on its surface. The affinity of the weaker Car9 tag located in the loop of the protein was in agreement with atomistic molecular dynamics simulations. In the coarse grained model, the contribution of protein-SiNP interactions from each surface bound protein to interparticle interactions was treated using an effective short range interaction potential approximated using a Lennard-Jones potential form, U_{PS} :

$$U_{PS} = 4\epsilon \left[\left(\frac{\sigma}{r - \sum R} \right)^{12} \left(\frac{\sigma}{r - \sum R} \right)^6 \right] \quad (6.1)$$

Here, ϵ is the depth of the interaction potential well, σ is the hard-sphere diameter of a molecule, r is the center to center interparticle distance, and $\sum R$ includes the radii of the protein and SiNP. As particles approach one another, the particle-particle becomes increas-

ingly sensitive to the separation distance. Therefore, it was critical to validate the true surface to surface distance as a result of bridging by sfGFP::Car9. This was accomplished by fitting USAXS curves of aggregated SiNP with a core-shell fractal model in which the thickness was fixed, as will be described in detail. Results from atomistic simulations of Car9 binding to flat silica surfaces could not be directly used to obtain ϵ because of particle curvature effects. A starting range of values for ϵ at a pH of 7.5 and 8.5 was obtained by accounting for the latter effect. However, the precision of this estimate had to be improved because a wide range of ϵ values were consistent with the experimentally observed reversible assembly. While ϵ cannot easily be varied experimentally, the molar ratio of protein to SiNP can serve as a proxy for changing the potential well in the effective short range interaction. Rigid-body simulations of protein decorated SiNP were carried out for pH 7.5 and 8.5, and a range of ϵ values. SAXS profiles from the resulting simulations were calculated and compared to experimental USAXS curves of samples prepared in the matching conditions (i.e. same pH and sfGFP:SiNP molar ratios) to narrow the value of ϵ .

The USAXS data and model fits are presented in fig. 6.4, and the resulting model parameters are shown in table 6.1. USAXS profiles of samples prepared at a pH of 8.5 at sfGFP:SiNP molar ratios of 1:1 and 3:1 were qualitatively similar to the scattering from fully dispersed SiNP with no protein present at a pH of 7.5. This was consistent with the expectation that aggregation of SiNP induced by sfGFP should be minimal at a pH of 8.5. Therefore, the SiNP suspension and the samples prepared at a pH of 8.5 with sfGFP:SiNP molar ratios of 1:1 and 3:1 were all fitted using a sphere form factor with a lognormal distribution of radii to account for polydispersity. The sphere radius was fixed at 4.7 nm and the radius polydispersity of the bare SiNP was fitted. This radius and radius polydispersity were then used for all samples. Note that the radius was comparable to that reported by the manufacturer (5 nm). The remaining USAXS data from aggregated particles were fitted using a fractal aggregate model with core-shell spheres as the building blocks as seen in fig. 6.3[189, 190]. This model represents the product of the form factor of a core-shell sphere, $P(q)$, and the structure factor of a fractal aggregate to represent inter-particle scattering

contributions, $S(q)$:

$$P(q) = \frac{\phi}{V_s} \left[3V_c(\rho_c - \rho_s) \frac{\sin(qr_c) - qr_c \cos(qr_c)}{(qr_c)^3} + 3V_s(\rho_s - \rho_{solvent}) \frac{\sin(qr_s) - qr_s \cos(qr_s)}{(qr_s)^3} \right] \quad (6.2)$$

$$S(q) = 1 + \frac{D_f \Gamma(D_f - 1)}{[1 + 1/(q\xi)^2]^{(D_f-1)/2}} \frac{\sin[(D_f - 1) \tan^{-1}(q\xi)]}{(qr_s)^{D_f}} \quad (6.3)$$

Where ϕ is the volume fraction of particles, V_s is the total volume of a core-shell particle, V_c is the volume of the core of a core-shell particle, $\rho_c, \rho_s, \rho_{solvent}$ are the scattering length densities of the core, shell, and solvent, respectively, r_c is the radius of the core, r_s is the radius of the total particle, D_f gives the fractal dimension, and ξ is the correlation length. In this model, the shell represents the layer of adsorbed protein and water that surrounds each SiNP and serves as a spacer for the aggregated particles. Given that the layer surrounding the SiNP is primarily composed of solvent, the X-ray scattering length density (SLD) of the shell is approximated as that of the solvent, $9.43 \times 10^{-6} \text{\AA}^{-2}$, whereas the X-ray SLD of the SiNP core is $16.99 \times 10^{-6} \text{\AA}^{-2}$. Thus, in the limit of a shell SLD that is equal to the solvent, the form factor of the core-shell sphere form-factor $P(Q)$ approaches that of a simple sphere. The structure factor on the other hand, remains sensitive to the thickness of the shell due to the interparticle spacing that is introduced between particles that are bridged by sfGFP adsorbed on the surfaces. The thickness of the shells in the model was fixed at 2.5 nm to account for the spacing of particles bridged by sfGFP, which is approximately 5 nm in the axial direction when also accounting for the SBPs. The fractal dimension, D_f , that is obtained from the model describes the distribution of mass inside a fractal. A schematic of the aggregates that form between the limits of $1 < D_f < 3$ is shown in fig. 6.3. For randomly aggregating mass fractals, D_f typically varies between approximately 1.5 and 2.5 and is correlated with the density of the distribution of subunits in the aggregate. Finally, the correlation length ξ is proportional to the maximum size of the fractal aggregates.

Aggregation was observed for all sfGFP:SiNP molar fractions which were prepared at a pH of 7.5. The value of D_f can also be linked to the kinetics of aggregation[191], where lower values correspond to fast, diffusion-limited aggregation, and conversely higher values

correspond to slow, reaction-limited aggregation. The sample prepared at a 5:1 sfGFP:SiNP molar fraction and a pH of 8.5 also showed aggregation, and had a similar fractal dimension (2.33) as the sample with the same molar fraction prepared at a pH of 7.5 (2.25). Similarly, whereas increasing the protein concentration led to formation of larger clusters for samples prepared at a pH of 7.5, the aggregates formed at a pH of 8.5 and 5:1 sfGFP:SiNP molar fraction were smaller ($\xi = 308\text{\AA}$) than those obtained at a pH of 7.5 ($\xi > 19998\text{\AA}$). The maximum size of the latter could not be determined due to a lack of a Guinier region in the q-range of the USAXS instrument. Together, these results indicate that the kinetics of aggregation and the final structure of the aggregate can be controlled by both the pH and the protein concentration. However, sampling of additional experimental conditions, in-situ USAXS kinetics experiments, and additional simulation efforts will be necessary to yield more predictive control over the final structure of SiNP aggregates.

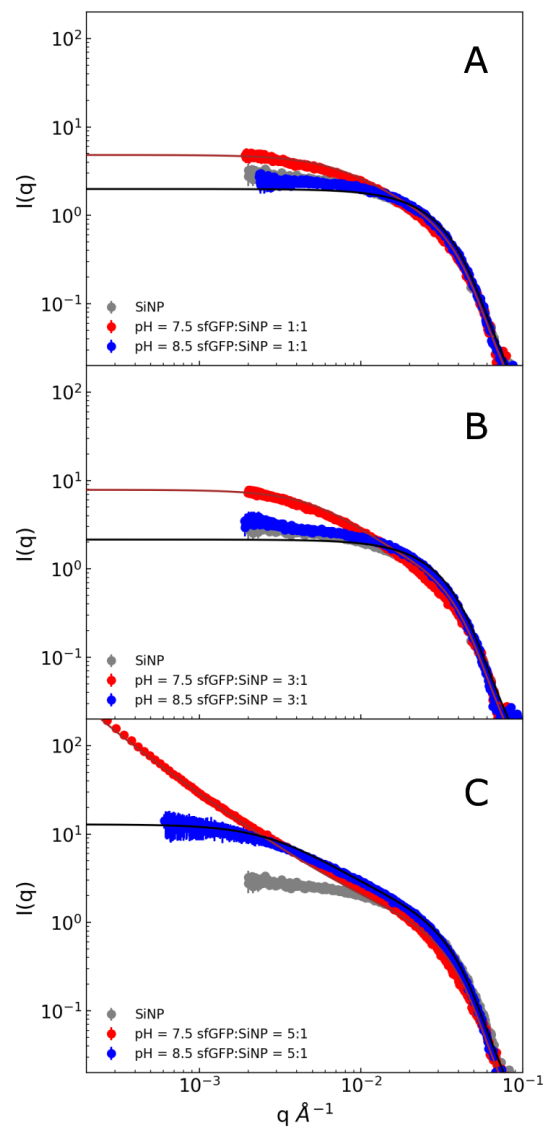


Figure 6.4: Smeared USAXS data of all sfGFP:SiNP molar fractions and pH conditions that were characterized. A,B, and C correspond to sfGFP:SiNP molar fractions of 1:1, 3:1, and 5:1, respectively. Scattering from SiNP measured at a pH of 7.5 at the same particle concentration with no protein present is plotted for reference. Solid lines correspond to the fitted model.

pH	sfGFP:SiNP Molar Ratio	Radius [Å]	Radius PD	Thickness [Å]	ρ_c [10^{-6}Å^{-2}]	ρ_s [10^{-6}Å^{-2}]	ρ_{solvent} [10^{-6}Å^{-2}]	D_f	ξ [Å]
8.5	5	47.0	0.30	25	16.99	9.43	9.43	2.33	308
8.5	3	47.0	0.38		16.99		9.43		
8.5	1	47.0	0.38		16.99		9.43		
7.5	5	47.0	0.38	25	16.99	9.43	9.43	2.25	19998
7.5	3	47.0	0.38	25	16.99	9.43	9.43	2.99	124
7.5	1	47.0	0.38	25	16.99	9.43	9.43	2.99	91
7.5	0	47.0	0.30		16.99		9.43		

Table 6.1: Results of fitting a fractal aggregate model composed of core-shell subunits, or a simple spherical model to all samples.

Chapter 7

CONCLUSION AND OUTLOOK

7.1 *Summary*

The numerous existing and envisioned applications of inorganic nanomaterials discussed in chapter 1 motivate the need to continue improving synthesis methods. Bioinspired approaches, such as the use of sequence defined molecules, offer numerous ways to modify the growth of nanoparticles. The design of sequence defined molecules, and the tangled relationship of experimental parameters in nanoparticle synthesis in general, are highly dimensional problems. In chapter 1, we grouped the former into three overlapping categories of approaches: rational design, computational methods, and directed evolution. The argument of this thesis is that design approaches need better integration with a diversity of experimental results. In other words, design cannot happen in a vacuum, and needs to be tested against a variety of experimental conditions. One of the ways that progress can occur is through improvements in experimental throughput, evaluation of datasets composed of high dimensional data, and methods for connecting these results back to the start of the design process. While directed evolution has been successful at identifying sequences with strong affinity towards solid binding surfaces, it cannot be used with biologically incompatible conditions, nor can it directly be used to screen for synthetic outcomes (i.e. control over nanoparticle structure requires more than just high binding affinity). Simultaneous optimization of both sequence design and experimental conditions by using high-throughput experimentation and genetic algorithms has already been demonstrated, and illustrates the potential that new experimental approaches can bring. In the next section, we will summarize the key results and advances in improving molecular design.

7.2 *Key Results and Perspectives*

In chapter 4 we used gold binding peptides (Z2 and AG3) identified in the literature to study how peptide presentation affected synthesis outcomes. Peptide presentation was used to connect the multivariate experimental design space of nanoparticle synthesis to the different ways that a peptide interacts with precursors and particles during synthesis. We made two sets of rational changes to Z2: substitution of methionine, and conjugation of a lipid tail. The first was chosen on the basis of understanding that methionine complexation with gold ions can modify the rate of reduction, and because it was evident from published computational studies that methionine was key to the strong binding energy of Z2 to gold surfaces. Conjugation of lipid tails of different lengths was used to make Z2 amphiphilic, and thereby change peptide-peptide and peptide-particle interactions. Nanoparticles were synthesized using 64 different reagent combinations with each of the 7 peptides, and UV-Vis spectroscopy was used as a proxy to characterize changes in synthesis outcomes. We created a data analysis pipeline to obtain a quantitative distance metric between the peptides on the basis of the latter. Our results were supported by lower-throughput, but more direct methods of structural characterization. Specifically, we identified that for this system changes in sequence had a more significant effect on synthesis outcomes than conjugation of a lipid tail, and that particle anisotropy increased as a result of methionine substitution. The significance of our experimental approach is that it can be used for a variety of other experiments which use spectral data to evaluate the outcome of an experiment.

Building on our observation that the amount of plate-like particles increased after methionine substitution, we sought to answer why this was happening and how it could be controlled in chapter 5. We used in-situ SAXS to study the growth of plate-like particles in the presence of Z2M246I, the Z2 variant with all methionines substituted for isoleucine. This experiment showed that 10 nm thick particles with two-dimensional symmetry are present within the first few minutes of synthesis, but limited information was gained about the lateral dimension of particles due to the lack of a Guinier region at low- q . Based on in-situ UV-Vis

spectra, particle clustering or aggregation which result in interparticle correlation could be a more likely explanation for the latter, rather than the presence of plate-like particles with lateral dimensions outside of the observable range of the SAXS configuration. Additionally, we used electron microscopy to identify that the basal facet of these plate-like particles was (111). Based on these results, and existing literature on facet selectivity of amino acids, we concluded that Z2M246I was selectively binding to and increasing the total surface area of (111) facets. However, the resulting materials were colloiddally unstable. We hypothesized that addition of Z2 to growing plate-like particles would decrease their lateral growth. A liquid handling robot was used to add Z2 to particles growing in the presence of Z2M246I, and we used UV-Vis spectroscopy to show that this decreased particle sedimentation, and gave control over the mean size of plate-like particles. In-situ studies of intervention with Z2 identified a second way in which methionine may play a role in controlling the growth of plate-like particles. Namely, that methionine complexation with Au(I) prevents secondary nucleation. The formation of spherical particles in addition to plate-like particles is a down side when evaluating this approach, but is also present in other methods. Careful evaluation of the proportion of spherical to plate-like particles will enable benchmarking of this approach against existing methods. The improved colloidal stability combined with the irregular edges of particles formed using this approach could be used for surface enhanced Raman spectroscopy studies in the future. Our underlying hypothesis that facet selectivity is key to explaining the appearance of plate-like particles in this system should be evaluated using computational methods. The significance of this work is that it provides in-situ characterization for the growth of thin metal nanomaterials with two-dimensional symmetry, which is seldom reported in the literature.

Insights into how solvent quality and pH on hierarchical self-assembly outcomes in the presence of sequence defined molecules were made in chapter 6. This was achieved by using SAXS results to support computational studies of peptoid self-assembly, and protein mediated self-assembly of silica nanoparticles. In the first work, we identified the self-assembly pathway of a nanosheet forming peptoid as a result of solvent evaporation in binary mix-

tures of organic solvent and water (typically 50/50 acetonitrile/water). By obtaining SAXS data at an intermediate concentrations of methanol and water, we found that structures with extended structures were present that had not previously been identified using imaging studies. The need for replacing acetonitrile with methanol was the strong scattering from solvent inhomogeneities relative to scattering from the peptoid. The thermodynamic stability of these structures was confirmed in both aqueous methanol and acetonitrile mixtures, and therefore proved that they were a plausible intermediate in nanosheet formation. While the 1D helical rods identified in this study were not shown to have a preference towards being left or right handed, we were interested in finding ways to control this chirality. The practical motivation of this is that chiral peptoid self-assemblies could be used as templates for growth of inorganic nanomaterials with inherent chirality. One way that we tried doing this is by replacing a part or all of the organic solvent with a chiral solvent. While some preliminary experiments appeared successful based on CD spectra and AFM counting results, we were not able to reproducibly achieve control over supramolecular chirality. In the future, another method for chiral amplification that could be used is addition of a small amount of a peptoid with a chiral side chain or end group.

In the second section of chapter 6, USAXS was used to support a computational framework for the reversible aggregation of silica nanoparticles in the presence of a homobifunctional linker (sfGFP::Car9). The strong, directionality of sfGFP::Car9 binding to SiNP at short separation distances of particles, coupled with long range interactions in which the contribution of the protein was negligible, were key to explaining the unique reversibility of the system. A rigid body model of particle aggregation was developed and validated by USAXS results in two ways. Interparticle attraction at close distances was highly sensitive to the surface to surface distance between SiNP, and the hypothesized orientation of sfGFP::Car9 needed to be accurate to ensure validity of simulations. Secondly, the strength of the interparticle potential as a result of Car9 at the surface was first approximated using atomistic molecular dynamics, and by accounting for the high curvature of SiNP. However, a range of minimum well potentials non-uniquely predicted the pH reversibility of SiNP aggregation.

By comparing USAXS profiles simulated from rigid-body simulations of SiNP aggregation in different protein:SiNP ratios and at both pH values, we were able to arrive at a more precise value for the minimum well potential.

BIBLIOGRAPHY

- [1] Dmitry I. Yakubovsky, Aleksey V. Arsenin, Yury V. Stebunov, Dmitry Yu. Fedyanin, and Valentyn S. Volkov. Optical constants and structural properties of thin gold films. *Optics Express*, 25(21):25574, oct 2017.
- [2] José G. Hernández-Cifre, Ricardo Rodríguez-Schmidt, Carmen M. Almagro-Gómez, and José García de la Torre. Calculation of the friction, diffusion and sedimentation coefficients of nanoplatelets of arbitrary shape. *Polymer*, 262(October):125467, 2022.
- [3] Vincenzo Amendola, Roberto Pilot, Marco Frasconi, Onofrio M. Maragò, and Maria Antonia Iatì. Surface plasmon resonance in gold nanoparticles: A review. *Journal of Physics Condensed Matter*, 29(20), 2017.
- [4] Maan Mahender Singh and Jitendra Satija. Enzyme-assisted metal nanoparticles etching based plasmonic ELISA: Progress and insights. *Analytical Biochemistry*, 654(March):114820, 2022.
- [5] Zelio Fusco, Kylie Catchpole, and Fiona J. Beck. Investigation of the mechanisms of plasmon-mediated photocatalysis: synergistic contribution of near-field and charge transfer effects. *Journal of Materials Chemistry C*, 10(19):7511–7524, 2022.
- [6] Erik C. Dreaden, Alaaldin M. Alkilany, Xiaohua Huang, Catherine J. Murphy, and Mostafa A. El-Sayed. The golden age: Gold nanoparticles for biomedicine. *Chemical Society Reviews*, 41(7):2740–2779, 2012.
- [7] Wenpei Fan, Peng Huang, and Xiaoyuan Chen. Overcoming the Achilles’ heel of photodynamic therapy. *Chemical Society reviews*, 45(23):6488–6519, 2016.
- [8] Daniel Franklin, Ziqian He, Pamela Mastranzo Ortega, Alireza Safaei, Pablo Cencillo-Abad, Shin Tson Wu, and Debashis Chanda. Self-assembled plasmonics for angle-independent structural color displays with actively addressed black states. *Proceedings of the National Academy of Sciences of the United States of America*, 117(24):13350–13358, 2020.
- [9] Sungi Kim, Jae Myoung Kim, Jeong Eun Park, and Jwa Min Nam. Nonnoble-Metal-Based Plasmonic Nanomaterials: Recent Advances and Future Perspectives. *Advanced Materials*, 30(42):1–24, 2018.

- [10] F. Pelayo García de Arquer, Dmitri V. Talapin, Victor I. Klimov, Yasuhiko Arakawa, Manfred Bayer, and Edward H. Sargent. Semiconductor quantum dots: Technological progress and future challenges. *Science (New York, N.Y.)*, 373(6555), 2021.
- [11] Loredana Protesescu, Sergii Yakunin, Maryna I. Bodnarchuk, Franziska Krieg, Riccardo Caputo, Christopher H. Hendon, Ruo Xi Yang, Aron Walsh, and Maksym V. Kovalenko. Nanocrystals of Cesium Lead Halide Perovskites (CsPbX₃, X = Cl, Br, and I): Novel Optoelectronic Materials Showing Bright Emission with Wide Color Gamut. *Nano Letters*, 15(6):3692–3696, 2015.
- [12] Son Tung Ha, Rui Su, Jun Xing, Qing Zhang, and Qihua Xiong. Metal halide perovskite nanomaterials: synthesis and applications. *Chemical Science*, 8(4):2522–2536, 2017.
- [13] Eli Yablonovitch. Lead halides join the top optoelectronic league. *Science*, 351(6280):1401, 2016.
- [14] Jerry B. Torrance, Philippe Lacorre, Chinnarong Asavaroengchai, and Robert M. Metzger. Why are some oxides metallic, while most are insulating? *Physica C: Superconductivity*, 182(4-6):351–364, nov 1991.
- [15] C. W. Knapp, G. E. Christidis, D. Venieri, I. Gounaki, J. Gibney-Vamvakari, M. Stillings, and E. Photos-Jones. The ecology and bioactivity of some Greco-Roman medicinal minerals: the case of Melos earth pigments. *Archaeological and Anthropological Sciences*, 13(10), 2021.
- [16] Mritunjaya Parashar, Vivek Kumar Shukla, and Ranbir Singh. Metal oxides nanoparticles via sol–gel method: a review on synthesis, characterization and applications. *Journal of Materials Science: Materials in Electronics*, 31(5):3729–3749, 2020.
- [17] T. Maier and H. C. Korting. Sunscreens - Which and what for? *Skin Pharmacology and Physiology*, 18(6):253–262, 2005.
- [18] Pei Jia Lu, Shou Chieh Huang, Yu Pen Chen, Lih Ching Chiueh, and Daniel Yang Chih Shih. Analysis of titanium dioxide and zinc oxide nanoparticles in cosmetics. *Journal of Food and Drug Analysis*, 23(3):587–594, 2015.
- [19] Akira Fujishima and Kenichi Honda. Electrochemical Photolysis of Water at a Semiconductor Electrode. *Nature*, 238(5358):37–38, jul 1972.
- [20] Kazuhiko Maeda. Photocatalytic water splitting using semiconductor particles: History and recent developments. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, 12(4):237–268, 2011.

- [21] Otto W. Flörke, Heribert A. Graetsch, Fred Brunk, Leopold Benda, Siegfried Paschen, Horacio E. Bergna, William O. Roberts, William A. Welsh, Cristian Libanati, Manfred Ettlinger, Dieter Kerner, Monika Maier, Walter Meon, Ralf Schmoll, Hermann Gies, and Dietmar Schiffmann. Silica. In *Ullmann's Encyclopedia of Industrial Chemistry*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, apr 2008.
- [22] Jane F. Bertone, Joel Cizeron, Rajeev K. Wahi, Joan K. Bosworth, and Vicki L. Colvin. Hydrothermal Synthesis of Quartz Nanocrystals. *Nano Letters*, 3(5):655–659, may 2003.
- [23] Sukalyan Dash, Soumyashri Mishra, Sabita Patel, and Bijay K. Mishra. Organically modified silica: Synthesis and applications due to its surface interaction with organic molecules. *Advances in Colloid and Interface Science*, 140(2):77–94, 2008.
- [24] Matthew R. Jones, Kyle D. Osberg, Robert J. Macfarlane, Mark R. Langille, and Chad A. Mirkin. Templated Techniques for the Synthesis and Assembly of Plasmonic Nanostructures. *Chemical Reviews*, 111(6):3736–3827, jun 2011.
- [25] Nguyen T. K. Thanh, N. Maclean, and S. Mahiddine. Mechanisms of Nucleation and Growth of Nanoparticles in Solution. *Chemical Reviews*, 114(15):7610–7630, aug 2014.
- [26] Xuelian Chen, Jan Schröder, Stephan Hauschild, Sabine Rosenfeldt, Martin Dulle, and Stephan Förster. Simultaneous SAXS/WAXS/UV-Vis Study of the Nucleation and Growth of Nanoparticles: A Test of Classical Nucleation Theory. *Langmuir*, 31(42):11678–11691, 2015.
- [27] Xuan Liu, Xiaoyi Zhang, Dmitry Khakhulin, Peiyuan Su, Michael Wulff, Francois Baudalet, Tsu-chien Weng, Qingyu Kong, and Yugang Sun. Deciphering Photochemical Reaction Pathways of Aqueous Tetrachloroauric Acid by X-ray Transient Absorption Spectroscopy. *The Journal of Physical Chemistry Letters*, 13(38):8921–8927, sep 2022.
- [28] Yoshiteru Mizukoshi, Ryuichiro Oshima, Yasuaki Maeda, and Yoshio Nagata. Preparation of platinum nanoparticles by sonochemical reduction of the Pt(II) ion. *Langmuir*, 15(8):2733–2737, 1999.
- [29] Yunhu Gao and Laura Torrente-Murciano. Mechanistic insights of the reduction of gold salts in the Turkevich protocol. *Nanoscale*, 12(4):2740–2751, 2020.
- [30] John Turkevich, Peter Cooper Stevenson, and James Hillier. A study of the nucleation and growth processes in the synthesis of colloidal gold, 1951.

- [31] Victor K. LaMer and Robert H. Dinegar. Theory, Production and Mechanism of Formation of Monodispersed Hydrosols. *Journal of the American Chemical Society*, 72(11):4847–4854, nov 1950.
- [32] Wuihs, Maria Chick, Alexander Birnbaum, Steffen Witte, Michael Sztucki, Ulla Vainio, Nicola Pinna, Klaus Rademann, Franziska Emmerling, Ralph Kraehnert, and Jörg Polte. Turkevich in New Robes: Key Questions Answered for the Most Common Gold Nanoparticle Synthesis. *ACS Nano*, 9(7):7052–7071, 2015.
- [33] Jörg Polte, T. Torsten Ahner, Friedmar Delissen, Sergey Sokolov, Franziska Emmerling, Andreas F. Thünemann, and Ralph Kraehnert. Mechanism of gold nanoparticle formation in the classical citrate synthesis method derived from coupled in situ XANES and SAXS evaluation. *Journal of the American Chemical Society*, 132(4):1296–1301, 2010.
- [34] Jörg Polte. Fundamental growth principles of colloidal metal nanoparticles - a new perspective. *CrystEngComm*, 17(36):6809–6830, 2015.
- [35] Nikhil R. Jana, Latha Gearheart, and Catherine J. Murphy. Wet chemical synthesis of high aspect ratio cylindrical gold nanorods. *Journal of Physical Chemistry B*, 105(19):4065–4067, 2001.
- [36] Jonas Hühn, Carolina Carrillo-Carrion, Mahmoud G. Soliman, Christian Pfeiffer, Daniel Valdeperez, Atif Masood, Indranath Chakraborty, Lin Zhu, Marta Gallego, Zhao Yue, Monica Carril, Neus Feliu, Alberto Escudero, Alaaldin M. Alkilany, Beatriz Pelaz, Pablo del Pino, and Wolfgang J. Parak. Selected Standard Protocols for the Synthesis, Phase Transfer, and Characterization of Inorganic Colloidal Nanoparticles. *Chemistry of Materials*, 29(1):399–461, jan 2017.
- [37] Christian Kuttner, Martin Mayer, Martin Dulle, Ana Moscoso, Juan Manuel López-Romero, Stephan Förster, Andreas Fery, Jorge Pérez-Juste, and Rafael Contreras-Cáceres. Seeded Growth Synthesis of Gold Nanotriangles: Size Control, SAXS Analysis, and SERS Performance. *ACS Applied Materials and Interfaces*, 10(13):11152–11163, 2018.
- [38] Leonardo Scarabelli, Ana Sánchez-Iglesias, Jorge Pérez-Juste, and Luis M. Liz-Marzán. A "Tips and Tricks" Practical Guide to the Synthesis of Gold Nanorods. *Journal of Physical Chemistry Letters*, 6(21):4270–4279, 2015.
- [39] Neyvis Almora-Barrios, Gerard Novell-Leruth, Peter Whiting, Luis M. Liz-Marzán, and Núria López. Theoretical description of the role of halides, silver, and surfactants on the structure of gold nanorods. *Nano Letters*, 14(2):871–875, 2014.

- [40] Younan Xia, Xiaohu Xia, and Hsin Chieh Peng. Shape-Controlled Synthesis of Colloidal Metal Nanocrystals: Thermodynamic versus Kinetic Products. *Journal of the American Chemical Society*, 137(25):7947–7966, 2015.
- [41] Soon Gu Kwon and Taeghwan Hyeon. Formation mechanisms of uniform nanocrystals via hot-injection and heat-up methods. *Small*, 7(19):2685–2702, 2011.
- [42] Ou Chen, Jing Zhao, Vikash P. Chauhan, Jian Cui, Cliff Wong, Daniel K. Harris, He Wei, Hee Sun Han, Dai Fukumura, Rakesh K. Jain, and Mounsi G. Bawendi. Compact high-quality CdSe-CdS core-shell nanocrystals with narrow emission linewidths and suppressed blinking. *Nature Materials*, 12(5):445–451, 2013.
- [43] C. B. Murray, D. J. Norris, and M. G. Bawendi. Synthesis and Characterization of Nearly Monodisperse CdE (E = S, Se, Te) Semiconductor Nanocrystallites. *Journal of the American Chemical Society*, 115(19):8706–8715, 1993.
- [44] Xiaogang Peng, J. Wickham, and A. P. Alivisatos. Kinetics of II-VI and III-V Colloidal Semiconductor Nanocrystal Growth: “Focusing” of Size Distributions. *Journal of the American Chemical Society*, 120(21):5343–5344, jun 1998.
- [45] Kameel Abdel-Latif, Fazel Bateni, Steven Crouse, and Milad Abolhasani. Flow Synthesis of Metal Halide Perovskite Quantum Dots: From Rapid Parameter Space Mapping to AI-Guided Modular Manufacturing. *Matter*, 3(4):1053–1086, 2020.
- [46] Nicolo Castro, Cécile Bouet, Sandrine Ithurria, Nicolas Lequeux, Doru Constantin, Pierre Levitz, Diego Pontoni, and Benjamin Abécassis. Insights into the Formation Mechanism of CdSe Nanoplatelets Using in Situ X-ray Scattering. *Nano Letters*, 19(9):6466–6474, 2019.
- [47] Miron V. Landau. Sol-Gel Process. In *Handbook of Heterogeneous Catalysis*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, jan 2008.
- [48] Werner Stöber, Arthur Fink, and Ernst Bohn. Controlled growth of monodisperse silica spheres in the micron size range. *Journal of Colloid and Interface Science*, 26(1):62–69, jan 1968.
- [49] Gareth J. Owens, Rajendra K. Singh, Farzad Foroutan, Mustafa Alqaysi, Cheol Min Han, Chinmaya Mahapatra, Hae Won Kim, and Jonathan C. Knowles. Sol-gel based materials for biomedical applications. *Progress in Materials Science*, 77:1–79, 2016.
- [50] J.N. Hasnidawani, H.N. Azlina, H. Norita, N.N. Bonnia, S. Ratim, and E.S. Ali. Synthesis of ZnO Nanostructures Using Sol-Gel Method. *Procedia Chemistry*, 19:211–216, 2016.

- [51] Chen Chi Wang and Jackie Y. Ying. Sol-gel synthesis and hydrothermal processing of anatase and rutile titania nanocrystals. *Chemistry of Materials*, 11(11):3113–3120, 1999.
- [52] Yandong Han, Ziyang Lu, Zhaogang Teng, Jinglun Liang, Zilong Guo, Dayang Wang, Ming Yong Han, and Wensheng Yang. Unraveling the growth mechanism of silica particles in the stöber method: In situ seeded growth model. *Langmuir*, 33(23):5879–5890, 2017.
- [53] Matthew B. Dickerson, Kenneth H. Sandhage, and Rajesh R. Naik. Protein- and peptide-directed syntheses of inorganic materials. *Chemical Reviews*, 108(11):4935–4978, 2008.
- [54] Honghua He, Erik J. Veneklaas, John Kuo, and Hans Lambers. Physiological and ecological significance of biomineralization in plants. *Trends in Plant Science*, 19(3):166–174, 2014.
- [55] Fabio Nudelman and Nico A.J.M. Sommerdijk. Biomineralization as an inspiration for materials chemistry. *Angewandte Chemie - International Edition*, 51(27):6582–6596, 2012.
- [56] Fiona C. Meldrum and Helmut Cölfen. Controlling mineral morphologies and structures in biological and synthetic systems. *Chemical Reviews*, 108(11):4332–4432, 2008.
- [57] Lukmaan A. Bawazer, Ciara S. McNally, Christopher J. Empson, William J. Marchant, Tim P. Comyn, Xize Niu, Soongwon Cho, Michael J. Mcpherson, Bernard P. Binks, Andrew Demello, and Fiona C. Meldrum. Combinatorial microfluidic droplet engineering for biomimetic material synthesis. *Science Advances*, 2(10), 2016.
- [58] Jos J. M. Lenders, Lukmaan A. Bawazer, David C. Green, Harshal R. Zope, Paul H. H. Bomans, Gijsbertus de With, Alexander Kros, Fiona C. Meldrum, and Nico A. J. M. Sommerdijk. Combinatorial Evolution of Biomimetic Magnetite Nanoparticles. *Advanced Functional Materials*, 27(10):1604863, mar 2017.
- [59] David A C Beck, James M Carothers, Venkat R Subramanian, and Jim Pfaendtner. Data science: Accelerating innovation and discovery in chemical engineering. *AIChE Journal*, 62(5):1402–1416, may 2016.
- [60] Bin Cai, Zhiliang Li, and Chun Long Chen. Programming Amphiphilic Peptoid Oligomers for Hierarchical Assembly and Inorganic Crystallization. *Accounts of Chemical Research*, 54(1):81–91, 2021.

- [61] Janak Prasad, Sébastien Viollet, Kargal L. Gurunatha, Agathe Urvoas, Agathe C. Fournier, Marie Valerio-Lepiniec, Cécile Marcelot, Bulent Baris, Philippe Minard, and Erik Dujardin. Directed evolution of artificial repeat proteins as habit modifiers for the morphosynthesis of (111)-terminated gold nanocrystals. *Nanoscale*, 11(37):17485–17497, 2019.
- [62] Li Shao, Jinrong Ma, Jesse L. Prelesnik, Yicheng Zhou, Mary Nguyen, Mingfei Zhao, Samson A. Jenekhe, Sergei V. Kalinin, Andrew L. Ferguson, Jim Pfaendtner, Christopher J. Mundy, James J. De Yoreo, François Baneyx, and Chun Long Chen. Hierarchical Materials from High Information Content Macromolecular Building Blocks: Construction, Dynamic Interventions, and Prediction. *Chemical Reviews*, 2022.
- [63] Xin Qi, Yundi Zhao, Kacper Lachowski, Julia Boese, Yifeng Cai, Orion Dollar, Brittny Hellner, Lilo Pozzo, Jim Pfaendtner, Jaehun Chun, François Baneyx, and Christopher J. Mundy. Predictive Theoretical Framework for Dynamic Control of Bioinspired Hybrid Nanoparticle Self-Assembly. *ACS Nano*, 16(2):1919–1928, 2022.
- [64] Feng Yan, Lili Liu, Tiffany R. Walsh, Yu Gong, Patrick Z. El-Khoury, Yanyan Zhang, Zihua Zhu, James J. De Yoreo, Mark H. Engelhard, Xin Zhang, and Chun Long Chen. Controlled synthesis of highly-branched plasmonic gold nanoparticles through peptoid engineering. *Nature Communications*, 9(1):1–8, 2018.
- [65] Alexander Thomas, James F. Mattheaei, and François Baneyx. A Self-Assembling Two-Dimensional Protein Array is a Versatile Platform for the Assembly of Multicomponent Nanostructures. *Biotechnology Journal*, 13(12):1–7, 2018.
- [66] Matthew Carter Childers and Valerie Daggett. Insights from molecular dynamics simulations for computational protein design. *Molecular Systems Design and Engineering*, 2(1):9–33, 2017.
- [67] Beau R. Pelle, Eric M. Krauland, K. Dane Wittrup, and Angela M. Belcher. Design Criteria for Engineering Inorganic Material- Specific Peptides. *Langmuir*, 21(15):6929–6933, jul 2005.
- [68] Yen Nee Tan, Jim Yang Lee, and Daniel I.C. Wang. Uncovering the design rules for peptide synthesis of metal nanoparticles. *Journal of the American Chemical Society*, 132(16):5677–5686, 2010.
- [69] Soumitra Mokashi-Punekar, Tiffany R. Walsh, and Nathaniel L. Rosi. Tuning the Structure and Chiroptical Properties of Gold Nanoparticle Single Helices via Peptide Sequence Variation. *Journal of the American Chemical Society*, 141(39):15710–15716, 2019.

- [70] Jacob N. Israelachvili. *Intermolecular and Surface Forces*. Elsevier, 2011.
- [71] Andrew Nelson and Lawrence H Friedman. Thermodynamically Stable Colloidal Solids: Interfacial Thermodynamics from the Particle Size Distribution. *The Journal of Physical Chemistry C*, 126(4):2161–2178, feb 2022.
- [72] John C. Berg. *An Introduction to Interfaces and Colloids*. WORLD SCIENTIFIC, Seattle, nov 2009.
- [73] Wenjing Xi, Hoa T. Phan, and Amanda J. Haes. How to accurately predict solution-phase gold nanostar stability. *Analytical and Bioanalytical Chemistry*, 410(24):6113–6123, 2018.
- [74] Ernest M. Hotze, Tanapon Phenrat, and Gregory V. Lowry. Nanoparticle Aggregation: Challenges to Understanding Transport and Reactivity in the Environment. *Journal of Environmental Quality*, 39(6):1909–1924, 2010.
- [75] K. Jiang and P. Pinchuk. Temperature and size-dependent Hamaker constants for metal nanoparticles. *Nanotechnology*, 27(34):1–9, 2016.
- [76] Young Shin Jun, Yaguang Zhu, Ying Wang, Deoukchen Ghim, Xuanhao Wu, Doyoon Kim, and Haesung Jung. Classical and Nonclassical Nucleation and Growth Mechanisms for Nanoparticle Formation. *Annual Review of Physical Chemistry*, 73:453–477, 2022.
- [77] James J. De Yoreo, Pupa U.P.A. Gilbert, Nico A.J.M. Sommerdijk, R. Lee Penn, Stephen Whitlam, Derk Joester, Hengzhong Zhang, Jeffrey D. Rimer, Alexandra Navrotsky, Jillian F. Banfield, Adam F. Wallace, F. Marc Michel, Fiona C. Meldrum, Helmut Cölfen, and Patricia M. Dove. Crystallization by particle attachment in synthetic, biogenic, and geologic environments. *Science*, 349(6247), 2015.
- [78] Jong K. Lee, Farid F. Abraham, and G.M. Pound. On the validity of the capillarity approximation in the rate theory of homogeneous nucleation. *Surface Science*, 34(3):745–758, feb 1973.
- [79] C. N. R. Rao, A Müller, and A. K. Cheetham, editors. *Nanomaterials Chemistry*. Wiley, jun 2007.
- [80] Dmitri V. Talapin, Andrey L. Rogach, Markus Haase, and Horst Weller. Evolution of an ensemble of nanoparticles in a colloidal solution: Theoretical study. *Journal of Physical Chemistry B*, 105(49):12278–12285, 2001.

- [81] Yingwen Cheng, Jinhui Tao, Guomin Zhu, Jennifer A. Soltis, Benjamin A. Legg, Elias Nakouzi, James J. De Yoreo, Maria L. Sushko, and Jun Liu. Near surface nucleation and particle mediated growth of colloidal Au nanocrystals. *Nanoscale*, 10(25):11907–11912, 2018.
- [82] Chao Zhu, Suxia Liang, Erhong Song, Yuanjun Zhou, Wen Wang, Feng Shan, Yantao Shi, Ce Hao, Kuibo Yin, Tong Zhang, Jianjun Liu, Haimei Zheng, and Litao Sun. In-situ liquid cell transmission electron microscopy investigation on oriented attachment of gold nanoparticles. *Nature Communications*, 9(1):421, jan 2018.
- [83] Biao Jin, Yanming Wang, Chuanhong Jin, James J. De Yoreo, and Ruikang Tang. Revealing Au₁₃as Elementary Clusters during the Early Formation of Au Nanocrystals. *Journal of Physical Chemistry Letters*, 12:5938–5943, 2021.
- [84] Brook R. Danger, Donna Fan, J. P. Vivek, and Ian J. Burgess. Electrochemical studies of capping agent adsorption provide insight into the formation of anisotropic gold nanocrystals. *ACS Nano*, 6(12):11018–11026, 2012.
- [85] Chin-yi Chiu, Hao Wu, Zhaoying Yao, Fei Zhou, Hua Zhang, Vidvuds Ozolins, and Yu Huang. Facet-Selective Adsorption on Noble Metal Crystals Guided by Electrostatic Potential Surfaces of Aromatic Molecules. *Journal of the American Chemical Society*, 135(41):15489–15500, oct 2013.
- [86] Chi Xiao, Bang An Lu, Peng Xue, Na Tian, Zhi You Zhou, Xiao Lin, Wen Feng Lin, and Shi Gang Sun. High-Index-Facet- and High-Surface-Energy Nanocrystals of Metals and Metal Oxides as Highly Efficient Catalysts. *Joule*, 4(12):2562–2598, 2020.
- [87] Kathryn M. Mayer and Jason H. Hafner. Localized surface plasmon resonance sensors. *Chemical Reviews*, 111(6):3828–3857, 2011.
- [88] Helmuth Horvath. Gustav Mie and the scattering and absorption of light by particles: Historic developments and basics. *Journal of Quantitative Spectroscopy and Radiative Transfer*, 110(11):787–799, 2009.
- [89] Craig F. Bohren and Donald R. Huffman. *Absorption and Scattering of Light by Small Particles*. Wiley, apr 1998.
- [90] K. Lance Kelly, Eduardo Coronado, Lin Lin Zhao, and George C. Schatz. The optical properties of metal nanoparticles: The influence of size, shape, and dielectric environment. *Journal of Physical Chemistry B*, 107(3):668–677, 2003.

- [91] Sean M. Meyer, Jacob Pettine, David J. Nesbitt, and Catherine J. Murphy. Size Effects in Gold Nanorod Light-to-Heat Conversion under Femtosecond Illumination. *Journal of Physical Chemistry C*, 125(29):16268–16278, 2021.
- [92] Christoph Langhammer, Bengt Kasemo, and Igor Zorić. Absorption and scattering of light by Pt, Pd, Ag, and Au nanodisks: Absolute cross sections and branching ratios. *Journal of Chemical Physics*, 126(19), 2007.
- [93] Kevin N. Dyer, Michal Hammel, Robert P. Rambo, Susan E. Tsutakawa, Ivan Rodic, Scott Classen, John A. Tainer, and Greg L. Hura. High-Throughput SAXS for the Characterization of Biomolecules in Solution: A Practical Approach. volume 1091 of *Methods in Molecular Biology*, pages 245–258. Humana Press, Totowa, NJ, 2014.
- [94] Dominic W. Hayward, Leonardo Chiappisi, Sylvain Prévost, Ralf Schweins, and Michael Gradzielski. A small-angle neutron scattering environment for in-situ observation of chemical processes. *Scientific Reports*, 8(1):1–11, 2018.
- [95] David S. Li, Yi Ting Lee, Yuyin Xi, Ivan Pelivanov, Matthew O'Donnell, and Lilo D. Pozzo. A small-angle scattering environment for: In situ ultrasound studies. *Soft Matter*, 14(25):5283–5293, 2018.
- [96] Erik W. Martin, Tyler S. Harmon, Jesse B. Hopkins, Srinivas Chakravarthy, J. Jeremías Incicco, Peter Schuck, Andrea Soranno, and Tanja Mittag. A multi-step nucleation process determines the kinetics of prion-like domain phase separation. *Nature Communications*, 12(1), 2021.
- [97] Andreas Haahr Larsen, Yong Wang, Sandro Bottaro, Sergei Grudinin, Lise Arleth, and Kresten Lindorff-Larsen. Combining molecular dynamics simulations with small-angle X-ray and neutron scattering data to study multi-domain proteins in solution. *PLOS Computational Biology*, 16(4):e1007870, apr 2020.
- [98] Andre Guinier and Gerard Fournet. *SMALL-ANGLE SCATTERING OF X-RAYS*. John Wiley & Sons, Inc., mar 1955.
- [99] Tao Li, Andrew J. Senesi, and Byeongdu Lee. Small Angle X-ray Scattering for Nanoparticle Research. *Chemical Reviews*, 116(18):11128–11180, 2016.
- [100] Boualem Hammouda. A new Guinier-Porod model. *Journal of Applied Crystallography*, 43(4):716–719, 2010.
- [101] Jan Ilavsky, Fan Zhang, Ross N. Andrews, Ivan Kuzmenko, Pete R. Jemian, Lyle E. Levine, and Andrew J. Allen. Development of combined microstructure and structure

- characterization facility for in situ and operando studies at the advanced photon source. *Journal of Applied Crystallography*, 51:867–882, 2018.
- [102] U. Bonse and M. Hart. Tailless X-Ray Single-Crystal Reflection Curves Obtained By Multiple Reflection. *Applied Physics Letters*, 7(9):238–240, nov 1965.
 - [103] Thomas Vad and Wiebke F.C. Sager. Comparison of iterative desmearing procedures for one-dimensional small-angle scattering data. *Journal of Applied Crystallography*, 44(1):32–42, 2011.
 - [104] J. A. Lake. An iterative method of slit-correcting small angle X-ray data. *Acta Crystallographica*, 23(2):191–194, 1967.
 - [105] Uriel N. Morzan, Gonzalo Díaz Mirón, Luca Grisanti, Mariano C. González Lebrero, Gabriele S. Kaminski Schierle, and Ali Hassanali. Non-Aromatic Fluorescence in Biological Matter: The Exception or the Rule? *Journal of Physical Chemistry B*, 126(38):7203–7211, 2022.
 - [106] K. Kalyanasundaram and J. K. Thomas. Environmental Effects on Vibronic Band Intensities in Pyrene Monomer Fluorescence and Their Application in Studies of Micellar Systems. *Journal of the American Chemical Society*, 99(7):2039–2044, 1977.
 - [107] Ingolf Warnke and Filipp Furche. Circular dichroism: Electronic. *Wiley Interdisciplinary Reviews: Computational Molecular Science*, 2(1):150–166, 2012.
 - [108] Minghua Liu, Li Zhang, and Tianyu Wang. Supramolecular chirality in self-Assembled systems. *Chemical Reviews*, 115(15):7304–7397, 2015.
 - [109] Goerg H. Michler, R. Godehardt, and V. Seydewitz. Electron Microscopy of Polymers. In *Electron Microscopy of Polymers*, chapter 2,4, pages 15–51, 87–120. Springer Berlin Heidelberg, Berlin, Heidelberg, 2008.
 - [110] Frances M. Ross, editor. *Liquid Cell Electron Microscopy*. Cambridge University Press, nov 2016.
 - [111] Qiubo Zhang, Xinxing Peng, Yifan Nie, Qi Zheng, Junyi Shangguan, Chao Zhu, Karen C. Bustillo, Peter Ercius, Linwang Wang, David T. Limmer, and Haimei Zheng. Defect-mediated ripening of core-shell nanostructures. *Nature Communications*, 13(1):1–10, 2022.
 - [112] Pablo Castro-Hartmann, Gerard Heck, Jose M. Eltit, Paul Fawcett, and Montserrat Samsó. The ArrayGrid: A methodology for applying multiple samples to a single TEM specimen grid. *Ultramicroscopy*, 135:105–112, 2013.

- [113] Martin Schorb, Isabella Haberbosch, Wim J.H. Hagen, Yannick Schwab, and David N. Mastronarde. Software tools for automated transmission electron microscopy. *Nature Methods*, 16(6):471–477, 2019.
- [114] Lehan Yao, Zihao Ou, Binbin Luo, Cong Xu, and Qian Chen. Machine Learning to Reveal Nanoparticle Dynamics from Liquid-Phase TEM Videos. *ACS Central Science*, 6(8):1421–1430, 2020.
- [115] Marc L. Mansfield, Jack F. Douglas, Saba Irfan, and Eun Hee Kang. Comparison of approximate methods for calculating the friction coefficient and intrinsic viscosity of nanoparticles and macromolecules. *Macromolecules*, 40(7):2575–2589, 2007.
- [116] Kacper J. Lachowski, Kiran Vaddi, Nada Y. Naser, François Baneyx, and Lilo D. Pozzo. Multivariate analysis of peptide-driven nucleation and growth of Au nanoparticles. *Digital Discovery*, 1(4):427–439, 2022.
- [117] Nicholas M. Bedford, Zak E. Hughes, Zhenghua Tang, Yue Li, Beverly D. Briggs, Yang Ren, Mark T. Swihart, Valeri G. Petkov, Rajesh R. Naik, Marc R. Knecht, and Tiffany R. Walsh. Sequence-Dependent Structure/Function Relationships of Catalytic Peptide-Enabled Gold Nanoparticles Generated under Ambient Synthetic Conditions. *Journal of the American Chemical Society*, 138(2):540–548, 2016.
- [118] Zhiliang Li, Bin Cai, Wenchao Yang, and Chun Long Chen. Hierarchical Nanomaterials Assembled from Peptoids and Other Sequence-Defined Synthetic Polymers. *Chemical Reviews*, 121(22):14031–14087, 2021.
- [119] Michelle A. Nguyen, Zak E. Hughes, Yang Liu, Yue Li, Mark T. Swihart, Marc R. Knecht, and Tiffany R. Walsh. Peptide-Mediated Growth and Dispersion of Au Nanoparticles in Water via Sequence Engineering. *Journal of Physical Chemistry C*, 122(21):11532–11542, 2018.
- [120] Catherine J. Munro and Marc R. Knecht. Solution Effects on Peptide-Mediated Reduction and Stabilization of Au Nanoparticles. *Langmuir*, 33(48):13757–13765, 2017.
- [121] Yue Li, Zhenghua Tang, Paras N. Prasad, Marc R. Knecht, and Mark T. Swihart. Peptide-mediated synthesis of gold nanoparticles: effects of peptide sequence and nature of binding on physicochemical properties. *Nanoscale*, 6(6):3165–3172, 2014.
- [122] Chen Zhang, Chengyi Song, H. Christopher Fry, and Nathaniel L. Rosi. Peptide conjugates for directing the morphology and assembly of 1D nanoparticle superstructures. *Chemistry - A European Journal*, 20(4):941–945, 2014.

- [123] Soumitra Mokashi-Punekar, Sydney C. Brooks, Camera D. Hogan, and Nathaniel L. Rosi. Leveraging Peptide Sequence Modification to Promote Assembly of Chiral Helical Gold Nanoparticle Superstructures. *Biochemistry*, 60(13):1044–1049, 2021.
- [124] Chun Long Chen, Peijun Zhang, and Nathaniel L. Rosi. A new peptide-based method for the design and synthesis of nanoparticle superstructures: Construction of highly ordered gold nanoparticle double helices. *Journal of the American Chemical Society*, 130(41):13555–13557, 2008.
- [125] Andrea D. Merg, Joseph Slocik, Martin G. Blaber, George C. Schatz, Rajesh Naik, and Nathaniel L. Rosi. Adjusting the Metrics of 1-D Helical Gold Nanoparticle Superstructures Using Multivalent Peptide Conjugates. *Langmuir*, 31(34):9492–9501, 2015.
- [126] Chengyi Song, Gongpu Zhao, Peijun Zhang, and Nathaniel L. Rosi. Expeditious synthesis and assembly of sub-100 nm hollow spherical gold nanoparticle superstructures. *Journal of the American Chemical Society*, 132(40):14033–14035, 2010.
- [127] Huachen Tao, Tianyi Wu, Matteo Aldeghi, Tony C. Wu, Alán Aspuru-Guzik, and Eugenia Kumacheva. Nanoparticle synthesis assisted by machine learning. *Nature Reviews Materials*, 0123456789:in press, 2021.
- [128] Jakob C. Dahl, Xingzhi Wang, Xiao Huang, Emory M. Chan, and A. Paul Alivisatos. Elucidating the Weakly Reversible Cs-Pb-Br Perovskite Nanocrystal Reaction Network with High-Throughput Maps and Transformations. *Journal of the American Chemical Society*, 142(27):11915–11926, 2020.
- [129] Kacper J. Lachowski, Kiran Vaddi, Nada Y. Naser, François Baneyx, and Lilo D. Pozzo. Gold Nanoparticles Synthesized in the Presence of Peptides - UV-Vis Spectra, Fluorescence, USAXS, Electron Microscopy, February 2022.
- [130] Lilo Pozzo, Kacper Lachowski, Sage Scheiwiller, and Edwin Antonio. pozzo-research-group/Automation-Hardware: v0.1.0 - Initial release for documenting liquid sample holder on Zenodo, January 2022.
- [131] Jan Ilavsky and Peter R. Jemian. *Irena*: tool suite for modeling and analysis of small-angle scattering. *Journal of Applied Crystallography*, 42(2):347–353, Apr 2009.
- [132] Maxim V. Petoukhov, Peter V. Konarev, Alexey G. Kikhney, and Dmitri I. Svergun. *ATSAS 2.1* – towards automated and web-supported small-angle scattering data analysis. *Journal of Applied Crystallography*, 40(s1):s223–s228, Apr 2007.

- [133] Rajesh R. Naik, Sarah J. Stringer, Gunjan Agarwal, Sharon E. Jones, and Morley O. Stone. Biomimetic synthesis and patterning of silver nanoparticles. *Nature Materials*, 1(3):169–172, nov 2002.
- [134] Thomas Hendel, Maria Wuthschick, Frieder Kettemann, Alexander Birnbaum, Klaus Rademann, and Jörg Polte. In Situ Determination of Colloidal Gold Concentrations with UV–Vis Spectroscopy: Limitations and Perspectives. *Analytical Chemistry*, 86(22):11115–11124, nov 2014.
- [135] Ahsan Habib, Masaaki Tabata, and Ying Guang Wu. Formation of gold nanoparticles by Good’s Buffers. *Bulletin of the Chemical Society of Japan*, 78(2):262–269, 2005.
- [136] Kangze Liu, Zhonglei He, James F. Curtin, Hugh J. Byrne, and Furong Tian. A novel, rapid, seedless, in situ synthesis method of shape and size controllable gold nanoparticles using phosphates. *Scientific Reports*, 9(1):1–13, 2019.
- [137] Jianping Xie, Jim Yang Lee, and Daniel I.C. Wang. Seedless, surfactantless, high-yield synthesis of branched gold nanocrystals in HEPES buffer solution. *Chemistry of Materials*, 19(11):2823–2830, 2007.
- [138] Wenjing Xi and Amanda J. Haes. Elucidation of HEPES Affinity to and Structure on Gold Nanostars. *Journal of the American Chemical Society*, 141(9):4034–4042, 2019.
- [139] Po Jung Jimmy Huang, Jeffy Yang, Kellie Chong, Qianyi Ma, Miao Li, Fang Zhang, Woohyun J. Moon, Guomei Zhang, and Juewen Liu. Good’s buffers have various affinities to gold nanoparticles regulating fluorescent and colorimetric DNA sensing. *Chemical Science*, 11(26):6795–6804, 2020.
- [140] Zhenghua Tang, J. Pablo Palafox-Hernandez, Wing Cheung Law, Zak E. Hughes, Mark T. Swihart, Paras N. Prasad, Marc R. Knecht, and Tiffany R. Walsh. Biomolecular recognition principles for bionanocombinatorics: An integrated approach to elucidate enthalpic and entropic factors. *ACS Nano*, 7(11):9632–9646, 2013.
- [141] Jiao Cao, Wendong Jia, Jinyue Zhang, Xiumei Xu, Shuanghong Yan, Yuqin Wang, Panke Zhang, Hong Yuan Chen, and Shuo Huang. Giant single molecule chemistry events observed from a tetrachloroaurate(III) embedded Mycobacterium smegmatis porin A nanopore. *Nature Communications*, 10(1), 2019.
- [142] R. Grantham. Amino Acid Difference Formula to Help Explain Protein Evolution. *Science*, 185(4154):862–864, sep 1974.

- [143] Benjamin Ricaud, Pierre Borgnat, Nicolas Tremblay, Paulo Gonçalves, and Pierre Vandergheynst. Fourier could be a data scientist: From graph fourier transform to signal processing on graphs. *Comptes Rendus Physique*, 20(5):474–488, 2019.
- [144] Aliaksei Sandryhaila and José MF Moura. Discrete signal processing on graphs: Graph fourier transform. In *2013 IEEE International Conference on Acoustics, Speech and Signal Processing*, pages 6167–6170. IEEE, 2013.
- [145] Michaël Defferrard, Lionel Martin, Rodrigo Pena, and Nathanaël Perraudin. Pygsp: Graph signal processing in python.
- [146] JO Ramsay and BW Silvermann. Functional data analysis. springer series in statistics, 1998.
- [147] Anuj Srivastava and Eric P Klassen. *Functional and shape data analysis*, volume 1. Springer, 2016.
- [148] Joseph B Kruskal. Multidimensional scaling by optimizing goodness of fit to a non-metric hypothesis. *Psychometrika*, 29(1):1–27, 1964.
- [149] Jerome Friedman, Trevor Hastie, and Robert Tibshirani. *The elements of statistical learning*, volume 1. Springer series in statistics New York, 2001.
- [150] Anthony Y. Fong, Lenson Pellouchoud, Malcolm Davidson, Richard C. Walroth, Carena Church, Ekaterina Tcareva, Liheng Wu, Kyle Peterson, Bryce Meredig, and Christopher J. Tassone. Utilization of machine learning to accelerate colloidal synthesis and discovery. *Journal of Chemical Physics*, 154(22), 2021.
- [151] M. Hussain Sangji, Hiroaki Sai, Stacey M. Chin, Sieun Ruth Lee, Ivan R Sasselli, Liam C. Palmer, and Samuel I. Stupp. Supramolecular Interactions and Morphology of Self-Assembling Peptide Amphiphile Nanostructures. *Nano Letters*, 21(14):6146–6155, 2021.
- [152] Sunjie Ye, Andy P. Brown, Ashley C. Stammers, Neil H. Thomson, Jin Wen, Lucien Roach, Richard J. Bushby, Patricia Louise Coletta, Kevin Critchley, Simon D. Connell, Alexander F. Markham, Rik Brydson, and Stephen D. Evans. Sub-Nanometer Thick Gold Nanosheets as Highly Efficient Catalysts. *Advanced Science*, 6(21), 2019.
- [153] Kiran Vaddi, Huat Thart Chiang, and Lilo D Pozzo. Autonomous retrosynthesis of nanoscale structures via spectral shape matching. 2022.

- [154] Judea Pearl. Causal inference in statistics: An overview. *Statistics surveys*, 3:96–146, 2009.
- [155] Karen Sachs, Omar Perez, Dana Pe’er, Douglas A Lauffenburger, and Garry P Nolan. Causal protein-signaling networks derived from multiparameter single-cell data. *Science*, 308(5721):523–529, 2005.
- [156] Zijian He, Hongmei Liu, Yiran Tian, Yong Jiang, Yixiang Xu, Pengjin Zhan, Mengqi Wang, Songfan Wang, Chuan Xia, Chenjie Gu, Ziqi Zhao, Tao Jiang, and Jun Zhou. Hollow Ag dendritic nanoplates with serrated inner surfaces for sensitive SERS-based detection. *Materials Research Express*, 6(10), 2019.
- [157] Matthew N. O’Brien, Matthew R. Jones, Kevin L. Kohlstedt, George C. Schatz, and Chad A. Mirkin. Uniform circular disks with synthetically tailorable diameters: Two-dimensional nanoparticles for plasmonics. *Nano Letters*, 15(2):1012–1017, 2015.
- [158] Bowen He, Yuntao Wang, Mengmeng Zhang, Yue Xu, Yonglong Zheng, Xi Liu, Hong Wang, and Hongyu Chen. Serrated Au Nanoplates via the Sharpening Etching Mode. *Chemistry of Materials*, 34(18):8213–8218, 2022.
- [159] Alberto Gobbo, Riccardo Marin, and Patrizia Canton. Seeded growth of gold nanorods: the effect of sulfur-containing quenching agents. *Journal of Nanoparticle Research*, 20(3):1–11, 2018.
- [160] Daniel A. Zweifel and Alexander Wei. Sulfide-arrested growth of gold nanorods. *Chemistry of Materials*, 17(16):4256–4261, 2005.
- [161] Xuan Liu, Dan Wu, Qing Chang, Jing Zhou, Yongyi Zhang, and Zhaona Wang. Grooved nanoplate assembly for rapid detection of surface enhanced Raman scattering. *Nanoscale*, 9(40):15390–15396, 2017.
- [162] Kamallesh Nehra, Senthil Kumar Pandian, Moram Sree Satya Bharati, and Venugopal Rao Soma. Enhanced catalytic and SERS performance of shape/size controlled anisotropic gold nanostructures. *New Journal of Chemistry*, 43(9):3835–3847, 2019.
- [163] Hayk Harutyunyan, Alex B.F. Martinson, Daniel Rosenmann, Larousse Khosravi Khorashad, Lucas V. Besteiro, Alexander O. Govorov, and Gary P. Wiederrecht. Anomalous ultrafast dynamics of hot plasmonic electrons in nanostructures with hot spots. *Nature Nanotechnology*, 10(9):770–774, 2015.
- [164] Zijian Li, Li Zhai, Yiyao Ge, Zhiqi Huang, Zhenyu Shi, Jiawei Liu, Wei Zhai, Jinzhe Liang, and Hua Zhang. Wet-chemical synthesis of two-dimensional metal nanomaterials for electrocatalysis. *National Science Review*, 9(5), 2022.

- [165] Yiming Wang, Nitya Sai Reddy Satyavolu, Hong Yang, and Yi Lu. Kinetic Reconstruction of DNA-Programed Plasmonic Metal Nanostructures with Predictable Shapes and Optical Properties. *Journal of the American Chemical Society*, 144(10):4410–4421, 2022.
- [166] Carla Abarca, M. Monsur Ali, Songtao Yang, Xiaofei Dong, and Robert H. Pelton. A Colloidal Stability Assay Suitable for High-Throughput Screening. *Analytical Chemistry*, 88(5):2929–2936, 2016.
- [167] Mathieu Doucet, Jae Hie Cho, Gervaise Alina, Ziggy Attala, Jurrian Bakker, Wim Bouwman, Paul Butler, Kieran Campbell, Torin Cooper-Benun, Celine Durniak, Laura Forster, Miguel Gonzalez, Richard Heenan, Andrew Jackson, Stephen King, Paul Kienzle, Jeff Krzywon, Ryan Murphy, Torben Nielsen, Lewis O’Driscoll, Wojciech Potrzebowski, Stewart Prescott, Ricardo Ferraz Leal, Piotr Rozyczko, Tim Snow, and Adam Washington. SasView version 5.0.4.
- [168] David Nečas and Petr Klapetek. Gwyddion: an open-source software for SPM data analysis. *Central European Journal of Physics*, 10:181–188, 2012.
- [169] Anvarhusein A. Isab and Peter J. Sadler. Reactions of gold(III) ions with ribonuclease A and methionine derivatives in aqueous solution. *BBA - Protein Structure*, 492(2):322–330, 1977.
- [170] Jitendra K. Sahu, Shahbaz Ahmad Lone, and Kalyan K. Sadhu. Methionine-Controlled Impediment of Secondary Nucleation Leading to Nonclassical Growth within Self-Assembled De Novo Gold Nanoparticles. *Langmuir*, 38(18):5865–5873, may 2022.
- [171] V. Germain, Jing Li, D. Inger, Z. L. Wang, and M. P. Pileni. Stacking faults in formation of silver nanodisks. *Journal of Physical Chemistry B*, 107(34):8717–8720, 2003.
- [172] Taixing Tan, Shun Zhang, Jie Wang, Yuqin Zheng, Haopeng Lai, Jialiang Liu, Fengqi Qin, and Cheng Wang. Resolving the stacking fault structure of silver nanoplates. *Nanoscale*, 13(1):195–205, 2021.
- [173] Hendrik Heinz, Barry L. Farmer, Ras B. Pandey, Joseph M. Slocik, Soumya S. Patnaik, Ruth Pachter, and Rajesh R. Naik. Nature of molecular interactions of peptides with gold, palladium, and Pd-Au bimetal surfaces in aqueous solution. *Journal of the American Chemical Society*, 131(28):9704–9714, 2009.
- [174] M. Reza Andalibi, Alexander Wokaun, Paul Bowen, and Andrea Testino. Kinetics and Mechanism of Metal Nanoparticle Growth via Optical Extinction Spectroscopy

- and Computational Modeling: The Curious Case of Colloidal Gold. *ACS Nano*, 13(10):11510–11521, 2019.
- [175] Caitlyn M. Wolf, Lorenzo Guio, Sage Scheiwiller, Viktoria Pakhnyuk, Christine Luscombe, and Lilo D. Pozzo. Strategies for the Development of Conjugated Polymer Molecular Dynamics Force Fields Validated with Neutron and X-ray Scattering. *ACS Polymers Au*, 1(3):134–152, 2021.
- [176] Masahiro Shimizu, Aya Okuda, Ken Morishima, Rintaro Inoue, Nobuhiro Sato, Yasuhiro Yunoki, Reiko Urade, and Masaaki Sugiyama. Extracting time series matching a small-angle X-ray scattering profile from trajectories of molecular dynamics simulations. *Scientific Reports*, 12(1):9970, jun 2022.
- [177] Mingfei Zhao, Kacper J. Lachowski, Shuai Zhang, Sarah Alamdari, Janani Sampath, Peng Mu, Christopher J. Mundy, Jim Pfaendtner, James J. De Yoreo, Chun Long Chen, Lilo D. Pozzo, and Andrew L. Ferguson. Hierarchical Self-Assembly Pathways of Peptoid Helices and Sheets. *Biomacromolecules*, 23(3):992–1008, 2022.
- [178] Ronald N. Zuckermann. Peptoid origins. *Biopolymers*, 96(5):545–555, 2011.
- [179] Madison Monahan, Bin Cai, Tengyue Jian, Shuai Zhang, Guomin Zhu, Chun Long Chen, James J. De Yoreo, and Brandi M. Cossairt. Peptoid-directed assembly of CdSe nanoparticles. *Nanoscale*, 13(2):1273–1282, 2021.
- [180] Gloria K. Olivier, Andrew Cho, Babak Sanii, Michael D. Connolly, Helen Tran, and Ronald N. Zuckermann. Antibody-mimetic peptoid nanosheets for molecular recognition. *ACS Nano*, 7(10):9276–9286, 2013.
- [181] Yuhan Wei, Jiliang Tian, Zekun Zhang, Chenhui Zhu, Jing Sun, and Zhibo Li. Supramolecular Nanosheets Assembled from Poly(ethylene glycol)- b-poly(N-(2-phenylethyl)glycine) Diblock Copolymer Containing Crystallizable Hydrophobic Polypeptoid: Crystallization Driven Assembly Transition from Filaments to Nanosheets. *Macromolecules*, 52(4):1546–1556, 2019.
- [182] Wei-Ren Chen, Paul D Butler, and Linda J Magid. Incorporating intermicellar interactions in the fitting of sars data from cationic wormlike micelles. *Langmuir*, 22(15):6539–6548, 2006.
- [183] Jan Skov Pedersen and Peter Schurtenberger. Scattering functions of semiflexible polymers with and without excluded volume effects. *Macromolecules*, 29(23):7602–7612, 1996.

- [184] Toshiyuki Takamuku, Masaaki Tabata, Atsushi Yamaguchi, Jun Nishimoto, Midori Kumamoto, Hisanobu Wakita, and Toshio Yamaguchi. Liquid structure of acetonitrile-water mixtures by x-ray diffraction and infrared spectroscopy. *J. Phys. Chem. B*, 102(44):8880–8888, 1998.
- [185] Yuyin Xi, Fan Zhang, Yuanchi Ma, Vivek M. Prabhu, and Yun Liu. Finely tunable dynamical coloration using bicontinuous micrometer-domains. *Nature Communications*, 13(1):1–7, 2022.
- [186] Fan Li, David P. Josephson, and Andreas Stein. Colloidal assembly: The road from particles to colloidal molecules and crystals. *Angewandte Chemie - International Edition*, 50(2):360–388, 2011.
- [187] Brittney Hellner, Sarah Alamdari, Harley Pyles, Shuai Zhang, Arushi Prakash, Kayla G. Sprenger, James J. De Yoreo, David Baker, Jim Pfaendtner, and François Baneyx. Sequence-Structure-Binding Relationships Reveal Adhesion Behavior of the Car9 Solid-Binding Peptide: An Integrated Experimental and Simulation Study. *Journal of the American Chemical Society*, 142(5):2355–2363, 2020.
- [188] Paul Kienzle, Scott Wu, Brian Benjamin Maranville, Stuart Prescott, Mathieu Doucet, David P. Hoogerheide, and Andrew Nelson. bumps/bumps: v0.7.11, 2018.
- [189] Steven R. Kline. Reduction and analysis of SANS and USANS data using IGOR Pro. *Journal of Applied Crystallography*, 39(6):895–900, 2006.
- [190] J. Teixeira. Small-angle scattering by fractal systems. *Journal of Applied Crystallography*, 21(6):781–785, 1988.
- [191] Anthony Y. Kim and John C. Berg. Fractal aggregation: Scaling of fractal dimension with stability ratio. *Langmuir*, 16(5):2101–2104, 2000.
- [192] Andrea E. Rawlings, Jonathan P. Bramble, Anna A.S. Tang, Lori A. Somner, Amy E. Monnington, David J. Cooke, Michael J. McPherson, Darren C. Tomlinson, and Sarah S. Staniland. Phage display selected magnetite interacting Adhirons for shape controlled nanoparticle synthesis. *Chemical Science*, 6(10):5586–5594, 2015.
- [193] Ian M. Pendleton, Gary Cattabriga, Zhi Li, Mansoor Ani Najeeb, Sorelle A. Friedler, Alexander J. Norquist, Emory M. Chan, and Joshua Schrier. Experiment Specification, Capture and Laboratory Automation Technology (ESCALATE): A software pipeline for automated chemical experimentation and data management. *MRS Communications*, 9(3):846–859, 2019.

- [194] J Derek Tucker, Wei Wu, and Anuj Srivastava. Generative models for functional data using phase and amplitude separation. *Computational Statistics & Data Analysis*, 61:50–66, 2013.
- [195] Paul R Halmos. What does the spectral theorem say? *The American Mathematical Monthly*, 70(3):241–247, 1963.
- [196] Lucas Piñeiro, Mercedes Novo, and Wajih Al-Soufi. Fluorescence emission of pyrene in surfactant solutions. *Advances in Colloid and Interface Science*, 215:1–12, 2015.
- [197] Jessica A. Hutchinson, Ian W. Hamley, Juan Torras, Carlos Alemán, Jani Seitsonen, and Janne Ruokolainen. Self-Assembly of Lipopeptides Containing Short Peptide Fragments Derived from the Gastrointestinal Hormone PYY 3–36 : From Micelles to Amyloid Fibrils. *The Journal of Physical Chemistry B*, 123(3):614–621, jan 2019.
- [198] Jan Skov Pedersen. Analysis of small-angle scattering data from colloids and polymer solutions: Modeling and least-squares fitting. *Advances in Colloid and Interface Science*, 70(1-3):171–210, 1997.
- [199] Gérard Fournet. Étude théorique et expérimentale de la diffusion des rayons X par les ensembles denses de particules. *Bulletin de la Société française de Minéralogie et de Cristallographie*, 74(1):37–172, 1951.
- [200] Lars Onsager. the Effects of Shape on the Interaction of Colloidal Particles. *Annals of the New York Academy of Sciences*, 51(4):627–659, 1949.
- [201] Jonathon Shlens. A tutorial on principal component analysis. *arXiv preprint arXiv:1404.1100*, 2014.
- [202] Arnim Henglein and Michael Giersig. Formation of Colloidal Silver Nanoparticles: Capping Action of Citrate Arnim Henglein. *Journal of Physical Chemistry B*, 103(44):9533–9539, 1999.
- [203] Ioannis Lignos, Stavros Stavrakis, Georgian Nedelcu, Loredana Protesescu, Andrew J. Demello, and Maksym V. Kovalenko. Synthesis of Cesium Lead Halide Perovskite Nanocrystals in a Droplet-Based Microfluidic Platform: Fast Parametric Space Mapping. *Nano Letters*, 16(3):1869–1877, 2016.
- [204] Ryan Kastilani, Brittany P. Bishop, Vincent C. Holmberg, and Lilo D. Pozzo. On-Demand Sonochemical Synthesis of Ultrasmall and Magic-Size CdSe Quantum Dots in Single-Phase and Emulsion Systems. *Langmuir*, 35(50):16583–16592, 2019.

- [205] Ferenc Liebig, Andreas F. Thünemann, and Joachim Koetz. Ostwald Ripening Growth Mechanism of Gold Nanotriangles in Vesicular Template Phases. *Langmuir*, 32(42):10928–10935, 2016.

Appendix A

MULTIVARIATE ANALYSIS OF PEPTIDE-DRIVEN NUCLEATION AND GROWTH OF AU NANOPARTICLES

A.0.1 Materials

Pyrene (98.2 % purity) was obtained from Sigma-Aldrich and used without further purification.

A.0.2 UV-Vis Spectra

Note that although Z2M6I contains an outlier in the 0 mM HEPES condition shown in Figure [A.1 J](#), the use of GFT on the transformed spectra would have treated this sample as noise.

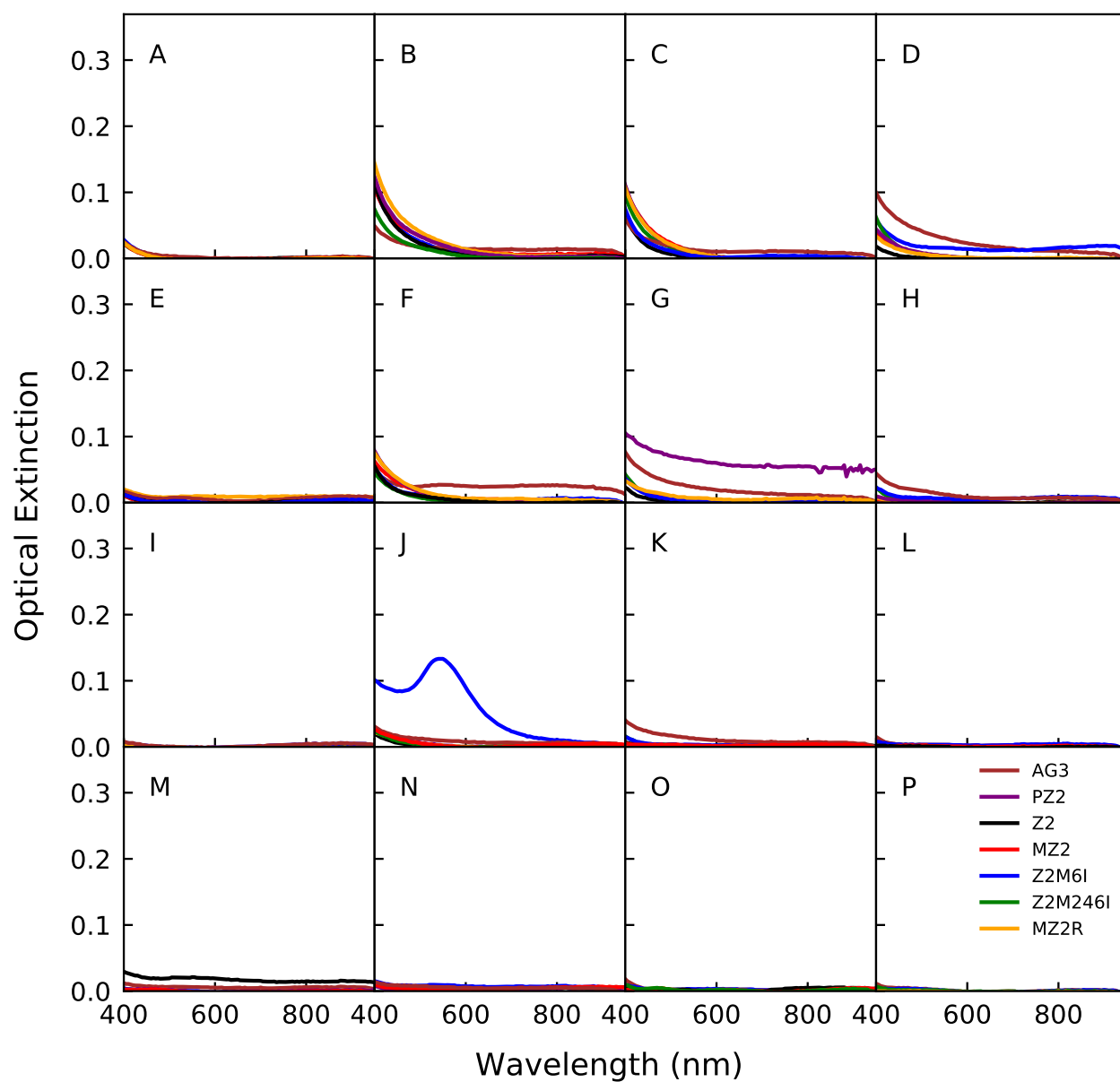


Figure A.1: UV-Vis spectra of Au Nanoparticles prepared in the presence of different peptides as indicated by the color labeling. HEPES concentration is 0 mM, HAuCl_4 concentration decreases from top to bottom (0.2, 0.117, 0.0684, 0.04 mM), and peptide concentration increases from left to right (0, 0.04, 0.0894, 0.2 mM).

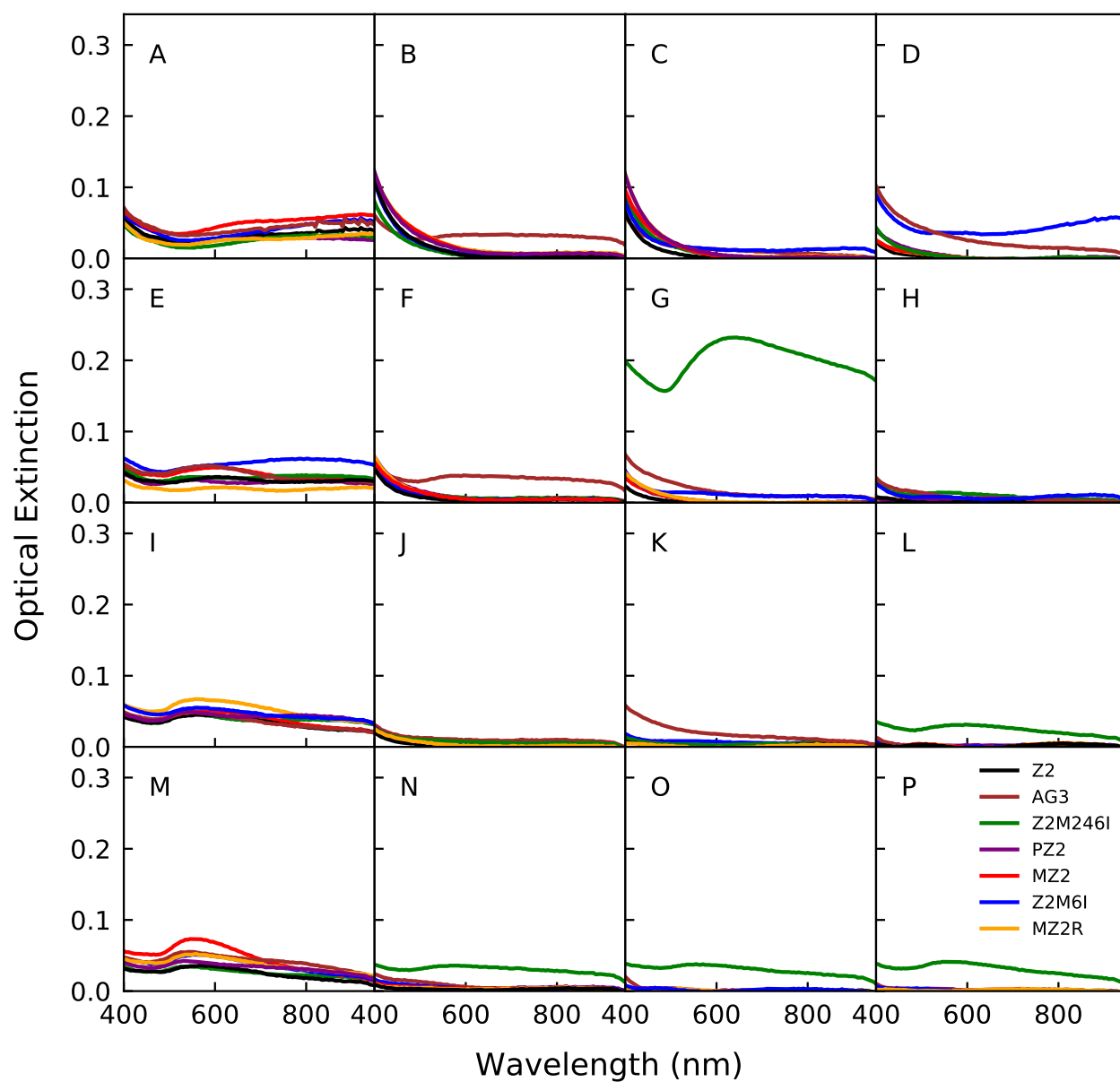


Figure A.2: UV-Vis spectra of Au Nanoparticles prepared in the presence of different peptides as indicated by the color labeling. HEPES concentration is 0.01 mM, HAuCl_4 concentration decreases from top to bottom (0.2, 0.117, 0.0684, 0.04 mM), and peptide concentration increases from left to right (0, 0.04, 0.0894, 0.2 mM).

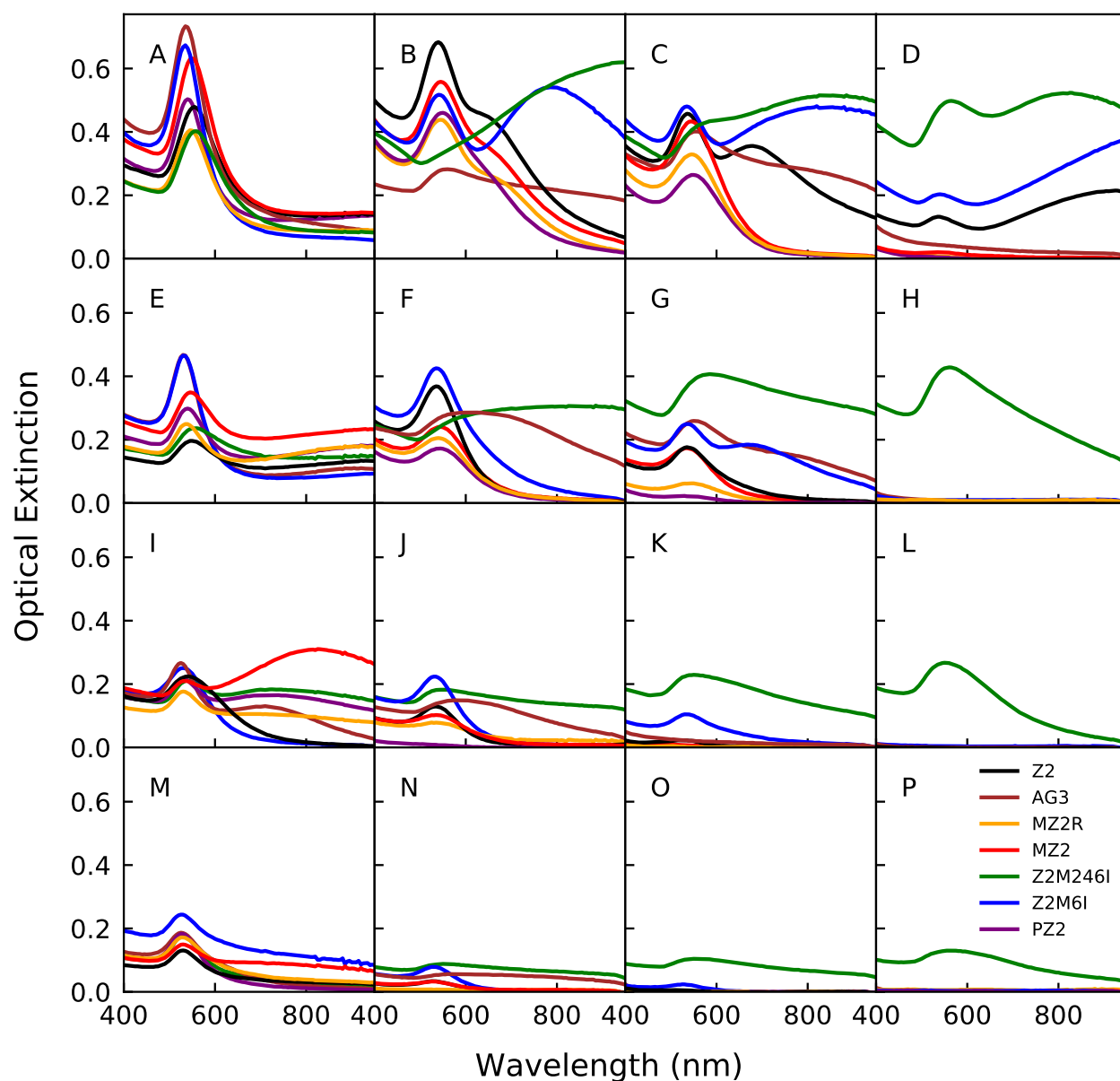


Figure A.3: UV-Vis spectra of Au Nanoparticles prepared in the presence of different peptides as indicated by the color labeling. HEPES concentration is 0.1 mM, HAuCl_4 concentration decreases from top to bottom (0.2, 0.117, 0.0684, 0.04 mM), and peptide concentration increases from left to right (0, 0.04, 0.0894, 0.2 mM).

A.0.3 Determination of Lipidated Peptide Critical Micelle Concentration

The pyrene stock was prepared by first preparing a 0.5 mM stock solution of pyrene in ethanol. The ethanol solution was diluted with deionized water to reach a pyrene concentration of 0.146 mg/mL. Note that the final ethanol concentration was 0.144 vol.%. Peptide stock solutions were prepared at 5×10^{-1} wt.% and 5×10^{-2} wt.%. Samples were prepared using an Opentrons OT-2 liquid handling robot. Solutions were prepared by transferring peptide, pyrene stock, and additional water into Caplugs Evergreen untreated 96-well microplates (black, flat bottom). The concentration of peptide in the samples was varied from 0.04 mM to 3 mM. Samples were mixed by pipetting up and down after addition of water, and were then characterized using a BioTek Synergy H1 microplate reader. Samples were excited at 334 nm and emission was obtained at 372 (**peak 1**) and 384 nm (**peak 3**).

Pyrene remains dissolved in samples where the peptide concentration is too low for formation of micelles. Above the critical micelle concentration (CMC), pyrene adsorbs to the peptide lipid tails inside micelles. The resulting change in the polarity of pyrene's environment leads to changes in its fluorescence emission. The ratio between emission **peaks 3** and **1** as a function of peptide concentration is used to estimate the critical micelle formation [106, 197, 196]. The CMC is estimated from the intersection of two lines in the plot of the ratio of the two fluorescence peaks as a function of the logarithm of peptide concentration. The order of the resulting CMC values are $MZ2 > MZ2R > PZ2$.

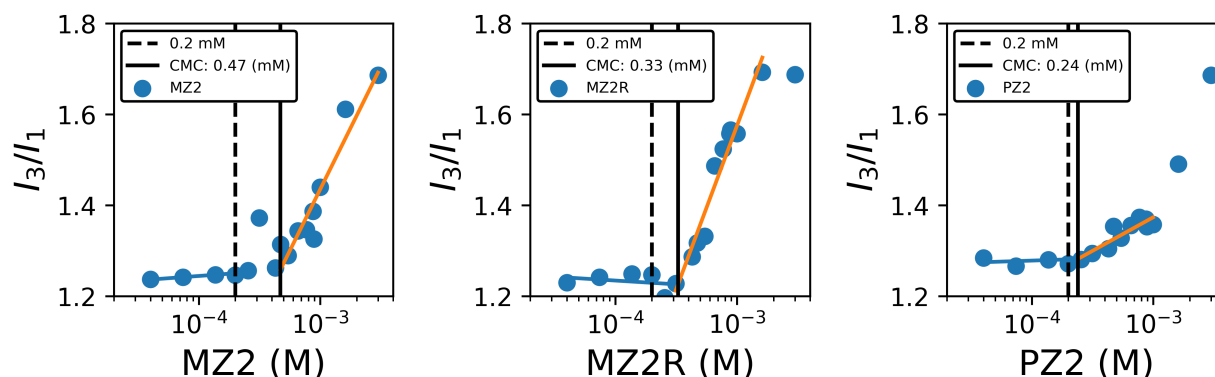


Figure A.4: Ratio of fluorescence intensity at 384 to 372 nm resulting from excitation at 334 nm as a function of peptide concentration. Adsorption of pyrene to micelles leads to a change in the fluorescence spectra which is used to estimate the CMC by finding intersection of two lines fitted to the data. The CMC of MZ2, MZ2R, and PZ2 were estimated to be 0.47 mM, 0.33 mM, and 0.24 mM, respectively.

A.0.4 Au Reduction Kinetics Assay

Samples were prepared by first adding all reagents except HAuCl_4 to well in a 48-well plate. Then the latter was added to each well, the solution was pipetted up and down, and the samples were moved into the plate reader for measurement.

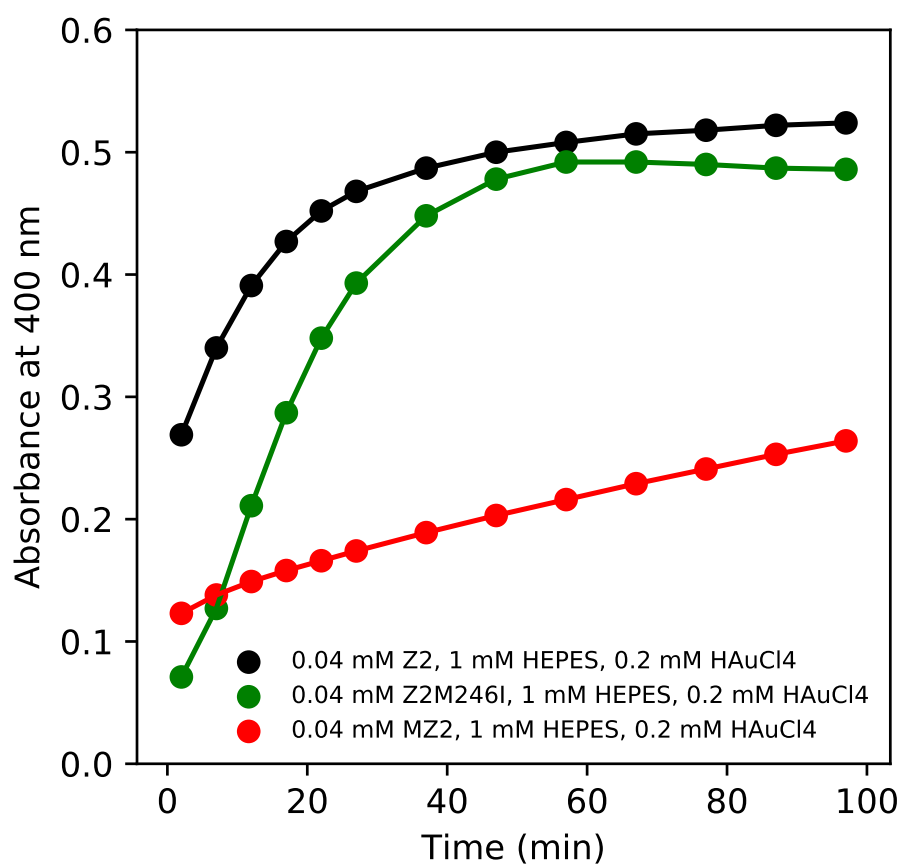


Figure A.5: Absorbance at 400 nm as a function of time (minutes) for Z2, Z2M246I, and MZ2.

A.1 USAXS Scattering Profiles and AUTORG Results

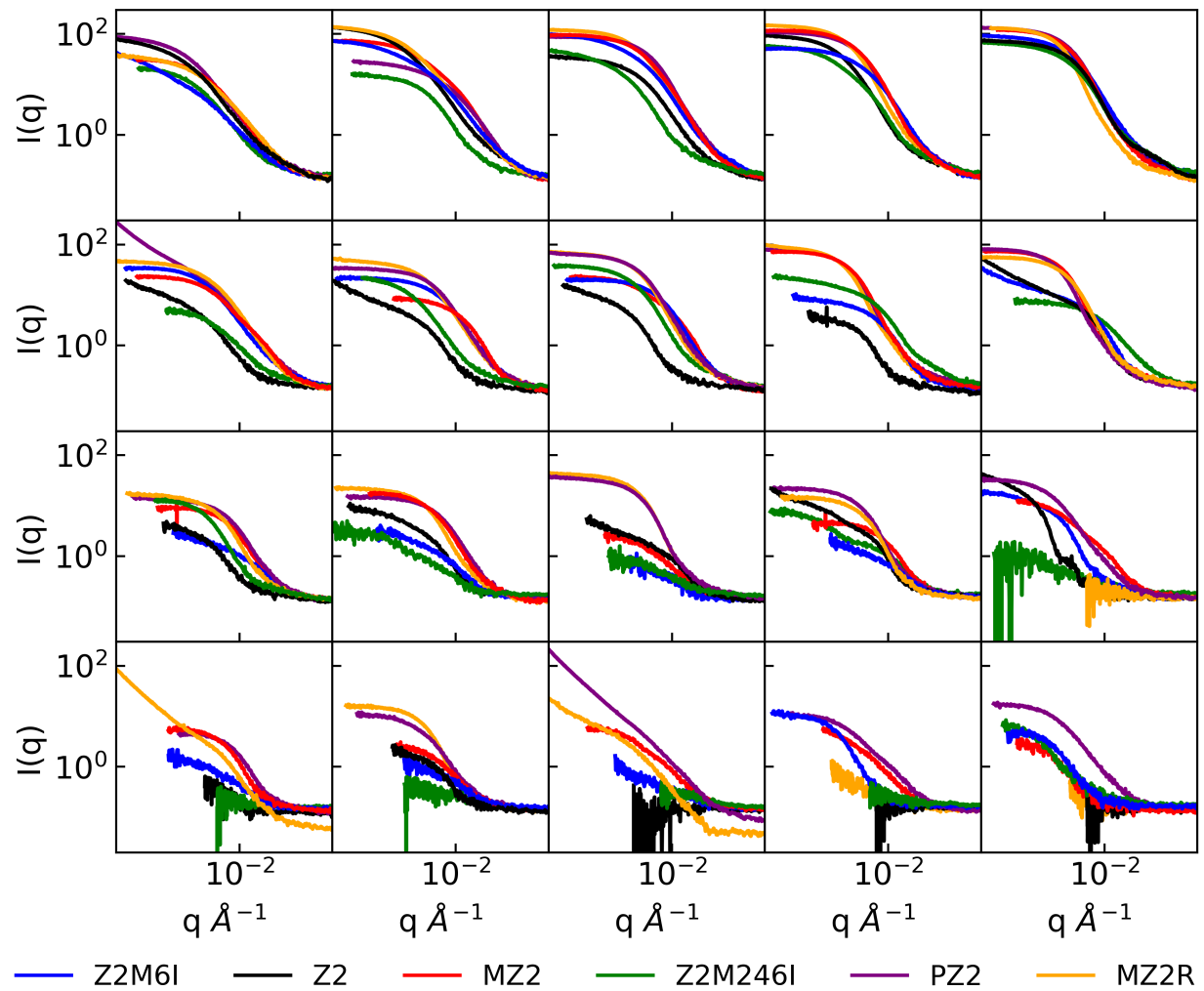


Figure A.6: Smeared USAXS data. Sample compositions: 0 mM HEPES; peptide left to right 0.040, 0.060, 0.089, 0.134, and 0.200 mM; HAuCl_4 top to bottom 0.200, 0.117, 0.068, and 0.040 mM.

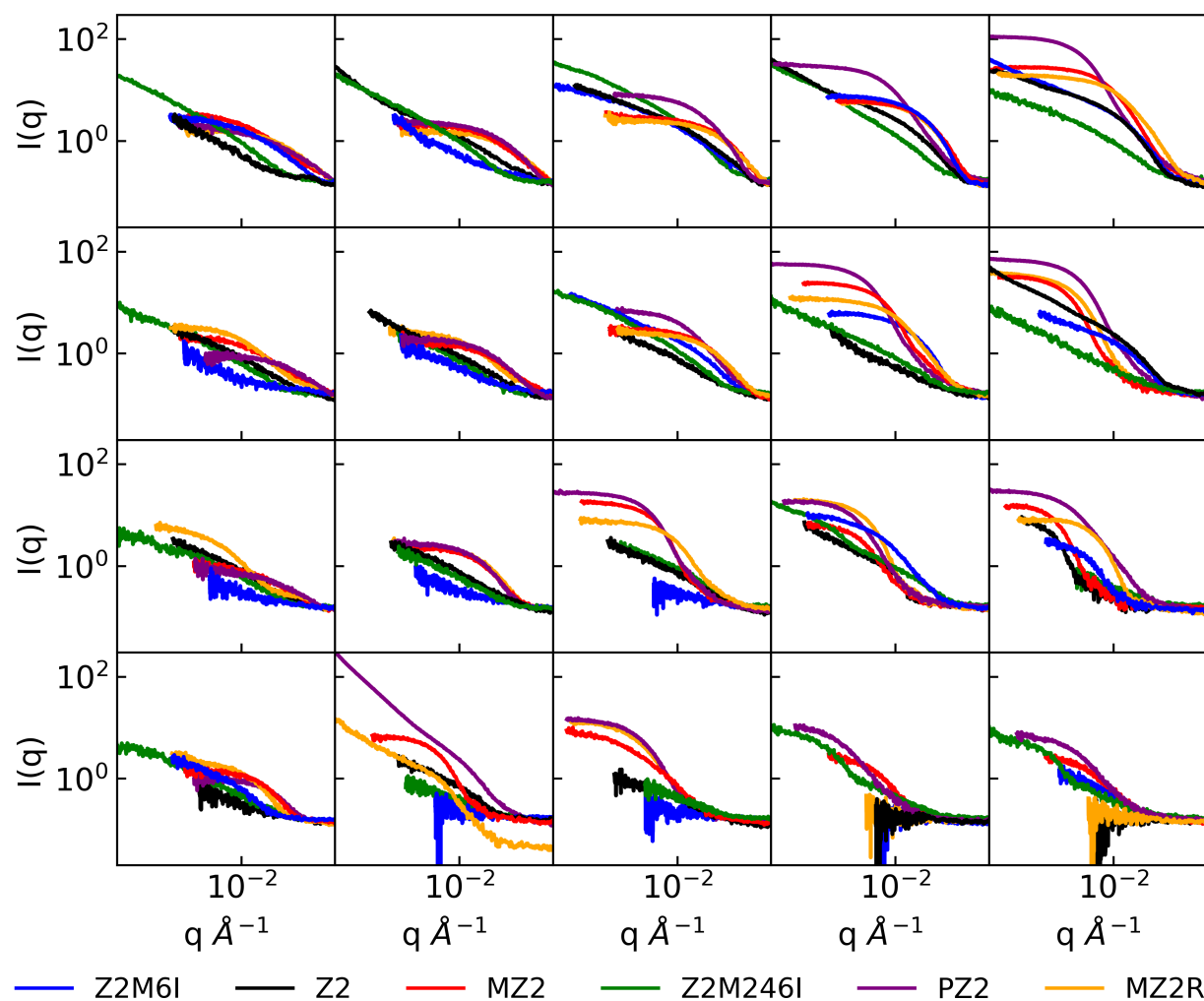


Figure A.7: Smeared USAXS data. Sample compositions: 1 mM HEPES; peptide left to right 0.040, 0.060, 0.089, 0.134, and 0.200 mM; HAuCl_4 top to bottom 0.200, 0.117, 0.068, and 0.040 mM.

The AUTORG results for lipidated and non-lipidated peptides are presented in Figure A.8 and Figure A.9, respectively. The lipidated and non-lipidated peptide- HAuCl_4 assemblies are of similar size across all of the studied conditions. However, the size of the lipidated variants appears to vary less as a function of the peptide: HAuCl_4 ratio when compared with

the non-lipidated variants. Note in Figure A.9 that the data for peptides which are lacking methionine showed similar scattering behavior to the Z2 peptide. We interpret this to mean that peptide-HAuCl₄ interactions that are responsible for the formation of the observed aggregates are not due to methionine-HAuCl₄ complexation.

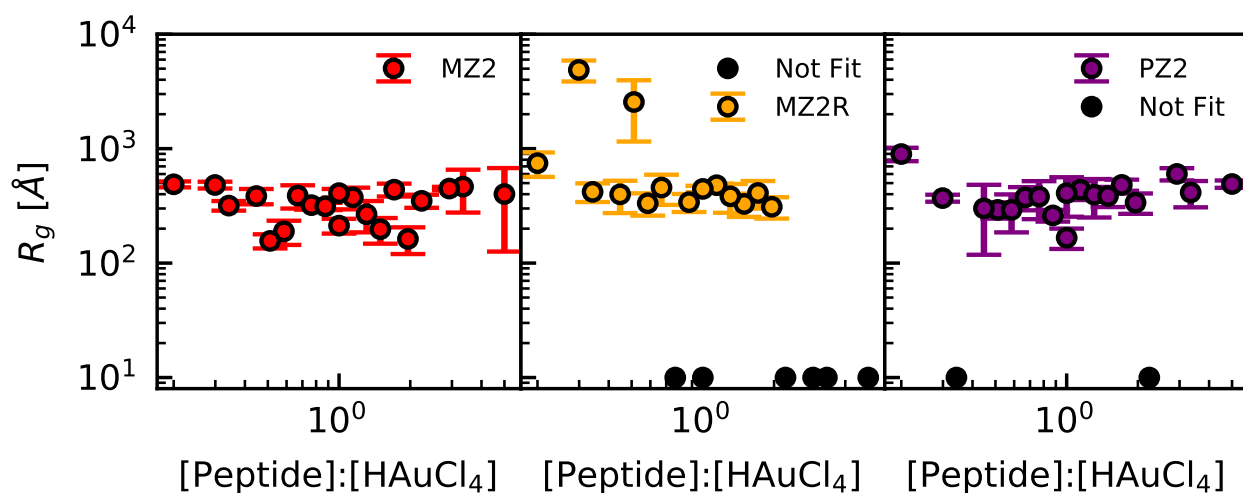


Figure A.8: Radius of gyration results obtained from AUTORG plotted as a function of peptide:HAuCl₄ molar ratio for all lipidated peptides and a HEPES concentration of 0 mM. Error bars indicate standard deviation. Profiles which did not contain a Guinier region that could be fitted were marked using black and plotted at a fixed R_g of 10 Å.

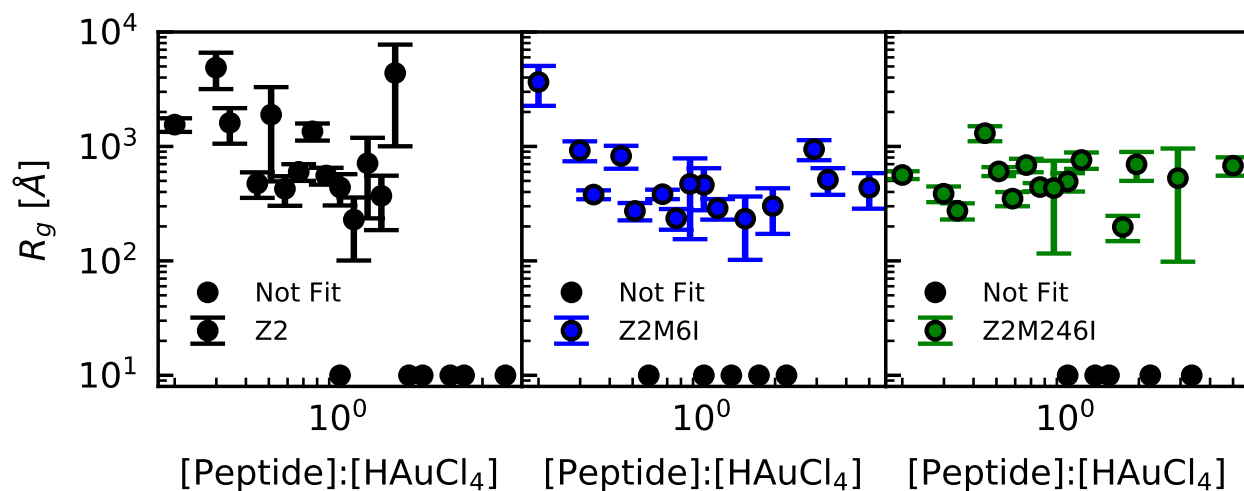


Figure A.9: Radius of gyration results obtained from AUTORG plotted as a function of peptide:HAuCl₄ molar ratio for non-lipidated peptides (except AG3) and a HEPES concentration of 0 mM. Error bars indicate standard deviation. Profiles which did not contain a Guinier region that could be fitted were marked using black and plotted at a fixed R_g of 10 Å.

A.1.1 SAXS

MZ2 (myristoylated RMRMKMK) was dissolved at a concentration of 4.2 mM in ultra pure water. The sample was flowed into a quartz capillary (1 mm outer diameter, Charles Supper Company, Natick, MA). The capillary was loaded inside a Kratky-type SAXS instrument (SAXSess, Anton Paar, Graz, Austria) with a Cu K_α source ($\lambda = 1.54$ Å). The slit-smeared scattering data was fit using a slit-smeared cylinder model using SASview (SasView v.5.0.1, www.sasview.org) [200, 199, 198]. The resulting radius of the fitted cylinder model was 5.2 nm. The length could not be determined from the scattering data due to the lack of a Guinier region in the q -range of the instrument. Therefore we can only conclude that the length of the extended, self-assembled MZ2 structures was in excess of 60 nm.

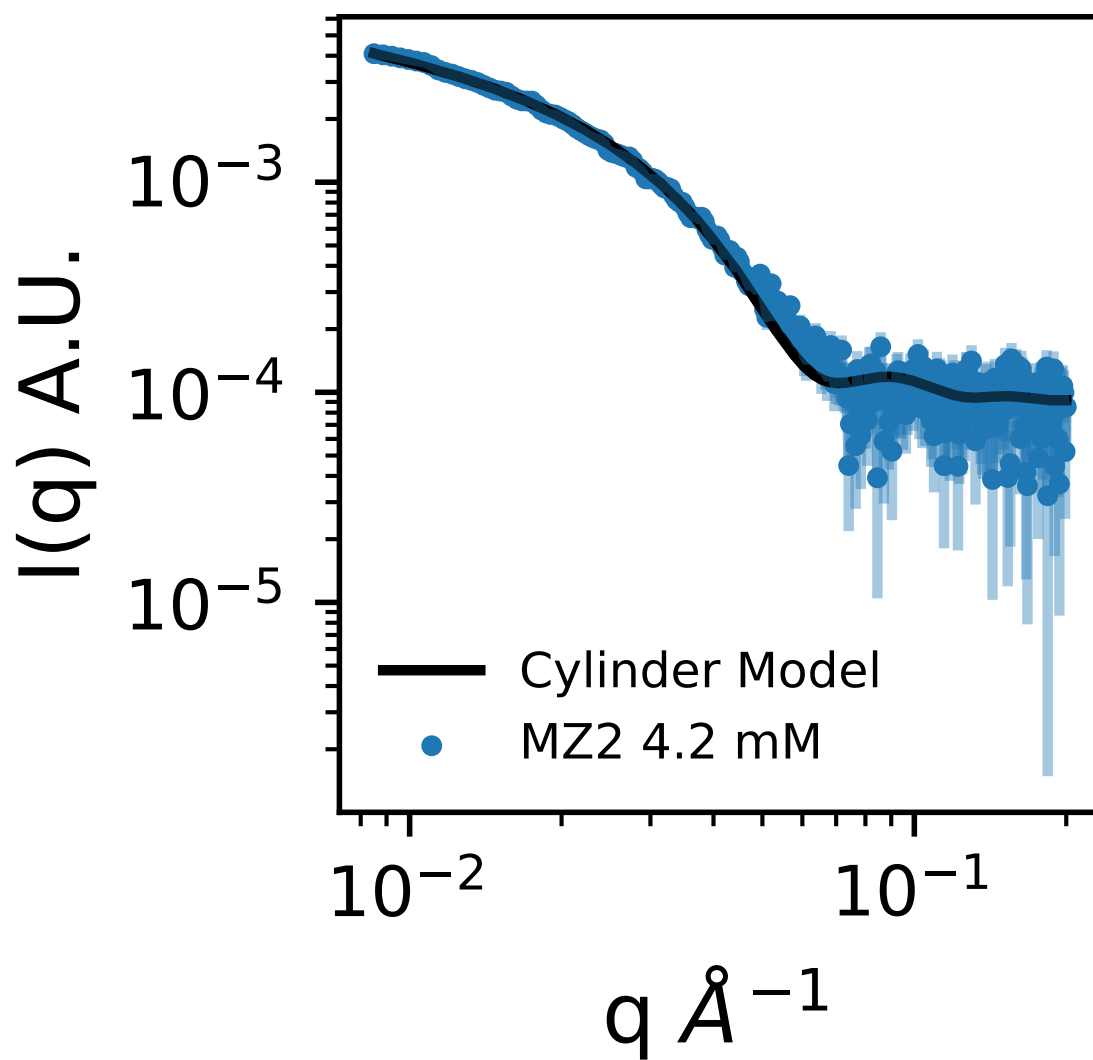


Figure A.10: Smeared small-angle scattering of MZ2 dissolved in water (4.2 mM) fit with a cylinder model (radius = 5.2 nm).

A.1.2 USAXS Sample Holder Assembly

The liquid sample holder used for USAXS experiments is a sandwiched well-plate style assembly which has the same profile as a standard well plate. Files and instructions for assembly have been shared online [130]. The process of assembly is as follows:

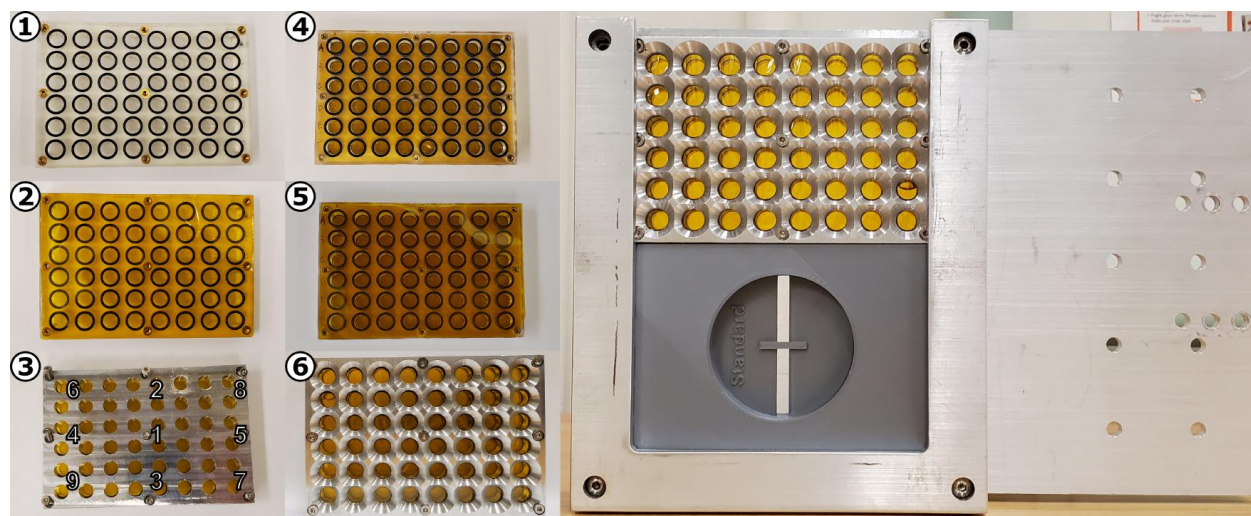


Figure A.11: Process of assembling USXAXS sample holder, and the frame which holds up to two plates during experiments. A 3D printed mock cartridge is used to hold silver behenate for USAXS calibration.

1. Insert o-rings into center piece face which will be facing the X-ray source.
2. Place Kapton sheet and then the aluminum cover piece which will be facing the source on top of the o-rings.
3. Insert screws and fasten in the order specified in the image.
4. Turn over the plate and insert o-rings.

Table A.1: List of items used per plate

Supplier	Material and Dimensions	Catalog Number	Part Name
Formlabs	Clear	RS-F2-GPCL-04	Center piece/main body
McMaster-Carr	Aluminum	9140T109	Cover plate source
McMaster-Carr	Aluminum	9140T109	Cover plate detector
McMaster-Carr	Kapton (0.0010" thick)	2271K401	Windows
McMaster-Carr	Buna-N (1.5 mm wide, 9 mm ID)	9262K126	O-rings
McMaster-Carr	Stainless steel M3 (8 mm Long, 0.5 mm Pitch)	90666A104	Screws
MiSUMi	Brass Cadmium-Free (M3x0.5 mm)	FB-305580	Threaded insert

5. Load liquid samples manually or with a liquid-handling system. Suggested volume is 450 μL .
6. Place second Kapton sheet and aluminum cover plate on top, and fasten using the same order of screws.

A.1.3 Opentrons Protocol Details

Code to control the OT2 is available online [\[129\]](#). The protocol uses an Opentrons P300 GEN2 and P50 GEN1. Deck layout is shown in Figure [A.12](#).

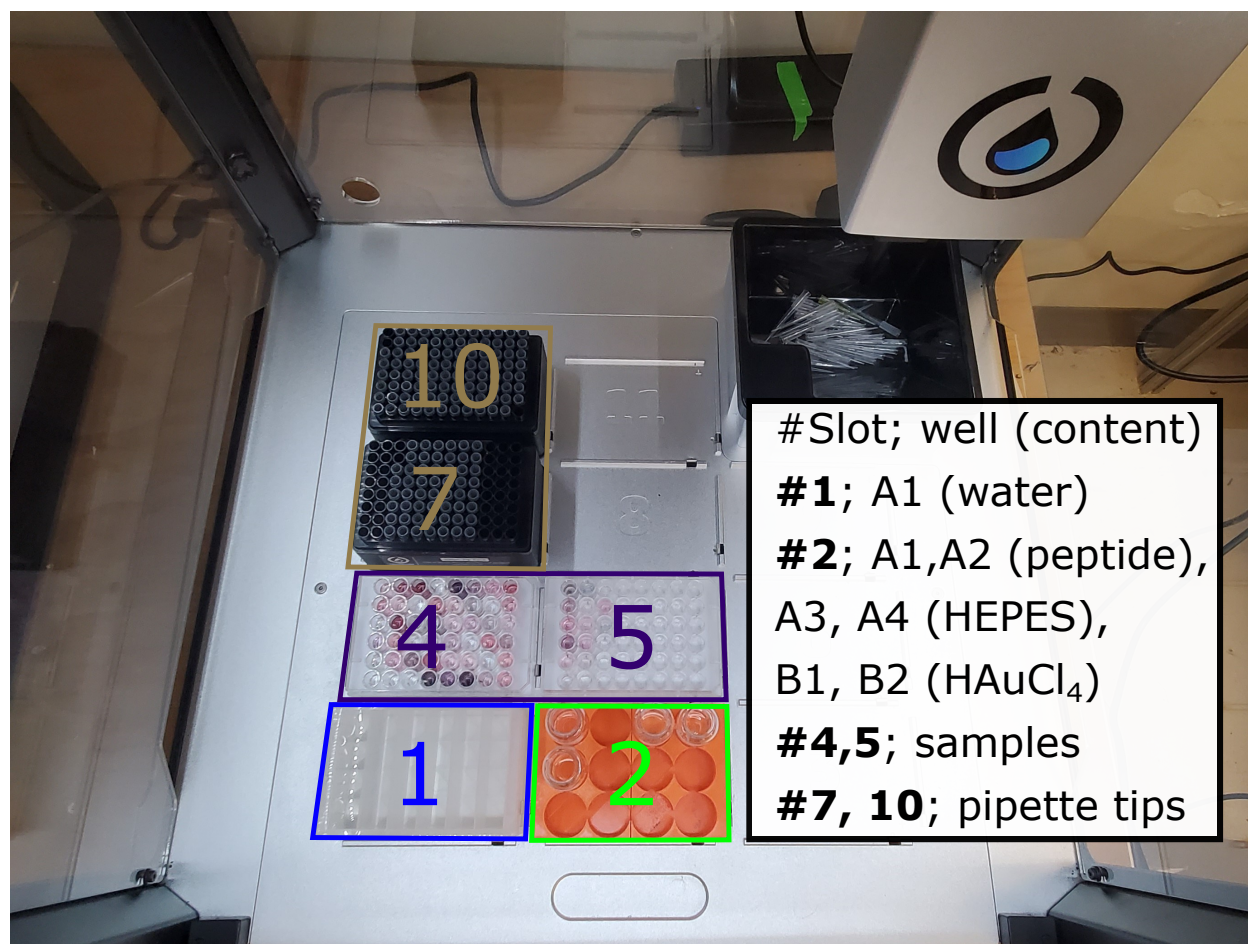


Figure A.12: Opentrons OT-2 deck layout: 1, Agilent 6 column reservoir, water; 2, stocks in 20 mL scintillation vials seated in 3D printed vial holder; 4 and 5, 48 well microplates; 7 and 10, 300 μL pipette tips. Stock distribution in slot 2: A1, peptide; A3 and A4, HEPES; B1, HAuCl₄

Appendix B

ANISOTROPIC GOLD NANOMATERIAL SYNTHESIS USING PEPTIDE FACET SPECIFICITY AND TIMED INTERVENTION

B.1 Liquid Handling Robot Program

All liquid handling was performed using an Opentrons OT-2 liquid handling robot. The robot's use enabled parallel sample preparation, resulting in decreased experiment time when mixing stock solutions with different time delays and orders of addition. The Python package (<https://github.com/pozzo-research-group/otto>) was used to create a schedule for the robot to execute in a short amount of time. In short, the script creates a Gantt chart of all the transfer volumes, and the time delay between each of the steps. Each row of this array corresponds to a sample, and each column corresponds to a one minute time interval, the time allotted for any pipette transfer and cleaning steps to occur. These start times of each sample are adjusted to minimize the total time of the experiment.

B.2 SAXS Sample Holder

Computer aided design was carried out using Autodesk (Mill Valley, CA) Fusion 360. The design features ca. 0.25 mm windows, and a nominal path length of 2.5 mm. Files were then sliced using Formlabs (Somerville, MA, USA) PreForm software, transferred to a Formlabs Form 3B+ stereo-lithographic printer, and printed using a clear photo-polymerizable resin from Formlabs. After completion, the finished part was processed by injecting ca. 30 mL of isopropyl alcohol (IPA) through each channel, washing for 20 minutes in an IPA bath, then another 10 minutes in a new IPA bath, drying the part using compressed air, and finally curing it at 60°C for 30 minutes in the Formlabs cure box. Connection to the sample holder

was made using a flangeless Delrin, 1/4"-28 flat-bottom fitting for 1/16" outer diameter tubing, part number XP-202 from IDEX Health & Science (Gig Harbor, WA, USA). The tubing used was polytetrafluoroethylene semi-clear tubing, 1/32" inner diameter, 1/16" outer diameter from McMaster-Carr (Elmhurst, IL, USA).

B.3 Atomic Force Microscopy Analysis

AFM imaging of samples prepared when Z2 was added after different delay periods are presented. Structures identified in fig. B.1 are sphere-like nanoparticles that form as a result of Z2 being added after no delay periods, based on their appearance and line profile measurements in fig. B.2. Both spherical and plate-like particles are identified when Z2 is added after 6 delay periods as seen in fig. B.3. Line profile measurements annotated as 1-7 in the figure appear to be nanotriangles with thicknesses of ca. 10-12 nm based on the fig. B.4 and fig. B.5. Additionally, a line profile measurement was obtained for one of two aggregates of nanotriangles seen in the image. Measurement 8, shown in fig. B.5, passes over either two or three nanotriangles or plate-like particles and has a maximum height of ca. 20 nm. Finally, plate-like particles obtained after Z2 was added after 10 delay periods and the corresponding line profile measurements are shown in fig. B.6 and fig. B.7. The thickness of these particles is approximately 12 nm.

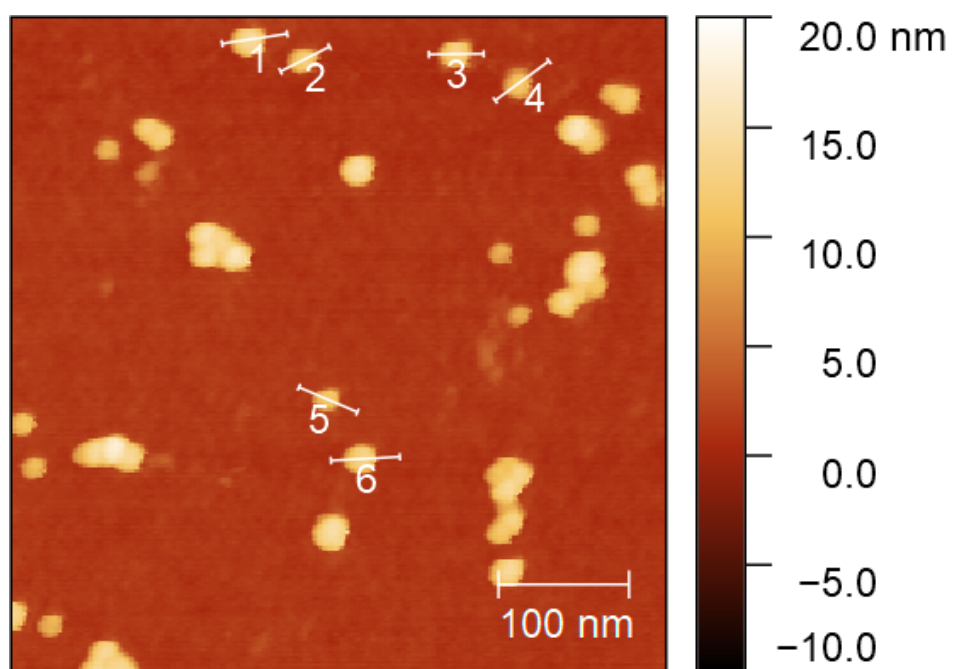


Figure B.1: AFM image of sample in which Z2 was added after 0 delay periods

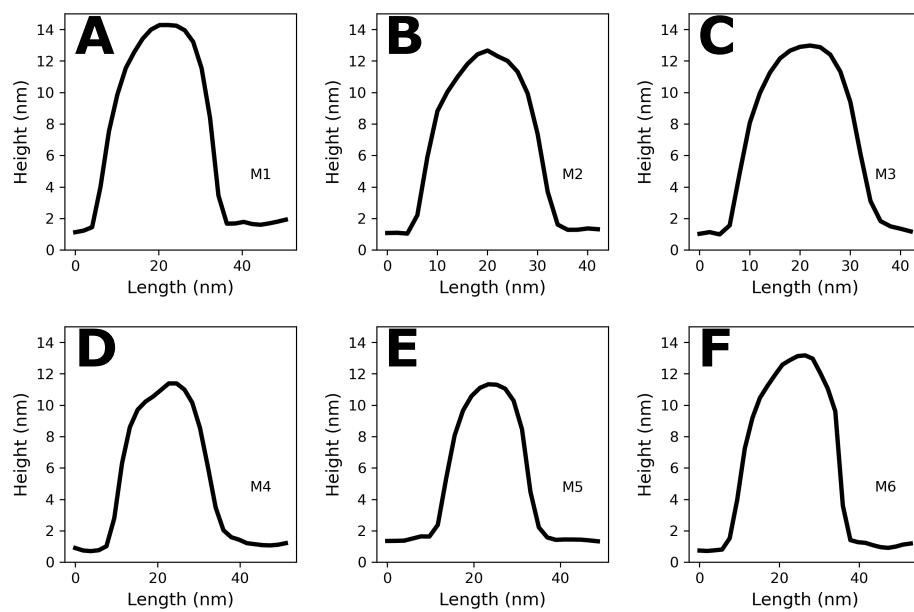


Figure B.2: Line profiles of measurements annotated in fig. B.1. A, B, C, D, E, and F correspond to measurements 1, 2, 3, 4, 5, and 6.

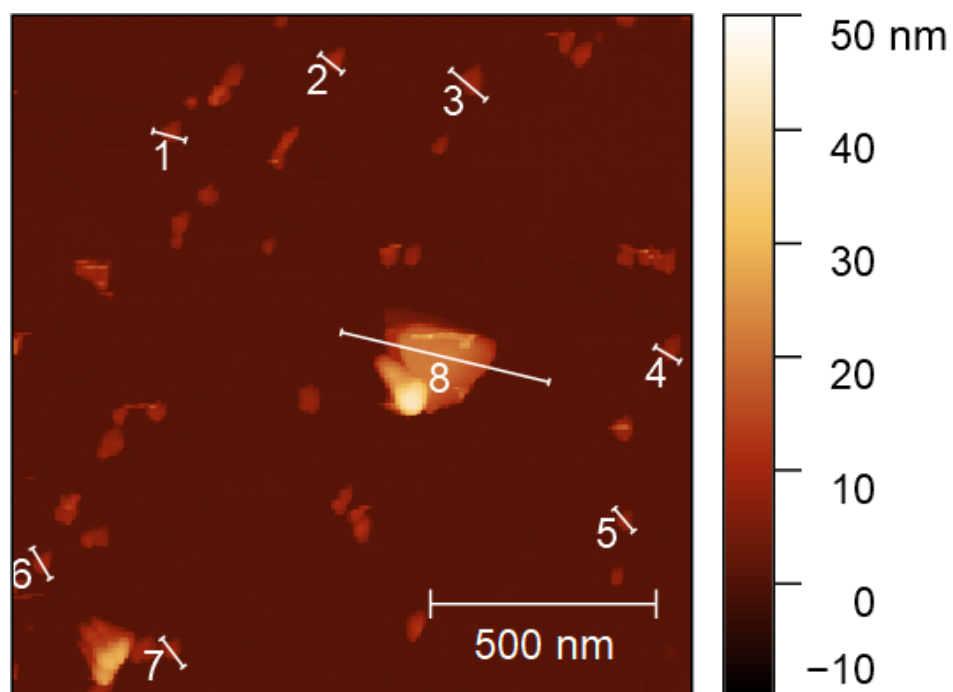


Figure B.3: AFM image of sample in which Z2 was added after 6 delay periods

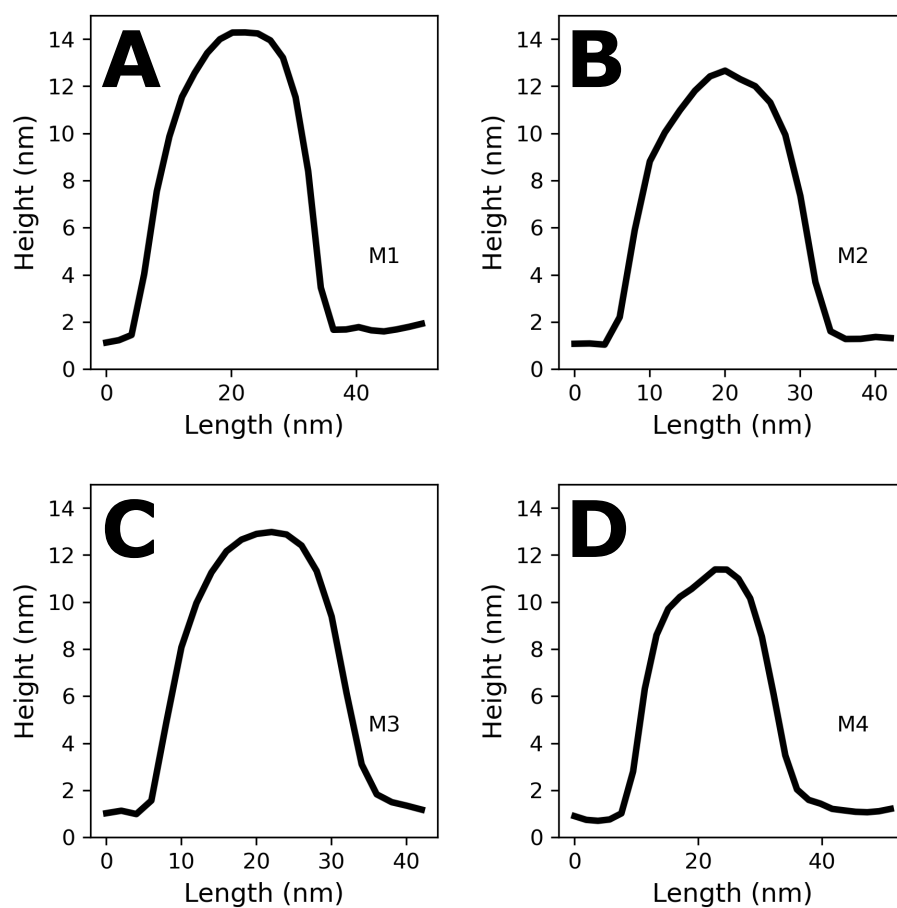


Figure B.4: Line profiles of measurements annotated in fig. B.3. A, B, C, D, E, and F correspond to measurements 1, 2, 3, and 4.

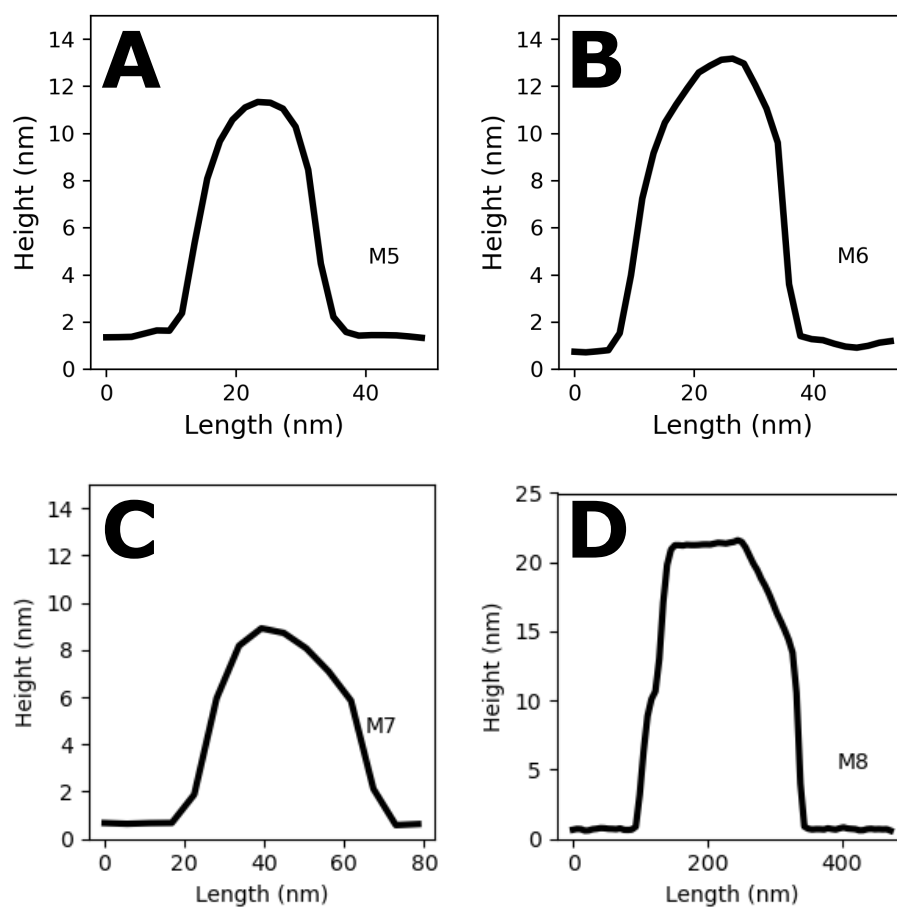


Figure B.5: Line profiles of measurements annotated in fig. B.3. A, B, C, and D correspond to measurements 5, 6, 7, and 8.

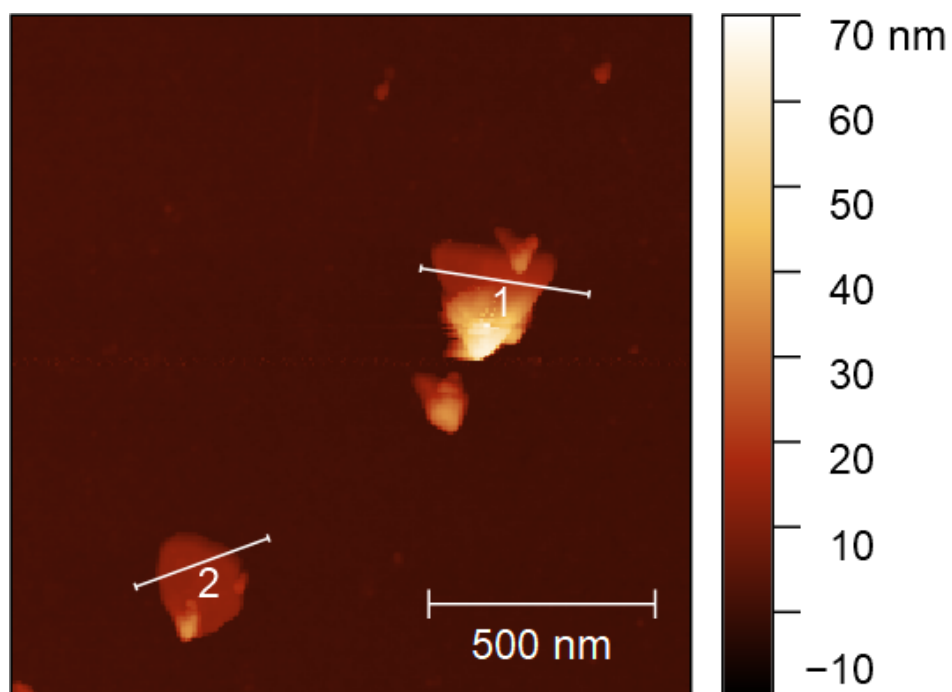


Figure B.6: AFM image of sample in which Z2 was added after 10 delay periods

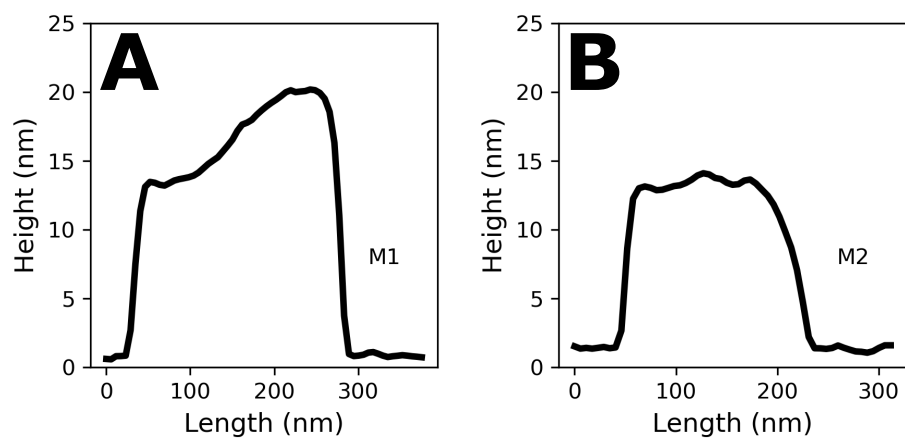


Figure B.7: Line profiles of measurements annotated in fig. B.6. A and B correspond to measurements 1 and 2.

B.4 Cuvette Based Colloidal Stability Assay

UV-Vis spectra of samples that were transferred to cuvettes and measured using UV-Vis over 3 days are shown in fig. B.8. Optical extinction at 400 nm and 1000 nm are used to characterize changes in Au(0) and the presence of plate-like particles, respectively. Images of samples in cuvettes are also presented in fig. B.9. Together, these results indicate that samples in which Z2 was added after 8 delay periods or later exhibited more sedimentation than those in which Z2 was added earlier, and that sedimentation in the control sample was most significant.

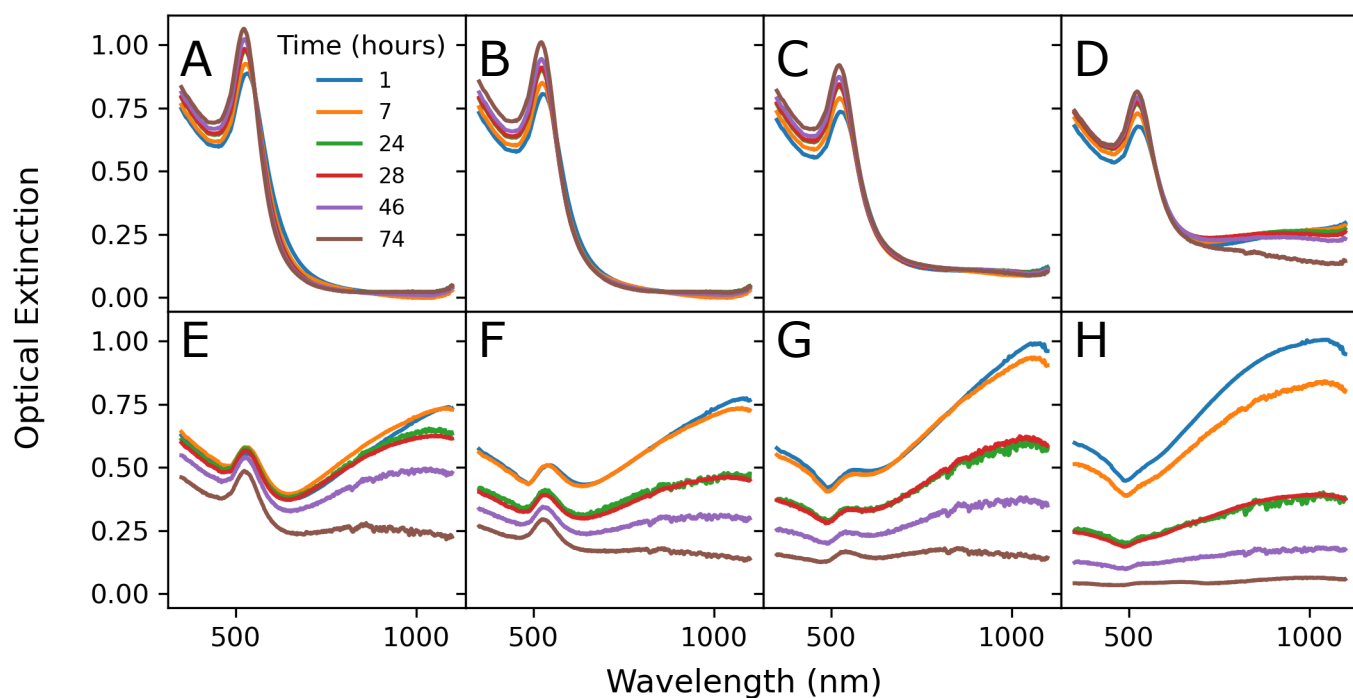


Figure B.8: UV-Vis spectra of samples prepared with Z2 added at different times, and a control with no Z2 added. A, B, C, D, E, F, and G correspond to samples with delay periods of 0, 2, 4, 6, 8, 10, and 12. H is the control sample with no Z2 added.

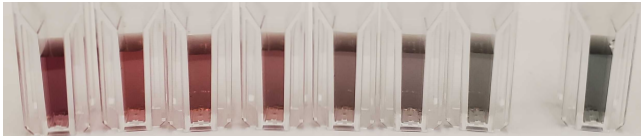
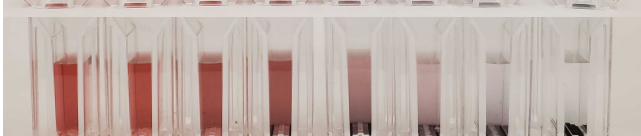
Delay Periods		0	2	4	6	8	10	12	No Z2	
Time (Hours)	1									
	7									
	24									
	28									
	46									
	74									

Figure B.9: Images of samples prepared with Z2 added at different times, and a control with no Z2 added.