Synthesis and Functionalization of Magnetic Particles

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Abstract

Magnetic nanoparticles are a complex mix including a magnetic inorganic core that is typically 30 nm or smaller and surrounding organic species bound to the surface all of which is typically dispersed in a solvent. Creating this structure in a well-controlled and reproducible manner is a topic that has been studied in great detail for the past few decades. This chapter will introduce the various materials and techniques utilized in creating these structures. The enormity of this topic requires a selective approach, and many topics are discussed in summary form, though references are provided for further reading on these topics. Emphasis is placed on the most common and promising approaches to forming and functionalizing magnetic nanoparticles for application in biotechnology. This means that the bulk of text is dedicated to chemical synthesis of magnetic nanoparticles as well as the surface functionalization of those particles with organic small molecules or polymers with or without further reactive or recognition sites.

6.1. Introduction

Nanoparticles are never the thermodynamic ground state of a system but contain a great deal of energy in the form of surface energy(Huber 2005). The system's overall energy would always be lowered by allowing nanoparticles to form macroscopic structures. They are then appropriately viewed as inherently unstable structures that are able to remain only because the pathway to lower their energy is blocked or slowed by the presence of a surfactant coating on the surface. Figure 6.1 shows a general sketch of the structure of a magnetic nanoparticle and the

species that are used to assemble it. Since nanoparticles are not the ground state, the method of their formation has a dramatic impact on the final product including such fundamental properties as size, size dispersity, crystallinity, and crystal phase. Reproducibility in preparation is a particular issue for nanoparticles, since they reside on a steeply sloped energy regime, where even small increases in size can lead to a dramatic decrease in surface energy. This is unlike most familiar preparation methods where the desired product is in a stable or at least meta-stable region of the energy landscape.

Insert Figure 6.1.

The preparation of nanoparticles is an exercise in the kinetic trapping of growing species in the desired size range, and while the details of the methods used to affect this trapping range widely, the general approaches have some fundamental similarities. There are two general approaches; one is to build up particles to the desired size and the other is to break them down to the desired size. In chemical approaches, we build particles up during the course of some chemical reaction, while, in what are termed physical approaches we begin with bulk material and, through the addition of tremendous amounts of energy, tear them down to the nanoscale.

While there are some physical methods of creating nanoparticles that will be addressed here, the majority of approaches are chemical in nature. Physical approaches are those that involve grinding, evaporations, or other processes where the material's size changes, but identity does not. In contrast, chemical methods involve chemical transformations that generate nanoparticles that consist of a chemical species that did not exist at the outset. There are a number of reasons why physical approaches are less used than chemical, but they generally come down to quality and quantity of materials. Approaches such as grinding are plagued by the inefficiency of grinding at the nanoscale, meaning that size distributions tend to be broad and

grinding times quite long(Kumar, Tiwary, and Biswas 2018). Evaporation techniques can quickly produce particle of narrow size distribution but are limited by the area of the surface onto which the particles are being deposited(Gangopadhyay et al. 1992). While there are exceptions, these difficulties have severely limited the usefulness of physical preparation of magnetic nanoparticles for biomedical applications.

In contrast, a large number of chemical approaches are successfully used to synthesize and functionalize magnetic nanoparticles(Yu et al. 2004, Park et al. 2004a, Kataby et al. 1999, Watt et al. 2017, McGrath et al. 2017, Smith and Wychick 1980). These reactions may be formal reductions, oxidations, decompositions, metatheses (A metathesis reaction is one where components are exchanged between compounds, but without a change in formal charge of any of the species.), or combinations of these(Huber 2005). In any case, the basic components are similar, as is the approach to size control. The reactions contain the same basic components, follow similar mechanisms, and yield similar, though far from identical, results. The real art and science are in the details of synthesis.

6.2. Materials Choice

The magnetic particles commonly used in biotechnology contain at least one of the three traditional magnetic elements: iron, nickel, and cobalt. While measurable magnetism has been demonstrated in a number of other materials ranging from noble metals to organic molecules, the effects tend to be too weak for practical use(Nealon et al. 2012, Miller 2014). Since the number of elements is so small, the question then becomes whether to use the metal, an alloy, or an oxide. Metals tend to have the strongest magnetism, though alloys come close and in some cases do exceed the magnetic properties of their parent metals, and oxides tend to be have magnetism that is suppressed relative to the parent metal. Finely divided metals , however, tend to be very

reactive, sometimes even pyrophoric(Huber 2005). As a practical matter, this sensitivity to oxygen and water precludes the use of pure magnetic metals in most biotechnology applications. Coatings to prevent or slow oxidation have been employed and include organic coatings(McGrath et al. 2017) and inorganic coatings(Herrmann et al. 2009), including a native oxide layer(Watt, Bleier, et al. 2018). This is still not an extremely popular approach as a coated metal nanoparticle often has magnetic properties comparable to or even weaker than an oxide particle. Alloying, however, can lead to real improvements. One common example is to use the more stable iron platinum in place of iron(Sun et al. 2000), though there are a number of other combinations of magnetic-noble metal alloys(Wu et al. 2016). These oxidatively stable alloys tend to have magnetism that is somewhat suppressed relative to the pure metal but are greater than the oxides.

By far the most common materials in biotechnology applications are metal oxides, particularly iron oxides. The oxides are readily synthesized, stable for long periods of time in physiological conditions, and are still strongly magnetic. Magnetite (Fe_3O_4) is the most commonly formed iron oxide at or near ambient temperatures, but maghemite (gamma-Fe_2O_3) is also reasonably common. Magnetite has the advantage of slightly higher saturation magnetization, while maghemite, being fully oxidized to iron (III), is more oxidatively stable. In the end, the choice between the two often is a question of which has a more convenient synthesis for a specific application. In contrast, cobalt and nickel oxides tend to be anti-ferromagnetic or paramagnetic and are not generally useful magnetic materials, though they find use as dopants in iron oxides(Fellows et al. 2018).

Iron oxides dominate the literature of magnetic particles in biological and medical applications, because of their ease of synthesis, their low cost, and especially, their

biocompatibility(Jain et al. 2008). Iron and its oxides are generally very well tolerated even in relatively large doses(Wang 2011, De Haro et al. 2015), while the other magnetic elements are of concern. A number of metal alloys are used in their bulk form in implants, the concern is that they are more bioavailable in fine particle form. For this reason, few researchers pursue these materials for in vivo use. In contrast, several iron oxide nanoparticle-based products have been granted regulatory approval for injection into humans(Wang 2011).

6.3. Physical Methods of Nanoparticle Formation

6.3.1. Mechanical Grinding

The standard approach for mechanically grinding materials into fine powders is ball-milling. In this method, the material to be ground is placed in a sealed vessel with spheres of a grinding medium. The vessel is then shaken vigorously for some proscribed length of time to reduce the particle size to the desired level. Ball milling can quickly and easily reduce materials to powders of about a micron, but to reduce the size further, in particular to the tens of nanometers of interest for most biotechnology applications, requires the input of tremendous energy. The plot of surface area versus size in Figure 6.2 pictorially demonstrates the increase in energy in the form of surface energy as the total surface energy in a system is proportional to the total surface area. Inputting this tremendous amount of energy mechanically undoubtedly changes the underlying material, so that crystalline order often suffers when high energy ball-milling is pursued. As a practical matter, inputting this energy mechanically can also require more vigorous shaking than is customary and is referred to as high-energy milling. Addition of surfactant is sometime used to reduce the amount of surface energy in the system and making it easier to reduce particle size in the nanoscale regime. This approach is termed surfactant assisted ball-milling(Chakka et al. 2006). While milling approaches can be tedious and time consuming, they are fairly straight forward for brittle oxides(Goya 2004), but can require more complicated

methods for ductile metals. Metals don't generally grind well, as the particles tend to stick and recombine under the applied pressure of the grinding medium. To overcome this issue, metals can be ground when mixed with an added brittle material such as alumina, to create a nanocomposite of the two. Metals can also be formed in situ by reacting an oxide with a more reactive metal (e.g. iron oxide milled with aluminum)(Pardavi-Horvath and Takacs 1995). Unfortunately, these methods for producing metals create a composite material that consists of large quantities of non-magnetic material. In the end, grinding is a difficult way to produce high quality magnetic nanoparticles for biotechnology applications and is therefore relatively rarely used.

Insert Figure 6.2 here.

6.3.2. Metal evaporation

Evaporating metal onto a surface is a popular method of forming supported thin films of metals, but if the coating is extremely thin, the metal generally dewets the surface and forms discreet nanoparticles. This has frequently been used to make supported nanoparticles. The difficulty is that if one wants to make appreciable quantities of particles that are solution, then evaporation onto a solid substrate is not useful. There are clever approaches to depositing metal directly into a solvent by either rotating a drum of viscous solvent to constantly refresh the surface and therefor allowing continuous deposition,(Nakatani et al. 1987) or by co-depositing frozen solvent with the metal to grow a frozen matrix of solution borne nanoparticles(Klabunde et al. 1974). Very good quality particles can be made in this way, including stoichiometrically controlled alloys or core-shell particles, but very specialized equipment is required, and the quantities produced are still fairly limited. To date these approaches have been used to producing very specialized structures for fundamental scientific studies and have not been broadly applied due to the difficulty and expense of producing particles in this manner.

6.3.3. Sonication

A newly reported physical approach to the formation of nanoparticles is the application of ultrasound to bulk metals. Ultrasound can be used to drive bubble formation and collapse in a solvent which leads to strong mechanical forces and temperatures that can be as high as thousands of degrees C. These forces are known to be capable of tearing particles into pieces and can easily generate micron scale particles. However, it has been shown that particles cannot be continuously broken into smaller and smaller pieces(Suslick and Price 1999). When particles reach a critical size, in the sub-micron range, the forces across the particle can no longer break it efficiently. Additionally, the same forces can smash particles together leading to the generation of an equilibrium size(Prozorov, Prozorov, and Suslick 2004).

It is also well known that when a bubble forms near a surface, that the surface can break the spherical symmetry of the bubble, leading to a flattened side near the surface. When this aspherical bubble collapses it generates a microjet that impinges on the surface(Suslick and Price 1999). This is the mechanism by which ultrasonic cleaners work. It has recently been demonstrated that when the surface of a bulk metal is coated with an appropriate surfactant, that metal can be ejected from the surface and directly captured as nanoparticles(Watt, Austin, et al. 2018). While this hasn't yet appeared in the literature as a method for synthesizing magnetic nanoparticles, given the simplicity of the approach, it can be expected to be seen very soon.

6.4. Chemical Methods of Nanoparticle Formation

Chemical synthesis has a wide range of applicability because there is such a wide choice of potential components. The basic mechanism of the vast majority of these approaches is similar and is described in some detail below. Similarly, there are a handful of basic component that make up a chemical synthesis of magnetic nanoparticles, including solvent, surfactant, and

metal precursor. This section will introduce the fundamental problems with nanoparticles synthesis, describe the popular approaches to mechanistically understand the reactions, discuss the various component that make up most reactions, then discuss specific approaches to nanoparticle synthesis.

As mentioned briefly before, nanoparticles are not the ground state of any reaction and instead are commonly referred to as a kinetic product. This means that the final product is determined by the kinetics of the reaction, not the thermodynamics. This contrasts with the way more tradition chemical reactions are described by high energy reagents that react to form a lower energy product, usually after the addition of some activation energy. The situation with nanoparticle generating reactions, shown graphically in Figure 6.3, is that the desired product has an energy that lies well above the thermodynamically favored product, a bulk material. In this generalized scheme we see the fundamental problem, that larger nanoparticles are lower energy than smaller particles. To produce nanoparticles of a desired size, one must prevent them from continuing to form a lower energy product. To do this, the kinetics of the reaction must be tuned to stop the reaction while the desired product is present. The details of how this is done fills the rest of this chapter, but there are some common themes. First is the use of a surfactant. Looking at the inset of Figure 6.3, we see that every atomic addition to a growing nanoparticle can be viewed as having its own small activation energy. Surfactants can be used to increase the energy barrier to add an atom to a nanoparticles, slowing the march down the curve toward larger sizes. While this doesn't directly exert kinetic control, it helps considerably. The other aspects to kinetic control are careful tuning of concentrations, temperatures, and times. The details of how

these parameters are tuned to desired effect are dependent upon the preferred outcome and the growth mechanism of the nanoparticle forming reaction.

Insert Figure 6.3 here.

6.4.1. The LaMer mechanism

LaMer and Dinegar published a paper discussing a proposed mechanism for a low size dispersity synthesis of an aqueous sulfur sol in 1950, that has become the basis for how we understand most nanoparticle syntheses.(Lamer and Dinegar 1950) The explanation is simple and elegant, and while it neglects certain effects, it qualitatively describes many common syntheses of narrow size dispersity nanoparticles. Figure 6.4 shows a modified version of LaMer's diagram that conceptually describes the three stages of a reaction. In the first stage, a reaction is occurring to generate a monomeric species that will eventually coalesce to form nanoparticles. In LaMer's paper this is referred to as dissolved sulfur species, but in our discussion could represent an iron oxide. The concentration is zero at time zero, but quickly grows and exceeds the solubility in the solvent becoming a supersaturated solution. Most systems, however, require some significant supersaturation to begin forming particles, so even upon crossing the saturation threshold, no stable particles are formed (transient particles below the minimum size for a stable nucleus may form but quickly dissolve).

Insert Figure 6.4 here.

The second stage of the reaction begins when the concentration crosses a critical nucleation concentration. This is the concentration above which the formation of stable nuclei is thermodynamically favored. This is the beginning of particle formation, and in most syntheses is coincident with a dramatic color change. During stage II the concentration may initially continue to increase but will eventually decrease as there is finally a mechanism, the formation

of particles, to partially relieve the supersaturation. The concentration eventually crosses below the critical concentration for nucleation, and the second stage of the reaction ends.

During the third stage, the concentration is below the concentration where nucleation is preferred so no new nuclei are formed, and the number of nanoparticles is constant. Still, the solution is supersaturated, so growth occurs on the existing particles which continues to relieve the degree of supersaturation. This final stage of the reaction then continues, with the particles continuing to grow without the formation of new particles until the reagents are consumed or the reaction is stopped.

The argument that LaMer made based upon this framework is that the key to narrow size dispersity is a short burst of nucleation followed by a relatively long period of growth without nucleation. The reason for this is that if the time between when the first and last nuclei are formed is relatively short, then they will both have had approximately the same amount of time to grow and should be approximately the same size. On the other hand, if there is a long period of nucleation, then the earliest nuclei will have had much longer to grow and would be expected to be much larger than those particles that were nucleated later, resulting in a broad dispersity in sizes. While the reaction that LaMer reported had this kind of behavior without altering the conditions during the synthesis, ensuring a period of growth without nucleation is sometimes done by lowering the temperature after nucleation(Dabbousi et al. 1997). This lowers the rate of formation of the monomeric species and allows the concentration to more quickly drop below the threshold for nucleation and remain there. Successful application of this approach is sometimes referred to as "separation of nucleation and growth" and while this is descriptive of the approach it should not be taken too literally. The two steps are never fully separated as growth cannot be avoided when nucleation is occurring, though growth can occur without nucleation.

6.4.2. Ostwald Ripening

While the LaMer mechanism established the requirement for temporal separation of the elementary steps of nucleation and growth to ensure low size dispersity, it did not explain the inhomogeneity that evolves in the system following the consumption of the monomeric species by the growing nanoparticles. In 1896, Wilhelm Ostwald first described the spontaneous process in a liquid suspension whereby smaller particles dissolve and redeposit onto larger particles(W. 1897). This phenomenon, termed Ostwald ripening, can be explained by considering the surface effects that play a critical role in many kinetic processes at the nanoscale. The atoms on the surface of a particle are energetically less stable than those that are well-ordered in the particle's interior. This means that smaller particles, with their greater surface area to volume ratio, are energetically less stable than larger particles. A simplified version of the Young-Laplace equation describes the relationship between the pressure that a particle in solution experiences as a function of its surface energy (J/m^2) or surface tension (N/m):

$$\Delta P = 2\gamma/r \qquad \text{Eq 6.1}$$

Where γ is the surface energy per unit area of the surface for a particle with radius r (Baldan 2002). From equation 6.1. it becomes apparent that smaller nanoparticles experience a higher internal pressure than larger nanoparticles resulting from their higher surface energy. In order to reduce the overall energy of the system, molecules on the surface of a small particle will tend to detach and diffuse through solution and attach to the surface of a larger particle.

The rate of Ostwald ripening for nanoparticles with an appreciable solubility in a solvent is determined by the concentration gradient at the particle-solution interface. For small particles, the concentration of molecules at this interface is larger than the concentration in bulk solution. This results in the flux of the molecular species from the particle to the solution, leading to shrinking of small particles. Conversely, for larger particles, the concentration of molecules at

the interface is less than that in the bulk solution, driving the diffusion of the molecular species from solution to the surface of the larger particles. The result is the complete dissolution of smaller particles in favor of the growth of larger ones.

Though it is typically associated with size-defocusing of nanoparticles in solution, ripening processes have been reported for increasing the average size of particles in a sample(Chen, Johnson, and Peng 2007, Zhang et al. 2015). However, this approach is typically less desirable than a process by which nanoparticle growth occurs from a continuous flux of the monomeric species in solution, as will be explored in the following section.

Other means to reduce the interfacial energy of a system include sintering and agglomeration. Sintering results from the merging of individual nanoparticles into larger, polycrystalline structures at high temperatures. While sintering of nanoparticles should generally be avoided in nanoparticle preparation, it only becomes a concern at temperatures greater than 70% of the melting point of a given material. Agglomeration of particles can also reduce the overall surface energy of a system without altering the crystalline structure of individual particles. Attractive forces between particles at the interface result in interactions that tend to be irreversible, though reversible agglomeration of nanoparticles in solution has been demonstrated as a method of size control in the synthesis of magnetic nanoparticles(Bleier et al. 2018). In this approach, magnetic nanoparticles nucleate and grow until a critical susceptibility is reached, after which, magnetic attraction between neighboring particles overcomes dispersive forces. This results in agglomeration and precipitation of the nanoparticles from solution, arresting nanoparticle growth. The magnetic agglomeration of the particles can be reversed by post-synthesis processing methods such as strong mixing, heating, sonication, ligand exchange, etc.

6.4.3. Extended LaMer

LaMer and Dinegar described a method by which nanoparticles with low size dispersity could be synthesized when a burst nucleation event is followed by a long growth period. However, achieving systematic size control of the nanoparticles with low size dispersity using this system presents a significant challenge. In this approach, the final size of the nanoparticles at the end of the reaction is ultimately determined by the number of nuclei formed during the nucleation event. For a given quantity of starting material, halving the number of nuclei would double the average volume of those particles when the reagent is fully consumed. Nucleation, however, is a chaotic, non-linear event that is extremely difficult to control systematically. Typical approaches to achieving reproducible control of nanoparticle size have focused on parameters reported to be influential for nucleation, such as the reaction temperature ramp rate(Guardia, Perez-Juste, et al. 2010, Park et al. 2004b). However, performance properties of commercially available temperature controllers make temperature ramp rate a difficult parameter to maintain reproducibly between reactions. Additionally, environmental conditions such as altitude and humidity may affect the onset of nucleation for reactions performed under atmosphere. To realize reproducible, systematic size control of nanoparticles while accepting that the nucleation event cannot be conveniently controlled, a different approach is required.

The LaMer method described a reaction in a closed system, though it is easy to conceive of an analogous process in an open system. This process, termed the Extended LaMer mechanism, uses a continuous addition of precursor to maintain a steady state concentration of the monomer species in solution while maintaining all other parameters constant. The result is a steady growth of particles with a predictable growth trajectory that can be altered by changing details such as addition rate and ligand concentration (Vreeland et al. 2015, Fellows et al. 2018).

The extended LaMer mechanism (Figure 6.5) follows the classic LaMer mechanism in stages I and II but deviates after the nucleation event. In stage III of the extended LaMer mechanism, the concentration of monomer decreases as growth of the nanoparticles occurs. However, the continuous addition of precursor means that production of the monomer species can be maintained indefinitely. The monomer reaches steady state concentration when the consumption of monomer by the growing nanoparticles becomes equal to the rate of production of monomer. This marks the beginning of stage IV, where steady state growth of nanoparticles occurs. The advantage of this approach is that this fourth stage can be extended for an arbitrarily long time,

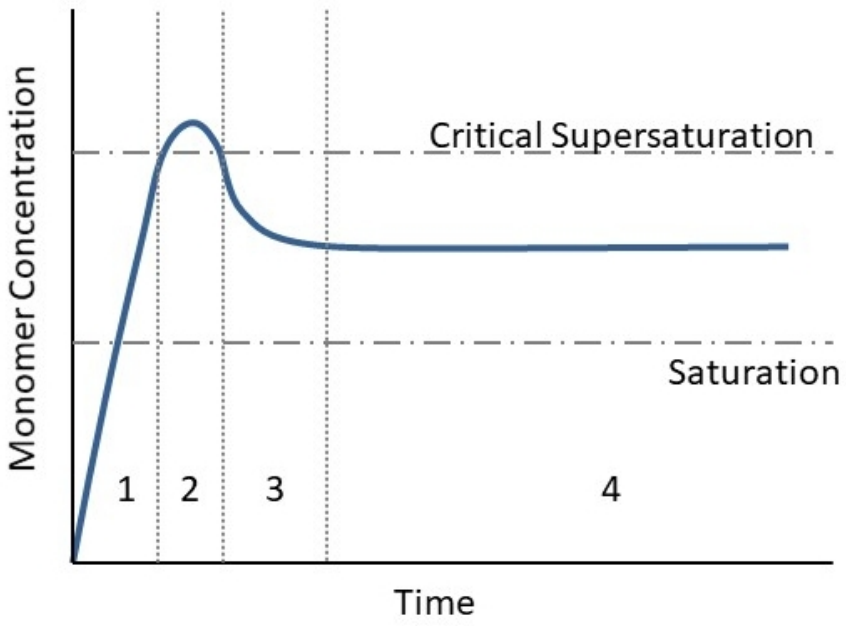


Figure 6.5. Diagram of the Extended LaMer mechanism with the four phases of the reaction labeled. The mechanism begins in a similar way to the traditional LaMer mechanism, but with important differences in the latter times due to the continuous addition of precursor. Phase 1 has an increase in monomer concentration without the formation of nanoparticles, while phase 2 is a burst of nucleation. Phase three is a transitional phase where the monomer concentration decreases until the consumption of monomer equals the formation of monomer from the continuously added precursor. Phase 4 sees steady state growth of the nanoparticles as monomer is continuously formed and consumed in the growth of the nanoparticles. In this phase the average volume of the nanoparticles increases linearly with time.

allowing the growth of a wide range of particle sizes while maintaining low size dispersity. The steady concentration of monomer species suppresses undesirable ripening processes. Further, maintaining the monomer level below the critical supersaturation limit ensure that no additional nucleation events will occur. This approach is applicable to a range of nanoparticle species, though the data here was derived during the synthesis of high quality iron oxide nanoparticles.

A stoichiometric quantity of iron precursor is slowly added to a reaction flask containing a heated mixture of aliphatic hydrocarbon solvent and a long-chain fatty acid. Real time, or near real time monitoring of the reaction provides a means by which a growth rate can be calculated and a desired reaction endpoint can be predicted. Though a number of optical techniques can be applied to monitor reaction progress, small angle X-ray scattering (SAXS) is a technique proves ideal for quantitative measurement of nanoparticle growth. Small aliquots withdrawn from the reaction at regular intervals can be measured in 15 minutes or less and an average nanoparticle diameter calculated. In the case of iron oxide nanoparticle growth, plotting the SAXS diameter as a function of time reveals that two growth regimes are present (Figure 6.6a). In the early stages of the reaction following nucleation, rapid, catalytic growth of nanoparticles occurs. The size dispersity also decreases sharply during this time, indicative of nanoparticle size focusing. This period of catalytic growth transitions to the steady state growth regime where the average particle volume grows linear with time. This is equivalent to diameter growing at time to the 1/3 power. Throughout this steady state growth, we can see that the low size dispersity is maintained.

We have seen that nanoparticles are a kinetically determined product, which makes reproducibility in nanoparticle synthesis a significant challenge. However, the simplified kinetics of the steady state growth stage in the extended LaMer mechanism allows for improved

batch-to-batch reproducibility. Examining three identical reactions, similar trajectories for nanoparticles are observed, with diameter growing as $t^{1/3}$ for all reactions (Figure 6.6b). Additionally, a low coefficient of variation in the size of the growing particles is observed between the three reactions that is comparable to the observed size dispersity of a single reaction. In fact, the data in the steady state growth regime can be so well fitted by the power law that this relationship can be used to accurately predict the final nanoparticle size with a high degree of confidence. As we discussed previously, the nucleation event is unpredictable, even with identical rates of precursor addition, and can yield differences in the number of particles produced between reactions. By taking measurements in the early stage of the steady state growth regime, any variation in the number of nuclei formed is accounted for and the growth trajectory of an individual reaction can be reliably predicted. This allows for nanoparticles of a desired size to be synthesized reproducibly with minimal error.

Insert Figure 6.6 here.

6.5. Reaction Components

6.5.1. Solvent

The majority component in nearly all chemical approaches to nanoparticle synthesis is the solvent. While solvents are often viewed as an inert ingredient in a reaction, this is far from true in nanoparticles synthesis. The solvent choice can be a critical one and is an important factor in the resultant properties such as size dispersity and shape of the nanoparticles formed. The solvent can also limit the reagents that can be used, the temperature of reaction, and the kind of stabilization that can be used to kinetically trap the particles. The initial and most fundamental choice is between water and an organic solvent.

Water is an outstanding solvent for many reactions and is an obvious choice for biomedical applications, where the end application is nearly always in water. There are many

metal sources for making magnetic particles that are freely soluble in water, including common salts such as halides, nitrates, acetates, and others. There are however a number of important drawbacks in the use of water. One is that the temperature of reaction is limited to 100°C unless hydrothermal conditions are used, where a high-pressure reactor allows the reaction to far exceed the boiling point of water (see below for details). In many cases, a low temperature reaction leads to less crystalline materials, whose magnetic response is lowered by the lesser crystallinity.

Magnetic materials, being metals and oxides, tend to be hydrophilic, which means that water will have strong, favorable interactions with the surfaces of growing particles. These strong interactions can differ based upon the crystal face, which results in preferred growth directions during the synthesis. The end result is often non-spherical particles that can anything from rods, to flakes to irregularly shaped, faceted particles. While there are certainly times when these shapes are acceptable or even desirable, spheres are often preferred. These interactions can also lead to increased size dispersity. There is one last concern with having strong favorable interactions between water and the particles, and that is the issue of passivating the particles. Chemists generally use some appropriate surfactant to kinetically trap the particles so that once they are formed, they remain stable. When the particles interact strongly with the solvent, any effective surfactant must interact much more strongly than the solvent, particularly given that it is generally present in orders of magnitude lower concentration. There are three common ways of dealing with this issue. One is to allow water to act as both solvent and surfactant. This is really only acceptable for a short time, as water does not present a strong barrier to agglomeration. Additionally, water passivated particles obviously cannot be dried or wholesale agglomeration can be expected. A second approach is to use a large or powerful surfactant to set up a barrier to agglomeration. A common example of this approach is the use of polar, water

soluble polymers that can have multiple favorable interactions with a particles surface to allow it to remain attached. The final approach is to forgo attachment of a surfactant and take advantage of charge stabilization. In this approach, the aqueous solution is maintained at a pH where the surface retains a strong charge that effectively repels any particle that approaches due to their like charges. In some cases, a bulky counterion (e.g. citrate) may be associated with the surface to provide additional stabilization through steric stabilization. Charge stabilization is very common in particle syntheses in water and can be highly effective, even for long periods of time. However, the pH and ionic strength must be maintained within a window of stability, or the particles' surfaces can be neutralized leading to a loss of stability. For this reason, charge stabilized particles are often coated to provide steric stabilization before being introduced into the complex and varied aqueous environments used in typical biotechnology applications.

Syntheses in non-polar organic solvents generally do not use charge stabilization since dissociation of ions is rare in these solvents. Instead, steric stabilization is very common and works quite well in these systems. Since the particles' surfaces are hydrophilic and the solvent hydrophobic, traditional surfactants with a polar end-group and a long, non-polar tail function extremely well. Oleic acid is a prototypical surfactant for synthesis of magnetic particles in non-polar solvents. The highly polar carboxylic end group can have a strong, chelating attachment to a metal atom on the surface of a particle, while the long alkyl chain shields the particle from the solvent and neighboring particles. The structure is reminiscent of an inverse micelle with its hydrophilic core and hydrophobic exterior. Extending this analogy can also explain the tendency of particles synthesized in non-polar solvents to be rounder that those synthesized in water. Micelles are round as this shape minimizes the contact area between the hydrophilic and hydrophobic entities, minimizing the energy of the system. Similarly, hydrophilic particles

synthesized in a hydrophobic solvent tend to be rounder than their counterparts made in water. This tendency, however, has its limits and at large enough size, preferred, lower energy facets do emerge destroying the roundness of the particles.

Since roundness and steric stabilization are generally beneficial attributes, it may appear that synthesis in organic solvents is the better route. While for some applications where roundness is crucial that is undoubtedly true, aqueous reactions have a number of benefits that can't be ignored. The lack of a hazardous solvent is a big advantage and means that aqueous chemistry is generally cheaper and more scalable. The chemistry can often be done at room temperature, sometimes on a benchtop without concern for fume removal. Reactions in organic solvents are often done at elevated temperatures, sometimes under blankets of inert gas, and generally in a chemical fume hood to manage hazardous gasses. In the end, both water and organic solvents are very common, with aqueous approaches more common in large scale and industrial applications.

6.5.2. Metal Precursor

The two primary concerns for choice of metal precursor is its solubility in the chosen solvent system, and whether there is a convenient reaction pathway under reasonable conditions. In practice, this means that ionic salts (in particular metal halides) are common in aqueous chemistry, while organometallic complexes are more often seen in non-polar solvents. It is also beneficial for reproducibility if the starting material is stable under ambient storage conditions and has a consistent stoichiometry. While this may not seem much to ask of a starting material, there are a number of popular metal sources that present difficulties. For example: Iron pentacarbonyl decomposes at all liquid temperatures (including standard freezers) losing carbon monoxide to form iron cluster compounds, iron oleate tends to form non-stoichiometric compounds with varying numbers of oleate ligands, and even a stable salt like iron (III) chloride

is hygroscopic and can absorb significant amounts of water that, unaccounted for, can render mole calculations inaccurate. These deficiencies can be handled by appropriate means such as freshly distilling iron pentacarbonyl, carefully washing iron oleate, and storing iron chloride in a desiccator, but one must be aware of the potential problems they may cause.

6.5.3. Surfactant

The surfactant choice is crucial for the control of the reaction kinetics, the short and long-term stability of the particles, and the solution properties of the particles in the final application. In general, the control of reaction kinetics and short term colloidal stability are the overriding concerns, because ligands can be and often are exchanged post-reaction.

So, if control of the reaction is the primary goal, then what are the criteria for a surfactant or mixture of surfactants? While the details depend upon the exact reaction envisioned, there are some general design principals. A stronger surfactant, one that partitions more strongly to the particles' surfaces, can be present in lesser amounts that weaker surfactants. Sometimes a coordinating solvent acts as both the solvent and a weak surfactant in a particle synthesis. Determining the optimum surfactant concentration is currently an inexact science, and with concentrations used that vary from millimolar to multi-molar, it can be difficult to decide where to start. Optimizing surfactant concentration for a specific reaction is unfortunately still an Edisonian process, as our predictive ability in reactions with such complex kinetics is still poor. A thorough reading of the literature, however can give us approximate values to begin our trial and error. Very strong surfactants are often present in concentrations below 5%, and occasionally much lower than this. Moderately strong surfactants tend to be present in the range of 5-30%, and a weak surfactant may require concentrations from 30% to greater than 90%. While these classes may sound, and really are, fairly arbitrary, it is not difficult to determine what class a surfactant belongs in. Strong surfactants partition very strongly to the surface of the

particles, generally due to the formation of a strong, covalent linkage to the surface. A familiar example would be alkanethiols serving as a surfactant for gold nanoparticle synthesis. This powerful surfactant can serve its function at low concentrations due to its very high affinity for the surface. Alkanethiols are also strong surfactants for the synthesis of the magnetic metals, but not the oxides. Strong surfactants are not often used for the synthesis of magnetic metal particles, because their very strong interactions can cause a large loss in magnetism in the particles(Huber 2005). Thiols(Katabi et al. 1996) and phosphines(Yee et al. 1999) are two of the more common strong surfactants for the synthesis of magnetic metal particles. The oxides, being much less reactive do not have any commonly used strong surfactants. Moderate strength surfactants are more common for the oxides and are those that form more ionic linkages with the particles' surfaces. These include common acids and bases such as carboxylic acids, phosphonic acids, alkyl amines, and a number of polymers that contain these groups. Additionally, functional groups that have weaker interactions can form moderate surfactants if they are multiply present on a surfactant molecule, such as the case of polymers. These polymers commonly include polyvinylpyrilidone, polyethers, polyamines, polyacrylates, polyacrylamides, and nearly any other polymer containing significant quantities of polar groups. The weak surfactants are generally small molecules containing a one or a few polar groups that form no strong bonds with the particles' surfaces. These can include a number of common functional groups like alcohol, ethers, or aprotic amines, and include a number of common solvents such as tetrahydrafuran (THF), dioxane, ethylene glycol dimethyl ether (glyme), and pyridine.

6.6. Methods to Synthesize Metal Oxide Nanoparticles

6.6.1. Aqueous precipitation

Aqueous precipitation of oxide particles is one of the most popular methods of forming magnetic particles. In particular, the coprecipitation of iron (III) and iron (II) salts in equal

amounts is a very common approach to making magnetite (Fe_3O_4) particles, which has is an oxide with equal amounts of Fe(II) and Fe(III). This reaction takes advantage of the varying solubility of iron ions as a function of pH. Iron is very soluble at low pH values and can be easily dissolved by relatively dilute (0.1 M) hydrochloric acid. This dissolution forms iron chloride, which is the species used in most coprecipitation reactions. Likewise, dissolution of iron chlorides in water forms an acidic solution upon dissociation of the iron and chloride ions. This low pH is critical to its solubility and rapid addition of base leads to an immediate precipitation of the iron as oxide particles. Size of the precipitate can be varied by addition of surfactants, initial and final pH, and concentration of reagents, including altering the ratio of iron(III) to iron(II).

Hematite (gamma-Fe_2O_3) is a magnetic iron oxide where the iron is all present as Fe(III) but forming it by aqueous precipitation is not as simple as neutralizing a solution of Fe(III) ions. This reaction typically forms a complex mix of phases with some hematite and some magnetite as well. Hematite is not the thermodynamically preferred phase of iron oxide at ambient temperature and pressure, magnetite is, and this makes it difficult to form phase pure hematite using room temperature aqueous chemistry. Thermolysis of iron salts in water has been shown to form hematite more effectively, as has hydrothermal reaction conditions (discussed below).

Aqueous precipitation is certainly the easiest, cheapest, safest, and most environmentally benign approach to the synthesis of magnetic particles, which has made it one of the most popular methods of forming magnetic particles industrially. In many applications these particles work extremely well and no other approach need be considered, however there are some applications where the odd shapes and poor size control are an issue. In general, those applications with more stringent requirements must simply use another synthesis approach, as

the kinetics of precipitation are not easy to control. The reason for this is that establishing a short nucleation event followed by a slow growth as prescribed my LaMer is extremely difficult in this type of precipitation reaction. When performing a neutralization, when nearing the equivalence point a very small addition of base leads to a very large change in pH. This of course means a very sudden loss of solubility occurs for the iron salts, so the entire reaction occurs over a very short amount of time and a short, chaotic reaction occurs to yield particles with substantial size dispersity. The rapidity of the reaction at relatively low temperature can also lead to particles that are irregularly formed and that are not fully crystalline, which can have detrimental effects on the magnetic properties.

6.6.2. Hydrothermal Synthesis

Hydrothermal synthesis is a method of aqueous synthesis where the reaction is heated above the boiling point of water by keeping the reaction in a sealed chamber. Specialized reaction equipment is designed for this kind of reaction where many atmospheres of pressure can be generated and safely contained for an extended period of time. While similar precursors may be used in this type of reaction, there are a number of important distinctions. Since they occur in a sealed reactor, hydrothermal reactions must occur without the addition of other species, so the precipitation reactions generally occur as precursors slowly react at elevated temperatures. The high temperatures and pressures also favor improved crystallinity, and the slow reaction times can allow subtle differences in the energetic of different facets to result in highly non-spherical particles. For example, rods are a frequent result of hydrothermal syntheses.

While all of this sounds very promising, hydrothermal syntheses are not without their difficulties. Obviously with sealed reactions reagents cannot be added nor can aliquots be withdrawn to monitor the reaction. Additionally, the stainless-steel vessels prevent any visual clues as to the progress of the reactions.

So, although other chemical methods may be more versatile in some respects, hydrothermal syntheses are important for the ability to yield highly crystalline material in an aqueous environment. This is particularly true for the synthesis of maghemite, as this is a material that is very challenging to produce as a single-phase material below 100°C.

It is worth noting that there is an analogous non-aqueous approach referred to as solvothermal synthesis. While the synthesis differs only in the choice of solvent, it has not found common use in the synthesis of magnetic particles. This may be because high boiling point non-polar solvents exist so that reactions can be conveniently run as high as 340°C at atmospheric pressure. Any reaction that is run higher than this will likely produce gaseous biproducts from thermal decomposition that could potentially lead to unsafe levels of autogenic pressure to be generated. In fact, it bears mentioning that any hydrothermal or solvothermal reaction should be performed only after careful considerations of the safety of the temperature/pressure state that will be achieved in the reaction. The specialized vessels used in these reactions are colloquially referred to as "bombs" for very good reason.

6.6.3. Thermolysis in organic solvents

In recent years thermolysis, or thermal decomposition, of organometallic compounds in organic solvents, has become a very popular method of synthesizing magnetic metal oxides. One of the most popular methods is the thermolysis of iron oleate in high boiling point solvents such as dioctyl ether or octadecene. These reactions can produce large quantities of narrow size dispersity particles that are round and often single crystalline. The quality of the particles produce in this method are truly outstanding, so it is worth discussing the reaction in some detail. While other iron carboxylates can produce nanoparticles of similar quality, it is the iron oleate reaction that has been most studied. The first step in this approach is to synthesize the iron oleate, as it is not currently commercially available. Perhaps one reason that it is not available is

the difficulty in producing it in the proper stoichiometry(Bronstein et al. 2007). The reaction itself is very easy. Oleic acid is neutralized with sodium hydroxide, and then the resultant sodium oleate is mixed with iron (III) chloride and stirred to produce iron (III) oleate and sodium chloride. Iron oleate does not crystallize, instead forming a viscous oil and upon cooling below ambient temperatures, a glass. Crystallizing is one of the simplest and most versatile methods of purifying a crude material, but without this option the most common approach is to wash repeatedly to remove unreacted oleic acid and iron chloride. The difficulty here is knowing when to stop washing, as excessive washing can continue to remove oleic acid producing a material that is deficient in the acid.

Once the iron oleate is synthesized, it is mixed with additional oleic acid and a high boiling point solvent and heated at a controlled rate and allowed to react a temperature near 300°C. Generally, a temperature in excess of 280°C is required for the reaction to occur, and temperatures as high as 360°C are not uncommon. Typical reaction times are from 30 to 90 minutes, and they are generally conducted under an inert atmosphere. Longer reaction times tend to allow faceting and wider size dispersities through ripening processes, while relatively short reactions yield uniform spheres of magnetite. While this approach can produce large quantities of particles that are very uniform, round, and highly magnetic such as those in **Error! Reference source not found.**, there are difficulties in controlling and reproducing the size in these reactions. Obviously, variations in the stoichiometry of the iron oleate can alter the final product, as can variations in the heating rate that a reaction sees. Both of these probably have their primary effects by altering the reaction in the early stages, which alters the amount of time that nucleation occurs. This in turn changes the number of nuclei, which, for a system with a fixed amount of precursor will naturally alter the size of the finished particles.

There are a number of other precursors that can be used to produce magnetic oxide particles, including various iron carboxylates(Bronstein et al. 2011), iron pentacarbonyl, and iron acetylacetonate(Masthoff et al. 2014, Guardia, Pérez, et al. 2010). The iron carboxylates are generally used in similar ways as the detailed description above of the most common member of this class, iron oleate. Iron pentacarbonyl can be used to make both metal and oxide particles depending upon the conditions chosen(Huber et al. 2004, Hufschmid et al. 2015). For the synthesis of iron oxide, generally magnetite, iron pentacarbonyl is heated in the presence of an oxidizing agent such as a carboxylic acid (The reaction is an oxidation, since the iron in iron pentacarbonyl is formally neutral). The decomposition reaction is generally conducted above 120°C, often as high as 200°C, to obtain rapid but controllable kinetics. The carboxylic acid is both the oxidant, and surfactant in this reaction. While this reaction produces high quality particles, it is not as popular as other methods since iron pentacarbonyl is a reactive, toxic, and volatile liquid. Iron acetylacetonate would then appear to be an ideal reagent as it is a stable solid that is not particularly hazardous. Its primary drawback is its lack of solubility in most common solvents at ambient temperature. This can be overcome by simply adding it at room temperature and heating the solvent until the material dissolves and decomposes. When heated in oleic acid, iron acetylacetonate decomposes to form iron oleate in situ, which further decomposes to form nanoparticles with low size dispersity and excellent magnetic properties(Vreeland et al. 2015). This approach provides stoichiometric control of the iron precursor while yielding a high quality nanocrystalline product from the iron oleate intermediate. In situ formation of iron oleate has the additional advantage of eliminating the processing and washing steps of the conventional method for preparing iron oleate that make reproducibility between reactions challenging. There also appears to be some debate in the literature as to

whether the main product of this decomposition is magnetite or wüstite (FeO). It is likely that the product depends upon the details of the specific reaction in question, but wüstite is generally not desirable as it is not strongly magnetic. As it is metastable in ambient conditions, wüstite can easily be converted to Fe_3O_4 with moderate heating under atmospheric oxygen.

Insert Figure 6.7 here.

6.6.4. Polyol synthesis

The polyol synthesis is a traditional method of making inert metal nanoparticles using molecules with multiple alcohol functionalities as a weak reducing agent at elevated temperatures. A typical reaction would be to heat a gold salt in the presence of a diol (e.g. ethylene glycol) in water and a surfactant to produce gold nanoparticles. When applying this reaction to iron salts, however, the reducing environment is not generally strong enough to produce pure iron nanoparticles. Instead polyol reactions with iron salts tend to yield oxide particles(Jungk and Feldmann 2000) or iron particles with oxidized surfaces(Joseyphus et al. 2007).

6.7. Methods to Synthesize Metal or Alloy Nanoparticles

6.7.1. Reduction of Salts

Metal salts can be reduced to form metallic nanoparticles using a wide array of reducing agents in either water or organic solvents. The solvent is an important consideration when choosing a reducing agent, as the stronger reducing agents are not compatible with some solvents. Water in particular is incompatible with most of the well-known hydride reducing agents. One important exception is sodium borohydride, which, though it slowly decomposes in water, can be used in aqueous solutions if they are freshly prepared and used immediately. This is probably the strongest reducing agent that can be used in an aqueous solution and is therefore one of the more popular. Aqueous syntheses of metal particles have some of the same shape and

size control problems seen in the oxide systems, so a compromise method is sometimes seen: the microemulsion route. In a microemulsion synthesis, an aqueous salt solution is reduced while encapsulated in water swollen surfactant micelles. This couples two advantages: the high solubility of metal salts in water and the tendency to form round particles in organic solvents. Still, the presence of water can be problematic. Water, is often avoided in the synthesis of magnetic metal nanoparticles, because none of the magnetic metals are stable in the presence of water, and they generally form an oxide relatively quickly(Sun et al. 2006). The oxides on the surface of metal nanoparticles don't contribute to the magnetic properties of the particle as they tend to be disordered and have poor magnetic responses. So, while they can be tolerated in larger particles, in small particles, even a thin oxide causes a large decrease in the magnetization. This is such a significant problem, that one often sees metal particles with lower saturation magnetizations than oxide particles.

Given the difficulties with water, the reduction of metal salts in anhydrous environments has been explored. In this approach, anhydrous salt, surfactant and solvent are mixed, and a strong reducing agent is added. One critical concern in this synthetic approach is the poor solubility of metal salts in non-polar solvents. This must be overcome by either using a coordinating solvent such as tetrahydrofuran or glyme that can provide sufficient solubility or using a surfactant that will solubilize the salts (alkylated polyethers work well(Martino et al. 1997)). While this approach can yield very good quality metal particles, the reaction tends to be very dilute so that the production of large quantities of nanoparticles is impractical.

6.7.2. Thermolysis /Sonochemical decomposition

Thermal decomposition to make magnetic metal nanoparticles proceeds very much like the methods to make oxides, the primary difference is in the choice of reagents and surfactants. Since the magnetic metals all form oxides quite easily, metal precursors employed generally

avoid structures that have oxygen metal bonds. During decomposition of compounds with these metals bound to oxygen, some of the oxygen generally remains with the metal to produce oxides as in the cases of iron oleate, or iron acetylacetonate. There is one popular class of precursors that lacks a metal oxygen bond, and also decomposes at convenient temperatures: the metal carbonyls. Carbonyls of all three magnetic metals exist and have convenient decomposition rates in the 100-200°C range. While nickel carbonyl, $Ni(CO)_4$ is well known, its high volatility and extreme toxicity has made it difficult to procure commercially, and its use is not recommended unless one is extremely skilled and well-versed in the handling of such acutely toxic compounds. Cobalt carbonyl, $Co_2(CO)_8$, and iron carbonyl, $Fe(CO)_5$ are both toxic, but are not of such extreme toxicity that they can't be handled using appropriate care in a well-equipped chemical laboratory. Iron and cobalt carbonyl have both been used to make metal nanoparticles individually, and as alloy particles. The general approach is to heat the materials in an appropriate high boiling point solvent such as dioctyl ether, and a surfactant(Huber et al. 2004). Oleic acid is commonly used, and while it coats the particles well, it also tends to form a thin oxide layer(Farrell et al. 2005).

While the thin oxide provided by oleic acid or some other appropriately mild oxidizer can provide some stability to the metal particles, their tendency to oxidize is so strong that additional alloying has been performed to stabilize them. Platinum and palladium alloying have been shown to stabilize both iron and cobalt towards oxidation. Iron platinum nanoparticles have been particularly well studied and have been used in a number of biotechnology applications. As formed, iron platinum occurs in a chemically disordered face centered cubic phase which has very good magnetic properties. While its saturation magnetization is below that of pure iron nanoparticles, it is higher than that of any iron oxide, and is higher than most oxide coated iron

nanoparticles as well. The most common approach is to make this material is to follow the approach of Sun et al, and simultaneously decompose iron carbonyl and platinum acetylacetonate in a high boiling point solvent in the presence of oleic acid and oleyl amine(Sun et al. 2003). The amount of platinum can be systematically varied in this system to alter the magnetic and oxidative properties of the resultant nanoparticles.

Another, closely related synthesis is the use of ultrasound irradiation to drive the decomposition of, for example, iron carbonyl. The reaction can be viewed as similar to the thermolysis of iron carbonyl, but the heating is decidedly non-uniform, as locally very high temperatures are created where cavitation and collapse occur in the liquid(Suslick et al. 1999). The resultant high cooling rate can actually create amorphous iron nanoparticles, a material that is generally very difficult to produce(Suslick et al. 1991).

Though very magnetic metal particles have been produced, precious few have found use in biotechnology. The primary exception is iron platinum alloy particles. These have good stability in physiological conditions and have excellent magnetic properties. The pure metals are rarely used as they tend to oxidize readily and lose their magnetic properties in water or buffers. Attempts have been made to coat the particles to prevent this from occurring without tremendous success. Overcoating with noble metal has generally been unsuccessful, though some particles with carbide coatings have shown greatly enhanced stability in water(Meffre et al. 2012).

6.8. Purification

At each step of nanoparticle synthesis and functionalization, purification steps are required to isolate particles from excess reagents in solution, as well as concentrate particles and perform solvent changes. The method chosen is dictated largely by the scale of separation desired and the solvent system required.

6.8.1. Precipitation and separation

A straightforward method for separating nanoparticles from a complex solution is precipitation of the nanoparticles, which generally requires the addition a poor solvent for the functionalized nanoparticles and rapid precipitation from suspension by centrifugation or by placing the suspension in close proximity to a magnetic field gradient. For instance, oleic acid functionalized magnetite nanoparticles suspended in a non-polar hydrocarbon solvent such as hexane can be precipitated from solution by the addition of acetone or ethanol to solution followed by centrifugation or magnetic separation(Vreeland et al. 2015). In this manner, small volumes of particles can be rapidly purified without complex experimental apparatus. However, practical use of this technique for production scale purification is limited by the difficulty of scaling this process to large volumes.

6.8.2. Liquid chromatography separation

Size exclusion chromatography (SEC) also known as gel permeation chromatography (GPC) is a liquid chromatographic technique typically used to separate macromolecules in solution. SEC columns are packed with porous media (either polymer or silica based) that separate molecules according to their hydrodynamic volume in solution. Large molecules explore less of the pore volume of the stationary phase, allowing them to elute from the column faster that smaller molecules. In a similar manner, a suspension of nanoparticles can be purified from excess reagents by passing the mixture through an SEC column and collecting the pure nanoparticle eluent(Davis et al. 2014). Depending on the surface functionalization of the nanoparticles, a broad selection of aqueous and organic mobile phase can be used with either polymer or silica based stationary phases, making this a flexible purification technique.

6.8.3. Hollow Fiber Diafiltration

In the hollow fiber diafiltration process, a nanoparticle suspension is circulated through a tubular porous membrane, where the pore size determines the retention or passage of the components in

solution(Sweeney, Woehrle, and Hutchison 2006). Repeated circulation of the solution with controlled replacement of the permeate results in a pure, concentrated nanoparticle solution. Commercially available membranes are best suited for use with aqueous-based suspensions. Additionally, the ability to parallelize the setup makes it an attractive option for production scale purification processes, whereby large volumes of nanoparticle solutions can be processed simultaneously with identical process parameters.

6.9. Functionalization Strategies

6.9.1. Introduction

While the details of a particle functionalization strategy will depend upon the ultimate application, functionalization of nanoparticles for biomedical applications generally has two broad tasks. These are to provide colloidal stability to the particles and to impart specific functionality. Colloidal stability is covered in much more detail in Chapter 4 of this text but will be discussed in a more concise manner here as necessary. Since most biotechnology is done in near-physiological conditions, stability in complex aqueous environments is usually critical. Ideally this would mean the particles remain well-dispersed in pure water or buffers of a wide range of pH and ionic strengths, and with or without the presence of proteins. For in vivo applications this is particularly important as the range of potential conditions that could be encountered is enormous, and significant agglomeration could have life threatening results. Shelf-life is also a concern, so maintaining this stability for the long term is also highly desirable. The first goal of almost any functionalization strategy then is to provide water solubility to the particles in a way that is robust enough to endure whatever pH, ionic strength, and temperature excursions the material can reasonably be expected experience.

It is also generally important to have a particle system that is biocompatible. This is critical in both in vitro and in vivo systems. The main concerns for in vitro systems is that the

particles will lose their colloidal stability and agglomerate in the presence of proteins of other biomolecules or that they will participate in non-specific adsorption of proteins or other biomolecules that is inappropriate for the application. For in vivo systems the concerns are more complex and include possibilities of provoking immune responses, agglomerating leading to an embolism, or having undesirable toxicity. In general, organic chemicals that are well hydrated, and have few or no charged species are the ones that are best tolerated in biological systems. These materials are often referred to as biocompatible or as non-fouling surfaces due to their tendency to avoid rapid, non-specific protein adsorption. The canonical example of an anti-fouling material is the water-soluble polymer poly(ethylene glycol), often abbreviated as PEG. This polymer may also be called poly(ethylene oxide) as polymers are generally named for the monomer from which they are synthesized and the polymerizations of ethylene glycol and ethylene oxide yield the same polymer. There is a trend in usage in that end-functionalized polymers and block copolymers are often named as poly (ethylene oxide) as the anionic polymerization of this monomer is more conducive to yielding these products.

Many applications will also have targeting species bound to particles, so in these cases the functionalization strategy must provide a method for attachment of these moieties in appropriate numbers. This adds to the complexity significantly as it demands multiple functionalities in the stabilization approach and care must be taken to use compatible chemistries in the attachment to the surface and subsequent binding to targeting species.

At the most basic level, the goal of the stabilization is to prevent the particles from agglomerating and thereby providing colloidal stability. This can be accomplished in two fundamentally different ways: charge stabilization and steric stabilization. Charge stabilization is a matter of maintaining a strongly charged particle surface, so that the like charges on the

particles repel neighboring particles. In steric stabilization, the particles maintain separation by having attached molecules whose steric bulk prevents the particles from touching. Both can be effective, but steric stabilization is most often used in biotechnology approaches as it is not as sensitive to pH and affords more obvious methods of attachment of targeting species.

It is worth noting that maintaining separation and colloidal stability is especially challenging in magnetic particles. There is a common misconception that superparamagnetic nanoparticles do not agglomerate magnetically because the constant realignment of their moments gives a time-averaged zero attraction. The situation is not so simple, as the moments in superparamagnets can align and interact, yielding a strong net attraction(Martin, Venturini, and Huber 2008). Of added concern is the fact that when agglomeration occurs, it can be particularly difficult to disrupt in magnetic particles as the interactions between particles can be very powerful. Agglomeration of particles is best avoided, and it is desirable to maintain colloidal stability at all times during the nanoparticle functionalization.

Following is a general discussion of the most common stabilization approaches and their relative merits. While only a subset of the approaches may be appropriate for any individual application, this should function as a general introduction to the most successful approaches.

6.9.2. Charge stabilization

Charge stabilization is perhaps the simplest possible method of maintaining colloidal stability as there is generally no functionalization of the particles required. Generally, all that must be done is to maintain the pH of the solution in a range where the inorganic surface of the particle is highly charged. In appropriate conditions, the surface charge is stable and provides stability indefinitely. While this may sound ideal, there are significant drawbacks. If the pH is moved towards the isoelectric point of the particles, where they bear no net charge, the charge stabilization obviously ceases to function. Additionally, there are numerous species that can act

as flocculants in systems like these by screening the surface charge or binding to multiple particles. Polyions in particular can be problematic in this respect, and proteins and many other biological species are polyions. Finally, since charge stabilization usually depends upon the strong charge that the inorganic surface bears, it generally precludes surface functionalization with biological targeting species.

The result of these caveats is that charge stabilization only works well in a well-controlled aqueous environment with no significant potential for pH excursions or the presence of complex biological fluids. The potential for the use of particles with colloidal stability that is purely charge stabilized is extremely limited in biotechnology. That being said, the phenomenon is critical in many aqueous synthetic approaches, and can contribute to the stability of particles that are functionalized with charged molecules. Decrease in charge stabilization should especially be suspected when particles unexpectedly lose their colloidal stability when pH is changed.

6.9.3. Steric Stabilization

Steric stabilization is a more common approach in biological applications, as it has considerably more versatility. The approach is to coat the inorganic surface with an organic coating that masks the attraction of the cores and has no attraction itself. The goal is to provide interactions between particles that have a net repulsion at all distances. Since the magnetic cores will have a strong attraction if they are in close proximity, there is a minimum thickness of the organic coating to prevent a potentially irreversible attractive interaction from occurring. The exact distance where the magnetic attraction is significant will depend upon the details of the system such as the magnetic material and the particles' sizes but it can be calculated if the system is well understood(Bleier et al. 2018). There is however a useful rule of thumb that can often be applied. In general, if magnetic nanoparticles are separated by a distance equal to or

greater than their radius, then their magnetic interactions are negligible. So, a coating that is half the radius of the particles will generally ensure that magnetic interactions are so weak that no magnetic agglomeration will occur in the absence of an external field. In practice, the coating may function even in the thickness is significantly lower than this, depending upon the details of the system. For a 5 nm particle, a small-molecule self-assembled monolayer could easily provide the separation necessary for colloidal stability, but for a 25 nm particle, a polymer layer is often required(Saville et al. 2014). Polymers are generally very popular for particle functionalization as they are cheap and easy to make, are commercially available with numerous functionalities, and are reasonably easy to attach to surfaces.

Note that the concept of a layer thickness is not simple when one is referring to a water-soluble polymer layer. For a small-molecule self-assembled monolayer, we often assume that the layer is as thick as the molecule is long in its all-trans conformation(Bleier et al. 2018). While this may not be precisely correct, it is generally very close. In the case of a polymer this would be woefully inaccurate. In polymer terminology, this all-trans length is referred to as the contour length and is generally much longer than the actual molecular dimension. Other length scales that are more appropriate are the radius of gyration (R_g) and the hydrodynamic radius (R_H), though even these do not generally precisely match the thickness of a polymer layer. Often, it is necessary to infer the thickness using things like changes in the size measured by dynamic light scattering.

6.9.4. Small molecule surface bound monolayers

As discussed above, nanoparticles are not the ground state of any system, and so a kinetic barrier to agglomeration is necessary at all times. Most systems will include some strongly bound small molecule surfactant on the surface of the nanoparticle. These are generally present when the particles are synthesized and often remain on the particle as it is further functionalized.

The continuous presence of a surface passivation layer prevents cores of the particles from making direct contact which can lead to particle fusion and irreversible agglomeration, a process that destroys the nanostructure of the material. A common surfactant on the surface of magnetic nanoparticles, such as magnetite, is oleic acid. This molecule is inexpensive, has a high boiling point, and has a well-documented affinity for iron oxides, making it an ideal surfactant for high temperature syntheses. It also forms a chelate with the surface, providing a reasonably strong linkage with the surface, as well as having a lengthy hydrophobic tail which provides a significant barrier that prevents the hydrophilic cores from coming into direct contact.

Still, oleic acid has its drawbacks. It is a monofunctional molecule, and with its carboxylic acid bonded to the surface, there is no reactive species to allow conjugation of biocompatible coatings, targeting species, or other biologically relevant molecules. This means that any functionalization that occurs on top of an oleic acid monolayer is by necessity performed using non-covalent means. Additionally, though oleic acid provides good stability in many environments, the oleic acid is somewhat labile and establishes an equilibrium with some molecules free in solution while others remain on the surface. This equilibrium behavior is characteristic of surfactant systems except those with the strongest covalent surface attachments. The result of this equilibrium behavior is that nanoparticles stored in a good solvent for oleic acid, that has had excess oleic acid removed, can show decreased stability and eventual agglomeration. If excess oleic acid is repeatedly removed, oleic acid is shed from the particles to re-establish an equilibrium leading to a more diffuse coating and the potential for core-to-core contacts.

The lability of the oleic acid bond is not an insurmountable problem. First, the loss of oleic acid only occurs when the particles are dissolved in a good solvent for oleic acid. In a final

biotechnology application, the solvent is generally water, in which oleic acid is insoluble. There is little need to be concerned about loss of oleic acid once the particle has been transferred to an aqueous solution. Second, the ease with which oleic acid can be removed provides an opportunity to perform a ligand exchange, where oleic acid is removed and replace with a more desirable surfactant. The new surfactant could provide a stronger bond to the surface or have additional functionality for covalent attachment of subsequent functionality.

Ligand exchanges must be done carefully to maintain stability of the nanoparticles at all times, as any agglomeration that occurs while ligands are being removed is likely to be difficult or impossible to reverse. The general approach for a ligand exchange is to remove an excess of the initial surfactant from solution, then add a large excess of the desired new surfactant. Heat or sonication can speed the ligand exchange, but merely agitating for several days can achieve the exchange as well. There is some evidence that a slow, mild exchange over several days can lead to less agglomeration. Ligand exchanges work particularly well when the new surfactant has a stronger bond than the initial, so very successful exchanges with phosphonates(Sahoo et al. 2001, Davis et al. 2014), catechols(Stone et al. 2013, Davis et al. 2014), and silanes(Ma et al. 2003) have been reported, though other carboxylic acids can also be exchanged onto the particles' surfaces, with more difficulty(Davis et al. 2016).

Regardless of the specific species chosen as the initial surfactant, there are several roles that it must fulfill. It is there to arrest agglomeration during synthesis of the nanoparticles and prevent their irreversible agglomeration during any subsequent purification or other treatment. It often provides either a covalent linkage for further functionalization or acts as an anchored base for non-covalent interactions that allow the adsorption of functional species.

6.9.5. Small molecule bilayer coatings

Small molecule coatings can be placed onto the initial coating of the nanoparticles using either covalent or non-covalent approaches. In practice the non-covalent coatings work so well, that covalent attachments are rare. The result of a non-covalent attachment is a structure that is essentially a bilayer structure. A typical structure then would have a hydrophilic layer in contact with the particle's surface, with a long hydrocarbon tail (typically in the range of 10-20 carbons) extending away from the surface. These hydrocarbon tails are then in contact with another layer of hydrocarbon tails of similar length that have their associated hydrophilic headgroups extending into solution providing water solubility. Almost any surfactant with the classical structure of an alkane tail and a hydrophilic headgroup can work in this type of system and will, under appropriate conditions, form a bilayer structure. The tail can be either a single tail or a double tail as in lipids, and the head can take a variety of forms that include ionic or non-ionic hydrophilic headgroups.

One important consideration though is that the surfactant should not have high solubility as single molecules in water, meaning they should have a low critical micelle concentration (CMC). Below the CMC every molecule in solution exists as a single isolated molecule, and at the CMC the solubility limit is reached and every additional molecule of surfactant enters into a micelle. In surfactant systems, coated particles act very much like micelles, and if the surfactant has a high CMC, then dilution of the coated nanoparticle solution can serve to strip the particles of their associated surfactants causing agglomeration. Fortunately, many commercial surfactants have low CMC values, as do naturally occurring lipids. Small molecule surfactants that are appropriate for this type of coating approach include lipids, alkylated oligoethers, alkylated sulfates, and many others.

An important limitation on this approach is the size of the surfactant layer that is produced. Small molecule bilayers are typically under 5 nm in thickness, meaning that for particles larger than about 10 nm, these kinds of structures may or may not provide protection from magnetic agglomeration for highly magnetic particles. For larger particles, it is often necessary to go to polymeric systems that may have similar bilayer structures but are considerably thicker.

Forming small molecule bilayer coatings to provide water solubility can be a very simple process. Commercial surfactants are typically very good micelle formers, and the desired structure is similar in shape and size to an aqueous micelle. The process can be as simple as adding the surfactant to the hydrophobic solvent that contains the particles to be coated, adding water, and shaking or stirring the solution until the nanoparticles transfer to the aqueous phase. The transfer is generally obvious as magnetic nanoparticles are generally darkly colored, and their transfer can be seen by eye. Any emulsion that forms can usually be broken by centrifuging or adding salt to the water to drive the phase separation, or both if needed.

6.9.6. Polymer coatings by "grafting to" approaches

Attachment of preformed polymers to a surface has a number of advantages for biotechnology applications. A wide range of functional and biocompatible polymers are commercially available, so they can be bought and attached to particles to yield exactly the desired properties. This can greatly simplify the functionalization chemistry. The drawback is that the density of the polymer layer that can be achieved by attaching preformed polymers to surfaces is somewhat limited. The reason for this limitation is that as a layer of polymer is forming on a surface, a concentration gradient begins to develop with a higher concentration of polymer near the surface. For further attachment to occur, each large polymer molecule must diffuse against this gradient before attaching to the surface(Huber et al. 1997). At some point,

the energy penalty for moving against this gradient becomes too high and further adsorption essentially ceases. Still, the graft densities achievable are often high enough to lend stability, as a density gradient that is high enough to prevent polymers from approaching the surface is likely to be dense enough to prevent other particles from approaching the surface as well.

There are two basic approaches to the attachment of the polymers to a surface: through single attachment point or multiple attachments. Single attachments are perhaps the canonical example but are not without problems. First, if a single attachment point is going to anchor a large polymer molecule, it must be a very strong bond, and covalent linkages are ideal. Second, the size of the polymer limits the kinetics of attachment, as a single reactive group on a large polymer molecule must find the surface for binding to occur. Very large polymers are rarely used for these single point attachments, but polymers up to about 100 kDa can be used with good results.

Polymers may be attached directly to the particle surface or may be attached through a reaction with a species already present on the surface. Attaching a polymer to the surface requires a displacement of the existing surfactant in a manner that is similar to small molecules. This leads to a familiar hierarchy in attachment efficiency where amines and carboxylic acids are relatively weakly binding, but phophonates and catechols bind more strongly. The stronger binding molecules displace more of the initial surfactant and attach more of the new polymeric surfactant(Davis et al. 2014).

When attaching a polymer to an existing functionality on a coating, a reaction with a low activation energy is highly desired, as excessive heating can change the underlying nanoparticles' morphology. The most common end terminations in these systems are probably amines and carboxylic acids. By having a surface coating that has one of these functional groups

and the other on the polymer, very stable amides can be formed using well known conjugation chemistry. There are a number of other chemistries that have been used successfully, including reactions between isocyanates and amines and or alcohols, epoxides and amines or alcohols, and isothiocyanates with amines(Hermanson 2013). Click chemistry approaches have also been applied including reacting thiols with alkenes, or the copper catalyzed reaction of azides with alkynes. These same reactions can be used to attach small molecules as well but are more commonly used with polymers and proteins.

Polymers that are bound through multiple attachment points do not require such strong attachments and can often be held by weaker van der Waals interactions between hydrophobic species. A good example is a diblock copolymer that has one block that it hydrophobic to provide water solubility and a second hydrophobic block that has favorable interactions with a hydrophobic surfactant on the particle surface. These molecules and the structures they form are very similar to what was discussed previously for small molecules but in this case just uses much larger molecules. There is no need for the attachment points to be all in one region of the molecule, so random copolymers can be used in this situation as well. For example: monomers with hydrophobic sidechains can be incorporated into a polymer to cause it to associate with an existing hydrophobic coating(Di Corato et al. 2008), or monomers containing carboxylic acids copolymerized into the polymer can provide multiple attachments directly to the particle core. Using carboxylic acids also has the added advantage that acids that don't succeed in attaching to the surface can provide reactive sites for further functionalization.

Pre-formed polymers can even be used as initial surfactants for nanoparticle syntheses, and polar polymers such as acid containing polymers or polyvinyl pyrrolidone have been used in this capacity. In some reactions, the polymer has even been shown to be catalytic for the particle

formation reaction(Smith and Wychick 1980). While synthesizing nanoparticles in the presence of polymer is a convenient approach, most reactions that have good size and property control are done in the presence of only small molecule surfactants.

6.9.7. "Grafting from" approaches

There are several advantages to growing a polymer in situ on the surface of a particle, but chief among these is the high grafting density that can be achieved by these methods. Grafting density has been shown to impact surface properties such as biocompatibility and biofouling properties, with coatings with high graft densities generally performing better(Huber et al. 2003). The drawbacks are that this approach is generally much more synthetically challenging that grafting a preformed polymer. If we take into account that this reaction is being performed on a set of particles that have been bought at great expense or synthesized with great effort, these reactions have the potential to be expensive failures if they don't perform well initially. Another problem is that it can be difficult to provide reactive groups for further functionalization reactions, though this can be achieved in well-designed reactions.

In principal, any polymerization reaction can be adapted to a surface bound reaction, but in practice, only chain growth polymerizations, where some reactive species can cause chains to grow bound to a surface, are common. Step growth polymerizations, which are typically condensation reactions, have a growth mechanism where monomers form dimers, dimers form trimers, etc. Because any molecule is equally likely to react, the majority of polymers form in solution and it is difficult to adapt to a surface except in very unique cases where a reaction can be made to prefer the surface(Whitesell and Chang 1993) or in a reaction that is done by alternately saturating the surface with species that react with each other but not themselves(Orendorff, Huber, and Bunker 2009).

Of the chain growth polymerizations, coordination polymerizations, which use a catalyst to drive the reaction, are rarely used for biotechnology. Catalysts tend to be poisoned by the polar, hydrophilic molecules of interest in the production of water-soluble particles. Ionic polymerizations are also fairly rare for these applications. They can also be poorly tolerant of the highly functional polymers of interest and also require extreme purity in the reagents that can be difficult to achieve in complex multicomponent systems such as these. The most common reactions by far are those that utilize radical chain growth polymerizations schemes.
Insert Figure 6.8. here.

Standard free radical polymerizations were once the dominant method of forming a polymer layer in situ. While they remain common, controlled radical approaches have grown tremendously in popularity in recent years. Both approaches have significant advantages and the best choice depends on the details of the system. In a surface bound free radical polymerization, a free radical initiator must be bound to a surface, and that initiator is typically heated to form the propagating free radical (though free radicals can also be initiated by illumination with UV light), which then reacts with a series of double-bond containing monomers to form a polymer. A number of approaches to binding the radical initiator to the surface can be used, and largely depend upon the functionality of the commercially available radical initiators, unless one wants to take on the fairly arduous task of custom initiator synthesis. Free radical initiators are commercially available with a number of convenient coupling chemistries including amines, carboxylic acids, alcohols, and isocyanate. Any of these can be coupled to a surface using appropriate room temperature chemistries (radical initiators are obviously temperature sensitive). Once attached to the surface, these radical initiators can be heated in the presence of monomer to initiate the polymerization reaction. Solutions are generally degassed as oxygen can behave as a

radical scavenger, and the solvent is largely a matter of choice, though dioxane and toluene are popular choices. It is also worth noting that when a surface bound initiator thermally decomposes, it forms two free radicals, only one of which remains on the surface. Free polymer always occurs in these reactions, and it should generally be separated from the particles at the end of the reaction. Centrifugation, filtration, and dialysis are all common methods of separating free polymers from surface bound polymers. This free polymer is generally of about the same molecular weight as the surface bound polymer, so analyzing its molecular weight by gel permeation chromatography or dynamic light scattering is useful to understand the properties of the bound polymer. The polydispersity index of these polymers is generally 2 or higher, and very high graft densities can be formed.

The molecular weight of the polymer formed is controlled by the details of the kinetics, so a brief discussion of polymerization kinetics is in order. There are three basic steps in a free radical polymerization: initiation, propagation, and termination as well as a fourth potential step: chain transfer. Initiation is defined as the formation of a free radical and addition of the first monomer. From this point on, an assumption is made that the rate of addition does not depend upon molecular weight (e.g. adding the second monomer occurs with the same rate constant as adding the 100^{th} monomer) which makes the kinetic equations much simpler. Of course, there is some dependence of reactivity on molecular size, but the effect is generally small enough that ignoring it does not cause significant error. This simple assumption allows us to use a single rate constant for an addition step, which is in fact a series of additions of monomers that forms the polymer. The addition continues until the growing chain is deactivated in one of two ways: termination or chain transfer. All free radical polymerizations experience termination and it is this step that destroys the radicals. There are two common ways that the chains undergo

termination: recombination and disproportionation. Recombination is when two radical bearing molecules form a new bond, thereby quenching both radicals, whereas disproportionation is the process of transferring a radical from one molecule to another radical-bearing molecule where they generally quench through the formation of a double bond. An important difference here is that recombination causes an increase in molecular weight, while disproportionation does not increase molecular weight but leads to a terminal double bond.

Finally, there is chain transfer, which in contrast to the other steps described, may or may not be a significant feature of a given polymerization reaction. Chain transfer occurs when a radical is transferred to another molecule and that molecule is able to initiate a polymerization. The mechanism for this to occur is generally that the radical on the growing chain abstracts a hydrogen atom from another molecule, leaving an unpaired electron on the second molecule. While chain transfer can be useful in lowering molecular weight, it is generally a nuisance in surface-initiated polymerization. This is because it generally transfers radicals from the surface, where they are useful, to the solution where they serve no useful purpose, but just create more unbound polymer. One exception to this is when chain transfer from solution to the surface is used to synthesize polymer layers. This approach is not in common usage but can be a convenient method of polymer growth in appropriate circumstances(Price and Huber 2013, Huber et al. 2003).

Insert Figure 6.8 here.

Having defined the various steps in a free radical polymerization, we can now see how the molecular weight is determined by the kinetics of the reaction. A free radical is a highly reactive, but short-lived species, with lifetimes that are typically less than a second(McIntosh, Eager, and Spinks 1960). When a free radical polymerization is conducted, high molecular

weight polymer begins to form immediately, with the number of polymer molecules slowly growing as the reaction progresses. The molecular weight of an individual chain is then determined by how many propagation reactions occur before one of the termination processes described above ends the chain's growth. From the kinetic equations in Figure 6.8 we can then determine what effect the reaction conditions have on the overall molecular weight. In general, increasing free radical concentration will yield a lower molecular weight, while increasing monomer concentration tends to lead to higher molecular weights. In addition to molecular weight, molecular weight dispersity is the other primary measure of the size of an ensemble of polymer molecules. Due to the random nature of the chain termination events, the lifetime of any particular growing chain varies significantly which ensures a substantial dispersity in final sizes, even if the propagation rate is unchanged throughout the reaction. For a standard free radical polymerization, we expect a dispersity of at least 1.5, though it can be significantly larger. For example, when the reaction depletes monomer during the course of the reaction, the propagation rate can decrease, leading to smaller polymers being grown in the latter stages of the reaction. This variation of average size during the course of the reaction leads to an increase in size dispersity and can yield dispersities of 10 or larger if a reaction is run until monomer is completely consumed.

There is another approach to radical polymerization termed "controlled radical polymerization", or more formally "reversible-deactivation radical polymerization"(Jenkins, Jones, and Moad 2010) which refer to a collection of methods that modifies the kinetics of a radical polymerization to yield a more controlled product. These reactions include the well-known techniques of atom-transfer radical polymerization (ATRP) and reversible-addition-fragmentation chain-transfer polymerization (RAFT), as well as a number of related techniques.

These reactions are often referred to as "living radical polymerizations" in the literature, although that term is no longer preferred.(Jenkins, Jones, and Moad 2010) These methods share a common approach to controlling the polymerization reaction, and all have a reversible reaction that deactivates the free radical. The precise details of the various approaches are described elsewhere(Matyjaszewski and Spanswick 2005) but can be envisioned simplistically as a free radical reacting with some capping species to form a metastable dormant species that periodically dissociates reforming the active radical. In a well-controlled reaction, the vast majority of the radicals are in the dormant, deactivated state at any given time, while a small proportion of the radicals are in an active state and are adding monomer (if it is available). Individual radicals freely convert between the dormant and active state, while maintaining a low, equilibrium concentration of the active state throughout the reaction.

The growth of a polymer molecule in this approach is very different from a free radical polymerization. Here, the growth is slowed by the periods of inactivity, making initiation a relatively fast event followed by a much slower growth. If we envision an ensemble of growing chains, they would each experience infrequent bursts of growth when they enter their active radical state, interspersed with lengthy periods of dormancy where no growth occurs. So, instead of a chain initiating, growing and terminating in less than a second, all of the chains initiate together near the outset of the reaction and grow slowly (through bursts of activity) over the course of hours, then are intentionally terminated at some point to conclude the reaction.

Controlled radical polymerizations offer a number of important advantages over traditional free radical polymerizations. First, the low concentration of active radicals at any time leads to a negligible amount of termination by recombination. Since this is the primary mode of termination in many reactions, in well-behaved systems there is an extremely small

amount of chain termination. This is what led to the use of the term "living" for these reactions, though its use is now regarded as inappropriate since termination still occurs to some small degree. Still, the termination is at such a low level that some of the advantageous features of living systems can still be present, such as low polydispersity and the ability to form diblock copolymers by addition of a second monomer after consumption of the first.

These advantages in control have led to an explosion in the use of these controlled radical polymerization techniques to functionalize surfaces. There are, however, some serious drawbacks. While free radical reactions are very versatile, and a standard initiator will work for a great many monomers, controlled radical polymerizations are generally tailored to an individual monomer or monomer class. Since there are so many unique approaches to controlled radical polymerizations, with more being developed all the time, many of the initiators used are not commercially available and require custom synthesis. Additionally, monomers that are highly functional or polar are particularly problematic, and unfortunately, these are the types of polymers used in biotechnology applications to impart water solubility and biocompatibility. These monomers tend to lead to undesirable side reactions including chain transfer and termination events. Techniques are being developed and refined all the time to improve on the controlled radical polymerization of these important materials, but the control is generally worse than in more traditional non-polar monomers.

6.9.8. Latex particle formation

One method of functionalization that has been popular for many years is the formation of latex particles containing magnetic nanoparticles. Though latex particles are very commonly made of polystyrene, the term latex does not refer to a specific material, but to a material made by an emulsion polymerization. (The exception here is the term natural latex that refers to the polymerizable sap from rubber plants that contains emulsified rubber that is used to make a

variety of consumer products and is also a well-known allergen.) An emulsion polymerization is one where a hydrophobic monomer and a hydrophobic initiator are dispersed in an aqueous matrix with the aid of surfactants. The emulsion is generally generated and maintained by constant, strong stirring, and the emulsion is heated (or occasionally exposed to UV light) to initiate the polymerization. Latex paints therefore contain water-borne hydrophobic polymers, latex films (e.g. gloves) are formed from these types of emulsions, and latex particles are formed by solidifying the droplets in an emulsion. Since the distribution of the sizes of droplets in an emulsion can be very small, latex spheres can be manufactured that are extraordinarily uniform and reproducible. For this reason, latex spheres have a long history of use as size calibration standards in, for example, electron microscopy.

Forming magnetic latex particles can be as simple as adding hydrophobic magnetic particles to a standard latex forming reaction. However, as the number of magnetic particles increase, several issues can arise. The addition of magnetic nanoparticles effects both the viscosity and the surface tension of the hydrophobic phase and can lead to difficulty in forming and maintaining high quality emulsions. Additionally, highly magnetic latex particles may have poor colloidal stability due to their strong magnetic attraction. Finally, magnetic interactions between the individual nanoparticles within a latex particle can lead to undesirable changes in magnetic properties such as a change from superparamagnetism to ferromagnetism. A combination of all of these effects causes a functional limit on the amount of magnetic material that can be loaded into a latex particle that is highly dependent on the specifics of the system. Still, loadings as high as 50% by mass are not unusual for systems like this.

This approach is different from previously described functionalization strategies in that it does not lead to individual nanoparticles with a thin coating but imbeds a large number of

particles into a mass of polymer. This leads to a particle in the hundreds of nanometer to micron scale that has the enhanced susceptibility of a magnetic nanoparticle, but a moment that could never be achieved with a single superparamagnetic nanoparticle. This can be highly advantageous for applications that require the application of a force using a modest magnetic field (e.g. magnetic separation of cells or biomolecules).

Another advantage of using a larger particle loaded with magnetic nanoparticles is that the surface area per volume and per moment is much lower. This means that if the goal is to magnetize a cell for collection using an antibody or other targeting molecule, less of the targeting molecule, which is typically the most expensive part of the formulation, can be used to provide a certain magnetization. An obvious disadvantage of this approach is the inability to consider in vivo applications of what are essentially small plastic beads.

As made, latex spheres are dispersed in water, so there is no awkward transfer from an organic phase to contend with, but long term-colloidal stability is not assured. Many latex dispersions are not thermodynamically stable dispersions and require vigorous agitation before use. Additional stability can be provided by functionalization with, for example, the addition of surface-bound water-soluble polymers. Biofunctionalization of latex particles is also commonplace to provide specific functionality to these particles. Many of the previously discussed approaches are used, with the obvious difference that the reactions are purely organic reactions, without the necessity of making an initial linkage to the inorganic magnetic material. The reactive functionality used for the further functionalization can be residual double-bonds from a cross-linker such as divinyl benzene, or a reactive species specifically added for later functionalization.

Another method to forming polymeric microspheres is through the use of microfluidic droplet chips. In a typical approach, a flow of polymer solution in an organic solvent meet aqueous flows on both sides then is forced through a narrow orifice. This "flow focusing" leads the organic fraction to break into small droplets which can then dry to form polymer spheres in the micron range that have very narrow size distributions. Addition of magnetic nanoparticles to the polymer solution leads to magnetic polymer spheres(Bokharaei et al. 2016). While the results of this method would not typically be called latex particles, they are very similar to the structured formed in a latex reaction.

6.9.9. Silica coating

Until now, we've focused on organic functionality of the inorganic magnetic nanoparticles, but there is one inorganic coating that is extremely useful: silica. Silica's many advantages include its chemical stability, lack of toxicity, low cost, and ease of further functionalization. The basic approach varies very little, but the details are critical to the final product. In general, the magnetic nanoparticles are dispersed in water or a water and alcohol mixture, and a silica precursor such as trimethoxysilane (TMOS) or triethoxysilane (TEOS) is added. The condensation reaction to form silica can be catalyzed by acid or base, though bases are more commonly used. The concentrations of all of these species determines the speed of the reaction, the thickness of the coating, and the likelihood that nanoparticles will be irreversible attached to each other by silica. Silica coatings can then be broadly divided into two classes: Those that encapsulate individual nanoparticles each in their own silica shell, and those that encapsulate multiple nanoparticles into a single silica particle. Keeping nanoparticles separate requires low concentrations of nanoparticles and, generally of the other species as well, while forming large agglomerates of particles occurs when particle and reagent concentrations are

high. Silica coating individual particles retains the advantages of individual nanoparticles, while silica coating agglomerates provides many of the advantages discussed above for latex particles.

Often, silica coating is only a first step in functionalization and is followed by functionalization with organic materials. The ease with which silica is functionalized with organics is a big reason why it is so commonly used. A great many silane coupling agents, capable of forming a strong bond with silica while providing a conveniently reactive second functionality, are commercially available. The reaction that binds silane coupling agents to silica is catalyzed similarly to the silica formation reaction, generally with added base. One exception is the use of aminopropyl silane which is known to be autocatalytic and requires no added base(White and Tripp 2000). It is worth noting that to maximize the strength of this bond, a high temperature treatment in anhydrous conditions is used to complete the condensation reaction. Without this treatment, the coating may be susceptible to degradation upon long standing in water or exposure to non-neutral pHs.

6.10. Biofunctionalization

Some in vivo applications do not require specific interactions. For example, nanoparticles that are used to image blood must only remain is circulation to be effective. Other nanoparticles can take advantage of the enhanced permeability and retention (EPR) effect in cancer tumors, though this effect and its use remains controversial(Wilhelm et al. 2016, Torrice 2016). For many systems, however, a key challenge to effective use is achieving selective delivery of systemically administered nanoparticles to a tissue of interest. This approach is attractive as it allows nanoparticles to be detected, imaged, or triggered to achieve a therapeutic effect, including localized heating or delivery of a medicine to a specific tissue. An ideal nanomedicine would target diseased tissues with high specificity, while evading uptake by healthy tissues or

rapid clearance by the reticuloendothelial system (RES)(Arami et al. 2015). Modification of the nanoparticle surface with a targeting ligand that enables selective binding is then critical, as is a comprehensive understanding of how the ligand will alter the relevant properties of the nanoparticle (hydrodynamic diameter, surface charge, etc.) and the resulting pharmacokinetics and biodistribution

An array of different organic molecules can be employed for biofunctionalization of a nanoparticle surface. These molecules can be grouped into a few general categories, including small molecules, polypeptides, nucleic acid-based aptamers, and proteins(Liu et al. 2011). Each of these classes of molecules has its associated advantages, disadvantages, conjugation chemistries, and will uniquely impact nanoparticle biodistribution. Small biomolecules and polypeptides can be used to target nanoparticles following the attachment procedures described in previous sections. Aptamers are short synthetic DNA molecules that are developed to bind to specific species and can also be attached using standard chemistries. Proteins, however required more careful handling as their intricate structures are susceptible to damage by high temperatures, organic solvents, and pH changes. Here, we focus on protein attachment, with emphasis on monoclonal antibodies whose high selectivity and specificity has contributed to their growing use in diagnostics and bio-therapeutics(Reichert 2014). The majority of antibodies used are immunoglobulin G antibodies (IGgs), which consist of two high molecular weight polypeptides (heavy chains) and two lower molecular weight polypeptides (light chains) assembled into what is typically depicted as a Y-shaped molecule(Harris et al. 1997). As shown pictorially in Figure 6.9, the two heavy chains are linked by disulfide bonds to form the Fc (shorthand for fragment, crystallizable) domain. Each heavy chain is also disulfide bonded to a light chain in the Fab (Fragment, antigen-binding) domain. The Fab domain contains two

antigen binding sites, found at the tips of the forked portion of the Y. The Fc domain, made up of heavy chains in the base of the Y, does not take part in antigen binding.

Insert Figure 6.9 here.

Perhaps the simplest approach to coat a nanoparticle with protein is nonspecific adsorption onto the nanoparticle surface. For example, nonspecific adsorption of albumin is a common approach to enhance biocompatibility in nanoparticles(Zhang et al. 2012). This approach takes advantage of the fact that albumin is the most common protein in blood and is therefore largely ignored by the immune system. Other proteins though have been used to drive delivery of the nanoparticles to a specific tissue(Rodrigues et al. 2018). Whether the protein is adsorbed in its native conformation or undergoes changes in conformation, including denaturation, upon adsorption can determine the difference between effective targeting agent and a highly immunogenic nanoparticle. Conformational changes are protein dependent and are typically driven by hydrophobic interactions between the protein and the nanoparticle surface following adsorption. Additionally, as the nanoparticle size becomes comparable to that of the protein, the radius of curvature of the nanoparticle and the size-dependent electrical double layer become increasingly important factors in driving conformational changes(Satzer et al. 2016). Moreover, protein adsorption can be reversible as a function of temperature, pH, and ionic strength of the environment. To improve the stability of the protein/nanoparticle interaction, covalent binding of the protein to the nanoparticle surface offers an attractive alternative.

The most popular approach for covalent linkage takes advantage of reactive side chains of amino acid residues found within proteins and conjugate them to appropriately functionalized nanoparticles. The most common of these include primary amines found in the lysine residue and the N-terminus of the protein, carboxylic acid in aspartic and glutamic residues and the C-

terminus of the protein, and sulfhydryl in the cysteine residue. Amines react with activated carboxylate moieties to form amide bonds, where sulfhydryl moieties react with haloacetyl, alkyl halide derivatives, maleimide, pyridyl disulfide, among others, to form thioester or disulfide linkages(Hermanson 2013). Crosslinker reagents are chosen for their reactivity to the functional moieties on proteins and nanoparticle surfaces. Homobifunctional crosslinkers have the same reactivity at both ends and have the potential to link neighboring molecules, resulting in protein polymerization or nanoparticle agglomeration. In this case, heterobifunctional crosslinkers may be chosen to reduce undesired reactions. The most common "zero-length" crosslinker of this type, EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), can be used to couple primary amines to carboxylic acid functionalized nanoparticles in an aqueous environment. In a simple one-step reaction, EDC is used to activate carboxylate moieties on particles, forming a reactive ester intermediate. This ester is reactive with primary amines, promoting the formation of a stable amide bond between the protein and the nanoparticle surface. NHS (N-hydroxysuccinimide) or sulfo-NHS are often used in conjunction with EDC to form a more stable NHS ester or sulfo-NHS ester intermediate that can improve reaction yield(Hermanson 2013). This approach is straightforward yet results in proteins bound to the surface in a variety of orientations. For conjugated antibodies, this means that a portion will have their active binding sites in the Fab region oriented in a way that will inhibit binding with the target. 'Directional' conjugation strategies have been developed to better control the orientation of immobilized antibodies by linking antibodies through the non-binding Fc region(Vijayendran and Leckband 2001). Antibodies typically contain glycosylated residues in the Fc region that can be oxidized under mild conditions to create a hydrazide reactive aldehyde moiety. A

hydrazide-terminated heterobifunctional linker can then be used to link the antibody to the nanoparticle surface(Kumar, Aaron, and Sokolov 2008).

Simply having the ability to attach proteins is not the complete story. For all of the attachment methods described above except the directional attachment of antibodies, there are multiple attachment points on the protein. This allows for the possibility of several non-ideal protein attachment motifs. Proteins may attach at multiple points leading to a distorted structure that could impair performance. A single protein could bridge between particles, which is essentially a cross-link between particles which will increase the hydrodynamic radius of the particles and if common enough could lead to wholesale agglomeration. In the case of homobifunctional attachment approaches, proteins can attach to other proteins leading to similar cross-linking and agglomeration issues. Generally, these concerns lead to conjugation reactions to be performed at the lowest concentration that is practical to limit the likelihood of these kinds of cross-links.

Attachment density is also an important consideration as active binding sites have a requisite amount of space around them to allow for interactions with the target species. The goal is generally to have a small number of these binding sites on every particle's surface. Exactly how to achieve this is a difficult problem that requires careful consideration of reaction conditions in general, but the relative concentrations of the species in particular. As is often the case in nanoparticle chemistry, the general concepts are reasonably straight forward, but the parameter space is enormous making control over the reaction extremely challenging.

6.11. Conclusions

This chapter has attempted to summarize the synthesis and functionalization of magnetic nanoparticles for applications in biotechnology. This is a topic of enormous breadth that

encompasses a tremendous number of approaches. Still, it is clear where the research is currently focused. Most magnetic nanoparticles are synthesized through wet chemical approaches, with thermolysis in organic solvents perhaps the most popular with precipitation in aqueous environments the other common approach.

Functionalization of the nanoparticle surfaces is still widely varied with both covalent and non-covalent attachment approaches being common. Perhaps the strongest trend is in what is being attached. There is a continuous movement toward more complex functionality. While studies on unfunctionalized magnetic nanoparticles were once common, currently we see a great deal of intentional treatment of the surface to drive specific functionality. While they are not yet common, we are now beginning to see multi-component coatings with each species having a specific function. For example, a coating may have a stealth polymer, like PEG, but also contain a targeting IgG as well as a drug payload. There is no reason to believe that this trend will not continue with ever more complex coatings with enhanced functionalities being created.

6.12. Acknowledgements

Sandia National Laboratories is a multimission laboratory managed and operated by National Technology & Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA0003525. This paper describes objective technical results and analysis. Any subjective views or opinions that might be expressed in the paper do not necessarily represent the views of the U.S. Department of Energy or the United States Government.

Figure captions:

- **Figure 6.1: Schematic representation of the various potential parts of a nanoparticle synthesized and functionalized specifically for a biotechnology application.**
- **Figure 6.2. Graph of the surface area to volume ratio for nanoparticles of varying diameter assuming spherical shape.**
- **Figure 6.3. Energy versus reaction coordinate for a generic synthesis of a nanoparticle. Reactants begin at relatively high energy and increase in energy as they are given activation energy, then begin to decrease in energy as they grow. Successful size control requires that the nanoparticles stop growing in an energy landscape where growing leads to a lower overall energy. The inset shows that small activation energies are required for each subsequent addition of an atom, which does provide some thermodynamic support for stopping a reaction before the thermodynamic, bulk product is reached.**
- **Figure 6.4. A diagram of the LaMer mechanism with the three phases of the reaction labeled. In phase 1 monomer concentration increases but no particles are formed. In phase 2 there is a burst of nucleation which is followed by phase 3 where nanoparticles grow without further nucleation occurring.**
- **Figure 6.5. Diagram of the Extended LaMer mechanism with the four phases of the reaction labeled. The mechanism begins in a similar way to the traditional LaMer mechanism, but with important differences in the latter times due to the continuous addition of precursor. Phase 1 has an increase in monomer concentration without**

the formation of nanoparticles, while phase 2 is a burst of nucleation. Phase three is a transitional phase where the monomer concentration decreases until the consumption of monomer equals the formation of monomer from the continuously added precursor. Phase 4 sees steady state growth of the nanoparticles as monomer is continuously formed and consumed in the growth of the nanoparticles. In this phase the average volume of the nanoparticles increases linearly with time.

- **Figure 6.6. a) A plot of nanoparticle diameter with time showing two distinct growth regimes, with a fast catalytic growth at early time and steady state growth at later times. b) A plot of the steady state growth regimes of three different reactions demonstrating the reproducibility of the reaction. Reproduced with permission from (Vreeland et al. 2015).**
- **Figure 6.7. Round magnetite nanoparticles synthesized through the thermal decomposition of iron oleate. Scale bare is 25 nm.**
- **Figure 6.8. Fundamental steps in a typical radical polymerization reaction. Initiation generally proceeds by the unimolecular decomposition (with rate constant k_d) which produces two radicals which are denoted here as a dot representing the unpaired electron residing on the organic group R. Initiation is no complete until the first monomer addition occurs yielding an R group terminated molecule with a radical on the other end prepared to propagate the reaction. Propagation proceeds by adding subsequent monomers in a chain reaction forming the polymer until a chain termination event occurs. While there are several mechanism of chain termination, recombination, the joining of two radicals into a stable molecule, is often dominant.**

- **Figure 6.9. Three common diagrammatic representations of IgG varying from stick figure representation to one base upon the actual structure as determined by X-ray diffraction.**

6.12. References

Arami, H., A. Khandhar, D. Liggitt, and K. M. Krishnan. 2015. "In vivo delivery, pharmacokinetics, biodistribution and toxicity of iron oxide nanoparticles." *Chem Soc Rev* 44 (23):8576-607. doi: 10.1039/c5cs00541h.

Baldan, A. 2002. "Review Progress in Ostwald ripening theories and their applications to nickel-base superalloys Part I: Ostwald ripening theories." *Journal of Materials Science* 37 (11):2171-2202. doi: 10.1023/A:1015388912729.

Bleier, G. C., J. Watt, C. K. Simocko, J. M. Lavin, and D. L. Huber. 2018. "Reversible Magnetic Agglomeration: A Mechanism for Thermodynamic Control over Nanoparticle Size." *Angew Chem Int Ed Engl* 57 (26):7678-7681. doi: 10.1002/anie.201800959.

Bokharaei, Mehrdad, Thomas Schneider, Silvio Dutz, Roland C. Stone, O. Thompson Mefford, and Urs O. Häfeli. 2016. "Production of monodispersed magnetic polymeric microspheres in a microfluidic chip and 3D simulation." *Microfluidics and Nanofluidics* 20 (1):1-14. doi: 10.1007/s10404-015-1693-y.

Bronstein, L. M., J. E. Atkinson, A. G. Malyutin, F. Kidwai, B. D. Stein, D. G. Morgan, J. M. Perry, and J. A. Karty. 2011. "Nanoparticles by Decomposition of Long Chain Iron Carboxylates: From Spheres to Stars and Cubes." *Langmuir*. doi: 10.1021/la104686d.

Bronstein, L. M., X. L. Huang, J. Retrum, A. Schmucker, M. Pink, B. D. Stein, and B. Dragnea. 2007. "Influence of iron oleate complex structure on iron oxide nanoparticle formation." *Chemistry of Materials* 19 (15):3624-3632. doi: 10.1021/cm062948j.

Chakka, V. M., B. Altuncevahir, Z. Q. Jin, Y. Li, and J. P. Liu. 2006. "Magnetic nanoparticles produced by surfactant-assisted ball milling." *Journal of Applied Physics* 99 (8). doi: 10.1063/1.2170593.

Chen, Y., E. Johnson, and X. Peng. 2007. "Formation of monodisperse and shape-controlled MnO nanocrystals in non-injection synthesis: self-focusing via ripening." *J Am Chem Soc* 129 (35):10937-47. doi: 10.1021/ja073023n.

Dabbousi, B. O., J. Rodriguez-Viejo, F. V. Mikulec, J. R. Heine, H. Mattoussi, R. Ober, K. F. Jensen, and M. G. Bawendi. 1997. "(CdSe)ZnS Core−Shell Quantum Dots: Synthesis and Characterization of a Size Series of Highly Luminescent Nanocrystallites." *The Journal of Physical Chemistry B* 101 (46):9463-9475. doi: 10.1021/jp971091y.

Davis, Kathleen, Brian Cole, Melanie Ghelardini, Brian A. Powell, and O. Thompson Mefford. 2016. "Quantitative Measurement of Ligand Exchange with Small-Molecule Ligands on Iron Oxide Nanoparticles via Radioanalytical Techniques." *Langmuir* 32 (51):13716-13727. doi: 10.1021/acs.langmuir.6b03644.

Davis, Kathleen, Bin Qi, Michael Witmer, Christopher L. Kitchens, Brian A. Powell, and O. Thompson Mefford. 2014. "Quantitative Measurement of Ligand Exchange on Iron Oxides via Radiolabeled Oleic Acid." *Langmuir* 30 (36):10918-10925. doi: 10.1021/la502204g.

De Haro, L. P., T. Karaulanov, E. C. Vreeland, B. Anderson, H. J. Hathaway, D. L. Huber, A. N. Matlashov, C. P. Nettles, A. D. Price, T. C. Monson, and E. R. Flynn. 2015. "Magnetic

relaxometry as applied to sensitive cancer detection and localization." *Biomed Tech (Berl)* 60 (5):445-55. doi: 10.1515/bmt-2015-0053.

Di Corato, Riccardo, Alessandra Quarta, Philomena Piacenza, Andrea Ragusa, Albert Figuerola, Raffaella Buonsanti, Roberto Cingolani, Liberato Manna, and Teresa Pellegrino. 2008. "Water solubilization of hydrophobic nanocrystals by means of poly(maleic anhydride-alt-1-octadecene)." *Journal of Materials Chemistry* 18 (17):1991-1996. doi: 10.1039/B717801H.

Farrell, D., Y. Cheng, S. Kan, M. Sachan, Y. Ding, S. A. Majetich, and L. Yang. 2005. "Iron nanoparticle assemblies: structures and magnetic behavior." In *Fifth International Conference on Fine Particle Magnetism*, edited by Q. Pankhurst, 185-195. Bristol: Iop Publishing Ltd.

Fellows, Benjamin D., Sarah Sandler, Jacob Livingston, Kristin Fuller, Lotanna Nwandu, Sarah Timmins, Kayla A. Lantz, Morgan Stefik, and O. Thompson Mefford. 2018. "Extended LaMer Synthesis of Cobalt-Doped Ferrite." *IEEE Magnetics Letters* 9:1-5. doi: 10.1109/lmag.2017.2787683.

Gangopadhyay, S., G.C. Hadjipanayis, B. Dale, C.M. Sorensen, K.J. Klabunde, V. Papaefthymiou, and A. Kostikas. 1992. "Magnetic properties of ultrafine iron particles." *Physical Review B (Condensed Matter)* 45 (17):9778-87.

Goya, G. F. 2004. "Magnetic interactions in ball-milled spinel ferrites." *Journal of Materials Science* 39 (16-17):5045-5049. doi: DOI 10.1023/B:JMSC.0000039183.99797.8d.

Guardia, P., J. Perez-Juste, A. Labarta, X. Batlle, and L. M. Liz-Marzan. 2010. "Heating rate influence on the synthesis of iron oxide nanoparticles: the case of decanoic acid." *Chem Commun (Camb)* 46 (33):6108-10. doi: 10.1039/c0cc01179g.

Guardia, Pablo, Nicolás Pérez, Amilcar Labarta, and Xavier Batlle. 2010. "Controlled Synthesis of Iron Oxide Nanoparticles over a Wide Size Range." *LANGMUIR* 26 (8):5843-5847. doi: 10.1021/la903767e.

Harris, Lisa J., Steven B. Larson, Karl W. Hasel, and Alexander McPherson. 1997. "Refined Structure of an Intact IgG2a Monoclonal Antibody." *Biochemistry* 36 (7):1581-1597. doi: 10.1021/bi962514+.

Hermanson, Greg. 2013. *Bioconjugate Techniques, 3rd Edition*: Academic Press.

Herrmann, Inge K., Robert N. Grass, Dmitry Mazunin, and Wendelin J. Stark. 2009. "Synthesis and Covalent Surface Functionalization of Nonoxidic Iron Core−Shell Nanomagnets." *Chemistry of Materials* 21 (14):3275-3281. doi: 10.1021/cm900785u.

Huber, D. L. 2005. "Synthesis, properties, and applications of iron nanoparticles." *Small* 1 (5):482-501. doi: 10.1002/smll.200500006.

Huber, D. L., K. E. Gonsalves, G. Carlson, and T. A. P. Seery. 1997. "The formation of polymer monolayers: From adsorption to surface initiated polymerizations." In *Interfacial Aspects of Multicomponent Polymer Materials*, edited by D. J. Lohse, T. P. Russell and L. H. Sperling, 107-122.

Huber, D. L., R. P. Manginell, M. A. Samara, B. I. Kim, and B. C. Bunker. 2003. "Programmed adsorption and release of proteins in a microfluidic device." *Science* 301 (5631):352-354. doi: 10.1126/science.1080759.

Huber, D. L., E. L. Venturini, J. E. Martin, P. P. Provencio, and R. J. Patel. 2004. "Synthesis of highly magnetic iron nanoparticles suitable for field structuring using a beta-diketone surfactant." *Journal of Magnetism and Magnetic Materials* 278 (3):311-316. doi: 10.1016/j.jmmm.2003.12.1317.

Hufschmid, R., H. Arami, R. M. Ferguson, M. Gonzales, E. Teeman, L. N. Brush, N. D. Browning, and K. M. Krishnan. 2015. "Synthesis of phase-pure and monodisperse iron oxide nanoparticles by thermal decomposition." *Nanoscale* 7 (25):11142-54. doi: 10.1039/c5nr01651g.

Jain, T. K., M. K. Reddy, M. A. Morales, D. L. Leslie-Pelecky, and V. Labhasetwar. 2008. "Biodistribution, clearance, and biocompatibility of iron oxide magnetic nanoparticles in rats." *Mol Pharm* 5 (2):316-27. doi: 10.1021/mp7001285.

Jenkins, Aubrey D., Richard G. Jones, and Graeme Moad. 2010. "Terminology for reversible-deactivation radical polymerization previously called "controlled" radical or "living" radical polymerization (IUPAC Recommendations 2010)." *Pure and Applied Chemistry* 82 (2):483-491. doi: 10.1351/pac-rep-08-04-03.

Joseyphus, R. Justin, D. Kodama, T. Matsumoto, Y. Sato, B. Jeyadevan, and K. Tohji. 2007. "Role of polyol in the synthesis of Fe particles." *JOURNAL OF MAGNETISM AND MAGNETIC MATERIALS* 310 (2, Part 3):2393-2395. doi: 10.1016/j.jmmm.2006.10.1132.

Jungk, H. O., and C. Feldmann. 2000. "Nonagglomerated, submicron α–Fe2O3 particles: Preparation and application." *Journal of Materials Research* 15 (10):2244-2248. doi: 10.1557/jmr.2000.0322.

Katabi, G., Y. Koltypin, X. Cao, and A. Gedanken. 1996. "Self-assembled monolayer coatings of iron nanoparticles with thiol derivatives." *Journal of Crystal Growth* 166 (1-4):760-762. doi: Doi 10.1016/0022-0248(96)00091-7.

Kataby, G., M. Cojocaru, R. Prozorov, and A. Gedanken. 1999. "Coating Carboxylic Acids on Amorphous Iron Nanoparticles." *Langmuir* 15 (5):1703-1708. doi: 10.1021/la981001w.

Klabunde, K. J., H. F. Efner, L. Satek, and W. Donley. 1974. "Preparation of an Extremely Active Magnesium Slurry for Grignard-Reagent Preparations by Metal Atom-Solvent Cocondensations." *Journal of Organometallic Chemistry* 71 (3):309-313. doi: Doi 10.1016/S0022-328x(00)95163-5.

Kumar, Nirmal, C. S. Tiwary, and Krishanu %J Journal of Materials Science Biswas. 2018. "Preparation of nanocrystalline high-entropy alloys via cryomilling of cast ingots." 53 (19):13411-13423. doi: 10.1007/s10853-018-2485-z.

Kumar, S., J. Aaron, and K. Sokolov. 2008. "Directional conjugation of antibodies to nanoparticles for synthesis of multiplexed optical contrast agents with both delivery and targeting moieties." *Nat Protoc* 3 (2):314-20. doi: 10.1038/nprot.2008.1.

Lamer, V. K., and R. H. Dinegar. 1950. "Theory, Production and Mechanism of Formation of Monodispersed Hydrosols." *Journal of the American Chemical Society* 72 (11):4847-4854. doi: DOI 10.1021/ja01167a001.

Liu, Rihe, Brian K. Kay, Shaoyi Jiang, and Shengfu Chen. 2011. "Nanoparticle Delivery: Targeting and Nonspecific Binding." *MRS Bulletin* 34 (06):432-440. doi: 10.1557/mrs2009.119.

Ma, Ming, Yu Zhang, Wei Yu, Hao-ying Shen, Hai-qian Zhang, and Ning Gu. 2003. "Preparation and characterization of magnetite nanoparticles coated by amino silane." *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 212 (2):219-226. doi: https://doi.org/10.1016/S0927-7757(02)00305-9.

Martin, James E., E. L. Venturini, and D. L. Huber. 2008. "Giant magnetic susceptibility enhancement in field-structured nanocomposites." *Journal of Magnetism and Magnetic Materials* 320 (18):2221-2227. doi: 10.1016/j.jmmm.2008.04.111.

Martino, A., M. Stoker, M. Hicks, C. H. Bartholomew, A. G. Sault, and J. S. Kawola. 1997. "The synthesis and characterization of iron colloid catalysts in inverse micelle solutions." *Applied Catalysis a-General* 161 (1-2):235-248. doi: Doi 10.1016/S0926-860x(97)00074-4.

Masthoff, I.-C., M. Kraken, D. Mauch, D. Menzel, J. A. Munevar, E. Baggio Saitovitch, F. J. Litterst, and G. %J Journal of Materials Science Garnweitner. 2014. "Study of the growth process of magnetic nanoparticles obtained via the non-aqueous sol–gel method." 49 (14):4705-4714. doi: 10.1007/s10853-014-8160-0.

Matyjaszewski, Krzysztof, and James Spanswick. 2005. "Controlled/living radical polymerization." *Materials Today* 8 (3):26-33. doi: https://doi.org/10.1016/S1369-7021(05)00745-5.

McGrath, Andrew J., Ciaran Dolan, Soshan Cheong, David A. J. Herman, Briar Naysmith, Fangrong Zong, Petrik Galvosas, Kathryn J. Farrand, Ian F. Hermans, Margaret Brimble, David E. Williams, Jianyong Jin, and Richard D. Tilley. 2017. "Stability of polyelectrolyte-coated iron nanoparticles for T 2 -weighted magnetic resonance imaging." *Journal of Magnetism and Magnetic Materials* 439:251-258. doi: 10.1016/j.jmmm.2017.04.026.

McIntosh, R. G., R. L. Eager, and J. W. T. Spinks. 1960. "Mean Lifetime of Free Radical Chains Determined by a Flow Technique." 131 (3405):992-992. doi: 10.1126/science.131.3405.992 %J Science.

Meffre, Anca, Boubker Mehdaoui, Vinciane Kelsen, Pier Francesco Fazzini, Julian Carrey, Sebastien Lachaize, Marc Respaud, and Bruno Chaudret. 2012. "A Simple Chemical Route toward Monodisperse Iron Carbide Nanoparticles Displaying Tunable Magnetic

and Unprecedented Hyperthermia Properties." *Nano Letters* 12 (9):4722-4728. doi: 10.1021/nl302160d.

Miller, Joel S. 2014. "Organic- and molecule-based magnets." *Materials Today* 17 (5):224-235. doi: 10.1016/j.mattod.2014.04.023.

Nakatani, I., T. Furubayashi, T. Takahashi, and H. Hanaoka. 1987. "Preparation and magnetic properties of colloidal ferromagnetic metals." *Journal of Magnetism and Magnetic Materials* 65 (2/3):261-4.

Nealon, Gareth L., Bertrand Donnio, Romain Greget, Jean-Paul Kappler, Emmanuel Terazzi, and Jean-Louis Gallani. 2012. "Magnetism in gold nanoparticles." *Nanoscale* 4 (17):5244-5258. doi: 10.1039/C2NR30640A.

Orendorff, C. J., D. L. Huber, and B. C. Bunker. 2009. "Effects of Water and Temperature on Conformational Order in Model Nylon Thin Films." *Journal of Physical Chemistry C* 113 (31):13723-13731. doi: 10.1021/jp901309y.

Pardavi-Horvath, M., and L. Takacs. 1995. "Magnetic nanocomposites by reaction milling." *Scripta Metallurgica et Materialia* 33 (10/11):1731-40.

Park, J., K. An, Y. Hwang, J.G. Park, H.J. Noh, J.Y. Kim, J.H. Park, N.M. Hwang, and T. Hyeon. 2004a. "Ultra-large-scale syntheses of monodisperse nanocrystals." *Nature Materials* 3 (12):891-895.

Park, Jongnam, Kwangjin An, Yosun Hwang, Je-Geun Park, Han-Jin Noh, Jae-Young Kim, Jae-Hoon Park, Nong-Moon Hwang, and Taeghwan Hyeon. 2004b. "Ultra-large-scale syntheses of monodisperse nanocrystals." *Nature Materials* 3 (12):891-895. doi: 10.1038/nmat1251.

Price, Andrew D., and Dale L. Huber. 2013. "Controlled polymer monolayer synthesis by radical transfer to surface immobilized transfer agents." *Polymer Chemistry* 4 (5):1565-1574. doi: 10.1039/C2PY20955A.

Prozorov, Tanya, Ruslan Prozorov, and Kenneth S. Suslick. 2004. "High Velocity Interparticle Collisions Driven by Ultrasound." *Journal of the American Chemical Society* 126 (43):13890-13891. doi: 10.1021/ja049493o.

Reichert, Janice M. 2014. "Global antibody development trends." *mAbs* 1 (1):86-87. doi: 10.4161/mabs.1.1.7645.

Rodrigues, Tasson C., Maria Leonor S. Oliveira, Alessandra Soares-Schanoski, Stefanni L. Chavez-Rico, Douglas B. Figueiredo, Viviane M. Gonçalves, Daniela M. Ferreira, Nitesh K. Kunda, Imran Y. Saleem, and Eliane N. Miyaji. 2018. "Mucosal immunization with PspA (Pneumococcal surface protein A)-adsorbed nanoparticles targeting the lungs for protection against pneumococcal infection." *PLOS ONE* 13 (1):e0191692. doi: 10.1371/journal.pone.0191692.

Sahoo, Yudhisthira, Hillel Pizem, Tcipi Fried, Dina Golodnitsky, Larisa Burstein, Chaim N. Sukenik, and Gil Markovich. 2001. "Alkyl Phosphonate/Phosphate Coating on Magnetite Nanoparticles: A Comparison with Fatty Acids." *Langmuir* 17 (25):7907-7911. doi: 10.1021/la010703+.

Satzer, P., F. Svec, G. Sekot, and A. Jungbauer. 2016. "Protein adsorption onto nanoparticles induces conformational changes: Particle size dependency, kinetics, and mechanisms." *Eng Life Sci* 16 (3):238-246. doi: 10.1002/elsc.201500059.

Saville, S. L., B. Qi, J. Baker, R. Stone, R. E. Camley, K. L. Livesey, L. F. Ye, T. M. Crawford, and O. T. Mefford. 2014. "The formation of linear aggregates in magnetic hyperthermia:

Implications on specific absorption rate and magnetic anisotropy." *Journal of Colloid and Interface Science* 424:141-151. doi: 10.1016/j.jcis.2014.03.007.

Smith, TW, and D Wychick. 1980. "Colloidal Iron Dispersions Prepared Via the Polymer-Catalyzed Decomposition of Iron Pentacarbonyl." *Journal of Physical Chemistry* 84 (12):1621-1629.

Stone, R., S. Hipp, J. Barden, P. J. Brown, and O. T. Mefford. 2013. "Highly scalable nanoparticle-polymer composite fiber via wet spinning." *Journal of Applied Polymer Science* 130 (3):1975-1980. doi: Doi 10.1002/App.39408.

Sun, S. H., S. Anders, T. Thomson, J. E. E. Baglin, M. F. Toney, H. F. Hamann, C. B. Murray, and B. D. Terris. 2003. "Controlled synthesis and assembly of FePt nanoparticles." *Journal of Physical Chemistry B* 107 (23):5419-5425. doi: Doi 10.1021/Jp027314o.

Sun, SH, CB Murray, D Weller, L Folks, and A Moser. 2000. "Monodisperse FePt nanoparticles and ferromagnetic FePt nanocrystal superlattices." *Science* 287 (5460):1989-1992.

Sun, Y. P., X. Q. Li, J. Cao, W. X. Zhang, and H. P. Wang. 2006. "Characterization of zero-valent iron nanoparticles." *Adv Colloid Interface Sci* 120 (1-3):47-56. doi: 10.1016/j.cis.2006.03.001.

Suslick, K. S., S. B. Choe, A. A. Cichowlas, and M. W. Grinstaff. 1991. "Sonochemical Synthesis of Amorphous Iron." *Nature* 353 (6343):414-416. doi: Doi 10.1038/353414a0.

Suslick, K. S., Y. Didenko, M. M. Fang, T. Hyeon, K. J. Kolbeck, W. B. McNamara, M. M. Mdleleni, and M. Wong. 1999. "Acoustic cavitation and its chemical consequences." *Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Sciences* 357 (1751):335-353. doi: DOI 10.1098/rsta.1999.0330.

Suslick, K. S., and G. J. Price. 1999. "Applications of ultrasound to materials chemistry." *Annual Review of Materials Science* 29 (1):295-326. doi: DOI 10.1146/annurev.matsci.29.1.295.

Sweeney, Scott F., Gerd H. Woehrle, and James E. Hutchison. 2006. "Rapid Purification and Size Separation of Gold Nanoparticles via Diafiltration." *Journal of the American Chemical Society* 128 (10):3190-3197. doi: 10.1021/ja0558241.

Torrice, M. 2016. "Does Nanomedicine Have a Delivery Problem?" *ACS Cent Sci* 2 (7):434-7. doi: 10.1021/acscentsci.6b00190.

Vijayendran, Ravi A., and Deborah E. Leckband. 2001. "A Quantitative Assessment of Heterogeneity for Surface-Immobilized Proteins." *Analytical Chemistry* 73 (3):471-480. doi: 10.1021/ac000523p.

Vreeland, Erika C., John Watt, Gretchen B. Schober, Bradley G. Hance, Mariah J. Austin, Andrew D. Price, Benjamin D. Fellows, Todd C. Monson, Nicholas S. Hudak, Lorena Maldonado-Camargo, Ana C. Bohorquez, Carlos Rinaldi, and Dale L. Huber. 2015. "Enhanced Nanoparticle Size Control by Extending LaMer's Mechanism." *Chemistry of Materials* 27 (17):6059-6066. doi: 10.1021/acs.chemmater.5b02510.

W., Ostwald. 1897. "Studien ber die Bildung und Umwandlung fester Körper." *Z. Phys. Chem.* 22:289-330.

Wang, Y. X. 2011. "Superparamagnetic iron oxide based MRI contrast agents: Current status of clinical application." *Quant Imaging Med Surg* 1 (1):35-40. doi: 10.3978/j.issn.2223-4292.2011.08.03.

Watt, J., M. J. Austin, C. K. Simocko, D. V. Pete, J. Chavez, L. M. Ammerman, and D. L. Huber. 2018. "Formation of Metal Nanoparticles Directly from Bulk Sources Using

Ultrasound and Application to E-Waste Upcycling." *Small* 14 (17):e1703615. doi: 10.1002/smll.201703615.

Watt, J., G. C. Bleier, M. J. Austin, S. A. Ivanov, and D. L. Huber. 2017. "Non-volatile iron carbonyls as versatile precursors for the synthesis of iron-containing nanoparticles." *Nanoscale* 9 (20):6632-6637. doi: 10.1039/c7nr01028a.

Watt, John, Grant C. Bleier, Zachary W. Romero, Bradley G. Hance, Jessica A. Bierner, Todd C. Monson, and Dale L. Huber. 2018. "Gram scale synthesis of Fe/FexOy core–shell nanoparticles and their incorporation into matrix-free superparamagnetic nanocomposites." *Journal of Materials Research* 33 (15):2156-2167. doi: 10.1557/jmr.2018.139.

White, L. D., and C. P. Tripp. 2000. "Reaction of (3-Aminopropyl)dimethylethoxysilane with Amine Catalysts on Silica Surfaces." *Journal of Colloid and Interface Science* 232 (2):400-407. doi: https://doi.org/10.1006/jcis.2000.7224.

Whitesell, J. K., and H. K. Chang. 1993. "Directionally aligned helical peptides on surfaces." *Science* 261 (5117):73-6. doi: 10.1126/science.261.5117.73.

Wilhelm, Stefan, Anthony J. Tavares, Qin Dai, Seiichi Ohta, Julie Audet, Harold F. Dvorak, and Warren C. W. Chan. 2016. "Analysis of nanoparticle delivery to tumours." *Nature Reviews Materials* 1 (5). doi: 10.1038/natrevmats.2016.14.

Wu, Liheng, Adriana Mendoza-Garcia, Qing Li, and Shouheng Sun. 2016. "Organic Phase Syntheses of Magnetic Nanoparticles and Their Applications." *Chemical Reviews*. doi: 10.1021/acs.chemrev.5b00687.

Yee, C., G. Kataby, A. Ulman, T. Prozorov, H. White, A. King, M. Rafailovich, J. Sokolov, and A. Gedanken. 1999. "Self-assembled monolayers of alkanesulfonic and -phosphonic

acids on amorphous iron oxide nanoparticles." *Langmuir* 15 (21):7111-7115. doi: Doi 10.1021/La990663y.

Yu, W. W., J. C. Falkner, C. T. Yavuz, and V. L. Colvin. 2004. "Synthesis of monodisperse iron oxide nanocrystals by thermal decomposition of iron carboxylate salts." *Chem Commun (Camb)* (20):2306-7. doi: 10.1039/b409601k.

Zhang, Bingbo, Xiaohui Wang, Fengjun Liu, Yingsheng Cheng, and Donglu Shi. 2012. "Effective Reduction of Nonspecific Binding by Surface Engineering of Quantum Dots with Bovine Serum Albumin for Cell-Targeted Imaging." *Langmuir* 28 (48):16605-16613. doi: 10.1021/la302758g.

Zhang, Z., Z. Wang, S. He, C. Wang, M. Jin, and Y. Yin. 2015. "Redox reaction induced Ostwald ripening for size- and shape-focusing of palladium nanocrystals." *Chem Sci* 6 (9):5197-5203. doi: 10.1039/c5sc01787d.

Figure 6-1

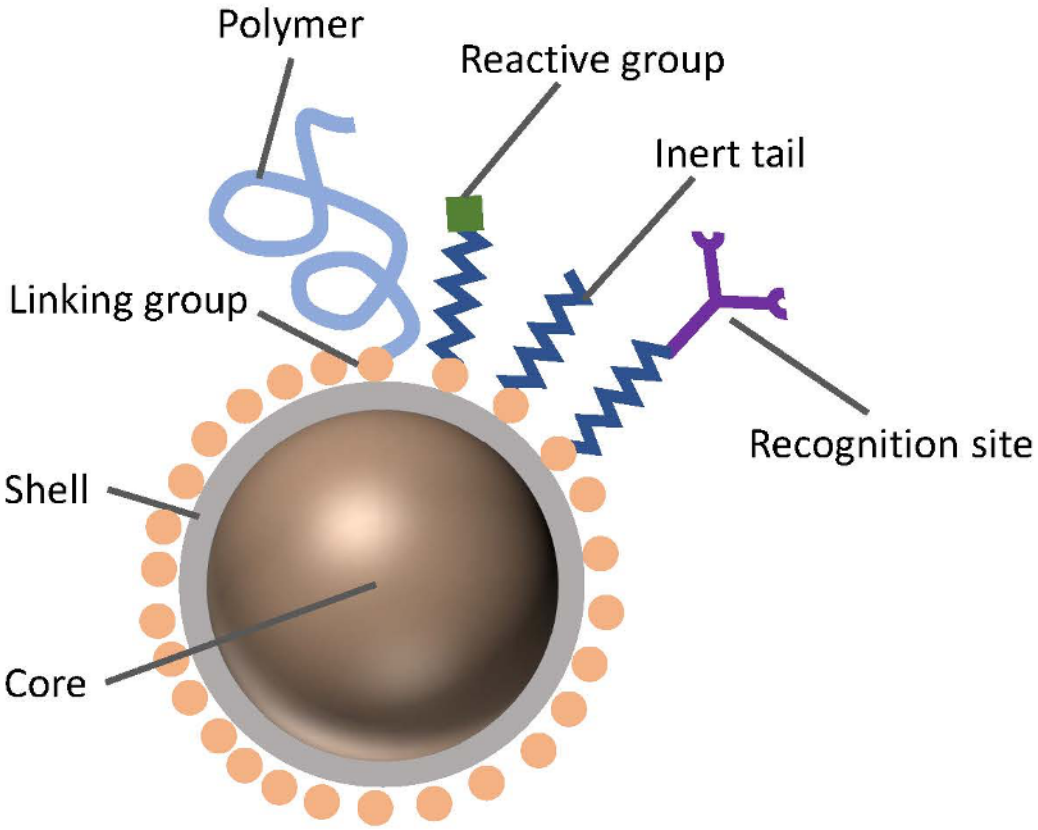

Polymer
Reactive group
Inert tail
Linking group
Recognition site
Shell
Core

Figure 6-2

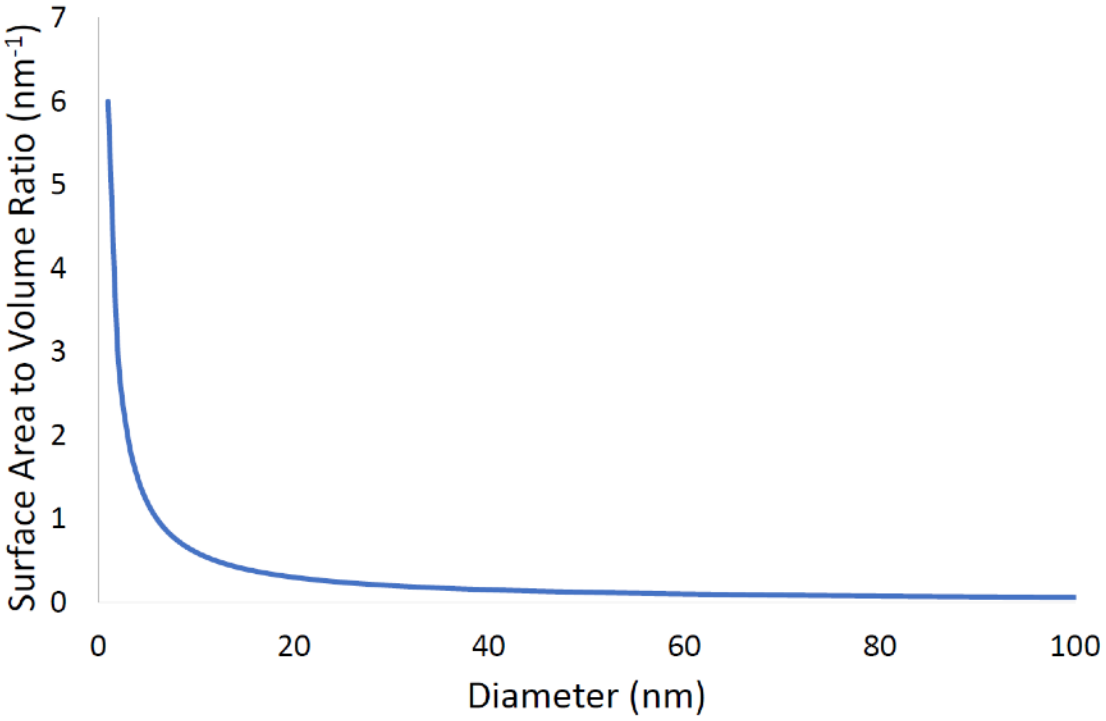

Surface Area to Volume Ratio (nm-1)
7
6
5
4
3
2
1
0
0
20
40
60
80
100
Diameter (nm)

Figure 6-3

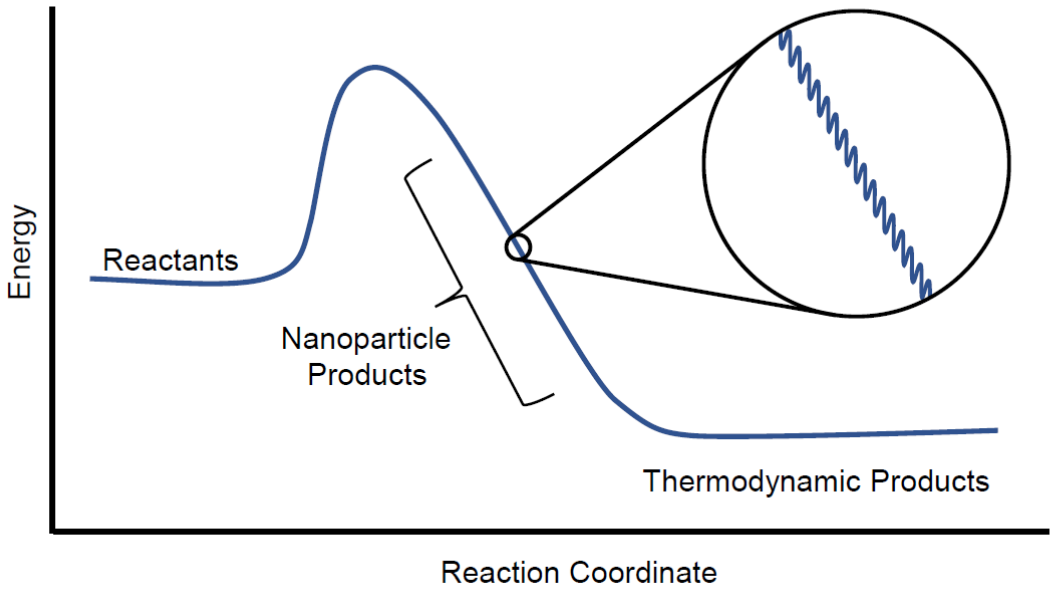

Energy
Reactants
Nanoparticle
Products
Thermodynamic Products
Reaction Coordinate

Figure 6-4

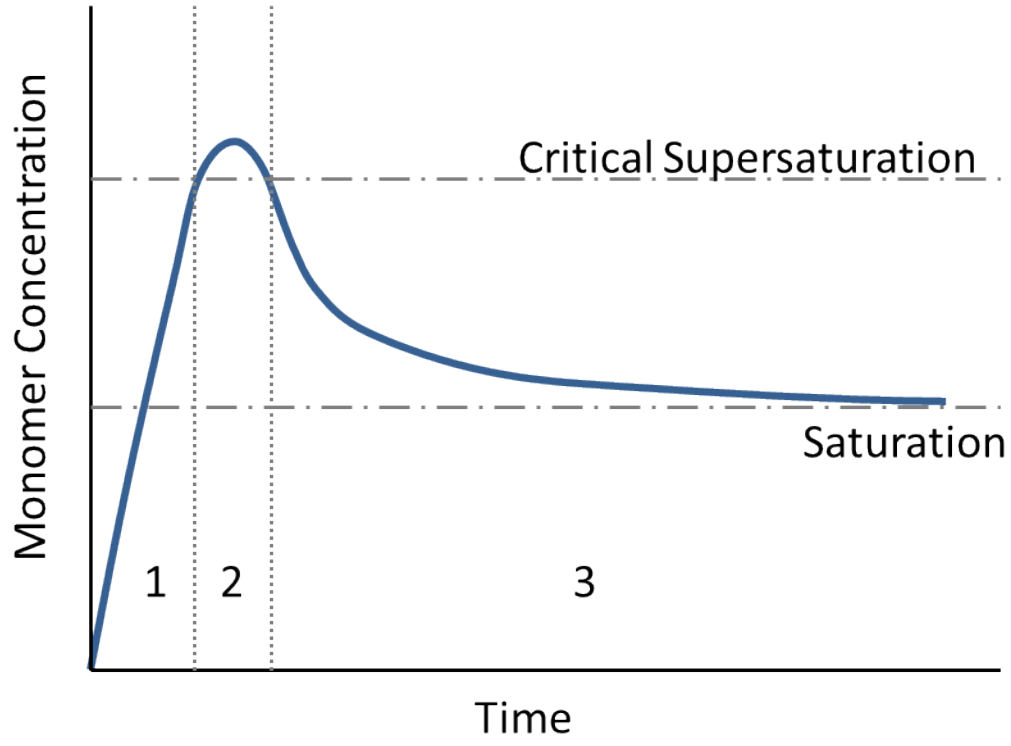

Monomer Concentration
Critical Supersaturation
Saturation
1
2
3
Time

Figure 6-5

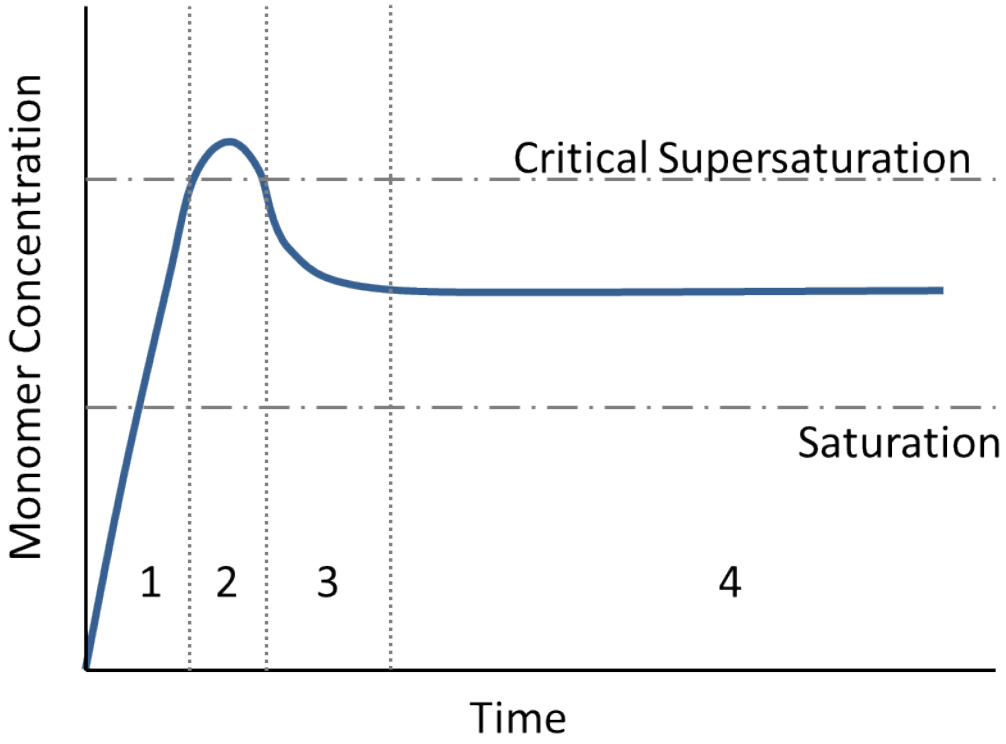

Monomer Concentration
Critical Supersaturation
Saturation
1
2
3
4
Time

Figure 6-6

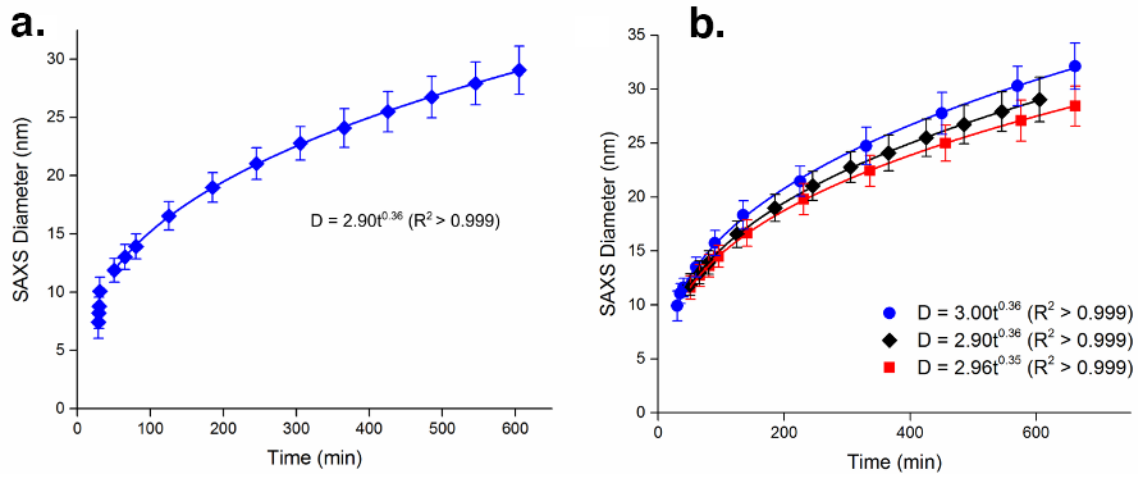

a.
SAXS Diameter (nm)
Time (min)
D = 2.90t^0.36 (R^2 > 0.999)
b.
SAXS Diameter (nm)
Time (min)
D = 3.00t^0.36 (R^2 > 0.999)
D = 2.90t^0.36 (R^2 > 0.999)
D = 2.96t^0.35 (R^2 > 0.999)

Figure 6-7

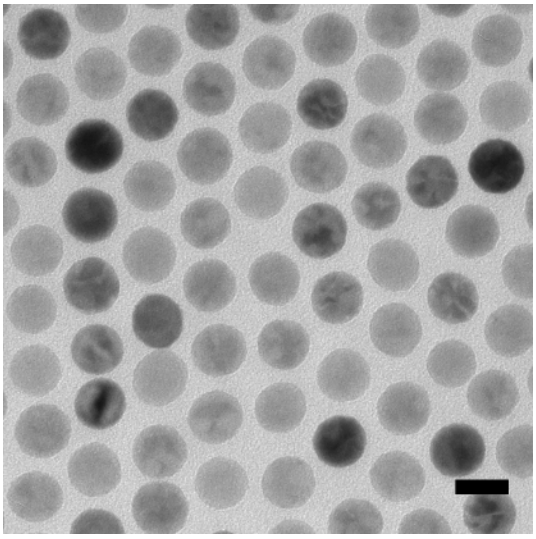


Figure 6-8

1. Initiation

$$I \xrightarrow{k_d} 2(R\cdot)$$

$$R\cdot + M \xrightarrow{k_i} R\text{-}M\cdot = M_1\cdot$$

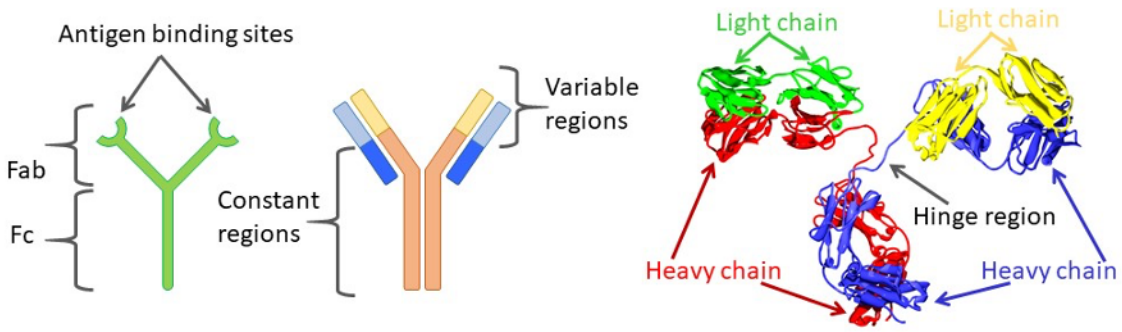
2. Propagation

$$M_n\cdot + M \xrightarrow{k_d} M_{n+1}\cdot$$

3. Termination

$$M_n\cdot + M_m\cdot \xrightarrow{k_t} M_{n+m}$$

Figure 6-9


Antigen binding sites
Fab
Fc
Constant regions
Variable regions
Light chain
Light chain
Hinge region
Heavy chain
Heavy chain