Directed Self-Assembly of Polymer-Decorated Nanoparticles

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ABSTRACT
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The use of nanoparticles has grown tremendously in the past 25 years in virtually all industries from alternative energy formulations to drug delivery development and from semiconductor fabrication to cosmetic research. The main goal of this thesis is to shed light on the surface behavior of these universally used components. The thesis explores controlling surface reactivity of nanoparticles to great detail and concludes with a proven method to organize the nanoparticles using self-assembly. The consistent and reproducible organization of the nanoparticles has tremendous value in all industries using nanoparticles in lowering production and processing costs and time.

The surface reactivity of the nanoparticles is found to be well-controlled. In Chapter 3, we show a method to control alkyne reactivity on nanoparticles using a mixture of organosilane monolayers. These surfaces have the unique ability to react with azide-terminated materials through the well-known copper catalyzed azide alkyne cycloaddition ‘click’ reaction. We also put to use a new method to ensure that the mixed surface formed is reactively homogeneous; this novel technique will sure help research endeavors as this has not been demonstrated on surfaces of nanoparticles before.

We extend our surface reactivity studies with the azide-functional surface in Chapter 4. Since we were unable to create a homogeneous surface using the methods described in Chapter 3, we looked to creating a mixed monolayer by kinetic control. This involved fabrication of a bromine-terminated surface and subsequent incomplete substitution of the bromide to azide. This
method of creating mixed monolayers was shown to be universally applicable to surfaces of
different chemical nature and different surface geometries with the same reaction kinetics. We
also contend that this method of creating mixed monolayers is novel and it provides for an
additional in the control of other surface reactivity groups.

Chapter 5 provides the grand finale of the thesis with the intended use of the mixed
monolayers surfaces to organize nanoparticles. We show that by carefully controlling the amount
of polymer chemisorbed onto a surface, the self-assembly behavior of the particles is changed. In
such a method we constructed a phase diagram showing how polymeric coverage controls self-
assembly. We also ensured that the phases formed were indeed equilibrated structures by
studying the formation of the phases under different preparation conditions. We encountered
sheets, rods, and agglomerates and were able to consistently create these structures as well as
study them using image analysis.
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Preface

In the past twenty years, nanoparticles have been focal in the development of many new technologies. Most commonly, nanoparticles are three dimensional objects with all three dimensions below 100nm. The particles may come in a variety of shapes, sizes, and chemical structures and vary widely in their methods of preparation. Particles have been used in virtually all fields and were instrumental in developing novel technologies such as solar panels, semiconductor devices, drug delivery vehicles, and solid state synthesis supports. As most applications call for solid-form products, it is no surprise that the interactions between nanoparticles and polymers have been heavily studied; indeed the common method for delivering nanoparticle-based products is in a polymer matrix. Recent work by our group has elucidated a methodology by which polymer matrices may be excluded while still maintaining their solid forms thereby reducing overall costs associated with preparation of nanoparticle-polymer mixtures.

Nanostructures owe their unique properties to their sizes; they are too small to exhibit bulk-like characteristics, yet large enough to bypass molecular interactions. Yet, when examining and studying nanoparticles, it is apparent that independently the particles do not produce meaningful properties and the character for which they are desired may only be realized when the particles form organized arrays. Unfortunately, a systematic method to bring about nanoparticle organization has been largely absent, despite many theoretical studies pointing to its potential. We present in this work a simple and elegant method to bring about nanoparticle organization through polymer-assisted self-assembly.

Self-assembly is the spontaneous self-organization of nanocomposites brought about by
favorable intermolecular interactions; the resulting structures may range from the nanoscale to mesoscopic to even macroscopic objects and the process is firmly stabilized thermodynamically. Some of the most complex structures are the result of self-assembly: proteins fold and unfold spontaneously as a response to a sole impetus such as temperature or pH; DNA double helix formation occurs spontaneously in cell division due to the interplay between hydrogen bonding and osmotic pressure. It is no surprise, then, that scientists and engineers have sought to control this process in creating materials and devices with long-range ordering. The design of the self-assembly process has been termed directed self-assembly (DSA) and has seen extensive applications in a variety of fields. While many objects indeed undergo self-assembly, efforts have focused on the organization of nanoparticle arrays due to the unique properties they may impart.

In designing nanoparticle-based DSA, colloidal packing forces must be modulated to preclude irreversible agglomeration. This is usually achieved by surface modification with capping agents, known as surface ligands, that may stabilize the particles in a disperse state. These stable colloids are then brought to a metastable state through the addition of a destabilizing effect; the particles are then free to coalesce but in a more controlled manner leading to self-organization. Destabilization may be achieved in one of two ways: (1) through the addition of a destabilizing agent such as a small molecule, or a polymer matrix that is able to interact with the surface ligands; or (2) by use surface ligands that may interact with one another without any intervening third parties. Additive agents have been used extensively in bringing about self-assembly but in all such applications, nanoparticle ordering is very short-ranged owing, in part, to the added interactions arising from the destabilizing agent. In the two-bodied system corresponding to surface-modified nanoparticles, there are three interaction energies to be
characterized whereas in the three-bodied system, there are six interaction energies. These additional interaction make the design of the self-assembly doubly as difficult as when the particles are dispersed alone. Additionally, self-assembly is complicated by the interaction between additive molecules which may agglomerate themselves thereby disrupting the intended organization. Thus, DSA performed this way results in quite short length scales of assembly and poor control of extant sizes.

By eliminating additional interactions and focusing exclusively on the nanoparticle-to-nanoparticle dynamics, large length scale assemblies have been created. It is with no surprise that most DSA experiments done to date have been attempted in this fashion. The degree of organization may be increased further through use of polymeric surface ligands since polymers are known to form long range ordering upon demixing. Indeed, many recent examples have made use of polymeric agents to effect self-assembly.

As we may show later in the contribution, this method of self-assembly comes with quite a caveat: organization arises from the balance between attractive packing forces between nanoparticle surfaces and repulsive osmotic interactions between surface groups. To fully take advantage of this methodology, the surface-bound polymers must be randomly mixed and their coverage carefully controlled. While several literary examples have attempted to control surface coverage, there is a significant lack in exhibiting true random mixing on the surface. In the absence of truly random mixed surface ligands, the particles may form large patchy areas that are prone to aggregation. Random mixing ensures full control of the surface and allows us to design the amount of surface coverage meaningfully. In addition, our control of the surface coverage allows us to explore a variety of self-organization hierarchies that would have been impossible to create otherwise. We will later show two novel methods by which mixing is ensured to be
random on the nanoparticle surface – a degree of control that could not have been achieved previously. The result of our careful surface analysis allowed for the creation of predictable organization architectures strictly based on the amount of the surface that was covered by polymer. We summarize these results in a universal phase diagram and proceed to show that the long-range ordering observed of the nanoparticles are at equilibrium and are independent of any destabilizing effects other than the surface ligands.

This thesis is divided into 5 chapters. Chapter 1 concerns introductory scientific material relevant to the main work and scope of this contribution. Chapter 2 highlights the physical background behind the instruments used to characterize nanocomposites; the chapter also includes an overall synthetic scheme that is common to the main body of the work. In Chapter 3, methods to fabricate and characterize well-formed mixed monolayers on both wafers and nanoparticles are discussed. Chapter 4 deals with kinetic control of click-groups on nanoparticle and wafer surfaces and provides for another route for mixed monolayer fabrication. The all-encompassing Chapter 5 deals with the use of mixed monolayer surfaces to drive self-assembly of nanoparticles. A phase diagram is developed and analyzed carefully showing the trends predicted and seen for nanoparticle DSA.
to my loving wife and best friend

to my family
Chapter 1: Introduction

1.1 Colloidal Dispersions\textsuperscript{1–3}

Two outcomes are apparent when two substances come in contact: they may either mix or stay apart. The degree to which two substances mix largely depends on their relative sizes and the forces between them. When the sizes of the two substances is on the molecular order, the two form a solution, or a homogeneous mixture wherein one substances solvates another through intermolecular interactions. The homogeneity of solutions masks the properties of the dispersed phase in favor of the continuous phase and two seem indistinguishable. Suspensions results when the size of the dispersed phase increases tremendously as compared the continuous phase. Sand in water may be exemplary: water molecules are too small to affect the stability of the micrometer-sized sand particles. As a result, sedimentation of the sand occurs unless external mixing is applied to maintain this heterogeneous mixture.

When nanometer-sized moieties are introduced into a continuous phase, they may stabilize and form homogeneous mixtures termed colloidal dispersions, or simply colloids (from Greek, meaning glue-like). In colloidal dispersions, the dispersed phase is in the shape of small drops or particles and is stabilized by surface interactions. Colloids can form regardless of the physical state of the solvent or the dispersing medium and as such have different nomenclature.

<table>
<thead>
<tr>
<th>Dispersing Phase</th>
<th>Solid</th>
<th>Liquid</th>
<th>Gas</th>
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<tr>
<td>Solid</td>
<td>Solid sol</td>
<td>Sol</td>
<td>Solid aerosol</td>
</tr>
<tr>
<td>Ex: opal</td>
<td>Ex: paint</td>
<td>Ex: smoke</td>
<td></td>
</tr>
<tr>
<td>Liquid</td>
<td>Gel</td>
<td>Emulsion</td>
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<td>Ex: butter</td>
<td>Ex: milk</td>
<td>Ex: clouds</td>
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<tr>
<td>Gas</td>
<td>Foam</td>
<td>Foam</td>
<td>All gases are miscible</td>
</tr>
<tr>
<td>Ex: wood</td>
<td>Ex: shaving foam</td>
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*Table 1-1: Terminology of colloidal mixtures*
Thermodynamics predicts all colloids to be unstable. If pressure-volume work and mechanical work are neglected, and only surface tension work is considered, then at constant pressure and temperature:

\[ dG = \delta W = \gamma d\sigma \]  \hspace{1cm} (1-1)

Since the surface area of the solvent decreases when particles are introduced, it follows that the \( dG \) must follow suit necessitating thermodynamic instability. The stability of colloids lies within the kinetic argument of coalescence, as will soon be apparent; bringing two particles together proves a difficult task kinetically allowing colloids to exist. Table 1-2 summarizes some common attractive potentials between two bodies. While attractive interactions may seem quite pervasive (e.g., dipole-dipole is long-range with \( D^{-6} \) dependence, where \( D \) is the surface-to-surface distance between two bodies) the overall sum of all attractive interactions depends only on \( D^2 \) and is deemed quite ineffective over long ranges. Opposing such interactions are hard sphere interactions and Coulombic interactions, which when taken together with the overall attractive potential generate an overall potential known as the Mie potential:

\[ V = \frac{C_n}{D^n} - \frac{C_m}{D^m} \]  \hspace{1cm} (1-2)

With \( n>m \). The first term on the right represents overall repulsive potential whereas the second term represents overall attractive potential. The well-known Lennard-Jones potential is retrieved for the special case when \( n=12 \) and \( m=6 \). This kinetic argument applies for all continuous media, not just colloids, and additional insight is required to shed light on the exact dynamics of colloidal systems, for which the Mie potential only serves as a crude approximation.
<table>
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<th>Interaction Type</th>
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<tr>
<td>Ion-Dipole</td>
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<tr>
<td>Overall Attractive</td>
<td>$\alpha D^{-2}$</td>
<td>N/A</td>
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*Table 1-2: Intermolecular interactions and their relative strengths.*

One of the earliest calculations to assess colloidal stability was developed by Boris Derjaguin and Lev Landau in 1941 and, independently, by Johannes Verwey and Theo Overbeek in 1948. Contributions from both groups led to the development of DLVO theory, named after the four scientists. The theory predicts the overall potential of a colloidal dispersion as the sum of the overall attractive potentials and the overall repulsive potentials, extending the idea of the Mie potential. The attractive potential is said to stem from van der Waals interactions, whereas the repulsive potential has its roots in the double layer theory.

*Figure 1-1: Double layer around a nanoparticle.*
The electric double layer describes the repulsions felt by particles dispersed in a solvent. Accordingly, when nanometer-sized particles are placed in a solvent of the molecular order (e.g., protein in organic solvent, nanoparticles in water), the particle acquires a charge. This charge is dependent on several factors, including solvent polarity and ionic strength and consists of firmly absorbed species. This acquired charge attracts a loose layer of oppositely charged species which serve to shield the surface charge. These absorbed species are typically ionic in character and are surface-specific. Diagram C1 portrays the double layer: the particle in the center is surrounded by two layers. The mobile layer is bounded between the radius of shear and the Debye screening length whereas the immobile charges are closer to the particles below the radius of shear. The repulsive potential takes the following form:\(^{4}\):

\[
V_{\text{rep}} = 64\pi\varepsilon_0\varepsilon_r \frac{\nu e\psi_1}{4kT} \tanh \left( \frac{\nu e\psi_2}{4kT} \right) \frac{r_1r_2}{r_1 + r_2} \left( \frac{kT}{\nu e} \right)^2 \exp(-\kappa D) \tag{1-3}
\]

Where \(\varepsilon_0\) and \(\varepsilon_r\) are the dielectric permittivity in the vacuum and in a solvent, respectively, \(\nu\) is valence of electrolyte on the surface of the particle, \(\psi\) is the surface potential, defined as the potential at the radius of shear (Figure 1-1), \(e\) is a charge, \(D\) is the distance between two surfaces, \(r_1\) and \(r_2\) are the radii of two particles, \(k\) is the Boltzmann constant, \(T\) is temperature, and \(\kappa\) is the inverse Debye length:

\[
\kappa = \sqrt{\frac{\varepsilon_0\varepsilon_r kT}{2N_A e^2 I}} \tag{1-4}
\]

Where \(N_A\) is Avogadro’s number, and \(I\) is the ionic strength.

The attractive potential between particles in a solvent was much discussed by Israelachvili\(^5\) and others\(^4\) and will be expanded upon in section 1.2 and 1.3.
Figure 1-2: Overall potential of a colloidal dispersion.

Figure 1-2 serves as an illustrative example for the overall potential of a colloidal dispersion (i.e., the sum of the attractive and repulsive potentials), with two regions of interest: R1, signifying coagulation, and R2, signifying flocculation. Coagulation occurs when the attractive potential exceeds the repulsive potential to a great extent and is an irreversible agglomeration. Flocculation, on the other hand, is the reversible agglomeration which occurs at high enough separation. This work exemplifies the significance of each in nanoparticle self-assembly. In chapter 5, coagulation is kept in mind when nanoparticles are synthesized. As such, care must be taken to stabilize the nanoparticles to avoid irreversible agglomeration. In chapter 4, different morphologies of nanoparticles are achieved through self-assembly, a reversible process which is described by flocculation.
1.2 Nanoparticles and Self-Assembly

Self-assembly has been used extensively for the past several decades in both scientific and industrial endeavors. The process refers to quite a fascinating phenomenon where unassociated, disorderly objects come together in some fashion to form organized structures without any external stimuli. These less energetic structures are typically held together by physical interactions and are, therefore, quite reversible. Nonetheless, self-assembled structures have been used across all scientific fields and size regimes. Molecular self-assembly has been used extensively, including the use of silica-based surfactants were used to create an artificial membrane in hollow aluminum skeletons, or to create “container molecules” that can be embedded. Nanoscale self-assembly has been used in the creation of block copolymers phases (See section), for nanoparticle organization, and in understanding the folding and unfolding behavior of biological compounds, such as DNA and proteins. Self-assembly has been also used for macroscopic structures. In keeping with the scope of this thesis paper, discussion will focus on the self-assembly of nanoparticles.

Within the exciting realm of nanotechnology, nanoparticles have emerged as promising platforms for a variety of applications. Nanoparticles have been in use since the time of antiquity, unbeknownst to the users, as dyes, for example in stained windows. With the advance of science, they have quickly been recognized for their interesting properties. Figure 1-3 exhibits the scattering properties of nanoparticles; since their sizes rival the wavelength of light, they are able to scatter it leading to different colors. As may be expected, individual nanoparticles are quite limited in practical applications. The properties which have given nanoparticles their panache are only apparent when they are clustered together in groups. Nanoparticle arrays may be used for the construction of semiconductors, through their plasmon resonance, membranes...
and filtration devices, through their nanovoids, for drug delivery purposes, and for catalysis, to name several applications. The focus of this thesis, as well as many other contributions, is to understand how to organize nanoparticles into predictable structures, with self-assembly of nanoparticles taking the center stage in this publication.

![Quantum Confinement](image)

*Figure 1-3: Size-dependence of light absorption as a function of nanoparticle size.*

The driving force for self-assembly of nanoparticle usually lies in the van der Waals interactions between them. While these interactions are most known for their description of molecular phenomena, they are present between any two bodies. Their strength may be illuminated by the gecko, whose feet cling to surfaces by mere van der Waals interactions. Many mathematical expressions have been derived to explain this interaction, all of which depend on the separation distance between the objects and the objects’ sizes. In the nanoscale, Israelachvili has developed many expressions to describe interactions, the most relevant of which is the intermediate forces between two spherical objects:
\[ E_{vdW} = -\frac{A}{12} \left[ \frac{4r^2}{D^2 - 4r^2} + \frac{4r^2}{D^2} + 2\ln \left( \frac{D^2 - 4r^2}{D^2} \right) \right] \] (1-6)

Where \( r \) is the radius of two nanoparticles separated by a distance, \( D \), apart, and \( A \) is the Hamaker constant, derived for several geometries\(^{18}\) and approximated\(^5\). Indeed, the validity of this expression has been verified experimentally\(^{19}\) through the direct self-assembly of polymer-loaded gold nanoparticles. When nanoparticles are not subject to directed self-assembly, van der Waals forces, alone, govern the organization and lead to the formation of simple hexagonal packing. Care must be taken when preparing such samples so as to avoid agglomeration of the particles.

Shevchenko et al. have found that when different size nanoparticles are employed, the two may form Bravais lattice structures with repeating “unit cells” and that the unit cells are predictable based on the sizes of the nanoparticles\(^{20-22}\). This entropically favorable organization has been extended to include over several dozen superlattice structures. Chapter 5 deals partly with our attempts to fabricate and understand these structures.

More typically, directed self-assembly is employed to effect particle organization. Thus, a repulsive interaction is introduced that counteracts the effects of van der Waals and packing forces and allows the particles to come together in a variety of shapes. Some methods to accomplish directed self-assembly include introduction of surface charges that would counteract the van der Waals interactions as was done by Frankamp\(^{23}\) et al. in functionalizing magnetic nanoparticles with polyamidoamine dendrimers. Once the nanoparticles have assembled, through a mediation between attractive van der Waals and magnetic properties and repulsive charge interactions, any pH change would directly lead to a change in center-to-center spacing of the nanoparticles. Another methodology to effect directed self-assembly was accomplished by
Sidhaye et al., who have functionalized gold nanoparticles with azobenzene derivatives, for which the cis-trans isomerization is controlled by light. The interparticle spacing is thus changed with the introduction of light. The most common way, however, and one which will be used extensively in this publication, involves functionalizing nanoparticles with large species, such as polymers or proteins. These large groups provide repulsive interactions due to the tremendous osmotic pressure involved in bringing two of them into the same space. Chapter 4 largely deals with the effects of polymeric coverage on nanoparticle. Unlike previous research in the field, the goal is to understand the forces involved by controlling the polymeric coverage on the nanoparticles. Nonexistent coverage leads to self-assembly, whereas partial coverage leads to strings and sheets.

1.3 Self-Assembly of Polymers

The self-assembly of polymer-decorated nanoparticles may be likened to the phase behavior of block copolymers since surface-tethered polymers are covalently attached to the particles. Whereas two incompatible materials may separate upon contact, as in the case of immiscible liquids, two chemically bound species may not. The separation of two unassociated species is referred to as macroseparation to distinguish from the microseparation experienced by block copolymers, a separation that must occur at the molecular level. In an attempt to minimize their energy, the attached polymers form predictable phases with long range ordering. The organization of nanoparticles may thus be envisioned by treating them as a block constituent.

To understand the phase behavior of block copolymers, it is instructive to examine the macroseparation observed upon mixture of homopolymers. This extension of solution theory was independently proposed by both Paul Flory and Maurice Huggins in the 1940s. This
revolutionary theory paved the path for the better understanding of polymer behavior; the details of which are summarized herein.

Four factors may be isolated that directly affect the mixing behavior of polymers: identity, architecture, length, volume fraction of the components. Whereas some applications call for mixture of the polymers, many others are based on the separation of the two into phases. Thus, it is important to choose the identity of the polymers mixed. Should mixing be desirable, two similar polymers may be chosen such as PEO and PVP, which may both undergo hydrogen bonding. In the case demixing is required, the two polymers chosen should be of conflicting chemical nature. As a general guideline, one may use the Hildrebrand solubility parameters in choosing the polymeric species. A more rigorous treatment may take into account the Flory-Huggins interaction parameter as defined:

\[ \chi \cdot kT = e_{AB} - \frac{e_{AA} + e_{BB}}{2} \]  

(1-6)

Where \( e_{ij} \) is the contact energy between component i and component j. A positive interaction parameter is said to be attractive between the two constituents whereas a negative value would predict complete separation of the constituents. It is instructive to note that the interaction parameter is in the order of zero for two nonpolar polymers since all interaction energies of the same magnitude.

Even when two polymers have quite conflicting chemical natures, intrinsic high viscosity and slow reptation will restrict their movement and separation may never be realized. Thus, in addition to their identity, the length and architecture of the polymers must be considered. Longer or branched ones may never reach the equilibrated state and be “frozen” in place. In the case where mixing is desirable, one should generally choose shorter, less branched polymers.
A phase diagram for polymer mixtures may be constructed that takes into account all four factors. In order to simplify the presentation, phase diagrams are usually constructed as functions of polymer volume fraction, or \( \varphi \). Typically, the ordinate is a combination of the polymer length, \( N \), and the interaction parameter, whereas the molecular architecture is not explicitly defined. As may be expected, a good starting point to define the phase diagram is the Gibbs free energy:

\[
\frac{dG}{kT} = dH_{mixing} - TdS_{mixing}
\]

For the polymer mixtures, the enthalpy and entropy may be defined as follows:

\[
dH_{mixing} = \varphi_A \cdot (1 - \varphi_A) \cdot \\
\text{and}
\]

\[
dS_{mixing} = \frac{\varphi_A \ln(\varphi_A)}{N_A} + \frac{(1-\varphi_A) \ln(1-\varphi_A)}{N}
\]

Where \( N_A \) is the degree of polymerization of polymer A, \( \varphi_A \) is the volume fraction of polymer A. Note that when \( N=1 \), the expression reduces to regular solution theory.

Three conditions are defined that allow for the construction of a phase diagram:

Equilibrium: \( \frac{\partial G(\varphi_A)}{\varphi_A} = \frac{\partial G(\varphi_A)}{\varphi_A} \)  

Stability: \( \frac{\partial^2 G}{\partial \varphi^2} = 0 \)  

Criticality: \( \frac{\partial^2 G}{\partial \varphi^3} = 0 \)

These lead to, in the case of similar polymer lengths, Figure 1-4.
Figure 1-4: Generic phase diagram based on thermodynamics conditions dictated by equations 1-10 through 1-12. Figure shows areas of system stability as a function of relative amounts.

Two mechanisms dominate phase separation and are represented in the phase diagram. Line y-y” represents nucleation and growth. Initial droplets of a second phase begin to form via diffusion in much the same way as melting or freezing occur (y-y’). Once nucleation reaches a certain size at y’, growth continues via Ostwald ripening, or the coalescence of the droplets (y’-y’”). The other mechanism by which separation may result is through spinodal decomposition (x-x’). Unlike nucleation and growth, no energy barrier needs to be overcome. Phase separation occurs spontaneously. Initial size of the spinodal structure is controlled by the length of the line x-x’.

In order to extend the Flory-Huggins model to block copolymer systems, we must change the equilibrium condition and set the lefthand side equal to zero, since block copolymers are
single component systems. A typical phase diagram for block copolymers is presented in Figure 1-5.

Studies of polymer phase behavior have been numerous. Almgren, et al.\(^\text{30}\), have studied the phase behavior of PEO-PPO-PEO structures in aqueous solvent and found no structures form when the weight content of the polymeric species is below 25%. The group found a significant increase in viscosity at higher temperatures and indicated that phase transitions occur more gradually than theoretically predicted. In a study by Chen and Hoffman\(^\text{31}\), PAA-PNIPAAm blocks were subjected to pH variation and a phase diagram was plotted. Early studies were conducted to exhibit the potential of this block in drug delivery applications, since the pH of various organs throughout the body is inherently different. Khandpur et al.\(^\text{32}\), found five phase formations for polyisoprene-polystyrene blocks with lower molecular weight structures only able to support two phases. This result is quite contradictory with theoretical calculations.

In our own experiments we chose several systems corresponding to regions where microphase separation is predicted. Poly (n-butyl acrylate) of three sizes were chosen to functionalize iron oxide and silica nanoparticles of different sizes. For each polymer-grafted nanoparticle system, the relative sizes are fixed as well as the interaction parameter; this leads to an apparent paradox if Figure 1-4 is consulted: the system seems to be dependent on one variable, alone, f. If we take one of these horizontal lines, however, we may see that this apparent one-dimensional dependency may be resolved into two components. Define f as:

\[
f = \frac{N_{\text{polymer}}V_{\text{polymer}}}{N_{\text{polymer}}V_{\text{Polymer}} + V_{\text{n nanoparticle}}} \tag{1-13}\]

Rearranging:

\[
N_{\text{polymer}} = \frac{V_{\text{n nanoparticle}}f}{V_{\text{polymer}}(1-f)} \tag{1-14}
\]
Figure 1-5: Phase diagram resolved into two components. The diagram shows clear phase behavior (where phases are indicated as the hyperbolic areas between graphs) where polymer-loading is plotted a function of the size ratio of particle ($R$) to polymer ($R_g$).

Thus a phase diagram as in Figure 1-5 may be plotted where the abscissa corresponds to a non-dimensionalized size ratio of the polymer and the nanoparticle and the ordinate as the number of polymers grafted on the nanoparticle. All three variables are experimentally measureable with sigma calculated from thermogravimetry, $R_g$ calculated from GPC and NMR data, and $R$ measured from TEM and DLS data. Additionally, this phase diagram may combine several horizontal lines in Figure 1-4 as long as the interaction parameter remains a constant.
between the polymer and the particle. Previous research in the field displays the phases in a manner related to Figure 1-5, and our data will follow this plot, as well.

1.4 Self-Assembled Monolayers

Self-assembled monolayers (SAMs) are molecular groups that easily absorb onto surfaces and form well-organized coatings around them. SAMs typically form by horizontal polymerization on surfaces such that the surface coverage is constant with a thickness of one molecule. Preparation of SAMs on surfaces is thus quite painstaking, with necessary precautions needed to preclude vertical polymerization, inconsistent coverage, and other possible complications. A variety of surface characterization techniques, which will be discussed in Chapter 2, have been developed to measure the quality of SAMs.

SAMs consist of a tail end with high affinity towards the surface they are to modify and a head group that imparts a certain character onto the surface. The head group may be reactive, thereby allowing additional layers to immobilize onto the surface, or it may be surface-active, changing surface properties such as wettability and adhesion. While SAMs may be prepared in a variety of fashions and on a variety of substrates, discussion will be limited to the preparation of organosilane-based SAMS from the liquid phase onto metal oxide nanoparticles and silicon wafers.

A generic organosilane is shown in Figure 1-6. Four groups surround the central silicon atom, one of which is organic in nature and the other three are typically anchoring sites that interact with a surface. Alkoxy and halogen groups are common anchoring moieties due their excellent leaving ability. A bond is formed between the silane and the substrate upon the removal of the anchoring sites. The number of anchoring groups may vary from silane to silane, but
previous results have shown that a well-formed monolayer takes shape for three leaving groups. However, many monolayers have been formed with one or two groups\textsuperscript{33}. The identity of the leaving group affects the reaction speed and how quickly the SAM forms with chlorine containing groups requiring a tenth the time to form than ethoxy anchoring groups\textsuperscript{34}.

The leaving groups are also susceptible to degradation in the presence of water; such conditions prior to surface functionalization yields polymerization of the silanes such that reduced surface anchoring occurs. However, it was found that some water concentration on the substrate is crucial to the formation of the monolayer\textsuperscript{35}. In the absence of water, the SAM does not form, whereas in the presence of too much water, multilayer formation persists. The solvent choice is thus crucial due to its inherent water content with toluene and benzene having the optimal amount of water to support SAM formation\textsuperscript{36}.

\begin{figure}[h]
\centering
\includegraphics[width=0.3\textwidth]{organosilane.png}
\caption{Typical organosilane with three leaving groups, denoted by R and typically –Cl, -OMe, or OEt, and an organic branch where A may be any functional group.}
\end{figure}

The organic portion of the silane usually contains the character which the surface takes upon modification. It was found that the length of the organic group changes the formation of the monolayer with shorter groups forming less dense monolayers than longer ones\textsuperscript{37}. Additionally, the polarity of the organic arm also affects the monolayer formation since polar groups may
interact with the substrate with amino-capped silanes, for example, forming SAMs more readily\textsuperscript{38}.

All substrates used in this publication have native hydroxyls on their surfaces with physisorbed water molecules. In an extensive study of silicon-based surfaces, the number of hydroxyl groups was found to be 5 for every nm\textsuperscript{2}, a number which is usually taken to be universal\textsuperscript{39}. Iron oxide has not seen such treatment but may usually be taken to have 8-10 hydroxyls for every square nanometer\textsuperscript{40}. This hydroxyl density allows one to calculate the amount of silane to be added to the surface; indeed iron oxide surfaces form denser monolayers as will be discussed later.

SAMs have been quite prevalent in functionalizing various surfaces across many disciplines. Organosilane SAMs have been on the surface of iron oxide as a precursor for magnetically-induced intercellular DNA transport\textsuperscript{41}. Silanes were uses to increase the wear resistance of alumina materials with applications in dental science\textsuperscript{42}. They have also been used to modify the dispersibility of titania in water\textsuperscript{43}. 
1.5 Copper Catalyzed Azide-Alkyne Cycloaddition (CuAAC)

One unifying tool in use throughout this work is the copper catalyzed cycloaddition reaction between a terminal alkyne and a terminal azide (CuAAC – Copper catalyzed azide-alkyne cycloaddition). This reaction, first studied by Huisgen in the 1960s\textsuperscript{44,45} has seen a surge in popularity after Barry Sharpless has included it in a breakthrough publication\textsuperscript{46}, one that led to a Noble prize award in chemistry in 2001. The concept behind the original Sharpless publication was to define a chemical toolbox that would allow researchers to emulate what nature does best: synthesize large molecules from smaller modular units. One may look to a variety of biological reactions as examples as DNA is made up of nucleic acid and proteins are assembled amino acids. Sharpless et al has included criteria for synthetic reactions which mimic this concept and branded them as click reactions. Click reactions are all modular, physiologically safe and stable, are orthogonal to other reactions, and occur to a great extent. Due to these demanding criteria, click reactions have seen tremendous use ranging from drug delivery, surface anchoring, and polymer synthesis.

While the list of click reactions is lengthy, perhaps the crowning jewel\textsuperscript{47} is the reaction between terminal azides and terminal alkynes, a reaction which has seen tremendous usage in this work as well as others. More specifically, CuAAC has been used to fabricate a biosensors on gold nanoparticles\textsuperscript{48}, synthesize HPLC stationary phase beads\textsuperscript{49}, create synthetic carbohydrates\textsuperscript{50}, fabricate organogels through crosslinking units such as diazides and dialkynes\textsuperscript{51}, and bioconjugating PEO to silica nanoparticles for drug delivery vehicle synthesis\textsuperscript{52}.

The most attractive features of the CuAAC reaction is its orthogonality and its very high yield: near full conversion is obtained when the reaction is catalyzed by copper in the presence of a polar solvent. The mechanism of the reaction has been studied extensively\textsuperscript{53} and is quite well
understood. In this work, surfaces are functionalized with alkyne groups to preferentially absorb azide-terminated polymers, a situation which was proven quite effective with relative ease.

The reactivity of azides and alkynes on nanoparticle surfaces is further explored in this work in the absence of copper catalyst. While preliminary data points to the nonlabile nature of this reaction in the absence of copper, it is thought that at elevated temperatures and pressures, the reaction may proceed. Indeed, previous work points to successful triazole formation for smaller species under pressure\textsuperscript{54} or under heat\textsuperscript{55}.

\begin{figure*}[h]
\centering
\includegraphics[width=\textwidth]{13dipcoadd.png}
\caption{Schematic of the 1,3-dipolar cycloaddition reaction.}
\end{figure*}


Chapter 2: Experimental Techniques

Part I: Instrumental Analysis

A variety of instruments have been used to collect data and to infer the structure and order of the nanoconstructs presented later in this manuscript. This portion examines the physics governing some of the more prominent instruments used for data collection and analysis, we refer the reader to several of the references for additional information.

2.1 Electron Microscopy

Electron microscopy is a powerful tool in the visualization and characterization of nano-sized objects. The ability to view such small objects revolutionized both physical and biological sciences and the technique proves useful in a variety of applications from viewing crystal defects and grain boundaries to visualizing cells. In our work, transmission electron microscopy (TEM) is used, which allows a visualization of polymer-decorated nanoparticles into a variety of morphologies (Chapter 4) and to check the quality of synthesized nanoparticles (Chapter 5). TEM directs electrons through a sample and the formed image is the result of the electrons that were not absorbed by the sample. Scanning electron microscopy, on the other hand, retrieves the image through electrons reflected from a sample.
Figure 2-1: Schematics of the components of a transmission electron microscope.²

The resolvability of electron microscopy is owed much to an increase in the diffraction limit as compared with traditional optical microscopy. Diffraction limit is the minimum distance, \( d \), from which two objects seem distinct to an observer and is defined as:

\[
d = \frac{\lambda}{2\sin\theta} \quad (2-1)
\]

Where \( n \) is the refractive index, \( \theta \) is half the angle of the cone of light that transmits through the sample, and \( \lambda \) is the wavelength of the source. The wavelength of a photon is defined as:

\[
\lambda_p = \frac{hc}{E} \quad (2-2)
\]

The wavelength of an electron is defined as:

\[
\lambda_p = \frac{h}{\sqrt{2m_eE}} \quad (2-3)
\]
Where \( h \) is Planck’s constant, \( c \) is the speed of light, \( E \) is the energy of the light source, and \( m_e \) the mass of an electron.

Assuming a source energy of 1 eV and \( \theta = 30^\circ \), the two wavelengths are \( \lambda_p = 1242 \text{ nm} \) and \( \lambda_e = 1.22 \text{ nm} \) exhibiting the tremendous superiority of electron microscopy in resolvability.

Quite generally, electron microscopes are able to resolve objects separated by several nanometers, and even picometers, whereas optical microscopy has a limit on the order of a micrometer. To further understand the instrumentation used in TEM, a basic introduction to optical microscopy will follow.

Microscopy is employed to enlarge small objects to the naked eye or to a computer. This is simplistically achieved by placing a specimen between a lens and a light source. Collimated light, or light whose rays are all parallel, pass through a specimen and onto a lens; the light is then refracted and an inverted image forms at the point of convergence of the refracted rays. The magnification of the image depends on the relative placements of the specimen and the lens and the material from which the lens is made. In the case when the refracted light rays diverge, a virtual image is formed between the lens and the specimen. Typically, microscopes include additional lenses for added accuracy and magnification: the condenser and the eyepiece. The condenser is a moveable series of lenses between the specimen and the objective lens whose function is to focus the light from the source and onto the specimen, reduce glare, and to increase the numerical aperture. The condenser is tied with the idea of focusing: if the condenser is modified to allow a different amount of light to pass through the specimen, the distance between the light source and the specimen may need to be adjusted to focus the image. An additional series of lenses, called the eyepiece, is placed between the objective lens and the viewer to magnify the image further and to cast the image onto the focal plane, or the plane viewable for
the user. The eyepiece may be replaced with a camera such that the image may be viewed electronically; in this case, the camera must be placed directly at the focal plane.

A schematic of a transmission electron microscope is seen in Figure 2-1. The main differences between TEM and an optical microscope are the source, which is electrons for TEM and photons otherwise, and the eyepiece. Instead of an eyepiece, two lenses and one aperture are used to project the final image onto plane viewable for the user. The objective aperture, between the specimen and the intermediate electron lens, acts to select electrons that will contribute to the final image and to improve its contrast. The intermediate electron lens focuses an initial image or a diffraction pattern onto a back focal plane from which the projector lens is used to magnify the image onto the final viewable plane.

2.2 Ellipsometry

Ellipsometry is one of the most powerful techniques in the study of surfaces and thin films. It is able to measure complex optical and surface properties of materials while remaining contactless and nondestructive. It has been used extensively to measure reflective indices, surface roughness, and surface thickness and may do so by measuring the phase difference of light reflected from a material. Since the instrument measures the difference in the polarization of light, as opposed to their absolute values, it is capable to transcend the diffraction limit and yields reproducible and highly accurate results. Meaningful measurements may only be obtained if the material is shown to be optically homogenous and isotropic.

Figure 2-2 demonstrates the setup of the ellipsometer. A light shines onto a sample and the reflected light is measured by the detector. Since light travels through at least two media, the use of Fresnel equations is necessary to describe the scenario. Briefly, when the incident light
hits the thin film of refractive index n₂, some of the light is reflected, whereas some is refracted. Thus, three angles are formed: \( \theta_i, \theta_r, \) and \( \theta_t \). Reflectivity, R, is defined as the fraction of light reflected at an interface, whereas transmittance, T, is the fraction of light that is refracted at an interface. Light is resolved into its two constituents: s-polarized and p-polarized and we may define the \( R_s \) as and \( R_p \) as:

\[
R_s = \frac{n_2 \cos \theta_1 - n_1 \cos \theta}{n_2 \cos \theta_1 + n_1 \cos \theta_2}
\]

(2-4)

And

\[
R_p = \frac{n_1 \cos \theta_2 + n_2 \cos \theta}{n_2 \cos \theta_1 - n_1 \cos \theta_2}
\]

(2-5)

The Brewster’s angle is the angle at which all light is refracted and no light is reflected and \( R_p = 0 \).

We define \( \rho \) as the ratio of the two reflectance values and assume it to be a complex number of the form \( n - ik \) (where k is the extinction coefficient and n is the refractive index); this is often the case for polymeric materials. The expression becomes complex and may be written as:

\[
\rho = \frac{R_p}{R_s} = \tan \psi e^{i\delta}
\]

(2-6)

While \( \delta \) and \( \psi \) will always be measured correctly, if the model is not set up well the calculated properties will be erroneous. It is important, therefore, to set up the correct model for the examined materials.
2.3 Infrared Spectroscopy

Fourier-transform infrared spectroscopy (FT-IR) has been used extensively in research. Its use comes at no surprise given its ability to not only identify unknown molecules, but to also quantify relative amounts within a mixture. As such, FT-IR has been at the forefront in identifying unknown compounds and monitoring kinetics.

In a typical experiment, infrared radiation shines onto a sample on its way to a detector. The sample molecules absorb the radiation and exhibit a net change in their dipole moments, manifested by an amplitude change in their molecular vibrations. The instrument detects the absorption within the molecules and reports the associated wavelength. This wavelength corresponds to a bond between molecule A and molecule B according to:

\[ \nu = \frac{1}{2\pi c} \sqrt{\frac{k}{\mu}} \]  

(2-7)

Where \( \nu \) is the absorbed wavelength in cm\(^{-1} \), \( c \) is the speed of light in cm sec\(^{-1} \), \( k \) is the spring constant of the A-B bond (generally, on the order of \( 10^0 \)-\( 10^1 \) N cm\(^{-1} \)), and \( m \) is the reduced mass of the two atoms (\( \mu = \frac{m_A m_B}{m_A + m_B} \)). Thus, the wavelength depends not only the type of bond
(single, double, etc.) but also on the identity of the bonded molecules, exhibiting the power of infrared spectroscopy in identifying molecules. An infrared spectrum reports the absorbed wavelengths in the forms of peaks and the resulting diagram is called a spectrograph.

The number of infrared peaks of a molecule depends on the number of vibrations within it, with larger molecules having more complicated spectra than do smaller ones. Generally, a linear molecule has $3N-5$ vibrations whereas a nonlinear molecule has $3N-6$ vibrations (where $N$ is the number of molecules in the molecule). Several complications arise that may reduce or augment the number of peaks of a molecule. In the case of symmetry, no dipole moment is observed reducing the vibrational freedoms of the molecules, for this reason nonpolar diatomic molecules may not be detected by infrared spectroscopy. Additionally, when two unassociated peaks absorb in the same region, the two peaks may overlap necessitating a method for resolving the two groups, previous research in our group has exemplified a method to do just that.

Figure 2-3: Schematics of the components of a FTIR. The left hand figure shows the function of the beamsplitter; the right hand figure exhibits how the moving mirror changes the magnitude of the resulting beam.
FT-IR instruments employ the basics of infrared spectroscopy but owe their quantitative superiority to their design. A laser source is first split into two paths, as seen in Figure 2-3. One of the beam goes to a fixed mirror, whereas the other to a moving mirror. The reflected beams recombine at the beam splitter and go on to radiate the sample and then to the detector. The moving mirror allows for variation in path length. If the moving mirror moves at a constant, known speed, outputs such as that in Figure 2-4a are typical in the absence of a sample, exhibiting interference pattern of light. Note that Figure 2-4 is a one-dimensional case whereas FT-IR handles three-dimensional interference patterns. In the presence of a sample, peaks are reduced due to absorbance as in Figure 2-4b. Fourier transform of the two graphs allows for a combined frequency domain spectrum.

![Interferograms of infrared windows](image)

*Figure 2-4: Interferograms of infrared windows in the absence of a sample (a) and in the presence of a sample (b). The spectrum of the sample is obtained by subtracting the two and performing a Fourier transform.*
2.4 Light Scattering

Light scattering is a powerful technique that allows characterization of the dispersed phase in suspensions. The technique is chiefly concerned with quantifying both the size and the morphology of the dispersed phase.

A typical light scattering schematics is presented Figure 2-5. An electric field originating in a laser is shining onto the scattering volume, which contains the sample. The molecules within the sample get charged and accelerate and the incident light is said to polarize the medium. Since accelerating charges radiate this polarization effects light and field emissions which may be measured by the detector. The molecules in the scattering volume are in continuous motion due to vibrations, rotations, and translations, and this leads to constant fluctuations in the scattered electric field. It is these fluctuations that provide information about the sample.

\[
E_i(r,t) = \eta E_0 e^{(k_i t - \omega_0 t)}
\]

\[
e(r,t) = \varepsilon_0 I + \delta \varepsilon(r,t)
\]

*Figure 2-5: Schematics of a typical light scattering instrument.*

Specifically, the fluctuations in the dielectric constant of the medium are measured. Due to Brownian motion, the dielectric constant is nonuniform across the medium. Define \(q = k_i - k_f\) where \(k_i\) and \(k_f\) are the wavelengths of the incident and scattered light, respectively. The wavelengths point in the direction of propagation of the incident light \((k_i)\) and the wave that reaches the detector \((k_f)\). Since scattering occurs due to variations in the dielectric constant, a
model that predicts these calculations is the focus of meaningful measurements. We may define q as:

\[ q^2 = k_f - k_i \left( k_f - k_i \right)^2 = 4k_i^2 \sin^2(\theta/2) \]  

Such that:

\[ q = 2k_i \sin(\theta/2) = \frac{4\pi n}{\lambda_i} \sin(\theta/2) \]  

\[ \mathbf{q} = \mathbf{k}_t - \mathbf{k}_f \]

\[ I(q, \omega_f, R) = \left( \frac{I_0 k_f^4}{16\pi^2 R^2 e_0^2} \right) \frac{1}{2\pi} \int_{-\infty}^{\infty} < \delta e_{ij}(q,0) \delta e_{ij}(q,t) > e^{i(\omega_f - \omega) \tau} dt \]  

\[ \delta e_{ij}(q,0) \delta e_{ij}(q,t) > e^{i(\omega_f - \omega) \tau} dt \]  

\[ (2-10) \]

Such that,
Thus, low wavelengths are more easily scattered, explaining the color of the sky.

In our work, dynamic light scattering is used to study sizes of nanoparticle dispersions both commercial and synthesized (Chapter 5). In Chapter 4, light scattering is used to complement TEM in quantifying the resulting geometries of polymer-decorated nanoparticles.

\[ I_f \propto \frac{k_f^4}{R^2 \lambda^4} \]  

\[ (2-11) \]

2.5 Thermogravimetric Analysis

Thermogravimetric analysis (TGA) is a technique used to probe thermal properties of materials. The instrument heats up samples while recording their weights producing a mass-time plot called a thermogram. TGA can be used to determine decomposition temperatures of proteins or polymers, to determine the organic or inorganic content of materials, to establish oxidation and reduction transitions, and to monitor kinetics. TGA is the most extensively used instrument in this publication and various calculations will be included in the various chapters.

The instrument consists of a very delicate balance, called a thermobalance, which is able to detect microgram changes in samples ranging from 1 mg to 1 g. This balance is housed within the instrument with the sample itself being stored within the furnace for the duration of the experiment. A thermocouple is employed to monitor temperatures, which can range from room temperature to 1000°C. A purge system is also included to allow the sample to be immersed in a variety of artificial atmospheres.

Samples are usually loaded using specially designed pans. Disposable aluminum pans are used for experiments not exceeding 600°C and provide for an alternative to platinum pans which
may withstand higher temperatures but are far more expensive. Care must be taken not to ramp the temperatures at high rates to avoid internal combustions. While any atmosphere may be employed, oxygen and nitrogen are typical choices for TGA experiments. Nitrogenous atmosphere proved useful in reduction and degradation studies, whereas oxygen is reserved for combustion and oxidation.

Weight calibrations are done using standard weights. To calibrate the thermocouple, magnetic materials are heated using a magnet at the base of the furnace. Once the material passes its Curie temperature the base magnet drops and the temperature is calibrated to the reported Curie temperature. These calibrations are extremely important for useful data to be gathered and are typically performed once a month.

**Part II: Synthetic Techniques**

Nanoparticle synthesis and characterization are common to all aspects of this thesis. We therefore focus our discussion on the theory behind nanoparticle synthesis and modification and proceed to present the synthetic routes taken to establish well-formed nanoparticle-polymer nanocomposites.

Any attempt to functionalize nanoparticles requires thorough understanding of their surface properties and size distributions. Such properties not only control particle stability in various solvents and the ability to be functionalized, but they are also critical in self-organization. As was expounded upon in the introductory chapter, intermolecular forces of nanoparticles are governed by size and surface considerations. Even slight change to the nanoparticle size could cause irreversible agglomeration thereby altering the circular shape of the
particle and increasing its size severalfold. Initial attempts to functionalize nanoparticles involved these considerations eventually leading to controlled synthesis of iron oxide nanoparticles and to a reliable commercial source for silica nanoparticles.

2.6 Nanoparticle Synthesis Theory

There has been increased interest in the use of nanoparticles for a variety of applications. Due to their small sizes and high surface areas, nanoparticles interact quite strongly with species around them. It comes as no surprise, then, that nanoparticles have been extensively used to form drug delivery vehicles with high loading of drugs or as product additives with a pronounced property, the reader is referred to Chapter 1 for detailed overview of applications. Synthesis of nanoparticles is prone to several problems that may lead to undesirable effects. Akin to polymer synthesis, nanoparticle formation occurs when monomers come together to form longer chains until a critical concentration is reached, after which nucleation commences; this process often causes disperse products with a large size distribution. Additionally, during nanoparticle synthesis, stabilization agents must be introduced to ensure the particles stay separated in solvent and do not form agglomerates.
Nanoparticle formation has begun early in the 1960s and continues to be perfected to this day. Early work by Stober\textsuperscript{5} has directed the field with the first ever monodisperse synthesis of silica. This contribution took into account controlled particle growth by electronic stabilization but was only able to produce particles in the order of several hundred nanometers in diameter. Stober has inspired a variety of groups to study silica synthesis, with, perhaps the best known example, work by Bogush, et al.,\textsuperscript{6} that extended Stober’s work to show the need for a systematic synthetic route to allow for reproducing monodispersity. Indeed, work in the 1990s and 2000s have produced elegant and simple methods to produce monodisperse silica that take into account steric and electronic considerations into modifying the Stober method. A well-known example was through the use of L-arginine to mediate the growth of the particles sterically and to prevent their flocculation, electronically.\textsuperscript{7} This route was replicated in our studies, as well, and will be discussed at length. Researchers around the globe are still hard at work to try to extend the monodispersity of silica synthesis to smaller sizes. In addition to silica, other researchers have expanded routes for synthesis of metal oxide nanoparticles such as iron oxide\textsuperscript{8} and alumina\textsuperscript{9} as
Nanoparticle growth may be seen schematically in Figure 2-7, which is a reproduction of the La Mer diagram\(^{10}\). As a monomer is introduced to the solvent of choice, growth slowly occurs with oligomers constantly forming and dissolving, this prenucleation period terminates once the concentration reaches a critical value \(C^\ast_{\text{min}}\) at which point nucleation commences. At this point, some growth occurs at the same time as nucleation and once the nucleation rate overtakes the growth rate, the concentration begins to dip until it reaches \(C^\ast_{\text{min}}\) again, at which point any additional growth occurs due to diffusion of monomers onto the nuclei. The process terminates when the concentration reaches its solubility value. In order to form monodisperse systems, the nucleation rate must be much higher than the growth rate during the nucleation period. Any lag in nucleation introduces the possibility for uneven growth. This may be achieved in a variety of ways such as temperature variation or a change in solvent.

Once the particles are formed, irreversible agglomeration must be avoided so that individual particles are still prevalent. This may be achieved by steric or electric stabilization. Nanoparticles often attract one another in the absence of the electric double layer (see Chapter 1). To ensure the double layer still exists, a system must be chosen with low ionic strength and a pH value consistent with the double layer. For example, silica surfaces lose their double layer at a pH of 7. Indeed, synthesis of particles is often carried in highly acidic environments as in the case of the Stober method.

Additives may be included to sterically stabilize the particles so as to ensure they never approach one another. Iron oxide nanoparticles are often synthesized with large carboxylic acids such as oleic acid AND, whereas copper oxide particles may be synthesized with a coverage OF. Permanent stabilization sets in when the particles are functionalized with organosilane groups.
Whether sterically or electrically stabilized, nanoparticles are often limited to the solvent from which they were synthesized.

An outline for the synthesis of iron oxide and silica nanoparticles will follow. Several routes were taken in an attempt to create monodisperse particles that may be functionalized by organosilanes of choice. In the case of silica nanoparticles, this situation was found to be unattainable as silica was required to be kept in aqueous environment to preclude agglomeration. However, in the case of iron oxide, a synthetic route is presented that allows surface functionalization with organosilane groups; this allowed us to meaningfully use the particles towards a variety of applications.

2.7 Iron Oxide

2.7a Problems with Commercial Sources

Commercial nanoparticles have been employed to try to establish a silane modification protocol. In the case of silica, inconsistent coverage has been observed with silane surface density ranging anywhere between 0.1 groups per nm$^2$ to 5 groups per nm$^2$. In the case of commercial iron oxide, silane coverage was rarely obtained, attributable to what was hypothesized to be oleic acid coverage on the particle surface. It was found that even when traditional methods of ligand exchange were employed, surface density rarely exceeded 0.5 groups per nm$^2$, far less than the 2-3 groups per nm$^2$ ensuring proper monolayer coverage$^{11-13}$. Synthetic route for iron oxide was then developed to avoid complications arising from commercial sources.
2.7b Synthetic Route of Monodisperse Nanoparticles

Iron oxide nanoparticles were prepared by a variation of the Massart Process\textsuperscript{14,15}. 3.9g Iron (II) chloride and 10.9g iron (III) chloride were dissolved in 435mL of deionized water in a two-neck round bottom flask. To ensure the salts were completely dissolved, the flask was ultrasonicated while stirred at 500rpm using a mechanical stirrer. The solution was subsequently heated to 80°C and kept under nitrogen after which 45mL of ammonium hydroxide was added quickly to afford a black precipitate that constituted magnetite nanoparticles. The stirring speed was then increased to 850rpm and the nanoparticles were let to grow for 15 minutes. The newly formed magnetite nanoparticles were magnetically separated from the solvents while being kept from contact with air. An oxidizing solution of 2M nitric acid (50mL) was subsequently added to the particles under vigorous agitation to afford a brown powder. The partially oxidized particles were separated by centrifugation after which an oxidizing solution of 0.33M iron (III) nitrate nonahydrate, previously heated to 100°C, was added. The system was allowed to vigorously mix for fifteen minutes, followed by centrifugation. The particles were then washed, once more, with 2M nitric acid to complete their oxidation at which point the particles appeared to be light brown. The newly formed maghemite nanoparticles were washed three times with acetone to remove residual nitrate ions after which the particles were dried and functionalized immediately. Particles were measured to be 10nm in diameter (TEM) and 9nm in diameter (DLS). The surface area was determined to be $130\pm0.2\text{m}^2\text{/g}$ (BET).
2.8 Silica

2.8a Problems with Commercial Sources

Silane-based SAMs have been extensively fabricated on the surface of both titania and silica nanoparticles and well-studied protocols for surface functionalization are widely available. Any attempts to duplicate these protocols on our commercial systems was futile and silane coverage often did not take hold; when silanization did occur, coverage was rarely consistent and never ideal, ranging anywhere from 0 to 8 groups per nm$^2$ despite constant initial ratios. These coverage values were obtained through thermogravimetric analysis. FT-IR has shown the lack of peaks associated with the silane; stretches for the alkyne, carbonyl, and silanol groups were all absent or, when present, relatively low compared to hydroxyl groups that define the unfunctionalized surface.

The nanoparticles were therefore characterized by TEM and shown to be irreversibly agglomerated and not circular in shape. Further, even for the case of some lone particles, size variation precluded their use for our applications. A quest for better suppliers commenced leading to the eventual use of Nissan Chemical nanoparticles.

2.8b Proposed Synthetic Route – Phase Transfer

Silica nanoparticles were synthesized via a modified Stober route. The nanoparticles formed were very monodisperse with a radius of 11.5nm with a typical size distribution shown in Figure 2-8. During the synthesis of the particles, TEOS slowly polymerizes; during nucleation, L-arginine stabilizes the particles and prevents them from growing further. Indeed, should the same procedure be repeated in the absence of arginine, much larger particles result with very wide size distribution$^{5,6}$. The stabilizing ability of arginine is thought to be due to its guanidinium
head group. The guanidinium group has a delocalized charge which is stabilized by three resonance structures. Since this stabilization is not permanent, it was desirable to displace the arginine groups with a chemisorbed monolayer of organosilane, which would, in addition to stabilizing the particles in solvent, allow for polymer tethering according to our established procedures.

![Image](image.png)

*Figure 2-8: Typical silica nanoparticles (d=23nm) prepared by a modification of the Stober Mechanism*

Evaporation of the solvent and its replacement with an optimal solvent for silanization, such as toluene, resulted in irreversible agglomeration of the particles; a typical result is shown in Figure 2-9. It is thought that once the aqueous solvent is removed, the electric association between arginine and the particles is severed and may not be reestablished. Indeed, even if the same aqueous solvent is introduced again, agglomeration persists. Precluding the removal of the
solvent, another method was devised in an attempt to introduce organosilanes onto the nanoparticle surfaces.

Figure 2-9: Typical reversible agglomeration of particles is seen when solvent is completely removed.

Addition of an amphiphilic species to the arginine-stabilized silica may serve to displace the arginine and allow the particles to stabilize in nonaqueous media. This method, dubbed as phase transfer, has been previously demonstrated\textsuperscript{16}. No direct route for silica particles has been established in the literature; therefore, three amphiphiles were selected that resemble arginine, specifically guanidinium-containing molecules. Additionally, since the point of zero charge for the silica surface occurs under neutral pH, any addition must not reduce the basic pH that persists in the solvent due to the presence of arginine. Thus, three potential candidates were selected for displacement. Two of the three species were not successful while the third one saw moderate success. The failure of the first two in phase transfer may be due to their low pKa values as
compared to arginine. The third one allowed silica to stabilize in toluene and subsequent silanization was successful. However, the process involved a total of three days to produce a very low yield of transfer. Since scaling up the process was deemed too expensive and time consuming, this method was eventually abandoned. Any future work should determine the effect of the pKa of the amphiphile on the yields so as to optimize the process further.


Chapter 3: Mixed Silane Monolayers for Controlling the Surface Areal Density of Click-Reactive Alkyne Groups: A Method to Assess Preferential Surface Adsorption on Flat Substrates and a Method to Verify Compositional Homogeneity on Nanoparticles

3.1 Introduction

Functional self-assembled monolayers (SAMs) have become the material of choice to provide reactive functionality on inorganic surfaces. Functional SAMs comprise short heterobifunctional molecules with a sticky foot on one end, selected to bond to the inorganic substrate, and a reactive functional group of interest on the other end. The two functional groups are joined by a spacer, typically a short chain hydrocarbon, to impart important in-plane interactions that drive the self-assembly process. During self-assembly, the sticky feet migrate to the inorganic surface thus orienting the reactive functional groups to the air interface. Functional SAMs have been formed on flat substrates as well as nanoparticles and serve as a versatile platform through which a wide variety of organic molecules have been covalently tethered to inorganic surfaces. The ability to tether organic molecules to inorganic surfaces has in turn enabled a wide range of applications ranging from directed cell growth\(^1\) to chemical separations\(^2\) to DNA sequencing\(^3\), and has enabled the fabrication of a myriad of important devices such as biosensors\(^4,5\), transistors\(^6\), drug delivery vehicles\(^7\), and extraction devices.\(^8\)

Because manipulation of the areal density of the surface tethered molecules is of critical importance to many of these applications, the control of areal density of functional groups in SAMs has become a topic of considerable interest. The most popular method for controlling surface areal density of reactive functional groups has been the use of mixed monolayers comprising a fraction of the functional heterobifunctional molecule and a fraction of a
chemically similar monofunctional molecule that lacks the reactive functional group. In the case of flat gold substrates, the areal density of reactive functional groups has been controlled by changing the ratio of monofunctional and heterobifunctional thiols in the mixed monolayer.\textsuperscript{9,7-10}. Mixed thiol SAMs have also been used to functionalize lead sulfide\textsuperscript{11} and silver\textsuperscript{10} and mixed silane SAMs have been used to functionalize the surfaces of mica\textsuperscript{12}, silica\textsuperscript{13,14}, alumina\textsuperscript{6}, and quartz.\textsuperscript{4}

While mixed monolayer SAMs have been employed extensively to control surface areal density of reactive functional groups on inorganic substrates, even the most seemingly compatible pair of monofunctional and heterobifunctional molecules can segregate upon self-assembly leading to phase separation of the two components.\textsuperscript{15} Indeed, several studies have shown complete phase separation in mixed SAMs.\textsuperscript{14,16} Mixed SAMs based on disulfides and unsymmetrical sulfides\textsuperscript{17} have been developed to minimize phase separation, however, it has sometimes proven difficult to detect phase separation in mixed SAMs, especially when formed on nanoparticle surfaces.

A variety of spectroscopic methods have been employed to characterize the homogeneity of mixed SAMs formed on flat substrates, including x-ray photoelectron spectroscopy\textsuperscript{18}, infrared spectroscopy\textsuperscript{7}, and Nuclear Magnetic Resonance\textsuperscript{19}. The success of these methods depends on the existence of some spectroscopic label that distinguishes the two molecules comprising the mixed SAM. Contact angle analysis has been applied to examine compositional uniformity at the surface, but this is an indirect method that reflects a variety of surface properties beyond surface composition and is limited to select moieties\textsuperscript{20}. The manifestation of phase separation can also be detected by optical microscopy if appropriate dyes are available that can bond to the reactive functional groups in the mixed SAM. The resolution of microscopic techniques is limited to
micron length scales associated with the wavelength of light used as well as the size of the reactive dye, which can be considerable for typical visible and fluorescent dye molecules. While mixed SAMs have been used extensively to functionalize nanoparticle surfaces, we know of no reports to date that have verified their homogeneity, in part due to lack of a method that is applicable for nanoparticles.

Preferential surface adsorption is a second phenomenon that can compromise the applicability of mixed monolayers. In this case, the composition of the monolayer is not the same as the composition of the solution used for deposition, necessitating the construction of calibration curves (i.e., adsorption isotherms) to achieve quantitative functionalization of surfaces. Preferential surface adsorption of one compound in mixed silane monolayers on silica was attributed to differences in absorption rates and solvent effects. Infrared spectroscopy (IR) was used to document preferential surface adsorption in mixed thiol monolayers formed on gold substrates. The relative concentration of the two thiols determined by IR showed a nonlinear dependence on the concentration of the deposition solution. Preferential adsorption in the latter case was attributed to differential salvation effects.

In recent years, the copper catalyzed alkyne-azide click reaction has emerged as a gold standard for surface modification applications due to its remarkable chemoselectivity, the ease with which surfaces can be functionalized with alkyne or azide groups and the high conversion of this click reaction. A variety of flat and nanoparticle substrates have subsequently been functionalized with azides and alkynes by our own and other research groups however, only a few of these investigations have employed mixed monolayers to control the areal density of surface functional groups and none of these studies have established that the SAMs were homogeneous.
The aim of our study was to develop a universal method to control the alkyne areal density on surfaces; the use of mixed SAMs has traditionally been the method of choice. Two novel analytical tools have been developed to characterize the integrity of a SAM formed from mixtures of alkyne-silane and alkane-silane: ATR-IR was used to preclude preferential adsorption of one component of the mixed SAM and TGA was used to ensure the SAM is homogeneous, that is, with no phase separation present. We provide herein a method to verify the integrity of any mixed SAMs regardless of the identity of a substrate; we also present an approach to fabricate materials with controlled ‘click’ functionality which may have ample use in the field.

3.2 Experimental Section

3.2.1 Materials. Toluene (Anhydrous, 99.8%, Sigma-Aldrich), acetone (>99.9%, Sigma-Aldrich), N,N-dimethylformamide (DMF, 99.9%, Sigma), bromoacetonitrile (97%, Sigma-Aldrich), acetonitrile (99.9%, Sigma-Aldrich), ethyl acetate (99.9% Sigma-Aldrich) copper(II) sulfate pentahydrate (CuSO₄, 99.999%, Aldrich), sodium L-ascorbate (NaAsc, >98%, Sigma), tetramethylrhodamine-5-carbonyl azide (TMRCA, Invitrogen), O-(propargyl)-N-(triethoxysilylpropyl) carbamate (OTPC, 90%, SIP6902.6 Gelest), (triethoxysilylpropyl) carbamate (TPC SIT8188.0, Gelest), silica nanoparticles (Nissan Chemicals). All materials were used as received without further modification and characterization of these materials was carried out as needed.

3.2.2 Nanoparticle Characterization. A suspension of 1mg particles per 1mL methanol remained in solution after sonicated confirming the stability of the nanoparticles in solvent. An aliquot was placed onto a Formvar covered copper grid (Electron Microscopy Sciences FF200-
Cu) and dried for 1 hour. Transmission electron microscopy images (JEOL 100CX Tokyo, Japan) were analyzed using ImageJ software (NIH, public domain). The average radius of the silica nanoparticles was 6.6±2.3nm and their specific surface area was 192.7±0.5 m$^2$/g.

3.2.3 Silanization of Silica Nanospheres. Silica nanospheres (1g) were placed in a 100mL round bottom flask equipped with a magnetic stirrer and covered with aluminum foil to exclude light. Anhydrous toluene (25mL) was added to the nanoparticles followed by flushing with nitrogen. A mixture of OTPC/TPC (1.1mL, 3mmol) was added and the reaction mixture was left to react overnight at 80°C. The reaction was stopped and the silica nanoparticles were washed three times with toluene.

3.2.4 Synthesis of Polymer-Modified Silica Nanoparticles. 100mg of silica nanoparticles functionalized with containing OPTC and 50mg of azide-terminated poly (n-butylacrylate) were added to a 50mL round bottom flask equipped with a magnetic stirrer. 0.1mL of 2.4mM sodium L-ascorbate and 0.1mL of 0.6mM copper sulfate pentahydrate were then added along with 10mL of DMF. The reaction vessel was left to react overnight at room temperature followed by two washes with water and two washes with toluene to remove catalyst and excess polymer.

3.2.5 Modified Nanoparticle Analysis. Thermogravimetric analysis (TGA) (TA Q50 New Castle, DE, USA) was used to measure the masses of silane and/or polymer per silica nanoparticle. Samples were loaded onto a platinum pan and heated under nitrogen to 150°C, held isothermally for 5 minutes to remove residual solvent, heated in oxygen at 10°C/minute to 800°C and held isothermally for 5 minutes. The mass lost between 150°C and 800°C was taken as the mass of any covalently bound organic layer.

3.2.6 Silane Modification of Silica Wafers. Silicon wafers were cut into 1cm squares, placed in a UV-ozone cleaning system for 30 minutes to remove residual surfactants and subsequently
washed progressively with water, toluene, and acetone. Cleaned and dried wafers were placed in a 20mL scintillation vial and flushed with nitrogen. Finally, a 0.1% 20mL solution of OTPC and TPC was added to the vial and the wafer was left to react for 1.5 hours. The wafer was then washed extensively with toluene to remove physisorbed silanes. Monolayer coverage was confirmed by ellipsometry (JA Woollam Alpha SE Lincoln, NE, USA).

3.2.7 Imaging SAM Morphology by Reaction with Fluorescent Dye (TMRCA). Three silicon wafers functionalized with 50% TPC by weight were reacted with 20mL of 0.2%wt solution of TMRCA in DMF in a 50mL Erlenmeyer flask in order to image the spatial distribution of surface alkyne groups. The flask was shielded from light and the solution was held under nitrogen for 24 hours after which the dye-functionalized surface was washed with copious amounts of DMF and water. The success of the reaction was confirmed by an increase in thickness measured by ellipsometry. The fluorescent activity of the dye was analyzed using 2-photon microscopy (Leica TCS SP5 Wetzlar, Germany). The correlation function between regions of dye labeling was calculated from the microscope image using ImageJ software in order to examine the randomness of the spatial distribution of surface-tethered fluorescent dye.
3.2.8 Cleaning and Activation of Germanium Surface. The methods used for cleaning and silanization of the germanium (Ge) substrates (i.e., crystals for attenuated total reflectance infrared spectroscopy) were reported previously. The Ge surface was degreased by sonicating in acetone, methanol and MilliQ water for 5 min. And then the surface was dried with N2 and exposed to UV-ozone under ambient conditions for 15 min. Finally the surface was abundantly rinsed with MilliQ water and dried under nitrogen flux.

3.2.9 Silane Modification of Germanium Surface. The activated Ge surface was immersed in a solution of a desired ratio of OTPC/TPC (volume ratio 1:0, 1:1, 3:1, 1:3) (total 20 µl) in anhydrous toluene (20 ml) in a glass vial and covered with aluminum foil to exclude light. The reaction mixture was left to react overnight at 80°C under nitrogen protection. Afterwards, the Ge substrate was sonicated in toluene, gently scrubbed with a toluene-soaked tissue to assist in
removal of any possible physisorbed multilayers that may form, and rinsed with toluene. Finally, the surface was dried under nitrogen flux.

3.2.10 Synthesis of Azidoacetonitrile. A suspension of bromoacetonitrile (5.3g) in water (9mL) was prepared and stirred vigorously. Sodium azide (5.2g) was added and the reaction was heated to 85°C for 2.5 hours during which the suspension turned black. After the mixture was cooled to room temperature it was added to 5mL ethyl acetate and the organic layer was separated. 5mL ethyl acetate was added to the remaining aqueous layer and shaken. The organic layer was once more separated into the same vial as the first extraction. The combined extract was vacuum dried at room temperature to remove the ethyl acetate giving 4mL of azidoacetonitrile. The compound was characterized by infrared spectroscopy. This procedure was slightly modified from another described elsewhere.36

Molecular modeling of azidoacetonitrile was carried out using SPARTAN 10 (Wavefunction, Inc.). After energy minimization, the molecule appeared to be cylindrical in shape and the projected area of its reactive face was about 0.2 nm².

Click Reaction of Silanized Germanium Wafers with Azidoacetonitrile. The silanized Ge surface was immersed in a solution of azidoacetonitrile (100 µl), CuSO₄·5H₂O (20 mg, 0.08 mmol) and sodium ascorbate (33.6 mg, 0.16 mmol), dissolved in a co-solvent of DMF (20 ml) and H₂O (200 µl) overnight. Afterwards, the Ge surface was washed with a copious amount of water to remove the excess catalyst, followed by rinsing with toluene to remove any residuals of the reactant solution, and was finally dried under nitrogen flux.

Attenuated Total Reflectance Infrared Spectroscopy. Infrared spectra for the Ge surfaces were obtained with a Nicolet 560 FTIR (MCT/A detector, broad range 4000-650 cm⁻¹, liquid N₂ cooled), coupled with an ATR accessory (Horizon™, Harrick Scientific Products Inc.) at a
resolution of 4 cm⁻¹ with an aperture of 100. The internal reflection element was a 50x10x2 mm trapezoidal Ge crystal with an aperture angle of 45° yielding 13 internal reflections. After each step of experiments, the modified Ge surface was characterized by ATR-IR.

3.3 Results and Discussion

3.3.1 Flat Substrate

A new infrared spectroscopy technique was developed to test for preferential surface adsorption in the mixed monolayers comprising alkyne-silanes and alkane-silanes. The basis of the method is titration of surface alkyne groups by reaction with an azide-functional molecule containing an infrared-active label. In the absence of preferential surface adsorption, the absorbance of the infrared label, determined by attenuated total reflectance spectroscopy (ATR-IR) measurements for SAMs formed on germanium ATR plates, will be linearly dependent on the fraction of alkyne-silanes in the solution used for silane deposition, provided that all alkyne groups are accessible for reaction. Satisfying the later caveat requires that the footprint of the azide-label molecule be smaller than the area per silane molecule so as to avoid steric hindrance. The number of molecules per unit area in a SAM is determined from the relation

\[ \sigma = \frac{N_A d \rho}{M} \]  

(3.1)

where \( d \) is the layer thickness, \( \rho \) is the density of the molecule with molecular weight \( M \), and \( N_A \) is Avogadro’s number. In the case of the alkyne-silane monolayer, ellipsometry measurements yield \( d = 1.2\text{nm} \) and its density is 1.0 g/cm³, leading to an areal density of 3 silanes per nm², or an area of 0.33 nm² per silane molecule. Azidoacetonitrile (AzAN) is an appropriate azide-functional molecule with a projected area smaller than this value as well as an
infrared-active nitrile group. The nitrile group has strong absorption bands in the windowpane region of the IR spectrum at 2100 cm\(^{-1}\) and 2250 cm\(^{-1}\) and the projected area of AzAN determined by molecular modeling is about 0.2 nm\(^2\). The nitrile absorbance will scale linearly with the concentration of alkyne in the deposition solution if there is no preferential surface adsorption.

The ATR-IR spectra for a mixed monolayer before and after reaction with AzAN are shown in Figure 3-2. The expanded region of interest spectra shown at right in the figure illustrate that the alkyne band disappears and the nitrile bands appear after reaction with AzAN. Complete disappearance of the alkyne band confirms that the projected area of the AzAN is small enough to allow for the click reaction to go to completion with no residual alkyne remaining.
Figure 3-2: ATR_IR spectra for mixed SAMs formed on germanium substrates before (lower spectra) and after (upper spectra) reaction with azidoacetonitrile. The arrows on the spectra indicate the locations of the alkyne and nitrile absorbance bands.
Figure 3-3: Normalized nitrile absorbance as a function of the fraction of alkyne-silane in the mixed monolayer. The absorbance is normalized by the absorbance of the carbonyl group, an internal standard, residing within the silane SAM.

Assuming that Beer’s law applies, the absorbance of the nitrile band can be taken as indicative of the areal density of surface alkyne groups. For quantitative analysis, the absorbance of the nitrile band is normalized to the absorbance of an internal standard, the carbonyl band emanating from the underlying SAM. The normalized nitrile absorbance is linearly dependent on the fraction of alkyne-silane, as shown in Figure 3-3, indicating that there is no preferential adsorption in the mixed silane SAM on germanium.

The micron scale homogeneity of the mixed monolayers was next examined by applying the fluorescent dye labeling technique described in Figure 3-4. Mixed monolayers formed on silicon wafers were reacted with an azide functional fluorescent dye, TMRCA. If the alkyne-silanes are randomly distributed, as depicted in the lower part of the figure, fluorescent dyes tethered to surface alkyne groups will also be randomly distributed. If phase separation is manifest in the
mixed SAM, however, as depicted in the upper part of the figure, the spatial distribution of surface-tethered dyes will reflect the phase separated morphology of the alkyne silanes in the SAM.

![Diagram of possible morphologies for mixed monolayers of alkyne-terminated and alkane-terminated silanes.](image)

_Figure 3-4. Possible morphologies for mixed monolayers of alkyne-terminated and alkane-terminated silanes. A reactive azide-functionalized fluorescent dye is coupled to the alkyne silanes to image the spatial distribution of alkyne groups in the monolayer. Upper figure: alkyne-silanes phase separate at the surface. Lower figure: alkyne-silanes in the mixed monolayer are randomly distributed._

Figure 3-5 shows the fluorescent microscopy image of a mixed monolayer comprising 50% alkyne-silane formed on a silicon wafer where the distribution of surface alkyne groups has been imaged by reaction with TMRCA. The reason that half of the image does not appear white even though half of the surface is covered by alkyne silanes, is that in many regions the two different silanes are well mixed at distances below the resolution of the microscope. Evidence for phase separation is not apparent in the micrograph, however, the spatial distribution of dye was characterized by correlation function analysis to provide a more quantitative assessment as shown in Figure 3-5.
Figure 3-5. Two-photon fluorescence microscopy image (top) of the surface of a silicon wafer functionalized with a 50:50 mixture of alkyne-silane and alkane-silane. The location of surface alkyne groups has been imaged by reaction with an azide-functionalized fluorescent dye. The graph at right shows the autocorrelation function determined from the image and a fit to the exponential functional expected for a random distribution of alkyne-silanes.

The correlation function corresponds well to an exponential function indicating that the dye molecules, as well as the underlying alkyne-silane molecules, are randomly distributed across the surface.37 This analysis allows us to conclude that, within the micron scale resolution of the fluorescence microscopy technique, the surface-tethered dye molecules and the underlying alkyne-silanes do not phase separate from the alkane-silanes when applied to the surface of a silicon wafer.

3.3.2 Nanoparticle Surface
Characterization of the homogeneity of mixed monolayers on nanoparticles required development of another new technique which is capable of detecting nanoscale heterogeneity. The technique is based upon the measurement of weight changes by thermogravimetric analysis (TGA) when surface alkyne groups on nanoparticles are reacted with azide molecules that are larger than the distance between silane molecules in the silane SAM. Azide terminated polymers are convenient probes for this purpose.

The first step in the experimental procedure is determining the number of silane ligands on each nanoparticle by thermogravimetric analysis of the weight change upon burning off the organic layer (see supplemental information for details of the analysis). We find the silane areal density to be independent of the ratio of alkyne-silanes to alkane-silanes at 2 groups per nm$^2$. The second step in the method is to apply TGA in similar fashion to measure the number of azide functional polymer molecules that can react to each nanoparticle as a function of the fraction of functional silanes on the surface. The ratio of reacted polymers to total number of ligands per nanoparticle measured in this fashion is referred to as the polymer-to-ligand ratio, or PLR.

Figure 3-6 illustrates schematically the method by which nanoscale segregation of reactive surface ligands may be determined for a curved nanoparticle surface. If the reactive azide used has a projected surface area that is smaller or equal to the surface area per silane group, all of the alkyne groups will be accessible for reaction regardless of the presence of phase separation, and the relationship between the PLR and the fraction of functional ligands, referred to as SF, will follow the dashed red line in Figure 3-6. This result is equivalent to what was presented in Figure 3-3 to test for preferential surface adsorption. If the reactive azide used has a projected area equal to that of 5 silane molecules, and the alkyne silanes are randomly distributed, the behavior will follow the solid red lines in Figure 3-6. The value of PLR for the case of 100% alkyne silane will
be 0.2, and PLR will remain constant at that value until SF falls to the same value, 0.2. Below SF=0.2 the behavior will correspond to the relation PLR=SF. The manifestation of phase segregation will always decrease the PLR because some functional alkyne groups will be shielded from reaction due to non-randomness. The behavior for mixed silanes that phase segregate will not follow the solid red line, but will show behavior represented approximately by the solid black line in Figure 3-6. The feature that distinguishes a randomly distributed mixed monolayer, following the red lines in Figure 3-6, is that the linear relationship terminates at the SF value equaling the plateau value in PLR (a value of 0.2 is illustrated in the figure) indicated by point A in the Figure. If phase segregation is manifest, the point where the linear relationship terminates will shift to a value of SF that is greater the plateau value, in other words, greater than 0.2 in the example illustrated in Figure 3-6, as indicated by point B.
Figure 3-6. Detection of Phase Separation on Nanoparticle Surfaces by TGA.
The nanoscale heterogeneity of mixed monolayers of alkyne-silanes and alkane-silanes was determined by grafting azide-terminated poly(n-butyl acrylate) onto the surfaces of silica nanoparticles for fractions of alkyne-silanes ranging from 0.1 to 1. The PLR values for these modified nanoparticles were then determined by TGA.

Graphs showing the dependence of PLR on SF for three different molecular weight polymers are shown in Figure 3-7. The results indicate that these polymers have projected surface areas in the range of 7-13 times the area of a silane molecule. Lower molecular weight polymers yield higher maximal loading as their projected areas are smaller. We see that the 6300Da PnBA has reacted with 13.85% of the silanes on the surface; this percentage decreases, as expected, to 9.97% for the 10500Da and 8.16% for the 16000Da polymers.

The dependence of the polymer loading on the size of the polymer may be expressed by considering the effect of size on the grafting density of the polymer. At high PLR values, the polymers form concentrated brushes on the surface of the particles. In this regime, the Flory radius is not characteristic of the polymer because the chain is stretched to accommodate a dense polymer brush. The height of the brush, h, exceeds the value of the Flory radius, while the distance between polymers, r, is far lower than it. The grafting density is related to \( r \) by

\[
\phi = \frac{1}{4} \frac{1}{r^2}
\]

and, indeed, when treating \( r \) as an equivalent of the Flory radius, an excluded volume factor of 3.48 is obtained indicating, as expected, that \( r << R_F \).

Studies of dense spherical brushes have often discussed the height of polymer chains. To our knowledge, there have been no studies linking polymer grafting density to size. However, we
may deduce a relationship by recognizing that at high loading $V=\text{h}\sigma$, the volume of the polymer chain. The height of a polymer brush has been shown$^{38}$ to vary as $h \sim \text{N}^{3/5}\sigma^{1/5}$. By inserting $V$, we may modify the relation to yield $\sigma \sim V^{5/6}\text{N}^{-1/2}$.

Grafting density is plotted against molecular weight in Figure 3-8 showing that $s \sim \text{N}^{-0.57}$. This agreement arises due to little variation in polymer volume over the narrow molecular weight range used and serves to show that the maximum PLR values obtained in our study correspond to the concentrated spherical brush regime.

![Figure 3-8](image)

Figure 3-8. Grafting density dependence on molecular weight signifying that at the maximum loading polymers are in the concentrated spherical brush regime.

3.4 Supporting Information

3.4.1 Nanoparticle Size Determination

Silica nanoparticles were purchased from Nissan Chemicals. The nanoparticles (Figure 3-9) were found to have an average diameter of 14nm by transmission electron microscopy.
(JEOL 100CX Tokyo, Japan) and 20nm by dynamic light scattering (Brookhaven BI-200SM Holtsville, NY)

![Image]

*Figure 3-9: TEM of typical silica nanospheres.*

**3.4.2 Determination of Polymeric Coverage on Nanoparticle Surface by Use of TGA**

A typical TGA plot is shown in Figure 3-10 for silane-grafted silica nanoparticles. To determine the exact loading of the silane on the surface, all thermographs were collected in the same manner. A sample was heated under nitrogen to 150°C in order to remove all residual solvent. Further heating was carried out under oxygen to burn off the organic layer. Heating was done slowly at a rate of 5°C/minute to 800°C to ensure complete combustion. The initial weight of the sample was taken at 150°C as soon as oxygen was introduced, denoted by point A on the graph; the final weight reading at 800°C is denoted by point B.
Figure 3-10: Typical TGA plot.

Point A denotes the weight of the nanoparticles with the organic layer attached to their surfaces whereas point B signifies the weight of the nanoparticles alone. By taking a 1g basis we may use the following to determine the silane coverage in silane groups per nm$^2$:

\[
\text{Silane Loading} = \left( \frac{m_T - m_{NP}}{m_{NP}} \right) \times \frac{N_A}{M_s S}
\]

(3.2)

Where:

- $m_T$ – mass of nanoparticles with organic layer (g)
- $m_{NP}$ – mass of nanoparticles (g)
- $M_s$ – molar mass of the organic portion of the silane (g/mol)
- $S$ – surface area of nanoparticles (g/nm$^2$).

The surface area of the nanoparticles was measured to be $192.7 \pm 0.5$ m$^2$/g using BET analysis (Quantachrome Autosorb-iQ Hartley Wintney, UK) The molar mass of the silane (M) in the
above equation simply denotes the mass of the silane that would be subject to combustion. When the silane forms a covalent bond with the surface of the particle, ethanol is eliminated from the silane backbone thus neither the silicon atom nor the ethoxy groups are included in the molar mass of Equation 3.2.

3.4.3 Use of TGA to set a baseline

In order to compare samples with different alkyne functionalities, it is crucial to ascertain that the silane loading is identical for all samples. Figure 3-10 clearly shows that the total silane loading on each sample is identical at 2.10±0.11 silanes per nm$^2$. By keeping the silane loading constant, the alkyne group density, and therefore the polymer density, is ensured to remain the same, allowing for meaningful comparison of the samples.

![Figure 3-11: Total silane areal density as a function of the silane composition.](image)

Polymeric loading was determined by a modification of Equation 3.2:
Polymer Loading = \left[ \left( \frac{m_f - m_{NP}}{m_{NP}} \right)_p - \left( \frac{m_f - m_{NP}}{m_{NP}} \right)_S \right] \times \frac{N_A}{M_p S} \tag{3.3}

where subscripts p and S denote the case for the polymer-grafted nanoparticle and its corresponding silane-grafted nanoparticles, respectively, and $M_p$ is the molar mass of the polymer. Thus, for a sample of a known alkyne coverage, the silane loading is determined first. Subsequent tethering of the polymer shell is then analyzed, and the data from the alkyne coverage is directly subtracted to arrive at the polymer loading.

### 3.4.4 Synthesis of Poly (n-Butyl Acrylate)

n-butyl acrylate (nBA, Sigma, >99%) (purified by passing through activated basic alumina), N,N,N’,N”,N”-pentamethyldiethylenetriamine (PMDETA, Sigma, 99%), copper (I) bromide (CuBR, Sigma, 98%), and tris(isopropyl silane) (TIPS) (synthesized as described elsewhere\textsuperscript{39}).

Briefly, a mixture of TIPS initiator, PMDETA, nBA and DMF were loaded in a Schlenk flask. After freeze-pump-thaw three times, CuBr was added and quickly dissolved. The flask was placed in oil bath at 70 °C to the desired reaction time. After the reaction, the catalyst was removed by passing through activated neutral alumina column. The polymers were obtained by concentrating the reaction mixture in vacuum to constant mass. In this fashion, three polymer molecular weights were synthesized of 6300Da, 10500Da, and 16000Da. Gel permeation chromatography of the three polymers is displayed in Figure 3-12.
3.4.5 Determination of SAM quality on silicon substrate

The thickness of the silane layer for the silicon substrate was determined by ellipsometry to be 1.03±0.07nm. After the tethering of the dye, the thickness increased to 22.4±3.3nm.

<table>
<thead>
<tr>
<th>% Alkyne on Surface</th>
<th>Ellipsometric Thickness (nm)</th>
<th>XPS Thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.15±0.10</td>
<td>1.17</td>
</tr>
<tr>
<td>25</td>
<td>1.17±0.10</td>
<td>1.18</td>
</tr>
<tr>
<td>50</td>
<td>1.17±0.10</td>
<td>1.18</td>
</tr>
<tr>
<td>75</td>
<td>1.16±0.10</td>
<td>1.17</td>
</tr>
<tr>
<td>100</td>
<td>1.20±0.10</td>
<td>1.20</td>
</tr>
</tbody>
</table>

Table 3-1: Thickness of mixed monolayer on Ge surface.
Atomic force microscope images of the specimen surfaces are shown in Figure 3-13. The root mean square value for the roughness was slightly below 0.6 for both specimens.

*Figure 3-13: AFM Images of germanium substrates for 50% alkyne functionality (left) and 100% alkyne functionality (right).*
3.4.6 Molecular simulation to determine areal coverages

The equilibrium conformation for azidoacetonitrile in DMF was simulated using SPARTAN (SPARTAN v 1.2.0, build 132, 2009). The resulting structure yielded a molecular area of 0.2nm$^2$ at the attachment site (the azide group). Figure 3-14 shows the molecular radius of azidoacetonitrile with red denoting carbon, blue denoting nitrogen, and gray denoting hydrogen.

Figure 3-14: Equilibrium structure of azidoacetonitrile in dimethylformamide.
References


Chapter 4: Universal Method to Fabricated Azide Mixed Monolayers Using Kinetic Control on Surfaces of Silica, Iron Oxide, and Germanium

4.1 Introduction

Much research has been directed to devise a way to control the exact composition of surfaces. Self-assembled monolayers (SAMs) have been at the forefront as modifying agents for curved and flat surfaces of ceramics, metal oxides, precious metals, semiconductors and other materials. To control surface composition, a mixture of SAMs is employed allowing for a variation in desired properties. By changing the end-group of one of the monolayers, a precise loading of a secondary layer can be achieved. This method has shown a wide range of applications in chemical separations\(^1\), DNA sequencing\(^2\), and protein extraction\(^3\) and has allowed the fabrication of biosensors\(^4\), transistors\(^5\), drug delivery vehicles\(^6,7\), polymerization routes\(^8,9\), and electronic devices\(^10\). More fundamentally, mixed SAMs have shed light on the mechanism by which silanes react with an oxide layer\(^11\) and thiols react with coinage metals\(^6,12\).

Several experiments have shown the use of mixture of two different thiols to functionalize a flat gold surface\(^4,6,14\). By changing the ratio of the two thiols and immersing the gold surface, different surface functionalities may be prepared. This same method was used with thiols on the surface of lead sulfide\(^15\) and silver\(^14\) or with silanes on the surfaces of mica\(^16\), silica\(^11,17\), alumina\(^5\), and quartz\(^18\). While this has traditionally been the most common method to fabricate mixed monolayers, Stranick et al.\(^19\) have shown that even the most compatible pair of monolayers segregates into two domains on the surface to a varying degree. Indeed, several studies have shown complete domain separation when two monolayers were mixed\(^17,20\) and much effort must be invested in ascertaining true mixing, especially when functionalizing nanoparticle
surfaces. Other methods of fabricating mixed SAMs on surfaces are scant but include use of disulfides and unsymmetrical sulfides\textsuperscript{21} which encounter the same difficulties in evaluating the uniformity of the surface. Others have used microprinting of flat surfaces\textsuperscript{22,23}.

A versatile method is reported herein which precludes the concerns of true monolayer mixing. Bromine-terminated silanes are used to completely cover the surfaces of $\gamma$-Fe$_2$O$_3$ and SiO$_2$ nanoparticles and of Ge flat surface. The bromine-end is partially converted to an azido-end through an $S_N2$ reaction. The kinetics of the substitution reaction was investigated by Fourier-transform infrared spectroscopy (FT-IR) using attenuated total reflectance (ATR) in the case of the germanium surface and a flow-equipped demountable liquid cell in the case of the nanoparticles. Regardless of the surface geometry and identity, the substitution reaction demonstrated identical kinetics exhibiting total conversion within 150 minutes, contrary to previously reported results showing complete conversion in 48-60 hours.\textsuperscript{20,24} The reaction can easily be stopped in the case of germanium by flushing the system at a desired time to achieve partial conversion of the end group. For the nanoparticle systems, use of nonsolvent allowed the immediate flocculation of the particles at a desired time. As a method of ascertaining partial conversion on the flat surface, the ratio of azide vibrational stretch to a fixed methylene vibration stretch is calculated. For the nanoparticles, polymer layers have been tethered to the end group using copper catalyzed azide-alkyne cycloaddition (CuAAC) and coverage was calculated by use of thermogravimetric analysis (TGA) to ascertain partial conversion to terminal azide.

Our method demonstrates complete independence of surface geometry and identity as well as silane areal density and provides for a universal method for the fabrication of truly-mixed monolayers in a way that has not been previously reported. The significance of the work is thus two-fold: first, it demonstrates complete conversion of bromine to azide occurs much faster than
previously thought. This drastically shortens synthesis time of ‘click’-functionalized surfaces from days to a few hours. Secondly, and more importantly, the work signifies a method by which surface composition can be tailored on any oxide surface regardless of its identity and provides for a universal method for the fabrication of truly-mixed monolayers.

4.2 Experimental Section

4.2.1 Materials. Toluene (Anhydrous, 99.8%, Sigma-Aldrich), acetone (>99.9%, Sigma-Aldrich), N,N-dimethylformamide (DMF, 99.9%, Sigma), 11-bromoundecyl trichlorosilane (BUTS, 95%, Gelest), 11-bromoundecyl trimethoxysilane (BUTMS, 95%, Gelest), undecyl trichlorosilane (UTS, Gelest) tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, 97%, Sigma-Aldrich), copper(II) sulfate pentahydrate (CuSO4, 99.999%, Aldrich), iron(II) chloride (FeCl2, 98%, Aldrich), iron(III) chloride (FeCl3, 97%, Sigma-Aldrich), ethylene diamine tetraacetic acid (EDTA, >99%, Sigma-Aldrich), ammonium hydroxide (>99.999%, Sigma-Aldrich), iron(III) nitrate nonahydrate (Fe(NO3)3, >99.999%, Aldrich), sodium L-ascorbate (NaAsc, >98%, Sigma), sodium azide (>99.5%, Sigma), hydrochloric acid (36.5-38.0%, Sigma), nitric acid (>99.5%, Sigma-Aldrich), tetrahydrofuran (THF, >99.9%, Sigma-Aldrich), 5-Carboxytetramethylrhodamine, Propargylamide (5CP, Invitrogen).

4.2.2 Functionalization of the Germanium Surface. The Ge surface was degreased by sonicatiing in acetone, methanol and MilliQ water for 5 min. And then the surface was dried with N2 and exposed to UV-ozone under ambient conditions for 15 min. Finally the surface was abundantly rinsed with MilliQ water and dried under nitrogen flux. The activated Ge surface was immersed in a solution of BUTS (0.08% v:v) in a mixture of undecane and carbon tetrachloride
(7:3 v:v). Afterwards, the Ge substrate was sonicated in toluene, gently scrubbed with a toluene-soaked tissue to assist in removal of possible physisorbed multilayers, and rinsed with toluene. Finally, the surface was dried under nitrogen flux.

**Figure 4-1: Treatment of the germanium surface.**

4.2.3 Functionalization of Silica. Silica (1g) was placed in a 100mL round bottom flask equipped with a magnetic stirrer. Anhydrous toluene (25mL) was added to the particles followed by a nitrogen flush. The vial was covered from light using aluminum foil and to it 11-bromoundecyl trichlorosilane (1.1mL, 3mmol) was added and let to react for 1.5 hours. The reaction was stopped and silica was washed three times with toluene. Coverage was calculated using TGA.
4.2.4 Functionalization of Iron Oxide. Maghemite (1g) was placed in a 100mL two-neck round bottom flask equipped with overhead stirring. Anhydrous toluene (25mL) was added followed by a nitrogen flush. The vial was covered from light using aluminum foil and to it 11-bromoundecyl trimethoxysilane (1.3mL, 4.25mmol) was added. Stirring was set to 250rpm and the vial was placed in sonication bath for 16 hours at 80°C. The reaction was then stopped and the particles were washed three times with toluene. Coverage was calculated using TGA.

4.2.5 Kinetic Control of Azide Monolayer. To each of the three substrates, a saturated solution of NaN₃ in DMF was added to effect substitution of the terminal bromine with an azide functional group. The kinetics of the reaction were monitored by infrared as per the following setups.

4.2.5.1 Germanium Substrate. The silanized Ge crystal was placed into a liquid ATR flow cell. A saturated solution of sodium azide in DMF (1mL) was injected into the cell whereupon it contacted the silanized Ge surface to initiate the azide substitution reaction. The reaction was performed at room temperature in air for 1 day with spectra recorded every 2 minute with 100 scans acquired. Background was collected with the bare Ge crystal. Automatic baseline correction methods were applied to all the spectra. The reaction was stopped by washing with a lot of DI water for three times and dried under nitrogen flux. Coverage was calculated upon the spectra.

4.2.5.2 Silica Substrate. Bromine-terminated silica (100mg) was dispersed in DMF (2mL) in a 20mL scintillation vial equipped with a magnetic stirrer and placed on a magnetic plate. The dispersion was fed to a liquid cell placed inside using a peristaltic pump. Sodium azide
(10mg) was added to the reaction vessel and measurements were then taken every minute for 4 hours.

**4.2.5.3 Iron Oxide Surface.** Bromine-terminated iron oxide (100mg) was dispersed in DMF (2mL) in a 20mL scintillation placed inside a sonication bath. The dispersion was fed to a liquid cell placed inside using a peristaltic pump. Sodium azide (10mg) was added to the reaction vessel and measurements were then taken every minute for 4 hours.

**4.2.6 Synthesis of Iron Oxide Nanoparticles.** Particles were made through the co-precipitation of FeCl$_3$ and FeCl$_2$. Briefly, FeCl$_3$ (10.9g, 39mmol) was dissolved in water (435mL) in a 500mL three-neck round bottom flask equipped with overhead stirring; stirring was maintained at 500rpm. FeCl$_2$ (3.9g, 19mmol) was dissolved in 1.5M HCl (22mL) and subsequently added to the reaction vessel, while stirring at 850rpm. After uniform color appeared, ammonium hydroxide (45mL) was added to the solution to afford a black precipitate. After 30 minutes, the reaction vessel was placed on a magnetic plate and the solvent was removed by aspiration, keeping the particles shielded from air. Thereupon, 2M nitric acid (75mL) was added to the particles and removed after three minutes in a sonication bath. Then, 0.33M Fe(NO$_3$)$_3$ (75mL) solution, preheated to 100°C, was poured onto the particles and let to mix. After 30 minutes, the solvent was removed and the particles were washed three times with acetone in a dispersion-centrifugation cycle.

**4.2.7 Modification of Silicon Wafer with Dye.** A silicon wafer was cut into a 4cm by 4cm square. The wafer was placed in a UV-ozone cleaning system to remove residual surfactants followed by an extensive wash with water, toluene, and acetone. The dry wafer was placed in a
20mL scintillation vial and flushed with nitrogen. Finally, a 0.1% 20mL solution of BUTS and UTS (50/50 by wt) was added to the vial and the wafer was left to react for 1.5 hours. The wafer was then washed extensively with toluene to remove physisorbed silanes. Monolayer coverage was confirmed by ellipsometry.

The bromine-terminated wafer was immersed in a saturated solution of NaN3 in DMF and left to react for 2 hours to yield a surface modified with a mixed azide-bromine monolayer. To visualize the spatial distribution of surface azide groups, the substrate was modified by reaction with 1mg 5CP dissolved in 2mL DMF with 0.5mg copper sulfate pentahydrate and 1mg of sodium ascorbate for 1 hour.

### 4.2.8 Polystyrene Tethering to Silica Nanoparticles

To test whether the azide substitution can be stopped effectively, alkyne-terminated polystyrene was reacted with partially converted silica. Bromine was exchanged for silica in a DMF solution saturated with sodium azide. Three reactions were stopped after 1 minute, 3 minutes, and 5 minutes using copious amounts of toluene and centrifuged. Excess sodium azide was washed with water and acetone. 100mg of the partially-converted particles and 25mg of alkyne terminated polystyrene were added to a 50mL round bottom flask equipped with a magnetic stirrer. To which 1mL of 2.4mM sodium L-ascorbate and 1mL of 0.6mM copper sulfate pentahydrate were added along with 12mL of DMF and TBTA (5mg, 10umol). Reaction vessel was flushed with nitrogen and let to react overnight at 80°C followed by several washes in THF, ammonia and EDTA in ammonia to remove excess copper and polystyrene. Coverage was calculated using TGA.
4.2.9 Surface Characterization. $S_N2$ reaction of terminal bromine to azide on nano surface was performed on a Nicolet Magna IR-560 Fourier transform infrared spectrometer equipped with a CaF$_2$ beam splitter, a liquid nitrogen cooled detector. Spectra for the nanoparticles were collected once a minute with 64 scans and a resolution of 8 cm$^{-1}$ using a liquid-cell holder to which samples were continuously flowing using a peristaltic pump.

Infrared spectra for Ge surface were obtained with a Nicolet 560 FTIR (MCT/A detector, broad range 4000-650 cm$^{-1}$, liquid N$_2$ cooled), coupled with an ATR accessory (Horizon™, Harrick Scientific Products Inc.) at a resolution of 4 cm$^{-1}$ with an aperture of 100. The internal reflection element was a 50×10×2 mm trapezoidal Ge crystal with an aperture angle of 45° yielding 13 internal reflections. The Ge crystal was incorporated within a flow through liquid cell.

Samples were loaded onto a platinum pan for thermogravimetric analysis (TGA) and heated under nitrogen to 150°C, held isothermally for 5 minutes to remove residual solvent and then heated using oxygen at 10°C/minute to 800°C and held isothermally for 5 minutes. The mass lost between 150°C and 800°C was taken as the covalently bound organic layer.

The thicknesses of SAMs on Ge surface were measured with a Beaglehole Spectroscopic and imaging ellipsometry (Beaglehole Instruments, Wellington, New Zealand) under angle mode (70°) with a fixed wavelength of 632.8 nm. The experimental ellipsometry data were analyzed using Film Wizard software.
4.3 Results

Figure 4-2. 2-Photon microscopy image of silicon surface modified by a 50/50 (volume%) mixture of azide-terminated silane and methyl-terminated silane, showing Aggregation of azide-terminated silanes.

As pursuant with Chapter 3, a silica wafer was initially modified using two organosilanes with differing terminal functionalities and identical backbone structure; as seen in Figure 4-2. The bromine-end was then substituted with an azido-end by an SN2 reaction and the surface was tethered with a fluorescent dye to evaluate the surface locations of the two structures. While the use of alkyne-terminated silanes have shown the silanes to completely disperse on the surface, the contrary is true in this case. The silanes preferentially adsorb onto the silica wafer creating domains rich with azido-terminated silanes, indicated by the location of the dye, and domains
rich with methyl-terminated silanes, indicated by the lack of a fluorescent signal. This finding supports previous literary reports for these two silanes.

Since initially the surface is covered with bromine-terminated moieties as a precursor to azide coverage, it was hypothesized that mixed coverage may be achieved by partially converting the terminal bromine groups to azide groups, which may be achieved by stopping the bromine-to-azide S_N2 reaction prior to full conversion. This scheme is summarized in Figure 4-3. This reaction may be monitored in situ using infrared spectroscopy to study the conversion kinetics, a technique useful to evaluate the cessation of the reaction, as well. ATR-IR is used for the flat germanium wafer, whereas FT-IR is employed for nanoparticles.

Figure 4-3: Schematic of azide partial conversion.
4.3.1 Bromine-to-Azide Conversion Kinetics

Bromine-to-azide conversion is measured in real time by collecting data every 30 seconds. To evaluate the amount of azide groups on the surface, the pure DMF spectrum is first subtracted. This is achieved by adjusting the weighting factor until DMF contributions are completely nulled indicated by the disappearance of its characteristic peak at 1700 cm$^{-1}$. The amount of the azide group is evaluated by monitoring the ratio of the unchanging methylene peak at 2950 cm$^{-1}$ to the increasing azide peak at 2150 cm$^{-1}$ in a manner shown in Figure 4-4. The reaction is said to be complete once the ratio of the two peaks plateaus to a constant value. The peak ratio is converted to azide areal density and plotted against time as shown in Figure 4-5.

The bromine-to-azide conversion proceeds as a first order reaction in bromine with a rate constant of 0.0226±0.0004 min$^{-1}$ on germanium, of 0.0252±0.003 min$^{-1}$ on silica, and 0.025±0.02
min$^{-1}$ on iron oxide. In all three systems, the reaction reaches completion after 150 minutes. These findings indicate that the reaction reproducibly proceeds by the same mechanism on all three substrates regardless of the substrate identity. We believe this suggests a universality of the reaction on any oxide layer, not only since this finding was reproducible but also since it was collected by different instruments using different methodologies. In the case of germanium, the reaction was left static and measured on an ATR-IR plate; for silica nanoparticles, the reaction was stirred and fed into the FT-IR by a peristaltic pump whereas the iron oxide reactions were placed in an ultrasonic bath that was connected to a peristaltic pump. In addition to this universality, this reaction also reproducibly terminated after about 2.5 hours, contrary to previous reports indicating the need for several days.

(a) Kinetic curve for the germanium substrate
(b) Kinetic curve for silica nanoparticles

(c) Kinetics curve for iron oxide nanoparticles

Figure 4-5. Kinetics curve of $S_N$ 2 substitution of bromine converted to azide on nano-surface: where $\sigma$ is the areal density of azide silane on surface in chains/nm$^2$, $x$ is the conversion of Br to azide, $t$ is reaction time in minutes.
4.3.2 Mixed Bromine-Azide Monolayers with Reaction Termination

In order to produce mixed monolayers on the surface, it is necessary to stop the azide conversion reaction prior to the reaction completion. In the case of germanium, this may easily be achieved since the substrate is immersed in the reaction mixture. The reaction is stopped by simply removing the germanium wafer from the solution and then washed, as a precautionary step. To confirm the reaction indeed ceased, the azide-to-methylene ratio was measured at sampling intervals and the conversion was compared to that expected if the reaction proceeded uninterrupted. Table 4-1 shows close agreement indicating the reaction is indeed stopped.

<table>
<thead>
<tr>
<th>Time stopped (minutes)</th>
<th>Calculated conversion</th>
<th>Kinetics curve conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.8±1.0%</td>
<td>Unmeasurable.</td>
</tr>
<tr>
<td>2</td>
<td>35.6±2.5%</td>
<td>43.4%</td>
</tr>
<tr>
<td>10</td>
<td>54.5±0.5%</td>
<td>54.3%</td>
</tr>
<tr>
<td>150</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Table 4-1: Spectral results compared with kinetic curve data showing agreement.*

The nanoparticle reactions could not be stopped in a similar way since the substrate is well-dispersed in the solvent. In order to fabricate a mixed monolayer containing the azide functional group, a method was developed to control the conversion of bromine on the surface. By introducing a solvent that would cause the nanoparticles to flocculate, we may stop the reaction
at any given time leading to a partially converted surface containing both the bromine and the azide groups.

As such, toluene was added at fixed time intervals to stop the reaction. When toluene was added, the nanoparticles settled out of the suspension very quickly and it was assumed that the reaction stopped. Azide-terminated PEO of 500 Da was then reacted with each of the samples and analyzed using TGA. Based on the amount of polymer on the surface, the conversion of bromine to azide may be deduced. Table 4-2 indicates the experimental result is consistent with that predicted by the kinetics curve on silica nanoparticle indicating the reaction indeed stops once toluene is added and that a mixed monolayer is achieved.

<table>
<thead>
<tr>
<th>Time Stopped (minutes)</th>
<th>Theoretical Percent of Converted Silanes</th>
<th>Actual Percent of Converted Silanes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10.9%</td>
<td>13.0±0.5%</td>
</tr>
<tr>
<td>6</td>
<td>27.8%</td>
<td>28.3±1.2%</td>
</tr>
<tr>
<td>10</td>
<td>29.4%</td>
<td>35.3±2.4%</td>
</tr>
</tbody>
</table>

Table 4-2. Thermogravimetric analysis of PEO-capped silica for which azide substitution reaction was stopped at the specified time.

### 4.3.3 Measuring the Quality of the Mixed Monolayer

The reaction was stopped on the germanium substrate after ten minutes corresponding to 50% azide conversion. The surface azide groups were allowed to react with an alkyne-terminated dye sing copper catalyzed azide-alkyne cycloaddition (CuAAC) and the resulting substrate was analyzed for fluorescent activity. The green dots in Figure 4-6(a) correspond to the dye, and thus,
to the azide on the surface. The spatial autocorrelation function for the dye is then calculated using the ImageJ software and fitted with a curve, as shown in (b). The randomness of the dye is verified as the curve fits with an exponential function to good agreement. This analysis allows us to conclude that, within the micron scale resolution of the fluorescence microscopy technique, the surface-tethered dye molecules and therefore the underlying azide monolayer do not phase separate from the bromine monolayer.

(a)
Figure 4-6. Fluorescence image of silicon surface modified by alkyne end-functional dye when the $S_N2$ reaction stopped at 10 min, showing no aggregation (a). Autocorrelation function determined from the image; the resulting curve is exponential indicating random distribution of azide silanes on the surface.
References


Chapter 5: Phase Diagram for Polymer-Decorated Nanoparticles

5.1 Introduction

Nanoparticles have unique properties arising from their small sizes\(^1\). They are too large to possess atomic properties, but too small to be considered bulk material. It is, thus, no surprise that they have been at the forefront of research and industry. While applications of nanoparticles are diverse and numerous, scant are those comprising of lone particles. As a group, nanoparticles have enhanced properties, and much research has been devoted to the understanding of nanoparticle organization\(^2,3\). Ordered arrays of nanoparticles have proven useful in many varied fields. Owing to their high surface areas, nanoparticles arranged to form nanowires with embedded dyes showed promise in the construction of solar cells\(^4\). Nanoparticle sheets have been used to guide electromagnetic waves so as to form novel focusing instruments that operate below the diffraction limit\(^5\). Others have used nanoparticle arrays as biodetectors\(^6\), for fuel cells\(^7\), and for drug delivery\(^8\). With many more applications, it would be very beneficial to understand nanoparticle organization at a fundamental level.

Controlling the packing forces of nanoparticles drives their self-assembly. Particle-particle interactions tend to be attractive in nature due to van der Waals forces between them\(^9\). Thus, energetically, the particles tend to come in contact with one another whenever possible. A variety of methods have been devised to try and finely control this attractive potential by introducing a repulsive interaction between the particles. By taking advantage of Coulombic interactions between charged surfaces, nanoparticles were shown to predictably arrange onto a thin-film substrate\(^10\). By varying the relative amounts of polymer to particle in polymer films, nanoparticles have been shown to self-assemble into two-dimensional and three-dimensional
structures. Recent work has shown that polymer-capped magnetic nanoparticles may self-assemble when subjected to an external magnetic field or external electric field. Controlling the self-assembly process may also be done by tethering functional groups that independently self-assemble such as DNA, proteins, and, as previously mentioned, polymers.

Oftentimes, the self-assembly of polymer-decorated nanoparticles is achieved by a mixture of two surface groups. The two groups may tailor the balance between the packing forces of the particles and the repulsion exerted by a grafted polymer layer. Previously, Janus nanoparticles covered with two different pH-responsive polymers exhibited the formation of phases. Varying the pH changed the electrostatic interactions between the particles which resulted in different particle morphologies. In another example, gold nanoparticles were functionalized with a mixture of hydrophobic and hydrophilic ligands in fixed proportions leading to several phases when the structures were dispersed in various solvents. While these methods have proven quite useful, there is a lack in fine morphological control when using multiple polymers on the surface of particles. The two polymers may segregate into regions rich with one and poor in the other resulting in inconsistent behavior. Unless shown to be randomly arranged on the surface of the particle, this surface treatment is unsuitable for controlled self-assembly of nanoparticles because exact control of the packing forces is not attained. If the loading of multiple polymer groups is well-understood, one may construct a phase diagram predicting nanoparticle arrangements based on polymer loading.

Polymer-capped nanoparticles self-assemble into different structures due to a balance between entropy and enthalpy. While particle surfaces wish to coalesce to minimize their energy, surface-grafted polymers must be stretched out to allow for this. This is entropically unfavorable since the polymer chain is restricted to less configurations than it would be if the particles did not
come together. Theoretical studies based on this premise were carried out in an effort to enrich the understanding of the self-assembly of these structures.\textsuperscript{20,21} The results were universal phase diagrams predicting nanoparticle organization based on the sizes of the polymers and particles and the grafting density of the polymers on the nanoparticle surfaces. It was found that when the density of the polymer on the surface is changed, four morphologies may form. A typical phase diagram from a previous study\textsuperscript{20} is presented in Figure 5-1.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{phase_diagram.png}
\caption{Theoretical prediction of the self-assembly behavior of nanoparticles as a function of the polymeric loading. Here, $n_p$ is the number of polymer groups on a nanoparticle, $R$ is the radius of the nanoparticle, and the $R_g$ is the melt radius of gyration of a generic polymer.\textsuperscript{12}}
\end{figure}

The amount of nanoparticle surface that is left uncovered with polymers dictates the degree of ordering seen in each phase. Three-dimensional ordering of nanoparticles is achieved in the case of incomplete polymer coverage where so much particle surface is uncovered that packing interactions are very strong resulting in agglomeration of particles. While previous research has shown that the growth of these structures is not predictable, others have found that
the sizes of agglomerates can be controlled. By increasing the polymeric coverage some more, areas of the patchy particles will be rich in polymer whereas some others will be left bare. When coming together, the particles will orient themselves to maximize the number of surface contacts leaving any polymer-covered surface exposed. Sheets may form if the particle has low polymer loading with the particles forming two-dimensional arrays with polymers extending orthogonally to the plane of the particles. Strings will form when the polymeric coverage is increased yet more to reduce the number of surface contacts such that one-dimensional array of particles results with the polymers arranged as they would on a cylindrical surface. Finally, when enough of the surface is covered with polymers, the attractive potential between the particles is completely shielded and particles may not come together at all resulting in the dispersed phase.

We report a method to control the self-assembly of nanoparticles in a matrix-free environment as an extension of the theoretical work presented in Figure 5-1. The surface reactivity of nanoparticles is controlled by using a mixture of silanes that have been shown by us to randomly arrange on the surface. This allows any tethered polymers to be randomly arranged on the surfaces eliminating the possibility that phases may form due to uneven polymeric coverage. This also allows us to freely travel across the phase space since we may control the polymeric loading on the surface. We proceed to construct our own phase diagrams that show agreement with theoretical studies. We also quantify the resulting phases to show the degree of ordering seen in each phase.

5.2 Experimental

5.2.1 Materials. Toluene (Anhydrous, 99.8%, Sigma-Aldrich), acetone (>99.9%, Sigma-Aldrich), N,N-dimethylformamide (DMF, 99.9%, Sigma), ethyl acetate (99.9% Sigma-Aldrich)
copper(II) sulfate pentahydrate (CuSO₄, 99.999%, Aldrich), sodium L-ascorbate (NaAsc, >98%, Sigma), O-(propargyl)-N-(triethoxysilylpropyl) carbamate (OTPC, 90%, SIP6902.6 Gelest), (triethoxysilylpropyl) carbamate (TPC SIT8188.0, Gelest), silica nanoparticles (Nissan Chemicals), iron (II) chloride tetrahydrate (>99.9%, Sigma-Aldrich), iron (III) chloride hexahydrate (>99%, Sigma-Aldrich), iron (III) nonahydrate (99.999%, Sigma-Aldrich), hydrochloric acid (36.5-38.0%, Sigma), ammonium hydroxide (28.0-30.0% NH₃, Sigma-Aldrich), nitric acid (2M, Sigma), TEM grid, Tetrahydrofuran (THF, >99%, Sigma), methanol (>99.9%, Sigma), ethanol (>99.5%, Sigma), t-butyl alcohol (99%, Sigma).

5.2.2 Preparation of iron oxide nanospheres. Iron oxide nanoparticles were prepared by a variation of the Massart Process²³,²⁴. 3.9g Iron (II) chloride and 10.9g iron (III) chloride were dissolved in 435mL of deionized water in a two-neck round bottom flask. To ensure the salts were completely dissolved, the flask was ultrasonicated while stirred at 500rpm using a mechanical stirrer. The solution was subsequently heated to 80°C and kept under nitrogen after which 45mL of ammonium hydroxide was added quickly to afford a black precipitate that constituted magnetite nanoparticles. The stirring speed was then increased to 850rpm and the nanoparticles were let to grow for 15 minutes. The newly formed magnetite nanoparticles were magnetically separated from the solvents while being kept from contact with air. An oxidizing solution of 2M nitric acid (50mL) was subsequently added to the particles under vigorous agitation to afford a brown powder. The partially oxidized particles were separated by centrifugation after which an oxidizing solution of 0.33M iron (III) nitrate nonahydrate, previously heated to 100°C, was added. The system was allowed to vigorously mix for fifteen minutes, followed by centrifugation. The particles were then washed, once more, with 2M nitric acid to complete their oxidation at which point the particles appeared to be light brown. The
newly formed maghemite nanoparticles were washed three times with acetone to remove residual nitrate ions after which the particles were dried and functionalized immediately. Particles were measured to be 10nm in diameter (TEM) and 9nm in diameter (DLS). The surface area was determined to be $130\pm0.2\text{m}^2/\text{g}$ (BET).

**5.2.3 Silane modification of iron oxide nanospheres.** 1g of maghemite nanoparticles was added to a 50mL round bottom flask and flashed with nitrogen gas. 20mL of anhydrous toluene was then added to the flask while keeping the contents under nitrogen. The flask was covered in aluminum foil and the particles were allowed to disperse in the solvent for a period of twenty minutes in a sonication bath. A 0.5mL mixture of OTPC/TPC was then added and allowed to react in the sonication bath overnight at 80°C. Subsequently, the particles were washed three times with toluene to afford silanized nanoparticles. Silane coverage was determined by TGA and IR.

**5.2.4 Silane modification of silica nanospheres.** 1g of silica nanoparticles was added to a 50mL round bottom flask equipped with a magnetic stirrer and flashed with nitrogen gas. 20mL of anhydrous toluene was then added to the flask while keeping the contents under nitrogen. The flask was covered in aluminum foil and the particles were allowed to disperse in the solvent for a period of twenty minutes under stirring in an oil bath. A 0.5mL mixture of OTPC/TPC was then added and allowed to react under stirring overnight at 80°C. Subsequently, the particles were washed three times with toluene to afford silanized nanoparticles. Silane coverage was determined by TGA and IR.

**5.2.5 Polymer-tethering to silica nanoparticles.** 100mg of silane-modified nanoparticles was added to a round bottom flask equipped with a magnetic stirrer containing 5mL of DMF. Excess PnBA was added to the flask to ensure maximum coverage. 0.1mL of a saturated copper sulfate
solution and 0.1mL of a saturated sodium ascorbate was added to the mixture. The contents were then left to stir overnight under ambient conditions. The nanoparticles were separated by centrifugation and washed with water so as to remove excess catalyst, and toluene to dissolve residual PnBA. Coverage was confirmed by TGA while morphology was investigated using TEM and SLS.

5.2.6 Polymer-tethering to iron oxide nanoparticles. 100mg of silane-modified nanoparticles was added to a round bottom flask containing 5mL THF. Excess PnBA was added to the flask to ensure maximum coverage. 0.1mL of a saturated copper sulfate solution and a 0.1mL of a saturated sodium ascorbate solution were added to the mixture. The contents were placed in a sonication bath and left overnight under ambient conditions. The nanoparticles were magnetically separated and washed with a 1:1 Water:THF solution several times to remove excess catalyst, and with toluene to remove residual PnBA. Coverage was confirmed by TGA and morphology investigated by TEM and SLS.

5.2.7 TEM preparations. Dry Casting. 1% (v/v) dispersions of polymer-tethered nanoparticles were prepared in methanol, ethanol, and toluene. Several drops were then placed on a TEM grid and left to dry for a period of 4 hours prior to use. Freeze Drying. TEM grids were submerged in 0.5mL of 1% dispersions of polymer-tethered nanoparticles in t-butyl alcohol. The setup was placed inside a scintillation vial fitted with a vacuum adapter. The vial was placed in an ice water bath to ensure freezing of the solvent and the vacuum was then turned on to remove the solvent by sublimation. Curing. Several drops of 1% dispersions of polymer-tethered nanoparticles were placed onto TEM grids in a petri dish. The dish was then covered and placed in a vacuum oven for temperatures ranging from 60°C to 200°C and for times ranging from 1h to 72h.

5.2.8 Dynamic Light Scattering. 0.1% (v/v) dispersions of polymer-tethered nanoparticles were
prepared in a variety of solvents in scintillation vials. The dispersions were triply filtered prior to use and the vials were cleaned on the inside and outside with triply filtered solvent. Care was taken not to touch the glass of the vial to eliminate contamination.

5.3 Results

Poly (n-butyl acrylate) of molecular weights 6.3kDa, 10.5kDa, and 15kDa were used to chemically bind to three partially reactive nanoparticles: iron oxide of size 3.4±0.6nm and two sizes of silica, one of 6.7±2nm and the other of 22.8±4nm. Thermogravimetric analysis was used to determine the precise polymer loading for each sample, which ranged from 0.007 to 0.24 groups per square nanometer. These ranges correspond to the theoretically determined loadings that allow for different morphologies of nanoparticles to form. These morphologies were indeed confirmed by transmission electron microscopy. Further analysis of the morphologies was carried out by image processing on ImageJ.

Typical morphologies of silica nanoparticles are seen in Figures 5.2 and 5.3. Four phases are observed, corresponding to predictions made in previous studies. While theoretical studies predict distinct phase formations for these systems, we often observe mixtures of two or more phases. We attribute these mixed morphologies to the size distributions of the nanoparticles and the polymers. The morphologies of iron oxide are shown in Figure 5-4. These smaller particles have a much narrower size distribution than the silica particles allowing for distinct phases to form. We later discuss the effect of size distribution on the mixed phases seen in the images.
Figure 5-2: Typical phases observed for silica nanoparticles with R=7.5nm corresponding to agglomerates (a) sheets (b) strings (c) and dispersed phase (d).
Figure 5-3: Typical phases observed for silica nanoparticles with R=20nm corresponding to agglomerates (a) sheets (b) strings (c) and dispersed phase (d).
Figure 5-4: Typical phases observed for iron oxide nanoparticles corresponding to agglomerates (a) sheets (b) strings (c) and dispersed phase (d).

The morphologies presented are in the absence of solvent as the samples were prepared by dry casting onto TEM grids. We later discuss the methodology required to prove these results reflect the morphologies found within the solvent and are not artifacts of solvent evaporation. Sample analysis also restricts us from directly viewing the polymers in the images with their locations predicted from the arrangements of the particles.

A phase diagram for the three particle systems was constructed based on the phases observed in the TEM images and is presented in Figure 5-5. The diagram clearly shows the effect of polymer coverage on nanoparticle self-assembly. On any horizontal line on the diagram
of constant polymer grafting density, several phases are observed. Going towards the right, the size ratio of nanoparticle to polymer increases suggesting that the packing forces will be more dominant since the polymer chains do not shield the packing forces as effectively. Indeed, the resulting phases tend to be aggregates on the extreme right of each horizontal line, but tend to be dispersed on the other extreme. The effect of polymer grafting density is seen on any vertical line in the diagrams. Lower polymeric coverage is not sufficient to overcome the attractive potential imparted by the particles, whereas higher coverage results in net repulsive potential of the particles. Aggregates tend to form at lower ordinate values whereas dispersed particles freely form at higher ordinate values. A balance between these two effects is seen in the diagram. Additionally, we see that lower polymeric coverage is required to create sheets than strings, supporting theoretical predictions as well as our description of the phases. When the actual values presented in Figure 5-5 are compared with theoretical values, we see close agreement. These values are compared in Table 5-1.
Figure 5-5: Phase diagram for all particle systems showing the effect of size and grafting density of polymers and particles on observed phases.

<table>
<thead>
<tr>
<th>Polymer Molecular Weight (kDa)</th>
<th>Surface Reactivity</th>
<th>Theoretical Prediction</th>
<th>Experimental Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0.25</td>
<td>Aggregated</td>
<td>Aggregated</td>
</tr>
<tr>
<td>16</td>
<td>0.9</td>
<td>String</td>
<td>Sheets</td>
</tr>
<tr>
<td>16</td>
<td>1.35</td>
<td>String</td>
<td>Sheets/String</td>
</tr>
<tr>
<td>16</td>
<td>1.8</td>
<td>String</td>
<td>String</td>
</tr>
<tr>
<td>16</td>
<td>2.4</td>
<td>Dispersed</td>
<td>String</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>Dispersed</td>
<td>Dispersed</td>
</tr>
<tr>
<td>10.5</td>
<td>0.25</td>
<td>Aggregated</td>
<td>Sheets</td>
</tr>
</tbody>
</table>
Table 5-1: Comparison of experimental and theoretical values show agreement.

5.4 Discussion

Autocorrelation functions of the four phases were constructed through the ImageJ software and are presented in Figure 5-6. As expected, the strongest correlation appears in the sheet-like phase whereas the dispersed phase is least correlated. The periodicity of the sheets stems from regular spacing of the particles; over long distances the correlation eventually asymptotes to a constant value akin to correlation functions seen in crystals. Within the dispersed phase the attractive potential between the particles is completely shielded and the particles’ placement within the solvent is most random mimicking an ideal gas. When fitted, the dispersed phase matches a random spatial distribution. Much less order is observed within the strings than the sheets because order is restricted to one dimension, which is inherently less organized. In the aggregated phase, the autocorrelation function is pseudo-three-dimensional as the images are mere projections of the actual structures which stretch in the third dimension. It is seen that the structures are quite correlated agreeing with previous research suggesting that aggregates form face-centered-cubic packing.
During dry casting, the nanoparticles are deposited onto a TEM grid simply by evaporating the solvent, which may affect the resulting structure. To ensure that the preparation method does not play a role in forming the observed phases, several samples were frozen in solvent with the solvent allowed to sublime under reduced pressure. When the sample is freeze-dried, the morphology of the particles in the solvent is preserved in the solid state. Sublimation of the solvent deposits the nanoparticles onto the TEM grid exactly as they appeared in the frozen solvent. Figure 5.7 shows that in tert-butanol string-like structures are formed when the solvent is evaporated at room temperature or freeze-dried at 0°C.

Figure 5-6: Autocorrelation of the various phases of the nanoparticles.
In order to address whether the images obtained show truly equilibrated structures, several samples were heated to allow for swifter mobility of the polymers. Figure 5.7 shows the heat treatment in tert-butanol does not alter its morphology.

*Figure 5-7: Phases seem independent of method of preparation. Pictured are the same sample under three separate conditions leading to identical results.*

Several of our silica samples have formed mixtures of sheets and strings contrary to theory which predicts strict phases. Perhaps the clearest reason to explain this resonates in the size distribution inherent to all nanoparticle and polymer systems. Both the larger silica nanoparticles and the smaller silica nanoparticles have wide size distributions as compared with the much smaller and nearly monodisperse iron oxide, as can be seen in the supporting information. Therefore, even though the polymer coverage on all nanoparticles in a given sample is assumed to be constant, larger particles will be more “patchy” than smaller particles. Within a given sample with a constant grafting density, the smaller nanoparticles have less surface area exposed than do the larger nanoparticles. Smaller nanoparticles should, therefore, agglomerate less than larger nanoparticles due to lower van der Waals surface attraction. As we have shown, strings are farther from the agglomerated state than are sheets, thus with less nanoparticle area
exposed, smaller particles should form strings whereas larger ones should form sheets.

Sheets and strings coexist in some samples as can be seen in Figure 5-8. Examining the image alone, one may see size segregation: the sheet-like structures are composed of larger particles than do the string-like structures. A size distribution confirms our prediction with the sheets composed of particles with radius 7.2±1.0nm and the strings composed of particles with radius 3.7±0.8nm. We have also shown that iron oxide does not exhibit mixed morphologies which can be attributed to the narrower size distribution of the particles.

![Figure 5-8: Multiple phases have been observed in some of the silica nanoparticles. Careful image analysis shows size segregation of phases.](image)

A less intuitive reason for size segregation, but significant nonetheless, lies in the stochastic distribution of polymers on the nanoparticles. While we are able to control the density of reactive groups on the surface, we cannot control their locations on each individual particle which are subject to a distribution. There is a nonzero probability that two reactive groups on the surface may be adjacent to one another reducing their availability to polymer tethering. This effect results in a distribution of the grafting density of the polymer. For lower density of reactive groups, the effect of this distribution is amplified. Recent work sheds light on the
distribution of ligand coverage on the nanoparticle surface supporting our notion that for lower grafting density, the distribution in grafting density is wider.

Additionally, the identity of the solvent plays no role in the formation of the phases, provided the solvent does not introduce charge into the interplay. The use of several different solvents is seen in Figure 5-9 supporting the system’s indifference towards the solvent. This finding supports the universality of the phases and their independence on environment and increases the usability of the formed structures in a variety of applications.

![Figure 5-9: Morphological formation is independent of the identity of the solvent.](image)

Based on our data, we see that the size distribution of the nanoparticle has the most significant effect on phase homogeneity. The monodisperse iron oxide particles are virtually without a mixture in phases despite being subject to the distribution of polymer size and grafting density. We may conclude, therefore, that monodisperse nanoparticles will form homogeneous phases given the polymer polydispersity index is less than 1.1.
5.5 Supporting Information

5.5.1 Particle Sizing

In order to arrive at the average size of the nanoparticles, several TEM images were analyzed. Figure 5-10 shows typical treatment of photos leading to a size histogram of the particles. ImageJ software was used to construct the histograms. Images were subject to color thresholds followed by removal binary grayscale. The software may then give the area of the particles in pixels. Each particle was examined by eye to ensure it is indeed a particle and the data point was discarded if the area given was for several aggregated particles or for a portion of a particle. In this manner, several TEM images were used to construct an average histogram based on at least 150 sample points. Sizing was also confirmed by DLS and, indirectly, by BET. This method was used to size all samples, regardless of polymer coverage.

Figure 5-10: Typical image analysis treatment for the nanoparticles.
References


(22) Chai, J.; Buriak, J. M. **2008**, *2*, 489–501.


